POSTGRADUATE DEGREE PROGRAMME (CBCS)

in

ZOOLOGY

(M.Sc.Programme)

SEMESTER-I

Ability Enhancement Compulsory Course

Basic Principles of methods used in AnimalBiology

ZAECCT-101

SELF LEARNING MATERIAL

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Ability Enhancement Compulsory Course (ZAECCT-101)

Basic Principles of methods used in Animal Biology

PAPER	Unit	Content	Credit	Page No.
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	VI	Methods in field biology: Methods of estimating population density of animals, ranging patterns through direct, indirect and remote observations, sampling methods in the study of behaviour, habitat characterization-ground and remote sensing methods.		96-110
Total counselling session 6 hours				

UNIT-I

Microscopy: Basic concepts of light and electron microscopy (magnification, resolution, limit of resolution, chromatic aberrations)

Objective: In this unit you will learn about different types of microscope such as Fluorescent microscope, confocal microscope, phase contrast microscope and dark field microscope. Besides optical imaging system you will also learn electronic imaging system, such as SEM ad TEM. Basic concept on flow cytometry will also be discussed here.

Introduction: A microscope is an optical instrument that is used for magnifying objects too small to be seen by naked eye. Investigations or studies of cell architecture by means of the microscope are called microscopy, and the person who pursuing the study is called microscopist.

History of Microscopy:

1590: The two Janssen brothers of Holland, Francis Janssen and Zacharias Janssen, who were spectacle makers built the first operational light microscope.
1611: Kepler built the first compound microscope.
1665: Robert Hooke developed the first laboratory microscope which has a magnification of 14-42 X. He observed small pores in sections of cork that he called cells.
1674: Leeuwenhoek discovered protozoa by his self built microscope with magnification of 270 X. He discovered bacteria for the first time 9 years later.
1905: Zsigmondy invented dark-field microscopy.

Properties of Microscope:

A microscope has dual property i.e. magnification and resolution. The usefulness of a microscope depends not so much on the degree of magnification but rather on the resolution. Resolution has nothing to do with the magnification.

(a) Magnification:

Magnification or magnifying power of a microscope is the degree of increase in size of optical image over the actual size of object being viewed.

Magnification = Size of retinal image seen with microscope/Size of retinal image with naked eye

Magnification of microscope is calculated by multiplying the magnification of the objective lens with that of the eye piece (ocular lens). For example, the magnification of eye piece is

10X and the magnification of objective lens is 40X, then the microscope magnifies the object by 10×40= 400 times i.e. magnification is 400X.

The human eye has no power of magnification, so magnifying glasses maybe used to magnify images up to about 10 times. A light microscope in which combination of lens used has a magnification of 100-2000 X. For higher magnification over400X, oil immersion lens can be used in which cedar wood oil placed between objective and the coverslip increase the light gathering properties of the lens.

Units of Measurement used in Microscopy:

1 metre (m) = 10^2 cm = 10^3 mm = 10^6 mm = 10^9 nm = 10^{10} A
1 centimeter (cm) = 1/100 metre (m) = 0.4 inch

1 millimetre (mm) =1/1000 metre = 0.001 m – 10^{-3} m = 10^{-3} mm = 10^6 nm = 10^7 A 1 micrometre (mm)** = 1 /1000 mm = 0.001mm = 10^{-3}mm = 10^{-6} m = 10^3 nm = 10^4 A 1 nanometer (nm) = 1/1000 mm = 0.001mm = 10^{-3} mm = 10^{-6} mm = 10^{-9} m = 10 A
1 angstrom $(A)^+$ = 1/10 nm = 0.1 nm = 10^{-1} nm = 10^{-7}mm = 10^{-10}m
Micrometers were formerly known as microns (μ), and nanometers as millimicrons (mμ).

The Angstrom is not an accepted measurement in the International system of Units. It is included here, however, because it was widely used in microscopey in the past.

(b) Resolution or Resolving power:

Resolution (= resolving power or resolving limit) of an optical device (eye or microscope) is its ability to distinguish between two very closely placed objects as separate objects. The resolving power of a microscope depends on (i) Wavelength of light (A) and (it) numerical-aperture (NA) of the lens system used. Resolution of a microscope can be calculated by Abbe Equation, after the name of German Physicist Ernst Abbe in 1876.

Resolution = $0.61\lambda/NA = 0.61\lambda/N \sin\theta$

0.61 = a trigonometric constant

λ = Wavelength of light used; 450-750 mm for visible light used in compound microscope, Blue light has shortest wavelength (λ =450nm) gives maximum resolution. Therefore, blue filter blue light commonly used in microscopy.

NA – N Sin θ; where N is the refractive index of the medium (usually air or oil) between the specimen and objective lens. For air N = 1.0 and for immersion oil N = 1.5.

θ or a- half angle of the cone of light entering the objective lens from the specimen. The maximum value of 6 for the best objective lens is 70° (Sin 70° = 0.94). The resolution of light microscope, using air and blue light, will be

Lm= 0.61x 450nm/ 1.0x 0.94= 292nm or – 0.3µm

If oil and blue light used, then Lm = 0.61x 450nm/ 1.0x 0.94= 194nmor – 0.2 µm

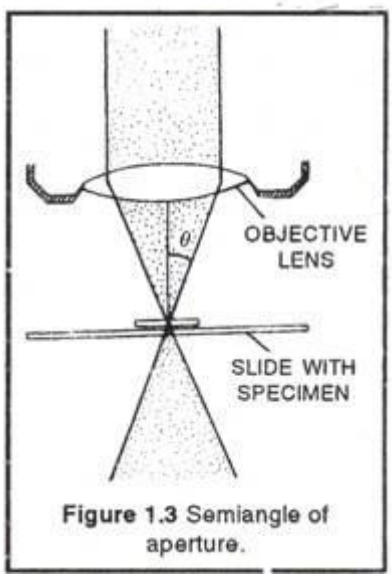


Figure 1.3 Semiangle of aperture.

Thus, light microscope can never resolve two closer particles less than about 0.2 nm apart, no matter how many times the image is magnified. The resolution of electron microscope is about 0.0005 µm whereas the human eye is about l00 µm. It should be noted that lower the value of Lm Higher will be resolution, which can be done by changing A, N or 6. Resolution will increase with a decrease in A and with an increase in NA; i.e.Lm is inversely proportional to A and Lm is proportional to NA. The numerical aperture (NA) is the light collecting ability of lens.

A. Light Microscope:

A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects, and enlarging them.They use lenses to focus light on the specimen, magnifying it thus producing an image. The specimen is normally placed close to the microscopic lens.Microscopic magnification varies greatly depending on the types and number of lenses that make up the microscope. Depending on the number of lenses, there are two types of microscopes i. e Simple light microscope (it has low magnification because it uses a single lens) and the Compound light microscope (it has a higher magnification compared to the simple microscope because it uses at least two sets of lenses, an objective lens, and an eyepiece). The lenses are aligned in that, they can be able to bend light for efficient magnification of the image.

The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent, to produce an image. The image is then passed through one or two lenses for magnification for viewing. The transparency of the

specimen allows easy and quick penetration of light. Specimens can vary from bacterial to cells and other microbial particles.

Principle of Light Microscope:

As mentioned earlier, light microscopes visualize an image by using a glass lens and magnification is determined by, the lens's ability to bend light and focus it on the specimen, which forms an image. When a ray of light passes through one medium into another, the ray bends at the interface causing **refraction.** The bending of light is determined by the **refractive index**, which is a measure of how great a substance slows the speed of light. The direction and magnitude of the bending of the light are determined by the refractive indexes of the two mediums that form the interface.

A medium with a lower refractive index such as glass to air, it normally speeds up the light penetration and making light bend away from the normal and when light is passed through a medium with a greater refractive index such as air to glass, it normally slows down and bends towards the normal, perpendicularly to the surface.

If an object is put between these two mediums i.e between water and air, in this case, a prism, the prism will bend the light at an angle. This is how the microscopic lenses work, they bend the light at an angle. The lens (convex) on receiving the light rays, it focuses the rays at a specific point known as the **focal point (F-point)**. The measure of distance from the center of the lens and the focal point is known as the **focal length.** A microscope uses lenses whose strength is predetermined, in that, the strength of a lens is directly related to the focal length i.e short focal length magnifies objects more than lenses with a long focal length.

Microscopy works strictly with a factor of resolution whereby resolution being the ability of a lens to be able to differentiate small objects that are closely packed together. The resolution of a light microscope is determined by a **numerical aperture** of its lens system and by the wavelength of the light it employs; a numerical aperture a definition of the light wavelengths produced when the specimen is illuminated. A minimum distance (d) between two objects that distinguishes then to be two separate entities, determined by the wavelengths of the light can be calculated by an Abbe equation using the wavelength of the light that illuminated the specimen (Lambda, **λ**) and the numerical aperture (NA, n sin Θ) i.e. **$d=0.5\ \lambda/n \sin \Theta$**

Types of light microscopes (optical microscope):

With the evolved field of Microbiology, the microscopes used to view specimens are both simple and compound light microscopes, all using lenses. The difference is simple light microscopes use a single lens for magnification while compound lenses use two or more lenses for magnifications. This means that a series of lenses are placed in an order such that, one lens magnifies the image further than the initial lens.

I. Brightfield Light Microscope (Compound light microscope):

This is the most basic optical Microscope used in microbiology laboratories which produces a dark image against a bright background. Made up of two lenses, it is widely used to view plant and animal cell organelles including some parasites such as *Paramecium* after staining with basic stains.Its functionality is based on being able to provide a high-resolution image, which highly depends on the proper use of the microscope. This means that an adequate amount of light will enable sufficient focusing of the image, to produce a quality image.
It is also known as a compound light microscope.

It is composed of:

- Two lenses which include the **objective lens** and the **eyepiece or ocular lens**.
- Objective lens is made up of six or more glasses, which make the image clear from the object
- The condenser is mounted below the stage which focuses a beam of light onto the specimen. It can be fixed or movable, to adjust the quality of light, but this entirely depends on the microscope.
- They are held together by a sturdy metallic curved back used as an **arm** and a stand at the bottom, known as the **base**, of the microscope. The arm and the base hold all the parts of the microscope.
- The stage where the specimen is placed, allowing movement of the specimen around for better viewing with the flexible knobs and it is where the light is focused on.
- Two focusing knobs i.e the fine adjustment knob and the coarse adjustment knob, found on the microscopes' arm, which can move the stage or the nosepiece to focus on the image. the sharpen the image clarity.
- It has a **light illuminator** or a **mirror** found at the base or on the microbes of the nosepiece.
- The nosepiece has about three to five objective lenses with different magnifying power. It can move round to any position depending on the objective lens to focus on the image.
- An aperture diaphragm also is known as the contrast, which controls the diameter of the beam of light that passes through the condenser, in that, when the condenser is almost closed, the light comes through to the center of the condenser creating high contrast. But when the condenser is widely open, the image is very bright with very low contrast.

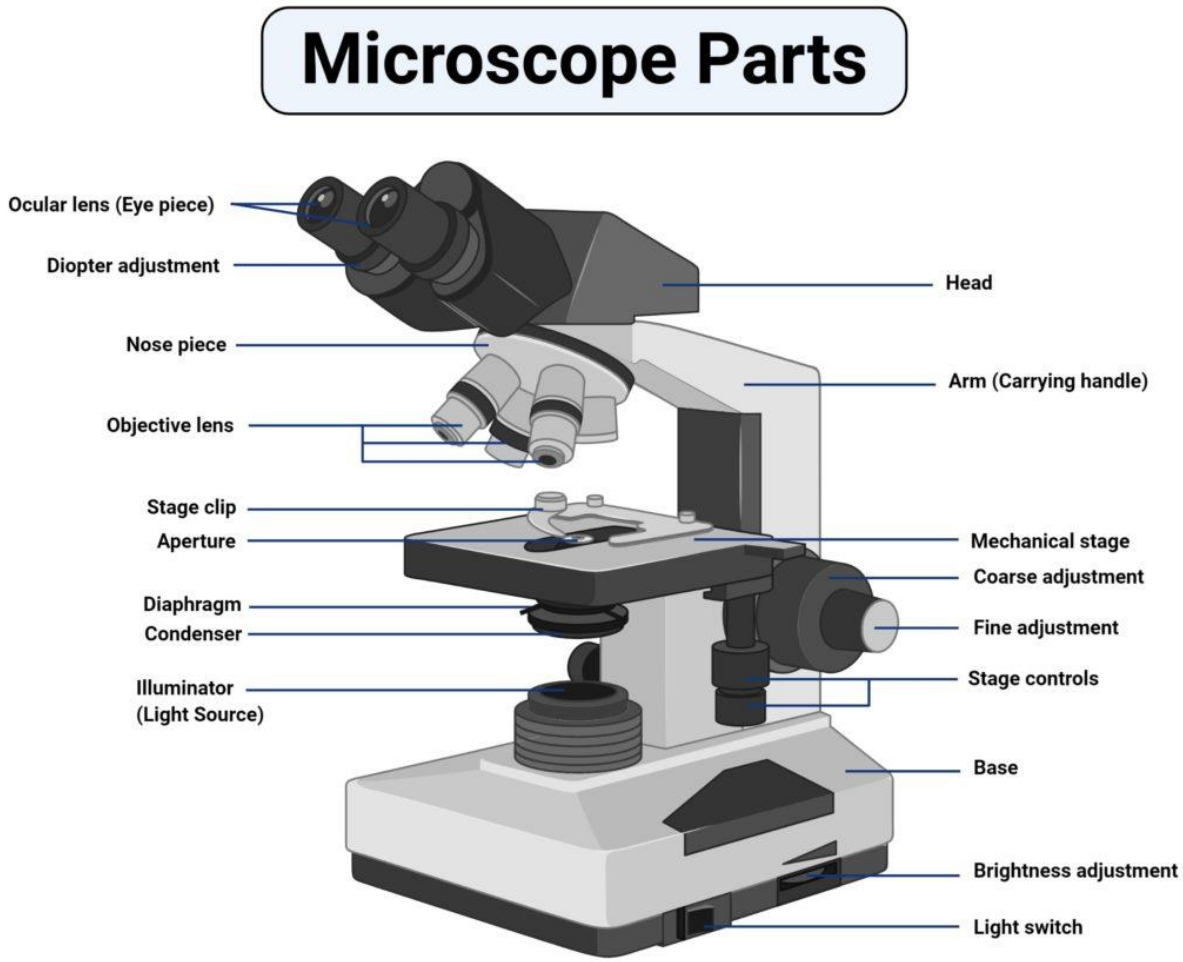


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Magnification by Bright field Microscope (Compound light microscope):

During visualization, the objective lens remains parfocal which means, when the objective lens is changed, the image still remains in focus. The objective lens plays a major role in focusing the image on the condenser forming an enlarged clear image within the microscope, which is then further magnified by the eyepiece to a primary image.

What is seen in the microscope as an enlarged clear image of the specimen is known as the virtual image. To calculate the magnification, multiply the objective and eyepiece objective magnification together. The magnification is standard, i.e not too high nor too low, and therefore depending on the magnification power of the lenses, it will range between 40X and 100oX.

Calculation of magnification = Magnification of objective lens/magnification of the eyepiece lens. The objective lens plays a vital role in not only enlarging the image but also making it clear for viewing, a feature known as **resolution.** Resolution according to Prescott, is the ability of a lens to separate or distinguish between small objects closely linked together. Whereas the eyepiece magnifies the image at the end of the viewing, its magnification range is lower than that of the objective lens at 8X-12X (10X standard) and that of the objective

lens at 40X-100X, magnification, and resolution of the microscope is highly dependant on the objective lens.

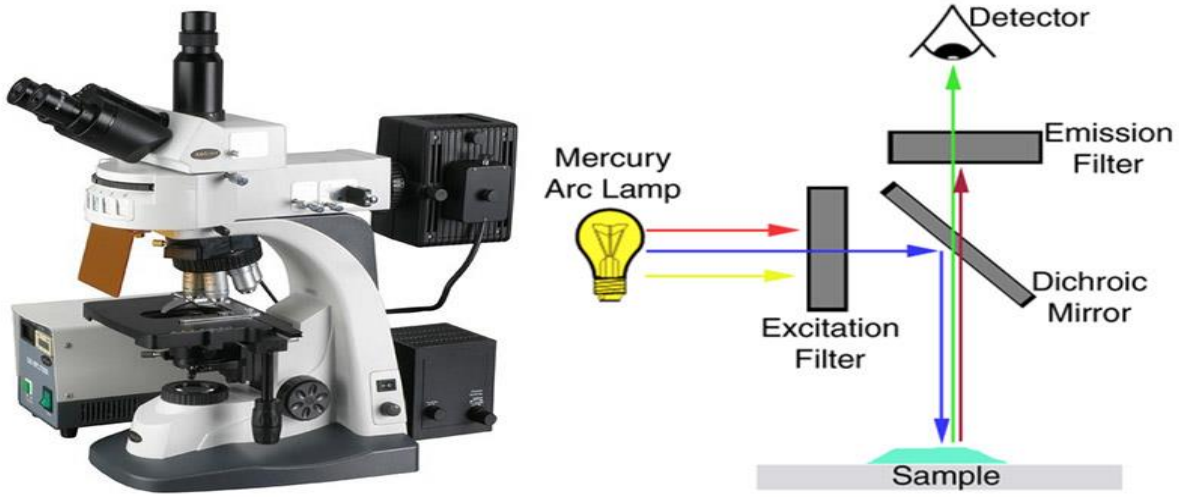
Applications of the Bright Field Light Microscope (Compound light microscope)
Vastly used in Microbiology this microscope is used to view fixed and live specimens, that have been stained with basic stains. This gives contrast for easy visibility under the microscope. Therefore it can be used to identify basic bacteria cells and parasitic protozoans such as *Paramecium.*

II. Fluorescent Microscopes:

A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances.
Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation while phosphorescence is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The fluorescence microscope was devised in the early part of the twentieth century by August Köhler, Carl Reichert, and Heinrich Lehmann, among others.

Principle of fluorescence microscope :

Most cellular components are colourless and cannot be clearly distinguished under a microscope. The basic premise of fluorescence microscopy is to stain the components with dyes. Fluorescent dyes, also known as fluorophores or fluorochromes, are molecules that absorb excitation light at a given wavelength (generally UV), and after a short delay emit light at a longer wavelength. The delay between absorption and emission is negligible, generally on the order of nanoseconds.
The emission light can then be filtered from the excitation light to reveal the location of the fluorophores. Fluorescence microscopy uses a much higher intensity light to illuminate the sample. This light excites fluorescence species in the sample, which then emit light of a longer wavelength. The image produced is based on the second light source or the emission wavelength of the fluorescent species rather than from the light originally used to illuminate, and excite, the sample.



Working

Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused on the detector by the objective. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light.

Forms

The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image. Most fluorescence microscopes in use are epifluorescence microscopes, where excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e. through the objective).

Typical components of a fluorescence microscope are:

a. Fluorescent dyes (Fluorophore)

A fluorophore is a fluorescent chemical compound that can re-emit light upon light excitation. Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.

Many fluorescent stains have been designed for a range of biological molecules. Some of these are small molecules that are intrinsically fluorescent and bind a biological molecule of interest. Major examples of these are nucleic acid stains like DAPI and Hoechst, phalloidin which is used to stain actin fibers in mammalian cells.

b. A light source

Four main types of light sources are used, including xenon arc lamps or mercury-vapor lamps with an excitation filter, lasers, and high- power LEDs.

Lasers are mostly used for complex fluorescence microscopy techniques, while xenon lamps, and mercury lamps, and LEDs with a dichroic excitation filter are commonly used for wide-field epifluorescence microscopes.

c. The excitation filter

The exciter is typically a bandpass filter that passes only the wavelengths absorbed by the fluorophore, thus minimizing the excitation of other sources of fluorescence and blocking excitation light in the fluorescence emission band.

d. The dichroic mirror

A dichroic filter or thin-film filter is a very accurate colour filter used to selectively pass light of a small range of colours while reflecting other colours.

e. The emission filter.

The emitter is typically a bandpass filter that passes only the wavelengths emitted by the fluorophore and blocks all undesired light outside this band – especially the excitation light.
By blocking unwanted excitation energy (including UV and IR) or sample and system autofluorescence, optical filters ensure the darkest background.

Advantages of fluorescence microscope:

1. Fluorescence microscopy is the most popular method for studying the dynamic behaviour exhibited in live-cell imaging.
2. This stems from its ability to isolate individual proteins with a high degree of specificity amidst non-fluorescing material.
3. The sensitivity is high enough to detect as few as 50 molecules per cubic micrometer.
4. Different molecules can now be stained with different colours, allowing multiple types of the molecule to be tracked simultaneously.
5. These factors combine to give fluorescence microscopy a clear advantage over other optical imaging techniques, for both in vitro and in vivo imaging.

Limitations of fluorescence microscope:

1. Fluorophores lose their ability to fluoresce as they are illuminated in a process called photobleaching. Photobleaching occurs as the fluorescent molecules accumulate chemical damage from the electrons excited during fluorescence.
2. Cells are susceptible to phototoxicity, particularly with short-wavelength light. Furthermore, fluorescent molecules have a tendency to generate reactive chemical species when under illumination which enhances the phototoxic effect.
3. Unlike transmitted and reflected light microscopy techniques fluorescence microscopy only allows observation of the specific structures which have been labelled for fluorescence.

III. ConfocalMicroscope:

Optical sections are produced in the laser scanning confocal microscope by scanning the specimen point by point with a laser beam focussed in the specimen, and using a spatial filter, usually a pinhole (or a slit), to remove unwanted fluorescence from above and below the focal plane of interest (Fig. 4.11). The power of the confocal approach lies in the ability to image structures at discrete levels within an intact biological specimen. There are two major advantages of using the LSCM in preference to conventional epifluorescence light microscopy. Glare from out-of-focus structures in the specimen is reduced and resolution is increased both laterally in the X and the Y directions (0.14 mm) and axially in the Z direction (0.23 mm). Image quality of some relatively thin specimens, for example, chromosome spreads and the leading lamellipodium of cells growing in tissue culture (<0.2 mm thick) is not dramatically improved by the LSCM whereas thicker specimens such as fluorescently labelled multicellular embryos can only be imaged using the LSCM.

For successful confocal imaging, a minimum number of photons should be used to efficiently excite each fluorescent probe labelling the specimen, and as many of the emitted photons from the fluorochromes as possible should make it through the light path of the instrument to the detector. The LSCM has found many different applications in biomedical imaging. Some of

these applications have been made possible by the ability of the instrument to produce a series of optical sections at discrete steps through the specimen (Fig. 4.12). This series of optical sections collected with a confocal microscope are all in register with each other, and can be merged together to form a single projection of the image (Z projection) or a 3D representation of the image (3D reconstruction).

Multiple-label images can be collected from a specimen labelled with more than one fluorescent probe using multiple laser light sources for excitation (Fig. 4.13, see also colour section). Since all of the images collected at different excitation wavelengths are in register it is relatively easy to combine them into a single multicoloured image. Here any overlap of staining is viewed as an additive colour change. Most confocal microscopes are able to routinely image three or four different wavelengths simultaneously. The scanning speed of most laser scanning systems is around one full frame percentage second. This is designed for collecting images from fixed and brightly labelled fluorescent specimens. Such scan speeds are not optimal for living specimens, and laser scanning instruments are available that scan at faster rates for more optimal live cell imaging. In addition to point scanning, swept field scanning rapidly moves a mm thin beam of light horizontally and vertically through the specimen.

Optical sectioning: Many images collected from relatively thick specimens produced using epifluorescence microscopy are not very clear. This is because the image is made up of the optical plane of interest together with contributions from fluorescence above and below the focal plane of interest. Since the conventional epifluorescence microscope collects all of the information from the specimen, it is often referred to as a wide field microscope. The ‘out-of-focus fluorescence’ can be removed using a variety of optical and electronic techniques to produce optical sections. The term optical section refers to a microscope’s

ability to produce sharper images of specimens than those produced using a standard wide field epifluorescence microscope by removing the contribution from out-of-focus light to the image, and in most cases, without resorting to physically sectioning the tissue. Such methods have revolutionised the ability to collect images from thick and fluorescently labelled specimens such as eggs, embryos and tissues. Optical sections can also be produced using high-resolution DIC optics, micro computerised tomography (CT) scanning or optical projection tomography. However, currently by far the most prevalent method is using some form of confocal or associated microscopical approach.

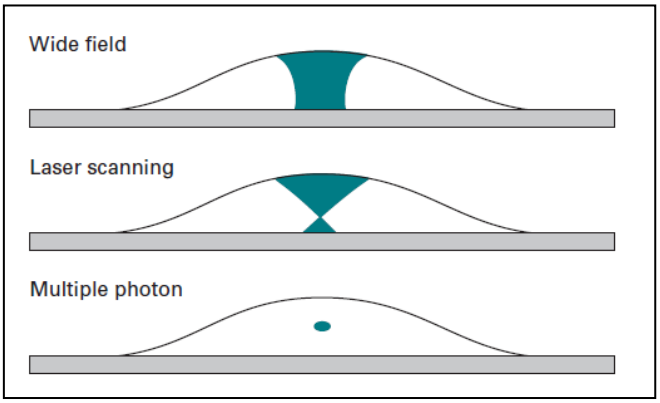


Figure: Illumination in a wide field, a confocal and a multiple photon microscope. The diagram shows a schematic of a side view of a fluorescently labelled cell on a coverslip. The shaded green areas in each cell represent the volume of fluorescent excitation produced by each of the different microscopes in the cell. Conventional epifluorescence microscopy illuminates throughout the cell. In the LSCM fluorescence illumination is throughout the cell but the pinhole in front of the detector excludes the out-of-focus light from the image. In the multiple photon microscope, excitation only occurs at the point of focus where the light flux is high enough.

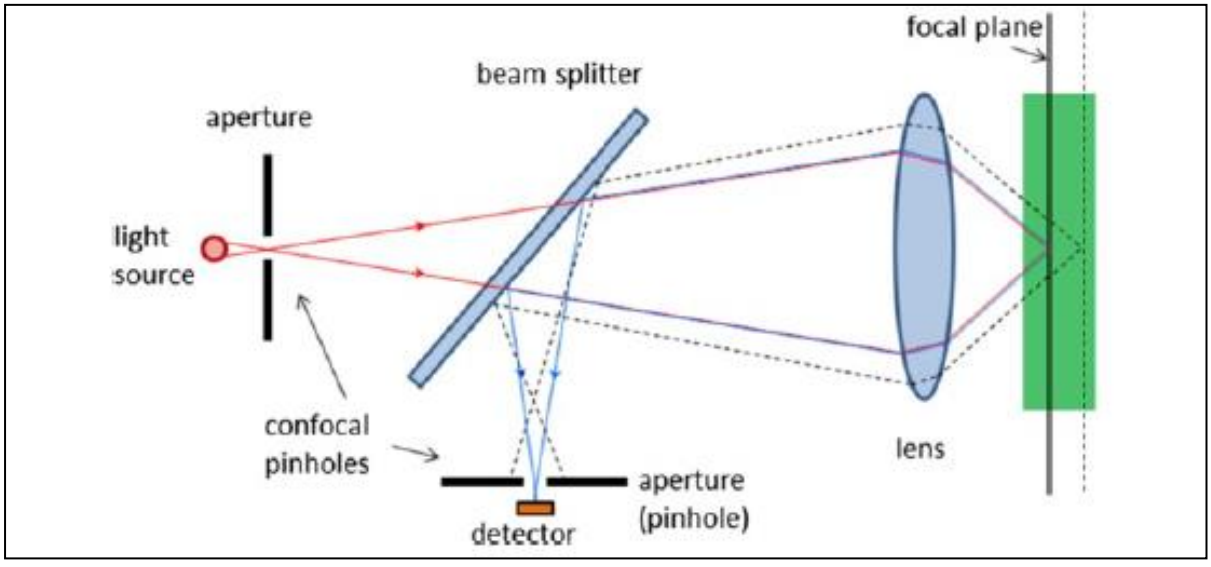


Figure: The principle of confocal microscopy. Only light reflected by structures very close to focal plane can be detected.

IV. Phase Contrast Microscope:

In recent years, remarkable advances have been made in the study of living cells (unstained) by the development of special optical techniques such as phase contrast and interference are highly transparent to visible light and they cause phase changes in transmitted radiations microscopy.

The biological specimens.

The phase contrast microscope has the same resolving power as the ordinary light microscope but it permits visualization of different parts of the cell due to differences in their refractive index (Refractive index is defined as the ratio of the velocity of light in a vacuum to its velocity in a transmitting medium).

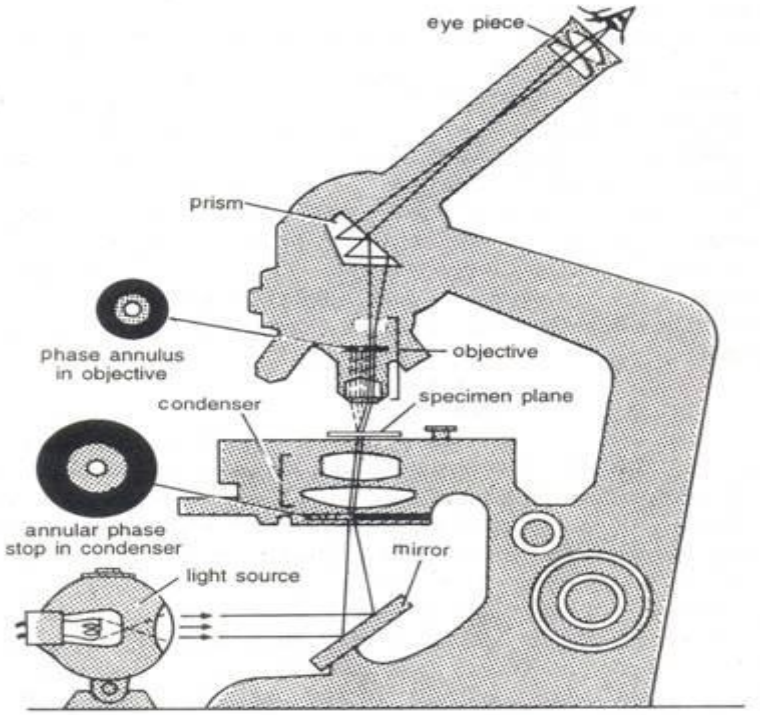


Fig. 2. The light path in a phase contrast microscope.

Because light is transmitted through a structure at a velocity inversely proportional to the refractive index of the structure, light waves emerging from structures with different refractive index will be out of phase with one another. The phase contrast microscope is able to convert these differences in phase to differences in light intensity, producing an image with good contrast. The phase-contrast microscope utilizes interference between two beams of light.

In the phase contrast microscope, the small phase differences are intensified. The most lateral light passing through the objective lens of the microscope is advanced or retarded by an additional l/4th wavelength (1/4λ.) with respect to the central light passing through the medium around the object, by an annular phase plate that introduces a 1/4 wavelength variation in the back focal plane of the objective.

In addition an annular diaphragm is placed in the substage condenser. The phase effect results from the interference between the direct geometric image given by the central part of the objective and the lateral diffracted image, which has been retarded or advanced to a total of 1/2 wavelength. In bright or negative contrast, the two sets of rays are added and the object appears brighter than the surroundings. In dark or positive contrast, the two sets of rays are subtracted making the image of the object darker than the surroundings. Because of this interference, the minute phase changes within the object are amplified and intensified.

A transparent object thus appears in various shades of gray, depending upon the thickness of the object and the difference between the refractive indices of the object and the medium. Phase microscopy is used to observe living cells and tissues. It is particularly valuable for observing the cells cultured in vitro during mitosis. In addition an annular diaphragm is placed in the substage condenser. The phase effect results from the interference between the direct geometric image given by the central part of the objective and the lateral diffracted image, which has been retarded or advanced to a total of 1/2 wavelength. In bright or negative contrast, the two sets of rays are added and the object appears brighter than the surroundings. In dark or positive contrast, the two sets of rays are subtracted making the image of the object darker than the surroundings. Because of this interference, the minute phase changes within the object are amplified and intensified.

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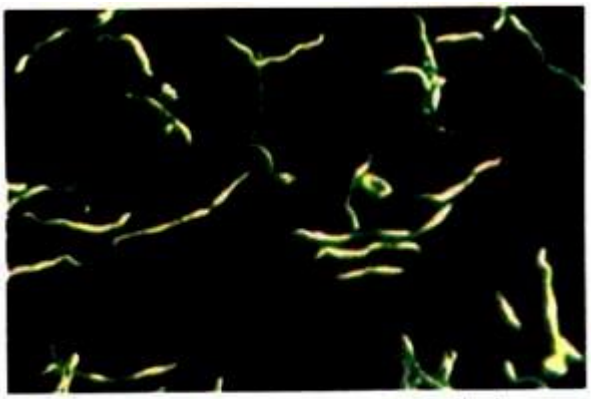
Principle of the phase-contrast microscope:

This is to convert small phase differences into differences in contrast that can be detected visually. An annular phase plate is placed in the objective of the microscope and an annular diaphragm is placed in the condenser as shown in the figure 2. As light is transmitted through the lenses, some of the rays pass through in a direct path while others are diffracted laterally. Diffracted light rays are, thus out of phase with the direct light, and an image of strong contrasts is produced. The annular diaphragm illuminates the object with a narrow cone of light, and the annular phase plate produces a variation of 1/4 A. between the diffracted lateral light and the direct light. The phase effect is the result of interference between the direct image in the centre of the objective and the diffracted lateral image. If the diffracted image is retarded, negative contrast results, whereas if it is advanced, positive contrast results. When the refractive index of the medium is greater than that of the object, the object is dark, and when the refractive index of the medium is less than that of the object, the object is bright.

V. Dark Field Microscope:

In this type of microscopy, a dark back ground is produced against which objects are brilliantly illuminated. For this purpose the light microscope is equipped with a special kind of condenser that transmits a hollow core of light from the source of illumination. Thus, if the aperture of condenser is allowed to open completely, and a dark field stop inserted below the condenser, the light rays reach the objects form a hollow core.

Any object within this beam of light will reflect some light into the objective and will be visible. This method of illuminating an object where the object appears self-illuminous against a dark field, called dark-field illustration.
The condensers used are Abbe condenser, paraboloid condenser and cardoid condenser. Dark field microscopy is particularly valuable for the examination of unstained microorganisms suspended in fluid wet mount and hanging drop operations.



Treponema pallidum, the spirochete that causes syphilis; dark-field microscopy.

B. Electron Microscopy:

In 1931 Knoll and Ruska, German scientists discovered electron microscopy. Von Borries and Ruska (1938) in Berlin constructed first practical electron microscope. The commercial instrument first came in around 1940.In electron microscope the source of illumination is electron beam. The construction and principle of electron microscope are easily related to those of light microscope. The range of wave length of visible light used in light microscope is 4000 Å – 7800 Å, while with an electron microscope employing 60-80 KV electron, the wave length is only 0.05 Å.
In the instrument as shown in the figure, the electron gun generates electron beam. These electrons are concentrated by other components of electron gun producing a fast moving narrow beam of electron.
Electrons are focused by electromagnetic lenses. Electromagnetic lens consists of wire encased in soft iron casing. When electric current is passed through the coil, it generates an electromagnetic field through which electrons are focused.
There are three general types of electromagnetic lenses. The one is placed between the source of illumination and the specimen. This focuses the beam of electron on specimen functions in a similar manner as that of light microscope. The other two lenses are on the opposite side of specimen which magnify the image in similar fashion as objective and ocular in light microscope.

General Principle of EM:

The fundamental principle of EM is similar to those of LM. In EM, a high velocity beam of electrons (instead of light) is used to travel in a vacuum tube. The beam of electrons is focused by a series of electromagnetic lenses analogous to the condenser, objective and eye piece lenses of the light microscope.

The object is placed between the condenser and objective. The magnified image of the object is formed on the fluorescent screen or on photographic film rather than being observed through eye piece. Since the image produced by electrons does not have the colour, the electron micrograph always has shades of black, grey and white.The objects under examination must be extremely thin and are treated with chemicals or dyes to enhance the contrast as such the live objects cannot be studied. Techniques like negative staining, shadow casting and tracers are commonly used to increase the contrast.

Theoretically, the maximum resolution of the EM is 0.005 nm which is less than the diameter of a single atom, or 40,000 times the resolution of the light microscope and 2 million times that of the naked eye. However, the practical resolution of modern EM is of 0.1 nm (1 A).

Construction of an Electron Microscope:

An electron microscope consists of an electric gun, microscope column, electromagnetic coils, a fluorescent screen and some other accessories described below:

(a) The electron gun is located at the top of the body of microscope. It is the source of electrons. It is made up of a tungsten filament surrounded by a negatively biased shield with an aperture. The electron beam is drawn off through this aperture.
(b) The microscope column or central column is made up of an evacuated metal tube. It protects the person operating the microscope from X-rays that are generated when the electrons strike the surface of the metal tube.
(c) The electromagnetic coils or lenses include projector coils, objective arid condenser. In each coil, the coils of electric wire are wound on a hollow metallic cylinder. The magnetic field, produced by passing the electric current through the magnetic coil, functions as a magnifying lens.
(d) The fluorescent screen is used for observing the magnified image of the object. It remains coated with a chemical which, on being excited, forms the image as on the screen of television.
(e) Some other essential accessories of the electron microscope include high voltage transformers (for developing high voltage current for the electron gun and electromagnetic coils), vacuum pumps (for maintaining high vacuum inside the microscope column), a water cooling system (for prevention from overheating of various parts), a circulating pump, a refrigeration plant and also a filter system.

All these parts require elaborate arrangements and contribute to the massive size of the electron microscope. The image formation in this microscope occurs by the scattering of electrons. The electrons strike the atomic nuclei and get dispersed. These dispersed electrons

form the electron image. By projecting on a fluorescent screen or photographic film, this electron image is converted into a visible image of the object. The electron beam in this microscope is made by accelerating electrons through a potential difference of from 1-1500 kV in an electron gun.

Only dried specimens are studied by electron microscope. Living cells cannot be studied with this microscope because they possess water which causes large scale scattering of electrons.

Ultrathin sections (10-50 nm thickness), which are more than 200 times thinner than those routinely used for light microscopy, are cut for electron microscopy. These are cut with the help of diamond or glass knives of an ultra-microtome.

Electron microscopes are of two types:

(1) Transmission electron microscope, and

2) Scanning electron microscope.

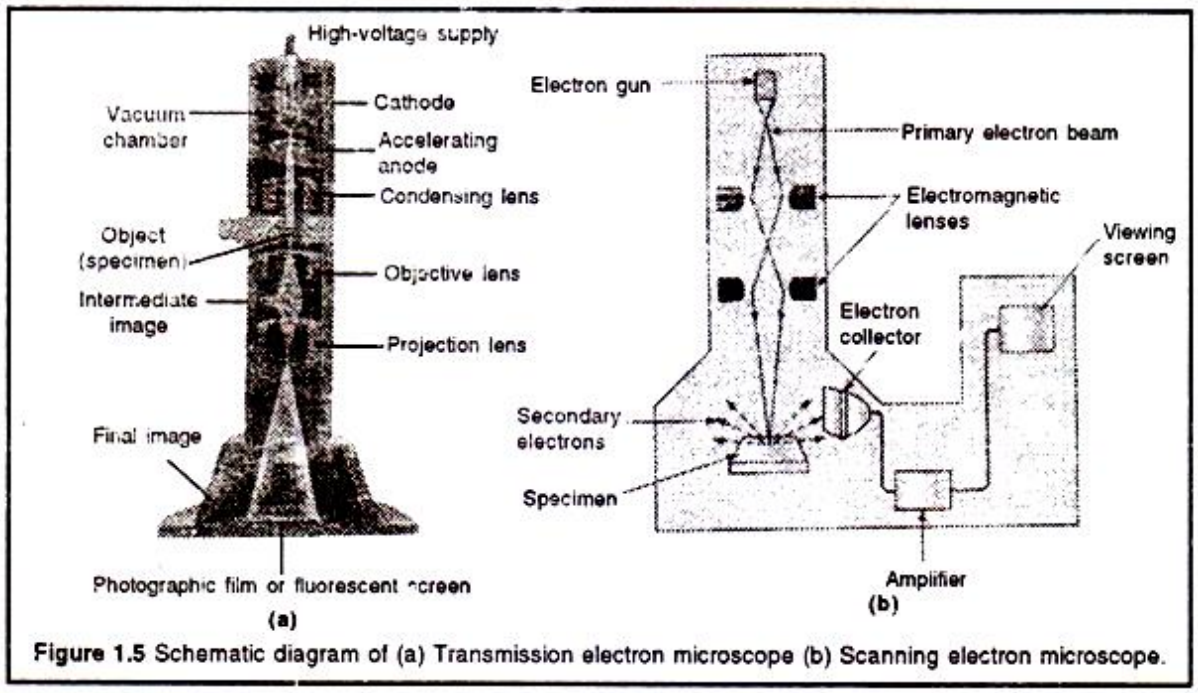


Figure 1.5 Schematic diagram of (a) Transmission electron microscope (b) Scanning electron microscope.

a. Transmission Electron Microscope:

This microscope forms an image of the specimen by the electrons that have passed through the specimen (Fig. 21.2). The components of the specimen that scatter electrons appear dark and are called **"electrons dense".** The part that have less ability of electron scattering appear light.

The electron scattering ability of the element with higher atomic number, such as, uranium, lead etc. is greater than those of lower atomic numbers. The biological molecules are composed of the elements with comparatively low atomic number, viz., hydrogen, carbon, nitrogen, oxygen, phosphorus and sulphur. These elements have poor electron scattering ability. Therefore, biological molecules are stained with metals of high atomic number such as uranium, lead and osmium.

The material is fixed in osmium tetrachloride, $KMnO_4$ or phosphotungstic acid. The fixed tissues are then embedded in hard plastic resin. Ultra-microtome is used to cut ultrathin sections (50-100 nm) of the material. These sections are examined under the electron microscope. Intact organelles and viruses are not sectioned. Followings are some techniques used to observe the materials by electron microscope.

Structural parts of a TEM- The structural parts of a TEM are as follows :

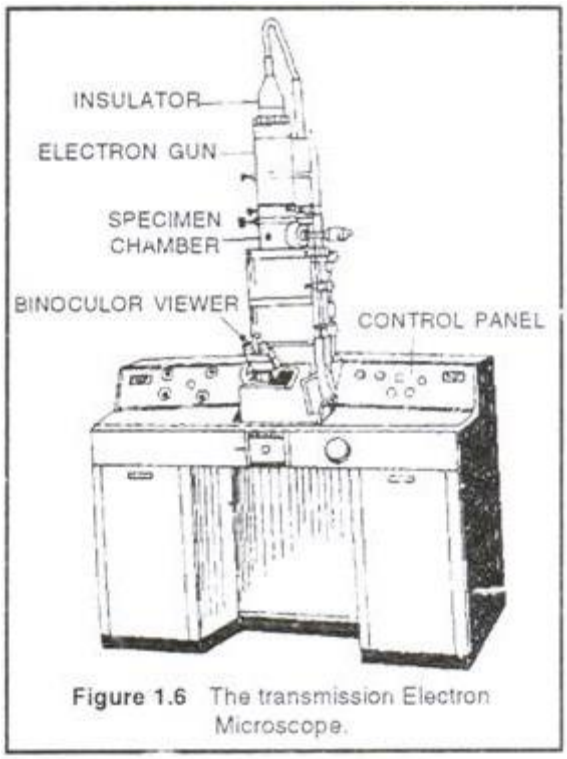


Figure 1.6 The transmission Electron Microscope.

(a) Electron gun:

It consists of a tungsten filament or cathode that emits electrons on receiving high voltage electric current (50,000-100,000 volts). Near the top of the tube is an anode which attracts electrons.

(b) Ray tube (Microscope Column):

It is a high vacuum metal tube (2mt. high) through which electrons travel.

(c) Condense lens:

It is the electromagnetic coil which focuses the electron beam in the plane of the specimen.

(d) Objective lens:

It is the electromagnetic coil which produces the first magnified image formed by the objective lens and produces the final image.

(e) Projector lens:

It is also an electromagnetic coil which further magnifies the first image formed by the objective lens and produces the final image. Each electromagnetic coil has a coil of wire encased by a soft iron casing.

(f) Fluorescent Screen or Photographic Film:

Since unaided human eye cannot observe electrons, therefore, a fluorescent screen is used for viewing the final image of the specimen. The final image can be captured on photographic film and die photograph obtained is called an electron micrograph.

Preparation of material for TEM:

The material to be studied under electron microscope must be well preserved, fixed, completely dehydrated, ultrathin and impregnated with heavy metals that sharpen the difference among various organelles. The material is preserved by fixation with glutaraldehyde and then with osmium tetroxide. The fixed material is dehydrated and then embedded in plastic (epoxy resin) and sectioned with the help of diamond or glass razor of ultra-microtome.

The sections are ultrathin about 50-100 nm thick. These sections are placed on a copper grid and exposed to electron dense materials like lead acetate, uranylacetate, palladium vapours, phosphotungstate etc. Now the sections can be viewed in the TEM. The coating with electron dense material enables the specimen to withstand electric bombardment.

Shadow Casting:

This technique is used to un-sectioned materials e.g., viral particles. The sample dried on a film supported by a grid is placed in an evaporation chamber. The chamber is evacuated. Heavy metal atoms projected from a glowing filament impinge at a predetermined angle on the film (Fig. 21.4).

The metal is deposited as a uniform electron opaque layer on the film. The metal is deposited on one side of the specimen, while the other side lacks the deposition.

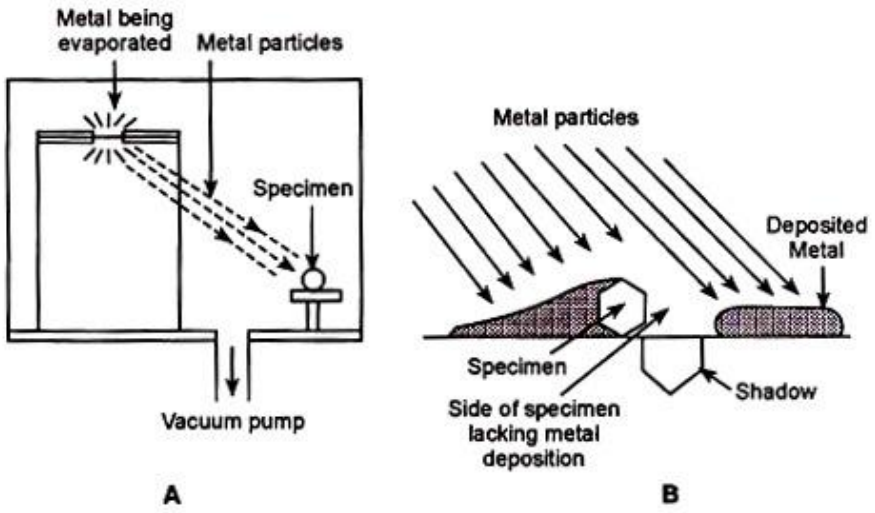


Fig. 21.4. Diagram showing method of shadow casting. **A.** Evaporation chamber. **B.** Shadow of specimen under electron microscope.

Examination under the electron microscope shows the "shadow" of the specimen in the place lacking the deposited metal. The size and shape of shadow provide the information on the 3-dimensional shape of the material.

Positive Staining:

The specimen is stained and than the excess of stain is removed. It gives an unstained background and stained object. Certain viruses can be stained by salts that become absorbed selectively. For example uranyl acetate stains the viral nucleic acid and other components. Abs conjugated to ferritin (electron opaque molecule) stains the protein.

Negative Staining:

Negative staining can be used to study the viral particles and organelles. The viral particles are mixed with salt, such as sodium phosphotungstate which is highly opaque to electrons. The mixture is spread on a carbon membrane and dried.

The regions of the particles which are not penetrated by salt form electron lucent area on an opaque background. Details of the surface structure is revealed by perpetration between protruding parts of the salt.

The Whole Mount Technique:

This technique is also used for the un-sectioned materials, but it does not involve staining or heavy metal deposition. The scattering of electrons from the object produces the image.

The Freeze-etch Technique:

By this technique, a unique picture of cells is viewed, especially where the membrane is involved. The cell is broken along and across the membranes and therefore, it shows the four views of the biological membrane, viz., protoplasmic surface, exoplasmic surface, protoplasmic fracture faces, and exoplasmic fracture faces.

The technique does not involve fixatives, stains and embedding agents and therefore, the cell structure is not deformed.
The material to be studied is frozen in liquid Freon in a vacuum. The cell function is instantly arrested due to rapid freezing. The frozen material becomes very hard, and when struck by a knife, it is broken along the lines of membranes.
Water is evaporated by placing the broken material in a vacuum. Water loss causes the "etching effect" i.e., details become much clearer. A heavy metal (e.g., platinum) is used for shadowing the fractured surface, and a replica is prepared by using a carbon film. A strong acid is used to remove the tissue and to leave the metal replica. This metal replica is viewed with the electron microscope.

b. Scanning Electron Microscope:

Scanning electron microscopes combine the mechanism of electron microscopy and television. SEM became commercially available in early 1960's and the researchers were Knoll, Von Ardenne, Zworytein etc.
In SEM, electrons are not transmitted through the very thin specimen from below but impinge on its surface from above. The specimen may be opaque and of any manageable thickness and size. If the specimen is an electron conductor, it needs only to be held on an appropriate support. If it is non-conductor, it is allowed to dry but if moist, freeze dried in liquid nitrogen is necessary. The specimen is then coated with metal vapour (gold) in vacuum. The electrons originate at high energy (20,000 V) from a hot tungsten or lanthanum hexoboride cathode "gun". These electrons are sharply focused, adjusted and narrowed by an arrangement of magnetic fields.
Instead of forming a broad inverted cone of rays, in SEM a needle sharp probe (about 5 – 10 mm in diameter) is made. This primary beam (probe) acts only as an exciter of image forming secondary electrons emerging from the surface of the specimen. The probe scans the specimen like that on a blank TV screen. The probe can impinge on depth and heights with equal speed and accuracy giving great depth of field and producing images with three dimensions. Images are elicited from wherever the probe strikes the metal coated areas of the specimen. Magnification is the ratio of final image to the diameter of area scanned.
Any of the secondary electrons with sufficient energy can emerge from the surface. Those that emerge not too far from the point of impact of the probe can be used to form an image. The useful secondary electrons are magnetically deflected to a collector or detector. Here, they produce a signal that represents at any single moment, only 5-10 mm area or spot of impingement of the probe on the specimen. The successive signals from the collector are amplified and transmitted to a cathode ray (TV) tube. The scanning beam and TV tube beam are synchronized.
The image scan by the eye on TV screen is thus the sequence of signals representing in araster pattern, the successive areas traversed by the primary probe beam. Exposure may range from a few second to one-half hour or more. The TV image may be photographed, video taped or processed in motion on a computer. This microscope shows 3-dimensional surface architecture of cells and organelles. The present day scanning electron microscopes have the resolution power of 10 nm which is less than the resolution power of transmission

electron microscopes. However, this resolving power can be increased by making further improvements. In this system (Fig. 21.5), a beam of electrons is used that moves back and forth across the specimen by a canning coil.

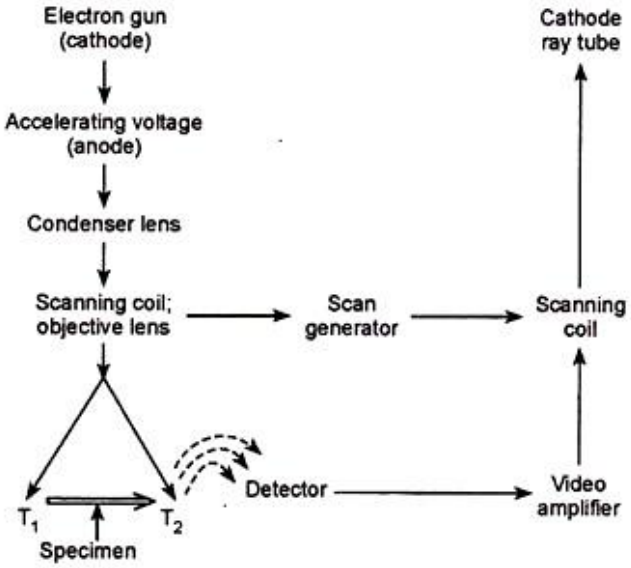


Fig. 21.5. Schematic view of the working system of a scanning electron microscope. The electron beam illuminates different points in the specimen at different times, T_1 and T_2. Movement of this beam is synchronized with beam in cathode ray tube by a scan generator. Detector picks the scattered electrons from specimen and modulates the beam in the cathode ray tube; an the scattered electrons from specimen and modulates the beam in the cathode ray tube; an image of the specimen is formed.

It illuminates different points on the surface of specimen at different times. The scan generator synchronizes the movement of this beam in a cathode ray rube (television tube). Electrons are deflected from the specimen and are picked up by a detector that modulates the beam in the cathode ray tube. A 3-dimensional structure of the surface of the cell or organelle is obtained.

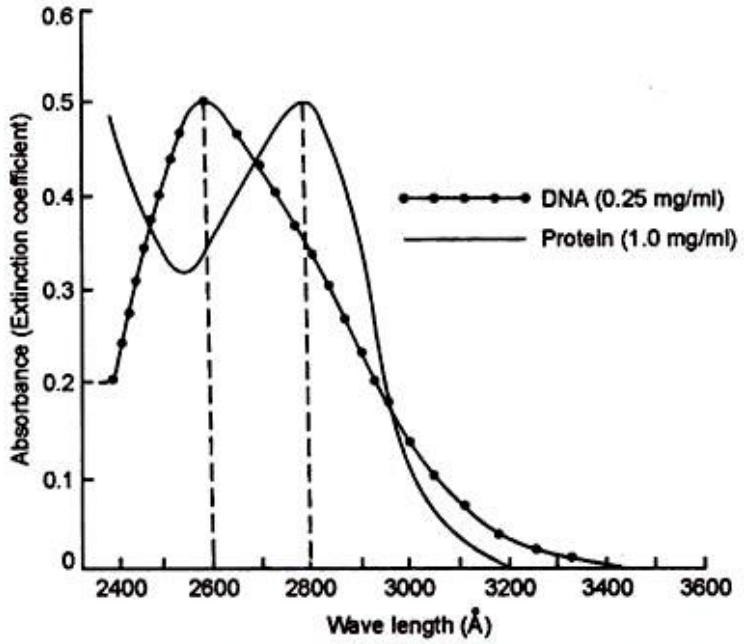


Fig. 21.6. Degree of ultraviolet absorption by DNA and proteins at different wavelengths (10Å = 1 nm).

Difference: Light Microscope vs electron Microscope:

Light Microscope :

1. Visible light is used in this microscope.
2. Source of illumination is situated at the bottom.
3. For magnification in this microscope the lens system consists of glass lenses.
4. The lenses are ocular, objective and condenser
5. The image is either seen with the eye or recorded on a photographic film with a camera in this microscope.

Electron Microscope:

1. Electrons are used in this microscope.
2. Source of illumination is situated at the top in this microscope.
3. The lens system consists of electromagnetic coils in this microscope.
4. This microscope has projector coils, an objective and a condenser.
5. The image in an electron microscope is either recorded on a fluorescent scent screen or recorded on a photographic film.

Difference between TEM and SEM:

Both SEM (scanning electron microscope/microscopy) and TEM (transmission electron microscope/microscopy) refer both to the instrument and the method used in electron microscopy.

There are a variety of similarities between the two. Both are types of electron microscopes and give the possibility of seeing, studying, and examining small, subatomic particles or compositions of a sample. Both also use electrons (specifically, electron beams), the negative charge of an atom. Also, both samples in use are required to be "stained" or mixed with a particular element in order to produce images. Images produced from these instruments are highly magnified and have a high resolution. However, an SEM and TEM also share some differences. The method used in SEM is based on scattered electrons while TEM is based on transmitted electrons. The scattered electrons in SEM are classified as backscattered or secondary electrons. However, there is no other classification of electrons in TEM.

The scattered electrons in SEM produced the image of the sample after the microscope collects and counts the scattered electrons. In TEM, electrons are directly pointed toward the sample. The electrons that pass through the sample are the parts that are illuminated in the image.

The focus of analysis is also different. SEM focuses on the sample's surface and its composition. On the other hand, TEM seeks to see what is inside or beyond the surface. SEM also shows the sample bit by bit while TEM shows the sample as a whole. SEM also provides a three-dimensional image while TEM delivers a two-dimensional picture.

In terms of magnification and resolution, TEM has an advantage compared to SEM. TEM has up to a 50 million magnification level while SEM only offers 2 million as a maximum level of magnification. The resolution of TEM is 0.5 angstroms while SEM has 0.4 nanometers. However, SEM images have a better depth of field compared to TEM produced images. Another point of difference is the sample thickness, "staining," and preparations. The sample in TEM is cut thinner in contrast to a SEM sample. In addition, an SEM sample is "stained" by an element that captures the scattered electrons.In SEM, the sample is prepared on specialized aluminium stubs and placed on the bottom of the chamber of the instrument. The image of the sample is projected onto the CRT or television-like screen.
On the other hand, TEM requires the sample to be prepared in a TEM grid and placed in the middle of the specialized chamber of the microscope. The image is produced by the microscope via fluorescent screens. Another feature of SEM is that the area where the sample is placed can be rotated in different angles. TEM was developed earlier than SEM. TEM was invented by Max Knoll and Ernst Ruska in 1931. Meanwhile, SEM was created in 1942. It was developed at a later time due to the complexity of the machine's scanning process.

Draw backs of EM:

(i) It is complicated and costly.

(ii) There is risk of radiation leak,

(iii) It requires very high voltage electric current.

(iv) A cooling system is required,

(v) The specimen or object has to be given special treatment including complete dehydration.

Probable Questions:

1. Write the basic principle of fluorescent microscope?
2. Describe different components of fluorescent microscope.
3. What are the advantages of fluorescent microscope ?
4. What are the limitations of fluorescent microscope ?
5. Describe basic principle of phase contrast microscope.
6. Describe basic principle of Dark field microscope.
7. Describe basic principle of confocal microscope.
8. Describe basic components of a electron microscope.
9. What is shadow casting in electron microscopy?
10. What is positive and negative staining in electron microscopy?
11. Write five differences between light microscope and electron microscope.
12. Differentiate between TEM and SEM.
13. Describe the working principle of FACS with suitable diagram.

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyay and Upadhyay.

UNIT-II

Common spectroscopic methods, Electromagnetic radiation, Principle of spectroscopy

Objective: In this unit we will discuss spectroscopy with special emphasis to spectrophotometer. We will also discuss different modes of pesticide formulation.

Principle of Spectroscopy:

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. When matter is energized (excited) by the application of thermal, electrical, nuclear or radiant energy, electromagnetic radiation is often emitted as the matter relaxes back to its original (ground) state.

The spectrum of radiation emitted by a substance that has absorbed energy is called an emission spectrum and the science is appropriately called emission spectroscopy.

Another approach often used to study the interaction of electromagnetic radiation with matter is one whereby a continuous range of radiation (e.g., white light) is allowed to fall on a substance; then the frequencies absorbed by the substance are examined.

The resulting spectrum from the substance contains the original range of radiation with dark spaces that correspond to missing, or absorbed frequencies. This type of spectrum is called an absorption spectrum. In spectroscopy the emitted or absorbed radiation is usually analyzed, i.e., separated into the various frequency components and the intensity is measured by means of an instrument called spectrometer.

The resultant spectrum is mainly a graph of intensity of emitted or absorbed radiation versus wavelength or frequency. There are in general three types of spectra: continuous, line, and band. The sun and heated solids produce continuous spectra in which the emitted radiation contains all frequencies within a region of the electromagnetic spectrum. A rainbow and light from a light bulb are examples of continuous spectra.

Line spectra are produced by excited atoms in the gas phase and contain only certain frequencies, all other frequencies being absent. Each chemical element of the periodic chart has a unique and, therefore, characteristic line spectrum. Band spectra are produced by excited molecules emitting radiation in groups of closely spaced lines that merge to form bands.

These categories of emission and absorption spectra contain tremendous amounts of useful information about the structure and composition of matter. Spectroscopy is a powerful and sensitive form of chemical analysis, as well as a method of probing electronic and nuclear structure and chemical bonding. The key to interpreting this spectral information is the knowledge that certain atomic and molecular processes involve only certain energy ranges. Fig. 12.1 shows the regions of the electromagnetic spectrum and the associated energy transitions that occur in atomic and molecular processes.

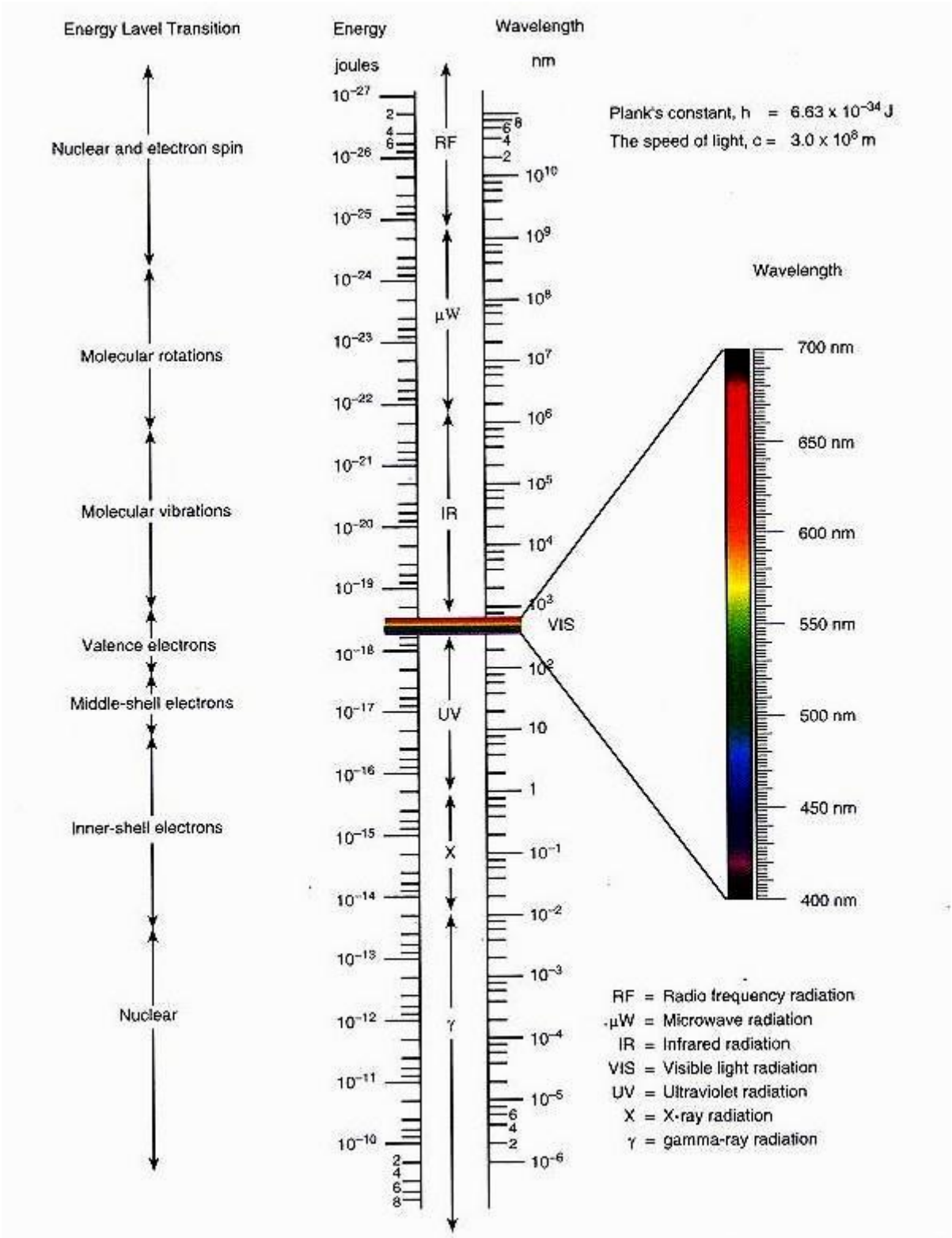


Fig. 12.1: The electromagnetic radiation ruler

Much of the scientific knowledge of the structure of the universe, from stars to atoms, is derived from interpretations of the interaction of radiation with matter. One example of the

power of these techniques is the determination of the composition, the velocities, and the evolutionary dynamics of stars.
The source of the incredible amount of energy produced by the sun is nuclear fusion reactions going on within the hot interior (temperature 40 × 106 K). Two fusion cycles, the carbon cycle and the proton cycle, convert hydrogen nuclei into helium nuclei via heavier nuclei, such as carbon 12 and nitrogen 14. The enormous radiation of energy from the hot core seethes outwards by convection.
This radiation consists of the entire electromagnetic spectrum as a continuous spectrum. Towards the surface of the sun (the photosphere), the different elements all absorb at their characteristic frequencies. The radiation that shoots into space toward the earth is a continuous emission spectrum with about 22,000 dark absorption lines present in it (Fraunhofer lines), of which about 70% have been identified. These absorption lines, i.e., missing frequencies, prove that more than 60 terrestrial elements are certainly present in the sun.

Classification of Spectroscopic Methods:

Different spectroscopic techniques have been classified mainly on two parameters, first what type of radiation is to be measured or by what measurement procedure is employed.

1. Nature of Radiation Measured:

This category of spectroscopy depends on the physical quantity measured. Normally, the quantity that is measured is an amount or intensity of something.

i. Electromagnetic spectroscopy involves interactions with electromagnetic radiation, or light. Ultraviolet-visible spectroscopy is an example.

ii. Electronic spectroscopy involves interactions with electron beams. Auger spectroscopy involves inducing the Auger effect with an electron beam.

iii. Mechanical spectroscopy involves interactions with macroscopic vibrations, such as phonons. An example is acoustic spectroscopy, involving sound waves.

iv. Mass spectroscopy involves the interaction of charged species with a magnetic field, giving rise to a mass spectrum. The term “mass spectroscopy” is deprecated in favour of mass spectrometry, tor the technique is primarily a form of measurement, though it doe- produce a spectrum for observation.

2. Measurement Process:

Most spectroscopic methods are differentiated as either atomic or molecular based on whether or not they apply to atoms or molecules.

Along with that distinction, they can be classified on the nature of their interaction:

i. Absorption spectroscopy uses the range of the electromagnetic spectra in which a sub stance absorbs. This includes atomic absorption spectroscopy and various molecular

techniques, such as infrared spectroscopy in that region and nuclear magnetic resonance (NMR) spectroscopy in the radio region.

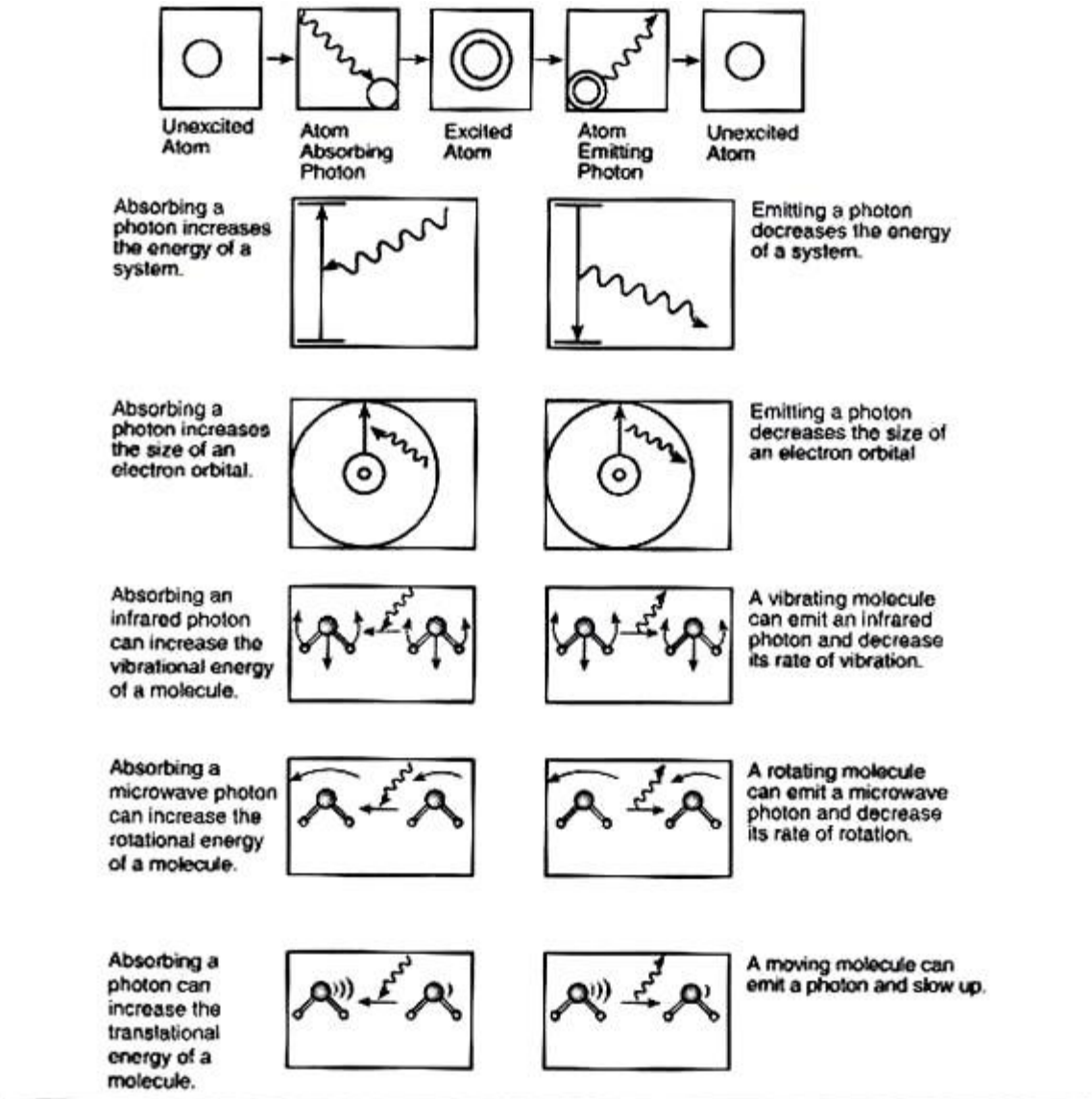


Fig. 12.2: Fundamental processes during spectroscopy

ii. Emission spectroscopy uses the range of electromagnetic spectra in which a substance radiates (emits). The substance first must absorb energy. This energy can be from a variety of sources, which determines the name of the subsequent emission, like luminescence. Molecular luminescence techniques include spectrofluorimetry.

iii. Scattering spectroscopy measures the amount of light that a substance scatters at certain wavelengths, incident angles, and polarisation angles. The scattering process is much faster than the absorption/emission process. One of the most useful applications of light scattering spectroscopy is Raman spectroscopy.

Spectrophotometer:

A **spectrophotometer** can be located in many studies, biology, chemistry, and industrial laboratories. The spectrophotometer is utilized for research and data evaluation in different scientific fields.

Some of the major fields in which a spectrophotometer is employed are physics, molecular biology, chemistry, and biochemistry labs. Generally, the title refers to Ultraviolet-Visible (UV-Vis) Spectroscopy.

What a spectrophotometer does is transmit and receive light. The spectrophotometer is utilized to evaluate samples of test material by passing light by means of the sample and studying the intensity of the wavelengths.

Different samples modify the light in numerous distinct ways and this allows researchers to obtain much more facts about the check content, by viewing the change in light conduct as it passes by way of the sample.

These final results must be precise or the researcher will just be throwing away time making use of a flawed instrument. The only way to make sure accuracy is by executing a spectrophotometer calibration.

Quantification of light absorption: The chance for a photon to be absorbed by matter is given by an extinction coefficient which itself is dependent on the wavelength l of the photon. If light with the intensity I0 passes through a sample with appropriate transparency and the path length (thickness) d, the intensity I drops along the pathway in an exponential manner. The characteristic absorption parameter for the sample is the extinction coefficient a, yielding the correlation $I = I_0 e^{-\alpha d}$ .The ratio $T = I/I_0$ is called transmission. Biochemical samples usually comprise aqueous solutions, where the substance of interest is present at a molar concentration c. Algebraic transformation of the exponential correlation into an expression based on the decadic logarithm yields the law of **Beer–Lambert:**

The **Beer-Lambert law (or Beer's law)** is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:

$$A = a(\lambda) * b * c$$

$$\lg \frac{I_0}{I} = \lg \frac{1}{T} = \varepsilon \times c \times d = A \qquad (12.2)$$

where $[d] = 1\ \text{cm}$, $[c] = 1\ \text{mol dm}^{-3}$, and $[\varepsilon] = 1\ \text{dm}^3\ \text{mol}^{-1}\ \text{cm}^{-1}$. ε is the molar absorption coefficient (also molar extinction coefficient) ($\alpha = 2.303 \times c \times \varepsilon$). A is the absorbance of the sample, which is displayed on the spectrophotometer.

where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, **b** is the path length, and **c** is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:

$$A = \varepsilon * b * c$$

where ε is the wavelength-dependent molar absorptivity coefficient with units of $M^{-1}\ cm^{-1}$. Data are frequently reported in percent transmission ($I/I_0 * 100$) or in absorbance [A = log

(I/I_0)]. The latter is particularly convenient. [common coefficients of near-ultraviolet absorption bands of some amino acids and nucleotides]
Sometimes the extinction coefficient is given in other units; for example,

$$A = E^{1\%} * b * c$$

where the concentration C is in gram per 100 ml of solution. This useful when the molecular weight of the solute is unknown or uncertain.
The Beer–Lambert law is valid for low concentrations only. Higher concentrations might lead to association of molecules and therefore cause deviations from the ideal behaviour. Absorbance and extinction coefficients are additive parameters, which complicates determination of concentrations in samples with more than one absorbing species. Note that in dispersive samples or suspensions scattering effects increase the absorbance, since the scattered light is not reaching the detector for readout. The absorbance recorded by the spectrophotometer is thus overestimated and needs to be corrected.

Deviations from the Beer–Lambert law

According to the Beer–Lambert law, absorbance is linearly proportional to the concentration of chromophores. This might not be the case any more in samples with high absorbance. Every spectrophotometer has a certain amount of stray light, which is light received at the detector but not anticipated in the spectral band isolated by the monochromator. In order to obtain reasonable signal-to-noise ratios, the intensity of light at the chosen wavelength (Il) should be 10 times higher than the intensity of the stray light (Istray). If the stray light gains in intensity, the effects measured at the detector have nothing or little to do with chromophore concentration. Secondly, molecular events might lead to deviations from the Beer–Lambert law. For instance, chromophores might dimerise at high concentrations and, as a result, might possess different spectroscopic parameters.

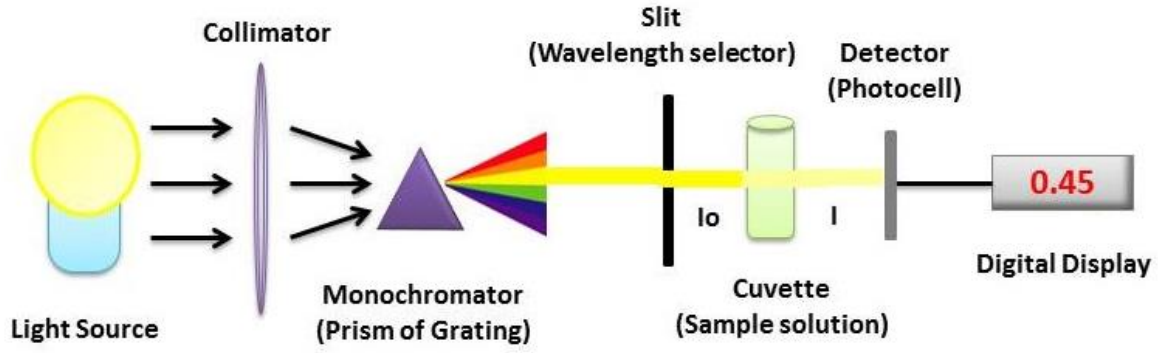
Definition: In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.[2] It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques.

Principle

The Spectrophotometer is a much more refined version of a colorimeter. In a colorimeter, filters are used which allow a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm. *The spectrophotometer* is useful for measuring the absorption spectrum of a compound, the absorption of light by a solution at each wavelength. This is the basic Principle of spectrophotometry in biochemistry.

Spectrophotometer Instrumentation

The essential components of spectrophotometer instrumentation include:



Basic Instrumentation of a Spectrophotometer

1. A Stable and cheap radiant energy source
2. A monochromator, to break the polychromatic radiation into component wavelength (or) bands of wavelengths.
3. Transport vessels (cuvettes), to hold the sample
4. A Photosensitive detector and an associated readout system

1. Radiant Energy Sources

Materials that can be excited to high energy states by a high-voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.

a. Sources of Ultraviolet radiation: The most commonly used sources of UV radiation are the hydrogen lamp and the deuterium lamp. Xenon lamp may also be used for UV radiation, but the radiation produced is not as stable as the hydrogen lamp.

b. Sources of Visible radiation: "Tungsten filament" lamp is the most commonly used source for visible radiation. It is inexpensive and emails continuous radiation in the range between 350 and 2500nm. "Carbon arc" which provides more intense visible radiation is used in a few commercially available instruments.

c. Sources of IR radiation: "Nernst Glower" and "Global" are the most satisfactory sources of IR radiation. Global is more stable than the nearest flower.

2. Wavelength Selectors: Wavelength selectors are of two types.

1. **Filters:** "Gelatin" filters are made of a layer of gelatin, coloured with organic dyes and sealed between glass plates.
2. **Monochromators:** A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands. The essential components of a monochromator are.
   - Entrance slip-admits polychromatic light from the source
   - Collimating device–Collimates the polychromatic light onto the dispersion device.

- Wavelength resolving device like a PRISM (or) a GRATING
- A focusing lens (or) a mirror
- An exit slip–allows the monochromatic beam to escape.
-

The kinds of resolving element are of primary importance

- PRISMS
- GRATINGS

PRISMS:

A prism disperses polychromatic light from the source into its constituent wavelengths by virtue of its ability to reflect different wavelengths to a different extent;

The degree of dispersion by the prism depends on upon

- The optical angle of the Prism (usually 60^0)
- The material of which it is made

Two types of Prisms are usually employed in commercial instruments. Namely, 60^0 cornu quartz prism and 30^0 Littrow Prism.

GRATINGS:

Gratings are often used in the monochromators of spectrophotometers operating ultraviolet, visible and infrared regions.

3. Sample Containers

Sample containers are also one of the parts of Spectrophotometer instrumentation. Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as “CUVETTES”.

Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz. Most of the spectrophotometric studies are made in solutions, the solvents assume prime importance.

The most important factor in choosing the solvent is that the solvent should not absorb (optically transparent) in the same region as the solute.

4. Detection Devices

Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it. Important requirements for a detector including

- High sensitivity to allow the detection of low levels of radiant energy
- Short response time
- Long-term stability
- An electric signal which easily amplified for a typical readout apparatus.

5. Amplification and Readout

Radiation detectors generate electronic signals which are proportional to the transmitted light. These signals need to be translated into a form easy to interpret. This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.

The above 5 major parts are the major part of Spectrophotometer instrumentation. Now let us see the Applications of Spectrophotometer.

Spectrophotometer Applications

How to use the spectrophotometer? There are uses of spectrophotometry in biochemistry which are listed below:

1. Qualitative Analysis

The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations. This is done by plotting absorption spectrum curves. Absorption by a compound in different regions gives some hints to its structure.

2. Quantitative Analysis

Spectrophotometer uses in the Quantitative analysis of Biochemistry practicals. Quantitative analysis method developing for determining an unknown concentration of a species by absorption spectrometry.

Most of the organic compounds of biological interest absorb in the UV-visible range of the spectrum.

Thus, several important classes of biological compounds may be measured semi-quantitatively using the UV-visible spectrophotometer. Nucleic acids at 254nm protein at 280nm provide good examples of such use.

The absorbance at 280nm by proteins depends on their “Tyrosine” and “Tryptophan” content.

- **Estimation of Proteins by Lowry method**
- **Estimation of Tyrosine by Folin-Ciocalteau Method**
- **Estimation of Blood Glucose level by Folin-Wu method**

3. **Enzyme** assay:

This is the basic application of spectrophotometry. This assay is carried out most quickly and conveniently when the substrate (or) the product is color (or) absorbs light in the UV range.

4. Molecular Weight Determination

Molecular weights of amine picrates, sugars and much aldehyde and ketone compounds have been determined by this method. Molecular weights of only small molecules may be determined by this method.

1. **Study of Cis-Trans Isomerism:** Geometrical isomers differ in the spatial arrangement of groups about a plane, the absorption spectra of the isomers also differs. The trans-

isomer is usually more elongated than its cis counterpart. Absorption spectrometry can be utilized to study Cis-Trans isomerism.

2. **Control of Purification:** Impurities in a compound can be detected very easily by spectrophotometric studies. “Carbon disulfide” impurity in carbon tetrachloride can be detected easily by measuring absorbance at 318nm where carbon sulfide absorbs. A lot many commercial solutions are routinely tested for purity spectroscopically.

5. Other Physiochemical Studies:

Spectrophotometry (UV-VIS) has been used to study the following physiochemical phenomena:

- Heats of formation of molecular addition compound and complexes in solution
- Determination of the empirical formula
- Formation constants of complexes in solution
- Hydration equilibrium of carbonyl compounds
- Association constants of weak acids and bases in organic solvents
- Protein-dye interactions
- Chlorophyll-Protein complexes
- Vitamin-A aldehyde–Protein complex
- Determination of reaction rates
- Dissociation constants of acids and bases
- Association of cyanine dyes

These are the basic spectrophotometer instrumentation and its applications.

Probable Questions:

1. What is the basic principle of spectroscopy?

2. Explain Lambert-Beer law.

3. What is molar extinction coefficient? How it is calculated?

4. Describe different components of a spectrophotometer?

5. Describe different applications of spectrophotometer?

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyay and Upadhyay.

UNIT-III

Histochemical and immunological techniques: Tissue processing, microtomy, fixatives (types and function), staining.

Objective: In this unit we will discuss about different kinds of microtomy and fixatives. We will also discuss production of antibody and antigen antibody interaction.

Microtome:A microtome is a tool that can be used to cut a slice of tissue that is so thin that it can be used for light or electron microscopy. Different steps of microtomy are as follows:

a. Section-Cutting:

Rotary microtomes are commonly used for section-cutting. Move the block holding the socket backward as far as possible with the help of the backward movement handle, which usually lies at the far side of the microtome. Adjust the thickness of sections.

Ordinarily, flower buds are cut at 14 µ and root tips at 12 µ thickness. Cut out one block from the large one cast previously. Trim it with a scalpel and/or blade, holding it gently with the left hand and obtain a perfect square or rectangle with the material at the centre. Place the block-holder vertically in a small tube. See that there is sufficient paraffin on it.Now hold the trimmed block gently with the thumb and forefinger of the left hand, moderately heat a scalpel, place it flatly on the block-holder, and rest the block on the scalpel and then remove the scalpel. Prick the four sides of the block where it has joined the holder with a hot needle. Submerge the block in cold water for a few seconds. Trim further, if necessary.

Now fix the block-holder in the block-holder socket with the help of its screws. Fix the razor in its socket. The block and razor should lie parallel to each other. The razor tip should be slightly tilted inwardly (about 8° angle). Bring the block and the razor as close as possible without touching each other. Now cut sections by moving the front wheel in a clockwise direction.The ribbon of sections will slip over the razor. Support it with a needle or brush held by the left hand. When the material is exhausted, dislodge the ribbon with a brush and place it carefully on a half foolscap piece of blotting paper or, preferably, on a large, glossy and somewhat stiff paper.

Precautions:

Microtome section cutting is a critical job and requires considerable practice to obtain perfection.

However, the following precautions should be noted:

1. The block-holding socket is moved backward as far as possible because, if the microtome gets stuck midway while section cutting, the ribbon will be disturbed. Resetting it may cause the loss of a portion of the material with the result that the entire material is not present in sections.

2. Thickness of sections depends upon the size of the cells. The idea is to get sections one cell in thickness.

3. Trimming the block is a very critical step. If it is trimmed into odd shapes the ribbon will not be straight and there may be difficulty in joining of the sections to form the ribbon. The excess paraffin should be trimmed gradually, there may be excess paraffin on one side but it should not be cut in one stroke.

The four sides should be trimmed in such a way that the material finally lies at the centre. Longitudinal sections are normally made in case of flower buds, and transverse sections in case of root tips. Obviously, trimming should be done accordingly. Another defect often noted in trimming is that the top of the block becomes narrow and the base progressively broader.This should be avoided. The block often needs a second trimming after mounting on the block-holder, as it sometimes bends due to pressure while mounting. For this, all the excess paraffin should not be trimmed away initially. After final trimming, the block should be of such a size that at least four ribbons can be accommodated on the slide.

4. If sufficient paraffin is not present on the block-holder, add some and melt it with a hot scalpel. This is necessary to ensure proper fixing of the block and, at the same time, reduce the chance of the metallic holder rubbing against the razor. While fixing the block, the scalpel method should be followed. As the paraffin on the holder and that of the block melt simultaneously it ensures perfect fixing. Pricking with a hot needle makes fixing doubly sure. It is all the more necessary because often the block gets detached while section cutting. Dipping the fixed block in cold water is also done for proper fixing.

5. Note carefully before section cutting that the block and the razor are perfectly parallel to each other; otherwise the ribbon will not be straight. Very often the ribbon becomes curved or dentate. This is due to faulty trimming or not keeping the block parallel to the razor (Fig. 3.4).

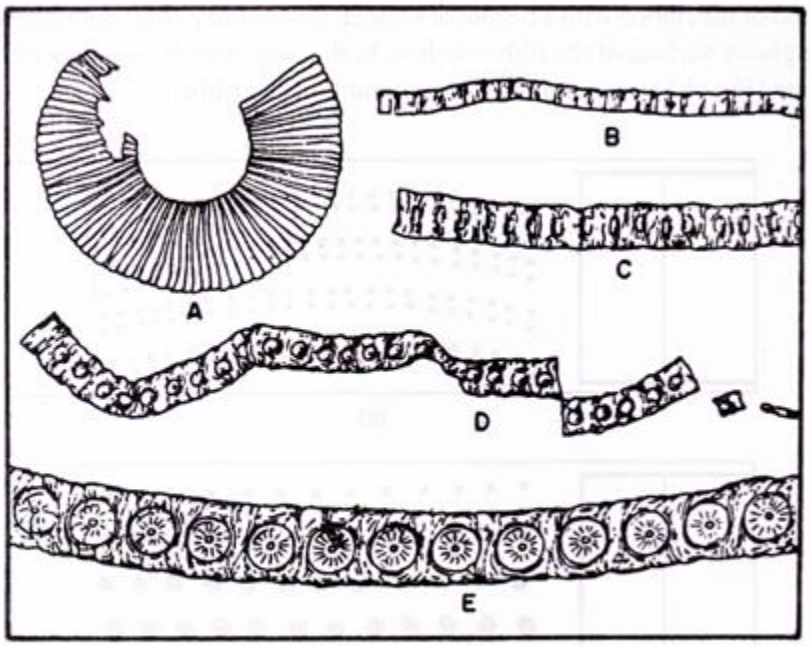


Fig. 3.4 : Types of ribbons — A, B, C and D showing defects of different types such as curling, tearing, wrinkling. etc.; E showing perfect ribbon

6. The razor is placed at a slightly inward angle to avoid rubbing of the block against the lower side of the razor. The lower side of the razor is quite thick; so if it is placed vertically, with every stroke the block will be pressed against this thick side of the razor and sections will not come out.

7. As the block strikes the razor a section is produced and the friction against the razor produces a little heat energy. When sections are cut in quick succession this energy is sufficient to join the very thin sections end to end to produce a ribbon. This is why paraffin of suitable melting point has to be taken while casting blocks. If the paraffin is too hard the heat energy produced by friction is not enough to join the sections.

If it is too soft the frictional energy makes the sections further soft and they become crumpled. Sometimes warming the block and the holder with slightly warm water or cooling with cold water helps to eliminate these defects. If the sections fail to form a ribbon reduce the thickness of the sections and, if it crumples, increase the thickness of the sections by a few microns. Sometimes this also helps in obtaining a ribbon.

8. For flower buds and L.S. of root tips cut the entire material into sections. While making T. S. of root tips, cut sections to fill one slide; divisions are not found beyond this region.

9. Never touch the edge of the razor with a needle or scalpel. Dislodge the ribbon at the end of section cutting with a brush. Friction with metal may cause minute dentation or folding of the edge of the razor. Once this happens, the razor cannot be used again without sharpening or polishing it.

Microtome razors are very costly. Remove the grease from the surface of the razor before using it and, after section cutting, clean its surface and apply grease. The microtome also needs cleaning and oiling from time to time for smooth running.

b. Mounting of Sections:

Cut the ribbon into 1½" (4 cm) long strips. Put a small drop of Meyer's fixative (white of egg 50 ml, Glycerol 50 ml; Sodium salicylate 1 gm.) on a clean grease-free slide. Rub it gently with the little finger all over the slide and drain off the excess fixative, if any. Flood the slide with a thin film of water with the help of a dropper. Dip the tip of a scalpel in cold water and touch the ribbon with it.

The ribbon will adhere to it. Free the other end of the ribbon with a needle or scalpel, if necessary. Shift the ribbon and place it on the slide, keeping the glossy surface of the ribbon below. In this way transfer as many ribbons to the slide as it can accommodate (Fig. 3.5), maintaining the continuity of the ribbon.

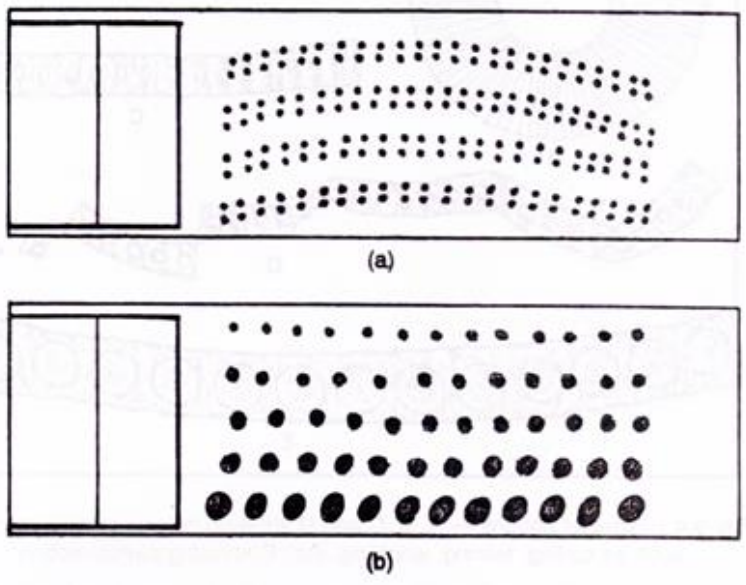


Fig. 3.5 : Arrangement of ribbons on slides — (a) Slightly defective as ribbons are not straight, (b) Perfect arrangement showing ribbons in straight lines and maintaining seriality

c. Stretching of Sections:

Transfer the slide on to a hot plate kept at 37-43°C, on a thin film of water. Wait for a few seconds. The ribbons will automatically stretch on being warmed. Take two needles and further stretch the ribbons by pulling in opposite directions. Dip the needles in cold water to keep cool.

d. Drying of Sections:

Take out the slide from the hot plate. Hold it with the thumb and forefinger of the left hand without touching the ribbons. With the help of a needle push the ribbons to one side of the slide and tilt the slide after placing the needle below them to drain off the excess water.

Adjust the ribbons with the needle in such a way that on one side of the slide about 2 cm open space and on the opposite side about 2-3 mm open space are left. Wipe off the under-surface of the slide and keep it on the hot plate for 3 hours to overnight for drying. The slides can now be stored indefinitely.

Precautions:

1. The size of the slide is 3″ × 1″ (75 × 25 mm) and that of a microtome cover-glass 2″ × 1″(approx.) (50 × 25 mm). Hence, if the ribbon is cut into 1½” (37 mm) long pieces, after stretching they will be 2″ (50 mm) long (approx.) to fill the cover glass completely. If, however, the ribbon is slightly crumpled, it will stretch more — so the initial pieces should be made smaller.

2. Meyer’s fixative is rubbed with the little finger, because it is the least used finger and, consequently, the cleanest. The slide is flooded with water to keep the ribbons floating, so that they can stretch when warmed.

3. The ribbons are transferred to the slide maintaining their continuity. In case of root-tips the ribbon-pieces are mounted as soon as the material comes in the ribbon. In case of flower buds the initial and final sections are discarded and only the middle portion of the ribbon is taken, because this is where the anther sections containing the spore mother cells are present. In both cases, usually only one slide is made.

4. The lower surface of the ribbon which remains in contact with the razor is glossy. While mounting, this surface should come in contact with the slide for better adherence to it.

5. Full stretching of the ribbon is of utmost importance. As the ribbon stretches, the sections also stretch, eliminating the possibility of minute folds remaining in the sections. Stretching has to be done under controlled temperature.

So a thermostatically controlled hot plate is most suitable for stretching. Direct heating over a burner should never be made, for it may melt away all the paraffin and the sections may get displaced and distorted due to over-heating. Heating over an alcohol flame may, however, be made with utmost care.

6. The ribbons are mounted on a thin film of water on the slide to keep them floating, so that they can stretch freely. A thin film of water is kept in-between the slide and the hot plate surface for intimate contact between the slide and the hot plate both of which are solid. This ensures uniform heating of the ribbons.

7. If the ribbons are found to be curved after mounting, touch the two inner edges of the curve with two needles and pull them upwards while stretching. This will make them straight.

8. The final size of the ribbons — after stretching — is limited by the length of the microtome cover glass, which is about 2″ (5 cm). At one side of the slide, 2-3 mm of empty space is left so that the ribbons or sections do not get damaged by friction with the slide box. The rest of the empty space is left at the other side of the slide for labelling.

e. Staining the Sections:

Microtome sections are stained in cylindrical or rectangular staining Jars or Coplin Jars provided with lids. Each jar should be filled up to about 2½" (6 cm) with the reagent so that the fingers are not dipped in the reagent while staining. A large number of jars are required and they are arranged in a row according to priority. Transferring a slide from one jar to another should be done slowly to keep the mixing of the reagents at a minimum; but not so slowly that the slides dry up. Slides can be stained singly or in pairs, taken in a back-to-back position. When the reagents become discoloured due to repeated use, they should be changed.

Both root tips and flower buds are stained in 1% aq. crystal violet solution. As the sections are embedded in paraffin, the first step is removal of this paraffin by xylol. Then xylol is replaced by alcohol and alcohol by water. The slides should be kept in xylol and abs. alcohol for as little time as possible, because both these reagents make the sections brittle.

The slides are stained after reaching down to water and then are mordanted in KI and iodine mordant for a few seconds. Mordanting increases the stain-retaining capacity of the chromosomes. Mordanting after staining is called post-mordanting. For some materials which do not stain easily, pre-mordanting is also done in 1 % chromic acid for overnight followed by washing in running water for 3 hours.It should be remembered that for crystal violet staining, a chromic fixative is always used. Chromium particles become impregnated on the

surface of the chromosomes and these adsorb the crystal violet stain. Mordanting is followed by quick dehydration in abs. alcohol and then differentiation in clove oil.

Crystal violet is highly soluble in alcohol. So dehydration in alcohol is done as quickly as possible; otherwise all the stain will be washed out. After differentiation, clove oil is cleared by xylol and, finally, the slides are mounted in Canada balsam.

f. Schedule of Staining the Section:

Pass the slide with paraffin sections through the following grades: Xylol I (Down), Xylol II (Down), Xylol III (Down) — 1 hr. in each Xylol — abs. ethyl alcohol mixture (1:1) — 1 hr.; absolute ethyl alcohol, 95%, 90%, 80%, 70%, 50% and 30% alcohol — 30 mins. in each. If required, the slides can be kept for overnight in 70% alcohol. Finally, keep in water at least 15 mins.

This downgrade is a long-drawn process. When so much time is not available, as during an examination, the durations of all the treatments can be reduced to half. But for a really good permanent preparation this should be avoided.Stain in 1% aq. crystal violet solution for 30 mins. Keep in Pot. Iodine mordant (1 gr KI and 1 gm. iodine crystals dissolved in 100 ml 80% alcohol) for 45 seconds. Pas through abs. ethyl alcohol I (UP), II (UP) and III (UP) — 2 to 3 dips in each.

At this stage a piece of blotting paper should be kept nearby and, after 2 to 3 rapid dips in each alcohol jar, the slides should be rapidly jerked against the wall of the reagent jar and then on the blotting paper.The entire process of dehydration in alcohol must be done as quickly as possible. Differentiate in clove oil I (2-5 mins.) and observe under the low power of microscope after placing a clean dry slide under the stained slide.

If cytoplasm and nucleus cannot be differentiated, put the slide back in clove oil I and again observe under microscope. After differentiation, keep the slide in clove oil II (10-15 min.). For differentiation, as long as the clove oil is fresh, less time is required; and more and more time is required as the clove oil gets more and more used. This has to be learnt by experience. Pass through xylolI (UP), II (UP) and III (UP) — 1 hr. in each. If required, the slides can be kept in xylol III (UP) for overnight. Mount in Canada balsam. Put 3 drops of Canada balsam on the slide immediately after taking it out of xylol III (UP). Dip the cover- glass in xylol and place it gradually on the slide. Press the cover-glass gently with a needle to bring out excess balsam and dry the slide on a hot plate.

Types of Microtomes:

The inherent defects of handmade sections can be eliminated by using a microtome.

Three types of microtomes are available:

Rotary,

Rocking and

Sliding Microtomes.

A Rotary microtome is worked by rotating a wheel fitted with a handle.

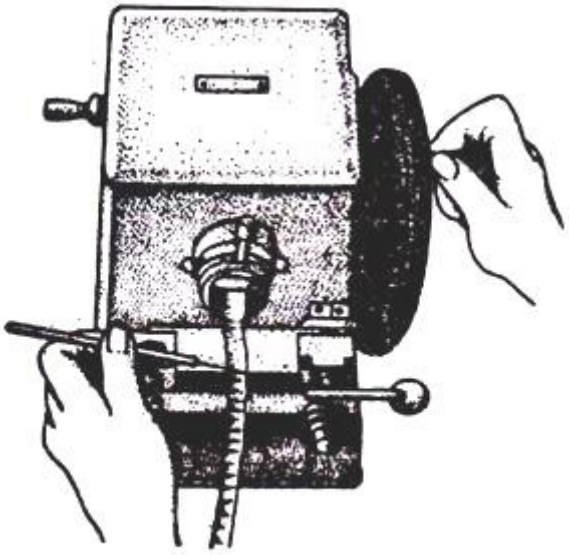


Fig. 3.1 : Rotary microtome

The razor is placed in front of the microtome in a razor- holder which is movable. The material, embedded in a paraffin block, is fixed on the block-holder which can be fixed to an adjustable socket. There is a scale indicating the thickness of the section in microns. The thickness is adjusted by moving a screw.

The block-holding socket can be moved forward or backward by rotating a handle placed at the back of the microtome. Nowadays, rotary microtomes are commonly used. A Rocking microtome has the same working principle. Sections are cut by moving a horizontal handle forward and backward. A Sliding microtome can be used for cutting sections of fresh materials, not embedded in paraffin.

Advantages of Using Microtome:

In free-hand sections, the thickness of the section cannot be regulated, it may be thick or thin, as also oblique. Moreover, the entire material cannot be obtained in sections, as the defective sections have to be thrown away.

When a microtome is used for section-cutting, the sections are of uniform thickness. They can be obtained at any desired thickness (10 or 15 or 20 n etc.). Sections are not oblique. The entire tissue can be retained in sections — this is particularly necessary for studying the development of a plant organ. However, microtome sectioning is a long-drawn and laborious process.

Fixatives:

A large number of chemicals such as ethyl alcohol, formalin, acetic acid, chloroform, mercuric chloride, chromic acid, picric acid, osmic acid, etc. are used singly or in combinations as fixatives for anatomical studies (For principle of fixation — see Cytology section). Amongst these, formalin and formalin-aceto-alcohol (FAA) are most commonly used in Anatomy.

a. Formalin:

A 5% to 10% aqueous solution of commercial formalin (35% to 40% concentration) is used for fixation. For making a 5% solution, 5 ml of comm. formalin is taken and dist. water is added to make the volume 100 ml (Plant Micro-technique by Johansen).Formalin, as a rule, penetrates slowly and is one of the best hardening agents. It is a powerful reducing agent. It does not precipitate proteins or render them insoluble in water. Formalin neither destroys not preserves fats but, more or less, preserves phospholipids.

b. Formalin-Aceto-Alcohol:

This fixative, commonly called FAA, is the standard fixative for plant tissues meant for anatomical studies.

A number of variations have been proposed, but the standard proportions are:

70% (or 50%) ethyl alcohol — 90 ml

Glacial acetic acid — 5 ml

Formalin (40% formaldehyde i.e. comm. formalin) — 5 mlor70% ethyl alcohol — 85 ml

Glacial acetic acid — 5 ml

Formalin — 10ml

The lower percentage of alcohol is more suitable for delicate materials, such as bryophytes. For hard and woody materials, it is better to decrease the amount of acetic acid and to increase the amount of formalin, i.e. use the second mixture, since formalin penetrates more slowly. The material may be left in FAA almost indefinitely without any appreciable damage.The minimum time of fixation is 18 hours. Materials to be sectioned are removed with forceps and washed in running water 1/2 to 1 hour and then handled freely. This is a corrosive liquid, and if it comes in contact with the skin it should be washed-off immediately.

Staining:

A large number of natural and synthetic dyes are used as anatomical stains, such as hematoxylin, brazilin, hematein, Bismarck brown, eosin, fast green, gentian violet, light green, safranin, Sudan IV, etc. (for principle of staining, see Cytology section).

a. Hematoxylin:

Hematoxylin is a chromogen obtained from the heartwood of the plant Hematoxylin campechianum. The dye solution itself has no affinity for tissues, unless iron or aluminium is present as a mordant. The colour effect of hematoxylin depends upon the pH of the solvent and after-treatment of the tissue.In acidic medium the colour is red and in alkaline medium it is blue. Hematoxylin stain can be prepared according to various schedules, such as Heidenhain's iron hematoxylin, Delafied'shematoxylin, Ehrlieh 's hematoxylin, etc.

b. Heidenhain's Iron Hematoxylin:

0.5% solution of hematoxylin in distilled water is used. Practically, a 10% solution in absolute ethyl alcohol is prepared and then diluted with distilled water to 0.5% when required. The solution is allowed to stand for a few days to ripen (into hematein). The solution may also be ripened more quickly following the method described under Ehrlieh'shematoxylin. On ripening, the solution attains a rich wine-red colour.

A more stable solution can be made by adding 5 ml of 10% absolute ethyl alcohol solution to 100 ml methyl cello solve, 50 ml distilled water and 50 ml tap-water that contains calcium compounds in solution.If, on shaking, the solution does not acquire a rich wine-red colour, add a pinch of sodium bicarbonate and shake vigorously. Ripening occurs almost immediately, and the solution can be used at once. This solution lasts longer than simple aqueous solutions and does not spoil at night temperature.Hematoxylin staining requires mordanting, which can be done by ferric ammonium sulphate (iron alum) or ferric chloride; 2 to 5% solutions are used for mordanting and 1 to 3% solutions for differentiation.

c. Ehrlieh'sHematoxylin:

This is extensive used with safranin on woody tissues.

It is prepared in the following way:

Distilled water — 50 ml Glacial, acetic acid — 5 ml

Absolute ethyl alcohol — 50 ml Hematoxylin crystals — 1 g

Glycerine C.P. — 50 ml Aluminium potassium sulphate — to excess.

Keep the solution in a dark place until it becomes deep red in colour. It may also be ripened or matured in 3 to 4 hours by exposure to a quartz mercury lamp. Place the solution in a wide and shallow evaporating dish at a distance of about 2 feet (61 cm) from the mercury lamp, stir the solution frequently during exposure.

d. Delafield's Hematoxylin:

This is used in mixture with safranin for staining woody plant tissues. To 400 ml of a saturated aqueous solution of ammonium aluminium sulphate add drop by drop a solution of 4 gm. of hematoxylin crystals in 25 ml of 95% ethyl alcohol. Expose this to light and air for 4 days.

Then add 10 ml G.P. glycerin and 100 ml methyl alcohol. Allow this solution to stand for 2 months, exposed to air, till the colour turns sufficiently dark. Alternatively, expose the solution to a quartz mercury lamp for 2 hours as described above.

e. Bismarck Brown Y:

This is a synthetic dye. It is obtained from coal-tar and belongs to the azo group. Bismarck brown is a basic dye. Its solubility is 1.36% in water and 1.08% in alcohol. A 1% solution in 70% ethyl alcohol is used. Cellulose and mucin walls are stained bright brown. The stain is permanent and it rarely over-stains. It is generally used for relatively soft plant tissues without lignin, such as bryophytes.

f. Eosin:

Eosin is available both in bluish and yellowish form. Yellowish eosin is more frequently used. It is a coal-tar dye, acidic and is a fluorine derivative. Solubility in water is 44.2% and in alcohol 2.18%. It is a valuable cytoplasmic stain, although not much used by botanists. Usually a 1% aqueous solution is used.

g. Light Green:

It is a coal-tar dye, acidic and belongs to diamino-triphenyl methane group. Solubility is 20.35% in water and 0.82% in alcohol. It is a very good stain for cytoplasm and cellulose walls. Staining occurs very rapidly. Irrespective of the solvent, a 0.2% to 0.5% solution is used.Usually the dye is dissolved in 90% or 95% alcohol. Sometimes it is dissolved in absolute alcohol and diluted with clove oil. By combining light green with alcoholic Sudan IV, cutinized and suberized tissues can be differentiated from lignified tissues.

h. Safranin O:

It is a coal-tar dye, basic in reaction and belongs to the azin group. Its solubility is 5.45% in water and 2.41% in alcohol. It is a very important stain for botanists. It stains lignified, suberized, cutinized and chitinized structures as also nucleoli, chromosomes and centrosomes.

Various formulae are available for making safranin solution; but generally a 1% solution in 50% alcohol is good enough for most plant materials. A 1% aqueous solution can be used for temporary staining and for this special water solution safranin is available; because although it is soluble in both water and alcohol, it dissolves better in alcohol than in water. Other stains will be mentioned as and when required.

Precautions for Staining:

1. While staining free-hand section, take a few drops of the required reagent in a watch glass staining cube) and, after transferring the sections, always keep it covered by another watch glass.

2. Transfer the section from one reagent to another with a section lifting spatula or a scalpel. Use of brush for transferring sections is not advisable because it causes transference of the reagent too. However, a fine brush may be used, blotting it each time after use. Needles should not be used as they may prick the sections and damage them.

3. Dehydration is influenced by atmospheric humidity. Hence it often becomes difficult to completely dehydrate sections during the rainy season. Fans should be switched-off during staining in order to reduce evaporation of alcohol during staining.

4. The watch glass should be placed on a piece of white paper while taking out sections from stain. They can be easily located against a white background.

5. If sections turn whitish on being transferred to xylol or balsam from absolute alcohol, it indicates incomplete dehydration. If staining is inadequate (either excess or less), it can be re-

stained. In all such cases the sections should be passed down through the alcohol grade in the opposite order as it has come up.

6. While mounting the sections in Canada balsam or any other mounting medium, the cover glass should be dipped in xylol and then placed over the section gradually with the help of a needle.If the balsam appears to be too thick, it should be diluted first with a little xylol. Ordinarily, balsam is neutral, and has a light straw colour. If the balsam is reddish, it indicates acidity. Sections should not be mounted in acidic balsam, because it fades the stain quickly.

7. After mounting, the sections should be kept on a hot plate (37° to 45°C) for at least 24 hours. It helps to drive away air bubbles and also dries the slides.

8. Clearing:

In many plant materials, various cell inclusions often interfere with staining and makes the stained preparation difficult to study. Such sections can be cleared by transferring them from 50% alcohol to a dish of water with a fine forceps or a brush.

Then, using a needle or forceps they are placed in a watch glass containing some sodium hypo-chlorate, household bleach or para-zone and kept for not more than 5 minutes. The whole section may dissolve if left long enough. Wash the sections thoroughly in water after bleaching, don't immerse the brush in the bleach, the bristles will dissolve.

Staining Schedules:

Two main types of stains are in use:

1. Temporary Stains – whose colour fades or which gradually damages the sections, and

2. Permanent Stains – whose colour lasts for many years.

1. Temporary Stains:

a. Methylene blue:

1% aqueous solution is used. The stain is usually mixed with glycerine, 10 ml of 1% aqueous stain is added to 90 ml of 50% glycerine. Sections are directly mounted into this medium. Macerated tissues can also be stained with this mixture.

A drop of washed macerate in water is mixed with a drop of the mixture on a slide and the cover glass is put on. All cell walls turn blue, except cutinized walls which remain unstained. The intensity of the blue colour depends upon the chemical and physical nature of the cell wall. Various wall layers stain differently.

b. Chlor-zinc-iodine solution (Schult's solution):

This solution consists of — Zinc chloride 30 g, potassium iodide 5 g, iodine crystals 1 g and distilled water 140 ml. Sections are placed on the slide and 1 or 2 drops of this solution are added. This is blotted off after 2-4 minutes and a drop of 50% glycerine is added. Alternatively, 50% glycerine is added directly and sections are mounted in the mixture. Cellulose wall turns blue, starch turns blue-black, lignin and suberin turn yellow and moderately lignified wall turns greenish-blue. This stain swells the cell walls and eventually dissolves them. So, observations must be made immediately.

c. Chlorazol black:

A saturated solution of the dye in 70% alcohol is used. Cell walls turn black or grey. It is particularly good for showing pitting.

d. Phloroglucin and conc. HCl:

Add a drop of saturated aqueous solution of phloroglucin to the section taken on a slide and then the HCl. Some prefer to use 20% HCl. Lignin turns red.

e. Sudan IV:

Stain in a saturated alcoholic solution for about 10 minutes and wash rapidly in alcohol. Mount in 50% glycerine. As fat is soluble in alcohol, keep in alcohol no more than necessary. Sudan IV is a specific stain for fat which turns orange. Lecithin, latex, wax, cutin and resins also stain. Chloroplasts are stained a dull red.

f. Iodine with potassium iodide:

An aqueous solution of 1 % iodine and 1 % potassium iodide is used. The sections are stained for a few minutes and observed directly or after mounting in 50% glycerine. An aqueous 1% iodine solution may also be used. Starch turns blue, cellulose, inulin deposits, proteins and alkaloids turn brown and pectin, cutin, callose and cork turn yellow.

g. Eosin:

An 1% aqueous solution of eosin is sometimes used. It gives general yellowish colour to the tissue.

2. Permanent Stains:

Permanent staining is always accompanied by complete dehydration and clearing followed by mounting in Canada balsam or euparal etc. (For details see Cytology section).

Depending upon the number of stains used, it is called:

(a) Single staining,

(b) Double staining, etc.

(a) Single Staining: Single staining is done generally in case of thallophytes and bryophytes where no lignified tissue is present and the cell wall material is mostly cellulose:

I. Heidenhain's Iron Hematoxylin:

1. Transfer the sections from water to a mordant (a mordant is any substance that combines with and fines an dye-stuff in material that cannot be dyed direct) containing iron, aluminium or copper, because hematoxylin cannot stain tissues without mordanting with one of these three metals.Ordinarily, ferric ammonium sulphate (iron alum) is used. The strength of the solution is usually 3% but varies from 2% to 4%; weaker solutions being more suitable for softer tissues such as algae. The solution should be freshly prepared before use.A permanent mordanting mixture can be prepared by mixing 500 ml. distilled water, 5 ml. glacial acetic acid, 0.6 ml. C.P. sulphuric acid and 15 g of clear violet coloured alum crystals. The time required for mordanting should not exceed 2 hours and, with thin sections, 1 hour is sufficient.

2. Wash thoroughly in running water for 5 minutes and then rinse in distilled water to remove salts present in tap-water, which interfere with staining.

3. Stain in 0.5% aqueous Heidenhain's hematoxylin solution. As a rule, the sections are left in the stain at least as long as they were left in the mordant; but, in most cases, 24 hours is the optimum time.

4. Wash off excess stain with water.

5. Destain in 2% ferric ammonium sulphate (or, ferric chloride). The time required for de-staining varies with the nature of the material, the thickness of sections, etc. Differentiate under a microscope. When the sections appear grayish-black, de-staining is complete.

6. Wash in running water for 30 minutes to 1 hour to remove all traces of the de-staining solution, which, if left, will gradually fade the stain.

7. Dehydrate in 50%, 70% and 95% alcohol, keeping at least 5 minutes in each. Give two changes in absolute alcohol at 5 minutes' interval.

8. Keep in absolute alcohol-xylem mixture (1: 1) for 5 minutes.

9. Give two changes in xylol at 5 minutes' interval.

10. Mount in Canada balsam and dry on a hot plate.

N. B.:
Although hematoxylin staining is rather cumbersome, the result is well worth it as different cellular components, including chromatin matter, take up various shades of stain.

II. Bismarck Brown Staining:

This is a suitable stain for soft and non-lignified plant tissues such as bryophytes and thallophytes.

The schedule is:
1. Take the sections from water and pass through 30% and 50% alcohol keeping 5 minutes in each. 30% alcohol may as well be omitted.

2. Stain the sections in Bismarck brown (1% solution in 70% ethanol) for 5 to 15 minutes. The duration of staining depends upon the concentration of the stain and the thickness of sections. If the stain appears to be relatively more concentrated, it may be diluted with a few drops of 70% alcohol. If the sections are rather thick, stain for a shorter period so that the stain remains light.

3. Pass through 80% (or 90%) and 95% alcohol keeping 5 minutes in each. Give two changes in absolute ethyl alcohol keeping at least 5 minutes in each.

4. Clear in xylol, preferably giving 2 changes of at least 5 minutes' duration.

5. Mount in Canada balsam, Euparol or DPX mountant. All cell walls turn brown.

III. Light Green Staining:

Single staining can also be done using light green instead of Bismarck brown. Staining is done after dehydration in 90% alcohol as light green is dissolved in 95% alcohol (1% solution in 95% alcohol). The rest of the staining schedule is the same as in Bismarck brown staining.

IV. Ehrlieh's Hematoxylin Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain in matured Ehrlieh's hematoxylin solution for 5 to 30 minutes.

3. Wash out excess stain with 50% alcohol.

4. Dehydrate, clear and mount in Canada balsam.

This is an excellent stain for algae, fungi and small bryophytes.

(b) Double Staining: Double staining is resorted to in case of all sections having both lignified and non-lignified tissues, i.e., in case of pteridophytes, gymnosperms and angiosperms. One of the two stains is specific for lignified tissues and the other stains the non-lignified tissues, i.e., mainly cellulose. The sections with two different stains show more contrast. There are numerous double staining schedules.

Some of these, which are usually followed in the class, are discussed:

I. Safranin and Delafield's Hematoxylin Staining:

1. Freshly mix safranin (1% in 50% alcohol) and matured Delafield's hematoxylin in the proportion of 1: 4. Filter the mixture. This stock mixture can be used up to a week, but should be filtered each time before use.

2. Stain the sections in this mixture for 2 to 6 hours. Some sections need less time. If it is more convenient to stain overnight, use safranin-hematoxylin mixture in the proportion of 94: 6.

3. Transfer the sections to 50% alcohol containing 2 to 3 drops of conc. HCl. This solution removes the stain, acting first on safranin. The duration of de-staining has to be learnt by experience. Generally, sections should be removed when they still appear slightly dark or over-stained under microscope.

4. Transfer the sections to 95% alcohol and keep for 5 minutes. This is followed by 5 minutes in absolute alcohol.

5. Keep the sections in alcohol-xylol mixture (1: 1) for 5 minutes.

6. Transfer to xylol and keep for 10 minutes.

7. Mount in Canada balsam and dry on a hot plate.

Lignin turns red, cellulose turns dark blue and cellulose walls with some lignin become purple.

II. Safranin and Fast Green Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain in a 1% solution of safranin in methyl cello solve 50% alcohol for 2 to 24 hours, or even 48 hours (Prepare safranin solution by dissolving 4 g of safranin in 100 ml each of 95% alcohol and distilled water followed by 4 g of sodium acetate and 8 ml of formalin. The acetate intensifies the stain and formalin acts as a mordant.).

3. Wash off the excess stain with running water for a few moments.

4. Differentiate and dehydrate with 95% alcohol to which 0.5% picric acid has been added. Ordinarily, about 10 seconds of treatment is sufficient for differentiation.

5. Stop action of the acid by immersing the slide in 95% alcohol to which 4 to 5 drops of ammonia per 100 ml of alcohol has been added. This treatment should not be continued for more than 2 minutes, as alcohol extracts stain.

6. Dehydrate in absolute alcohol for 10 seconds.

7. Counterstain in fast green for not more than 15 seconds (Prepare a nearly saturated solution of fast green in equal parts of methyl cello solve and absolute alcohol and 75 parts clove oil.). This stain can be used repeatedly.

8. Pour the fast green stain back into dropping bottle and rinse off the excess stain with clove oil. Used clove oil may be used.

9. Clear in a mixture of 50 parts clove oil, 25 parts absolute alcohol and 25 parts xylol — for a few seconds (Actually this reagent mixture, after use, is collected in a bottle and used in step 8.).

10. Remove the clearing mixture by treating for a few seconds in xylol (Add 3 to 4 drops of absolute alcohol to this xylol to take care of any moisture that may be inadvertently brought over.).

11. Give two changes in pure xylol for at least 10 minutes' interval and then mount in Canada balsam.

The safranin appears a brilliant red in chromosomes, nuclei and ii} lignified and cutinized cell walls; while the fast green gives a bright green colour to cellulose cell walls and cytoplasm. Both the colours are permanent and they persist for many years.

III. Safranin and Light Green Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain the sections in 1% solution of safranin in 50% alcohol for 30 minutes. The exact duration of staining depends upon the intensity of the dye solution. Some anatomists (Johansen etc.) prefer to make a 1% solution of the dye in 95% alcohol. A part of this stock solution is diluted with an equal volume of distilled water when needed for use. If this proves to be a strong solution, it may be further diluted with 50% alcohol.

3. Dehydrate in 70%, 80% and 90% alcohol, keeping 5 minutes in each.

4. Counterstain in 1% light green in 95% alcohol, for 15 seconds to 1 minute. The exact duration of staining is to be determined by experience. It depends on the intensity of the dye solution. If required, the staining solution may be further diluted by adding more 95% alcohol.

5. Dehydrate in absolute alcohol for 5 minutes.

6. Pass through alcohol xylol (1: 1) mixture keeping the sections in it for 5 minutes.

7. Clear in xylol for at least 10 minutes.

8. Mount in Canada balsam and dry on a hot plate.

Lignified walls turn red and cellulose walls green. Often the entire section turns green indicating over-staining in light green and under-staining in safranin. In such cases, the duration of safranin staining should be increased, while reducing the duration of light green staining.The light green solution may also be further diluted. Sometimes, both the stains appear to be rather fade. To counteract this, the duration of treatment in different alcohol grades should be reduced. Although both the stains are permanent, light green appears to fade rather quickly.

Probable Questions:

1. What are section cutting? What are the precautions?
2. How mounting, stretching and drying is done in microtome?
3. What are the types and uses of microtomes?
4. Describe different types of stains and fixatives.

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyay and Upadhyay.

UNIT-IV

Antibody generation, antigen-antibody interaction and immunohistochemistry

Objective: In this unit we will discuss about antibody antigen interaction, how antibodies are generated and also about immunohistochemistry.

Introduction:

Antigen (Ag) antibody (Ab) reactions occur when an antigen combines with a corresponding antibody to produce an immune complex. Therefore, an antigen-antibody reaction is thus a bimolecular association which is similar to an enzyme-substrate interaction but the only difference is that antigen-antibody reaction does not lead to an irreversible chemical interaction.

The basis for antigen-antibody reactions are the non-covalent interactions like hydrogen bonds, ionic bonds, van der Waal interactions, hydrophobic interactions, etc. These interactions are individually weak, therefore, a large number of such interactions work together in an antigen-antibody reaction. The in vitro study of antigen antibody reactions is known as serology.The principle for all diagnostic immunological tests is serological reactions. The binding of an antibody with an antigen of the type that stimulated the formation of the antibody, results in agglutination, precipitation, complement fixation, greater susceptibility to ingestion and destruction by phagocytes, or neutralization of an exotoxin.

The main use of antigen-antibody reactions is in the determination of blood groups for transfusion, serological ascertainment of exposure to infectious agents, and development of immunoassays for the quantification of various substances.

Schematically an Antigen-Antibody Reaction can be represented as:
Ag + Ab [Ag-Ab] → Aggregation → Precipitation/Agglutination/Neutralization

For diagnostic immunological tests, the serological tests must possess high specificity and sensitivity. Specificity is the ability of an antibody to recognize a single specific antigen. There is a high degree of specificity in antigen-antibody reactions.

Antibodies can distinguish differences in:

i. Primary structure of an antigen,

ii. Isomeric forms of an antigen, and

iii. Secondary and tertiary structure of an antigen.

Therefore specificity implies that:

a. Antibody is specific for a single and specific antigen.

b. Antibody wills not cross-react with other antigens.

c. It will not give false positive results.

Sensitivity means the lowest amount of antigen that can be detected. If in a diagnostic test an antibody is capable of detecting a single antigen molecule, then such a test possesses highest sensitivity. The amount of antigen detected in a test is directly proportional to the amount of antibody used. Enzyme Linked Immuno Sorbent Assays (ELISA) is the most sensitive serological tests.

The following points highlight the important antigen-antibody reactions.

1. Precipitation Reactions:

The reaction of soluble antigens with IgG or IgM antibodies to form a large interlocking aggregates (lattices) is called precipitation reaction. The precipitates formed by antibodies are known as precipitins.

The precipitation reactions occur in two stages:

(i) Rapid interactions within a second between antigen and antibodies and formation of complex.

(ii) Slow rate of reaction completing even within a few minutes or hours and forming lattices from antigen-antibody complexes.

When the antibodies and antigens are in proper ratio, precipitation reactions normally occur. When there is excess amount of either of two, no visible precipitate is formed.One can produce the optimal ratio of these two by putting antigens and antibody adjacent to each other and waiting for their diffusion together. In precipitation test, a precipitation ring appears which display the creation of optimal ratio. This zone is known as the zone of equivalence (Fig. 22.21).

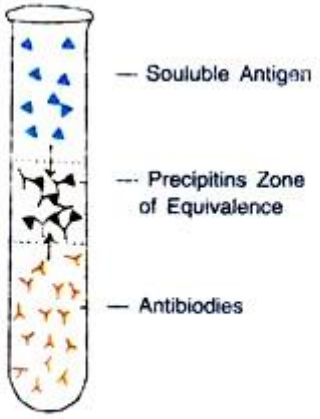


Fig. 22.21: The precipitation ring test.

2. Immunodiffusion Test (IDT):

Immunodiffusion tests are performed in a gelled agar medium. One of the IDTs is Ouchterlony test (Fig. 22.22). In Ouchterlony test wells are cut, into which a purified antiserum (a serum containing antibodies) is added, and to each surrounding well, soluble form of test antigens are added.Thereafter, a line of visible precipitate is formed between the wells where after diffusion optimal ratio of antigen-antibody is formed. Through the Ouchterlony test, the presence of antibodies in the serum against more than one antigen at a time can be demonstrated. Through this test, identical, partially identical and different types of antigens can also be found out.

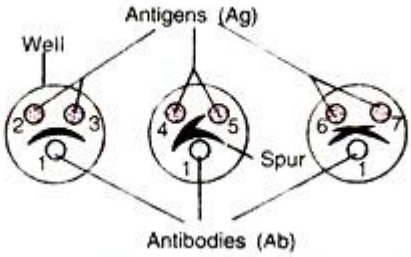


Fig. 22.22: The Ouchterlony immunodiffusion test. 1, antiserum filled in wells, 2-7, different types of antigens; A, line of identity (diffusion of antigens and antibodies does not occur, therefore, they react and precipitate to form the dark line); B, lines of partial identity (antigens are not identical but they share many antigenic determinant sites and related to each other); C, line of non-identity (antiserum contains antibodies against antigens in wells). The antigens are not related because they diffuse across each others' zone of precipitation.

3. Counter Current Immunoelectroptioresis Test (Counter Immunoelectroptioresis (CIE)):

CIE not only depends entirely on diffusion of antigen and antibody in a gel, but also uses electrophoresis for their rapid movement (Fig. 22.23). By using this method protein can be

separated within an hour. CIE is useful for the diagnosis of bacterial meningitis and the other diseases.

The principle of CIE is based on the movement of antigens and antibodies to opposite poles after applying electric current in buffers of correct electric strength and pH, because some of the antigens and antibodies have the opposite charges. If a reaction occurs, a precipitation line appears within an hour.

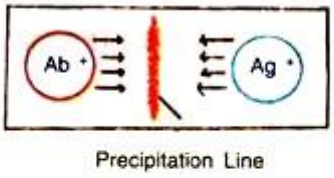


Fig. 22.23: Counter immunoelectro-phoresis (CIE). Ag, antigen; Ab, antibodies.

4. Agglutination Reactions:

Agglutination is the process of linking together of antigens by antibodies and formation of visible aggregates. Agglutination reactions involve particulate antigens i.e. soluble antigens adhering to particles. Agglutination reactions are very sensitive, readable and available in several varieties.

It is of two types, direct and indirect agglutination tests:

A. Direct Agglutination Test:

Direct agglutination test diagnoses antibodies against a large number of cellular antigens such as RBCs, bacteria and fungi. This test is carried out in plastic microtiter plates that have several small shallow wells. Each well acts as small test tube (Fig. 22.24).

Previously this test was done in test tubes. However, each well contains an equal amount of particulate antigen (e.g. RBCs) but the amount of antibodies in the serum is serially diluted in successive wells so that their concentration may be half of the previous well.

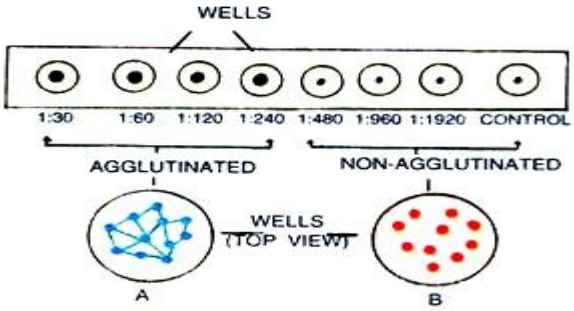


Fig. 22.24: Direct agglutination test.

If one starts with more antibodies, more dilutions will be required to lower the amount to a point at which agglutination does not occur. This is the measure of titer or concentration of serum antibody.

In a positive reaction, agglutination occurs, and sufficient antibodies are present in the serum to link the antigen together. This results in formation of antibody-antigen mat which sinks to bottom of well (A). However, in the negative reaction, agglutination does not occur and insufficient antibodies are present to cause the linking of antigens.The particulate antigens roll down the sloping sides of the well, and form a pellet at the bottom. In this example the antigen titer is only 80 since the well with a 1: 80 concentration is the most dilute concentration that gives a positive reaction. It may also be demonstrated that before illness, blood of persons does not have any antibody, whereas titer develops significantly with the progress of disease. This change in titer is called servotiter.

B. Indirect (Passive) Agglutination Tests:

These type of diagnostic tests are very rapid particularly for the detection of streptococci. If the antigens are adsorbed onto particles (e.g. RBCs, latex beads, bentomile clay), soluble antigens can respond to agglutination test. Antibody reacts with the soluble antigen adhering to the particles. Therefore, the particles agglutinate with each other as these do in the direct agglutination tests.

C. Haemagglutination:

Haemagglutination is the phenomenon of clumping of RBCs. When the RBCs are agglutinated by certain viruses such as those causing mumps, measles, influenza, etc. it is called viral haemagglutination. In the serum of a person, certain antibodies act against the antigens (of these viruses), the antibodies neutralize them after reaction. The haemagglutination test is widely used for the diagnosis of a number of viruses including those as above.

5. Complement Fixation Reactions:

A group of 20 or more serum protein is collectively known as complement. During reaction, the complement binds to antigen-antibody complex and is used up or fixed. This process of complement fixation may be used to measure even very small amount of antibody that does not produce a visible reaction such as precipitation or agglutination. Therefore, it is necessary to use indicator system.This method is used in diagnosis of diseases such as leptospirosis, mycoplasmal pneumonia, Q fever, polio, rubella, histoplasmosis, coccidiodomycosis and streptococcal infections. The test requires patient's serum, test antigen, complement from guinea pig and antibodies of sheep RBCs to determine whether sheep RBCs may be lysed by guinea pig complement.

The test is accomplished in the following two stages:

Stage 1:

The patient's serum is heated at 56°C for 30 minutes so that the complement should be inactivated. The heated serum is diluted and then added to known amount of specific antigen and complement (Fig. 22.24). The test antigen may correspond to the diseases.

For example, if a patient is suffering from a disease caused by streptococci the test antigen would be the streptococcal antigen. If the patient's serum contains antibodies against streptococci, the test antigen will form complement sequence. This mixture is again incubated for about 30 minutes. At this point, no antigen-antibody reaction occurs.

Stage 2:

In stage 2, the complement fixed by antigen-antibody reaction is detected by an indication system. This system consists of sheep RBCs containing specific antibodies attached to their surfaces.

When these are added to complement, haemolysis of RBCs occurs that impart changes in colour of the mixture. This shows that the complements have not been fixed during the first stage; therefore, these become available to cause haemolysis (Fig. 22.25). This indicates that the patient has no streptococcal pneumonia.

However, if the guinea pig complements are destroyed, they will not be able to cause the lysis of RBCs. On the other hand, if the complements are fixed (by antigen- antibody reaction) during the first stage, these will not be available to cause haemolysis during the second stage. This indicates that the patient has the infection of streptococci.

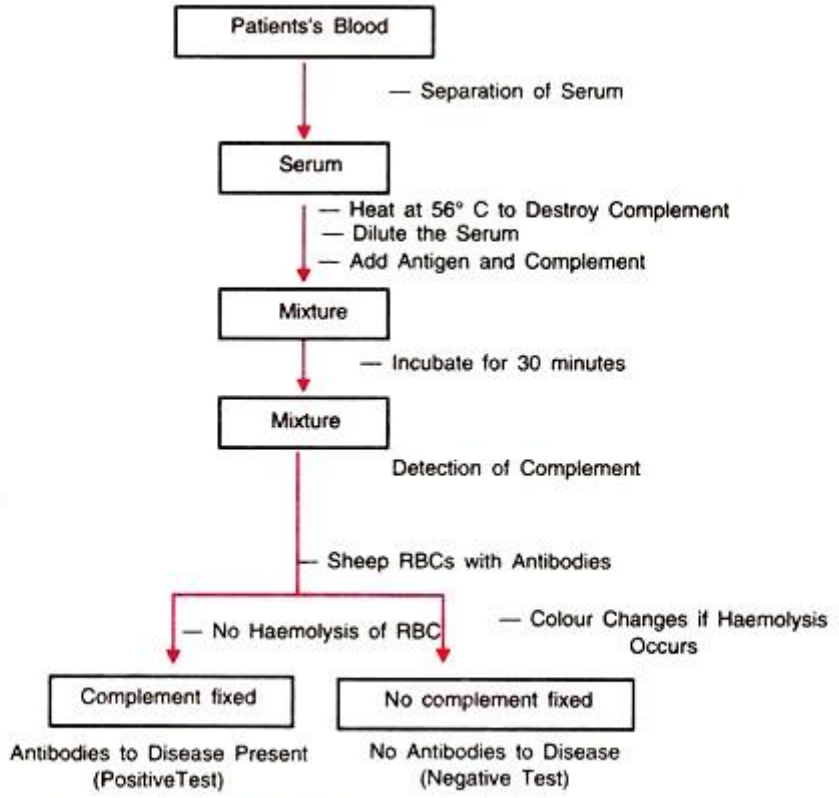


Fig. 22.25: Procedure for complement fixation test.

6. Neutralization Reactions:

The neutralization reactions are the reactions of antigen- antibody that involve the elimination of harmful effects of bacterial exotoxins or a virus by specific antibodies. These neutralizing substances i.e. antibodies are known as antitoxins. This specific antibody is produced by a host cell in response to a bacterial exotoxin or corresponding toxoid (inactivated toxin).The antitoxin reacts with exotoxin and neutralizes it. These antitoxins can be artificially induced in animals such as horses. Thus, the antitoxin of animal sources in turn can be injected into human which provides a passive immunity against a toxin present in human body produced by the pathogens causing diphtheria, tetanus, etc.

a. Diagnosis of Viral Infections:

Neutralization test is very useful in diagnosis of viral infections in humans. After introduction of a virus, antibodies are produced in response and bind to receptor sites present on the viral surfaces. After binding of antibodies, viral particles fail to reach to the cells. Thereafter, the virus is destroyed.

Artificially, the virus is capable of destroying their cell-damaging effect in cell culture or embryonic eggs can be used to determine the presence of antibodies against them. However, when serum contains antibodies against a particular virus, the antibodies will not allow the virus to infect the cell in cell culture; consequently the cells will not be damaged.

b. Schick Test:

Schick test measures the level of immune system of a person to the infection of diphtheria. When testing the status of immunity, a small amount of diphtheria exotoxin is inoculated in the skin of a person. Depending on ability and quantity of antitoxin, positive or negative responses develop.If serum antitoxin in body would be in sufficient amount to neutralize the exotoxin, no visible reaction will occur. In control, when serum antitoxin is in insufficient amount the exotoxin will damage the tissues at the site where incision was made, and will produce a swollen and reddish area which is converted into brown within 4 or 5 days. This shows that the immune response is not present to a satisfactory level.

7. Radioimmunoassay (RIA):

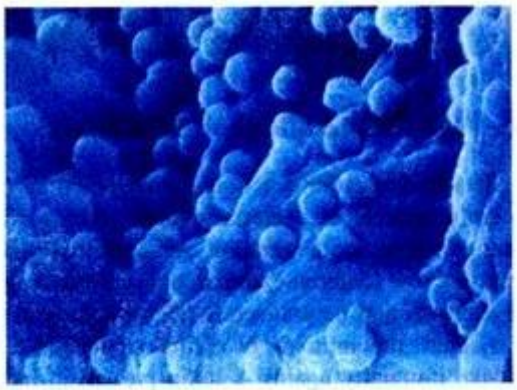
It is such a technique which is highly sensitive and can measure even the less concentration (i.e. 0.001 µg/ml) of antigen or antibody. In 1960, for the first time this technique was developed by S.A. Berson and R. Yalow when they were engaged in determining the concentration of insulin and anti-insulin complexes in diabetics. Thereafter, Berson died, and significance of this technique was realised. In 1977, Yalow was awarded a Nobel Prize.

There are two methods of measuring RIA: the liquid phase and the solid phase RIAs.

1. Liquid Phase RIA:

The liquid phase RIA is based on competitive binding of radiolabeled antigen and un-labelled antigen, to a high affinity antibody. The antigen labelled with ^{125}I is mixed with such a concentration of antibody that can just saturate the antibody. Therefore, the increasing amount of antigen (un-labelled) of unknown concentration is added. The two types of antigens now compete for available sites of the antibody.

The antibody does not differentiate the labelled antigen from the un-labelled one. Upon gradually increasing concentration of un-labelled antigen, the labelled antigen could be displaced from the binding sites available on antibody. The labelled antigens are made free in the solution. The amount of labelled antigen in solution is measured, and the concentration of un-labelled antigen can be determined.



ELISA kits being developed to test for AIDS antibodies, the disease caused by HIV. The viral particles bud out of an infected lymphocyte.

2. Solid Phase RIA:

In solid phase RIA, either antigen or antibody is immobilized on a solid phase matrix. It is simple and easy in handling as compared to liquid phase RIA.

8. Enzyme-Linked Immunosorbent Assay (ELISA):

The principle of ELISA is similar to RIA, but differs slightly. In RIA radiolabelled antigen is used, whereas in ELISA enzyme is used that reacts with a colourless substrate and develops a coloured reaction product. There is a large number of enzymes such as alkaline phosphatase, horse radish peroxidase, and p-nitro-phenyl phosphatase which are employed in ELISA. As compared to RIA, this assay is both cheaper and safer.On the basis of known concentration of antigen or antibody a standard curve is prepared from which the unknown concentration of sample is measured. A microliter plate with numerous shallow wells is used in this method. It is very useful in testing for AIDS antibodies. However, now-a days a number of ELISA kits have been developed and are in current use.

A. Indirect ELISA:

It is used to measure antibody. Known antigen is coated on the plastic lining of the wells of microtiter plate which is made up of polystyrene latex. To test for the presence of antibodies against this antigen in the patient, his blood serum is added to the wells (Fig. 22.26A). If the patient's serum contains antibody specific to antigen, the antibody will bind to the absorbed antigen otherwise not.

After incubation the wells are washed and the enzyme, labelled with antihuman gamma globulin (anti-Hgg), is added to the wells. Anti-Hgg can react with antigen antibody complex. The mixture of wells is washed to remove the excess of unbound labelled anti- Hgg. Finally the correct substrate for the enzyme is added which is hydrolysed by the enzyme and develops a colour. Varying concentrations of antibody in serum shows changes in the intensity of colour. This method is very useful in detection of antibodies to HIV, Salmonella, Yersinia, Brucella, Treponema and streptococci.

B. Double Antibody Sandwich ELISA:

This method detects antigen. In this case antibody (antiserum) is immobilised on the surface of wells of microtiter plate (Fig. 22.26B). A test antigen is added to each well and allowed to react with the bound antibody. It is incubated during this period. If antigen combines specifically with antibody absorbed to wells, the antigen will be retained even after washing and unbound antigen would be made free.

Thereafter, a second enzyme-linked antibody (e.g. alkaline phosphatase tagged to antibody) is added to react with bound antigen. It is again incubated for a few seconds, the enzyme labelled antibody reacts with the antigen-antibody complex already formed in the wells and results in the development of a "sandwich'.The mixture in wells is washed again to remove the excess of labelled enzyme. A chromogenic substrate e.g. nitro-phenyl phosphate is added which reacts with enzyme and develop yellow colour.

The reaction can be stopped simply by changing the pH or denaturing the enzyme. The change in colour is measured visually or spectrophotometrically. Change in colour shows the presence of desired antigen in the sample. This technique is useful in detection of toxins of Vibrio cholerae, E. coli, Staphylococcus enterotoxin-A and antigens of rotavirus.

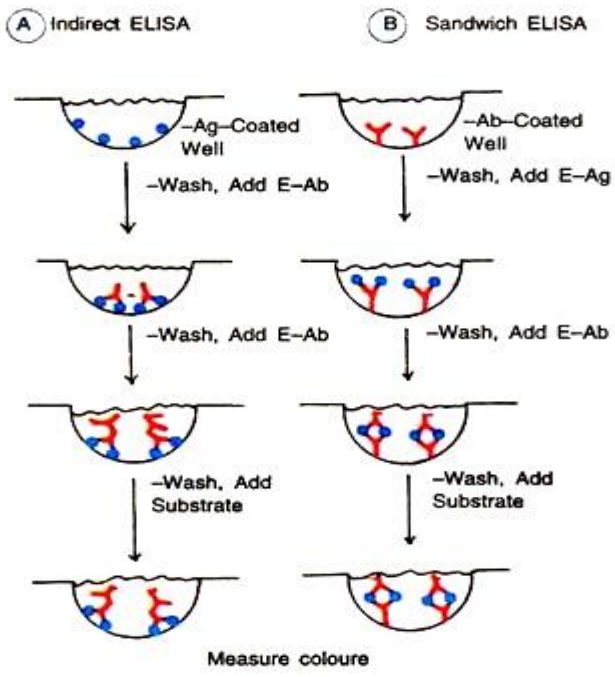


Fig. 22.26: Enzyme-linked immunosorbent assay (ELISA) technique for detection of antibody (A) and antigen (B).

9. Fluorescent Antibody (FA) Technique:

The FA technique is used to detect the microorganisms present in clinical specimens, and specific antibodies present in serum. If the antibodies bind to cell or tissues, it can be

observed by tagging the antibody with a fluorescent dye such as fluorescein isothiocyanate and rhodamine.

Both the dyes can conjugate the FC region of antibody without affecting the specificity and make the antibody fluorescent when exposed to UV light. Fluorescein absorbs blue light (490 nm) and emits yellow green fluorescence (517 nm). Similarly, rhodamine absorbs the yellow green light (515 nm) and emits deep red fluorescence (546 nm). The FA technique is very useful in testing for rabies within a few hours with 100% accuracy.There are two methods of FA test, direct FA test and indirect FA test. Direct FA test is used to identify the microorganisms present in clinical specimen. The specimen containing antigen is fixed onto a slide and, thereafter, fluorescein-labelled antibodies are added on the specimen. It is incubated for a few minutes.The slide is washed to remove unbound antibody and observed under the UV microscope for yellow-green fluorescence. The indirect FA test is useful for the detection of specific antibodies in serum formed by a microorganism.

This method follows the following steps:
(a) Fix a known antigen onto a slide,

(b) Add a test serum (microorganism-specific antibody reacts with antigen and forms a bound complex,

(c) Add fluorescein-labelled anti-Hgg to the slide,

(d) Incubate and wash the slide,

(e) Examine the slide under fluorescence microscope.

The development of fluorescence confirms the presence of antibody specific to antigen fixed on slide.

Immunocytochemistry:

Immunocytochemistry is a procedure that is done in a laboratory that visualizes proteins and peptides. Samples are obtained from sections of tissues, mouth swabs, blood, or any other type of sample from humans, plants, and animals. Biomolecules or antibodies are applied to samples which react with the protein (antigen) that is being tested, causing a change in color to identify the protein. The antibody is associated with a reporter such as an enzyme, fluorescent dye, or fluorophore. The reporter changes the color of the protein which is seen by using a microscope.

Phil has his equipment and is now taking time to familiarize himself with the samples he is testing. The type of sample being tested determines if there needs to be an incubation period

before the staining can begin, which type of reporter will be used, and what microscope is needed for visualization.

Immunochemistry is the study of the chemistry of the immune system. This involves the study of the properties, functions, interactions and production of the chemical components (antibodies/immunoglobulins, toxin, epitopes of proteins like CD4, antitoxins, cytokines/chemokines, antigens) of the immune system. It also include immune responses and determination of immune materials/products by immunochemical assays.

In addition, immunochemistry is the study of the identities and functions of the components of the immune system. Immunochemistry is also used to describe the application of immune system components, in particular antibodies, to chemically labelled antigen molecules for visualization.Various methods in immunochemistry have been developed and refined, and used in scientific study, from virology to molecular evolution. Immunochemical techniques include: enzyme-linked immunosorbent assay, immunoblotting (e.g., Western blot assay), precipitation and agglutination reactions, immunoelectrophoresis, immunophenotyping, immunochromatographic assay and cyflometry.

Immunocytochemistry Uses

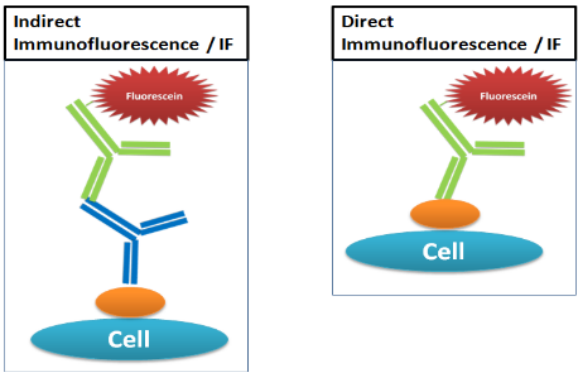
The body is made up of many different types of proteins, peptides, and antigens that help carry out functions of the immune system. **Antigens** are single molecules that combine to form peptides. **Peptides** are called **proteins** when they contain more than fifty amino acids. The identification of proteins in cells is important in recognizing the body's immune response to various antibodies. **Antibodies** are used to fight infections in the body. Immunocytochemistry helps researchers determine which antibodies will bind to specific antigens and build immunity.Phil is now ready to start applying immunocytochemistry to his samples. Some samples require direct detection while others require indirect detection. He gathers the samples that are ready after necessary incubation time.

There are three concepts which include immunohistochemistry, immunocytochemistry and immunofluorescence. The assay of immunohistochemistry is relatively easy to confirm and distinguish from other two assays. From the scope of the concept, the range of coverage of immunofluorescence its larger and immunofluorescence include immunohistochemistry and immunocytochemistry. Immunohistochemistry is an assay for confirming the expression and expression location of proteins in nature tissue section. Immunofluorescence can be used on cultured cell lines, tissue sections, or individual cells. Immunofluorescence may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules in cells or tissues. Immunocytochemistry is performed on sample of intact cells.

Immunocytochemistry (ICC) is a common laboratory assay that can confirm the expression and location of target peptides or protein antigens in the cell via specific combination of antibodies and target molecules. These bound antibodies can then be detected using several different methods. Immunocytochemistry (ICC) allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an

immunopositive signal is found, ICC also allows researchers to determine which sub-cellular compartments are expressing the antigen.

There are also two different immunocytochemistry assay which include indirect nd direct immunocytochemistry technique. For indirect immunocytochemistry assay, the protocol mainly include preparation and culture of cells, cells fixation, serum blocking, primary antibody incubation, marked second antibody incubation, staining, result judgment and imaging. For direct immunocytochemistry assay, there are only marked primary antibody been incubated without second antibody and other steps are same with indirect immunocytochemistry.



Principle of Immunofluorescence (IF)

Antibody Production:
(a) Polyclonal Antibodies:

It is possible to produce antibodies which bind to proteins, peptides, carbohydrate and nucleic acids. In general, most immunochemical methods are devised for use with antibodies that recognize proteins and peptides.

Choice of species for antibody production depends upon the amount of antigen available, amount of antiserum required and quality of antiserum desired. In some case use of closely related species or different strains within single species for derivation of antigen and production of antibody can provide antibodies with specific properties.

The most important consideration for immunogenicity is differences in amino acid sequences. Immunogenicity tends to increase with size. Peptides with molecular weight less than 2000 are poor antigens while with more than 10000 are good antigens as long as they are recognized as foreign in responding animal.Antibodies that bind to smaller peptides can be produced by linking (conjugating) these substances to larger proteins called as carrier

proteins. Thus the Antisera produce will recognize carrier protein too along with peptide. Substances that are not immunogenic alone, but are when conjugated are known as heptane's. Such substances potentiate the immune response by forming a slow-release depot of antigen, by stimulating T cell help or by aiding antigen presentation.

Single immunization results in production of antisera but it is usually suboptimal, containing antibodies of low avidity and a high proportion of IgM, therefore several "booster" immunizations with the antigen is done so as to boost the antibody production by making the animal hyper-immune. Such "hyper-immune sera" are usually the polyclonal reagent of choice for immunochemical techniques.

(b) Monoclonal Antibodies:

Monoclonal antibodies are secreted by cloned, i.e., monoclonal cells. Mature, antibody secreted lymphocytes from immunized animals can be cloned, but these survive only for very few period in culture, and therefore, do not provide useful amount of antibody.However, procedures have been developed to separate antibodies from continuously growing (immortal) cells. These involve generation of hybrid cells, transformation of lymphocytes with virus or recombinant DNA.

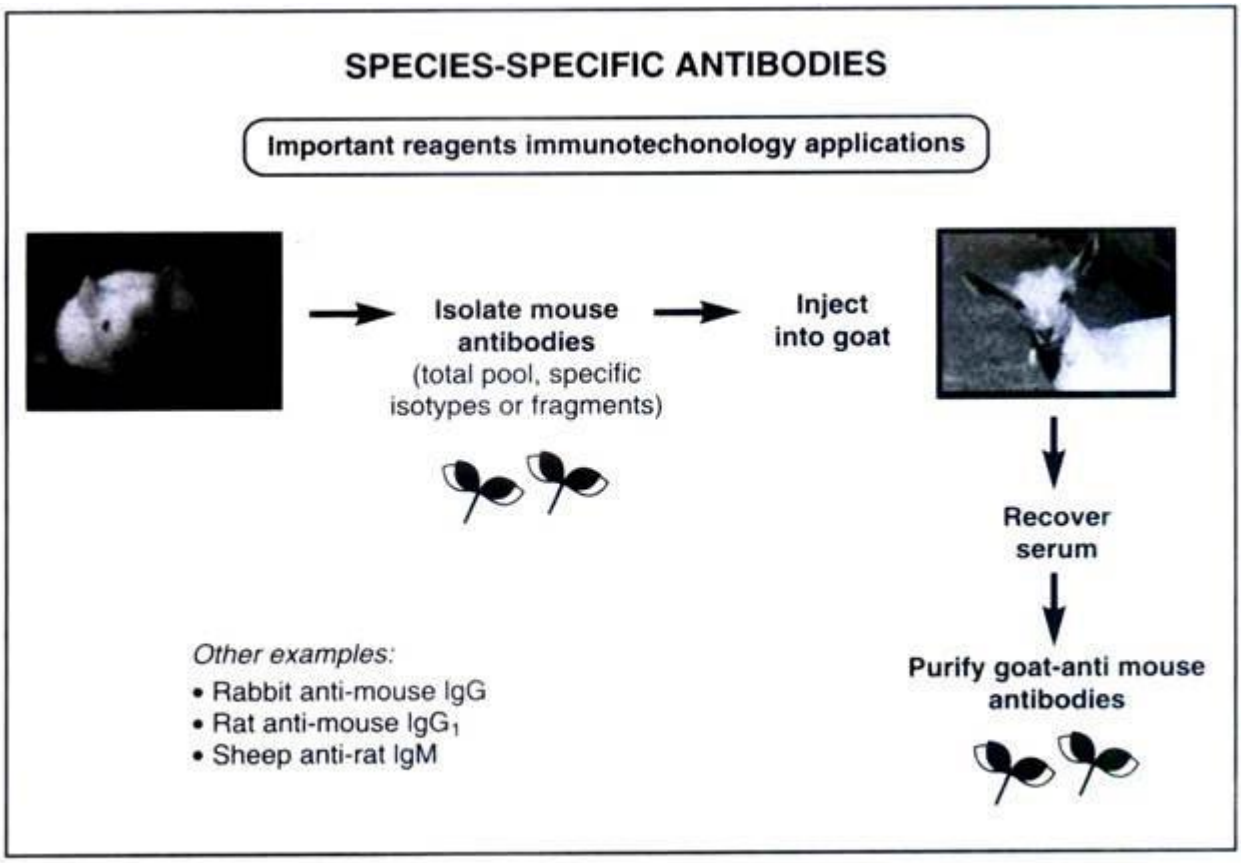


Fig. 6.1: Figure showing the development of species specific antibodies

Antibody Labelling:

Like the fluorochrome as described in above section, antibodies can also be tagged with enzymes, or carbohydrate or biotin for different purposes. Labelling antibodies with enzymes, fluorochromes, or biotin provides a signal for visualization or quantitation of the target molecule. Antibody bound to agarose is useful for separating a target antigen from a complex mixture.To avoid excessive background staining and to improve sensitivity, only purified antibodies should be used for staining. At the minimum, the IgG fraction, which contains naturally occurring immunoglobulin as well as specific antibody, should be isolated from the antiserum.

This can be done by various methods, usually involving a combination of fractionation and chromatography. Preferably, the antibody should be affinity isolated on a column containing the antigen bound to a solid support. This will eliminate all serum proteins, including immunoglobulin's that do not specifically bind to the antigen.

Labelling with Enzymes:

Antibodies may be labelled with various enzymes to provide highly specific probes that both visualize the target and amplify the signal by acting on a substrate to produce a coloured or chemiluminescent product. Horseradish peroxidase and alkaline phosphatase are the most commonly used enzymes for this purpose. Antibody-enzyme conjugates may be used for detecting proteins in immuno-histology and immuno-cytology, immuno-blotting, and ELISA.

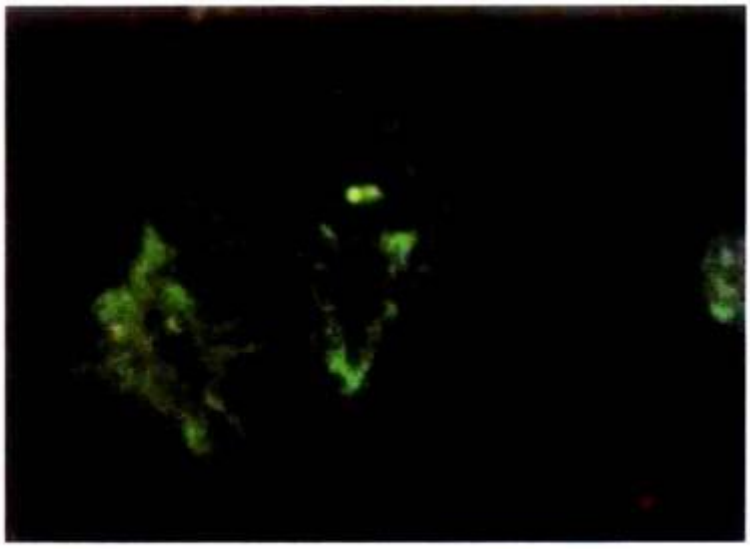


Fig. 6.12: Direct immunofluorescence of cytoskeletal protein actin in human platelets by phalloidin labelled with FITC (Courtesy: Dr D Dash, Deptt. of Biochemistry, IMS, BHU)

Labelling with Fluorochromes:

Fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), and R-phycoerythrin (PE), represent the fluorescent probes most commonly cited. Labelling proteins with fluorochromes provides a coloured reagent that can be observed directly. The intensity and narrow wavelength of the fluorochromes make them useful in immunohistochemistry and immunocytochemistry, using both visible and fluorescence microscopy, and in flow cytometry.

Labelling with Biotin:

Biotinylated antibodies are widely used in systems where signal amplification is desired. Biotin binds avidin with a high degree of affinity and specificity. Avidin, ExtrAvidin®, or streptavidin labelled with enzymes or fluorochromes can bind biotinylated antibodies, amplifying the signal and allowing detection of antigens present in small amounts. This system may be used in immunohistochemistry and immunocytochemistry, immuno-blotting, ELISA, and flow cytometry.

Labelling with Gold:

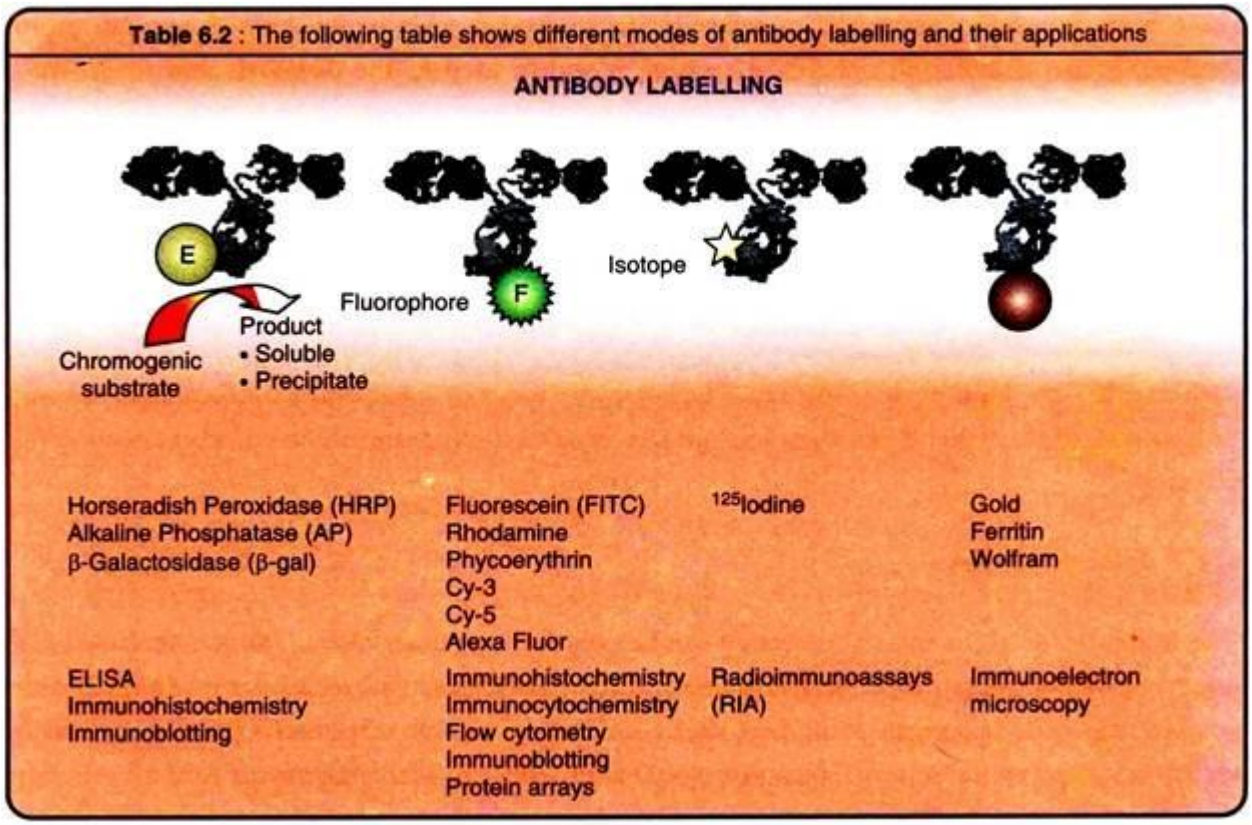
Antibodies conjugated to colloidal gold are used primarily in electron microscopy (EM) because of the electron-dense nature of the gold particles. Gold-antibody conjugates may also be used for immunohistochemistry and immunocytochemistry, and immuno-blotting. In some cases silver enhancement may be used to amplify the signal.

In general, 5 nm particles are recommended for intracellular staining because they are able to penetrate the cell membranes more easily, and for high resolution EM because the small size allows more exact localization of antigen. 10 nm particles are recommended for cell surface staining and for light microscopy because the larger size makes the stain more visible. 20 nm particles are recommended for blotting and for some histochemical applications. These are suggestions only — the end user will have to determine the best particle size for each application.

Attaching Antibodies to Agarose:

Antibodies attached to agarose have a variety of applications. They can be used to isolate proteins and other compounds from sera or from cell and tissue homogenates by affinity chromatography for quantitation or further analysis. They have also been used for immuno-precipitation of proteins from cell lysates, and for reduction of serum immunoglobulin in autoimmune diseases and transplantation experiments.

Table 6.2 : The following table shows different modes of antibody labelling and their applications



Horseradish Peroxidase (HRP) Alkaline Phosphatase (AP) β-Galactosidase (β-gal)	Fluorescein (FITC) Rhodamine Phycoerythrin Cy-3 Cy-5 Alexa Fluor	^{125}Iodine	Gold Ferritin Wolfram
ELISA Immunohistochemistry Immunoblotting	Immunohistochemistry Immunocytochemistry Flow cytometry Immunoblotting Protein arrays	Radioimmunoassays (RIA)	Immunoelectron microscopy

Antibody Fragmentation:

In some assays it is preferable to use only the antigen binding (Fab) portion of the antibody. For these applications, antibodies may be enzymatically digested to produce either an Fab or an F (ab')$_2$ fragment of the antibody. To produce an F (ab')$_2$ fragment, IgG is digested with pepsin, which cleaves the heavy chains near the hinge region.

One or more of the disulphide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation F (ab')$_2$. The light chains remain intact and attached to the heavy chain. The Fc fragment is digested into small peptides. Fab fragments are generated by cleavage of IgG with papain instead of pepsin.

Papain cleaves IgG above the hinge region containing the disulphide bonds that join the heavy chains, but below the site of the disulphide bond between the light chain and heavy chain. This generates two separate monovalent (containing a single antibody binding site) Fab fragments and an intact Fc fragment. The fragments can be purified by gel filtration, ion exchange, or affinity chromatography.

Affinity and Avidity:

Antibody Affinity is a Quantitative Measure of Binding Strength:

The combined strength of the non-covalent interactions between a single antigen-binding site on an antibody and a single epitope is the affinity of the antibody for that epitope. Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer.

The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation:

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab$$

where k_1 is the forward (association) rate constant and k_{-1} is the reverse (dissociation) rate constant. The ratio k_1/k_{-1} is the association constant K_a (i.e., $k_1/k_{-1} = K_a$), a measure of affinity. Because K_a is the equilibrium constant for the above reaction, it can be calculated from the ratio of the molar concentration of bound Ag-Ab complex to the molar concentration of unbound antigen and antibody at equilibrium as follows:

$K_a = [Ag\text{-}Ab] / [Ab][Ag]$

The value of K_a varies for different Ag-Ab complexes and depends upon both k_1, which is expressed in units for litres/mole/second (1/mol/s), and $k_{-1;}$ which is expressed in units of 1/second (inverse second). For small haptens, the forward rate constant can be extremely high; in some cases, k_1 can be as high as 4×10^8 1/mol/s, approaching the theoretical upper limit of diffusion-limited reactions (10^9 1/mol/s). For larger protein antigens, however, k_1 is smaller, with values in the range of 10^5 1/mol/s.

The rate at which bound antigen leaves as antibody's binding site (i.e., the dissociation rate constant, k_{-1}) plays a major role in determining the antibody's affinity for an antigen.

For some purposes, the dissociation of the antigen-antibody complex is of interest:

$Ag\text{-}Ab \leftrightarrow Ab + Ag$

The equilibrium constant for that reactions is K_d, the reciprocal of K_a

$K_d = [Ab]\,[Ag]/[Ab\text{-}Ag] = 1/K_a$

and is a quantitative indicator of the stability of an Ag-Ab complex; very stable complexes have very low values of K_d, and less stable ones have higher values. The affinity constant, K_a, can be determined by equilibrium dialysis or by various newer methods. Because equilibrium dialysis remains for many the standard against which other methods are evaluated, it is described here. This procedure uses a dialysis chamber containing two equal compartments separated by a semipermeable membrane.

Antibody is placed in one compartment, and a radioactively labelled ligand that is small enough to pass through the semipermeable membrane is placed in the other compartment (Fig. 6.17). Suitable ligands include haptens, oligosaccharides, and oligopeptides.

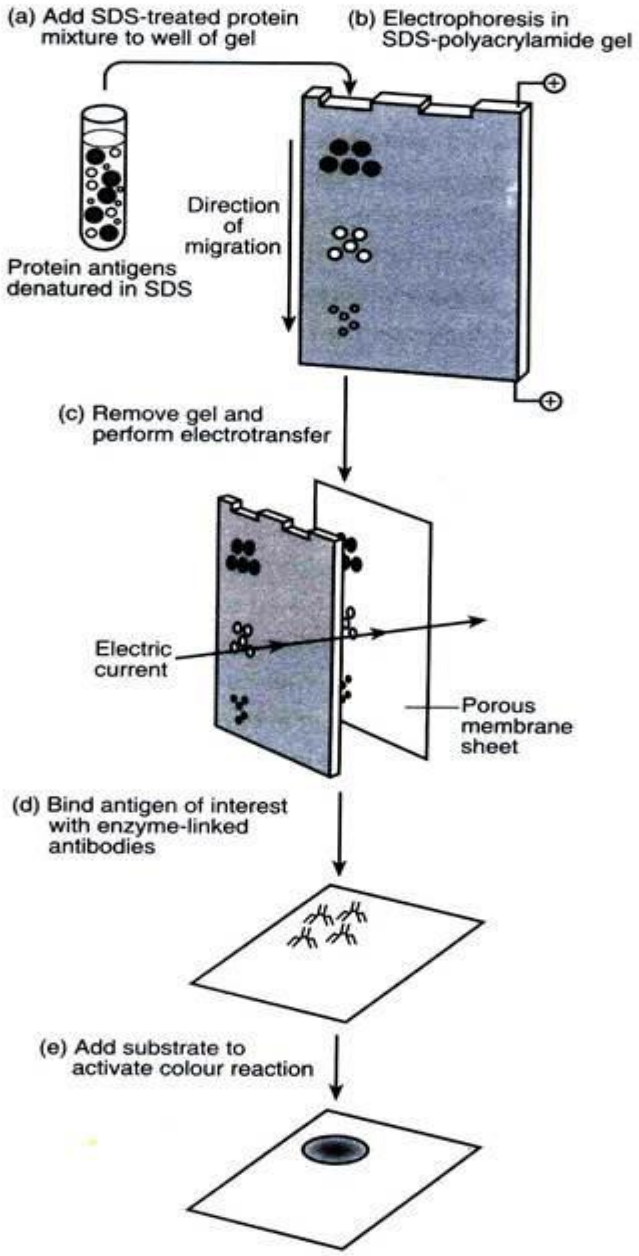


Fig. 6.17: Stern blotting, a protein mixture is (a) treated with SDS, a strong denaturing detergent, (b) then separated by electrophoresis in an SDS polyacrylamide gel (SDS-PAGE) which separates the components according to their molecular weight; lower molecular weight components migrate farther than higher molecular weight ones. (c) The gel is removed from the apparatus and applied to a protein-binding sheet of nitrocellulose or nylon, and the proteins in the gel are transferred to the sheet by the passage of an electric current. (d) Addition of enzyme-linked antibodies detects the antigen of interest, and (e) the position of the antibodies is visualized by means of an ELISA reaction that generates a highly coloured insoluble product that is deposited at the site of the reaction. Alternatively, a chemiluminescent ELISA can be used to generate light that is readily detected by exposure of the blot to a piece of photographic film.

In the absence of antibody, ligand added to compartment B will equilibrate on both sides of the membrane (Fig. 6.17a). In the presence of antibody, however, part of the labelled ligand

will be bound to the antibody at equilibrium, trapping the ligand on the antibody side of the vessel, whereas unbound ligand will be equally distributed in both compartments.

Thus the total concentration of ligand will be greater in the compartment containing antibody (Fig. 6.17b). The difference in the ligand concentration in the two compartments represents the concentration of ligand bound to the antibody (i.e., the concentration of Ag-Ab complex). The higher the affinity of the antibody, the more ligand is bound.

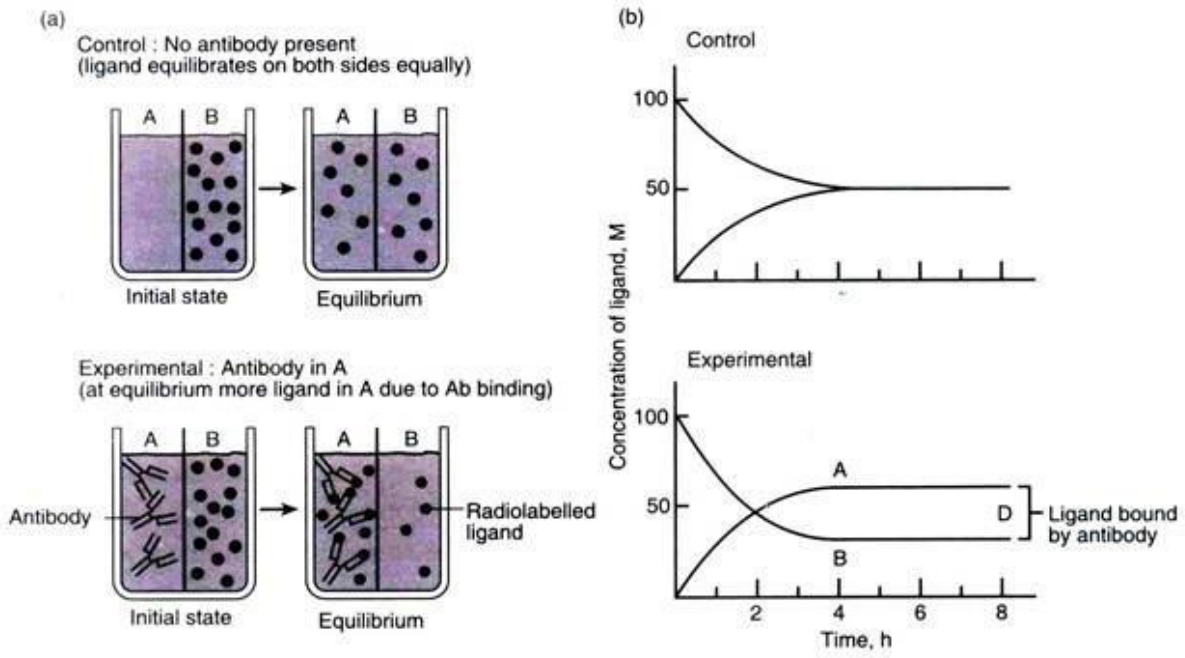


Fig. 6.18: Determination of antibody affinity by equilibrium dialysis. (a) The dialysis chamber contains two compartments (A and B) separated by a semipermeable membrane. Antibody is added to one compartment and a radiolabelled ligand to another. At equilibrium, the concentration of radioactivity in both compartments is measured. (b) Plot of concentration of ligand in each compartment with time. At equilibrium, the difference in the concentration of radioactive ligand in the two compartments represents the amount of ligand bound to antibody.

Since the total concentration of antibody in the equilibrium dialysis chamber is known, the equilibrium equation can be rewritten as:

$K_a = [Ab\text{-}Ag] / [Ab]\,[Ag] = r/c(n - r)$,

where r equals the ratio of the concentration of bound ligand to total antibody concentration, c is the concentration of free ligand, and n is the number of binding sites per antibody molecule. This expression can be rearranged to give the Scatchard equation:

$r/c = K_a n - K_a r$.

Values for r and c can be obtained by repeating the equilibrium dialysis with the same concentration of antibody but with different concentrations of ligand. If K_a is a constant, that is, if all the antibodies within the dialysis chamber have the same affinity for the ligand, then a Scatchard plot of r/c versus r will yield a straight line with a slope of —K_a (Fig. 6.18a). As the concentration of unbound ligand c increases, r/c approaches 0, and r approaches n, the valency, equal to the number of binding sites per antibody molecule.

Most antibody preparations are polyclonal, and K_a is, therefore, not a constant because a heterogeneous mixture of antibodies with a range of affinities is present. A Scatchard plot of heterogeneous antibody yields a curved line whose slope is constantly changing, reflecting this antibody heterogeneity (Fig. 6.19b).

With this type of Scatchard plot, it is possible to determine the average affinity constant, K_0, by determining the value of K_a, when half of the antigen-binding sites are filled. This is conveniently done by determining the slope of the curve at the point where half of the antigen binding sites are filled.

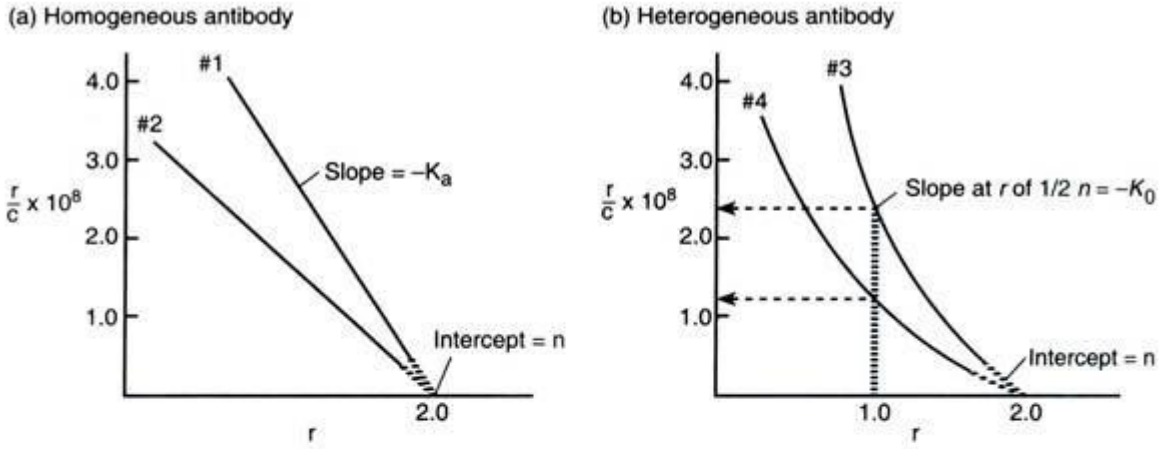


Fig. 6.19: Scatchard plots are based on repeated equilibrium dialyses with a constant concentration of antibody and varying concentration of ligand. In these plots, r equals moles of bound ligand/ mole antibody and c is the concentration of free ligand. From a Scatchard plot, both the equilibrium constant (K_a) and the number of binding sites per antibody molecule (n), or its valency, can be obtained. (a) If all antibodies have the same affinity, then a Scatchard plot yields a straight line with a slope of $-K_a$. The x intercept is n, the valency of the antibody, which is 2 for IgG and other divalent Igs. For IgM, which is pentameric, n =10, and for dimeric IgA, n = 4. In this graph, antibody #1 has a higher affinity than antibody #2. (b) If the antibody preparation is polyclonal and has a range of affinities, a Scatchard plot yields a curved line whose slope is constantly changing.The average affinity constant K_0 can be calculated by determining the value of K_a when half of the binding sites are occupied (i.e., when r =1 in this example). In this graph, antiserum #3 has a higher affinity ($K_0 = 2.4 \times 10^8$) than antiserum #4 ($K_0 = 1.25 \times 10^8$). Note that the curves shown in (a) and (b) are for divalent antibodies such as IgG.

Antibody Avidity Incorporates Affinity of Multiple Binding Sites:

The affinity at one binding site does not always reflect the true strength of the antibody-antigen interaction. When complex antigens containing multiple, repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of

an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the avidity.

The avidity of an antibody is a better measure of its binding capacity within biological systems (e.g., the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites. High avidity can compensate for low affinity. For example, secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

Probable Questions:

1. Define immunochemistry? What are its uses?
2. Describe different types of ELISA.
3. Describe different types of agglutination.
4. Describe the precipitation reaction with diagram.
5. Describe the process RIA.

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyay and Upadhyay.

Unit V

Radio labelling techniques: Properties of different types of radioisotopes normally used in biology, their detection and measurement; incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material, safety guidelines

Objective:

In this unit we will discuss about Radio labelling techniques: Properties of different types of radioisotopes normally used in biology, their detection and measurement; incorporation of radioisotopes in biological tissues and cells, molecular imaging of radioactive material, safety guidelines.

Introduction:

Isotopes are chemical elements that have the same atomic number (i.e., the number of protons in the nucleus of the atom) but different atomic masses (i.e., the sum of the number of protons and neutrons in the nucleus).

Certain isotopes are unstable and undergo spontaneous nuclear changes (called transmutations) accompanied by the emission of particulate and sometimes also electromagnetic radiations.

These atoms are said to be radioactive and are called radioisotopes or radionuclides. Radioactive atoms may readily be detected by instruments sensitive to their radiations.

Generally, an organism cannot distinguish between the stable and radioactive forms of the same element so that both are metabolized in an identical manner.

It is for this reason that radioisotopes have proven extremely useful to biologists, as these elements may be conveniently employed as tracers. That is, the fate of a given element (or molecule) in an organism or individual cell may be studied by introducing the radioactive form of that element and following the uptake and subsequent localization of the radioactivity.

If the radioisotope is initially a part of a molecule, then the fate of all or part of that molecule may similarly be followed. The use of radio-isotopically labelled compounds is particularly desirable when the compound to be administered is a normal constituent of the cell or organism and would be impossible to distinguish from stable molecules already present.

Many organic and inorganic compounds of biological interest may now be obtained that have one or more specific atomic positions occupied by radioisotopes. Because of the extremely high sensitivities of many radiation detectors, the quantities of radioisotopes employed in

tracer studies can be kept small enough to preclude significant damage to cell constituents by the radiation.

Properties of Radioactive Isotopes:

Most radiations emitted by radioisotopes are the result of changes in the unstable atomic nuclei. Whether a given atomic nucleus is stable-depends in turn on the numbers of neutrons (N) and protons (Z) that it contains. The relationship between nuclear stability and the neutron: proton composition of the nucleus is shown in Figure 14-2.

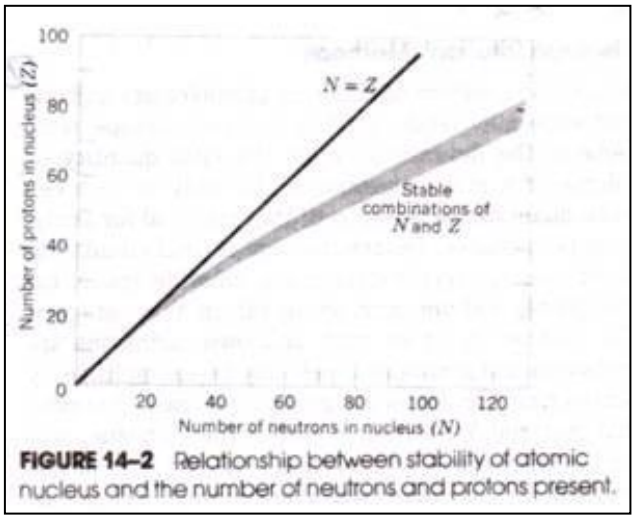


FIGURE 14–2 Relationship between stability of atomic nucleus and the number of neutrons and protons present.

Fig 14.2: Relationship between stability of atomic nucleus and the number of neutrons and protons present

All stable (and a number of unstable) isotopes have N and Z values that fall within the shaded zone. Note that for the lighter elements, nuclear stability exists when N=Z, whereas in the stable, heavier elements, the number of neutrons exceeds the number of protons, with the allowable neutron excess increasing with atomic number.

Isotopes with N and Z numbers outside of the shaded zone (as well as some that occur within the zone) shown in Figure 14-2 undergo spontaneous changes in which neutrons and protons are inter converted. The resulting change establishes a stable combination of N and Z values. These intra-nuclear changes are accompanied by the emission of particulate and sometimes electromagnetic radiation.

Advantages of the Radioisotope Technique:

1. Results obtained from experiments in which radioisotopes are used are quantitative, because the amount of radioactivity present and available for detection is directly proportional to the radioisotope content of the sample being analyzed. Moreover, numerous biological studies carried out on a routine basis with radioisotopes can only be performed

with great difficulty or are virtually impossible without them. Some examples are cited to illustrate the value of the radioisotope technique.

Radioactive isotopes have many useful applications.

2. In medicine, for example, cobalt-60 is extensively employed as a radiation source to arrest the development of cancer. Other radioactive isotopes are used as tracers for diagnostic purposes as well as in research on metabolic processes. When a radioactive isotope is added in small amounts to comparatively large quantities of the stable element, it behaves exactly the same as the ordinary isotope chemically; it can, however, be traced with a Geiger counter or other detection device. Iodine-131 has proved effective in treating hyperthyroidism. Another medically important radioactive isotope is carbon-14, which is used in a breath test to detect the ulcer-causing bacteria *Heliobacter pylori*.

3. In industry, radioactive isotopes of various kinds are used for measuring the thickness of metal or plastic sheets; their precise thickness is indicated by the strength of the radiations that penetrate the material being inspected. They also may be employed in place of large X-ray machines to examine manufactured metal parts for structural defects. Other significant applications include the use of radioactive isotopes as compact sources of electrical power—e.g., plutonium-238 in spacecraft. In such cases, the heat produced in the decay of the radioactive isotope is converted into electricity by means of thermoelectric junction circuits or related devices.

Half-life:

The number of atoms in a sample of radioisotope that disintegrate during a given time interval decreases logarithmically with time and is unaffected by chemical and physical factors that normally alter the rates of chemical processes (i.e., temperature, concentration, pressure, etc.). Radioactive decay is therefore a classic example of a first-order reaction. A convenient term used to describe the rate of decay of a radioisotope is the physical half-life, T_p. This is the amount of time required to reduce the amount of radioactive material to one-half its previous value.

Each radioactive isotope decays at a characteristic rate and therefore has a specific half-life (see Table 14-1). For example, the amount of radioactivity arising from a sample of ^{59}Fe is reduced to one-half its original value in 45.1 days, to one-fourth in 90.2 days, to one-eighth in 135.3 days, and so on. The amount of decay occurring in the course of a tracer experiment must be taken into account when radioisotopes of short physical half-life such as ^{24}Na, ^{32}P, ^{42}K, ^{59}Fe, ^{131}I, and ^{125}I are used. Of course, this is not a problem in tracer experiments employing ^{3}H and ^{14}C (Table 14-1) because the length of the experiment is insignificant in comparison with the half-life.

When radioisotopes are used in vivo experiments of extended duration, the turnover rate of the element in the body (or in the cell) must also be considered, for the rate of decrease of radioactivity will be a function in both radioactive decay and metabolic turnover. In these instances, a more useful term is the effective half-life, T_e, which is the amount of time

required to reduce the radioisotope content of the body (or cell) to one-half its original value by the combined effects of decay and turnover; it is determined using the relationship

Where

T_e = effective half-life,

T_p = physical half-life, and

T_b = biological half-life and is defined as the normal amount of time required for the turnover of one-half of the body content of a given element (radioactive or nonradioactive).

The physical, biological, and effective half-lives of several elements are compared in Table 14-3. It should be noted that T_e can never be greater than T_p and that the slower the rate of turnover of an element, the closer T_e approaches T_p.

TABLE 14–3 PHYSICAL, BIOLOGICAL AND EFFECTIVE HALF-LIVES OF SOME RADIOISOTOPES

Element	Radio-isotope	Half-life		
		Physical	Biological	Effective
Hydrogen	^{3}H	12.3 yr	19 days	19 days
Carbon	^{14}C	5570 yr	180 days	180 days
Phospohorus	^{32}P	14.3 days	3 yr	14.1 days
Calcium	^{45}Ca	164 days	73 yr	163 days
Iron	^{59}Fe	45.1 days	3.4 yr	43.5 days
Cobalt	^{60}Co	5.3 yr	8.1 days	8.1 days
Iodine	^{125}I	60 days	156 days	43.3 days
Iodine	^{131}I	8.1 days	156 days	7.7 days
Radium	^{226}Ra	1620 yr	104 days	104 days

Detection and Measurement of Radiation:

The selection of instruments for the detection and measurement of radioisotopes is based primarily on the type and energy of the emitted radiation. The most commonly used detectors are: (1) Geiger- Muller counters, which are employed primarily with isotopes emitting beta particles of intermediate or high energy (E_{max} above 0.2 MeV) and which may also be used at low efficiency for the measurement of gamma radiation; (2) solid scintillation counters, which are generally employed with gamma ray- emitting isotopes; and (3) liquid scintillation counters, which are used with isotopes emitting low- energy beta particles (E_{max} below 0.2 MeV).

- ### Geiger-Muller Counters:

The most widely used instrument for the detection and measurement of radiation is the Geiger-Muller (or G-M) counter. The detector itself, called a G-M tube, consists of a cylinder several inches long containing two electrodes and filled with a readily ionizable inert gas such as helium or argon.

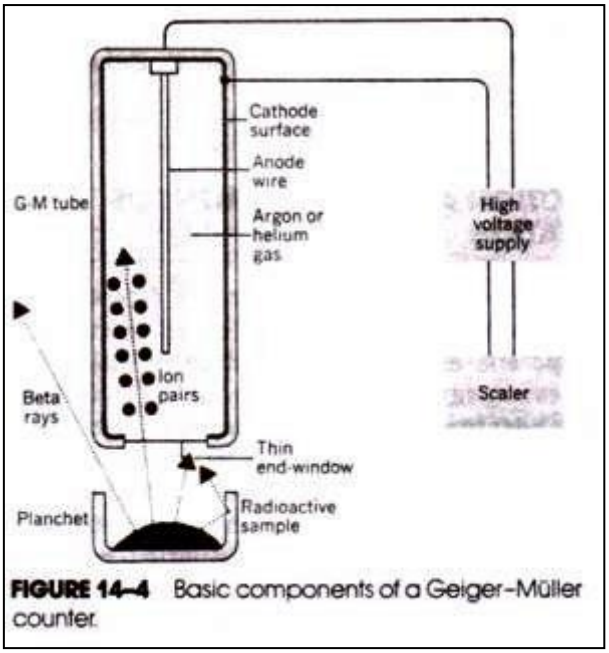


FIGURE 14–4 Basic components of a Geiger–Müller counter.

The insulated metallic internal surface of the cylinder serves as the cathode and a narrow wire passing down the center of the tube serves as the anode (Fig. 14-4). One end of the G-M tube is covered by a thin material such as mylar plastic or mica and is called the end-window. The anode and cathode terminals at the other end of the tube are connected to a source of high voltage and a scaler, a device that simply counts electrical pulses.

When a radioactive sample (usually deposited on a small metal disk called a planchet) is placed near the end-window, radiation enters the G-M tube, ionizing some of the gas molecules and forming a number of ion pairs (i.e., positively charged argon or helium atoms and electrons). If a sufficiently high electrical potential is applied to the electrodes, the ion pairs will migrate toward the appropriate electrode. During this migration, the ions collide with and ionize additional gas molecules, so that the passage of a single beta particle through the gas results in a large number of ions being collected at the electrodes.

These events produce an electrical pulse that is recorded by the scaler as a count. Ideally, each ionizing ray entering the G-M tube is registered as a count and the amount of radioactivity is expressed as counts per minute (cpm). Because radiation is emitted in all directions from a radioactive source, it is apparent that only a small percentage of the rays arising from the sample are directed toward the end-window. Therefore, even if all the rays entering the G-M tube are detected and counted, the cpm recorded for the sample is only a fraction of the true rate of disintegration (i.e., disintegrations per minute, dpm) of the isotope.

This does not pose a serious problem when the relative isotope contents of a number of samples are to be determined (this is generally the case) and if constant geometric conditions are maintained for each sample (i.e., distance of the sample from the end-window, volume of sample, etc.). For some radioisotopes such as ^{3}H, ^{14}C; ^{35}S, and others of low E_{max}, much or all of the energy of the emitted beta particles may be dissipated before the ray enters the ionizing gas. For example, the energy may be expended within the sample itself (called self-absorption), in the air between the sample and the end-window, or in the material of the end-window.

Even with radioisotopes emitting beta particles of high E_{max} (Table 14-2), beta particles in the low region of the energy spectrum may go undetected. Because of the low specific ionization of gamma rays and the low density of the gas in the G-M tube, gamma rays may pass through the tube without causing ionizations and therefore go undetected. For these reasons, Geiger-Muller counters are usually not suitable for the detection and measurement of radioisotopes emitting gamma rays or beta particles of low E_{max}.

Even when no radioactive sample is placed below the end-window of the G-M tube, a small count is recorded. This is known as the background count and results from cosmic radiation (primarily gamma rays), naturally occurring radioisotopes in laboratory materials (such as ^{40}K in glass and naturally occurring ^{14}C and ^{3}H in organic compounds), radioactive samples left in the vicinity of the detector and electronic "noise" within the components of the counting system.

Therefore, the background count must always be subtracted from the count obtained for a radioactive sample. The magnitude of the background count may be reduced by placing lead shielding around the detector so that much of the cosmic radiation and radiations from other sources are absorbed before reaching the detector.

The total amount of radioisotope present in a sample at any instant in time may be determined from its rate of disintegration, that is, its dpm; the basic unit of measurement is the curie (Ci) and is defined as that quantity of radioisotope undergoing 2.22×10^{12}dpm. (Note that the curie content of a radioactive sample decreases exponentially with time at a rate determined by the physical half-life of the radioisotope.)

In most tracer experiments, the quantity of radioisotope used is generally at the millicurie (mCi) or microcurie (μCi) level. The curie content of a labeled compound is generally provided at the time of purchase so that the efficiency of the counting system may be determined by comparing the recorded cpm of an aliquot of the isotope with its known dpm.

Generally, this value is 10% or less for G-M counters but is much higher in solid and liquid scintillation systems. Once the efficiency of the counting system is known, then the specific activity of a radioactive sample (which we may now define as the number of curies per unit mass of element or compound) collected during the course of a tracer experiment may be calculated from its observed counting rate and composition. G-M counters are effectively employed in tracer experiments involving ^{24}Na, ^{32}P, $^{36}C1$, and other "hard beta" emitters but generally are not used with ^{3}H and ^{14}C, which emit "soft beta" rays.

- ## Solid Scintillation Counters:

Solid scintillation counters are used to detect and measure radioisotopes emitting gamma rays. The detector (Fig. 14-5) consists of a large crystal of thallium-activated sodium iodide and a photomultiplier tube encased in aluminium housing; the latter is interfaced with a preamplifier, a source of high voltage, and a scaler.

The radioactive sample to be analyzed is placed either against the end of the detector containing the crystal or, for greatly improved counting efficiency, into a well-shaped opening drilled into the crystal's surface (Fig. 14-5).

Because of its high density, the crystal absorbs much of the energy of the gamma rays, causing excitation of electrons of atoms composing the crystal and raising them to higher energy orbitals. As these electrons return again to their lower energy orbitals, flashes of light or scintillations are emitted and these are proportional in number to the number and energy of the gamma rays exciting the crystal.

The light photons are converted by the photomultiplier tube into electrical pulses of corresponding magnitude and frequency and these are relayed to the scaler. Because the magnitude of the electrical pulses produced is proportional to the energy of the gamma rays, and because gamma rays are mono- energetic, the inclusion of the appropriate circuitry in the counting system (i.e., a pulse-height analyzer) allows different gamma ray-emitting isotopes to be distinguished.

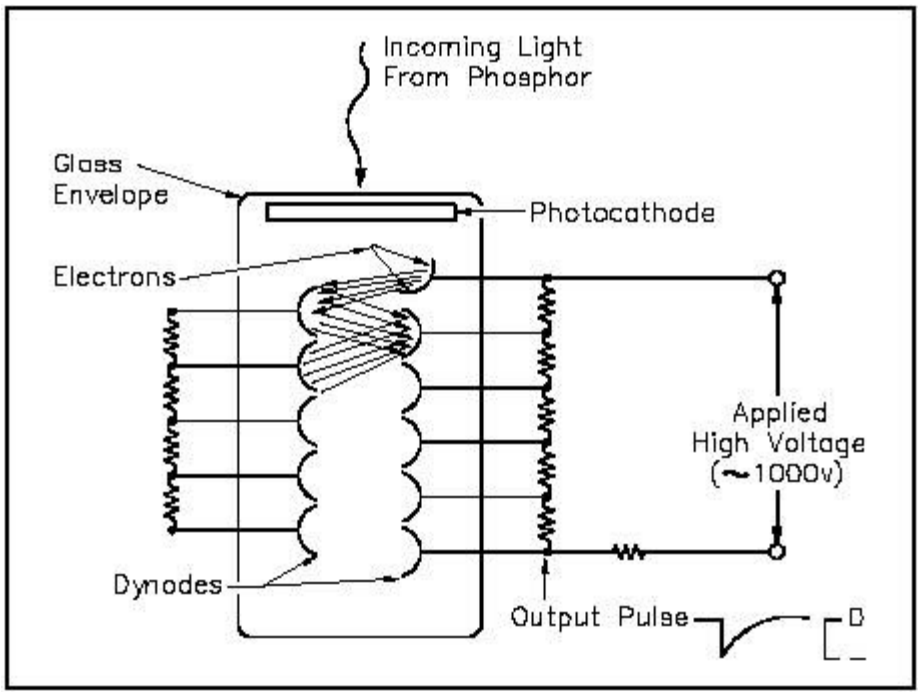


Fig 14.5: Basic components of solid scintillation counter

In contrast to G-M counters, few or no problems involving self-absorption and end-window absorption are incurred when solid scintillation methods are used with gamma ray-emitting isotopes. However, the use of constant geometry and lead shielding around the detector to reduce the magnitude of the background count is important.

Incorporation of radioisotopes in biological tissues and cells

- ### Incorporation

After inhalation, ingestion, or wound contamination, small radioisotope particles may be transported via blood or lymphatics into cells, tissues, and organs.

Isotopes can be alpha-, beta-, or gamma-emitting.

Radioisotopes can be incorporated into one or more organs specific for that isotope, (e.g. thyroid, lungs, kidneys, bones/bone marrow, or liver/spleen) resulting in exposure at that site.

Medical countermeasures called decorporation agents or other procedures (e.g., diuresis) may be needed to remove radioisotopes that have been incorporated into tissues.

Toxic effects of radioisotopes may be due to their chemical and/or radiological properties.

- ### How to diagnose

If after external decontamination, an appropriate radiation survey meter continues to identify significant residual radioactivity, suspect internal contamination.

Swab each nostril separately to help estimate level of internal (lung) contamination

Collect ≥70 mL spot urine sample for isotope measurement

Instructions for sample collection, labeling, packaging and shipping (HHS/CDC)

Consider total body radiation survey with modified hospital nuclear medicine equipment

Molecular imaging of radioactive material

Molecular imaging is a type of medical imaging that provides detailed pictures of what is happening inside the body at the molecular and cellular level. Where other diagnostic imaging procedures—such as x-rays, computed tomography (CT) and ultrasound—offer pictures of physical structure, molecular imaging allows physicians to see how the body is functioning and to measure its chemical and biological processes.

Nuclear imaging, also called molecular imaging, includes positron emission computed tomography (PET) and single photon emission computed tomography (SPECT) imaging. This section includes radiopharmaceuticals and tracers, PET-CT, SPECT-CT, and PET-MRI. Molecular imaging includes the field of nuclear medicine, which uses very small amounts of radioactive materials, or radiopharmaceuticals, to diagnose and treat disease.

Molecular imaging includes the field of nuclear medicine, which uses very small amounts of radioactive materials (radiopharmaceuticals) to diagnose and treat disease. In nuclear medicine imaging, the radiopharmaceuticals are detected by special types of cameras that work with computers to provide very precise pictures of the area of the body being imaged. Nuclear medicine can also be used to treat certain types of cancer and other diseases.

Molecular imaging offers unique insights into the human body that enable physicians to personalize patient care. In terms of diagnosis, molecular imaging is able to:

- provide information that is unattainable with other imaging technologies or that would require more invasive procedures such as biopsy or surgery
- identify disease in its earliest stages and determine the exact location of a tumor, often before symptoms occur or abnormalities can be detected with other diagnostic tests

How do they help patients?

As a tool for evaluating and managing the care of patients, molecular imaging studies help physicians:

- determine the extent or severity of the disease, including whether it has spread elsewhere in the body
- select the most effective therapy based on the unique biologic characteristics of the patient and the molecular properties of a tumor or other disease
- determine a patient's response to specific drugs
- accurately assess the effectiveness of a treatment regimen
- adapt treatment plans quickly in response to changes in cellular activity
- assess disease progression
- identify recurrence of disease and help manage ongoing care

When are they used?

Molecular imaging procedures—which are noninvasive, safe and painless—are used to diagnose and manage the treatment of cancer, heart disease, brain disorders such as Alzheimer's and Parkinson's disease, gastrointestinal disorders, lung disorders, bone disorders, kidney and thyroid disorders, and more.

Safety Rules and Procedures for Working with Radioisotopes

General Rules

1. Keep the laboratory locked when not in use and keep unauthorized persons out of the laboratory.
2. Do not eat, drink, smoke and apply cosmetics in the laboratory.
3. Always wear appropriate personal protective equipment and avoid direct contact with radioactive materials. Never pipette solutions by mouth.

4. Effectively contain radioactive materials at all stages of handling and use a fume hood whenever possible for work with open sources. Always use a fume hood for work with boiling or evaporating sources.
5. Use appropriate shielding when working with radioactive materials.
6. Do not use refrigerators or freezers designated for storage of radioisotopes for food storage. Glassware and other equipment used for radioactive work must not be used for other purposes.
7. Clearly mark containers of radioactive materials with warning symbols indicating the nature and amount or radioactivity. Mark all other equipment that has been contaminated with radioisotopes and store in appropriately shielded locations in the radioisotope lab. This equipment must not be removed from the lab.
8. Store radioactive waste and sources in a safe and secure place. Use shielding to ensure that the surface radiation does not exceed 2.5 Sv/h (0.25 mR/h).
9. When work is complete, clean and/or isolates contaminated supplies and equipment; monitor and decontaminate trays and working surfaces. Floors and working surfaces should be wipe checked daily when the lab is in use.
10. Wash hands, monitor clothes, shoes and hands before leaving the laboratory.

Personal protective equipment

1. **Gloves**
   Disposable gloves must be worn when working with open radioactive sources. Gloves should be checked frequently during the experiment to detect small punctures that may have developed, especially at the fingertips. Disposable gloves must never be worn outside the laboratory. For work with iodine, a minimum of two pairs of gloves is recommended, with the outer pair being changed frequently.
2. **Lab Coats**
   It must be worn when working with radioactive materials. Button completely, with sleeves rolled down fully and the cuffs sealed with gloves. Lab coats should not be worn outside the lab and never in areas where food is consumed. Coat hooks are provided in the lab for storage of your lab coat.
3. **Clothing**
   It is recommended that long pants be worn to provide splash protection for the lower legs. Do not wear rings when working with open sources as contamination can become trapped under the band. It may be impossible to decontaminate a piece of jewellery, in which case it could never be worn again.
4. **Shoes**
   Shoes that cover the entire foot are required. Sandals or thongs do not provide adequate coverage in the event of a spill, nor do they provide protection from falling objects.

5. Eye protection

Safety glasses, goggles or face guards should be worn, especially if there is any hazard of splashing material in the eyes. It is also good practice to wear glasses as shielding when working with high energy beta emitters to reduce the external radiation dose to the eyes.

6. Remote handling devices

Such as forceps or tongs, should be used when handling stock solution vials or other sources that produce a significant radiation field. A glove box should be used when working with dry radioactive powders.

7. The Radioisotope Lab

Open source radioactive materials may be used and stored only in licensed locations (i.e. Room 370A). This room must be locked at all times when not in use and only authorized personnel will be permitted entry. The room will be labelled with signs: Caution - Radiation Area and In case of Emergency Call... All storage areas, contaminated sites and decay cupboards, etc. Must be labelled Caution - Radioactive Materials. The room must also display the No Eating, Drinking or Smoking and Rules for Working with Radioisotopes signs.

8. When radioisotopes are being used, all personnel in the radiation area should be informed and precautions taken that the maximum allowable working field of 2.5 Sv/h in any direction from the source is not exceeded.

9. Working surfaces should be covered with an absorbent covering, such as plasticized paper or incontinence pads, to prevent contamination.

10. Label all material used for radioactive work with radiation stickers. Signs and labels should be removed when the equipment has been shown to be free of contamination and will no longer be used for isotope work.

11. The sink should be clearly labelled with a radiation sign.

12. If there is a possibility of producing airborne radioactivity (aerosols, dust, vapours) work should be performed in an absorbent paper-lined fume hood. The hood should be labelled clearly with a radioactive sign. All work with 125I must be performed in the fume hood. If the fume hood stops working, report it to the RSO immediately.

13. Store open source radioisotopes in the refrigerator marked with a radiation sign. The refrigerator will be kept locked. On a routine basis the refrigerators should be defrosted, cleaned and wipe tested. Food or beverages must not be stored in the same refrigerator with radioisotopes.

Probable Questions:

1. What do you mean by isotope?
2. What do you mean by radio labelling?
3. Write down the properties of radioactive isotopes.
4. Discuss the advantages of the radioisotope technique.
5. What do you mean by half life?
6. Discuss the measurement technique of radiation by Geiger-Muller Counters.
7. Discuss the procedure of Incorporation of radioisotopes in biological tissues and cells
8. What do you mean by molecular imaging?
9. Discuss about the Personal protective equipment for Working with Radioisotopes.
10. Discuss in detail about the Safety Rules and Procedures for Working with Radioisotopes.

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyaya and Upadhyaya

Unit VI

Methods in field biology: Methods of estimating population density of animals, ranging patterns through direct, indirect and remote observations, sampling methods in the study of behaviour, habitat characterization-ground and remote sensing methods.

Objective:

In this unit we will discuss about Methods in field biology: Methods of estimating population density of animals, ranging patterns through direct, indirect and remote observations, sampling methods in the study of behaviour, habitat characterization-ground and remote sensing methods.

Introduction:

Field techniques refer to the standardized methods employed to select, count, measure, capture, mark, and observe individuals sampled from the target population for the purpose of collecting data required to achieve study objectives. The term also includes methods used to collect voucher specimens, tissue samples, and habitat data. The choice of field techniques to use for a particular species or population is influenced by five major factors:

1. Data needed to achieve inventory and monitoring objectives
2. Spatial extent and duration of the project
3. Life history and population characteristics
4. Terrain and vegetation in the study area
5. Budget constraints

- ## Data Requirements

The types of data required to achieve inventory or monitoring objectives should be the primary consideration in selecting field techniques. Four categories of data collection are discussed below along with some suggestions for electing appropriate field techniques for each.

- ## Occurrence and distribution data

For some population studies, simply determining whether a species is present in an area is sufficient for conducting the planned data analysis. For example, biologists attempting to conserve a threatened salamander may need to monitor the extent of the species' range and

degree of population fragmentation on a land ownership. One hypothetical approach is to map all streams in which the salamander is known to be present, as well as additional streams that may qualify as the habitat type for the species in the region. To monitor changes in salamander distribution, data collection could consist of a survey along randomly selected reaches in each of the streams to determine if at least one individual (or some alternative characteristic such as egg mass) is present. Using only a list that includes the stream reach (i.e., the unique identifier), the survey year, and an occupancy indicator variable, a biologist could prepare a time series of maps displaying all of the streams by year and distinguish the subset of streams that were known to be occupied by the salamander. Such an approach could support a qualitative assessment of changes in the species distribution pattern, thereby attaining the program' objectives, and generate new hypotheses as to the cause of the observed changes.

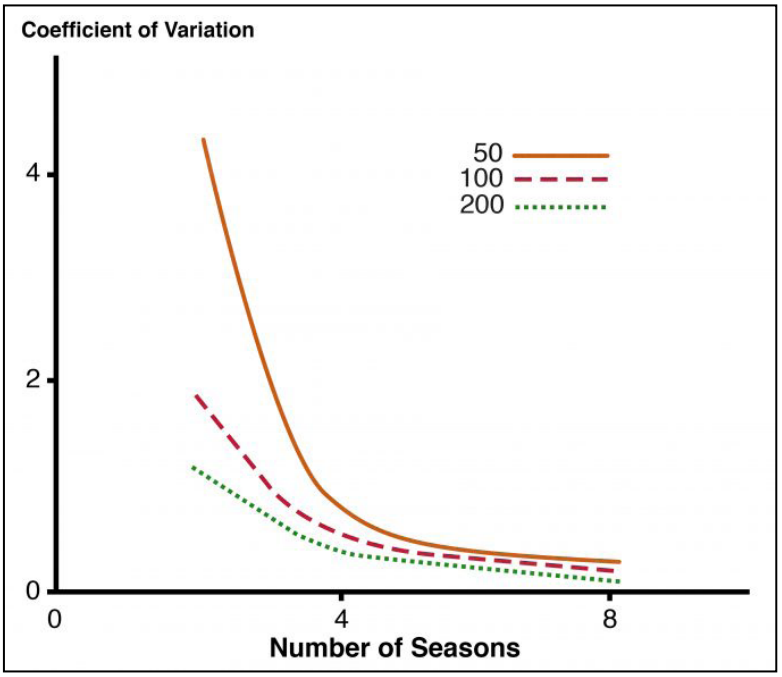


Figure 8.1. Simulation-based coefficient of variation for estimated trend in occupancy (on the logistic scale) where 50, 100, or 200 landscapes are each surveyed 3 times per season, for multiple seasons (redrafted from MacKenzie 2005). Estimates of occupancy can be facilitated by use of computer programs such as PRESENCE (MacKenzie et al. 2003).

It is far easier to determine if there is at least one individual of the target species on a sampling unit than it is to count all of the individuals. Determining with confidence that a species is not present on a sampling unit also requires more intensive sampling than collecting count or frequency data because it is so difficult to dismiss the possibility that an individual eluded detection. Probability of occurrence can be estimated using approaches such as those described by MacKenzie and Royale (2005). MacKenzie (2005) offered an excellent overview for managers of the trade-off between number of units sampled per year and the number of years (or other unit of time) for which the study is to be conducted. The variation in the estimated trend in occupancy decreases as the number of years of data

collection increases (Fig. 8.1). A similar level of precision can be achieved by surveying more units over fewer years vs. surveying fewer units over a longer period.

- ## Population size and density

National policy on threatened and endangered species is ultimately directed toward efforts to increase or maintain the total number of individuals of the species within their natural geographic range (Suckling and Taylor 2006). Total population size and effective population size (i.e., the number of breeding individuals in a population; Lande and Barrowclough 1987) most directly indicate the degree of species endangerment and effectiveness of conservation policies and practices. Population size or more accurately density per unit area is usually used as the basis for trend analyses because changes in density integrate changes in natural mortality, exploitation, and habitat quality. In some circumstances, it may be feasible to conduct a census of all individuals of a particular species in an area to determine the population density. Typically however, population size and density parameters are estimated using statistical analyses based on only a sample of population members. Population densities of plants and sessile animals can be estimated from counts taken on plots or data describing the spacing between individuals (i.e. distance methods) and are relatively straightforward. Population analyses for many animal species must account for animal response to capture or observation, observer biases, and different detection probabilities among sub-populations. Pilot studies are usually required to collect the data necessary to address these factors in the analysis. Furthermore, mark-recapture studies, catch-per-unit effort surveys, and other estimation methods require multiple visits to sampling units (Pradel 1996). These considerations increase the complexity and cost of studies designed for population parameter estimation.

- ## Abundance indices

The goals and objectives of some biological inventories and monitoring studies can be met with indices of population density or abundance, rather than population estimators. The difference between estimators and indices is that the former yield absolute values of population density while the latter provide relative measures of density that can be used to compare indices to populations among places or times. Indices are founded on the assumption that index values are closely associated with values of a population parameter, although the precise relationship between the index and parameter usually is not quantified. Examples of abundance or density indices are: plant canopy cover, numbers of individuals captured per 1000 trap nights, counts of individuals observed during a standardized unit of time, among many others. From a data collection perspective, density indices often require less sampling intensity and complexity than population estimation procedures. However, population indices are not comparable among different studies unless field techniques are strictly standardized. Furthermore, the assumption that an abundance index closely approximates population density is rarely tested (Seber 1982).

- **Fitness data**

For rare or declining populations, estimates of survival in each life stage as well as reproductive rates are required. These data not only provide useful trigger points for estimating rates of decline (lambda) they also allow trigger points for removal of a species from a threatened or other legal status. Collecting these sorts of data is often labor intensive and expensive. In a study on northern spotted owls, for instance, millions of dollars have been spent collecting these types of data (Lint 2001). This is not particularly surprising as the types of data that would be necessary to understand the population dynamics of a bird are numerous and complicated to generate. Nest densities, clutch sizes, hatching rates, fledging rates, and survival rates to maturity and survival rates as reproductive adults would be a minimum data set. New approaches to estimating individual contributions to population growth and changes in distributions of quantitative traits and alleles include genetic analyses, which can lead to even more detailed understanding of the potential for a population to adapt to variations in environmental factors (Pelletier et al. 2009).

- **Research studies**

Studies of habitat relationships or cause-and-effect responses require coordinated sampling of the target population and environmental measurements or stressors to which the population may respond. Data collection efforts tend to be complex, requiring multiple sampling protocols for the target population, study site attributes, and landscape pattern metrics. The funding required to conduct research studies typically limits their application to species or populations in greatest need of management planning such as those listed as threatened or endangered. Manipulative studies are often carried out to generate the necessary data, but when these focus on a threatened species, ethical questions regarding the conduct of the experiment placing the species at even great risk, at least locally, often emerge. Hence it is often monitoring of both environmental conditions and aspects of population density or fitness that are used to assess associations in trends between population parameters and environment parameters.

Frequently Used Techniques for Sampling Animals

The array of techniques available to sample animals is vast and summarized elsewhere in techniques manuals (e.g., Bookhout 1994). We summarize a few examples of commonly used techniques, but strongly suggest that those of you developing monitoring plans do a more complete literature search on sampling of the species that are of most concern in your monitoring program.

Here we provide a brief overview of some of the commonly used techniques. Kuenzi and Morrison 1998 ,Tags Birds/Mammals Nietfeld et al. 1994

	Observational Mode	**Technique**	**Target Groups of Species**	**References**
	Direct	Quadrats; fixed-area plots	Sessile or relatively immobile organisms	Bonham 1989
		Avian point counts	Bird species that sing or call on territories	Ralph et al. 1995
		Spot mapping & nest searches	Territorial bird species	Ralph et al. 1993
		Line transect	Large mammals, birds	Anderson et al. 1979
		Call playback response	Wolves, ground squirrels, raptors, woodpeckers	Ogutu and Dublin 1998
		Standardized visual searches	Large herbivores,	Cook and Jacobsen 1979
		Census	Cave-dwelling bats; large herbivores	Thomas and West 1989
	Observational Mode	**Technique**	**Target Groups of Species**	**References**
	Animal Sign	Foot track surveys	Medium-large mammals	Wilson and Delahay 2001
	Pellet & scat counts	Medium-large mammals	Fuller 1991	
		Food cache searches	Large carnivores	Easter-Pilcher 1990

		Structures (e.g., dens, nests)	Arboreal mammals; fossorial mammals; bears	Healy and Welsh 1992
	Remote Sensing	Track plates	Medium-large mammals	Wilson and Delahay 2001
	Photo & video stations	Medium-large mammals	Morruzzi et al. 2002	
		Ultrasonic detectors	Bats	Thomas and West 1989
		Audio monitoring	Frogs	Crouch and Paton 2002
		Hair traps	Small-medium mammals, large carnivores	McDaniel et al. 2000
		Radio telemetry	Limited by animal body size (>20 g) ?	USGS 1997
	Observational Mode	**Technique**	**Target Groups of Species**	**References**
		GPS telemetry	Limited by animal body (>2000 g) ?	Girard et al. 2002
		Marine radar	Bats, migrating birds	Harmata et al. 1999
		Harmonic radar	Bats, amphibians, reptiles	Pellet et al. 2006
Capture	Passive	Pitfalls	Salamanders, lizards, small mammals	Enge 2001, Mengak and

				Guynn 1987
		Snap traps	Small mammals	Mengak and Guynn 1987
		Box traps	Small-medium mammals	Powell and Proulx 2003
		Funnel-type traps	Snakes, turtles	Enge 2001
		Leg-hold & snares	Large mammals	Bookhout 1994
		Mist nets		
	Active	Drives to an enclosure	Medium-large mammals with predictable flight response	deCalesta and Witmer 1990
	Observational Mode	**Technique**	**Target Groups of Species**	**References**
		Cannon nets	Medium-large mammals	Bookhout 1994
		Immobilizing agents	Large mammals	Bookhout 1994
		Hand capture	Salamanders	Kolozsvary and Swihart. 1999
Marking				
		Mutilation	Small mammals	Wood and Slade 1990

		Pigments	Small mammals	Lemen and Freeman 1985
		Collars & Bands	Birds/mammals	Nietfeld et al. 1994

Table 8.1. Field techniques for inventories and monitoring studies of terrestrial and semi-aquatic vertebrates. References can be found in the Literature Cited section.

➢ Ranging patterns through direct, indirect and remote observations

The process of fixing or establishing intermediate points to facilitate measurement of the survey lines are called as Ranging. The intermediate points are located by means of ranging rodes, offset rods and ranging poles.

Ranging Out Survey Lines

While measuring the survey lines, the chain or the tape has to be stretched along the survey line along that joins two terminal stations. When the line to be measured has a smaller length compared to the chain, then the measurement goes smooth. If the length of the line is greater, the survey lines have to be divided by certain intermediate points, before conducting the chaining process. This process is called ranging.

The process of ranging can be done by two methods:

1. Direct Ranging
2. Indirect Ranging
3.

1. Direct Ranging

Direct ranging is the ranging conducted when the intermediate points are intervisible. Direct ranging can be performed by eye or with the help of an eye instrument.

Ranging by Eye

As shown in figure-1 below, let A and B are the two intervisible points at the ends of the survey line. The surveyor stands with a ranging rod at the point A by keeping the ranging rod at the point B. The ranging rod is held at about half metre length.

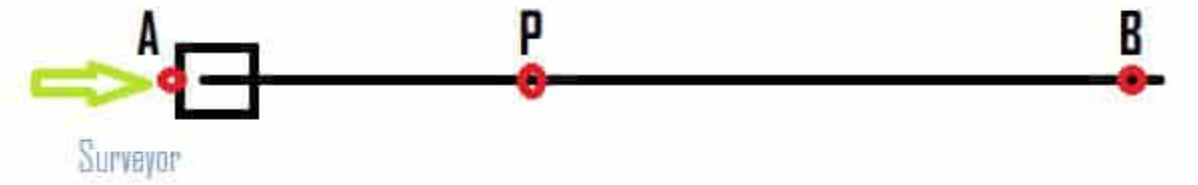


Fig.1.Direct Ranging

The assistant then takes the ranging rod and establishes at a point in between AB, almost in line with AB. This is fixed at a distance not greater than one chain length from point A.

The surveyor can give signals to the assistant to move traverse till the rod is in line with A and B. In this way, other intermediate points are determined.

Ranging by Line Ranger

The figure-2 below shows a line ranger that has either two plane mirror arrangement or two isosceles prisms that are placed one over the other. The diagonals of the prism are arranged and silvered such that they reflect incident rays.

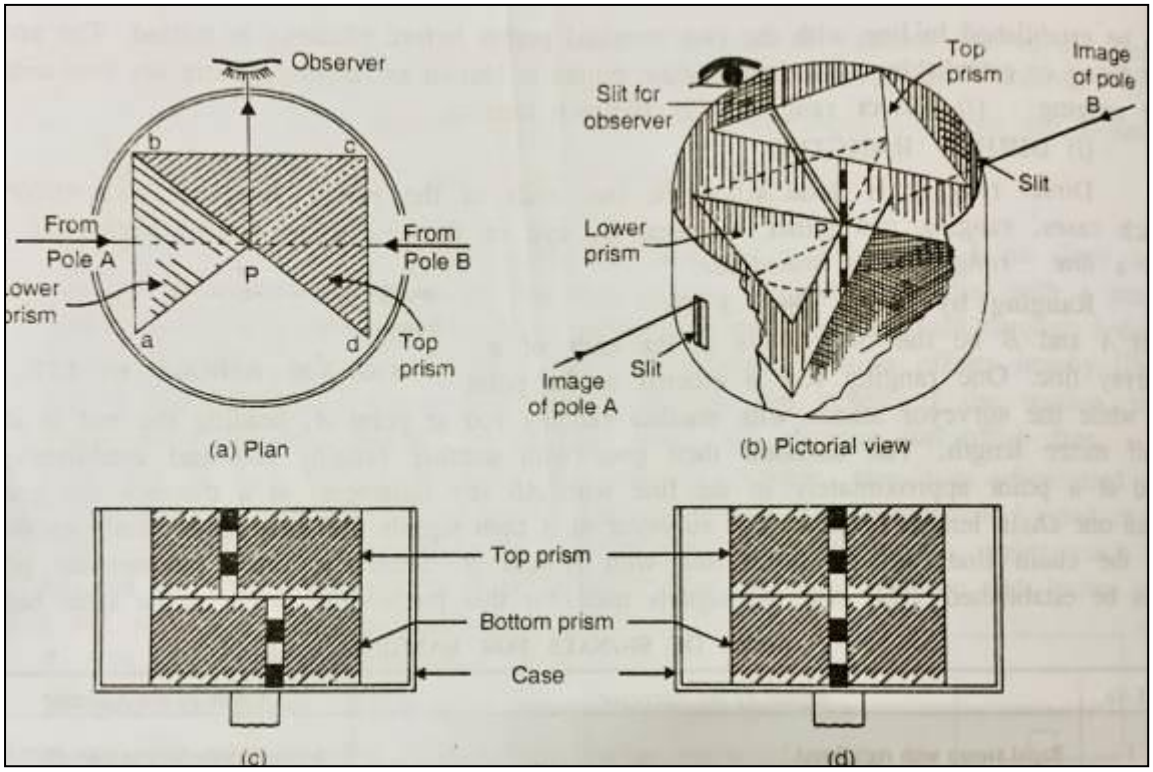


Fig.2. Ranging by Line Ranger

In order to handle the instrument in hand a handle with hook is provided. The hook is to enable a plumb- bob to help transfer the point to the ground.

In order to range the point 'P', initially two rods are fixed at points A and B. By eye judgment, the surveyor holds the ranging rod at P almost in line with AB.

The lower prism abc receives the rays coming from A which is then reflected by the diagonal ac towards the observer. The upper prism dbc receives the rays from B which is then reflected by the diagonal bd towards the observer. Hence the observer can see the images of the ranging rods A and B, which might not be in the same vertical line as shown in figure-2(c).

The surveyor moves the instrument till the two images come in the same vertical line as shown in figure-2(d). With the help of a plumb bob, the point P is then transferred to the ground. This instrument can be used to locate the intermediate points without going to the other end of the survey line. This method only requires one person to hold the line ranger.

2. Indirect Ranging

Indirect ranging is employed when the two points are not intervisible or the two points are at a long distance. This may be due to some kind of intervention between the two points. In this case, the following procedure is followed.

As shown in figure-3, two intermediate points are located M1 and N1 very near to chain line by judgment such that from M1, both N1 and B are visible & from N1 both M1 and A are visible.

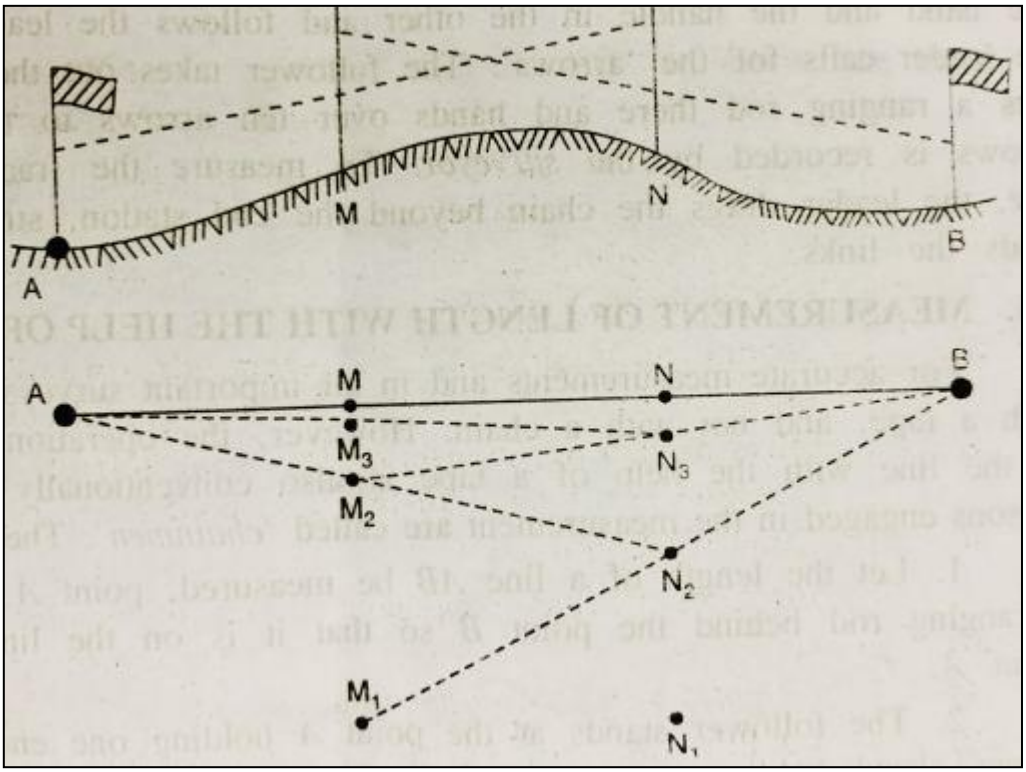


Fig.3. Indirect Ranging

At M1 and N1 two surveyors stay with ranging rods. The person standing at M1 directs the person at N1 to move to a new position N2 as shown in the figure. N2 must be in line with M1B.

Next, a person at N2 directs the person at M1 to move to a position M2 such that it is in line with N2A. Hence, the two persons are in points are M2 and N2.

The process is repeated until the points M and N are in the survey line AB. Finally, it reaches a situation where the person standing at M finds the person standing at N in line with NA and vice versa. Once M and N are fixed, other points are fixed by direct ranging.

Remote observations

Remote observing with the 30m telescope is now routinely possible. The telescope is controlled in real time via vnc-viewers. Remote observations are restricted to experienced 30m observers.

Using dedicated stations for remote observations is not mandatory any longer. However, fully-equipped remote stations like the one at IRAM/Granada offer the advantage of large screens to accomodate the various displays necessary for the command interface and to monitor the observations. Other advantages are the readily available documentation and a phone, last-not-least local help is usually available. Please address your contact at the remote station well in advance.

Sampling methods in the study of behaviour

Animals are always behaving. From the point following conception, when movement can first be detected, until death they perform a continuous stream of behaviour. It is very difficult and unnecessary to completely record major portions of the stream of behaviour in order to answer research questions. Because behaviour does not occur randomly, the relative frequency and duration of behaviours can be approximated through sampling. Because samples are only estimates of the true behaviour (Crockett, 1991), protocols must be used for sampling that provide valid answers to particular research questions.

Variables affecting sampling method selection

The appropriate sampling method will be determined, in part, by how the research questions (Lehner, 1987) focus the study on particular variables (Table 1), regardless of whether the study is descriptive or experimental. An experiment tests a hypothesis and requires that independent variables be held constant, be allowed to vary naturally, or be manipulated and measurements mad e on dependent variables (behaviours). In preparing a descriptive ethogram for a species, observations and measurements of the behaviour are made under the widest range of conditions for the variables in Table 1.

Table 1. Some variables used to help select behaviour sampling

Variable	Description and examples
What	Type of behaviour (e.g., feeding, mating, and agonistic behaviour)
Who	Individuals (e.g., sex, age, social rank, and genotype)
When	Temporal (e.g., season, time-ofday, pre- and posttreatment, and response latency)
Where	Spatial (e.g., geographic, in building, outside, and distance from food, water, or other individuals)
Environment Abiotic	methods (e.g., temperature, humidity, wind speed, and photoperiod)
Biotic	(e.g., animals, vegetation, and observers)

A question that should be addressed initially is: What behaviour is of interest? Is the behaviour known or unknown? A determination of the behaviours that occur under specified conditions may be desired. For example, Wood-Gush (1956) released cocks singly into a pen containing a hen and recorded all the males' movements in relation to the hen in order to determine and describe their courtship behaviour. In contrast, specific behaviours, such as how ingestion, agonistic, and fearful behaviours of laying hens are affected by cage configuration may be of interest (Anderson and Adams, 1991). Secondly, a decision must be made whether who performs the behaviour is important. The individuals of interest can differ along several parameters (Table 1).

The third variable of concern is the temporal aspect of a study. The time when the behaviours occur in response to manipulated stimuli (i.e., treatments) or natural variation in environmental factors may be of interest. For example, Tanaka and Hurnik (1991) measured behavioural responses (e.g., eating) of hens to simulated dawn and dusk. Further, Tanaka and Hurnik (1991) determined where the hens were located spatially (e.g., on the ground, floors, or perches). In this study, they measured the behavioural responses of the hens to changes in the abiotic environment (light), just as Murphy and Wood-Gush (1978) measured the behavioural responses of chickens of two different genotypes to being placed in a strange physical environment (a sound-proof room). The role of the biotic environment was the focus of Kratzer and Craig's (1980) study of the effects of group size and density on mating behaviour of cockerels.

Behaviour sampling methods

There are eight common behaviour sampling methods (Table 2). However, sampling always consists of using either Focal Animal (Pair, Group) or All Animals Sampling paired with another method (e.g., Focal Animal and All Occurrences). Some methods are used consecutively or simultaneously

Table 2. Behaviour sampling methods

Method	**Description**
Focal animal (pair, group)	Restricts data recording during a sample period to one animal, pair, or group.
All animals	Data is gathered from all observable animals.
One of the above methods is paired with one of the methods below	
Ad-libitum	Opportunistic sampling with no constraints.
Continuous recording	
All occurrences	Record all occurrences of selected behaviour(s).
Sequence	Sampling is restricted to all occurrences of selected intra- or interindividual sequences of behaviour
Sodometric matrix	Record results of interactions between individuals.
Time sampling	
One-zero	Record the occurrence (one) or nonoccurrence (zero) of selected behaviour(s) during sequential sample intervals
Instantaneous or scan	Record behaviour of an individual (instantaneous sample) or group of individuals (scan sample) at sequential, predetermined points in time.

Other factors to consider when selecting a sampling method

Duration of Behaviour

A state is an ongoing, duration meaningful behaviour. An event is a momentary behaviour that happens so rapidly that it is normally recorded only as an occurrence and not a duration. Events are often changes in states, such as a hen flying (state), landing on the ground (event), and then walking (state).

Scale of Measurement

The four scales of measurement are: nominal, ordinal, interval, and ratio. Nominal data are counts (frequencies) of occurrences of behaviours that differ qualitatively. For example, seven waltzes, ten feather rufflings, and twelve wing-flaps might be recorded during a Focal Animal and All Occurrences sample of a cock's agonistic behaviour. Ordinal data are ranked by a common qualitative property. The relative order of the ranks is known, but the distance between them is neither continuous nor known.

Habitat characterization-ground and remote sensing methods

Remote sensing

At its simplest definition, remote sensing is obtaining information about an object by a device that is not in contact with the object. In ecology remote sensing usually involves sensors on satellite platforms or airplanes. Most devices have a series of sensors that record the intensity of **electromagnetic radiation** in particular segments of the spectrum for each point, or pixel, in an image. These sensors are designed to collect data in the visible wavelength as well as in other portions of the electromagnetic spectrum (such as the infrared region) that are needed to examine specific aspects of the physical world.

In addition to collecting data from a large part of the electromagnetic spectrum, remote sensing systems collect data over large areas. For instance, the U.S. *Landsat* satellites record continuous data over an area 71.4 square miles (185 square kilometers) wide. Since some satellites have been in orbit since the mid-1970s scientists can effectively "collect data" from this time period. Therefore, remote sensing offers scientists a wide spectral, spatial, and temporal data range.

For remote sensing to be of use to ecologists the spectral data must be related to some ground-based measurement such as land cover type or vegetation characteristics (biomass or net primary production, **evapotranspiration** rates, water stress, vegetation structure). Most work in ecology is done at the scale of a small plot, or piece of a field or forest. It can be difficult to extrapolate these small-scale measurements to larger, **heterogeneous** areas. Because sensors record continuous data over large areas, remote sensing can be used to "scale-up" plot-based measurements to examine landscape or even regional patterns. For example, ecologists have used remote sensing data to determine the rate at which rainforest in Brazil is being converted to agricultural land. In North America, scientists using satellite data have determined that one of the most endangered **ecosystems** , the tall grass prairie, is being replaced by woody vegetation at an alarming rate.

Another set of questions that can be addressed with remote sensing data involves landscape heterogeneity. In these analyses, any of a number of spatial statistics can be applied to the original spectral data. Also, the original bands can be recombined to create indices. The most common of these is the Normalized Difference Vegetation Index, a ratio of red to near infrared bands, which has been useful in quantifying vegetation in numerous locations around the world.

Spectral data can be analysed directly (total infrared reflected) or a classification can be performed on the data. With this method, the spectral data are analysed and each pixel is assigned to a land cover type: forest, grassland, or urban. For instance, forests reflect less infrared than grasslands. These land cover data can then be incorporated into a Geographical Information System (GIS) for further analysis. A GIS is a computer-based system that can deal with virtually any type of information that can be referenced by geographical location.

Once the land cover types are identified and GIS coverage is generated, additional data such as soil type, elevation, and land use history can be entered into the GIS. Ecologists can then ask questions about landscape-level patterns such as the average patch size of a certain land cover type or its dispersion across the landscape. This information can then be related to some ecological process such as the movement or dispersal of animals.

Probable Questions:

1. Discuss the methods of estimating population density of animals.
2. Briefly discuss about the Field techniques for inventories and monitoring studies of terrestrial and semi-aquatic vertebrates.
3. Discuss about direct ranging with diagram.
4. What do you mean by remote observation?
5. Discuss about different Variables affecting sampling method selection in the study of behaviour.
6. Write short notes on Remote sensing

Suggested Readings:

1. Wilson and Walker: Principle and technique of biochemistry and molecular biology, 7th Edition.
2. Biophysical Chemistry by Upadhyay and Upadhyay

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The study materials of this book have been collected from books, various e-books, journals and other e-source

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

NON-CHORDATE BIOLOGY AND INSECT ORGANIZATION

ZCORT-101

Self-Learning Material

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HARD CORE THEORY PAPER (ZCORT – 101)

Group A (Non-Chordate Biology)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT – 101 (Non-Chordate Biology and Insect Organization)	**I**	Origin & Evolution of Metazoans.	**3**	8-17
	II	Cell organelles in Protozoa: Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle.		18-25
	III	Cell association and cellular differentiation in Protozoa.		26-27
	IV	Osmoregulation in Protozoa and Nematodes.		28-33
	V	Sense organs and their importance - Chemoreception, photoreception and mechanoreception; sensory organelles and reaction for stimuli in protozoa.		34-43
	VI	Organs and process of reproduction in major invertebrate groups.		44-59
	VII	Structural organization of respiratory organs in major invertebrate groups; factors affecting respiration.		60-71
	VIII	Photogenic organs in insects: structure, mechanism and significance of light production		72-77
	IX	a) Trochophore larva: Structure and evolutionary significance. b) Evolutionary significance of minor invertebrate phyla.		78-95
	X	Insect flight: types; structure concerned; functional mechanism; evolution of flight patterns in different insect groups.		96-105
	Group B (Insect Organization)			
	XI	Modern classification of insects		107-121
	XII	General organization, segmentation, division of body: i) Head and mouth-parts in general		122-135

ZCORT – 101 (Non-Chordate Biology and Insect Organization)		ii) Thorax and thoracic appendages. Modification of legs and wings. iii) Abdomen and abdominal appendages.	**3**	
	XIII	Integument: Basic structure and functions; modification in different insect groups.		136-144
	XIV	Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber.		145-155
	XV	Structure and functions of the central nervous system in insects.		156-162
	XVI	Insect visual organs, their structure and functional mechanisms		163-172
	XVII	Insect blood: composition, functions, morphology of circulatory system.		173-177
	XVIII	Structure and function of sound producing organs in insects; types of insect sound; significance of sound production		178-187
	XIX	Exocrine glands: Lac gland, Wax gland, Silk gland, Labial gland		188-195
	XX	Metamorphosis in insects: hormonal regulation; factors affecting metamorphosis; diapause		196-209
	Total counseling session 18hrs.			

Group-A : NON CHORDATE BIOLOGY

UNIT I

Origin and evolution of metazoans

Objective:

In this unit we will discuss about the Origin & Evolution of Metazoans. Here Cell organelles in Protozoa: Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle also be discussed.

Introduction:

The unicellular protozoans are highly versatile and successful organisms that show remarkable organization and division of labour within the confines of the single cell. This diversity is achieved by varying the structure of their organelles at the sub-cellular level. The Metazoa or the multicellular animals have achieved their structural diversity by varying their cells that have become specialized to perform different functions. These cells are normally incapable of independent existence.

Characteristics of Metazoa:

Let us list out some of the features that characterize metazoans.

1. Members of Metazoa possess a complex multicellular structural organization which may include the presence of tissues, organs and organ systems.

2. In the life history of metazoans, typically a fertilized egg passes through a blastula stage in the course of its early embryonic development before changing into an adult.

3. Since metazoans are multicellular they are relatively larger in size than unicellular protozoans. Naturally, their nutritional requirements are more and they have to search for food. Consequently, locomotion in metazoans is highly developed and for this purpose they have evolved contractile muscular elements and nervous structures.

4. The ability for locomotion has influenced the shape of the metazoan animals which in turn has conferred specific types of symmetries to metazoan groups.

5. Most of the metazoans show differentiation of the anterior end or head (cephalization); associated with cephalisation, there is the centralization of the nervous system in the head region.

Although all metazoans share some characteristic features, their body plans differ in symmetry, internal organization, developmental patterns and modes of formation of, body cavity. These differences provide us a means of grouping them or organizing them

into different phyla. Lets discuss these features one by one.

Theories Regarding the Origin of Metazoa

1. Syncytial-Ciliate Theory:

The origin of metazoans probably took place through the growth, nuclear division and cellularisation of a single protozoan. Depending on this idea, Hadzi (1953, 1963) proposed the syncytial-ciliate theory. His version supported by Steinbock (1958, 1963) and Hanson (1958, 1963, 1977). Hadzi proposed that the first metazoan evolved from a primitive multinucleated protozoan of a ciliate type.

The multinucleate ciliate represented either *Paramoecium* or *Opalina*. These animals are syncytial in nature, i.e., the nuclear division takes place without cytoplasmic division. In course of evolution the multinucleated cytoplasm developed boundary cell membranes between the nuclei and in course of time metazoans have developed from these ciliated protozoans (Fig. 22.2).

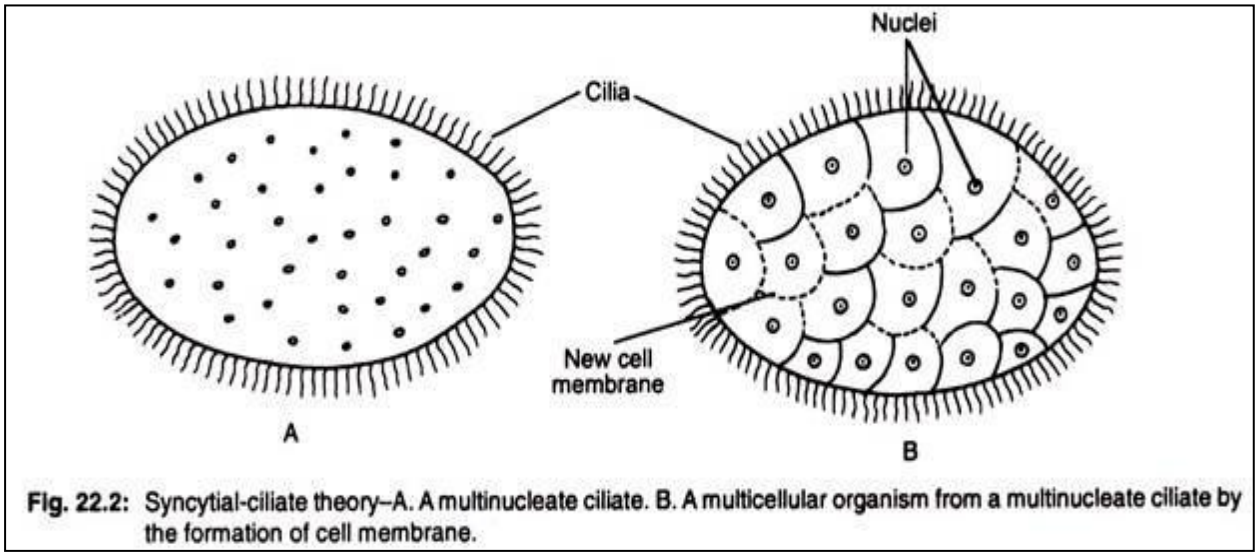


Fig. 22.2: Syncytial-ciliate theory–A. A multinucleate ciliate. B. A multicellular organism from a multinucleate ciliate by the formation of cell membrane.

A bilateral ancestor could give rise to the acoel turbellarians which is devoid of gut. But Hadzi believed that midgut tissue of acoels is probably syncytial in nature and it may be believed that acoels would be the ancestral stock from which all other metazoans, such as Mollusca, Annelida, Arthropoda, Echinodermata and Chordata, have originated except poriferans which could have originated from flagellated protozoans in a separate line.

Remark:

It may be assumed that flatworms and spiralian protostomes may have derived from opalinids because they belong to multiciliated metazoans but sponges and cnidarians are monociliated, so could not develop from the multiciliated acoel ancestor.

Spiralian protostomes include molluscs, annelids and arthropods and they are called for the spiral cleavage during embryonic development.

2. Colonial Flagellate Theory:

This theory was proposed by a German scientist Haeckel (1874, 1875) and supported by Lankester (1873, 1877), Metschnikoff (1886) and Hyman (1940).

Morphological data indicate to the understanding of the transitional stages of the Metazoa suggest a colpnial origin of Metazoa. The recent popularity of colonial theory was due to the acceptability of Hyman who updated the theory. According to this theory, the most primitive metazoan was originated from colonial flagellated protozoa.

Haeckel stated that the colonial ancestor of Metazoa was spherical, hollow, volvox-like colonial flagellate. Lankester proposed that the ancestral colonial protozoa were solid, flagellated protozoan-like animal (e.g. *Pandorina*). Metschnikoff has also stated that the ancestral colonial choanaflagellate was solid and resembles the existing Proterospongia.

The outer layer of the colonial Proterospongia possesses collared cells help in locomotion and the inner layer is filled with amoeboid cells. When the flagella and the collares of the Proterospongia are lost, these cells move into the central area like amoeboid cells (Fig. 22.3).

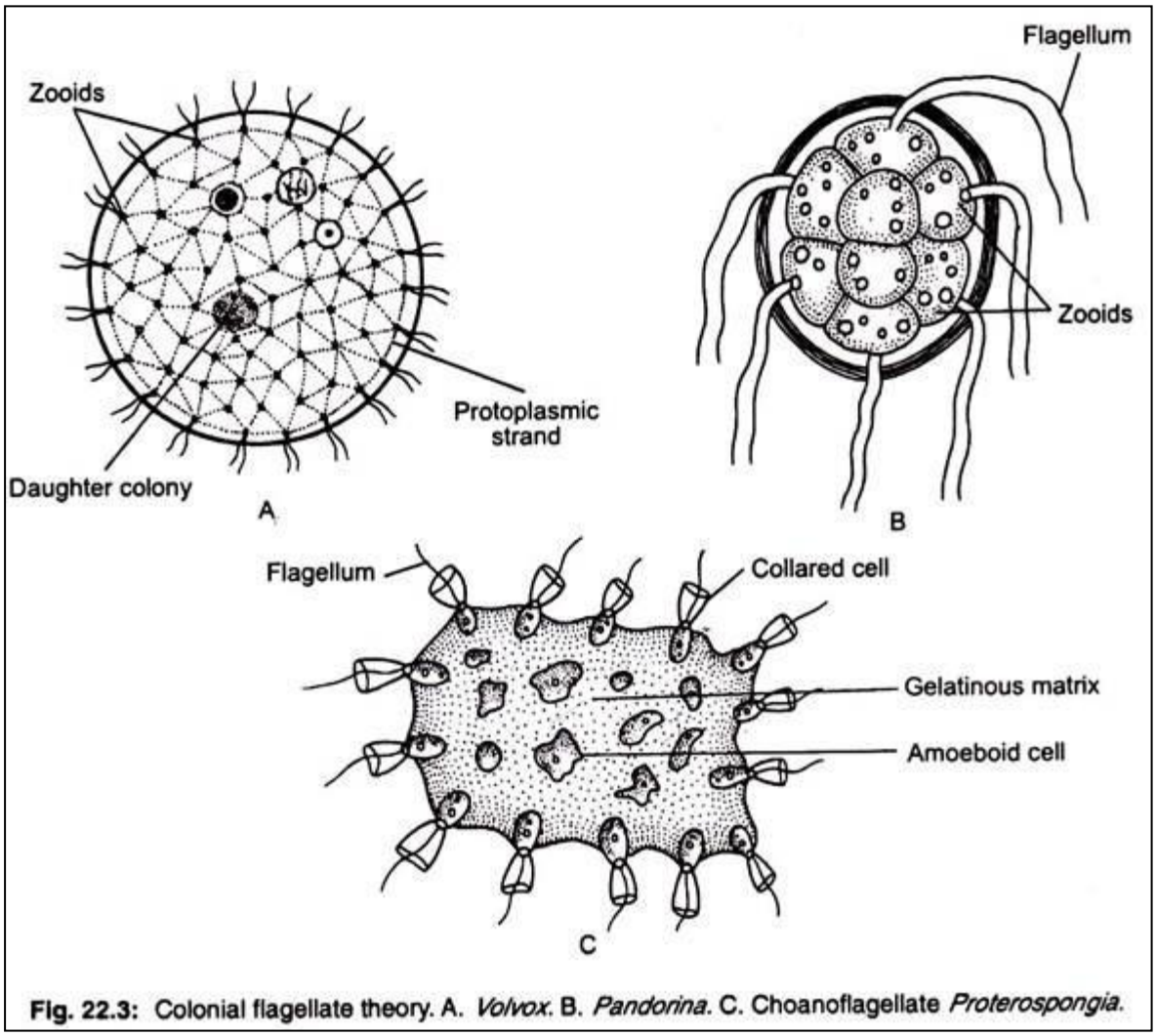


Fig. 22.3: Colonial flagellate theory. A. *Volvox*. B. *Pandorina*. C. Choanoflagellate *Proterospongia*.

3. Colonial Blastea and Planula Theories:

It is presumed that the primitive metazoans first originated from a spherical, hollow, colonial flagellate (Fig. 22.4).

The evidences in support of this statement are:

(i) Sperm cells are flagellated in all metazoans, and

(ii) In lower metazoans such as sponges, in many cnidarians, the monociliated cells occur within the body.

The cells of the colony bear collar-like structure around a single flagellum and possess anterior-posterior axis and would swim by placing the anterior pole forward. The cells were differentiated into somatic (non-reproductive) and reproductive cells. Haeckel proposed this hypothetical ancestral stage, the blastaea as a first stage in metazoan origins.

Blastea may be compared with the existing blastula stage of the multicellular animals and resemble the present day Volvox colony. The next evolutionary stage is a solid, non-evaginated structure of the archemetazoan which is called parenchymula or planaea (also called planula).

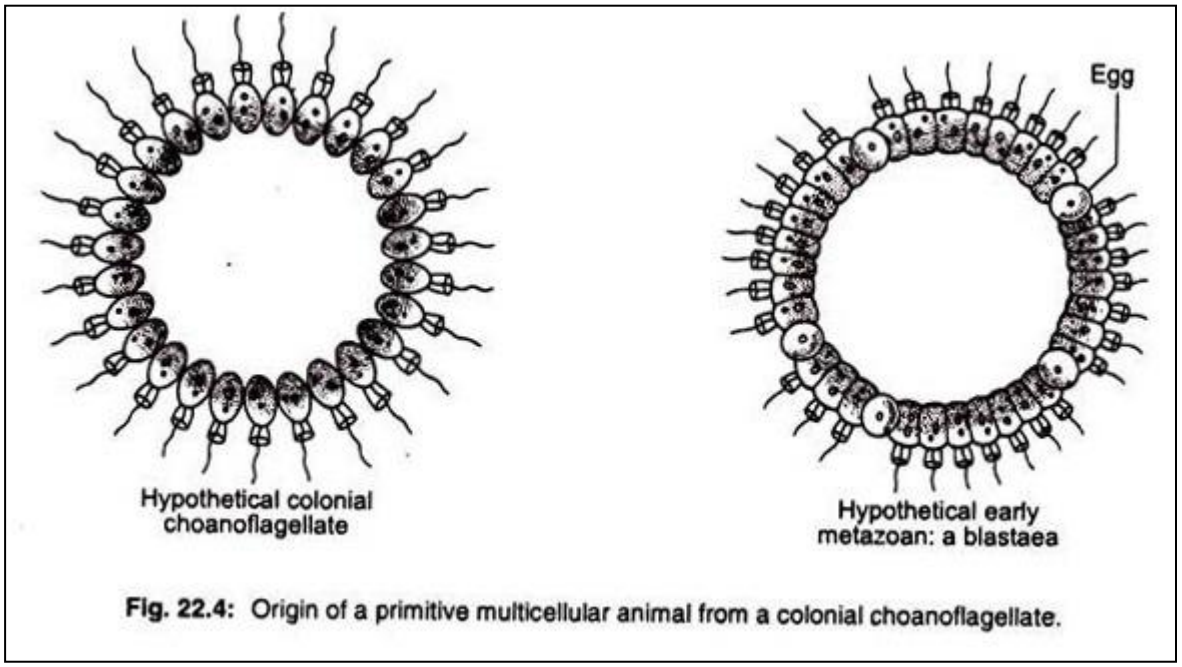


Fig. 22.4: Origin of a primitive multicellular animal from a colonial choanoflagellate.

The planula ancestor is represented as a small, ovoid, pelagic animal with radial symmetry containing solid mass of cells and the internal cell mass is to be considered as the migratory cells of the blastula stage.

The planula stage gave rise to cnidarians and ctenophores by separate lines and it is also presumed that in course of evolution, the benthic, bilateral primitive flatworms (e.g., Turbellarian-acoels) have evolved from the sexual planulae.

4. Gastraea Theory:

This theory was proposed in somewhat modified form by Metschnikoff. He proposed a solid double walled cup-like structure, called the gastraea which formed by the invagination of the blastaea at the posterior pole. The gastraea represents the gastrula stage of development in the existing multicellular organisms and proposed as a metazoan ancestor.

The outer layer of the gastraea is called ectoderm and an inner layer is called endoderm. The space which is enclosed by the endoderm is called primitive gut. The gut communicates to the exterior by the mouth (protostome) (Fig. 22.5).

Later on, colonial theories have been made up-to-date by Hand (1963), Ivanov (1968) and Reisinger (1970), etc. Greenberg (1959) has pointed out that planula larva is seen not only in many cnidarians but also in a few sponges and at least in a single ctenophore.

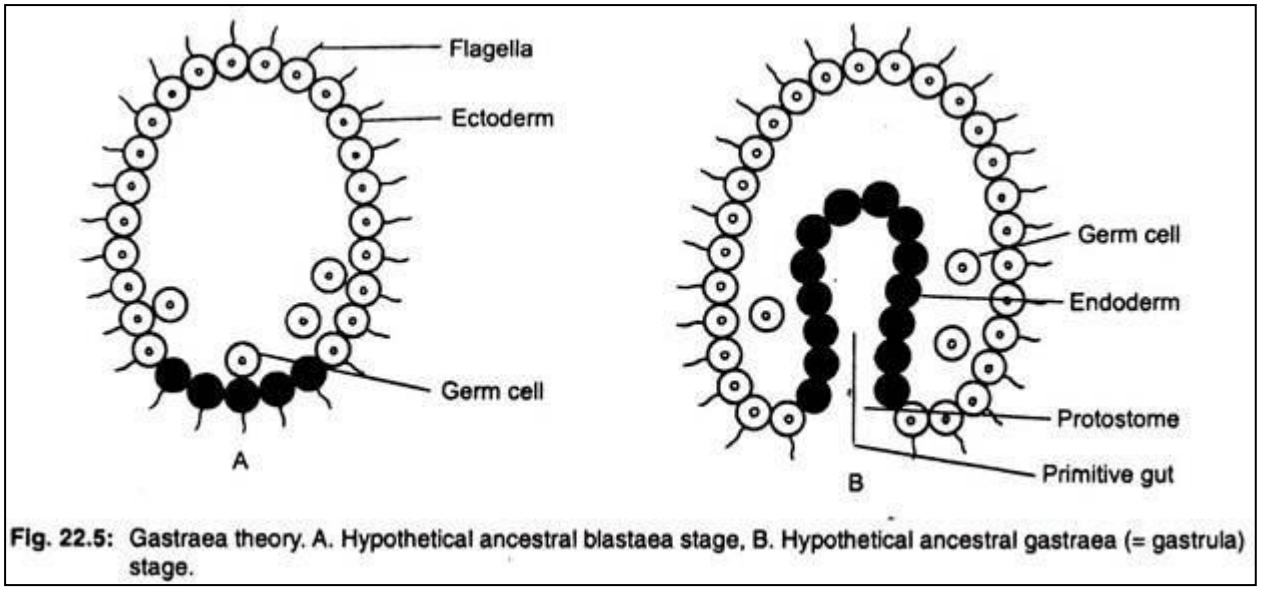


Fig. 22.5: Gastraea theory. A. Hypothetical ancestral blastaea stage, B. Hypothetical ancestral gastraea (= gastrula) stage.

5. Monophyletic Origin of Metazoans:

Muller (2001) reported that the adhesion molecules are found in the sponges and also present in the major metazoan phyla. So it may be considered that all metazoans originated from a single common ancestor who may be called Urmetazoa.

6. Polyphyletic Origin of Metazoans:

Recent molecular studies based on rDNA have suggested that the origins of Metazoans are polyphyletic and some of them are described below:

i. Greenberg (1959):

Greenberg (1959) has stated that metazoans have originated from two ancestors, such as sponges and cnidarians have evolved separately from colonial flagellates and ctenophores and flatworms from the ancestral ciliates.

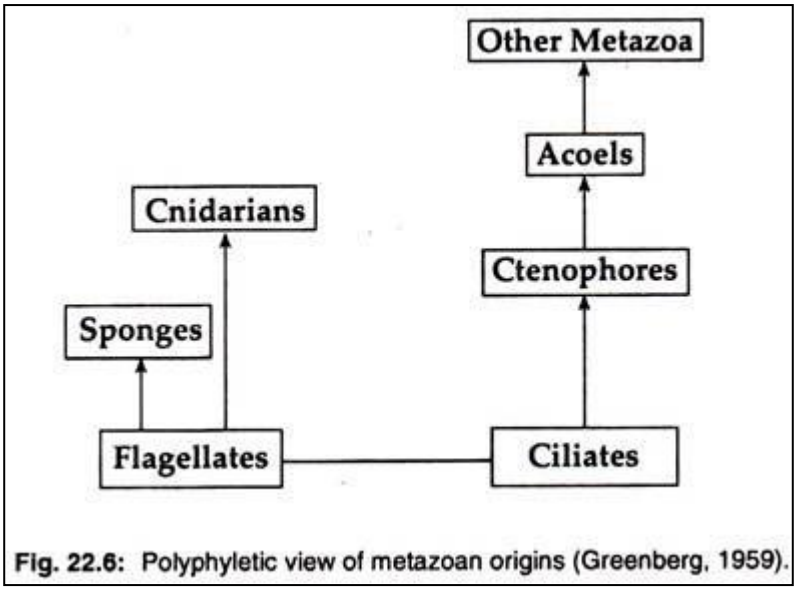


Fig. 22.6: Polyphyletic view of metazoan origins (Greenberg, 1959).

ii. Nursall (1962):

Nursall (1962) has proposed that different multicellular invertebrate phyla have evolved independently from different protists.

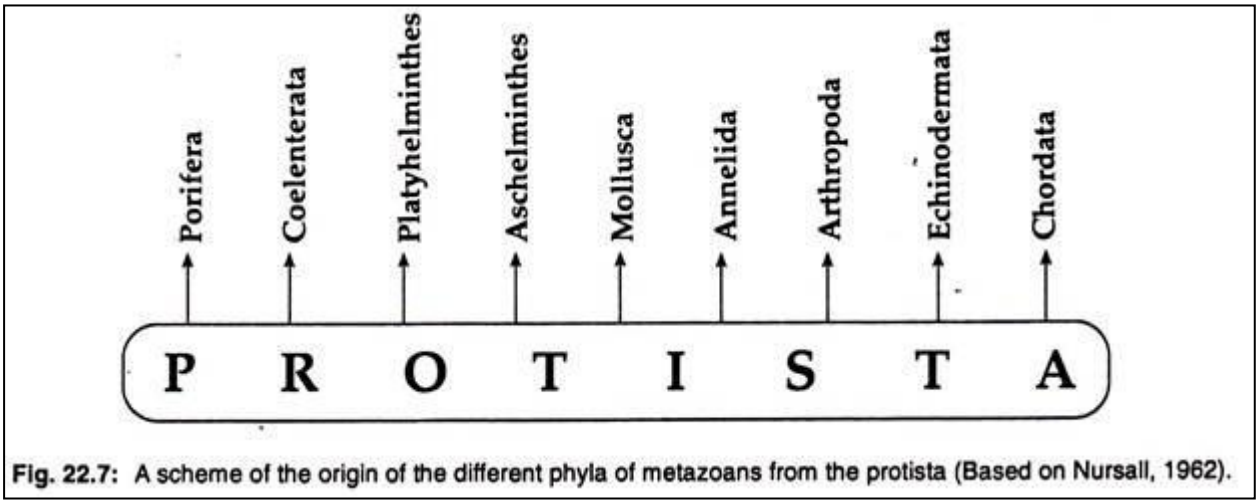


Fig. 22.7: A scheme of the origin of the different phyla of metazoans from the protista (Based on Nursall, 1962).

iii. Hanson (1977):

Hanson (1977) advocated that a colonial amoeboid form which is considered as the ancestor of cnidarians, and platyhelminthes is derived from syncytial ciliates.

iv. Hanson (1977) and Sleigh (1979):

Hanson (1977) and Sleigh (1979) have proposed that sponges and cnidarians have evolved separately from flagellated protists and other group-ciliates has originated

from flagellated protists and ciliates have given rise to Acoel-platyhelminthes and other metazoan phyla have evolved from Acoel-platyhelminthes.

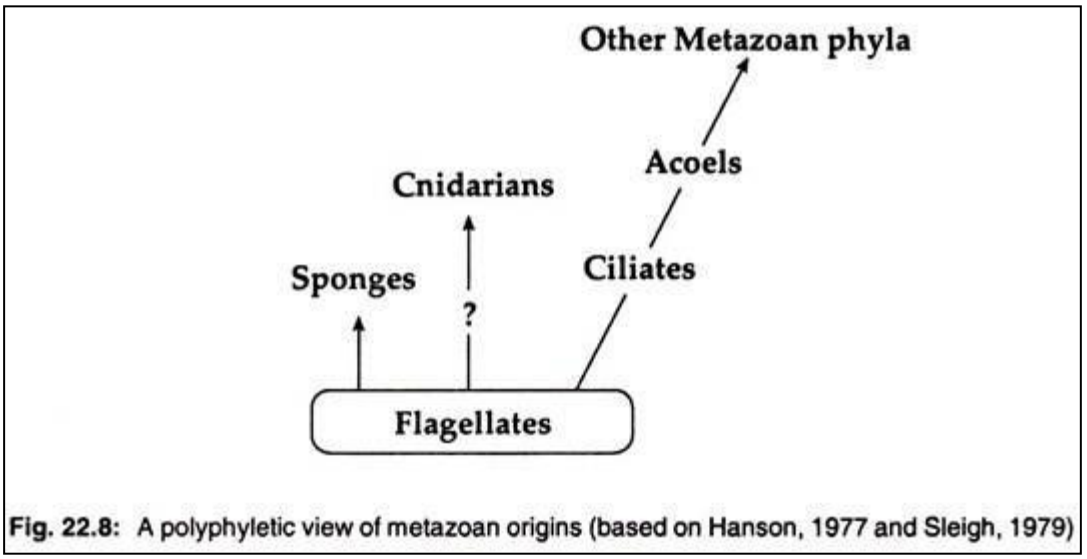


Fig. 22.8: A polyphyletic view of metazoan origins (based on Hanson, 1977 and Sleigh, 1979)

v. Valentine (1973):

Valentine (1973) has created 5 coelomate super-phyla with deuterostomes and Tentaculata (lophophores) being joined by three new groups which split up from the usual protostome assemblage.

This division is largely based on segmentation and these superphyla are:

(i) Metameria or Polymeria included annelids and arthropods.

ii) Amera or Sipunculata included only unsegmented sipunculan worms and

(iii) Mollusca included creeping animals and unsegmented coelom.

Valentine's scheme is the establishment of superphyla rather than traditional morphological criteria.

vi. Anderson (1982):

Anderson (1982) has stated that Cnidaria and Ctenophora have evolved from choanoflagellates, and deuterostomes and other groups of minor phyla have evolved from different protist groups independently.

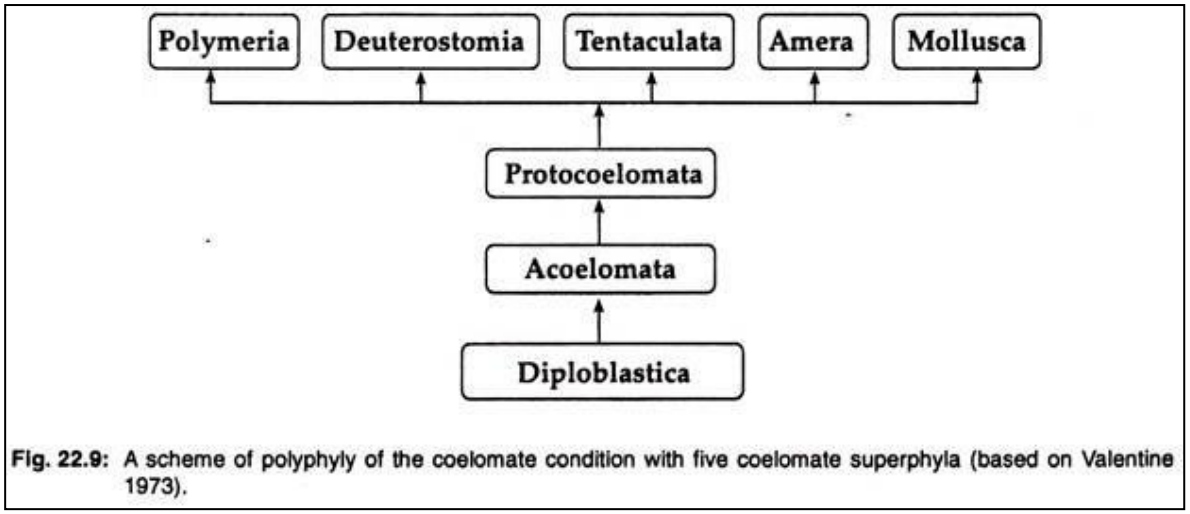


Fig. 22.9: A scheme of polyphyly of the coelomate condition with five coelomate superphyla (based on Valentine 1973).

vii. Nielsen and Norrevang (1985):

Nielsen and Norrevang (1985) forwarded the trochaea theory in which the pelagic trochophore larva (trochaea) transforms into blastaea and gastraea, to form the ancestor of spiralians, and in turn gives rise to a pelagic tornaea as the ancestor of deuterostomes.

Evolution of Metazoa

The sponges, coming under phylum Porifera are the closest to Protista, and can perhaps be regarded even as a colony of protists rather than being multicellular. No other group has evolved from them. The coelenterates consisting of the Cnidaria and the Ctenophora, which are both diploblastic and primarily radially symmetrical, can be regarded as truly the most. primitive of the Metazoa. They have evolved from the ancesteral planuloid metazoans as an offshoot: The Platyhelminthes (flatworms) have also evolved from phuloid ancestor. They do not have a coelomic cavity (acoelomates) but the mesoderm is cellular, they are triploblastic and are bilaterally symmetrical. This group and all higher metazoa can be grouped as Grade Bilateria. (See chart in Sec. 3.8) The pseudocoelomate phyla which include the nematodes and the rotifers are supposed to have evolved as an offshoot from the flatworms. The Eucoelomata constitute the.remainder of the Metazoa. The acoeloid flatworm like ancestors has given rise to two main stocks: i) The molluscan - annelidan, arthropodan stock with schizocoelous coelom. ii) The echinoderm, hemichordate, chordate stock with enterocoelic coelom. The coelom serves as skeleton in the more primitive of the coelomates. Such as the polycheate worms, earthworms-etc. In arthropods and molluscs the coelom becomes reduced to the point of being represented by the cavity of the gonads. The other space in arthropod body is only a haemocael which is a mere space in the tissue filled with blood. The phylogeny of Metazoa can be sumrnarised in following figure.

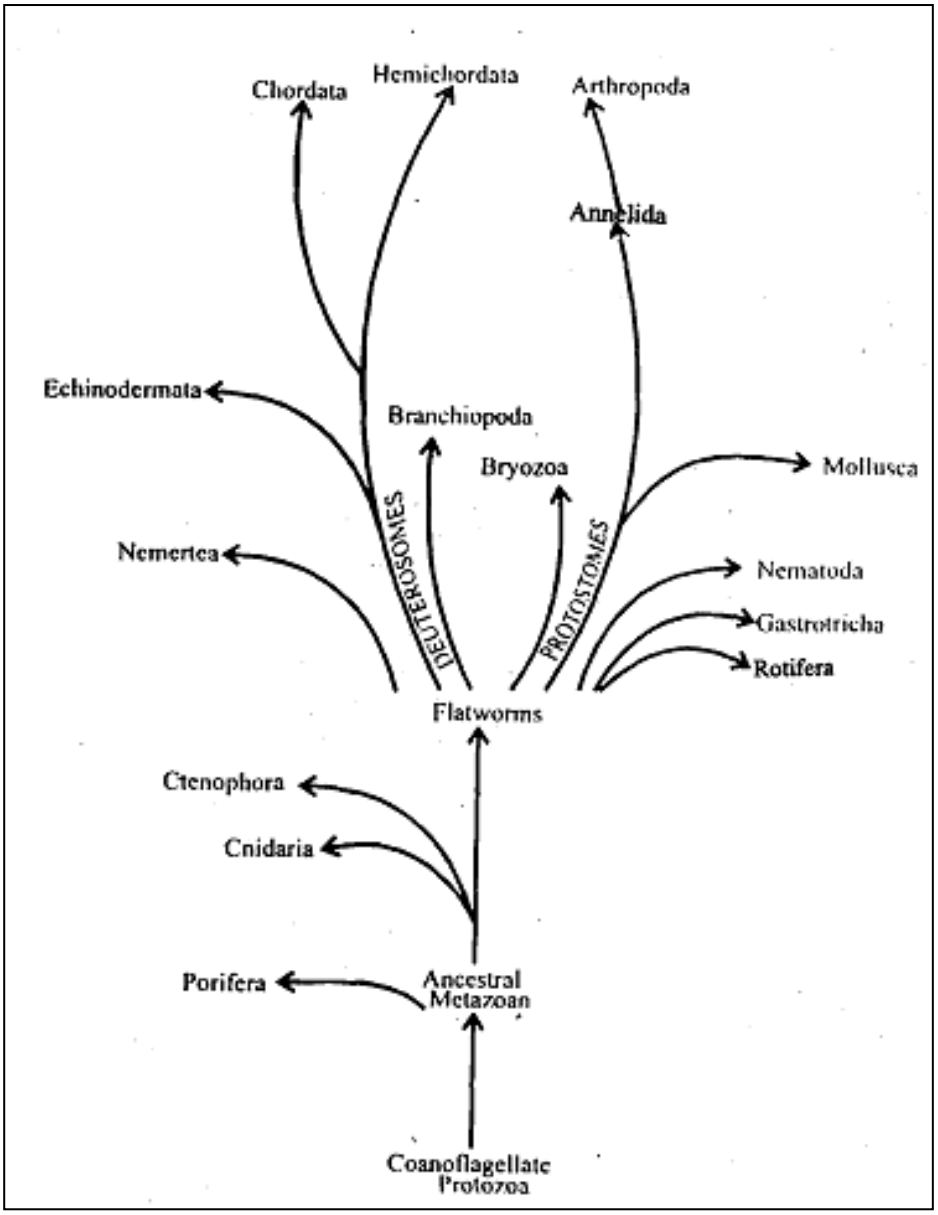


Fig: evolution of Metazoa

Probable Questions:

1. Describe the characteristics features of Metazoa.
2. Write short notes on Syncytial-Ciliate Theory.
3. Describe the different theories regarding the origin of metazoa.
4. Describe polyphyletic origin of metazoans.
5. Explain the evolution of metazoa.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
6. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 1931

UNIT II

Cell organelles in Protozoa: Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle

Objective:

In this unit we will discuss about Cell organelles in Protozoa: Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle.

Introduction:

The Protozoa may be defined as 'microscopic, acellular animalcules existing singly or in colonies, without tissues and organs, having one or more nuclei**.** When in colonies, they differ from Metazoa in having all the individuals alike except those engaged in reproductive activities'.

General Characters of Protozoa:

1. The protozoans are small, generally microscopic animalcules.

2. Simplest and primitive of all animals with very simple body organisation, i.e., protoplasmic grade of organisation.

3. Acellular animals, without tissues and organs.

4. Body naked or covered by pellicle but in some forms body is covered with shells and often provided with internal skeleton.

5. Protozoans are solitary or colonial; in colonial forms the individuals are alike and independent.

6. Body shape variable; it may be spherical, oval, elongated or flattened.

7. Body protoplasm is differentiated into an outer ectoplasm and inner endoplasm.

8. Protozoans may have one or more nuclei; nuclei may be monomorphic or dimorphic, vesicular or massive. Vesicular nuclei are commonly spherical, oval or biconvex, consist of a central body, the endosome (nucleolus) encircled by a zone of nuclear sap.

9. Locomotory organelles are pseudopodia, flagella, cilia or none.

10. Nutrition may be holozoic (animal-like), holophytic (plant-like), saprozoic or parasitic. Digestion intracellular, takes place inside the food vacuoles.

11. Respiration occurs by diffusion through general body surface.

12. Excretion also occurs through general body surface but in some forms through a temporary opening in the ectoplasm or through a permanent pore, the cytopyge.

13. Contractile vacuoles perform osmoregulation in freshwater forms and also help in removing excretory products.

14. Reproduction asexual or sexual; asexual reproduction occurs by binary fission, multiple fission, budding or sporulation and sexual reproduction is performed by gamete formation or conjugation.

15. Life cycle often exhibits alternation of generation, i.e., it includes asexual and sexual phases.

16. Encystment usually occurs to tide over the un-favourable conditions and it also helps in dispersal.

17. The single celled body of Protozoa performs all the vital activities of life and, therefore, no physiological division of labour is exhibited by them.

18. The protozoans exhibit mainly two modes of life, free-living inhabiting freshwater, saltwater and damp places, and parasitic living as ecto- and endoparasites. They are also commensal in habit.

- **Golgi apparatus**

Presence of Golgi bodies in the form of compact, flattened and plate-like vesicles has been reported in Amoeba and Pelomyxa with certainty.

The Golgi apparatus is considered to be a definite cellular inclusion in the Protozoa, although there is still considerable disagreement regarding identification, morphology, distribution, and function in the different classes. The osmic techniques of Weigl (Mann-Kopsch) and Kolatchew, followed by bleaching in hydrogen peroxide or turpentine, are specific for the Golgi material in most organisms. The silver methods of *Cajal* and Da *Fano are seldom successful in the Protozoa.* The application of the ultra-centrifuge has proved that the neutral red bodies, i.e. the 'vacuome', are not identical with the Golgi bodies. In the Mastigophora the parabasal bodies of many forms are considered to represent the Golgi apparatus, though the homology may not be said yet to be fully proved. In other flagellates the cortex of the contractile vacuole i s impregnated b y the osmic Golgi techniques and its behaviour during division in many organisms is similar to that of the metazoan apparatus. These criteria seem sufficient to identify it as Golgi material. In the Sporozoa there is general agreement that the Golgi apparatus is represented by scattered globules or dictyosomes, often possessing osmiophile and osmiophobe regions. In some cells it shows the juxta-nuclear position so characteristic of the Golgi apparatus of many metazoan cells. The homology is also supported by the fact that in the centrifuged sporozoan the osmiophile material occupies the same position relative to the other inclusions as does the Golgi material in higher animal cells. There is still much confusion as to the exact nature of the Golgi apparatus in the Khizopoda, and there is little agreement as to its identity in any of the types studied. Some workers have described the Golgi apparatus as being represented by scattered osmiophilic globules or granules. Others have failed to identify any Golgi material in

members of this class. In the Ciliata most workers agree that the osmiophile cortex to the contractile vacuole found in many forms represents the Golgi apparatus. In some instances scattered Golgi bodies are distributed throughout the cytoplasm. These are often present in combination with the Golgi cortex. There is no convincing evidence that any Golgi material is present in seawater ciliates, the freshwater form *Stytonychia*, or the parasitic *Nyctotherus. Nassonow's view* that the *osmiophile material together ivith* the *contractile vacuole represented* the *Golgi apparatus* is not supported by modern workers. The osmiophile material alone is believed to represent this inclusion. There is much evidence to suggest that there is a single type of granular Golgi body, from which both the osmiophile cortex type and the dictyosome type are formed. It is Likely that t he Golgi apparatus arose in connexion with the base of the flagellum, later becoming associated with the vacuolar system in many forms. In view of the great diversity in form of t he protozoan Golgi apparatus any single hypothesis regarding function is inadequate to explain all the facts. There is much evidence, however, to indicate that it is concerned w with the mechanism of excretion or secretion, and possibly also with the osmoregulation of the organism. In the Sporozoa it may take part in fat metabolism.

- Situated near the nucleus
- Consist of many sacs / cistern like structures.
- Receives proteins from ER & sorts, pack & deliver by secretary vesicles internally orexternally

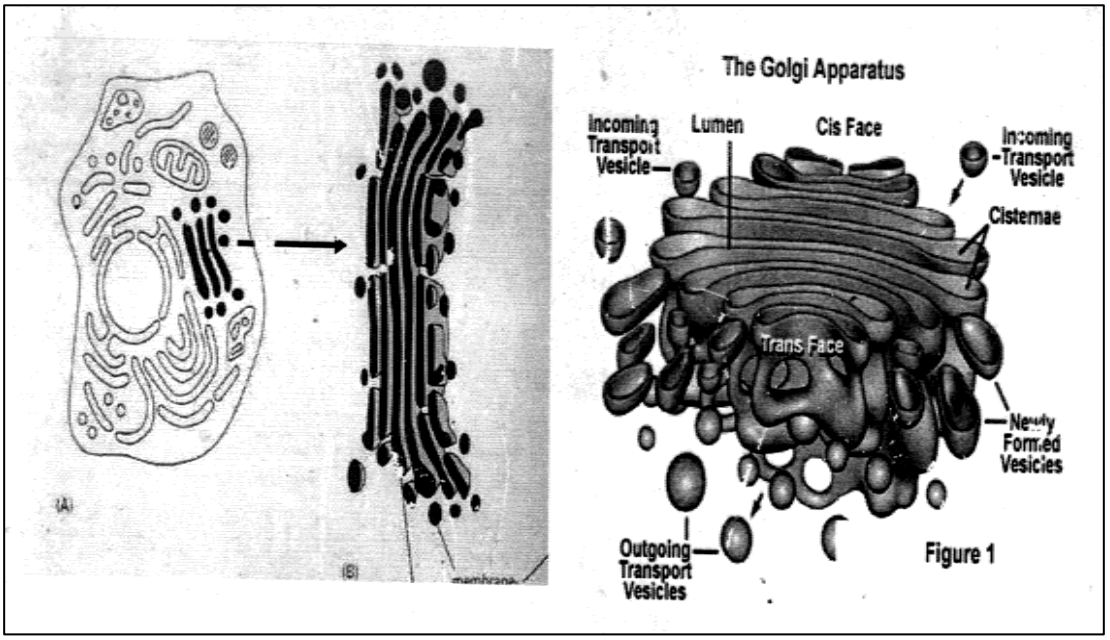


Fig: Structure of Golgi apparatus

- **<u>Mitochondria</u>**

Mitochondria are present in all aerobic species. Number of mitochondria present in an organism is dependent on the volume of that particular organism. In *Tetrahymena* there

are many mitochondria while in avian malaria parasite *Plasmodium lophurae* there are one or two mitochondria.

Structurally and functionally the Protozoan mitochondria differ very little from that of higher animals. The mitochondria occur as small spherical, oval, rod-shaped or filamentous bodies. They may be evenly distributed in the cytoplasm or may be localised in position as they are arranged between the kinetostomes of cillia in *Opalina* and *Paramocium*.

- Spherical or rod shaped.
- Consist of double membrane.
- Power house of cell due to ATP production.
- Have their own DNA

Almost all protozoans contain double-membrane mitochondria; the inner membrane forms flattened, tubular, or discoidal extensions (cristae) into the mitochondrial interior in order to increase the surface area of the respiratory machinery, and the outer membrane forms the boundary of the organelle. Mitochondria are the sites of cellular respiration in most eukaryotes. Species that do not require oxygen (anaerobes), such as those that live in the intestinal tract of their hosts or those that occupy special anaerobic ecological niches, lack mitochondria. Instead, they have energy-generating organelles, such as hydrogenosomes and mitosomes, which belong to the family of organelles called microbodies. These oblong or spherical membrane-bound organelles, about 1–2 micrometres (μm; 1 micrometre = 3.9 × 10^{-5} inch) in length, are believed to be the site of fermentative processes. They contain enzymes that oxidize pyruvate to acetate and carbon dioxide, resulting in the release of hydrogen sulfide under anaerobic conditions.

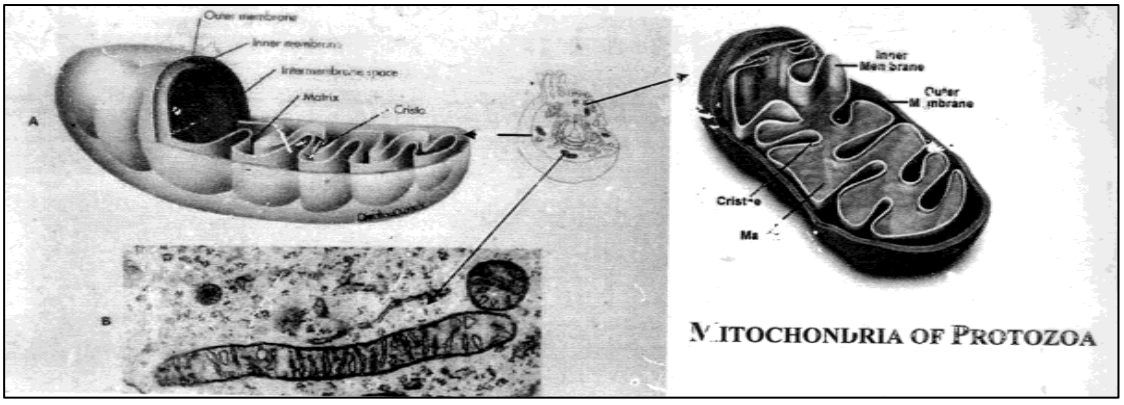


Fig: Structure of Mitochondria

- **<u>Pellicle -Protective or Supporting Organelles</u> :**

Outside the plasma membrane many protozoa have a differentiated pellicle, i.e., a continuous covering which may be more or less flexible. The thick pellicles often show surface decorations in the form of ridges, papillae or pits. The pellicle in ciliates is

perforated through which cilia and trichocysts emerge. The chief component of pellicle in case of *Amoeba* is polysaccharide and in *Euglena* the principal component is protein.

Unlike plants, fungi and most types of algae, protozoans do not typically have a rigid cell wall, but are usually enveloped by elastic structures of membranes that permit movement of the cell. In some protozoans, such as the ciliates and euglenozoans, the cell is supported by a composite membranous envelope called the "pellicle." The pellicle gives some shape to the cell, especially during locomotion. Pellicles of protozoan organisms vary from flexible and elastic to fairly rigid. In ciliates and Apicomplexa, the pellicle is supported by closely packed vesicles called alveoli. In euglenids, it is formed from protein strips arranged spirally along the length of the body. Familiar examples of protists with a pellicle are the euglenoids and the ciliate Paramecium. In some protozoa, the pellicle hosts epibiotic bacteria that adhere to the surface by their fimbriae (attachment pili)

In other Protozoa, *Euglena*, the body covering is in the form of a differentiated pellicle, which is somewhat thicker and firm. It is underlined by plasma membrane and is formed of proteins. The rigidity of pellicle gives definite shape to the body. The thickened pellicle in some of the more specialized Protozoa is variously ridge and sculptured as in *Paramoecium*, *Coleps* etc.

The pellicle is a thin layer supporting the cell membrane in various protozoa, such as ciliates, protecting them and allowing them to retain their shape, especially during locomotion, allowing the organism to be more hydrodynamic. The pellicle varies from flexible and elastic to rigid. Although somewhat stiff, the pellicle is also flexible and allows the protist to fit into tighter spaces. In ciliates and Apicomplexa, it is formed from closely packed vesicles called alveoli. In euglenids, it is formed from protein strips arranged spirally along the length of the body. Familiar examples of protists with a pellicle are the euglenoids and t he ciliate *P*aramecium. In some protozoa, the pellicle hosts epibiotic bacteria that adhere to the surface by their fimbriae or "attachment pili". Pellicle (biology), a thin layer supporting the cell membrane in various protozoa. Pellicle mirror, a thin plastic membrane which may be used as a beam splitter or protective cover in optical systems. Pellicle (dental), the thin layer of salivary glycoproteins deposited on the teeth of many species through normal biologic processes.

Pellicle, the protective cover which can be applied to a photomask used in semiconductor device fabrication. The pellicle protects the photomask from damage and dirt. Pellicle (cooking), a skin or coating of proteins on t he surface of meat, fish or poultry, which allow smoke t o better adhere the surface of the meat during the smoking process. Pellicle (material), a brand name for a very resistant synthetic material used for covering different surfaces, such as that of the Aeron chair.

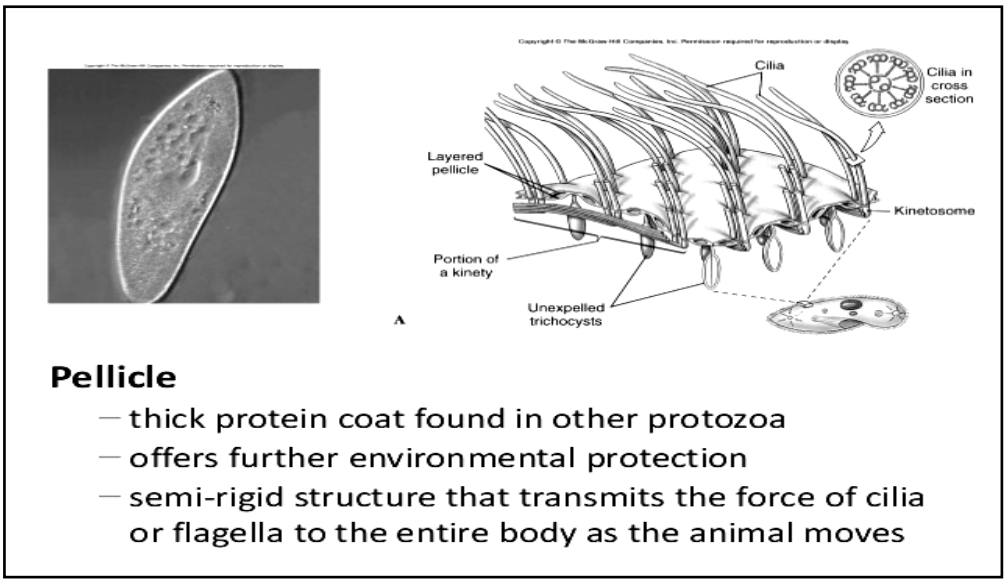


Fig: Structure of Pellicle

- **Cuticle:**

Some invertebrates like rotifers have cuticles that are thin and elastic. In crustaceans, arachnids, insects, cuticles are thick and rigid and support the body. Such cuticles consist of chitin and proteins in rigid plates that a flexible membrane links together. Cuticles retard growth. Thus some of invertebrates like arthropods periodically undergo molting or ecdysis. In Cnidarians like *Hydra* the epidermis is only few cell layers thick. Other cnidarians (e.g. corals) have mucous glands that secrete calcium carbonate ($CaCO_3$) shell. Outer covering of parasitic flukes and tapeworms in a complex syncytuim called tegument (i.e. to cover) host enzymes cannot digest it.

The secreted coatings of protozoans exhibit all grades between soft forms (as in *Amoeba*) and forms with a cuticle that may be proteinaceous (as in *Monocystis*) or composed of cellulose (as in the plantlike flagellates). Other protozoans have definite shells, composed of protein incorporating various foreign bodies, such as siliceous plates or calcium carbonate (in most foraminiferans), or cellulose (in the resting stages of slime molds). The radiolarians have an internal lattice of silica that is laid down inside the cell—a kind of internal skeleton, or endoskeleton.

- **Kinetoplast:**

A kinetoplast is a network of circular DNA (called kDNA) inside a large mitochondrion that contains many copies of the mitochondrial genome. The most common kinetoplast structure is a disk, but they have been observed in other arrangements. Kinetoplasts

are only found in excavates of t he class Kinetoplastida. The variation in the structures of kinetoplasts may reflect phylogenic relationships between kinetoplastids. A kinetoplast is usually adjacent to the organism's flagellar basal body, suggesting that it is tightly bound to the cytoskeleton. In *Trypanosoma brucei* this cytoskeletal connection is called the tripartite attachment complex and includes the protein p166.

The kinetoplast contains circular DNA in two forms, maxicircles and minicircles. Maxicircles are between 20 and 40kb in size and there are a few dozen per kinetoplast. There are several thousand minicircles per kinetoplast and they are between 0.5 and 1kb in size. Maxicircles encode the typical protein products needed for the mitochondria which is encrypted. Here in lies the only known function of the mini circles - producing guide RNA (gRNA) to decode this encrypted maxi circle information, typically through the insertion or deletion of uridine residues. The network of maxicircles and minicircles are catenated to form a planar network that resembles chain mail. Reproduction of this network then requires that these rings be disconnected from the parental kinetoplast and subsequently reconnected in the daughter kinetoplast. This unique mode of DNA replication may inspire potential drug targets.

The best studied kDNA structure is that of *Crithidia fasciculata*, a catenated disk of circular kDNA maxicircles and minicircles, most of which are not supercoiled. Exterior to the kDNA disk but directly adjacent are two complexes of proteins situated 180° from each other and are involved in minicircle replication.

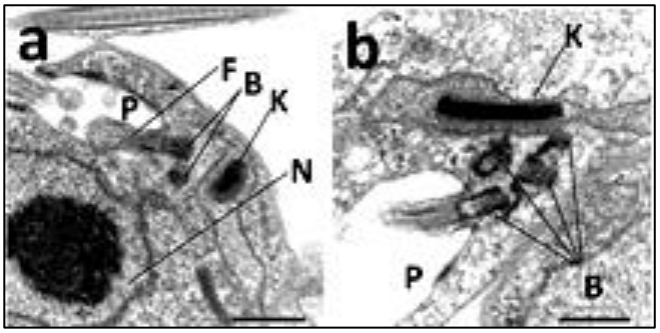


Figure: Electron micrograph of normal kinetoplast (K) of *Trypanosoma brucei*

Probable Questions:

1. What are cristae?
2. Describe the ultra structure of mitochondria?
3. How mitochondria function in the body of a protozoan cell?
4. What do you mean by kinetosome?
5. What is a Golgi body?
6. State the ultra structure of Golgi bodies.
7. What is cistern?
8. State the function of Golgi bodies.
9. What is Pellicle?
10. How it protect a protozoan cell?
11. Describe the electron microscopic structure of pellicle?
12. What is cuticle?

Suggested reading:

7. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
8. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
9. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
10. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
11. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
12. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 1931

UNIT III

Cell association and cellular differentiation in Protozoa

Objectives:

In this unit we will discuss about Cell association and cellular differentiation in Protozoa.

Cell Association and Cellular Differentiation in Protozoa:

It was said at the outset that the word "protozoan" does not necessarily imply a unicellular condition. If the Protozoa are nevertheless generally unicellular, this is based on the nature of their organization. They do not form the physiological unity of a centrally controlled multicellular confederation. However, there are first indications of some tendencies towards a cell association. Colonial associations occur in the flagellates *Chrysomonadina, Phytomonadina* and *Protomonadina*. Some genera of the Radiolaria (*Collozoum, Sphaerozoum, Collosphaera*) form 4 -6 cm swimming colonies. Many eritrichou sciloiates, too form colonies. These associations generally provide greater protection to the individual cells and increase the water current and the procuring of food by means of the action of the flagella and cilia. The protective effect is increased still more when as in *Carchesium* and *Zoothamnium*, a contact stimulus can be transmitted by one individual to the entire colony, thereby leading to a united escape reaction by contraction.

A further advance conducting to organism survival is cell differentiation. This occurs temporally in some amoebae, the Acrasiae, for example in *Dictyostellium discoideum*. When environmental conditions are unfavourable, individual amoebae secretes a hormone, acrasin, which diffuses into the medium and stimulates the amoebae to mass together into a conus structure. The conus tips over; all the constituent amoebae shift their position and finally pile up a gain t o form t he so-called sporophore. Cell differentiation occurs in this structure; the amoebae constituting the stalk fuse together into a compact tissue, while the amoebae in the head of the sporophore survive as cysts. In the flagellates, the individuals f orming an association of cells usually retain their capacity for independent development. However, in the Pgytomonadina, some volvocidae show differentiation.

The individual colonies contain cell which are still all the same and which can all divide, but in *Pleodorina califonica* there are two types of cell which can be distinguished by the difference in size. The small somatic cells have lost the capacity to divide, so that only the larger generative cells are still capable of forming new colonies. In *Volvox aureus* and *V. globator* only a few generative individuals remain. A corresponding condition is found in the peritrichous ciliate *Zoothamnium alternaus*. Only a few macrozoid

individuals can detach themselves and are still able to divide, while the many somatic microzoids have lost the ability to produce daughter colonies. The Cnidosporidia have a particular form of cell differentiation during the production of spores.

The site formation of the spores in the Myxosporidia is multinuclear at first and becomes temporarily multicellular. In this relatively short phase of development, the physiological unity related to that of the metazoan cells is still less marked that in, for example, the colonies of the Phytomonadina. In the Phytomonadina (Volvocidae) not only agamous reproduction but also sexuality is sometimes limited to a few individuals scattered within the colony. This differentiation into pure mortal soma cells and the cells of the path achieves at highest development in the genus *Volvox*. A parallel intracellular development occurs in the macronuclei of the eucilliates and Foraminifera which also have only as somatic function. These examples reveal a certain tendency in the Protozoa towards the subsequent development of the Metazoa, but the fact remains that they do not yet have the physiological unity of a multicellular organism as possessed by the true Metazoa.

Probable Questions:

1. Write a short note on Cell Association and Cellular Differentiation in Protozoa.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
   Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 1931

UNIT IV

Osmoregulation in Protozoa and Nematodes

Objectives:

In this unit we will discuss about Osmoregulation in Protozoa and nematodes.

Osmoregulation in Protozoa

Water is vital to the chemistry of life. Therefore, must attain a water balance within the body water balance systems are based on three processes: Diffusion, osmosis and active transport.

The fresh water Protozoa animals will face a problem with excess of water that enters into the body by osmosis. The process by which water balance in the body is maintained is called Osmoregulation. In protozoans animal's osmoregulation is carried on by contractile vacuoles.

In marine protozoans and parasitic protozoans contractile vacuole is absent. In Amoeba osmoregulation is carried out by contractile vacuoles. The contractile vacuole will absorb excess of water in the body then it reaches the surface and ruptures. This sends out the excess water. Now-a-days "Duncan Wigg, EC. Bovee, T L Jahn' in-1967 worked on the contractile vacuole of Amoeba and came to the conclusion this contractile vacuole has no contraction capacity. It will rupture only to send out the excess water. So they called the contractile vacuole as *Water pulsating vesicle'*.

In *Paramoecium* two contractile vacuoles are present. They are surrounded by 6 to 10 radiating canals. The two contractile vacuoles will work alternately and perform osmoregulation.

Maintenance of constant internal environment is called **homeostasis**. This word is coined by W.B Cannon, in Protozoans homeostasis or Osmoregulation is carried on by contractile vacuoles.

What is Osmosis?

Osmosis is the movement of water across a partially (semi) permeable membrane from an area of high water potential (low solute concentration) to an area of low water potential (high solute concentration). The solute may be something like salt.

A semipermeable membrane is a barrier that permits the passage of some substances but not others. Cell membranes are described as selectively permeable because not only do they allow the passage of water but also allow the passage of certain solutes (dissolved substaces). It is a passive process, requiring now energy and is a special case

of diffusion. A weak or dilute solution with little dissolved solute in it is a hypotonic solution. A strong or concentrated solution is a hypertonic solution.

Two solutions with the same concentration (i.e. the same concentration of water and solute)are **isotonic** solutions. Some examples of osmosis are:

- Absorption of water by plant roots.
- Reabsorption of water by the proximal and distal convoluted tubules of the nephron.
- Reabsorption of tissue fluid into the venule ends of the blood capillaries.
- Absorption of water by the alimentary canal — stomach, small intestine and thecolon.

Diffusion

Diffusion is the process by which molecules spread from areas of high concentration, to areas of low concentration. When the molecules are even throughout a space - it is called Equilibrium. A concentration gradient is a difference between concentrations in a space. The rate (speed) at which the substance can diffuse in and out of cells can be affectedby a number of factors such as temperature and surface area.

- The medium - diffusion is faster in gases than in liquids
- The difference between the concentration of a substance inside and outside the cell(concentration gradient)
- The size of the molecules - smaller diffuse quicker
- Temperature - molecules move faster when heated which will increase the rate ofdiffusion.

In addition to these factors, the surface area to volume ratio can determine the amount of nutrients that can reach the centre of a cell. When you increase the surface area (like in the lungs and the digestive tract), but maintain the volume, you can increase the rate at which nutrients can diffuse throughout a cell. Cells can be different shapes to increase their surface area to volume ratio. E.g. the villi on the cells that line the intestines give more area for nutrient absorption.

Multicellular organisms use transport epithelia to control water loss and excretion

Platyhelminthes: Protonephridia (flame cells) collect excess water in addition to nitrogenous wastes, empty into nephridiopore, excretes NH_3.

Annelida: Metanephridia organized on a per segment basis collect waste from coelom via the nephrostome, counters water uptake by epidermis, excretes NH_3.

Insecta: Malpighian tubules collect nitrogenous wastes from haemocoel, excretes Uric Acid

Contractile Vacuole and Osmoregulation in Protozoa

(i) Shape, Size and Occurrence:

The contractile vacuoles are usually large, colourless, pulsatile fluid-filled organelles found in majority of protozoans. These vacuoles are nearly always found in freshwater Flagellata, Sarcodina and Ciliata. The contractile vacuoles are also found in some marine ciliates but these are not at all found in parasitic protozoans.

The contractile vacuoles are found in their simplest form in Sarcodina like Amoeba. In this case, these are usually spherical vesicles or sometimes irregular and bounded by a limiting membrane. These vacuoles are found surrounded by a circlet of mitochondria which provide energy for their pulsating activity.

In Flagellate like Euglena, the contractile vacuole is somewhat complicated and surrounded by a large number of accessory contractile vacuoles. In Ciliata like Paramecium, the contractile vacuoles are much complicated and found surrounded by 5 to 12 radiating canals or feeding canals which collect water from the various parts of the body.

The number of radiating canals varies in different ciliates. The radiating canals discharge their contents in the main contractile vacuole, thus, serving as feeders.

(ii) Situation and Number:

The position of contractile vacuole is not definite in Sarcodina and, therefore, can be found anywhere in the endoplasm. In Flagellata, e.g., Euglena the contractile vacuole is found situated near the anterior end at the side of reservoir. In Ciliata like Paramecium, the contractile vacuoles are usually two in number, situated one at each end of the body.

The number of contractile vacuoles varies in the different groups of Protozoa but its number remains constant, in the same species. However, it is single in Amoeba, single in Euglena, two in Paramecium but these are many in Radiolaria and Heliozoa.

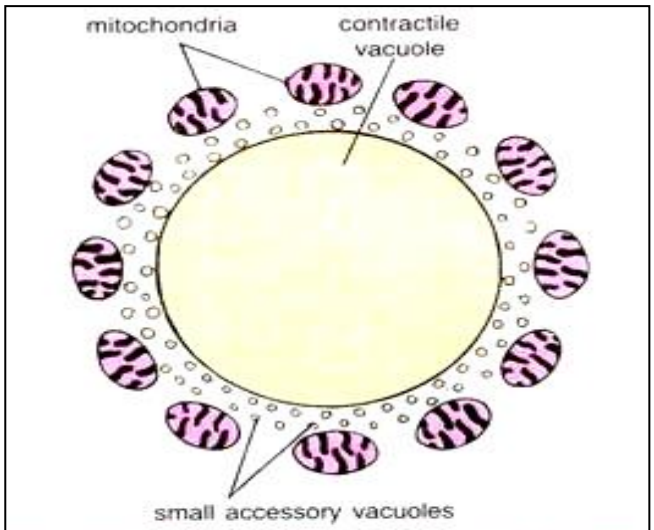


Fig. 23.8. Contractile vacuole of *Amoeba* surrounded by small accessory vacuoles and mitochondria

(iii) Structure:

Contractile vacuole is an empty space filled with fluid. Electron microscopic studies have revealed that its limiting membrane is lipoprotein in nature, like that of the plasma membrane.

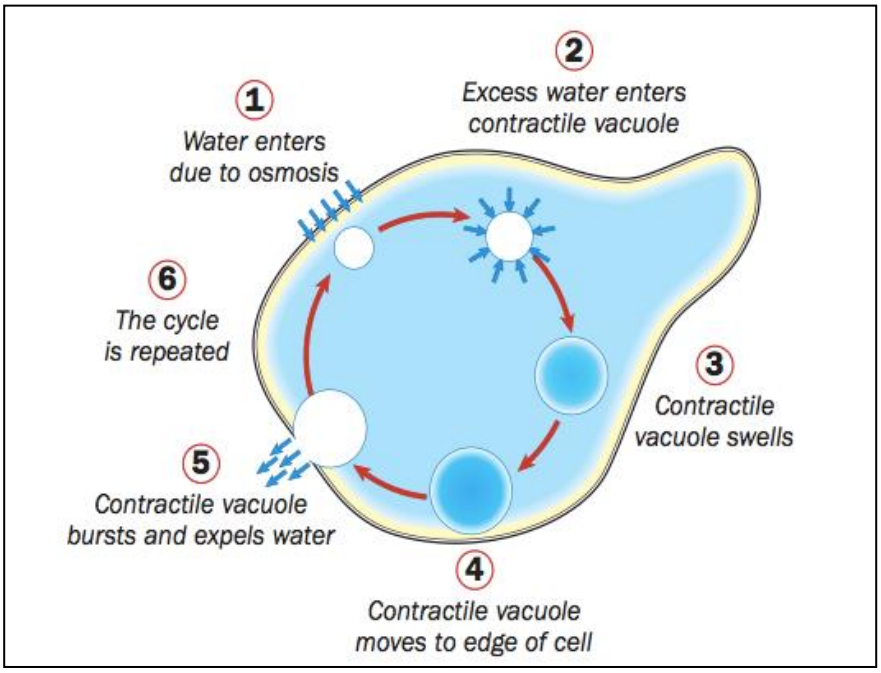


Fig: Osmoregulation in Protozoa

(iv) Mode of Working:

The mode of working of contractile vacuole includes two steps, the diastole and the systole. The diastole is the phase of enlargement of the contractile vacuole to its maximum size and systole is the phase of its contraction to expel its contents.

A contractile vacuole is usually formed by the fusion of a large number of very small droplets in the area where contractile vacuole is to be formed (*Amoeba*) or around the mitochondria contractile vacuole (*Euglena*).

The systole occurs by the sudden burst of the contractile vacuole in Sarcodina but in Flagellata like *Euglena*, it empties in reservoir and so is the case with other forms. As referred, the energy required for the working of contractile vacuole is furnished by the mitochondria surrounding the vacuole.

However, the exact mechanism of working of contractile vacuole is not yet understood, even then the following theories have been put forth to explain its working:

1. Osmotic theory:

This theory explains that the water from the surrounding cytoplasm enters into the contractile vacuole by osmosis.

2. Filtration theory:

This theory explains that the water from the cytoplasm is forced into the contractile vacuole through its membrane due Fig- to internal hydrostatic pressure. Kitching has, however, contradicted this theory.

3. Secretion theory:

This theory states that the water is actively secreted into the vacuole during diastole through the vacuole wall. This theory, too, is not widely accepted.

(v) Function and Significance:

Contractile vacuole performs the function of osmoregulation by removing excess of water content from the body. In addition to its water regulatory function, the contractile vacuole is also believed to be excretory in function.

It has been observed that the water from the surrounding media continuously enters in the body of freshwater protozoans, therefore, water content of the protoplasm increases, i.e., there is an increase in the internal hydrostatic pressure.

This increased water content or hydrostatic pressure of the protoplasm inhibits the normal functioning of the body and if it continues to increase then a time may come when the body of the individual may burst.

Thus, the contractile vacuole helps in removing the excess water content of the protoplasm, i.e., it helps in maintaining the internal hydrostatic pressure.

Actually, the body fluid is hypertonic to the surrounding medium in freshwater forms. In case of marine and parasitic protozoans the surrounding media is nearly isotonic and, therefore, no excess water enters in the body. Hence, contractile vacuole is usually absent in these forms.

Probable Questions:

1. Define Osmosis. Write notes on role of osmosis in Protozoa.
2. Define Diffusion. Write notes on role of diffusion in Protozoa.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
    a. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 1931

UNIT V

Sense organs and their importance: Chemoreception, photoreception and mechanoreception; sensory organelles and reaction for stimuli in protozoa

Objective:

In this unit we will learn about Sense organs and their importance: Chemoreception, photoreception and mechanoreception; sensory organelles and reaction for stimuli in protozoa.

Chemoreception, process by which organisms respond to chemical stimuli in their environments that depends primarily on the senses of taste and smell. Chemoreception relies on chemicals that act as signals to regulate cell function, without the chemical necessarily being taken into the cell for metabolic purposes. While many chemicals, such as hormones and neurotransmitters, occur within organisms and serve to regulate specific physiological activities, chemicals in the external environment are also perceived by and elicit responses from whole organisms. All animals and microorganisms such as bacteria exhibit this latter type of chemoreception, but the two commonly recognized chemosensory systems are the senses of taste, or gustation, and smell, or olfaction.

Photoreception, any of the biological responses of animals to stimulation by light.

In animals, photoreception refers to mechanisms of light detection that lead to vision and depends on specialized light-sensitive cells called photoreceptors, which are located in the eye. The quality of vision provided by photoreceptors varies enormously among animals. For example, some simple eyes such as those of flatworms have few photoreceptors and are capable of determining only the approximate direction of a light source. In contrast, the human eye has 100 million photoreceptors and can resolve one minute of arc (one-sixtieth of a degree), which is about 4,000 times better than the resolution achieved by the flatworm eye.

Mechanoreception, ability of an animal to detect and respond to certain kinds of stimuli—notably touch, sound, and changes in pressure or posture—in its environment. Sensitivity to mechanical stimuli is a common endowment among animals. In addition to mediating the sense of touch, mechanoreception is the function of a number of specialized sense organs; some found only in particular groups of animals. Thus, some mechanoreceptors act to inform the animal of changes in bodily posture, others help

detect painful stimuli, and still others serve the sense of hearing.

Slight deformation of any mechanoreceptive nerve cell ending results in electrical changes, called receptor or generator potentials, at the outer surface of the cell, and this in turn induces the appearance of impulses ("spikes") in the associated nerve fibre. Various laboratory devices are used to record and observe these electrical events in the study of mechanoreceptors. In addition to electrophysiological studies, mechanoreceptive functions are also investigated more indirectly—i.e., on the basis of behavioral responses to mechanical stimuli. These responses include bodily movements (e.g., locomotion), changes in respiration or heartbeat, glandular activity, skin colour changes, and (in the case of humans) verbal reports of mechanoreceptive sensations. The behavioral method sometimes is combined with partial or total surgical elimination of the sense organs involved. Not all the electrophysiologically effective mechanical stimuli evoke a behavioral response; the central nervous system (brain and spinal cord) acts to screen or to select nerve impulses from receptor neurons.

Humans experience pain as a result of stimulation of pain receptors (nociceptors), which are located in the skin and other tissues. Pain receptors respond to three different types of harmful (noxious, or nociceptive) stimuli: mechanical, thermal, and chemical. The pain sensation may be acute, involving a short-lived intense feeling of pain that subsides to dull throbbing, or chronic, involving long-lasting pain that often is associated with disease. The stimulation of pain receptors is characterized by a range of physiological and psychological responses, including an effort to withdraw from the stimulus. The reflex withdrawal of the hand from a flame, for example, may begin even before the person becomes conscious of the pain sensation.

Aversive responses to noxious stimuli nevertheless have a major adaptive role in avoiding bodily injury. Without them, the animal may even become a predator against itself; bats and rats, for instance, chew on their own feet when their limbs are made insensitive by nerve cutting. Some insects normally show no signs of painful experience. A dragonfly, for example, may eat much of its own abdomen if its tail end is brought into the mouthparts. Removal of part of the abdomen of a honeybee does not stop the animal's feeding. If the head of a blow fly (*Phormia*) is cut off, it nevertheless stretches its tubular feeding organ (proboscis) and begins to suck if its chemoreceptors (labellae) are brought in touch with a sugar solution; the ingested solution simply flows out at the severed neck.

At any rate, responsiveness to mechanical deformation is a basic property of living matter; even a one-celled organism such as an *Amoeba* shows withdrawal responses to touch. The evolutionary course of mechanoreception in the development of such complex functions as gravity detection and sound-wave reception leaves much room for speculation and scholarly disagreement.

Sensory organelles and reaction for stimuli in protozoa

• Chemoreception in different organisms

○ Single-celled organisms

Many microorganisms are known to remain in favourable chemical environments and to disperse away from unfavourable environments. This implies that microorganisms have a chemical sense, but, because they are so small, they are unable to detect chemical gradients by simultaneous comparison of the chemical concentration at two parts of the body. Instead, microorganisms exhibit differential responses to temporal differences in concentration, implying that they have the capacity to "remember" whether the concentration previously experienced was higher or lower than the current concentration. Movement in these organisms consists of periods of movement in a straight line interrupted at intervals by a turn, or "tumble." The organisms swim smoothly up the concentration gradient of an attractant and begin to accumulate in areas of high concentration of the attractant. Accumulation is reinforced by the organisms' own secretion of attractant chemicals. Organisms that leave the aggregation tumble and the direction of the turn and of the new path relative to the original appear to be random. The rate of tumbling varies, with organisms tumbling most in the absence of attractants and in the presence of repellents. Organisms that tumble away from an aggregation typically swim in a straight line back to the attractant. The bacterium *Escherichia coli* accumulate in high concentrations of sugars and some amino acids. This is also true of the ciliate protozoan *Paramecium*, which accumulates in areas with high concentrations of folate or biotin—compounds that are released by bacteria, the food of these animals. However, *Paramecium* disperses when it encounters quinine or potassium hydroxide.

As in multicellular organisms, perception of chemicals often involves the possession of receptor proteins in the cell membrane that activate second-messenger systems within the cell. However, unlike with multicellular organisms, the second messengers of single-celled organisms cause changes in the effector mechanisms of the cell, such as the flagellum or cilium, that modify the cell's movement. This causes the organism to move appropriately, relative to the stimulus. The receptor proteins of the yeast *Saccharomyces* and the slime mold *Dictyostelium* both have seven transmembrane domains, similar to the olfactory receptors of higher organisms, although belonging to different gene families. However, in the bacterium *E. coli* the receptor proteins have only two transmembrane domains, perhaps reflecting the fact that bacteria, as prokaryotes (lacking distinct nuclei), predate the evolution of eukaryotes (having membrane-bound nuclei).

The number of different types of receptor proteins is limited in single-celled organisms compared with multicellular organisms. This appears to be the result of limited space

available on the surface of a single cell. In *E. coli* there are five types of receptor proteins involved in positive responses. One receptor responds to serine, an amino acid (this receptor is also sensitive to temperature and pH); a second receptor responds to aspartate and ribose, an amino acid and a sugar, respectively; a third receptor responds to galactose and maltose, both sugars; a fourth receptor responds to dipeptides; and a fifth receptor responds to oxygen and changes in reduction-oxidation potential in the cell. Metallic ions, organic acids, inorganic acids, and glycerol produce negative responses, but it is not clear whether these molecules act via receptors or via an alternative mechanism. *Paramecium* has membrane receptor proteins that respond to favourable compounds such as biotin and to aversive compounds such as quinine. Several hundred of each receptor type is present on the cell surface, and they may be differentially distributed; for example, *Paramecium* has more quinine receptors at its front end than at its back end. In *E. coli* a difference in concentration producing a change in the occupancy of only a single receptor site is sufficient to produce a change in behaviour.

In addition to receptor-mediated responses, environmental chemicals may act on intracellular processes by entering the cell. In bacteria, for example, sugars and some other compounds act intracellularly, and, in *Paramecium*, ammonium ions enter the cell as ammonia, changing the pH of the cytoplasm and affecting the membrane potential. Inside the cell these effects are integrated with effects produced via cell membrane receptors. Therefore, the overall effect in *Paramecium* is to change the cell membrane potential, with favourable stimuli causing slight hyperpolarization (the potential difference across the cell membrane is increased), which increases the frequency of ciliary beating and reduces the frequency with which the organism makes turns, and aversive substances producing slight depolarization (a reduction in the potential difference across the cell membrane). In flagellates, changes in flagellar movement do not depend on general membrane effects. In species with a single flagellum, changes in direction are induced by reversals in the direction of flagellar rotation from counterclockwise to clockwise. The several flagella of *E. coli* normally rotate counterclockwise, and, when the flagella all have the same rotation, they form a bundle that drives the organism in a straight path. However, when one or more flagella rotate in the opposite direction, the unity of the bundle is destroyed, and the bacterium tumbles.

Sperm of all animals are faced with the problem of locating an egg, whether the eggs are free in the environment, such as those released from sea urchins and toads, or are contained within the female ducts, such as the eggs of humans. In toads and humans, sperm have been shown to make directed movements toward eggs, and there is evidence that they move up the concentration gradient of a small protein released by the egg. In sea urchin sperm, comparable small proteins are detected by receptors in the cell membrane, and this is probably true of all species.

- **Specialized chemosensory structures**

Many invertebrates have chemoreceptor cells contained in discrete structures called sensilla that are located on the outside of the body. Each sensillum consists of one or a small number of receptor cells together with accessory cells derived from the epidermis. These accessory cells produce a fluid (analogous to vertebrate mucus) that protects the nerve endings from desiccation and provides the constant ionic environment necessary for nerve cells to function properly. In some animals the sensillum and accessory cells form a physical structure around the receptor cells. Chemicals in the environment reach the receptor cells through one or more pores in this protective covering. In some invertebrates sensilla are found all over the body, including on the legs, cerci, and wing margins. In polychaetes the sensilla are often borne on tentacles.

The number of chemoreceptor cells in nematodes is very limited. *Caenorhabditis elegans*, a small soil-inhabiting species, has only 34 chemosensory cells arranged in eight sensilla near the head. This organism also has four sensory cells in the tail, although it is not known whether these cells function as chemoreceptors.

• Photoreception

Eyespot, also called **stigma,** a heavily pigmented region in certain one-celled organisms that apparently functions in light reception. The term is also applied to certain light-sensitive cells in the epidermis (skin) of some invertebrate animals (e.g., worms, starfishes).

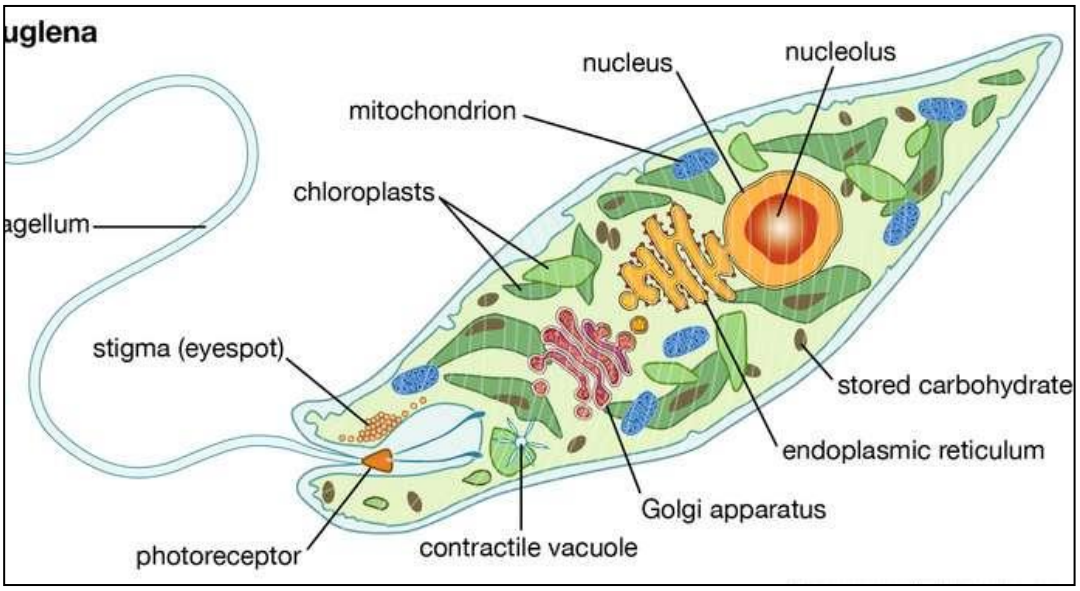


Fig: Position of stigma (eyespot) in *Euglena* sp.

In the green one-celled organism *Euglena,* the eyespot is located in the gullet, at the base of the flagellum (a whip like locomotory structure). A cup-shaped mass of pigment rods shields a sensitive area of the flagellar base from light coming from the direction of the

opposite end of the organism. The light-sensitive region apparently influences flagellar motion in such a manner that the organism moves toward light.

A stigma occurs in many green flagellates and a few of their colorless relatives. Observations on behavior have suggested that the stigma functions in orientation with relation to a light source. Also, stigmaless mutants (*Chlamydomonas*) respond to light more erratically than the green parent stock. Whether the stigma is a photoreceptor or merely a shield for a photosensitive organelle is debatable.

A typical stigma shows globules containing carotenoid pigment. The organelle lies on the wall of the reservoir, as in Euglena, or may be adherent to or lie within a chromatophore, as in the phytomonads. The pigment globules (100 nm or larger) are packed into one or more plates forming the stigma. In experimentally bleached Euglena, the fate of the stigma is not closely correlated with that of the chromatophores. A more complex ocellus of certain dinoflagellates shows a lenslike body in a pigmented cup.

- **Microscopic structure:**

Under the light microscope, eyespots appear as dark, orange-reddish spots or *stigmata*. They get their color from carotenoid pigments contained in bodies called pigment granules. The photoreceptors are found in the plasma membrane overlaying the pigmented bodies.

The eyespot apparatus of *Euglena* comprises the paraflagellar body connecting the eyespot to the flagellum. In electron microscopy, the eyespot apparatus appears as a highly ordered lamellar structure formed by membranous rods in a helical arrangement.

In *Chlamydomonas*, the eyespot is part of the chloroplast and takes on the appearance of a membranous sandwich structure. It is assembled from chloroplast membranes (outer, inner, and thylakoid membranes) and carotenoid-filled granules overlaid by plasma membrane. The stacks of granules act as a quarter-wave plate, reflecting incoming photons back to the overlying photoreceptors, while shielding the photoreceptors from light coming from other directions. It disassembles during cell division and reforms in the daughter cells in an asymmetric fashion in relation to the cytoskeleton. This asymmetric positioning of the eyespot in the cell is essential for proper phototaxis.

Eye spot proteins:

The most critical eyespot proteins are the photoreceptor proteins that sense light. The photoreceptors found in unicellular organisms fall into two main groups: flavoproteins and retinylidene proteins (rhodopsins). Flavoproteins are characterized by containing flavin molecules as chromophores, whereas retinylidene proteins contain retinal. The photoreceptor protein in *Euglena* is likely a flavoprotein. In contrast, *Chlamydomonas* phototaxis is mediated by archaeal-type rhodopsins.

Besides photoreceptor proteins, eyespots contain a large number of structural, metabolic and signaling proteins. The eyespot proteome of *Chlamydomonas* cells consists of roughly 200 different proteins.

• Mechanoreception

Gravity is most suitable in providing animals with cues to their position in space because of the constancy of its magnitude and direction,. The sense organs involved (statoreceptors) usually have the structure of a **statocyst**, a fluid-filled vesicle containing one or more sandy or stonelike elements (statoliths). The statocyst is a balance sensory receptor present in some aquatic invertebrates, including bivalves, cnidarians, ctenophorans, echinoderms, cephalopods, and crustaceans. A similar structure is also found in Xenoturbella.

Sensory cells in the wall of the vesicle have hairs that are in contact with the statolith, which always weighs vertically down. Hence, depending on the animal's position, different sense cells will be stimulated in statocysts with loose statoliths, or the same sense cells will be stimulated in different ways in statocysts with a statolith loosely fixed to the sense hairs.

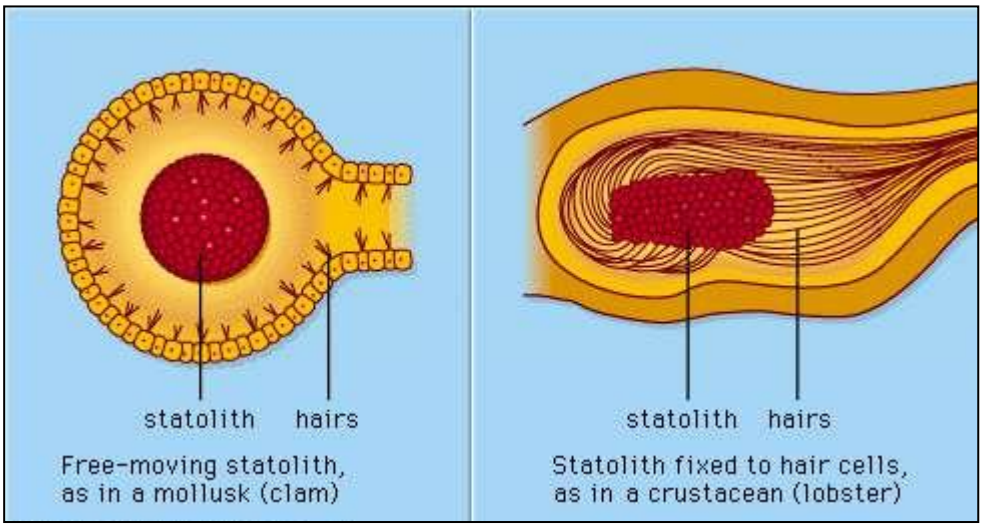


Fig: Structure of statocyst

Statocysts are found in representatives of all of the major groups of invertebrates: jellyfish, sandworms, higher crustaceans, some sea cucumbers, free-swimming tunicate larvae, and all the mollusks studied thus far. Analogous receptors that occur generally in vertebrates are the ear's utriculus and probably (to a degree) also two other otolith organs (sacculus and lagena) of the ear (labyrinth). Statocysts (including vertebrate labyrinthine statoreceptors) develop embryologically from local invaginations of the body surface. In primitive evolutionary forms, the interior of the statocyst is in open communication with the surrounding sea and thus is filled with water; statoliths usually are sand particles taken up from outside. In a few animal groups, this developmental stage is only found during the larval phase, the initial

opening to the exterior being closed in the adult animal. In more advanced forms, the liquid content (statolymph) and the statoliths are produced by cells in the wall of the organ. This specialized type of closed statocyst is found in many snails, in all the cephalopods such as the squid (except *Nautilus*), and in the vast majority of vertebrates (from bony fishes up to and including mammals).

Statocyst function may be studied by observing compensatory reflexes under experimental conditions. When the position of a laboratory animal is appropriately changed, movements of such body parts as the eyes, head, and limbs can be observed. Such movements tend to counteract the imposed change and to restore or to maintain the original position. Evidence of statoreceptor function is provided if these reflexes are abolished after surgical elimination of both statocysts. Many animals exhibit locomotion that is gravitationally directed vertically down or up (positive or negative geotaxis, respectively). Geotactic behaviour may be experimentally altered by whirling the animal in a centrifuge to change the direction and to increase the intensity of the force exerted on the sensory hairs by the statoliths. Molting crustaceans shed the contents of their statocysts along with their exoskeleton. If such an animal is placed in clean water containing iron filings, it takes up new iron statoliths instead of the usual sand grains. By moving a magnet to vary the direction of the force exerted by the metal statoliths, the animal can be made to adopt any resting position, even to stay upside down. Statoliths can be washed out of the open statocysts of a shrimp without damaging the sensory hairs. When the hairs are pushed in different directions with a fine water jet, the shrimp exhibits compensatory reflexes. In this way, it has been shown that each statocyst signals a change of position around the animal's long axis; the same reaction is found to occur after removal of the statocyst on one side only. Electrical impulses in the statocyst nerve can be recorded while the animal is in different spatial positions, or during experimental deflection of the sensory hairs. Such experiments reveal that both vertebrates and decapod crustaceans (e.g., shrimp) exhibit spontaneous and statolith-induced neural activity in the lining (epithelium) of the gravity receptor.

Spontaneous activity

The sensory epithelium of a statocyst is spontaneously active, initiating a continuing series of impulses directed toward the central nervous system (even when the statoliths are experimentally removed from the statocyst). This resting frequency of neural activity is fairly constant and completely independent of the animal's position in space. In vertebrates and in crustaceans, spontaneous activity of the left statocyst affects the central nervous system to produce a tendency of the animal to roll to the right about its long axis; spontaneous activity of the right statocyst prompts a tendency to roll to the left. Normally, these rolling tendencies neutralize each other in the central nervous system, not becoming manifest unless the statocyst on one side of the body is functionally eliminated by complete surgical removal, by destruction of its sensory epithelium, or by cutting its nerve. This intervention permits the influence of the spontaneous activity generated in the remaining statocyst to be felt, and the animal tends to roll toward the operated side. Unilateral (one-sided) removal of the statoliths

alone, however, does not produce such an effect so long as the sensory cells in the epithelium remain intact. The rolling tendency of a unilaterally operated animal usually diminishes little by little in the course of hours or days, until it finally disappears completely. If the remaining statocyst is then removed, rolling occurs again, but this time to the other (last operated) side. This tendency also diminishes and disappears with time. Apparently the unbalancing effect of the spontaneous influx from a statocyst is gradually counteracted in some unknown way by the central nervous system.

Statolith influences

Vertebrates and crustaceans have statoliths that are loosely connected to the sensory hairs by a sticky substance. With such a mechanical arrangement, the statolith stimulates the sensory cells by parallel (shearing) motion rather than by pressure or pull at right angles to the epithelium. The effects are demonstrable in experiments with fish, based on the dorsal-light orientation noted above. In a laboratory darkroom, if light shines at a fish from one side, the animal assumes an oblique position. While the fish tends to turn on its side (with its back side to the light), gravity tends to keep it vertical; the oblique position is the result. In a whirling centrifuge, the pressure exerted by the statoliths may be increased. When this is done, the fish rights itself almost precisely to the degree that the shearing force exerted by the statoliths is held constant.

Among the invertebrates, most statocyst research has been done with such decapod crustaceans as lobsters. The working mechanism of their statocysts conforms with the physiological principles of vertebrate statoreception discussed above. The results of electrophysiological investigations support the conclusions drawn from behavioral observations. In some crustacean statocysts (for example, in the lobster, *Homarus*), special statoreceptors are found that signal the same bodily position differently, depending on the direction of movement through which it was reached (hysteresis effect). The part played by the statocyst in equilibration has been investigated in several other invertebrate groups, among them jellyfish, sandworms, and such mollusks as scallops, common snails, sea hare, and octopus. Each sensory cell from the vertical macula in a statocyst of the octopus bears up to 200 kinocilia, and all the cilia of each cell are polarized in the same direction. On the macula as a whole, there is a radiating polarization pattern, the activating direction pointing everywhere from the centre to the margin. Compensatory eye reflexes resulting from tilting the animal head down or head up around a transverse axis reveal a hysteresis effect. After unilateral-statocyst removal, mollusks do not tend to roll toward the operated side (as do vertebrates and crustaceans) but toward the side of the remaining statocyst.

The almost complete absence of statocysts in insects is remarkable in view of evidence that many of them have a high degree of sensitivity to the direction of gravity. Receptors involved are specialized tufts of tactile hairs at the external body surface; in the honeybee, such groups of hairs are notably found between head and thorax and between thorax and abdomen. The adaptive function of these static (gravity) receptors becomes manifest in the honeybee “dance language” performed on a vertical comb in

the hive. The angle between the dancing bee and the perpendicular seems to direct other bees to sources of nectar and pollen.

Probable Questions:

1. Define chemoreception. Write notes on role of chemoreceptor.
2. Define photoreception. Write notes on role of photoreceptor.
3. Write a short note on statocyst.
4. What is the function of statocyst?
5. Write the function of stigma.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
6. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 193

UNIT VI

Organs and process of Reproduction in major invertebrate groups

Objective: In this unit we will learn about Organs and process of Reproduction in major invertebrate groups

Reproduction is the process by which plants and animals give rise to offspring and which fundamentally consists of the segregation of a portion of the parental body by a sexual or an asexual process and its subsequent growth and differentiation into a new individual.

1. Protozoa

A. ASEXUAL REPRODUCTION:

The mode of reproduction in which there is no union of gametes. In such a case, only one animal can produce new individuals. Protozoa usually reproduces asexually by binary fission and multiple fission.

I. Binary Fission:

The animal divides and two individuals are produced from one:

The micronucleus divides into two by a simplified form of mitosis.

2. The macronucleus divides into two by amitosis.

3. The cytoplasm divides into two equal halves by a constriction.

4. The daughter individuals can reconstruct the wanting structures which it does not obtain from the parent. Asymmetrical structures like gullet, peristome of Paramoecim cannot be equally shared by both the daughter individuals.

Binary fission is again of three types:

a. Transverse fission. The animal divides transversely into two. Examples: Amoeba, Paramoecium, etc.

b. Longitudinal fission. The animal splits into two along the long axis of the body. Examples: Euglena, Vorticella, etc.

c. Oblique binary fission. The plane of fission is oblique. Examples: Dinoflagellata, Ceratium, Cochliodinium,. etc.

II. *Multiple Fission or Sporulation:*

Many individuals are produced from one at a time. Examples: Some Amoebae, Euglena, Polystomella, etc.

1. The animal becomes encysted, the nucleus divides repeatedly and a large number of minute daughter nuclei are produced.

2. The cytoplasm fragments and a small bit of it surrounds each daughter nucleus and, thus, many minute animals are formed.

3. Under favourable circumstances the cyst bursts and these small animals come out and grow to the adult stage

SEXUAL REPRODUCTION:

Sexual reproduction is one when it takes place by the union of two entire individuals or it involves merely the nuclear exchange and their subsequent fusion.

In Protozoa the sexual reproduction occurs by the following processes:

A. Syngamy or Copulation: Syngamy is the complete fusion of two sex cells or gametes, resulting in the formation of zygote.

Depending upon the degree of differentiation displayed, by the fusing gametes, syngamy is of the following types:

(a) Hologamy: The two ordinary mature protozoan individuals do not form gametes but themselves behave as gametes and fuse together to form zygote. It occurs in a few Sarcodina and Mastigophora.

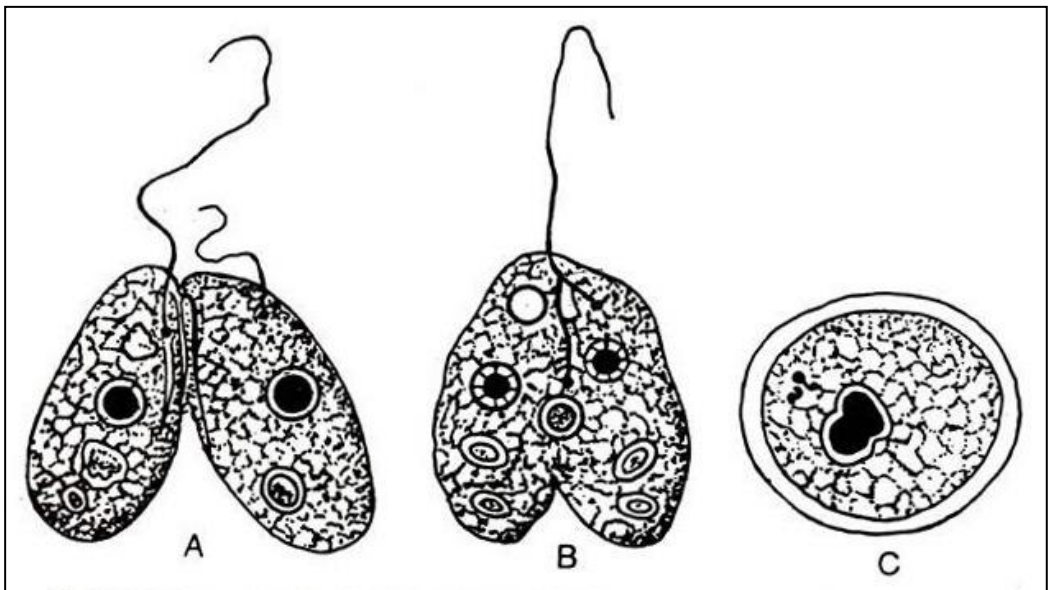


Fig. 18.16. Hologamy in a flagellate capromonas sp. A-B. Fusion of two individuals. C. Encysted zygote

(b) Isogamy: When two fusing gametes are similar in size and shape and cannot be morphologically distinguished from each other though there exist physiological differences, they are called isogametes and their union, isogamy. The isogametes are generally produced by multiple fission. Isogamy is common in Forminifera, Gregarines and Phytomonadina like Copromonas.

(c) Paedogamy: When the fusing pronuclei are present in two different cells derived from a single parent cell, the process is called paedogamy. The process has been observed in Actinophrys sold by Blar (1922) and in some Myxosporidia.

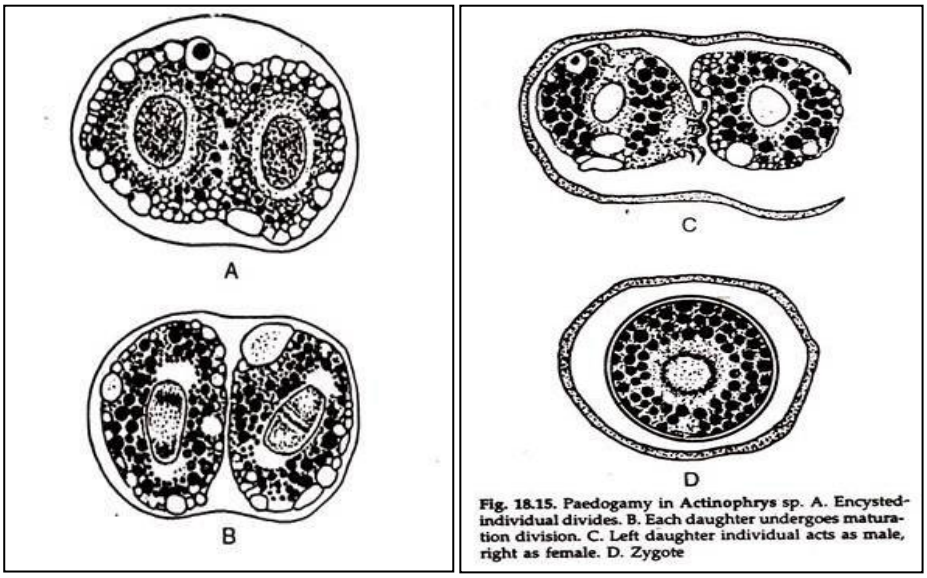


Fig. 18.15. Paedogamy in Actinophrys sp. A. Encysted-individual divides. B. Each daughter undergoes maturation division. C. Left daughter individual acts as male, right as female. D. Zygote

(d) Anisogamy: It is fusion of dissimilar gametes. The copulating sex units are dissimilar in size, form and behaviour. The large and non-motile unit is called female or macrogamete and the small mobile one is termed male or microgamete in such fusion. They widely occur in Phytomonadina and Sporozoa, e.g.,*Plasmodium*.

(e) Oogamy: In this case the gametes are quite dissimilar. The female gamete is non- motile egg and the male is a flagellate and motile sperm. It is found in *Volvax*.

B. *Conjugation:*

The conjugation is the temporary union of two mating types of individuals of the same species to facilitate exchange of nuclear materials. They retain their distinct individuality and separate out after nuclear exchange. The pairing gametes are known as conjugants. The conjugants may be either isogamous (*Paramecium*) or anisogamous (*Vorticella*).

Conjugation is considered to be an episode in reproduction and not a mode of multiplication. In conjugation (i) reorganization of a fresh meganucleus occurs to accelerate the metabolic activities, (ii) rejuvenation and revival of lost vigour, (iii) new nuclear combinations and new hereditary combinations arise.

C. Other modes of reproduction:

1. Plasmogamy:

Two or more individuals may fuse by their cytoplasm to form a plasmodium and separate out unchanged with their distinct nuclei. This sexual phenomenon is known as Plasmogamy and occurs in certain Rhizopoda and Mycetozoa.

2. Regeneration:

The regeneration and replacement of lost parts among free-living and few parasitic protists is widespread. A proper proportion of cytoplasm and nucleus can regenerate into an entire individual.

3. Parthenogenesis:

The gametes which fail to fertilize start their development parthenogenetically. Examples: Actinophrys, Chlamydomonas, etc.

2. Porifera:

Sponges reproduce both asexually and sexually and they also possess the power of regeneration due which it is almost impossible to kill a sponge. Their sexual reproduction is similar to higher animals even though their body organization is primitive type.

A. ASEXUAL REPRODUCTION

I. Regeneration

All sponges possess a remarkable ability to regenerate lost parts. A piece cut from the body of a sponge is capable of growing into a complete sponge. If a sponge is cut into small pieces and squeezed through a fine silken mesh to separate cells, the separated amoebocytes will reunite and in a few days will develop canals, flagellated chambers and skeleton and grow up into a new sponge. This power of regeneration helps the sponges to repair the damage caused in the harsh environment.

II. Budding & Branching

In budding, numerous archaeocytes gather near the surface resulting in a small outgrowth on the pinacoderm. The bud thus formed grows outward to produce a small individual, which either remains attached with the parent individual or gets detached and attached to a nearby rock to grow into an independent colony. Stolon of the sponge grows by branching and secondary branching and many small vertical buds grow out of it.

III.Fission & Fragmentation

In some sponges multiplication takes place by developing a line of fission and throwing off parts of the body which later can develop into a new sponge. Sponges can break into several pieces along several lines of weakness and breaking into

fragments that are capable to tide over unfavourable environmental conditions and grow into complete sponges in the following favourable season.

IV. Reduction bodies

Many fresh water and marine sponges disintegrate in adverse environmental conditions particularly in winter, leaving small rounded balls called reduction bodies. Each body consists of an internal mass of amoebocytes, covered externally by a pinacoderm and spicules. When favourable conditions return, these reduction bodies grow into complete new sponges.

V. Gemmules

Fresh water sponges such as *Spongilla* as well as some marine forms such as *Ficulina, Suberites,* and *Tethya* possess the remarkable ability to produce specialised bodies called *gemmules*, which survive during unfavourable conditions and germinate to produce new sponges. For gemmule formation, archaeocytes laden with food material in the form of glycoprotein or lipoprotein get aggregated into a mass. Amoebocytes surround the central mass of archaeocytes and secrete a thick hard chitinous inner layer and an outer membranous layer over it. Scleroblasts secrete amphidisc spicules between the inner and outer membranes. A fully formed gemmule is a small hard ball having a mass of food laden archaeocytes enclosed in a double layered tough envelope with amphidisc spicules in between. There is a small opening the micropyle through which the cells come out during development in favourable conditions.

In autumn fresh water sponges die and disintegrate, leaving behind a large number of gemmules, which remain viable throughout the winter. Same thing happens during summer when water available is low. In favourable conditions with abundance of water the gemmules begin to hatch and their living contents escape through micropyles and develop into new sponges by collecting themselves together.

B. SEXUAL REPRODUCTION

Sexual reproduction involves formation of sperms and ova. The sex cells arise either from archaeocytes or choanocytes. Although most sponges are hermaphrodite but cross-fertilization is the rule because eggs and sperms are produced at different times.

Oocytes are produced inside the body and remain inside mesogloea waiting for fertilization. In sperm formation, archaeocyte and trophocyte cells are involved and in demospongiae choanocytes form sperms and leave the body of sponge through osculum in large numbers.

Sperms from water enter the body of another sponge through canal system and reach the flagellate chambers, where choanocytes trap them. Acting as nurse cells, choanocytes transport the sperm body without tail to the mature ova that wait in the

mesogloea. The sperm nucleus then fuses with the nucleus of ovum, ensuring internal fertilization.

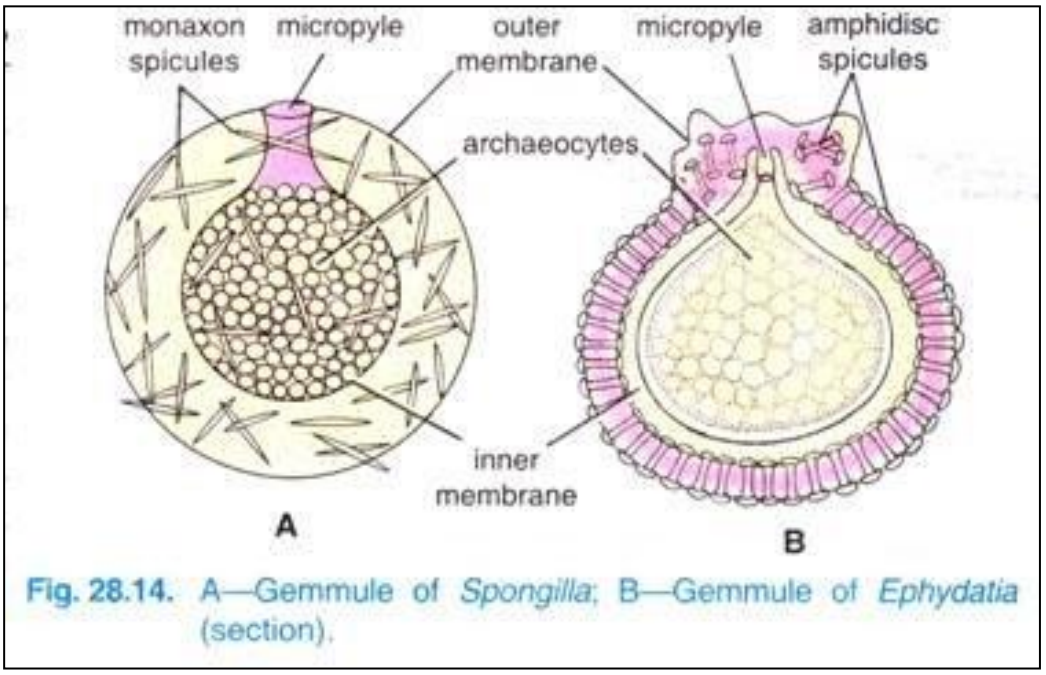


Fig. 28.14. A—Gemmule of *Spongilla*; B—Gemmule of *Ephydatia* (section).

3. Cnidaria

Reproduction is both by asexual (budding) and sexual methods. Both gonads and buds arise from the interstitial cells. The power of regeneration is also developed.

Metagenesis:

In Obelia, polyps reproduce medusae asexually and medusae form the polyps sexually. Such alternation of asexual and sexual phases in the life cycle of Obelia is called metagenesis. It should not be confused with alternation of generations as found in plants where one phase is haploid and other is diploid. Here both phases are diploid.

4. Platyhelminthes

With very few exceptions, platyhelminthes are hermaphroditic. Male and female reproductive organs in each individual are separate and open externally by their own pores or by a common genital aperture. In some cases the gonoducts open into the digestive tract and the sex cells are liberated through the mouth. Their reproductive systems are generally complex. Numerous testes but only one or two ovaries are usually present in these flatworms.

Male reproductive organs consist of testes which in primitive condition are numerous and scattered. The numbers of testes are reduced to one or two in many and in Acoela definite gonads and ducts are lacking. The vasa efferentia, when present, correspond to

the number of testes present. In general, there is a pair of vasa deferentia which unite and open into the complicated copulatory apparatus.

The copulatory apparatus consists of an eversible cirrus or a protrusible penis armed with spines or hook-lets. Various glands are associated with it. Vesicle in single or paired condition is often present and acts as the reservoir for storing sperms.

The female reproductive system is unusual in that it is separated into two structures: the ovaries and the vitellaria, often known as the vitelline glands or yolk glands. The cells of the vitellaria form yolk and eggshell components. In some groups, particularly those that live primarily in water or have an aqueous phase in the life cycle, the eggshell consists of a hardened protein known as sclerotin, or tanned protein. Most of this protein comes from the vitellaria. In other groups, especially those that are primarily terrestrial or have a terrestrial phase in their life cycle, the eggshells are composed of another protein, keratin, a tougher material that is more resistant to adverse environmental conditions.

In the tapeworms, the tapelike body is generally divided into a series of segments, or proglottids, each of which develops a complete set of male and female genitalia. A rather complex copulatory apparatus consists of an evertible (capable of turning outward) penis, or cirrus, in the male and a canal, or vagina, in the female. Near its opening the female canal may differentiate into a variety of tubular organs. Fertilized eggs are often stored in a saclike uterus, which may become greatly distended; in tapeworms, it may fill a whole segment.

Each male and female reproductive system may have its own external opening, or gonopore, or the terminal regions of each system may join to form a common genital atrium, or passage, and a genital pore.

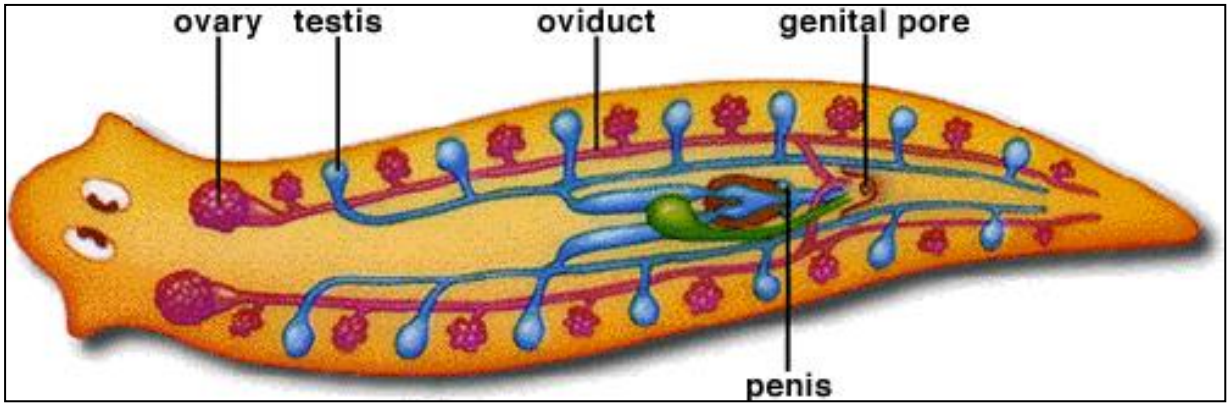


Fig: Reproductive structure of *Planaria*

Either cross-fertilization (i.e., involving two individuals) or self-fertilization may occur; self-fertilization is probably more common. Some free-living flatworms perform a type of copulation known as hypodermic impregnation, whereby the penis of

one animal pierces the epidermis of another and injects sperm into the tissues. Some forms reproduce asexually through budding.

5. Annelida

A. Asexual Reproduction:

Amongst the annelids asexual reproduction is encountered in many polychaetes. In forms, like Filigrana and Salmacina, transverse fission occurs near the posterior end of the body dividing the animals into two unequal parts. The anterior part of the half regenerates a new pygidium and the posterior half regenerates new cephalic regions.

The sexes of the two individuals produced by such fission are always identical. That means metagenesis or alternation of generation is lacking. In some forms, like Syllis hyalina, a constriction occurs somewhere in the middle of the body. The constriction deepens and ultimately two individuals are formed.

The anterior half with the original head behaves like a non-sexual part and regenerates anal region. While the posterior half develops a new head and becomes an independent male or female individual. That means metagenesis or alternation of generation is eminent in this case because sexual worms are being formed out of non-sexual worms.

In Autolytus and Myrianida a zone of proliferation exists between the anterior non-sexual part and posterior sexual part. This zone gives rise to a series of zooids which remain arranged in a linear fashion. The posterior-most zooid in the chain is oldest and most developed.

The sexes of the individuals in the chain are always identical. Syllis ramosa lives a sedentary life inside the canal system of some deep sea sponges. In this type some of the parapodia become transformed into buds which grow laterally and form a colony.

Some branches from the bud develop parapodia, head and gonad and ultimately leave the parent body to form sexual individuals. In Trypanosyllis buds come out from the undersurface of last two segments.

Asexual reproduction is not very common in oligochaetes and Hirudinea.

B. Sexual Reproduction:

Most polychaetes are dioecious, but sexual dimorphism is seldom encountered. Well-formed gonads occur only in few polychaetes. The reproductive cells mature in clumps on the walls of the coelom and it is believed that they arise from specially determined cells and not from coelomic epithelium.

These clumps form gonads during reproductive season. Gonads may occur in most segments or they may remain limited in some posterior segments. On maturity, the peritonium covering the gonads bursts and sperms or ova, as the case may be, are liberated into the coelomic fluid. Finally the sperms or ova come to the outside of the body through body wall or through ducts.

Often nephridial ducts become transformed temporarily into genital ducts to liberate the reproductive cells outside. In Phyllodoce, genital funnels appear and get connection with the nephridia only when the gonads become mature. Fertilization is external but in some forms copulation may occur as the females of these species are provided with receptacles.

All oligochaetes are hermaphrodite and have well-defined gonads which remain limited to few segments only. Testes are one to four pairs and lie in adjacent segments.

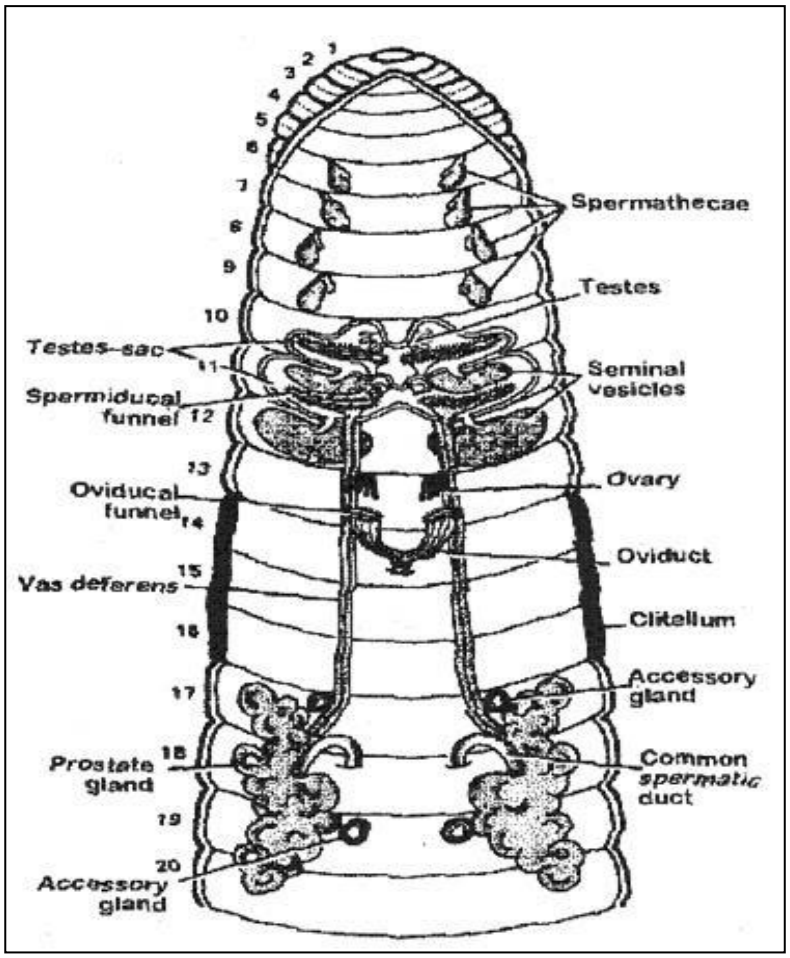


Fig: Reproductive structure of Earthworm

The testes remain encased in special sacs, called seminal vesicles. Male- reproductive cells are detached early from the testes and they are nourished in these vesicles. The sperm ducts bear funnels at their tips and traverse several segments posteriorly, before opening to the outside through the gonopore.

Ovaries are strictly one pair and are located posterior to the testes bearing segments. Each ovary is followed by an oviduct and the two oviducts unite before opening to the outside through gonopore. Though hermaphrodite, the oligochaetes practise cross-fertilization. Reciprocal copulation enables the worm to pass on sperms of one worm to the other. The sperms thus received remain stored in spermatheca.

Hirudinea, like the oligochaetes, are hermaphrodite. Female reproductive organs consist of several rows of reproductive cells encased in a pair of ovarian tubes. The ovarian tubes are short in Gnathobdella, long in Rhyncobdella and looped in Pharyngobdella. The anterior end of the tube is prolonged as oviduct. The two oviducts often fuse to form a vagina which opens to the outside in the 11th segment.

Male reproductive system consists of 4- 17 pairs of metamerically arranged testis sacs. There is a pair of longitudinal ducts, called vas deferens. The vas deferens widens at the tip to form the seminal vesicle. Each seminal vesicle opens into a median atrium. The atrium opens to the outside through the 11th segment. The atrium often remains provided with an intermittent organ, called penis.

6. Arthropoda

Arthropods reproduce mainly by sexual reproduction. However, some arthropods are hermaphroditic, *meaning they have the organs of both sexes, such as in barnacles*. Few crustaceans and insects can reproduce by means of parthenogenesis. They revert to reproducing sexually when conditions become more favorable again.

Arthropods that live in aquatic habitats often reproduce sexually typically by means of external fertilization. Some Crustaceans and Opiliones reproduce sexually by using their specialized appendages that serve as penises or *gonopods*. All terrestrial arthropods reproduce by *internal fertilization*. They do so usually by males producing packets of sperms called *spermatophores*, which the females take into their bodies.

In arthropods, sperm are commonly transferred to the female within sealed packets known as spermatophores. In this method of transfer the sperm are not diluted by the surrounding medium, in the case of aquatic forms, nor do they suffer from rapid desiccation on land. Among some arachnids, such as scorpions, pseudoscorpions, and some mites, the stalked spermatophore is deposited on the ground. Either the female is attracted to the spermatophore chemically or the deposition of the spermatophore occurs during the course of a nuptial dance, and the male afterward maneuvers the female into a position in which she can take up the spermatophore within her genital opening. **Centipedes** also utilize spermatophores with accompanying courtship behaviour. Among insects there are some primitive wingless groups, such as **collembolans** and **thysanurans**, in which the spermatophore is deposited on the ground, but in most insects the spermatophores are placed directly into the female genital opening by the male during copulation. Many other invertebrates, including several gastropods and chaetognaths, also use spermatophores. Many arthropods transfer free sperm rather than spermatophores. These include many crustaceans, millipedes, some insects (such as dipterans and hemipterans), spiders, and some mites.

Arthropod eggs are usually rich in yolk, but in all groups there are species whose eggs have little yolk. Some specialized methods of reproduction found among certain arthropods include the development of unfertilized eggs (parthenogenesis), the birth of

living young (viviparity), and the formation of several embryos from a single fertilized egg (polyembryony).

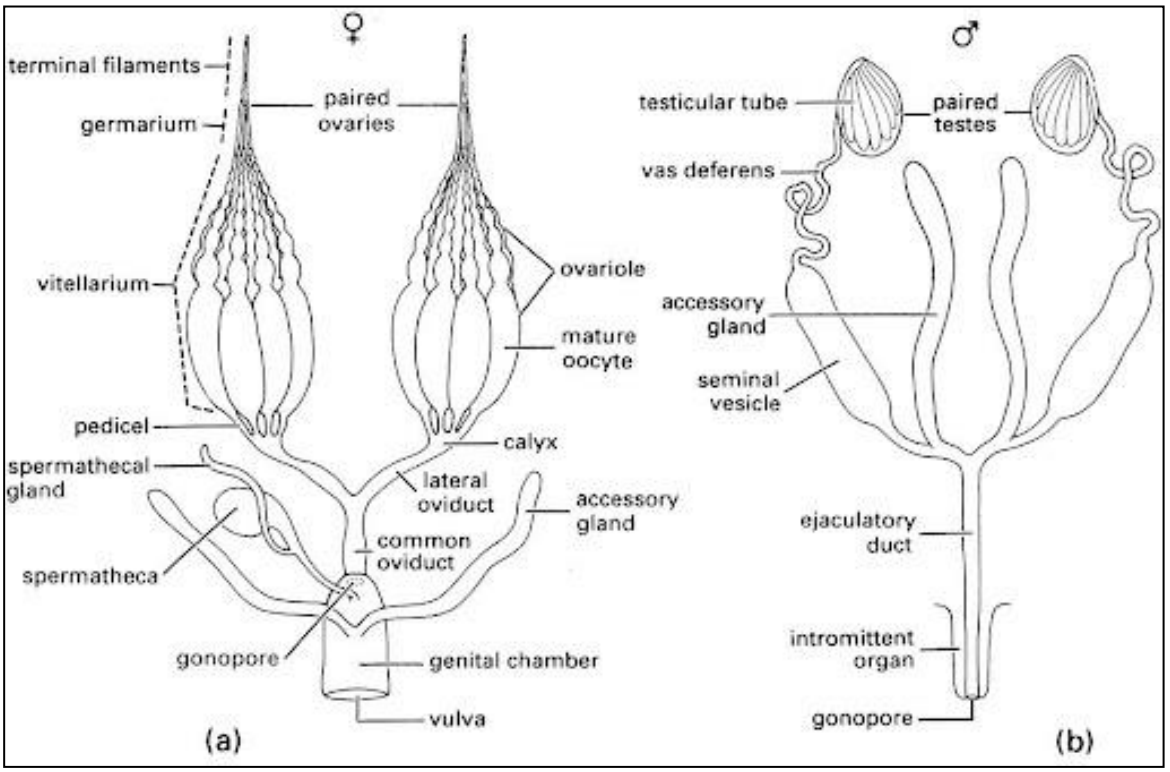


Fig: Reproductive structure in Insect

The eggs of many crustaceans hatch into larvae which have fewer segments than the adult. The earliest larval hatching stage is a minute nauplius larva, which possesses only the first three pairs of appendages. Additional segments and appendages then appear at regular intervals with molting. There are several advantages of larval stages in the development of aquatic animals: Currents disperse the larvae, enabling some to settle in different locations from the parents; because many larvae are capable of feeding, less yolk is required in the egg; and, moreover, planktonic larvae do not compete with benthic adults.

In most **chelicerates** and **insects**, almost all of the segments are present at hatching, although in insects the body form may differ from that of the adult. Primitive insects, such as collembolans, have the adult form on hatching. Many insects, such as grasshoppers, crickets, and true bugs, hatch as nymphs, which superficially resemble the adult but lack wings. They gradually acquire these adult features during the nymphal instars. Other insects, such as beetles, butterflies, moths, flies, and wasps, hatch as larvae (grubs, caterpillars, maggots) that differ markedly from the adult. The larvae inhabit different environments and eat different foods than the adults. In these insects a pupal stage with metamorphosis bridges the gap between the larva and the adult form.

Myriapods have the general body form of the adult on hatching though they may lack some of the segments. Most millipedes hatch with only seven trunk segments. Some centipedes hatch with all of the adult trunk segments, but others have fewer than the adult.

The young of most arachnids are similar to the adult. The female scorpion gives birth to her young, which immediately climb onto her back. Female wolf spiders also carry their young, and prior to hatching they carry the white egg case attached to the posterior spinnerets. Unlike other arachnids, mites and ticks hatch as six-legged larvae, which acquire the fourth pair of legs at a later molt.

7. Mollusca

Mollusks are primarily of separate sexes, and the reproductive organs (gonads) are simple. Reproduction via an unfertilized gamete (parthenogenesis) is also found among gastropods of the subclass Prosobranchia. Most reproduction, however, is by sexual means. Eggs and sperm are released into the water by members of some (primitive) species, and fertilization occurs there. In prosobranch gastropods, water currents may cause a simple internal fertilization within the mantle cavity, or males may fertilize eggs internally using a muscular penis. Both male and female reproductive organs may be present in one individual (hermaphroditism) in some species, and various groups exhibit different adaptations to this body form. For example, in hermaphroditic bivalves and prosobranch gastropods, male and female gonads are functional at separate times and in rhythmic and consecutive patterns (successive hermaphroditism). Conversely, male and female gonads are functional at the same time (simultaneous hermaphroditism) in solenogasters and many other gastropods.

Fertilization by transfer of capsules containing sperm (spermatophores) typically occurs in cephalopods and some gastropods. In cephalopods, transfer of spermatophores is usually combined with copulation by a modified arm, or hectocotylus. Copulation in solenogasters, often by means of a special genital cone, may be supported by copulatory stylets. Various penis formations, in part with copulatory stylets, or darts, are widely found in gastropods.

Eggs are deposited singly or in groups, generally on some hard surface and often within jelly masses or leathery capsules. Squids of the suborder Oegopsida and some gastropods have eggs that are suspended in the water. Fertilized eggs commonly undergo spiral cleavage, as in annelids and a number of other “protostome” phyla. The eggs of cephalopods, on the other hand, possess a large amount of yolk, which displaces the dividing cells and causes a characteristic type of development.

Many mollusks develop into free-swimming larvae; these larvae are either feeding (planktotrophic) or nonfeeding (lecithotrophic). The larva in primitive bivalves is a pericalymma (test cell) larva in which the embryo is protected below a covering (test) of cells provided with one to four girdles of cilia, at the apex of which is a sensory plate of ciliated cells. After the developing juvenile has grown out apically of the test (which

then is lost), the animal settles and develops into an adult. The test in other lecithotrophic larvae is restricted to a preoral girdle of ciliated cells (the prototrochus) and is called the trochophore larva. Trochophores are encountered in the development of many marine annelid species (phylum Annelida). In more advanced mollusks (such as in marine gastropods and bivalves), the trochophore larva develops into a veliger larva. In these generally planktotrophic larvae, the girdle of ciliated cells widens to form a velum that entraps food and also propels the microscopic mollusk through the water. As the larva continues to develop, the shell, mantle cavity, tentacles, and foot appear. After a specific amount of time, which varies according to species and environmental conditions, the larva loses the velum and metamorphoses into an adult. A substantial change in shell morphology usually marks the transition to adult form.

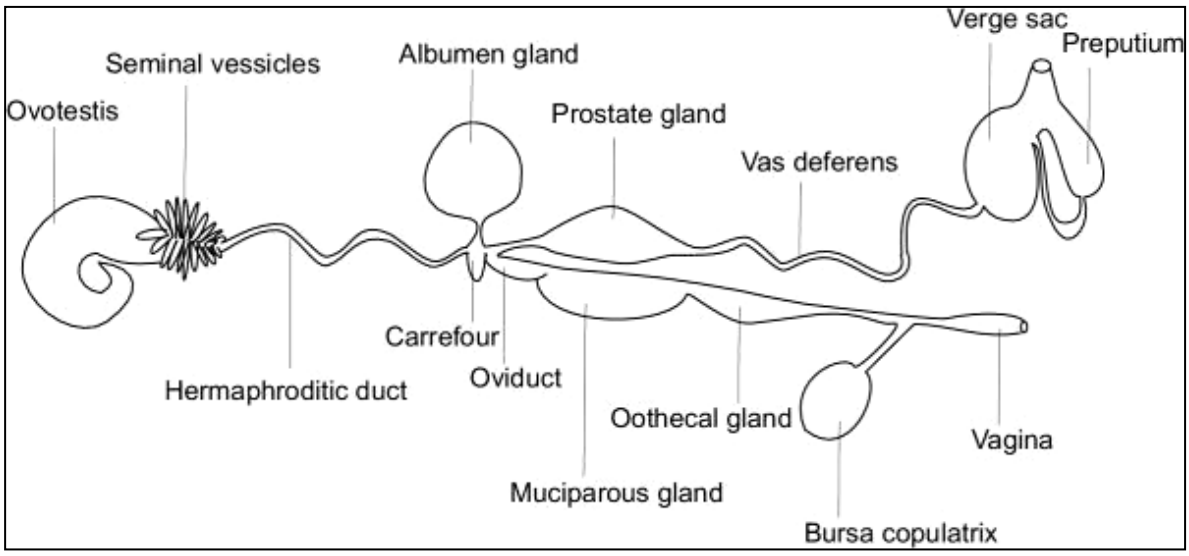


Fig: Reproductive structure of Mollusca

Secondary (newly evolved) larvae have developed among some freshwater bivalves and some cephalopods. Maternal protection of the developing eggs (brood) is not unexceptional behaviour in solenogasters, bivalves, and certain **gastropod** adults. Direct development without a larval stage or the bearing of live young from a yolky egg, or both, are typical in cephalopods and most nonmarine (and many marine) gastropods. Many species go through two breeding seasons per year, whereas in some **cephalopod** species **mating** or egg laying appears to be rapidly followed by death effected by **hormones**.

8. Echinodermata

Echinoderms are sexually dimorphic and release their eggs and sperm cells into water; fertilization is external. In some species, the larvae divide asexually and multiply before they reach sexual maturity. Echinoderms may also reproduce asexually, as well as regenerate body parts lost in trauma.

A. Asexual reproduction

In echinoderms usually involves the division of the body into two or more parts (fragmentation) and the regeneration of missing body parts. Fragmentation is a common method of reproduction used by some species of asteroids, ophiuroids, and holothurians, and in some of these species sexual reproduction is not known to occur. Successful fragmentation and regeneration require a body wall that can be torn and an ability to seal resultant wounds. In some asteroids fragmentation occurs when two groups of arms pull in opposite directions, thereby tearing the animal into two pieces. Successful regeneration requires that certain body parts be present in the lost pieces; for example, many asteroids and ophiuroids can regenerate a lost portion only if some part of the disk is present. In sea cucumbers, which divide transversely, considerable reorganization of tissues occurs in both regenerating parts.

The ability to regenerate, or regrow, lost or destroyed parts is well developed in echinoderms, especially sea lilies, starfishes, and brittle stars, all of which can regenerate new arms if existing ones are broken off. Echinoderm regeneration frustrated early attempts to keep starfishes from destroying oyster beds; when captured starfishes were chopped into pieces and thrown back into the sea, they actually increased in numbers. So long as a portion of a body, or disk, remained associated with an arm, new starfishes regenerated. Some sea cucumbers can expel their internal organs (autoeviscerate) under certain conditions (i.e., if attacked, if the environment is unfavourable, or on a seasonal basis), and a new set of internal organs regenerates within several weeks. Sea urchins (Echinoidea) readily regenerate lost spines, pincerlike organs called pedicellariae, and small areas of the internal skeleton, or test.

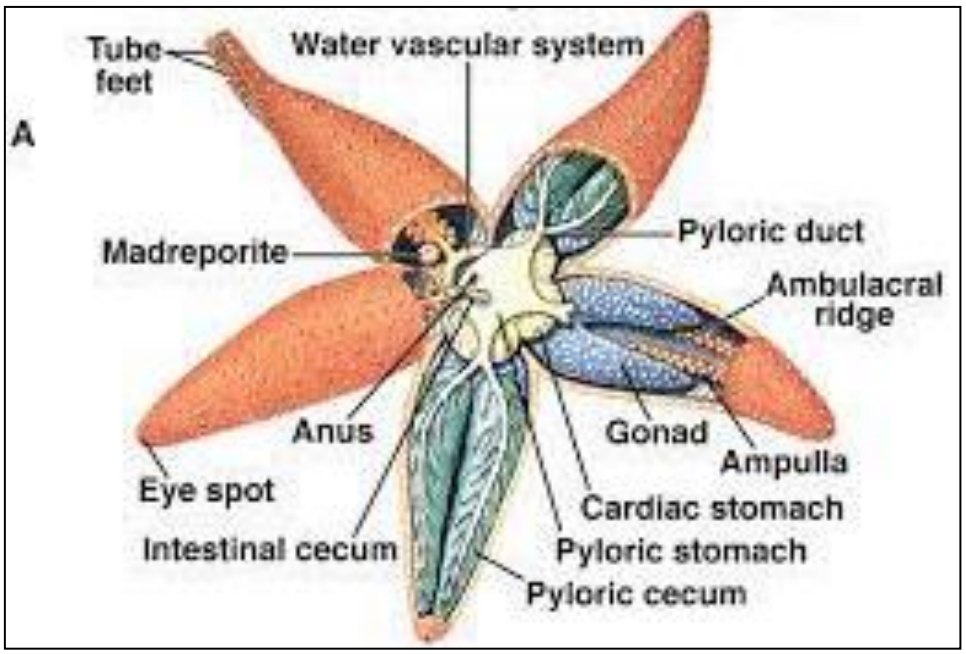


Fig: reproductive structure of star fish

B. Sexual Reproduction

In sexual reproduction, eggs (up to several million) from females and spermatozoa from males are shed into the water (spawning), where the eggs are fertilized. Most echinoderms spawn on an annual cycle, with the spawning period normally lasting one or two months during spring or summer; several species, however, are capable of spawning throughout the year. Spawn-inducing factors are complex and may include external influences such as temperature, light, or salinity of the water. In the case of one Japanese feather star (Crinoidea), spawning is correlated with phases of the Moon and takes place during early October when the Moon is in the first or last quarter. Many echinoderms aggregate before spawning, thus increasing the probability of fertilization of eggs. Some also display a characteristic behaviour during the spawning process; some asteroids and ophiuroids raise the centre of the body off the seafloor; holothurians may raise the front end of the body and wave it about. These movements are presumably intended to prevent eggs and sperm from becoming entrapped in the sediment.

Probable Questions:

1. What is binary fission?
2. Define syngamy and hologamy.
3. What is paedogamy?
4. What is gemmeul?
5. What is regeneration?
6. What is metagenesis?
7. Describe the reproductive structure of arthropod.
8. Function of albumen gland.
9. Describe the sexual reproduction of annelid.
10. Describe the asexual reproduction in Echinodermata,

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
6. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 193

Unit VII

Structural organization of respiratory organs in major invertebrate groups; factors affecting respiration.

Objective:

In this unit we will learn about Structural organization of respiratory organs in major invertebrate groups; factors affecting respiration.

Introduction:

Respiration is an essential physiological process in all living organisms by which they obtain energy for carrying out all the metabolic activities of the body. The term respiration has several meanings. In Latin, from which it has been derived, it means 'to breathe' or 'to exhale' and in this sense, respiration was initially applied to the exchange of gases between an organism and its environment. It meant the activities of breathing or their equivalent. We can say that respiration is a process in living organisms involving the production of energy, typically with the intake of oxygen and the release of carbon dioxide from the oxidation of complex organic substances. As years rolled by, it became apparent that the fundamental exchanges occurred at the cellular level and consequently the term internal respiration was applied to this phase of gaseous exchange. The exchange of gases between the body surface and the environment then came to be called external respiration.

✓ Characteristic feature of respiration

The characteristic feature of respiration is the intake of oxygen and the output of the carbon dioxide. The oxygen taken in is used in the oxidation of digested food in the cell to liberate energy. Carbon dioxide is produced as a consequence of oxidation of food materials. Its presence in the body is harmful, and hence it is removed from the body during this process.

In lower organisms such as protazoans, sponges, and platyhelminthes, oxygen is taken directly from the surrounding aquatic medium and carbon dioxide is given out directly into the surrounding medium. In larger animals, most of the cells are deprived of direct contact with the external environment because of their complex structure and larger size. Hence they require the help of respiratory and circulatory systems so as to facilitate exchange of gases and distribution of oxygen in all parts of the body. The process of respiration in these animals comprises the following phases.

i) External respiration is generally described as breathing. It involves organs which bring oxygen from the environment to the respiratory surface of the body. The system also serves the purpose of removing carbon dioxide from the body to the

environment. The respiratory surface may often be within the body of the animal. The respiratory surface may be integument, gills, tracheae or lungs.

ii) The second phase of respiration comprises the transportation of oxygen from respiratory surface to the body tissues and carbon dioxide from the tissues to the respiratory surface. In higher animals the transportation of respiratory gases is often carried out through blood.

iii) During this phase oxygen is consumed by the cells and carbon dioxide is produced by them as a result of oxidative processes which liberate energy 'for physiological activities of the body. It is the sum of enzymatic reactions, both oxidative and non-oxidative processes, by which energy is made available to maintain the vital activities. This phase is internal respiration or tissue respiration.

Respiratory organs in major invertebrate groups

In non-chordates various types of respiratory organs are found. These organs may differ in their structure but all are provided with a large surface of contact with the surrounding environment and are richly supplied with blood vessels and capillaries that ensure rapid gaseous exchange between the external environment and the blood. The external environment is usually water. Even when it is air, there is a thin film of water covering the respiratory surface and exchange of gases takes place through this aqueous film. The respiratory organs which work in water are gills; those which work in air, are lungs. They may project outwards (gills) of they may be invaginations (lungs). The kind of respiratory organs found in different phyla of non-chordates are as follows.

I. Protozoa

General Body Surface In protozoans respiration takes place by diffusion. Oxygen diffuses into the cytoplasm through general body surface and carbon dioxide diffuses outwards.

II. Porifera

Sponges do not have special organs of respiration; they have a canal system which brings water to the interior of the body. Gases diffuse through the surface of the cells. Sponges prefer places where water is rich with oxygen. If kept in foul water or water with less oxygen content the sponges undergo reduction in size and ultimately die. Similar results are obtained if the dermal pores are clogged, which prevent water current. The oxygen consumption is dependent upon the rate of water current (Fig.1).

Thus if the oscula are partially closed it is reduced, but this is compensated when the oscula are filly open. Also in coelenterates no definite organs of respiration are present. However, there is water current through the gastrovascular cavity of these animals. So their epidermis and gastrodermis are necessarily in contact with water. Gaseous interchange necessary for respiration takes place by diffusion (Fig 1).

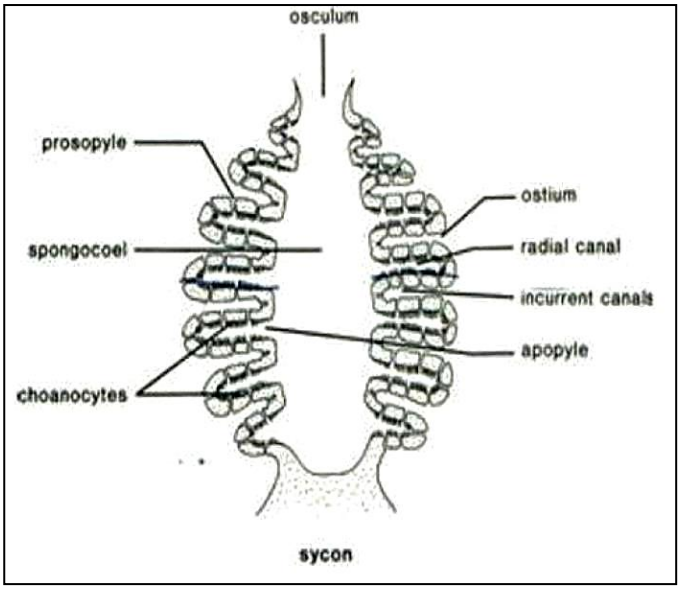


Fig 1. Canal syslem in *Sycon*

III. Helminthes

Free living flatworms are usually small and they respire by simple diffision through body surface. Respiration of the helminth parasites is quite different. They usually live in an environment whose oxygen content is very low or nil as in the case of gut parasites. So they have no access to air or oxygen. They are anaerobic. Similarly most nematode parasites also resort to anaerobic respiration. Lactic acid is the end product in such anaerobes. Generally speaking, the amount of oxygen the endoparasites use depends upon its availability.

IV. Annelida

In annelids respiration takes place through the entire body surface which is enormously increased by thin flattened parapodia of many polychaetes (e.g. *Nereis*). Within the parapodia there is an extensive capillary network. The dorsal and ventral tody walls are provided with numerous such networks of capillaries, which lie very close to the surface (Fig.2).

While passing through them, blood receives oxygen from the surrounding water and gives up carbon dioxide collected from the tissues. The oxygen carrying capacity of the blood of many annelids is increased by the presence of haemoglobin or other similar blood pigments. Usually these blood pigments are found in the plasma (fluid) instead of being contained within the cell. Gills are common among the polychaetes, but they vary greatly in both structure and location indicating that they have arisen independently within the class. Gills are never enclosed within the protective chambers. Many species which possess gills are already protected because they live in tubes and burrows. Gills are lacking in those polychaetes which are very small or which possess long.

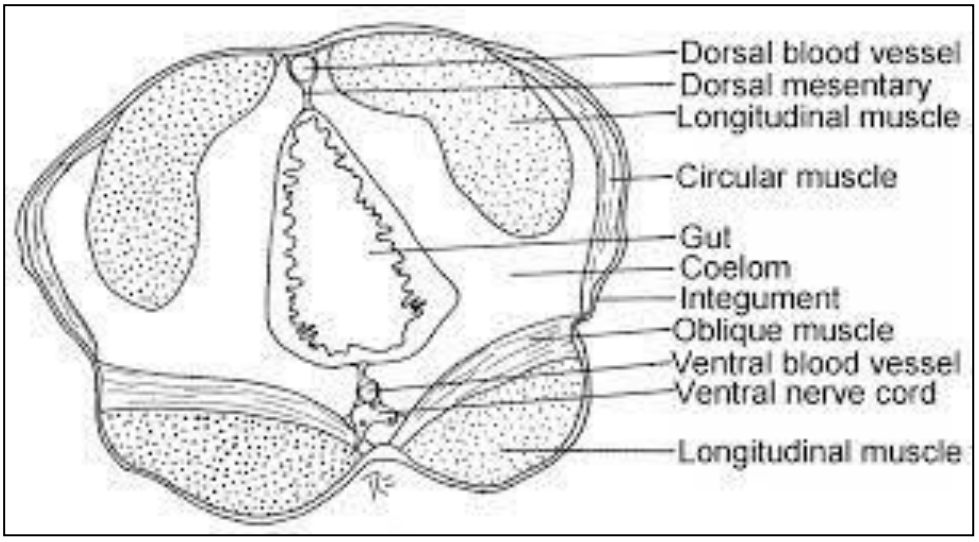


Fig. 2: Section of *Nereis*

Cilia present on the dorsal surface create a current of water flowing posteriorly beneath the elytra. In felt covered sea mouse (*Aphrodita*) cilia are lacking, but dorsal water current is produced by the animal that tilts the elytra upward and then rapidly brings them down in sequence. Most commonly the gills are associated with parapodia and in many cases these gills are modified parts of the parapodium. The notopodium sometimes possesses a flattened branchial lobe, which acts as a gill as in case of nereids (Fig. 3).

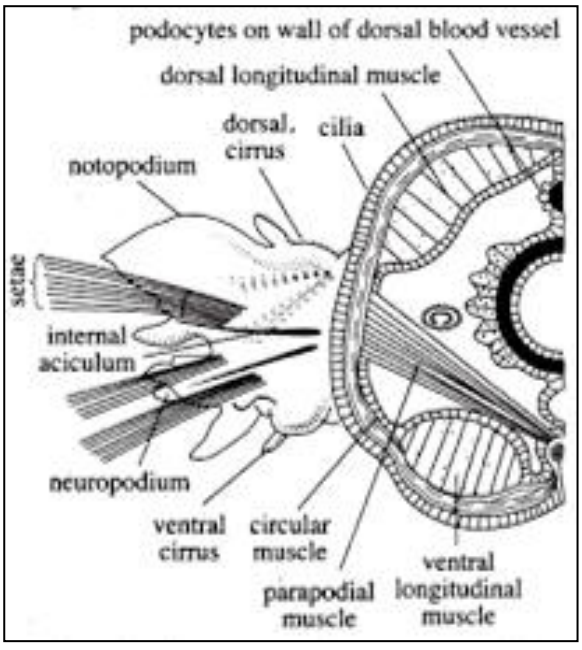


Fig.3: Cross section of Polychaete trunk

Generally, the dorsal citrus of the parapodium is modified to serve as a gill or the gills may arise from the base of the dorsal cirrus. Cirratulids have long, contractile, thread like gills, each attached to the base of the notopodium. The gills are not always

associated with parapodia. Many sedentary species have gills at the anterior ends near the opening of the tubes or burrows. For examp!e, the gills of some terebellids such as Amphitrite are arborescent and are situated on the dorsal surface of the anterior segments. The bipinnate radioles composing the fans serve as sites of gas exchange in the fan worms sabellids (*Sabella*, Fig. 4) and serpulids. Ventilation may be provided by gill cilia or by gill contractions. But many burrowing and tube dwelling polychaetes drive water through their burrows or tubes by undulating or peristaltic contractions of the body e.g. *Arenicola*, *Chaetopterus.*

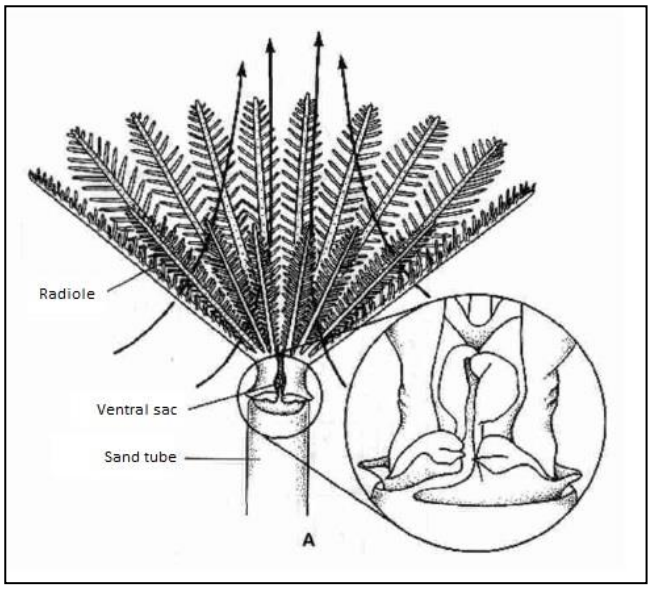


Fig.4: Anterior end of the fan worm, *Sabella*, showing the filter-feeding currents and tube building.

V. Arthopoda

For the process of respiration, there are special respiratory organs present in these organisms. These different structures are present in them for the adaptation to facilitate the passage of oxygen throughout their body.

These structures are:

- **Gills:** In prawns and crabs these structures are present. As these insects live in water so they have the presence of gills in them.
- **Book-Gills:** They are a modified version of gills. Lamellae are present in them. The lamellae are a plate-like structure and these lamellae lie parallel to each other and in this course, they are arranged in the form of a book, therefore they are called book gills. These structures are present in the Limulus.
- **Book-Lungs:** These are the modified structures of book-gills. An example is book lungs in scorpion. This helps us to understand book lungs meaning.

- **Tracheal System**: This comprises the major respiratory system in the insects. Elastic air tubes that are called the trachea are present in them. Some pores that are known as spiracles are present on the outside of their body. This system is present in cockroaches and mosquitoes.

The respiratory organ, trachea, is a hallmark of insects. It is made up of a system of branching tubes that deliver oxygen to, and remove carbon dioxide from, the tissues, thereby obviating the need for a circulatory system to transport the respiratory gases (although the circulatory system does serve other vital functions, such as the delivery of energy-containing molecules derived from food). The pores to the outside, called spiracles, are typically paired structures, two in the thorax and eight in the abdomen. Periodic opening and closing of the spiracles prevents water loss by evaporation, a serious threat to insects that live in dry environments. Muscular pumping motions of the abdomen, especially in large animals, may promote ventilation of the tracheal system.

Although tracheal systems are primarily designed for life in air, in some insects modifications enable the tracheae to serve for gas exchange under water. Of special interest are the insects that might be termed bubble breathers, which, as in the case of the water beetle *Dytiscus*, take on a gas supply in the form of an air bubble under their wing surfaces next to the spiracles before they submerge. Tracheal gas exchange continues after the beetle submerges and anchors beneath the surface. As oxygen is consumed from the bubble, the partial pressure of oxygen within the bubble falls below that in the water; consequently oxygen diffuses from the water into the bubble to replace that consumed. The carbon dioxide produced by the insect diffuses through the tracheal system into the bubble and thence into the water. The bubble thus behaves like a gill.

The respiratory structures of spiders consist of peculiar “book lungs,” leaflike plates over which air circulates through slits on the abdomen.

The book lungs contain blood vessels that bring the blood into close contact with the surface exposed to the air and where gas exchange between blood and air occurs. In addition to these structures, there may also be abdominal spiracles and a tracheal system like that of insects.

Since spiders are air breathers, they are mostly restricted to terrestrial situations, although some of them regularly hunt aquatic creatures at stream or pond edges and may actually travel about on the surface film as easily as on land. The water spider (or diving bell spider), *Argyroneta aquatica*—known for its underwater silk web, which resembles a kind of diving bell—is the only species of spider that spends its entire life underwater. Using fine hairs on its abdomen, where its respiratory openings lie, the water spider captures tiny bubbles of air at the water’s surface, transports them to its silk web, which is anchored to underwater plants or other objects, and ejects them into the interior, thereby inflating the underwater house with air. Research has shown

that the inflated web serves as a sort of gill, extracting dissolved oxygen from the water when oxygen concentrations inside the web become sufficiently low to draw oxygen in from the water. As the spider consumes the oxygen, nitrogen concentrations in the inflated web rise, causing it to slowly collapse. Hence, the spider must travel to the water's surface for bubble renewal, which it does about once each day. Most of the life cycle of the water spider, including courtship and breeding, prey capture and feeding, and the development of eggs and embryos, occurs below the water surface. Many of these activities take place within the spider's diving bell.

Many immature insects have special adaptations for an aquatic existence. Thin-walled protrusions of the integument, containing tracheal networks, form a series of gills (tracheal gills) that bring water into close contact with the closed tracheal tubes. The nymphs of mayflies and dragonflies have external tracheal gills attached to their abdominal segments, and certain of the gill plates may move in a way that sets up water currents over the exchange surfaces. Dragonfly nymphs possess a series of tracheal gills enclosed within the rectum. Periodic pumping of the rectal chamber serves to renew water flow over the gills. Removing the gills or plugging the rectum results in lower oxygen consumption. Considerable gas exchange also occurs across the general body surface in immature aquatic insects.

The insect tracheal system has inherent limitations. Gases diffuse slowly in long narrow tubes, and effective gas transport can occur only if the tubes do not exceed a certain length. It is generally thought that this has imposed a size limit upon insects.

➢ Structure of Book lung

T.S. of book lung of an arachnid (Scorpion) (Fig.5)

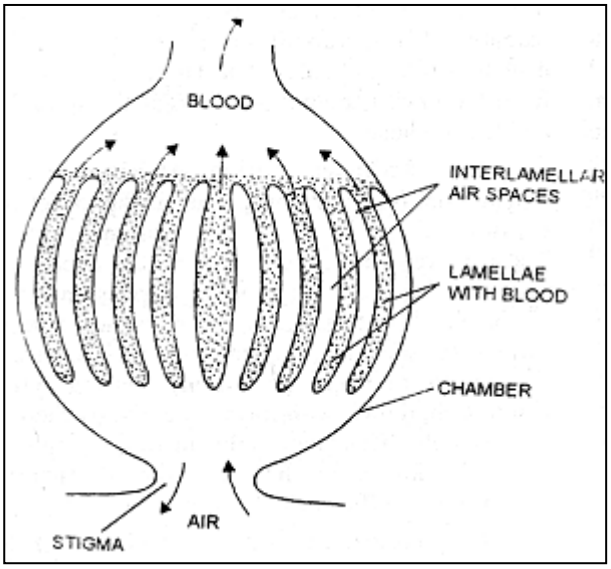


Fig. 5: Structure of Book lung

1. The book lungs are respiratory organs and are always in pair.

3. Each book lung is comprised of numerous (100-130) leaflets arranged like leaves of the book.

4. Each leaflet is divided into compartments – the blood spaces through partitions of blood vessels.

5. The leaflets are separated from each other through air spaces.

The leaflets remain enclosed in a closed chamber, which is divided by the leaflets into an upper pulmonary chamber and a lower atrial chamber.

7. The atrial chamber communicates outside through stigmata or stigma.

8. Air enters the atrial chamber through stigma and after aerating the leaflets goes out through pulmonary chamber. Thus, oxygenation of blood takes place.

➢ Trachea:

This is the most important organ for aerial respiration. This chitin-lined tube is seen in almost all land arthropods, such as insects, centipedes, millipedes and many arachnids.

Structures of trachea and associated parts (Fig.6):

i. Each trachea is a tube with walls made up of polygonal cells.

ii. The wall of trachea is composed of three layers—these are the internal layer, called intima, a middle layer of epithelium and an outer layer of basement membrane.

iii. The intima is lined by spiral cuticular ridges, called taenidea, that prevent collapse.

iv. The tracheal cuticle contains the same layers as the surface cuticle except the cement layer and wax layer.

v. The tracheae open externally by small openings, called spiracles or stigmata.

vi. These spiracles are located along the sides of the body.

vii. Each spiracle opens into a chamber, called atrium and the spiracle is placed on a plate, called penetrene.

viii. Each spiracle has two lids for opening and closing.

ix. Within the chamber foreign particles are eliminated by a filtering apparatus, containing either special bundles of setae or a kind of sieve-like membrane.

x. Some parts of tracheae are dilated to form air-sacs. They help as reservoirs of air.

xi. The finer branches of tracheae are called tracheoles which are without inner taerridial ridges. A tracheole may be 1μ in diameter and reaches every cell of the body.

xii. The end of a finer tracheae is immersed in a fluid through which gaseous exchange takes place.

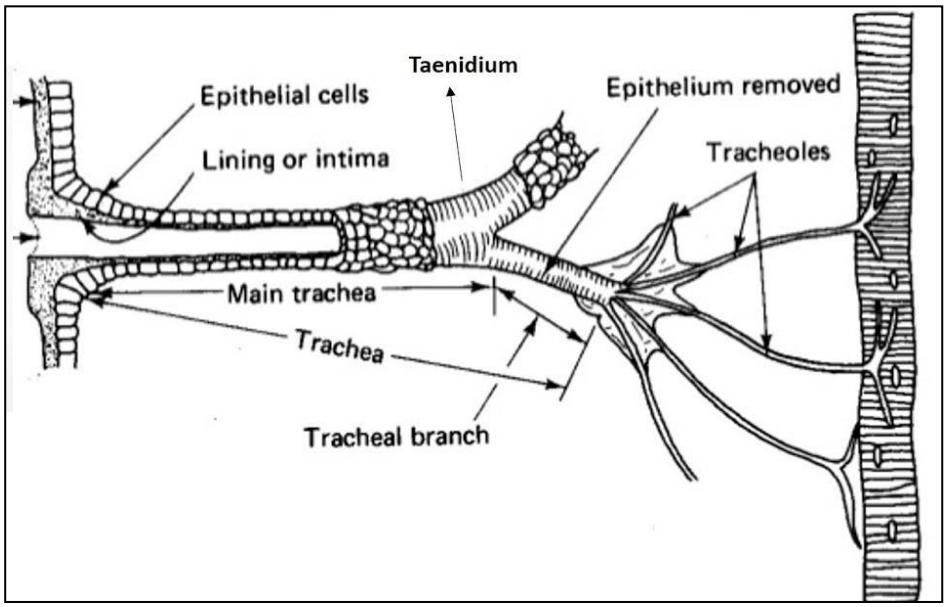


Fig.6: Structure of insect trachea

Mechanism of tracheal respiration:

The trachea ramifies into a number of fine networks of tracheoles which terminate into tissues where exchange of gases takes place by diffusion. Air is drawn in and forced out through the spiracles by the alternate contraction and expansion of the body. The spiracles remain closed most of the time and exchange of gases is probably due to diffusion and ventilation.

Recent studies indicate that the spiracles open very briefly but not all at a time due to reduction of haemocoelomic pressure. The spiracles are closed by valves, thus control the water loss, and opening of the spiracles is related to the high CO_2 concentration.

Gaseous exchange through the tracheae takes place by diffusion primarily and tracheoles are permeable to water and remain fluid-filled. This fluid is believed to be involved in the final O_2 transport to the tissues.

Again it is reported that the movement of trachea is facilitated by the alternate contraction and relaxation of the body sclerites. In the bed bugs, rigid and convex sternum does not take part in the respiratory movement, which is done only by the elastic tergum. In cockroaches the tergum and sternum of the segments are separated by intersegmental membrane which bulges out during respiration.

➢ Structure of gills in Crustacea:

A typical gill (Fig. 7) is crescent- shaped. It consists of a central axis or rod, on each side of which are arranged blade-like gill filaments, called lamellae. One end of each filament or lamella remains connected with the rod or central axis and the other end of the

filament is blind. Through the central axis of each gill runs an afferent and an efferent branchial channel.

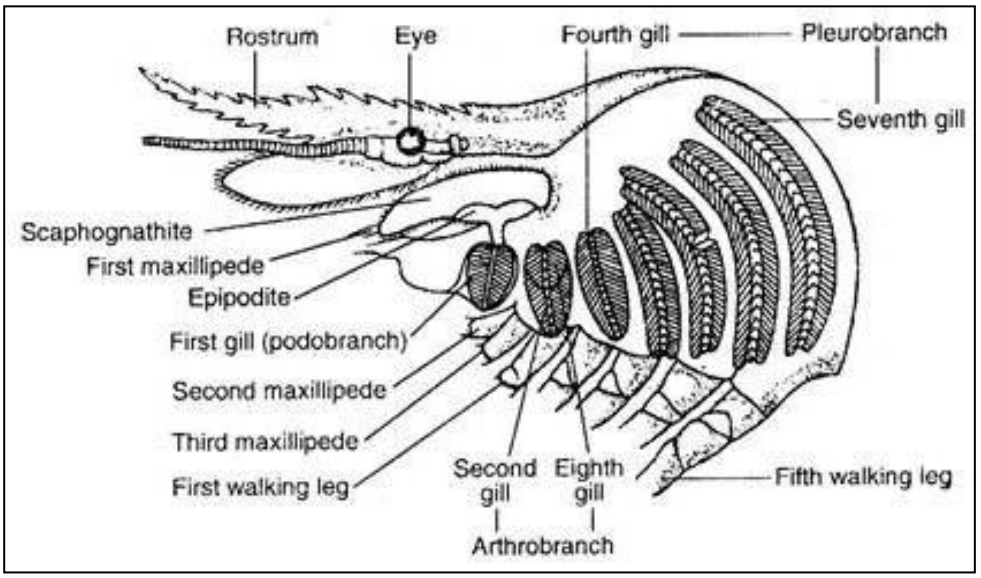


Fig.7: Structure of Book gill in crustacean

Mechanism of gill respiration:

In most Crustaceans, the gills are not covered within a special gill chamber. But in Decapods, the carapace extends laterally over the gills to house them in a special chamber.

In such forms with chamber, current of water enters through one end and after bathing the gills, passes out through another direction. In Crustaceans and Xiphosurids, gaseous exchange takes place in the gills between the blood and the water. But in Insects, after diffusion the oxygen passes to the tracheal tubes.

➢ Other devices of aquatic respiration:

Branchiostegites:

In Crustacea the gill-chamber is covered by the lateral extension of carapace, called gill cover or branchiostegite. The inner lining of the branchiostegite is thin, membranous and richly supplied with blood. It is in direct contact with water current and exchanges gases between the blood and the water.

Epipodites:

These are small highly vascularised leaf-like membranous outgrowths of integument on the outer side of coxa of the maxillipeds in first three thoracic segments. These epipodites being present in the anterior part of each gill-chamber (e.g., Crustacea) carry out respiratory functions.

Branchial basket:

The immature Odonates (Insects) have their rectum modified into a branchial basket. Its wall is contractile and richly supplied with the branches of tracheae. This kind of respiration is often referred to as anal respiration.

VI. Echinodermata

In echinodermata, there are finger like evaginations of the coelomic cavity called dermal papulae (dermal branchiae or branchial papulae) that serve as respiratory organs (Fig. 8). A major part of respiration takes place in echinoids, through ten branched gills situated in the area surrounding the mouth (Peristomial gills), one pair in each angle between the ambulacral plates. The tube feet in echinoderms are also respiratory in function.

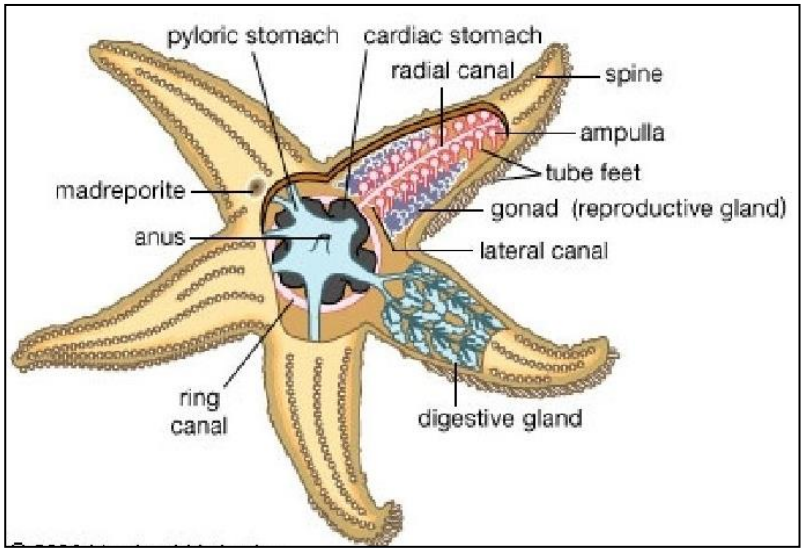


Fig. 8: The general plan of the water vascular system

Factors affecting respiration

- The process of respiration is influenced by a number of external and internal factors.
- The main external factors are temperature, light, oxygen supply, water supply, CO2 concentration, toxic and stimulating substances and disease and injury.
- The internal factors include the amount of respirable material and respiratory enzymes, acidity of the cell sap and activity of the organism.

Probable Questions:

1. Describe the characteristics features of respiration.
2. What is external respiration?
3. Describe the respiratory structure in phylum Porifera.
4. What is osculum?
5. What is notopodium?
6. Describe the structure of boo gill.
7. What is epipodite?
8. Describe the factors affecting respiration.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001

UNIT VIII

Photogenic organs in insects: structure, mechanism and significance of light production

Objective:

In this unit we will learn about photogenic organs in insects: structure, mechanism and significance of light production.

Introduction:

Bioluminescence in insects: The ability of certain organism to produce light is known as Bioluminescence. This phenomenon exists in more than half of Zoological Phyla. The light production is a result of some chemical reaction that does not involve heat production and is brought about half of suitable substrate and an enzyme system.

In many cases the luminescence occurs due to some symbiotic bacteria, such as luminescence in some marine fish. But in case of self luminescence, the emersion of light is purely depend on chemical reactions. This luminescence mechanism is highly developed in insects and the production of light in insects is always a phenomenon of Chemiluminescence.

Examples-

- *Onychiurusarmatus*- Subclass: Collembola (Subclass-Apterygota) emits their general from their whole body;
- *Fulgora lanternaria* –Suborder: Homoptera, light organ is on their head;
- Order: Coleoptera, the insects from family: Lampyridae (fireflies) emits their light from abdominal segments. In case of male, the light organ is situated on the ventral side of 7^{th} and 8^{th} abdominal segments, in female only in 7^{th} segment and in larvae 1^{st} to 7^{th} sengments;
- Order: Coleoptera, Family: Elateridae, Some insects from this family emits their light fromthree organs,

  A) One is in the lateral side of the prothorax.

  B) Other two organs are in the dorsal side of the 1^{st} abdominal segment;
- Order: Coleoptera, Family: Phenogodidae, they have 11 pairs if dorsolateral side of thorax, abdomen and another on head.

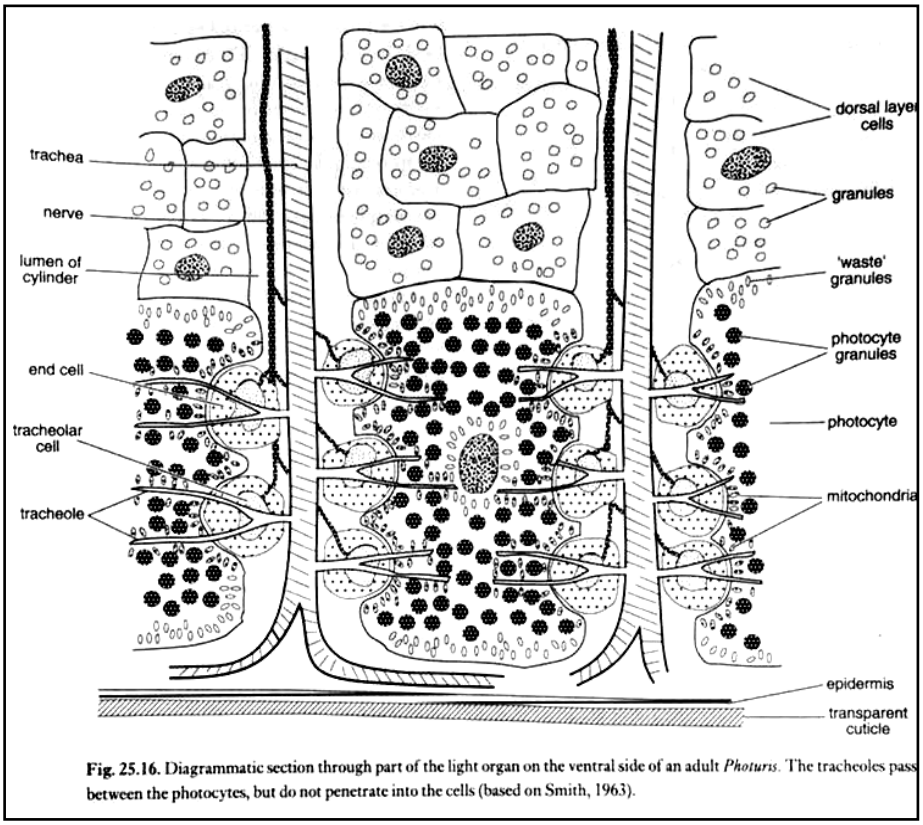


Fig. 25.16. Diagrammatic section through part of the light organ on the ventral side of an adult *Photuris*. The tracheoles pass between the photocytes, but do not penetrate into the cells (based on Smith, 1963).

Types of Light Emission:

1. **Continuous Glow**–mostly in larvae
2. **Intermittent glow**–*Photinus* sp.(Order : Coleoptera, Family:Lampyridae)
3. **Pulsation glow**–*Pyroicoelias*p. (Order : Coleoptera, Family : Lampyridae)- 6-13 per second & stop
4. **Flush glow** – *Luciola*sp. (Order: Coleoptera, Family: Lampyridae) - one time high intensity light) Different colour of light: Some of the insects can change the colour of the light and the changing of the pH of enzyme is responsible for the colour change.

   a) Yellow green or Blue Green
   b) Pure Green
   c) Pure Red

   } High intensity

Figure: *Luciola* sp. emitting luminescence.

Image source: Chapman, R .F. (2000). The insects structure and function. *Fourth ed. Cambridge University press*.

Structure of photocytes

1. Each light producing organ consists of number of large special type of cells the photocytes.
2. Photocytes lie just beneath the epidermis and are backed by several layers of cells called the dorsal layer cells.
3. The cuticle overlying the light organ is transparent.
4. The photocytes from a series of cylinders at right angles to the cuticle.
5. Tracheae and nerve run through the core of each cylinder.
6. Each trachea gives of f branches at right angles and as these enter the region of photocytes they break into tracheoles, which run between the photocytes parallel withcuticle.
7. Active ventilation – The tracheoles are spaced 10-15 µm apart and as the photcytes are only 10µm thick the diffusion path for oxygen is short.
8. The origin of the tracheoles is enclosed within a large tracheal end cell, the inner membrane of which is complexly folded where it bounds the tracheolar cell.
9. Mitochondria are sparsely distributed except where the cell adjoins the end cell tracheoles.
10. Prasence of mitochondria i.e. cells very active.
11. Photocytes are packed with photocyte granular, each containing a cavity connectingwith outside cytoplasm via a neck.
12. Photocute granules pour its secretion (Luciferase) for this oxidation is necessary,

so,tracheal supply is necessary.

13. There are two types of nerve ending

    Big vesicle (larger nerve ending) - Neurosecratory function.

    Small vesicle (Small nerve ending) - Secretion of enzyme, Cholin esterase, which is necessary to form pyrophosphate.

14. Dorsal layer cells contain urate crystal de posit. So, these cells become darker and functions as reflective site.

Mechanism of light production in insect

It was William Mcelroy and his colleagues at John Hopkins University first isolated the principal biochemical componenets involved in the firefly light production. Luciferin, a complex carboxylic acid and luciferase is the enzyme.

Basically light is produced by the oxidation of luciferin, in the presence of enzyme luciferase. Luciferin is first act ivated by ATP in the presence of magnesium and luciferase to produce adenylluciferin. This remains tightly bound to the enzyme and is oxidized to form excited oxyluciferin, which de cays spontaneously with the production of light. The reaction is very efficient, some 98% of energy involved being released as light

1) Initial Reaction:

Mg^{2+}

$E+LH_2+ ATP \longrightarrow \boxed{E\ LH_2 - AMP} + pp$

2) Final Reaction:

$\boxed{E\ LH_2\text{-}AMP} + O_2 \longrightarrow E\text{-}L{=}O\text{-}AMP + light + H_2O$

pp

3) Additional Reaction:

$$E\text{-}L{=}O\text{-}AMP \xrightarrow{Mg^{2+}} E\text{-}L{=}O + AMP$$

E- Enzyme Luciferase

LH_2- Luciferin

L=O – Oxyluciferin

LH_2-AMP = Adenyl luciferin

L=O-AMP= Adenyl oxyluciferin

Light production stop

Significance of bioluminescence:

1. Light production generally related to mating -In Lampyridae, female is sedentary and attracts the male b y producing light in a species specific manner. They also release some Phenomenon with light; In case of *Photinus pyratis* generally male on ground climbs steeply at the time of light production after flashing it hovers for about two seconds, then female f lash on grasses, it flies towards the female and flashes a gain and repeated flashing sequences bring the male to female.
2. Food Capturing device- The luminescence of *Bolitophila* larvae serves as a trap, attracting small insects into networks of glutinous silk threads on which they become trapped.

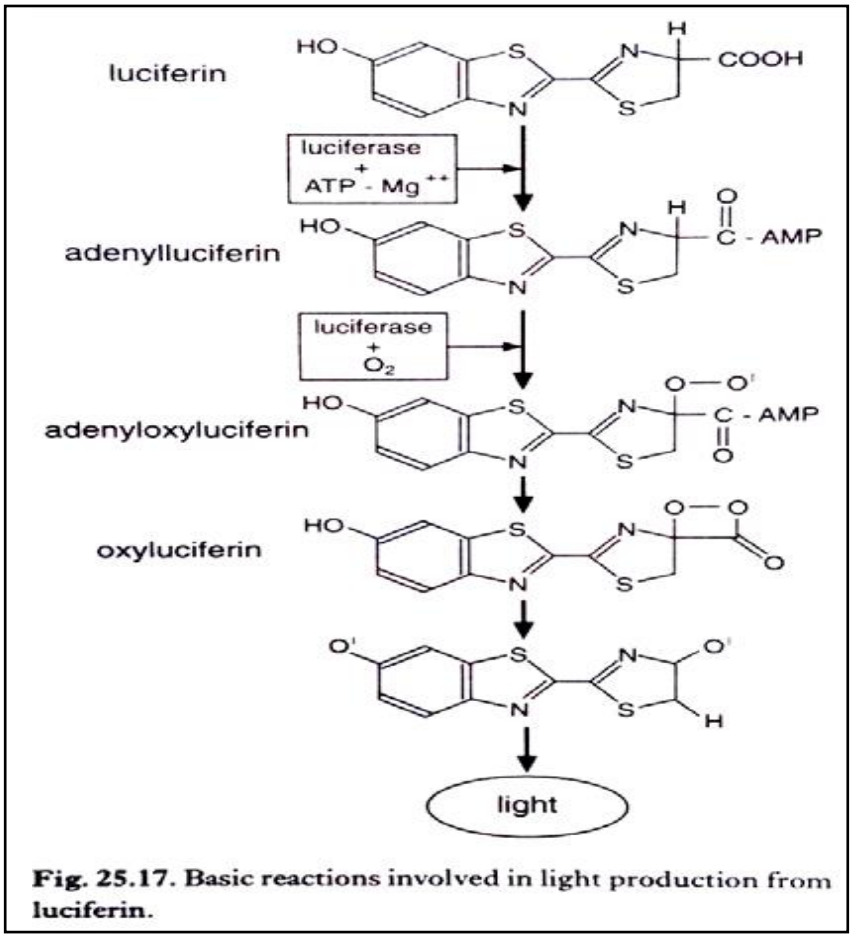


Fig. 25.17. Basic reactions involved in light production from luciferin.

Probable Questions:

1. What is Bioluminescence?
2. Define Chemiluminescence.
3. Describe different type of light emission?
4. Describe the structure of photocyte.
5. Discuss the mechanism of light production in insect?
6. Discuss the significance of bioluminescence?

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
5. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
6. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 193

UNIT VIII

Trochophore larva: Structure and evolutionary significance and Evolutionary significance of minor invertebrate phyla

Objective:

In this unit we will learn about trochophore larva: structure and evolutionary significance and evolutionary significance of minor invertebrate phyla.

Introduction:

When development is indirect there is a larval stage through metamorphosis which differs from the adulthood. Advancement from free living to sessile or sedimentary requires many stages. If the food coexisting member of a habitat, habit scenario limits the larval form and adults are more taxa shoes a significant larva. As in case phylogeny points out their origin to be a monophylatic one. Such as larva are theories in bilateral over radial symmetry.

Occurrence:

1. A characteristics larval stage of annelids, egg hatches from Trochophore larva- the first larval stage.

2. Also found in Mollusca and some other phyla also.

• Structures of the Trochophore Larva (Loven's Larva of Polygordius):

All the archiannelids (Polygordius and other species) are known to pass through the larval stage—the trochophore.

1. Marine planktonic and most are lecithotrophic larvae.

2. The anterior end of the body is broader than the posterior end and it exhibits bilateral symmetry.

3. It has mouth, alimentary canal and anus.

4. The mouth is situated near the mid-ventral line of the body and leads into the sac-like stomach and proceeds as a narrow alimentary canal. Both the walls of the alimentary canal extending from mouth to anus are lined by cilia.

5. The canal ends in an anal aperture at the posterior tip of the body.

6. Two prominent bands of cilia encircle the body and in certain forms a third band may be present. These ciliary bands are used in locomotion and feeding.

7. A circlet of strong locomotor ciliary band, called the pre-oral circlet or Prototroch (Fig. 17.12A), encircle the body around the middle and lies above the mouth, and the metatroch which is a ciliary transverse ring behind the mouth.

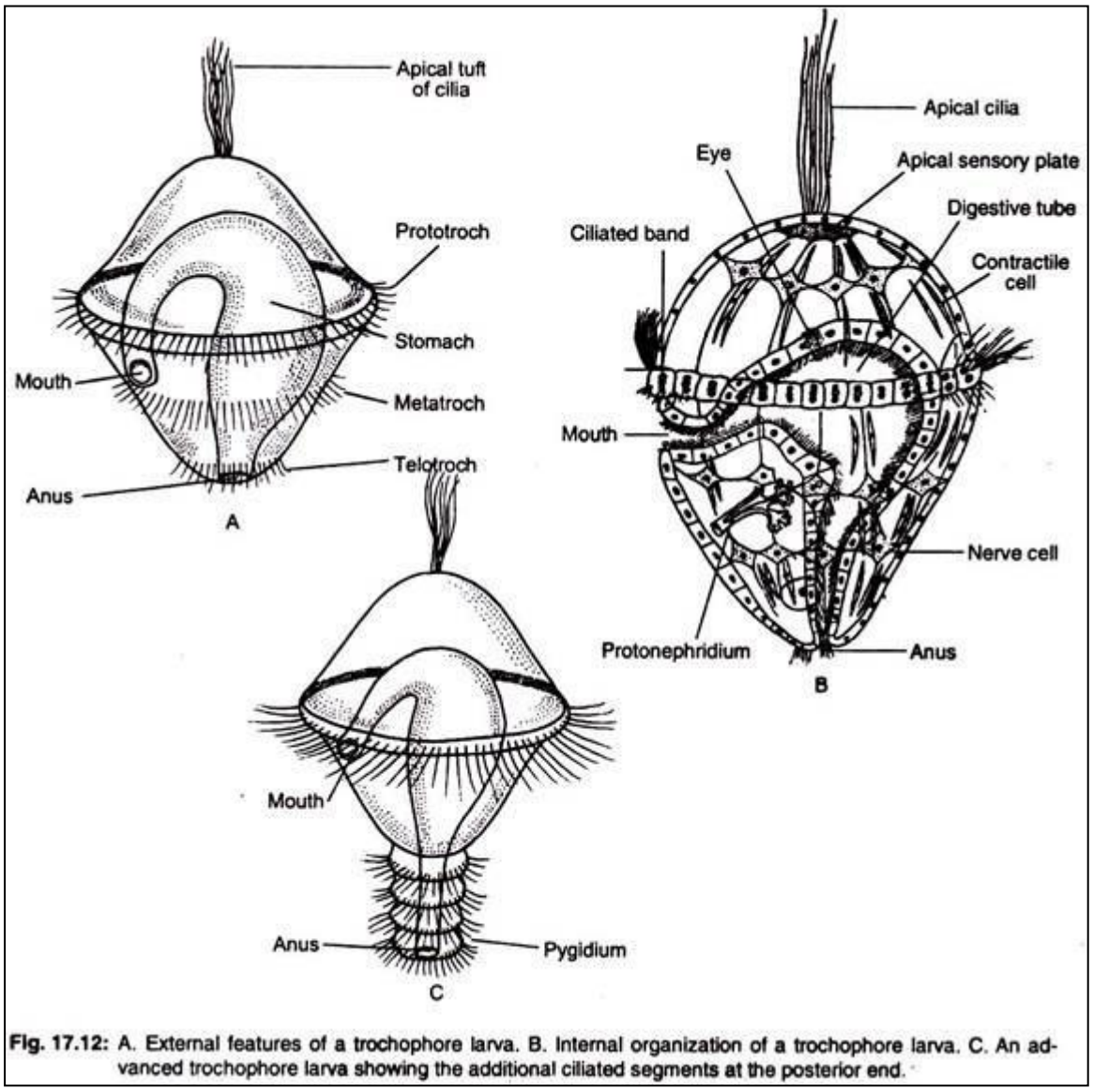


Fig. 17.12: A. External features of a trochophore larva. B. Internal organization of a trochophore larva. C. An advanced trochophore larva showing the additional ciliated segments at the posterior end.

8. The prototroch arises from special cells, called trochoblasts.

There is often a second circlet of cilia around the pygidium or anus, called the telotroch.

10. A longitudinal band along the mid- ventral part of the body is also present in some cases and is called a neurotroch.

11. Trochophore exhibits no metamerism and the rudiment of future adult trunk is seen as a small region at the posterior pole.

12. There is no coelom at this stage but only a spacious blastocoel encloses the gut.

Within blastocoel, a pair of protonephridia, certain amount of mesenchyme and larval muscles is present.

14. The nephridia are made of two hollow cells; each contains a flame of cilia. One nephridium lies on each side of the gut.

15. The upper pole possesses an ectodermal, thickened area, called apical sensory plate which contains cells that are the primordia of the cerebral ganglia.

16. At the apical end, a number of long cilia emerge from the apical plate, called apical tuft of cilia.

Many trochophores bear sense organs, such as ocelli (eye spots) below the apical plate region.

18. The fully developed trochophore larva may be divided into three regions, such as the pretrochal region which includes the area above the prototroch. The posterior region of the larva is called pygidium which includes telotroch and anal area. The middle region is called growth zone which includes the area between the mouth and telotroch.

- **Biology and Metamorphosis of Trochophore Larva:**

The free-swimming trochophore larva of some forms (e.g., Echiurans, some polychaetes, Polygordius, phyllodocids, serpulid fan worms) feeds on the plankton and other microscopic marine organisms and the trochophore is called planktotrophic larva and has long free-swimming life.

In some other groups (e.g., Sipunculans, nereids and eunicids of polychaetes), the trochophore larvae are lecithotrophic larvae. They do not take any food from external sources and mainly feed upon the yolk originally laid down in the egg. The lecithotrophic larvae lead a short planktonic life. Metamorphosis of the larvae is best seen in Polygordius

The first sign of metamorphosis is marked by the segmentation of mesodermal bands. Later the posterior region elongates rapidly and is externally marked with segmentation. The area above the prototrochal ring becomes prostomium and the prototrochal area differentiates as peristomium.

The apical sense organ area becomes the cerebral ganglion which is joined with the ventral nerve cord. Internally the mesodermal band splits to produce coelomic sacs.

The mouth shifts forward and the anal organization changes gradually. The ciliary bands disappear and the larva grows in size and length with addition of the new segments. After metamorphosis the young worm sinks to the bottom of the sea and becomes the burrowing adult worm.

- **Structures of the Trochophore Larva in Different Classes:**

Class Polychaeta:

1. The larva of Neanthes (= Nereis) is similar to the typical trochophore but with a pair of eye spots.

2. Larvae with no blastocoel but the ectoderm and endoderm are in contact except where they are separated by the larval mesoderm, e.g., larva of Psygmobranchus.

Cilia are either rarely distributed over the whole surface of the body or are not confined to special circlets, called atrochal larva, e.g., larva of Lumbriconereis.

4. In the larva of Nephthys, two circlets of cilia are seen, one at each end of the body, the pre-oral (anterior) and perianal (posterior), called telotroch larva.

5. The complete rings when may be present on both dorsal and ventral surfaces, called amphitrochal larva.

6. In the larva of Chaetopterus where one or more rows of cilia surrounds the middle of the body, called mesotrochal larva. In mesotrochs the pre-oral and peri-anal rings are absent.

7. In the larva of Ophryotrocha, there are many ciliary circlets and each develops on a true mesodermal segment hence called polytrochal larva.

8. In the larva of Mitraria, long provisional setae are found which are replaced by permanent structures. The older larva of Nereis possesses parapodial-like lateral flattened structures with setae.

Class Oligochaeta:

No free larval stage is noticed.

Class Hirudinea:

There is no larval stage and the development is direct.

- **Affinities of Trochophore Larva (Evolutionary significance):**

This larval form exhibits remarkable similarities with several other larval forms. As a consequence the phylogenetic status of Trochophore warrants serious consideration.

- **Affinities of Ctenophora:**

The aboral sense organ (Statocyst) of a ctenophore is compared with the apical sensory plate of trophophore. The sub-ectoder- mal radiating nerves are comparable. The prototroch is derived from fourth group of ciliated cells. Both of them have pear-shaped body.

Despite the similarities the fundamental organisation portrays many diversities. The cleavage pattern is different in both the cases. The anus is absent in ctenophores. So the trophophore larva cannot be regarded as related to ctenophores.

- **Affinities with Muller's larva:**

The Muller's larva of Turbellarians especially that of Planocera, shows similarities with the trochophore larva. Similarity in developmental stage, similarity in the disposition of ciliated bands and presence of eye spots at the aboral end of the two larval forms led many workers on this line to draw parallelism between the two groups. But due to undernoted dissimilarities the parallelism cannot be justified.

The dissimilarities are:

(i) Absence of anus in Muller's larva,

(ii) The enter on opening into one opening in Muller's larva,

(iii) Difference in the embryonic differentiation of mesoderm and

(iv) The existence of tuft of cilia at the caudal end of Muller's larva.

- **Affinities with Pilidium (Nemertini) larva:**

The pilidium larva of Nemertini exhibits certain similarities with the trochophore larva.

The similarities are:

(i) Both have helmet-shaped body,

(ii) The ciliated ring between aboral and oral ends of pilidium larva represents the prototroch of trochophore,

(iii) Similarities in the disposition and distribution of nerve ring,

(iv) The stomodaeum shows similarities,

(v) The schizocoelic mode of formation of coelom in both.

But the absence of anus in pilidium and the dissimilarities in the formation of mesoderm stand on the way to draw any relationship between them.

- **Affinities with Rotifera:**

Trochosphaera, a rotifer, shows some similarities with the trochophore larva of annelid. Trochosphaera resembles trochophore in many respects, viz., ciliated girdles, disposition of nervous system ('Brain') and the sense organ, placement of anus, nephridia and curvature of intestine. But the resemblances are mostly superficial in nature and need critical examination to draw any phylogenetic relationship.

- **Affinities with Veliger larva:**

The pre-oral ciliated ring, ciliated tuft of flagella and apical plate of the veliger larva of mollusca are similar with that of trochophore larva. The similarities between the trochophore and veliger larva are possibly due to remote phylogenetic convergence.

Phylogenetic Significance of Trochophore Larva:

In the evolutionary dynamics of invertebrates the trophophore larva occupies a prominent status. It shows similarities with many invertebrate groups. The affinities throw light on the emergence of bilateral groups from the animals having radial symmetry (Fig. 1).

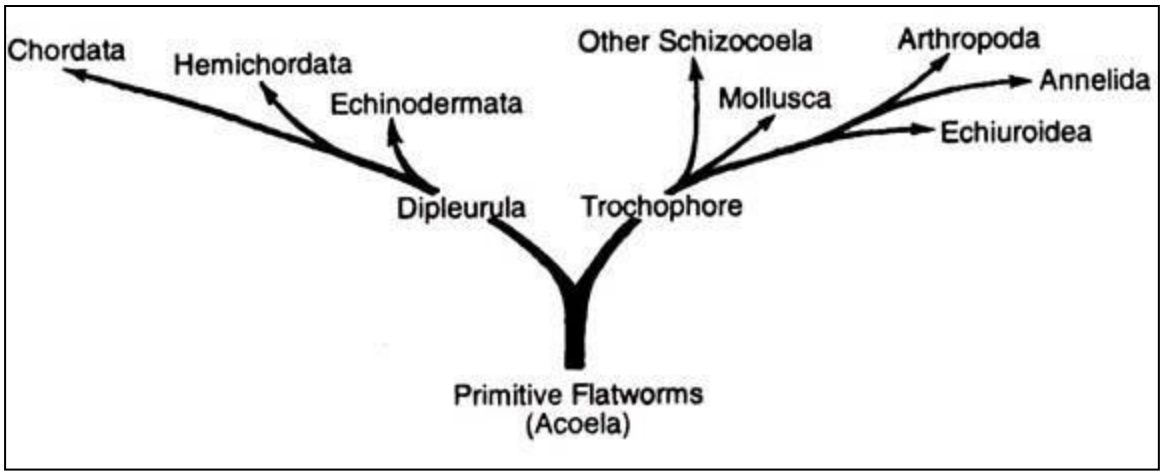


Fig.1: Phylogenetic relationship of Trochophore larva.

It is claimed that the trochophore represents a transitional stage in the line of emergence of the bilateral groups (e.g., Rotifers) from the radial groups (Ctenophores). Similarities between the trochophore and the echinoderm larva (Bipinnaria and Pluteus) and Tornaria larva of Balanoglossus added more weight to this contention.

Many workers are of the opinion that the Trochophore larva serves as a bridge between radial and bilateral symmetry. They have opined that the bilateral symmetry has evolved from the radial one.

Regarding their views, there are many theories which may be given below:

1. Ctenophore—Polyclad theory (proposed by Lang, 1881)

2. Ctenophore—Trochophore theory, later modified by Hatschek (1878)

3. Planuloid—Coeloid theory (proposed by L. Vongraff, 1882).

Discussion:

Second theory is more or less acceptable by the workers. According to this theory Trochophore larva arose from the hypothetical animal—Trochozoon. Again, Salvini—Plawen L. (1973) has stated that annelids and echiurans are closely related by their larval stage (trochophore larva) whereas flatworms, nemerteans and entoproct larvae are unrelated.

Evolutionary significance of minor invertebrate phyla

Introduction:

Minor phyla are often referred to as enigmatic or problematic, are usually of uncertain affinity, and generally are treated superficially in invertebrate texts. Minor phyla are considered to be of little consequence to mainstream animal evolution, usually because they are not well represented in present day macrofauna This is a major error, since the modern day or paleontological prominence of a taxon does not necessarily reflect its role, or the role of its ancestors in the metazoan radiation. If we use the questionable definition of a phylum as a taxon with a distinctly unique body plan and leave aside the requirement of monophyly, then minor phyla represent the majority of nature's experimentation with animal body plans. In contrast, the "major" phyla are a small number of groups that are prominent among modern macrofauna and are composed of annelids, arthropods, chordates, cnidarians, echinoderms, molluscs, and perhaps platyhelminths. Several kinds of minor taxa are important to this discussion. Some groups such as mesozoans and placozoans have been considered to be representatives of the stem lineage leading to triploblastic animals. Other groups have uncertain affinities, appear to have simple body plans, and are generally small in size. Many of these historically have been lumped together into the "Aschelminthes" {e.g., Rotifera, Acanthocephala, Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Gastrotricha), based on the dubious assessment that each possesses a pseudocoelom. Other minor phyla appear to be sister taxa to larger and more well defined groups. For example, echiurans, sipunculids, pogonophorans and vestimentiferans have long been considered to be protostomes, possibly allied with annelids or molluscs.

1. Rotifera

Characteristics of the Phylum Rotifera:

1. Phylum Rotifera are microscopic animals, mainly found in freshwater, rarely in marine or parasitic.

2. Body wall of Phylum Rotifera generally lacks a cuticle and thickened into stiff plates or lorica into which the head may retreat.

3. Anterior end with a ciliated organ called corona helps in swimming and feeding.

4. Posterior foot of Phylum Rotifera has two toes; foot with cement glands.

5. Cuticle secreted within epidermis and never moulted.

6. Digestive system with a highly muscular pharynx called mastax lined internally with cuticle and within mastax is a rigid structure or jaws called trophi used for grasping and grinding the prey.

7. Pseudocoelomate animals.

8. Eutelic condition is seen in Phylum Rotifera.

9. Excretory organs are protonephridia with flame cells. The protonephridia function in osmoregulation.

10. Nervous system includes cerebral ganglion with longitudinal nerve cords.

11. Sexes in Phylum Rotifera are separate (gonochoristic).

12. Parthenogenesis is largely present in Phylum Rotifera.

13. Spiral cleavage.

14. No larval stage in the life cycle.

- ## Affinities of Phylum Rotifera (Evolutionary significance):

Since their discovery, rotifers have a very uncertain systematic status. Rotifers exhibit superficial similarities with many invertebrate groups, namely Arthropoda, Annelida and Platyhelminthes

A. Affinities with Arthropoda:

Resemblances:

(i) Body covered by a cuticle.

(ii) Superficial metamerism.

(iii) Presence of two jaws (trophi)

(iv) Movable bristle bearing arms of pedalia suggest the appendages of a crustacean larva.

Remark:

As the similarities are superficial, that the relationship can't be drawn.

B. Affinities with Annelida:

The annelidan relationship of rotifers as advocated by Hatschek (1878) is based on structural resemblance between Trochophore larva of annelids and a peculiar rotifer, Trochosphaera. The ciliary girdle, bent intestine and excretory organs of Trochosphaera are similar to the corresponding parts of trochophore.

Remark:

For the above resemblances Hatschek propounded his famous 'Trochophore theory' which proposes that living rotifers are closely related to the ancestral Mollusca,

Annelido and certain other groups. The annelid theory concludes that the rotifers are simply annelids that have remained in a larval condition.

But this theory fails because Trochosphaera is merely a peculiar rotifer with a modified girdle-type corona.

It is assumed that such similarities are regarded as a case of coincidence without having any phylogenetic significance.

C. Affinities with Platyhelminthes:

Similarities:

(i) Primitive type of corona may have been derived from a complete or ventral ciliation in turbellarians.

(ii) Formation of trophi is also common in turbellarians.

(iii) The protonephridial system with flame cells is identical with that of rhabdocoels.

(iv) The retrocerebral organ is probably homologous with the frontal organs of turbellarians.

Dissimilarities:

(i) Presence of an anus in rotifers.

(ii) Lack of sub-epidermal continuous muscles.

(iii) Lack of epidermal nerve plexus.

Remark:

The above resemblances do not suggest that they are closely related to turbellarians than that of other groups. The study of development of rotifers suggests that they are of primitive forms, not a degenerate group.

D. Affinities with Nematoda:

Similarities:

(i) A syncytial epidermis.

(ii) Pseudocoelomic body cavity in both cases.

(iii) Presence of a gut with mouth and anus.

(iv) Lacking a circulatory system in both cases.

(v) Cleavage determinate type in both.

(vi) No larval stage in the life cycle.

2. Acanthocephala

Characteristics of the Phylum Acanthocephala:

The Acanthocephala are endoparasitic worms of slender cylindroid or slightly flattened form and hollow construction. They live as adults in the intestine of vertebrates and as larvae in arthropods. The diagnostic feature of the phylum is the organ of attachment consisting of an invaginable proboscis that forms the anterior end.

This proboscis is armed with rows of recurved hooks. The body wall consists of cuticle, syncytial epidermis permeated with spaces and sub-epidermal musculature. In connection with the proboscis apparatus the epidermis forms two elongated bodies termed lemnisci that hang down into the trunk.

Mouth, anus and digestive tube are completely wanting. There is no circulatory system. Excretory organs when present are of the nature of protonephridia and open into the terminal part of the reproductive system.

The nervous system consists of a ganglion near the proboscis and two lateral cords extend posteriorly from the ganglion along with numerous minor nerves. The sexes are separate, the females are generally larger than the males, the males are provided with a copulatory apparatus and the terminal part of the female apparatus is also somewhat complicated.

The eggs develop within the maternal body into a larva that requires an intermediate invertebrate host for its further development. There are over 500 known species. Echinorhynchus is the chief genus of Acanthocephala and it is described here in detail.

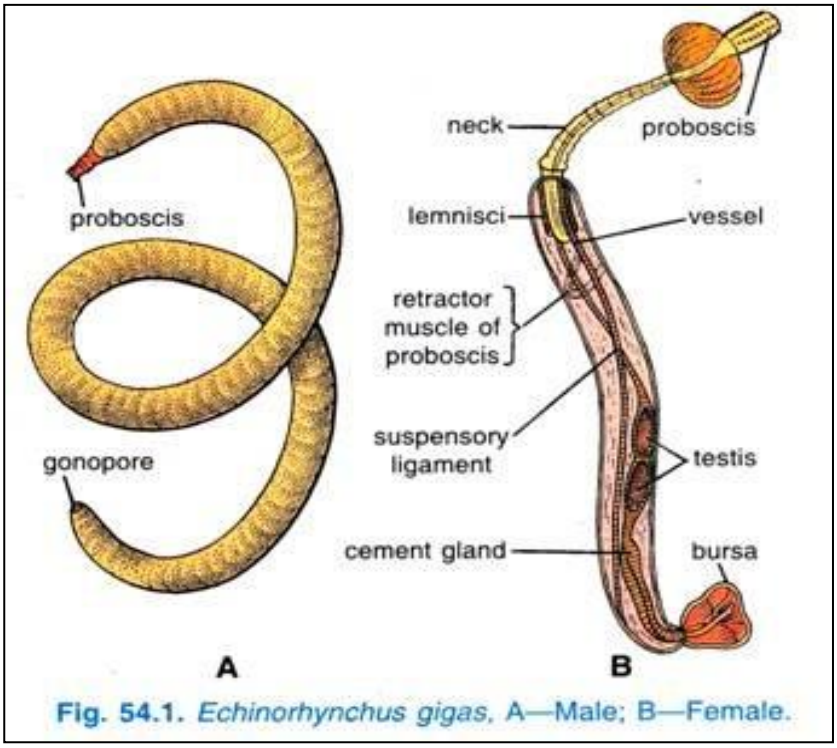


Fig. 54.1. *Echinorhynchus gigas*, A—Male; B—Female.

- **Affinities of Phylum Acanthocephala:**

Because of many peculiarities of structure of Acanthocephala and the parasitic degeneration or alternation of some of their systems, determination of their affinities poses a difficult problem. The acanthocephalan worms were noticed about the beginning of the 18th century but were clearly distinguished from other intestinal worms until 1771 when Koelreuther proposed the name Acanthocephalus for one from fish.

In 1776, Zoega and O.F. Muller without knowledge of Koelreuther's work gave the name Echinorhynchus to a similar fish parasite. Zeder (1803) gave these worms a common name **"Haken wurmer"** (hooked worms) and Rudolphi (1809) changed this into the form Acanthocephala (Greek, akantho = spiny; kephalo = pertaining to the head) by which the group has since been known.

Cuvier included the acanthocephalan worms with the flatworms in his group of parasitic worms. Vogt (1851) first clearly distinguished the flatworms from the roundworms upon which Gegenbaur gave the name Nemathelminthes to the roundworms. The position of Acanthocephala, however, has remained uncertain.

At the present time there are only two views that need be seriously considered, those associating the Acanthocephala with the Platyhelminthes or with the Aschelminthes. The best way to decide between these two possibilities is to discuss, system by system, the similarities and dissimilarities between the three groups, something that has also been done by Chitwood (1940) and Van Cleave (1941).

A. Similarities with Platyhelminthes:

The Acanthocephala resemble with the Platyhelminthes in the following respects:

(1) An armed and protrusible proboscis occurs in certain cestodes and the proboscides of Trypanorhyncha and Acanthocephala is similar so far as the shape and arrangement of hooks is concerned.

(2) Presence of cuticle and syncytial nucleated epidermis.

(3) Musculature with circular as well as longitudinal fibres.

(4) Protonephridia of flame-bulbs are present.

(5) Reproductive system, particularly in male, resembles that of many flatworms.

(6) Embryology is like that of cestodes.

(7) Serological tests indicate a relationship of Acanthocephala with cestodes rather than nematodes.

B. Similarities with Aschelminthes:

The following similarities are noteworthy:

(1) The division of the body into the presoma and the trunk as in priapulids and the gordiacean larva.

(2) An armed proboscis is found among the Aschelminthes in echinoderids, priapulids and gordiacean larva.

Superficial segmentation, sometimes involving musculature, is also conspicuous in rotifers, echinoderids, priapulids and nematodes, etc.

(4) Clothing of body with cuticle and syncytial nucleated epidermis.

(5) Division of pseudocoel by partitions and tissues resembling mesenteries.

(6) Reduction of gut to a strand is found in male rotifers.

(7) Flame-bulb protonephridia present.

(8) A close relationship of nephridial and reproductive systems is common in priapulids and rotifers.

C. Differences from Nematoda:

Acanthocephala, however, differs from the Nematoda in the following points:

(1) Presence of proboscis.

(2) Absence of digestive tract.

(3) Presence of circular muscles.

(4) Presence of ciliated excretory organs.

(5) Peculiarities and complexities of reproductive system.

The foregoing comparisons do not furnish a decisive answer to the question whether the Acanthocephala are allied to the Platyhelminthes or to the Aschelminthes. The general structure is rather on the aschelminthic side, whereas the embryology presents more points of resemblance with the Platyhelminthes.

Chitwood (1940) and Van Cleave (1941) favour a platyhelminth affinity, and on this basis Chitwood (1940) has proposed a superphylum Parenchymata to embrace the flatworms, nemertines and acanthocephalans.

Leaving apart the fact that the Acanthocephala are not parenchymatous animals, one doubts the utility of the superphylum concept. On present evidence, raising the Acanthocephala to the rank of an independent phylum appears the best disposition of the group.

3. Gastrotricha

Characteristics of the Phylum Gastrotricha:

1. Phylum Gastrotricha is microscopic, un-segmented, worm-like animals.

2. Body length ranges 50-1000 μm

3. Cephalic region slightly swollen and caudal region forked with glands.

4. Dorsal and lateral surfaces of Phylum Gastrotricha is covered with spines, scales and warts.

5. Ventral surface with abundant cilia, hence called Gastrotricha.

6. Head with a number of sensory bristles.

7. Locomotion by cilia and muscular action. They glide over the bottom on ventral cilia.

8. Phylum Gastrotricha has multi-layered cuticle.

9. Epidermis with monociliated cells with gland cells and glandular adhesive tubes in some species as are gnathostomulids.

10. Circular and longitudinal muscles are present in the body wall of Phylum Gastrotricha.

11. The space between the body wall and gut is filled with loose organs and mesenchyme that indicate the acoelomate condition. Blastocoel forms during development but does not persist in the adult stage.

12. Digestive tract complete; mouth surrounded by bristles; pharynx triradiate and muscular.

13. Circulatory and respiratory systems are absent in Phylum Gastrotricha.

14. Nervous system consists of two cerebral ganglionic masses at the anterior of the pharynx with two lateral nerve cords.

15. Excretory organs of Phylum Gastrotricha are protonephridia with ciliated flame cells.

16. Phylum Gastrotricha are eutelic animals.

17. Hermaphrodite or parthenogenetic with simple gonads. Marine species are mostly hermaphroditic and freshwater species are parthenogenetic.

18. Fertilization is internal in Phylum Gastrotricha.

19. Development with determinate cleavage as in rotifers and other aschelminthes.

20. Blastopore forms both mouth and anus.

21. No larval stage occurs in the life cycle of Phylum Gastrotricha.

- **Affinities of Phylum Gastrotricha:**

A. Similarities with Rotifers:

1. Simple musculature.

2. External ciliation.

3. Forked caudal region.

4. Presence of adhesive gland,

5. Protonephridia with flame cells.

6. Eutelic animals.

Remark:

The similarities do not bear any close relationship between the two groups.

B. Similarities with Nematoda:

1. Cuticular structure.

2. Structure of the pharynx.

3. Muscle innervation.

4. Similar nature of the brain.

5. Cleavage pattern.

Remark:

From the above similarities, the gastrotrichs are more closely related to Nematoda than other groups of invertebrates. Hyman (1951) stated that gastrotrichs, nematodes along with rotifers would have arisen from a turbellarian-ancestor. Ruppert and Barnes (1994) considered that gastrotrichs and Nematoda both constitute a monophyletic group.

Some Indian gastrotrichs:

1. Chaetonotus similis,

2. Chaetonotus brevispinosus and

3. Chaetonotus trachyneusticus.

4. Nematomorpha

Characteristics of the Phylum Nematomorpha:

1. Body of Phylum Nematomorpha is long, heir-like, un-segmented and worm-like.

2. Length of the body ranging from 0.5 m to 1.0 m.

3. External collagenous cuticle.

4. Cuticle with small papillae, moulted periodically.

5. Absence of locomotory cilia in Phylum Nematomorpha.

6. Presence of only longitudinal body wall muscles.

7. Gut reduced, non-functional in adult stage.

8. Pseudocoel is mostly fluid-filled body cavity and filled with parenchyma.

9. Circulatory, respiratory and excretory systems are absent in Phylum Nematomorpha.

10. Nervous system of Phylum Nematomorpha consists of cerebral ganglion with an epidermal midventral nerve cord.

11. Absence of constancy of cell numbers (eutelic condition).

12. Sexes gonochoristic (= dioecious).

13. Simple gonads.

14. The sperm ducts open into a cloaca but there are no penial spicules (Fig. 15.16A).

15. Fertilization is internal in Phylum Nematomorpha.

16. Bilateral cleavage pattern.

17. The larvae bear a protrusible proboscis which bears spines (Fig. 15.17B).

18. Juveniles parasitize arthropods (e.g., crickets, grasshoppers and other insects) but adults are free-living.

19. Free-living, aquatic animals or live in damp soil.

- **Affinities of Phylum Nematomorpha:**

A. Affinities of Phylum Nematomorpha with Nematoda:

The nematomorphs bear many characteristics similar to nematodes, kinorhynchs and priapulids.

The following features show resemblances to the nematodes:

1. Un-segmented worm-like body as in nematodes.

2. Collagenous external cuticle in both nematodes and nematomorphs.

3. Only longitudinal muscles in the body wall as in nematodes.

4. Both respiratory and circulatory system absent in nematodes and nematomorphs.

5. Sexes separate as in nematodes.

6. Nature of body as in nematodes.

B. Affinities of Phylum Nematomorpha with Kinorhyncha:

Similarities:

1. Absence of respiratory and circulatory systems.

2. The cuticle of juveniles in kinorhynchs moults periodically as in nematomorphs.

3. Sexes separate in both groups.

4. Nature of body cavity.

C. Affinities of Phylum Nematomorpha with Priapulida:

Similarities:

1. External cuticle moults periodically in both groups.

2. Extensive body cavity in both groups (The body cavity is either a pseudocoel or eucoelom has not clearly understood).

3. Both groups are gonochoristic.

Remarks:

Considering the above similarities to the three groups the Nematomorpha are more closely related to the Nematoda rather than Kinorhyncha and Priapulida.

5. Loricifera

Characteristics of the Phylum Loricifera:

1. Phylum Loricifera are microscopic, un-segmented body, and about 200 to 300 μm in length.

2. Major part of the body called abdomen, covered by 6 overlapping protective plates comprising by rigid cuticle called lorica. So the name of the phylum is 'Loricifera' or lorica bearing.

3. Cone-shaped anterior region (introvert) can be retracted into the abdomen and bears recurved spines on the lateral sides, called scalids.

4. Rigid plated cuticle moulted periodically.

5. Mouth is situated at the end of the introvert and encircled by 8 stylets.

6. Neck composed of several segments.

7. External cilia is absent in Phylum Loricifera.

8. Body cavity of Phylum Loricifera is probably pseudocoelome.

9. Bulb-like pharynx with both circular and longitudinal muscles, and long midgut.

10. Protonephridial excretory system.

11. Gonochoristic (sexes separate).

12. Fertilization is probably internal in Phylum Loricifera.

13. Development not yet known.

14. A free-swimming larval stage called Higgins larva (Fig. 15.23B) is found which differs from the adult having a pair of un-jointed swimming appendages (toes) at the posterior end. The introvert lacks the stylets and thorax lacks the spines.

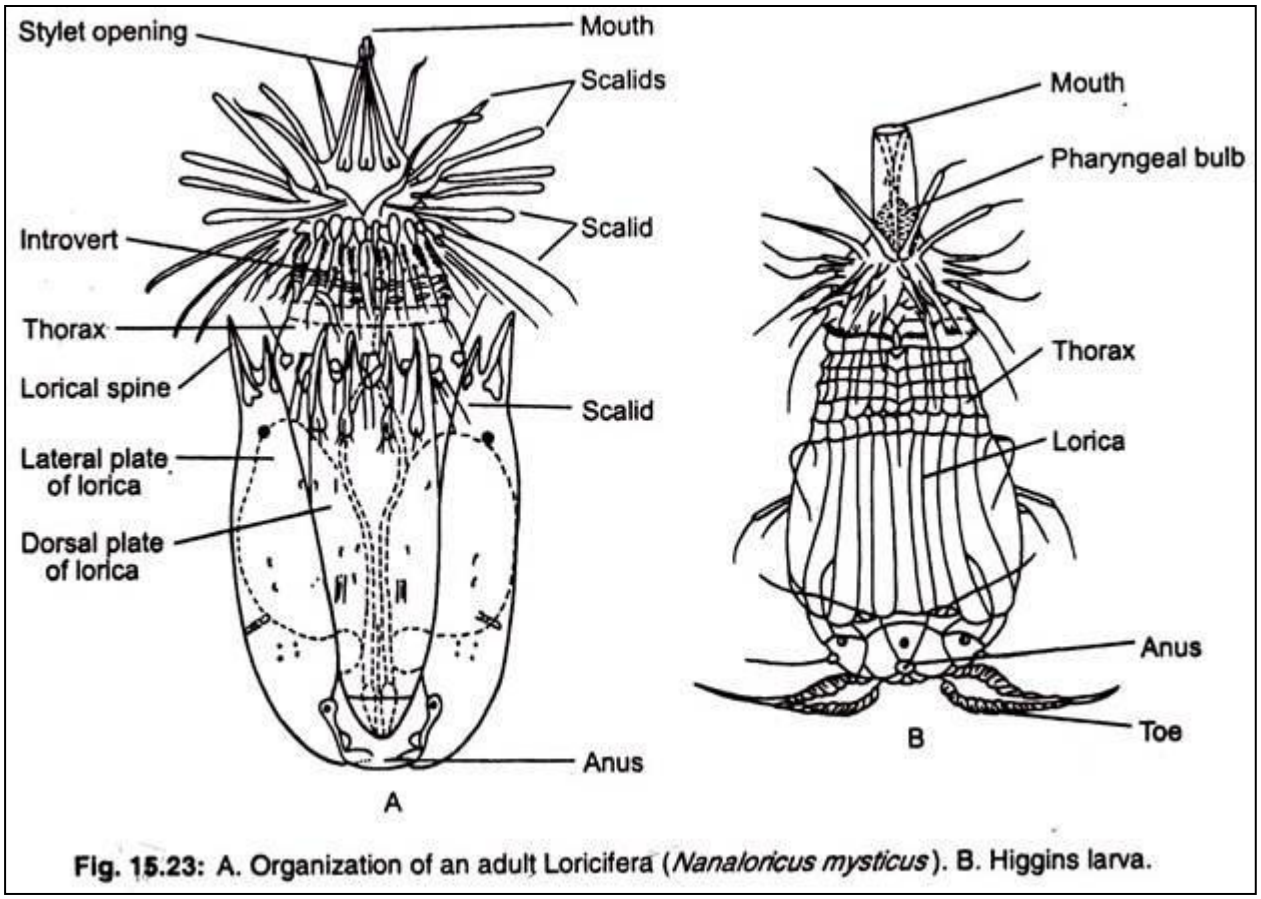


Fig. 15.23: A. Organization of an adult Loricifera (*Nanaloricus mysticus*). B. Higgins larva.

- **Phylogenetic Relationship of Phylum Loricifera:**

The relationships among the different phyla consider between the two groups. One group includes gastrotrichs, nematodes and nematomorphs and the other group considers rotifers and acanthocephalans.

Structure of cuticle, pharynx, nervous system and developmental pattern help to establish the relationship between the gastrotrichs and nematodes. Organization of epidermis and development of sperm show the similarities between the Rotifers and Acanthocephalans. The similarities among the rest phyla are not clear.

On the basis of moulting, the different phyla under Aschelminthes are divided into two main groups. One group includes rotifers and acanthocephalans which do not moult.

Morphological characters and molecular data indicate that the two phyla are closely related and should be placed in one phylum and may be called Syndermata.

The other group which includes Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Loricifera, Onychophora, Tardigrada and Arthropoda should be considered as Ecdysozoa that refers to the fact that the members of these phyla shed their cuticle during growth, though several phyla (such as Priapulida, Onychophora, Tardigrada and Arthropoda) are not included under Aschelminthes.

Probable Questions:

6. Describe the structure of Trochophore larva?
7. Write short note on metamorphosis of Trochophore larva.
8. Discuss evolutionary significance of trochophore larva?
9. Write the name of minor invertebrate phyla.
10. Discuss the evolutionary significance of phylum Rotifera?
11. Discuss the general characteristics of phylum Acanthocephala?

Suggested reading:

7. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
8. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
9. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
10. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972
11. Moore: An Introduction to the Invertebrates, Cambridge University Press, 2001
12. Kudo: Hand Book of Protozoology, Charles C Thomas Publishers, 193

UNIT X

Insect flight: types; structure concerned; functional mechanism; evolution of flight patterns in different insect groups

Objective:

In this unit we will discuss about Insect flight: types; structure concerned; functional mechanism; evolution of flight patterns in different insect groups.

Introduction:

Wings in living insects serve a number of functions, including active flying, gliding, parachuting, altitude stability while jumping, thermoregulation, and sound production.

Understanding the evolution of wings requires an understanding of the adaptive value of the intermediate or transitional stages in their development.

A. Structure of a wing:

The wings are not appendages; these are extensions of the body wall. In general, the insects possess one pair of wings on the metathorax. The wings develop as wing buds which may be either internal or external. Each wing is supported by an arrangement of branching ribs, called nervures.

Each rib carries the branches of tracheae, which remain functional up to the completion of the development of wing. The shape, pattern and number of wings differ in various insects. In Lepidoptera, both the wings are large and are covered by numerous brilliantly colored scales. In beetles, the anterior part is hard and known as elytra.

At the time of rest, it covers the membranous metathoracic wings. In Diptera, the anterior pair is well-developed but the posterior pair are shortened and known as the halters or balancers. In the Strepsiptera, the anterior wings are halters and posterior wings are well-developed. In the bee-parasites, the anterior pair is vestigial and the posterior pair is membranous. The wings are entirely absent in lice and fleas.

- **Cross section through the wing –**The wing membrane consists of two layers of integument. Veins include the nerves, blood space and tracheae. Wings do not containmuscle.
- **Venation -**Irregular network of veins are found in primitive insects. Longitudinal veins with limited cross-veins are common in many pterygote groups. Extreme reduction of all veins is common in small insects. Longitudinal veins are concentrated and thickened towards the anterior margin of the wing. This gives increased efficiency and support during insect flight.

The arrangement of veins in an insect's wing is called venation. It is an important means for the identification of species. The veins of a generalized wing are named as follows (Fig. 1):

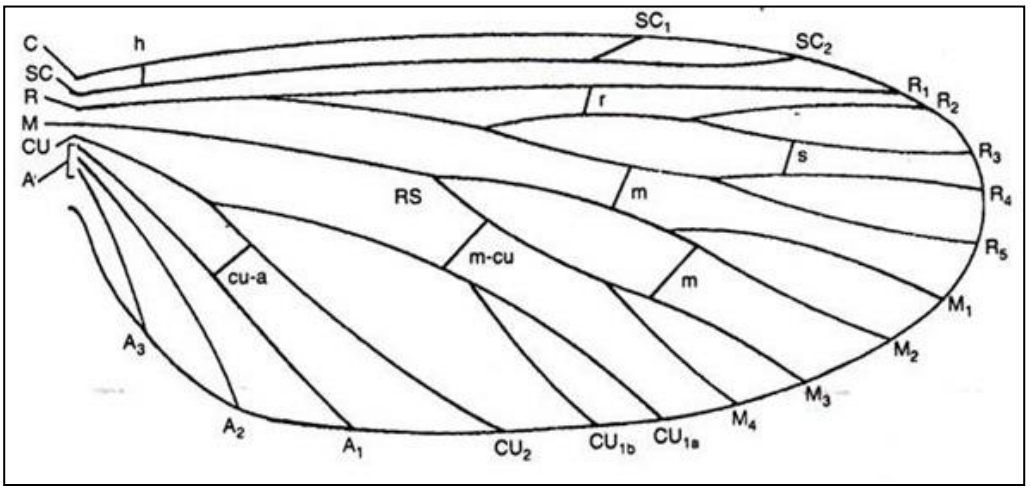


Fig.1: Venetion of wing

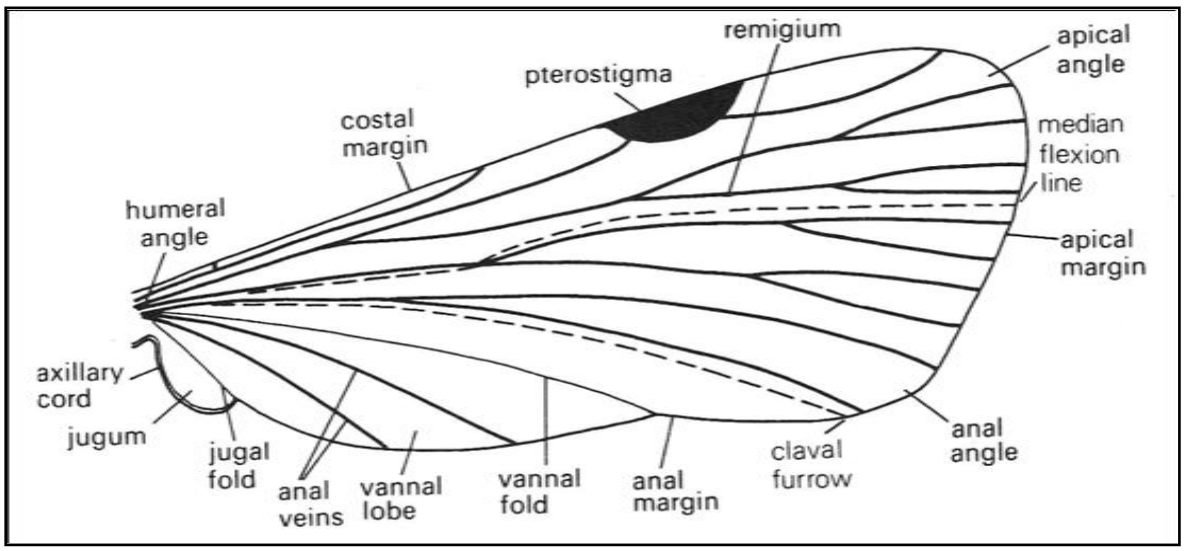


Figure: Diagram of a typical insect wing

I. **Costa (C):** It is a thick, un-branched vein that forms the anterior margin of the wing.

II. **Subcosta (Sc):** It is also another un-branched vein which lies below the costa (C).

III. **Radius (R):** It is a branched vein lying below the subcosta (Sc).

IV. **Media (M):** It is a branched vein that runs medially and lies below the radius (R).

V. **Cubitus (CU):** It is usually a branched vein and lies below the media (M).

VI. **Anal veins (A):** These are short, un-branched veins which lie below the cubitus (CU).

All these veins run longitudinally and are linked by cross veins which are in different lengths. The areas between the longitudinal and cross veins in the wings are called cells. The cells are numerous in May flies and dragon flies.

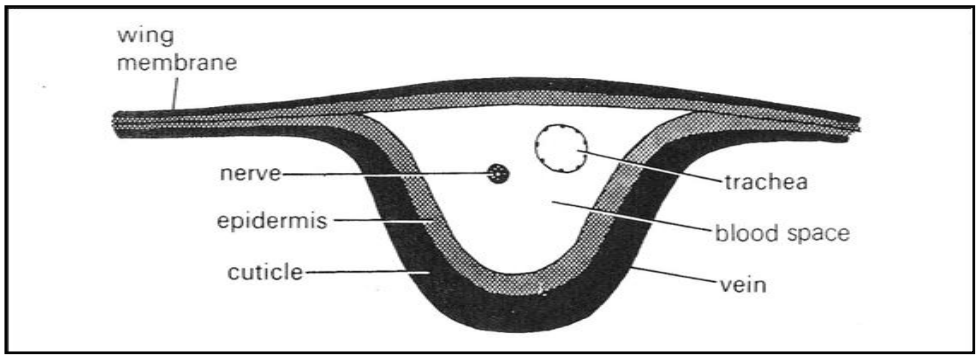


Figure: Diagrammatic section through part of a wing including the TS of a vein.

- **Pterostigma -** Darkened area on forewing i n Hymenoptera, Pscoptera, Megaloptera and Mecoptera and on both wings in Odonata. Function as inertial mass in f light. Reduces wing flutter during gliding in odonates and thereby increases flight efficiency. Provide passive control of angle of attack in small insects which enhances efficiency during flapping flight.
- **Wing folding -** Flexion lines reduce passive deformation and enhances wing as anaerofoil. Fold lines used in folding of wings over back.

B. Wing movement:

- **Wing coupling -** Orthoptera and Odonata wings are not anatomically coupled. Coordination of forewings and hindwings in flight is accomplished by pattern-generator neurons in the central nervous system. Detail of anatomical wing-coupling varies among taxonomic groups, suggesting that it evolved independently several times.
- **Types of Coupling:**

  a) Primitive Mecopteran Pattern,

  b) Jugate coupling,

  c) Frenate coupling in female and

  d) Frenate coupling in male

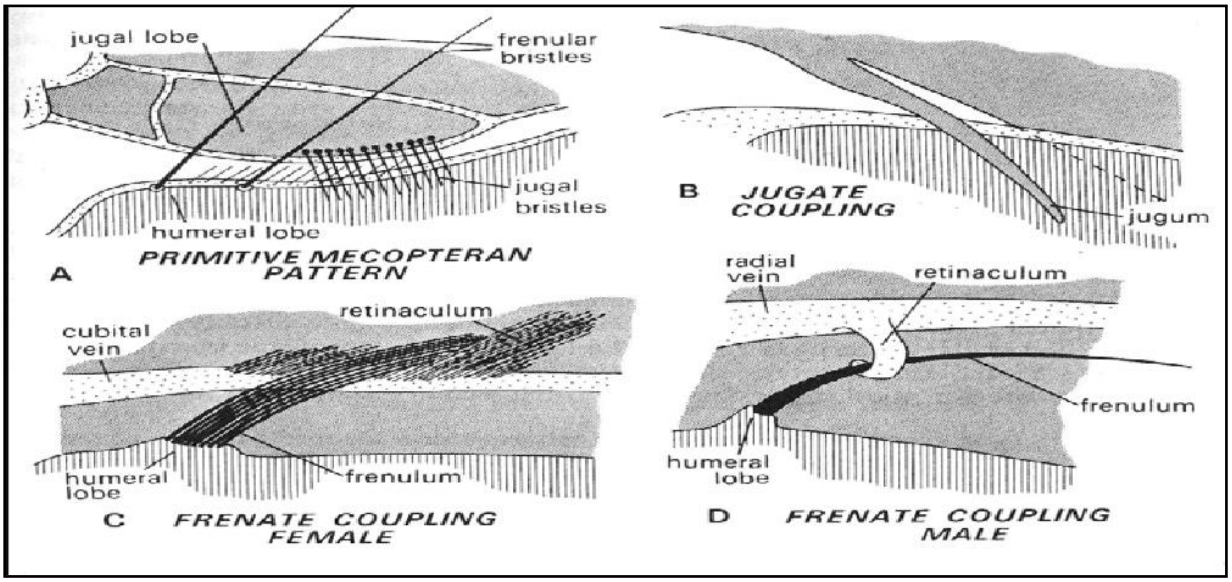


Figure: Types of wing coupling

- **Halteres in Diptera -** Derived from the hindwings. Functions to maintain stability inflight.

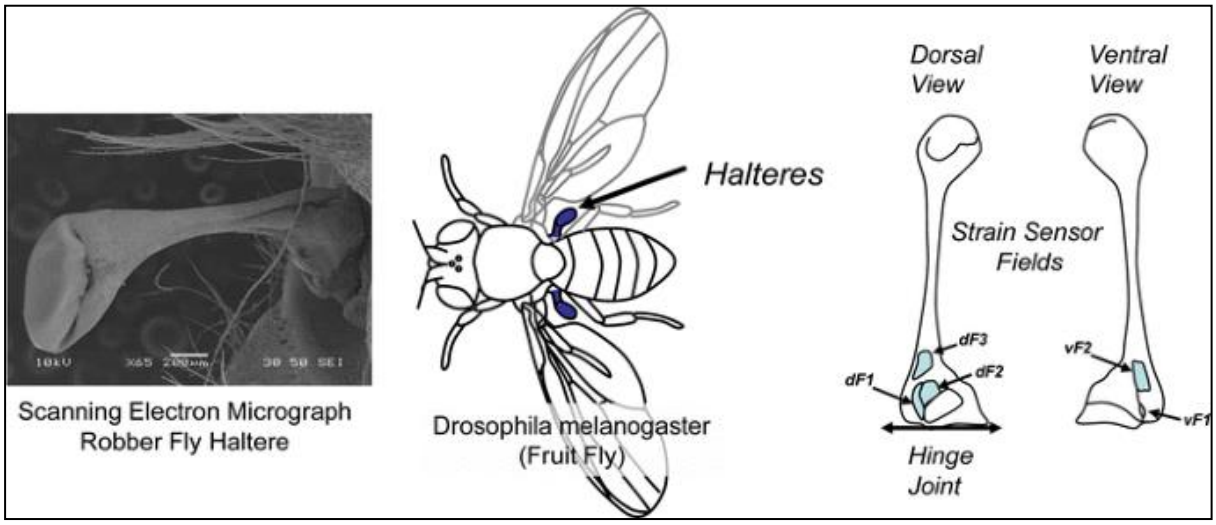


Figure: Halteres in Dipteras

C Two general mechanisms of wing movement:

I. **Direct mechanism-** Downward movement of the wing is the result of the contraction of muscles attached directly to the wing. This flight mechanism is under the control of **synchronous flight muscle**. Because each wing beat is control by a nervous impulse, the direct mechanism of insect flight is said to be **neurogenic** in origin.

II. **Indirect mechanism-** Downward movement of the wing is the indirect result of the contraction of muscles attached to the thorax. This flight mechanism is under

the control of **asynchronous flight muscle**. Because several too many wing beats occur for every nervous impulse, the indirect mechanism of insect flight is said to be **myogenic** in origin.

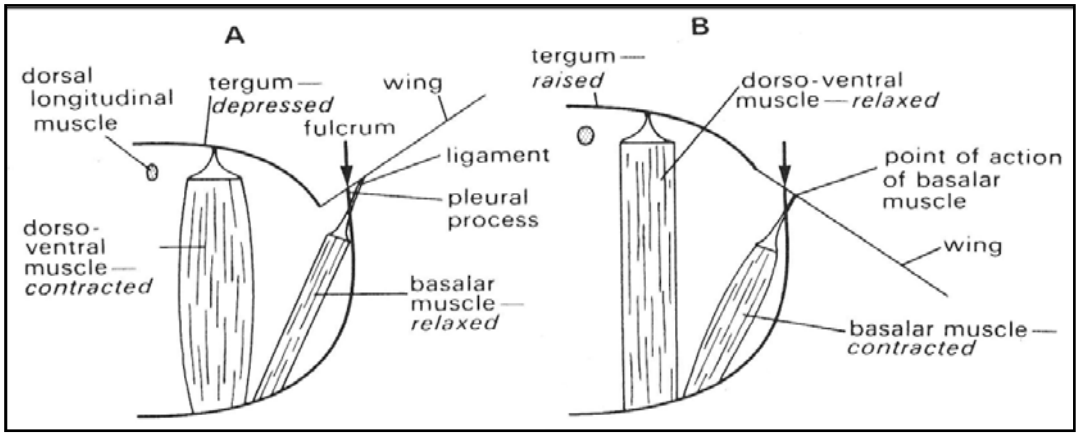


Figure: Diagrammatic cross section of the thorax illustrating the wing movements in an insect, in which the direct wing muscles cause depression of the wings.

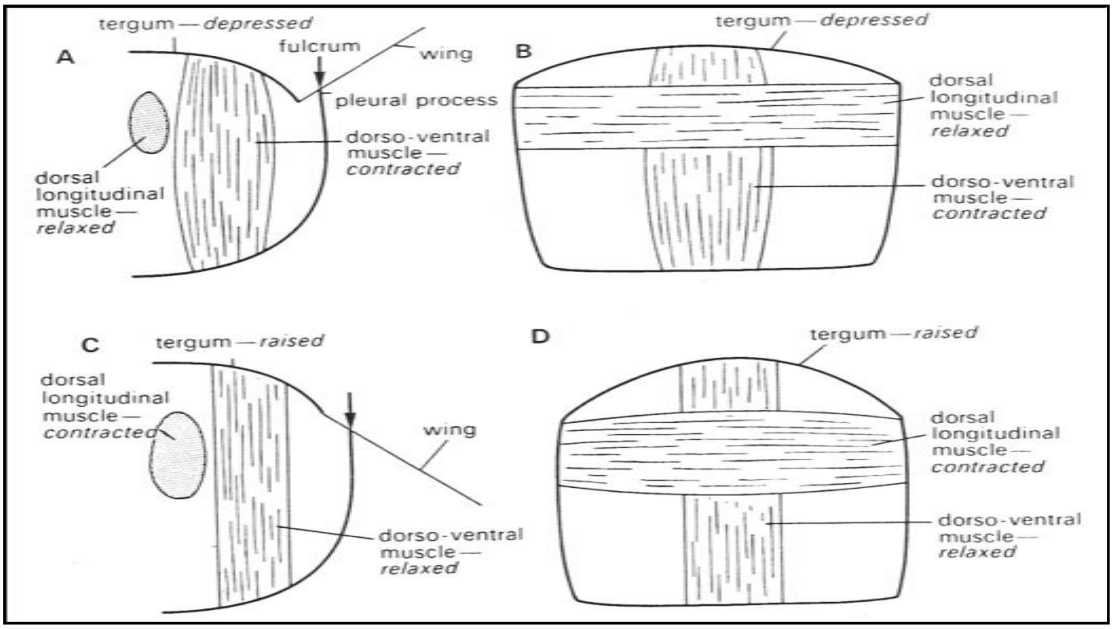


Figure: Diagrams illustrating the movement of wings in an insect in which both up and down movement of the wings are produced by indirect muscles (A and C). Cross section of the thorax, (B and D).

- **Taxonomic distribution of direct and indirect flight mechanism –**

Direct mechanism of wing movement is found in the Palaeoptera and the Blatteria. Indirect mechanism of wing movement is found in the Hymenoptera (bees), Diptera, some Coleoptera and some Hemiptera. Other groups (some Coleoptera and Orthoptera)

use a combination of direct andindirect mechanisms to move wings.

Efficiency of flight production - Muscles used in flight arise in the coxa in many insects and also f unction in leg movement during terrestrial locomotion. Elastic properties of wing hinges, wing muscles and thorax greatly enhance flight efficiency. Elasticity of these structures is due to the presence of the protein resilin. In locust, 86% of the energy used in the upstroke can by recovered during the down stroke. Elasticity of the thorax means that wings are in stable position only at the top of the upstroke or at the bottom of the down stroke.

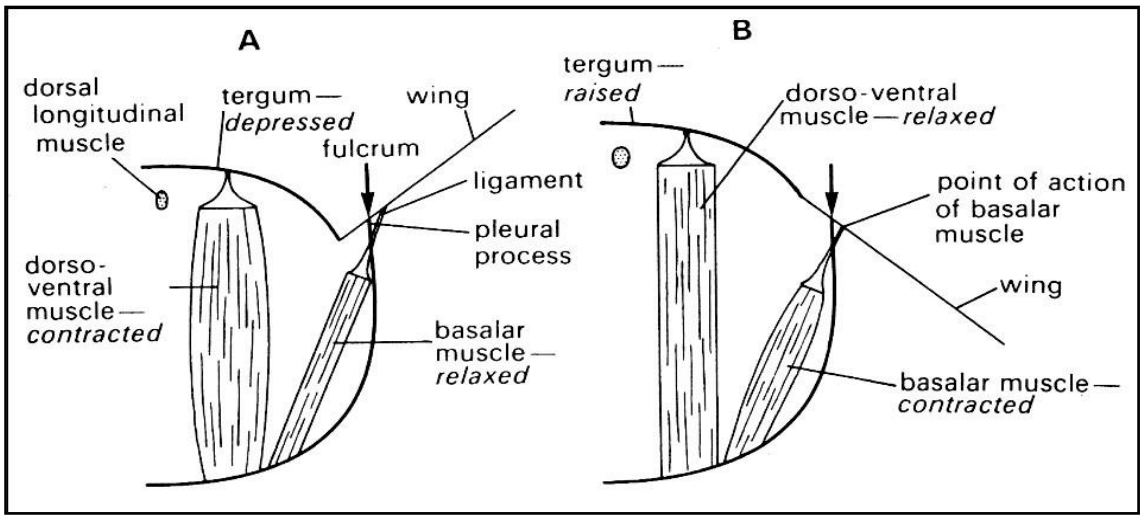


Figure: Diagrammatic cross-section of the thorax illustrating the wing movements in an insect in which the direct wing muscles cause depression of the wings.

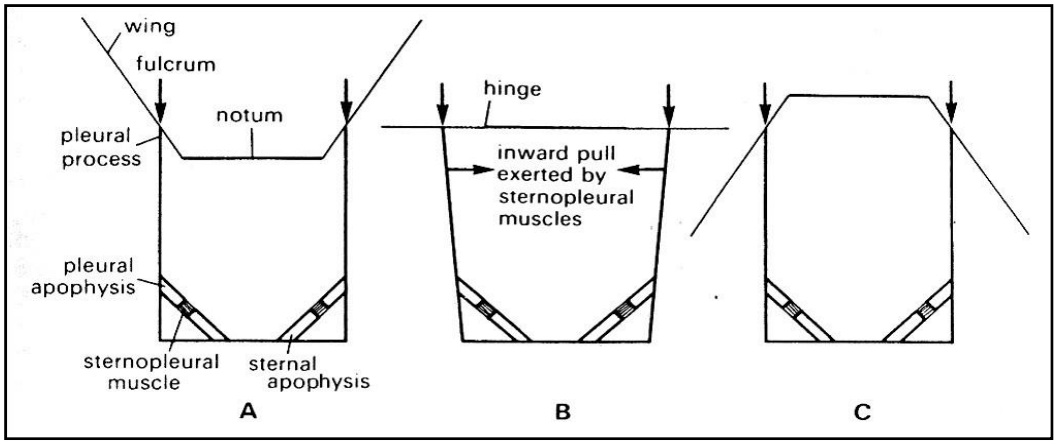


Figure: Diagrammatic cross-section of the insect thorax illustrating the distortion of the thorax produced by wing movement. A. Wings stable in the up position, B. Unstable position due to inward pull of the sternopleural muscles, C. Wings stable in the down position.

Stroke plane is the plane in which wings move relative to the long axis of the body. Stroke plane determines the rate of forward movement during flight. Insects control turning movements by changing the stroke plane on one side of the body relative to that on the other side of the body. Stroke plane in locust averages about 30°. Hovering

requires an average stroke plane of 0°.

Amplitude of wing beat is the distance in degrees travelled by the wing tip from the top ofthe up stroke to the bottom of the downstroke. Greater amplitude produces greater power output.

Insect control turning movements by varying the amplitude of the wing beat on both sides of the body.

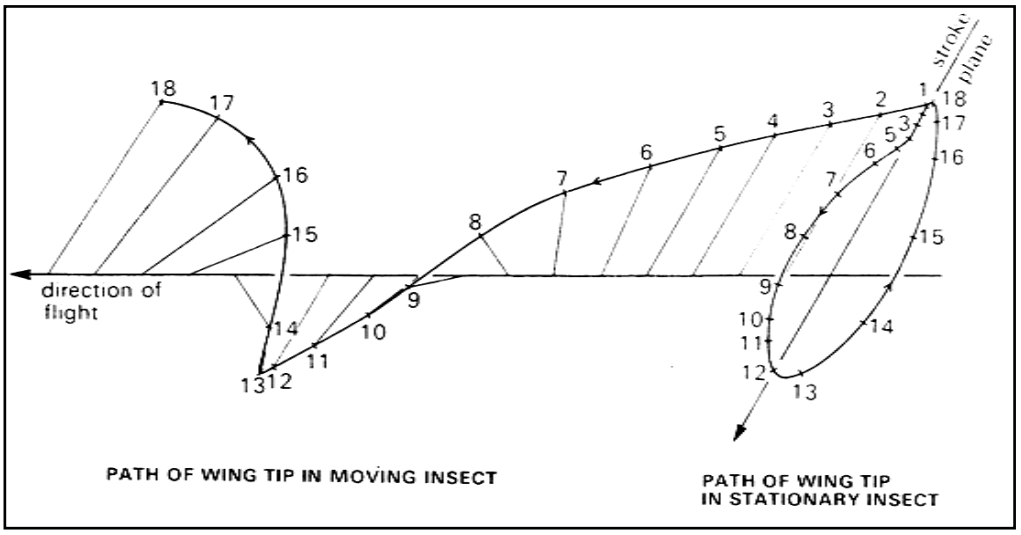


Figure: Movement of the tip of the forewing in a typical insect. The numbers indicate the positions of the wing at regular time intervals throughout the stroke and the lines joining the wing-tip path to the flight axis show the angle which the long axis of the wing makes with thebody at different stages of the stroke.

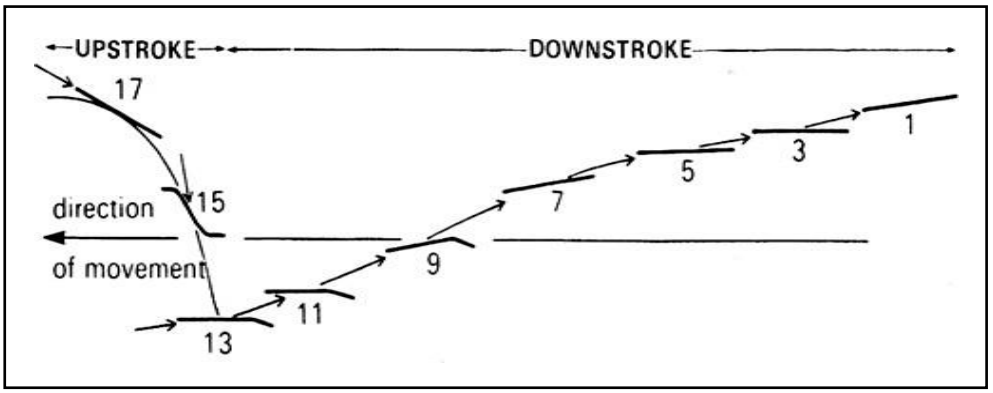


Figure: Changes in the twisting of the mid section of the forewing.

Wing beat frequency is the number of wing beats per second (Hz). Insects with synchronous flight muscles have low wing beat frequencies (≤ 50 Hz) relative to insects with asynchronous flight muscle (100-1000 Hz). Wing beat frequency is also negatively correlated with body size. The greater the wingbeat frequency the greater will be the

power out put and the greaterthe lift production.

Wing twisting occurs when the relative position of the leading and tailing edges of the wing changes du ring the wing beat. Both passive and active (=muscular) forces are responsible for

changing wing twisting. Wing twisting controls the angle of attack which control lift and forward movements of the insect in space.

Relative wind is the movement of air relative to the wing. Its two components are due to 1) the air speed of the insect and 2) the velocity of the wing in the stroke plane.

Angle of attack is the angle at which the relative wind strikes the chord of the wing. Insects control the angle of attack by active and passive twisting of the wing. Changes in the angle of attack are used to control the force of relative wind.

- **Force of relative wind** has two components:

Lift is the vertical force produced by relative wind. This force iswhat gets insects into the airand keeps them there.

Thrust is the horizontal force produced by relative wind. This force moves insect forward through the air.

Forward thrust is resisted by **profile drag** (the cross-sectional area t he insect presents to theair) and mostly by **induced drag (**development of vortices at the wing tips.)

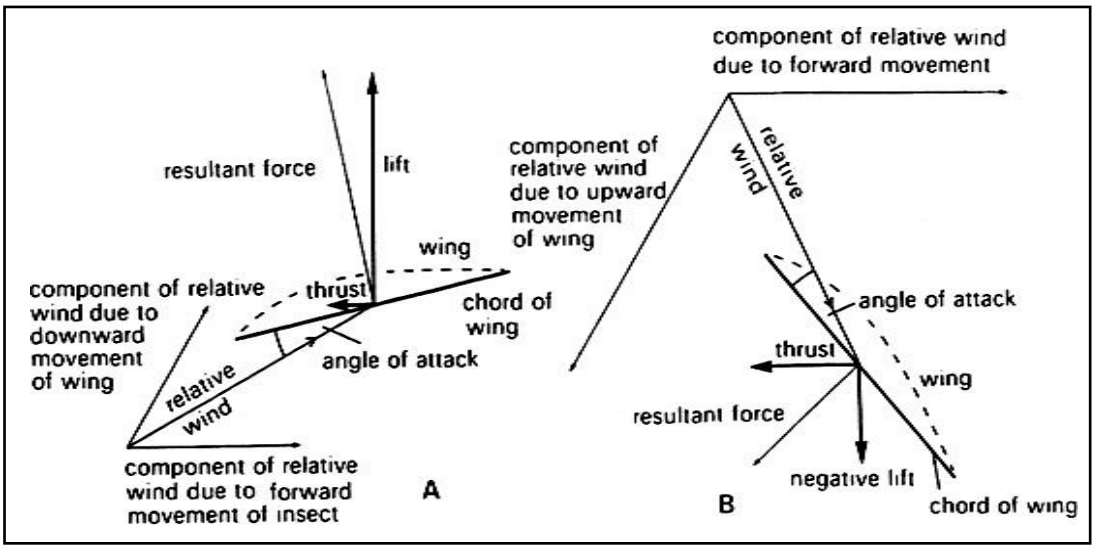


Figure: Diagrams illustrating the forces acting at the mid -point of the wing a t different phases of the wing beat corresponding roughly with positions 7 and 15 in earlier Figure. A. Down stroke of wing with positive angle of attack. B. Upstroke of wing with negative angle of attack.

➢ Types of flight:

1) **Hovering** in flight is accomplished by changing the stroke plane to nearly horizontaland maintaining a positive angle of attack throughout the wing beat.

2) **Gliding** requires a high lift-to drag ratio. This is accomplished mostly by changing theangle of attack to maximize thrust and minimize drag and negative lift.

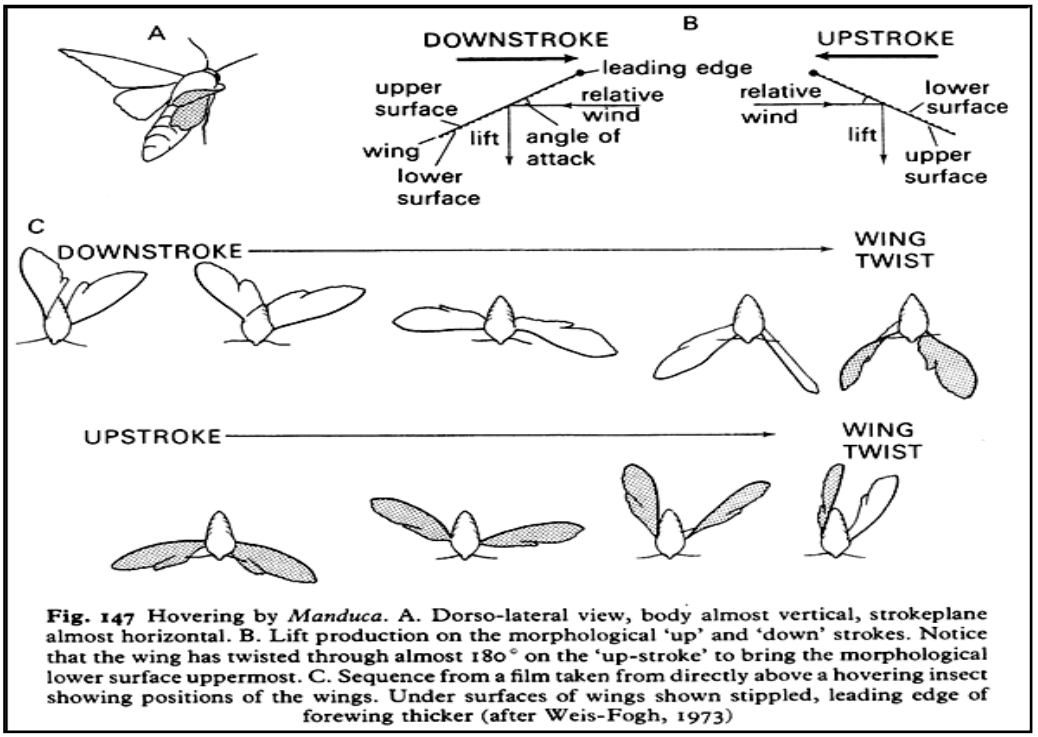


Fig. 147 Hovering by *Manduca*. A. Dorso-lateral view, body almost vertical, strokeplane almost horizontal. B. Lift production on the morphological 'up' and 'down' strokes. Notice that the wing has twisted through almost 180° on the 'up-stroke' to bring the morphological lower surface uppermost. C. Sequence from a film taken from directly above a hovering insect showing positions of the wings. Under surfaces of wings shown stippled, leading edge of forewing thicker (after Weis-Fogh, 1973)

Figure: Hovering in insect. A) Dorso-lateral view, body almost vertical, stroke plane almost horizontal. B) Lift production on t he morphological "up" and "down" strokes. C) Sequence from a film taken directly above a hovering insect showing positions of the wings.

Probable questions:

1. Describe the structure of a typical insect wing with proper diagram.
2. What is pterostigma? State its function.
3. Name the different veins of an insect wing.
4. Describe the two general mechanisms of wing movement in insect with diagrammaticillustrations.
5. Describe different types of wing coupling in insects. State the evolutionary significance of wing coupling.
6. What do you mean by stroke plane in regard to insect flight?
7. What do you mean by wing beat frequency and wing twisting?
8. What are the two components of relative wind which forms the angle of attack?
9. Describe different types of insect flight.
10. Write short notes on: Taxonomic distribution of flight mechanism in insects.
11. Write short notes on: Efficiency of flight production in different insects.

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-WesleyPubl. Co., 436 p.
3. Gullan, P .J & Cranston, P .S. (2010). T he Insects: A n ou line of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O .W. & Davies, R .G. (1977). IMMS' General textbook o f Entomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R . E. (1935). Principles o f Insect Morphology. Mcgraw-Hill B ook Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

HARD CORE THEORY PAPER (ZCORT – 101)

Group B (Insect Organization)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT– 101 (Non-Chordate Biology and Insect Organization)	**XI**	Modern classification of insects	3	107-121
	XII	General organization, segmentation, division of body: i) Head and mouth-parts in general ii) Thorax and thoracic appendages. Modification of legs and wings. iii) Abdomen and abdominal appendages.		122-135
	XIII	Integument: Basic structure and functions; modification in different insect groups.		136-144
	XIV	Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber.		145-155
	XV	Structure and functions of the central nervous system in insects.		156-162
	XVI	Insect visual organs, their structure and functional mechanisms		163-172
	XVII	Insect blood: composition, functions, morphology of circulatory system.		173-177
	XVIII	Structure and function of sound producing organs in insects; types of insect sound; significance of sound production		178-187
	XIX	Exocrine glands: Lac gland, Wax gland, Silk gland, Labial gland		188-195
	XX	Metamorphosis in insects: hormonal regulation; factors affecting metamorphosis; diapause		196-209

UNIT XI
MODERN CLASSIFICATION OF INSECTS

Objective:

In this Unit we will discuss about Modern Classification of Insects.

Introduction:

Insects are a very large, highly diverse group of small, mostly terrestrial and aquatic animals. They have a three-part body plan with a head, thorax, and abdomen, where six legs and (in most species) four wings are attached to the thorax, and the head has a pair of compound eyesand a pair of antennae.

Insects have by far the largest number of species of all known living things and make up the vast majority of species in Kingdom Animalia. Over a million have been described by science so far. Their relatedness t o each other can get rather convoluted, which makes the scientific classification of insects into a Linnaean-style taxonomic tree a challenge.

Characteristics of Class Insecta:

1. Insects are air breathing mostly terrestrial and rarely aquatic arthropods.

2. Body is divided into three distinct regions head, thorax and abdomen.

3. Head consists of six fused segments and bears a pair of compound eyes, a pair of antennae and mouth parts adapted for chewing, biting, piercing, sucking, siphoning or sponging type.

4. Thorax comprises three free segments, each bearing a pair of legs and two pairs of wings borne on the second and third segments.

5. Abdomen comprises 7-11 segments and devoid of appendages.

6. Liver is absent but salivary glands are usually present.

7. Heart is elongated, tubular and is divided into eight chambers situated in the abdomen.

8. Respiration by branched tracheae.

9. Excretion by Malphigian tubules.

10. Sexes are separate.

11. Development is sometimes direct, more usually complicated by metamorphosis.

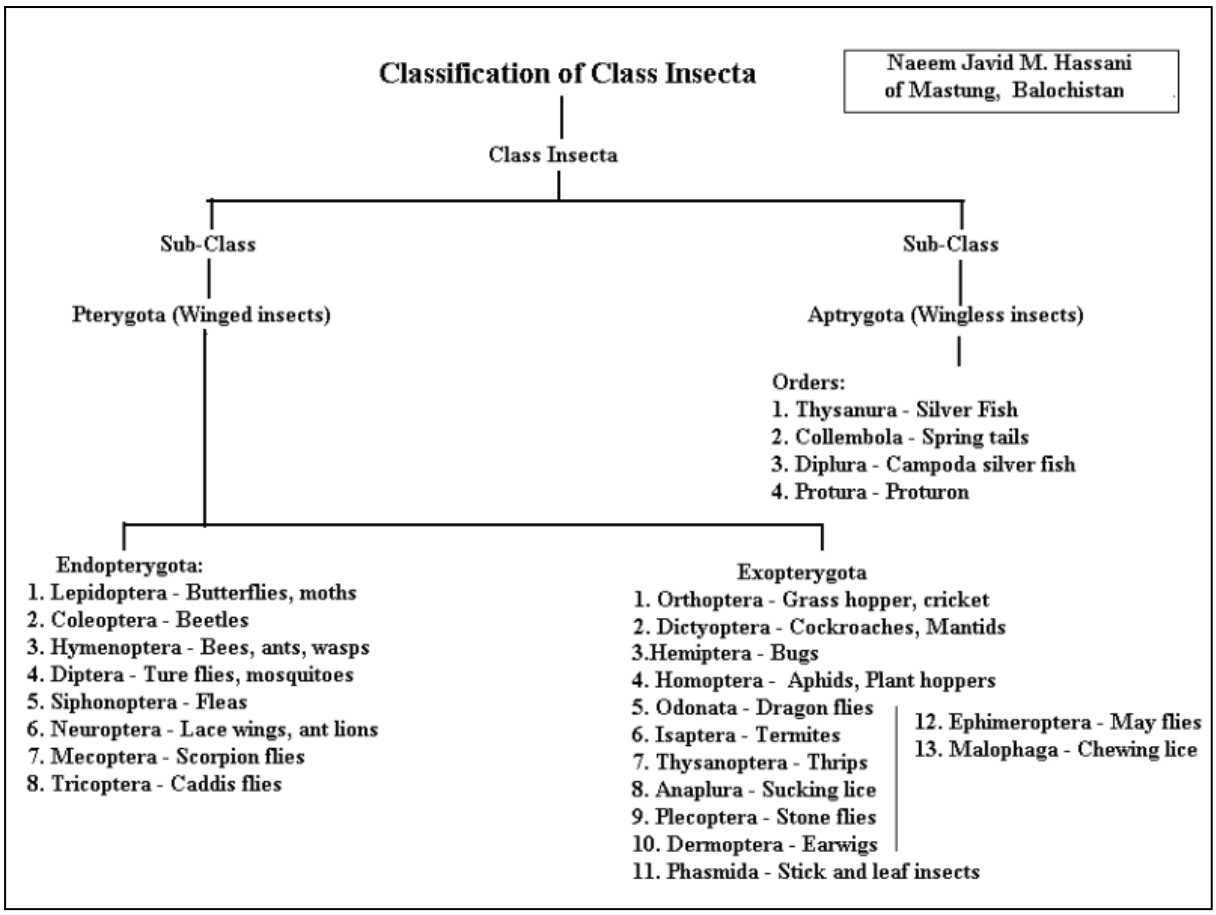


Fig: Schemative diagram of Insect classification (approx)

Subclass I. Apterygota (Gr., a = without ptera = wings):

1. Primitive wingless insects.

2. Metamorphosis is little or absent.

Order 1. Protura:

1. Minute soft bodied insects.

2. Antennae and compound eyes are absent.

3. Mouth parts are biting.

4. Abdomen twelve segmented.

5. Tracheae are absent.

6. Metamorphosis little.

Example:

Acerentomon.

Order 2. Thysanura:

1. Small primitive insects.

2. Body is elongated, flattened and naked or scaly.

3. Mouth parts biting; antennae long and well developed.

4. Abdomen eleven segmented.

5. A pair of cerci many segmented and a median caudal filament long.

6. Primitive metamorphosis.

Example:

Lepisma (Silver fish).

Order 3. Aptera:

1. Small, white or pale blind insects.

2. Body flattened.

3. Mouth parts biting.

4. Antennae long, many segmented.

5. Caudal filament absent.

6. Metamorphosis absent.

Example:

Campodea.

Order 4. Collembola:

1. Minute insects; body naked or covered with hair or scales.

2. Antennae four to six segmented.

3. Eyes absent.

4. Mouth parts biting,

5. Abdomen six segmented and usually with a ventral tube, tenaculum and spring.

6. Metamorphosis absent.

Examples: Spring tails, Snow flies.

Subclass II. Pterygota (Gr., ptera = wings):

1. Wings are usually present.

2. Abdomen devoid of appendages except genitalia and cerci.

3. Metamorphosis simple or complex.

Division I. Exopterygota:

1. Metamorphosis primitive or simple.

2. Young stages are nymphs.

3. Wings develop externally.

Order 5. Orthoptera:

1. Medium sized to large insects.

2. Usually with a pair of narrow leathery forewings or tegmina and a pair of well developed membranous hind wings.

3. Mouth parts strong biting and chewing.

4. Compound eyes and usually two or three ocelli.

5. Short or long, simple or segmented cerci.

6. Simple metamorphosis.

Examples:

Locusts, Grasshoppers Crickets, etc.

Order 6. Gryllobiattodea:

1. Small thysanuri form insects.

2. Wings absent, mouth parts biting type.

3. Antennae filiform, many segmented.

4. Compound eyes are small, ocelli absent.

5. Simple metamorphosis.

Example:

Grylloblatta.

Order 7. Blattaria:

1. Medium to large sized insects

2. Wings present or absent.

3. Antennae long, many segmented and filiform.

4. Mouth parts biting and chewing type.

5. Cerci prominent and segmented.

6. Metamorphosis simple.

Examples:

Periplaneta, Blatta.

Order 8. Phasmida:

1. Large leaf or stick-like insects.
2. Antennae usually long, filiform and may segmented.
3. Compound eyes are small, ocelli two or three or absent.
4. Mouth parts biting type.
5. Wings present or absent.
6. Cerci small and segmented.

Examples:

PhyIlium, Carausius (Stick insect).

Order 9. Mantodea:

1. Medium to large insects.
2. Head small, triangular and bears large compound eyes with three or more ocelli.
3. Mouth parts biting type.
4. Prothorax greatly elongated.
5. Fore-legs modified for capturing and holding the prey.
6. Wings large, small or absent. Metamorphosis is simple.

Example:

Mantis (Praying mantis).

Order 10. Dermaptera:

1. Small to medium sized insects.
2. Body is elongated with smooth or tough or chitinous covering.
3. Mouth parts biting type.
4. Abdomen is provided with force-like cerci at the posterior end.
5. Simple metamorphosis.

Example:

Forficula.

Order 11. Diploglossata:

1. Very small insects.
2. Body depressed covered with short hairs.
3. Mouth parts biting type.
4. Eyes and wings absent.

5. A pair of unsegmented cerci present.

6. Simple metamorphosis.

Example:

Hemimerus.

Order 12. Plecoptera:

1. Meidum to large sized, elongated somewhat flattened and soft bodied insects.

2. Head broad and bears a pair of long filiform 25 to 100 segmented antennae.

3. Compound eyes are moderate in size with two or three or no ocelli.

4. Mouth parts biting type.

5. Two pairs of equal many veined wings, hind pair with large anal area.

6. Abdomen eleven segmented, last segment reduced and bears long filiform multi-segmented cerci.

7. Simple or hemimetabolous metamorphosis.

8. Larvae are known as naiads which are aquatic.

Examples:

Stone flies, Salmon flies.

Order 13. Isoptera:

1. Social and polymorphic insects living in colonies under a caste system.

2. Small to medium sized mostly soft bodied and pale coloured insects.

3. Head small to very large, bears moniliform short or long many segmented antennae.

4. Mouth parts biting and chewing type.

5. Compound eyes vestigial with two or no ocelli.

6. Wings when present, two pairs, similar in size, shape and venation.

7. Cerci short and simple or 2-8 segmented.

8. Metamorphosis simple.

9. A colony contains both winged and wingless sexual individuals and wingless sterile workers and soldiers.

Examples:

Termites, White ants.

Order 14. Zoraptera:

1. Minute winged or wingless insects.
2. Antennae moniliform, nine segmented.
3. Compound eyes and ocelli are present in winged forms, absent in wingless forms.
4. Mouth parts biting type.
5. Cerci short and un-segmented.
6. Metamorphosis simple.

Example:

Zorotypus.

Order 15. Embioptera:

1. Small slender insects with somewhat flattened body.
2. Head large bears filiform antennae.
3. Compound eyes small in female, often large in male, ocelli absent.
4. Mouth parts biting chewing type.
5. Thorax nearly as long as abdomen.
6. First tarsal segment of fore-legs enlarged to contain glands and spinnerets.
7. Wings when present two pairs, membranous, similar in size and venation.
8. Metamorphosis simple or hemimetabolous.

Example:

Oligotoma.

Order 16. Corrodentia:

1. Small compact winged or wingless insects.
2. Head large and free, bears short or long filiform antennae.
3. Compound eyes are usually large or without ocelli.
4. Mouth parts modified biting type.
5. Cerci absent.
6. Metamorphosis simple.

Examples:

Book lice, Bark lice, Dust lice.

Order 17. Mallophaga:

1. Small wingless ectoparasitic insects.

2. Body is broad or elongated, flattened dorsally, tough and well chitinised.

3. Head hypognathus without setiform or clavate antennae.

4. Compound eyes are reduced, ocelli absent.

5. Mouth parts biting type.

6. Legs short with stout claws specialised for clasping hairs and feathers.

7. Cerci absent.

Example:

Bird lice.

Order 18. Anoplura:

1. Minute to small sized, permanently ectoparasitic on mammals.

2. Body is flattened, elongated without wings.

3. Head small with short 3-5 segmented, setiform antenne.

4. Compound eyes reduced or absent, ocelli absent.

5. Mouth parts piercing and sucking type.

6. Legs short with single claw specialised for grasping hairs.

7. Cerci absent.

Example:

Pediculus (Human louse).

Order 19. Ephemerida:

1. Small to medium sized, soft bodied delicate slender insects.

2. Antennae short setiform.

3. Mouth parts vestigial.

4. Well developed compound eyes and three ocelli present.

5. One or two pairs of fragile many veined wings.

6. Cerci are very long, filiform and multisegmented with a similar median caudal filament.

7. Metamorphosis simple or hemimetabolous.

8. Naiad (nymphs) are aquatic with abdominal tracheal gills.

Example: Ephemera (May fly).

Order 20. Odonata:

1. Medium sized to large, slender, swift flying predaceous insects.

2. Head mobile, bears large compound eyes and ocelli.

3. Mouth parts biting type.

4. Two pairs of similar long narrow net- veined wings.

5. Naiads (nymphs) are aquatic with or without exterior gils.

6. Metamorphosis simple or hemimetabolous.

Examples:

Dragon flies, Damsel flies.

Order 21. Thysanoptera:

1. Minute, slender, terrestrial insects.

2. Body somewhat compressed dorsally or almost cylindrical.

3. Antennae short six to nine segmented.

4. Compound eyes with three ocelli present.

5. Mouth parts modified for piercing, chafing and sucking.

6. Wings when present two pairs of long and narrow with few veins.

7. Abdomen 10-11 segmented with an ovipositor.

8. Simple metamorphosis.

Example:

Thrips.

Order 22. Hemiptera:

1. Minute to large, oval or elongated and dorso-ventrally flattened terrestrial or aquatic insects.

2. Antennae 2 to 10 or rarely 25 segmented.

3. Eyes large with or without ocelli.

4. Mouth parts piercing and sucking type.

5. Wings when present two pairs, fore pair usually thickened basally and membranous apically in Heteroptera and wholly membranous in Homoptera.

6. Cerci absent.

7. Anal respiratory filaments present in some aquatic forms and anal filaments in male coccids.

8. Metamorphosis simple.

9. Mostly phytophagous, some are predaceous.

Examples:

Belostoma, Aphids, Cicadas, Scale insects.

Division II. Endopterygota:

1. Metamorphosis is complex (complete).

2. Young stages are known as larvae.

3. Wings develop internally.

Order 23. Megaloptera:

1. Medium to large sized insects.

2. Head prognathus, bears many segmented slender antennae.

3. Mouth parts biting type.

4. Wings two pairs, similar in shape, size and venation.

5. Cerci absent.

6. Metamorphosis complex including aquatic larvae.

Examples:

Sialis, Corydalus.

Order 24. Neuroptera:

1. Minute to medium sized, mostly terrestrial insects.

2. Head hypognathus, bears large and widely separated compound eyes.

3. Antennae usually filiform.

4. Mouth parts biting type.

5. Wings two pairs, similar in shape, size and venation.

6. Cerci absent.

7. Complex metamorphosis, carnivorous.

Example:

Chrysopa.

Order 25. Raphidiodea:

1. Small to medium sized, elongated, fragile terrestrial insects.

2. Antennae setiform, many segmented.

3. Compound eyes prominent with three or none ocelli.

4. Mouth parts biting type.

5. Two pairs of similar wings.

6. Female with long slender ovipositor.

7. Metamorphosis is complex.

Examples:

Snake flies, Serpent flies.

Order 26. Mecoptera:

1. Small to medium sized, slender, predaceous insects.

2. Head usually elongated and vertical.

3. Antennae long, filiform and many segmented.

4. Compound eyes are large and widely separated, ocelli three or none.

5. Mouth parts biting type.

6. Wings two pairs, similar in shape, size and venation.

7. Cerci short, simple or two segmented.

8. Metamorphosis complex.

Example:

Panorpa (Scorpion flies).

Order 27. Trichoptera:

1. Small to medium sized, diurnal and nocturnal moth-like insects.

2. Antennae long, filiform and many segmented.

3. Compound eyes are well developed with three or none ocelli.

4. Mouth parts biting type.

5. Wings two pairs, covered with hairs and scales.

6. Cerci one or two segmented.

7. Metamorphosis complex or holometabolic.

8. Larva aquatic.

Example:

Philopotamus (Caddis fly).

Order 28. Lepidoptera:

1. Medium to large sized flying terrestrial insects.

2. Body and wings are completely covered by flat overlapping, scales and hairs.

3. Antennae variable often clavate or serrate, hooked or knobbed or plumose.

4. Compound eyes large with two or no ocelli.

5. Mouth parts siphoning type with long coiled proboscis.

6. Wings two pairs, fore pair often largest.

7. Metamorphosis complex.

8. Larvae caterpillars with three pairs of thorcic legs and two to four pairs of abdominal prolegs.

Examples:

Butterflies, Moths.

Order 29. Coleoptera:

1. Minute to large insects with leathery integument.

2. Antennae variable in shape and size, usually 11 segmented.

3. Eyes conspicuous but cecelli absent.

4. Mouth parts biting and chewing type.

5. Wings two pairs, fore wings or elytra similar to body texture and useless in flight.

6. Abdomen usually 10 segmented.

7. Cerci absent.

8. Metamorphosis complex.

Examples:

Beetles, Weevils.

Order 30. Strepsiptera:

1. Minute free living and endoparasitic dimorphic insects.

2. Antennae short flabellate.

3. Eyes conspicuous but ocelli absent.

4. Mouth parts atrophied biting type.

5. Fore-wings small white, hind-wings large fan-shaped.

6. Metamorphosis complex and hyper metamorphosis.

Example:

Stylops.

Order 31. Hymenoptera:

1. Minute to large sised, social or parasitic insects

2. Antennae 12 segmented in male and 13 segmented in female.

3. Ocelli generally present.

4. Mouth parts sepcialised for biting, chewing lapping arid sucking.

5. Wings two pairs, membranous and narrow.

6. Abdomen always with basal pedicel.

7. Female with conspicuous ovipositor modified into saw, drill or sting.

8. Metamorphosis complex.

Examples:

Wasps, Bees, Ants.

Order 32. Diptera:

1. Minute to medium sized, diurnal, nocturnal, terrestrial and aquatic insects.

2. Body slender with fragile integument.

3. Antennae variable, simple or with arista.

4. Eyes large and separate with three ocelli.

5. Mouth parts modified for sucking, lapping piercing and sponging.

6. Fore-wings developed for flight, while- hind-wings reduced to halteres.

7. Metamorphosis complex.

8. Larvae limbless and vermiform known as maggots.

Examples:

Houseflies, Mosquitoes, Midges.

Order 33. Siphonoptera:

1. Minute to small sized insects ectoparasitic on birds and mammals.

2. Body laterally compressed.

3. Head small with or without eyes and ocelli.

4. Antennae short and capitate.

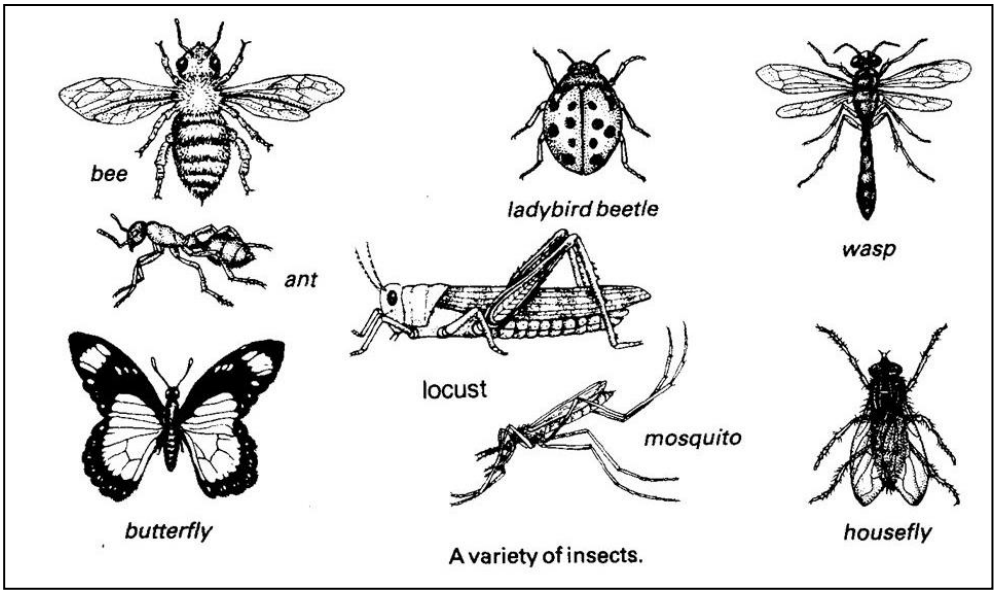
5. Mouth parts piercing and sucking type.

6. Cerci absent.

7. Metamorphoses complex.

Example:

Fleas.



A variety of insects.

Order (Species)	Description	Example	Order (Species)	Description	Example
Diptera (7058)	sucking mouthparts two wings	Black Fly	**Odonata** (194)	carnivorous, hard chewing mouthparts	Damselfly
Hymenoptera (6028)	social, some have stingers	Hornet	**Hemiptera** (3079)	sucking mouthparts, wings overlap abdomen	Aphid
Coleoptera (6748)	hard body parts and wings, chewers	Beetle	**Trichoptera** (546)	aquatic, hairy wings	Caddisfly
Siphonaptera (180)	sucking mouthparts, no wings	Louse	**Orthoptera** (133)	plant eating, chewing mouthparts	Grasshopper
Neuroptera (75)	membranous wings, biting mouthparts	Lacewing	**Lepidoptera** (4692)	scales on wings, caterpillar larvae	Butterfly
Ephemeroptera (301)	large triangular wings, non-funtional mouthparts	Mayfly	**Plecoptera** (250)	two pairs of long wings, acquatic	Stonefly
Thysanoptera (102)	plant eating, bristled wings	Thrip	**Megaloptera** (20)	freshwater, 2 pairs of wings, chewers	Alderfly

Probable questions:

1. Write down the characteristic features of class Insecta. Who are endopterygotes?
2. Classify class Insecta upto living orders.
3. Write down the characteristic features of the following orders and cite examples.

a) Coleoptera b) Lepidoptera c) Orthoptera d) Diptera c) Heteroptera

4. Write down the evolutionary pattern of insect classification.
5. Insects of w hich or der are know n as t rue f liers? State their advantage over insects belonging to other orders.
6. State the characteristic features of the following insect orders.

   Trichoptera b) Hymenoptera c) Dictyoptera c) Siphonaptera

Suggested readings:

1. Gullan, P .J & C ranston, P .S. (2010). The Insects: A n out line of E ntomology (4thEdition). Wiley-Blackwell, 584 p.
2. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
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UNIT XII

General organization, segmentation and division of body in insect

Objectives:

In this unit we will discuss about General organization, segmentation and division of body ininsect

I. Head and mouth parts in general

Head of insect:

The head is entirely encased in rigid exoskeleton (head capsule) which supports the mouth parts, antennae and eyes and within it lies the brain.

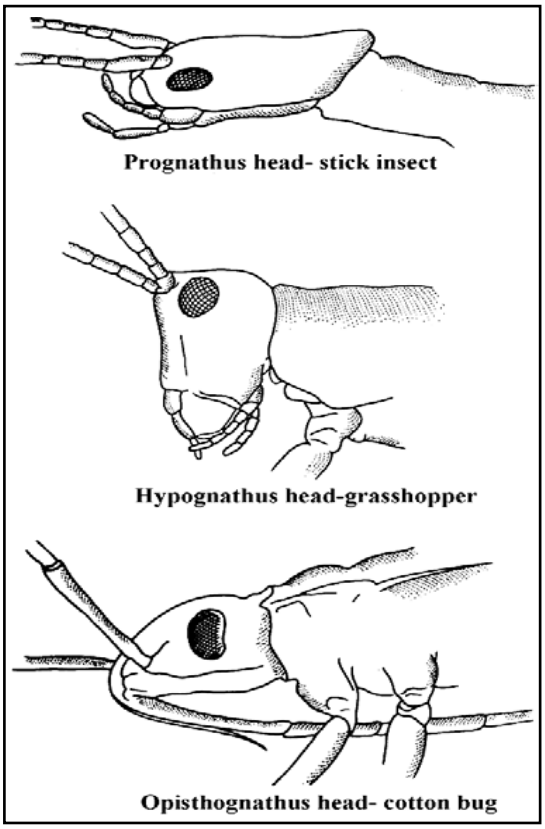
- **Orientation of the head:**

Based on the inclination of the long axis and the position of mouth parts, the heads of insects are oriented into three types:

i. **Prognathus**- The long axis of the head is horizontal, or s lightly inclined ventrally and this is in line with the long axis of the insect's body. The mouth parts are pointed forward. E.g. Stick insects, mostly Coleoptera.

ii. **Hypognathus-** The long axis of the head is at right angle (vertical) to the long axis of the body. The mouth parts are directed downwards ventrally. E.g. Cricket, Grasshoppers

iii. **Opisthognathus-** The long axis of the head is horizontal and the mouth parts are directed backward so that they arise between the anterior legs. E.g. Plant sucking bug.



- **Structural components of insect head:**

The head has lost all the traces of segmentation and forms a strongly sclerotized capsule. However, it is a well known fact that at least 6 anterior segments are fused in

the head. In the head of a generalized insect the following structures are clearly recognized:

i. **Frons**- It is a large unpaired upper sclerite that occupies the frontal area of the head which extends from the frontal sutures to the clypeus and it laterally extends up to the eyes and to the genal suture running from the eyes to the anterior condyle of the mandible. The frons bears the median ocellus.

ii. **Clypeus-** It is separated from the frons by the epistomial or fronto- clypeal or clypeal suture and the upper lip or labrum is attached along its anterior border.

iii. **Labrum**- It is an unpaired sclerite movably articulated with the clypeus with the clypeo- labral suture.

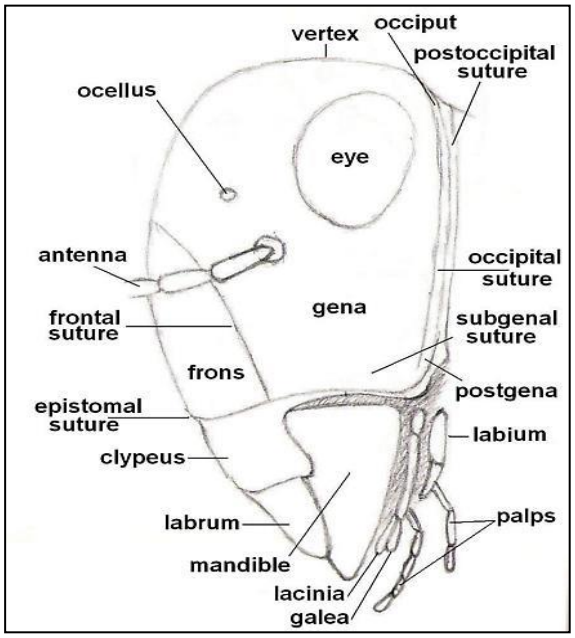


Fig: Lateral view of head of a cricket

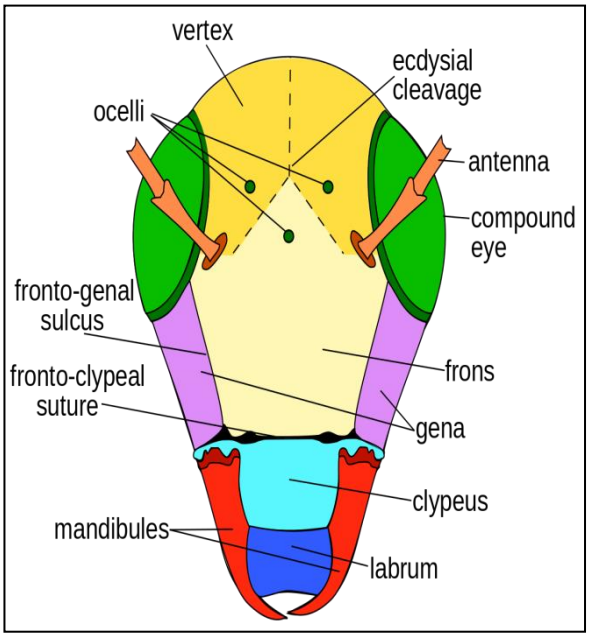


Fig: Anterior view of head of a cricket

iv. **Vertex**- It is the upper part of the head which lies between the compound eyes and behind the frontal suture. This area includes the dorsal portion of the two sclerites separated by the coronal suture or ecdysial cleavage line. These lateral sclerites are called the parietals, which extend back to the occipital sclerite and down each side of the head to include the compound eyes and the genae.

v. **Occiput-** It is a narrow, arching, sclerite lying behind the vertex and extending downward on each side to form postgenae. Behind the occiput lies a narrow rim which attaches the head to the neck membrane. A few small cervicle sclerites are present in the neck membrane.

vi. **Genae**- These represent the parietals or lateral areas that extend below the eye and between the genal suture and the post genae.

vii. **Post genae**- The part of the occiput below the level of the compound eyes and

behind the occipital suture is known as post genae.

viii. **Compound eyes**-These are located in the lateral areas (parietal) and each eye is surrounded by a narrow ocular sclerite.

ix. **Ocelli-** These are simple eyes, 3 in number. The median ocellus is located in the frons and thelateral ocelli are placed near the compound eyes.

x. **Antennae**- These arise in front of the eyes. Each is set in a membranous area, the margin of which is reinforced by a ridge, the antennal ridge.

xi. **Tentorium**- It is an endoskeletal structure which braces the head internally. The tentorium is composed of 2 pairs o f invaginations which unite within the head t o form a strong frame, overlying on t he ventral nerve cord and supporting the foregut which passes above it. It has anterior, posterior and dorsal arms. The anterior arms arise as invagination at each end of the clypeal suture, the posterior arms arise from the lower part of the post occipital suture and the dorsal arms extend from the anterior arms to the bases of the antennal sclerite.

Mouth parts of insects:

The structures on t he lower part of the head are known as mouth parts, which are variously adapted to the diet of the insects. The mouth parts are formed from modified appendages of head segments. They surround the mouth opening and in most cases are quite exposed and visible. However in a few insect groups eg. Collembola the mouth parts are enclosed by the lower p art of t he head. Mouth parts in a generalized insect comprise of the followingstructures:

i. **Labrum**- flap like structure attached to the clypeus and is capable of a limited up and down movement; it acts as upper lip and helps to pull the food into the mouth.

ii. **Epipharynx**- it is continuous with the roof of the mouth and t o the oesophagus. It is asensitive area which contains the organs of taste.

iii. **Mandible-** important structure in biting and chewing type of mouth parts; are stiff, solid and compact structures articulating with the head possessing about three faces and are pyramidal shaped. The upper or outer surface is articulated and connected with a gena, the inner face is provided with large number of teeth and the hinder ends are articulated by a round process that fits into a socket of the genae, post genae or subgenae. They are regarded as 1st pair of jaws and they move from side to side in a transverse plane. They also help in carrying the food particles and cutting of leaf or tissues; they act as organ of offence and defense.

iv. **Maxillae**- 2nd pair of jaws, work side to side just like mandible; shape varies with the kind of food and mode of feeding, composed of cardo, stripes and palpifer. The palpifer forms the maxillary palp which is 1-7 segmented and antennae like in shape. The palp functions as olfactory and gustatory organ. Maxilla is distally composed of two lobes- and

Outer lobe galea and an inner lobe lacinia, the inner border of lacinia is spined and often modified for cutting, grasping and grinding the food.

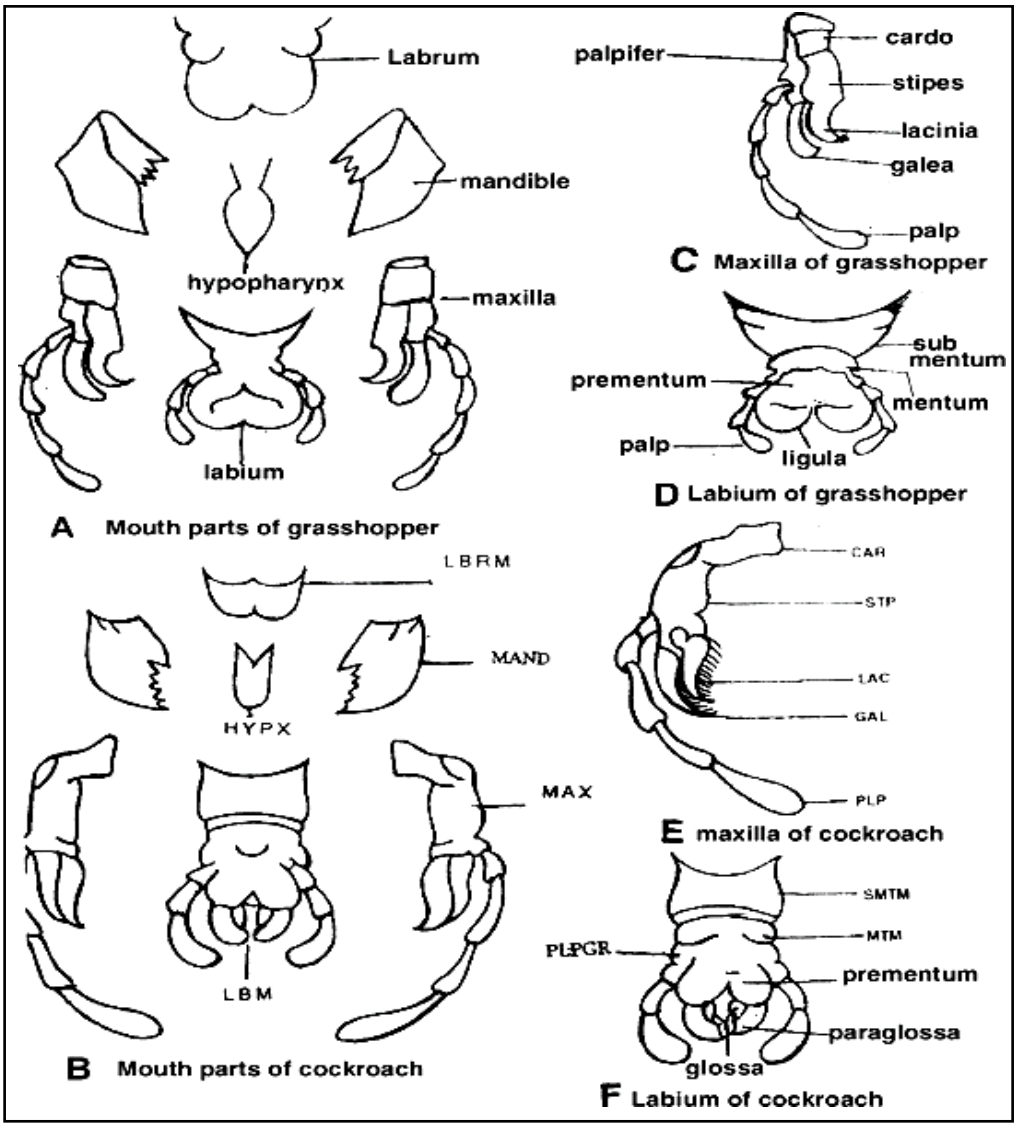


Fig: Mouth parts of Cockroach

v. **Hypopharynx** - it is a fleshy tongue, arising from the floor of mouth or preoral cavity and is usually attached to the inner wall of the labium. It is more or less covered with hairs. The salivary glands open through it.

vi. **Labium -** it is formed by the fusion of a pair of appendages homologous to maxillae. It form the lower lip o f t he mouth. The labium is divided into two primary regions- proximal- postmentum and a distal-prementum. The line of division between the two portions is known as labial suture. On either side near the base of prementum is the labial palp consisting of 1- 4 segments and functioning as sensory organs. From the distal margin of prementum arise 2 pairs of lobes which collectively constitute the ligula. Ligula comprises 4 unsegmented appendages- a median or inner pair of glossae and a lateral or outer pair of paraglossae.

II. Thorax and thoracic appendages, modification of legs Thoracic segments:

Thorax is the 2nd region of the body of the insects. It i s connected to the head by a membranous neck region that help in the movement of the head. Thorax comprises of three segments: Prothorax, Mesothorax and Metathorax. Each of these segments consists of a dorsal sclerite called the **tergum** or **notum**, a ventral sclerite called the **sternum** and on the either side is the **pleuron** which is membranous. Three pairs of jointed legs are attached to the thorax, one pair to each segment. 2 pairs of wings arise from the 2nd and 3rd segments of the thorax. Within the thorax are the powerful muscles which operate the wings and legs.

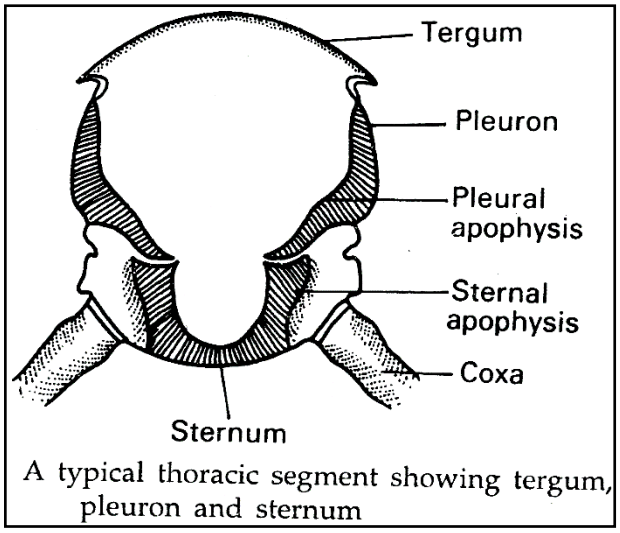


Fig: Diagram of thoracic segments

i)Insect leg:

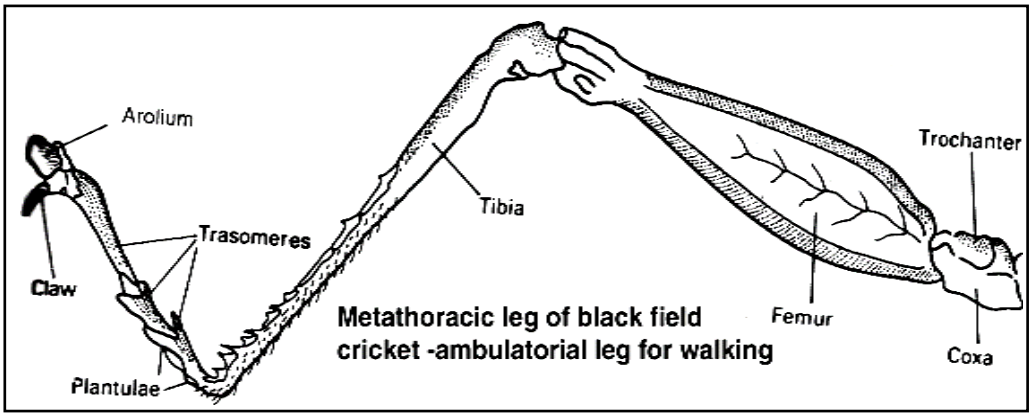


Fig: Structure of Insect leg

Insect legs have great structural variations adapted to life habits of insects. However the basic plan of all the legs is similar. The fore-legs are located on the prothorax, the mid-legs on the mesothorax, and the hind legs on the metathorax. Each leg has six major components, listed here from proximal to distal: coxa (plural coxae), trochanter, femur (plural femora), tibia (plural tibiae), tarsus (plural tarsi), pretarsus.

Modification of insect legs:

Like the mouthparts and antennae, insect legs are highly modified for different functions, depending on the environment and lifestyle of an insect.

The functions for which the legs have become modified include jumping, swimming, grasping, digging, sound production and cleaning.

- Saltatorial - jumping
- Raptorial - seizing
- Fossorial - digging
- Natatorial - swimming
- Cursorial - running
- Ambulatory- walking

1. Ambulatory legs:

Ambulatory legs are used for walking. The structure is similar to cursorial (running) legs. Examples: Bugs (order Hemiptera), leaf beetles beetles (order Coleoptera).

2. Saltatorial legs:

Saltatorial hind legs adapted for jumping. These legs are characterized by an elongated femur and tibia. Examples: Grasshoppers, crickets and katydids (order Orthoptera).

3. Raptorial legs:

Raptorial fore legs modified for grasping (catching prey). Examples: Mantids (order Mantodea), ambush bugs, giant water bugs and water scorpions (order Hemiptera).

4. Fossorial legs:

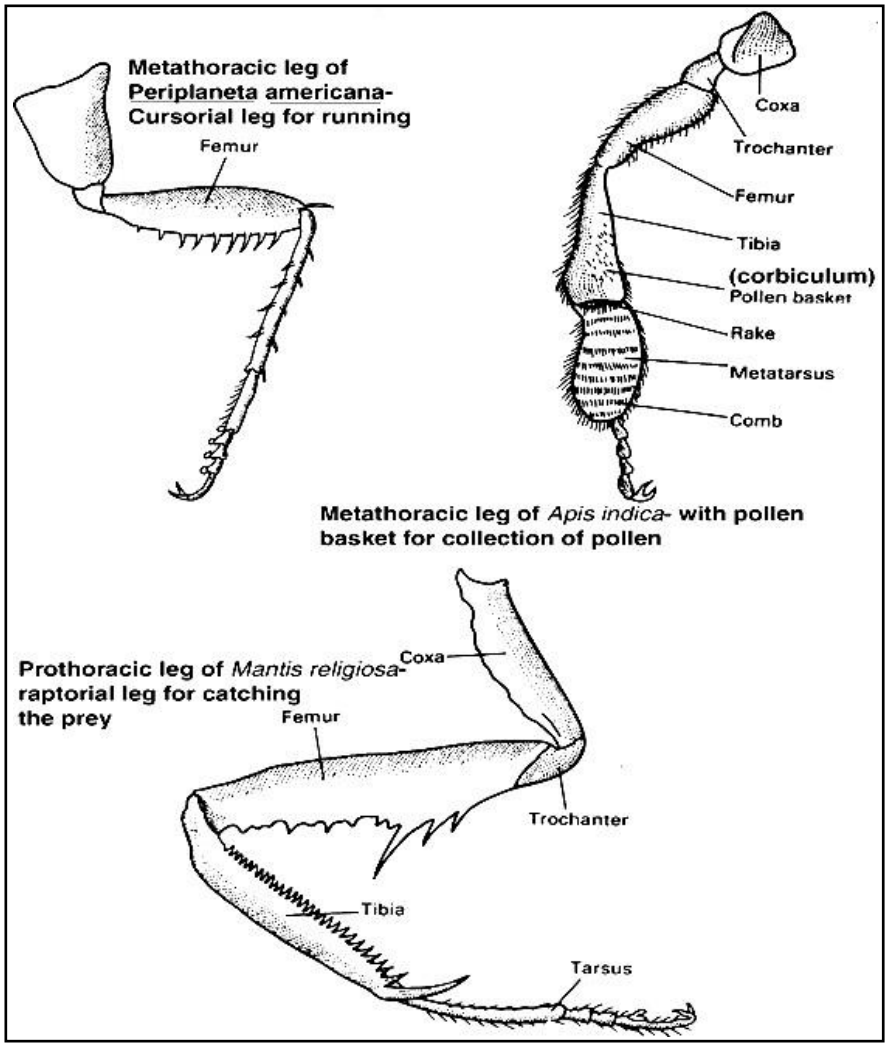
Fossorial fore legs are modified for digging. Examples: Ground dwelling insects; mole crickets (order Orthoptera) and cicada nymphs (order Hemiptera).

5. Natatorial legs:

Natorial legs are modified for swimming. These legs have long setae on the tarsi. Examples: Aquatic beetes (order Coleoptera) and bugs (order Hemiptera).

6. Cursorial legs:

Cursorial legs are modified for running. Note the long, thin leg segments. Examples: Cockroaches (order Blattaria), ground and tiger beetles (order Coleoptera).



Different types of wings:

1. Tegmina: Forewings are leathery and tough. They protect the membranous hind wings. e.g.: forewings wings of cockroach, grasshopper

2.Elytra: Hard, shell like without clear venation. They form horny sheet and protect the membranous hind wings and abdomen. e.g. Forewings beetles

3.Hemelytra: The base of the wing is thick like elytra and the remaining half is membranous. This thickened portion is divided in to corium, clavus,cuneus and embolium. They are useful of protection and flight e.g. Fore wings of bugs

4. Membranous: Naked thin with clear venation. Always useful of flight e.g.: Both

the wings of Dragonflies, bees and wasps, Hind wings of grasshopper, beetles etc.

5. Scaly wings: Wings thin , membranous but covered with unicellular scales all over the surface .They are useful for flight e.g.: Both the wings of moths and butterflies

6. Fringed wings: Wings are highly reduced with reduced venation. The wings are fringed with long marginal hairs giving a feather like appearance e.g.: Both the wings of thrips

7. Fissured wings: Forewings are longitudinally divided twice forming a fork like structure whereas hindwings are divided twice in to three arms. All the forks possess small marginal hairs . They are useful for flight. e.g.: Both the wings of plume moth

8. Halteres: The hind wings of houseflies are modified in to small microscopic structures called halteres and are divided in to three regions namely scabellum ,pedicel and capitellum.They act as balancers. eg. Hind wings housefly and front wings of male stylopids

9. Pseudohalteres: They are short and modified into pseudohalteres which are dumbbell shaped. Eg: Front wings of Strepsiptera

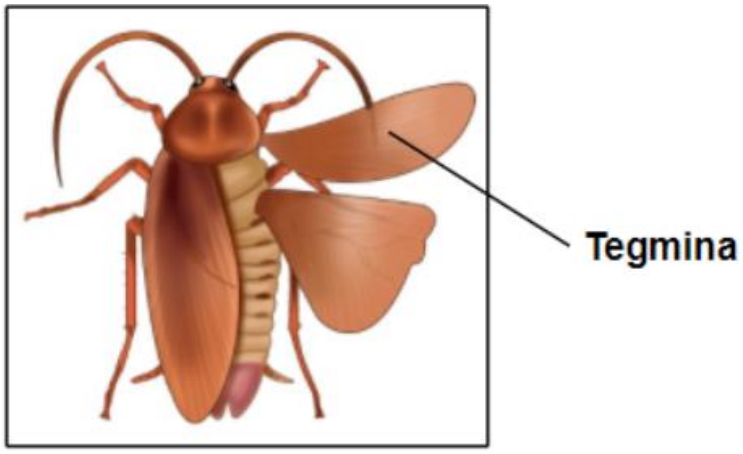


Fig: Elytra

III. Abdomen and abdominal appendages

The 3rd division of the insect body, the abdomen, consists of 10-11 segments. In most insects the actual number of segments present is less and it is not possible to recognize more than 5-6 segments (reduction a t t he posterior end). Each abdominal segment consists of a tergum or dorsal plate, a sternum or ventral plate, separated by a membranous area at each lateral side. The dorsal and ventral plates are joined together by membranous intersegmental membranes. There are small dark openings, the spiracles, set into the soft membranous area along each side of the abdomen. There is 1 pair in each segment.

Abdominal appendages:

i. **Cerci:** These tapering appendages arise from the tip of the abdomen (generally segment 11). Paired cersi occur in Diptera, Ephemeroptera, Zygoptera, Plecoptera, Dictyoptera. They are elongate multi segmented structures that function as sense or gans. In *Periplanet a americana* (Dictyoptera), cerci are simple, jointed and clothed in fine hair while in Dermaptera, these are s lerotized and form unjointed forceps. The cerci of nymphs of Zygoptera are modified to form t he lateral caudal lamellae w here as in adult m ale t he cer ci form claspers for grasping the female during copulation.

ii. **Styli:** Styli occur on most abdominal segments of Microcoryphia, Zygentoma and Diplura and on t he 9 th sternum of s ome ma le O rthopteroids. In some br istletails the s tyli are articulated with a distinct coxal plate and helps in locomotion but generally the original coxal segment is fused with the sternum

iii. **Eversible vesicles:** they are short cylindrical structures found on some pregenital segments of apterygotes. They are closely associated with the styli when present and believed to have the ability to take up water from the environment.

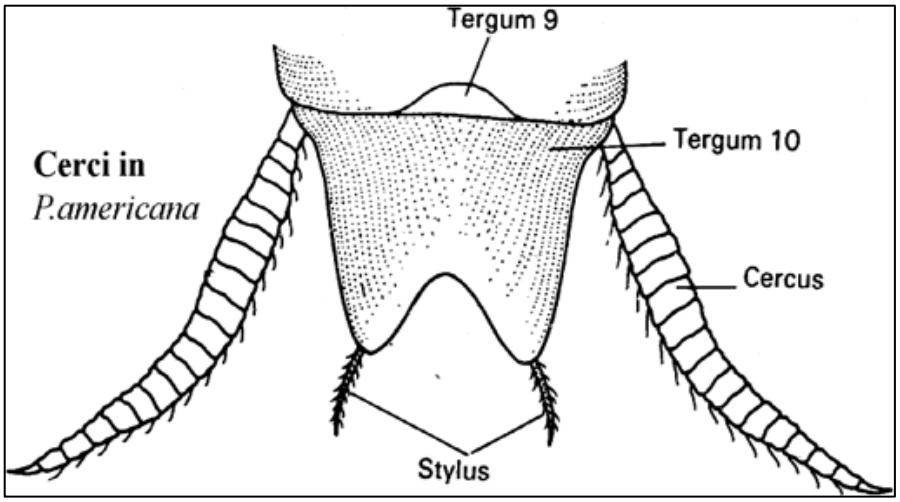
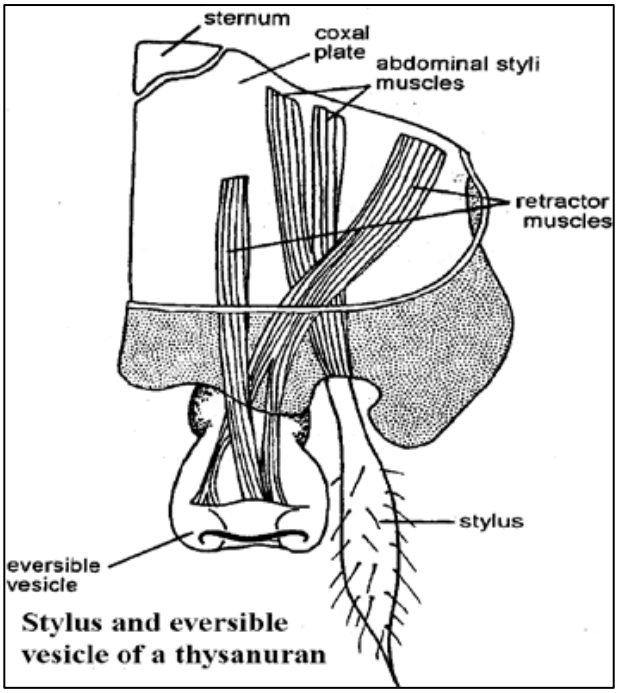


Fig: Parts of abdominal appendages



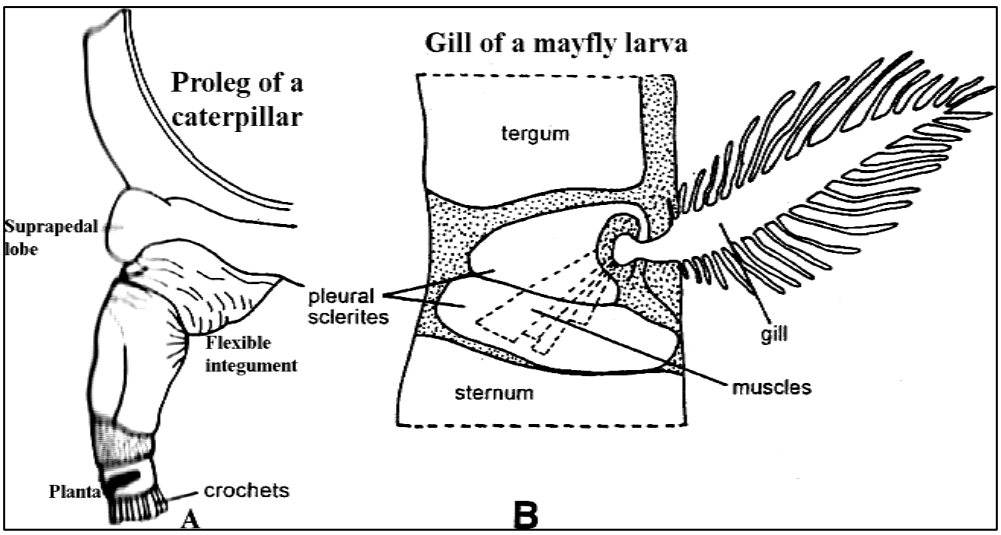
Stylus and eversible vesicle of a thysanuran

iv. **Prolegs (pseudopods or larvapods):** Segmentally arranged, leg like structures are present on the abdomen of many endopterygote larvae. Their structure is varied, though typically (eg. in caterpillars) three regions can be distinguished; a basal membranous articulation, followed by a longer section having a sclerotized plate on the outer wall and an apical protractile lobe, planta, which bears claws, crochets, peripherally. The planta is protracted by means of blood pressure. Above the leg there is a swollen area in the body wall, suprapedal lobe.

v. **Gills:** A large number of aqua tic larvae possess segmentally arranged gills on a varied number of abdominal segments. These are flattened, filamentous structures, frequently articulated at the base.

vi. **Non-segmental appendages**: These are typically a mediodorsal projection on the last abdominal segment. eg. The median lamella of Zygopteran larvae and the caudal filament of Microcoryphia, Zygentoma and Ephemeroptera. Occasionally these structures are paired (e.g. the urogomphi of some larval Coleoptera) and are easily mistaken for cerci. The anal papillae of certain dipteran larvae also fit in this category.

vii. **External Genitalia or reproductive appendages:** The 9^{th} abdominal segment in the male and the 8^{th} and 9^{th} in the female insects form the genital apparatus.



viii. **Male genitalia:** Helps in distinguishing species. In a typical male Pterygote insect, the complete external genitalia are com posed of – Penis; a pair of parameres or t he inner processes; and a pair of claspers. The penis is formed b y the fusion of paired outgrowths and it be ars the m ale gonopore. T he original paired condition is f ound only in Ephemeroptera and in certain Dermaptera. In several apterygote and pterygote insects, gonopodsare present which are unmodified limb bases bearing either long styli but perform no genital functions as in Thysanura, or short styli as in Dictyoptera. In many insects e.g. Ephemeroptera, Mecoptera and Trichoptera, the styli along with their limb bases form functional clasping organs. Absent in Coleoptera.

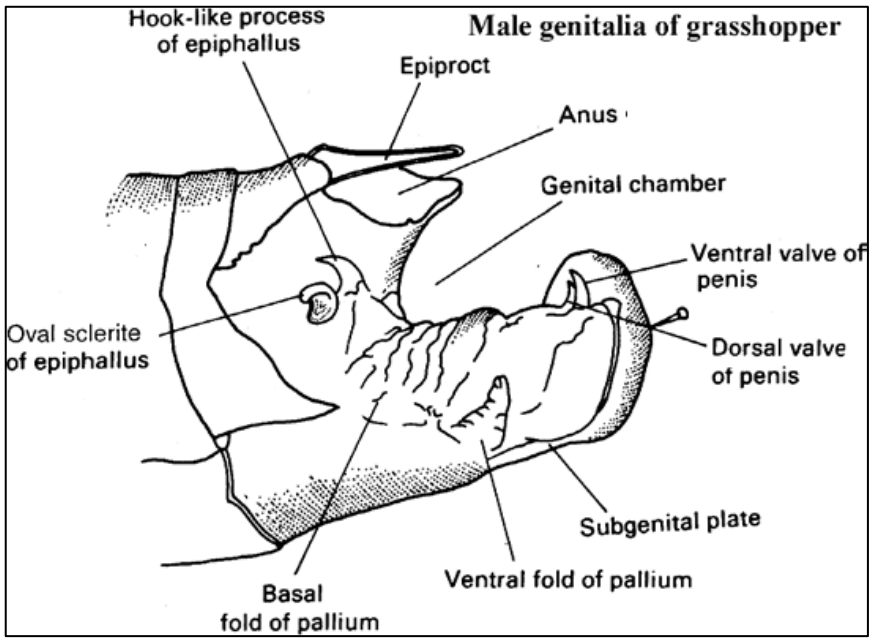


Fig: Male genital appendages

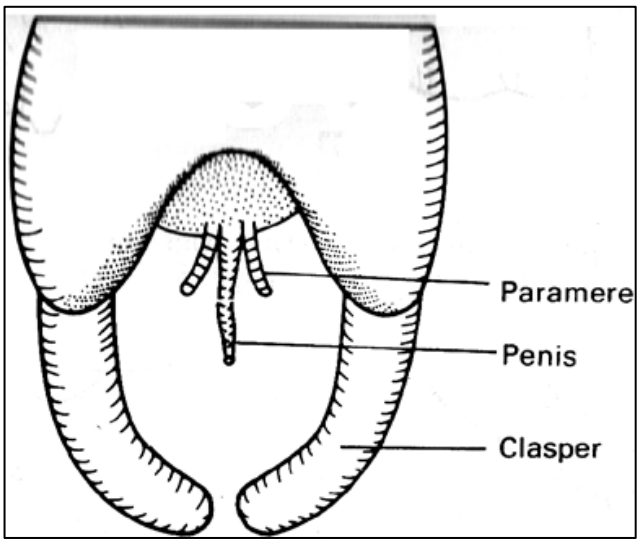


Fig: Male genital appendages

ix. **Female genitalia:** They take t he form of a sword-like projection, the ovipositor, directed backwards. Ovipositor is composed of 3 pairs of valves- anterior or ventral (1st pair); posterior or inner (2nd pair); and lateral or dorsal (3rd pair). The 1st pair arises from the valvifer- 1 of the 8th segment, 2nd pair is the median outgrowths of the valvifer-2 of the 9th segment and the 3rd pair arise from the valvifer-3 of the 9th segment. These three pairs of valves are variously modified in several insects and are well developed in grasshoppers, crickets and cicadas. It is used for depositing eggs (in s oil) which are squeezed along its length between the shafts. In some insect groups such as Diptera, Lepidoptera and Coleoptera, the hind end of the abdomen is telescopic in form and acts as an ovipositorinstead. In higher Hymenoptera such as wasps, bees the ovipositor has become modified to form a poison-injecting apparatus, the sting, instead of lying eggs. At rest the sting lies in a pocket within 7th abdominal segment, from which it is exerted when in use. There are 3 pairs of plate-like sclerites- the quadrate, oblong and triangular plates at the base of the sting whicharticulate with each other.

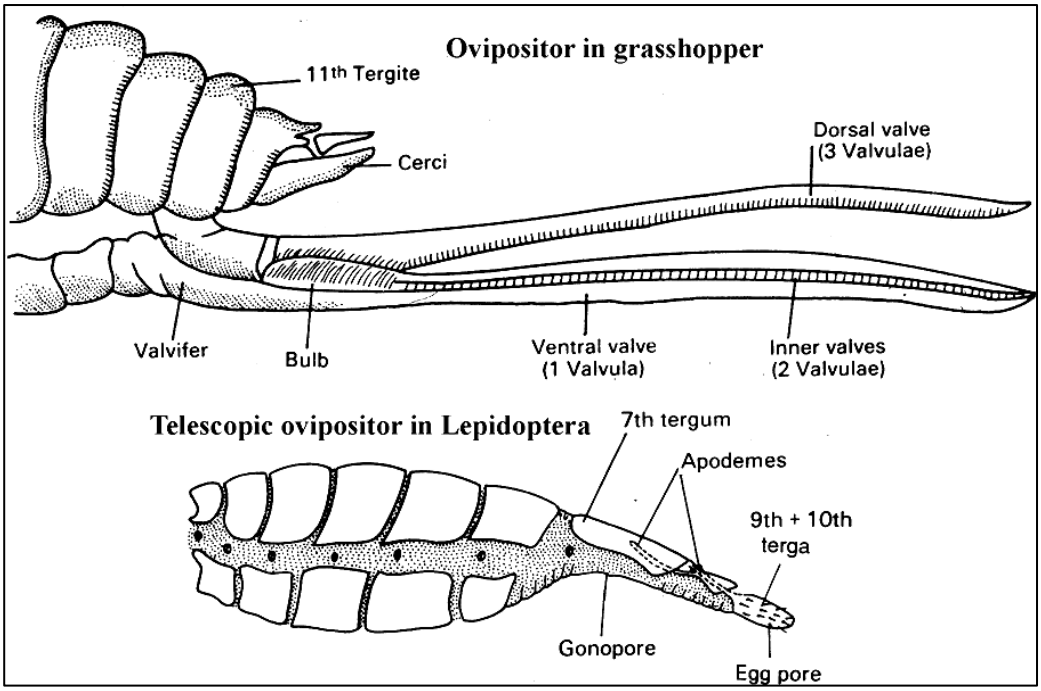
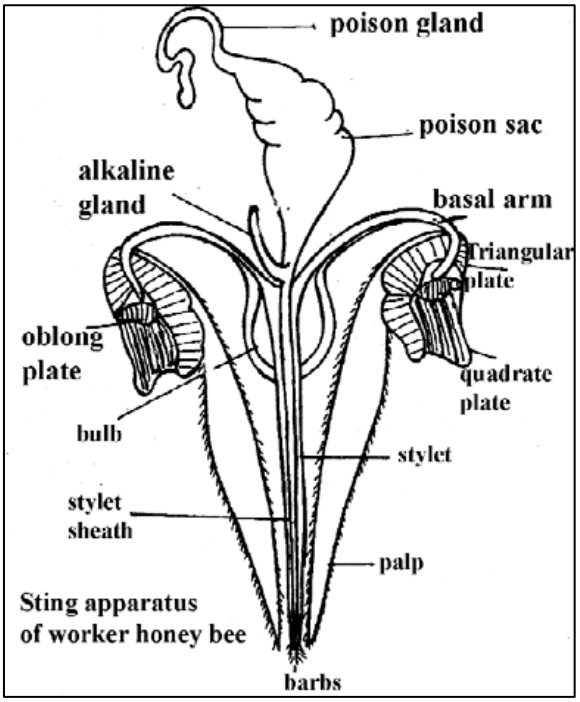


Fig: Female genital appendages



Probable Questions:

1. Classify orientation of insect head.
2. Describe different structural components of insect head.
3. Describe different components of insect mouth parts.
4. Write down the structure of insect leg.
5. Describe different types of abdominal appendages of insect.

Suggested Reading:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
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UNIT-IX

Integument: Basic structure and functions; modification in different insect groups

Objectives:

In this unit we will discuss about Integument: Basic structure and functions; modification in different insect groups

Introduction:

Insect body wall is called as Integument or Exoskeleton. It is the external covering of the body which is ectodermal in origin. It is rigid, flexible, lighter, stronger and variously modified in different body parts to suit different modes of life.

The outer most layer of insect covering of the whole insect body. It forms a composite structure which forms the skeleton of the insect body and ectodermal in origin. It provides area for muscle attachment; protection from desiccation, physical /mechanical injuries and shape, strength to the body and its appendages.

Body wall of insect consists of 3 layers

1. Cuticle

2. Epidermis (or) hypodermis

3. Basement membrane

1. Cuticle:

It is outermost thick layer of integument secreted by epidermis. It is divided in to two layer A) Epicuticle B) Procuticle

A. Epicuticle: It is a thin outermost layer varying in thickness from 1-4µ. Chitin is absent in epicuticle. epicuticle consists of the following 4 layers.

1. Cement layer: It is secreted by dermal glands and is composed of lipoprotein. It protects the body from external damage. It is give the size and shape of insect body.

2. Wax layer: It is prominent layer, 0.25µ in thickness, consisting of long chain hydrocarbons, esters of fatty acids and alcohols. It is water proof layer preventing water loss from the body

3. Polyphenol layer: It is a non-static layer containing various types of phenols which are mainly used in the formation of the proteins. It is resistant to acids and organic solvents.

4. Cuticulin layer: It is an amber coloured thin layer over the surface of the epidermis which is strengthened by outer polyphenol layer.

B. Procuticle: It is differentiated in to exo and endocuticle after sclerotization process.

1. Exocuticle: It is darkly pigmented, hard and sclerotized. It offers rigidity to the cuticle and consists mainly chitin and a hard protein called sclerotin.

2. Endocuticle It is soft, light coloured and unsclerotized. It contains more chitin but lacks hard protein sclerotin.

Pore canals: These are numerous fine vertical channels traversing both exo and endocuticle measuring from < 1μ (0.1 0.15μ) in diameter. They run perpendicularly ful in epicuticle throughout the length of the cuticle. They are use transportation of cuticular material and enzymes to the outer pro and epicuticle parts.

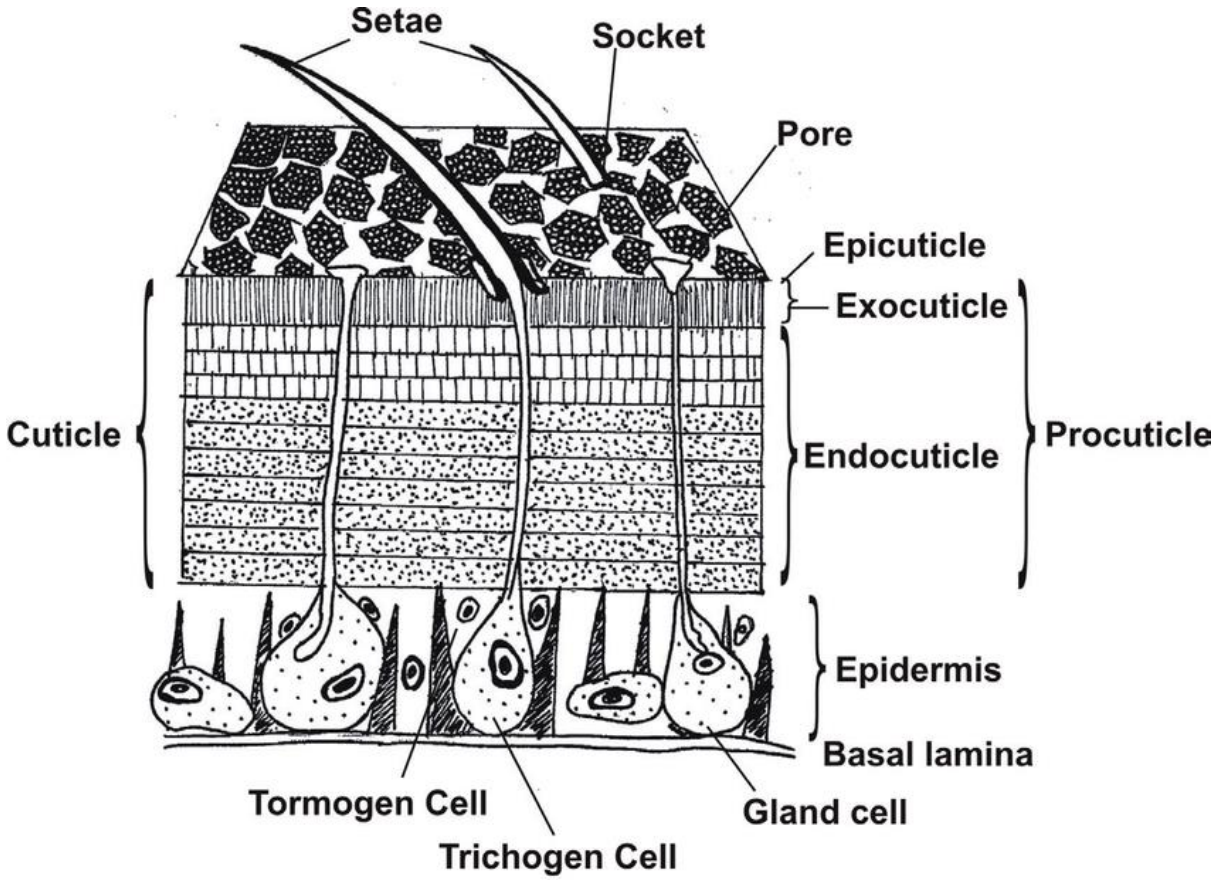

Fig: T.S of body wall of cockroach

Two major components of insect cuticle are Chitin and Proteins

i) Chitin: It is a nitrogenous polysaccharide. $(C_8H_{13}O_6N)_x$. It accounts for 25-60 per cent of the dry weight of the cuticle. It is named by Odier in 1834. It consists of high molecular weight polymer of anhydro-N-acetyl glucosamine residues joined by β-glycosidic linkages. It is embedded with proteins in the procuticle to form glycoproteins. It is insoluble in water, alcohol, organic solvents dilute acids and concentrated alkalies, but soluble only in concentrated mineral acids and sodium hypochlorite.

ii) Proteins: Cuticle has 3 types of proteins

(a) Arthropodin: It is soft water soluble protein present in endocuticle. The conversion of arthopodin in to sclerotin is known as sclerotization or tanning.

(b) Sclerotin: It is also called tanned protein which is amber coloured and present only in exocuticle.

(c) Resilin: It is a rubber like elastic protein which is colourless and present in joints such as wing hinge ligaments, leg joints, clypeolabral joints or suture and tergosternal joints

2. Epidermis or Epidermal Cell

It is an unicellular layer formed from polygonal cells which modifies in to cuboidal or columnar during the process of moulting. These cells consist of well developed nucleus and other cytoplasmic contents. Adjacent epidermal cells are held together by means of certain cytoplasmic processes.

All the epidermal cells are glandular and secrete cuticle and the enzymes involved in production and digestion of old cuticle during moulting. The epidermal cells get differentiated in to following types based on the function they perform and may modify in to

a) Dermal glands producing cement layer

b) Trichogen cell producing hair like seta or trichome.

c) Moulting glands secreting moulting fluid which digests the old cuticle

d) Peristigmatic glands around the spiracles in case of Dipteran larvae

3. Basement membrane:

It is the basal part of the body wall formed from degenerated epidermal cells and appears as non-living amorphous (shapeless) granular layer of integument. It is about 0.5µ in thickness and consists of fibrous protein, glycosaminoglycans which are polymers of disaccharides. The basement membrane forms a continuous sheet beneath the epidermis, where muscles are attached and become continuous with sarcolemma of the muscles.

Oenocytes:

The oenocytes are large polyploid cells associated with the basement membrane.

– some oenocytes might be involved in the production of cuticular lipid that ar e d eposited inthe epicuticle.

– other types of oenocytes may secrete ecdysteroid hormones.

ENDOSKELETON

Cuticular in growth of body wall providing space for muscle attachment is known as endoskeleton. There are two types

i.**Apodeme:** Hollow invagination of body wall.

ii.**Apophysis:** Solid invagination of body wall.

• Cuticular/ Integumental modifications

A. Cuticular Out growths

- **Non-cellular:** Non-cellular appendages have no epidermal association, but rigidly attached. e.g. minute hairs and thorns.
- **Cellular:** Cellular appendages have epidermal association. These are the outgrowths of the cuticle / integument connected with it by means of a membranous joint. They arise from modified epidermal cells. These are classified in to setae and spurs.

  Seta - Commonly known as hairs and arise from a cup like alveolus or pit. Setae are hollow structures developed as extension of exocuticle and are produced by a single enlarged hypodermal cell called 'trichogen' cell. Articular membrane is usually produced by a second hypodermal cell called 'tormogen' cell. Setae have role of taxonomic importance and vary with species to species.

a) Glandular seta. e.g. caterpillar
b) Sensory setae - associated with sensory neuron or neurons

   Spurs: It is a multicellular structure. It occurs on the legs of many insects and differs from setae in being multicellular in origin.

- **Other Unicellular structures:**

c) Clothing hairs, plumose hairs. e.g. Honey bee. Bristles. e.g. flies.
d) Scales - flattened out growth of body wall e.g. Moths and butterflies

B.Cuticular processes:

They have no membranous articulation. They are of two types

(1) Microtrichia / fixed hairs / aculei: These are minute hair like structures found on wings of Mecoptera and certain Diptera.

(2) Spines: Outgrowths of the cuticle which are more or less thorn like in form.

Spurs Spines Cuticular appendages Cuticular processes Movable, multicellular structures and thick walled

GLANDS

Cuticular glands are either unicellular or multicellular. Following are some of the examples.

i. Wax gland - e.g. Honey bee and mealy bug
ii. Lac gland - e.g. Lac insects
iii. Moulting gland secreting moulting fluid.
iv. Androconia or scent scale - e.g.moth
v. Poison gland - e.g. slug caterpillar

Functions of the Integument:

i. It provides the Structure, size and shape of the insect body.
ii. Acts as external armour and strengthen external organs like jaws and ovipositor
iii. Internally protects the vital organs, foregut, hindgut and trachea.
iv. Provides shape to the body.
v. Prevents water loss from the body.
vi. Cuticular sensory organs help in sensing the environment.
vii. Cuticular pigments give colour.
viii. It provides the Water resistance/ balance of insect.
ix. It protects the insect organs against physical harm.
x. Provides the space for muscle attachment.
xi. It helps to prevent the entry of harmful microbes and chemicals.
xii. It serves as interface between insect and environment.
xiii. It helps to maintain an ionic balance in insect body.

MOULTING (Ecdysis)

The insect cuticle is hard and forms unstretchable exoskeleton and it must be shed from time to time to permit the insects to increase their size during growth period. Before the old cuticle is shed new one has to be formed underneath it, this process is known as moulting.

Moulting is a complex process which involve 3 process

1) Apolysis 2. Ecdysis 3) Sclerotization

1) Apolysis : [Apo = formation ; Lysis = dissolution] The dissolution of old cuticle and formation of new one is known as apolysis.

2) Ecdysis : The stage where the insect has both newly formed epi and procuticle and old exo and epicuticle is known as pharate instar. The ecdysial membrane starts

splitting along the line of weakness due to muscular activity of the inner developing insect and also because of swallowing of air & water resulting in the distention of the gut. The breaking at the ecdysial membrane is also due to the pumping of blood from abdomen to thorax through muscular activity. After the breakage of old cuticles which is known as exuviae, the new instar comes out bringing its head followed by thorax, abdomen and appendages.

3) Sclerotization : After shedding of old cuticle the new cuticle which is soft, milky white coloured becomes dark and hard through the process known as tanning (or) sclerotization. The process of hardening involves the development of cross links between protein chains which is also known as sclerotization. This tanning involves the differentiation of procuticle in to outer hard exocuticle and inner soft endocuticle.

The time interval between the two subsequent moulting is called as **Stadium** and the form assumed by the insect in any stadium is called as **Instar**.

Steps in moulting

1. Behaviroual changes: Larva stops feeding and become inactive.
2. Changes in epidermis: In the epidermis cell size, its activity, protein content and enzyme level increases. Cells divide mitotically and increase the tension, which results in looseningof cells of cuticle.
3. Aolysis: Detachment of cuticle from epidermis
4. Formation of Sub cuticular space
5. Secretion of moulting g el in t he sub cuticular space which is rich with chitinase andprotease.
6. New epicuticle formation: Lipoprotein layer (cuticulin) is laid over the epidermis.
7. Procuticle formation: Procuticle is formed below the epicuticle.
8. Activation of moulting gel: Moulting gel is converted into moulting fluid rich in enzymes.This activates endocuticle digestion and absorption.
9. Wax layer formation: Wax threads of pore canals secrete wax layer.
10. Cement layer formation: Dermal glands secretes cement layer (Tectocuticle).

I. **Rupturing of old cuticle:** Insect increases its body volume through intake of air or water which enhances the blood f low to head and thorax. There by the old cuticle ruptures along prede-termined line of weakness known as **ecdysial line**

II. **Removal of old cuticle:** Peristaltic movement of bod y and lubricant action of moulting fluid he lps i n t he r emoval of ol d c uticle. During each m oulting t he cuticular c overings discarded are the cuticular of legs, internal linings of foregut and hindgut and trachea.

III. **Formation of exocuticle**: The upper layer of procuticle develops as exocuticle through addition of protein and tanning by phenolic substance.

IV. **Formation of endocuticle:** The lower layer of procuticle develops as endocuticle through addition of chitin and protein. This layer increases in thickness.

Control of Moulting:

It is controlled by endocrine gland like prothoracic gland which secrete moulting hormone. Endocrine glands are activated by prothoracico-tropic hormones produced by neurosecretory cells of brain.

Three types of hormones involved in the process of moulting which are as follows

JH: Juvenile Hormone: Produced from corpora allata of brain that helps the insects to be in immature stage.

MH: Moulting hormone: Produced from prothoracic glands of brain that induces the process of moulting

Eclosion Hormone: Released from neurosecretory cells in the brain that help in the process of ecdysis or eclosion.

Modification of integument in different insect groups

The exoskeleton attains its most elaborate forms in the arthropods (for example, crustaceans and insects). The insect epidermis lies on a basement membrane and secretes a tough cuticle, the bulk of which is composed of fibres of a material known as chitin embedded in a matrix of protein. Peripheral to this is an epicuticle. Chitin is a high-molecular-weight polysaccharide containing amino groups. It is synthesized within the epidermis from sugars and amino sugars.

In the integument of caterpillars chitin forms a cuticle that is tough but flexible. But in most arthropods the segments of the body or of the limbs are in the form of rigid plates that form a true exoskeleton linked to adjacent segments by flexible membranes. Such cuticles are hard and may be dark in colour. They are said to be tanned, or sclerotized, and in some species they are also mineralized.

Sclerotization involves the molecular stabilization of the protein chains of the cuticles by establishment of cross-links. Sclerotin, the product of sclerotinization, is a kind of natural plastic. In its horny consistency it closely resembles keratin; both are cross-linked, or polymerized, proteins, but the chemical nature of the linkage is different in the two substances. It is probable that other skeletal proteins in invertebrates, such as the spongin of sponges and the conchiolin of mollusks, are also tanned proteins allied to sclerotin.

In many crustaceans—crabs and lobsters, for example—much of the cuticle is rendered hard by the incorporation of calcareous substances such as aragonite or calcite. But

sclerotin is actually harder than calcite, and those parts of crustaceans that need to be of maximum hardness, such as the mandibles and the tips of the claws, are in fact composed of sclerotin.

Besides functioning as a skeleton, the cuticle of terrestrial arthropods must act as a waterproof covering in order to prevent these small animals from drying up. This waterproofing is effected by the secretion of a layer of wax on the surface of the cuticle. Such a wax layer, if exposed in an unprotected state, would be excessively fragile. It is commonly protected by a thin layer of a cementlike substance that is poured over its surface by small dermal glands.

The cuticle of arthropods, pierced by ducts of dermal glands that pour out secretions over the surface, is a living structure; it can produce tactile bristles, pigment-bearing scales, claws, wings, and other structures. In some insects it shows brilliant metallic colours that result from the presence of multiple thin plates or ridges in the cuticle. In order that the arthropod may grow, the old cuticle is shed from time to time after a new and larger cuticle has been laid down beneath it. This process is termed molting, or ecdysis. During the time when the new cuticle is hardening, the arthropod is in a very vulnerable condition.

Probable questions:

1. Describe the structure of insect integument with proper diagram and comment on theroles of different regions.
2. Write down the chemical composition of cuticle. How exocuticle and endocuticle areformed?
3. Describe the different steps of Moulting. Add a note on control of moulting.
4. What are apodemes and apophysis?
5. What are cuticular appendages? Name different cuticular appendages of insects.
6. Write down the functions of integument.
7. What are oenocytes? State their function.
8. Name the different glands of the cuticle and state their function.
9. What are pore canals? State their function.

Suggested Reading:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-WesleyPubl. Co., 436 p.
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UNIT- XIV

Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber

Objectives:

In this section we will discuss about Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber

Introduction:

An insect uses its digestive system to extract **nutrients** and other substances from the food it consumes. Most of this food is ingested in the form of macromolecules and other complex substances (such as proteins, polysaccharides, fats, nucleic acids, etc.) which must be broken down by catabolic reactions into smaller molecules (i.e. amino acids, simple sugars, etc.) before being used by cells of the body for energy, growth, or reproduction. This break-down process is known as **digestion**.

Insects of different groups consume an astonishing variety of foods. There are four major feeding specializations that can be identified depending on whether the food is solid or liquid or of plant or animal origin. Hence gut morphology and physiology relate to these dietarydifferences in the following ways:

Insects that take solid food typically have wide, straight, short gut with strong musculature and obvious protection from abrasion especially in the midgut which has no cuticular lining.

e.g. Plant feeding caterpillars.

Insects feeding on blood sap or nectar usually have long, narrow, convoluted guts to allow maximal contact with the liquid food. It requires the mechanism for removing excess water toconcentrate nutrient substances prior to digestion. e.g. Hemiptera

From a nutritional view point most plant feeding insects need to process large a mounts of food because nutrient levels in leaves and stems are often low. The gut is usually short and without storage areas as food is available continuously.

A diet of animal tissue is nutrient rich and well balanced. However the food may be availableonly intermittently and hence gut has large storage capacity.

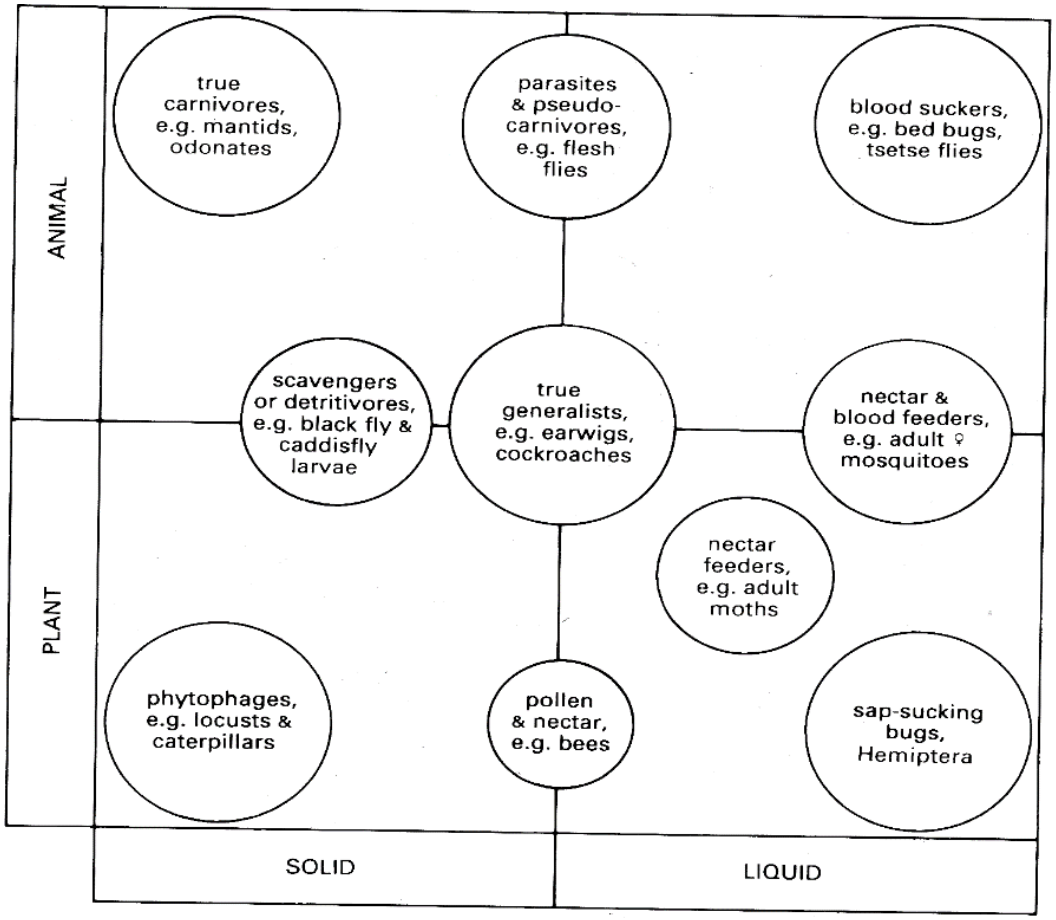

Fig: Different Food types consumed by different insects

All insects have a **complete digestive system**. This means that food processing occurs within a tube-like enclosure, the **alimentary canal**, running lengthwise through the body from mouth to anus. Ingested food usually travels in only one direction. This arrangement differs from an **incomplete digestive system** (found in certain lower invertebrates like hydra and flatworms) where a single opening to a pouch-like cavity serves as both mouth and anus. Most biologists regard a complete digestive system as an evolutionary improvement over an incomplete digestive system because it permits **functional specialization** — different parts of the system may be specially adapted for various functions of food digestion, nutrient absorption, and waste excretion. In most insects, the alimentary canal is subdivided into three functional regions: foregut (stomodeum), midgut (mesenteron), and hindgut (proctodeum).

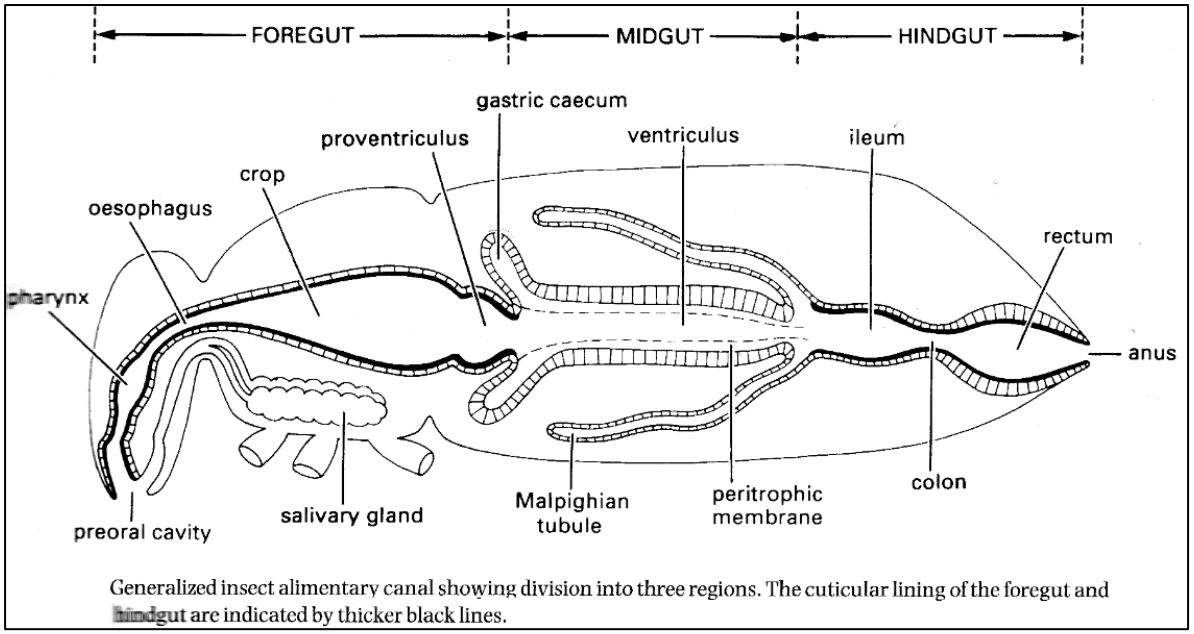

Fig: Generalised insect elementary canal

- **Alimentary Canal of Cockroach:**

The alimentary canal starts from mouth and it consists of the preoral cavity, pharynx, oesophagus, crop and gizzard forming the foregut or stomodaeum; the mesenteron forming the midgut and the ileum, colon and rectum constituting the hindgut or proctodaeum.

The stomodaeum and proctodaeum are ectodermal in origin and lined internally by the continuation of the exoskeletal cuticle, while the mesenteron is endodermal in origin and without cuticular lining.

Foregut:

The so-called mouth is situated at the base of the pre-oral cavity or buccal chamber, also known as cibarium. The buccal chamber is a space in front of the mouth into which the food is received. This cavity is bounded in front by the labrum, posteriorly by the labium and on each side by a mandible and a maxilla. Inside this cavity a large tongue-like hypo pharynx is present.

The mouth opens behind into a short tubular pharynx which passes vertically upwards, and then it bends backward into an oesophagus. The oesophagus is a narrow tubular passage which passes through the neck and gradually expands in the thorax, finally taking the shape of sac-like structure in abdomen. This sac-like structure is called crop which is thin-walled and muscular. The crop is followed by a gizzard or pro-ventriculus.

The gizzard is a round, thick-walled bulb-like structure. Structurally, it has an outer thick layer of circular muscles and its lumen gets considerably reduced due to the in-

folding of its wall. The gizzard can be divided into anterior armarium and posterior stomodaeal valve.

The cuticular lining of armarium is thickened to form six highly chitinised plates called teeth. Behind the teeth, there are thin less chitinised plates which bear cushion-like pads covered with backwardly directed bristles. The deep grooves are also provided with fine bristles.

The teeth help in grinding the food, while bristles help in straining the food to allow only well crushed food to pass on. The hind part of the gizzard projects into the midgut as a funnel, called stomodaeal valve which prevents the passage of food from midgut into the gizzard.

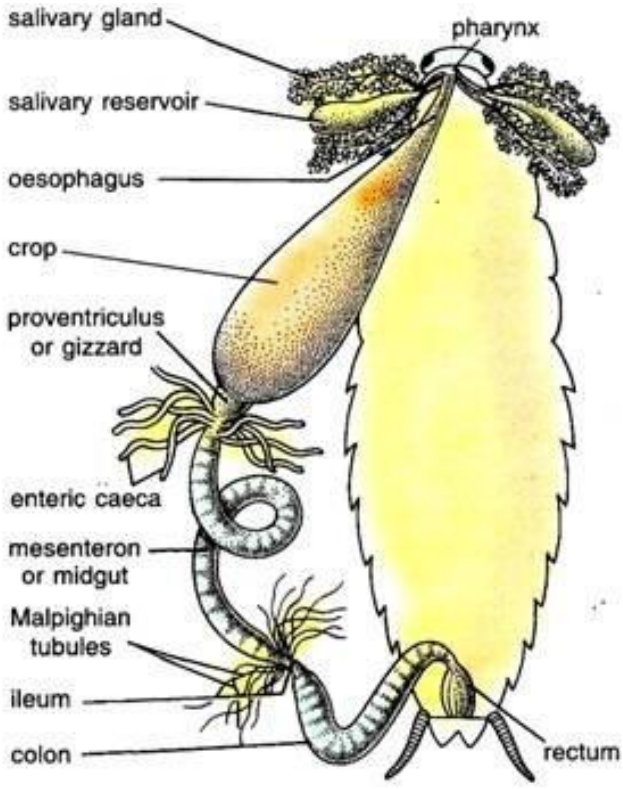

Fig. 73.13. *Periplaneta.* Digestive system.

Midgut:

The gizzard is followed by a narrow tube of uniform diameter representing the midgut or mesenteron. Its junctional region with the gizzard, which actually surrounds the stomodaeal valve, is called cardia. From this region, arise eight finger-like tubular blind processes called hepatic caeca or enteric caeca or mesenteric caeca. The midgut is formed of tall columnar endodermal cells which are glandular in nature. The internal lining of the midgut is thrown out into small but several folds forming villi and covered by a very thin layer of transparent peritrophic membrane.

The peritrophic membrane is secreted by the anterior end of the cardia and it is permeable for enzymes and for the end products of digestion. It also protects the wall of the midgut. The process of digestion is completed in this region of the alimentary canal and the digested food is also absorbed in it.

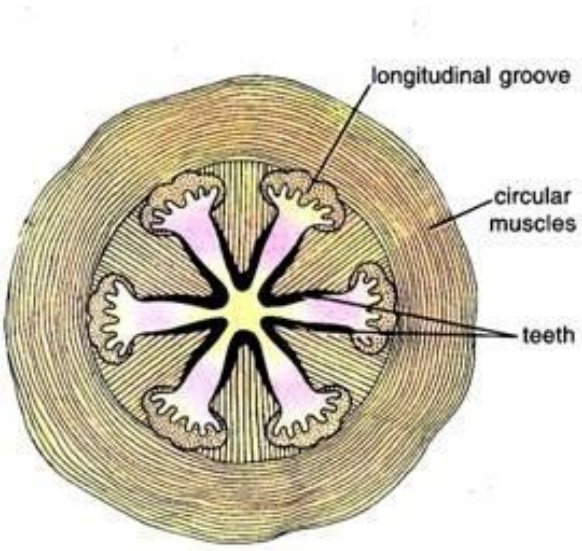

Fig. 73.14. *Periplaneta*. T.S. of gizzard.

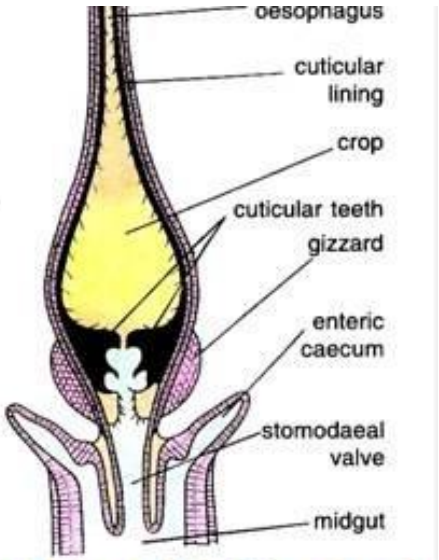

Fig. 73.15. *Periplaneta*. L.S. of foregut and anterior part of midgut.

Fig: Diagram of T.S of gizzard and L.S. of foregut and anterior part of midgut

Hindgut:

The junction of midgut and hindgut is marked by the presence of nearly sixty to one hundred fifty long, filamentous and blind Malpighian tubules which are not related with digestion but with excretion. The hindgut is relatively broader than the midgut. It is ectodermal and lined internally with the cuticle. Its anterior region following the midgut is called ileum which is followed by a long and coiled colon.

The colon ends in a broad rectum which opens by an anus lying posteriorly below the 10th tergum. The lining of the colon is wrinkled and that of the rectum forms six thick longitudinal folds called rectal papillae. The cuticle covering the papillae is very thin but its underlying epithelium is thick, this is perhaps an adaptive device for absorbing maximum water from the passing out faeces.

- **Digestive Glands:**

Digestive glands of cockroach generally include the salivary glands, the glandular cells of the midgut and hepatic caeca.

1. **Salivary Glands:**

A pair of salivary glands (Fig. 73.16), one on either side of the crop in the thorax, is found associated with the alimentary canal. Each gland has two glandular portions and a bag-like diverticulum or receptacle or reservoir in which saliva is stored. From the glandular portions of the two sides arise salivary ducts which unite to form a common duct. Likewise, two ducts from the reservoirs also join to form another common duct.

The two common ducts join to form an efferent salivary duct which opens in the pre-oral cavity at the base of the hypo pharynx. The ducts of glands and reservoir are peculiar in having a spirally thickened cuticular lining like trachea.

Each salivary gland is made of several secreting lobules or acini. Each acinus is formed of two types of cells—zymogenic cells, packed with secretory granules and ductule containing cells with very less secretory granules but having plenty of mitochondria, rough endoplasmic reticulum and vesicular bodies.

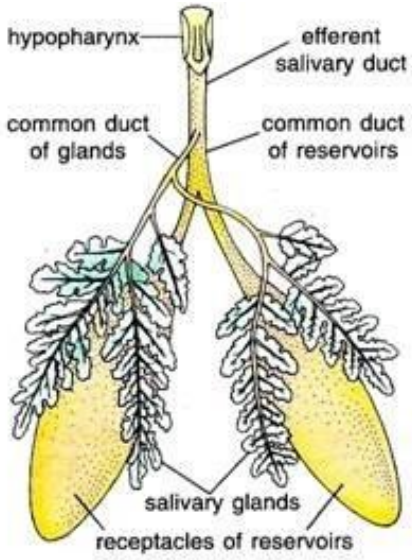

Fig. 73.16. *Periplaneta*. Salivary apparatus.

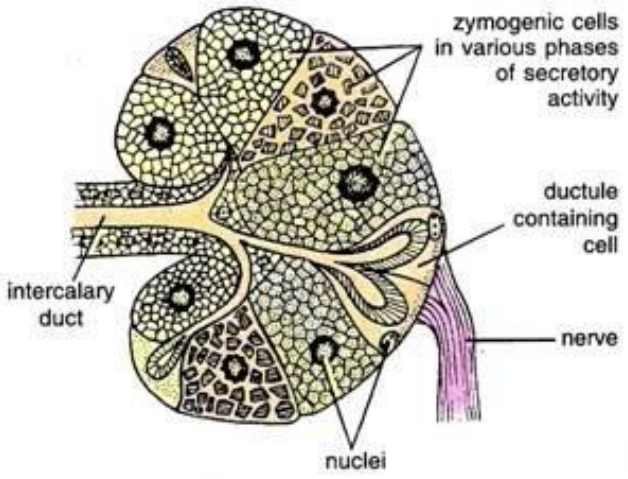

Fig. 73.17. *Periplaneta*. A salivary acinus in section.

The glandular cells of the internal lining of the midgut and hepatic caeca also produce juice containing digestive enzymes.

- **Food and feeding of Cockroach:**

As referred earlier, cockroach is omnivorous; it feeds on every type of animal and plant materials, though it prefers to feed on sugary and starchy substances but tastes almost everything it comes across. The presence of food is detected by the sensory receptors present on the antennae and maxillary palps.

The maxillae pick up and bring food to the mandibles for mastication. During the act of mastication the teeth of the mandibles bite and chew the food.

The labrum and labium work like lips to prevent the loss of food from the mandibles at the time of mastication. The chewed food is pushed into the pre-oral cavity by maxillae, prostheca of mandibles and labium from where it is swallowed into the mouth. The function of hypo pharynx is not certain in this connection.

- **Physiology of Digestion:**

Since salivary glands open by their common duct in the pre-oral cavity at the base of the hypo pharynx, hence, saliva mixes with the food during mastication. The saliva contains enzymes like amylase, chitinase and cellulase which hydrolyse different carbohydrates; some of them are converted into glucose. The saliva also moistens the food for its easy transport in the alimentary canal.

However, such food is swallowed by the mouth and transported through the pharynx and oesophagus into the crop. After reaching into the crop, the digested carbohydrate in the form of glucose is absorbed and remaining food comes across the secretion of the glandular cells of the midgut.

Actually, the digestive juice secreted by the glandular cells of the midgut ascends into the crop through the gizzard. This juice contains amylolytic enzymes like invertase, maltase and lactase to complete the carbohydrate digestion; proteolytic enzymes like trypsin, proteases and peptidases to digest proteins into amino acids; lipolytic enzymes for the digestion of fats into fatty acids and glycerine.

Thus, maximum digestion occurs in crop and then the food descends down through the gizzard into the midgut. The gizzard grinds and crushes the food particles into finer ones and again it filters the food with the help of the fine bristles present at its posterior region. Thus, food coming into the midgut is very fine paste-like.

The stomodaeal valves check the backward passage of food from the midgut into the crop. The inner lining of the midgut—the peritrophic membrane is permeable to digestive enzymes and digestive nutrients, therefore, the digestive enzymes meet the food to complete the digestion which is already going on.

Absorption:

The end products of digestion, i.e., proteins as amino acids, fats as fatty acids and glycerine and carbohydrates as glucose are absorbed by the lining cells of the midgut

and hepatic caecae and transported to the different parts of the body for their use. The excess food material is stored in the fat body as glycogen, fat and probably albumen.

Egestion:

The undigested food passes into the hindgut. In the rectum, maximum of water is absorbed from it, hence, the undigested residue which remains here is almost solid. This is egested out in the form of small dry pellets through the anus.

• Structure and Function of Peritrophic membrane

The epithelial cells of the mid gut are devoid of cuticle, but in most insects they are protected from the contents of the gut by a delicate detached sheath called peritrophic membrane (PM). This consists of a mucoprotein in which chitin fibrils are arranged irregularly or in hexagonal or orthogonal arrays.

According to Hansen and Peters (1997), the peritrophic membranes are formed by secretion of the midgut epithelium. They consist of a network of chitin-containing microfibrils embedded in a matrix of proteins, glycoproteins, and proteoglycans. The thickness of the peritrophic envelope is 280 nm.

PM protect the midgut from abrasion by food particles and are said to be absent from most insects feeding on a liquid diet eg .the Hemiptera except Corixidae and many adult Lepidoptera and blood-sucking insects. However t hey occur in Cicadella, mosquitoes andGlossina.

The PM has been considered to arise in two ways:

- In some Lepidoptera, Diptera and Dermaptera it is secreted by cells near the junction of fore and mid gut and extruded in tubular form by a muscular press in this region.
- In other insects it arises by delamination from part or all of the general surface of the mid gut and aseries of concentric membranes, formed by successive delaminations is often pr esent. In A pis and P eriplanata a m embrane f ormed pr edominantly i n t he anterior part of the mid gut is supplemented by delamination further back.

Function of PM-

Protect the mid gut from abration by hard fragments in the foods.

Provides permeability to the dies with l arge colloidal particles such as congo red or Berlin blue.

- **Structure and Function of Filter chamber**

Many homopteran insects feed on low concentrate plant sap. Their digestive tract shows a complex system called the **'filter chamber'** where the extra solute water is made to flow directly from the initial to the terminal part of the midgut and the proximal regions of the **'malpighian tubules'**. The filter chamber of **Cicadella viridis** shows the intra membrane particles presence on the whole surface of the microvilli and of basal membrane infolded cells. The inner surface of the membrane is covered with particles which are protruding into the cytoplasm. They correspond to the numerous number of the observed intra membrane particles on the P fracture face of the membrane. The outer membrane surface has a regular network which responds to observed on the E fracture face. SDS-PAGE analyses performed on the filter chamber's membranes of **C.viridis** and **Philaenus spumarious** .in both cases 2 major components **25kDa** and **75kDa**, were found. These 2 major components showed for the filter chambers when they can't be found in membranes which were isolating from the midgut. The membrane of filter chambers would be **water –shunting complexes**, which have structural and biochemical complexes which should be related to **water permeability**.

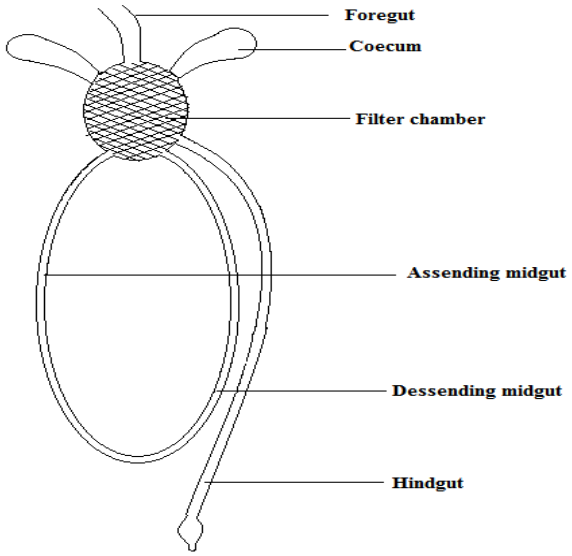

Fig: Filter chamber in insect digestive system

Importance of filter chamber/Significance of filter chamber:

The role the filter chamber is very important in insect digestive system. Many homopteran insects like leaf-hoppers (**Eurymela distinct** and **Eurymela fenestrata**) feed on low concentrate plant sap of Eucalyptus spp. In the filter chamber osmotic pressure of both haemolymph and gut fluids involved in a filtration mechanism, passive osmosis. In this is process water is shunting rapidly from foregut to hindgut in the comparatively simple filter chamber. The main role of this process is the osmotic gradient which cause the system is mostly produced by the secretion of sodium (Na)

and potassium (K) into both the Malpighian tubules and posterior midgut. The osmotic pressure is higher in all parts of the system.

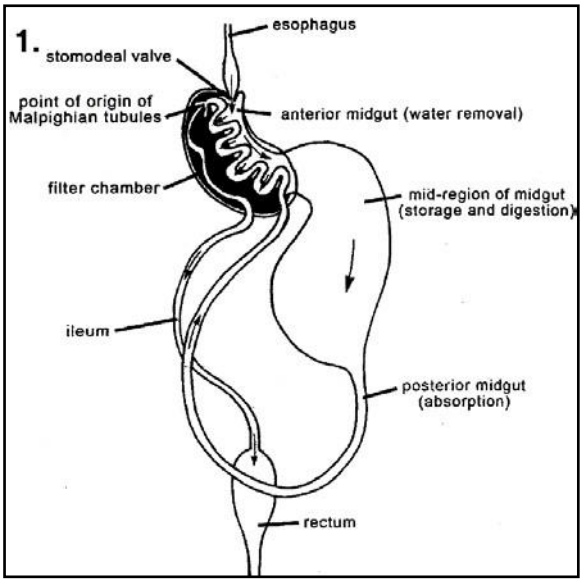

Figure: Alimentary canal of cercopid (cercopoidea) showing filter chamber arrangement.

In many homopterans, which feed on plant sap, the mid gut is modified both morphologically and anatomically so that excess water present in the food can be removed, thus preventing dilution of the hemolymph. Though details vary among different groups of homopterans, the anterior end of the midgut or the posterior part of the oesophagus is brought into close contact with the posterior region of the midgut or anterior hindgut and the region of contact becomes enclosed within a sac called the 'Filter chamber'.

Such an arrangement facilitates rapid movement of water by osmosis from the lumen of the anterior midgut across the wall of the posterior midgut and possibly also the malpighian tubules and thus only little of the original water in the food actually passes a long the fulllength of the midgut.

Probable Questions:

1. Give the structure of generalized digestive system of an insect.
2. Explain the structural and functional significance of filter chamber found in the mid gut of insect.
3. Explain with proper diagram the various structural components of a typical insect mouth parts.
4. What is petitrophic membrane?
5. Write down the importance of filter chamber.

Suggested Reading:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-WesleyPubl. Co., 436 p.
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UNIT XV

Structure and functions of the central nervous system in insects

Objective:

In this unit we will duscuss about Structure and functions of the central nervous system in insects.

Introduction:

An insect's nervous system is a network of specialized cells (called **neurons**) that serve as an "information highway" within the body. These cells generate electrical impulses (action potentials) that travel as waves of depolarization along the cell's membrane. Every neuron has a nerve cell body (where the nucleus is found) and filament-like processes (dendrites, axons, or collaterals) that propagate the act ion potential. Signal transmission is always unidirectional -- moving toward the nerve cell body along a dendrite or collateral andaway from the nerve cell body along an axon.

Like most other arthropods, insects have a relatively simple central nervous system with a dorsal brain linked t o a ventral nerve co rd that consists of paired segmental ganglia running along the ventral midline of the thorax and abdomen. Ganglia within each segment are linked to one another by a short medial nerve (commissure) and also joined by intersegmental connectives to ganglia in adjacent body segments. In general, the central nervous system is rather ladder-like in appearance. Commissures are the rungs of the ladder and intersegmental connectives are the rails. In more "advanced" insect orders there is a tendency for individual ganglia to combine (both laterally and longitudinally) into larger ganglia that serve multiplebody segments.

Individual nerve cells connect with one another through special junctions, called **synapses**. When a nerve impulse reaches the synapse, it releases a chemical messenger (neurotransmitter substance) that diffuses across the synapse and triggers a new impulse in the dendrite(s) of o ne or m ore connecting neurons. Acetylcholine, 5-hydroxytryptamine, dopamine, and noradrenaline are examples of neurotransmitters found in both vertebrate andinvertebrate nervous systems.

Nerve cells are typically found grouped in bundles. A nerve is simply a bundle of dendrites or axons that serve the same part of the body. A ganglion is a dense cluster of interconnected neurons that process sensory information or control motor outputs.

Neurons are usually divided into three categories, depending on their function within the nervous system:

i. **Afferent (sensory) neurons** — these bipolar or multipolar cells have dendrites that are associated with sense organs or receptors. They always carry information toward the central nervous system.

ii. **Efferent (motor) neurons** — unipolar cells that conduct signals away from the central nervous system and stimulate responses in muscles and glands.

iii. **Internuncial (association) neurons** — unipolar cells (often with several collaterals and/or branching axons) that conduct signals within the central nervous system.

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Nerve cells are typically found grouped in bundles. A nerve is simply a bundle of dendrites or axons that serve the same part of the body. A ganglion is a dense cluster of interconnected neurons that process sensory information or control motor outputs.

The central nervous system

An insect's **brain** is a complex of six fused ganglia (three pairs) located dorsally within the head capsule. Each part of the brain controls (innervates) a limited spectrum of activities in the insect's body.

- **Protocerebrum:**

The first pair of ganglia is largely associated with vision; they innervate the compound eyes and ocelli. The **optic lobes** of the fly (an insect with particularly good vision) contain about 76% of the brain's neurons. The optic lobe connects directly to the sensory bodies') are best developed in social insects, making up 20% of the brain of the bee and 50% of the brain of worker ants (*Formica*). These are thought to function as higher centres responsible for the most sophisticated computations occurring in the insect brain. Each consists of a topmost cap and a stalk or peduncle (which branches into at least two lobes). The cap consists of a pair of cup-like structures, the medial calyx and the lateral calyx (plural of calyx is calyces). The mushroom bodies receive sensory inputs from the lobula of the optic lobe and from the antennal lobes of the deutocerebrum. Most sensory inputs enter the MB through the calyx. There are about 1000 to 100 000 specialised neurons, called **Kenyon cells**, in each mushroom body. These neurons have tree-like branching dendrites which receive inputs in the calyces of the MB, a single axon which extends down the stalk of the

MB and then gives of branches to two lobes of the MB. Dragonfly mushroom bodies have no calyces and no Kenyon cells. The mushroom bodies are also involved in learning, and in the honeybee have been shown to process memories, transferring data from **short-term memory (STM)** into **long-term memory (LTM)**. The **central body** receives inputs from the mushroom bodies and integrates sensory inputs from different sensory modalities (such as smell and vision)-so-called **multimodal sensory perception**. It functions as an **activating centre**, switching on appropriate locomotor activity patterns which are **central programs** located in the thoracic ganglia. That is it instructs the thoracic ganglia which programs to run - programs that control the legs and wings. These hard-wired programs are sometimes called **central pattern generators** and require no sensory input for their execution, though sensory inputs may start and stop these programs or modify them slightly.

The **pars-intercerebralis** is a mass of cell bodies; **including neurosecretory cells** which send their axons to the pair of corpora cardiaca (see the neuroendocrine system in insect development). The corpora cardiac are sometimes fused into a single medial ganglion. They send out nerves to innervate the dorsal blood vessel, forming a **cardio-aortic system**, which controls the rate of heart beat, as well as having a secretory hormonal function.

Biological Clocks

Another function associated with the protocerebrum is time-keeping. Insect activity is timed with the daily light/dark cycle - the **circadian cycle** ('ciracdian' means 'about a day', the exact time being set each day according to environmental cues such as the length of daylight). This timing is due to internal clocks within the insect, which update themselves according to external cues from the environment (**zeitgeibers** or time-givers) such as the number of hours of light and dark.

- ## Deutocerebrum:

The second pair of ganglia process sensory information collected by the antennae. This consists of two nerve centres - the main **antennal lobe (AL)** and the smaller **antennal mechanosensory and motor centre (AMMC)** or dorsal lobe. The AL receives inputs from the third (terminal) antennal segment (the flagellum, which is made-up of sub-segments called flagellomeres) via the antennal nerves. It contains from less than 10 to more than 200 sub-centres called **glomeruli** (singular glomerulus). Inputs to the AL appear to be mainly or exclusively from chemoreceptors (i.e.chemical sensors - olfactory and gustatory, smell and taste) on t he flagellum. Each antenna sends signals to the AL on the same side of the head (ipsilateral pathways) although some may also send signals to the AL on the opposite side (contralateral pa thways). Each glomerulus is a region of neuropil (nerve cell processes and synapses) w here computations oc cur. It i s thought that each glomerulus may, in some species at least, receive inputs from as

pecific class of receptor (sensor) on the antenna. For example, in the males of some species there is a specially large glomerulus, called the **macroglomerular complex (MGC)** which receives inputs from pheromone olfactory sensors on the antenna.The AL does not receive one input line from each chemoreceptor, as sensors of the same type converge - their axons fuse into a smaller number of axons in the antennal nerve (typically inputs from 15 s sensors are combined, a 15: 1 ratio). These sensory input axons, and also input axons from the CB of the protocerebrum, synapse with local interneurones within the AL (amacrine cells). Outputs from the AL are carried along the axons of out put neurons to the MB of the protocerebrum.The AMMC receives mechanosensory inputs from mechanosensors (mechanoreceptors) on the first two antennal segments (scape and pedicel) via the antennal nerves. It also sends motor outputs to the muscles of the scape. It also receives inputs from mechanosensors on the labial palps, some tegument (body w all) mechanosensors, and some inputs from the flagellum (possibly from the mechanosensors found on the flagellum). The antennal nerve is therefore a mixed nerve - containing bothsensory and motor axons. Some of the antennal mechanoreceptors also send outputs to the SOG, the protocerebrum and the thoracic ganglia.

- ## Tritocerebrum and Stomatogastric System:

The third pair of ganglia innervates the labrum and integrates sensory inputs from proto- and deutocerebrums. They also link the brain with the rest of the ventral nerve cord and the stomodaeal nervous system (see below) that controls the internal organs. The commissure for the tritocerebrum loops around the digestive system, suggesting that these ganglia were originally located behind the mouth and migrated forward (around the esophagus) during evolution.

Located ventrally in the head capsule (just below the brain and esophagus) is another complex of fused ganglia (jointly called the subesophageal ganglion)? Embryologists believe this structure contains neural elements from the three primitive body segments that merged with the head to form mouthparts. In modern insects, the subesophageal ganglion innervates not only mandibles, maxillae, and labium, but also the hypopharynx, salivary glands, and neck muscles. A pair of circumesophageal connectives loop a round the digestive system to link the brain and subesophageal complex together.

In the thorax, three pairs of thoracic ganglia (sometimes fused) control locomotion by innervating the legs and wings. Thoracic muscles and sensory receptors are also associated with these ganglia. Similarly, abdominal ganglia control movements of abdominal muscles. Spiracles in both the thorax and abdomen are controlled by a pair of lateral nerves that arise from each segmental ganglion (or by a median ventral nerve those branches to each side). A pair of terminal abdominal ganglia (usually fused to form a large **caudal ganglion**) innervates the anus, internal and external genitalia, and sensory receptors (such as cerci) located on the insect's back end.

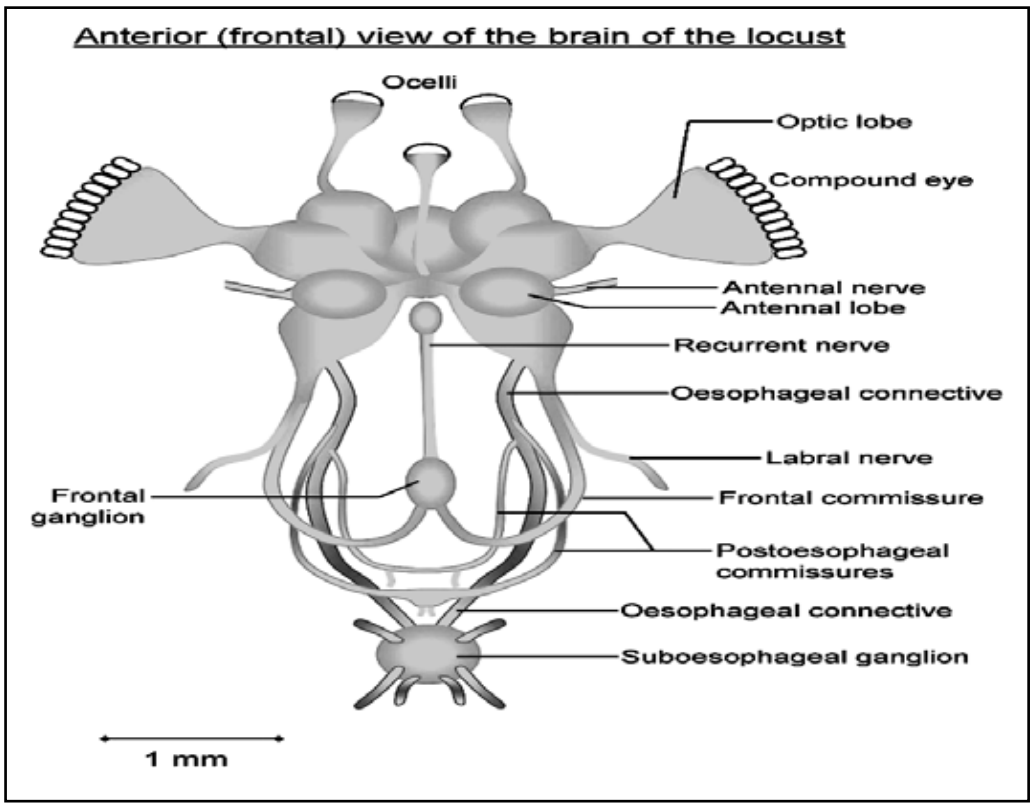

Figure: Insect Brain- frontal view.

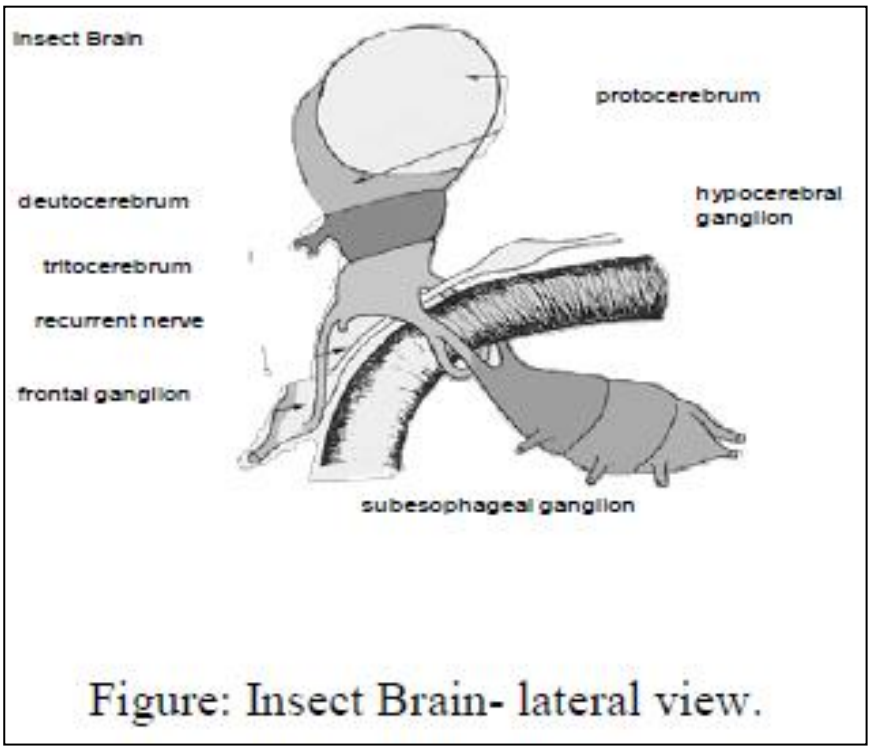

Figure: Insect Brain- lateral view.

The Stomodaeal Nervous System

An insect's internal organs are largely innervated by a stomodaeal (or stomatogastric) nervous system. A pair of frontal nerves arising near the base of the tritocerebrum links the brain with a frontal ganglion (unpaired) on the anterior wall of the esophagus. This ganglion innervates the pharynx and muscles associated with swallowing. A recurrent nerve along the anterio- dorsal surface of the foregut connects the frontal ganglion with a hypocerebral ganglion that innervates the heart, corpora cardiaca, and portions of the foregut. Gastric nerves arising from the hypocerebral ganglion run posteriorly to ingluvial ganglia (paired) in the abdomen that innervates the hind gut.

In comparison to vertebrates, an insect's nervous system is far more decentralized. Most overt behavior (*e.g.* feeding, locomotion, mating, etc.) is integrated and controlled by segmental ganglia instead of the brain. In some cases, the brain may stimulate or inhibit activity in segmental ganglia but these signals are not essential for survival. Indeed, a headless insect may survive for days or weeks (until it dies of starvation or dehydration) as long as the neck is sealed to prevent loss of blood!

Suboesophageal Ganglion

The suboesophageal ganglion (SOG) and the segmental ganglia of the double ventral nerve- cord each send out pairs of nerves, one of which innervate t he pair of spiracles on that segment and so help regulate breathing. (In some insects the segmental ganglia are absent,

e.g. in *Dytiscus*, in which case the lateral abdominal nerves send out nerves to innervate the spiracles). The SOG is a composite ganglion; formed by fusion of the ganglia from the mandibular, maxillary and labial segments of the head and the SOG also sends out nerves to the mouthparts (mandibles, palps, etc.) and so controls feeding behaviours.

The Ventral Nerve Cord

From the suboesophageal ganglion two connectives or nerve cords run back along the ventral side (underside) of t he insect. These connect to the thoracic ganglion of the first thoracic segment, T1, which is actually a pair of ganglia, more-or-less fused into a single structure. T1 then g ives off two connectives to the second thoracic ganglion, T2 and the sequence continues with a chain of connected ganglia running throughout the length of the insect, in the basic plan. Thus, we say that insects have a double ganglionated ventral nerve cord (VNC). Each ganglion functions as a local processor, regulating the functions of its body segment. The thoracic ganglia are especially well-developed as they have to carry out complex computations to generate patterns of movement in the legs and wings. These **output patterns or cen tral programs** are contained in the ganglia, but the brain is normally required to switch them on and off. Sensory inputs have little effect on the basic patterns, but do modify them. For example,

stress sensors in the wings feedback information to allow fine-adjustments to the wings and control of the angle of attack and wing-twisting. Typically, however, the basic pattern of movement is pre-coded.

Probable questions:

1. Name the different parts of insect brain and draw a labeled diagram of the frontal view.
2. Describe the protocerebrum of insect brain and state the functions of different cells ofthis region.
3. Describe the deutocerebrum of insect brain and state the functions of different centresof this region.
4. Describe the tritocerebrum of insect brain and state the functions of different centres ofthis region.
5. What is stomodaeal nervous system? Describe stomodaeal nervous system with properdiagram.
6. What do you mean by AMMC & AL in respect of insect brain? State their function.
7. Describe the structure of insect ventral nerve cord and state its function.

Suggested Reading:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
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8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

UNIT XVI

Insect visual organs, their structure and functional mechanisms

Objective:

In this unit we will discuss about Insect visual organs, their structure and functional mechanisms.

Introduction:

Most adult insects have a pair of **compound eyes**, one on either side of the head, which bulge out to a greater or lesser extent so that they give a wide field of vision in all directions. The compound eyes may, however, be reduced or even absent in some parasitic insects.

As the name suggests, compound eyes are composed of many similar, closely-packed facets (called **ommatidia**) which are the structural and functional units of vision. The number of ommatidia varies considerably from species to species: some worker ants have fewer than six while some dragonflies may have more than 25,000.

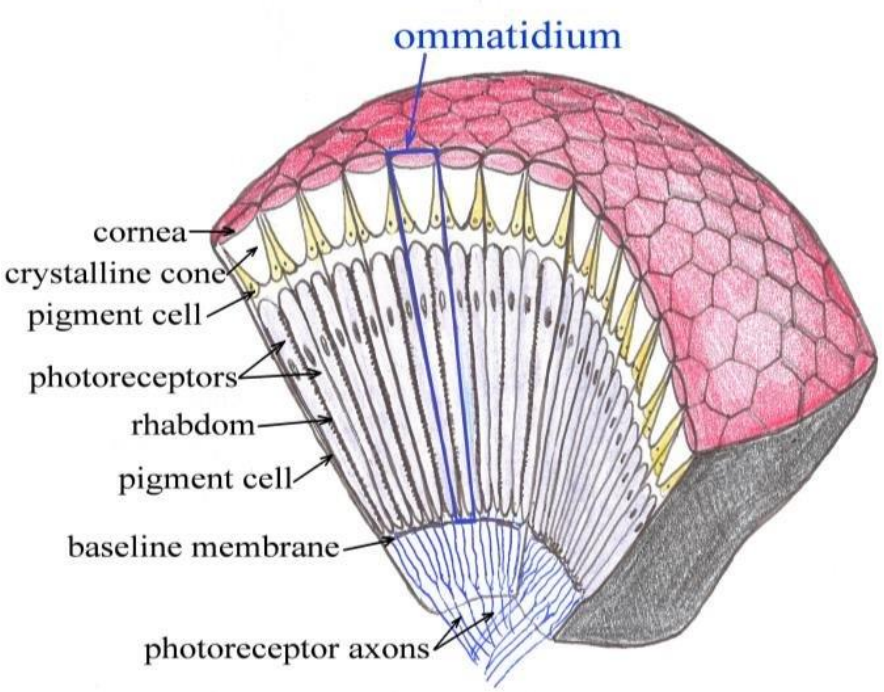


Fig: Structure of compound eye

Externally, each ommatidium is marked by a convex thickening of transparent cuticle, the corneal lens. Beneath the lens, there is often a crystalline cone secreted by a pair of semper cells. Together, the lens and the crystalline cone form a dioptric apparatus that refracts incoming light down into a receptor region containing visual pigment. Each ommatidium is composed of a number of cells arranged end to end along a central axis.

However, it comprises the following structures:

(i) Cornea:

The outermost layer of an eye is the transparent cuticle forming cornea which is divided into a large number of square-like facets. These facets are thickened in the centre to give them the appearance of a biconvex lens. Thus, each corneal facet behaves like a lens and sheds off at the time of moulting, again secreted by the underlying cells.

(ii) Corneagen cells:

Each corneal facet is followed by a group of two cells; these cells are modified epidermal cells called corneagen cells. Their function is to secrete cornea when it is moulted off.

(iii) Cone cells or vitrellae:

These are a group of four much elongated cells situated beneath the corneagen cells. These cells secrete and enclose a transparent and refractile crystalline cone which works like a second lens. The inner end of cone cells is long and tapering. The cornea, corneagen cells and cone cells together constitute the dioptrical region, whose function is to focus the light rays on the inner sensitive region.

(iv) Retinal cells:

The cone cells are followed by a group of seven elongated cells forming the proximal part of the axis of an ommatidium. These cells are elongated and provided with distally placed dilations having nuclei.

(v) Rhabdome or optic rod:

It is an elongated, spindle-shaped and transversely striated body which is secreted and fully enclosed by the retinal cells.

(vi) Basement membrane:

It is the innermost layer of a thin fenestrated membrane that marks the internal boundary of the ommatidia in a compound eye. The ommatidia are innervated by optic nerve fibres, coming from optic ganglia, through the fenestrae in the basement membrane. The retinal cells and rhabdome up to the basement membrane constitute the receptor region; its function is to receive the light rays focused by the dioptrical region.

Pigment sheath:

Each ommatidium is cut off from its neighbouring ommatidia by a sheath of dark pigment formed by the surrounding amoeboid chromatophores which are arranged in two groups. The proximal group surrounding the rhabdome forms the retinal pigment and the distal group surrounding the crystalline cone forms the iris pigment. The amoeboid pigment cells take up different positions according to the changes in the intensity of light.

- ## Working of the compound eye:

The working of the compound eye is very complex. In the formation of an image, several adjacent ommatidia take part and light enters through them.

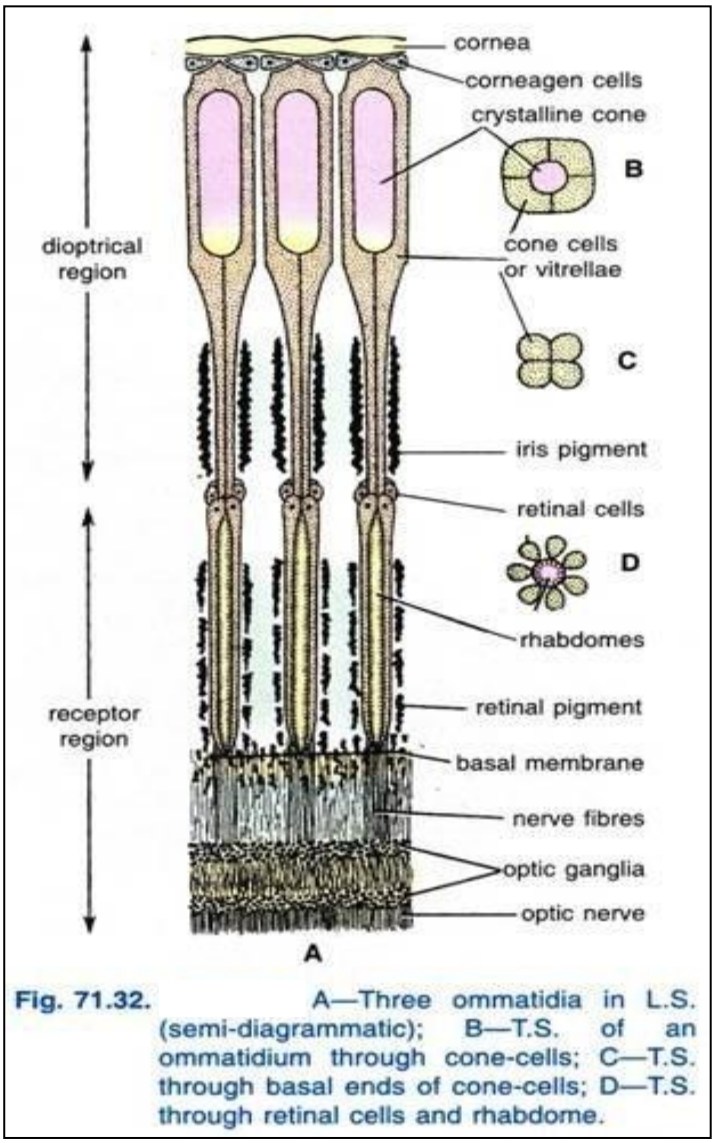


Fig. 71.32. A—Three ommatidia in L.S. (semi-diagrammatic); B—T.S. of an ommatidium through cone-cells; C—T.S. through basal ends of cone-cells; D—T.S. through retinal cells and rhabdome.

Each ommatidium is capable of producing a separate image of a small part of the object seen. Therefore, the whole image formed in a compound eye is actually made of several small pieces contributed by the several adjacent ommatidia. On this account the vision effected through a compound eye is called mosaic vision.

These small parts, placed together like the parts in a mosaic, form the image of the entire object. This is known as a mosaic vision in which the rays are received simultaneously by distinctly separate visual elements, i.e., ommatidiaand the image is made up of several components placed in juxtaposition. Such an image is called an apposition image.

The sharpness of the image depends upon the number of ommatidia involved and the degree of their isolation from one another ; the larger number of ommatidia and more complete their isolation from one another, the sharper the image.

However, an eye adapted for this type of image formation functions best at short distances only; it is, therefore, most of the arthropods are short-sighted. Such arthropods are usually night blind, e.g., butterflies.

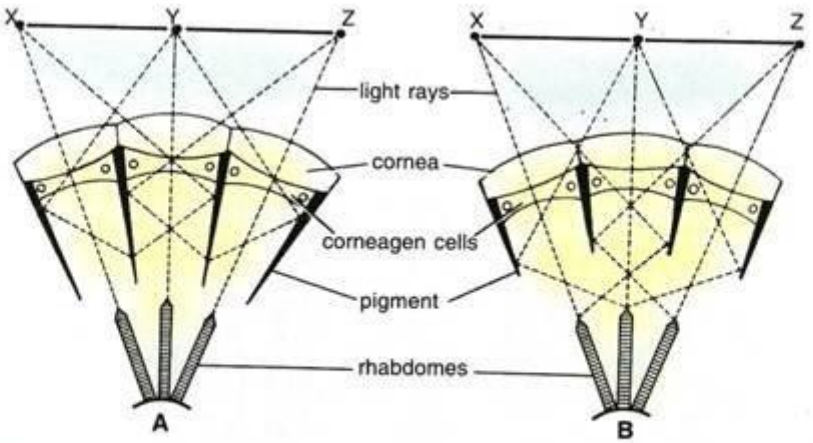


Fig. 71.33. Diagrammatic representation of image formation by a compound eye. A—Apposition or mosaic image; B—Superposition image.

Formation of superposition image:

In the dim light, the pigment cells migrate towards the distal and basal parts of the ommatidia and the neighbouring ommatidia work in unison. In this condition, even the oblique rays of the light are capable of forming a point of image, after passing through a number of ommatidia.

As a result, an overlapping of the adjacent points of image takes place and, thus, a continuous image is formed. Such an image is called superposition image.

In this case, the vision is not distinct but the animal is able to have some sort of idea of its surrounding objects, especially of their movements.

In some insects, like fire-flies and some moths, the eyes are permanently set in the way that they are adapted for vision in the dim light, i.e., at night but they are day-blind, e.g., moths and fireflies. It is probable that the *Palaemon malcolmsonii* like most of the arthropods can adjust its eyes so as to form both the types of images according to the intensity of light available.

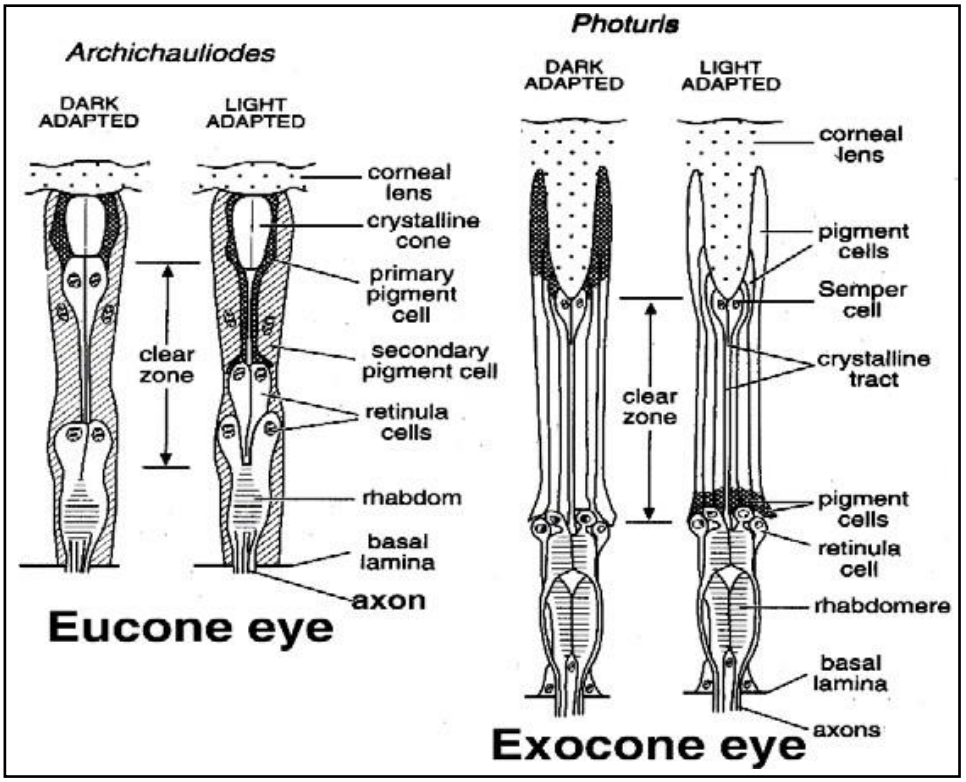
- **Types of compound eye: according to the presence and nature of the crystalline cone.**

Eucone eyes- each ommatidium contains a hard, refractive, conical body secreted with in the crystalline c one c ells a nd f orming pa rt of t he di optric a pparatus. O ccur i n T hysanura, Ephemeroptera, Odonata, Orthoptera, some Hemiptera, Lepidoptera, clear zone is bridged by retinula cells]

Pseudocone eyes- the cone cells are filled with a transparent viscous liquid. Ex Brachyceran and Cyclorrhaphan Diptera

Acone eyes- the long, transparent crystalline cone cells do not secrete any refractive material.Eg. Dermaptera, some Hemiptera and Coleoptera.

Exocone eye s- in which the crystalline body is replaced by a cone shaped extension of the inner s urface of t he cornea, l ying di stal t o t he unm odified c rystalline c one c ells. E x i nDermestidae, Elateridae, Byrrhidae, clear z one is crossed by a c rystalline t ract f ormed fromthe semper cells.



- **Variations of compound eye in different insects**

The number and size of the facets vary greatly. For ex ample: in the worker ant, *Ponera sp.* each eye is a ingle facet while among t he workers of other ant species, it m ay vary from 100-600. In *Musca sp*.- 4000; Odonata- 10000-28000.In most insects facets are very closely packed together and hexagonal but where they are fewer and less closely compacted, they are circular. In male *Tabanus*, they are often larger over the anterior and upper part of the eye and the two fields are not sharply demarcated. In males of other D iptera such as *Bibio* and *Simulium*, the two areas of di fferent s ized facets ar e very distinctly separated, each eye appearing double. In certain Coleoptera, *Gyrinus* and Ephemeroptera, *Cloeon*, the two parts of the eye are separated so that the insect appears to possess two pairs of compound eyes. In *Cloeon* the anterior division of e ach eye is elevated on a pillar like outgrowth of the head while the posterior division is normal.

- **Ocelli — Simple eyes**

Two types of "simple eyes" can be found in the class Insecta: **dorsal ocelli** and **lateral ocelli (=stemmata)**. Although both types of ocelli are similar in structure, they are believed to have separate phylogenetic and embryological origins.

1. Dorsal ocelli (simple eye)

Dorsal ocelli are commonly found in adults and in the immature stages (nymphs) of many hemimetabolous species. They are not independent visual organs and never occur in species that lack compound eyes. Whenever present, dorsal ocelli appear as two or three small, convex swellings on the dorsal or facial regions of the head. They differ from compound eyes in having only a single corneal lens covering an array of several dozen rhabdom-like sensory rods. These simple eyes do not form an image or perceive objects in the environment, but they are sensitive to a wide range of wavelengths, react to the polarization of light, and respond quickly to changes in light intensity. No exact function has been clearly established, but many physiologists believe they act as an "iris mechanism" — adjusting the sensitivity of the compound eyes to different levels of light intensity.

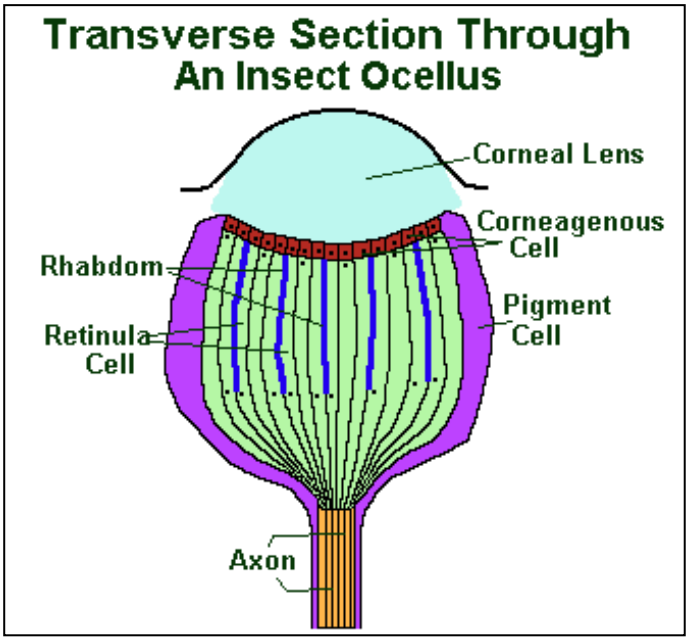


Fig: T.S. of insect ocelli

Functions of Dorsal ocelli

The visual fields of the dorsal ocelli are overlapped by those of the compound eyes, this is because the principal focal plane of t heir lens falls below the level of t he retinal layer and because of the convergence of the many retinal cells on to a much smaller number of ocellar nerve fibers. They signal the level of changes in the light intensity. Occluding the ocelli usually leads the insects to move less rapidly, specially in bright light. This is probably because, the dorsal ocelli may be 'stimulatory organs' serving to rise the ex citatory level of the insects with respect to other visual stimuli perceived through the compound eyes. Another view is that the o celli a nd compound eyes interactin mediating the phototactic behavior of insects. In *Locusta* and *Gryllus*, they act together in promoting m ore accurate directional orientation; in dim light the dorsal ocelli and eyes interact synergistically while in brighter light they behave antagonistically.

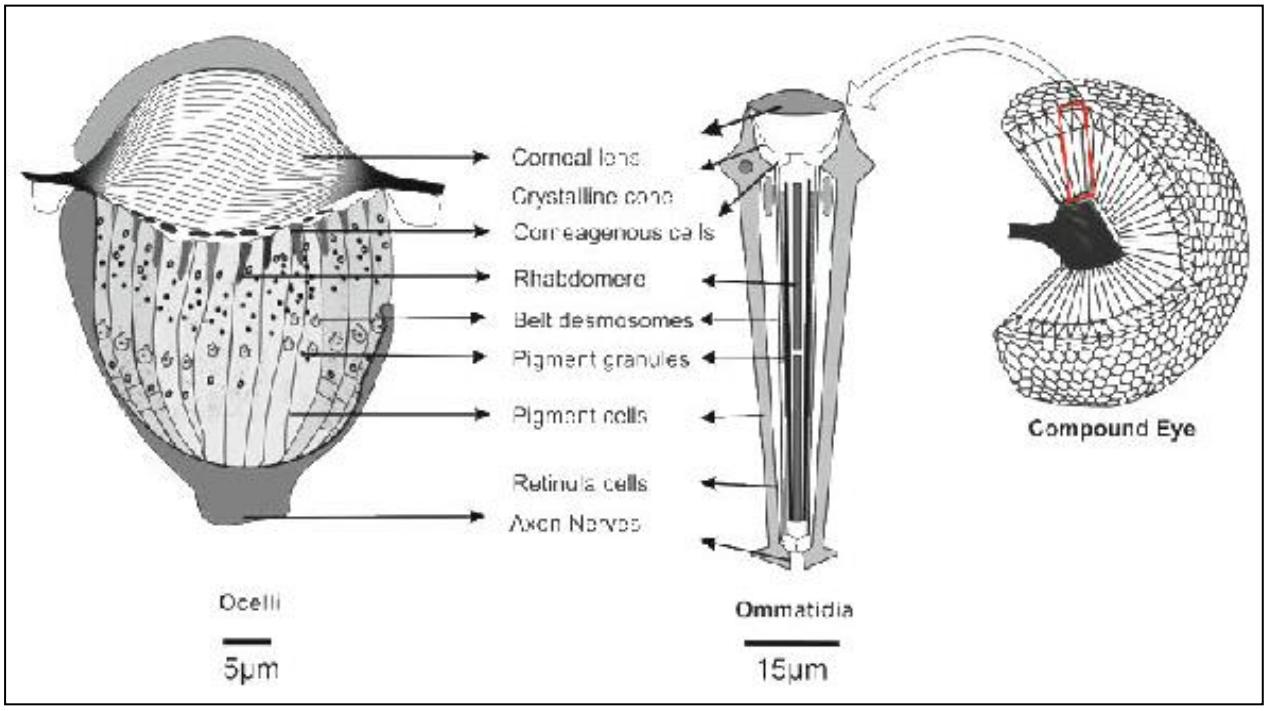


Fig: Structure of Dorsal ocelli and compound eye

2. Lateral ocelli (=stemmata)

Lateral ocelli (=stemmata) are the sole visual organs of holometabolous larvae and certain adults (*e.g.* Collembola, Zygentoma, Siphonaptera, and Strepsiptera). Stemmata always occur laterally on the head, and vary in number from one to six on each side. Structurally, they are similar to dorsal ocelli but often have a crystalline cone under the cornea and fewer sensory rods. Larvae use these simple eyes to sense light intensity, detect outlines of nearby objects, and even track the movements of predators or prey. Covering several ocelli on each side of the head seems to impair form vision, so the brain must be able to construct a coarse mosaic of nearby objects from the

visual fields of adjacent ocelli.

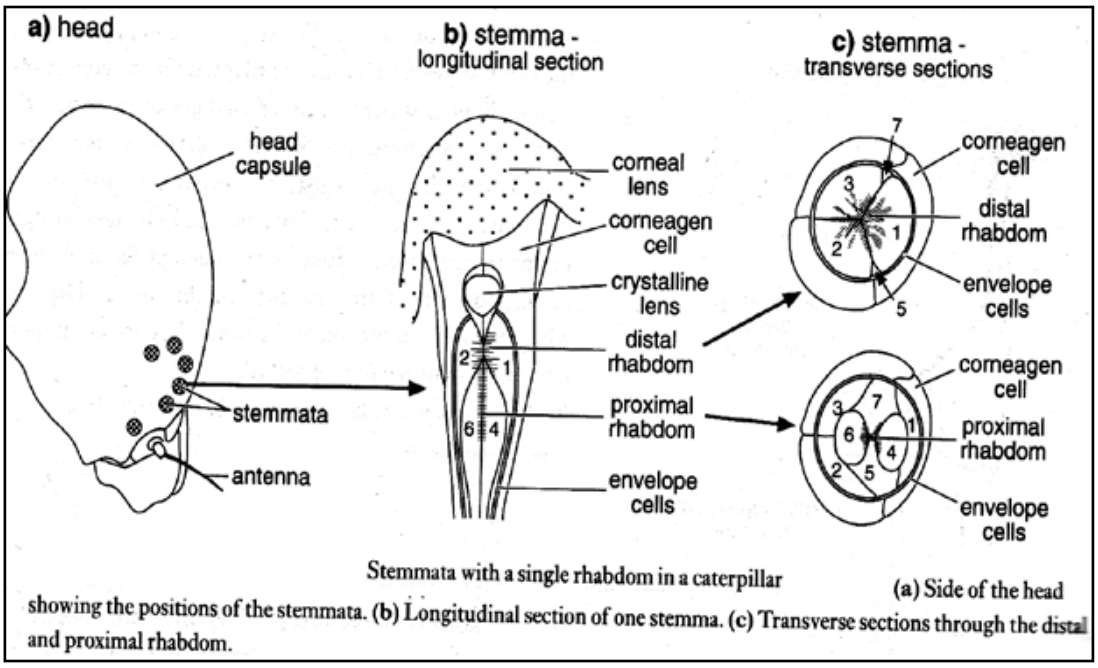


Fig: Diagram of different position of stemmata

Functions of stemmata

Their functions appear very similar to those of compound eyes. They form a relatively focused image and are important in colour vision, predator avoidance, prey capture etc. They can also detect the plane of polarized light, though no functional significance has yet been reported. Found in Mecoptera, most Neuroptera, Lepidoptera and Trichoptera

- **Types of stemmata found in various insects**

✓ In Saw fly and beetle larvae each stemmata one on either side of the head, comprises a single cuticular lens lying beneath which are groups of photosensitive cells with a central rhabdom.

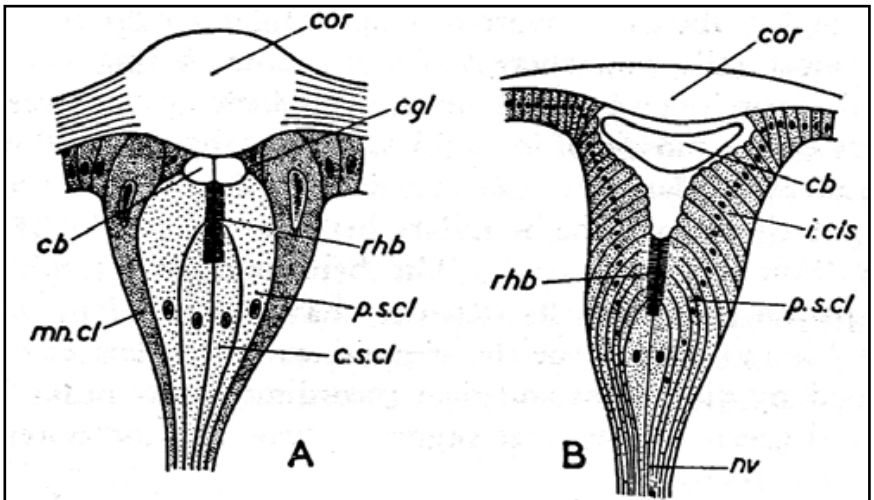
✓ In *Dytiscus, Euroleon* and *Sialis*, a lens like crystalline body is secreted beneath the cornea but the structure otherwise resembles the first type.

✓ In the Lepidoptera and Trichoptera, each larval ocellus has a cornea and crystalline body with seven retinal cells, forming a single retinula with the rhabdomeres constituting a single axial rhabdom. The resulting organ is strikingly similar to each ommatidium of a compound eye.

✓ In larval Symphytans (Hymenoptera) and tiger beetle (*Cicindela*), stemmata with multiple rhabdoms are reported. In the larvae of cyclorrhaphan Diptera there are no

external signs of stemmata, but a pocket of photosensitive cells occurs on each side of the pharyngeal skeleton.

- ✓ In the unpigmented ocellus of *Chironomous* the vitreous cells are absent and a single retinula lies directly beneath unmodified cuticle. In *Aedes aegypti* larvae the retinular cells have the kind of ultrastructure expected in insect eyes, with RER, rhabdomeric microvilli and a zonula adherent junction between contiguous cells. In *Musca* there is a small group of light-sensitive cells on each side of the pharyngeal sclerites and invisible externally. They are most sensitive to green light and apparently unable to perceive red.



Two types of lateral ocelli. A. Lepidopteran larva. B. *Dytiscus* larva (*after* Snodgrass, 1935)

cb, crystalline body; *cgl*, corneagen cell; *cor*, corneal lens; *c.s.cl*, central retinal cells; *i.cls*, pigmented iris cells; *mn.cl*, mantle cell; *nv.*, ocellar nerve; *p.s.cl.*, peripheral retinal cells; *rhb*, rhabdom.

Fig: Different types of lateral ocelli

Probable Questions:

1. What are ommatidia?
2. What is the function of compound eye?
3. Name different types of compound eyes?
4. What is the function of stemmata?
5. Write short notes on lateral ocelli.
6. Discuss different types of stemmata found in various insects.

Suggested Readings / References:

1. Insect Vision: Ommatidium Structure and Function; You tube presentation by ProfLarry Keeley, Emeritus, Department of Entomology, Texas A & M University
2. Structure and functions of Photoreceptors: Encyclopaedia Britanica.
3. Richards & Davies, Imms general textbook of entomology, 10th Edition
4. The Principles Of Insect Physiology, by Wiggles Worth V.B.
5. The Insects: Structure and Function 5th Edition, by R. F. Chapman

UNIT XVII

Insect blood: Composition, functions, morphology of circulatory system

Objective:

In this unit we will learn about Insect blood: Composition, functions, morphology of circulatory system

Introduction:

Insects, like all other arthropods, have an open circulatory system which differs in both structure and function from the closed circulatory system found in humans and other vertebrates. In a closed system, blood is always contained within vessels (arteries, veins, capillaries, or the heart itself). In an open system, blood (usually called hemolymph) spends much of its time flowing freely within body cavities where it makes direct contact with all internal tissues and organs.

The circulatory system is responsible for movement of nutrients, salts, hormones, and metabolic wastes throughout the insect's body. In addition, it plays several critical roles in defense: it seals off wounds through a clotting reaction, it encapsulates and destroys internal parasites or other invaders, and in some species, it produces (or sequesters) distasteful compounds that provide a degree of protection against predators. The hydraulic (liquid) properties of blood are important as well. Hydrostatic pressure generated internally by muscle contraction is used to facilitate hatching, moulting, expansion of body and wings after moulting, physical movements (especially in s oft-bodied larvae), reproduction (e.g. insemination and oviposition), and evagination of certain types of exocrine glands. In some insects, the blood aids in thermoregulation: it can help cool the body by conducting excess heat away from active f light muscles or it can warm the body by collecting and circulatingheat absorbed while basking in the sun.

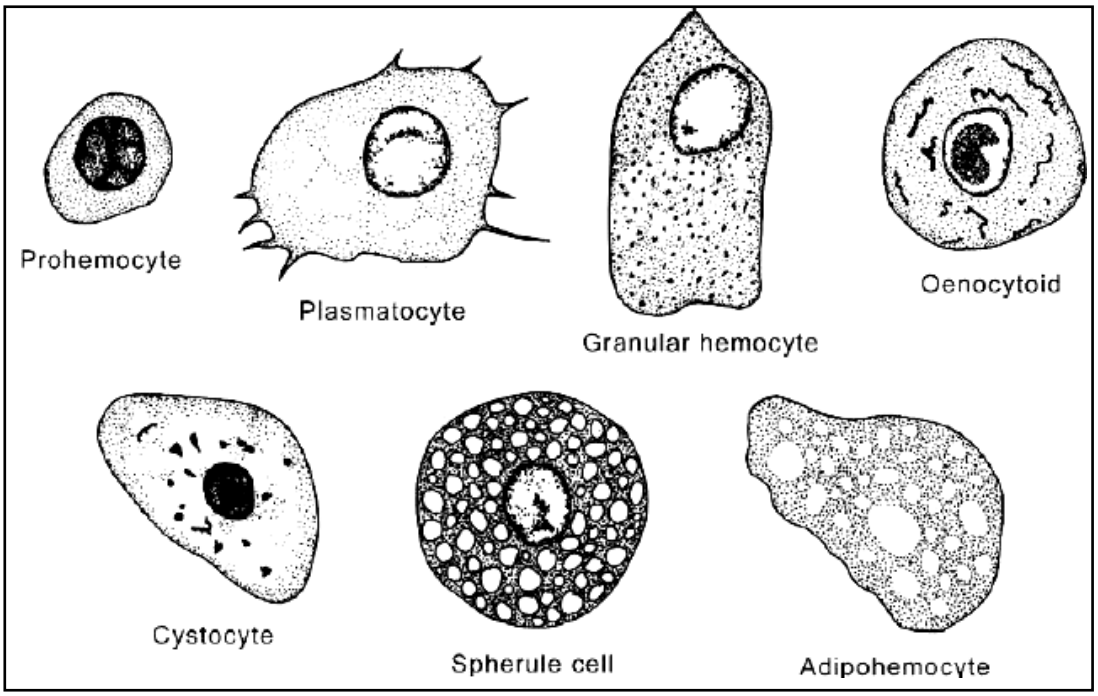
Composition:

About 90% of insect hemolymph is **plasma**: a watery fluid — usually clear, but sometimes greenish or yellowish in color. Compared to vertebrate blood, it contains relatively high concentrations of amino acids, proteins, sugars, and inorganic ions. Overwintering insects often sequester enough ribulose, trehalose, or glycerol in the plasma to prevent it from freezing during the coldest winters. The remaining 10% of hemolymph volume is made up of various cell types (collectively known as **hemocytes**); they are involved in the clotting reaction, phagocytosis, and/or encapsulation of foreign bodies. The density of insect hemocytes can fluctuate from less than 25,000 to more than 100,000 per cubic millimeter, but this is significantly

fewer than the 5 million red blood cells, 300,000 platelets, and 7000 white blood cells found in the same volume of human blood. With the exception of a few aquatic midges, insect hemolymph does NOT contain hemoglobin (or red blood cells). Oxygen is delivered by the tracheal system, not the circulatory system.

The circulating hemolymph, or blood, is not important in respiration but functions in transporting nutrients to all parts of the body and metabolic waste products from the organs to the malpighian tubules for excretion. It contains free cells called hemocytes, most of which are phagocytes that help to protect the insect by devouring microorganisms. An important tissue bathed by the hemolymph is the fat body, the main organ of intermediary metabolism. It serves for the storage of fat, glycogen, and protein, particularly during metamorphosis. These materials are set free as required by the tissues for energy production or for growth and reproduction.

Types of haemocytes:



Function of haemocytes: Insect hemocytes originate from mesodermally derived stem cells that differentiate into specific lineages identified by morphology, function, and molecular markers.

1. Phagocytosis is the most common function of blood cells-ingesting foreign matter, bacteria etc.
2. Haemocytes help in formation of fat bodies.
3. Transport of nutrient and waste
4. Temperature control.
5. Protection against parasites and diseases.

6. For maintaining hydrostatic skeletal element.

- **Composition and function of hemolymph in cockroach**

The haemolymph is the circulatory media in cockroach. It has colourless plasma and many corpuscles called haemocytes. No respiratory pigment is found in it. Hence, it does not help in the transport of respiratory gases. Its sole function is to transport various nutrients from one part of the body to the other and to carry nitrogenous waste substances from the tissues to the organ of excretion.

The plasma contains about 70% water and a large number of organic molecules like free amino acids, uric acid, proteins, sugars and fats, etc. The haemocytes are of three types, i.e., pro-haemocytes, transitional haemocytes and large haemocytes.

According to some workers, only two types of haemocytes are found in *P. americana*, the plasmatocytes and coagulocytes or cytocytes. The plasmatocytes are polymorphic and constitute nearly 60-95% of the total haemocytes. The haemocytes are phagocytic in nature, help in coagulation and haemocoel for healing the wounds.

Structural component of insect circulatory system

Insects, like all other arthropods, have an **open circulatory system** which differs in both structure and function from the **closed circulatory system** found in humans and other vertebrates. In a closed system, blood is always contained within vessels (arteries, veins, capillaries, or the heart itself). In an open system, blood (usually called **hemolymph**) spends much of its time flowing freely within body cavities where it makes direct contact with all internal tissues and organs.

The circulatory system is an open one, with most of the body fluid, or hemolymph, occupying cavities of the body and its appendages. The one closed organ, called the dorsal vessel, extends from the hind end through the thorax to the head; it is a continuous tube with two regions, the heart or pumping organ, which is restricted to the abdomen, and the aorta, or conducting vessel, which extends forward through the thorax to the head. Hemolymph, pumped forward from the hind end and the sides of the body along the dorsal vessel, passes through a series of valved chambers, each containing a pair of lateral openings called ostia, to the aorta and is discharged in the front of the head. Accessory pumps carry the hemolymph through the wings and along the antennae and legs before it flows backward again to the abdomen.

The circulatory system is responsible for movement of nutrients, salts, hormones, and metabolic wastes throughout the insect's body. In addition, it plays several critical roles in defense: it seals off wounds through a clotting reaction, it encapsulates and destroys internal parasites or other invaders, and in some species, it produces (or sequesters) distasteful compounds that provide a degree of protection against

predators. The hydraulic (liquid) properties of blood are important as well. Hydrostatic pressure generated internally by muscle contraction is used to facilitate hatching, molting, expansion of body and wings after molting, physical movements (especially in soft-bodied larvae), reproduction (e.g. insemination and oviposition), and evagination of certain types of exocrine glands. In some insects, the blood aids in thermoregulation: it can help cool the body by conducting excess heat away from active flight muscles or it can warm the body by collecting and circulating heat absorbed while basking in the sun.

A dorsal vessel is the major structural component of an insect's circulatory system. This tube runs longitudinally through the thorax and abdomen, along the inside of the dorsal body wall. In most insects, it is a fragile, membranous structure that collects hemolymph in the abdomen and conducts it forward to the head

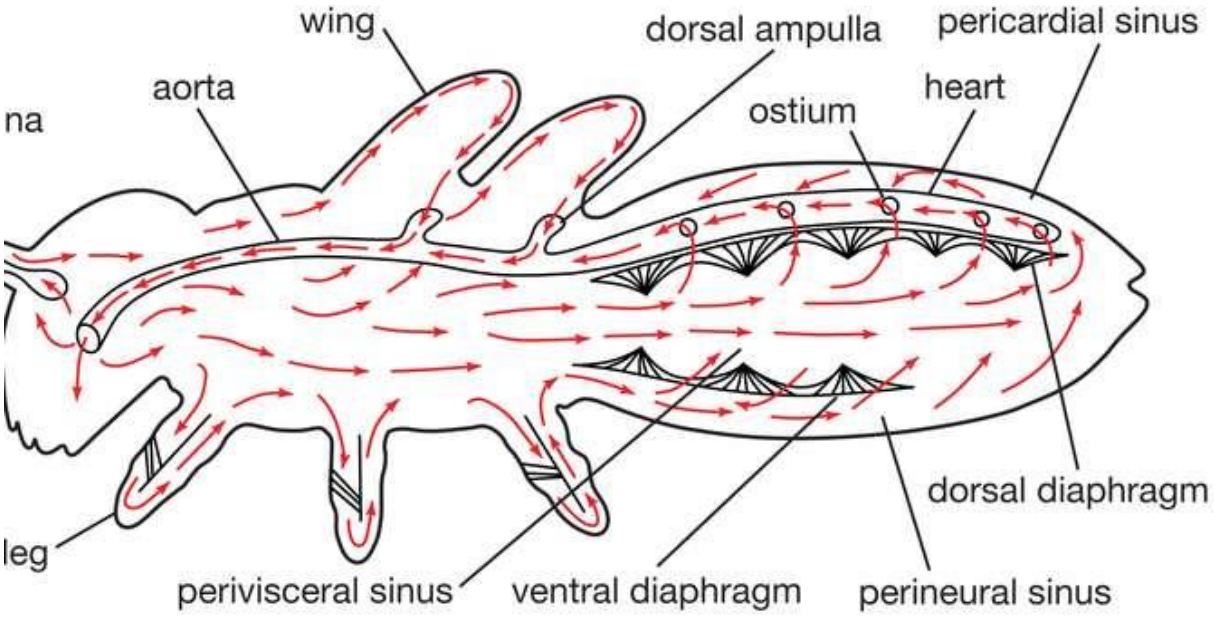


Fig: Circulatory system of a generalized insect.

In the abdomen, the dorsal vessel is called the heart. It is divided segmentally into chambers that are separated by valves (ostia) to ensure one-way flow of hemolymph. A pair of **alary muscles** is attached laterally to the walls of each chamber. Peristaltic contractions of these muscles force the hemolymph forward from chamber to chamber. During each diastolic phase (relaxation), the ostia open to allow inflow of hemolymph from the body cavity. The heart's contraction rate varies considerably from species to species — typically in the range of 30 to 200 beats per minute. The rate tends to fall as ambient temperature drops and rise as temperature (or the insect's level of activity) increases.

In front of the heart, the dorsal vessel lacks valves or musculature. It is a simple tube (called the aorta) which continues forward to the head and empties near the

brain. Hemolymph bathes the organs and muscles of the head as it emerges from the aorta, and then haphazardly percolates back over the alimentary canal and through the body until it reaches the abdomen and re-enters the heart.

To facilitate circulation of hemolymph, the body cavity is divided into three compartments (called blood sinuses) by two thin sheets of muscle and/or membrane known as the dorsal and ventral diaphragms. The dorsal diaphragm is formed by alary muscles of the heart and related structures; it separates the pericardial sinus from the perivisceral sinus. The ventral diaphragm usually covers the nerve cord; it separates the perivisceral sinus from the perineural sinus.

In some insects, **pulsatile organs** are located near the base of the wings or legs. These muscular "pumps" do not usually contract on a regular basis, but they act in conjunction with certain body movements to force hemolymph out into the extremities.

Probable Questions:

1. What is hemolymph?
2. What is the compositon of hemolymph in cockroach?
3. What is the function of hemolymph?
4. What is open circulatory system?
5. What is alary muscle?
6. What is perivisceral sinus?

Suggested Readings / References:

1. Insect Vision: Ommatidium Structure and Function; You tube presentation by ProfLarry Keeley, Emeritus, Department of Entomology, Texas A & M University
2. Structure and functions of Photoreceptors: Encyclopaedia Britanica.
3. Richards & Davies, Imms general textbook of entomology, 10th Edition
4. The Principles Of Insect Physiology, by Wiggles Worth V.B.
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UNIT-XVIII

Structure and functions of sound producing organs in insects

Objectives:

In this section we will discuss about Structure and functions of sound producing organs in insects.

Introduction:

It has long been believed that at least some insects can hear. Chief attention has been given to those that make distinctive sounds (e.g., katydids, crickets, and cicadas) because it was naturally assumed that these insects produce signals for communication purposes. Organs suitable for hearing have been found in insects at various locations on the thorax and abdomen and, in one group (mosquitoes), on the head.

Among the many orders of insects, hearing is known to exist in only a few: Orthoptera (crickets, grasshoppers, katydids), Homoptera (cicadas), Heteroptera (bugs), Lepidoptera (butterflies and moths), and Diptera (flies). In the Orthoptera, ears are present, and the ability to perceive sounds has been well established. The ears of katydids and crickets are found on the first walking legs; those of grasshoppers are on the first segment of the abdomen. Cicadas are noted for the intensity of sound produced by some species and for the elaborate development of the ears, which are located on the first segment of the abdomen. The waterboatman, a heteropteran, is a small aquatic insect with an ear on the first segment of the thorax. Moths have simple ears that are located in certain species on the posterior part of the thorax and in others on the first segment of the abdomen. Among the Diptera, only mosquitoes are known to possess ears; they are located on the head as a part of the antennae.

All the insects just mentioned have a pair of organs for which there is good evidence of auditory function. Other structures of simpler form that often have been considered to be sound receptors occur widely within these insect groups as well as in others. There is strong evidence that some kind of hearing exists in two other insect orders: the Coleoptera (beetles) and the Hymenoptera (ants, bees, and wasps). In these orders, however, receptive organs have not yet been positively identified.

Types of insect auditory structures

Four structures found in insects have been considered as possibly serving an auditory function: hair sensilla, antennae, cercal organs, and tympanal organs.

1. **Hair sensilla**

Many specialized structures on the bodies of insects seem to have a sensory function. Among these are hair sensilla, each of which consists of a hair with a base portion containing a nerve supply. Because the hairs have been seen to vibrate in response to tones of certain frequencies, it has been suggested that they are sound receptors. It seems more likely, however, that the sensilla primarily mediate the sense of touch and that their response to sound waves is only incidental to that function.

2. **Antennae and antennal organs**

Many sensory functions have been attributed to the antennae of insects, and it is believed that they serve both as tactual and as smell receptors. In some species, the development of elaborate antennal plumes and brushlike terminations has led to the suggestion that they also serve for hearing. This suggestion is supported by positive evidence only in the case of the mosquito, especially the male, in which the base of the antenna is an expanded sac containing a large number of sensory units known as scolophores. These structures, found in many places in the bodies of insects, commonly occur across joints or body segments, where they probably serve as mechanoreceptors for movement. When the scolophores are associated with any structure that is set in motion by sound, however, the arrangement is that of a sound receptor.

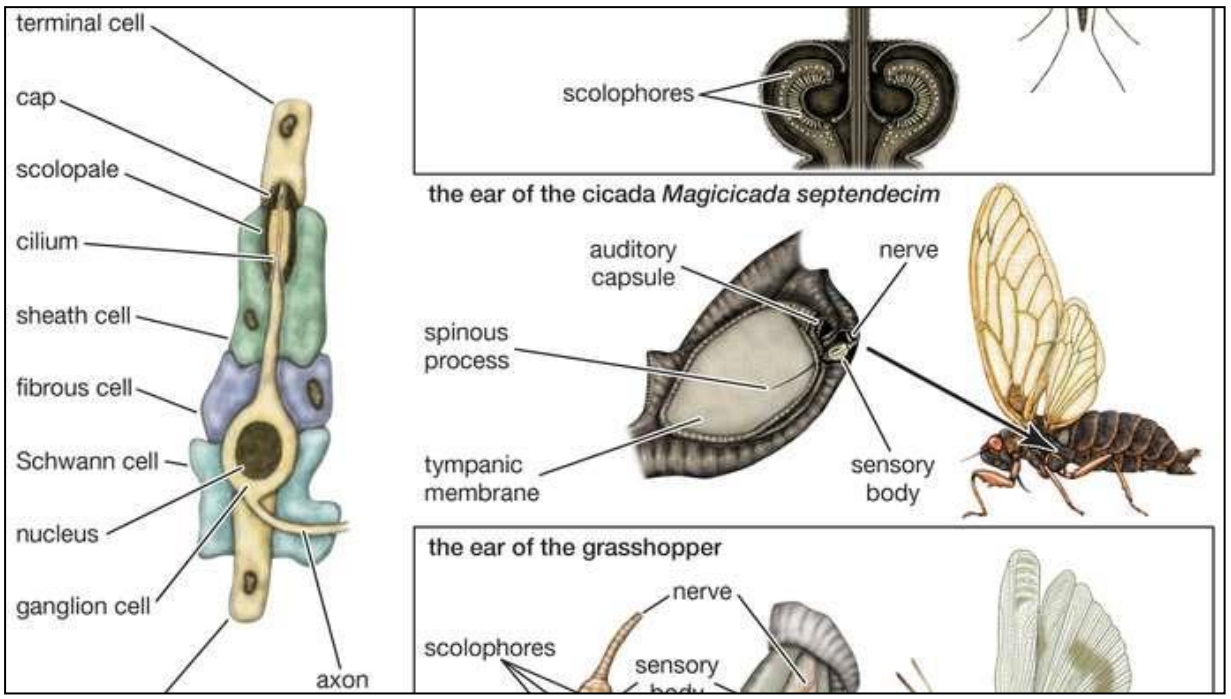

Fig: Auditory mechanisms in insects. (Left) A scolophore organ. (Top right) The mosquito ear. (Centre right) The ear of the cicada *Magicicada septendecim*. (Bottom right) The ear of the grasshopper

In the basic structure of the scolophore, four cells (base cell, ganglion cell, sheath cell, and terminal cell), together with an extracellular body called a cap, constitute a chain. Extending outward from the ganglion cell is the cilium, a hairlike projection that, because of its position, acts as a trigger in response to any relative motion between the two ends of the chain. The sheath cell with its scolopale provides support and protection for the delicate cilium. Two types of enclosing cells (fibrous cells and cells of Schwann) surround the ganglion and sheath cells. The ganglion cell has both a sensory and a neural function; it sends forth its own fibre (axon) that connects to the central nervous system.

In the mosquito ear the scolophores are connected to the antenna and are stimulated by vibrations of the antennal shaft. Because the shaft vibrates in response to the oscillating air particles, this ear is of the velocity type. It is supposed that stimulation is greatest when the antenna is pointed toward the sound source, thereby enabling the insect to determine the direction of sounds. The male mosquito, sensitive only to the vibration frequencies of the hum made by the wings of the female in his own species, flies in the direction of the sound and finds the female for mating. For the male yellow fever mosquito, the most effective (i.e., apparently best heard) frequency has been found to be 384 hertz, or cycles per second, which is in the middle of the frequency range of the hum of females of this species. The antennae of insects other than the mosquito and its relatives probably do not serve a true auditory function.

3. Cercal organs

The cercal organ, which is found at the posterior end of the abdomen in such insects as cockroaches and crickets, consists of a thick brush of several hundred fine hairs. When an electrode is placed on the nerve trunk of the organ, which has a rich nerve supply, a discharge of impulses can be detected when the brush is exposed to sound. Sensitivity extends over a fairly wide range of vibration frequencies, from below 100 to perhaps as high as 3,000 hertz. As observed in the cockroach, the responses to sound waves up to 400 hertz have the same frequency as that of the stimulus. Although the cercal organ is reported to be extremely sensitive, precise measurements remain to be carried out. It is possible, nevertheless, that this structure, which is another example of a velocity type of sound receptor, is primarily auditory in function.

4. Tympanal organs

The tympanal organ of insects consists of a group of scolophores associated with a thin, horny (chitinous) membrane at the surface of the body, one on each side. Usually the scolophores are attached at one end by a spinous process to the tympanic membrane (eardrum); the other ends rest on an immobile part of the body structure. When the membrane moves back and forth in response to the alternating pressures of sound waves, the nerve fibre from the ganglion cell of the scolophore transmits impulses to the central nervous system. Because the tympanic membrane is activated by the pressure of sound waves, this is a pressure type of ear.

Simple tympanal organs, such as those found in moths, contain only two or four elements, or scolophores. In cicadas, on the other hand, these organs are highly developed; they include a sensory body (a number of scolophores in a capsule) that may contain as many as 1,500 elements.

With 80 to 100 scolophores, the grasshopper ear, which has been studied more thoroughly than any other insect ear, is structurally between that of moths and cicadas. Ordinarily, the tympanic membrane is hidden beneath the base of the insect's wing cover. A bundle of auditory nerve fibres runs from one side of the sensory body, which lies on the inner surface of the membrane, and joins other nerve fibres of the region to form a large nerve extending to a ganglion (nerve centre) in the thorax.

• Evidence of hearing and communication in insects

A. Behavioral observations

That the insect ear serves an auditory purpose has been proved by a large number of experimental observations, particularly those that have dealt most extensively with katydids and crickets. Males of these groups produce sounds by stridulation, which usually involves rubbing the covers of the wings together in a particular way. One wing has a serrated surface (a "file") that runs along an enlarged vein; the other wing has a sharp edge over which the file is scraped. The scraping causes the wing surfaces to vibrate; the natural resonances of the vibrations and the particular rhythm and repetition rate of the scraping movements determine the nature of the song, which varies with each species. Most females are silent, but those of a few species have a poorly developed stridulatory apparatus, and weak sounds have been reported. Both males and females have tympanal organs for sound reception.

The observation that the males of many insect species produce repeated stridulatory sounds during the mating season led to the inference that the primary purpose of these noises was to attract a female. That this is indeed the case was first established by the extensive observations of the Yugoslavian entomologist Ivan Regen, who worked over the period 1902–30 mostly with a few species of katydids and crickets. In one of his earliest experiments, Regen proved (1913–14) that a male katydid of the species *Thamnotrizon apterus* responds to the sound of another male by chirping. The first male responds in turn to the second male's chirp, and the two insects then set up an alternating pattern of chirping. Although this pattern had been observed earlier, Regen was the first to prove by a series of experiments that it depends upon the sense of hearing. After removing the forelegs, on which the tympanal organs are located, of certain males, he found that even though these insects continued to stridulate, they did so only in individual rhythms that were not affected by the sounds of other males. Any alternation of chirping between deafened males, or between a deafened and a normal male, occurred only rarely, for brief times, and by chance.

A long series of check experiments by Regen showed that other stimuli, such as light, odours, and surface vibrations, did not affect the chirping behaviour. In these experiments the insects were placed in separate rooms, and their sounds were transmitted by telephone.

Further experiments carried out by Regen on field crickets (*Liogryllus campestris*) demonstrated the reactions of females to chirping males. In the most elaborate of these experiments, 1,600 sexually receptive females were released around the periphery of a large enclosed area in the middle of which had been placed a cage containing one or more chirping males. Precise data concerning the frequency with which the females moved toward the cage were obtained by surrounding the cage site with an array of traps in which the females were caught as they moved inward. The results were statistically significant. Normal females (those with intact tympanal organs) moved toward the cage and eventually reached it. The removal of one foreleg and its tympanal organ, however, caused difficulty; the movements were more random and the approaches fewer, although some females did succeed in reaching the cage. When both tympanal organs were removed or if the male failed to chirp, the performance of the females was reduced to chance. They also failed to exhibit the seeking performances if the male's stridulatory organ was modified, as by removing the file, so that little or no sound was produced.

In 1926 Regen returned to his study of the alternating chirping pattern of katydids and succeeded in having males react to an artificial sound, one that Regen himself produced. He also found that the alternation could be demonstrated with a suitably active male by using a variety of sounds—whistles, percussion noises, and sounds made with his mouth. It was never altogether clear, however, what changes Regen had made in his signals that finally brought success; probably the secret lay in the particular rhythm and timing of the signals. At any rate, this method made possible a study of the general nature of the auditory sensitivity of these insects and the range of sound frequencies to which they responded. It was shown that katydids are most sensitive to the very high frequencies, those that are beyond the limit of the human ear. The instruments available to Regen at the time, however, did not permit a precise measurement of intensity thresholds. (A threshold is the lowest point at which a particular stimulus will cause a response in an organism.)

Although the work of Regen and others established the basic character of sound reception in insects and its role in communication and mating, other details had to await the introduction of electrophysiological methods in this field as well as the development of electronic methods for the precise production, control, and measurement of sound stimuli.

B. Electrophysiological observations

When making electrophysiological observations of an auditory mechanism, an electrode (one terminal, generally a fine wire, in an electric circuit) is placed on a nerve or some other sensory structure in the mechanism. Sounds, presented at different frequencies

and intensities, produce neural or sensory changes, which are actually electrical discharges or changes in electrical potential of extremely small magnitude. The impulses are picked up by the electrode and transmitted to an instrument with which they can be amplified, observed, and recorded. In both behavioral and electrophysiological observations, the auditory sensitivity of an animal to sounds of different frequencies can be illustrated by a curve.

The electrophysiological method was first used in research on the insect ear in 1933, with observations mainly on two katydid and one cricket species. The tympanal organ of these insects is located on one of the segments of the foreleg; its nerve goes to a ganglion in the thorax. When an electrode is placed on this nerve, its threshold sensitivity and overall frequency range can be determined by varying the intensity and frequency of the sounds applied to the tympanic membrane. It has been found that the tympanal organ of these insects responds poorly to low tones (those of low frequency) but improves rapidly as the frequency increases to a maximum sensitivity around 3,000 to 5,000 hertz. For higher frequencies the sensitivity declines, until a limit is reached at 30,000 hertz. It is likely that the insect's identification of its own species by means of song is primarily in terms of intensity and time patterns, with the rapid changes of intensity playing a prominent part. The possibility of frequency also entering into the pattern, however, cannot be ruled out.

• Sound production in Beetels

Sound production

Many beetles produce sound, usually by rubbing one part of the body (a scraper) against another part (the file). These stridulating organs are generally present in both sexes and probably serve for mutual sex calling. Some beetles have a filelike area on the head that is rasped by the front margin of the prothorax. Among the cerambycids, sound is produced either by rubbing the rear margin of the prothorax over a grooved area on the mesothorax or by rubbing the femurs of the hind legs against the margins of the elytra.

Stridulation, however, is not confined to adult beetles; it occurs also in certain larvae. Some larvae of the Scarabaeoidea, for example, have a series of ridges, or tubercles, on the coxal segment of the middle pair of legs, and the hind legs are modified in various ways as rasping organs. In the larvae of some chafers (Melolonthinae), a ridged area on the mandible is rasped by a series of teeth on the maxillae. Stridulation in larvae is independent of sex and may be used to warn neighbouring larvae to avoid getting in each other's way.

Insects are preadapted to be noisy animals. With a hard and sclerotised exoskeleton, the segmented form of the body and jointed limbs will inevitably cause vibrations in t he surrounding environment when an insect moves. It will be very difficult for insects to move silently without making a noise. It is not surprising that m any groups have developed specialised systems of sound production and associated receptors which are used in communication within and between species.

- ## General mechanisms of sound production:

Many authors have attempted to classify sound producing mechanisms in insects. The most useful is probably the entirely m echanistic on e in which five categories of sound producing mechanisms are recognized. These are:

1. Vibration
2. Percussion
3. Stridulation
4. Click mechanisms
5. Air expulsion

These categories are not completely exclusive and some insects m ay use combinations of them.

1. Vibration — Including Tremulation

All animal sounds result from the vibration of some structures. However, in this category are included sound emissions which result from vibrations of relatively unspecialized parts of the insect body, most usually oscillations of the abdomen, either dorso-ventrally or laterally. The term tremulation is useful for this type of sound production and differentiates it from the very general term "vibration". Such sounds are usually transmitted through the legs to the substrate on which t he insect is walking or standing. These w ill therefore usually be detected as substrate transmitted vibrations. Such signals have been documented in various insects, but are well known in lacewing flies and their allies.

Vibrations of other body parts may be important in insect signaling, most obviously the wings. Sounds are inevitably produced as byproducts of flapping flight, but m any insects have developed the use of wing vibrations in communication. The flight sounds in swarming mosquitoes are known often t o be species-specific and t of unction in part, for species recognition. The use of low frequency wing vibration in the courtship dances of Drosophila species is better known. When in close proximity, the pulsed songs of these flies stimulate antennal receptors of other individuals by air particle vibration in the vicinity.

2. Percussion

Tremulation does not involve percussion either of the substrate or of other body parts. This is regarded as a separate mechanism. Percussion of one body part against another may develop as a communication system.

Signaling by percussion of the substrate with the tip of the abdomen is well known in various insect groups, for example in termites and particularly stoneflies. An unusual example among the bus h crickets (Tettigoniidae) i s t he species of Meconema. It is a group otherwise w ell known f or t he pr oduction of l oud s tridulatory signals. M ales of Meconema lack the distinctive stridulatory mechanism typical of the family, but actively stamp the substrate with one of their hind legs and produce patterned signals in that way.

3. Stridulation

The term stridulation has sometimes been used as a general term for any mechanism of sound production in insects, but that negates the utility of the term. It is more usually confined to sounds produced by frictional mechanisms, involving the movements of two specialized body parts against each other in a regular patterned manner. This is an extremely widespread and relatively well-studied mechanism. Such systems have been described in at least s even different insect orders, in most of which it has evolved separately on numerous occasions, as for example i n t he Coleoptera. Almost all body parts which it is possible to bring into juxtaposition have been modified as stridulatory mechanisms in one group or another. The mechanisms i n t he groups of Orthoptera sensulato are particularly w ell-known and documented.

4. Click Mechanisms

These rely on t he deformation of a modified area of cuticle, usually by contraction and relaxation of special musculature within the body. This results in a series of clicks which may be repeated rapidly in distinctive patterns. Such signals may be amplified in a variety of ways in different insects. The specialized area of cuticle, as exemplified most obviously in the loud singing cicadas (Hemiptera, Cicadidae), is known as a tymbal. Such mechanisms are now well known, though not necessarily well understood, in many other groups, including most, if not all other Auchenorrhyncha, many Heteroptera and various families of Lepidoptera.

5. Air Expulsion

This is an unusual and rare mechanism within the Insecta. Various authors have described in a number of insect's exhalatory sounds, often expelled via the tracheal spiracles, but little is known about any function. The best-known example is t he large and spectacular European hawkmoth, the Death's Head Hawk, Acherontiaatropos, which expels air forcibly through the mouthparts t o make a distinctive pi ping sound. Thus m any sound producing mechanisms have been described f or a wide variety o f insects, but m any exist only as possible mechanisms based simply on surmise from morphological evidence.

Neural regulation of sound production:

The patterns of muscular activity resulting in sound production are generated by pattern generators in the central nervous system. In crickets, where the sound is produced by movements of the forewings, the pattern generator is in the mesothoracic ganglion; in grasshoppers, where stridulation involves movements of the hind legs, the generator is in the metathorax. The pattern generators are presumed to be networks of interneurons whose output regulates the activity of the motor neurons controlling the muscles.

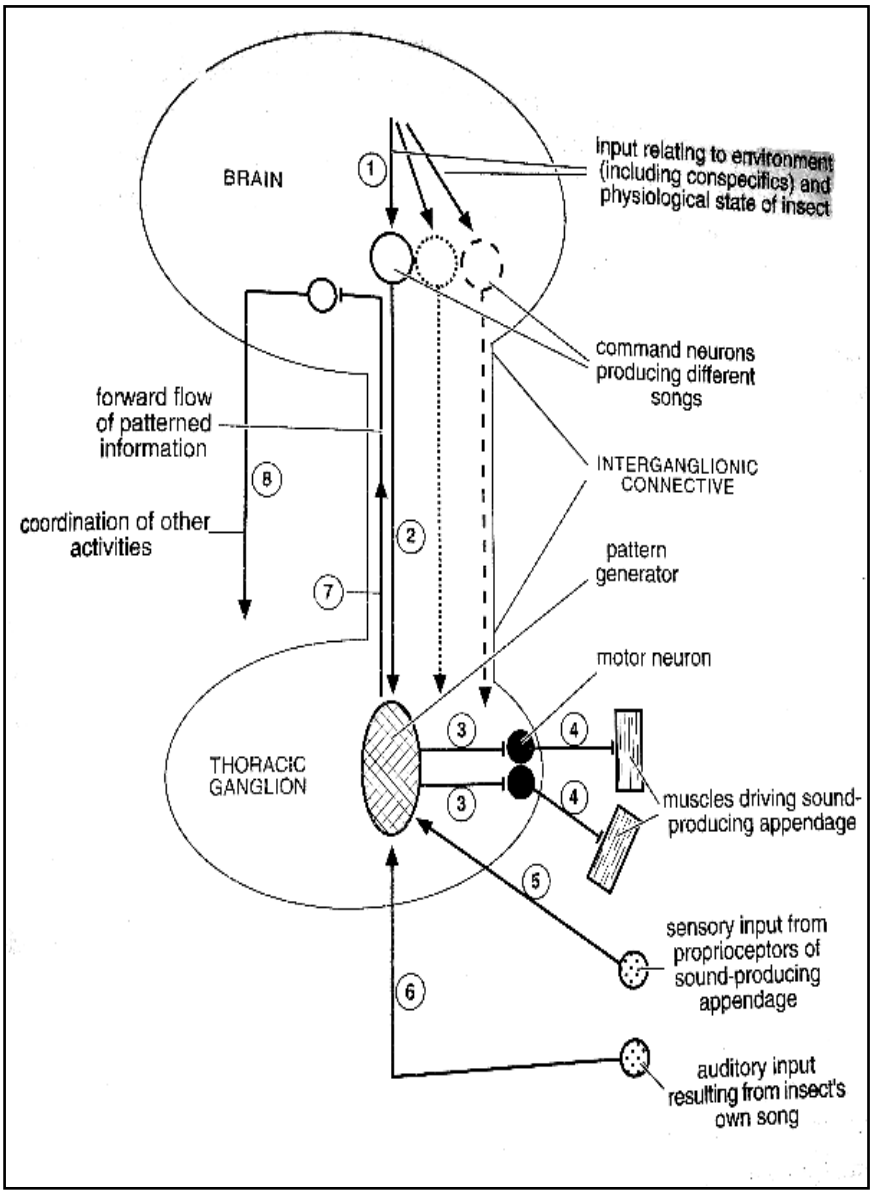

Figure: Neural control of sound production in Orthoptera. Numbers indicate the sequence of neural events.

Probable questions:

1. Discuss the different mechanisms of sound production by insects.
2. What is stridulation? Mention any stridulatory apparatus of insect and write down themechanism by which it produces.
3. What do you mean by percussion in regard to insect sound production?
4. What do you m ean b y vibration in regard t o insect sound production? Mention t heorgans by which insect produce this type of sound.
5. Discuss the neural regulation of sound production in insects.
6. What are tymbal organs? State their functions.

Suggested readings:

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4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
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UNIT XIX

Exocrine glands: Lac gland, Wax gland, Silk gland, Labial gland

Objective:

In this unit we will learn about insect Exocrine glands: Lac gland, Wax gland, Silk gland, Labial gland

Introduction:

The variety of chemical signals and defenses vary among different group of insects. These chemicals are produced by t he exocrine glands which show immense diversity in location, structure and f unction. Some of these glands are the modified labial/salivary glands whileothers are dedicated glands (wax, poison) having specific functions.

Types of Insect exocrine glands:

1. Lac gland

Lac is a resinous exudate secreted from glands situated beneath the skin of lac insects. On chemical analysis it has been found that lac contains resin, carbohydrate, protein, water soluble salts, sand particles, volatile oils and pigments.

The lac insects Tachardia belong to the order Hemiptera of the class insecta. Lac insects and its economic importance are known to mankind for a long time. The Atharba Veda contains an account and description of female lac insects. In Ain-E-Akbari specific account of the economic importance of lac insect is given.

Two species under the genus Tachardia namely T. lacca and T. chinensis are very common. T. lacca is the common Indian variety. India tops the lac producing countries and Thailand comes next to India. Lac insects live on certain specific trees like Kusum, Khair and Ber.

The commercial value of lac is great.

It is used for the following:

(a) Varnishes and polishes,

(b) Lithographic ink,

(c) Sealing wax,

(d) Insulating material,

(e) Shoe polish, toys and ornaments etc.

- **Structure:**

The integument of the lac insect consists of an external layer of cuticle, underlying which there is the syncytial hypodermis, many of whose cells are modified into lac glands which secrete the resin of commerce.

The lac glands are of two types. The first type includes pleuricellular glands which are restricted in their distribution to the perivaginal pore clusters. To the second type belong unicellular glands with separate ducts and prominent nuclei. Some of these are very much limited in their distribution, being present only along six serpentine areas of marginal duct clusters. These are flask-shaped in appearance, and have wide necks and prominent nuclei and nucleoli. Others belonging to the same category are distributed diffusedly all over under the integument, excepting the six serpentine areas, and possess a round fundus with filiform ducts.

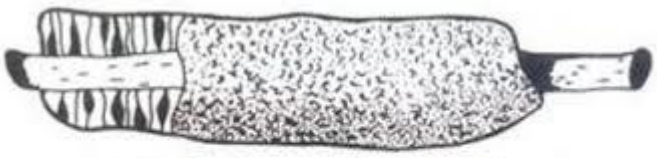

Fig. Lac incrustation on a twig

2. Wax gland

Glands that secrete wax, located in the integument of insects. They are frequently present in herbivorous species, such as bees, bumblebees, scale insects, and aphids. Wax glands sometimes consist of a single hypodermal cell, for example, in aphids.

Wax glands are located on sternites 4, 5, 6 and 7. On each sternite there is two wax mirrors. They consist of modified epidermal cells. The cells are larger when the gland is active. The wax is secreted as liquid and it hardens forming a wax scale. The wax scale is moved by hind leg from wax mirror to mandibles. Production of 1 kg of wax requires consumption 8.4 kg of honey. The wax glands are active only in workers who were fed with pollen during first 5-6 days of their life.

The worker bees secrete wax from glands situated in the abdomen. The secretion is exuded between the segments of the underside of the abdomen and scales of wax can be noticed as a result of hardening of this secretion. These scales are detached from the body by the setae of tarsi and passed onwards to the mouth, wherein they are chewed and made plastic to be used in building the comb walls.

This wax is isolated and forms an important base for an important industry concerned with the manufacture of toilet goods and cosmetics. A large quantity is utilised in pressing comb foundations and returned to the bees-hive wherever artificial methods of rearing is carried out.

Several thousand mounds bees-wax is used in preparing candles, shaving creams, cold creams, cosmetics, polishes, castings of models, carbon paper, cryons, electrical and other products.

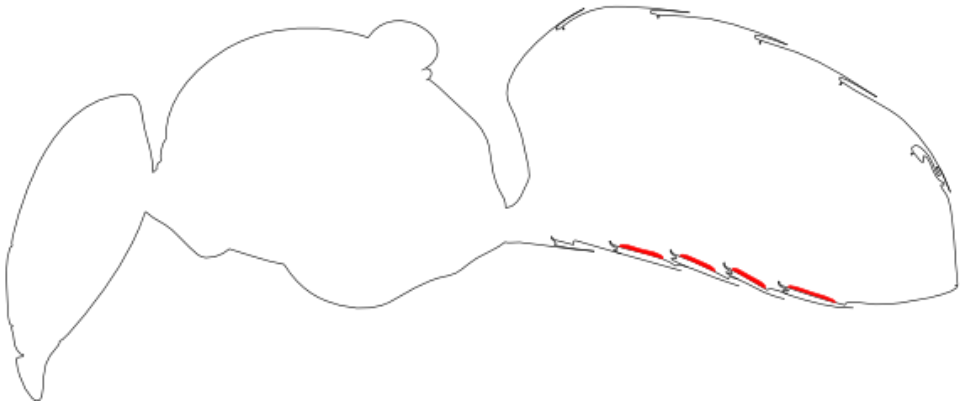

Fig: Wax glands (marked red) of honey bee worker. After Rösch (1927)

3. Silk gland

The silk or modified labial glands are tubular glands with characteristically branched nuclei, situated on the ventrolateral sides of the mid intestine (Fig. 8.2). Partially uncoil the tubular glands with a needle at the anterior end up to the spinneret. Anteriorly, the paired ducts unite and open into the spinneret.

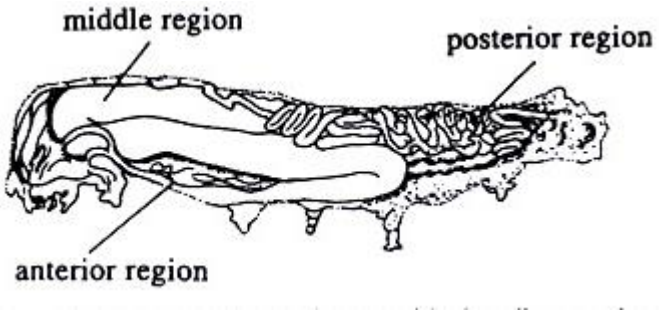

Fig 8.2: Position of silk gland

The silk gland (Fig. 8.3) is a paired structure, divided into three distinct regions: anterior, middle and posterior.

i) Anterior region:

Two straight tubes joining at the fore end open in the spinneret. Posteriorly the tubes open into the middle region. The thin anterior region of silk gland has no secretory role and only transports the assembled silk to the spinneret.

Spinneret:

It is a projection of the median part of the labium, which draws the silk out in the form of fine filament. The secreted silk comes out as a thread or filament as it passes through

silk press which resembles a typical salivary pump. The two filaments coming out of two sides are called brins. The sericin (gum) layer of the two brins then bind together into a single filament or bave.

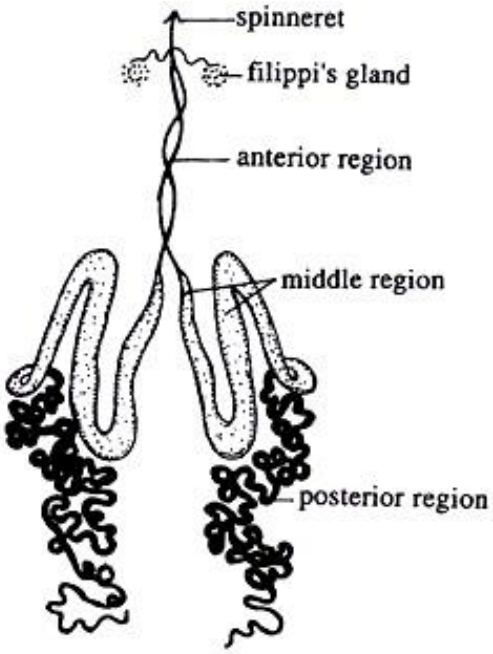

Fig. 8.3 : Bombyx mori. Silk gland

ii) Middle region:

Most prominent and widest part of silk gland. It remains folded in a W-shaped structure and thus has 3 limbs — posterior, middle and anterior limbs. The posterior arm secretes sericin-I. It gets surrounded by serecin-II secreted from the middle limb. This sericin again gets surrounded by sericin- III secreted from the anterior limb. The middle region of silk gland also acts as the reservoir of fibroin where the later gets mature during the storage period.

The beginning of the middle region is narrow but widens suddenly; the mid-part is the widest and the end of the rear part is narrow. The posterior part is crooked and curved between the tracheae and dorso-visceral muscles.

iii) Posterior region:

A narrow coiled tube ending blindly. This part remaining coiled in mid portion to posterior portion of the body, above and sides of the gut.

This part secretes fibroin as fibrinogen which converted to fibroin upon extrusion.

- **Histologically the entire gland has 3 layers:**

(1) The outer tunica propia with uniform thickness;

(2) The middle glandular layer with gland cells which increase in size during later instar stages of larval development and

(3) The inner tunica intima: It has varying thickness.

In the anterior region of the gland, this layer is very thick and is shed at each moult. In other regions of silk gland, it is thin and not shed at each moult.

4. Labial gland/ Salivary gland

The salivary glands of most insects are labial glands. There is great variation in the structure of these glands considering the variation in mode of feeding (e.g., chewing, piercing-sucking, non-piercing-sucking, sponging, etc.) and types of food consumed by different insect species. Several aspects of structure and function are common to most or all variations of insect labial salivary glands. The glands occur in pairs, and the ducts from each gland usually join to form a single common duct that opens to the oral cavity at a single orifice. Even though the glands originate in the labial segment, the orifice usually occurs just behind or on the hypopharynx, and the glands often extend back into the thorax and even as far back as the abdomen. The glands are suspended in the hemocoel and are constantly bathed in hemolymph. The glands generally have at least two regions: a secretory region and a reabsorptive region. Generally, the lumen of the salivary duct is lined with cuticle, at least at the end closest to its opening.

The secretory region produces the primary saliva. The major component of saliva is water. Water is transported from the hemo-lymph a cross cells of the salivary gland and into t he lumen of the gland. Movement of water from the blood to the gland lumen is accomplished by active transport of potassium or sodium ions from the hemolymph to the lumen, causing water to move from the hemolymph to the lumen down an osmotic gradient. Cells responsible for water transport generally have deep infoldings of the cell membrane and/or dense microvilli on the side of the cell adjacent to the lumen of the gland. This serves to greatly increase the cell's luminal surface area, and also serves t o enclose very narrow extracellular spaces into which ions are pumped. The enclosed nature of the spaces helps contain the ions to keep their concentration high, thus facilitating the osmotic movement of water from the cell into the space. The infoldings and microvilli usually are associated with abundant mitochondria to provide the energy for the ion pumps. The secretory region of the gland also synthesizes proteins, such as salivary enzymes and other organic components of the saliva. Cells responsible for secretion of these components generally possess extensive endoplasmic reticulum, Golgi bodies, and secretory granules that synthesize and transport (intracellularly) the secretions. There may be one or several different types of cell in the secretory region. It should be noted that salivary components are not necessarily produced by the salivary glands themselves, but may be produced elsewhere in the body and transported tothe salivary glands via the hemolymph.

The reabsorptive region of the salivary gland reabsorbs potassium or sodium ions from the saliva and transports them back into the hemolymph. As a result, potassium and sodium ions are conserved, and the saliva is usually hypotonic to the hemolymph. Reabsorptive cells often have infoldings, especially on their basal side (hemolymph side), to increase surface area. These infoldings, however, tend not to be tightly enclosed

(unlike the lumen side of water- secreting cells in the secretory region), to facilitate movement of secreted ions into the hemolymph and away from the cells, thus reducing the osmotic gradient, which would cause the cells to lose water. Reabsorptive cells also have abundant mitochondria to power the active transport of ions from saliva to hemolymph.

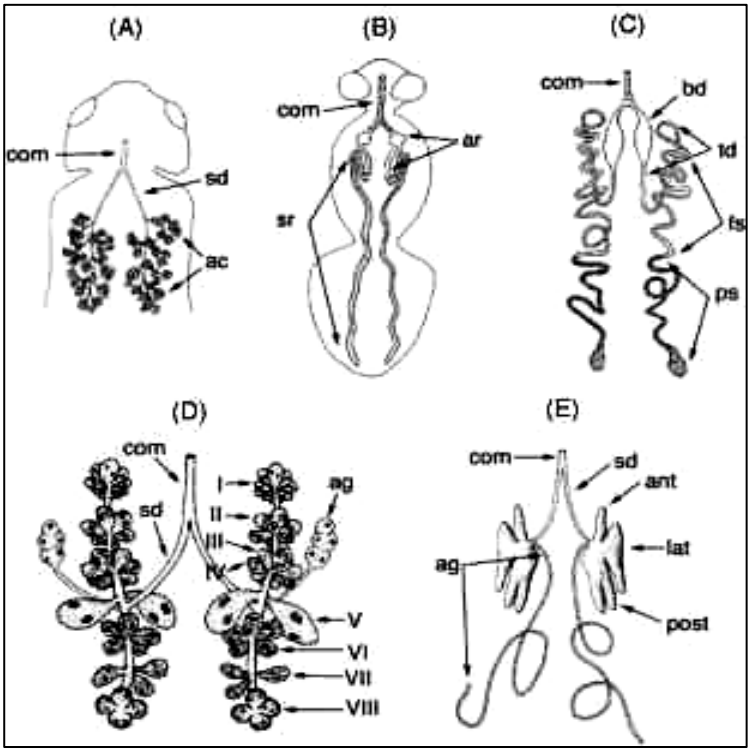

Figure: Salivary glands of representative insects. (A) The locust (B) A full blow fly (C) The tobacco hornworm moth (D) The beet leaf hopper showing cell types (E) The large milkweed bug

Abbreviations: ac, ac ini; a g, accessory gland; ant, anterior lobe; ar, absorptive region; bd, bulbous duct region; com, common salivary duct; fs, fluid secretion region; lat, lateral lobe; post, posterior lobe; ps , protein secretion region; s d, salivary duct; sr , secretoryregion; td, thin duct region.

- **Salivary glands of cockroach**

1 . These glands are paired and white structures and are situated one on either side of crop in the prothoracic region.

3. The salivary glands, as a matter of fact, are comprised of a glandular part and a hollow receptacle or reservoir.

4. The receptacle is situated between paired glands of each side and remains connected through a duct.

5. The glands are compressed, made up of hexagonal glandular cells and each of them opens into a small narrow duct.

6. The ducts of two glands of one side join into a salivary duct and the salivary ducts from two sides join into a common duct.

7. The common duct receives the ducts from two receptacles and transforms into a spacious efferent salivary duct.

8. The efferent salivary duct opens at the base of hypo pharynx, which is situated on the floor of buccal cavity.

9. The glands secrete digestive enzymes which are stored in reservoir till they are required for digestion of food.

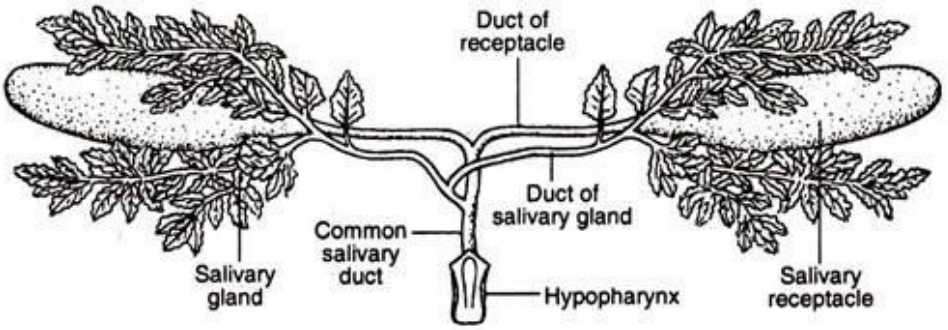

Fig. 18.55: Salivary apparatus of *Periplaneta americana*.

Probable questions:

1. Describe the structure of labial glands of insects and state their role in different insectorders.
2. Describe the structure of salivary gland in cockroach with diagram.
3. Discuss different types of silk glands found among insects. Cite examples.
4. What is the function of silk gland?
5. What is a wax gland? State its location and function.

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-WesleyPubl. Co., 436 p.
3. Gullan, P .J & Cranston, P .S. (2010). T he Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.

Unit-XX

Metamorphosis in insects: hormonal regulation; factors affecting metamorphosis; diapauses

Objective:

In this unit we will discuss about Metamorphosis in insects: hormonal regulation; factors affecting metamorphosis; diapauses.

Introduction:

Metamorphosis can be defined as "a rapid and complete transformation from an immature larval life to a sexually adult form involving morphology, function and habitat changes".

Ecdysis or moulting is the periodic shed- ding off the old exoskeleton. The duration of the period between two successive moults of a developing insect is called stadium. The form of the developing insect between two moults is called instar.

A larva is a motile, immature feeding stage in arthropods which is morphologically different from the adult stage. The larvae of hemimetabolous insects are called nymphs. The adult stage of holometabolous insects is called imago.

- **Types of Metamorphosis:**

On the basis of degree of changes there are 5 basic types of metamorphosis seen in insects.

They are:

(1) Ametabolous development or Ametamorphic

(2) Gradual metamorphosis or Paurometabolous development

(3) Incomplete metamorphosis or Hemimetabolous development

(4) Complete metamorphosis or Holometabolous development and

(5) Hypermetamorphosis or Hypermetabolous development.

(1) Ametabolous Development or Direct Development:

Ametabolous type of development is called when the insects undergo little or no metamorphosis. Here the young's emerge from the eggs resemble the adults in all respects except in size and sexual structures. It grows only in size by replacing its old skin through a process, called moulting.

The young which emerges from the egg resembles a miniature adult, called nymph. In nymph the reproductive organs are undeveloped, and after several moults the nymph

becomes an adult. This type of development is seen in apterygotan (wingless) insects (e.g., Lepisma, Fig. 18.134 and spring tails or Collembola, etc.).

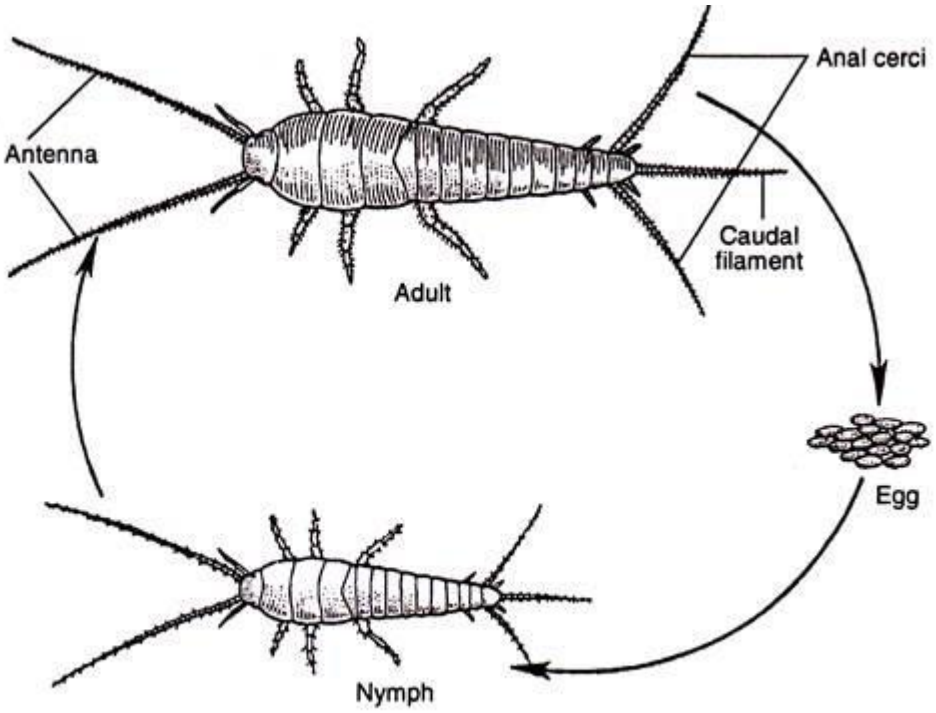

Fig. 18.134: Ametabolous development in *Lepisma*.

(2) Gradual Metamorphosis or Paurometabolous Development:

This type of metamorphosis is seen in less primitive forms like cockroaches, grasshoppers (Fig. 18.135), mantis and white ants, etc.

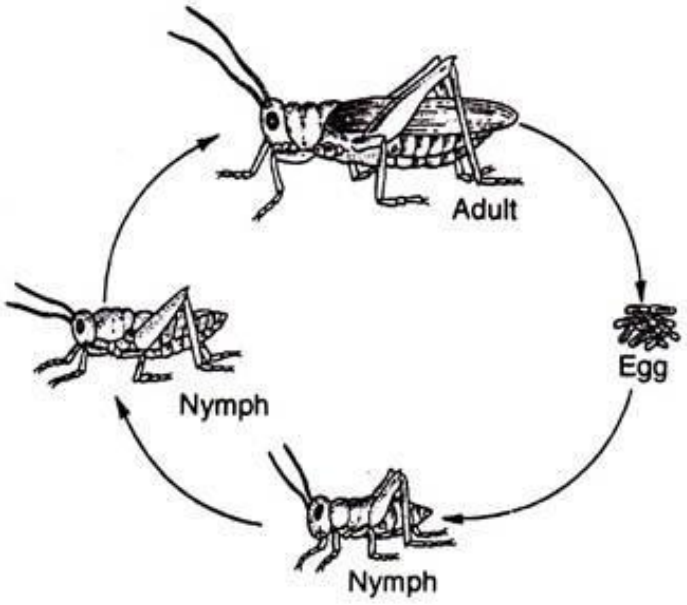

Fig. 18.135: Paurometabolous development in grasshopper.

Here the newly young which comes out of egg closely resembles the adult in general body form, habits and habitat but many adult features, i.e., wings and reproductive organs are undeveloped and their relative proportions of the body also differ. The young's re called nymphs.

The wings develop as wing pads in the second and third thoracic segments at early age and gradually increase in size by mitosis in each moult. The external genitalia develop gradually at each moult. These nymphs lead an independent life and attain adult form through several moults.

This type of metamorphosis is called gradual metamorphosis or paurometabolous development because the young undergoes slow but steady change in each moult and attains the adult form.

Sometimes the gradual metamorphosis or paurometabolous development is included under hemimetabolous development. In each moult the proportion of the head gradually becomes smaller and the abdomen becomes longer.

In the life cycle of these insects there are three stages, e.g., egg → nymph → imago (adult). There is no pupal stage.

(3) Incomplete Metamorphosis or Hemimetabolous Development:

The insects which attain adult forms by gradual morphological change with successive moults are called incomplete metamorphosis or hemimetabolous development.

In many insects, e.g., dragonflies (Fig. 18.136), mayflies and damselflies, the different stages of the life cycle resemble to paurometabolous development except the nymphs are called naiads which are aquatic and respire by external gills but the adults are terrestrial.

When these nymphs are ready to be adult they come out of water and adult winged forms are released. The wings and genitalia develop externally but are not fully formed until adulthood. No further moulting takes place after the formation of wings, only exception in mayfly where winged form comes out of aquatic nymph and rests on a tree to undergo another moulting to become an adult.

In the naiads of hemimetabolous insects there are 3 pairs of thoracic legs, a head with compound eyes, antennae and small abdomen with posterior tracheal gills.

The naiads when attain adult stage after several moults, the head of the adult becomes proportionately smaller but the abdomen becomes larger. The tracheal gills are lost and spiracles appear for aerial breathing. Many insects live for longer period as nymphs and the adult stage is short, the chief purpose of which is multiplication.

The best example is the may fly where adult stage lasts only for a day but nyphs take one year to grow.

From the egg → nymph → adult cycle of incomplete metamorphosis, it is evident that insects which are advanced from the primitive Lepisma like forms, have started to explore two types of environments. But success in such attempts is achieved in forms with complete metamorphosis.

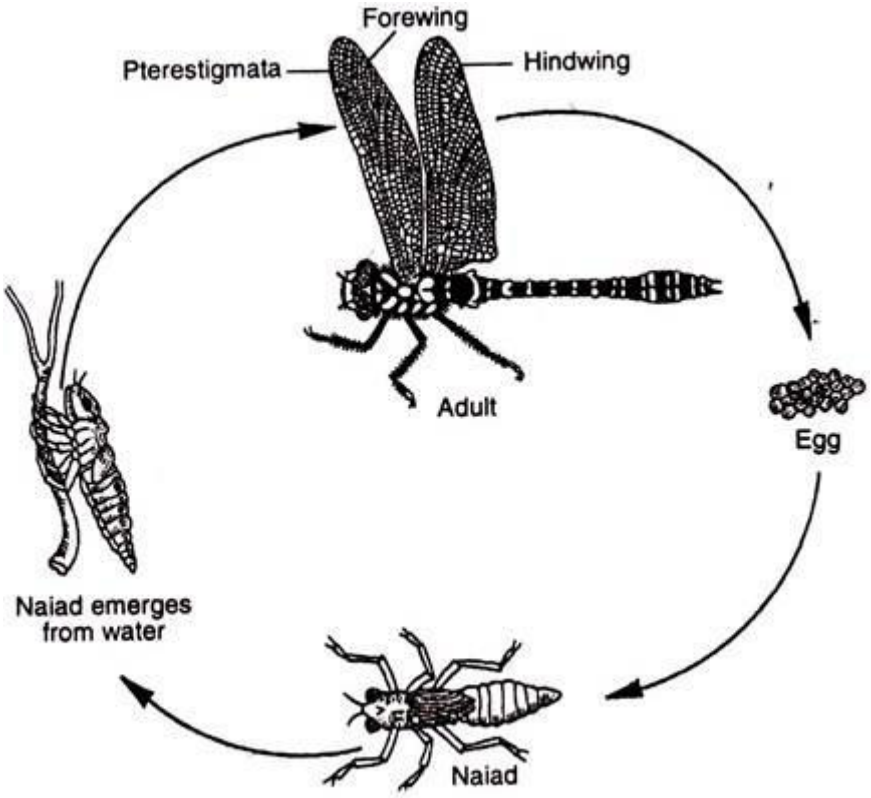

Fig. 18.136: Hemimetabolous metamorphosis in dragon fly.

This climax of double life has been attained through a cycle of egg → larva → pupa adult; larva existing in an altogether different environment than the adult. Nearly 87% of known insects develop through this cycle which involves two changes of form—one is from egg to caterpillar and the other from caterpillar to pupa and the adult.

(4) Complete Metamorphosis or Holometabolous Development:

Complete metamorphosis or holometabolous development is a kind of rapid morphological change during post embryonic transformation in some forms of insects where larva has no similarity with the adult and there is always a pupal stage. Complete metamorphosis takes place in beetles, caddis- flies, butterflies, moths, mosquitoes, flies, bees and wasps (Fig. 18.137).

In the house-fly (order Diptera) the larva is worm-like and devoid of appendages. It is called maggot. The mature larva is about 12 mm long. The head is indistinct and with a pair of oral lobes and hooks.

In case of beetle (Order Coleoptera) the larva is known as grub. The body of the grub is thick and with thoracic legs and well-developed head. They are usually sluggish in nature.

In the moths and butterflies (Order Lepidoptera), the larva is known as Caterpillar, which possesses a distinct head with powerful mandibles and three pairs of jointed thoracic legs.

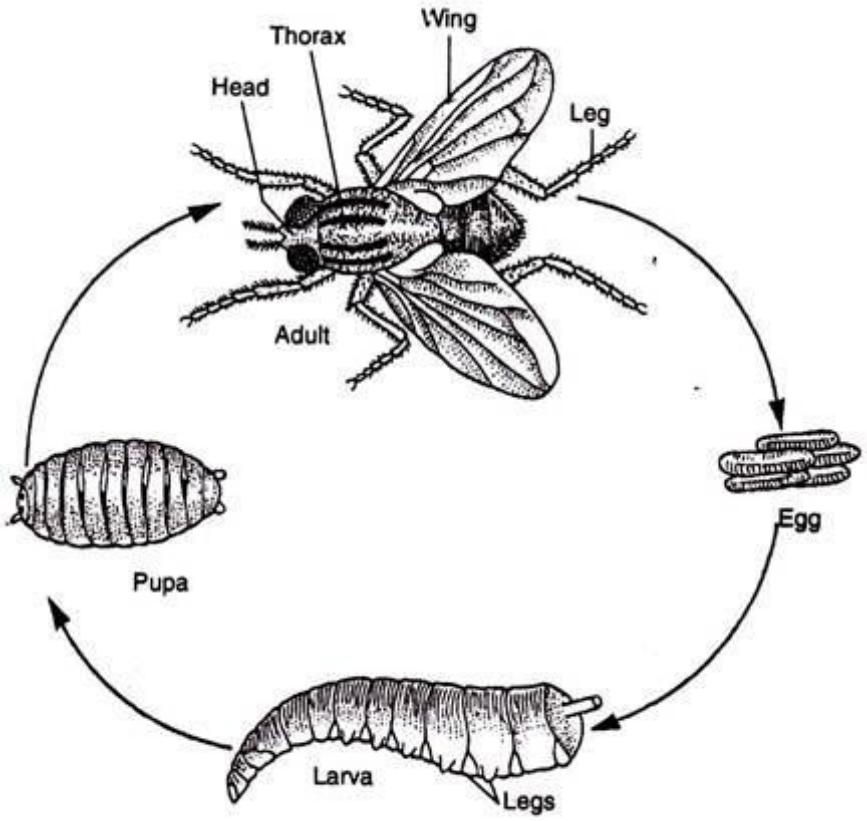

Fig. 18.137: Illustration of holometabolous development in fly.

The abdomen possesses four or five pairs of un-jointed, short abdominal legs or also called pseudo-legs or prolegs. The caterpillars are often with protective colour or defensively shaped. These larvae eat voraciously and grow rapidly with several moultings. After sometime the larva is transformed into a stage, called pupa.

Again, these above mentioned larvae are also included under three categories, such as the maggot is called apodous larva for the absence of appendages on thorax and abdomen and segmented body with a small head with sense organs. The larvae among beetles are also called campodeiform or oligopod larvae for the resemblance to apterous Campodea (Order Thysanura).

The larvae are characterised by without abdominal appendages except cerci and the skin of the body is thick, provided with thoracic legs and sense organs. The caterpillar type larva is also called polypod or eruciform larvae (Fig. 18.138) which is characterised by a fleshy body with a thin skin and prolegs on the abdomen and six legs on the thorax.

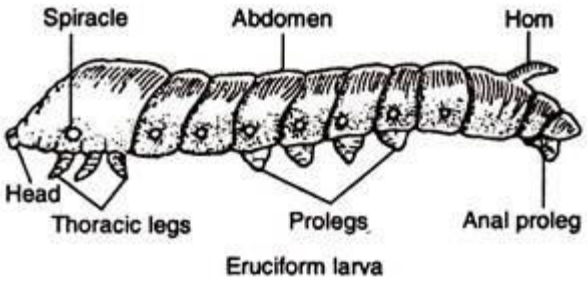

Fig. 18.138: Eruciform larva.

The pupa is the third stage in the life of holometabolous insects and usually immobile and often remain within a protective covering from predators, called cocoon. In the mosquito the pupa is very active but does not eat anything. Though the pupal stage considers as a quiescent stage but it undergoes many internal changes. There are three types of pupa among the holometabolous insects.

They are:

(i) Exarate pupa:

This type of pupa is very common and found in almost all holometabolous insects (Fig. 18.139A) except Lepidoptera. The pupa is characterized by the presence of free legs and appendages and the abdomen is capable of movement. This type of pupa is called free pupa.

(ii) Obtect pupa:

This type of pupa is seen among moths and butterflies and is characterized by the wings, and appendages are not moved and fixed to the body by a moulting fluid. The pupa of butterflies is called chrysalis and it possesses a slender stalk at the top by which the pupa remains attached to the twigs.

(iii) Coarctate pupa:

This type of pupa is seen among dipterans (Fig. 18.139B). The pupa of house-fly is enclosed by a hard barrel-shaped chitinous case, called puparium. The puparium is segmented externally and the spiracles remain projected outwardly. The pupa of mosquitoes is comma-shaped and contains broad anterior cephalothorax.

The dorsal side of thorax bears a pair of small respiratory trumpets; the openings are guarded by numerous hairs. The abdomen is nine segmented and a pair of paddles on the eighth segment by which the pupa swims. After a period of pupal existence, the young insect emerges out by breaking or dissolving the pupal case. The holometabolous insects include 4 stages in their life cycle.

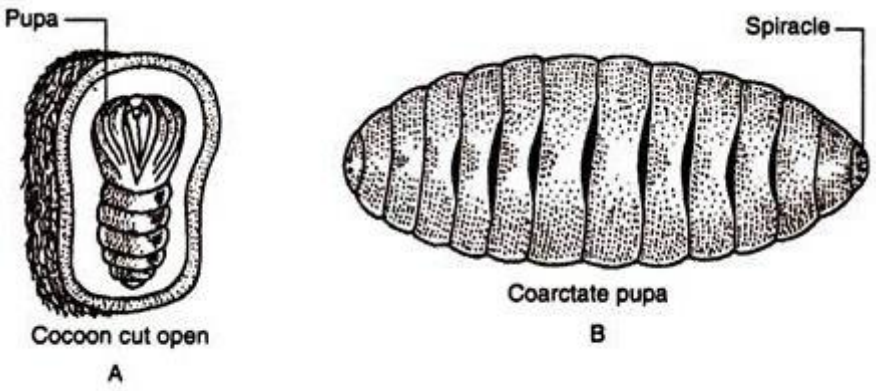

Fig. 18.139: A. Exarate pupa. B. Coarctate pupa.

Egg → Larva → Pupa → Imago (adult):

(iv) Prepupa:

In the holometabolous insects, a stage is seen before the pupal stage, called prepupa. During this stage the feeding usually stops and sometimes a cocoon is produced. The prepupa resembles the larva but it is often shrunken and less pigmented.

(5) Hypermetamorphosis or Hypermetabolous Development:

It is a kind of metamorphosis in which there are two or three distinct types of larval instars with different habits and structures found in certain insects. This type of metamorphosis is seen in blister beetles (Fig. 18.140).

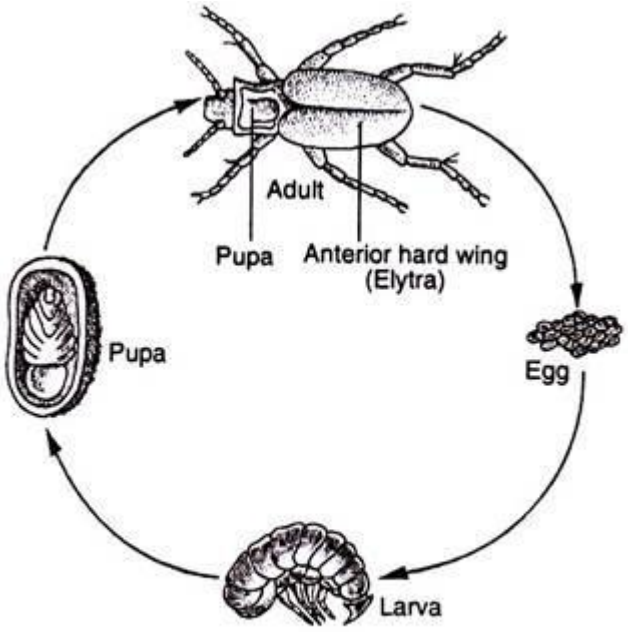

Fig. 18.140: Illustration of hypermetabolous development in blister beetle.

- **Events of Metamorphosis:**

In recent years considerable amount of work has been done to understand the events of metamorphosis. It has been found that at the time of early development, in the developing egg the cells are segregated into two groups—one group for working at the larval life and the second group to take charge during pupal and adult life.

In a growing larva, the larval cells increase only in size but never undergo division. The second group of cells, called imaginal buds and discs, remain inactive in the body of larva. When the larva is full-grown, second group of cells take over the charge. Within the apparently inactive pupa tremendous activities go on at cellular level. Imaginal buds grow by division.

During metamorphosis, most of the larval organs in the pupa except the central nervous system and developing reproductive organs are broken down by enzymes and the process of disintegration of the larval organs is called histolysis and these larval disintegrated cells die and is used up by the imaginal cells.

In certain insects, within larva, pupal cells become fluid in consistency and imaginal cells continue to form the adult structures. The imaginal buds are the groups of formative cells but remain inactive in the larva but form the rudiments of future organs by mitosis.

These formative cells set aside in the pupa and reach functional organs by differentiation in the imago (adult). The process of formation of tissues and organs from the imaginal buds, called histogenesis. The wings, mouth parts, internal organs, muscles and legs develop from the imaginal buds.

- **Role of Hormones during Metamorphosis:**

It has also been well established that the moulting and metamorphosis in insects are controlled by hormones (Fig. 18.141). The secretions of three organs are related to this process.

These organs are:

(i) The brain (protocerebrum)

(ii) The prothoracic gland and

(iii) The corpora allata.

(i) The Brain (protocerebrum):

In the brain there are four groups of neurosecretory cells. Of these two groups lie on the midline and another two group's lie on the sides-one group on each side. The neurosecretory cells secrete a kind of protein hormone, called prothoracotropic (PTTH)

or brain hormone that activates the prothoracic glands which in turn produce moulting hormone.

The protocerebrum sends the neurosecretory axons to the corpora cardiaca, a pair of small glands which lie posterior to the brain. Prothoracotropic hormone passes to the corpora cardiaca along the axons where it is released to the blood.

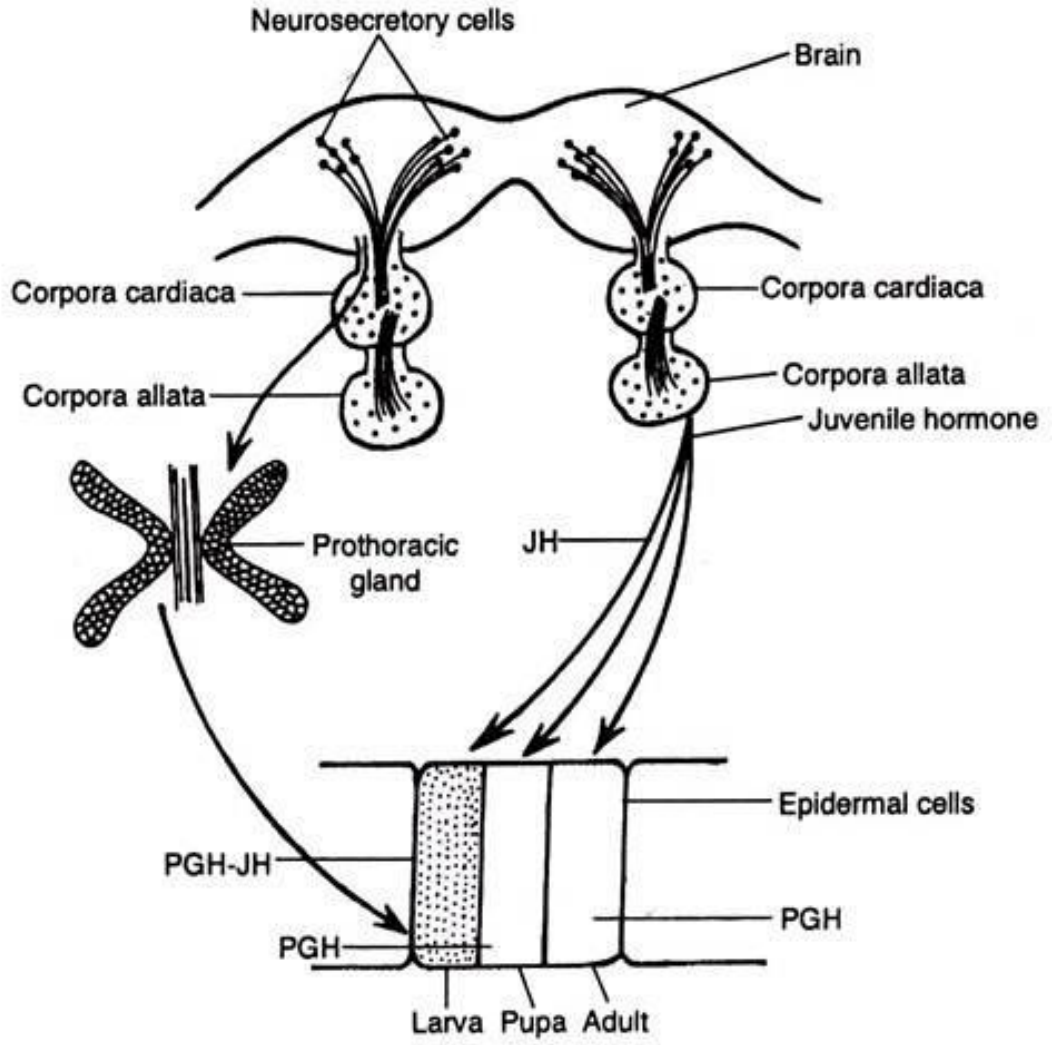

Fig. 18.141: Endocrine glands of insects and their influence during moulting.

(ii) Prothoracic Glands (PG):

There are a pair of glands located in the prothoracic region. These are also called moult gland or ecdysial gland. Each gland appears V-shaped and is a mass of glandular tissue of non- nervous origin.

It produces a hormone, known as ecdysone. It is steroid in nature. Butenandt and Karlson (1951) first isolated from the pupa of silkworm. Its empirical formula is $C_{27}H_{44}O_6$. The hormone stimulates growth and initiates the process of moulting and

shedding the old cuticle of the larva and the new cuticle is formed beneath the old cuticle.

(iii) Corpora Allata:

They are paired non- nervous secretory cells and situated behind the brain posterior to corpora cardiaca. The corpora allata secrete another fat soluble hormone, called juvenile hormone (JH). Chemically it is related to ecdysone and also a steroid in nature. The juvenile hormone keeps the larval cells active and also controls the qualitative changes in the body during metamorphosis.

As long as the juvenile hormone is secreted from the corpora allata, the pupa and imago (adult) stages are not developed. After a certain period the production of ecdysone instructs to stop the flow of juvenile hormone on one hand and on the other hand triggers the imaginal buds to be active.

The absence of juvenile hormone causes the death of larval cells and they are used as nutrients for the growing imaginal buds. If the amount of juvenile hormone (JH) becomes lower in the blood, the moulting from larva to pupa takes place and absence of juvenile hormone in the blood, there occurs from pupa to adult moult. So it has been determined that the process of moulting is under hormonal control.

- **Factors controlling metamorphosis in insects**

For a larval moult to be successful all parts of the body must take part in the process and carry it out at the same time. This indicates that a common factor acts on all parts of the body. The existence of a common factor or cause is even more apparent in metamorphosis, in which the involvement of both external and internal organs may be more radical and farreaching. This common factor may be external or internal.

External factors

In some cases an external factor may be responsible for initiating moulting, as for instance in the blood sucking *Rhodnius* the intake of food (blood meal) is such a factor. Another example in which external factor is essential to initiate a moult is the case of the pupa moth *Platysamia cecropia*. After pupation the insect falls into a quiescent state with a reduced rate of metabolism - diapause - which continues throughout winter. It is essential that during this time the pupa be exposed to cold; otherwise the diapause is prolonged indefinitely. The diapause may be broken precociously if the pupa is exposed to cold (3' to 5' C) for at least two weeks. The temporary cooling activates the vital processes in the pupa and on return to a warmer environment development is completed, the pupa moults and the imago emerges.

In other insects factors such as humidity, population density etc., appear to initiate metamorphosis. However in the majority of insects no external cause of any moult has been detected and moults follow one another at intervals which appear to be determined entirely by the internal processes in the animal. Similar to the amphibian metamorphosis, moulting and metamorphosis in insects has been found to be initiated

internally by hormones. Hormonal control involves at least three organs of endocrine secretions . They are (1) brain (protocerebrum) (2) corpora allata (3) prothoracic gland.

Diapause

Diapause can be defined as the physiological state of dormancy or developmental arrest where most life processes are shut down. It is initiated during unfavourable conditions and is most commonly observed in insects, especially in arthropods.

Diapause is a period of suspended or arrested development during an insect's life cycle. Insect diapause is usually triggered by environmental cues, like changes in daylight, temperature, or food availability. Diapause may occur in any life cycle stage—embryonic, larval, pupal, or adult—depending on the insect species.

Insects inhabit every continent on Earth, from the frozen Antarctic to the balmy tropics. They live on mountaintops, in deserts, and even in the oceans. They survive frigid winters and summer droughts. Many insects survive such extreme environmental conditions through diapause. When things get tough, they take a break.

Diapause is a predetermined period of dormancy, meaning it is genetically programmed and involves adaptive physiological changes. Environmental cues aren't the cause of diapause, but they may control when diapause begins and ends. Quiescence, in contrast, is a period of slowed development that is triggered directly by environmental conditions, and that ends when favorable conditions return.

Diapause is a predetermined period of dormancy, meaning it is genetically programmed and involves adaptive physiological changes. Environmental cues aren't the cause of diapause, but they may control when diapause begins and ends. Quiescence, in contrast, is a period of slowed development that is triggered directly by environmental conditions, and that ends when favorable conditions return.

• Organisms that Exhibit Diapause

The longest ever diapause to be reported belongs to the Prodoxus Y-inversus, a moth which is found in the United States and South Western New Mexico. It can remain dormant for as long as 19 years.

Diapause during the embryological stage can be observed in many arthropods such as the flesh fly, tobacco hornworm and the Southwestern corn borer.

In fishes, a species called mummichog exhibit diapause during their embryo phase. Even the silkworm exhibits diapause between the late embryonic stage and late larval stage.

• Types of Diapause

Diapause can be either obligatory or facultative:

- Insects with **obligatory diapause** will undergo this period of arrested development at the predetermined point in their life cycle, regardless of the environmental conditions. Diapause occurs in every generation. Obligatory diapause is most often associated with univoltine insects, meaning insects that have one generation per year.
- Insects with **facultative diapause** undergo a period of suspended development only when conditions require it for survival. Facultative diapause is found in most insects and is associated with bivoltine (two generations per year) or multivoltine insects (more than two generations per year).

Additionally, some insects undergo **reproductive diapause**, which is a suspension of reproductive functions in adult insects. The best example of reproductive diapause is the monarch butterfly in North America. The migrant generation of late summer and fall goes into a state of reproductive diapause in preparation for the long journey to Mexico.

• Stages of Diapause (Exclusive to insects)

✓ Induction:

Induction occurs much before the onset of unfavourable environmental conditions. Essentially, certain environmental stimuli, called "token stimuli" triggers the diapause behaviour. The stimuli themselves do not negatively affect any of the factors, though they signify an impending transformation in the environmental conditions.

✓ Preparation

The induction phase is followed by the preparation phase. This phase is characterized by the insects storing molecules such as carbohydrates, proteins and lipids to sustain themselves during the diapause

✓ Initiation

One of the most important phases in the stage, the initiation stage is characterized by cessation of morphological development. These include a change in colour, moulting into a diapause stage, or release of special enzymes.

For example, some insects like the firebug accumulate certain chemicals which help in lowering the freezing point.

✓ Maintenence

Maintenance is characterised by the reduced metabolism and arrest of embryological development. Furthermore, sensitivity to certain stimuli is increased, especially the ones that prevent the termination of the diapause is decreased.

✓ Termination

Termination, as the name implies, terminates the diapause. However, certain insects rely on specific environmental stimuli to trigger the termination of diapause.

✓ Post-diapause quiescence

Termination is followed by the state of quiescence, where the insect can resume development, provided the environmental conditions become quite favourable.

• Environmental Factors

Diapause in insects is induced or terminated in response to environmental cues. These cues may include changes in the length of daylight, temperature, food quality and availability, moisture, pH, and other factors. No single cue solely determines the start or end of diapause. Their combined influence, along with programmed genetic factors, controls diapause.

i. **Photoperiod:** A photoperiod is the alternating phases of light and dark in the day. Seasonal changes to the photoperiod (such as shorter days as winter approaches) cue the start or end of diapause for many insects. Photoperiod is the most important.

ii. **Temperature:** Along with photoperiod, changes in temperature (such as an extreme cold spell) can influence the start or end of diapause. The thermoperiod, alternating phases of cooler and warmer temperatures, also influences diapause. Some insects require specific thermal cues to end the diapause phase. For example, the woolly bear caterpillar must endure a period of chilling to trigger the end of diapause and continuation of the life cycle.

iii. **Food:** As the growing season ends, the diminishing quality of their food sources may help trigger a diapause phase in an insect species. As potato plants and other hosts turn brown and dry, for example, Colorado potato beetle adults enter a state of diapause.

Probable Questions:

1. What do you mean by metamorphosis?
2. Write short note on hemimetabolous development.
3. Discuss Hypermetabolous Development.
4. What is Coarctate pupa?
5. Discuss the Role of Hormones during Metamorphosis.
6. What is obligatory diapause?
7. Describe Stages of Diapause in insects.

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas,Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-WesleyPubl. Co., 436 p.
3. Gullan, P .J & Cranston, P .S. (2010). T he Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.

Disclaimer:

The study materials of this book have been collected from various books,e-books, journals and other e-sources.

Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

PARASITOLOGY, ECOLOGY, ENVIRONMENT AND WILDLIFE BIOLOGY

ZCORT-102

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia West
Bengal, India

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HARD CORE THEORY PAPER (ZCORT – 102)

Group A (Parasitology)				
Module	**Unit**	**Content**	**Credit**	**Page No.**
ZCORT – 102 (Parasitology, Ecology, Environment and Wildlife Biology)	**I**	Concept of parasitism, symbiosis commensalism and mutualism	3	8-15
	II	Host-parasite interaction: immunopathological consequences in parasitic infections.		16-29
	III	Classification of Protozoa and Helminths.		30-41
	IV	*Entamoeba* and Blood Flukes in Humans.		42-58
	V	Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture.		59-62
	VI	Mode of transmission of *Plasmodium*, *Trypanosoma* and Piroplasm.		63-77
	VII	Zoonosis with particular reference to *i) Toxoplasma* and *ii) Schistosoma.*		78-94
	VIII	Life cycle, biology, pathogenesis, epidemiology and control of medically important helminthes *i) Diphyllobothrium latum,* *ii) Paragonimus westermani,*		95-105
	IX	Life cycle, biology, pathogenesis, epidemiology and control of medically important helminthes *i) Trichinella spiralis* and *ii) Wuchereria bancrofti*.		106-117
	X	Salient features of plant parasitic nematode and life cycle patterns of *i) Meloidogyne hapla*, *ii)Anguina tritici*.		118-127
	Group B (Ecology, Environment and Wildlife Biology)			
	XI	The Ecosystem: Gaia hypothesis, cybernetic nature and stability of	3	130-159

ZCORT – 102 (Parasitology, Ecology, Environment and Wildlife Biology)		the ecosystem, ecosystem management and optimization. Macroecology: concept and consequences. Principles of Thermodynamics, energy flow and ecological energetics.		
	XII	Niche theory: Concept of habitat and niche; niche width and overlap; fundamental and realized niche; resource partitioning; character displacement. (Lotka-Volterra model, Isoclines, Niche prediction)		160-177
	XIII	Community: Structure and Gradient analysis, Structure of biotic community. Community patterns: diversity and stability. Community boundary: Ecotone and edge types, Edge effect and edge species, Leibig's Law of the minimum.		178-191
	XIV	Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate, gross reproductive rate, vital index. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.		192-201
	XV	Metapopulation: Concept, models, structure and dynamics		202-210
	XVI	Major terrestrial biomes: Theory of island biogeography, Biogeographical zones of India		211-224
	XVII	Concept of Environment: Structure, radiation balance, UN movements on environment.		225-234
	XVIII	Theory and analysis of conservation: Stochastic perturbations, population viability		235-254

		analysis, recovery strategy for threatened species: Different approaches for conservation – in-situ and ex-situ, In-situ conservation- problems and prospects; Sanctuaries, National parks, Community Reserves and Conservation Reserves; Biosphere Reserve, EIA, EIS.		
	XIX	Conservation biology of important wild animals: Conservation status, habit & habitat, threats and conservation management of the following animals: i) Olive Ridley Turtle ii) Greater one-horned *Rhinoceros* iii) Ganges river dolphin.		255-266
	XX	Basic Concept of Wildlife Biology: Wildlife wealth of India; Threatened wildlife and IUCN status - Concept of Extinct, Critically Endangered, Endangered, Vulnerable and rare species; concept of corridor.		267-273
	Total counselling session 18hrs.			

Group-A: Parasitology

Unit-I

Concept of parasitism, symbiosis commensalism and mutualism.

Objectives:

In this section we will discuss on concept of parasitism, symbiosis commensalism and mutualism.

Parasitism

Parasitism is described as the hetero- specific association in which one partner derives all possible benefits and the other is affected adversely. The benefitted member is called a parasite and the losing member accommodating a parasite is recognised as the host. Between the two partners, the parasite is always smaller in size and metabolically dependent on the host.

Origin and Evolution of Parasitism:

Parasitism dates back to ancient geological time and arose soon after the differentiation of life began in the world. Parasitism is a secondary mode of life. It has arisen from free living way of life. This get together of different individuals started with comparatively smaller animals occasionally taking shelter temporarily on the body of some larger forms.

When this temporary shelter was repeatedly used, the association of two organisms became more intimate and the smaller organisms got not only shelter, but also free transport and a bit of nourishment from the host, of course, without doing any harm to the host body.

Still later, the weak organism started living within the body of the host without doing any harm to the host. Finally they started to feed on the body tissues of the host so that the latter has to suffer with some harm. This association has been called parasitism.

It may be presumed that the parasite first confines itself to the external surface of the body and is called ectoparasite. The ectoparasites may gradually change to endoparasites when they get their entire nourishment from the host.

Nature of parasitism:

Crofton (1977) lists the following features of parasitism:

(i) That the parasite is physiologically dependent upon the host;

(ii) That the parasite has a greater reproductive potential than the host;

(iii) That it is capable of killing the heavily infected host, and

(iv) That the infection process tends to increase the chance of dispersion of the parasites within the host population.

Types of parasites:

Generally there are two types of parasites on the basis of relationship:

(i) Temporary or partial parasites and

(ii) Permanent parasites.

(i) Temporary or partial parasites:

The organisms which spend only a part of their life-cycle as a parasite. For examples, the duration of the feeding of mosquitoes and leeches on the host's blood. Another example may be provided as the glochidium larvae of some freshwater bivalves which spend the earlier part of life on the skin of fish as a parasite and after few weeks the larvae transform as young ones and lead an independent life.

(ii) Permanent parasites:

When the organisms spend their entire life as parasites. The adult liver flukes (*Fasciola hepatica*) as permanent parasites spend in the bile duct of the liver of sheep, cows and pigs and occasionally found in man.

Based on their location in relation to host, the permanent parasites are divided into the following:

(i) Ectoparasites:

When the parasites spend on the outside of their host as in the case of sucking lice (e.g., *Pediculus* sp.) of mammals, fleas (e.g., *Pulex, Ctenocephalus*) on birds and mammals, *Gyrodactylus*, a monogenean ectoparasite of fishes and frogs.

(ii) Endoparasites:

When the parasites live within the body of host, such as intestine, body cavities, organs or in the blood. The members of trichomonad flagellates (e.g., *Trichomonas, Tritrichomonas*), opalinids (e.g. *Opalina*), sporozoans (e.g., *Monocystis, Gregarina, Eucoccidium, Eimeria, Plasmodium,* etc.), Myxosporideans, Microsporideans, digenetic trematodes, cestodes, nematodes, etc. are endoparasites.

- **Incidental parasite:**

When a free-living animal meets accidentally an unnatural host and leads a parasitic life for certain period.

- **Periodic or Sporadic parasite:**

An organism which visits its host intermittently to obtain some metabolic requirements, is called periodic or sporadic parasite.

- **Facultative parasite:**

An individual leads a parasitic life if he gets a chance or he spends a free-living life as usual.
An example of this in humans is *Naegleria fowleri* - this excavate amoeba species is a free-living bacterivore, but occasionally it successfully infects humans with an often fatal result

- **Obligatory parasite:**

If a parasite fails to meet a suitable host to complete its life cycle, the parasite must die.

- **Hyperparasitism:**

When a parasitic species is parasitized by an another parasite species. Such parasite is called hyperparasite, as in the case of *Nosema notabilis* which is a hyperparasite on a myxosporidian, *Sphaerospora polymorpha*, a parasite of the urinary bladder of the toad fish.

- **Sexual Parasitism:**

When a male becomes a parasite on the opposite sex or vice versa. For example, the female Bonellia gives the shelter of minute male Benellia in its uterus. The male is dependent entirely in females for food and shelter.

Phoresis:

The term is derived from Greek phoros or phoron which means bearing or bearer. It is a symbiotic relationship in which one member of the association that is usually smaller, is mechanically carried by another organism (that is usually larger) of a different species.

In this relationship the smaller one is called 'phoront' and the larger one is called 'host'. An example of phoresis is the transport of a crustacean ectoparasite, *Bopyrus* on the gills of sea fish.

Zoonosis:

It is an infectious disease which is transmissible from vertebrates or invertebrates (sometimes by a vector) to humans.

Types of Host:

- **Definitive or primary host**:

An organism in which the parasite reaches the adult stage and reproduces sexually, if

possible. This is the final host. Example: Dog for *Ancylostoma caninum*, Cattle for *Fasciola gigantica*

- **Secondary or intermediate host**:

An organism that harbors the sexually immature parasite and is required by the parasite to undergo development and complete its life cycle. It often acts as a vector of the parasite to reach its definitive host. Depending upon the species of the parasite there may be first, second, third intermediate host. Example: Snails in trematodes.

- **Reservoir hosts:**

The animals which are infected and serve as a source from which another animal can be infected. It does not cause any harm to host animal. The source animals are called reservoir hosts. Example: *Balantidium coli* occur in pigs and horses.

- **Transfer or paratenic host:**

An individual that gives a temporary shelter to a parasite and helps the parasite in reaching to a definitive host called the transfer or paratenic host.

- **Natural host:**

They are the host in which a particular parasite can live and develop easily. It is due to host specificity. Example: dog for *Ancylostoma canninum*.

- **Accidental host**

An individual that accidentally harbors an organism that is not ordinarily ordinarily parasitic in the particular species.

Vector

It is a Latin word which means bearer. The vector of a parasite or disease agent is an arthropod, mollusc or other agent which transmits the parasite from one vertebrates to another. It is very much like host intermediate host.

It is of two types. (i) Biological vector and (ii) Mechanical vector

- **Biological vector:**

Those vectors in which the parasite develops or multiplies and makes it infective for susceptible host. All intermediate hosts are biological vector but all biological vectors are not necessarily intermediate host. Example: Mosquitoes parasites, for malarian parasites. Ticks for *Theileria, Babesia, Anaplasma*.

- **Mechanical vector:** Those vectors in which the parasite does not develop or multiply and transmit the parasites in the short period. Example: Tabanid flies for *Trypanosoma evensi*.

Symbiosis

A symbiosis is an evolved interaction or close living relationship between organisms from different species, usually with benefits to one or both of the individuals involved. Symbioses may be 'obligate', in which case the relationship between the two species is so interdependent, that each of the organisms is unable to survive without the other, or 'facultative', in which the two species engage in a symbiotic partnership through choice, and can survive individually. Obligate symbioses are often evolved over a long period of time, while facultative symbioses may be more modern, behavioral adaptions; given time, facultative symbioses may evolve into obligate symbioses.

Endosymbiosis is a symbiotic relationship, occurring when one of the symbiotic partners lives within the body of the other. Endosymbiosis can take place either within the *cells* (intercellular symbiosis) of the 'host' organism, or outside the cells (extracellular symbiosis). On the other hand, *ectosymbiosis* is a symbiotic relationship in which one organism lives on the body surface of the host, including the lining of the digestive tract, or *exocrine* glands such as mucus or sweat glands.

Types of Symbiosis

1. Mutualism
2. Commensalism
3. Ammensalism
4. Parasitism
5. *Mutualism*

Mutualisms

Mutualisms are a form of symbiosis in which both symbiotic partners benefit from the interaction, often resulting in a significant *fitness* gain for either one or both parties. Mutualisms can take the form of resource-resource relationships, service-resource relationships, or service-service relationships.

Resource-resource mutualisms (also known as 'trophic mutualisms') happen through the exchange of one resource for another between the two organisms involved. Resource-resource mutualisms most often occur between an autotroph (a photosynthesizing organism) and a heterotroph (an organism which must absorb or ingest food to gain energy). Most plants have a trophic mutualism called a mycorrhizal association, which is a symbiosis between the roots of the plants and a fungus. The fungus colonizes the plants roots and is provided with carbohydrates, sucrose and glucose. In exchange, the plant benefits from the fungi's higher water and mineral absorption capabilities.

Service-resource mutualisms occur when the symbiotic partner provides a service in exchange for a resource reward. One of the best known examples of this is the exchange between plants and their pollinators. While visiting the plants to gain a supply of

energy-rich nectar, the pollinator (insects, birds, moths, bats, etc.), provides the plant with the service benefit of being pollinated, while ensuring their own pollen is distributed when the pollinator visits more plants of the same species.

A rare form of mutualistic symbiosis comes in the form of service-service interactions. As the name suggests, both of the symbiotic partners receive a service, such as shelter or protection from predators. For example, the close relationship between anemone fish (family: *Pomacentridae*) and sea anemones provides both partners with protection from predators. The anemone fish, which have evolved an extra thick mucus layer on their skin to prevent them from being stung by the anemone's *nematocysts*, are provided with shelter from predators and a place to breed, while aggressively chasing away other fish which may try to bite the ends off the nutrient-rich tentacles. However, it is argued that there are very few truly service-service mutualism as there is usually a resource component to the symbiosis. In the case of the anemone-anemone fish mutualism, the nutrients from the anemone fish waste provide food for the symbiotic algae, which live within the tentacles of the anemone and provide energy to the anemone through photosynthesis. In this way, symbioses are shown to be highly complex and indicative of the delicate balance within ecosystems.

Mutualism is of two types:

(i) Facultative mutualism and

(ii) Obligatory mutualism.

(i) Facultative mutualism:

In this mutualistic relationship one species can live normally independent of another.

(ii) Obligatory mutualism:

In this type of mutualism the relationship is obligatory for the existence of single or both species.

Various examples of mutualism can be cited by plant-plant association, plant- animal association and animal-animal association. In plant-plant association, the various species of algae and fungi are closely associated to form lichens in which algae synthesize certain organic compounds and these compounds are utilized by the fungi.

In return, the fungi provide the algae with water, minerals and protection. This is an example of obligatory mutualism because if the two species are separated they will die.

Another example of mutualistic relationships (plant-animal) can be cited by the unicellular green algae (*Zoochlorellae*) and brown or yellow algae (*Zooxanthellae*) which live in the protective ectoderm of certain hydras (*Hydra* sp.), molluscs and worms.

The algae can manufacture their own food by photosynthesis and give off O_2 benefitting the animals which is taken by the animals for the respiration and in turn supply CO_2 and

N_2 to the plants that are benefitted to the host. This is an example of plant-animal's mutualistic relationship.

Another example of animal-animal mutualism is the relationship between flagellated protozoans (e.g., *Trichonympha*) living in the gut of wood eating termites. The protozoans live entirely depending on carbohydrate, collected from wood chips which are ingested by their host-termites. But the host cannot digest these wood chips because they have no cellulase-digesting enzyme.

For this the hosts depend on totally Trichonympha which are capable of digesting wood chips by secreting cellulose digesting enzyme-cellulase. In return the flagellates get food and protection. Here the protozoans act as mutualists and termites act as hosts.

Commensalism

Commensalism is a symbiosis in which one organism benefits from, and is often completely dependent on, the other for food, shelter, or locomotion, with no obvious effect on the host. The relationship between whales and barnacles is an example of commensalism. The barnacles attach themselves to the tough skin of whales, and benefit from widespread movement and exposure to currents, from which they feed, while the whale is seemingly unaffected by their presence.

Commensals are of two types:

(i) Ecto-commensals and

(ii) Endo- commensals.

An example of commensalism can be cited as the relationship between remora fish (the ectocommensal) and the sharks and rays (the hosts). The dorsal fin of remoras (*Remora* sp.) or shark suckers (*Echeneis* sp.) is modified to form a dorsal sucker and is found to be attached to the host. The shark suckers or remoras feed on copepods that are found on the host's body and also the residue of the prey of host's periodically.

Other than shark and rays, the tarpons, barracudas, sail fishes, marlins, sword fishes, sunfish, sea turtles, whale and dolphins are associated with shark suckers and remoras. Certain commensals (shark suckers and remoras) have a preference toward certain hosts. The pea crab, pinnotheres lives within mantle cavity of sea mussels. The pea crab as an endocommensal collects food particles from the host mussels.

Amensalism

On the opposing side of commensalism is amensalism. This occurs when one organism is inhibited or damaged by the presence of the other, who does not benefit. Amensalism may involve competition, in which a larger, more powerful, or environmentally better adapted organism excludes another organism from its food source or shelter; for example, one plant shades out another while growing at its normal speed and height.

Alternatively, *antibiosis*, where one organism secretes chemicals as by-products that kill or damage the other organism, but do not benefit the other, can be seen commonly in nature.

Probable questions:

1. What is paratism? Give example.
2. What is symbiosis? Give example.
3. What is commensalism? Give example.
4. What is vector? Give example.
5. What is accidental parasite? Give example.
6. What is mutualism? Give example.
7. Describe the types of mutualism with example.

Suggested Reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
2. Noble, E. R. and Noble G. A. (1989). Parasitology. The Biology of animal Parasites. 6th ed.
3. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology. 9th ed. McGraw-Hill International.

Unit II

Host-parasite interaction: immunopathological consequences in parasitic infections

Objective:

In this unit we will learn about Host-parasite interaction: immunopathological consequences in parasitic infections.

Introduction:

Parasitism is an association or a situation in which two organisms of different taxonomic positions live together where one enjoys all sorts of benefits (like derivation of nourishment, reproduction etc. which are basic requirements for existence) at the expense of the other. The benefited organism is called the parasite and the organism harbouring the parasite is called the host.

Hosts are not hospitable to parasites. Instead they consider parasites as foreign bodies and want to exterminate or overpower them by operating various devices like: producing antibodies, increased peristalsis, diarrhoea, mucus secretion, encystation by host tissues etc. Parasites to avoid host's reaction for existence develop many specialities like increased fecundity, polyembryony, safe-habitat, production of special enzymes, a good deal of transmission etc.

Definition:

Due to close contact/intimate association, the responsive reactions and resistance displayed by a host to its parasite and the protective devices adopted by a parasite in response to its host's reactions in order to establish them in their respective environments are called host-parasite-interactions. Parasitism is a very broad term and different types of parasites are recognised on different basis.

In the course of their life cycle, parasite may become associated with more than one host. In many cases the life cycle is characterized by numerous very rigid requirements. Whenever a parasite is able to live and reproduce within a host—the result is an elaborate host- parasite interactions.

Basis of classification	Types of parasites	Definition and example
Duration	Temporary or Partial	Visits its host for a short period, e.g. adult dog flea.
	Permanent or Total	Leads a parasitic life throughout the whole period of its life e.g., life cycle of *Trichina* worm.
Degree of dependence	Facultative or Optional	Lives a parasitic life when opportunity arises e.g. *Ancylostoma*.
	Obligatory or Compulsory	Cannot exist without a parasitic life, e.g. *Taenia solium*.
Position	Ectoparasites or Ectozoa	Lives outside on the surface of the body of the host e.g. lice.
	Endoparasites or Endozoa	Lives inside the body of the host, i.e. in blood or in digestive tract, e.g. *Taenia saginata*.

- ## Host Specificity of Parasites:

In mature condition a given parasite is quite often found in limited number of hosts. In extreme condition, distribution of a parasite may be restricted to a single host—mono-specific parasite. Even when poly-specific the different hosts are phylogenetically related. This host specificity is a function of physiological specialization and evolutionary age.

It is broadly divided into two parts:

(a) Ecological specificity:

The parasites are capable of making room in a foreign host but normally never reach another host due to ecological barriers. Such parasites are able to develop in more host-species under laboratory conditions than in nature.

(b) Physiological specificity:

The parasites are physiologically incapable of surviving and reproducing in a foreign host, e.g., Taenia solium in dog survives but never achieves reproductive ability. If the parasites find the conditions suitable for their development—then it is said to be compatible with that of the host. If not, it is said to be incompatible.

- ## Host and Parasite: A Mutual Relation

In the course of time a mutual adjustment or relation or tolerance frequently develops between the two which permits them to live together as a sort of compound organization without very serious effect or damage to either.

The virulant types, however, try to eradicate the hosts. But it is essential to keep the host alive and not to kill it by causing a high degree of pathogenecity. By killing the host it will ultimately lead to death of itself also. Accordingly Natural Selection leads to the elimination of most virulent species and maintains the less virulant ones.

- **Effects of Parasites on Hosts:**

The effects of parasitism on the hosts are intimately associated to the effect of host on the parasites. These effects depend on several factors, such as—age, diet, genetic factors, susceptibility of the hosts, the size, number and virulence of the parasites, their mortality, migration, and method of feeding.

A. Destruction of Host's Tissues:

Time and degree of damage vary greatly:

1. Time of injury:

i. Some parasites injure the host's tissue during the process of entry, e.g. hookworms like Ancylostoma duodenale, whose infective larvae inflict extensive damage to cells and underlying connective tissue while penetrating the host's skin.

ii. Some inflict tissue damage after they have entered, e.g., larvae of Ascaris lumbricoides while passing through lungs of human host cause physical damage to lung-tissue, leading to pneumonia.

iii. Others induce to histopathology changes by eliciting cellular immunologic response to their presence, e.g. Entamoeba histolytica actively lyses the epithelial cells lining the host's large intestine and liver causing large ulcerations by the action of secreted enzymes.

2. Types of cell damage:

Three major types:

i. Parenchymatous or albuminous degenerative cells become swollen and packed with albuminous or fatty granules and pale cytoplasm. This type of damage is characteristic of liver, cardiac muscle and kidney cells.

ii. Fatty degeneration cells are filled with an abnormal amount of fat deposits, e.g. liver cells.

iii. Necrosis means any type of persistent cell degeneration which finally die, e.g. as the result of encystment of Trichinella spiralis in mammalian skeletal muscles; necrosis of surrounding tissue is followed by calcification.

3. Tissue changes:

Four main types:

(a) Hyperplasia:

i. Refers to an increased rate of cell division resulting from an increased level of cell metabolism.

ii. Leads to a greater total number of cells but not in their sizes.

iii. This commonly follows an inflammation and is the consequence of an excessive level of tissue repair.

For example—thickening of bile duct in presence of *Fasciola* sp. is the result of hyperplasia.

(b) Hypertrophy:

i. Refers to an increase in cell size.

ii. Commonly associated with intracellular parasites.

For example in Erythrocytic phase of *Plasmodium vivax*, the parasitized RBC's are commonly enlarged. Spermatogonial cells of *Polymnia nebulosum* (an Annelid) when parasitized with *Caryotropha mesnili* (a Protozoan), are enlarged.

(c) Metaplasia:

i. Refers to the changing of one type of tissue into another without the intervention of embryonic tissue.

ii. The encapsulating epithelial cells and fibroblasts of the fluke, *Paragonimus westermani* in human lungs are transformation of certain other type of cells in the lungs.

(d) Neoplasia:

i. This is the growth of cells in a tissue to form a new structure, e.g., a tumour.

ii. Neoplastic tumour is not inflammatory.

iii. This is not required for the repair of organs.

iv. It does not conform to a normal growth pattern.

v. It may be benign or malignant.

Example:

Eimeria sp. causes tumor in rabbit liver, *Schistosoma mansoni* in human intestine and liver, *Echinococcus granulosus* in human lungs etc.

B. Competition for host's nutrients:

i. Endoparasites with a great density cause nutritional deficiency in host by absorbing sugars, vitamins, amino-acids etc.

ii. Malnourished hosts are more proned to disease and infection.

Example:

Diphyllobothrium latum (a fish tapeworm) in human causes anaemia by absorbing profuse Vitamin B_{12} (as much as 10 to 50 times more than do other tape-worms). Vitamin B_{12} plays an important role in blood formation, thus its uptake by *D. latum* results in anaemia.

C. Utilization of host's non-nutritional materials:

Parasites in some cases also feed on host substances, other than stored or recently acquired nutrients. Ectoparasites and endoparasites feed on host's blood, 500 human hookworms can cause a loss of about 250 ml blood/day, leading to anaemia.

4. Mechanical interferences:

Mechanical interferences by parasite cause injuries to hosts, e.g. elephantiasis or filariasis in humans is caused by *Wuchereria bancrofti*. Increased number of those adult worms in lymph vessels coupled with aggregation of connective tissue may result in complete blockage of lymph flow.

Excess fluid behind the blockage seeps through the walls of lymph ducts into the surrounding tissues, causing edema and ultimately with scar tissues—the elephantiasis of limbs, breasts, scrotum etc.

5. Effects of toxins, poisons and secretions:

Specific poisons or toxins egested, secreted or excreted by parasites cause irritation and damage to hosts, e.g.

i. Antienzymes produced by intestinal parasites counteract host's digestion.

ii. Allergin, a toxin as the body fluid of nematodes—*Parascaris equorum* and other ascarids, irritates the host's cornea and nasopharyngeal mucous membrane.

iii. Toxin of pathogenic *Entamoeba histolytica* produces toxic symptoms in parasitized mammalian hosts and creates ulcerations within the large gut of man.

iv. *Schistosome cercarial* dermatitis is the result of an allergin reaction against an irritating parasitic secretion from the fluke.

v. Haemozoin pigment produced by trophozoites of *P. vivax* exerts toxic effect in infected persons and the patients suffer from periodic effect of high fever with chilliness and shivering.

6. Other parasite-induced alterations:

(a) Sex reversals:

Gonads of parasitized hosts may change, leading to sex reversals; e.g. crab when parasitized by *Sacculina* (a crustacean) display sex reversals. Parasitized male crab acquired secondary female characteristics like broad abdomen, appendages modified to grasp eggs, chelae become smaller, testes with testicular cells at various stages of degeneration.

Parasite-removed male develops into hermaphrodite by regeneration of rest testicular cells. Parasitized female crab shows ovarian degeneration but does not show hermaphroditism on removal of parasite, as ovarian tissue cannot regenerate.

(b) Parasitic castration:

i. It refers to destruction of host's gonadal tissues by a parasite.

ii. Reduces egg and/sperm production in host's body or becomes sterile.

iii. The mudflat snail—Ilyanassa obsoleta are directly castrated by the trematode—*Zoogonus lasius*. Sporocysts of *Z. lasius* secrete a molecule that causes the destruction of host reproductive cells as well as inhibits same to genesis.

The freshwater snail, *Lymnaea stagnalis* is indirectly castrated by larvae (Sporocysts) of *Trichobilharzia ocellata* (a trematode). These larvae do not possess mouth and thus destroy the gonadal tissue by chemical means.

(c) Enhanced growth of host:

An interesting aspect of parasite induced change in hosts is responsible for enhanced growth; e.g.

i. Workers of the ant, *Pheidole commutula* become much larger when parasitized by the nematode, *Mermis* sp.

ii. Fresh water snail, *Lymnaea ariculata* infected with trematode larvae is larger than un-infected ones.

iii. Mice infected with larvae of *Spirometra mansonoides* (a tapeworm) grows faster than non-parasitized one.

iv. Rats when parasitized by *Trypanosoma lewise* increase their weight more rapidly than non-parasitized one.

The enhanced growth of the host is due to stimulation of growth-promoting molecules secreted by the parasites.

• Host reaction:

In immuno-parasitology, the animal is the host and the parasite is either self (by molecular memory) or non-self (foreign).

When a host recognizes the parasite as non-self, it generally reacts against the invader in two ways:

1. Cellular (or cell mediated) reactions:

Where specialised cells become mobilised to arrest and eventually destroy the parasite as usual.

2. Humoral reactions:

Where specialised molecules in circulatory system (antibodies/ immunoglobulin's in case of vertebrates) interact with the parasite, usually resulting in its immobilization and destruction.

- **Internal defense mechanisms:**

The internal defense mechanisms of animals, both invertebrates and vertebrates, are of two types:

I. Innate (or natural) and

II. Acquired.

Theoretically each of them again can be of two types—cellular and humoral.

Invertebrate immunity:

I. Innate internal defense mechanism:

Cellular factors:

This includes the following chief categories:

(a) Phagocytosis:

When a foreign parasite (small enough to be phagocytosed) invades into an invertebrate host, it is usually phagocytosed by the host's leucocytes, primarily the granulocytes.

Phagocytosis consists of three phases:

i. Attraction of phagocytes to the non-self material, commonly by chaemotaxis.

ii. Attachment of foreign material to the surface of the phagocyte, usually involving a specific chemical binding site.

iii. Internalization of the foreign substance i.e. engulfment by the phagocyte.

Fate of phagocytosed parasites:

i. May be degraded intracellularly.

ii. May be transported by phagocytes across epithelial borders to the exterior.

iii. May remain undamaged within the phagocytes and some may even multiply within host cells.

(b) Encapsulation:

1. Parasites, that are too large to be phagocytosed, are encapsulated as invading non-self mass enveloped by cells and/or fibres of host origin, as found in insect and molluscan hosts.

2. Encapsulation consists of:

(i) First leucocytosis (increase in number of leucocytes);

(ii) Migration—many of these cells migrate by the process of chaemotactic movement towards the parasite and form a capsule of discrete cells around it, as found in insects and in other cases (i.e., in molluscs).

Host cells synthesize fibrous material which becomes deposited inter-cellularly and concentrically in layers around the parasite.

3. Encapsulation of *Tetragonocephalum* (a tapeworm) in the American oyster, *Crassostrea virginica*.

Fate of encapsulated parasite:

Destroyed and disintegrated parasite's tissues are phagocytosed by host's granulocytes.

(c) Nacrezation (i.e. pearl formation):

1. Nacrezation is another type of cellular defense mechanism, known in molluscs.

2. As certain helminth parasites, e.g., *Meiogymnophallus minutus* (a trematode) occurs between the inner surface of the shell (nacreous layer) and the mantle of marine bivalves. Now the mantle is stimulated to secrete nacre that becomes deposited around the parasite. In so doing, a pearl is formed and the enclosed parasite is killed.

(d) Melanization:

1. The process involves deposition of the black-brown pigment, melanin around the invading parasite.

2. Melanization is chemically the result of enzymatic oxidation of polyphenol by tyrosinase.

3. This is detrimental to the parasite and may lead to its death by interfering with such vital activities like hatching, moulting or feeding.

4. Melanization of the nematode, Heterotylenchus autumnalis in haemocoel of larval housefly—*Musca domestica*.

II. Humoral factors:

These fall into two categories:

(a) Innate humoral factors:

These are two types:

i. Those are directly parasitocidal, e.g. several marine molluscan species contain a constituent in their tissue extract that is lethal to Cercariae of the trematode, *Himasthla quissetensis*.

ii. Those that enhance cellular reactions, e.g., naturally occurring agglutinins or lectins. These glycoprotein molecules enhance phagocytosis of the non-self-material.

(b) Acquired humoral factors:

These are also of two types:

i. Lysosomal enzyme:

When challenged with non-self-parasites, some invertebrate's granulocytes (haemocytes) hypersynthe size certain lysosomal enzymes and subsequently release them into some parasites. When they come in contact with elevated enzymes are killed either directly or indirectly whereas the parasite's body surface by action of lysosomal enzyme undergoes chemical alteration and thus is recognized as non-self-material and consequently get attacked by host's haemocytes.

ii. Antimicrobial molecules:

When challenged with micro-organisms, some insects synthesize antimicrobial molecules which are quite different from vertebrate antibody but kill the microorganisms, e.g.; the synthesis of two small basic proteins (PgA and PgB) by the moth, *Hyalophora cearopia* when challenged with *E. coli* and these proteins kill the bacterium.

Vertebrate Immunity:

Immunity refers to resistance against disease caused by a foreign agent. This is based on antigen-antibody interaction. In vertebrates this reaction is very specific. Antigen is the only foreign substance (Proteins, glycoproteins, nucleoproteins etc.) which on introduction induces the synthesis of antibody under some appropriate conditions. All zoo-parasites theoretically contain multiple antigens.

These are chiefly of two types:

i. Somatic antigens molecules comprising some of parasites.

ii. Metabolic antigen molecules are associated with secretion and excretion, e.g., moulting fluid of nematode is highly antigenic.

Antibody—Refers to proteins synthesized by host tissue in response to the administration of an antigen and which specifically react with that antigen to immobilize and destroy it.

Mechanism of antigen-antibody interactions:

i. Antigens on introduction are able to bind with specific cell surface receptor of lymphocytes (both B and T-lymphocytes).

ii. Host lymphocytes are now stimulated to proliferate and differentiate.

iii. As a consequence, clones of progeny lymphocytes are formed.

iv. In the process of proliferation, some progeny differentiate into effector cells (the functional end products of the immune response). Plasma cells are B-lymphocyte

effector cells that secrete antibodies. Killer T-cells are such T-lymphocyte effector cells that eliminate foreign cells simply by contact.

v. As soon as immunoglobulins are produced, immunogens are coated with such antibodies and are rapidly destructed and / or phagocytosed.

The parasites try to establish itself within the host while the latter tries to destroy it which results in dynamic state of equilibrium. The reaction of the host in the presence of a parasite is termed as resistance. If resistance is sufficiently high to prevent parasite reproduction, it is known as absolute resistance and if parasite is able to overcome it and still reproducing it is called partial resistance.

(a) In case of larger parasites there is considerable damage to host tissues where histamin is released, macrophages are attracted and a primary stage of inflammation is set up.

(b) In the second stage, the cells of the lymphoid macrophage system elaborate antibodies. The immunoglobulin's appears in various molecular forms differing in properties and actions. The macroglobulin's (IgM) is the first to appear in an infected animal. This is followed by the appearance of gamma-globulin and alpha-globulin (IgA). The properties and number of antibodies vary from individual to individual parasitic infections.

(c) Interferons also play an important role in the immunity reaction of the host. These are known to operate in malaria and other viral reactions by rendering the host cells unfit for habitation by intercellular parasites.

Categories of antigen-antibody interactions:

These are of three types:

1. Primary interaction:

Refers to the basic event during which the antigen is bound to and/or more available sites on the antibody molecule.

2. Secondary interactions:

Include agglutination, precipitation, complement-dependent reaction, neutralisation, immobilisation etc.

i. Agglutination reaction:

Antibodies (agglutinins) clump microbes representing antigens and visible conglomerates are formed. This is referred to as agglutination reaction.

ii. Lysin and lysis reaction:

Lysin (antibodies) dissolves or lyses antigens. The reaction occurs in the presence of complement, a substance in normal serum representing a system of enzymes.

Complement is sensitive to heat, chemical substances, ultraviolet rays, long-term storage etc.

iii. Complement-dependent reaction:

In the first phase of this reaction mutual adsorption of antigen-antibody takes place and precipitation occurs. In the second phase of reaction the fixation of complement by antigen-antibody occurs which is used for detecting many of the parasitological infections.

iv. Precipitin reaction:

Precipitin is the antibody that brings about the formation of a minute deposit (precipitation) when interacted with specific antigen (precipitinogen). While in agglutination the entire microbial bodies act as antigen, in precipitin the antigen will be the results of breakdown of microbial bodies or their products. This precipitin reaction is used for detecting infections like plague, anthrax, tularaemia etc.

v. Phagocytosis:

Refers to the engulfment of non-self material by host cell like macrophages. In vertebrates, this is introduced through the action of antigen, antibody and complement.

This occurs in two ways:

(1) Accumulation of leucocytes through complement sequence and

(2) Certain antibodies called opsonins become coated on to the foreign materials and they enhance phagocytosis.

Opsonins are antibodies occurring in normal as well as in immune sera which inhibit microbes making them more amenable to phagocytosis.

3. Tertiary interaction:

Refers to in vivo expressions of antigen-antibody reactions. At times these may be of survival value to the host, but at other times they may lead' to a disease through immunologic injury.

Immunity to parasites:

Vertebrate hosts always develop some degree of acquired immunity in the presence of parasites.

This is usually of two types:

i. Concomitant immunity:

Where immunity, either complete or partial, may be maintained only while the parasites are present.

ii. Sterile immunity:

Where immunity persists long after the complete disappearance of the parasites.

Protozoan blood parasites:

i. Cattle infected with certain species of *Babesia* shows premonition.

ii. While cattle, long after *Theileria parva* has disappeared, shows sterile immunity.

iii. For malaria-causing parasites—Premonition usually occurs in case of avian- infecting species of *Plasmodium* while sterile immunity can be produced with the rodent malaria causing agents *P. berghei* and *P. vinckei* (Fig. 16.3).

iv. Certain breeds of cattle develop partial immunity against *Trypanosoma*.

Protozoan tissue parasites:

(a) Antibodies are produced by host cells when they come in contact with antigens of parasites. Thus,

i. When *Entamoeba histolytica* resides in host's intestinal lumina, no detectable antibody occurs, but when the Amoeba invades the mucosa and other tissues, antibodies are evident.

ii. For *Leishmania tropica*, if the sores produced by this parasite are allowed to heal spontaneously, the host becomes totally immuned to reinfection.

iii. Immunity to *Toxoplasma gondii* is evident in adult humans who show the antibodies, while congenital infection with *T. gondii* leads usually to death and those who survive, show a hydrocephalus condition. Immunity can control the intracellular stages only if the macrophage becomes successfully activated, which requires production of cytokines by T-cells.

iv. The trophozoites of gregarines (*Gregarina* sp) exhibit an eerie gliding movement. Various theories have been put forward. Similar movement has been noticed for the trophozoites of *Plasmodium* and *Eimeria*. There is little evidence of an effective immunity against merozoites of *Eimeria* sp and *Gregarina* sp.

Experimental studies have shown that immunity can directly affect intracellular developmental stages in both initial and subsequent infections. This immunity relies on the production of cytokine interferon, presumably activating an intracellular defence mechanism that is capable of preventing sporozoite and merozoite development.

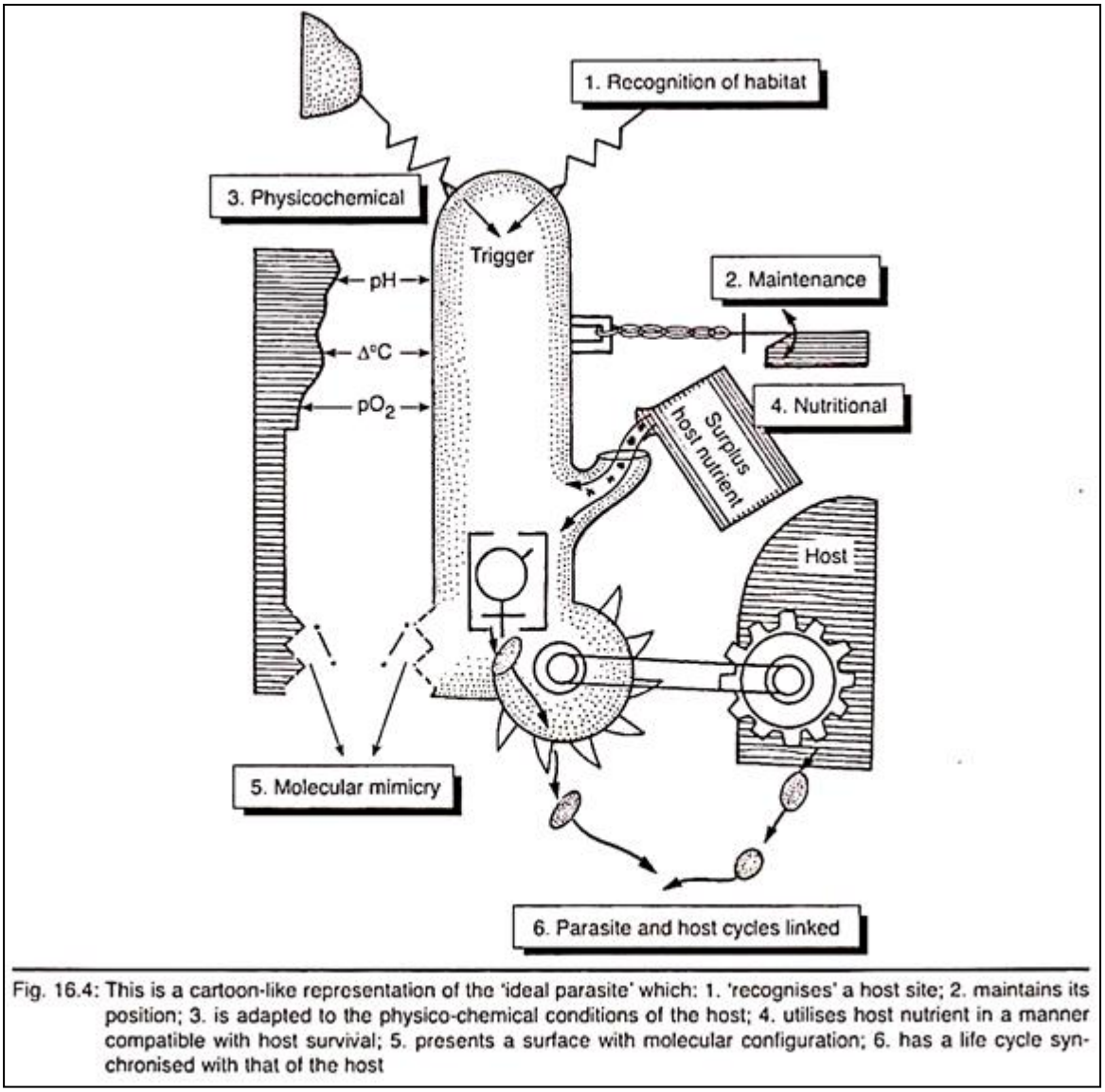

Fig. 16.4: This is a cartoon-like representation of the 'ideal parasite' which: 1. 'recognises' a host site; 2. maintains its position; 3. is adapted to the physico-chemical conditions of the host; 4. utilises host nutrient in a manner compatible with host survival; 5. presents a surface with molecular configuration; 6. has a life cycle synchronised with that of the host

Hosts and Parasites: A mutual tolerance

In course of time, a mutual adjustment or tolerance frequently develops between the two host and parasite, which results to lead their life as a sort of compound organisms, without any serious effect to either. It may not" be in the best interest of the parasites to destroy its host for it would do it—invariably it would destroy itself.

Thus, parasitism is a specialized mode of living within a broader ecological category—symbiosis. In parasitism—the host and the parasite have a very intimate association where all the benefits are derived by the parasites from its prey—the host and the two (2) systems constantly interacting with each other.

Thus the criticism of the story—"Host- Parasite Interaction" is one of the compromise—a key (parasite) to unlock the box (host) of an unrevelled mysterious entity—in which the parasites making elaborate efforts to overcome the match against the host while the host making attempts to keep the ball in the goal of parasites, thus trying to eradicate it.

Probable questions:

1. Discuss the effect of parasite in host.
2. What is hypertrophy?
3. What is neoplasia?
4. What is parasitic castration?
5. Discuss host immunity reaction when it encounters a parasite.

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Unit-III

Classification of Protozoa and Helminths

Objectives:

In this section we will discuss on Classification of Protozoa upto class and Helminths upto class.

Classification of Protozoa

Introduction:

Anton Van Leeuwenhoek was the first to observe protozoa (Vorticella convellaria) under a microscope. He called them animalcules. Gold fuss coined the term Protozoa which in Greek means first animals (Proto= first; zoans=animals). Hyman and other zoologists preferred to call them as acellular animals. The body of protozoans is unicellular. They are generally referred to as acellular rather than unicellular as the so called single cell performs all the life activities. Though it is structurally equivalent to a single cell of the metazoan body, it is functionally equivalent to the whole metazoan animals.

General Characteristic Features:

1. There are about 50,000 known species of Phylum Protozoa.
2. Protozoans exhibit mainly two forms of life; free-living (aquatic, freshwater, seawater) and parasitic (ectoparasites or endoparasites). They are also commensal in habitat.
3. They are small, usually microscopic, not visualize without a microscope.
4. They are the simplest and primitive of all animals.
5. They have a simple body organization. i.e. with a protoplasmic grade of organization.
6. The body is unicellular (without tissue and organs).
7. They have one or more nuclei which are monomorphic or dimorphic.
8. Body naked or bounded by a pellicle, but in some forms may be covered with shells and often provided with an internal skeleton.
9. They are solitary (existing alone/single) or colonial (individuals are alike and independent).
10. Body shape variables may be spherical, oval, elongated or flattened.
11. Body symmetry either none or bilateral or radial or spherical.
12. Body form usually constant, varied in some, while changing with environment or age in many.

13. Body protoplasm is differentiated into an outer ectoplasm and inner endoplasm.
14. The single-cell body performs all the essential and vital activities, which characterize the animal body; hence only subcellular physiological division of labor.
15. Locomotory organs are fingers like pseudopodia, whip-like flagella, hair-like cilia or none.
16. Nutrition may be holozoic (animal-like), holophytic (plant like), saprozoic or parasitic.
17. Digestion occurs intracellularly which takes place inside the food vacuoles.
18. Respiration occurs by diffusion through the general body surface.
19. Excretion occurs through the general body surface, but in some forms through a temporary opening in the ectoplasm or through a permanent pore called cytopyge.
20. Contractile vacuoles perform osmoregulation in freshwater forms and also help in removing excretory products.
21. Reproduction asexual (binary or multiple fission, budding, sporulation) or sexual (conjugation (hologamy), game formation (syngamy).
22. The life cycle often complicated with alternation of asexual and sexual phases (alternation of generation).
23. Encystment commonly occurs to resist unfavorable conditions of food, temperature, and moisture, and also helps in dispersal.
24. The single-celled individual not differentiated into somatoplasm and germplasm; therefore, exempt from natural death which is the price paid for the body.
25. Protozoans exhibit mainly two forms of life; free-living (aquatic, freshwater, seawater) and parasitic (ectoparasites or endoparasites). They are also commensal in habitat.

Classification in Outline:

Previously, the protozoan classification was done mainly on the basis of locomotor organs but recently the electron microscopic findings have added new dimension to the study of Protozoa and the scheme of protozoan classification has been changed considerably. The conventional scheme followed by Hyman (1940), Hickman (1961) and Storer (1965), etc. recognizes two subphyla on the basis of organs of locomotion and 5 classes as follows:

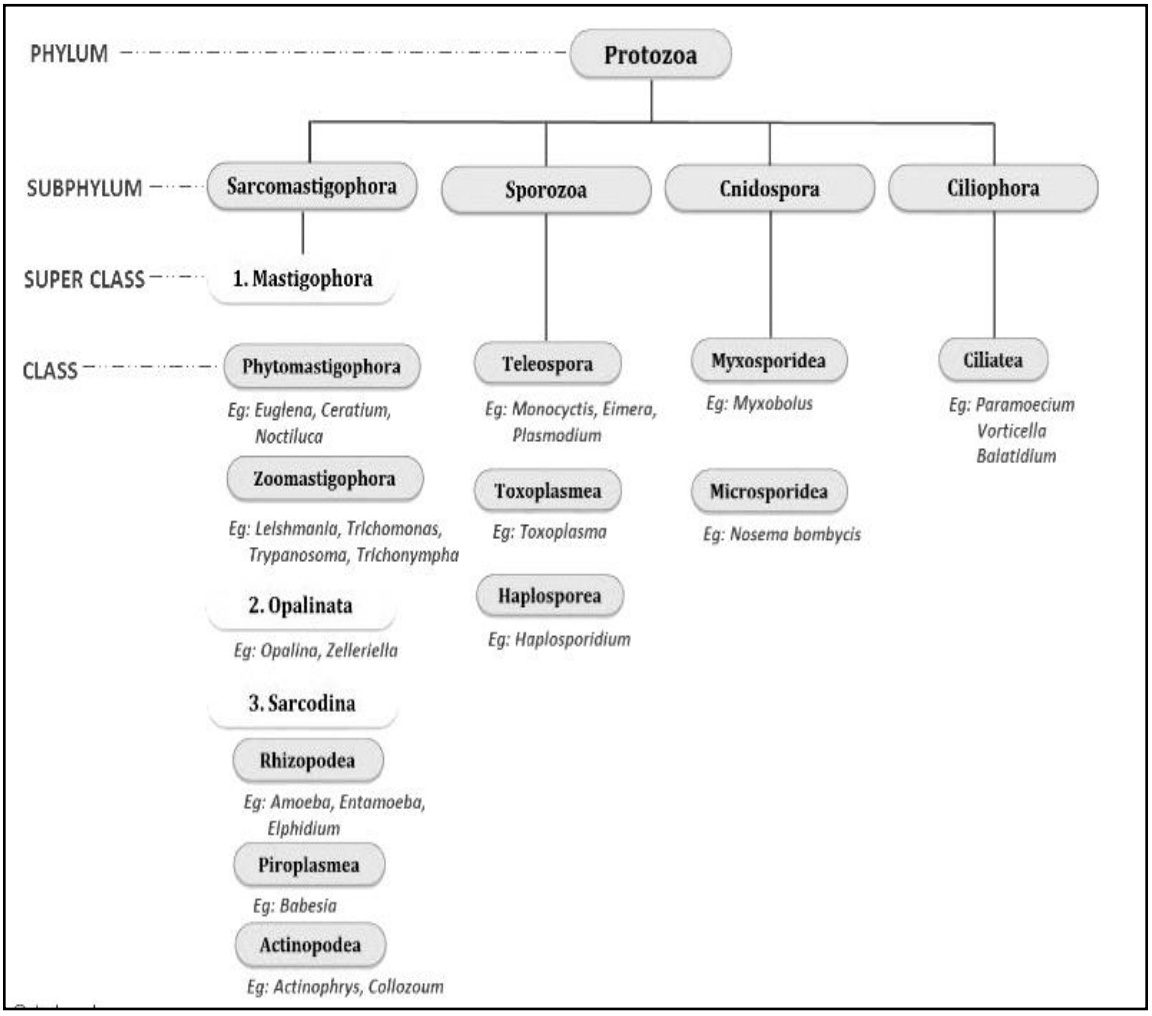

Fig: The conventional scheme of classification of Phylum Protozoa followed by Hyman (1940), Hickman (1961) and Storer (1965), etc.

Classification with Characters:

A. Subphylum : Sarcomastigophora

Features:

1. Locomotory structures are present either in the form of flagella, pseudopodia or both.
2. Nucleus is usually of single type (excepting the developmental stages of certainForaminiferida).
3. No spore formation.
4. Sexual reproduction when present is through syngamy.

It includes three superclasses—Mastigophora, Opalinata and Sarcodina.

a) Superclass: Mastigophora

Features:

1. They are commonly called flagellates.
2. Locomotory organelles are flagella in adults.

3. The body is covered by a pellicle.
4. Binary fission is longitudinal.
5. They are mostly free-living though some are parasitic.
6. Nutrition is autotrophic or heterotrophic or both.

It is subdivided into two classes— Phytomastigophorea and Zoomastigophorea.

I. Class: Phytomastigophorea

Features:

1. Usually possess chromatophores which may be secondarily lost.
2. Presence of one or two emergent flagella.
3. In some groups amoeboid forms occur.
4. Most members are free-living and certain forms exhibit sexual reproduction. There are ten orders in this class.

II. Class: Zoomastigophorea

Features:

1. Usually live in association.
2. Chromatophores are absent.
3. Presence of one to many flagella.
4. Amoeboid forms, when present, may not have flagella.It includes nine orders.

b) Superclass: Opalinata

Features:

1. Presence of cilium-like organelles in oblique rows over entire body surface.
2. Cytostome is absent, more than one nucleus of same type.
3. Sexual reproduction happens through the production of anisogamous flagellated gametes.
4. Always live in association. Examples. *Opalina, Zelleriella*.

c) Superclass: Sarcodina

Features:

1. Usually free-living locomotor organella in the form of pseudopodia.
2. Flagella appear in some forms during development.
3. Cortical cytoplasm is undifferentiated.
4. Body may or may have various types or exo or endo-skeleton.
5. Asexual reproduction occurs by fission.
6. Sometimes sexual reproduction with flagellate or amoeboid gametes is noted.

The members of this superclass (Sarcodina) are again subdivided into three classes— Rhizopodea,Piroplasmea and Actinopodea.

III. Class Rhizopodea:

Features:

1. Nutrition is phagotrophic.

2. Pseudopodia may be lobopodia, filo-podia or reticulopodia.

IV. Class: Piroplasmea

Features:

1. Small forms of various shapes.
2. Spores, flagellal and cilia absent.
3. Locomotion by gliding.
4. Binary fission takes place.
5. Lives as parasite in vertebrate blood and are carried by ticks. Examples. *Theileria, Babesia*.

V. Class: Actinopodea

Features:

1. Usually floating with spherical body and delicate pseudopodia.
2. Pseudopodia may be axopodia, filose or reticulate.
3. Usually naked, when test present it is either membranous or chitinoid or silicious orstrontium.
4. Both asexual and sexual reproduction occurs.
5. Gametes are flagellated.

B. <u>Subphylum: Sporozoa</u>

Features:

1. Simple spores without polar filaments carry one to many sporozoites.
2. Cilia absent but flagellated gametes may occur.
3. Sexual reproduction, when occurs, is syngamous.
4. All the forms live in association.

It is subdivided into three classes— Telosporea, Toxoplasmea and Hoplosporea.

VI. Class: Telosporea

Features:

1. Spores are seen.
2. Both asexual and sexual reproduction take place.
3. Locomotion by gliding or body flexion.
4. Pseudopodia are usually absent but sometimes used only for food capture.
5. Microgametes are flagellated in some.

VII. Class: Toxoplasmea

Features:

1. No spore formation.
2. Asexual reproduction by binary fission.
3. Locomotion is effected by gliding or body flexion.
4. Structures like pseudopodia and flagella are absent.
5. Cysts include naked trophozoites.Examples. *Toxoplasma, Sarcocystis*.

VIII. Class: Haplosporea

Features:

1. Presence of spores, only asexual reproduction takes place.
2. Schizogony is present.
3. Though pseudopodia may appear in some cases, yet flagella are absent.
   Examples. *Haplosporidium, Coelosporidium*.

C. Subphylum: Cnidospora

Features:

1. Presence of spores having one or more spore filaments and sporoplasms.
2. All the members live as parasite.

Two classes—Myxosporidea and Microsporidea are present.

IX. Class: Myxosporidea

Features:

1. Multicellular state gives rise to spore.
2. Presence of one or more sporoplasms and more than one valve.
   Examples. *Myxobolus*

X. Class: Microsporidea

Features:

1. Spores originate from a single cell.
2. Presence of single sporoplasm, valve and an elongated tubular polar filament.
3. Parasites in invertebrates.
   Examples. *Caudospora, Nosema*.

D. Subphylum: Ciliophora

Features:

1. The locomotory organelle of all the animals of this subphylum is cilia.
2. These cilia also help in feeding at some stage of the life cycle of the animals. The nuclei are dimorphic.
3. Macronucleus is vegetative and polyploid.
4. Micronucleus is reproductive and diploid.
5. Asexual reproduction takes place by binary fission.
6. Sexual reproduction takes place by conjugation.

Single class Ciliate is present.

XI. Class: Ciliatea

Features:

1. Free living forms with cilia or ciliated organelle at least in some part of the life cycle.
2. Sub-pellicular infra-ciliature always present, even during the absence of cilia.
3. Usually two types of nuclei are seen.

4. Both asexual and sexual reproductions occur.
5. Sexual reproduction involves either conjugation or autogamy or cytogamy.
6. Nutrition is heterotrophic.
   Examples. *Paramoecium, Vorticella, Balantidium*

Classification of Helminths

Introduction:

The classification and identification of helminths are dependent on numerous factors including body shape, body cavity, body covering, digestive tubing, sex and type of attachment organs.

Platyhelminths (flatworms) include both trematodes (flukes) and cestodes (tapeworms). Specifically, tapeworms are characterized using the above criteria and are organized in a segmented plane. They lack a body cavity and have a *tegument* body covering. Tapeworms lack a digestive tube and are hermaphroditic. They utilize suckers or *bothridia*, and *rostellum* with hooks for an attachment organ.

Trematodes are characterized by an unsegmented plane for body shape. They also lack a body cavity and have a tegument for body covering. However, the digestive tube for trematodes ends in the cecum. Trematodes are hermaphroditic and utilize oral suckers, ventral suckers or *acetabulum* for attachment organs.

Nematodes are characterized by a cylindrical body shape and do indeed have a body cavity. Its body covering is a cuticle and the digestive tube ends in the anus. The sex of nematodes is *dioecious* (distinct male and female organisms). Lastly, their attachment organs range from lips, teeth, filariform extremities and dentary plates.

General Characteristic Features of Platyhelminthes:

1. Bilaterally symmetrical, dorso-ventrally flat, triploblastic animals without true segmentation.
2. Body covered with epidermis which is soft and ciliated (Turbelleria) or covered by cuticle and provided with external suckers or hooks or both for attachment to the host (Trematoda, Cestoidea).
3. Digestive system incomplete, having a mouth but no anus and much branched (Absent in Cestoda).
4. No body cavity; space between internal organs and body wall filled with loose parenchyma; muscle layers well developed.
5. Excretory system characteristic; consisting of numerous flame cells distributed

in the parenchyma and connected to excretory ducts opening to outside.

6. Apair of anterior nerve ganglia or a nerve ring connected with 1 to 3 pairs of longitudinal nerve cords with transverse commisures.
7. Sexes united; fertilization internal; eggs numerous, minute yolky, development direct. (Turbellaria, Monogena) or indirect and complicated (Digena).
8. Circulatory, respiratory or skeletal systems absent.

Classification of Platyhelminthes:

The classification adopted here is from Rupart and Barnes, 1994 (6^{th} edition). Phylum Platyhelminthes is divided into four classes on the basis of body shape, mouth position and habitat.

I. Class: Turbellaria (L., turbella = a little string)

i. Mostly free-living flatworms but some ectocommensals and endocommensals or parasitic.
ii. Body unsegmented.
iii. Body covered with a cellular or syncytial epidermis usually with mucous cells and which is usually partly ciliated.
iv. Adhesive organs abundantly present.
v. Digestive system usually consists of mouth, pharynx and intestine, anus not found.
vi. Excretory system consists of protonephridia, the flame cells.
vii. Sense organs consist of tango receptors and chemoreceptors.
viii. Mostly hermaphrodite.
ix. Reproduction sexual, asexual and by regeneration.
x. Life cycle simple.

Example: *Ectocotyla, Planeria, Dugesia, Bipalium*

II. Class: Trematoda (Gr., trematodes = having pores)

i. Ectoparasitic or endoparasitic forms, commonly called flukes.
ii. Body shape usually leaf-like, dorsoventrally flattened.
iii. Body wall without epidermis and cilia.
iv. Body undivided and covered with a cuticle.
v. Well developed suckers usually present.
vi. Digestive tract incomplete consisting of mouth, pharynx and two forked or many branched intestine; anus absent.
vii. Protonephridial excretory system consisting of flame cells.

viii. Mostly hermaphrodite (monoecious).

ix. Ovary single, testes two to many.

x. Life history simple or complicated.

Example: *Polystoma, Gryodactylus, Aspidogaster, Fasciola, Schistosoma, Bucephalus, Opisthorchis* (=Clanorchis).

III. Class: Monogenea

i. Monogeneans are mostly ectoparasites of aquatic vertebrates, particularly fishes, but amphibians, reptiles and cephalopod molluscs are also hosts.

ii. Body dorsoventrally flattened and have a large, posterior attachment organ, the haptor, which bears hooks and suckers.

iii. Anterior end has also adhesive glands.

iv. A gut is present but mouth lacks a sucker. The pharynx secretes a protease that digests the host's skin.

v. They respire aerobically, being ectoparasites.

vi. Monogeneans have inconspicuous protonephridia for excretion

vii. All members are hermaphrodite.

viii. Life cycle simple and having no intermediate host.

ix. One egg by way of a ciliate larva (Oncomiracidium) gives rise to only one adult worm and hence the name monogenea meaning "one generation"

Examples: *Polystoma* (bladder of frogs), *Polystomoidella* (Urinary bladder of turtles), *Dactylogyrus* (gills of freshwater fishes)

IV. Class: Cestoidea (Gr., kestos = girdle + eidos = form):

i. Endoparasites in the intestine of vertebrates.

ii. Commonly called tapeworms.

iii. Body without epidermis and cilia but covered with cuticle.

iv. Body usually divided into few to many segments (proglottids), rarely undivided.

v. Anterior end (scolex) is provided with adhesive structures (hooks, suckers) except in Cestodaria.

vi. Mouth and digestive tract totally absent.

vii. Excretory system consists of protonephridia with typical terminal flame bulbs.

viii. Nervous system usually comprises a pair of ganglia and two lateral longitudinal nerve cords.

ix. Each segment (proglottis) contains one or two sets of complete hermaphroditic reproductive system.

x. Life cycle complicated usually involving two or more hosts.

xi. Embryos possess hooks.

Example: *Amphilina, Gyrocotyle, Echinobothrium, Taenia, Echinococcus, Hymenolepis.*

General Characteristic of Aschelminthes:

1. Body of Phylum Nematoda is un-segmented, bilaterally symmetrical, elongated and tapering at both ends.
2. Triploblastic animals with perivisceral cavity are more extensive than that of Platyhelminthes.
3. Body of of Phylum Nematoda is generally covered with thick, flexible multi-layered collagenous cuticle and often bears cuticular setae (hairs), spines or annulations.
4. Cuticle moulted periodically.
5. Epidermis or hypodermis syncytial; i.e., the nuclei are not separated from each other by cell membranes.
6. Only longitudinal body-wall muscles; no circular body-wall muscles.
7. Body cavity of of Phylum Nematoda is pseudocoel filled with parenchyma in most cases.
8. Alimentary canal provided with distinct mouth and anus (complete digestive tract). Muscular pharynx and the inner surface of the gut usually not lined by cilia. Extracellular digestion.
9. Mouth of of Phylum Nematoda is surrounded by six lips.
10. Blood vascular system and respiratory system are absent in of Phylum Nematoda.
11. Haemoglobin sometimes present in the pseudocoelomic fluid.
12. Excretory system without nephridia and flame cells. In the class Adenophorea glandular renette cells with a duct or in the class Secernentea excretory canal system without flame cells act as excretory system.
13. Dorsal and ventral nerve cords in the epidermis.
14. Chemosensory organs are small cuticular projections called amphids which are situated on the lips, derived from cilia and opening to the exterior through a small pore, and lined with modified non-motile cilia called sensillae.
15. Sexes of of Phylum Nematoda are separate (gonochoristic).
16. Tubular gonads are present in of Phylum Nematoda.
17. Amoeboid sperm cells.
18. Fertilization is internal in of Phylum Nematoda.
19. They are free-living or phytoparasitic or zooparasitic.

Classification of Aschelminthes:

Following the scheme of Chitwood (1933), the phylum Nematoda is divided into two classes:

1. Adenophorea or Aphasmida and
2. Secernentea or phasmidea.

I. Class: Adenophorea or Aphasmida

i. Most species possess caudal adhesive glands and epidermal glands.
ii. Phasmids (caudal papillae bearing pores connecting with glandular pouch called phasmids which are thought to be chemosensory in function) are absent.
iii. Amphids are post labial and variously shaped such as pouch-like or tube-like, rarely pore-like.
iv. Coelomocytes well developed.
v. Excretory organs are only renette cells but without collecting tubules.
vi. Males usually without caudal alae.
vii. Usually two testes in males.
viii. Mostly marine, and include both free- living and parasitic species. The free- living species include both terrestrial, freshwater, and major marine forms.

Examples: *Enoplus, Anticoma, Metonchdiamus, Xiphinema, Trichodoris, Desmoscolex, Odontophora.*

II. Class: Secernentea or Phasmida

i. Caudal phasmids present.
ii. Labial amphids pore-like.
iii. Excretory system canal-like and comparatively more complex.
iv. Epidermal and caudal adhesive glands absent.
v. Males with a single testis.
vi. Mostly parasitic.
vii. Free-living species are largely terrestrial.

Examples: *Rhabditis, Heterodera, Bunonema, Ancylostoma duodenale (Hookworm), Strongylus, Trichostrongylus (Hair worm), Ascaris (Ascaris lumbricoides, Ascaris megalocephala, Ascaris suillas), Parascari, Toxocara, Spirura, Wuchereria bancrofti (Filaria), Loa loa (Eye worm), Brugia, Onchocerca, Trichuris (Parasites of mammals), Trichinella spiralis (Trichinia worm), Camallanus, Dracunculus* etc.

Probable questions:

1. Discuss the characteristics of phylum protozoa.
2. Discuss the systematic position of *Paramoecium.*
3. Elaborate the classification of Phylum protozoa up to class with example.
4. Write down the characteristics of class ciliate with example.
5. Discuss the characteristics of phylum platyhelminthes.
6. Discuss the systematic position of *Fasciola.*
7. Elaborate the schematic diagram of phylum Aschelminthes up to class with example.
8. Write down the characteristics of class phasmida with example.

Suggested reading:

1. Barnes: Invertebrate Zoology (Holt-Saunders International, 4th edition, 1980)
2. Barnes: The Invertebrates – A synthesis, 3rd edition, Blackwell, 2001
3. Hunter: Life of Invertebrates, Collier Macmillan Pub. 1979
4. Marshall: Parker & Haswell Text Book of Zoology, Vol. I, 7th edition, Macmillan, 1972

Unit IV

Entamoeba and Blood Flukes in Humans

Objectives:

In this section we will discuss about *Entamoeba* and different blood flukes present in human blood; their life cycle, biology, pathogenesity, epidemiology and control. Beside this we will also discuss about the Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture

Entamoeba sp.

Entamoeba histolytica was first of all reported by a Russian Zoologist, Losch (1875), as *Amoeba coli* from the faeces of a patient suffering from dysentery. The genus *Entamoeba* was established by Cosagrandi and Barbagallo (1895); it should not, be confused with the genus *Endamoeba* which was established by Leidy (1879). Concilman and Loffteur (1901) worked out the pathogenecity of amoebiasis and amoebic ulcers.

The species *Entamoeba histolytica* was established by Schaudinn (1903) and he differentiated the pathogenic and non-pathogenic types. But, Walker and Sellards (1930) are credited for attributing pathogenic effects of this parasite. Craig (1962) has estimated that more than 10 percent of world population is suffering from the infection of this parasite.

Systematic position:

Kingdom : Protista
Phylum : Sarcomastigophora
Sub phylum : Sarcodina
Super class : Rhizopoda
Class : Lobosa
Genus : *Entamoeba*

Distribution of *Entamoeba histolytica*:

Entamoeba histolytica is world-wide (cosmopolitan) in distribution. But it is commonly found in epidemic form in tropical and sub-tropical regions than in the temperate region. The more epidemic condition of this parasite is reported from Mexico, China, India, Philippines, South America and Thailand.

Its incidence is relatively higher in rural and densely-populated urban areas particularly in those areas where the sanitary conditions are poor. In India, its effect is on higher level in humid climates as compared to dry and cold climates. The children and adults

are more frequently infected; surprisingly, the males are more commonly infected than the females.

Habit and Habitat of *Entamoeba histolytica*:

Entamoeba histolytica is microscopic and lives as an endoparasite in the upper part of the large intestine, i.e., colon of man. It inhabits the mucous and sub-mucous layers of the large intestine. It feeds mainly on the tissues of the intestinal wall and often produces severe ulcers and abscesses.

In chronic cases, it may enter the blood circulation to reach the liver, lungs, brain and other organs. It causes a serious and often fatal disease known as amoebic dysentery or amoebiasis.

Geographic Distribution

Worldwide, with higher incidence of amebiasis in developing countries. In industrialized countries, risk groups include male homosexuals, travellers and recent immigrants, and institutionalized populations.

Structure of *Entamoeba histolytica*:

Entamoeba histolytica exists in two distinct forms the magna or trophozoite form and the minuta or precystic form.

Trophozoite:

The mature parasite or active and motile adult is known as trophozoite. The trophozoites of E. *histolytica* are large, hence, called magna, usually 20-30 *Entamoeba histolytica* trophozoites, microns in diameter. It is feeding form which is pathogenic to man.

It resembles *Amoeba* in all structural details. The cytoplasm of trophozoite is differentiated into two distinct portions, the outer ectoplasm and inner endoplasm. The ectoplasm is clear, non-granular and hyaline, while the endoplasm is granular and fluid-like.

The pseudopodia may be short, broad and rounded, long and finger-like, mainly composed of ectoplasm. During locomotion, the rapidly advancing end of the body consists of a single clear pseudopodium, i.e., monopodial. With this single pseudopodium, it moves in a crawling fashion like garden slug, hence, the movement is also sometimes called limax-type movement.

The endoplasm contains the nucleus and food vacuoles containing the tissue fragments, erythrocytes, leucocytes and ingested bacteria, etc.

The nucleus is rounded, 4-6 microns in diameter and vesicular. The nucleus is

composed of a delicate membrane, small peripheral chromatin granules and a centrally located small dot-like nucleolus or endosome or karyosome and chromatin granules arranged in spoke-like striations.

The nucleolus is surrounded by an indefinite clear area called halo. The presence of red blood corpuscles (RBCs) in food vacuoles is an important characteristic feature of this parasite, as the capacity of ingesting RBCs. is not seen in other intestinal amoebae of man.

Contractile vacuole is entirely absent because *E. histolytica* lives in an environment which is isotonic. Since, the osmotic concentration of its body remains equal to its surroundings and, hence, no water enters in its body by osmosis. Therefore, there is no need of contractile vacuole.

The magna or trophozoic form of *E. histolytica* develops from small minuta form; it enters into the mucosa and sub-mucosa layers of the intestinal wall by dissolving its tissues. Thus, it makes small wounds in the intestinal lining which later develop into ulcers. After reaching into the intestinal tissues, ingests RBCs and grows up to 60 microns in size.

Minuta:

It is the pre-cystic form which is smaller, spherical, non-feeding, non-motile and non-pathogenic. It measures to about 7-10 microns in diameter and resembles the trophozoite form in its structure except that it is smaller in size having no pseudopodium and contractile vacuole. It lives only in the lumen of intestine and rarely found in the tissues. It undergoes encystation and helps in the transmission of the parasite from one host to another.

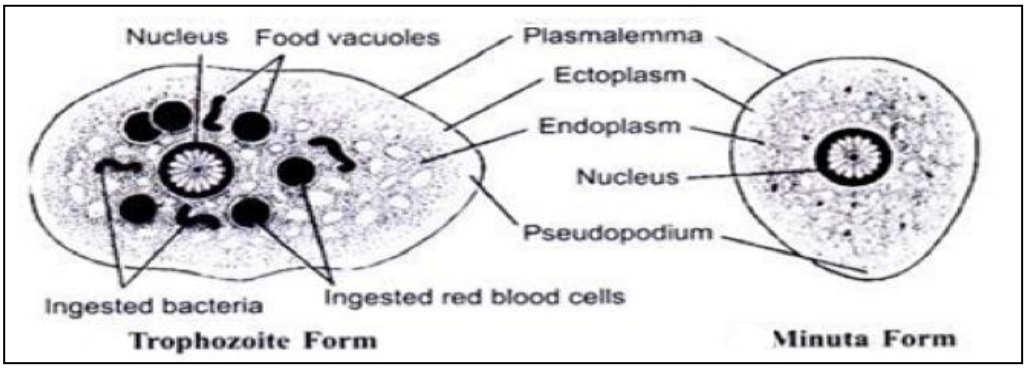

Fig: Structure of different forms of *E. histolytica*

Nutrition of *Entamoeba histolytica*:

In *Entamoeba histolytica*, the nutrition is holozoic. It feeds mainly upon the blood corpuscles, other host elements, bacteria and yeasts. It also absorbs substances saprozoically from the surrounding medium.

Reproduction:

The trophozoite of *Entamoeba histolytica* reproduces normally by a process of simple binary fission in the intestinal wall and by a modified form of mitosis. The exact nature of the division of the nucleus is controversial but it is believed by many authors that it is probably a modified type of mitosis. Kofoid and Swezy observed six chromosomes in it. *Entamoeba histolytica* also has the capacity to encyst.

In fact, the nucleus divides by mitosis but without the disappearance of the nuclear membrane. It is, then, followed by the division of the cytoplasm (cytokinesis) resulting into two daughter *Entamoeba*. These start feeding upon bacteria and host tissues, grow in size and again multiply by binary fission. Some of these forms may invade fresh intestinal tissues, while some of them become precystic or minuta form.

Life Cycle of *Entamoeba histolytica*:

Entamoeba histolytica is monogenetic (Gr., mono = single; genos = race), i.e., its life cycle is completed in one host only; the host being the man.

Encystment:

The pre-cystic forms, under certain circumstances which are not well understood, remainsmall (7-10 microns in diameter) and live only in the intestinal lumen.

They undergo encystment but before encystment, the parasites round up, eliminate food vacuoles and accumulate considerable amount of food materials in the form of glycogen and black rod-like chromatoid granules. Soon each parasite secretes a thin, rounded, resistant, colourless and transparent cyst wall around it.

The cysts of *Entamoeba histolytica* vary in size from 10-20 microns (average 12 microns) in diameter. Its cytoplasm is clear and each cyst is mononucleate at this stage. Presence of chromatoid bodies is the characteristic of the cysts of *Entamoeba histolytica*. They occur either singly or in the multiples of two or more.

There is a controversy about the exact nature of these bodies. Some authorities consider them as nutrient material of the cysts, while others believe them as excess of chromatin thrown off during nuclear division. The chromatoid bodies are found in the early stages of the cysts but they disappear in the mature tetranucleate cysts.

Pitelka (1963) has suggested that the chromatoid bodies are made of ribonucleoprotein and Neal (1966) believes that the disappearance of chromatid granules occurs because of the dispersion of their nucleoprotein in the substance of mature tetranucleate cystic form.

The nucleus of the cysts divides twice so that each cyst now becomes tetranucleate. At this stage, the cyst is infective to a new host. Encysted forms pass out with the faecal matter of thehost.

Transfer to new host:

The infective cysts remain viable for a considerable length of time outside the human intestine, if environmental conditions are favourable. Infection of fresh human host takes place by swallowing the infective cysts with contaminated food and drinks. Contamination of food and drinks is brought about by houseflies, cockroaches and food-handlers.

Houseflies generally carry the cysts from the faeces to the foods. Cockroaches have also been found to transport cysts from faeces to food. Food-handlers are also sometimes responsible for the contamination of food through touch by dirty fingers carrying the cysts under the nails. Through contaminated food or drinks, the infective cysts pass into the lower portion of the small intestine of the new host.

Excystment:

The excystment of cysts and metacystic development have been observed and studied specially by Dobell (1924) and Cleveland and Sanders (1930) in cultures. According to Dobell, in the process of excystation, a single tetranucleate amoeba (metacystic form) emerges from a cyst through a minute pore in the cyst wall.The tetranucleate metacystic form produces a new generation of trophozoites by a diverse series of nuclear and cytoplasmic divisions which result in the production of eight uninucleate amoebulae.

These are called metacystic trophozoites. No sexual phenomena have been observed during these changes. The metacystic trophozoites feed on the contents of the intestine and grow in size to form the trophozoites of the next generation. The trophozoites stay in the lumen of the intestine for a particular period when they may attack the wall of the intestine and start the life cycle again.

Cysts and trophozoites are passed in feces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, and both stages are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (noninvasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients the trophozoites invade the intestinal mucosa (intestinal

disease), or, through the bloodstream, extraintestinal sites such as the liver, brain, and lungs (extraintestinal disease), with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent two separate species, respectively *E. histolytica* and *E. dispar*. These two species are morphologically indistinguishable unless *E. histolytica* is observed with ingested red blood cells (erythrophagocystosis). Transmission can also occur through exposure to fecal matter during sexual contact (in which case not only cysts, but also trophozoites could prove infective).

A very important point to note is that both excystation and encystation are not reproductive processes. Encystation and excystation can take place in the same host; another host is required only for the perpetuation of the species.

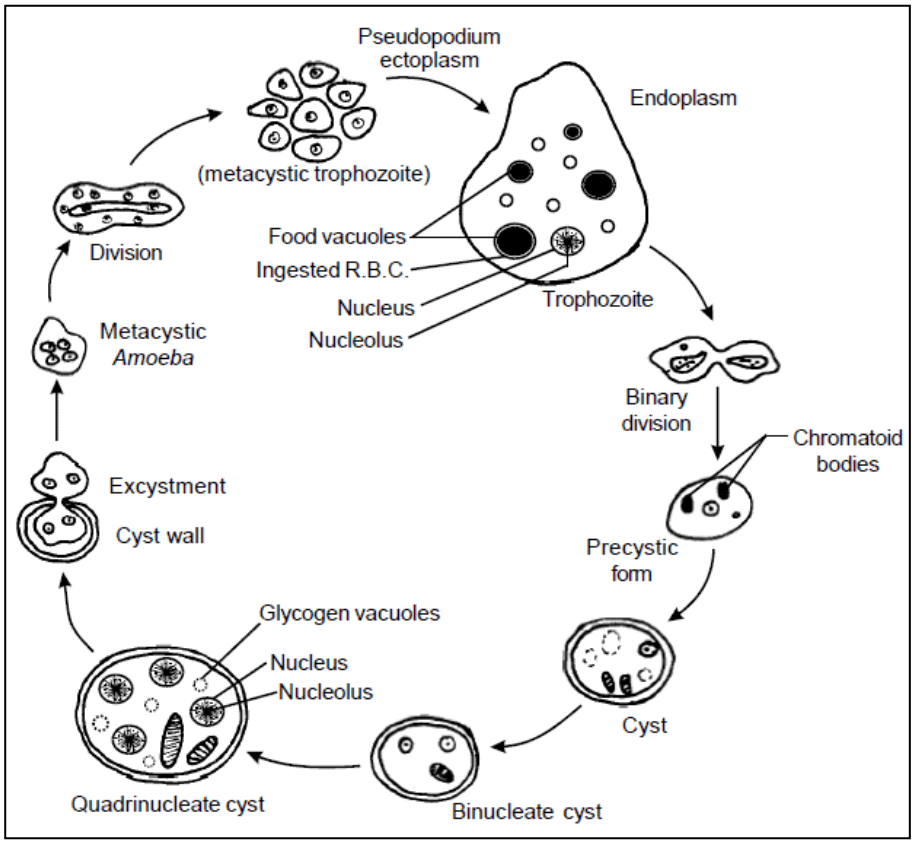

Fig: *Entamoeba histolytica* and its life cycle

Mode of Infection: faecal-oral route

Infective Stage: Mature Quadrinucleate cyst

Source of Infection: Carriers of *Entamoeba histolytica* are of two types:

1. Contact carriers: People who have never suffered from amoebic dysentery and their health remains unaffected. They are healthy carriers of *E. histolytica*. They can shed cysts for many years
2. Convalescent carriers: Persons who have recovered from acute amoebic dysentery

Transmission

- ✓ Fruits, raw vegetables and food contaminated by faeces containing quadrinucleate cyst
- ✓ Contaminated water
- ✓ Unhygienic habits
- ✓ Mechanical vectors like houseflies and cockroaches. Houseflies may act as carrier of the cysts from faeces to unprotected food and water. The droppings of cockroaches too have been found to contain mature cysts and thus they also serve as a source of infection.
- ✓ Sexual transmission of *Entamoeba histolytica* has been reported in about 20-30% homosexuals.

Pathogenicity (Pathogenic Effects) of *Entamoeba histolytica*:

Entamoeba histolytica causes amoebic dysentery, abscesses in liver, lungs and brain and non-dysenteric infections.

1. Amoebic Dysentery:

Entamoeba histolytica secretes a tissue dissolving enzyme (probably of histolysin nature) that destroys the epithelial lining of the colon and causes its necrosis and forms the abscesses (small wounds) which later become flask- shaped bleeding ulcers. The cavity of these ulcers is generally filled with mucus, bacteria, amoeba and cell debris. The abscesses pour their contents into the lumen of the intestine.

The ulcers vary greatly in number and size; in severe cases almost the entire colon is undermined. The ulceration of colon may produce severe dysentery. In amoebic dysentery, the stools are acidic and contain pure blood and mucus, in which swarms of amoeba and blood corpuscles are usually present. The patient feels discomforted due to the rectal straining and intense gripping pains with the passage of blood and mucus stools every few minutes.

2. Abscesses in Liver, Lungs and Brain:

Sometimes *Entamoeba histolytica* may be drawn into the portal circulation and carried to the liver. In liver the parasites settle, attack the liver tissue and form abscesses. The patient has pain in liver region, fever and high leucocyte number, a condition referred to as amoebic hepatitis.

Lung abscesses are fairly frequent; these are usually caused by direct extension from a liver abscess through the diaphragm. The lung abscesses usually rupture into a bronchial tube and discharge a brown mucoid material which is coughed out with the sputum. Sometimes the parasite also forms abscesses in the brain. Abscesses elsewhere are rare.

3. **Non-Dysenteric Infections:**

Although amoebiasis is usually thought of as the cause of dysentery with blood and mucus containing stools or of liver abscesses, these conditions are actually the exception rather than rule and some workers have reported that as many as 90% of dysentery cases in temperate climates are apparently symptomless. Even in tropics, dysentery is exceptional.

Although about 10% of the general population is infected with *Entamoeba histolytica*, yet most of them are carriers or passers. The symptoms commonly associated with chronic amoebiasis are abdominal pain, nausea, and bowel irregularity, with headaches, fatigability and nervousness in minority of cases.

Symptoms, Diagnosis and Treatment of Infection Caused by *Entamoeba histolytica*:

As referred, the infection of *Entamoeba histolytica* causes amoebiasis. The common symptoms are the passing out of stool with blood and mucus, abdominal pain, nausea,flatulence and bowel irregularity with headache and fatigability, etc.

Diagnosis of *Entamoeba histolytica*:

The microscopic examination of the stool of an infected man shows the presence of trophozoites and cysts in it. The presence of stone-shaped, white coloured crystals of Charcot- Leyden suggests the infection of *Entamoeba histolytica*.

Treatment of *Entamoeba histolytica*:

For prompt relief of acute or sub-acute dysentery the injections of Emetin are given. But certain antibiotics, such as Fumagillin, Terramycin, Erythromycin and Aureomycin are more effective and may be given orally.
For eradication of intestinal infections or in chronic cases, certain arsenic compounds (Carbarsone, Thiocarbarsone and Vioform) and a number of iodine compounds (Yatren, Diodoquin and Vioform) are effective. For amoebiasis of liver or lungs, Chloroquine is quite effective. The most significant advancement in the treatment of amoebiasis is the use of Metronidazole and Tinidazole as both luminal and tissue amoebicide.

Prevention (Prophylaxis) of Infection Caused by *Entamoeba histolytica*:

Following measures are essential in the prevention of the disease:

1. Sanitary disposal of faecal matter.
2. Perfect sanitation and protection of water and vegetables from pollution.

3. Washing of hands with antiseptic soap and water before touching the food.
4. Cleanliness in preparing the food.
5. Protection of foods and drinks from houseflies, cockroaches, etc.
6. Raw and improperly washed and cooked vegetables should be avoided.

Entamoeba gingivalis

This is the first amoeba to be described in man by Gros in 1849 in the soft tartar collected from the teeth of human. It has only the trophozoite stage, cystic stage is absent in them. The trophozoite structure is same as that of *Entamoeba histolytica* except that the food vacuole does not contain RBC. The food vacuole however contains cellular debris, bacteria and ingested leucocytes. It is a commensal present in the margins of the gum and sometimes in the crypts of the tonsils. The infection is transmitted from the mouth of an infected person during the act of kissing (oral-oral contact). Trophozoite moves rapidly with the help of numerous blunt pseudopodia.

Entamoeba coli

A commensal present in the lumen of the large intestine. A non-invasive *Entamoeba* that exists in all the three forms, trophozoite, pre-cyst and cyst. The life cycle of *Entamoeba coli* is same as *Entamoeba histolytica*.

Blood Flukes in Humans

Introduction:

Fluke**,** also called blood fluke or trematode**,** any member of the invertebrate class Trematoda (phylum Platyhelminthes), a group of parasitic flatworms that probably evolved from free-living forms millions of years ago. There are more than 10,000 species of flukes. They occur worldwide and range in size from about 5 millimetres (0.2 inch) to several centimetres; most do not exceed 100 millimetres (4 inches) in length.

Flukes parasitize members of all vertebrate classes but most commonly parasitize fish, frogs, and turtles; they also parasitize humans, domestic animals, and invertebrates such as mollusks and crustaceans. Some are external parasites (ectoparasites); some attach themselves to internal organs (endoparasites); others are semi-external, attaching themselves to the lining of the mouth, to the gills, or to the cloaca (the end of the digestive tract). Some attack a single host, while others require two or more hosts.

Schistosoma, fluke genus (phylum Platyhelminthes), three members of which are well known for causing the disease schistosomiasis (*q.v.*) in humans.

Schistosoma sp.

Structure:

Schistosoma is commonly known as the blood fluke. It is a dioecious parasite commonly found in the human hepatic portal or pelvic veins. Some species are parasites in vein of birds and mammals. It is peculiar in having separate males and female, but the two are found together in pairs.

The female, a bit longer than the male, lives in the ventral groove of its male. The male is thicker than the female and permanently carries its female in the gynaecophoric canal. Body surface is rough and spiny, and both have an oral sucker and an acetabulum. There is no pharynx, and the two branches of the intestine reunite in the middle of the body. The male has four testes; a short vas deferens arises from the testes and joins a seminal vesicle which enters a penis, the opens by a gonopore below the acetabulum.

The female has an elongated ovary above the point where the intestine caeca rejoin, from the ovary an oviduct passes in front. In the posterior part of the female are vitelline glands from which a vitelline duct joins the oviduct, the oviduct meets an ootype surrounded by Mehlis' gland. From the ootype arises a straight uterus containing a few capsules, it opens by a female gonopore below the acetabulum.

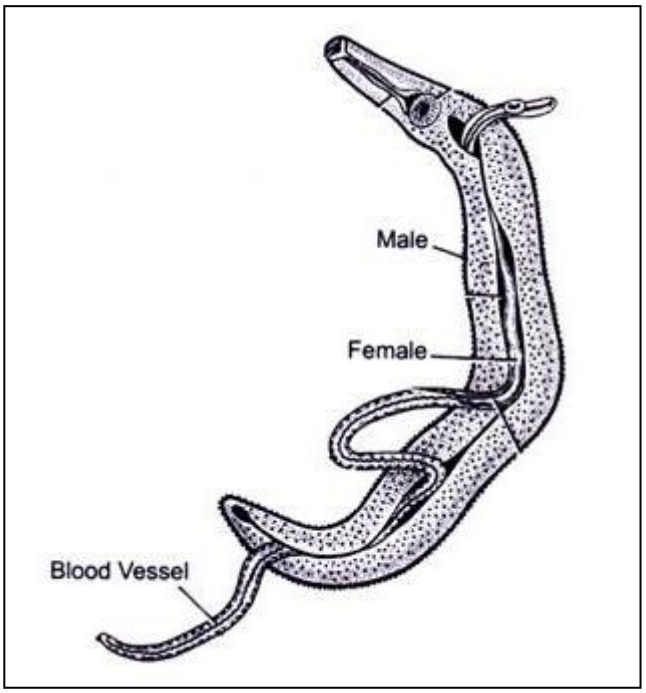

Fig: *Schistosoma* male and female in pair

There are three species of Schistosoma parasitic on human beings:

1. *S. haematobium*

2. *S. mansoni*

3. *S. japonicum*

Bilharziosis is a tropical illness caused by infection of *Schistosoma*.

Schistosomiasis

Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. Although the worms that cause schistosomiasis are not found in the United States, more than 200 million people are infected worldwide. In terms of impact this disease is second only to malaria as the most devastating parasitic disease. Schistosomiasis is considered one of the Neglected Tropical Diseases (NTDs).

The parasites that cause schistosomiasis live in certain types of freshwater snails. The infectious form of the parasite, known as cercariae, emerges from the snail, hence contaminating water. You can become infected when your skin comes in contact with contaminated freshwater. Most human infections are caused by *Schistosoma mansoni, S.haematobium*, or *S. japonicum*.

Epidemiology and Risk Factors

Schistosomiasis is an important cause of disease in many parts of the world, most commonly in places with poor sanitation. School-age children who live in these areas are often most at risk because they tend to spend time swimming or bathing in water containing infectious cercariae.

If you live in, or travel to, areas where schistosomiasis is found and are exposed to contaminate freshwater, you are at risk.

Areas where human Schistosomiasis is found include:

1. ***Schistosoma mansoni***
    - Distributed throughout Africa: There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Sudan and Egypt
    - South America: including Brazil, Suriname, Venezuela
    - Caribbean (risk is low): Dominican Republic, Guadeloupe, Martinique, and Saint Lucia.
2. ***Schistosoma haematobium***
    - Distributed throughout Africa. There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Egypt and the Mahgreb region of North Africa.
    - Found in areas of the Middle East.

3. S. japonicum

Found in Indonesia and parts of China and Southeast Asia.

4. S. mekongi

Found in Cambodia and Laos.

5. S. intercalatum

Found in parts of Central and West Africa.

Biology

Causal Agents:

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium, S. japonicum,* and *S. mansoni.* Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum.* In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis inhumans.

Life Cycle:

Eggs are eliminated with feces or urine. Under optimal conditions the eggs hatch and release miracidia, which swim and penetrate specific snail intermediate hosts. The stages in the snail include 2 generations of sporocysts and the production of cercariae. Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host, and shed their forked tail, becoming schistosomulae. The schistosomulae migrate through several tissues and stages to their residence in the veins. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively. Pathology of *S. mansoni* and *S. japonicum* schistosomiasis includes: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord. Pathology of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord. Human contact with water is thus necessary for infection by

schistosomes. Various animals, such as dogs, cats, rodents, pigs, horses and goats, serve as reservoirs for *S. japonicum*, and dogs for *S. mekongi*.

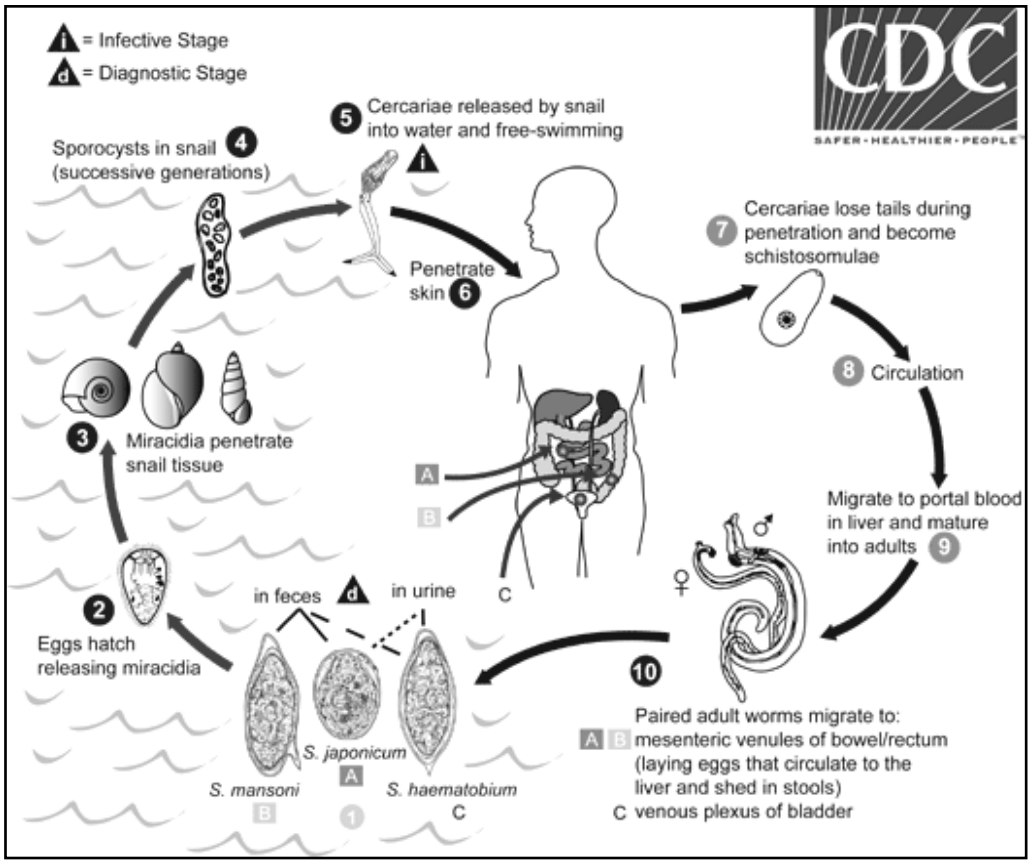

Figure: General life cycle of *Schistosoma* sp.

Disease

Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 hours. *Schistosoma* parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Children who are repeatedly infected can develop anemia, malnutrition, and learning difficulties. After years of infection, the parasite can also damage the liver, intestine, spleen, lungs,

and bladder.

Symptoms:

Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

- *In acute stage*

Symptoms can take between 14 and 84; approximately 3 to 8 weeks after infection, the person may experience:

  - a rash
  - fever
  - headache
  - body aches, or myalgia
  - breathing difficulties

- *In chronic stage*

Many people do not show symptoms at the early stage, but they may develop symptoms as the disease progresses. These later symptoms again depend on the type of parasite.
If the parasites affect the liver or intestines, symptoms may include:

  - diarrhea and constipation
  - blood in the feces
  - intestinal ulcers
  - liver fibrosis
  - portal hypertension, or high blood pressure around the digestive system

If the parasites affect the urinary system, there may also be:

  - blood in the urine
  - painful urination
  - higher risk of bladder cancer

Over time, anemia can develop. In rare cases, the parasite may affect the central nervous system.

Children may have stunted growth and a reduced capability to learn, according to the WHO.

Chronic schistosomiasis

Without treatment, schistosomiasis can persist for years. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, blood in the stool or blood in the urine, and problems passing urine. Chronic infection can also lead to increased risk of bladdercancer.

Rarely, eggs are found in the brain or spinal cord and can cause seizures, paralysis, or spinal cord inflammation.

Diagnosis

Stool or urine samples can be examined microscopically for parasite eggs (stool for *S. mansoni* or *S. japonicum* eggs and urine for *S. haematobium* eggs). The eggs tend to be passed intermittently and in small amounts and may not be detected, so it may be necessaryto perform a blood (serologic) test.

The worm takes about 40 days to mature. A blood sample may not show reliable results until at least 6 to 8 weeks after exposure.

Treatment

No vaccine is available for schistosomiasis, but treatment can help reduce the impact of infection.

Safe and effective medication is available for treatment of both urinary and intestinal schistosomiasis. Praziquantel, a prescription medication, is taken for 1-2 days to treat infections caused by all *Schistosoma* species.

If a person's test result is positive, a short course of a medication called praziquantel is usually effective as long as the individual has not experienced significant damage or complications.

Praziquantel can help, even at an advanced stage, but it does not prevent reinfection.

Prevention and Control

Prevention

No vaccine is available. The best way to prevent schistosomiasis is to take the following steps if you are visiting or live in an area where schistosomiasis is transmitted:

- ✓ Avoid swimming or wading in freshwater when you are in countries in which schistosomiasis occurs. Swimming in the ocean and in chlorinated swimming pools is safe.
- ✓ Drink safe water. Although schistosomiasis is not transmitted by swallowing contaminated water, if your mouth or lips come in contact with water containing the parasites, you could become infected. Because water coming directly from canals, lakes, rivers, streams, or springs may be contaminated with a variety of infectious organisms, you should either bring your water to a rolling boil for 1 minute or filter water before drinking it. Bring your water to a rolling boil for at least 1 minute will kill any harmful parasites, bacteria, or viruses present. Iodine treatment alone will not guarantee that water is safe and free of all parasites.
- ✓ Water used for bathing should be brought to a rolling boil for 1 minute to kill any cercariae, and then cooled before bathing to avoid scalding. Water held in a storage tank for at least 1 - 2 days should be safe for bathing.

- ✓ Vigorous towel drying after an accidental, very brief water exposure may help to prevent the *Schistosoma* parasite from penetrating the skin. However, do not rely on vigorous towel drying alone to prevent schistosomiasis.

Control

In countries where schistosomiasis causes significant disease, control efforts usually focus on

i. Reducing the number of infections in people and/or
ii. Eliminating the snails that are required to maintain the parasite's life cycle.

For all species that cause schistosomiasis, improved sanitation could reduce or eliminate transmission of this disease. In some areas with lower transmission levels, elimination of schistosomiasis is considered a "winnable battle" by public health officials.

Control measures can include mass drug treatment of entire communities and targeted treatment of school-age children. Some of the problems with control of schistosomiasis include:

1. Chemicals used to eliminate snails in freshwater sources may harm other species of animals in the water and, if treatment is not sustained, the snails may return to those sites afterwards.
2. For certain species of the parasite, such as *S. japonicum*, animals such as cows or water buffalo can also be infected. Run off from pastures (if the cows are infected) can contaminate freshwater sources.

Probable questions:

9. Discuss the trophozoit Structure of *Entamoeba histolytica*.
10. Discuss the life cycle of *Entamoeba histolytica*
11. Elaborate Excystment process of *Entamoeba histolytica*.
12. Write down the Pathogenicity of *Entamoeba histolytica*.
13. Discuss the structure of *Schistosoma* sp.
14. Discuss the systematic position of *Schistosoma* sp.
15. Elaborate the Symptoms of *Schistosoma* sp.

Suggested reading:

1. Chakraborty P. 2016.. Textbook of Medical parasitology, 3rd edition. New Central Book Agency.
2. Chatterjee K D. 2009. Parasitology: Protozoology and Helminthology. XIII Edition, CBS Publishers
3. Paniker CKJ, Ghosh S. 2013. Paniker's Text Book of Medical Parasitology. Jaypee
4. Gerald D, Schimdt & larrey S. Roberts' Foundations of Parasitology, 9th Edition

Unit V

Microspora: Structure and life history of *Nosema bombycis* - impact onsericulture

Objective:

In this unit we will discuss about Microspora: Structure and life history of *Nosema bombycis* - impact onsericulture

Nosema Bombycis:

Nosema is a genus of microsporidian parasites. The genus, circumscribed by Swiss botanist Karl Wilhelm von Nägeli in 1857, contains 81 species.Most parasitise insects and other arthropods, and the best-known *Nosema* species parasitise honeybees, where they are considered a significant disease by beekeepers, often causing a colony to fail to thrive in the spring as they come out of their overwintering period. Eight species parasitize digeneans, a group of parasitic flatworms, and thus are hyperparasites, i.e., parasites of a parasite.

Pébrine, or "pepper disease," is a disease of silkworms, which is caused by protozoan microsporidian parasites, mainly *Nosema bombycis* and, to a lesser extent, *Vairimorpha*, *Pleistophora* and *Thelohania* species. The parasites infect eggs and are therefore transmitted to next generation.

The silkworm larvae infected by pébrine are usually covered in brown dots and are unable to spin silkworm thread. Louis Pasteur was the first one to recognize the cause of this disease when a plague of the disease spread across France.

Nosema bombycis is a microsporidium that kills all of the silkworms hatched from infected eggs and comes from the food that silkworms eat. If silkworms acquire this microsporidium in their larval stage, there are no visible symptoms; however, mother moths will pass the microsporidium onto the eggs, and all of the worms hatching from the infected eggs will die in their larva stage. Therefore, it is extremely important to rule out all eggs from infected moths by checking the moth's body fluid under a microscope.

Life cycle stages

Nosema bombycis, like other microsporidians, has two major life cycle stages,

i. a spore stage, and
ii. a vegetative stage

In most cases, the spore is ingested by the host, infecting host cells in the gut lumen and

the Malpighian tubules. During reproduction and proliferation the parasite spreads within the host. Fresh spores are then released into the environment via feces or a decaying host.

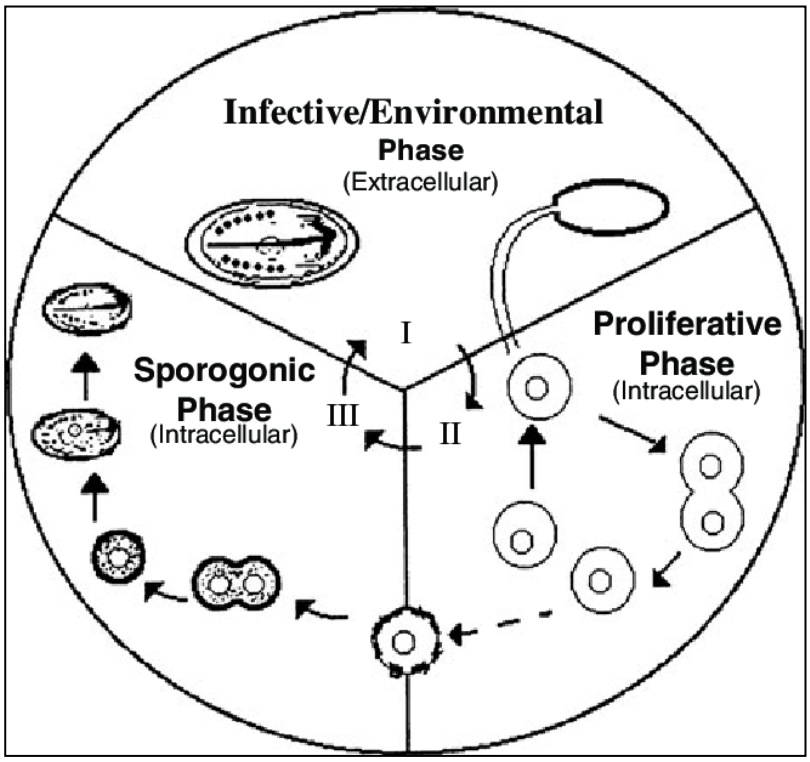

Fig: General Microsporidial development

Transmission

The parasite is believed to rely mainly on horizontal transmission between colonies via infected workers contaminating shared food sources such as pollen or nectar, but there is some evidence that it may also be transmitted vertically. Males may transmit the infection to new queens during mating. *N. bombycis* infection prevalence has been reported to vary widely over time. For example, Manlik et al. (2017) reported that *N. bombycis* infection prevalence in buff-tailed bumblebees (*Bombus terrestris*) fluctuated between 2% (2010) and 81% (2003) in Neunforn, Switzerland.

i. The infective form of microsporidia is the resistant spore and it can survive for an extended period of time in the environment.

ii. The spore extrudes its polar tubule and infects the host cell.

iii. The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule.

iv. Inside the cell, the sporoplasm undergoes extensive multiplication eitherby merogony (binary fission) or schizogony (multiple fission).

v. This development can occur either in direct contact with the host cell cytoplasm (*E. bieneusi*) or inside a vacuole called a parasitophorous vacuole (*E. intestinalis*). Either free in the cytoplasm or inside a parasitophorous vacuole,

microsporidia developby sporogony to mature spores.

vi. During sporogony, a thick wall is formed around the spore, which provides resistance to adverse environmental conditions. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and releasesthe spores to the surroundings.

vii. These free mature spores can infect new cells thus continuing the cycle

Effects on host

Studies have found conflicting results as to the effects of *N. bombycis* on bumble bee health. Some field studies have found little to no negative effects on colony size or success, while others have found that infected queens produce smaller colony sizes and reduced sexual offspring.Under laboratory conditions, the fungus has been found to affect the survival and efficiency of adult individuals as well as the sperm counts of male offspring.

Impact on Silk industry

The commercial rearing of polyphagous Indian tasar silkworm*, Antheraea mylitta* Drury being practiced on naturally grown primary food plants like *Terminalia arjuna* (Arjun) *Terminalia tomentosa* (Asan), and *Shorea robusta* (Sal) available in the tropical forests of central India , at times, is seriously affected by the disease- Pebrine, caused by *Nosema* sp., a microsporidian pathogen. The present investigation on comparative larval, silk gland weight and also cocoon parameters in Pebrine-free and Pebrine-infected ecorace of tasar silkworm Antheraea mylitta Drury (Daba TV), illustrates the tasar silkworm larvae infected with pebrine disease causing heavy losses to the economy of the silk industry. It plays a major role in improving socio-economic status of tribal, weaker sections, landless people andwomen.

The tasar food plant leaf quality in terms of nutrition can influence the health and growth of larvae, effective rate of rearing (ERR) and crop yields as it has correlation with the weights of cocoon, shell and silk ratio and can influence the crop economics. The leaf nutrient status of tasar food plant is fundamental not only for silk productivity, but also for its metamorphosis during life cycle and subsequent parental moth reproductive efficiency. The larval feeding status of any polyphagous commercial insect has impact on food storage and budgeting for biological activities so as to combat the adverse or to excel during favourable conditions. The diseases in silkworm are the major constraintsin tasar culture, which adversely affect the economics of this culture by causing 35-40% crop loss. Among the diseases, pebrine is causing most devastating effect on the rearing of the tasar silkworm accounts for 20-25% yield loss Even though some work has been done on the breeding aspects of Tasar silkworm, not much work has been published so far on pathological aspects of tasar silkworm.

Tasar silkworm is often infected with the intracellular parasite of the genus *Nosema*. Pebrine can be acquired from the mother moth (primary infection) or from the environment through food (secondary infection). Infected larvae show black pepper like spots on the integument. These infected hypodermal cells become enlarged and vacuolated and blackened due to the formation of melanin. Larvae infected with *Nosema* sp. show extended development period, reduced size and larval weight in comparison to uninfected one.

Probable questions:

1. Discuss the life cycle stages of *Nosema Bombycis.*
2. What do you mean by Pébrine?
3. What is the impact of *Nosema Bombycis* on Impact on Silk industry
4. Write down the transmission process of *Nosema Bombycis*.

Suggested reading:

1. Chakraborty P. 2016.. Textbook of Medical parasitology, 3rd edition. New Central Book Agency.
2. Chatterjee K D. 2009. Parasitology: Protozoology and Helminthology. XIII Edition, CBS Publishers
3. Paniker CKJ, Ghosh S. 2013. Paniker's Text Book of Medical Parasitology. Jaypee
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Unit VI

Mode of transmission of *Plasmodium*, *Trypanosoma* and Piroplasm

Objective:

In this unit we will discuss about the Mode of transmission of *Plasmodium*, *Trypanosoma* and Piroplasm.

1. Mode of transmission of *Plasmodium*

Introduction:

Plasmodium vivax is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring (Benign tertian) malaria; *P. vivax* is one of the five species of malaria parasites that commonly infect humans. Although it is less virulent than *Plasmodium falciparum*, the deadliest of the five human malaria parasites, *P. vivax* malaria infections can lead to severe disease and death, often due to splenomegaly (a pathologically enlarged spleen). *P. vivax* is carried by the female *Anopheles* mosquito, sinceit is only the female of the species that bites.

Epidemiology:

Plasmodium vivax is found mainly in Asia, Latin America, and in some parts of Africa. *P. vivax* is believed to have originated in Asia, but latest studies have shown that wild chimpanzees and gorillas throughout central Africa are endemically infected with parasites that are closely related to human *P. vivax.* These findings indicate that human *P. vivax* is of African origin*. Plasmodium vivax* accounts for 65% of malaria cases in Asia and South America. Unlike *Plasmodium falciparum*, *Plasmodium vivax* is capable of undergoing sporogonic development in the mosquito at lower temperatures. It has been estimated that 2.5 billion people are at risk of infection with this organism.

Although the Americas contribute 22% of the global area at risk, high endemic areas are generally sparsely populated and the region contributes only 6% to the total population at risk. In Africa, the widespread lack of the Duffy antigen in the population has ensured that stable transmission is constrained to Madagascar and parts of the Horn of Africa. It contributes 3.5% of global population at risk. Central Asia is responsible for 82% of global population at risk with high endemic areas coinciding with dense populations particularly in India and Myanmar. South East Asia has areas of high endemicity in Indonesia and Papua New Guinea and overall contributes 9% of global population at risk.

P. vivax is carried by at least 71 mosquito species. Many vivax vectors live happily in temperate climates—as far north as Finland. Some prefer to bite outdoors or

during the daytime, hampering the effectiveness of indoor insecticide and bed nets. Several key vector species have yet to be grown in the lab for closer study, and insecticide resistance is unquantified.

Systematic Position:

Phylum – Protozoa

Class – Sporozoa

Order – Haemosporidia

Genus – *Plasmodium*

Discovery:

Charles Laveran (1880) discovered Plasmodium in the blood of a malarial patient. In the year 1895 Ronald Ross, an Indian army doctor discovered the oocyte of plasmodium in the stomach of the female *Anopheles* mosquito.

Mode of Infection:

When an infected female anopheles mosquito bites a healthy person to suck blood, it injects the sporozoites (infective stage) into the human blood along with its saliva. Sporozoites are inoculated in thousands into the human blood. Asporozoite is microscopic, slender and sickle shaped animalcule.

Its body length is about 14 μ and breadth is 1μ Body is covered by a thin, elastic cuticle which gives a definite shape to its body. In the absence of any locomotory organelles the sporozoites show gliding movement. Two secretory glands are present at the anterior ends which are believed tso help in penetration into the cell.

Causes

Malaria happens when a bite from the female *Anopheles* mosquito infects the body with Plasmodium. Only the *Anopheles* mosquito can transmit malaria. The successful development of the parasite within the mosquito depends on several factors, the most important being humidity and ambient temperatures. When an infected mosquito bites a human host, the parasite enters the bloodstream and lays dormant within the liver. The host will have no symptoms for an average of 10.5 days, but the malaria parasite will begin multiplying during this time.

The new malaria parasites are then released back into the bloodstream, where they infect red blood cells and multiply further. Some malaria parasites remain in the liver and are not released until later, resulting in recurrence. An unaffected mosquito becomes infected once it feeds on an infected individual. This restarts the cycle.

Life cycle and Transmission routes:

The life cycle of *Plasmodium* is completed inside the body of the two hosts such as male and female *Anopheles* mosquito. Hence, the life cycle is digenetic. Man is the primary or

definitive host and female *Anopheles* mosquito is the secondary or intermediate host or vector.

The asexual life cycle in man is called schizogony. It is divided in to 3 phases (a) Pre-erythrocytic schizogony, (b) exo-erythrocytic schizogony, (c) erythro-cytic schizogony. The sporozoite is the infective stage. While sucking blood an infected mosquito injects the sporozoites into the body of man. The sporozoites enter the liver to carry on pre and exo-erthrocytic schizogony.

Through these two cycles large number of merozoites is produced. They enter R.B.C. to carry on erythrocytic schizogony. This erythrocytic cycle is repeated in every 48 hours which coincides with the appearance of the symptoms of malaria. Some of the merozoites produced by erythrocytic cycle never attack fresh R.B.C. They wait for the mosquito to be sucked. They are of two types: male and female gametocytes. They carry on sexual reproduction inside the stomach of mosquito.

The main mode of transmission of the disease is by bites from infected *Anopheles* mosquitoes that have previously had a blood meal from an individual with parasitemia. The human infection begins when an infected female anopheles mosquito bites a person and injects infected with sporozoites saliva into the blood circulation. That is the first life stage of *plasmodium* (stage of infection).

The next stage in malaria life cycle is the one of asexual reproduction that is divided into different phases:the pre-erythrocytic (or better, exoerythrocytic) and the erythrocytic phase. Within only 30- 60 minutes after the parasites inoculation, sporozoites find their way through blood circulation to their first target, the liver. The sporozoites enter the liver cells and start dividing leading to schizonts creation in 6- 7 days. Each schizont gives birth to thousands of merozoites (exoerythrocytic schizogony) that are then released into the blood stream marking the end of the exoerythrocytic phase of the asexual reproductive stage. The liver phase occurs only once while the erythrocytic phase undergoes multiple cycles; the merozoites release after each cycle creates the febrile waves. A second scenario into the RBCs is the parasite differentiation into male and female gametocytes that is a non pathogenic form of parasite. When a female anopheles mosquito bites an infected person, it takes up these gametocytes with the blood meal (mosquitoes can be infected only if they have a meal during the period that gametocytes circulate in the human's blood). The gametocytes, then, mature and become microgametes (male) and macrogametes (female) during a process known as gametogenesis. The time needed for the gametocytes to mature differs for each plasmodium species: 3- 4 days for *P. vivax* and *P. ovale*, 6- 8 days for *P. malariae* and 8-10 days for *P. falciparum*.

In the mosquito gut, the microgamete nucleus divides three times producing eight nuclei; each nucleus fertilizes a macrogamete forming a zygote. The zygote, after the fusion of nuclei and the fertilization, becomes the so- called ookinete. The ookinete, then, penetrates the midgut wall of the mosquito, where it encysts into a formation called oocyst. Inside the oocyst, the ookinete nucleus divides to produce thousands of

sporozoites (sporogony). That is the end of the third stage (stage of sexual reproduction/ sporogony). Sporogony lasts 8- 15 days.

The oocyst ruptures and the sporozoites are released inside the mosquito cavity and find their way to its salivary glands but only few hundreds of sporozoites manage to enter. Thus, when the above mentioned infected mosquito takes a blood meal, it injects its infected saliva into the next victim marking the beginning of a new cycle.

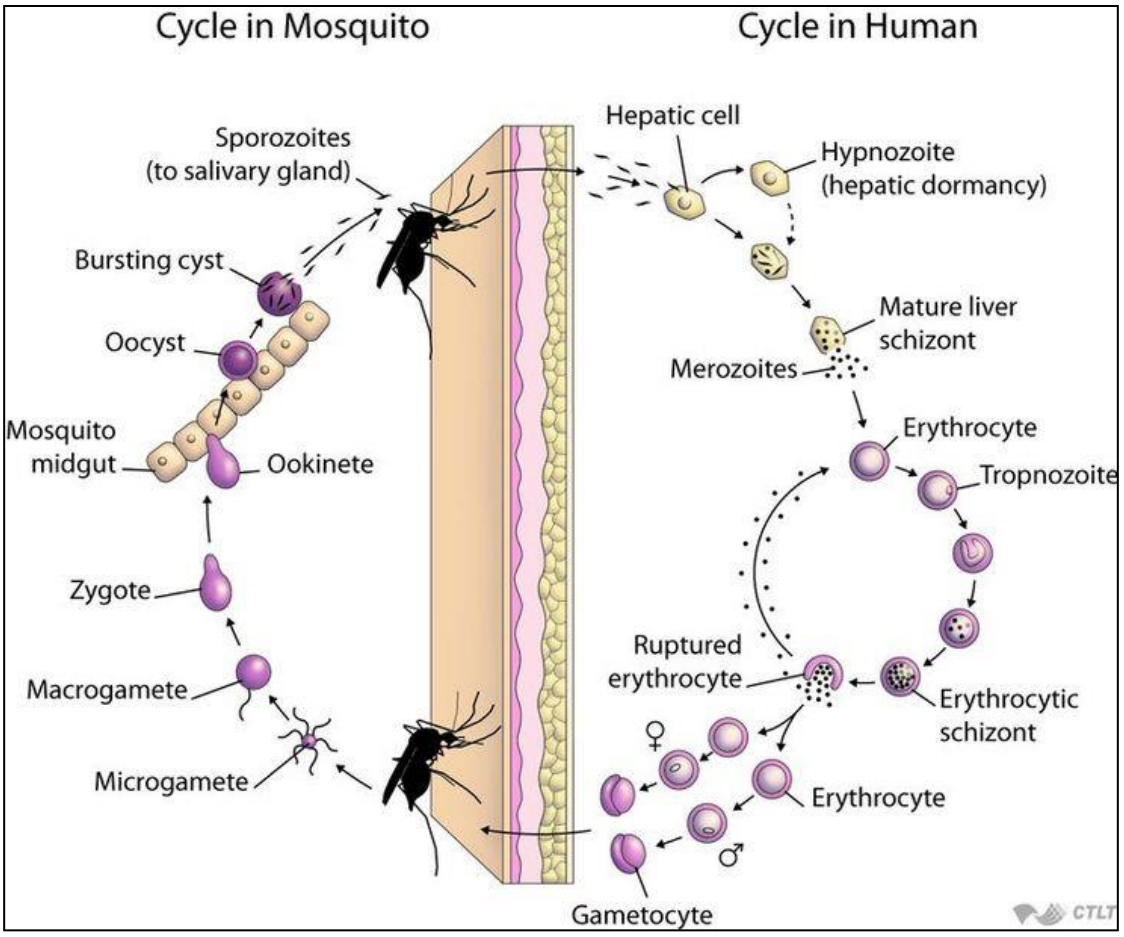

Fig: Life cycle of *Plasmodium* sp.

Clinical representation:

Pathogenesis results from rupture of infected red blood cells, leading to fever. Infected red blood cells may also stick to each other and to walls of capillaries. Vessels plug up and deprive tissues of oxygen. Infection may also cause the spleen to enlarge.

Unlike *P. falciparum*, *P. vivax* can populate the bloodstream with sexual-stage parasites—the form picked up by mosquitoes on their way to the next victim—even before a patient shows symptoms. Consequently, prompt treatment of symptomatic patients doesn't necessarily help stop an outbreak, as it does with falciparum malaria, in which fevers occur as sexual stages develop. Even when symptoms appear, because they are usually not immediately fatal, the parasite continues to multiply.

The parasite can go dormant in the liver for days to years, causing no symptoms and remaining undetectable in blood tests. They form what are called hypnozoites (the name derives from "sleeping organisms"), a small form that nestles inside an individual liver cell. The hypnozoites allow the parasite to survive in more temperate zones, where mosquitoes bite only part of the year.

Sign and Symptoms:

A single infectious bite can trigger six or more relapses a year, leaving sufferers more vulnerable to other diseases. Other infectious diseases, including falciparum malaria, appearto trigger relapses

Uncomplicated malaria

This is diagnosed when symptoms are present, but there are no signs to indicate severe infection or dysfunction of the vital organs. This form can become severe malaria if left untreated, or if the host has poor or no immunity. Symptoms of uncomplicated malaria typically last 6 to 10 hours and recur every second day. Some strains of the parasite can have a longer cycle or cause mixed symptoms. As symptoms resemble those of flu, they may be undiagnosed or misdiagnosed in areas where malaria is less common. In uncomplicated malaria, symptoms progress as follows, through cold, hot, and sweating stages:

i. A sensation of cold with shivering
ii. Fever, headaches, and vomiting
iii. Seizures sometimes occur in younger people with the disease
iv. Sweats, followed by a return to normal temperature, with tiredness

In areas where malaria is common, many patients recognize the symptoms as malaria andtreat themselves without visiting a doctor.

Severe malaria

In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction. Symptoms of severe malaria include:

i. fever and chills
ii. impaired consciousness
iii. prostration, or adopting a prone position
iv. multiple convulsions
v. deep breathing and respiratory distress
vi. abnormal bleeding and signs of anemia
vii. clinical jaundice and evidence of vital organ dysfunction

Prevention

The main way to prevent malaria is through vector control. There are mostly three mainforms that the vector can be controlled:

(1) insecticide-treated mosquito nets,

(2) indoor residual spraying and

(3) antimalarial drugs.

Long-lasting insecticidal nets (LLNs) are the preferred method of control because it is the most cost effective. The WHO is currently strategizing how to ensure that the net is properly maintained to protect people at risk. The second option is indoor residual spraying and has been proven effective if at least 80% of the homes are sprayed. However, such method is only effective for 3-6months. A drawback to these two methods, unfortunately, is that mosquito resistance against these insecticides has risen. National malaria control efforts are undergoing rapid changes to ensure the people are given the most effective method of vector control. Lastly, antimalarial drugs can also be used to prevent infection from developing into a clinical disease. However, there has also been an increase resistance to antimalarial medicine.

In 2015 the World Health Organization (WHO) drew up a plan to address vivax malaria, as part of their Global Technical Strategy for Malaria.

Diagnosis

P. vivax and *P. ovale* that has been sitting in EDTA for more than 30 minutes before the blood film is made will look very similar in appearance to *P. malariae*, which is an important reason to warn the laboratory immediately when the blood sample is drawn so they can process the sample as soon as it arrives. Blood films are preferably made within 30 minutes of the blood draw and must certainly be made within an hour of the blood being drawn.Diagnosis can be done with the strip fast test of antibodies.

Treatment

Chloroquine remains the treatment of choice for *vivax* malaria,except in Indonesia's Irian Jaya (Western New Guinea) region and the geographically contiguous Papua New Guinea, where chloroquine resistance is common (up to 20% resistance). Chloroquine resistance is an increasing problem in other parts of the world, such as Korea and India.

When chloroquine resistance is common or when chloroquine is contraindicated, then artesunate is the drug of choice, except in the U.S., where it is not approved for use. Where an artemisinin-based combination therapy has been adopted as the first-line treatment for *P. falciparum* malaria, it may also be used for *P. vivax* malaria in combination with primaquine for radical cure. An exception is artesunate plus sulfadoxine- pyrimethamine (AS+SP), which is not effective against *P. vivax* in many places. Mefloquine is a good alternative and in some countries is more readily available. Atovaquone-proguanil is an effective alternative in patients unable to tolerate chloroquine. Quinine may be used to treat *vivax* malaria but is associated with inferior outcomes.

32–100% of patients will relapse following successful treatment of *P. vivax* infection if a radical cure (eradication of liver stages) is not given.

Eradication of the liver stages is achieved by giving primaquine. Patients with glucose-6- phosphate dehydrogenase risk haemolysis. G6PD is an enzyme important for blood chemistry. No field-ready test is available. Recently, this point has taken particular importance for the increased incidence of vivax malaria among travelers. At least a 14-day course of primaquine is required for the radical treatment of *P. Vivax.*

2. Mode of transmission of *Trypanosoma*

Trypanosomiasis:

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease caused by protozoa which belongs to the genus *Trypanosoma*. The parasite is classified into three subspecies: *Trypanosoma brucei gambiense, T. brucei rhodesiense,* and *T. brucei brucei*. The later subspecies is not pathogenic to humans. These subspecies cannot be differentiated morphologically. *T. b. gambiense* is distributed in western and central Africa which causes chronic disease whereas, *T. b. rhodesiense* found in eastern and southern Africa and responsible for acute severe disease.

The epidemiology of these parasite species also differs and depends on the distribution of their vectors, *Glossina palpalis* and *Glossina morsitans* respectively. The *G. palpalis* is found in the areas of vegetation near rivers or cultivated fields. The *G. morsitans* mainly depends on wild animals in savannah which are far away from human settlements. The disease is transmitted to humans by tsetse fly which gets their infection from humans or from wild animals harboring the parasites. Some species of Trypanosomes are non pathogenic and does not cause any harm to the humans while other species such as *Trypanosoma gambiense* are pathogenic and causes severe disease in human and animals. In this module we will discuss about morphology, life cycle and pathogenesis of *Trypanosoma gambiense*. We will also take a glance at diagnosis and treatment of the disease.

Morphology:

T. gambiense are microscopic, elongate, flattened and have fusiform body pointed at both ends and covered by a membranous pellicle which maintains the form of body . It measures about 10 µm to 40 µm in length and 2.5 µm to 10 µm in width. A single flagellum arises from a basal body situated near the posterior end and curves in a spiral form round the body forming undulating membrane, thrown into 3 or 4 folds depending upon the length of the parasite. The undulating membrane is believed to be an adaptation for locomotion in the blood. The flagellum is free at the anterior end. The nucleus is large and oval, situated in the centre of the body and the cytoplasm contains numerous greenish refractile

granules called volutin granules. These granules store food particles mainly glycogen and phosphate. At the base of the flagellum is located the basal granule or blepharoplast close to which is another granule, the parabasal body.

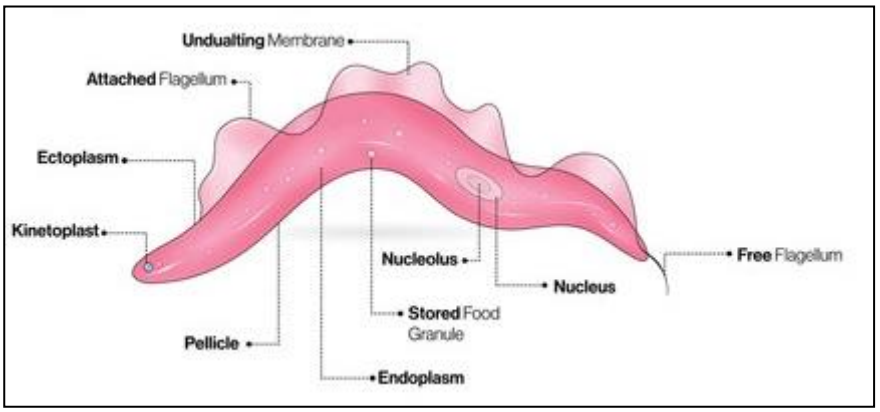

Fig: Morphology of *Trypanosoma gambiense*

Life cycle:

T. gambiense is a digenetic parasite which requires two hosts for completing the life cycle (figure 6). The primary host is humans and the intermediate host is blood sucking insect Tsetse fly of the genus *Glossina*. The mammals like pig, antelopes and buffaloes often act as reservoir host harbouring the parasite. When Tsetse fly sucks the blood from infected individual or wild mammal, it carries Trypanosomes to its mid gut where they divide asexually by longitudinal binary fission. Here the parasite changes their morphology and give rise to metacyclic forms which are short and stumpy. At this stage, the fly is said to be infective. When the infected tsetse fly bites a healthy human host, it releases these metacyclic trypanosomes in the blood stream of host and repeats the life cycle. Sexual reproduction is unknown in *T. gambiense*. It is essentially a parasite of connective tissue in human where it multiplies readily. It consumes large amount of glucose and invades the regional lymph nodes through the lymphatic systems and also invades the blood system causing parasitaemia. It finally localizes in the brain. It is to be noted that African sleeping sickness is a disease which affects the central nervous system.

Life cycle in human:

Infection: The infection by parasite is initiated when tsetse fly harboring the infective metacyclic form, bite the healthy individual. When the fly bites, it releases trypanosomes into blood stream which develop into long slender form and multiply asexually by binary longitudinal fission at the site of innoculation. These become 'stumpy' via 'intermediate' forms. Consequently the parasites invade the blood stream and causes parasitaemia. The trypomastigote forms, mainly the short stumpy forms are taken up by the tsetse fly along with its blood meal and

undergo a series of complex biological development inside the insect host before becoming infective to man**.**

Multiplication: All stages of parasites in humans are extracellular as they are present in the blood cells. In human blood, the metacyclic forms which are devoid of free flagellum become transformed into long slender forms equipped with long flagella. These stages can freely swim by beating of their free flagellum along with the vibratile movements of the undulating membrane. They multiply asexually by longitudinal binary fission and obtain energy by anaerobic process of glycolysis.

Metamorphosis: When absorption of glucose ceases due to antibodies which are produced in blood, is hampered glycolysis. As a result, the trypanosomes stop dividing and shrink to short stumpy forms, which are lacking free flagellum. These stumpy forms do not feed and ultimately die if they are not sucked up by tsetse fly along with the blood meal from infected human.

Relapse of infection: It has also been reported that some of the long and slender forms of trypanosomes do not undergo any transformation, but change their antigen in blood to which the host has produced the antibodies. These unaltered slender forms continue to survive and multiply in blood leading to future relapses of the infection

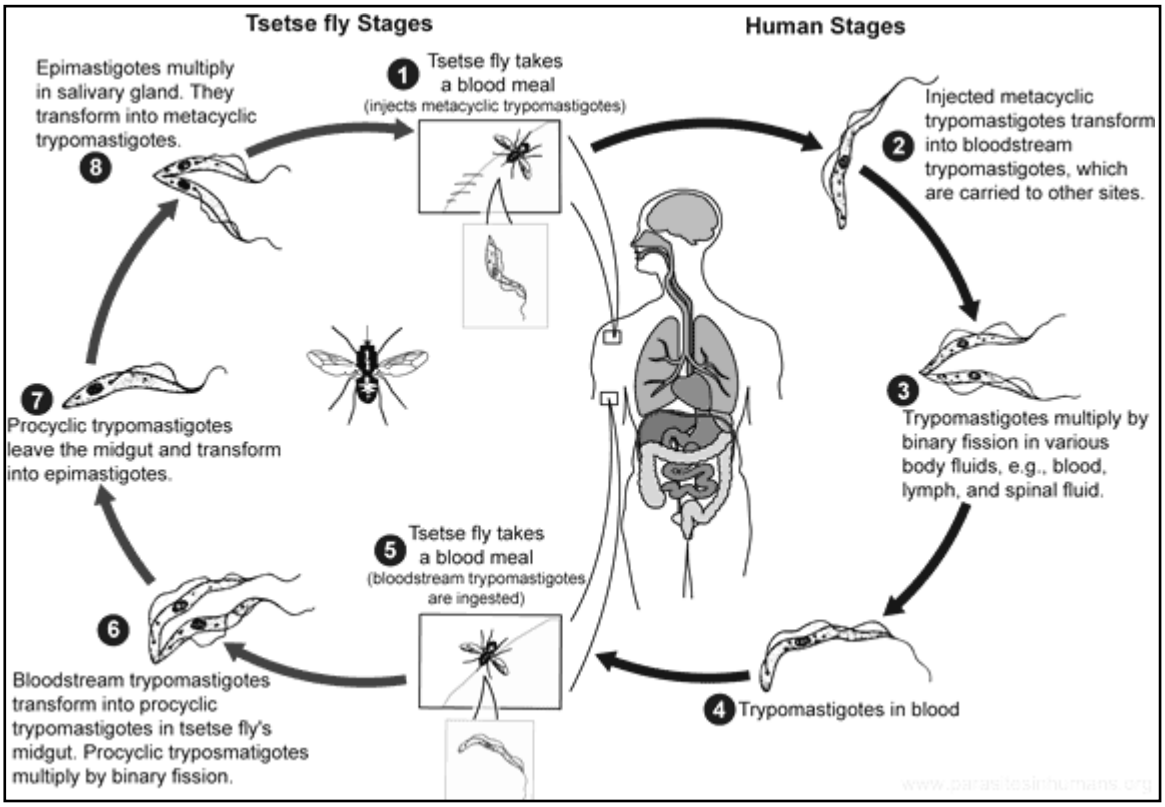

Fig: Life cycle of *Trypanosoma* sp.

Life cycle in tsetse fly:

Transfer to tsetse fly: When tsetse fly feed on the blood of an infected person, it also sucks short stumpy forms of parasite along with the blood. It is the stumpy forms which continue development in the vector. Development in mid gut: Further developments of stumpy forms proceeds in the insect mid gut within peritrophic membrane. In the mid gut parasite transforms into long slender form and multiply asexually by longitudinal binary fission. The kinetoplast moves farther from the posterior end of body. The energy yielding process is related to mitochondrial oxidation of pyruvic acid. Development in salivary gland: After sometime, the long slender forms migrate into salivary glands via oesophagus and mouthparts of insects. Here, they metamorphose into the crithidial forms with shortened body, reduced free flagellum and the kinetoplast in front of the nucleus. The mitochondria develop an extensive network of cristae and parasite respires more economically as blood glucose gradually declines. The crithidial forms multiply in the lumen of salivary glands and transform into slender metacyclic forms. When the tsetse fly bites a healthy person, it transfers the metacyclic stage into his blood where they initiate another infection. Sleeping sickness: *T. gambiense* causes the disease of West African sleeping sickness. It is different from American sleeping sickness or Encephalitis which is caused by filterable virus.

Mode of infection:

Inoculative method: by the bite of the infective tsetse fly, *Glossina*: Both male and female suck the blood and can transmit the infection. They bite by daylight, usually in the early morning and evening. The metacyclic stage is introduced by the tsetse fly with the saliva into the subcutaneous pool of blood on which it feeds. Some of the parasites may enter the blood stream directly and majority of them entangled in the tissue space. The initial growth of trypomastigotes occurs in the tissue space which form a favourable nidus or possibly here the organisms can escape the action of antibodies which might be developed. It is to be noted that while the trypomastigotes are multiplying in the subcutaneous tissue, the organisms are either absent or present in small numbers only in the peripheral blood. It has been suggest that although unlikely yet the connective tissue damage caused by the trypomastigotes may be due to an exaggerated immune response (autoimmune reaction or massive release of kinin) rather than to any direct effect (mechanical damage due to motility) of this relatively non toxic organism. The presence of trypomastigotes in the subcutaneous connective tissue excites host's immune response in two ways.

a) By producing large amount of non specific immunoglobulins which are however not capable of sensitizing the antigen. Antibodies are produced in response to the secretion of an exo-antigen of the trypomastigotes.

b) By heavily infiltrating the site of infection with macrophages, the cells competent to deal with the invaders. The neutrophils take peculiarly little interest

in the defense and are therefore not much in evidence.

Thus it will be seen that there is no lack of mobilization of the hosts defensive mechanism but it is the cellular defense which plays the dominant role. The macrophages could be seen to remove the living trypomastigotes in the tissue space. The release of kinins may help to attract macrophages, it also increases the capillary permeability of tissues and may explain the oedematous swollen subcutaneous tissue at the site of infection. Furthermore, trypanosomes are surrounded by a coat that is composed of variant surface glycoproteins (VSG). These proteins act to protect the parasite from any lytic factors that are present in human plasma. The host's immune system recognizes the glycoproteins present on the coat of the parasite leading to the production of different antibodies (IgM and IgG). These antibodies will then act to destroy the parasites that circulate around the blood. However, from the several parasites present in the plasma, a small number of them will experience changes in their surface coats, resulting in the formation of new VSGs. Thus, the antibodies produced by the immune system will no longer recognize the parasite leading to proliferation until new antibodies are created to combat the novel VSGs. Eventually the immune system will no longer be able to fight off the parasite due to the constant changes in VSGs and infection will arise.

Clinical features:

Bite of tsetse fly causes local irritation which subsides after few days. A trypanosomal chancre may develop at the site of inoculation of trypomastigotes introduced by the bite of the infected tsetse fly. It is a hard painful nodule and fluid withdrawn from it contains actively dividing trypomastigotes. It subsides in a week or two without suppurating. The symptom can appear after several months or a year in Gambian form but symptoms may appear after two weeks in case of Rhodesian form. It is characterized by the infection of blood stream, involvement and enlargement of lymph nodes and eventually invasion of the central nervous system. The early symptoms are fever, loss of nocturnal sleep, severe headache, and feeling of oppression. A fleeting circulate erythematous rash may appear on the chest and shoulder. Lymph node enlargement, particularly of the posterior triangle of the neck is a feature of Gambian disease whereas invasion of CNS is very rapid in case of 'rhodesian' form. As the CNS is involved, the symptoms of meningo-encephalitis develop resulting in classical sleeping sickness. In due course, the patient fall asleep, first at regular interval and then lies prostrate in coma. Finally, the patient becomes thin and exhausted, accompanied by signs of malnutrition. Disruption of the sleep cycle is an important symptom of this stage that gave the disease the name 'sleeping sickness'.

The person infected from disease experience unsystematic and uneven 24-hour rhythm of the sleep-wake cycle. The patient sleeps in daytime and at night time shows periods of wakefulness. Other neurological symptoms of the disease include

tremor, confusion, paralysis, general muscle weakness, hemiparesis and paralysis of a limb. Parkinson like movements may also arise due to non-specific movement and speech disorders. The person infected from sleeping sickness may also exhibit psychiatric signs like aggressive behaviour, irritability, psychotic reactions or apathy which can sometimes dominate the clinical diagnosis. If the disease is not treated, it can invariably become fatal, with progressive mental deterioration that leads to coma, systemic organ failure and finally death. In case of T*. b. rhodesiense*, an untreated infection will leads to death within few months; however, infection with T. b. gambiense will lead to death of the patient after several years when left untreated. Tryptophol is a chemical compound which stimulates sleep in humans. It is the chemical that is produced by the trypanosomal parasite in sleeping sickness. The major mode of transmitting the disease is the bite of an infected tsetse fly but there are several other ways through which people are infected with sleeping sickness.

The infection can be spread from pregnant mother to her child because the trypanosomes are able to cross the placenta and cause the disease to the fetus.

The mechanical transmission is also possible through other blood sucking insects. However, assessment of epidemiological impact of transmission is very difficult.

Accidental infections may also be possible in the laboratories due to pricks from contaminated needles.

Disease management:

Disease management can be done in three major steps.

i. The first step is the screening for potential infection which can be done by serological tests (only available for *T. b. gambiense*) and confirms major symptoms such as swollen cervical glands.

ii. The second stage is to diagnose the presence of the parasite.

iii. The last step is staging, which is done to find out the state of disease progression. This involves collection and examination of cerebro-spinal fluid from lumbar region which helps in determining the course of treatment.

Treatment:

The Gambian or African trypanosomiasis or sleeping sickness can be treated during early stage but once the parasites enter into the cerebrospinal fluid of central nervous system, it is very difficult to control the disease. There are a number of drugs that are used to control the disease. Drugs such as suramin sodium, Bayer 205, Atoxyl and Tryparsamide have been useful in early stages of infection. Other drugs such as Parsenophenyl butyric acids, germanin and pentamidine are also effective in treatment of early cases. Orsanine is quite effective in cases where the Central nervous system is involved. Melarsen oxide is rapid in action and is less toxic.

Prophylaxis (Prevention):

Prevention depends upon the eradication of vector i.e., the tsetse fly. It can be eradicated by destruction of the habitat of the vector. It is supplemented by the use of insecticides such as spraying of DDT over bushy areas in the vicinity of villages. Game destruction program to eliminate the blood meal of the fly and isolation of the human population from areas known to harbor infective animals can be done to prevent the spreading of the disease. This is much more important in Gambian disease

3 Mode of transmission of Piroplasm

Piroplasmosis:

Piroplasmosis a disease of horses and other equids, is caused by one of two protozoan parasites: *Theileria equi* or *Babesia caballi*, says Glen Scoles, PhD, a research entomologist with the U.S. Department of Agriculture (USDA) Animal Disease Research Unit (ADRU). These organisms can be transmitted by ticks or through contaminated blood from infected horses, whether transmitted iatrogenically or via blood transfusions. Though biologically different, the two parasites share similar pathologies, life cycles and tick vector relationships.

T. equi and *B. caballi* must undergo sexual-stage development in ticks to complete their life cycle, making ticks the definitive hosts and vectors of the disease-causing parasites. Though relatively few species of ticks can support *T. equi* and *B. caballi*, competent tick vectors inthe United States include:

- *Amblyomma mixtum* (the Cayenne tick, formerly known as *Amblyomma cajennese*) isprobably one of the primary U.S. vectors for *T. equi*.
- *Dermacentor variabilis* (the American dog tick) transmits *T. equi*.
- *Dermacentor nitens* (the tropical horse tick) transmits *B. caballi*.
- *Dermacentor albipictus* (the winter tick) transmits *B. caballi*.

Incubation Period

Incubation period can last anywhere between 1-6 weeks.

Life Cycle

T. equi undergoes four stages of development. First, asexual replication occurs in the equine host's peripheral blood mononuclear cells (PBMCs), followed by asexual replication in the host's erythrocytes. Once a tick obtains erythrocytes infected with *T. equi* during blood feeding, the parasites sexually reproduce in the the tick's midgut, followed by a round of asexual replication in the tick's salivary glands. Sporozoites

develop in the tick's salivary glands and are transferred to the horse during the feeding process, thereby infecting it with piroplasmosis.

According to Scoles, infection is transmitted in one of two ways: transtadial (or interstadial) transmission or intrastadial transmission. In the former, larval or nymphal ticks take on infected erythrocytes when they feed on an infected host. The ticks then drop off the equine host, molt, and find and feed on a new host once they reach their next development stage. In intrastadial transmission, an adult male tick feeds on an infected host before moving to another horse to transmit.

The cycle begins when an infected tick sends sporozoites into a mouse while taking a blood meal. The sporozoites then go into red blood cells, where they asexually reproduce by budding. The babesia then differentiates into male and female gametes. The gametes are once again ingested by the tick, where they join and undergo the sporogonic, producing sporozoites. Vertical transmission occurs in some types of Babesia, but not *B. microti*.

The life cycle of *B. caballi* is similar to that of *T. equi* in many respects, but there are some fundamental differences, Scoles says. For example, *B. caballi* does not replicated in the equine host's PBMCs, and it invades the tick's ovaries rather than its salivary glands. *B. caballi* is therefore transmitted when a female tick that has fed on an infected host lays infected eggs—a process called transovarial transmission. Within the tick embryo, the parasite invades the salivary glands. After the larvae hatch, the parasites develop into sporozoites that are then shed into the saliva during blood feeding to infect naive equines.

Because horses are social animals that tend to cluster together, Scoles says, male ticks, which take blood in smaller amounts than females (females engorge themselves before dropping off to lay eggs), are easily able to move from horse to horse, primarily in search of mates. Once on another horse, they may transmit the infection while feeding. When an infected tick bites a human for a blood meal, *Babesia* sporozoites are introduced into the human. Just as in the mouse, sporozoites then go into erythrocytes, where they asexually reproduce by budding. As the parasites multiply within the blood, the disease begins to clinically manifest itself. Once within the human, the parasite cycle cannot continue, and is only transmitted human-to-human by blood transfusions.

Signs

B. caballi may clear their infection in three to five years, such horses are reservoirs of infection during that period.

Diagnostics

The number of parasites present in infected horses is often too low to detect on blood smears, but there are other methods of diagnosis, Scoles says. For example, infected animals develop an immune response that can be detected using a serological assay. However, the presence of an immune response doesn't necessarily mean the horse is

currently infected, Scoles says. It could just mean that the animal was infected and has cleared the infection without a change toits serology results.

That's why Scoles also uses polymerase chain reaction (PCR) assay to detect the presence of a certain parasite DNA sequence. According to Scoles, PCR is a method for chemically amplifying that sequence many times so you can get enough to detect. "PCR confirms the presence of parasite DNA," Scoles says, "but not necessarily living parasites. However, if the animal is seropositive *and* positive by PCR, the combined results may confirm that you have an active parasite infection present."

Probable questions:

1. Elaborate the mode of infection of *Plasmodium*?
2. What is the symptom of malaria?
3. What is spleenomegaly?
4. Discuss the treatment of malaria.
5. What is trypanosomiasis?
6. Discuss the life cycle of *Trypanosoma*.
7. Elaborate the pathology of Trypanosomiasis.
8. What is the causative agent of piroplasmosis?
9. What is Piroplasmosis?

Suggested reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
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Unit VII

Zoonosis with particular reference to *Toxoplasma* and *Schistosoma*

Objective:

In this chapter we will learn about zoonosis with particular reference to *Toxoplasma* and *Schistosoma.*

Zoonosis

A zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans. Animals thus play an essential role in maintaining zoonotic infections in nature. Zoonosis may be bacterial, viral, or parasitic, or may involve unconventional agents. As well as being a public health problem, many of the major zoonotic diseases prevent the efficient production of food of animal origin and create obstacles to international trade in animal products.

Animals provide many benefits to people. Many people interact with animals in their daily lives, both at home and away from home. Pets offer companionship and entertainment, with millions of households having one or more pets. We might come into close contact with animals at a county fair or petting zoo, or encounter wildlife while enjoying outdoor activities. Also, animals are an important food source and provide meat, dairy, and eggs.

Zoonosis refers to diseases that can be passed from animals to humans. They are sometimes called zoonotic diseases.

Animals can carry harmful germs, such as bacteria, fungi, parasites, and viruses. These are then shared with humans and cause illness. Zoonotic diseases range from mild to severe, and some can even be fatal.

Zoonotic diseases are widespread both in the U.S. and worldwide. The World Health Organization (WHO) estimates that 61 percent of all human diseases are zoonotic in origin, while 75 percent of new diseases discovered in the last decade are zoonotic. Before the introduction of new hygiene regulations around 100 years ago, zoonotic diseases such as bovine tuberculosis, bubonic plague caused millions of deaths. They arestill a major problem in developing countries.

However, some animals can carry harmful germs that can be shared with people and cause illness – these are known as zoonotic diseases or zoonosis. Zoonotic diseases are caused by harmful germs like viruses, bacteria, parasites, and fungi. These germs can cause many different types of illnesses in people and animals ranging from mild to serious illness and even death. Some animals can appear healthy even when they are

carrying germs that can make people sick.

Zoonotic diseases are very common, both in the United States and around the world. Scientists estimate that more than 6 out of every 10 known infectious diseases in people are spread from animals, and 3 out of every 4 new or emerging infectious diseases in people are spread from animals. Every year, tens of thousands of Americans will get sick from harmful germs spread between animals and people. Because of this, CDC works 24/7 to protectpeople from zoonotic diseases.

How do germs spread between animals and people?

Because of the close connection between people and animals, it's important to be aware of the common ways people can get infected with germs that can cause zoonotic diseases. Thesecan include:

* **Direct contact**: Coming into contact with the saliva, blood, urine, mucous, feces, or other body fluids of an infected animal. Examples include petting or touching animals, andbites or scratches.
* **Indirect contact**: Coming into contact with areas where animals live and roam, or objects or surfaces that have been contaminated with germs. Examples include aquarium tank water, pet habitats, chicken coops, plants, and soil, as well as pet food and water dishes.
* **Vector-borne**: Being bitten by a tick, or an insect like a mosquito or a flea.
* **Foodborne**: Each year, 1 in 6 Americans get sick from eating contaminated food. Eating or drinking something unsafe (such as unpasteurized milk, undercooked meat or eggs, or raw fruits and vegetables that are contaminated with feces from an infected animal).

Types

Common zoonotic illnesses include:

1. **Rabies**

Rabies is a disease that affects the nervous system of mammals. It is usually caused by a virusand is transmitted if an infected animal bites a person or other animal.

Rabies is almost always fatal once symptoms appear. However, rabies vaccines exist and arecommonly available.

2. **Lyme disease and Rocky Mountain spotted fever**

Lyme disease is transmitted through tick bites. Symptoms can range from mild to severe, butit can be treated using antibiotics.

3. Dengue, malaria, and chikungunya

These are mosquito-borne diseases and are more common in certain areas, such as the Caribbean. Symptoms include fever, vomiting, and headaches. It is vital to treat these conditions as soonas possible, as they can be fatal.

4. *Salmonella* infection

Salmonella is often caused by handling reptiles or amphibians that carry *Salmonella*, or by handling baby chicks or ducks.

The illness usually lasts for between 4 and 7 days, and symptoms include diarrhoea, fever, and abdominal cramps. People can usually recover without medical treatment, although conservative measures are recommended.

5. *E. coli* infection

This infection is often caused by touching infected animals or handling contaminated food. Cows also have *E. coli* germs on their udders.

Often associated with food poisoning, salmonella can cause vomiting, abdominal cramps, anddiarrhoea. It is essential that infected people rest and drink plenty of fluids.

6. Psittacosis

Also known as ornithosis or parrot fever, psittacosis is a bacterial disease that most often affects birds. Humans can get it from feathers, secretions, and droppings. Symptoms include fever, headache, and dry cough. In serious cases, it may cause pneumonia and require a hospital visit.

7. Other types

There are hundreds of zoonotic diseases, but many are rare. Other well-known types include:

- Anthrax
- avian influenza or bird flu
- bovine tuberculosis
- brucellosis
- cat scratch fever
- Ebola
- West Nile virus
- leprosy
- Zika fever
- trichinosis
- swine influenza
- histoplasmosis

Populations at increased risk

Any person who comes into contact with an infected animal, vector, or contaminated area can become infected with a zoonotic disease. However, the risk of acquiring disease, the clinical signs of disease, and the risk of death are not uniformly distributed across individuals. The proportion of people who remain asymptomatic and the case fatality rate (proportion of ill persons who die) vary with certain risk factors. For example, age often is associated with disease severity. Of those infected with *Escherichia coli* O157:H7 from contact with animals or their environment, very young children and the elderly are more likely to develop potentially fatal hemolytic uremic syndrome (HUS) than are older children and healthy adults. By contrast, Hantavirus appears to be especially deadly among fit young adults and middle-aged individuals, possibly owing to the increased likelihood of those individuals' coming into contact with the infectious agent. The risk of becoming infected with a zoonotic disease is increased in persons affected by immunosuppression from a preexisting disease or medication. For example, cryptosporidiosis caused by *Cryptosporidium parvum*, which is transmitted to humans following contact with calves, their manure, or manure-contaminated objects or food, can occur as a coinfection with acquired immunodeficiency syndrome (AIDS). Normally a self-limiting disease, in those with AIDS cryptosporidiosis can cause serious illness, sometimes ending in death. Persons without a functioning spleen have an increased risk of illness and death from *Capnocytophaga canimorsus* infection, which can be acquired through contact with cats or dogs (particularly through dog bites). Persons who take chloroquine for malaria prophylaxis concurrently with rabies preexposure immunizations are less likely to develop a sufficient immunologic response to survive a rabies exposure. Other populations at risk include those who are cognitively impaired; such individuals, for example, may not be able to recognize or report bites from rabid bats. Pregnant women are at risk of fetal congenital malformations with lymphocytic choriomeningitis virus (LCMV) infection. Solid-organ transplant recipients have died from rabies and LCMV infections transmitted from donors.

Zoonotic disease control

Zoonotic diseases are difficult to control, particularly because of their animal reservoirs. Indeed, unlike diseases such as smallpox and polio, most zoonotic diseases cannot be eradicated through intensive human vaccination campaigns. Their successful control relies instead on strategies aimed at reducing the burden of disease among wild animals. In the case of rabies, for example, the distribution of baits containing oral rabies vaccine has led to the near-elimination or eradication of variant rabies (e.g., the Arctic fox and red fox variants) from regional wildlife reservoirs (e.g., foxes and raccoons).

Zoonotic disease risk is increased when humans live in close proximity to domestic animals such as poultry and livestock. Although the practice allows for the efficient use of limited land resources and constant care and protection of the animals, it also

increases the risk of humans' becoming infected with disease agents such as HPAI (highly pathogenic avian influenza—e.g., H5N1 virus). Pets, which often live in human homes, are common sources of zoonotic disease. For example, *Salmonella* infections (sometimes with multidrug-resistant strains) can occur as a result of contact with pet reptiles and amphibians (e.g., turtles, iguanas, and snakes), exotic pets (e.g., hedgehogs and sugar gliders), pocket pets (e.g., hamsters, mice, and rats), pet birds (e.g., chicks and ducklings), and dogs and cats. Pet treats and other pet-associated environmental factors may also be sources of *Salmonella*.

Limiting contact between humans and wild animals is critical to reducing the risk of zoonotic disease transmission. Many human rabies deaths are due to bites from bats, frequently in home settings. Although the human immunodeficiency virus (HIV), which causes AIDS, is not zoonotic, it is thought to have evolved from similar monkey viruses that jumped to humans through the practice of hunting and consuming bush meat (monkeys). Contact with rodent feces is associated with hantavirus infection, and plague infection is associated with activities that bring people into contact with wild rodents and their fleas. The risk of zoonotic disease in humans can be further reduced by limiting contact between wild and domestic animals.

Because zoonotic disease agents can be found in humans, animals, the environment, and vectors, management requires the collaboration of many types of health and disease-control specialists. Disease control may include vector-control programs for ticks, fleas, or mosquitoes, and environmental cleanup or protection may be required to address disease agents that remain viable from days to years on surfaces, in soils, or in the water. In most state health agencies, public health veterinarians are available to assist in disease-control coordination.

Zoonotic diseases continue to be of significant concern for public health. In the early 21st century, an estimated 60 percent of novel human pathogens were zoonotic in origin, and increasing numbers of zoonotic diseases were spreading into areas where they previously had not occurred. In addition, several zoonotic disease agents were identified as candidates for use in bioterrorism attacks.

Toxoplasmosis

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. In the United States it is estimated that 11% of the population 6 years and older have been infected with *Toxoplasma*. In various places throughout the world, it has been shown that up to 95% of some populations have been infected with *Toxoplasma*. Infection is often highest in areas of the world that have hot, humid climates and lower altitudes.

Toxoplasmosis is not passed from person-to-person, except in instances of mother-to-child (congenital) transmission and blood transfusion or organ transplantation. People

typically become infected by three principal routes of transmission.

I. Food borne
II. Animal-to-human (zoonotic)
III. Mother-to-child (congenital)
IV. Rare instances

I. Foodborne transmission

The tissue form of the parasite (a microscopic cyst consisting of bradyzoites) can be transmitted to humans by food. People become infected by:

- Eating undercooked, contaminated meat (especially pork, lamb, and venison).
- Accidental ingestion of undercooked, contaminated meat after handling it and not washing hands thoroughly (*Toxoplasma* cannot be absorbed through intact skin).
- Eating food that was contaminated by knives, utensils, cutting boards, or other foods thathad contact with raw, contaminated meat.

II. Animal-to-human (zoonotic) transmission

Cats play an important role in the spread of toxoplasmosis. They become infected by eating infected rodents, birds, or other small animals. The parasite is then passed in the cat's feces in an oocyst form, which is microscopic.

Kittens and cats can shed millions of oocysts in their feces for as long as 3 weeks after infection. Mature cats are less likely to shed *Toxoplasma* if they have been previously infected. A *Toxoplasma*-infected cat that is shedding the parasite in its feces contaminates the litter box. If the cat is allowed outside, it can contaminate the soil or water in the environmentas well.

People can accidentally swallow the oocyst form of the parasite. People can be infected by:

Accidental ingestion of oocysts after cleaning a cat's litter box when the cat hasshed *Toxoplasma* in its feces.

- ✓ Accidental ingestion of oocysts after touching or ingesting anything that has come intocontact with a cat's feces that contain *Toxoplasma.*
- ✓ Accidental ingestion of oocysts in contaminated soil (e.g., not washing hands aftergardening or eating unwashed fruits or vegetables from a garden).
- ✓ Drinking water contaminated with the *Toxoplasma* parasite.

III. Mother-to-child (congenital) transmission

A woman who is newly infected with *Toxoplasma* during pregnancy can pass the infection to her unborn child (congenital infection). The woman may not have symptoms, but there can be severe consequences for the unborn child, such as diseases of the nervous system and eyes.

IV. Rare instances of transmission

Organ transplant recipients can become infected by receiving an organ from a *Toxoplasma*- positive donor. Rarely, people can also become infected by receiving infected blood via transfusion. Laboratory workers who handle infected blood can also acquire infection through accidental inoculation.

Causal Agent:

Toxoplasma gondii is a protozoan parasite that infects most species of warm blooded animals,including humans, and can cause the disease toxoplasmosis.

Life Cycle:

The only known definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces . Although oocysts are usually only shed for 1-2 weeks, large numbers may be shed. Oocysts take 1-5 days to sporulate in the environment and become infective. Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts. Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. Cats become infected after consuming intermediate hosts harboring tissue cysts. Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans can become infected by any of several routes:

- eating undercooked meat of animals harboring tissue cysts .
- consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat) .
- blood transfusion or organ transplantation .
- transplacentally from mother to fetus .

In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens. Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR.

Healthy people (nonpregnant)

Healthy people who become infected with *Toxoplasma gondii* often do not have symptoms because their immune system usually keeps the parasite from causing illness. When illness occurs, it is usually mild with "flu-like" symptoms (e.g., tender lymph nodes, muscle aches, etc.) that last for weeks to months and then go away. However, the parasite remains in their body in an inactive state. It can become reactivated if the

person becomes immunosuppressed.

Mother-to-child (congenital)

Generally if a woman has been infected before becoming pregnant, the unborn child will be protected because the mother has developed immunity. If a woman is pregnant and becomes newly infected with *Toxoplasma* during or just before pregnancy, she can pass the infection to her unborn baby (congenital transmission). The damage to the unborn child is often more severe if the transmission occurs earlier in pregnancy. Potential results can be

- a miscarriage
- a stillborn child
- a child born with signs of toxoplasmosis (e.g., abnormal enlargement or smallness of thehead)

Infants infected before birth often show no symptoms at birth but may develop them later in life with potential vision loss, mental disability, and seizures.

Persons with ocular disease

Eye disease (most frequently retinochoroiditis) from *Toxoplasma* infection can result from congenital infection or infection after birth by any of the modes of transmission discussed on the epidemiology and risk factors page. Eye lesions from congenital infection are often not identified at birth but occur in 20-80% of infected persons by adulthood. However, in the U.S. <2% of persons infected after birth develop eye lesions. Eye infection leads to an acute inflammatory lesion of the retina, which resolves leaving retinochoroidal scarring. Symptoms of acute disease include

- eye pain
- sensitivity to light (photophobia)
- tearing of the eyes
- blurred vision

The eye disease can reactivate months or years later, each time causing more damage to the retina. If the central structures of the retina are involved there will be a progressive loss of vision that can lead to blindness.

Persons with compromised immune systems

Persons with compromised immune systems may experience severe symptoms if they are infected with *Toxoplasma* while immune suppressed. For example, a person who is HIV- infected and who has reactivated *Toxoplasma* infection can have symptoms that include fever, confusion, headache, seizures, nausea, and poor coordination. Persons who acquire HIV infection and were not infected previously with *Toxoplasma* are more likely to develop asevere primary infection.

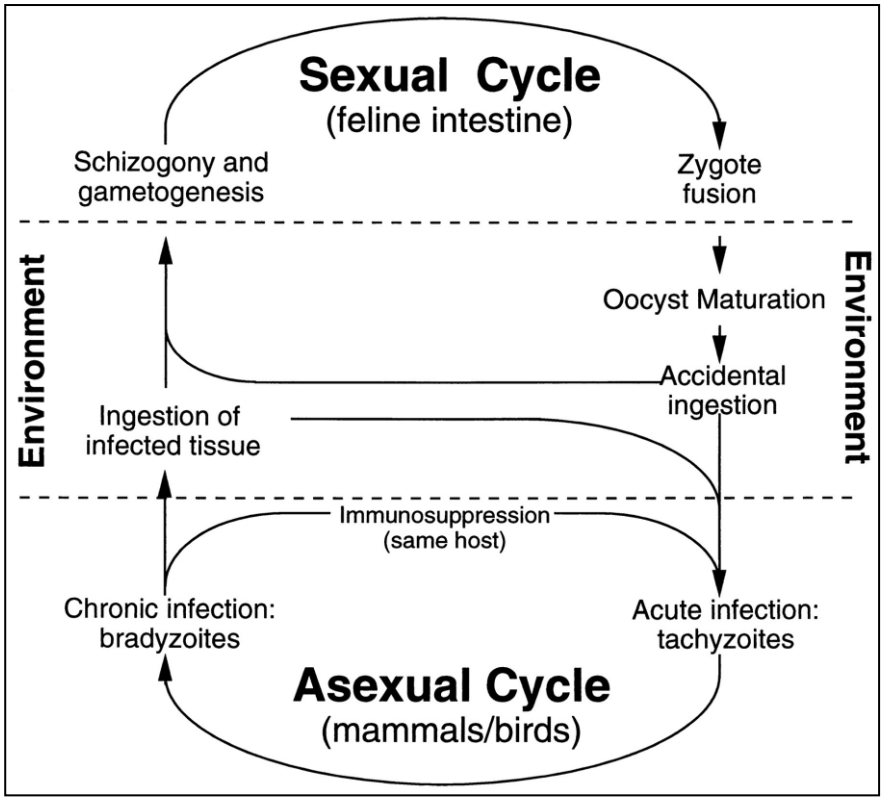


Fig: Life cycle of *Toxoplasma gondii*

Immunocompromised persons who were infected with *Toxoplasma* at some point before they become immunosuppressed are particularly at risk for developing a relapse of toxoplasmosis. *Toxoplasma* infection can reactivate in immunocompromised pregnant women who were infected with *Toxoplasma* before their pregnancy and this can lead to congenital infection.

Diagnosis

The diagnosis of toxoplasmosis is typically made by serologic testing. A test that measures immunoglobulin G (IgG) is used to determine if a person has been infected. If it is necessary to estimate the time of infection, which is of particular importance for pregnant women, a test which measures immunoglobulin M (IgM) is also used along with other tests such as an avidity test.

Diagnosis can be made by direct observation of the parasite in stained tissue sections, cerebrospinal fluid (CSF), or other biopsy material. These techniques are used less frequently because of the difficulty of obtaining these specimens.

Parasites can also be isolated from blood or other body fluids (for example, CSF) but this process can be difficult and requires considerable time.

Molecular techniques that can detect the parasite's DNA in the amniotic fluid can be useful incases of possible mother-to-child (congenital) transmission.

Ocular disease is diagnosed based on the appearance of the lesions in the eye,

symptoms, course of disease, and often serologic testing.

Treatment

- **Healthy people (nonpregnant)**

Most healthy people recover from toxoplasmosis without treatment. Persons who are ill can be treated with a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid.

- **Pregnant women, newborns, and infants**

Pregnant women, newborns, and infants can be treated, although the parasite is not eliminated completely. The parasites can remain within tissue cells in a less active phase; their location makes it difficult for the medication to completely eliminate them.

- **Persons with ocular disease**

Persons with ocular toxoplasmosis are sometimes prescribed medicine to treat active disease by their ophthalmologist. Whether or not medication is recommended depends on the size of the eye lesion, the location, and the characteristics of the lesion (acute active, versus chronicnot progressing).

- **Persons with compromised immune systems**

Persons with compromised immune systems need to be treated until they have improvement in their condition. For AIDS patients, continuation of medication for the rest of their livesmay be necessary, or for as long as they are immunosuppressed.

Prevention and Control

People who are healthy should follow the guidelines below to reduce risk of toxoplasmosis. If you have a weakened immune system, please see guidelines for Immunocompromised Persons.

Reduce Risk from Food

- **To prevent risk of toxoplasmosis and other infections from food:**

Cook food to safe temperatures. A food thermometer should be used to measure the internal temperature of cooked meat. Do not sample meat until it is cooked. USDA recommends the following for meat preparation.

- **For Whole Cuts of Meat (excluding poultry)**

Cook to at least 145° F (63° C) as measured with a food thermometer placed in the thickest part of the meat, then allow the meat to rest for three minutes before carving or consuming.

- **For Ground Meat (excluding poultry)**

Cook to at least 160° F (71° C); ground meats do not require a rest time.

- **For All Poultry (whole cuts and ground)**

Cook to at least 165° F (74° C), and for whole poultry allow the meat to rest for three minutes before carving or consuming. According to USDA, "A 'rest time' is the amount of time the product remains at the final temperature, after it has been removed from a grill, oven, or other heat source. During the three minutes after meat is removed from the heat source, its temperature remains constant or continues to rise, which destroys pathogens."

- Freeze meat for several days at sub-zero (0° F) temperatures before cooking to greatly reduce chance of infection.
- Peel or wash fruits and vegetables thoroughly before eating.
- Wash countertops carefully.
- Wash cutting boards, dishes, counters, utensils, and hands with hot soapy water after contact with raw meat, poultry, seafood, or unwashed fruits or vegetables.

The U.S. Government and the meat industry continue their efforts to reduce *T. gondii* inmeat.

Reduce Risk from the Environment

To prevent risk of toxoplasmosis from the environment:

- Avoid drinking untreated drinking water.
- Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain *Toxoplasma*. Wash hands with soap and warm water after gardening or contact with soil or sand.
- Teach children the importance of washing hands to prevent infection.
- Keep outdoor sandboxes covered.
- Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.
- Change the litter box daily if you own a cat. The *Toxoplasma* parasite does not become infectious until 1 to 5 days after it is shed in a cat's feces.

If you are pregnant or immunocompromised:

- Avoid changing cat litter if possible. If no one else can perform the task, wear disposable gloves and wash your hands with soap and warm water afterwards.
- Keep cats indoors.
- Do not adopt or handle stray cats, especially kittens. Do not get a new cat while you are pregnant.

Schistosomiasis

Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. Although the worms that cause schistosomiasis are not found in the United States, more than 200 million people are infected worldwide. In terms of impact this disease is second only to malaria as the most devastating parasitic disease. Schistosomiasis is considered one of the Neglected Tropical Diseases (NTDs).

The parasites that cause schistosomiasis live in certain types of freshwater snails. The infectious form of the parasite, known as cercariae, emerges from the snail, hence contaminating water. You can become infected when your skin comes in contact with contaminated freshwater. Most human infections are caused by *Schistosoma mansoni, S.haematobium*, or *S. japonicum*.

Epidemiology and Risk Factors

Schistosomiasis is an important cause of disease in many parts of the world, most commonly in places with poor sanitation. School-age children who live in these areas are often most at risk because they tend to spend time swimming or bathing in water containing infectious cercariae.

If you live in, or travel to, areas where schistosomiasis is found and are exposed to contaminated freshwater, you are at risk.

Areas where human Schistosomiasis is found include:

i. Schistosoma mansoni

- Distributed throughout Africa: There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Sudan and Egypt
- South America: including Brazil, Suriname, Venezuela
- Caribbean (risk is low): Dominican Republic, Guadeloupe, Martinique, and Saint Lucia.

ii. S. haematobium

- Distributed throughout Africa. There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Egypt and the Mahgreb region of North Africa.
- found in areas of the Middle East.

iii. S. japonicum

- found in Indonesia and parts of China and Southeast Asia.

iv. S. mekongi

- found in Cambodia and Laos.

v. S. intercalatum

- found in parts of Central and West Africa.

Biology

Causal Agents:

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium, S. japonicum,* and *S. mansoni.* Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum.* In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis inhumans.

Life Cycle:

Eggs are eliminated with feces or urine. Under optimal conditions the eggs hatch and release miracidia, which swim and penetrate specific snail intermediate hosts . The stages in the snail include 2 generations of sporocysts and the production of cercariae. Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host, and shed their forked tail, becoming schistosomulae. The schistosomulae migrate through several tissues and stages to their residence in the veins. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively. Pathology of *S. mansoni* and *S. japonicum* schistosomiasis includes: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord. Pathology of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord. Human contact with water is thus necessary for infection by schistosomes. Various animals, such as dogs, cats, rodents, pigs, hourse and goats, serve as reservoirs for *S. japonicum*, and dogs for *S. mekongi*.

Disease

Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 hours. *Schistosoma* parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Children who are repeatedly infected can develop anemia, malnutrition, and learning difficulties. After years of infection, the parasite can also damage the liver, intestine, spleen, lungs, and bladder.

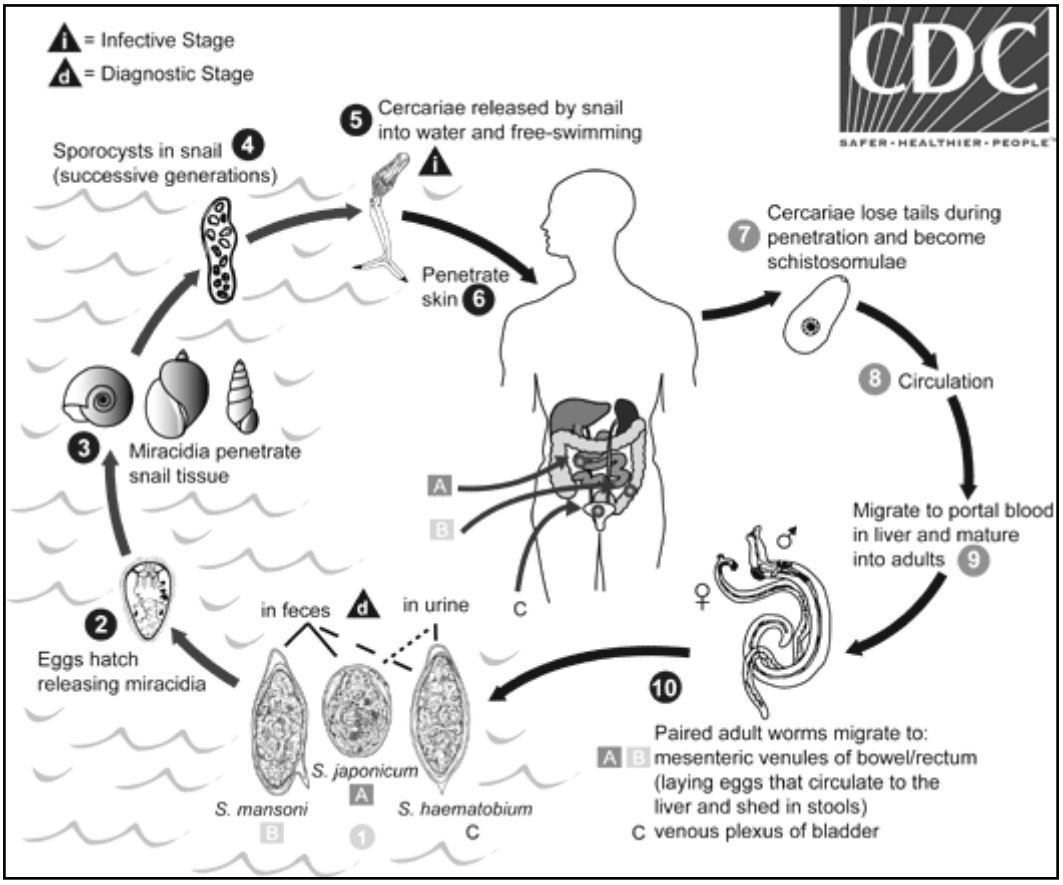


Figure: General life cycle of *Schistosoma* sp.

Common Symptoms

Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

Chronic schistosomiasis

Without treatment, schistosomiasis can persist for years. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, blood in the stool or blood in the urine, and problems passing urine. Chronic infection can also lead to increased risk of bladdercancer.

Rarely, eggs are found in the brain or spinal cord and can cause seizures, paralysis, or spinal cord inflammation.

Diagnosis

Stool or urine samples can be examined microscopically for parasite eggs (stool for *S. mansoni* or *S. japonicum* eggs and urine for *S. haematobium* eggs). The eggs tend to be passed intermittently and in small amounts and may not be detected, so it may be necessaryto perform a blood (serologic) test.

Treatment

Safe and effective medication is available for treatment of both urinary and intestinal schistosomiasis. Praziquantel, a prescription medication, is taken for 1-2 days to treat infections caused by all *Schistosoma* species.

Prevention and Control

Prevention

No vaccine is available.

The best way to prevent schistosomiasis is to take the following steps if you are visiting or live in an area where schistosomiasis is transmitted:

- ✓ Avoid swimming or wading in freshwater when you are in countries in which schistosomiasis occurs. Swimming in the ocean and in chlorinated swimming pools is safe.
- ✓ Drink safe water. Although schistosomiasis is not transmitted by swallowing contaminated water, if your mouth or lips come in contact with water containing the parasites, you could become infected. Because water coming directly from canals, lakes, rivers, streams, or springs may be contaminated with a variety of infectious organisms, you should either bring your water to a rolling boil for 1

minute or filter water before drinking it. Bring your water to a rolling boil for at least 1 minute will kill any harmful parasites, bacteria, or viruses present. Iodine treatment alone will not guarantee that water is safe and free of all parasites.

- ✓ Water used for bathing should be brought to a rolling boil for 1 minute to kill any cercariae, and then cooled before bathing to avoid scalding. Water held in a storage tank for at least 1 - 2 days should be safe for bathing.
- ✓ Vigorous towel drying after an accidental, very brief water exposure may help to prevent the *Schistosoma* parasite from penetrating the skin. However, do not rely on vigorous towel drying alone to prevent schistosomiasis.

Those who have had contact with potentially contaminated water overseas should see theirhealth care provider after returning from travel to discuss testing.

Control

In countries where schistosomiasis causes significant disease, control efforts usually focuson:

- reducing the number of infections in people and/or
- eliminating the snails that are required to maintain the parasite's life cycle.

For all species that cause schistosomiasis, improved sanitation could reduce or eliminate transmission of this disease. In some areas with lower transmission levels, elimination of schistosomiasis is considered a "winnable battle" by public health officials.

Control measures can include mass drug treatment of entire communities and targeted treatment of school-age children. Some of the problems with control of schistosomiasis include:

- Chemicals used to eliminate snails in freshwater sources may harm other species of animals in the water and, if treatment is not sustained, the snails may return to those sites afterwards.
- For certain species of the parasite, such as *S. japonicum*, animals such as cows or water buffalo can also be infected. Runoff from pastures (if the cows are infected) can contaminate freshwater sources.

Probable questions:

1. What is Zoonosis? Give example.
2. How do germs spread between animals and people in Zoonotic disease?
3. How Zoonotic disease can be controlled?
4. What is Toxoplasmosis?
5. What is the prophylaxis of Toxoplasmosis?
6. What is the causative agent of Schistosomiasis?
7. Discuss the epidemiology of Schistosomiasis.

Suggested reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
2. Noble, E. R. and Noble G. A. (1989). Parasitology. The Biology of animal Parasites. 6th ed.
3. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology. 9th ed. McGraw-Hill International.

Unit VIII

Life cycle, biology, pathogenesis, epidemiology and control of medically important helminthes - *Diphyllobothrium latum, Paragonimus westermani*

Objectives:

In this Unit we will discuss on Life cycle, biology, pathogenesis, epidemiology and control of important human and veterinary helminthes - *Diphyllobothrium latum, Paragonimus westermani.*

- ***Diphyllobothrium latum***

Diphyllobothrium is a genus of tapeworms which can cause diphyllobothriasis in humans through consumption of raw or undercooked fish. The principal species causing diphyllobothriasis is *Diphyllobothrium latum*, known as the broad or fish tapeworm, or broad fish tapeworm. *D. latum* is a pseudophyllid cestode that infects fish and mammals.

D. latum is native to Scandinavia, western Russia, and the Baltics, though it is now also present in North America, especially the Pacific Northwest.

Causal Agent

The cestode *Diphyllobothrium latum* (the fish or broad tapeworm), the largest human tapeworm. Several other *Diphyllobothrium* species have been reported to infect humans, but less frequently; they include *D. pacificum*, *D. cordatum*, *D. ursi*, *D. dendriticum*, *D. lanceolatum*, *D. dalliae*, and *D. yonagoensis*.

Geographic Distribution

Diphyllobothriasis occurs in the Northern Hemisphere (Europe, North America, and Asia) and in South America (Uruguay and Chile). Freshwater fish infected with *Diphyllobothrium* sp. larva may be transported to and consumed in geographic areas where active transmission does not occur, resulting in human diphyllobothriasis. For example, cases of *D. latum* infection associated with consumption of imported fish have beenreported in Brazil.

Morphology of *Diphyllobothrium Latum* (Fish Tapeworm):

The adult worm is ivory or yellowish grey in colour, measuring 3-10 metres in length. The head (scolex) is small, spatulated or spoon shaped, has a pair of slit grooves (bothria) ventrally and dorsally and has no rostellum (a beaklike projection on the head) and no hooklets. Scolex is followed by "**neck**" and 3,000 segments. A single worm may discharge as many as one million eggs (Fig. 107.3) per day.

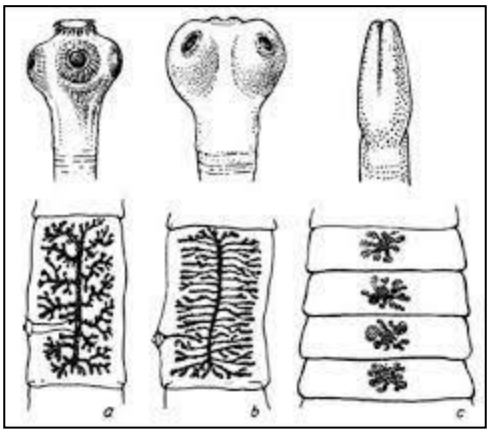


Fig: Morphology of *Diphyllobothrium latum*

Life Cycle:

Adult tapeworms may infect humans, canids, felines, bears, pinnipeds, and mustelids, though the accuracy of the records for some of the nonhuman species is disputed. Immature eggs are passed in feces of the mammal host (the definitive host, where the worms reproduce). After ingestion by a suitable freshwater crustacean such as a copepod (the first intermediate host), the coracidia develop into procercoid larvae. Following ingestion of the copepod by a suitable second intermediate host, typically a minnow or other small freshwater fish, the procercoid larvae are released from the crustacean and migrate into the fish's flesh where they develop into a plerocercoid larvae (sparganum). The plerocercoid larvae are the infective stage for the definitive host (including humans).

Because humans do not generally eat undercooked minnows and similar small freshwater fish, these do not represent an important source of infection. Nevertheless, these small second intermediate hosts can be eaten by larger predator species, for example trout, perch, walleye, and pike. In this case, the sparganum can migrate to the musculature of the larger predator fish and mammals can acquire the disease by eating these later intermediate infected host fish raw or undercooked. After ingestion of the infected fish, the plerocercoids develop into immature adults and then into mature adult tapeworms which will reside in the small intestine. The adults attach to the intestinal mucosa by means of the two bilateral grooves (bothria) of their scolex. The adults can reach more than 10 m (up to 30 ft) in length in some species such as *D. latum,* with more than 3,000 proglottids. One or several of the tape-like proglottid segments (hence the

name tape-worm) regularly detach from the main body of the worm and release immature eggs in fresh water to start the cycle over again. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) and are passed in the feces. The incubation period in humans, after which eggs begin to appear in the feces is typically 4–6 weeks, but can vary from as short as 2 weeks to as long as 2 years. The tapeworm can live up to 20 years

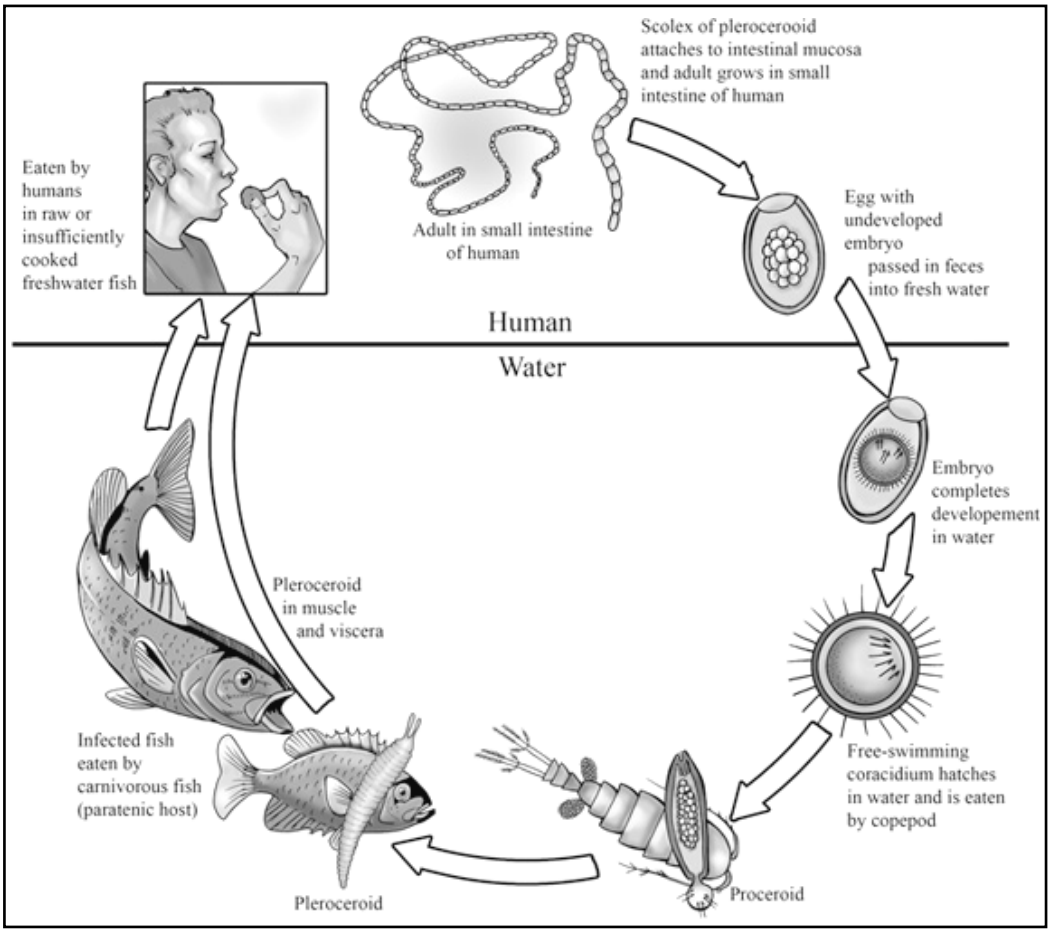


Figure: Life Cycle of *Diphyllobothrium Latum*

Immature eggs are passed in feces. Under appropriate conditions, the eggs mature (approximately 18 to 20 days) and yield oncospheres which develop into a coracidia . After ingestion by a suitable freshwater crustacean (the copepod first intermediate host) the coracidia develop into procercoid larvae. Following ingestion of the copepod by a suitable second intermediate host, typically minnows and other small freshwater fish, the procercoid larvae are released from the crustacean and migrate into the fish flesh where they develop into a plerocercoid larva (sparganum). The plerocercoid larvae are the infective stage for humans. Because humans do not generally eat undercooked minnows and similar small freshwater fish, these do not represent an important source of infection. Nevertheless, these small second intermediate hosts can be eaten by larger predator species, e.g., trout, perch, walleyed pike . In this case, the sparganum can migrate to the musculature of the larger predator fish and humans can acquire the disease by eating these later intermediate infected host fish raw or

undercooked. After ingestion of the infected fish, the plerocercoid develop into immature adults and then into mature adult tapeworms which will reside in the small intestine. The adults of *D. latum* attach to the intestinal mucosa by means of the two bilateral groves (bothria) of their scolex. The adults can reach more than 10 m in length, with more than 3,000 proglottids. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) and are passed in the feces. Eggs appear in the feces 5 to 6 weeks after infection. In addition to humans, many other mammals can also serve as definitive hosts for *D. latum*.

Signs and symptoms

Most persons with diphyllobothriasis are asymptomatic. In symptomatic persons, the following are the most common symptoms:

- Abdominal pain
- Indigestion or dyspepsia
- Passage of proglottids

Other, less common, symptoms include the following:

- Fatigue
- Diarrhea
- Dizziness
- Weakness (rare)
- Numbness of extremities
- Sensation of hunger
- Pruritus ani

Most patients with diphyllobothriasis have no signs of illness. Rare physical findings thatmay be noted include the following:

- Pallor
- Glossitis
- Dyspnea
- Tachycardia
- Weakness
- Hypoesthesia
- Paresthesias

Disturbances of movement and coordination, loss of vibratory sense and proprioception
In patients who present with obstruction, the following physical findings may be noted:

- Abdominal tenderness
- Abdominal distention
- Peritoneal signs

Diagnosis

Diagnosis is usually made by identifying proglottid segments, or characteristic eggs in the feces. These simple diagnostic techniques are able to identify the nature of the infection to the genus level, which is usually sufficient in a clinical setting. However, when the species needs to be determined (in epidemiological studies, for example), restriction fragment length polymorphisms can be effectively used. PCRcan be performed on samples of purified eggs, or native fecal samples following sonication of the eggs to release their contents. Another interesting potential diagnostic tool and treatment is the contrast medium, Gastrografin, introduced into the duodenum, which allows both visualization of the parasite, and has also been shown to cause detachment and passing of the whole worm.

Clinical Features

Diphyllobothriasis can be a long-lasting infection (decades). Most infections are asymptomatic. Manifestations may include abdominal discomfort, diarrhea, vomiting, and weight loss. Vitamin B12 deficiency with pernicious anemia may occur. Massive infections may result in intestinal obstruction. Migration of proglottids can cause cholecystitis or cholangitis. The presence of adult worm in the intestinal tract causes no symptom, but sometimes, non-specific abdominal symptoms have been ascribed. If the worms attach themselves to the jejunum, clinical vitamin B_{12} deficiency develops. In the laboratory, mircoscopical examination of the faeces will reveal the eggs; sometimes proglottids can be observed in the stool.

Pathogenesis

Infection with *Diphyllobothrium latum* is often asymptomatic and long-lasting (decades). Infected persons may experience abdominal pain, vomiting, diarrhoea, and weight loss. In some infections there may be a severe Vitamin B_{12} deficiency and anaemia caused by obstruction of Vitamin B_{12} absorption coupled with high absorption rates by the tapeworm.

Treatment

Quinacrine hydrochloride, niclosamide and paromomycin are found effective. Perniciousanaemia can be treated with folic acid.

Prophylaxis:

(1) Thorough cooking of suspected fresh water fish is important;

(2) Pollution of water can be prevented by efficient .disposal of sewage;

(3) In endemic areas of infection, dogs and cats should not be given fish

- ***Paragonimus westermani***

Paragonimus westermani is the major species of lung fluke that infects humans, causing paragonimiasis. The species sometimes is called the **Japanese lung fluke** or **oriental lung fluke**. Human infections are most common in eastern Asia and in South America. *Paragonimus westermani* was discovered when two Bengal tigers died of paragonimiasis in zoos in Europe in 1878. Several years later, infections in humans were recognised in Formosa

In size, shape, and color, *Paragonimus westermani* resembles a coffee bean when alive. Adult worms are 7.5 mm to 12 mm long and 4 mm to 6 mm wide. The thickness ranges from 3.5 mm to 5 mm. The skin of the worm (tegument) is thickly covered with scalelike spines. The oral and ventral suckers are similar in size, with the latter placed slightly pre- equatorially. The excretory bladder extends from the posterior end to the pharynx. The lobed testes are adjacent from each other located at the posterior end, and the lobed ovaries are off-centered near the center of the worm (slightly postacetabular). The uterus is located in a tight coil to the right of the acetabulum, which is connected to the vas deferens. The vitelline glands, which produce the yolk for the eggs, are widespread in the lateral field from the pharynx to the posterior end. Inspection of the tegumental spines and shape of the metacercariae may distinguish between the 30-odd species of *Paragonimus spp.* but the distinction is sufficiently difficult to justify suspicion that many of the described species are synonyms.

- **Eggs:** *Paragonimus westermani* eggs range from 80 to 120 µm long by 45 to 70 µm wide. They are yellow-brown, ovoid or elongate, with a thick shell, and often asymmetrical with one end slightly flattened. At the large end, the operculum is clearly visible. The opposite (abopercular) end is thickened. The eggs are unembryonated when passed in sputum or feces.
- **Cercaria**: Cercariae are often indistinguishable between species. There is a large posterior sucker, and the exterior is spined.
- **Metacercaria:** Metacercariae are usually encysted in tissue. The exterior is spined and has two suckers
- **Adults:** Adult flukes are typically reddish brown and ovoid, measuring 7 to 16 mm by 4 to 8 mm, similar in size and appearance to a coffee bean.They are hermaphroditic, with a lobed ovary located anterior to two branching testes. Like all members of the Trematoda,they possess oral and ventral suckers.

Causal Agent

More than 30 species of trematodes (flukes) of the genus *Paragonimus* have been reported which infect animals and humans. Among the more than 10 species reported to infecthumans, the most common is *P. westermani*, the oriental lung fluke.

Epidemiology

Paragonimus spp. is a common parasite of crustacean-eating mammals such as tigers, leopards, domestic cats, dogs, mongooses, opossums and monkeys (reservoir final hosts). The adult flukes live in the lungs and lay eggs that are coughed up through the airways and either expectorated in the sputum or swallowed and defecated. When they reach freshwater, the eggs develop into miracidia that penetrate various species of aquatic snails, where they further develop and reproduce asexually, giving rise to cercariae (larvae).

Cercariae released into water swim to penetrate suitable species of freshwater crabs, crayfish and other crustaceans and encyst the gills, liver and muscles as metacercariae. When such animals are eaten, the metacercariae hatch in the intestine: young worms penetrate the intestinal wall and the peritoneum, then the diaphragm and the pleura; they finally reach the lungs, where they live in pairs surrounded by a capsula, thus completing the cycle.

Habitat: - adult worm live in respiratory tract (lung) of man.

• Definitive Hosts: - Man and Domestic animals (usually host in Asia are the tiger & leopard)

• Intermediate Host: - First Host:- A fresh-water snail of the genus Melania Second Host:- A fresh-water crayfish or a crab

Life Cycle

Paragonimus has a quite complex life-cycle that involves two intermediate hosts as well as humans. Eggs first develop in water after being expelled by coughing (unembryonated) or being passed in human feces. In the external environment, the eggs become embryonated.

The eggs are excreted unembryonated in the sputum, or alternately they are swallowed and passed with stool. In the external environment, the eggs become embryonated and miracidia hatch and seek the first intermediate host, a snail, and penetrate its soft tissues. Miracidia go through several developmental stages inside the snail: sporocysts, rediae, with the latter giving rise to many cercariae, which emerge from the snail. The cercariae invade the second intermediate host, a crustacean such as a crab or crayfish, where they encyst and become metacercariae. This is the infective stage for the mammalian host. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through

the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults (7.5 to 12 mm by 4 to 6 mm). The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place

completion of the life cycles is not achieved, because the eggs laid cannot exit these sites. Time from infection to oviposition is 65 to 90 days.

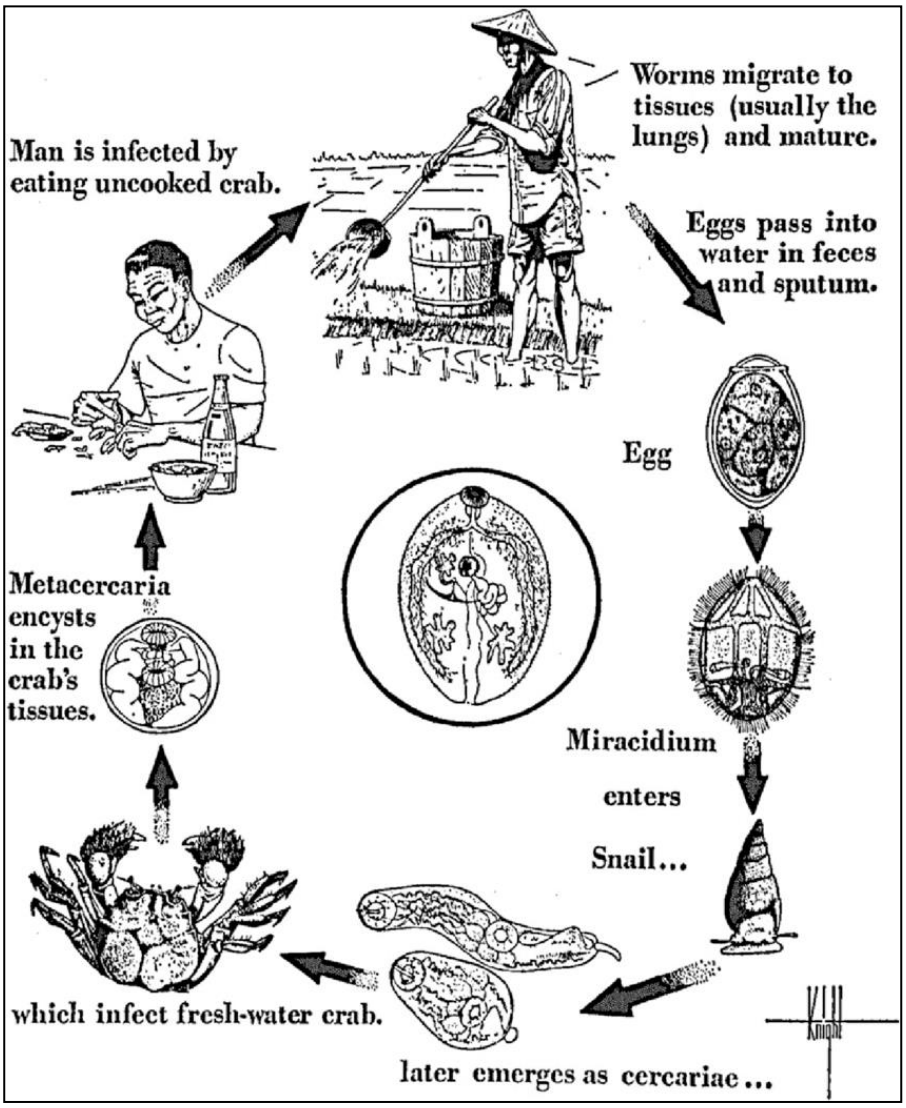


Fig: Life cycle of *Paragonimus westermani*

In the next stage, the parasite miracidia hatch and invades the first intermediate host such as a species of freshwater snail. Miracidia penetrate its soft tissues and go through several developmental stages inside the snail but mature into cercariae in 3 to 5 months. Cercariae next invade the second intermediate host such as crabs or crayfish and encyst to develop into metacercariae within 2 months. Infection of humans or other mammals (definitive hosts) occurs via consumption of raw or undercooked crustaceans. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults. The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place completion of the life cycles is not achieved, because the eggs laid cannot exit

these sites.

Infections may persist for 20 years in humans. Animals such as pigs, dogs, and a variety of feline species can also harbor *P. westermani*.

Disease

Humans may substitute reservoir hosts in the transmission cycle when they eat raw, salted, pickled, smoked, marinated, dried, partially cooked or poorly processed crustaceans, thus ingesting the metacercariae. In humans, the earliest stages of paragonimiasis may present an elusive clinical picture, and be asymptomatic or scarcely symptomatic. Conversely, when worms reach the lungs, symptoms may be significant and typically include chronic cough with blood-stained sputum; chest pain with dyspnoea and fever; pleural effusionand pneumothorax are possible complications.

Symptoms and signs mimic those of tuberculosis, and paragonimiasis should always be suspected in patients with tuberculosis who are non-responsive to treatment. Ectopic paragonimiasis may result from erratic migration of the juvenile worms: the most frequent locations include the abdominal cavity and subcutaneous tissues and, most frequently, the brain: cerebral paragonimiasis is a severe condition that may be associated with headache, visual impairment and epileptic seizures.

Pathology

Once in the lung or ectopic site, the worm stimulates an inflammatory response that allows it to cover itself in granulation tissue forming a capsule. These capsules can ulcerate and heal over time. The eggs in the surrounding tissue become pseudotubercles. If the worm becomes disseminated and gets into the spinal cord, it can cause paralysis; capsules in the heart can cause death. The symptoms are localized in the pulmonary system, which include a bad cough, bronchitis, and blood in sputum (hemoptysis).

Diagnosis, treatment and control

Diagnosis of paragonimiasis is suspected on the basis of the clinical picture, on the anamnestic recall of consuming raw crustaceans, on the detection of eosinophilia, and on typical findings of ultrasound, X-ray, computed tomography or magnetic resonance imaging scans. Tests to rule out tuberculosis should always be conducted. Confirmation ofdiagnosis relies on different types of diagnostic techniques:

i. **parasitological techniques** to detect *Paragonimus* eggs in sputum or stool samples.; the cost and sensitivity of these techniques may vary according to the type of technique used; they can only be employed once worms have reached the lungs and started laying eggs; some quantify the intensity of infection (allowing an estimation of the severity of the infection);

ii. **immunological techniques** to detect worm-specific antibodies in serum samples or worm-specific antigens in serum or stool samples; these techniques are usually more sensitive than the commonly used parasitological techniques; detection of antibodies does not distinguish between current, recent and past infections; their ability to quantify intensity of infection is disputed; these techniques are still at an experimental stage;

iii. **Molecular techniques** such as the polymerase chain reaction are also still at an experimental stage.

Triclabendazole, 20 mg/kg, in two divided doses of 10 mg/kg, to be administered on the same day, and praziquantel 25 mg/kg of body weight, 3 times a day for 3 days, are both WHO-recommended medicines for treatment of paragonimiasis. The former is preferred for the simplicity of its regimen, which ensures higher compliances to treatment.

The most basic public health measure that should be implemented is making triclabendazole or praziquantel available at peripheral health centres in all endemic areas for clinical management of confirmed cases.

In areas where cases appear to be clustered, treatment should be also offered to people with suspected paragonimiasis. Suspected cases are defined as individuals coming from an endemic district with a history of consuming raw crustaceans who present with any of the following characteristics:

- cough lasting for more than 3 weeks;
- bloody or rusty sputum;
- clinically or radiologically diagnosed tuberculosis with a negative sputum smear (smear-negative tuberculosis);
- poor or no response to tuberculosis treatment.

In communities and villages where cases of paragonimiasis appear to be significantly clustered, mass drug administration with triclabendazole should also be considered. The recommended regimen is 20 mg/kg of body weight in a single administration.

Complementary interventions such as information, education and communication on safe food practices, improved sanitation and veterinary public health measures should also be implemented in order to decrease rates of transmission.

Probable questions:

1. Describe the Morphology of *Diphyllobothrium Latum.*
2. What are the symptoms of diphyllobothriasis?
3. Describe the pathogenecity of diphyllobothriasis.
4. Describe the life cycle of *Paragonimus westermani.*
5. Discuss the Diagnosis, treatment and control of *Paragonimus* infection.

Suggested reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
2. Noble, E. R. and Noble G. A. (1989). Parasitology. The Biology of animal Parasites. 6th ed.
3. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology. 9th ed. McGraw-Hill International.

Unit IX

Life cycle, biology, pathogenesis, epidemiology and control of medically important helminthes - *Trichinella spiralis* and *Wuchereria bancrofti*

Objective:

In this unit we will discuss about Life cycle, biology, pathogenesis, epidemiology and control of medically important helminthes - *Trichinella spiralis* and *Wuchereria bancrofti*

- ## *Trichinella spiralis*

Trichinella spiralis is an ovoviviparous nematode parasite, occurring in rodents, pigs, horses, bears, and humans, and is responsible for the disease trichinosis. It is sometimes referred to as the "pork worm" due to it being typically encountered in undercooked pork products. Itshould not be confused with the distantly related pork tapeworm.

Description

Trichinella species, the smallest nematode parasite of humans, have an unusual lifecycle, and are one of the most widespread and clinically important parasites in the world. The small adult worms mature in the small intestine of a definitive host, such as a pig. Each adult female produces batches of live larvae, which bore through the intestinal wall, enter the blood (to feed on it) and lymphatic system, and are carried to striated muscle. Once in the muscle, they encyst, or become enclosed in a capsule. Humans can become infected by eating infected pork, horsemeat, or wild carnivores such as fox, cat, or bear.

Morphology

Males of *T. spiralis* measure between 1.4 and 1.6 mm long, and are more flat anteriorly than posteriorly. The anus can be found in the terminal end, and they have a large copulatory pseudobursa on each side. The females of *T. spiralis* are about twice the size of the males, and have an anus found terminally. The vulva is located near the esophagus. The single uterus of the female is filled with developing eggs in the posterior portion, while the anterior portion contains the fully developed juveniles.

Life Cycle

Trichinella spiralis can live the majority of its adult life in the intestines of humans. To begin its life cycle, *Trichinella spiralis* adults will invade the intestinal wall of a pig, and produce larvae that invade the pig's muscles. The larval forms are encapsulated as a small cystic structure within a muscle cell of the infected host. When another animal

(perhaps a human) eats the infected meat, the larvae are released from the nurse cells in the meat (due to stomach pH), and migrate to the intestine, where they burrow into the intestinal mucosa, mature, and reproduce. Juveniles within nurse cells have an anaerobic or facultative anaerobic metabolism, but when they become activated, they adopt the aerobic metabolism characteristics of the adult.

Female *Trichinella* worms live for about six weeks, and in that time can produce up to 1,500 larvae; when a spent female dies, she passes out of the host. The larvae gain access to the circulation and migrate around the body of the host, in search of a muscle cell in which to encyst. The migration and encystment of larvae can cause fever and pain, brought on by the host inflammatory response. In some cases, accidental migration to specific organ tissues can cause myocarditis and encephalitis that can result in death.

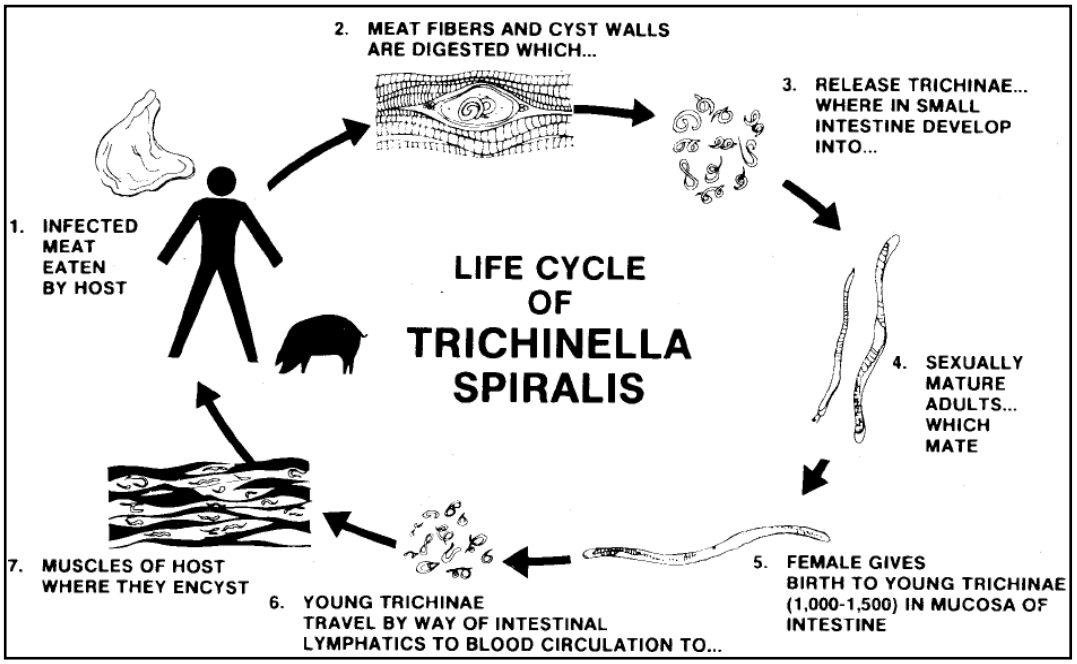


Figure: Life cycle of *Trichinella spiralis*

Trichinellosis is acquired by ingesting meat containing cysts (encysted larvae) of *Trichinella*. After exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the small bowel mucosa where they develop into adult worms (female 2.2 mm in length, males 1.2 mm; life span in the small bowel: 4 weeks). After 1 week, the females release larvae that migrate to the striated muscles where they encyst. *Trichinella pseudospiralis*, however, does not encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle. Rats and rodents are primarily responsible for maintaining the endemicity of this infection. Carnivorous/omnivorous animals, such as pigs or bears, feed on infected rodents or meat from other animals. Different animal hosts are implicated in the life cycle of the different species of *Trichinella*. Humans are accidentally infected when eating improperly processed meat of these carnivorous

animals (or eating food contaminated with such meat).

Infectious dose, incubation, and colonization

The infectious dose of trichinosis is still unknown, however predictions estimate consuming between 100 and 300 of live cysts will result in the disease. A severe infection may result from the ingestion of 1,000 larvae; however these estimates have also not been scientifically proven.

Development and colonization of *T. spiralis* occurs entirely within the host during two larval stages and one adult stage . When a host ingests the pathogen, the larvae will spend between one to seven days developing into an adult in the gastrointestinal tract. Gastric symptoms of the disease will occur during this time frame. Incubation period of larvae in the host tissue may range from one week to eight weeks, however encapsulated larvae may persist in the host for months or even years. Incubation periods vary depending on the severity and initial infectious dose. Initial colonization begins as the host ingests cysts containing larvae. During digestion, acidic compounds such as hydrochloric acid dissolve the cystic shell. The liberated larvae will pass through the stomach and invade and occupy epithelial tissues of the small intestine. Once established, the larvae will undergo four molting events to become an adult worm and mate. The female will potentially produce between 500-1,500 larvae before being expelled from the body in the stool . The larvae will then move across the intestinal wall and migrate to the rest host by lymphatic blood vessels, specifically the striated muscles. Exact mechanisms of this transfer across the intestine are unknown. Once *T. spiralis* larvae invade individual muscle cells, they adjust cellular function to accommodate their nutrient requirements thereby turning the host cell into a nurse cell. These mechanisms are still unknown today. The nurse cell will stop its lifecycle as the worm stimulates new blood vessel formation around the host cell for nutrients. *T. spiralis* larvae will also encapsulate itself during development . This incubation period generally lasts 15 days, however this is also based on severity of the infection. If left untreated, these larvae will remain in nurse cells for as long as 40 years.

Epidemiology

Outbreaks of trichinosis occur worldwide; however infections are is more common in Europe, Asia, and Southeast Asia. This disease is now endemic in Japan and China. Outbreaks are generally linked to cultural dietary preferences. The number of cases globally is estimated to be approximately 10,000, however these statistics are possibly higher due to unreported cases in countries without proper identification techniques. The mortality rate for trichinosis is approximately 0.2%. Rates of infection have dramatically decreased in the United States over the past decades. In the late 1940s, the CDC reported 400 cases of Trichinosis yearly. Based on statistics from 2010, the number of cases yearly has dropped to 20. This change can be attributed to increased

awareness of the pathogen as well as strict regulations enacted about meat preparation as well the adoption of safer pig raising methods. Currently, most reported cases of trichinosis in the United States stem from personal preparation of wild game instead of commercial production error. Outbreaks have recently occurred in Europe within the last 20 years. In 2003, Poland experienced an outbreak that caused 124 people to be hospitalized. Romania experienced a similar outbreak in 2008 that caused 108 people to be hospitalized. These outbreaks were all linked to contaminated and undercooked meat

Clinical Features

Severity and signs of symptoms vary on the number of larvae ingested by host. If the infection is minor based on the low amount of larvae ingested, *T. spiralis* may not be able to colonize and the immune system can free the infection. Symptoms may never appear or slowly intensify as larvae move to the muscles. If infectious dose is large enough, within the first week following infection gastrointestinal problems may arise including diarrhea, vomiting, cramps, or abdominal pain. As larvae migrate through the lymphatic system during the second week of infection, symptoms may include muscle pain, fever, swelling of the face or eyes, weakness, constipation or diarrhea, and splinter hemorrhages under the fingernail. These symptoms may persist for up to eight weeks with no medical attention, however larvae may survive in cells for up to 40 years. Many patients do not seek treatment due to symptoms being similar to the flu. Severe trichinosis may also become extremely debilitating. Loss of motor functions including walking, swallowing, and breathing can result from this agonizing pain . In the case of severe trichinosis, complications may arise because migrating larvae have access to the entire body through the blood stream. *T. spiralis* larvae stimulate inflammation at major organ sites including the brain, lungs, and the heart. This may result in life threatening conditions such as myocarditis, encephalitis, meningitis, nephritis, pneumonia, or bronchopneumonia.

Diagnosis

Diagnosis of the parasite during the first week of infection is challenging due to the similar secretion of enzymes, including creatine kinase and lactate dehydrogenase, which also elevate during other infections. Levels of eosinophil cells also elevate, however this is also nonspecific to trichinosis and could indicate other parasitic infections or even allergies.

Detection of antibodies developed to this parasite through tests, such as indirect immunofluorescence and latex agglutination, are the least invasive tests available. A muscle biopsy, however, is the most effective testing in diagnosing trichinosis. The deltoid muscle is most commonly used to test for *T.spiralis* larvae formation. The biopsy can also indicate the severity and stage of the disease. Complications with this test

include an incubation period between 17 to 24 days while the larvae develop, as well as a false negative test if infection rates are too low.

Treatment

Depending on the severity of the infection, trichinosis may not require medical intervention. Moderate to severe infections require medication. Within the first week of infection, the main goal of medical intervention is to limit the spread of the larvae which would lead to a systemic infection. Patients are generally administered albendazole, mebendazole, or thiabendazole, an anti-parasitic medication. This treatment is less effective after larvae invasion of the muscles. If this occurs, pain relievers may be prescribed for sore muscles or antipyretics to counteract fever. The main purpose for medication after larvae have migratedis to control and decrease muscle tissue damage.

In a case where this infection results in an allergic reaction due to chemicals being released in the muscles after larvae death, corticosteroids are usually prescribed. While steroids are helpful in regards to controlling inflammation, they may delay the expulsion of the adult worms in the intestinal lining which would result in a longer infection.

Prevention

Due to meat being the main vector of transfer, meat preparation must be monitored and controlled. This would require careful preparation of meat as well as animal feed which would include heating to higher temperatures to ensure larvae death. According to standards set by the USDA, a food thermometer should be used when cooking whole cut meats excluding wild game chicken in order to ensure the temperature reaches 145° F. This would also require a rest time of three minutes where the meat should be set out to before consumption in order to kill all pathogens as the temperature remains constant. For ground meat and wild that also excludes poultry, the meat should be cooked above 160° F with no rest time required afterwards. Poultry should be cooked above 165 °F with a three minute rest time before consumption. Regulations about freezing meats are also in place. Deep freezing meat for three weeks is shown to deactivate larvae, however larvae in bear meat are more resistant to this method. Other methods of preserving meat including curing or smoking will not ensure the meat is pathogen free. Heating meat is the most effective way to ensure larval death.

Animals destined for human consumption should be carefully managed to ensure they are not in contact with wild animals such as rats, which would put them at risk of contracting trichinosis.

▪ *Wuchereria bancrofti*

Wuchereria bancrofti is a filarial nematode that causes Wuchereriasis or filariasis (commonly called elephantiasis) in human beings. The name of this worm is given *Wuchereria bancrofti* in honor of the two scientists **Wucherer** and **Bancroft** who made a considerable contribution in studying the disease caused by these worms.

Habit and habitat

- *Wuchereria bancrofti* (Filarial worm) is a dreaded **endoparasite** of humans.
- It is a **digenetic parasite** completing its life cycle in 2 hosts. The final host is man harboring the adult worms, while the intermediate host is blood-sucking insects, the female mosquitoes of genus *Culex, Aedes*, or *Anopheles.*
- Adult worms live coiled up in the lymph glands and lymph passage of man, where they often obstruct the flow of lymph.
- The larvae i.e. microfilariae are found in the peripheral blood, occasionally they are also found in chylous urine or in hydrocele fluid.

Geographic Distribution of *Wuchereria bancrofti*:

Wuchereria bancrofti is largely confined to the tropical and sub-tropical countries of the world. However, it occurs in India, West Indies, Puerto Rico, Southern China, Japan, Pacific Islands, West and Central Africa and South America. In India, the parasite is chiefly distributed along the sea coast and along the banks of big rivers (except Indus); it has also been reported from Rajasthan, Punjab, Delhi and from various vicinities of Uttar Pradesh.

Morphology

1. Adult worm

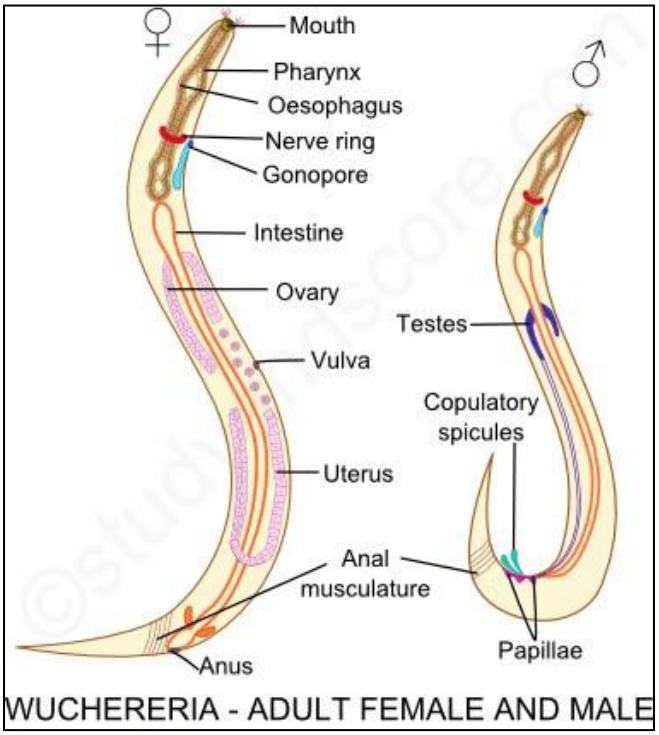
i. These are long, hair-like, transparent, translucent, thread-like worms with smooth cuticle and tapering ends.

ii. These are filiform and cylindrical in shape with both ends tapering.

iii. Sexes are separate with distinct **sexual dimorphism.**

iv. The female is larger (70–100 × 0.25 mm) than the male (25–40 × 0.1 mm).

v. The posterior end of the female worm is narrow and pointed that bears an anus, while that of the male is curved vertically and contains numbers of genital papillae two **copulatory spicules** of unequal length.



WUCHERERIA - ADULT FEMALE AND MALE

vi. Males and females remain coiled together usually in the abdominal and inguinal lymphatics and in the testicular tissues.

vii. The head end is slightly enlarged.

viii. Mouth aperture is simple, without lips.

ix. The pharynx is divisible into an anterior muscular portion and a posterior glandular portion. An oesophageal bulb is lacking.

x. The intestine is simple.

xi. The vulva of the female is located ventrally in the pharyngeal region and provided with a pyriform ejector mechanism.

xii. The female worm is viviparous and directly liberates sheathed microfilariae into the lymph.

xiii. The adult worms live for many years, probably 10–15 years or more.

2. Microfilaria

i. The first stage larva is called **microfilaria**.

ii. They are colorless, transparent bodies with blunt anterior ends and pointed tails.

iii. They are very active and can move forward and backward within the sheath which is much longer than the embryo.

iv. They are microscopic and measure about250–300 μm in length and 6–10 μm in thickness.

v. Its body is covered with a **hyaline sheath** followed by a **cuticula** being lined by flattened **subcuticular cells** or **epidermis a**nd an inner column of cytoplasm containing nuclei. Its cuticle has well-marked striations.

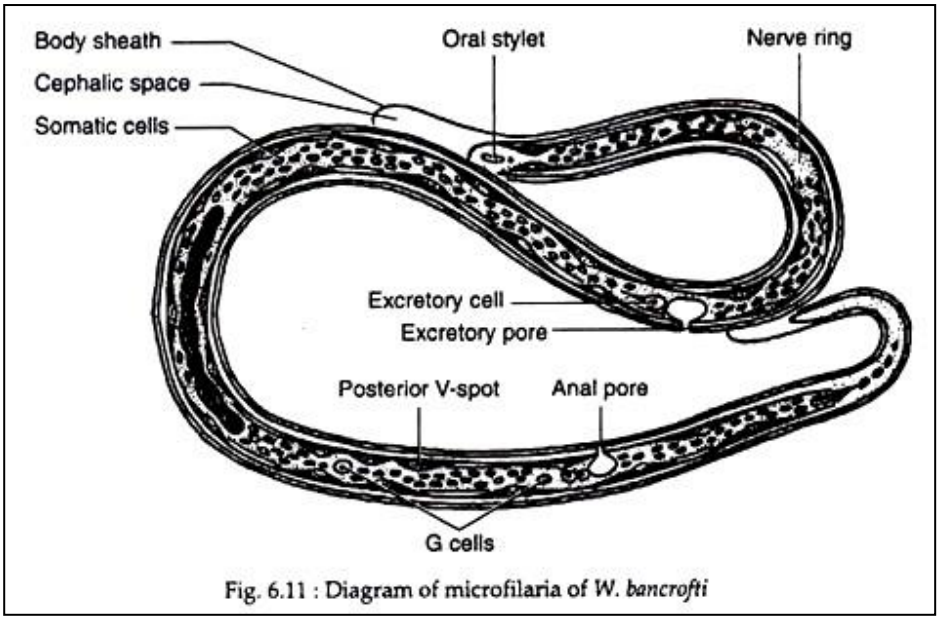


Fig. 6.11 : Diagram of microfilaria of *W. bancrofti*

vi. Somatic cella or Nuclei appear as granules in the central axis of the body extended from head to tail except for the 5% terminal end of the tip.

vii. Their head-end has clear space devoid of granules known as **cephalic space.**

viii. Important structures from the anterior end downwards are future mouth or oral stylet, nerve ring: oblique area devoid of granules, nephridiopore, renette cell, dark-colored inner mass, and 4 cells of the future anus.

ix. They do not undergo further development in the human body unless they are taken up by their suitable host (mosquitoes).

x. Their life span in the human body is probably 70 days.

3. Third stage larva (infective form)

i. The third stage larva is the infective form of the parasite is found only in mosquitoes.

ii. They are elongated, filariform, measures1.5mm in length, and 18-23 µm in diameter.

Periodicity of Microfilariae:

- ✓ The microfilariae circulate in the bloodstream.
- ✓ In India, China, and many other Asian countries, they show a **nocturnal periodicity** in peripheral circulation; being seen in large numbers in peripheral blood only at night (between 10 pm and 4 am), but they disappear inside during the rest of the day. It is believed that during the daytime they retire inside the deeper blood vessel. This correlates with the night biting habit of the vector

Culex mosquito.

- ✓ In the Pacific islands and some parts of the Malaysian archipelago, the microfilariae are **nonperiodic** or **diurnal subperiodic**, such that they occur in peripheral circulation at all times, with a slight peak during the late afternoon or evening. This is related to the day-biting habits of the local vector mosquitoes.

Life cycle

- *Wuchereria bancrofti* is **digenetic** i.e. its life history is completed in two hosts
- **Definitive host**: Man. No animal host or reservoir is known for *W*. *bancroft*i
- I**ntermediate host**: Female mosquitoes, belonging to genus Culex, Aedes, and Anopheles.
- **Infective form:** Actively motile third-stage filariform larva is infective to man.
- **Mode of transmission**: Humans get the infection by the bite of a mosquito carrying a filariform larva.

a. Copulation

Copulation takes place when individuals of both sexes are present in the same lymph gland.

b. Larval development in man

- The female worm is **viviparous** or **ovoviviparous**, it releases numerous larvae called microfilariae into the bloodstream.
- The microfilariae are born in a very immature stage. However, they find their way into the lymph vessels, soon enter into blood vessels and circulate with blood showing active movements.
- They do not undergo further development until sucked by the intermediate host. i.e. female mosquitoes.
- Microfilariae show nocturnal and diurnal periodicity in the blood of man.
- During the daytime, the microfilariae tend to stay in the deep blood vessels of man. But, at night time they travel near the surface in peripheral or superficial blood vessels. This behavior enables them to get ingested by the night biting mosquito(*Culex* and *Aedes*) which serves as an intermediate host.
- These microfilariae circulate in the blood for 6 months to 2 years and then die if not taken by a mosquito.

c. Development in mosquito

- Microfilariae are sucked from the peripheral blood of man.
- Microfilariae lose their sheath within 2 to 6 hours in the stomach of the mosquito

and then they penetrate the stomach wall and within 4 to 17 hours migrates to thoracic muscles or wing musculature where they undergo metamorphosis and grow.

- In the next 2 days, they become short and thick like sausages having short spiky tails and measures 124 to 250µm in length and 10 to 17µm in diameter, also possess rudimentary digestive tract. These are **first-stage larvae (L1).**
- Within the next 3 to 7 days they grow rapidly and moult once or twice to become the **second stage larvae (L2)** which measure 225 to 33oµm in length and 15 to 3o µm in diameter.
- Metamorphosis finally completes by 10-11 days into **third-stage filariform larvae (L3)** which measure about 1500 to 2000 µm in length and 18-23 µm in diameter.
- The third stage larvae are actively motile and infective.
- These larvae migrate through the hemocoel to the mosquito's labium (proboscis).

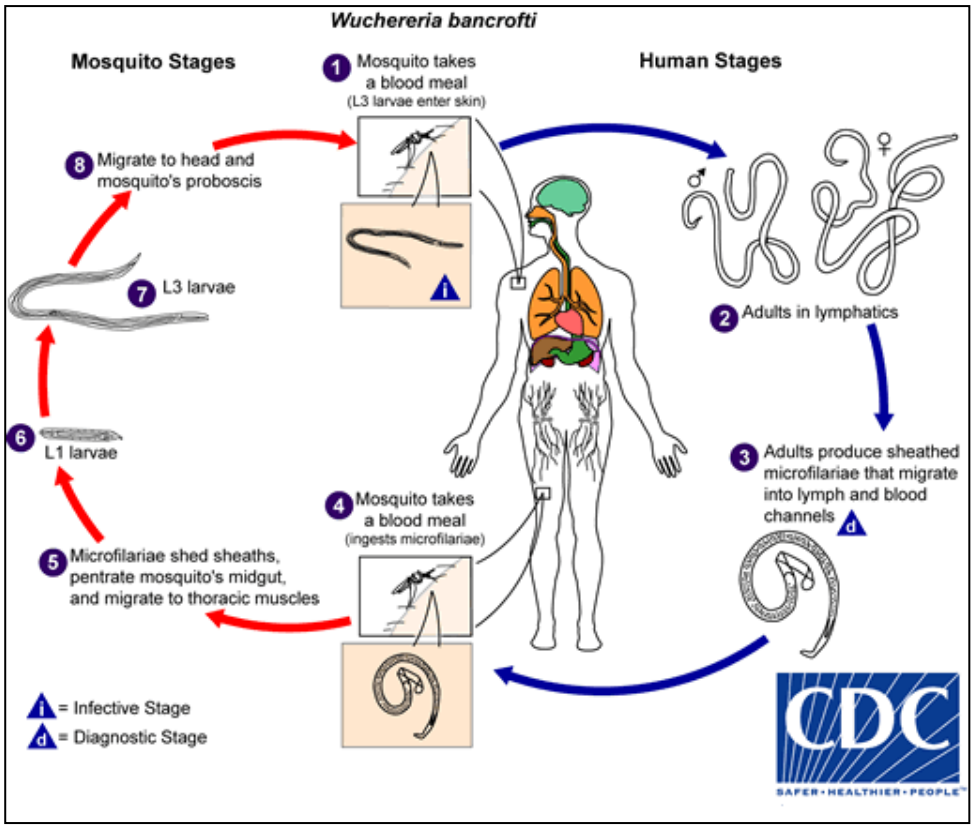


Fig: Life cycle of *Wuchereria bancrofti*

d. *Infection of the new human host*

- When this infected mosquito pierces its proboscis in the warm and moist skin of man, the larvae creep out of labium to human skin,
- Then, it penetrates the skin and finally come to settle down into lymphatics.
- In the lymph vessels, they grow and become fully adult and sexually mature within a

period of 5 to 18 months.

- The sexually mature worms start reproduction to repeat life history.

Pathogenesis of *Wuchereria bancrofti*:

The pathogenic effect of *Wuchereria bancrofti* is produced by the adult either living or dead. Light infection produces no serious symptoms. It causes filarial fever, head ache and mental depression. In heavy infection, the adult, living or dead blocks lymphatic vessels and glands, resulting in various pathological conditions. When the disease has caused the enlargement of such organs as the scrotum, breasts or legs, it is called elephantiasis. The adult worm causes an inflammatory reaction of lymphatic system or lymphangitis, obstruction of lymph in the organs results in their enlargement called lymphedema. The enlargement of lymphatic glands also leads to lymphadentis. Adult and microfilariae produce lesions in lymph node and also granulomas in spleen.

Occasionally hyperplasia of muscle fibres may be observed. Symptoms of filarial infection include fever, tenderness of infected parts, eosinophilia inflammation and transient swelling. Anxiety caused by fear is of considerable importance. Thick blood smears are preferable in examinations for microfilariae.

Treatment of Disease Caused by *Wuchereria bancrofti*:

No proper medicine is available to eradicate the filaria worms. However, following filarial drugs can be used.

1. Mel, W. It is an arsenical preparation effective on adult worms.

2. Heterazan compound (Diethyl carbamaide) can be used to check microfilariae.

3. MSb (Paramelaminyl phenyl stibonate) is effective on infective larvae and immature adult worm.

Control of Disease Caused by *Wuchereria Bancrofti*:

1. Eradication of mosquito by insecticide or by other control measures.

2. Reducing infection amongst mosquitoes.

3. Treatment of carriers by using heterozon and cyanine.

4. Movement from areas of infection.

5. Protection from mosquitoes, using mosquito net or mosquito repellent while sleeping in night, and

6. Control of breeding of mosquitoes.

Probable questions:

1. What do you mean by ovoviviparous nematode?
2. Write down the life cycle of *Trichinella spiralis.*
3. What is the incubation process of *Trichinella spiralis?*
4. What is trichinosis?
5. *What is the causative agent of trichinosis?*
6. *Discuss the structure of microfilaria.*
7. Write down epidemiology of *Wuchereria bancrofti.*
8. How elephantiasis can be controlled?

Suggested reading:

1. Chakraborty P. 2016.. Textbook of Medical parasitology, 3rd edition. New Central Book Agency.
2. Chatterjee K D. 2009. Parasitology: Protozoology and Helminthology. XIII Edition, CBS Publishers
3. Paniker CKJ, Ghosh S. 2013. Paniker's Text Book of Medical Parasitology. Jaypee
4. Gerald D, Schimdt & larrey S. Roberts' Foundations of Parasitology, 9th Edition

Unit X

Salient features of plant parasitic nematodes and life cycle patterns of *Meloidogyne hapla* and *Anguina tritici*

Objectives:

In this Unit we will discuss on salient features of plant parasitic nematodes and life cycle patterns of i) *Anguina tritici*, ii) *Meloidogyne hapla*.

Introduction:

Nematodes are the most highly developed of the pseudocoelomates. They belong to the phylum Nematoda, previously named Phylum Nemathelminthes or Phylum Aschelminthes. They are widely present in the soil, fresh water or marine water.

Nematodes are the only plant parasites belonging to the animal kingdom which are studied in plant pathology Nematodes, sometimes called eelworms, are worm-like in appearance but quite distinct taxonomically from the true worms- Numerous species of nematodes attack and parasitize man and animals and cause various diseases. Several hundred species are known to feed on living plants as parasites and cause a variety of plant diseases.

- ***Anguina tritici***

Anguina tritici (ear-cockle nematode, seed-gall nematode, seed and leaf gall nematode, wheat gall nematode, wheat seed gall nematode, wheat seed-gall nematode, wheat seed and leaf gallnematode) is a plant pathogenic nematode.

Hosts:

Emmer (*Triticum monococcum*), rye (*Secale cereale*), spelt (*T. spelta*), and wheat (*T. aestivum*). Barley (*Hordeum vulgare*) is a very poor host. There is no evidence that this nematode reproduces on oats (*Avena sativa*) and other grasses.

Geographical Distribution:

Reported from Afghanistan, Australia, Brazil, Bulgaria, China, Egypt, Ethiopia, Hungary, India, Iran, Iraq, Israel, Lithuania, New Zealand, Pakistan, Poland, Romania, Russian Federation, Russian Far East, Syria, Switzerland, Turkey, and Yugoslavia. Early records of nematode detection in the US include California, Georgia, Maryland, New York, North and South Carolina, Virginia and West Virginia. Recent surveys of the wheat seed gal nematode in stored grain harvested from states with records of this nematode have not

provided any evidence that nematodes are still occurring in the USA.

Morphology

It is a large nematode, ranging from 3–5 mm in length. *Anguina tritici has a three part* esophagus and the esophageal glands do not overlap with intestine. The female body tends to be thickened and curved ventrally. It has a short stylet (8-11 µm). Females have one ovary and the vulva located posterior. Males possess small spicules and small bursae or alae.

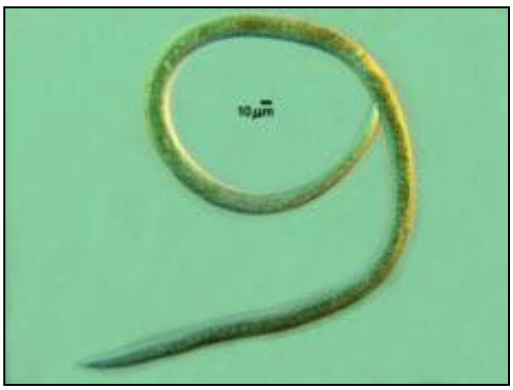


Fig: Morphology of *Anguina tritici*

Notes on Taxonomy and Biology

Anguina tritici female show a well developed anterior branch of the ovary which is folded in two or more flexures and a conoid tail, tapered to an obtuse or round tip. This species is closely related to *A. funesta* and *Subanguina wevelli*. The morphological separation of these three species is difficult. Recent molecular diagnostic techniques have facilitated the separation of these three species. J2 emerge from the seed galls in the soil and crawl onto the newly germinated seedlings. They establish infection sites between young leaves where they feed as an ectoparasite causing leaf distortion and crinkling. Later, they penetrate the flower buds at the time of flower bud initiation. J2 stimulate the formation of galls in floral tissues in place of seed development. Juvenile development is completed inside the galls. Newly formed females deposit eggs, which hatch producing J2, which remain, encased in the galls (cockle) and perpetuate plant infection in following years. Dried cockles are harvested with developed seeds. *Anguina tritici* vectors a bacterium *Clavibacter tritici*, which is the causal agent of yellow ear rot or tonduof wheat. Freshly harvested infected wheat cockles containing the bacterium are toxic to cattle and sheep.

Juveniles find host and move up the plant in a film of water, they invade meristems and penetrate inflorescence. Once in the developing seed they molt, become adults, mate, and reproduce. Eggs laid by the female develop and hatch as J2 within the seed gall where they desiccate and become dormant. Dormant J2 overwinter in the seed galls

until spring. They are released when galls come in contact with moist soil and hydrate. Total life cycle is completedin 113 days.

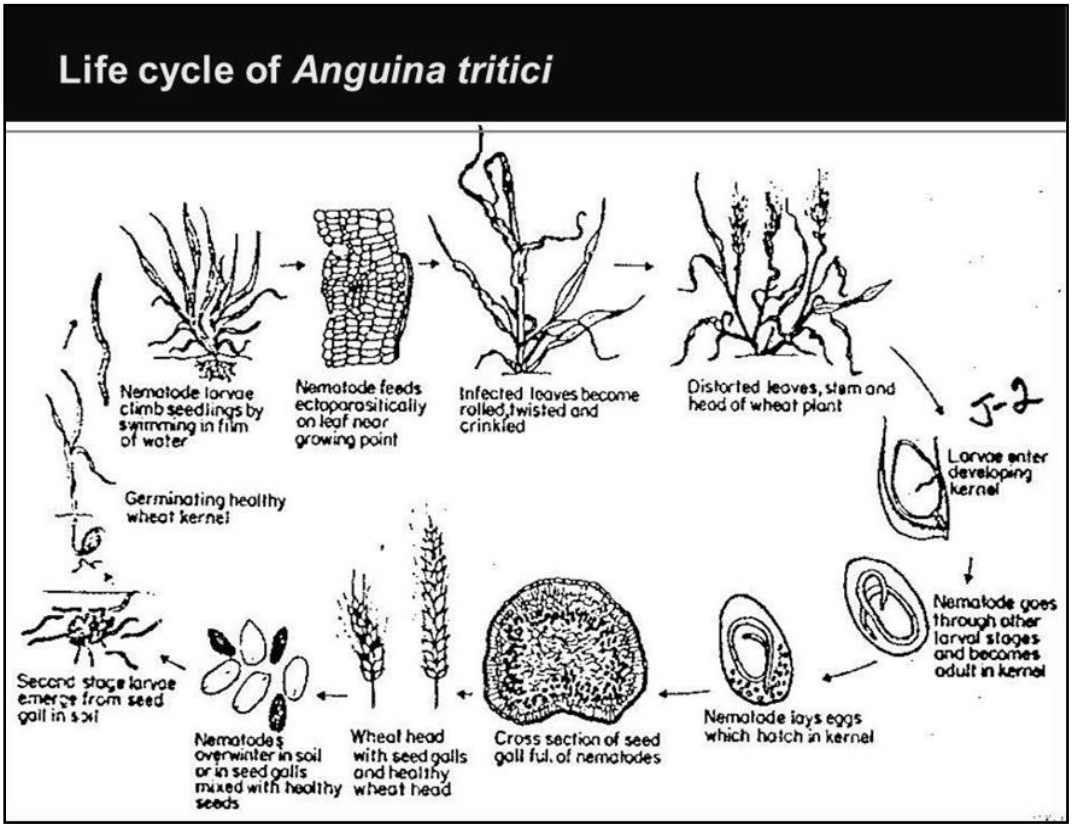


Fig: Life cycle of *Anguina tritici*

Host parasite relationship

It is an ectoparasite that becomes endoparasitic invading inflorescence and developing seeds. It causes a disease called “ear-cockle”, “gout” or seed gall on wheat and rye. It is not a host of oat, maize and shorgum. On wheat it causes stunted plants, distorted leaves, seeds are transformed into galls which contain a dried mass of nematodes. If compared to normal wheat seeds, galls are smaller in size, lighter, and their color ranges from light brown to black(normal wheat seeds are tan in color).

Symptoms

The absence of symptoms does not mean absence of *A. tritici*. Slight elevations occur on the upper leaf surface with indentations on the lower side. Other symptoms include wrinkling, twisting, curling of the margins towards the midrib, distortion, buckling, swelling and bulging. A tight spiral coil evolves, and dwarfing, loss of colour or a mottled, yellowed appearance and stem bending may also occur. In severe infection, the entire above-ground plant is distorted to some degree and a disease problem is usually obvious. Heads (spikes).Wheat heads are reduced with glumes protruding

at an abnormal angle exposing the galls to view. This does not occur in rye heads.

Galls

Young galls are short-thick, smooth, light to dark green, turning brown to black with age, 3.5-4.5 mm long and 2-3 mm wide. Rye galls are small, buff-coloured and longer than wide, 2-4.5 mm long by 1-2.5 mm wide.

Prevention and Control

Ear-cockles are the only source for perpetuation of the disease and their removal from contaminated seed lots can completely eradicate the disease. *A. tritici* has been eliminated, or reduced to a minimal number of infestations, in Europe and the USA by seed cleaning, crop rotation and fallow.

Cultural Control and Sanitary Methods of Seed cleaning:

Salt brine method: seed is poured into a salt solution (8 lbs salt in 5 gallons water) and stirred vigorously. Sound galls sink, and debris and galls float to the surface. The galls and debris are skimmed from the surface and steamed, boiled or chemically treated to kill the nematodes. The salt solution is drained into another container and the cleaned seed is rinsed several times in fresh water to remove salt and then spread in thin layers on a clean surface to dry. The cleaned seed is ready to sow when dry. It is important that the seed is washed two or three times in plain water after brine treatment to remove salt particles which may impair germination.

- **Crop rotation**

A. tritici cannot survive in soil for more than 1 year if the soil is left fallow or planted to anon-host crop. The pest will be eliminated in more than a year.

- **Physical Control**

i. Hot-water treatments:

Hot-water treatments may be used to eradicate *A. tritici* from seed lots. Marcinowski (1910) demonstrated that nematode galls in a seed lot could be destroyed by keeping the mixture in water at 54-56°C for 10-12 minutes. Pre-soaking the seeds before the hot-water treatment hasalso been advocated.

Another hot-water treatment involves pre-soaking the seed at 21-27°C for 2-4 hours, then placing them in water for 30 minutes at 50°C. The seeds are rinsed in tap water, then spreadin thin layers on a clean surface till dry.

ii. Mechanical separation:

Jones et al. (1938) developed an indented cylinder machine which separated oval wheat seeds from globular nematode galls; the device was claimed to be 98% effective in removing the seed galls. Chu (1945) also designed a machine to separate nematode galls

from healthy grain.

iii. Nematicidal Plants

Nematicidal plants are not as effective as the clean seed or fallow method and offer little hopeas an effective method of controlling *A. tritici*.

- **Biological Control**

There are few reports concerning biological control of *A. tritici*.

iv. Host-Plant Resistance

A large number of plants have been evaluated for resistance to *A. tritici* over a period of more than 60 years. A few resistant plants have been found, such as the wheat cultivar however, resistance does not appear to be a viable solution to the problem of seed gall nematodes.

Crop Losses:

Nematode damage is negligible in countries adopting modern mechanical and cleaning procedures to separate the nematode galls from visible wheat seeds. The use of high quality seeds has nearly eradicated this nematode from developed countries. However, the nematodes causes severe crop losses to rye (35- 65%) and wheat (20-50%) in 3rd world countries, where poor agricultural practices, monoculture, and the use of poor quality seeds are widespread. In spite of the insignificant damage caused by the nematode in modern agricultural production systems of developed countries, their ability to export grains in the international markets is severely hampered if historical records still exist of the presence of this pest in grain production areas due to the quarantines imposed by many countries because of this pest.

Means of Movement and Dispersal: Through the characteristic dark seed galls harboring the nematode juveniles in harvested grains.

▪ *Meloidogyne hapla* (Root knot nematode)

Meloidogyne hapla was first described from the USA by Chitwood (1949). The type host was *Solanum tuberosum* and the type locality was Long Island, New York, USA. No synonyms are known, although some of the records attributed to this species refer to other species such as *Meloidogyne chitwoodi*. **Root-knot nematodes** are plant-parasitic nematodes from the genus ***Meloidogyne***. They exist in soil in areas with hot climates or short winters. About 2000 plants worldwide are susceptible to infection by root-knot nematodes and they cause approximately 5% of global crop loss.[1] Root-knot nematode larvae infect plant roots, causing the development of root-knot galls that drain the plant's photosynthate and nutrients. Infection of young plants may be lethal, while infection of mature plants causes decreased yield. **Northern root-knot nematode**

(*Meloidogyne hapla*) is a species of vegetable pathogens which produces tiny galls (a sore caused by friction and abrasion) on around 550 crop and weed species. They invade root tissue after birth. Females are able to lay up to 1,000 eggs at a time in a large egg mass. By surviving harsh winters, they can survive in cold climates (hence, the name, **Northern**).

Distribution

M. hapla is widely distributed, particularly in temperate regions and the cooler, higher altitude areas of the tropics. According to Whitehead (1969), *M. hapla* only flourishes at high altitudes above 6000 feet in East Africa (Kenya, Tanzania and Uganda), despite the abundance of host plants at lower altitudes. In Queensland, Australia, *M. hapla* was not found as far north as *M. javanica* (Colbran, 1958). Taylor and Buhrer (1958) reported that in the USA, *M. hapla* was the commonest root-knot nematode north of 39°N.

The distribution in this summary table is based on all the information available. When several references are cited, they may give conflicting information on the status. Further details may be available for individual references in the Distribution Table Details section which can be selected by going to Generate Report.

Description

Female: Body pyroid with short neck. Cuticle becoming thicker in posterior half of body, sometimes considerably. Head with two annules behind head-cap. Spear knobs rounded, inconspicuous. Excretory pore 14-20 annules behind head, hemizonid just posterior to pore. Posterior cuticular pattern roughly circular, composed of closely spaced smooth or slightly wavy striae. Dorsal arch low. Lateral fields may be unmarked, may be marked only by slight irregularities in the striae, or dorsal and ventral striae may meet at a slight angle along the fields. Some forking of striae at lateral fields may also occur. In some cases ventral striae may extend laterally on one or both sides to form 'wings' which the dorsal striae meet almost at right angles. Tail with few striae but distinct punctuations forming a stippled area between the anus and tail terminus. Sometimes the stippling may be more diffuse over the inner part of the pattern. Phasmids fairly widely spaced.

Male: Numerous in some populations, absent in others. Head not offset, a truncate cone to hemispherical in outline. Usually only one annule behind head-cap. Spear slender, spear knobs rounded and not offset. Anterior cephalid on second body annule, posterior cephalid just anterior to level of relaxed spear. Hemizonid 45-58 annules behind head, 0-4 annules anterior to excretory pore. Lateral field with four incisures. Tail terminus bluntly rounded; phasmids at about cloacal level. One or two testes. Spicules slightly curved, with small sharp processes projecting from the spicule wall at the junction of head and shaft into the spicule head. Gubernaculum crescentic, proximal end thicker

than distal end.

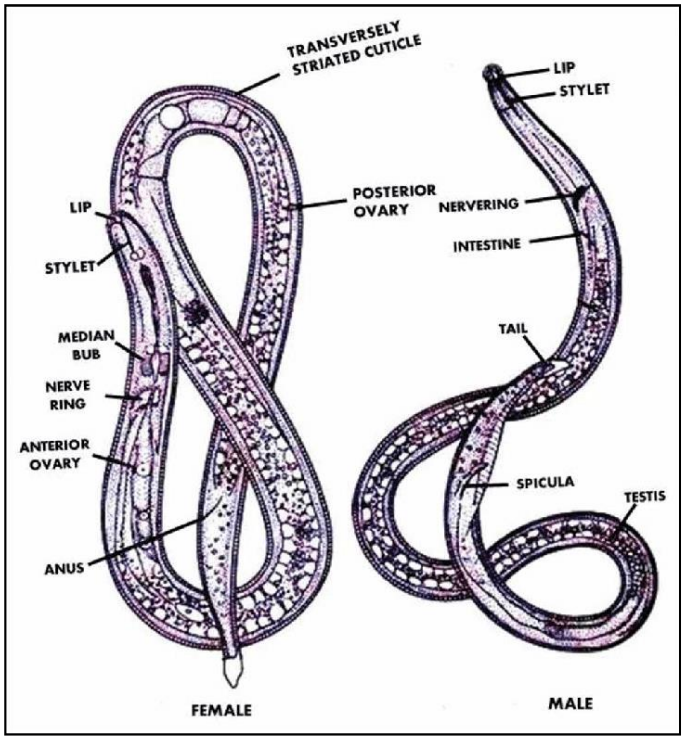


Fig: Morphology of *Meloidogyne* sp.

Biology and Ecology

All nematodes pass through an embryonic stage, four juvenile stages (J1–J4) and an adult stage. Juvenile *Meloidogynes* parasites hatch from eggs as vermiform, second-stage juveniles (J2), the first moult having occurred within the egg. Newly hatched juveniles have a short free-living stage in the soil, in the rhizosphere of the host plants. They may reinvade the host plants of their parent or migrate through the soil to find a new host root. J2 larvae do not feed during the free-living stage, but use lipids stored in the gut.

An excellent model system for the study of the parasitic behaviour of plant-parasitic nematodes has been developed using *Arabidopsis thaliana* as a model host. The *Arabidopsis* roots are initially small and transparent, enabling every detail to be seen. Invasion and migration in the root was studied using *M. incognita*. Briefly, second stage juveniles invade in the root elongation region and migrate in the root until they became sedentary. Signals from the J2 promote parenchyma cells near the head of the J2 to become multinucleate to form feeding cells, generally known as giant cells, from which the J2 and later the adults feed Concomitant with giant cell formation, the surrounding root tissue gives rise to a gall in which the developing juvenile is embedded. Juveniles first feed from the giant cells about 24 hours after becoming sedentary.

After further feeding, the J2s undergo morphological changes and become saccate. Without further feeding, they moult three times and eventually become adults. In females, which are close to spherical, feeding resumes and the reproductive system

develops. The life span of an adult female may extend to three months, and many hundreds of eggs can be produced. Females can continue egg laying after harvest of aerial parts of the plant and the survival stage between crops is generally within the egg.

The length of the life cycle is temperature-dependent. The relationship between rate of development and temperature is linear over much of the root-knot nematode life cycle, though it is possible the component stages of the life cycle, e.g. egg development, host root invasion or growth, have slightly different optima. Species within the *Meloidogyne* genus also have different temperature optima. In *M. javanica*, development occurs between 13 and 34 °C, with optimal development at about 29 °C.

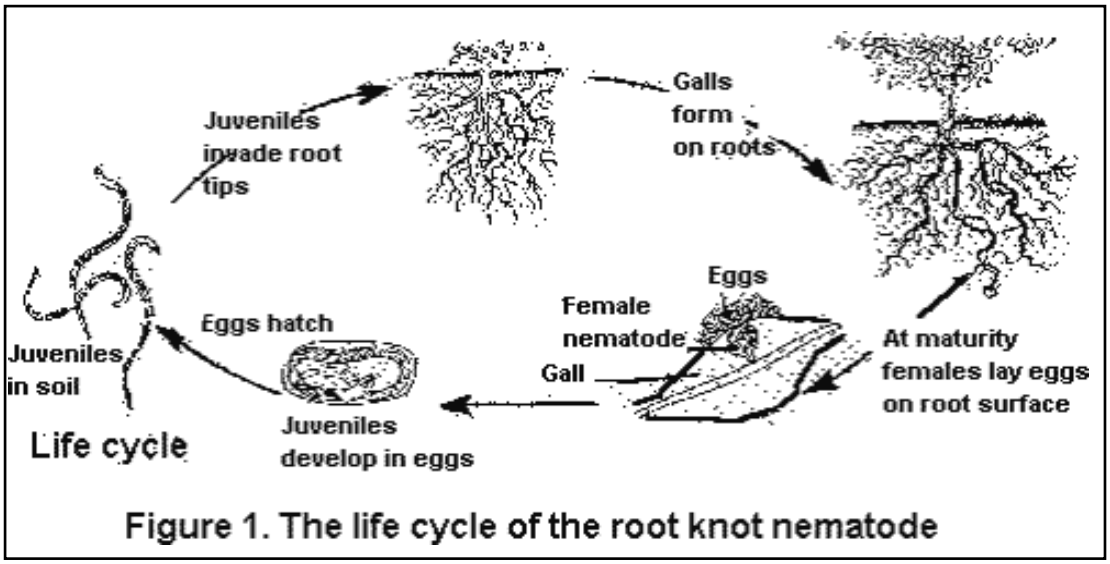


Fig: Life cycle of root knot nematode

Symptoms/signs

Leaves- abnormal colours

Roots- galls along length Roots- reduced root system

Whole plant- dwarfing and early senescence

Typical symptoms of attack include a galling of the root system, the galls being relatively small and subspherical, often with a marked proliferation of small roots at the site of the gall (this is in contrast to the symptoms caused by other common species of *Meloidogyne*). In potato tubers, brown spots appearing in the tubers after the females commence egg production may identify infection sites. Severe attack by *M. hapla* results in impaired root function and concomitant stunting of the above ground parts leading to a reduction in yield.

Prevention and control

Various methods have been used to cleanse planting material, including hot water treatment and a range of nematicidal drenches. Treatment in the field also relies upon the application of nematicides although frequent rotation with cereals or other graminaceous non-host crops may also be efficacious. Glasshouse soils may be fumigated to eradicate the pest. Many crops have potential for development of resistant or tolerant varieties.

Economic Impact

Root-knot nematodes (*Meloidogyne* spp.) are one of the three most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed worldwide, and are obligate parasites of the roots of thousands of plant species, including monocotyledonous and dicotyledonous, herbaceous and woody plants. The genus includes more than 90 species, with some species having several races. Four *Meloidogyne* species (*M. javanica, M. arenaria, M. incognita, and M. hapla*) are major pests worldwide, with another seven being important on a local basis. *Meloidogyne* occurs in 23 of 43 crops listed as having plant-parasitic nematodes of major importance, ranging from field crops, through pasture and grasses, to horticultural, ornamental and vegetable crops. If root-knot nematodes become established in deep-rooted, perennial crops, control is difficult and options are limited.

Meloidogyne spp. were first reported in cassava by Neal in 1889. Damage on cassava is variable depending on cultivar planted, and can range from negligible to serious. Early-season infection leads to worse damage. In most crops, nematode damage reduces plant health and growth; in cassava, though, nematode damage sometimes leads to increased aerial growth as the plants try to compensate. This possibly enables the plant to maintain a reasonable level of production. Therefore, aerial correlations to nematode density can be positive, negative or not at all. Vegetable crops grown in warm climates can experience severe losses from root-knot nematodes, and are often routinely treated with a chemical nematicide. Root-knot nematode damage results in poor growth, a decline in quality and yield of the crop and reduced resistance to other stresses (e.g. drought, other diseases). A high level of damage can lead to total crop loss. Nematode-damaged roots do not use water and fertilisers as effectively, leading to additional losses for the grower. In cassava, it has been suggested that levels of *Meloidogyne* spp. that are sufficient to cause injury rarely occur naturally. However, with changing farming systems, in a disease complex or weakened by other factors, nematode damage is likely to be associated with other problems.

Probable questions:

1. Describe the structure of root knot nematode.
2. Describe the life cycle of root knot nematode.
3. Describe the control measures of ear cockles.
4. Discuss the pathogenesity of Ear cockles.

Suggested reading:

1. Cheng, T. C. (1986). General Parasitology. 2nd ed. Academic Press, Inc. Orlando.U.S.A.
2. Noble, E. R. and Noble G. A. (1989). Parasitology. The Biology of animal Parasites. 6th ed.
3. Roberts, L. S., Janovy, J. and Nadler S. (2013) Gerald D. Schmidt &Lary S. Roberts'Foundation of Parasitology. 9th ed. McGraw-Hill International.

HARD CORE THEORY PAPER (ZCORT – 102)

ZCORT – 102 (Parasitology, Ecology, Environment and Wildlife Biology)	**Group B (Ecology, Environment and Wildlife Biology)**			
	XI	The Ecosystem: Gaia hypothesis, cybernetic nature and stability of the ecosystem, ecosystem management and optimization. Macroecology: concept and consequences. Principles of Thermodynamics, energy flow and ecological energetics.	**3**	130-159
	XII	Niche theory: Concept of habitat and niche; niche width and overlap; fundamental and realized niche; resource partitioning; character displacement. (Lotka-Volterra model, Isoclines, Niche prediction)		160-177
	XIII	Community: Structure and Gradient analysis, Structure of biotic community. Community patterns: diversity and stability. Community boundary: Ecotone and edge types, Edge effect and edge species, Leibig's Law of the minimum.		178-191
	XIV	Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate, gross reproductive rate, vital index. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.		192-201
	XV	Metapopulation: Concept, models, structure and dynamics		202-210
	XVI	Major terrestrial biomes: Theory of island biogeography, Biogeographical zones of India		211-224

	XVII	Concept of Environment: Structure, radiation balance, UN movements on environment.		225-234
	XVIII	Theory and analysis of conservation: Stochastic perturbations, population viability analysis, recovery strategy for threatened species: Different approaches for conservation – in-situ and ex-situ, In-situ conservation- problems and prospects; Sanctuaries, National parks, Community Reserves and Conservation Reserves; Biosphere Reserve, EIA, EIS.		235-254
	XIX	Conservation biology of important wild animals: Conservation status, habit & habitat, threats and conservation management of the following animals: iv) Olive Ridley Turtle v) Greater one-horned *Rhinoceros* vi) Ganges river dolphin.		255-266
	XX	Basic Concept of Wildlife Biology: Wildlife wealth of India; Threatened wildlife and IUCN status - Concept of Extinct, Critically Endangered, Endangered, Vulnerable and rare species; concept of corridor.		267-273

Group-B:

Ecology Ecology, Environment and Wildlife Biology

Unit XI

The Ecosystem: Gaia hypothesis, cybernetic nature and stability of the ecosystem, ecosystem management and optimization. Macroecology: concept and consequences. Principles of Thermodynamics, energy flow and ecological energetic

Objective:

In this chapter we will discuss about The Ecosystem: Gaia hypothesis, cybernetic nature and stability of the ecosystem, ecosystem management and optimization. Here we will also discuss about Macroecology: concept and consequences. Principles of Thermodynamics, energy flow and ecological energetic

Introduction:

The ecosystem is the basic functional unit in ecology, since it includes both biotic and abiotic environment each influencing the properties of the other & both necessary for maintenance oflife as well as we have it on the earth.

Some examples of the natural ecosystems are ponds, lakes, oceans, grasslands, forests, deserts tundra & so on. An ecosystem be it a pond, forest, desert or at tundra has the following four components:

1. **The non living environment:**

Which is called abiotic environment includes air, water, soil and the basic elements. There, substances enter into the body of the environment and performs metabolic activity and again return to the environment. Abiotic factor could be divided into three-

a. Physical (climate) factors: Such as temperature, relative humidity.
b. In organic substances like water, C, N_2, S, P and so on.
c. Organic substances: such as proteins, carbohydrates, lipids and humic substances.

The organic substances linked the living body with the biotic and abiotic components. In the terrestrial ecosystem the diversity of the organisms determined by the climate, soil etc. In high altitude the availability of O_2 is important. In aquatic ecosystem temperature, salinity, dissolved gases, chemical substances etc determine the specific

diversity. In arctic & tropics climatic factors greatly affect the distribution of the organisms.

2. Producers:

Producers are energy transducers which convert solar energy into chemical energy with the help of inorganic substances such as water, CO_2, and enzymes. These producers are autotrophic (auto-self, troph - nourishing) organisms mainly green plants like trees, grasses, phytoplanktons etc. They possess a green pigment (Chlorophyll) and transducts solar energy and are known as photoautotrophs, while the chemoautotrophs use energy generated in an oxidation reduction process. eg: S-bacteria

3. Consumers:

Which are heterotrophic (hetero = other, trophic = nourishing) organisms. Depending upon their food habits consumer may be herbivores (Plant eaters) and Carnivores (Flesh eaters).

Macro consumers or phagotrophs (Phago = to eat) ingest other organisms or particulate matters.

Micro consumers are saprotrophs (sapro = to decompose) or osmotrophs (Osmo = to pass through membrane). They are mainly bacteria & fungi, which breakdown the complex compounds of dead. Their protoplasm absorb some of the decomposition products.

Herbivores include insects, zooplanktons, deer, cattle elephants etc. The carnivores are grouped into 1st order, 2nd order and so on depending upon their food habit.

4. Decomposer:

Decomposers are actually the micro consumers. They breakdown complex organic matter like cellulose, hemicelluloses, chitin etc of plant and animal body and fungi. But some invertebrates like protozoa, oligochaetes (earthworm) euchytraeid etc use the dead organic matter for their food and hence they are also grouped into decomposers.

Another two category subdivisions for heterotrophs suggested by Wiegert and Owens (1970) is as follows.

i. Biophages, organisms consuming other living organisms.

ii. Saprophages, organisms feeding on dead organic matter.

From the functional standpoint an ecosystem may be conveniently analysed in terms offollowing:

i. Energy circuits.

ii. Food chain.

iii. Diversity patterns in time & space.

iv. Nutrient cycles.

v. Development and evolution.
vi. Cybernetics.

The Gaia hypothesis

The Gaia means The Greek goddess “Mother Earth”. James Lovelock was the pioneer of the concept. But James Lovelock and Redfield contributed independently the gaia concept. Lovelock published a readable little book entitled “Gaia: A New look at life on earth” which is his own words, is a personal account of journey through space & time in search of evidenceto substantiate this model of earth.

In develops word, “The Gaia hypothesis states that the biosphere is a self regulating entity with the capacity to keep our planet healthy by controlling the chemical and physical environment.” In other words, the earth is a super-ecosystem with numerous interacting functions and general feedback loops that tolerate extremes of temperature and oceans relatively constant.

Feedback Loop

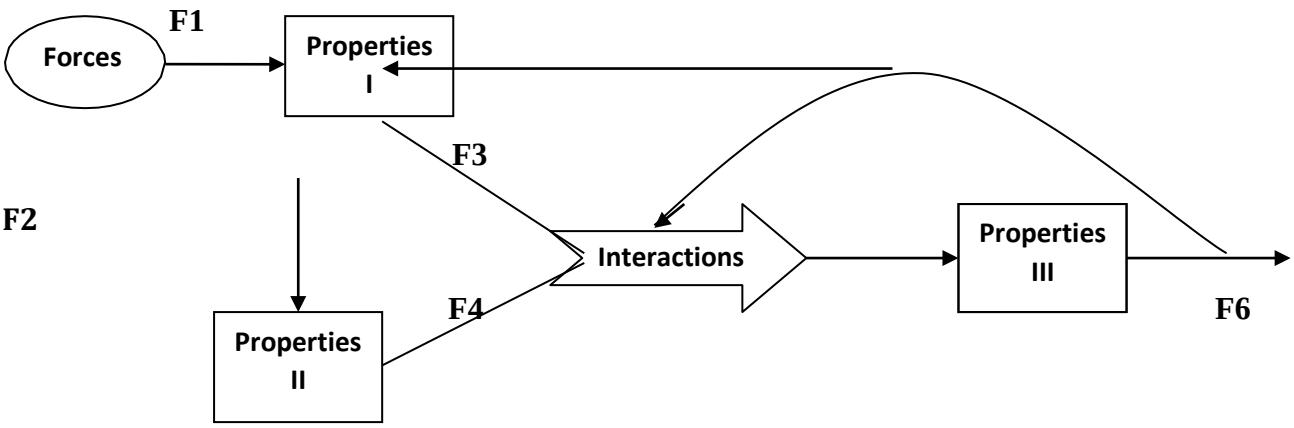


Figure: Diagram showing the five basic components that are of primary intern is modelling ecosystems.

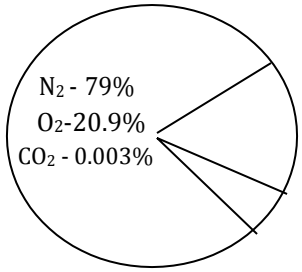
The most controversial past of the hypothesis is that the biotic community plays the major role in biosphere homeostasis and organisms began to establish soon after the first life appeared more than a billion years ago. The contrary hypothesis is that purely geological (abiotic) processes produced condition favorable for life, which then merely adapted to these conditions.

The question is that did physical conditions evolve first then life or did both evolve together? The primary atmosphere was formed from gases (Outgas rising from the hot core of the earth by the process called outgassing).

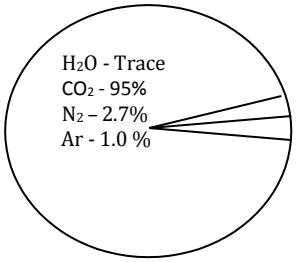
While volcanoes continue to affect climates, the secondary atmosphere is produced and according to Gaia hypothesis, is a biological product.

This reconstruction, as it were, began with the first life and thus the primitive microbes are that do not require oxygen – the anaerobes. When the green anaerobic microbes began to put oxygen into the air, the plants and animal that require gaseous oxygen, the aerobes, evolved. The anaerobes retreated to the O_2 less depth of soils & sediments, where they continue to thrive & play a major role in various ecosystems. The cooling of earth that resulted from the removal of CO_2 from the atmosphere by lime-stone forming marine organisms.

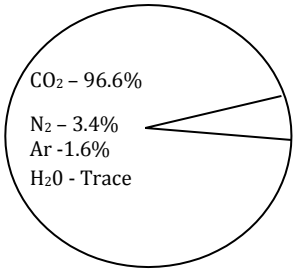
Comparison of the atmosphere of the earth with that of the planets Venus & Mars, where of there in life it is restricted to anaerobic microbes, provides strong indirect evidence for the Gaia hypothesis.



Earth



Mars



Venus

The low CO_2, High O_2 and N_2 atmosphere of the earth is completely opposite from the conditions of the nearby planets. Since photosynthesis, which evolved soon from after the first appearance of life, removes CO_2 and add O_2 in the atmosphere? It is logical to conclude that the biotic community is responsible for the building up of O_2 & reduction in CO_2 over time. Recently, many geochemical geochemist assumed without much direct evidence that O_2 came solely from the breakdown of water vapour & the escape of H_2 into space, leaking an excess of O_2 behind.

It is also difficult to explain how gaseous N_2 could accumulate in the atmosphere in absence of life. But biological transformations, nitrogen would go its most stable form, nitrate ion dissolved in the oceans. The N_2 cycle clearly demonstrated that biotic community does not just borrow gases from the atmosphere and return them unchanged but after their chemistry inways that are beneficial to life.

A variety of special microorganisms (N_2-fire and denitrification) play major roles in keeping the vital compounds (such as NH_3, H_2O, N_2 & H_2) moving in an orderly manner between biotic and abiotic states. Without the critical buffering activities (to the acedic environment) of early life forms and the continued coordinated activities of plants & microbes that dampen fluctuations in physical factors, conditions on earth, according to Lovelok & Margulis, would be similar to current conditions on Venus: very hot, with no O_2 in the atmosphere.

Cybernetic Structure & Stability of the Ecosystem

Ecosystems are capable of self maintenance and self regulation as their component populations and organisms. Thus cybernetics (from kybernetes = pilot or governor). The science of control has important application in ecology especially since man increasingly tends to disrupt natural controls or attempts to substitute artificial mechanisms for natural ones. Homeostasis (Homeo = same, Stasis = Standing) is the term generally applied to the tendency for biological systems to resist change and to remain in a state of equilibrium.

According to the Gaia hypothesis, the biosphere is a highly integrated self organized cybernetic or controlled system. But cybernetics at the biosphere level is not accomplished by external goal oriented set point controls (like thermostat, chemostat or other mechanical feedback) but the control is internal & diffuse, involving hundreds of thousands of feedback loops & synergistic interactions in subsystems such as the microbial network controls the N_2 cycle.

Waddington (1975) coined the term HOMEORHESIS (from the Greek meaning "Maintaining the flow") to denote evolutionary & ecological stability as opposed to homeostasis, the widely used term for physiological stability at the organism level. The simplest form of control system consists of two black boxes (A black box may be defined as any unit whose functions may be evaluated without specifying the internal contents).

Set point or "STANDARD"

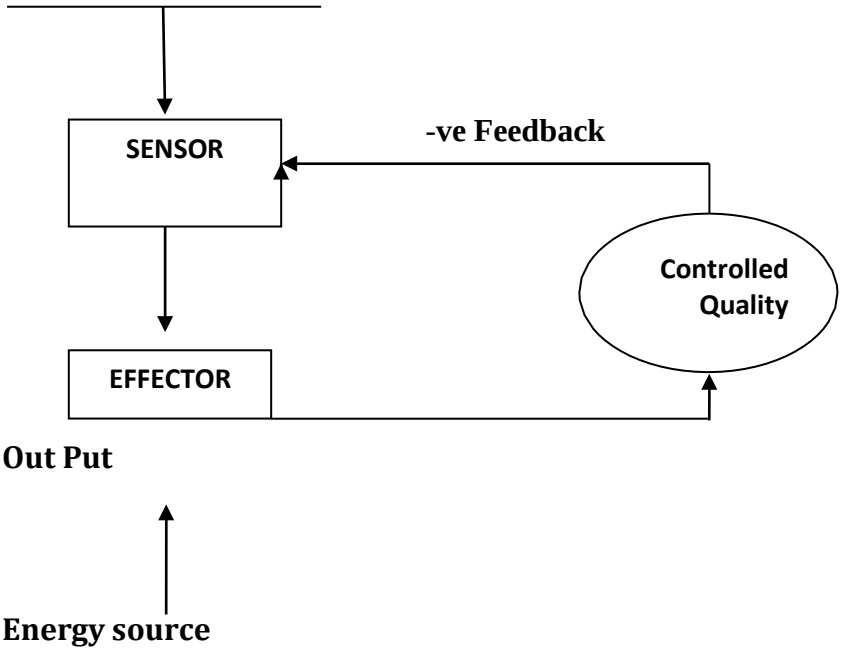


Figure: A simple control system, analogus to a house hold thermostat, in which some of the output is used as –ve feedback to maintain some kind of equilibrium into controlled quantity.

And a controlled quantity interconnected by output & input circuit or signals. When this feedback input is +ve. The quantity grows which is necessary for growth & survival of organisms. Again –ve feedback and the limits of homeostatic control can be plotted.

The +ve feedback involved in the expansion of knowledge power and productivity threatens the quality of human life and environment unless adequate –ve feedback controls can be found. The science of controls or cybernetics, thus becomes one of the most important subjects in practice.

Some population are regulated by density which "feedback" by way of behavioral mechanisms to reduce or increase the reproductions rate (the effectors) and thus maintain the population size (controlled quality) within set limits. But in other populations like human do not seem to be capable of self limitation but are controlled by outside factors.

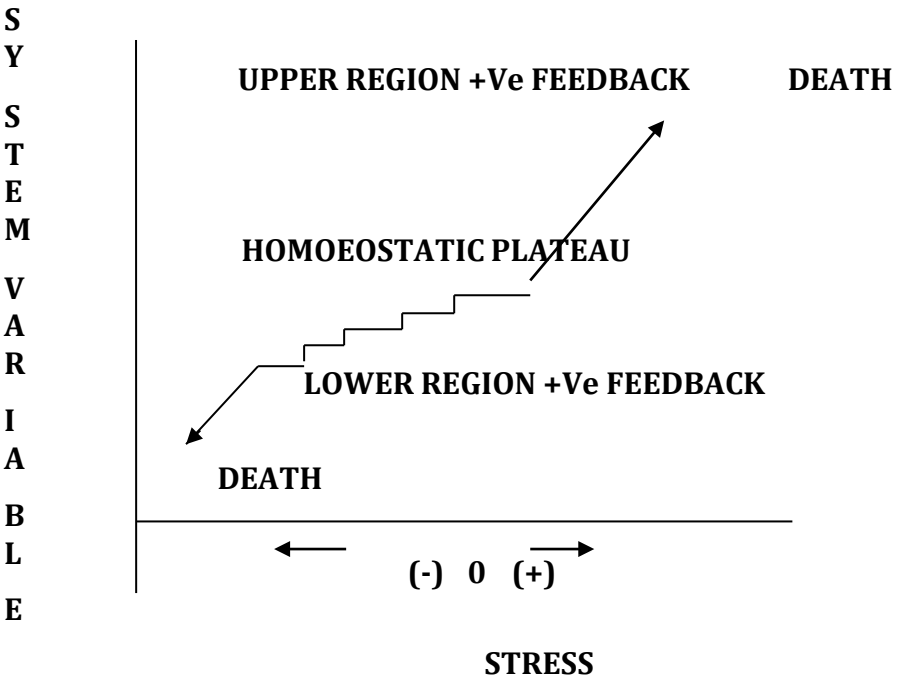


Figure: The concept of the homeostatic plateau within which relative constancy is maintained by –ve feed back despite tendency of stress to cause deviation. Beyond limits of homeostasis +ve feed back results in rapid destruction of the system (Hardin 1963).

The enter play of material cycles (storage & release of nutrients) and production & decomposition of organic substances) and energy flows in large ecosystems generate & a self correcting homeostasis with no outside control or required set point.

The homeostatic mechanisms have limits beyond which unrestricted +ve feedback leads to death. There is a series of levels of steps in the homeostatic plateau.

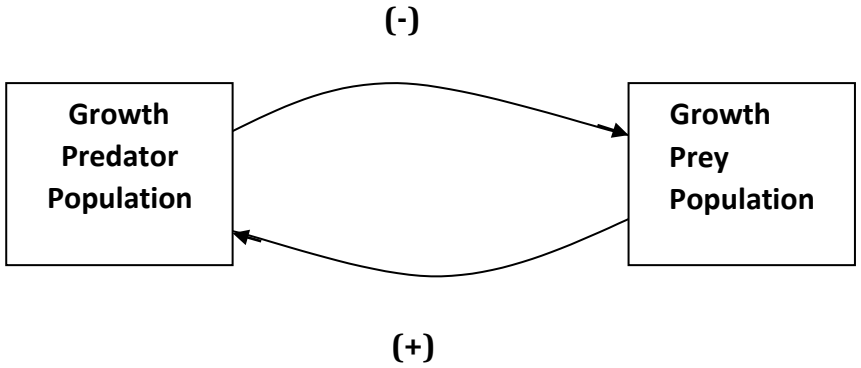


Figure: The interaction of +ve & -ve feedback in a predator prey feedback loop system.

Really good homeostatic control comes only after a period of evolutionary adjustment and thus the system actually becomes stable. New ecosystems or new predator prey assemblage tend to oscillate violently.

As a result of the evolution of the central nervous system, mankind has gradually become the most powerful organism and is able to modify the operation of ecosystem.

Because of the absence of set point controls, many scientists are doubting that ecosystems and the biosphere really function as cybernetic systems, although most accept the concept that organisms play major roles in the control of the chemistry of the atmosphere and the oceans (Kerr 1988). The fact that catastrophic events, such as come to crushing into the earth, massive volcanic eruptions, and glaciers, have occurred from time to time rises questions about global homeostasis. Yet despite a loss of species during these geological & cosmic upheavals, life has not only persisted but has continued to diversify & play a role in restoring favorable conditions for it self. However, just become the biosphere has exhibited the resilience stability to recover in past ages is no reason to be complacent or satisfied about the resilience (flexibility) of our current life support system. Humans as a species might not survive a human-made catastrophic such as nuclear war or fortification (defense) of the oceans, and even if we did survive, all our hardened cultural & life style gains would bewiped out.

Ecosystem Management & Optimization

The proper functioning of natural ecosystem as interacting & indicated biological units in liable to artificial change and disruption as a consequence of various anthropogenic activities such as agriculture, forestry and fisheries. Man made activities can cause changes in the nature and extent of the 4 dynamic phenomena of organic productions and breakdown energy flow, nutrient cycling and water movement involved in the ecosystem functioning. As a result of man's activities the plant, animal and microbial communities of a given ecosystem can be significantly modified and show also the environment characteristic of the system. For example – soil erosion is a main consequence of indiscriminate deforestation.

Proper management of the ecosystem is often hampered by lack of adequate knowledge of the functional relationships within ecosystems and also of their numbers form and kind etc. Most ecosystems at some stages on the other are subjected to actual or man made shocks, calamities, traumatic stresses etc. Those who can survive such stresses are the one's ended within the capacity to tolerate these shocks, that is they manifest a kind of internal resilience. This resilience is a determinate of the magnitude of the disturbance that can be tolerated by the ecosystem before it would shift into a basically different behavior.

The concept of persistence or resistance & resilience are the component parts of the concept of ecosystem stability. Desert ecosystem in general show low persistence or resistance but high resilience. Ecosystem stability constitutes one of the main unifying concepts of ecology. There are several features which contribute to stability of ecosystem.

i. Tolerance to extreme & harsh conditions.
ii. Ability for rapid recovery upon recurrence of favorable growth condition.
iii. Flexible & opportunistic feeding habits.
iv. Nomadic migrations of animals etc.

Likewise some destabilizing features include:

i. Sensitivity to damage to reserves.
ii. Sensitivity to lagging components.
iii. Low density, biomass & productivity.
iv. Sensitivity to soil erosion.

Of course biotic & human features are highly significant in affecting the stability of theecosystem.

Optimizations:

The term 'optimizations' implies that as a consequence of Natural Selection & evolution, organisms tend to have a combination of form & function, that is optimal for growth & reproduction in their particular environment. So, the principle of optimization may not however become translated into reality in all cases. Parkhurst & Loucks (1972) have listed 3 reasons that may possibly explain such a failure to attain optimization-

i. Inadequate time in a given environment.
ii. The environment does not remain constant for a long enough period of time.
iii. Omission or lack of certain important variables.

According to Parkhurst & Loucks (1972) optimization works mostly at the organic level but according to Cody (1974) it is applicable at the level of the ecosystem. In fact it is only to be expected that Natural selection would ensure survival of the fittest phenotypes. Cody's model to explain optimization explains the following areas:

i. Higher resource availability tends to favour specialist feature over generalist where as lower resources favour generalist over specialist.
ii. A mix of similar resources favors generalist over specialist, where as dissimilarresources favor specialist.
iii. The addition of a competing species favors a wide range of specialist.
iv. At higher premium or breeding greater co-existence occurs.

Holling (1973) believes that natural system could have multiple domex (habitats) or attraction and that system persistence possibly is more dependent on the ability to move from one domain to another rather than on the dynamics of a given domain.

The principle of optimization is however not universally accepted based on work of

bioenergetics and dynamics of birds in a grassland biome. Innis (1974) finds it difficult to rationalize optimization and states. The determination of an objective function such that observed phenomena optimize that function subjected to can strains is not a 'vacuous enterprise'. On the other hand Katz and Batnick (1974) distinguished between dynamic long term and static (instantaneous) optimizations in ecosystems, the former differing from the later in that it considers the effect of decisions made at one time upon the status of the system at a later time and optimizes a long term objectives by choosing these variables which change throughout the long period of time. In static optimizations, only a single time period is considered and the relationship between this particular time period and other time period is ignored. Fisheries, pest management and phorasing are the example of dynamic optimizations.

Resistance stability indicates the ability of an ecosystem to resist perturbation.

Resilience stability indicates ability to recover when the system disrupted by a perturbation.

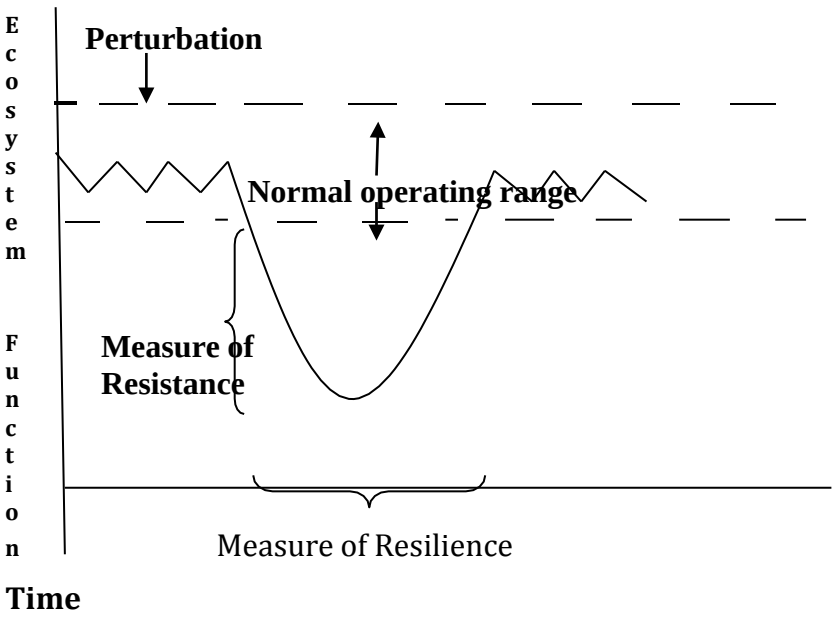


Figure: Resistance & resilience stability

When a perturbation causes a major ecosystem function to deviate from the normal operations range, the degree of deviation is a measure of relative resistance. While time required for recovery is a measure of relative resilience.

Principles of Thermodynamics, energy flow and ecological energetic

Study of Thermodynamics:

The study of thermodynamics is assemblage of matter and energy that are called systems. Classical thermodynamics enquires into the alterations in the content of energy and its distribution that take place when a system passes from an initial state into a terminal state at equilibrium.

The state of a system can be defined in terms of its temperature, pressure and composition, and when both temperature and pressure of a system are kept constant, energetic changes are directly related to changes in composition.

Two most important systems of classical thermodynamics are:

(i) The isolated system, and

(ii) The closed system.

An isolated system is completely insulated from its surroundings and is both materially and energetically self-contained. A closed system though materially self-contained, is freely able to exchange energy with its surroundings. In closed system temperature and pressure (T and P) are constant.

In biology classical thermodynamics is concerned with the reaction taking place at constant temperature and pressure. The systems plus the surroundings constitute the universe.

Units of Measuring Energy:

The most common unit of energy used in thermodynamics is the calorie (1 cal = 4.184 J). The SI unit employed in thermodynamics is the Joules (J) or kilo-joules (1 kJ = 1000J).

The energy content of a system is related to the standard quantity of the compound, i.e., 1 mol., as both are directly proportional. Values of energy terms expressed in cal/mole are converted to values in J mol^{-1} by multiplying by 4.184. Conversely, to convert) mol^{-1} to cal/mole it is divided by 4.184.

The Laws of Thermodynamics (Conservation of Energy):

(i) First Law of Thermodynamics:

It states that the total energy of an isolated system is constant though within the system it may change its form. Or, in all chemical and physical changes, energy is neither created nor destroyed but is only transformed from one form to another. Or, in any process the total energy of the system and its surroundings remains constant. Or, nobody can get something for nothing.

Thus change in an isolated system can neither lead to an increase nor a decrease in the intrinsic energy of the system, but it can only redistribute the intrinsic energy in different forms.

A closed system involves both re-distribution of energy within the system and transfer of energy between the system and its surroundings. Thus whatever reaction takes place in a closed system, the total internal energy content of that system and its surroundings must be constant.

If a closed system (T and P constant) having initially a total internal energy content E_1, undergoes some change resulting in a different intrinsic energy E_2 the change in energy $\Delta E = (E_2 - E_1)$, is an equal but opposite change in the intrinsic energy content of its surroundings.

The energetic interaction between the system and its surroundings is achieved by (i) thermal transfer, and (ii) performance of work. If the reaction in a closed system brings a change in its volume, then by its volume change (ΔV m^{-3}), the system obligatorily performs work on the surroundings equal to $- w_{obligatory} = - P\Delta V$ J mol^{-1}.

The minus sign is introduced as it conventionally considers that energy is released from the system to its surroundings that are utilized for the work done. Various other types of work may optionally be performed by the reaction, so that the total energy lost by the system in the performance of all types of work (both obligatory and optional) may be summed in one term as – w J mol^{-1}.

The thermal transfer between the system and its surroundings is designated as q J mol^{-1}. Thus in a closed system (T and P constant), any change in intrinsic energy (ΔE_{system} J mol^{-1}) is the outcome of heat exchanged and work performed; i.e.

$\Delta E_{system} = E_2 - E_1 = (q - w)$ J mol^{-1}.

Heat is a reflection of random molecular motion, whereas work is defined as the distance moved under the influence of force associated with organised motion.

Force may be of different forms like the gravitational force exerted by one mass on another, the expansional force exerted by a gas, the fissional force exerted by a spring or muscle fibre, the electrical force of one charge on another, and the dissipative forces of friction and viscosity.

The intrinsic energy (E) of a system is the characteristic of the system that depends on the present state and is independent of its previous history. So the intrinsic energy is the so-called function of state. There are three further energetic functions of state of the system and its surroundings namely, enthalpy, entropy and free energy.

Enthalpy:

Enthalpy is a new thermodynamic quantity (**Greek:** enthalpein, to warm in) and is abbreviated H. It is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. For a reaction in closed system (T and P constant) it has been established that,

$$\Delta E = (q_p - w)$$

where, q_p = heat acquired by the system from the surroundings at constant pressure

$-w$ = work done by the system on its surroundings

$= -(w_{obligatory} + w_{optional})$

Now, $w_{obligatory} = -P\Delta V$, when $-w_{optional} = 0$.

So, $\Delta E = (q_p - P\Delta V)$

or, $q_p = (\Delta E + P\Delta V)$

This $(\Delta E + P\Delta V)$ is the change in enthalpy (H), i.e.

$$(\Delta E + P\Delta V) = \Delta H.$$

which is defined as the quantity of heat absorbed by a closed, isothermal system when at constant pressure it undergoes a change of state without performing any work associated with its change in volume. Thus enthalpy of a system consists of the internal energy (E), plus the absolute pressure (P) multiplied by the volume (V).

When a chemical reaction releases heat into the surroundings, it is said to be exothermic. The heat content of the products is less than that of the reactants and ΔH has a negative value.

Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH. For a chemical reaction, the sign and magnitude of ΔH is largely attributable to the energy changes associated with the making and breaking of chemical bonds.

The value of ΔH depends only on the initial and final states of the system and is totally independent of the mechanism of the reaction. This is the basis of Hess's Law of Constant Heat Summation, which states that the thermal transfer in a particular reaction is the same whether it is accomplished in one or several stages.

For example, the value of ΔH for the reaction P → S is – 4.5 kJ mol^{-1} when this is taking place at certain fixed temperature and pressure. If the same reaction takes place in a stepwise manner, the total magnitude of ΔH for the contributory reactions will equal the value of ΔH for the overall reaction. Thus if,

$$P \rightarrow Q \qquad \Delta H = (a)$$
$$Q \rightarrow R \qquad \Delta H = (b)$$
$$R \rightarrow S \qquad \Delta H = (c)$$

since ΔH = – 4.5 kJ mol^{-1} for the overall reaction P → S, then (a) + (b) + (c) = – 4.5 kJ mol^{-1}.

(ii) Second Law of Thermodynamics:

It states that any system, and its surroundings, tends spontaneously toward increasing disorder. Or, heat cannot be completely converted into work without changing some part of the system. Or, no real process can be 100 per cent efficient. Or, in any energy conversion some energy is transferred to the surroundings as heat.

Entropy:

The measure of randomness or disorder is termed entropy (S) coined first by Rudolf Clausius in 1851. The term literally means **"a change within".** It is the outcome of the second law. It is a function of state, and any change in state of a system is associated with a change of entropy AS = (S_{final} – $S_{initial}$). Entropy is essentially a mathematical function with no physical analogue involving statistical and probability considerations.

It can be defined as S = k In w, where k is a proportionality constant (Boltzmann's constant), and In w is the natural logarithm of w, the thermodynamic probability.

It is the number of ways in which the system can be microscopically arranged without changing its macroscopic features. The S value of an isolated system is an index of its intrinsic stability. The greater the entropy of the system the more stable is this and the smaller is its capacity for spontaneous change.

Entropy is state or condition not only of energy status but also of matter. For example, an aerobic organism extracts free energy from glucose, taken from its environment. The energy is released from the glucose molecule through oxidation with molecular oxygen, also obtained from the surroundings.

The oxidized products, CO_2 and H_2O, are returned to the surroundings leading to an increase in entropy, whereas the organism itself remains in a steady state without changing its internal order. The entropy arises from heat dissipation as well as from molecular disorder.

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

One molecule of glucose and 6 molecules of oxygen are more randomly dispersed into 12 molecules ($6CO_2 + 6H_2O$) through oxidation. Thus through chemical reactions number of molecules increases. When a solid substance is converted into liquid or gas, they have more freedom to move or fill space than a solid.

(iii) Third Law of Thermodynamics:

It assumes that perfect crystals of all compounds possess zero entropy at the absolute zero (OK). The entropy of each compound increases with the increase in temperature. The quantity is measured in JK^{-1} mol^{-1}. If the entropy of a system and its surroundings increases during a process, then it is a spontaneous process.

The criterion for spontaneous process in a closed system can be revealed as

$\Delta S_{system} + \Delta S_{surroundings}$ = a positive value.

If the value is zero (0) the total (isolated) system remains throughout at equilibrium, i.e., when the entropy reaches its maximum level for a system and its surroundings, the system is said to be at equilibrium.

The spontaneous processes are irreversible without an input of energy into the system that increases entropy in the surroundings. For example, perfume molecules do not spontaneously condense back into an open bottle. All natural processes that take place at a significant rate are thermodynamically irreversible.

Free Energy:

The entropy changes are of limited usefulness in predicting the direction and the equilibrium position of chemical reactions.

The 2nd Law's criterion of spontaneity, i.e., feasibility of independent occurrence of reaction in a closed system (T and P constant) is inconvenient as it compels us to measure the entropy changes in both the system itself and its surroundings. But entropy cannot always be directly measured or calculated in chemical processes.

A more useful criterion than the entropy change has been derived for predicting the direction and the equilibrium position of chemical reactions, namely, the change in free energy, that form of energy capable of doing work under constant temperature and pressure.

The thermodynamic measure of the maximum energy available for conversion to work (at constant temperature and pressure) is called the Gibbs free energy symbolized as G, after John Willard Gibbs who drew attention to its significance in systems maintained at constant pressure.

The free-energy change of a reacting system related the entropy (at constant temperature and pressure), through the following equation:

$\Delta H - T\Delta S = \Delta G$

where ΔG is the free energy change of the system, ΔH is the change in enthalpy, T is the absolute temperature, and AS is the change in entropy.

The criteria of spontaneity of reaction in a closed system can briefly be expressed as follows:

(a) When the reaction proceeding at measurable rates under conditions of thermodynamic irreversibility, i.e., the system is not already at equilibrium

$\Delta G < 0$ (i.e., negative)

(b) In the conditions of thermodynamic reversibility, i.e., at equilibrium

$\Delta G = 0$

So, in any closed system (T and P constant) which is not at equilibrium, only exergonic reactions (-ΔG) can occur spontaneously. If under given conditions a reaction A + B → C + D is endergonic (+ ΔG), then the reverse reaction C + D → A + B will be exergonic (-ΔG) and could proceed spontaneously.

Standard free energy change can be determined by the following equation:

$\Delta G^\circ = - RT \ln K_{eq} = - 2.3 RT \log K_{eq}$

where, ΔG^o = standard free energy change in joules (J) or calories (cal)

R = the ideal gas constant (8.304 J mol^{-1} K^{-1})

T = absolute temperature (K)

In = natural logarithm

K_{eq} = equilibrium constant; the subscript **"eq"** denotes reactant and product concentration at equilibrium:

= concentration of products multiplied together/concentration of reactants multiplied together

When one mole of each reactant is converted to one mole of each product, maximum standard free energy change occurs. In other words, the standard free energy change, ΔG°, is the change in free energy when the concentration of reactants and products is 1M.

The above equation is one of the most useful links between thermodynamics and biochemistry and has a host of application. The equation is easily modified to allow computation of the change in free energy for concentrations. In the reaction A + B ↔ C + D, the actual change in free energy, ΔG, is given by the equation

$$\Delta G = \Delta G^{\circ} + RT \ln \frac{[C][D]}{[A][B]}$$

where the brackets refer to the concentrations at the time of the reaction. The value of ΔG is a function of the displacement of the reaction from equilibrium. It is central to an understanding of bioenergetics.

The equilibrium constant depends on temperature that can be sure by rearranging the equation:

$$\ln K_{eq} = \frac{-\Delta H^{\circ}}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^{\circ}}{R}$$

where, H° and S° represent enthalpy and entropy in standard state. The above equation has the form y = mn + 6, the equation for a straight line. A plot of In K_{eq} versus 1 /T is known as Van't Hoff plot. From the measurements of K_{eq} at different temperatures the values of ΔH°, ΔS° and ΔG° can be determined more easily than measuring by calorimetry.

In biochemistry the standard free energy changes of reactions are conventionally symbolized by ΔG°' instead of ΔG° used in physical chemistry. If a reaction includes neither H_2O, H^+, nor an ionizable species, then ΔG°' = G°.

Living organisms are open systems and can never be at equilibrium. They take up nutrients from the environment and release waste products and generate work and

heat. They continuously ingest high enthalpy and low entropy nutrients which they convert to low enthalpy, high entropy waste products.

The free energy released in this conversion powers the cellular activities that produce the high degree of organizational characteristics of life. If the flow of conversion processes is interrupted anyhow, the system ultimately reaches equilibrium causing death.

So, the living organisms maintain a steady state meaning that all the flows in the system are constant so that the system does not change with time. Similarly energy flow in the biosphere is an example of a system in a steady state. In all living systems the flow of energy is always downhill ($\Delta G < 0$).

Example 1:

The hydrolysis of ATP liberates its terminal phosphate group. At 309K and pH 7 in the presence of Mg^{+2} ions, it was calculated that

ΔH was – 20.08 kJ mol^{-1}, ΔS was + 35.21 JK^{-1} mol^{-1}. Calculate the corresponding value of ΔG of the reaction.

For an isothermal reaction at constant pressure in a closed system,

$\Delta G = \Delta H - T\Delta S$

For the given reaction,

ΔG =?

ΔH = – 20080 J mol^{-1}

T = 309K

ΔS = + 35.21 J K^{-1} mol^{-1}

Substituting these values in the above equation,

ΔG = – 20080 – (309 x 35.21) J mol^{-1}

or, ΔG = – 20080 – 10880 J mol^{-1}

= – 30.95 k J mol^{-1}

Example 2:

ΔG^o of glucose and ethanol in aqueous solution equals to – 917.0 and – 181.6 k J mol^{-1} respectively, and ΔG_f° of carbon dioxide as a gas is – 394.5 k J mol^{-1}. Deduce ΔG° for the net reaction of alcoholic fermentation.

Glucose → 2 Ethanol + 2 CO_2

In aqueous solution (at 298K) with the evolution of gaseous CO_2.

Since the value of ΔG° are additive,

ΔG^o = (sum of values of ΔG_f° of products) – (sum of values of ΔG_f° of reactants).

Therefore,

$$\Delta G^\circ = (2 \times \Delta G_f^\circ \text{ ethanol in aqueous solution} + 2\Delta G_f^\circ \text{ of } CO_2 \text{ as gas}) - (\Delta G_f^\circ \text{ glucose in aqueous solution})$$

$$= \{(2 \times -181.6) + (2 \times -394.5)\} - (-917.0) \text{ k J mol}^{-1}$$

$$= -1152.2 + 917.0 \text{ k J mol}^{-1}$$

$$= -235.2 \text{ k J mol}^{-1}$$

$$\therefore \Delta G^\circ \text{ for the net reaction} = -235.2 \text{ k J mol}^{-1}$$

Energy flow

Energy has been defined as the capacity to do work. Energy exists in two forms potential and kinetic.

Potential energy is the energy at rest {i.e., stored energy) capable of performing work. Kinetic energy is the energy of motion (free energy).

It results in work performance at the expense of potential energy. Conversion of potential energy into kinetic energy involves the imparting of motion.

Energy flow in Ecosystems:

Living organisms can use energy in two forms radiant and fixed energy. Radiant energy is in the form of electromagnetic waves, such as light. Fixed energy is potential chemical energy bound in various organic substances which can be broken down in order to release their energy content.

Organisms that can fix radiant energy utilizing inorganic substances to produce organic molecules are called autotrophs. Organisms that cannot obtain energy from abiotic source but depend on energy-rich organic molecules synthesized by autotrophs are called heterotrophs. Those which obtain energy from living organisms are called consumers and those which obtain energy from dead organisms are called decomposers (Fig. 3.7).

When the light energy falls on the green surfaces of plants, a part of it is transformed into chemical energy which is stored in various organic products in the plants. When the herbivores consume plants as food and convert chemical energy accumulated in plant products into kinetic energy, degradation of energy will occur through its conversion into heat. When herbivores are consumed by carnivores of the first order (secondary consumers) further degradation will occur. Similarly, when primary carnivores are consumed by top carnivores, again energy will be degraded.

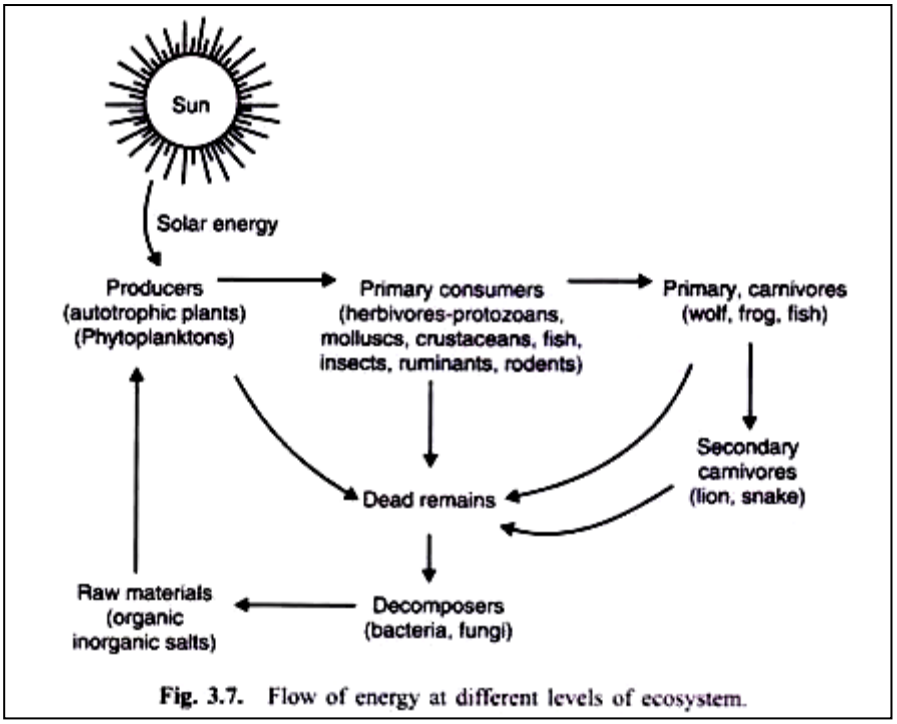


Fig. 3.7. Flow of energy at different levels of ecosystem.

Ecological energetic

The flow of energy through an ecosystem is called ecological energetic. Energy is the ability to do some work. The sun emits light and other types of radiation. Some of these radiations fall on the earth. The light radiation falls on some object. The Object absorbs light and become warm. Thus light energy is transferred into heat energy. Land and oceans absorb heat. It produces heat and cold areas on the earth. It causes flow of air and clouds.

The source of energy on the earth is the sun. A small amount of the radiant energy is absorbed by the green plants. They change this energy into chemical energy in the form of glucose. All the other living organisms obtain this chemical energy from the plants. A large amount of energy is lost in the form of heat.

Only a small part of the solar radiation is used during photosynthesis. This stored energy provides energy to living components of the ecosystem. Green plants absorb blue and red part of the spectrum. However, only I to 5% of the absorbed energy is converted into food energy. The energy used once by an organism is soon lost in the form of heat. There is always one way flow of energy. The flow of energy through ecosystem follows laws of thermodynamics.

It has been estimated that about 57% of the solar energy is absorbed in atmosphere. It is scattered in space. About 36% is absorbed by water or land. About 8% falls on plants. 80 to 85% is absorbed by green plants and rest of it is reflected. About 50% of the absorbed energy is used in photosynthesis i.e. it is stored in the form of potential energy of organic compounds of green plants.

The bioenergetics is discuss under three headings; (a) Productivity of ecosystem, (b) Food chain, Trophic levels and food web and (c) Ecological pyramids

Trophic level:

The producers and consumers in ecosystem can be arranged into several feeding groups, each known as trophic level (feeding level). In any ecosystem, producers represent the first trophic level, herbivores present the second trophic level, primary carnivores represent the third trophic level and top carnivores represent the last level.

Food Chain:

In the ecosystem, green plants alone are able to trap in solar energy and convert it into chemical energy. The chemical energy is locked up in the various organic compounds, such as carbohydrates, fats and proteins, present in the green plants. Since virtually all other living organisms depend upon green plants for their energy, the efficiency of plants in any given area in capturing solar energy sets the upper limit to long-term energy flow and biological activity in the community.

The food manufactured by the green plants is utilized by themselves and also by herbivores. Animals feed repeatedly. Herbivores fall prey to some carnivorous animals. In this way one form of life supports the other form. Thus, food from one trophic level reaches to the other trophic level and in this way a chain is established. This is known as food chain.

A food chain may be defined as the transfer of energy and nutrients through a succession of organisms through repeated process of eating and being eaten. In food chain initial link is a green plant or producer which produces chemical energy available to consumers. For example, marsh grass is consumed by grasshopper, the grasshopper is consumed by a bird and that bird is consumed by hawk.

Thus, a food chain is formed which can be written as follows:

Marsh grass → grasshopper → bird → hawk

Food chain in any ecosystem runs directly in which green plants are eaten by herbivores, herbivores are eaten by carnivores and carnivores are eaten by top carnivores. Man forms the terrestrial links of many food chains.

Food chains are of three types:

1. Grazing food chain

2. Parasitic food chain

3. Saprophytic or detritus food chain

1. Grazing food chain:

The grazing food chain starts from green plants and from autotrophs it goes to herbivores (primary consumers) to primary carnivores (secondary consumers) and

then to secondary carnivores (tertiary consumers) and so on. The gross production of a green plant in an ecosystem may meet three fates—it may be oxidized in respiration, it may be eaten by herbivorous animals and after the death and decay of producers it may be utilized by decomposers and converters and finally released into the environment. In herbivores the assimilated food can be stored as carbohydrates, proteins and fats, and transformed into much more complex organic molecules.

The energy for these transformations is supplied through respiration. As in autotrophs, the energy in herbivores also meets three routes respiration, decay of organic matter by microbes and consumption by the carnivores. Likewise, when the secondary carnivores or tertiary consumers eat primary carnivores, the total energy assimilated by primary carnivores or gross tertiary production follows the same course and its disposition into respiration, decay and further consumption by other carnivores is entirely similar to that of herbivores.

Thus, it is obvious that much of the energy flow in the grazing food chain can be described in terms of trophic levels as outlined below:

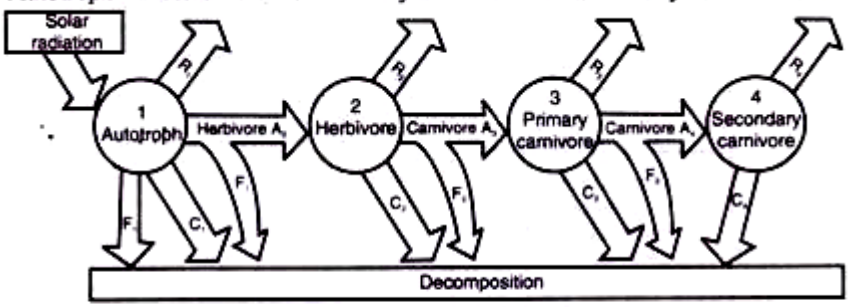


Fig. 3.8. Diagrammatic representation of a grazing food chain showing input and losses of energy at each trophic level. Trophic levels are numbered and used as subscripts to letters indicating energy transfer. A—assimilation of food by the organisms at the trophic level; F—energy lost in the form of faeces and other excretory products; C—energy lost through decay; and R—energy lost to respiration.

A schematic representation of grazing food chain showing input and losses of energy has been presented in Fig. 3.8.

2. Parasitic food chain:

It goes from large organisms to smaller ones without outright killing as in the case of predator.

3. Detritus food chain:

The dead organic remains including metabolic wastes and exudates derived from grazing food chain are generally termed detritus. The energy contained in detritus is not lost in ecosystem as a whole, rather it serves as a source of energy for a group of organisms called detritivores that are separate from the grazing food chain. The food chain so formed is called detritus food chain (Fig. 3.9).

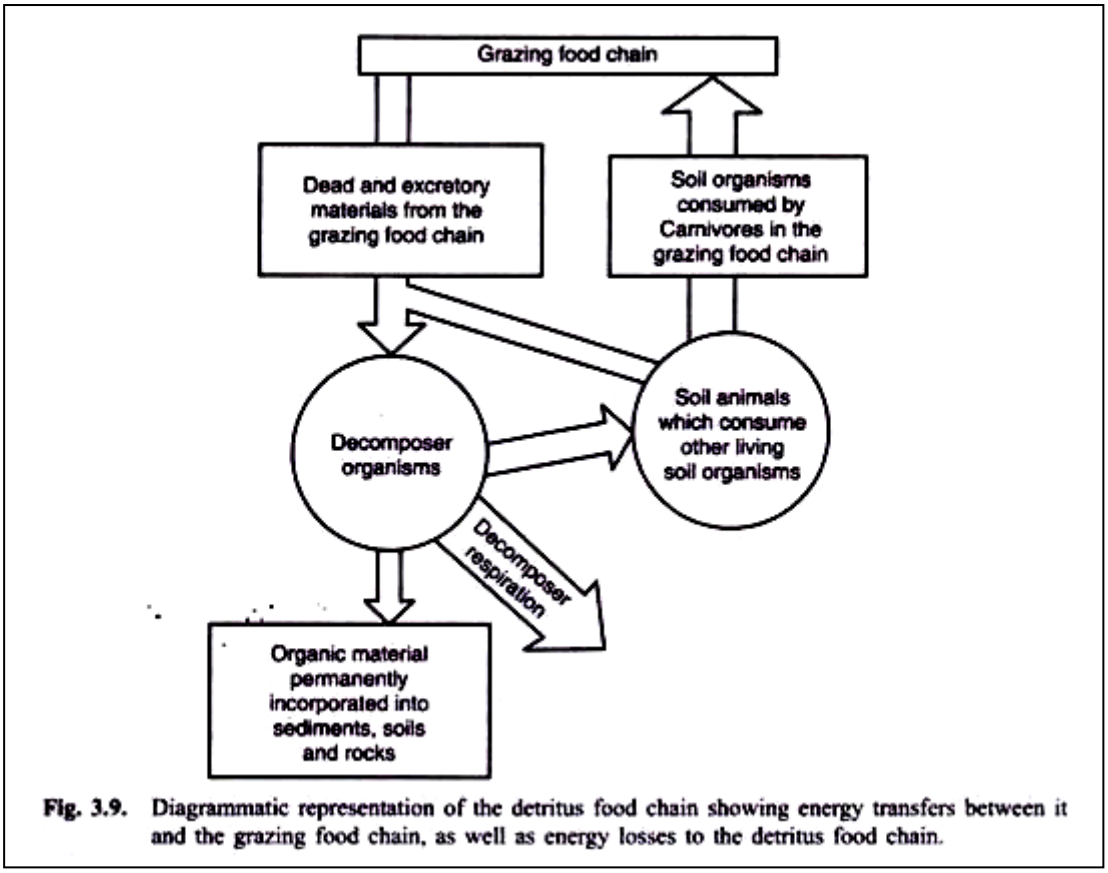


Fig. 3.9. Diagrammatic representation of the detritus food chain showing energy transfers between it and the grazing food chain, as well as energy losses to the detritus food chain.

In some ecosystems more energy flows through the detritus food chain than through grazing food chain. In detritus food chain the energy flow remains as a continuous passage rather than as a stepwise flow between discrete entities. The organisms in the detritus food chain are many and include algae, fungi, bacteria, slime moulds, actinomycetes, protozoa, etc. Detritus organisms ingest pieces of partially decomposed organic matter, digest them partially and after extracting some of the chemical energy in the food to run their metabolism, excrete the remainder in the form of simpler organic molecules.

The waste from one organism can be immediately utilized by a second one which repeats the process. Gradually, the complex organic molecules present in the organic wastes or dead tissues are broken down to much simpler compounds, sometimes to carbon dioxide and water and all that are left are humus. In a normal environment the humus is quite stable and forms an essential part of the soil. Schematic representation of detritus food chain is given in Fig. 3.9.

Food web:

Many food chains exist in an ecosystem, but as a matter of fact these food chains are not independent. In ecosystem, one organism does not depend wholly on another. The resources are shared specially at the beginning of the chain. The marsh plants are eaten by variety of insects, birds, mammals and fishes and some of the animals are eaten by several predators.

Similarly, in the food chain grass → mouse → snakes → owls, sometimes mice are not eaten by snakes but directly by owls. This type of interrelationship interlinks the individuals of the whole community. In this way, food chains become interlinked. A complex of interrelated food chains makes up a food web. Food web maintains the stability of the ecosystem. The greater the number of alternative pathways the more stable is the community of living things. Fig. 3.10. illustrates a food web in ecosystem.

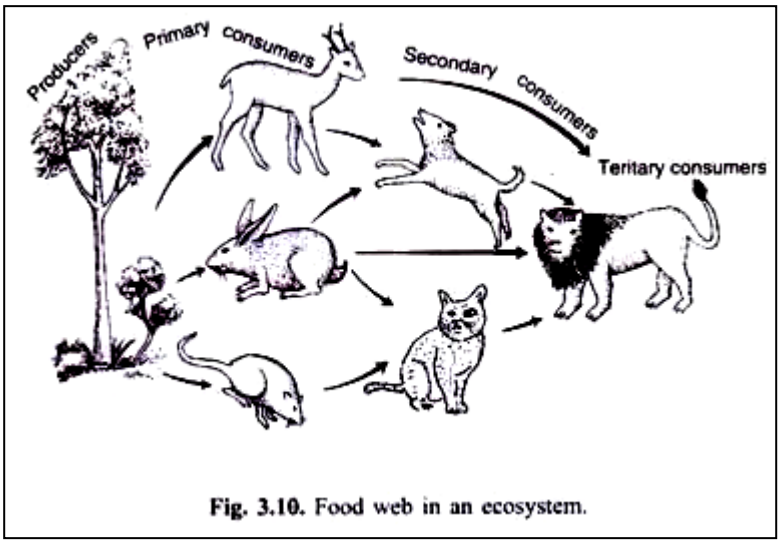


Fig. 3.10. Food web in an ecosystem.

Advantages of food web

There are varieties of pathways in a food web. These pathways maintain the stability of the ecosystem. For example, owls prey on rabbits and mice. If a disease reduces the rabbit population, a fewer plants are consumed. The larger plant population produces more fruits and seeds. Thus these plants support a larger mice population. The increased number of mice becomes the major food source for the owls. The rabbit population gradually increases, and these primary consumers again become a food source for the owls. Thus nature maintains a balance.

Ecological pyramid:

The trophic structure of an ecosystem can be indicated by means of ecological pyramid. At each step in the food chain a considerable fraction of the potential energy is lost as heat. As a result, organisms in each trophic level pass on lesser energy to the next trophic level than they actually receive. This limits the number of steps in any food chain to 4 or 5. Longer the food chain the lesser energy is available for final members. Because of this tapering off of available energy in the food chain a pyramid is formed that is known as ecological pyramid. The higher the steps in the ecological pyramid the lower will be the number of individuals and the larger their size.

The idea of ecological pyramids was advanced by C.E. Elton (1927). There are different types of ecological pyramids. In each ecological pyramid, producer level forms the base and successive levels make up the apex. Three types of pyramidal relations may be found among the organisms at different levels in the ecosystem.

These are as follows:

1. Pyramid of numbers,

2. Pyramid of biomass (biomass is the weight of living organisms), and

3. Pyramid of energy.

1. Pyramid of numbers:

It depicts the numbers of individuals in producers and in different orders of consumers in an ecosystem. The base of pyramid is represented by producers which are the most abundant. In the successive levels of consumers, the number of organisms goes on decreasing rapidly until there are a few carnivores.

The pyramid of numbers of an ecosystem indicates that the producers are ingested in large numbers by smaller numbers of primary consumers. These primary consumers are eaten by relatively smaller number of secondary consumers and these secondary consumers, in turn, are consumed by only a few tertiary consumers (Fig. 3.11, 3.12a).

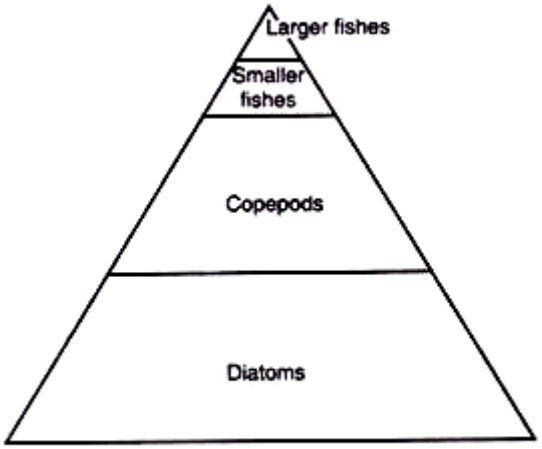


Fig. 3.11. A pyramid of numbers of a lake ecosystem.

This type of pyramid is best presented by taking an example of Lake Ecosystem. In this type of pyramid the base trophic level is occupied by producer elements—algae, diatoms and other hydrophytes which are most abundant. At the second trophic level come the herbivores or zooplanktons which are lesser in number than producers.

The third trophic level is occupied by carnivores which are still smaller in number than the herbivores and the top is occupied by a few top carnivores. Thus, in the ecological pyramid of numbers there is a relative reduction in number of organisms and an increase in the size of body from base to apex of the pyramid. In parasitic food chain starting from tree, the pyramid of numbers will be inverted (Fig. 3.12).

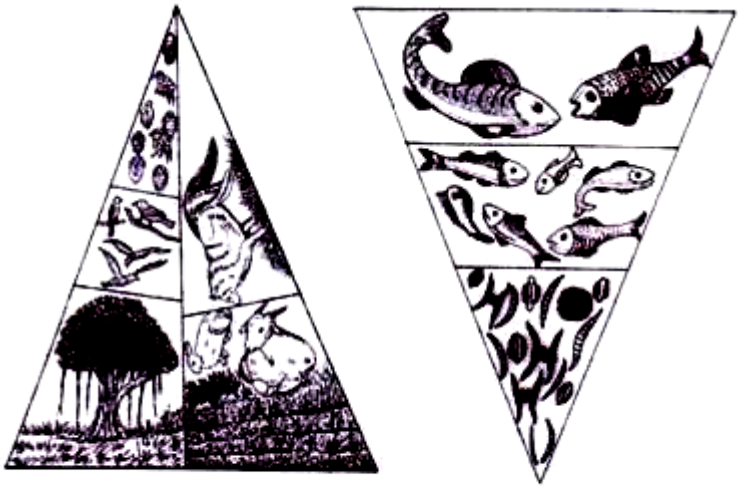


Fig. 3.12 (*a* & *b*). Pyramids of number
(*a*) Fig. 3.12 a & b. Up-right Pyramids of numbers in a grassland and cultivated field
(*b*) Pyramid of numbers (inverted) of diseased tree (Parasitic ecosystem)

Pyramid of biomass of organisms:

The living weights or biomass of the members of the food chain present at any one time form the pyramid of biomass of organisms. This indicates, by weight or other means of measuring materials, the total bulk of organisms or fixed energy present at one time. Pyramid of biomass indicates the decrease of biomass in each tropic level from base to apex, e.g., total biomass of producers is more than the total biomass of the herbivores.

Likewise, the total biomass of secondary consumers will be lesser than that of herbivores and so on (Fig. 3.13, 3.14 a, b). Since some energy and material are lost in each successive link, the total mass supported at each level is limited by the rate at which the energy is being stored below. This usually gives sloping pyramid for most of the communities in terrestrial and shallow water ecosystems. The pyramid of biomass in a pond ecosystem will be inverted as shown in Fig. 3.13 b.

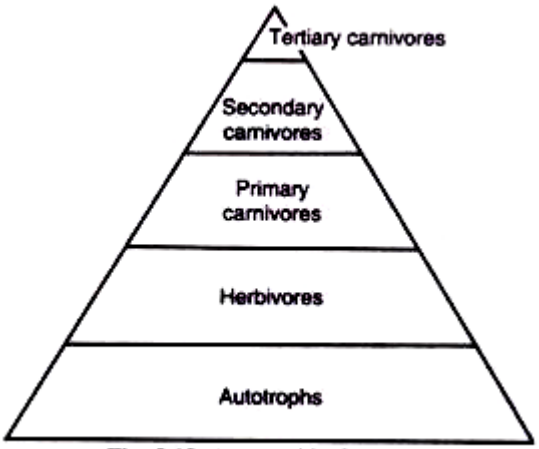


Fig. 3.13. A pyramid of biomass

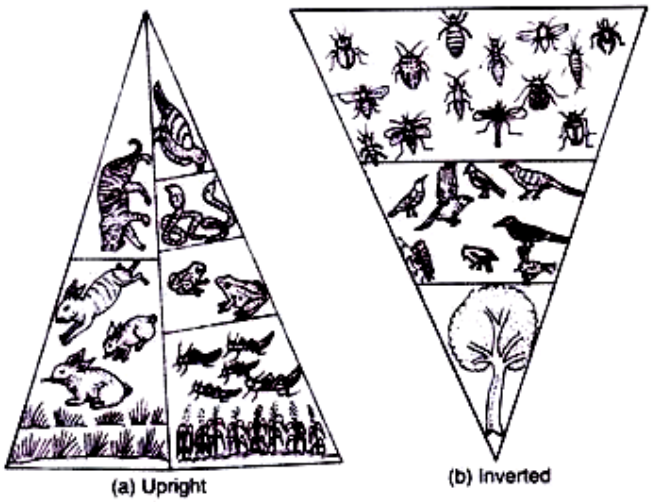


Fig. 3.14. (*a* & *b*). Pyramids of biomass
(*a*) A grassland ecosystems showing upright-triangular
(*b*) Inverted pyramid of biomass of an aquatic ecosystem.

3. Pyramid of energy:

This depicts not only the amount of total energy utilized by the organisms at each trophic level of food chain but more important, the actual role of various organisms in transfer of energy. At the producer level the total energy will be much greater than the energy at the successive higher trophic level.

Some producer organisms may have small biomass but the total energy they assimilate and pass on to consumers may be greater than that of organisms with much larger biomass. Higher trophic levels are more efficient in energy utilization but much heat is lost in energy transfer. Energy loss by respiration also progressively increases from lower to higher trophic states (Fig. 3.15).

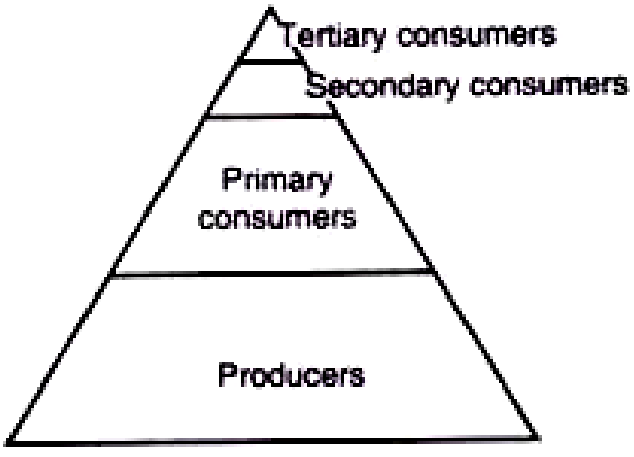


Fig. 3.15. A pyramid of energy

In the energy flow process, two things become obvious. Firstly there is one way along which energy moves i.e. unidirectional flow of energy. Energy comes in the ecosystem from outside source i.e. sun. The energy captured by autotrophs does not go back to the sun, the energy that passes from autotrophs to herbivores does not revert back and as it

moves progressively through the various trophic levels, it is no longer available to the previous levels.

Thus due to unidirectional flow of energy, the system would collapse if the supply from primary source, the sun is cut off. Secondly, there occurs a progressive decrease in energy level at each trophic level which is accounted largely by the energy dissipated as heat in metabolic activities.

Productivity:

The relationship between the amount of energy accumulated and the amount of energy utilized within one trophic level of food chain has an important bearing on how much energy from one trophic level passes on to the next trophic level in the food chain. The ratio of output of energy to input of energy is referred to as ecological efficiency.

Different kinds of efficiencies can be measured by the following parameters:

(i) Ingestion which indicates the quantity of food or energy taken by trophic level. This is also called exploitation efficiency.

(ii) Assimilation indicates the amount of food absorbed and fixed into energy rich organic substances which are stored or combined with other molecules to build complex molecules such as proteins, fats etc.

(iii) Respiration which indicates the energy lost in metabolism.

Primary Productivity:

The fraction of fixed energy a trophic level passes on to the next trophic level is called production. Green plants fix solar energy and accumulate it in organic forms as chemical energy. Since it is the first and basic form of energy storage, the rate at which the energy accumulates in the green plants or producers is known as primary productivity.

Primary productivity is the rate at which energy is bound or organic material is created by photosynthesis per unit area of earth's surface per unit time. It is most often expressed as energy in calories / cm^2 / yr or dry organic matter in g / m^2 / yr (g/m^2 x 8.92 = lb / acre). The amount of organic matter present at a given time per unit area is called standing crop or biomass and as such productivity, which is a rate, is quite different from biomass or standing crop.

The standing crop is usually expressed as dry weight in g/m^2 or kg/m^2 or t/ha (metric tons) or 10^6g/hectare. Primary productivity is the result of photosynthesis by green plants including algae of different colours. Bacterial photosynthesis or chemosynthesis, although of small significance may also contribute to primary productivity. The total solar energy trapped in the food material by photosynthesis is referred to as gross primary productivity (G.P.P.).

A good fraction of gross primary production is utilized in respiration of green plants. The amount of energy bound in organic matter per unit area and time that is left after

respiration in plants is net primary production (N.P.P.) or plant growth. Only the net primary productivity is available for harvest by man and other animals. Net productivity of energy = gross productivity—energy lost in respiration.

Secondary Productivity:

The rates at which the heterotrophic organisms resynthesize the energy-yielding substances is termed as secondary productivity. Secondary productivities are the productivities of animals and saprobes in communities. The amount of energy stored in the tissues of consumers or heterotrophs is termed as net secondary production and the total plant material ingested by herbivores is grass secondary production. Total plant material ingested by herbivores minus the materials lost as faeces is equal to Ingested Secondary Production.

Environmental factors affecting the production processes in an ecosystem are as follows:

1. Solar radiation and Temperature

2. Moisture. Leaf water potential, soil moisture and precipitation fluctuation and transpiration.

3. Mineral nutrition. Uptake of minerals from the soil, rhizosphere effects, fire effects, salinity, heavy metals, nitrogen metabolism.

4. Biotic activities. Grazing, above ground herbivores, below ground herbivores, predators and parasites, diseases of primary producers.

5. Impact of human population. Pollutions of different sorts, ionizing radiations like atomic explosions, etc.

There are three fundamental concepts of productivity:

1. Standing crop

2. Materials removed

3. Production rate.

1. Standing crop:

It is the abundance of the organisms existing in the area at any one time. It may be expressed in terms of number of individuals, as biomass of organisms, as energy content or in some other suitable terms. Measurement of standing crop reveals the concentration of individuals in the various populations of ecosystem.

2. The materials removed:

The second concept of productivity is the materials removed from the area per unit time. It includes the yield to man, organisms removed from the ecosystem by migration, and the material withdrawn as organic deposit.

3. The production rate:

The third concept of productivity is the production rate. It is the rate at which the growth processes are going forward within the area. The amount of material formed by each link in the food chain per unit of time per unit area or volume is the production rate.

All the three major groups of organisms—producers, consumers and reducers are the functional kingdoms of natural communities. The three represent major directions of evolution and are characterised by different modes of nutrition. Plants feed primarily by photosynthesis, animals feed primarily by ingesting food that is digested and absorbed in the alimentary canal and the saprobes feed by absorption and have need for an extensive surface of absorption. The principal kinds of organisms among saprobes are the unicellular bacteria, yeasts, chytrids or lower fungi and higher fungi with mycelial bodies.

In terrestrial communities as much as 90% of net primary production remains un-harvested and are utilized as dead tissue by saprobes and soil animals. The saprobes have a larger and more essential role than animals in degrading dead organic matter to inorganic forms and in such ecosystems, secondary production by reducers (decomposers) should exceed that by consumers, though the former is even more difficult to measure than the latter.

Biomass of decomposers with their microscopic cells and filaments embedded in food sources is also difficult to measure and that is small in relation to their productivity and significance for the ecosystem. Small masses of reducers degrade and transform larger masses of organic matter to inorganic remnants. In so doing decomposers disperse back to the environment the energy of photosynthesis accumulated in the organic compounds that are decomposed.

Thus they have a major role in the energy flow of ecosystems. A community or ecosystem, like an organism, is an open energy system. The continuous intake of energy in photosynthesis replaces the energy dissipated to environment by respiration and biological activity and the system does not run-down through the loss of free energy to maximum entropy.

If the amount of energy entrapped is greater than the energy dissipated, the pool of biologically useful energy of organic bonds increases. This results in increase of community biomass and consequently the community grows; such is the case in succession. If energy intake is lesser than energy dissipation, the community biomass will decrease and it must, in some sense, retrogress. If energy intake and loss are in balance, the pool of organic energy is in steady state; such is the case in climax communities.

Three aspects of this steady state may be recognized:

(i) The steady state of population of climax communities, in which equal birth and death rates in population keep the number of individuals relatively constant,

(ii) The steady state of energy flow,

(iii) The steady state of the matter of community, where addition of material by photosynthesis and organic synthesis is balanced by loss of material through respiration and decomposition.

Probable questions:

1. Describe the Gaia hypothesis.
2. Describe the feed back loop.
3. Discuss the Cybernetic Structure & Stability of the Ecosystem.
4. Elaborate the law of Thermodynamics.
5. Discuss the first law of Thermodynamics.
6. What is entropy?
7. What is enthalpy?
8. What is free energy?
9. What do you mean by food chain?
10. What is the importance of food web?

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XII

Niche theory: Concept of habitat and niche; niche width and overlap; fundamental and realized niche; resource partitioning; character displacement. (Lotka-Volterra model, Isoclines, Niche prediction)

Objective:

In this unit we will discuss about Niche theory: Concept of habitat and niche; niche width and overlap; fundamental and realized niche; resource partitioning; character displacement. (Lotka-Volterra model, Isoclines, Niche prediction)

Niche concept:

Introduction- For a species to maintain its population, its individuals must survive and reproduce. Certain environmental conditions are necessary for individuals of each species to tolerate the physical environment, obtain energy and nutrients, and avoid predators. The total requirements of a species for all resources and physical conditions determine where it can live and how abundant it can be at any one place within its range. These requirements are termed abstractly the ecological niche.

Definition of Niche- G.E. Hutchinson (1958) suggested that the niche could be modelled as an imaginary space with many dimensions, in which each dimension or axis represents the range of some environmental condition or resource that is required by the species. Thus, the niche of a plant might include the range of temperatures that it can tolerate, the intensity of light required for photosynthesis, specific humidity regimes, and minimum quantities of essential soil nutrients for uptake.

Ecological Niche-

An ecological niche is the role and position a species has in its environment; how it meets its needs for food and shelter, how it survives, and how it reproduces. A species' niche includes all of its interactions with the biotic and abiotic factors of its environment. Biotic factors are living things, while abiotic factors are non-living things. It is advantageous for a species to occupy a unique niche in an ecosystem because it reduces the amount of competition for resources that species will encounter.

In otherwords ecological niche is all of the physical, chemical and biological conditions required by a species for survival, growth and reproduction. Two further abstractions of this concept are the fundamental niche and the realized niche. A useful extension of the niche concept is the distinction between fundamental and realized niches. The fundamental niche of a species includes the total range of environmental conditions that are suitable for existence without the influence of interspecific competition or predation

from other species. The realized niche describes that part of the fundamental niche actually occupied by the species.

Habitat

Habitat refers to a specific place where a species normally lives. For example, habitat of a tiger is the forest, of a shark is the sea, and of Plasmodium are the red blood cells. More than one animal or plant may live in the same habitat. For example, tiger, deer, wolf, fox, lion, etc. may be found in the same forest.

Animals exhibit habitat specificity and require specific environmental conditions to live. For example, a fish lives in an aquatic habitat, but a river fish can live only in freshwater, while a sea fish can live only in a marine habitat. Some organisms are more tolerant than the other.

A habitat can be subdivided into regions with different environmental conditions. These subdivisions are called microhabitat. For example, in a pond, some organisms are surface dwellers while some others are bottom dwellers.

Difference between Niche and Habitat: Niche vs habitat

Every organism in the planet has a significant role to play in the ecosystem of the earth. They have their own places to live, functions, and amazing ways of reproduction to multiply their species. To help maintain the biological world people must be informed, not only on the physical aspect of it, but on a deeper level of how they live as one.The ecological study or the way of learning about the way these organisms interact with each other in the ecosystem is through studying its ecological niche and habitat. The niche and the habitat may have common attributes, but they are totally different from each other. To have a deeper knowledge of the biological world, it is best that these two be defined and differentiated.

The most basic and the most difficult thing to understand in this study is the ecological niche. This is the study of how of adaption of these organisms to their ecological community is also very important in studying the ecological niche concept. The habitat, on the other hand, is focused more on the location or the places in the organisms relate or fit in the ecosystem that it belongs to. The physical structure of the organisms is one of the very important factors for this concept or study. The physical attributes of the organisms are called the morphology. The psychological and behavioural ways environment where the organisms live. The resources available to each species, whether physical or biological, are very important in this study or aspect.

The niche is the study that is more focused on the response of the organism to the limited resources of the environment and their competitors. On the other hand, the habitat is more focussed on where the organisms live and how their environment affects them. To describe niche easier, it is the study of how an organism makes a living

in the ecological community it belongs to, while the habitat is defined as where the organisms live. With this simple definition, it will be easy to differentiate the two. Niche and habitat is correlated in the study of biodiversity. By studying these two, people may be able to help keep the homes of different organisms healthy and ultimately maintaining the numbers of the organisms in the ecosystem. In today's fast developing world, there are tendencies that the bio-diversities of this world are taken for granted. This is why studying the concept of niche and habitat is very important in today's modern world.

Summary of Niche vs habitat:

1. The study or the concept of the ecological niche is more difficult to understand than that ofthe habitat.
2. The niche is more on how the organisms behave in the places where they belong, while thehabitat is focussed on the places where each organism belongs.
3. The niche is more on how organisms react to their environment while habitat is more onhow their environment affects them.
4. The short definition for niche is how an organism makes a living in the place it belongs,while the habitat is where the organisms live.

Examples of Niche:

A. Pandas

Giant panda bears (*Ailuropoda melanoleuca*) are niche specialists. They have a very limited diet, 99% of which consists of bamboo. They have evolved specially adapted thumbs, which allow them to grip the bamboo. Bamboo does not provide much nutrition, and so the pandas must spend most of their time eating, consuming around 70lbs of bamboo every day to support their large bodies.

To conserve energy, pandas do not move very far, and so have a home range of around only 3 miles, near streams so that fresh drinking water is available, and where there are caves suitable for raising their young. Their highly specialized diet means that they do not usually encounter interspecific competition, and they live solitary lives so as to avoid intraspecific competition. Adults have no natural predators, so their populations are kept to suitable levels through their life cycles; in the wild they reproduce only once every two years, which means natural populations do not get too dense and intraspecific competition does not occur frequently. Because the niche of pandas is so specialized, they are exceptionally vulnerable to human impact and their populations have experienced dramatic declines.

The biggest threat has been deforestation for farmland, mining and logging, which has destroyed most of their habitat. They are now restricted to the humid bamboo forests of a few mountains in South Western China where they prefer the cool temperatures at high altitudes of around 4,000 to 10,000 ft.

B. The Birds of New Zealand

The island of New Zealand is a geographically isolated landmass off the southeast coast of Australia. Because of the great distance between New Zealand and any other large landmass, the only organisms that were able to colonize the land were those which were able to fly or float across the sea. Because migration on to the island was so difficult, the island community completely lacked mammals, except for three species of bat and mammals that were able to swim, such as seals and sea lions.

In the absence of mammals, the native animals filled ecological niches of predation, scavenging and grazing, which are filled by mammals in most other ecosystems. This resulted in a diverse set of morphologically distinct birds, insects and reptiles, which are like no others seen on Earth. For example, the South Island takahē (*Porphyrio hochstetteri*) and the Kakapo Parrot (*Strigops habroptilus*) evolved to assume the role of grazers such as sheep, feeding on grass, shoots and leaves. The Giant Moa (*Dinornis robustus* and *Dinornis novaezelandiae*), although now extinct, were large birds, growing up to around 12ft tall and over 500lb in weight. These birds fed on twigs, leaves and other various plant parts, assuming the niche that in other parts of the world is filled by deer and other ungulate browsing herbivores. The Kiwi, a nocturnal bird of the genus Apteryx, assumes the niche that small mammals such as mice and moles usually fill, feeding on seeds, fruit, invertebrates and grubs. The only predators on the island were flying birds, so in the absence of land-based predators, and as a result of their terrestrial niche roles, many of New Zealand's birds evolved to become flightless; even the bats spend most of their time on the ground.

When humans first arrived on the islands around 700 years ago, they brought with them rats and dogs, and later, Europeans brought stoats, pigs, dogs, cats, sheep, cattle, deer and many other animals. This has been devastating to the native wildlife; they are either outcompeted by other organisms that are adapted to fill particular niches or they are directly predated on because they lack protection against land-based predators. The case of New Zealand's birds is not entirely an anomaly. Islands that have been isolated for a long time often develop their own unique flora and fauna which have evolved to fill niches in the absence of other organisms. For example, the lemurs of Madagascar evolved diverse adaptions to fill many niches not usually filled by primates, due to Madagascar's varied terrains and habitats and a lack of other types of mammal. Another famous example of niche adaption is Darwin's finches on the Galapagos Islands. These birds evolved different beak shapes and body sizes on each island, according to the types of food that was available.

Importance of Niche:

Niche concept has several significances. In ecology, a niche is a term describing the relational position of a species or population in an ecology and how a population responds to the abundance of its resources and enemies (e. g., by growing when resources are abundant, and predators, parasites and pathogens are scarce) and how it

affects those same factors (e. g., by reducing the abundance of resources through consumption and contributing to the population growth of enemies by falling prey to them).

1) The niche includes a biotic or physical environment which is also a part of the ecosystem.
2) More formally, the niche includes niche because it influences how populations affect, and are affected by, resources and enemies.
3) The description of a niche may include descriptions of the organism's life history, habitat, and place in the food chain.
4) According to the competitive exclusion principle, no two species can occupy the same niche in the same environment for a long time.
5) A species' niche is determined by the traits that allow it to gather resources, evade enemies and any other factor that influences its relative birth and death rates.
6) The niche is often heralded as one of the most important concepts in community ecology, studies of the niche are often considered tautological, owing to the seemingly insurmountable task of defining the dimensions of the niche, especially those that help maintain species diversity.
7) Data on niches can be used to:

   (i) Make comparisons of the composition and organizationof communities.

   (ii) Examine shifts in the behaviour or ecology of one species in response to another species. (In particular, niche shifts are commonly used to study interspecific competition, based on Gause's Principle of Competitive Exclusion).

Types of Niche - Niche is generally four types. Such as-

A] Fundamental Niche,

B] Realized Niche

C] Spatial Niche and

D] Multidimensional Niche

A. Fundamental Niche:

Every species has a role that it plays in nature. That role is defined by a combination of the organism's behaviours, habitat, and interaction with other species. For example, a garden spider is a predator that hunts for prey among plants, while an oak tree grows to dominate a forest canopy, turning sunlight into food. The role that a species plays is called its ecological niche.A niche includes more than what an organism eats or where it lives. Environmental factors, such as climate, soil chemistry, and elevation, also play a

role in defining a niche. Sometimes other species will compete for the same niche. Lions on the African savanna compete with hyenas for food. Daisies and dandelions in a field compete for sunlight and soil. Competition from other species for the same niche is called interspecific competition. In order to better understand an organism, ecologists try to determine what sort of niche it would have in the absence of such competition. They might ask, 'How would a lion behave if there were no other predators competing for a zebra's flesh?' or 'What would a stand of water lilies look like if there was no duckweed living in the same area of the pond?' A fundamental niche is the term for what an organism's niche would be in the absence of competition from other species. Generally, however, there are competitors for the same lifestyle. Rabbits compete with groundhogs for food. Grasses compete with shrubs for soil, and bacteria compete with mold for nutrients among the leaf litter. The niche that a species actually inhabits, taking into account interspecific competition, is its realized niche.

Definition of fundamental niche

The full range of environmental conditions and resources an organism can possibly occupy and use, especially when limiting factors are absent in its habitat. The fundamental niche describes the potential area and resources an organism is capable of using. But the presence of limiting factors such as direct competition with other organisms, the organism tends to occupy a niche narrower than this.

In another word the fundamental niche is the theoretical niche of an organism given that there are no limiting factors on the environment or resources the organism can use/inhabit.

Explanation of the above definition:

A fundamental niche is the niche the organism would have if there were no limiting factors, such as predators, competitors, parasites, and disease. A fundamental niche differs from a realized niche because limiting factors often exist in the real world. The niche the organism actually occupies in the wild is called the realized niche.

For example, let's say an animal has a diverse, omnivorous diet. It can travel in many habitats and find sufficient food. However, if a predator is introduced in one of those habitats, the animal will avoid that area and no longer feed in that habitat. Thus, its realized niche differs from its fundamental niche.

Below we have an example where competition is the limiting factor for a species of barnacle:

Examples of Fundamental Niches

The male red-winged blackbird's mating call can be heard in the marshes in early spring. At that time, they hold the prime real estate in the marsh. However, as the season progresses, the more aggressive tri-color blackbirds move in. The tri-colors take over the best territory and force the red-wings to choose the leftovers. The entire marsh represents the red-winged blackbirds' fundamental niche

Spartina alterniflora is a grass species that is very tolerant of salt. It lives in salt marshes along the eastern North American coast. These marshes go underwater at high tide. While it is found all along the coast, it does not live in nearby freshwater environments.

Tolerance to Environmental Conditions-

Each species is tolerant of a range of certain environmental conditions, such as temperature, light and moisture, which are essential for their survival mechanisms and for growth, reproduction and feeding.

Organisms which live in the littoral zone of a coastal habitat are exposed to extreme changes in these conditions on a daily basis. A study on two species of barnacle within the littoral zone demonstrates how the niche of two organisms could be affected by slight changes in their environmental tolerance, in the presence of another species. are species removed, the larger *Semibalanus balanoides* is a species of large barnacle, adapted to living within deep water where it is rarely exposed to air. *Chthamalus stellatus* is a slightly smaller species, living in both deep and shallow water and capable of withstanding exposure to air which causes dehydration. Systematic removal of each species from an area is able to show that the smaller species could occupy shallow and deep zones in the absence of the large species. However, when smaller species do not occupy the shallow zone. In the natural environment when both species are present, the larger *S.balanoides* outcompete the smaller *C.stellatus* for resources and space, and the smaller species do not inhabit the deeper zone; this is called competitive exclusion. The fundamental niche of the two barnacles overlaps in the deeper zone, although the actual niche of the smaller species can only be realized in the shallow zone. In contrast, the fundamental niche of the large species is met in its realized niche.

B. Realized niche:

The role that an organism plays in nature is called ecological niche. For an animal, that niche includes things like its behavior, the food it eats, and whether it is active at night or in theday. For a plant, it includes how much direct sunlight it can tolerate and the sort of soil on which it thrives.

Every organism, whether an apex predator like the lion or a bacterium living in a hippo's intestinal tract, is likely to face competition from other species. The most direct form of competition comes from those organisms that try to make a living in almost the exact same way. For example, a lion does not worry much about the presence of a mongoose, but it is certainly concerned when a pack of hyenas moves in. This direct form of competition for an ecological niche is called interspecific competition.

If all the lions' competitors, such as hyenas, leopards, and hunting dogs, were to leave the African savanna, the lions would have the prey to themselves. Their niche would be wide open, limited only by their adaptations to their habitat and lifestyle. This ideal niche that would exist in the absence of competition from other species is called a

species' fundamental niche. However, organisms like the lion are generally forced to play a more limited role thanks to competition. The actual niche that a species fills in the face of interspecific competition is called its realized niche.

Definition

The realized niche is the part of fundamental niche that an organism occupies as a result of limiting factors present in its habitat.The presence of competing species in an environment is one example of a limiting factor that restrains or narrows an organism's ecological niche. In a realized niche, the organism tends to occupy and play an ecological role where it is mostly highly adapted.

Realized niche width

It is a phrase relating to ecology defining the actual space that an organism inhabits and the resources it can access as a result of limiting pressures from other species (e.g. superior competitors).

Niche width vs. realized niche width

The niche width of an organism refers to a theoretical range of conditions that a species could inhabit and successfully survive and reproduce with no competition. The niche width is defined as the parameters of this range which are determined by biotic and abiotic factors such as appropriate food sources and suitable climate respectively.

The niche width often differs from the area that a species actually inhabits, with the area a species actually persists in referred to as its realized niche width. This is due to interspecific competition with other species within their ecosystem and other biotic and abiotic limiting factors. A species realized niche is usually much narrower than its theoretical niche width as it is forced to adapt its niche around superior competing species.

The physical area where a species lives, is its "habitat." The abstract hypercube that defines the limits of environmental features essential to that species' survival is its "niche." (ECOLOGY, Begon,Harper,Townsend).

Example in comparison to Fundamental Niche:

To mention just one issue, there is a difference between fundamental niches and realized niches. What does that mean? Well, it could be that a plant, for example, could happily grow under wet, mesic and dry conditions if it were the only plant around. However, there is another plant that can also grow under mesic conditions but it is much more competitive than the first plant. That means that if they occur in different areas, the first plant will grow in all three habitats and the second one only in the mesic habitat, but if they happen to occur in the same area, you will find the first species in wet and dry places and the second one inthe mesicplaces where it excludes the first.

For illustration, this is how well both species do along a moisture gradient, which we could call their fundamental niches:

And this is how many individuals of each species you would really be able to find along the moisture gradient if they both occur together, which would show their realized niches:

What this shows is first that no organism should be expected to come with a clearly defined role but that it has to settle into one depending on what other organisms are around. Also, which of these two is the niche relevant for the Ecological Species Concept?

If we go with the realized niche, for example because we have so far only observed the species together in nature and don't realize that the orange species would feel even happier under mesic conditions than where it is actually forced to live, we would probably intuit that the orange populations in dry and the wet habitats are two Ecospecies.

C. Spatial Niche

Spatial resource partitioning occurs when two competing species use the same resource by occupying different areas or habitats within the range of occurrence of the resource. Spatial partitioning can occur at small scales (microhabitat differentiation) or at large scales (geographical differentiation).

Definition:

Spatial ecology represents the ultimate distributional or spatial unit occupied by a species. If a particular habitat is shared by several species, each of the species is usually confined to its own micro habitat or spatial niche because two species in the same general territory cannot usually occupy the same ecological niche

D. Multidimensional Niche

Hutchinson (1957) included in a species' (fundamental) niche "all ecological factors", "all environmental variables, both physical and biological, relative to [the] species", that is, both abiotic conditions, abiotic and/or biotic resources, and (though this may not have been his intention) other biotic factors (predators, parasites, competitors, etcetera). Thus, Hutchinson's niche concept is very comprehensive: it says just about everything about the ecology of a species. It is probably for that reason that the concept has become so popular. In my view, however, it is inadequate as a niche concept. In order to show this, I will have to extend a bit on modern niche theory and in particular competition theory.

Fundamental vs Realized Niche:

Fundamental niche is the entire set of conditions under which an animal (population, species) can survive and reproduce itself. Realized niche is the set of conditions actually

used by given animal (population, species), after interactions with other species (predation and especially competition) have been taken into account. Sometimes FN and RN are termed precompetitive and postcompetitive niches, reflecting a traditional focus on interspecificcompetition's effect on niches.

Note that:

1. FN ≥ RN

RN for different populations of same species may differ, because of differences in competitors and predators between locations.

Summary:

1. Fundamental and realized niche refers to the environmental conditions or positions of different species in an ecosystem.
2. A fundamental niche can be defined as the range of environmental conditions in which each of the species survives.
3. The realized niche can be termed as the range of environmental conditions in which a species is really found.
4. The fundamental niche is larger than the realized niche. The realized niche can be called a subset of the fundamental niche. It can be said that as the realized niche grows, the fundamental niche also grows accordingly.
5. While a fundamental niche elaborates on the various roles of species, the realized niche elaborates on what the species actually do.
6. It is in the realized niche that a species will be well adapted, and so this niche is where the species actually exist.
7. The fundamental niche refers to a range of conditions, roles, and resources under which a species survives, grows, and reproduces.

Niche differentiation:

The term niche differentiation (synonymous with niche segregation, niche separation and niche partitioning), as it applies to the field of ecology, refers to the process by which competing species use the environment differently in a way that helps them to coexist. The competitive exclusion principle states that if two species with identical niches (i.e., ecological roles) compete, then one will inevitably drive the other to extinction. When two species differentiate their niches, they tend to compete less strongly, and are thus more likely to coexist. Species can differentiate their niches in many ways, such as by consuming different foods, or using different parts of the environment.

As an example of niche partitioning, several anole lizards in the Caribbean islands share common food needs—mainly insects. They avoid competition by occupying different

physical locations. For example, some live on the leaf litter floor while others live on branches. Species that live in different areas compete less for food and other resources, which minimizes competition between species. However, species who live in similar areas compete strongly.

Types of Niche differentiation

Below is a list of ways that species can partition their niche. This list is not exhaustive, but illustrates several classic examples.

Resource partitioning:

When species use different resources, this can help them to coexist. For example, some lizard species appear to coexist because they consume insects of differing sizes. Alternatively, species can coexist on the same resources if each species is limited by different resources, or differently able to capture resources. For example, different types of phytoplankton can coexist when different species are differently limited by nitrogen, phosphorus, silicon, and light. In the Galapagos Islands, finches with small beaks are more able to consume small seeds, and finches with large beaks are more able to consume large seeds. If a species' density declines, then the food it most depends on will become more abundant (since there are so few individuals to consume it). As a result, the remaining individuals will experience less competition for food.Although "resource" generally refers to food, species can partition other non-consumable objects, such as parts of the habitat. For example, warblers are thought to coexist because they nest in different parts of trees. Species can also partition habitat in a way that gives them access to different types of resources. As stated in the introduction, anole lizards appear to coexist because each uses different parts of the forests as perch locations. This likely gives them access to different species of insects.

Predator partitioning:

Predator partitioning occurs when species are attacked differently by different predators (or natural enemies more generally). For example, trees could differentiate their niche if they are consumed by different species of specialist herbivores, such as herbivorous insects. If a species density declines, so too will the density of its natural enemies, giving it an advantage. Thus, if each species is constrained by different natural enemies, they will be able to coexist. Early work focused on specialist predators however, more recent studies have shown that predators do not need to be pure specialists, they simply need to affect each prey species differently. The Janzen–Connell hypothesis represents a form of predator partitioning.

Conditional differentiation

Conditional differentiation (sometimes called temporal niche partitioning) occurs when species differ in their competitive abilities based on varying environmental

conditions. For example, in the Sonoran Desert, some annual plants are more successful during wet years, while others are more successful during dry years. As a result, each species will have an advantage in some years, but not others. When environmental conditions are most favourable, individuals will tend to compete most strongly with member of the same species. For example, in a dry year, dry-adapted plants will tend to be most limited by other dry-adapted plants.This can help them to coexist through a storage effect.

Competition-predation trade-off

Species can differentiate their niche via a competition-predation trade-off if one species is a better competitor when predators are absent, and the other is better when predators are present. Defences against predators, such as toxic compounds or hard shells, are often metabolically costly. As a result, species that produce such defences are often poor competitors when predators are absent. Species can coexist through a competition-predation trade-off if predators are more abundant when the less defended species is common, and less abundant if the well-defended species is common.This effect has been criticized as being weak, because theoretical models suggest that only two species within a community can coexist because of this mechanism.

Niches and competition

Humans compete with other humans all the time – for jobs, athletic prizes, dates, you name it. But do we compete with other species? If you've ever gone camping and had you food stolen by an enterprising raccoon, bear, or other critter, you've had a little taste of interspecific competition – competition between members of different species that use overlapping, limited resources. Resources are often limited in a habitat, and many species may compete to get ahold of them. For instance, plants in a garden may compete with each other for soil nutrients, water, and light. The overall effect of interspecific competition is negative for both species that participate (a -/- interaction). That is, each species would do better if the other species weren't there.In this article, we'll look at the concept of an ecological niche and see how species having similar niches can lead to competition. We'll also see how species can evolve by natural selection to occupy more different niches, thus divvying up resources and minimizing competition.

A species' niche is its ecological role or "way of life," which is defined by the full set of conditions, resources, and interactions it needs. Each species fits into an ecological community in its own special way and has its own tolerable ranges for many environmental factors. For example, a fish species' niche might be defined partly by ranges of salinity (saltiness), pH (acidity), and temperature it can tolerate, As we'll see, two organisms with exactly the same niche can't survive in the same habitat (because they compete for exactly the same resources, so one will drive the other to extinction).

However, species whose niches only partly overlap may be able to coexist. Also, over long periods of time, they may evolve to make use of more different, or less overlapping, sets of resources. as well as the types of food it can eat.

Competitive exclusion principle

The competitive exclusion principle tells us that two species can't have exactly the same niche in a habitat and stably coexist. That's because species with identical niches also have identical needs, which means they would compete for precisely the same resources.

A famous example of the competitive exclusion principle is shown in the Figurebelow, which features two types of single-celled microorganisms, *Paramecium aurelia* and *Paramecium caudatum*. When grown individually in the lab, both species thrive. But when they are grown in the same test tube (habitat) with a fixed amount of nutrients, both grow more poorly and P. *aurelia* eventually outcompetes *P. caudatum* for food, leading to *P. caudatum's* extinction.

In nature, it's rarely the case that two species occupy exactly identical niches. However, the greater the extent to which two species' niches overlap, the stronger the competition between them will tend to be.

Resource partitioning

Competitive exclusion may be avoided if one or both of the competing species evolves to use a different resource, occupy a different area of the habitat, or feed during a different time of day. The result of this kind of evolution is that two similar species use largely non- overlapping resources and thus have different niches. This is called resource partitioning, and it helps the species coexist because there is less direct competition between them. The anole lizards found on the island of Puerto Rico are a good example of resource partitioning. In this group, natural selection has led to the evolution of different species that make use of different resources. Each species lives in its own preferred habitat, which is defined by type and height of vegetation (trees, shrubs, cactus, etc.), sunlight, and moisture, among other factors.

Niche width and overlap

Identifying the niche width and overlap of a species depends on the distribution of the speciesacross.

Resource availability: A species that can live on diverse resources normally will have a wider width. In natural systems organisms prefer to have smaller, realized width as this ensures better resource within a small area. This assists in improving the ecological efficiency of the species. Niche overlap is the collective utilization of resources by

two or more species in the same region. Overlap brings in interspecific competition. Organisms constantly try to minimize competition in sharing resources either by temporal or spatial variation. Niche breadth is the "distance through" a niche along some particular line in niche space. Niche overlap is simply the joint use of a resource, or resources, by two or more species. The measures of niche breadth and overlap we will discuss are all based on the distribution of individual organisms, by species, within a set of resource states. The table formed by using species as rows, and resource states as columns will be called the "resource matrix." A heterogeneous habitat, for example, might be subdivided into sunny-dry, sunny-wet, shady-dry, and shady-wet resource states, or alternatively, into unnamed random quadrats, which would then be considered the resource states. When two distinct habitats merge each other one can observe more number of plant and animal species this is known as ecotone. This zone having different types of food/ different stages of mineralized food supporting saprophytes, autotrophs and phagotrophs. This is an example of resource partitioning.

Conclusion:

Ultimately we can say that niche is one of the most important part of ecology. The term niche was for the first time used by Joseph Grinnel (1971) to explain microhabitats of organsims.It has various types, difference and specific importance in ecology. Niche overlap, competition and other term like resource partitioning are related with eachother.While habitat and niche are not same but they both are related with each other. In very simple way we can define niche asIn ecology, a niche is a term describing the relational position of a species or population in an ecosystem. The description of a niche may include descriptions of the organism's life history, habitat, and place in the food chain. According to the competitive exclusion principle, no two species can occupy the same niche in the same environment for a long time.

Character displacement:

Character displacement is defined as the divergence in the characteristics of two otherwise similar species where their ranges overlap, caused by the selective effects of competition between the species in the area of overlap.

In the phenomenon of character displacement, the character traits of two closely related species differ more when they occur in sympatry (two species coexisting within the same geographic area) than in allopatry (when their distributions do not overlap, they are said to be allopatric). The ground finches (*Geospiza* spp.) of Galapagos Islands, first described by Darwin, furnish an example of character displacement. Character displacement occurs when inter-specific competition results in natural selection causing changes in the morphology of two closely related species.

Lotka- Volterra Predator- Prey Model:

The Lotka- Volterra predator- prey model, first presented by Lotka (1925) and Volterra (1926), a simple mathematical model representing the interaction between predators and their prey.

However, the model makes three simplifying assumptions:

(i) There is only one predator and one prey species involved in the interaction;

(ii) Prey numbers increase if the number of predators falls low a threshold, and decrease if there are more predators; and

(iii) Predator numbers increase if die number of prey rises above a threshold and decrease if there are fewer prey.

This model provides a basis for understanding how predator and prey populations sometimes come to oscillate with respect to one another. Despite the simplicity of this model, the pattern shows clear similarities to the cycling observed in the snowshoe hare-lynx example.

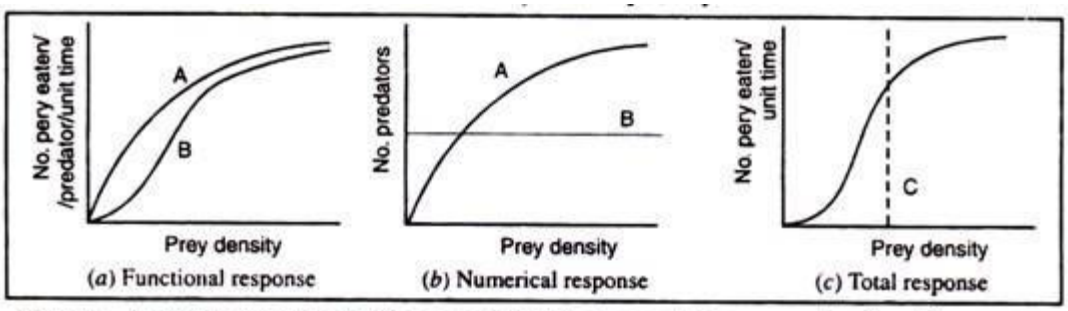


Fig. 3.8. Alternative ways to obtaining a sigmoid total response between prey density and prey eaten. The total response curve may be obtained by combining functional response A or B with numerical response A, or combining functional response B with any numerical response. Below prey density C the predator may control the density of the prey. (After Hassell, 1981)

Lotka and Volterra expressed the rate of growth of both predator and prey populations by the following differential equations:

dP/dt = g(H, P)———– for the predator

dH/dt = f (H,P)————- for the prey

where P = predator population size

H = prey population size

Thus, the rate of growth of prey population, dH/dt, is some joint function (f) of the size of prey population (H) and the size of predator population (P). It may be recalled that the general form of these models is the same as that of the population growth models in which the change in population size (dN/dt) was shown as some function (f) of N,f (N). The Lotka – Volterra predator- prey model is a simple elaboration of this idea. What has been included in the functions (H, P) and g (H, P) are terms that account for how

consuming and being consumed add and remove individuals from the populations of predator and prey.

Isoclines:

A line joining points on a graph at which combinations of resource levels or population densities produce a similar rate of population growth for a particular species.

Isoclines can be used as a graphical method of solving an ordinary differential equation. The term is also used to refer to points on maps of the world having identical magnetic inclinations.

Isoclines are often used as a graphical method of solving ordinary differential equations. In an equation of the form y' = f(x, y), the isoclines are lines in the (x, y) plane obtained by setting f(x, y) equal to a constant.

Meta population:

Meta population is a set of local population occupying an array of habitat patches and connected to one another by the movement of individuals among them.

Theory of metapopulation:

The term metapopulation was first used by Richard Levins, 1970. A metapopulation of species is thought of as a set of small populations occupying an array of similar small habitat patches situated in a matrix of uniform suitable habitat. At any particular time, some suitable habitat patches may contain no individual of the species and thus may be subjected to colonization from other patches that are inhabited.

The local populations are assumed to be small. Local catastrophes and chance fluctuations in number of individuals have important effects on population dynamics, so there is a high probability of extinction of a local population during a particular time interval. Metapopulation dynamics indicate a balance between extinction and colonization.

Simple metapopulation model:

It is conceptualized as a group of local population each having a density of either zero (extinct) or K (equilibrium density), where “K” is the patch carrying capacity. The carrying capacity of patch is the number of individuals that can be supported by the recourses in the patch for a indefinite period of time. At any time, some proportions (P) of the total number of patches in the metapopulations will be occupied and the remaining fraction (1-P), will be unoccupied or become extinct.

$$dP/\,dt \quad = [(1 - P)] - eP$$

Where, m = rate of patch

e =rate of patch extinction.

When the rate of change of occupancy ~~is~~ zero dP/dt ~~=~~ 0 and P= 1- e/m is the equilibrium proportion of occupied populations.

The main prediction of this mo~~d~~el is that species will not perish i.e p < 0 when extinction rate is greater than colonization rate, m is the metapopulation and to put it in an way metapopulation persistence require e/m < 1.

Probable questions:

1. What is fundamental niche?
2. What is realized niche?
3. What is importance of niche?
4. What do you mean resource partitioning?
5. Discuss Lotka- Volterra predator- prey model?

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XII

Community: Structure and Gradient analysis, Structure of biotic community. Community patterns: diversity and stability. Community boundary: Ecotone and edge types, Edge effect and edge species, Leibig's Law of the minimum.

Objective:

In this unit we will learn about Community: Structure and Gradient analysis, Structure of biotic community. Community patterns: diversity and stability. Community boundary: Ecotone and edge types, Edge effect and edge species, Leibig's Law of the minimum.

Introduction:

The group of species that occupy a given area, interacting either directly or indirectly, is called a community. The definition of community also recognizes that species living in close association may interact. They may compete for a shared resource, such as food, light, space, or moisture. One may depend on the other as a source of food. They may provide mutual aid, or they may not directly affect each other at all. A community also has several attributes. These attributes include the number of species, their relative abundances, the nature of their interactions (mutualism, commensalism, symbiosis, parasitism, predation and competition) and their physical structure (defined primarily by the growth forms of the plant componentsof the community).

Community Structure:

A community is composed of individuals and populations. The species that assemble to make up a community are determined by dispersal constraints, environmental constraints and internal dynamics (Belyea& Lancaster, 1999). Different ecological communities can be pretty different in terms of the types and numbers of species they contain. For instance, some Arctic communities include just a few species, while some tropical rainforest communities have huge numbers of species packed into each cubic meter. One way to describe this difference is to say that the communities have different structures. Community structure is essentially the composition of a community, including the number of species in that community and their relative numbers. It can also be interpreted more broadly, to include all of the patterns of interaction between these different species. Some ecologists also use the term "community structure" for the pattern of a community in space – how the populations that make it up are distributed in the physical environment. For instance, a community may have sharp edges or may

blend gradually into the neighboring community. Also, different populations may occupy different areas inside the community's boundaries.

Ecologists use to describe the composition of a community by two important measures i.e.species richness and species diversity.

- **Species richness:**

Species richness is the number of different species in a particular community. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distribution. For instance, if we found 100 species in one community and 1000 species in another community, the second community would have much higher species richness than the first. Communities with the highest species richness tend to be found in areas near the equator, which have lots of solar energy (supporting high primary productivity), warm temperatures, large amounts of rainfall, and little seasonal change. Communities with the lowest species richness lie near the poles, which get less solar energy and are colder, drier, and less amenable to life. This pattern is illustrated below for mammalian species richness (species richness calculated only for mammal species, not for all species). Many other factors in addition to latitude can also affect a community's species-richness.

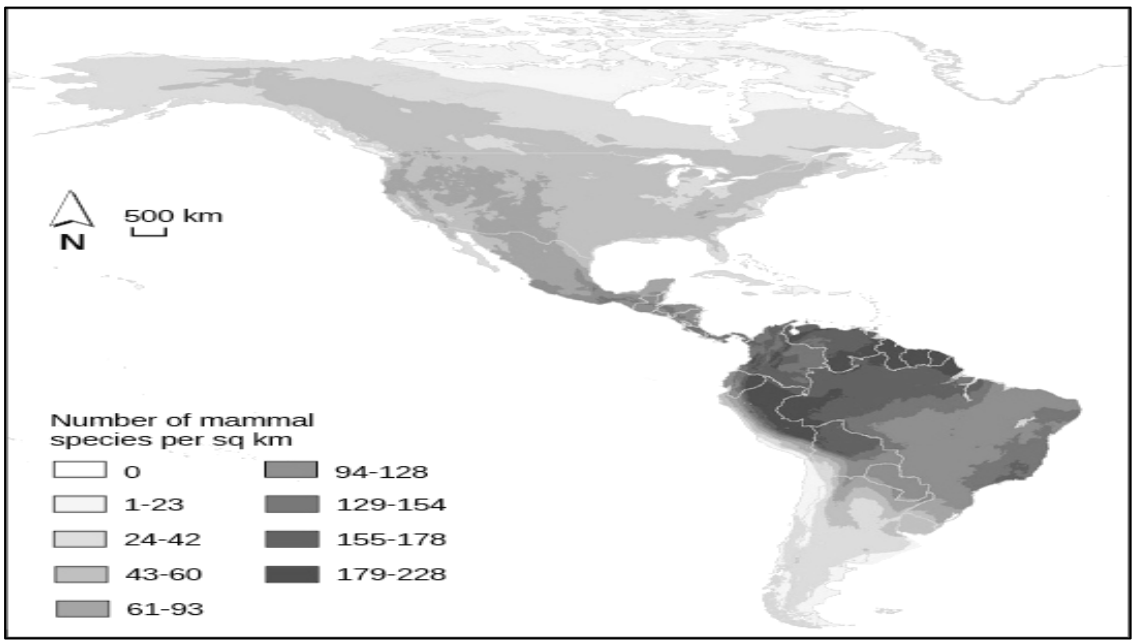


Figure: Map shows the spatial distribution of mammal species richness in North and South America. The highest number of mammal species, 179-228 per square kilometer, occurs in the Amazon region of South America. Species richness is generally highest in tropical latitudes, and then decreases to the north and south, with zero species in the Arctic regions.

Global species richness as calculated for mammal species.

- **Species diversity:**

Species diversity is a measure of community complexity. It is a function of both the

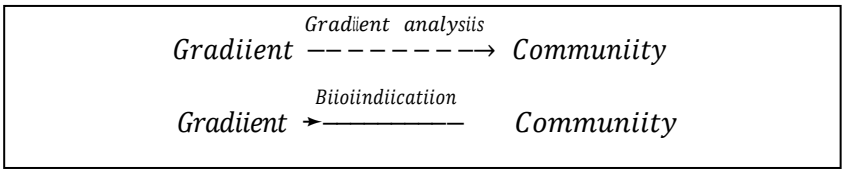
number of different species in the community (species richness) and their relative abundances (species evenness). Larger numbers of species and more even abundances of species lead to higher species diversity. For example, a forest community with 200500 different kinds of trees would have greater species diversity than a forest community with only 5000 kinds of trees. Communities in which the species are all more or less equal in abundance exhibit evenness, whereas communities with one or a few abundant species and many rare one show dominance.

Gradient analysis:

Introduction:

It is often difficult to determine where one community ends and the next community begins. Many communities grade continuously into each other with no sharp boundaries. The zone of vegetation separating two different types of communities called ecotone. It is also known as a transition zone. Ecotone is a region where the influence of two different patterns of environment works together and hence the vegetation of ecotone is highly specialized. A general characteristic of ecotone is that it has sufficiently greater number of species and high diversity as compared to neighbouring communities. The phenomenon of increased species diversity at the boundary (edge) is called the edge effect. Species that uses the edges for the purposes of reproduction or survival termed as edge species. Gradient analysis is the small step on the way to the objective description of the communities. Choice of gradient is always subjective. The environmental factor that is chosen as a gradient is more or less loosely correlated with whatever really matters in the lives of the species involved.

So, Gradient analysis is the relation of the species and environmental variation or gradients.



Types of Gradients: Austin, Smith (1989) discussed the current issues in continuum theory and offered a new model. The authors distinguish three types of gradients:

Indirect or complex gradients – These are complex gradients such as elevation gradient, for example. The influence of elevation is indirect, through variables like temperature and precipitation, which have direct effect on plant growth. These variables, depending on the site location, are in complex correlation with the elevation. Relationships between vegetation and these environmental gradients cannot be extrapolated beyond the studied territory.

Resource gradients –These are variable resources consumed by plants for their growth. For the autotrophy plants these resources are with limited number: light,

water, CO_2, O_2, nutrients. Plant reaction to the resource gradients is relaxed. Plants react only to the toxic levels, exceeding normal concentrations in the environment.

Direct gradients – These are gradients having direct physiological effect on plant growth but are not consumed by plants, for example, air temperature and soil pH. Direct gradients regulate plant growth and support plant physiological integrity. Plant physiological response shows different adaptations in different temperature regimes and their fundamental niches aredispersed along the gradient.

Different Approaches in Gradient Analysis:

Goodall (1954) first introduced the term 'ordination', relevant to methods, which arrange samples or species in 'multidimensional series'. Ordination in vegetation ecology is the process of sample (or species) arrangement along one or more environmental gradients, or along abstract axes, which may represent important environmental gradients.

Two different approaches are used-

Direct gradient analysis or **ecological ordination**:

In this technique vegetation data are investigated graphically or mathematically in the context of selected environmental gradients. This method is a simple graphical technique for representation of species distribution along the environmental gradients, which are *a priori* considered as important for plants.

Indirect gradient analysis or vegetation ordination:

In this gradient analysis mathematical methods are used for summarizing the emergent data structure of species and samples in a few abstract dimensions. In indirect gradient analysis there is not *a priori* assumptions about which are the most important environmental gradients influencing vegetation. Graphical result from the analysis shows which samples are located in extreme and intermediate parts of the environmental gradient. Next, appropriate statistical tests are used, which on the basis of statistically significant correlations, show the environmental variables having sense in the species and sample arrangement.

Structure of biotic community

Origin of Biotic Community:

All the living things make the biotic system of the earth. Neither organisms nor species populations exist by themselves in nature, but are always part of an assemblage of different species populations living together in the same area. Any assemblage of populations of living organisms in the prescribed area or habitat is termed biotic community.

For example, the different species of organisms occurring in a pond constitute the pond community. Some other communities such as swamps, deserts, large lakes, grass lands and many others of different dimensions.

Similarly, the interdependent species in any environment, such as a forest, a lawn or a desert track, constitute biotic communities. A community may be composed primarily of animals, or primarily of plants, but most communities consist of both animals and plants. The factors that influence the living organisms are known as biotic factors. In the natural world there is interdependence of one form of life on another. A population of single species is not a viable entity by itself. It will not be able to survive for long.

For example, the sparrows require worms, insects or seeds or some other kind of food. Thus the most appropriate definition of biotic community— "a biotic community is a naturally occurring assemblage of plants and animals that live in the same environment, are mutually sustaining and interdependent, and are constantly fixing and dissipating energy."

However, the different species in a biotic community share a common environment and their relationships are based on direct or indirect functional interactions. The nature of relationship is determined by the requirements of the members of the community. Biotic community organization results from interdependence and interaction among populations of different species in a habitat. Large number of biotic communities found in nature due to two specific reasons.

They are as follows:

(i) Existence of diverse habitats with characteristic environmental conditions and

(ii) Co-occurrence of different species whose tolerance ranges overlap with the environmental condition obtained in that habitat. When similar habitat conditions are repeated at another location, the same biotic community gets established there.

Characteristics of Biotic Community:

Each biotic community consists of very diverse organisms belonging to different kingdoms of living things. The number of species and abundance of population in communities also vary greatly. The organisms in a community depend upon each other as well as upon the non-living environment for food, shelter and reproduction.

I. **Species Composition:**

The kinds of plants and other organisms present in a community indicate its species composition, which differs from one community to another. Sometimes, in the same community, there may be seasonal variation in plant species.

Each species of community has got definite range of tolerance towards the physical and biological environmental conditions of the habitat. The range of environment a species can tolerate is called its ecological amplitude. The nature of community of a particular

habitat is determined by the species, and physical and biotic influences prevailing in the locale of community.

II. **Dominance:**

A biotic community may have major categories of growth forms, such as trees, shrubs, herbs and mosses. Out of hundreds of species present in the community, relatively only a few exert a major controlling influence due to their large size, numbers of activities.

The phenomenon is called dominance. **"Dominant species are those which are highly successful ecologically and which determine to a considerable extent the conditions under which the associated species must grow."**

The dominance in the community may be the result of co-action between two or more species. Different communities are generally recognized and named on the basis of dominant species occurring in them. For example, a forest community in which pine trees are dominant is called pine forest.

Grassland represents a community which has grass species dominating over the other herbs. Sometimes, communities are named after environmental factors, such as desert community, marine community, mangrove vegetation, etc.

III. **Physiognomy:**

General appearance of vegetation is referred to as physiognomy. It constitutes general stature, shape and life-forms of the species comprising the vegetation and actually the classification of vegetation types has been done on the basis of physiognomy.

The species of a community can be grouped into several life-forms on the basis of general appearance and growth. The physiognomy is the total effect created by the combination of vertical structure and architecture of dominant species of vegetation.

For example, the high physiognomy of a forest differs distinctly from a low physiognomy of a grassland. However, several communities though possess similar physiognomy, yet differ markedly on the basis of species composition and dominants, e.g., different types of forests.

IV. **Stratification:**

Every biotic community has a vertical layering or stratification of organisms or environmental conditions. A number of examples can be cited to support the concept of community stratification from different habitats.

Diversity Index:

A diversity index is a mathematical measure of species diversity in a community. It

takes into account the number of species present (species richness), as well as the abundance of each species (evenness). Brief outlines of the two types of diversity indices of biodiversity are discussed here. The two types are: (1) Dominance Indices, and (2) Information-Statistic Indices.

1. Dominance Indices:

Dominance indices are weighted toward the abundance of the commonest species. A widely used dominance index is Simpson's diversity index. It takes into account both richness and evenness.

- **Simpson's Diversity Indices:**

The term "Simpson's diversity index" can actually refer to any one of 3 closely related indices.

- **Simpson's Index (D):**

Simpson's index measures the probability that any two individuals drawn at random from an infinitely large community will belong to same species. There are two versions of the formula for calculating D. The value of D ranges between 0 and 1. With this index, 0 represents infinite diversity and 1, no diversity. That is, the bigger the value of D, the lower the diversity. This does not sound logical, so to get over this problem, D is often subtracted from 1 or the reciprocal of the index is taken.

- **Simpson's Index of Diversity 1-D:**

This index represents the probability that two individuals randomly selected from a community will belong to different species. The value of this index also ranges between 0 and 1, but here, the greater the value, the greater the diversity.

The name Simpson's diversity index is often very loosely applied and all three related indices described above (Simpson's index, Simpson's index of diversity and Simpson's reciprocal index) have been quoted under term, depending on authors.

As an example, let us consider the following table:

Species	Number(n)	n(n – 1)
A	2	2
B	8	56
C	1	0
D	1	0
E	3	6
Total (N)	15	64

Putting the values into the formula for Simpson's index:

$$D = \frac{\sum n(n-1)}{N(N-1)} = \frac{64}{15 \times 14} = 0.3 \text{ (Simpson's index)}$$

Then, Simpson's index of diversity 1 – D = 0.7 and Simpson's reciprocal index 1/D = 3.3.

All these three values represent the same biodiversity. It is, therefore, important to ascertain which index has actually been used in any comparative studies of biodiversity. The disadvantage of Simpson's index is that it is heavily weighed toward the most abundant species, as are in all dominance indices.

The addition of rare species with one individual will fail to change the index. As a result, Simpson's index is of limited value in conservation biology if an area has many rare species with just one individual.

2. Information-Statistic Indices:

Information-statistic indices can take into account rare species in a community. Information- statistic indices are based on the rationale that diversity in a natural system can be measured in a way that is similar to the way information contained in a code or message is measured.

By analogy, if we know how to calculate the uncertainty of the next letter in a coded message, then we can use the same technique to calculate the uncertainty of the next species to be found in a community.

- **Shannon Index:**

A widely used diversity index is Shannon index.

The Index is given by:

$$H_s = \sum_{i=1}^{s} p_i \ln p_i$$

where, p_i is the proportion of individuals found in the i^{th} species and In denotes natural logarithm.

The following table gives an example:

	Species	Abundance	p_i	$p_i \ln p_i$
	A	50	0.5	– 0.347
	B	30	0.3	– 0.361
	C	10	0.1	– 0.230
	D	9	0.09	– 0.217
	E	1	0.01	– 0.046
Total	5	100	1.00	– 1.201

Putting the values into the formula for Shannon index, $H_s = 1.201$

Even the rare species with one individual (species E) contributes some value to the Shannon index, so if an area has many rare species, their contributions would accommodate. Shannon index has a minus sign in the calculation, so the index actually becomes 1.201, not-1.201. Values of Shannon index for real communities are often found to fall between 1.5 and 3.5. The value obtained from a sample is in itself of no significance. The index becomes useful only while comparing two or more sites.

For example, humming birds and other nectar-feeding birds form a guild of species that exploit the common resource of flowering plants in a similar fashion. Likewise, seed-eating birds could be grouped into another feeding guild within the broader community. Because species within a guild draw upon a shared resource, there is potential for strong interactions between the members.

Factors influencing community structure:

The structure of a community is the result of many interacting factors, both abiotic (non- living) and biotic (living organism-related). Here are some important factors that influence community structure:

i. The climate patterns of the community's location.
ii. The geography of the community's location.
iii. The heterogeneity (patchiness) of the environment.
iv. The frequency of disturbances, or disruptive events.
v. Interactions between organisms.

A community's structure can also be shaped by the chance events that happened during its history. For instance, suppose that a single seed blows into the dirt of a particular area. If it happens to take root, the species may establish itself and, after some period of time, become dominant (excluding similar species). If the seed fails to sprout, another similar species may instead be the lucky one to establish itself and become dominant.

Foundation Species:

A **foundation species** plays a unique, essential role in creating and defining a community. Often, foundation species act by modifying the environment so that it can support the other organisms that form the community.

Example:

Kelp (brown algae) is a foundation species that forms the basis of the kelp forests off the coast of California. Kelps create environments that allow the survival of other organisms that make up the kelp forest. The corals of a coral reef are another foundation species. The exoskeletons of living and dead coral make up most of the reef

structure, which protects other species from waves and ocean currents. Beavers, which modify their environment by buildingdams, can also be seen as a foundation species.

Image: Kelp (brown algae) of kelp forests of California.

Image: Beaver building dams.

Keystone species:

A **keystone species** is a species that has a disproportionately large effect on community structure relative to its biomass or abundance. Keystone species differ from foundation species in two main ways: they are more likely to belong to higher trophic levels (to be top predators), and they act in more diverse ways than foundation species, which tend to modify their environment. Keystone species are species that function in a unique and signify cant manner through their activities and their removal initiates changes in community structure and often results in a significant loss of diversity. Their role in the community may be to create or modify habitats or to influence the interactions among other species.

Example:

The intertidal sea star *Pisastero chraceus*, which is found in the northwestern United States, is perhaps the most famous example of a keystone species. In a classic experiment of community ecology, the sea stars were experimentally removed from the intertidal zone where they lived. As a result, populations of their prey (mussels) increased, altering the species composition of the community and sharply reducing species diversity. When the sea stars were present, about 25 species of barnacles and algae were found in the lower part of the intertidal zone, but when they were missing, the mussel population expanded downward and almost entirely replaced these other species. This type of sharp reduction in diversity or collapse of community structure commonly occurs when a keystone species is removed. In this case, the loss of diversity happened because the mussels crowded out other species, which could normally persist because the sea stars kept the mussels in check.

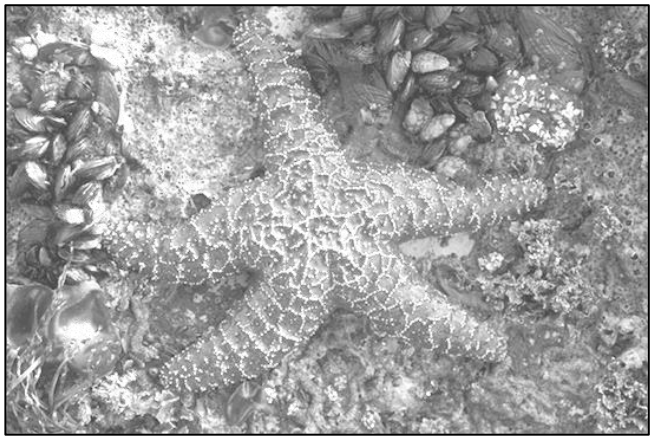


Figure: Photograph of sea star.

Functional groups within a community:

Guilds:

The grouping of species into trophic levels is a functional classification, defining groups of species that derive their energy in a similar manner. Another approach is to subdivide each trophic level into groups of species that exploit a common resource in a similar fashion; thesegroups are termed guilds.

Community boundaries:

Ecotone

- ✓ An ecotone is a zone of junction or a transition area between two biomes (diverse ecosystems).
- ✓ Ecotone is the zone where two communities meet and integrate.

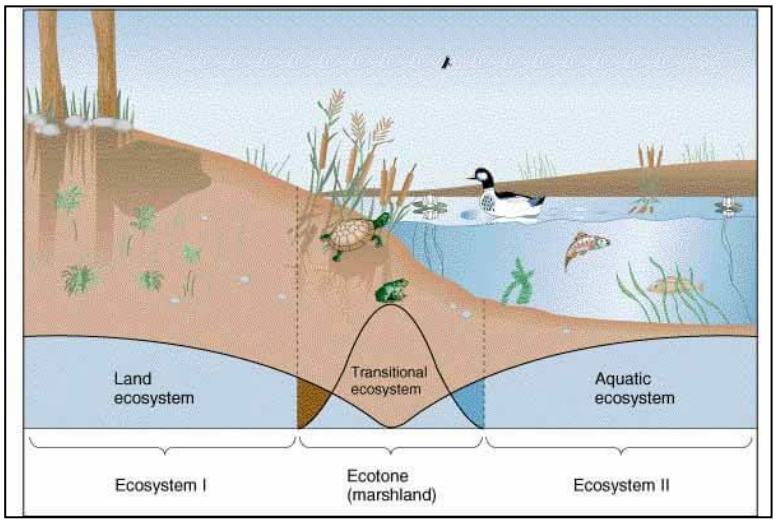
For e.g. the **mangrove forests** represent an ecotone between marine and terrestrial ecosystem.

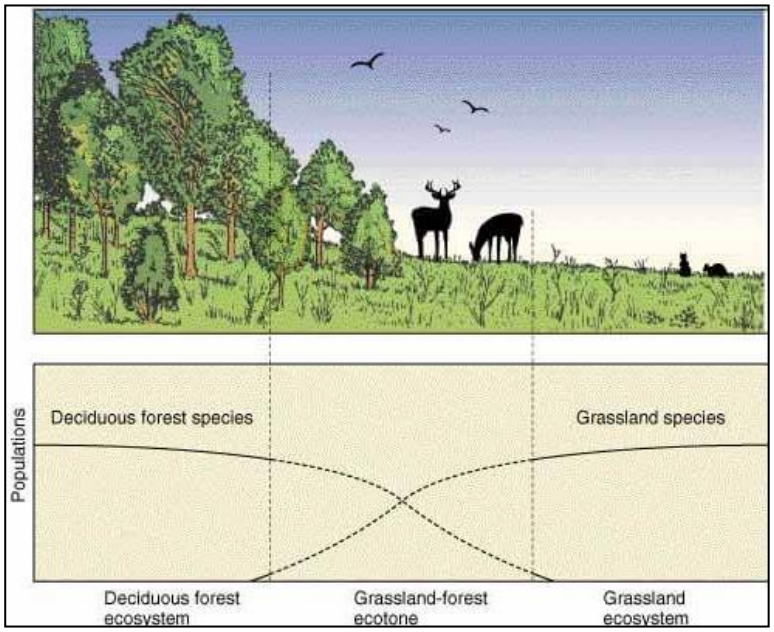
Other examples are **grassland** (between forest and desert), **estuary** (between fresh water and salt water) and **riverbank or marshland** (between dry and wet).

Characteristics of Ecotone

- ✓ It may be narrow (between grassland and forest) or wide (between forest and desert).
- ✓ It has **conditions intermediate** to the adjacent ecosystems. Hence it is a **zone of tension**.
- ✓ Usually, the number and the population density of the species of an outgoing community decreases as we move away from the community or ecosystem.

- ✓ A well-developed ecotone contains some organisms which are entirely different from that of the adjoining communities.





Edge Effect – Edge Species

- ✓ Edge effect refers to the changes in population or community structures that occur at the boundary of two habitats (ecotone).
- ✓ Sometimes the number of species and the population density of some of the species in the ecotone is much greater than either community. This is called **edge effect**.
- ✓ The organisms which occur primarily or most abundantly in this zone are known as **edge species**.
- ✓ In the terrestrial ecosystems edge effect is especially applicable to birds.
- ✓ For example, the density of birds is greater in the ecotone between the forest and

the desert.

Liebig's law of the minimum

In the 19th century, the German scientist Justus von Liebig formulated the "Law of the Minimum," which states that if one of the essential plant nutrients is deficient, plant growth will be poor even when all other essential nutrients are abundant.

This law states that the growth is regulated by a limiting factor, i.e. the scarcest resource, rather than by the total resources available. In biology and ecology, this means that the growth of a population is restricted by the factors that are scarcest and not by the factors that are abundant. This was based on the observation of crop growth. Accordingly, the addition of nutrients in abundance did not result in increased growth. Conversely, the addition of nutrients that are scarce, which in this case is the limiting factor, did lead to increased crop growth. This means that even if some of the nutrients in the soil are abundant but if the other nutrients are limiting or relatively fewer then crop growth will not increase. Applying this principle to other biological populations, this implicates growth occurring only as dictated by the most limiting factor. This principle was used by William Cumming Rose as a basis in identifying the amino acids that were labeled as *essential*.

Liebig's Law has been extended to biological populations (and is commonly used in ecosystem models). For example, the growth of an organism such as a plant may be dependent on a number of different factors, such as sunlight or mineral nutrients (e.g. nitrate or phosphate). The availability of these may vary, such that at any given time one is more limiting than the others. Liebig's Law states that growth only occurs at the rate permitted by the most limiting. For instance, in the equation below, the growth of population O is a function of the minimum of three Michaelis-Menten terms representing limitation by factors I, N and P.

$$\frac{dO}{dt} = min\left(\frac{I}{k_I + I}, \frac{N}{k_N + N}, \frac{P}{k_P + P}\right)$$

It is limited to a situation where there are steady state conditions, and factor interactions are tightly controlled.

How It Works

It states that growth is controlled not by the total originality applied to plant growth, where it was found that increasing the amount of plentiful nutrients did not increase plant growth. Only by increasing the amount of the limiting nutrient (the most scarce) in relation to "need", was the growth of the plant improved.

Importance of Micronutrients

The Law of the Minimum takes on added importance when fertilizer prices — especially of nitrogen (N) and phosphate (P2O5) products — are high. This may tempt some

growers to reduce or even eliminate applications of micronutrient or secondary nutrient fertilizers that provide balanced potassium (K), magnesium (Mg) and sulfur (S). But von Liebig's "Law" tells us clearly that if a soil is deficient in, say, Mg, yields will be depressed regardless of how much N-P-K product you apply.

Probable Questions:

1. What is species richness?
2. What is ecotone and edge effect?
3. What is importance of edge species?
4. What do you mean by Physiognomy?
5. Discuss Simpson's index?
6. What is key stone species?
7. *Describe Liebig's* law of the minimum.

Suggested reading:

9. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
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11. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
12. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XIV

Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate, gross reproductive rate, vital index. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.

Objective:

In this unit we will discuss about Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate, gross reproductive rate, vital index. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.

Introduction:

A population is a group of individual of same species that inhibit a particular geographical area and have the capability of interbreeding. Population ecology is the study of populations (especially population abundance) and how they change over time.

Population Attributes

A population has several characteristics or attributes which is a function of whole group not of the individual. Different population can be compared by measuring these attributes. These attributes are population density, natality, mortality, survivorship, age structure, growth forms, emigration, immigration etc. The study of group characteristics or parameters of the population, their changes over time and prediction of future changes is known as demography.

A population has certain attributes that an individual organism does not have. Some of them are given below:

(i) Population Size or Density:

It is the number of individuals of a species per unit area or volume

$$\text{Population Density (PD)} = \frac{\text{Number of individuals in a region (N)}}{\text{Number of unit area in the region (S)}} \text{PD} = \frac{N}{S}$$

(ii) Birth Rate (Natality):

It is the rate of production (birth rate) of new individuals per unit of population per unit time. For example, if in a pond, there are 20 lotus plants last year and through reproduction, 8 new plants are added, taking the current population to 28. Then, birth rate = 8/20 = 0.4 offspring per lotus per year.

(iii) Death Rate (Mortality):

It is the rate of loss of individuals (death rate) per unit time due to death or due to the different environmental changes, competition, predation, etc. For example, if individuals in a laboratory population of 40 fruit flies died during a specified time interval. Then, the death rate = 4/40 = 0.1 individuals per fruit fly per week.

(iv) Sex Ratio:

An individual is either a male or a female but a population has a sex ratio like 60% of the population are females and 40% are males.

Age Pyramid:

Population at any given time is composed of individuals of different ages. When the age distribution (per cent individuals of a given age or age group) is plotted for the population, this is called age pyramid.

The age pyramids of human population generally show the age distribution of males and females in a combined diagram.

The growth status of the population is reflected by the shape of the pyramids.

That whether it is:

(i) Growing Expanding

(ii) Stable

(iii) Declining

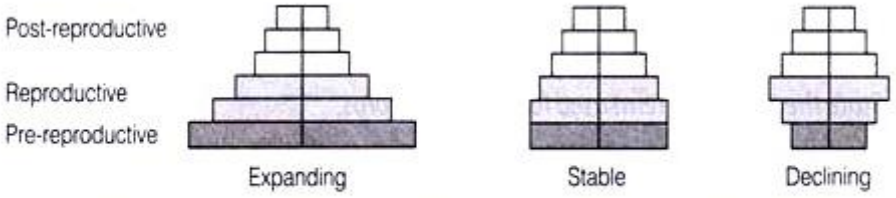


Fig. 13.3 Representation of age pyramids for human population

Population Growth:

The size of a population for any species is not a static parameter, it keeps changing with time.

It depends on the following factors:

(i) Food availability

(ii) Predation pressure

(iii) Weather

The density of a population in a given habitat during a given period, fluctuates due to the four basic processes:

(a) Natality refers to the number of births during a given period in the population that are added to initial density.

(b) Mortality is the number of deaths in the population during a given period.

(c) Immigration is the number of individuals of the same species that have come into the habitat from elsewhere during the time period under consideration.

(d) Emigration is the number of individuals of population who left the habitat and moved elsewhere during a given period of time.

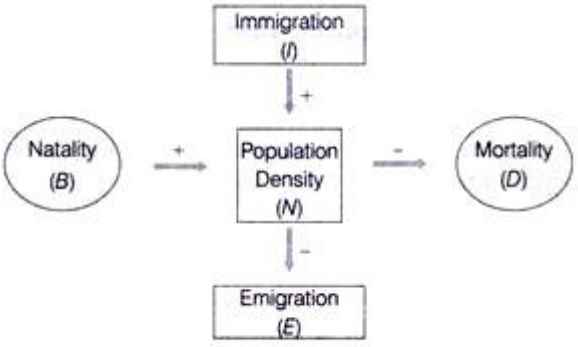


Fig. 13.4 Factors influencing population density

Out of these four, natality and immigration contribute an increase in population density while mortality and emigration contribute to the decrease in population density.

So, if N is the population density at time t, then its density at time t +1 is

$N_{t+1} = N_t + [(B + I) - (D + E)]$

Where, N = Population density

t = Time,

B = Birth rate,

I = Immigration,

D = Death rate,

E = Emigration

From the above equations, we can see that population density will increase if, (B + I) is more than (D + E).

Growth Models:

Studying about the behaviour and pattern of different animals can help us to learn a lesson on how to control the human population growth.

There are following two models of population growth:

Exponential Growth:

Availability of resources (food and space) is essential for the growth of population. The unlimited availability results in population exponential. The increase or decrease in population density (N) at a unit time period (t) is calculated as (dN/dt)

Let dN/dt = (b – d) XN

Let (b-d) = r, then, dN/dt = rN

Where, N is population size, b is birth per capita

d is death per capita, t is time period

and r is intrinsic rate of natural increase.

r, is an important parameter that assess the effects of biotic and abiotic factors on population growth. It is different for different organisms.

It is 0.015 for Norway rat and 0.12 for flour beetle. The above equation results in J-shaped curve as shown in graph.

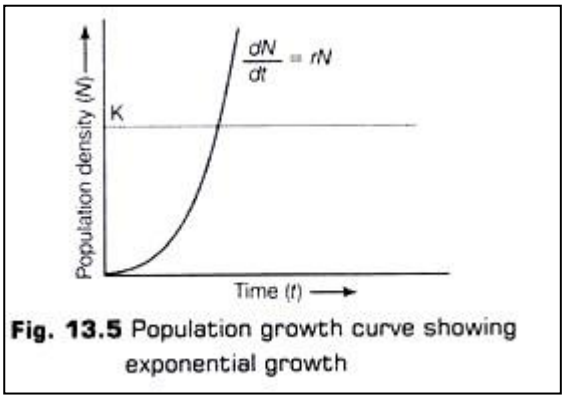


Fig. 13.5 Population growth curve showing exponential growth

Integral form of exponential growth is $N_t = N_0 e^{rt}$

Where,

N_t = Population density after time t,

N_0 = Population density at time zero (beginning),

r = Intrinsic rate of natural increase,

e = Base of natural logarithms (2.71828).

Any species growing exponentially under unlimited resource conditions, without any checks can reach enormous population densities in a short time.

Logistic Growth:

Practically, no population of any species in nature has unlimited resources at its disposal. This leads to competition among the individuals and the survival of the fittest. Therefore, a given habitat has enough resources to support a maximum possible number, beyond which no further growth is possible.

This is called the carrying capacity (K) for that species in that habitat. When N is plotted in relation to time t, the logistic growth show sigmoid curve and is also called Verhulst-Pearl Logistic Growth and is calculated as

$dN/dt = rN (K – N/K)$

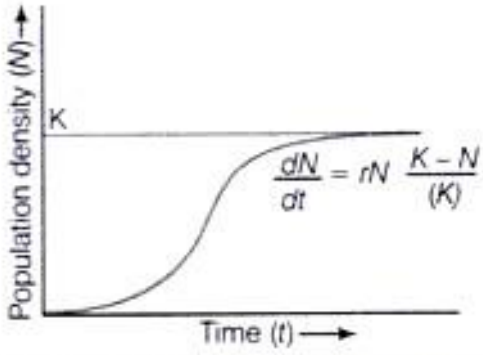


Fig. 13.6 Population growth curve showing logistic growth

Where, N is population density at time t K is carrying capacity and r is intrinsic rate of natural increase.

This model is more realistic in nature because no population growth can sustain exponential growth indefinitely as there will be completion for the basic needs.

Human population growth curve will become S-shaped, if efforts are being made throughout the world to reduce the rate of population growth and make it stationary.

Note:

Human population growth curve is not J-shaped.

Life Table

Life history tables, or life tables, are a method of quantifying population structure that addresses all of the above population traits. Life tables provide age-specific information on survival and fecundity rates for a particular population. An ecologist can collect two very different types of life history data for individuals in a population, which can lead to two kindsof life tables:

Cohort (horizontal or dynamic) life tables require ecologists to follow all the individuals of a single cohort in a population from birth to death. A cohort is a group of individuals all born during the same time interval. Construction of cohort life tables frequently depends on the recapture of marked individuals for mobile species or

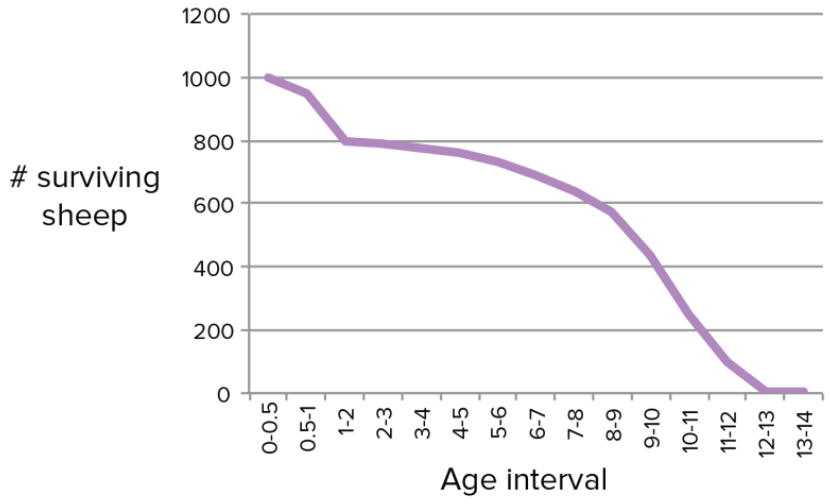
repeated, representative samples of sessile species. Since individuals must be followed from birth to death, the horizontal life table technique is not well suited for the study of long-lived individuals.

Static (vertical or time-specific) life tables consist of data on individuals of all ages in a population from a single point in time. In vertical life table studies, it is important to work with a large, random sample of individuals to ensure that the data is representative of the entire population. For example, the age distribution of your sample of individuals should reflect the age distribution of the whole population. Non-destructive sampling methods are particularly useful for the construction of vertical life tables as they minimize the impact of large sampling efforts on population dynamics.

Both cohort and static observations of population structure can be used 1) to quantify the age structure of a population; 2) to estimate an optimal age of sexual maturity; and 3) to predict population growth rates.

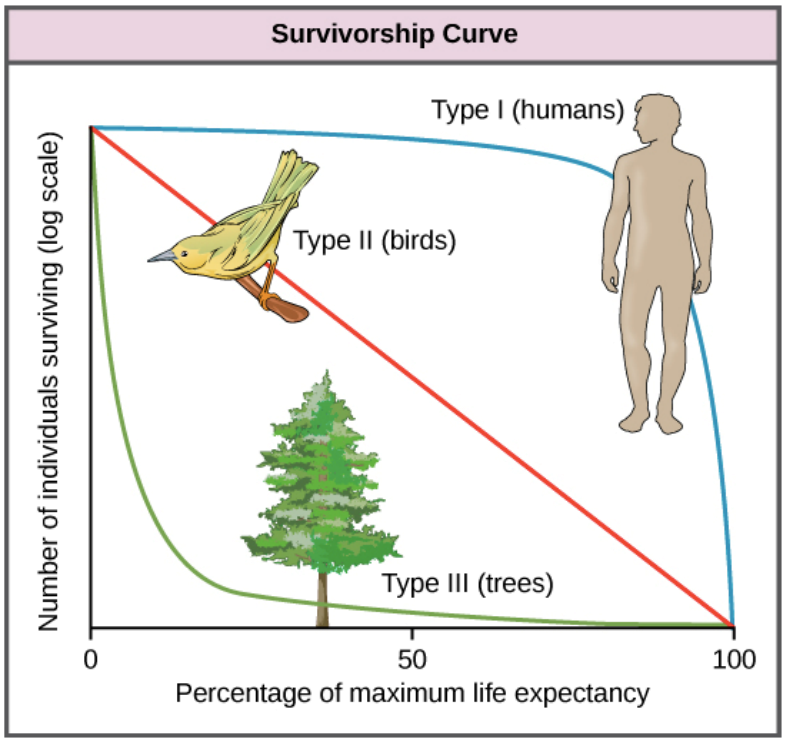
Survivorship curves

A survivorship curve shows what fraction of a starting group is still alive at each successive age. For example, the survivorship curve for Dall mountain sheep is shown below:



The graph makes it nice and clear that there's a small dip in sheep survival early on, but most of the sheep die relatively late in life.

Different species have differently shaped survivorship curves. In general, we can divide survivorship curves into three types based on their shapes:



- **Type I.** Humans and most primates have a Type I survivorship curve. In a Type I curve, organisms tend not to die when they are young or middle-aged but, instead, die when they become elderly. Species with Type I curves usually have small numbers of offspring and provide lots of parental care to make sure those offspring survive.
- **Type II.** Many bird species have a Type II survivorship curve. In a Type II curve, organisms die more or less equally at each age interval. Organisms with this type of survivorship curve may also have relatively few offspring and provide significant parental care.
- **Type III.** Trees, marine invertebrates, and most fish have a Type III survivorship curve. In a Type III curve, very few organisms survive their younger years. However, the lucky ones that make it through youth are likely to have pretty long lives after that. Species with this type of curve usually have lots of offspring at once—such as a tree releasing thousands of seeds—but don't provide much care for the offspring.

Net Reproductive Rate (R_0)

The net reproductive rate is the reproductive potential of the average female, adjusted for survival. We can calculate it by multiplying the standardized survivorship of each age (lx) byits fecundity (bx), and summing these products:

$R_0 = \sum l_x b_x$

Assuming survival and fertility schedules remain constant over time (an unreasonable assumption in most situations), if $R_0 > 1$, then the population will grow exponentially, if

R0 < 1, the population will shrink exponentially, and if R_0 = 1, the population size will not change over time. You may be tempted to conclude that R_0 = r. However, this is not quite correct: r measures population change in absolute units of time (e.g., years); R_0 measures population change in terms of generation time.

Gross reproductive rate

The gross reproduction rate is the average number of daughters that would be born alive to a hypothetical cohort of women if they lived to the end of their reproductive years and if they experienced the some age-specific fertility throughout their lives that women in each age group experience in a given year or period of years. Although the gross reproduction rate purports to describe the fertility experience of a generation of women, the rates presented in this table are actually based on the fertility reported or estimated for a given reference period, usually a single year or a five-year period.

The gross reproduction rate is not affected by the age structure of the population because it is, in effect, an age-standardized fertility rate with each age given a weight of one. In addition, it can also be thought of as the ratio between female births in two successive generations assuming that there are no deaths before the end of the reproductive period, or it may be considered as the ratio between the number of females in one generation at a given age and the number of their daughters at the same age, assuming that there is no mortality during the child-bearing years.

In a female population unaffected by mortality and migration, and assuming the age-specific fertility rates of the reference period do not change, a gross reproduction rate of one indicates exact replacement, a rate of less than one indicates that the population is not replacing itself while a rate of more than one means that the population is more than replacing itself. Because of the impact of mortality, gross reproduction rates somewhat in excess of one are needed to achieve replacement.

Vital index

Vital index is defined as the ratio of births to deaths within a population during a given time.

Birth rate or natality rate is defined as number of births per 1,000 individuals of a population per year.

Death rate or mortality rate is generally expressed as number of deaths per 1,000 individuals of a population per year.

Therefore, the formula of vital index is Natality/Mortality x 100.

Generation Time

The generation time is the average time between two consecutive generations in the

lineagesof a population. Generation time is calculated as

$G = \sum l_x b_x x / \sum l_x b_x$

For organisms that live only 1 year, the numerator and denominator will be equal, and generation time will equal 1 year. For all longer-lived organisms, generation time will be >1 year, but exactly how much greater will depend on the survival and fertility.

Life History strategies

Life history strategy is correlated with many aspects of an organism's reproductive strategy and life history, as well as with demographic variables such as generation time, life span and population density and population dynamics. The concept of r-strategies and k-strategieslinks population dynamics to life history.

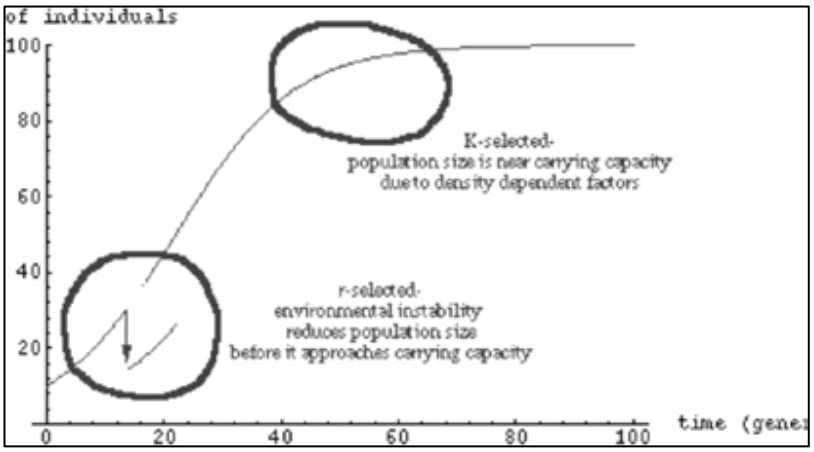
A high intrinsic growth rate r which is achieved by a distinctive strategy consisting of rapid development, a small body size, early reproduction, semelparity and a short life span (less than a year). Species with this reproductive pattern overcome the massive loss of their offspring by producing so many unprotected young that a few will survive to reproduce to begin the cycle again. Species with these characteristics are called r-strategists or r-selected species. Algae, bacteria, rodent, annual plants (such as dandelions) and most insects are r- selected species.

On the other hand, relatively constant or predictable environmental conditions allow a population to reach its carrying capacity k and thus a high average population density. The concept says that these conditions select for high k, which is achieved by slow development (associated with great competitive ability), a large body size, delayed reproduction, iteroparity and a long life span. Species with these characteristics are called k-strategists or k-selected species.

Table 1: Differences between r-selection and k-selection

Characteristics	r-selection	k-selection
Environment	Variable and unpredictable	Fairly constant or predictable
Survivorship	Often type III	Usually types I and II
Population size	Variable in time, non-equilibrium; Usually well below carrying capacityof environment	Fairly constant in time, equilibrium; at or near carrying capacity of the environment

Selection favors	Rapid development Early reproduction High rate of increaseSmall body size Semelparity: single reproduction Many offsprings Low level of social organization	Slower development Delayed reproductionLow rate of increase Larger body size Iteroparity: repeated reproductionFewer and larger offsprings High level of social organization
Life span	Short, less than one year	Longer, usually more than one year
Successional stage	Early	Late, climax



Source: Pianka ER (1970) r- and k-selection. American Natuaralist, 102: 592-597

Figure: r and k selection of life history strategies (Ref: Smith and Smith)

Evolution of life history traits

Life history traits includes such factors as the number, size and sex ratio of offspring, the timing of reproduction, age and size at maturity and growth pattern, longevity, and so on. All of these are heritable to some degree and thus subject to natural selection.

Clutch Size

Clutch size refers to the number of eggs produced by birds, amphibians or reptiles, often at a single time, particularly those laid in a nest. Clutch size differs not only among major taxonomic groups of birds among species, but also among populations and individuals of the same species.

Probable Questions:

1. Probable questions:
2. What is age pyramid?
3. What is life table?
4. What is survivorship curve?
5. What is generation time?
6. Discuss vital index.

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XV

Metapopulation: Concept, models, structure and dynamics Population Biology

Objective:

In this unit we will discuss about Metapopulation: Concept, models, structure and dynamics Population Biology

Meta population:

Meta population is a set of local population occupying an array of habitat patches and connected to one another by the movement of individuals among them.

Theory of metapopulation:

The term metapopulation was first used by Richard Levins, 1970. A metapopulation of species is thought of as a set of small populations occupying an array of similar small habitat patches situated in a matrix of uniform suitable habitat. At any particular time, some suitable habitat patches may contain no individual of the species and thus may be subjected to colonization from other patches that are inhabited.

The local populations are assumed to be small. Local catastrophes and chance fluctuations in number of individuals have important effects on population dynamics, so there is a high probability of extinction of a local population during a particular time interval. Metapopulation dynamics indicate a balance between extinction and colonization.

Simple metapopulation model:

It is conceptualized as a group of local population each having a density of either zero (extinct) or K(equilibrium density), where "K" is the patch carrying capacity. The carrying capacity of patch is the number of individuals that can be supported by the recourses in the patch for a indefinite period of time. At any time, some proportions (P) of the total number of patches in the metapopulations will be occupied and the remaining fraction (1-P), will be unoccupied or become extinct.

= [(1-)]-

Where m = rate of patch

e =rate of patch extinction.

When the rate of change of occupancy is zero $dP/dt = 0$ and $P = 1 - e/m$ is the equilibrium proportion of occupied populations.

The main prediction of this model is that species will not perish i.e $p < 0$ when extinction rate is greater than colonization rate, m is the metapopulation and to put it in another waymetapopulation persistence require $e/m < 1$.

Characteristics of metapopulation:

The major characteristics of metapopulation are patch size & density and compensation of patch size and density.

Patch size and density:

The dynamics of metapopulation are affected not only by the extinction and colonization rates but also by the relationship of those rates to the spatial arrangement and density of the habitat patches for a metapopulation to persist the overall colonization rate must be greater than the extinction rate (1- $^{e}/m$). But successful colonization requires that individuals move from an occupied site to one that is unoccupied and such movements may be prevented if there is a great distance between the occupied and unoccupied sites. The distance barrier may be overcome in time if potential colonized arise continuously from an occupied site. Thereby increasing the chance that one will make it to the distant unoccupied site. But, in order for this to happen, the colonizing population must be persist. In general, the population persistence is related to population size. Small population suffer a higher risk of extinction than the large populations.

Compensation of patch size and density: Patch size and density may interact in a compensatory way to affect population persistence. This interaction can be shown with a modification of a basic metapopoulation model.

Suppose, migration rate (m) dependent on degree to which a patch is isolated, measured as some distance (D), that is migration to an unoccupied patch that is isolated from a colonising patch [high(D)] is less likely than if the patch is in the proximity of the colonizing patch(low D).

The exact relationship between “E” and “m” is not that important so long as “m” declines as “D” increases. One possible relationship between two is a negative potential function.

$m = m_0 e^{-\alpha D}$

Fluctuation in meta population:

The metapopulation are characterized by frequent extinctions of population in patches followed by the colonization of those patches (turn over). Thus at any point in time, some patches area unoccupied. In metapopulation, it is the level of patch occupancy and the rate of turnover (extinction, colonization events) that are important rather than the density of populations in particular patch.

Migration & Demographic feature of Metapopulation:

The more isolated patches in a metapopulation are less likely to be colonized. The reason is that the greater distance represents a barrier to the movement of individuals. Although receives a reasonable basic relationship, other factors such as patch-quality, environment & demo graphic patterns affect the immigration & emigration of the individuals in a patch.

Rescue effect in Metapopulation:

One of the assumptions of simple metapopulation model, is that local patches either contain a population (occupied) or do not (extinct). Some population model relay this assumption and take into consideration of demographic of the individual population.

This more advance model gave us an avenue for understanding the fluctuation or stability of local populations, in the context of regional process.

Stacey et. al (1992) identified a natural population that appear to show rescue effects. In the case of checker spot butterflies (*Euphytryas*) of North America, this distribution reflects the patchy distribution of planets of plant on which the butterflies suggest that relatively high level of heterozygocity are maintained in local populations.

Differences in habitat quality among patches, rather than patches on population size, may produce a similar rescue effects, giving rise to source and sink populations (of habitat patches). Source populations are defined by their ability to maintain a positive growth rate ($\pi>0$), whereas sink populations cannot support negative population growth ($\pi<0$), because oftheir four qualities-

The suitable habitat occurs in discrete patches that may be occupiedby local breedingpopulations.

1. Even the largest populations have a substantial risk of extinction.
2. Habitat patches must not be too isolated to prevent recolonization after local extinction;and
3. The dynamics of the local populations are not synchronized.

Metapopulation dynamics:

The fundamental idea of metapopulation persistence is a dynamic balance between the extinction of local populations and recolonization of empty habitat patches. Levins proposed a simple model of metapopulation dynamics, where metapopulation size is defined by the fraction of (discrete) habitat patches (P) occupied at any given time (t). Within a given time interval, each subpopulation occupying a habitat patch has a probability of going extinct (e). Therefore, if P is the fraction of patches that is occupied during the time interval, the rate at which subpopulations will go extinct (E) is defined as

$$E = eP$$

The rate of colonization of empty patches (C) depends on the fraction of empty patches ($1 - P$) available for colonization and the fraction of occupied patches, providing colonists (P), multiplied by the probability of colonization (m), a constant that reflects the rate of movement (dispersal) of individuals between habitat patches. Therefore, the colonization rate will be $C = [mP (1 - P)]$

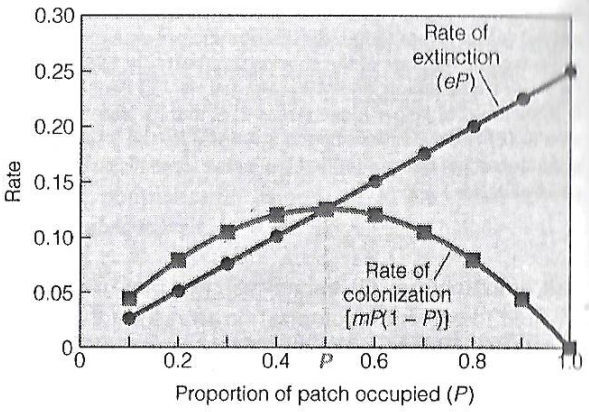


Figure | The rates of extinction and colonization as a function of patch occupancy (P, the proportion of available habitat patches that is occupied) under the Levins model of metapopulation dynamics: $\Delta P/\Delta t = [mP(1 - P)] - eP$. Values of m (probability of colonization) and e (probability of extinction) were set at 0.5 and 0.25, respectively. Note that the equilibrium value of patch occupancy (P) is 0.5. This is the value at which the rate of extinction is equal to the rate of colonization. At values of P above 0.5, the rate of change is negative, and the value of P declines. When values of P are below the equilibrium value (<0.5), the rate of change is positive, and P increases with time.

Figure: Metapopulation dynamics (Ref: Smith and Smith)

We can think of metapopulation growth in a manner analogous to the normal population growth, where the change in the population (ΔN) over a given time interval (Δt) can be expressed as the difference between the rates of birth and death ($^{\Delta N}/_{\Delta t} = b - d$). The change in metapopulation, defined as the fraction of habitat patches occupied by local population through time ($\Delta P/\Delta t$), can therefore be defined as the difference between the rates of colonization (C) and extinction (E) :

$$\frac{\Delta P}{\Delta t} = C - E$$

Or

$$\frac{\Delta P}{\Delta t} = [\mathrm{m}(1 - P)] - eP$$

The model of metapopulation growth functions in a manner similar to the logistic model, in that growth is regulated in a density dependent fashion. For any given values of "*e"* and "*m",*we can plot the rates of extinction (*E*) and colonization (*C*) as a function of the proportion of habitat patches occupied (P). The rate of extinction increases linearly with *P,* and the colonization rate forms a convex curve, initially rising with the proportion of patches occupied, then declining as the proportion approaches 1 (all patches are occupied). The value of *P* where the lines cross represents the equilibrium value, ***P*** and at this value of *P,* the extinction and colonization rates are equal (*E* = *C*) and the metapopulation growth rate is zero ($\frac{\Delta P}{} = 0$). It is an equilibrium value because when the fraction of patches occupied (*P*) is below this value (***P***), the rate of colonization exceeds the rate of extinction and the number of occupied habitat patches increases. Conversely, if the value of *P* exceeds *P* (total patches) **,**the rate of extinction exceeds the rate of colonization and the size of the metapopulation (number of occupied patches) declines. So just as in the logistic model—in which the population density (*N*) tends to the equilibrium population size represented by the carrying capacity (*K*)—in the metapopulation model, the metapopulation density, *P* (proportion of patches occupied), will tend to the equilibrium metapopulation size represented by *P.* The equilibrium value of *P* is a function of the probabilities of extinction (*e*) and colonization (*m*):

$$P = 1 - \frac{e}{m}$$

Probable Questions:

7. What is metapopulation?
8. What four conditions are necessary for the term metapopulation to be applied to asystem of local populations?
9. Discuss the role of local extinction and colonization on metapopulation dynamics.
10. Describe briefly the simple metapopulation model.
11. What are the major characteristics of a metapopulation?
12. How fluctuation in metapopulation occurs?
13. What is rescue effect in metapopulation?
14. Write short notes on metapopulation dynamics.

Suggested reading:

5. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
6. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
7. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
8. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XVI

Major terrestrial biomes: Theory of island biogeography, Biogeographical zones of India

Objective:

In this unit we will learn about Major terrestrial biomes: Theory of island biogeography, Biogeographical zones of India

Theory of island biogeography

Introduction:

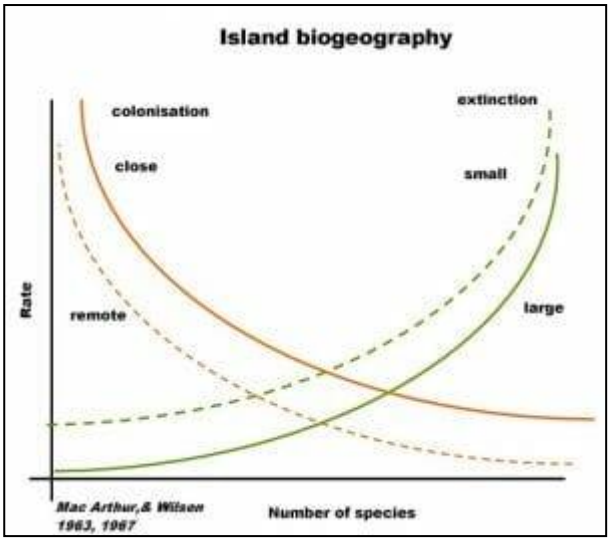
Think of a large office building. It can hold many different people, possibly even several different companies. A smaller office building will hold far fewer people and may be limited to only one or two different companies. The limit is due to the amount of space - a larger space can hold more people and more companies, while a smaller space can't hold as many.

The same idea applies to island biogeography. The theory of island biogeography simply says that a larger island will have a greater number of species than a smaller island. For this theory, an island is any ecosystem that is remarkably different from the surrounding area. So, this could refer to an actual island in the ocean, or it may be an oasis that is surrounded by a desert.

Theory of Island Biogeography:

Island biogeography (also called insular biogeography) provides some of the best evidence in support of natural selection and the theory of evolution. The term describes an ecosystem that is isolated by being surrounded by different ecosystems. For the purposes of this theory, an island is defined as more than just a piece of land surrounded by water. It includes mountain peaks, a lake surrounded by a desert, a patch of woodland or even a national park. The theory provides a model to explain the richness and uniqueness of species, both plants and animals, found in an isolated area.

The two events that determine how many species are found in an isolated ecosystem are immigration and extinction. Research as shown that how big the island is and how far it is from the mainland have a great influence on the number (richness) of species that are found there (see image below). Once species have established themselves on an island, the rate at which they will go extinct depends on the size of the island, with there being less likelihood of extinction on larger islands. This is called the species-area relationship. This relationship is not just observable, but it can also be predicted mathematically. By the same theory, the farther an island is from the mainland, the fewer species it tends to have. This is referred to as the species-distance relationship.



The image above shows how the size of an island and its distance from the mainland interact with immigration (colonization, the orange lines) and extinction events (green lines) to influence the richness of species.

Influences on Species Diversity

When trying to understand the species diversity within any of these ecological 'islands,' you will need to consider three main factors. First is **immigration**, which is the number of new species that move to the island. When there is a higher rate of immigration, there will be a higher number of species in the island ecosystem. However, immigration rates tend to slow when species diversity becomes higher on the island because of competition.

Next is **emigration**, which is the number of species that leave the island. Emigration produces results opposite of immigration. As more species emigrate, there is a lower species diversity on the island, and as fewer species emigrate, there will be a higher species diversity.

The third factor is **extinction**, which is the number of species on the island that become extinct. Extinction rates are related to the size of the island. The smaller the island, the higher the rate of extinction. This is because larger islands contain more resources and habitats, and are thus able to support more life.

Island Biogeography Examples

- ***Australia***

Marsupials like the kangaroo and the wallaby are only found in Australia. If marsupials were found all over the world, then that would mean they did not come into existence by means of natural selection and the evolutionary process. Additional evidence for this evolutionary model is shown by the fact that about 75% of the plant and mammal species in Australia are found only on that continent. There are some exceptions,

however, due to continental drift when animals were able to wander around large land masses before they separated.

- ***The Galapagos and Cape Verde Islands***

When he visited the Galapagos and Cape Verde Islands in 1835, Charles Darwin encountered species that are found nowhere else on Earth like the Galapagos Tortoise, the Flightless Cormorant, and the Blue-footed Boobie and, of course, the famous Darwin's Finches. But it's not just the fact that these isolated environments gave rise to unique species—each island also had its own unique species. In addition, Darwin noted at the time that none of the species from these islands were found in similar climates anywhere in the world. Darwin wondered why there were distinct species on each of the islands when the climates are virtually identical, and why there appeared to be closely related species on the nearest mainland continent. These observations led to his hypothesis (at the time) that the islands had broken off separately from the main continent sometime in the distant past, resulting in two identical populations that evolved separately over time.

Major terrestrial biomes:

The word biome is formed from two Greek words: bios = life and oma = group or mass. The biome as a biological unit which is a type of vegetation, climate, soil and altitude of that specific place. The word was first used with this meaning by the American ecologist Clements in 1916.

Definition:

A biome is a community of plants and animals that have common characteristics for the environment they exist in. They can be found over a range of continents. Biomes are distinct biological communities that have formed in response to a shared physical climate.

Terrestrial Biomes:

Terrestrial biomes are usually distinguished on the basis of the major components of their mature or climax vegetation, while aquatic biomes, especially marine ones, are often characterized by their dominant animals.

8 major terrestrial biomes are -

1. Tundra
2. Northern Conifer Forest
3. Temperate deciduous forest
4. Tropical rain forest
5. Chaparral
6. Tropical Savannah
7. Grassland
8. Desert

I. Tundra:

The literal meaning of word Tundra is north of the timberline. The tundra extends above 60°N latitude. It is almost treeless plain in the far northern parts of Asia, Europe and North America. Tundra consists of plains characterised by snow, ice and frozen soil most of the year. The permanent frozen soil of tundra is called permafrost.

Winters are very long on the tundra with little daylight. In contrast summers are short but there are many daylight hours. Precipitation is low, amounting to only 25 cm or less per year, because cold air can hold relatively little moisture.

The ground is soggy in the summer because moisture cannot soak into the permanently frozen ground. Ponds, small lakes and marshes are abundant due to the nearly flat terrain.

There are no upright trees on the tundra. Only trees such as dwarf willows and birches, which grow low to the ground, can escape the drying effect of the wind which upright trees would experience. This biome consists mainly of mosses, grasses, sedges, lichens and some shrubs. Seasonal thawing of the frozen soil occurs only up-to a few centimetres depth, which permits the growth of shallow rooted plants.

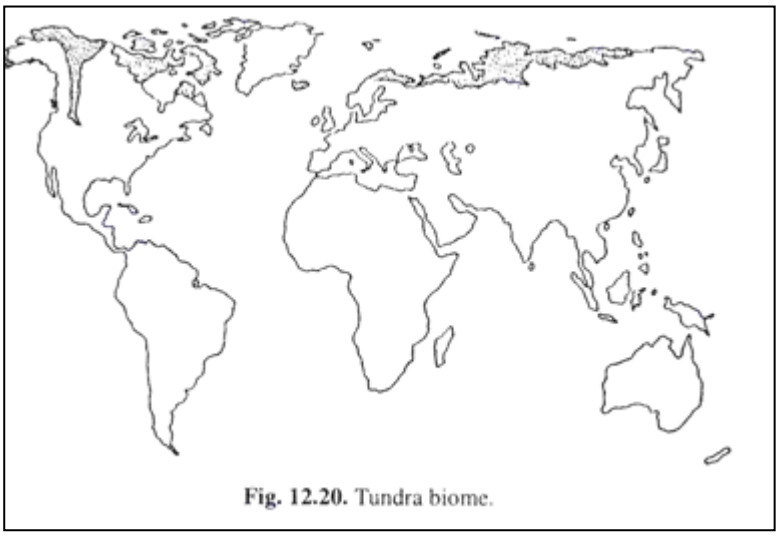


Fig. 12.20. Tundra biome.

Carbon, arctic hare and musk ox are important herbivores of tundra biome. Some important carnivores that prey on the herbivores are the arctic fox, arctic wolf, bobcat and snowy owl. Polar bears live along coastal areas, and prey on seals.

Because of the severe winters, many of the animals are migratory and move from one region to another with the change in seasons. Many shorebirds and water fowls, such as ducks and geese, nest on the tundra during the summer but migrate south for the winter. The tundra makes a very delicate ecosystem, and may be recovered from any disturbance very slowly.

II. Northern Conifer Forest:

The northern coniferous forest or taiga is a 1300-1450 km wide band south of the tundra. This extends as an east-west band across North America, Europe and Asia. This

area also has long, cold winters, but summer temperatures may reach 10-12°C, and the summer and the growing season are longer than in the tundra. Precipitation is higher than in the tundra, ranging from 10 to 35 cm annually.

The moisture is the combined result of summer rains and winter snows. Lakes, ponds and bogs are abundant. The duration of growing period of plants is only about 150 days. Since five physical conditions are variable, the organisms are resistant to fluctuations of temperature.

The taiga makes really a northern forest of coniferous trees such as spruce, fir, pine, cedar and hemlock. In disturbed areas, deciduous trees such as birch, willow and poplar are abundant. In certain areas the trees are so dense that little light may reach the floor of the forest. Vines, maple and spring wild flowers are common. Mosses and ferns also grow in moist areas.

The common smaller mammals are herbivores, such as squirrels, snowshoe hare, and predatory martens. Important migratory herbivores include moose, elk, deer and carbon. Moose and carbon migrate to the taiga for winters and to the tundra for summers.

Important predators are the timber wolf, grizzly bear, black bear, bobcat and wolverine. Many insects are found during the warmer months. Migratory shore birds and waterfowls are abundant during summer months.

III. Temperate Deciduous Forests:

The deciduous forests are found in the temperate regions of north central Europe, East Asia and the eastern United States, that is, south of the taiga in the Northern Hemisphere. Such forests occur in regions having hot summers, cold winter, rich soil and abundant rain. Annual rainfall is typically around 100 cm per year.

Common deciduous trees are the hardwoods such as beech, maple, oak, hickory and walnut. They are broad-leaved trees. The trees shed their leaves in the late fall so the biome has an entirely different appearance in the winter than in the summer.

The fallen leaves provide food for a large variety of consumer and decomposer populations, such as millipedes, snails and fungi living in or on the soil. The temperate deciduous forest produces flowers, fruits and seeds of many types which provide a variety of food for animals.

The common herbivores of this biome are deer, chipmunks, squirrels, rabbits and beavers. Tree-dwelling birds are abundant in number and diversity. Important predators are—black bears, bobcats, and foxes. Predatory birds are also found, such as hawks, owls and eagles. The coldblooded or ectothermic animals, such as snakes, lizards, frogs, and salamanders are also common.

The temperate deciduous forest makes a very complex biome. Many changes take place during the year, and a large variety of species inhabit the soil, trees and air.

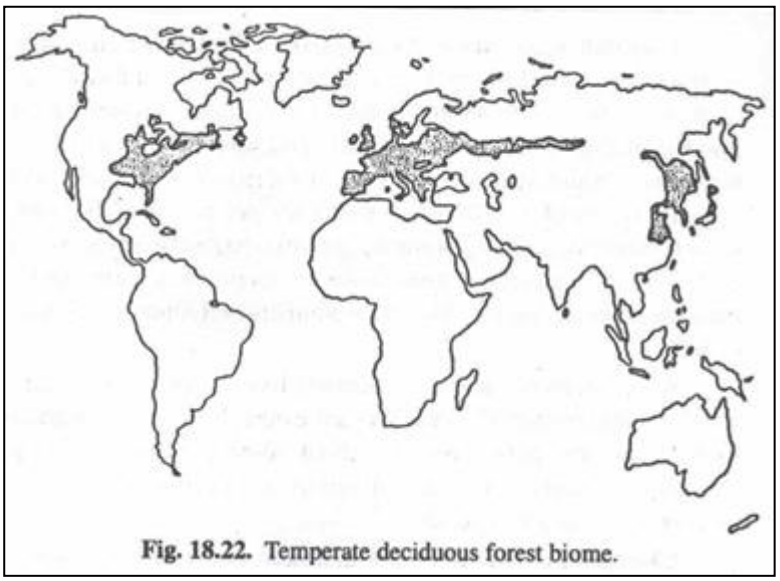


Fig. 18.22. Temperate deciduous forest biome.

IV. Tropical Rain Forest:

This biome is situated in the equatorial regions having the annual rainfall more than 140 cm. However, the tropical rain forest makes an important biome across the earth as a whole. This biome is found in Central America, the Amazon Basin, Orinocon Basin of South America, Central Africa, India and Southeast Asia.

Tropical rain forests have high rainfall, high temperature all year, and a great variety of vegetation. Plant life is highly diverse reaching up-to a framework of 200 species of trees per hectare. The warm, humid climate supports broad- leaved evergreen plants showing peculiar stratification into an upper storey and two or three understoreys.

The tallest trees make an open canopy, but the understoreyed plants block most of the light from the jungle floor. The climbers and lianas reach the highest level of the trees in search of light.

An enormous variety of animals lives in the rain forest, such as insects, lizards, snakes, monkeys and colorful birds. The ant eaters, bats, large carnivorous animals, and a

variety of fish in the rivers are quite common. About 70-80 per cent of the known insects are found in tropical rain forests. Such rich animal diversity is linked to plant-animal interaction for pollination and dispersal of fruits and seeds.

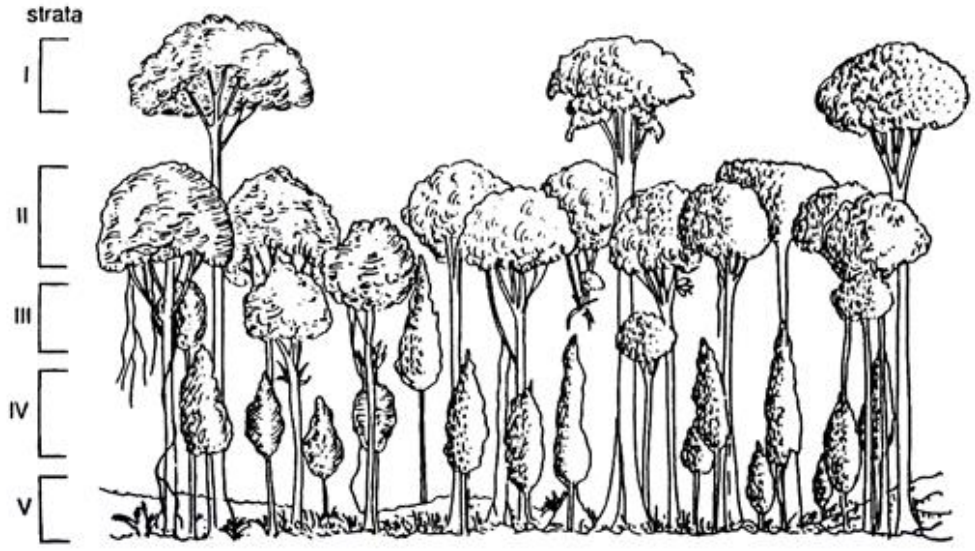


Fig. 12.23. Stratification in a tropical rain forest strata : 1. Tallest trees; II. dense canopy of trees ; III. short trees ; IV. shrubs ; V. herbs and decaying.

V. Chapparal:

This biome is also known as mediterranian scrub forest. This is marked by limited winter rain followed by drought in the rest of the year. The temperature is moderate under the influence of cool, moist air of the oceans. The biome extends along the mediterranian.

Pacific coast of North America, Chile, South Africa and South Australia. This biome has broad-leaved evergreen vegetation. The vegetation is generally made up of fire resistant resinous plants and drought-adapted animals. Bush fires are very common in this biome.

VI. Tropical Savannah:

The savannahs are warm climate plants characterized by coarse grass and scattered trees on the margins of tropics having seasonal rainfall. Primarily they are situated in South America, Africa and Australia. However, there is no savannah vegetation in India. The average total rainfall in such regions is 100 to 150 cm. There is alternation of wet and dry seasons.

Plants and animals are drought tolerant and do not have much diversity. The animal life of tropical savannah biome consists of hoofed herbivorous species, such as giraffe, zebra, elephant, rhinoceros and several kinds of antelope. Kangaroos are found in the savannahs of Australia.

VII. Grassland:

Some grassland occurs in temperate areas of the earth and some occur in tropical regions. Temperate grasslands usually possess deep, rich soil. They have hot summers cold winters and irregular rainfall. Often they are characterized by high winds. The main grasslands include the prairies of Canada and U.S.A., the pampas of South America, the steppes of Europe and Asia, and the veldts of Africa.

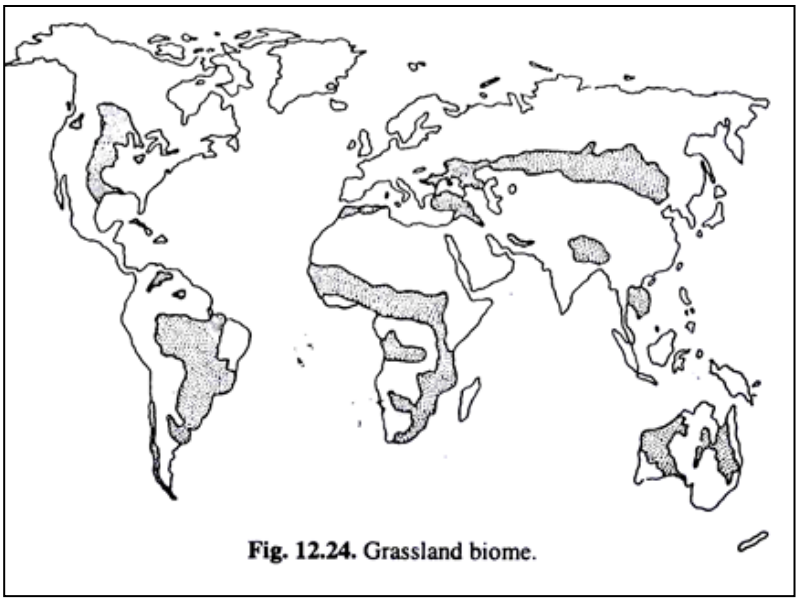


Fig. 12.24. Grassland biome.

The dominant plant species comprise short and tall grasses. In tall-grasses prairies in the United States, important grasses are tall bluestem, Indian grass and slough grass. Short-grass prairies generally have blue grama grass, mesquite grass and bluegrass. Many grasses have long, well-developed root systems which enable them to survive limited rainfall and the effects of fire.

The main animals of this biome are-the prong-horned antelopes, bison, wild horse, jack rabbit, ground squirrel and prairie dogs. Larks, the burrowing owl and badgers are also found. Important grassland predators include coyotes, foxes, hawks and snakes.

VIII.Desert:

The desert biome is characterised by its very low rainfall, which is usually 25 cm per year or less. Most of this limited moisture comes as short, hard showers. Primarily the deserts of the world are located in the south-west U.S.A., Mexico, Chile, Peru, North Africa (Sahara desert), Asia (Tibet Gobi Thar) and central Western Australia. Deserts generally have hot days and cold nights, and they often have high winds.

The reason for the difference of temperature between day and night is due to the lack of water vapour in the air. Deserts are characterised by scanty flora and fauna. Desert organisms must meet some initial requirements if they are to survive. The plants must be able to obtain and conserve water.

In order to meet these requirements, many adaptations have been made by desert plants. Such adaptations are—reduced leaf surface area, which reduces evaporation

from the plants, loss of leaves during long dry spell; small hairs on the leaf surfaces, and the ability to store large amount of water.

The examples of important desert plants are—yuccas, acacias, euphorbias, cacti, many other succulents and hardy grasses. Many of the small plants are annuals.

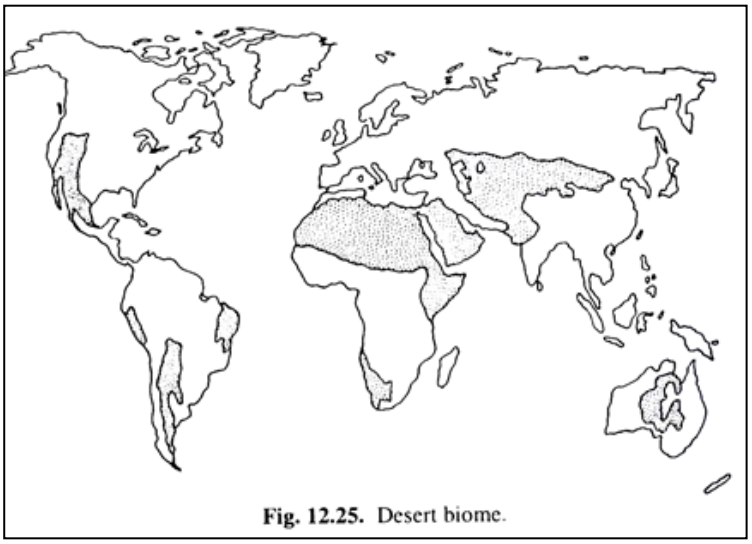


Fig. 12.25. Desert biome.

Animals also must meet the requirements of heat, cold and limited water. Many desert animals are nocturnal in habit, and are active mainly at night. Many reptiles and small mammals burrow to get away from the intense heat of midday. The other common desert animals are the herbivorous kangaroo, rat, ground squirrel, and jack rabbit.

The important predators are—coyotes, badgers, kit fox, eagles, hawks, falcons and owls. Ants, locusts, wasps, scorpions, spiders, insect-eating birds, such as swifts and swallows, seed-eating quails, doves and various cats are other common desert animals.

Major biogeographical zones of India

Biogeographic zones of India are the division of India according to biogeographic characteristics. Biogeography is the study of the distribution of species, organism, and ecosystems in geographic space and through geological time.

India is a megadiverse country with only 2.4% of the total land area of the world, the known biological diversity of India contributes 8% to the known global biological diversity. Biogeographically, India has been divided into 10 biogeographic zones as follows:

a. Trans-Himalayan region
b. Himalayan zone
c. Indian Desert Zone
d. Semi-Arid Region
e. Western Ghats
f. Deccan Peninsula/ Plateau
g. Gangetic Plain

h. North-East Region
i. Coastal region
j. Andaman and Nicobar Islands

a) Trans-Himalayan region:

The Himalayan ranges immediately north of the great Himalayan ranges are called the trans-himalayan.it constitutes 5.6% of the total geographical area, includes the high altitude, cold and arid mountain areas of Kazakh, Jammu and Kashmir, North Sikkim,lahaul and spirit areas of Himachal Pradesh. This zone has sparse alpine steppe vegetarian that harbors several endemic species and is a favorable habitat for the biggest population of wild sheep and goats in the world and other rare fauna that includes Snow Leopard and the migratory Black necked Crane (Grus nigricollis).The cold dry desert of this zones represent an extremely fragile ecosystem.

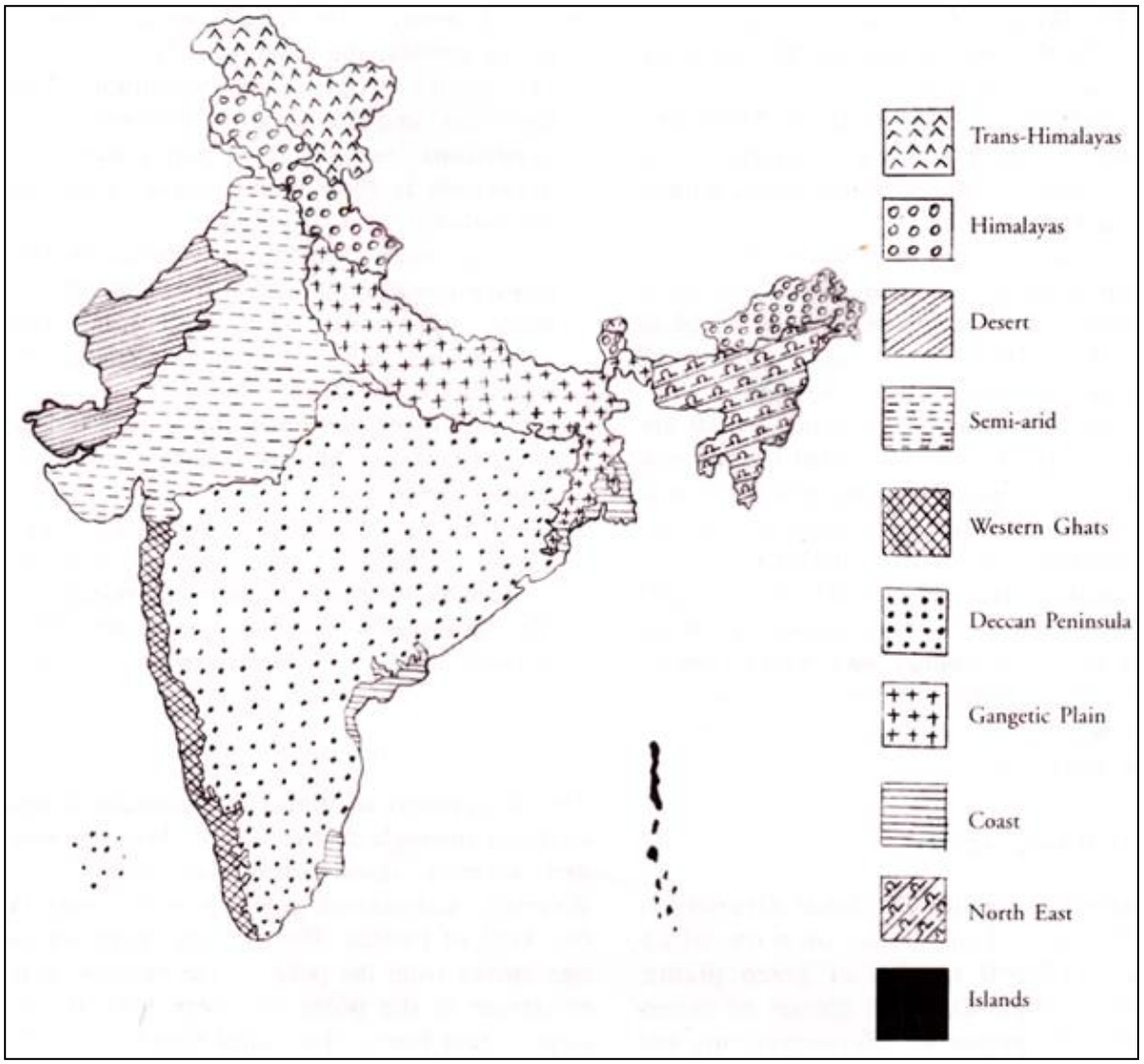


Fig: Biogeographical region of India

b) Himalayan zone:

It constitutes 6.4% of the total geographical areas includes some of the highest peaks in the world. The Himalayan zone makes India one of the richest areas in terms of habitats and species.

The Himalayas consists of the youngest and loftiest mountain chains in the world .The Himalayas have attained a unique personality owing to their high altitude ,steep gradient and rich temperate flora. The forests are very dense with extensive growth of grass and evergreen tall trees.Oaktu,chestnut,conifer,ash,pine,said at are abundant in Himalayas. Chief species includes wild sheep, mountain goats, ibex, shrew, Tapir, Panda and snow leopard.

The alpine and sub-alpine forests ,grassy meadows and moist mixed deciduous forests provide diverse habitat for endangered species of voids such as Bharal (*Pseudois nayaur*), Ibex (*captain ibex*), Markhor(*Capra falconeri*), Himalayan Tahr (*Hemitragus jemlabicus*), and Takin (*Budoreas taxicolor*). Other rare and endangered species restricted to this zone include Hangul (*Verbis elfineldi*) and Mask Deer (*Moschus mpschiferus*).

c) Indian Desert Zone:

It constitutes 6.6% of the total geographical areas, includes the Thar and the Kutch deserts and has large expanses of grassland. The climate is characterized by very hot and dry summer and cold winter. Rainfall is less than 70cm.The plants are mostly Xerophytic. Bavul, Korar, wild plants grows in areas of moderate rainfall.

It supports several endangered species of mammals such as Wolf (*Canis lupus*), Caracal (*Felix caracal*), Desert Cat (*Felix libyca*) and birds of conservation interest viz., Houbara Bustard (*Chamydotis undulate*) and the Great Indian Bustard (*Ardeotis nigriceps*).

d) Semi-Arid Region:

It constitutes 16.6% of total geographical area,is a transition zone between the desert and the dense forest's of western Ghats.

Peninsula India has two large regions, which are climatically semi-arid. This region has several artificial and natural lakes and marshy lands.

The dominant grass and palatable shrub layer in this zone supports the highest wildlife biomass. The cervix species of samber (*Cervix unicolor*) and Chital (*Axis axis*) are restricted to the better wooded hills and mioster valley areas respectively. The Lion (*Leo parsica*), an endangered species, Caracal (*Felis caracal*), Jackal (*Canis aureus*) and Wolf (*Canis lupus*) are some of the endangered species that are characteristic of this region.

e) Western Ghats:

It constitutes 4.0% of the total geographic area. The mountains along the Western Ghat extend from the southern tip of the peninsula (8 degree N) northwards about 1600km to the mouth of the tapti (21 degree N).

It is one of the major tropical evergreen forest region in India and represents one of the two biodiversity 'hotspots'. Western Ghats are home to viable populations of most of the vertebrate species found in peninsular India, besides an endemic faunal element of its own.

Significant species endemic to this region include Nilgiri langur (*Presbytis jobni*), Lion tailed macaque (*Macaca silenus*), Grizzled Giant Squirrel (*Ratufa macroura*), Malabar Civet (*Viverricula megaspila*), Nilgiri Tahr (*Hemitragus bylocriud*) and Malabar Grey Hornbill (*Ocycerous griseus*).The Travancore Tortoise (*Indotestudo forstem*) are two endangered taxa restricted to a small area in central Western Ghats.

f) Deccam Plateau:

Beyond the ghats is Deccan Plateau, a semi- arid region lying in the rain shadow of the Wester Ghats. This is India is largest biogeographic region, constitutes 42% of the total geographical area.

This zone of peninsular India is most extensive zone, covering India's finest forests, particularly in the states of Madhya Pradesh, Maharashtra and Odisha.

The highlands of the plateau are covered with different types of forests, which provide a large variety of forest products. Majority of the forests are deciduous in nature but there are regions of greater biological diversity in the hill ranges. The zone comprising of deciduous forests, thorn forest and degraded scrubland support diverse wildlife species.

Species found in this region are Chital (*Axis axis*),Sambar(Cervus unicolor), Nilgai (*Boselapbus tragocamelus*) and Chousingha (*Tetracerus quadricornis*) .Barking deer (*Muntiacus muntjak*),Gaur (*Antilope cervicapra*), Elephant (*Elephus maximus*) in Bihar-Orissa and Karnataka-Tamil Nadu belts, wild Buffalo (*Bubalus bubalis*) in a small area at the junction of Orissa, Madhya Pradesh and Maharashtra and the hard ground swamp Deer (*Cervus duvauceli*), now restricted to a single locality in Madhya Pradesh.

g) Gangetic Plain:

Gangetic plain constitutes around 10.8% of the total geographical area. In the north is the Gangetic plain extending upto the Himalayan foothills. This is the largest unit of the Great Plain of India. Ganga is the main river after whose name this plain is named. The tree belonging to these forests are are teak, sal, shisham, mahua, khair etc.

The characteristic fauna of this region include Rhino (*Rhinoceros unicornis*), Elephant (*Elephas maximus*), Buffalo (*Bubalus bubalis*), Swamp Deer *(Cervus duvauceli*), Hog-Deer (*Axis porcinus*) and Hispid Hare (*Carprolagus bispidus*).

h) North-East Region:

It constitutes 5.2% of the total geographical area, represents the transition zone between the India, Indo- Malayan and Indo-Chinese biogeographical regions as well as being a melting point of the Himalayan Mountains and peninsular India. Thus North-East is the 'gateway' for much of India's flora and fauna and also a biodiversity hotspot (Easter Himalaya). It is one of the richest flora region in the country It has several

species includes orchids, bamboos, ferns and other plants. Many species are either restricted to this biological diversity or to the smaller region itself or to the Kashi Hills of the smaller localized areas.

i) Coastal region:

It constitutes 2.5% of the total geographical area which includes sandy beaches, mangroves, mud flats, coral reefs and marine angiosperm pastures make them the wealth and health zones of India. The coastline extending from Gujrat to Sundarbans is estimated to be 5,500 km long. Total of 25 islets constitutes the Lakshadweep, which are of coral origin, and have a typical reef lagoon system, rich in biodiversity .However the densely populated Lakshadweep islands virtually have no natural vegetation.

The backwaters are the characteristic features of south cost and east coast are broader due to depositional activities of the eat -flowing river owning to the change in their base levels .

j) Andaman and Nicobar Islands:

This constitutes 0.3% of the total geographical area. It is one of the three tropical moist evergreen forests zones in India. The islands house an array of fauna and flora not found elsewhere.

These islands are centers of high endemism and contain some of India's finest evergreen forests and support a wide diversity of corals. In India endemic island biodiversity is found only in the Andaman and Nicobar Islands. Some of the Island is fringed with coral and some of the endemic fauna of Andaman and Nicobar Islands include Narcondam hornbill, south Andaman krait etc.

Probable Questions:

1. Define Biome.
2. State the characteristics of each biome.
3. Describe the characteristics of different biogeographical zones of India.
4. Describe the flora and fauna of desert.
5. Describe the climatic character of tundra biome.

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XVII

Concept of Environment: Structure, radiation balance, UN movements on environment

Objective:

In this unit we will learn about Concept of Environment: Structure, radiation balance, UN movements on environment.

Structure of environment:

Earth is the only planet in our solar system that supports life as we know it, and its distance from the Sun provides favourable temperatures for life. Planet Earth is a sphere about 25,000 miles (40,000 kilometers) in circumference, the fifth largest in the solar system. About 70 percent of its surface is covered by large bodies of salty water called oceans, which are never still, but flow and change all the time. About 30 percent is dry land. The great land masses, known as continents, are surrounded by the oceans. The formation and evolution of the Earth and its environment have occurred over the long, long course of millennia. The principal components of the planet are:

The atmosphere:

The mass of air surrounding the Earth like a transparent wrapping.

The lithosphere: the solid outer crust of rocks about 80 km thick, which is the outer solid shell of the planet body. This also includes the pedosphere, where the soils are present andthe soil-forming processes occur.

The hydrosphere: the water portion of Earth as distinguished from the solid part (the lithosphere), and from the gaseous outer envelope (the atmosphere). This part of the environment also includes the cryosphere, which is that part of the Earth's body that is predominantly frozen and mainly consists of different forms of ice.

The biosphere: the life zone of the planet which permeates all the above as life is widely spread around the planet. The biosphere includes the lower atmosphere, the whole hydrosphere, the pedosphere, and the outer portion of the lithosphere to a depth of about 2 km; in short, all regions in which living organisms exist. These structural components are interlinked and unified into a holistic concept of the Earth system. While the hydrosphere is included here to provide a complete overview of the physical environment as a whole, the subject is considered in far greater detail in other themes. Besides covering the hydrological cycle, the hydrosphere topic includes articles on the oceans, freshwater (surface water and groundwater), and the linkages between surface water and groundwater. The cryosphere topic deals with all forms of ice on Earth. This has an important effect on the global climate, which is also considered in

other themes. In the topic on the lithosphere, discussions deal with the genesis of the zone, the geologic processes occurring there, and the mineral resources it contains. Although the pedosphere is the outer layer of the lithosphere, it is discussed here as a separate entity because of the special circumstances of its genesis and its important role in the functioning of many life-supporting systems.

i) Atmosphere:

In everyday life and activities, human society interacts closely with the atmosphere through climate and weather, which are intimately related to the state of the atmosphere. Quite minor in its mass, as compared to that of the whole planet (it amounts to only about one millionth of the latter) the atmosphere is an absolutely indispensable environment for all life forms. Without it the Earth would be a lifeless planet. Weather has a powerful effect on agricultural productivity; it controls human needs in the production and consumption of all forms of energy, and it is critical for aviation safety and the efficiency of ground transportation. Many aspects of human activities are critically affected by sharp changes in weather and the oscillations of climate. History knows many cases when severe winters, or extensive summer droughts afflicting large territories, brought catastrophe to the economies of whole countries. The atmosphere—an air envelope of our planet—is studied in the science of meteorology, which considers atmospheric processes in all their complexity, including the interaction of the atmosphere with the hydrosphere and the lithosphere (the Earth's surface), and investigates the origin and causes of various atmospheric phenomena, partly with the objective of developing forecasting techniques.

Structure of Atmosphere:

Troposphere:

Almost 70% of the mass of the atmosphere is contained in this layer. It is more or less homogeneous in composition where there is no air pollution. The water content here also depends on the hydrological cycle. The troposphere is a region of turbulence. This is because of the global energy flow that arises from imbalances of heating and cooling rates between the equator and the poles. The air is far from uniform, depending on density and temperature. In the troposphere, temperature decreases uniformly with increasing altitude. Near the ground level, the air is heated by radiation from the earth. At the top of the troposphere is the tropopause. This is the cold layer (-56°C) which marks temperature inversion, i.e. transition from negative to positive lapse rates.

Stratosphere:

This is the quiescent layer marked by a positive lapse rate. The temperature increases with increase in altitude. At the upper limit of the stratosphere it is -2°C. This region has the maximum concentration of Ozone. The gas absorbs ultraviolet radiation of the sun, raising the temperature and causing a positive lapse rate.

The importance of ozone lies in the fact that it acts as a protective shield for life on earth and from the detrimental effects of the scorching ultraviolet rays. It also supplies the heat for dividing the earth into a quiescent stratosphere and turbulent troposphere.

Since mixing in the stratosphere is very slow, the molecules or particles here persist for a long time. Therefore, once the pollutants are able to reach the stratosphere, they pose long- term global hazards, compared to their impact in the more dense troposphere.

Mesosphere:

This layer has been so named because of its situation in the middle of the five layers that is divided according to the thermal conditions of the atmosphere. The mesosphere stretches at altitudes between 50 and 80 km. It is also known as upper Troposphere since, due to the absence of ultraviolet absorbing species, temperature falls with increasing altitude, i.e. negative lapse rate prevails, basically similar to what happens in the troposphere.

During summer, the temperature of the mesopause over Arctic region can drop to as low as - 100°C. The upper part of this layer remains colder while the lower part is warm. Due to this, there arises a connective motion of air. A significant feature of this layer is the formation of noctilucent cloud or an ice-cloud, formed by the deposition of a very small amount of water vapour on the nickel containing cosmic dust (by depositing, it means here the process by which water vapour turns into ice-crystals directly on the condensation nuclei).

The noctilucent cloud is silver-white and light blue in colour. It is cirrus-like in shape and appears over higher latitudes before sunset and disappears after sunset. A series of intense photochemical reactions, i.e. dissociations and recombination's of different elements of the atmosphere, take place in the mesosphere under the action of the sun's ultraviolet radiation.

In the layer below the mesosphere, the air is homogeneously mixed since both horizontal and vertical motions are present. For this reason, the atmosphere up to 90 km is termed as the homogeneous atmosphere.

Termosphere:

This layer that extends from the mesopause up to about 800 km is noted for two striking features:

Temperature rises with increasing altitude i.e. positive lapse rate prevails here. Satellite data shows that at 200 km temperature stays around 700°C, and at 300 km it exceeds 1,000°C. The high temperature results from the heat released by the dissociation of oxygen and nitrogen molecules and atoms. The dissociation is caused by the sun's ultraviolet radiation with wavelengths shorter than 0.175 microns that can absorb the gases in the layer.

The air is highly ionized. The gases present here, especially nitric oxide and oxygen, split

into atoms and undergo ionization, following absorption of solar radiation in the far ultraviolet region. Over higher latitudes, the resplendent aurora appears in the Thermosphere. It is the outcome of the collision of some of the charged particles thrown out of the sun with the molecules and atoms of gases in the thermosphere, under the action of the earth'smagnetic field.

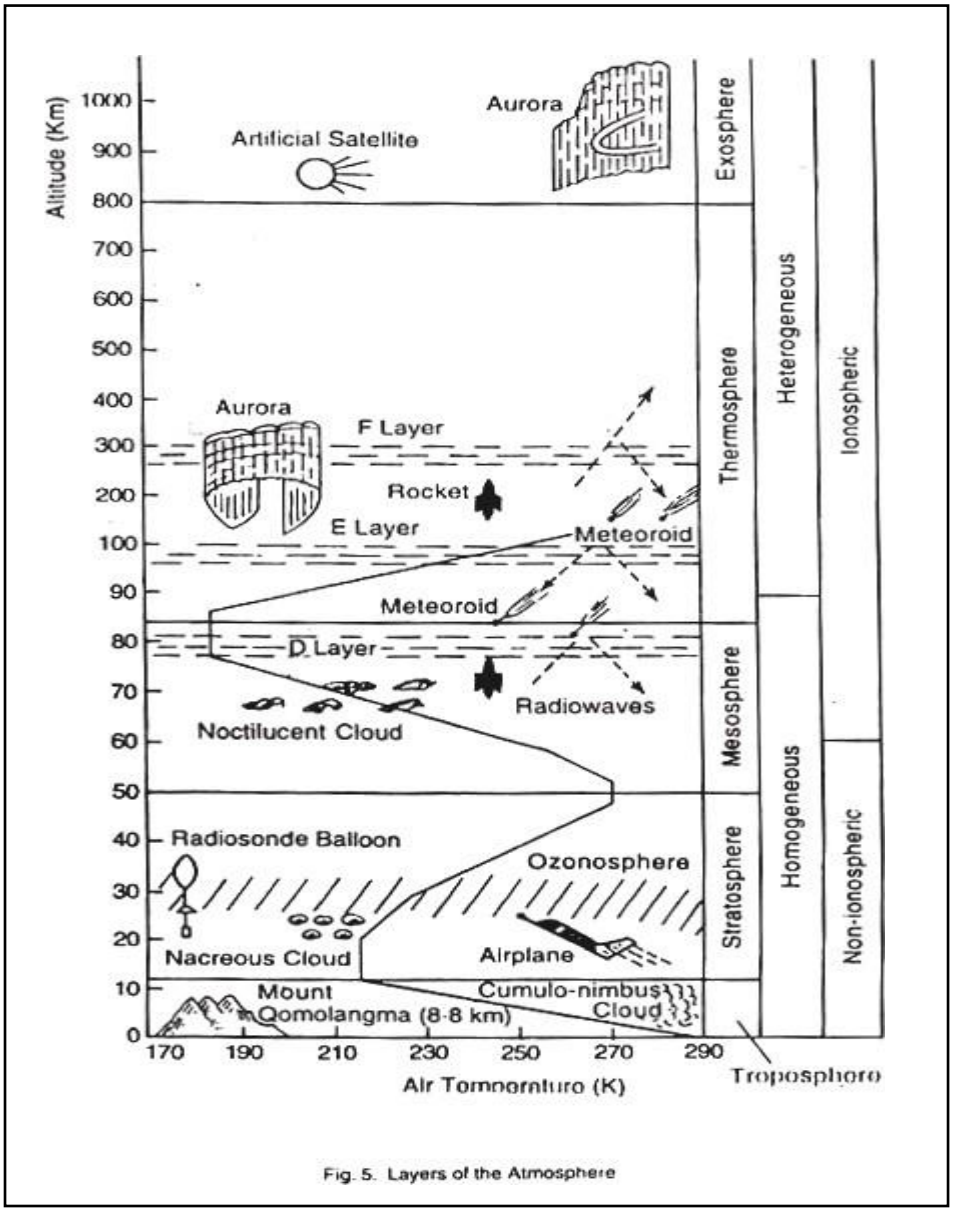


Fig. 5. Layers of the Atmosphere

Exosphere:

This is the outermost layer of the atmosphere, and also the transition zone between the earth's atmosphere and interplanetary space. The air is inconceivably thin and almost completely ionized. It consists of helium and hydrogen, the lightest constituents of air.

Based on its electric properties, the atmosphere can be divided into two layers:

a) The non-ionosphere is the section of the atmosphere up to 60 km. Here, the

constituents remain in neutral state.

b) The Ionosphere is the section of the atmosphere between 60 and 2,000 km. The ultraviolet radiation from the sun ionizes the air and produces ions and free electrons. The air thus becomes a mixture of charged particles and non-ionized neutral particles. These free electrons act as mirror and reflect certain types of radio-waves.

ii) Hydrosphere:

It includes water in the oceans, lakes, ponds, etc., and covers about 73% area of the earth's surface. Water is the major inorganic nutrient needed by all living organisms, hence, water is essential to all life. First life originated in water.

The means of obtaining and conserving water have shaped the nature of terrestrial life; means of living within the water have the overwhelming influence on aquatic life. Water is one of the main agents in pedogenesis and is also the medium for several different ecosystems.

Water continuously circulates between atmosphere and the earth's surface; this cycle is referred to as the water cycle. The energy for driving the cycle and, thus, ensuring a constant supply of fresh water on land comes from the sun. Solar heat evaporates water from the oceanwhich is the great reservoir of water.

A lesser amount of water is also evaporated from the surface of the land from plants, a process known as evapotranspiration. All this vaporized water forms clouds which moved by winds, may pass over land where they are cooled enough to precipitate the water as rain or snow. Some of the precipitated water soaks into ground, some runs off the surface into streamand goes directly back to the seas.

The ground water is returned to the surface by springs, by pumps and the transpiration—the movement of water in plants from roots to leaves. Water inevitably ends up back in the sea, but it may become incorporated into the bodies of several different organisms, one after another, en route.

iii) Lithosphere:

The solid component of earth is called lithosphere. It is multilayered and includes following three main layers: crust, mantle and core (outer and inner). The core is the central fluid or vapourized sphere having diameter of about 2500 km from the centre and is possibly composed of nickel-iron. The mantle extends about 2900 km above the core. This is in a molten state.

The crust is the outermost solid zone of the earth and it is about 8 to 40 km above mantle. The crust is very complex and its surface is covered with the soil supporting rich and varied

Cross section of earth showing different layers, biotic communities. The soil provides

food, shelter, anchorage and concealment from predators to living organisms. The soil is the most characteristic feature of terrestrial environment. It is the top layer of earth's crust and is the mixture of weathered rock materials (i.e., minerals) and organic detritus, both of which are formed through the physical, chemical and biological processes occurring slowly and slowly for a long period at the earth's surface.

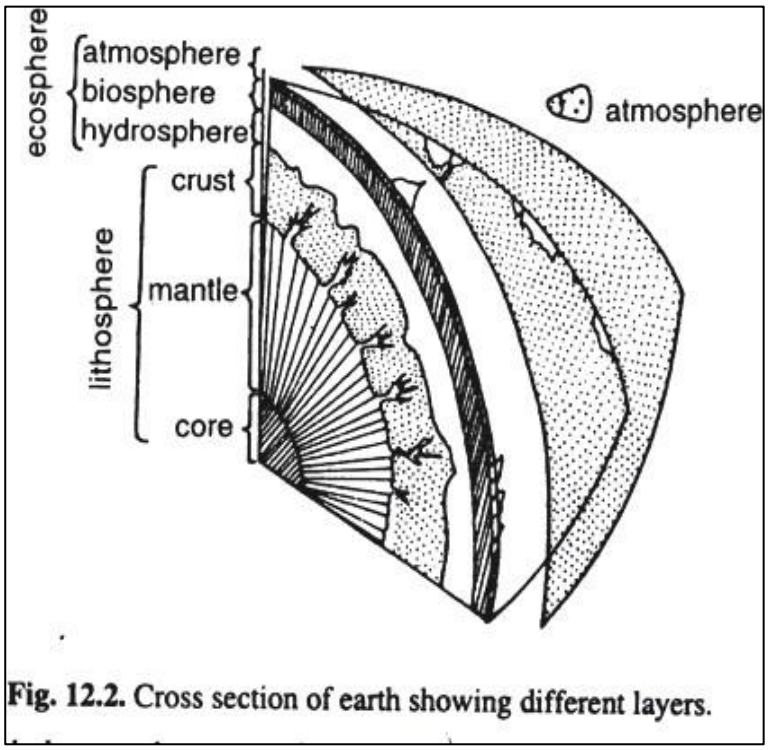


Fig. 12.2. Cross section of earth showing different layers.

Besides being the source for the entry of nutrients and water in plants, the soil is the medium for the detritus food chain: nutrients released in detritus are decomposed by various soil microbes like bacteria, algae, fungi, protozoa, etc., bound in or on soil particles and taken back into plants through their roots.

Soil (mud) is also the main source of nutrients for all aquatic plants; rooted, submerged or free-floating. In addition, soil is the means to support for all terrestrial organisms: plants are anchored to the soil by their root systems; animals walk upon it and are supported by it, as many animals like nematodes, polychaetes, arachnids, insects, rodents, etc., live under the soil.

However, the chemical composition of earth's crust has been shown in Table below. In addition, soil also contains organic matters like humus, etc.

Table 12.2. Composition of dry atmosphere.

S.No.	*Constituent*	*Percentage*
1.	Nitrogen (N_2)	78.084
2.	Oxygen (O_2)	20.947
3.	Argon (Ar)	0.934
4.	Carbon dioxide (CO_2)	0.0314*
5.	Other gases	0.003

* These constituents are highly variable.

Thus, these three ecological components and the biotic component of the world constitute the biosphere. All these four components, thus, represent the four major global components ofthe world ecosystem.

These four spheres continuously exchange matter with one another in a cyclical manner. Thus, biosphere is that part of the earth in which life exists. More specifically, the sum of those portions of the hydrosphere, lithosphere and atmosphere into which life penetrates, is the biosphere.

Table 12.3. Chemical composition of earth's crust.

S.No.	*Constituent*	*Percentage*
1.	Oxygen (O)	46.6
2.	Silicon (Si)	27.7
3.	Aluminium (Al)	8.1
4.	Iron (Fe)	5.0
5.	Calcium (Ca)	3.6
6.	Sodium (Na)	2.8
7.	Potassium (K)	2.6
8.	Magnesium (Mg)	2.1
9.	Compounds of elements like B, Mn, Cu, Zn, Mo, Co, I, F, etc.	1.4

However, together with the geological, chemical features of the totality of our habitats, these (air, water, earth and organisms) are sometimes grouped under the term ecosphere. Biosphere or ecosphere may be thought as a biochemical system capable of capturing, converting, storing and utilizing the energy of the sun.

Approximately three hundred thousand species of green plants and microorganisms are recognized as primary producers which utilize inorganic elements and compounds to synthesize the organic minerals of life. Their productivity is consumed by more than a million other species of organisms which convert this organic store-house into animal form, adding to the beauty, and value of the biosphere as well as its complexity. Still other species, primarily bacteria and fungi, accomplish the recycling process by returning plant and animal wastes and residues to inorganic form so the process may be renewed.

In this cyclic process of life, many elements are shared form a common global pool and are converted from inorganic to organic form and back again. Examples of such elements which commonly shuttle among the air, water, earth and organism are carbon, oxygen, hydrogen, nitrogen, phosphorus, sulphur, sodium, potassium, calcium, magnesium, iron, manganese, cobalt, copper, and zinc.

In physical terms, the biosphere is a relatively thin and incomplete envelope covering most of the world. It represents a mosaic of different biotic communities from simple to complex, aquatic to terrestrial, and tropical to polar. It does not exist in the extremities of the Polar Regions, the highest mountains, the deepest ocean troughs, the most extreme deserts, or the most highly polluted areas of land and water.

Its total thickness, including all portions of the earth where living organisms can exist, is less than 26 kilometres. Its zone of active biological production, in terms of photosynthesis, is much narrower, and varies from a few centimetres to over 100 metres.

This zone would, for instance, be only a few centimetres in muddy or turbid water, whereas in very clear ocean water, it could be more than 100 metres in thickness. On land, the zone of biological production might be only a few millimetres in a desert or rock environment, whereas it might again be more than 100 metres in a sequoia or tropical rain forest.

Living organisms can exist, of course, beyond the range of active biological production; some insects or birds may be airborne to altitudes above 20.000 feet, and viable spores, seeds of plants, and microorganisms may be found in the atmosphere and mountain tops above 25,000feet.

In the ocean depths, many animals can exist well below a thousand feet—one hydra-like animal has been photographed at a depth of 15,900 feet in the south Atlantic.

However, in both extreme altitude and extreme depth, the organisms depend upon the much thinner zone of active biological production, that portion of the system which converts the energy of sunlight into the chemical and physical energy of living organisms.

Radiation Balance:

Apart from a small amount of energy that comes from inside the Earth, the energy that feeds the climate system of our planet comes mainly from the Sun. In fact the Earth receives the radiant energy of the Sun (i.e. transported by electromagnetic radiation), about half of which is visible light, a small part is ultraviolet light, and the remaining part is infrared light. The solar radiation that hits the Earth's surface in one hour is equal to approximately 342 w/m^2; out of which only 235 w/m^2 are actually absorbed by the Earth's surface, while the remaining 107 are immediately reflected into space. The percentage of total incident radiation reflected from the Earth's surface is known as albedo. The Earth's albedo, therefore, is equal to 30% (342/107=30%). Out of these 107 w/m^2, 77 are reflected by the clouds, by gases and by the micro-particles that are present in the atmosphere (aerosol), while the remaining 30 w/m^2 return to the atmosphere as they are reflected by light-coloured surfaces present on the Earth, consisting prevalently of glaciers, snow and deserts. Snow has a very high albedo, equal to 0.9, which means that 90% of the radiation that hits the snow is reflected.

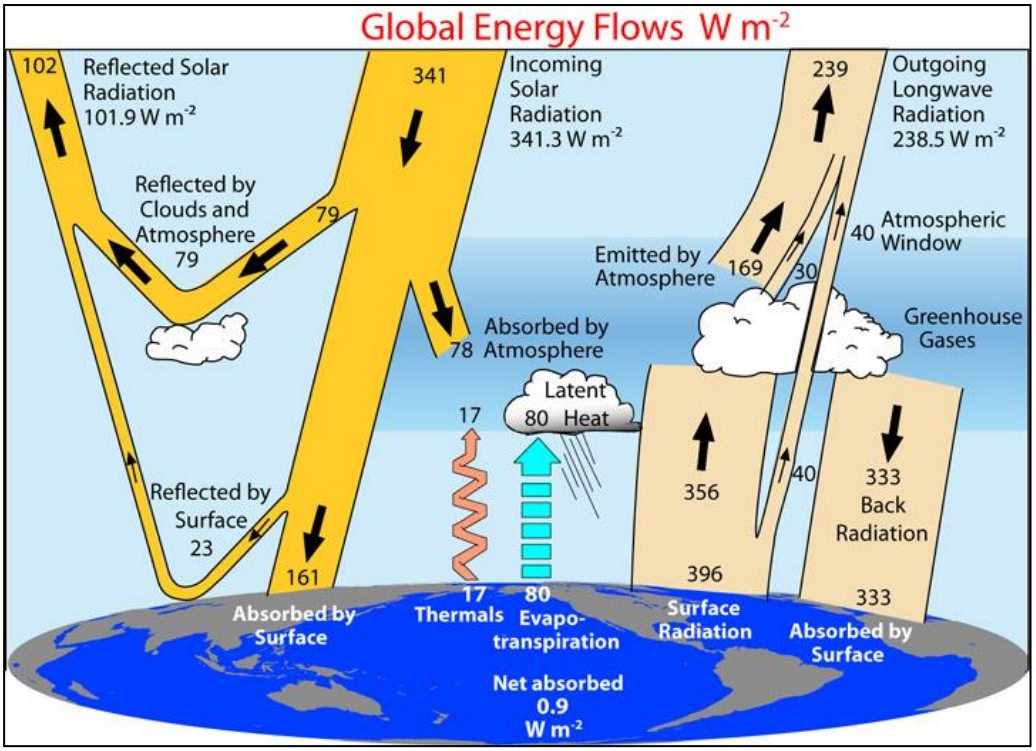


Figure: Earth's Energy Budget.

The energy that is not reflected into space, equal to 235 w/m^2, is absorbed by the Earth's surface and by the atmosphere, and is re-emitted in the form of infrared radiations (heat). The atmosphere, which consists prevalently of nitrogen and oxygen, that are transparent to infrared thermal radiation, let these radiations escape into space. However there are some gases (known as greenhouse gases) that absorb thermal radiation and prevent their dispersion, and this causes a warming of the atmosphere. This natural physical phenomenon, called greenhouse effect is very important for life on Earth because it allows the Earth's surface to have an average temperature of 14°C instead of -18°C which would be the case without greenhouse gases in the atmosphere.

Probable questions:

1. What is atmosphere? Describe the different layers of Atmosphere.
2. How hydrosphere is formed?
3. What is radiation balance?
4. Describe the zonation in lithosphere.
5. Name some of the oscillations in climate cycle. What is the effect of climate cycle?
6. What do you mean by biosphere?
7. Describe the atmospheric structure in details.
8. Write short note on lithosphere?

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XVIII

Theory and analysis of conservation: Stochastic perturbations, population viability analysis, recovery strategy for threatened species: Different approaches for conservation – in-situ and ex-situ, In-situ conservation-problems and prospects; Sanctuaries, National parks, Community Reserves and Conservation Reserves; Biosphere Reserve, EIA, EIS

Objective:

In this unit we will learn about Theory and analysis of conservation: Stochastic perturbations, population viability analysis, recovery strategy for threatened species: Different approaches for conservation – in-situ and ex-situ, In-situ conservation-problems and prospects; Sanctuaries, National parks, Community Reserves and Conservation Reserves; Biosphere Reserve, EIA, EIS

Stochastic perturbations

Simultaneous perturbation stochastic approximation (SPSA) is an algorithmic method for optimizing systems with multiple unknown parameters. It is a type of stochastic approximation algorithm. Stochastic perturbations or fluctuations refer to changes in populations owing to chance events. This is felt more strongly in small populations (Shaffer 1981, Pimm et al. 1988).

What is the principle of perturbation theory?

The principle of perturbation theory is to study dynamical systems that are small perturbations of `simple' systems. Here simple may refer to `linear' or `integrable' or `normal form truncation', etc. In many cases general `dissipative' systems can be viewed as small perturbations of Hamiltonian systems.

Types

These can be divided into two types –

1. *Demographic Stochasticity:*

Stochastic (random) variations in birth and death rates that occurs in populations from year to year are called demographic stochasticity. They cause populations to deviate from the predictions of population growth based on the deterministic models.

2. *Environmental Stochasticity:*

Random variations in the environment, such as annual variations in climate (temperature and precipitation) or the occurrence of natural disasters, and drought, are referred to as environmental stochasticity. They can directly influence the birth and death rates within the population.

Population viability analysis (PVA)

Population viability analysis (PVA) is a species-specific method of risk assessment frequently used in conservation biology. It is traditionally defined as the process that determines the probability that a population will go extinct within a given number of years. More recently, PVA has been described as a marriage of ecology and statistics that brings together species characteristics and environmental variability to forecast population health and extinction risk. Each PVA is individually developed for a target population or species, and consequently, each PVA is unique. The larger goal in mind when conducting a PVA is to ensure that the population of a species is self-sustaining over the long term

Uses:

Population viability analysis (PVA) is used to estimate the likelihood of a population's extinction and indicate the urgency of recovery efforts, and identify key life stages or processes that should be the focus of recovery efforts. PVA is also used to identify factors that drive population dynamics, compare proposed management options and assess existing recovery efforts. PVA is frequently used in endangered species management to develop a plan of action, rank the pros and cons of different management scenarios, and assess the potential impacts of habitat loss.

History:

In the 1970s, Yellowstone National Park was the centre of a heated debate over different proposals to manage the park's problem grizzly bears (*Ursus arctos*). In 1978, Mark Shaffer proposed a model for the grizzlies that incorporated random variability, and calculated extinction probabilities and minimum viable population size. The first PVA is credited to Shaffer.

PVA gained popularity in the United States as federal agencies and ecologists required methods to evaluate the risk of extinction and possible outcomes of management decisions, particularly in accordance with the Endangered Species Act of 1973, and the National Forest Management Act of 1976.

In 1986, Gilpin and Soulé broadened the PVA definition to include the interactive forces that affect the viability of a population, including genetics. The use of PVA increased dramatically in the late 1980s and early 1990s following advances in personal computers and software packages.

Example:

The endangered Fender's blue butterfly (*Icaricia icarioides*) was recently assessed with a goal of providing additional information to the United States Fish and Wildlife Service, which was developing a recovery plan for the species. The PVA concluded that the species was more at risk of extinction than previously thought and identified key sites where recovery efforts should be focused. The PVA also indicated that because the butterfly populations fluctuate widely from year to year, to prevent the populations from going extinct the minimum annual population growth rate must be kept much higher than at levels typically considered acceptable for other species

Following a recent outbreak of canine distemper virus, a PVA was performed for the critically endangered island fox (*Urocyon littoralis*) of Santa Catalina Island, California. The Santa Catalina Island fox population is uniquely composed of two subpopulations that are separated by an isthmus, with the eastern subpopulation at greater risk of extinction than the western subpopulation.

PVA was conducted with the goals of

1) Evaluating the island fox's extinction risk,

2) Estimating the island fox's sensitivity to catastrophic events, and

3) Evaluating recent recovery efforts which include release of captive-bred foxes and transport of wild juvenile foxes from the west to the east side. Results of the PVA concluded that the island fox is still at significant risk of extinction, and is highly susceptible to catastrophes that occur more than once every 20 years. Furthermore, extinction risks and future population sizes on both sides of the island were significantly dependent on the number of foxes released and transported each year

PVAs in combination with sensitivity analysis can also be used to identify which vital rates have the relative greatest effect on population growth and other measures of population viability. For example, a study by Manlik *et al.* (2016) forecast the viability of two bottlenose dolphin populations in Western Australia and identified reproduction as having the greatest influence on the forecast of these populations. One of the two populations was forecast to be stable, whereas the other population was forecast to decline, if it isolated from other populations and low reproductive rates persist. The difference in viability between the two studies was primarily due to differences in reproduction and not survival. The study also showed that temporal variation in reproduction had a greater effect on population growth than temporal variation in survival.

Future direction: Improvements to PVA likely to occur in the near future include:

1) creating a fixed definition of PVA and scientific standards of quality by which all PVA are judged and

2) incorporating recent genetic advances into PVA.

Recovery strategy for threatened species

What is an endangered or threatened species?

Under the Endangered Species Act, a species is considered "endangered" if it is in danger of extinction throughout all or a significant portion of its range. A species is considered "threatened" if it is likely to become endangered within the foreseeable future.

What does it mean to recover an endangered or threatened species?

Recovery is the process of restoring endangered and threatened species to the point where they no longer require the safeguards of the Endangered Species Act.

To determine if an endangered or threatened species has recovered, NOAA Fisheries reviews the best available data about the species. This information is gathered from the states, members of the public, the scientific community, and various other sources. We evaluate this information against the definitions of threatened and endangered and the species' recovery criteria, where identified, to determine if the species is still in danger of extinction or likely to become endangered in the future. Some factors that managers may consider when determining if a species is eligible for downlisting (i.e., from endangered to threatened) or delisting (i.e., removal from the list) are population increases, mechanisms to conserve a species and its habitat, and reduction of threats. Once a species is determined to be recovered, it can then be removed from the list of endangered and threatened species. NOAA Fisheries and partners monitor a delisted species status for at least five years afterward to ensure its recovery is sustained.

Why is it important to recover endangered and threatened marine and Anadromous species?

Each plant, animal, and their physical environment is part of a complex web of life. The extinction of a single species can affect many other species, especially humans, who depend on marine, estuarine, and riverine environments for food, commerce, medicine, and recreation. In addition to these practical benefits, the wide variety of species found in our oceans and coasts provide inspiration, beauty, and solace to many. The Endangered Species Act is a tool to help focus conservation efforts and preserve the diversity of the planet in an effort to maintain this natural legacy for future generations.

What threats are preventing species recovery?

Endangered and threatened species face many threats, including habitat destruction; the effects of pollution, disease, and competition from invasive species; a changing climate; and intense or illegal harvesting pressures. For some listed species, while the major threats no longer exist today, there still are not enough members of the species to sufficiently minimize extinction risk the point where the species is no longer in danger of extinction.

What are we doing to recover endangered and threatened species?

Once a species is listed as endangered under the Endangered Species Act, it becomes illegal to "take" that species (harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or attempt to do these things). Similar prohibitions may also be extended to species listed as threatened under the ESA. Critical habitat must also be designated for the conservation of the species. In addition to the protections associated with their listing under the ESA, we also take the following management actions:

- Develop and implement recovery plans for listed species where such plans would promote conservation.
- Monitor and evaluate the status of listed species.
- Provide grants to states and tribes for species conservation.
- Consult on any federal government actions that may affect a listed species or its designated critical habitat to minimize possible adverse effects.
- Enter bilateral and multilateral agreements with other nations to encourage conservation of listed species.
- Investigate violations of the ESA.
- Cooperate with non-federal partners to develop conservation plans, safe harbor agreements, and candidate conservation agreements with assurances for the long-term conservation of species.
- Issue permits that authorize scientific research to learn more about listed species, or activities that enhance the propagation or survival of listed species.
- Designate experimental populations of listed species to further the conservation and recovery of those species.
- Issue determinations regarding the pre-listed or antique status of ESA species parts.

What is a recovery plan and how does it help listed species?

A primary role for NOAA Fisheries in recovering endangered and threatened species is to set goals for each species' recovery comeback through the development of recovery plans. A recovery plan serves as a road map for species recovery. The plans help organize, coordinate, and prioritize recovery actions among the many management agencies, nonprofit organizations, tribal entities, stakeholders, and citizens that undertake recovery efforts. Focused implementation of actions outlined in a recovery plan also ensures effective use of resources. Recovery plans are guidance documents, not regulatory, and the Endangered Species Act clearly envisions recovery plans as the central organizing tool guiding each species' progress toward recovery.

How can a person individually help to prevent species from going extinct and aid in their recovery?

Here are some ways you can help recover endangered and threatened species:

- Learn about endangered and threatened species in your area and the threats they face.
- Watch wildlife responsibly.
- Volunteer for restoration projects and take other actions to protect habitat.
- Report marine mammals or sea turtles in distress.
- Report wildlife harassment to NOAA's Office of Law Enforcement 24-hour hotline: (800) 853-1964.
- Never purchase anything made from an endangered or threatened species.
- Reduce your water consumption.
- Reduce the amount of pollution you generate.

Conservations of Biodiversity: In-Situ Conservation and Ex-Situ Conservation

Conservation is the protection, preservation, management, or restoration of wildlife and natural resources such as forests and water. Through the conservation of biodiversity and the survival of many species and habitats which are threatened due to human activities can be ensured. There is an urgent need, not only to manage and conserve the biotic wealth, but also restore the degraded ecosystems. Humans have been directly or indirectly dependent on biodiversity for sustenance to a considerable extent. However, increasing population pressure and developmental activities have led to large scale depletion of the natural resources. Conservation is the protection, preservation, management, or restoration of wildlife and natural resources such as forests and water. Through the conservation of biodiversity and the survival of many species and habitats which are threatened due to human activities can be ensured. There is an urgent need, not only to manage and conserve the biotic wealth, but also restore the degraded ecosystems.

Types of Conservation:

Conservation can broadly be divided into two types: 1. In-situ conservation 2. Ex-situ conservation

Ex-situ conservation

Ex-situ conservation literally means, "off-site conservation". It is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may

be a wild area or within the care of humans. While ex-situ conservation comprises some of the oldest and best known conservation methods, it also involves newer, sometimes controversial laboratory methods. Ex situ conservation, using sample populations, is done through establishment of gene banks, whih include genetic resources centres, zoo‟s, botanical gardens, cultue collections etc.

Advantages of ex-situ conservation

The conservation of biodiversity can be achieved through an integrated approach balancing in situ and ex situ conservation strategies. The preservation of species in situ offers all the advantages of allowing natural selection to act, which cannot be recreated ex situ. The maintenance of viable and self-sustainable populations of wild species in their natural state represents the ultimate goal, but habitat destruction is inevitable and endangered species need to be preserved before they become extinct. Ex situ conservation provide the opportunity to study the biology of, and understand the threats to, endangered species in order to eventually consider successful species recovery programmes, which would include restoration and reintroduction. It also has the advantage of preserving plant 45material and making it available for research purposes, without damaging the natural populations. Their conservation ex situ is therefore complementary to in situ conservation and can act as an "insurance policy" when species are threatened in their natural habitats It is the process of protecting an endangered species of plant or animal by removing part of the population from a threatened habitat and placing it in a new location, which may be a wild area or within the care of humans.

Ex-situ conservation has several purposes

i. Rescue threatened germplasm.
ii. Produce material for conservation biology research.
iii. Bulk up germplasm for storage in various forms of ex situ facility.
iv. Supply material for various purposes to remove or reduce pressure from wild collecting.
v. Grow those species with recalcitrant seeds that cannot be maintained in a seed store.
vi. Make available material for conservation education and display.
vii. Produce material for reintroduction, reinforcement, habitat restoration and management.

Conventional methods of ex-situ conservation

Ex-situ conservation is the preservation of components of biological diversity outside their natural habitats. This involves conservation of genetic resources, as well as wild and cultivated or species, and draws on a diverse body of techniques and facilities.

Such strategies include establishment of botanical gardens, zoos, conservation strands and gene, pollen seed, seedling, tissue culture and DNA banks.

i. Seed gene bank: These are cold storages where seeds are kept under controlled temperature and humidity for storage and this is easiest way to store the germ plasma of plants at low temperature. Seeds preserved under controlled conditions (minus temperature) remain viable for long durations of time.

ii.Gene bank: Genetic variability also is preserved by gene bank under normal growing conditions. These are cold storages where germ plam are kept under controlled temperature and humidity for storage; this is an important way of preserving the genetic resources.

iii. Cryopreservation: This is the newest application of technology for preservation of biotic parts. This type of conservation is done at very low temperature (196°C) in liquid nitrogen. The metabolic activities of the organisms are suspended under low temperature, which are later used for research purposes.

iv. Tissue culture bank: Cryopreservation of disease free meristems is very helpful. Long term culture of excised roots and shoots are maintained. Meristem culture is very popular in plant propagation as it's a virus and disease free method of multiplication.

v. Long term captive breeding: The method involves capture, maintenance and captive breeding on long term basis of individuals of the endangered species which have lost their habitat permanently or certain highly unfavorable conditions are present in their habitat.

vi. Botanical gardens: A botanical garden is a place where flowers, fruits and vegetables are grown. The botanical gardens provide beauty and calm environment. Most of them have started keeping exotic plants for educational and research purposes.

vii. Animal Translocation: Release of animals in a new locality which come from anywhere else.

I. Translocation is carried in following cases:
II. When a species on which an animal is dependent becomes rare.
III. When a species is endemic or restricted to a particular area.
IV. Due to habit destruction and unfavorable environment conditions.
V. Increase in population in an area.

viii. Zoological Gardens: In zoos wild animals are maintained in captivity and conservation of wild animals (rare, endangered species). The oldest zoo, the Schonbrumm zoo which exists today also, was established in VIENNA in 1759. In India, the 1st zoo came into existence at BARRACKPORE in 1800. In world there are about 800 zoos. Such zoos have about 3000 species of vertebrates. Some zoos have undertaken captive breeding programmes.

Advantages of ex-situ preservation:

1. It is useful for declining population of species.

2. Endangered animals on the verge of extinction are successfully breeded.

3. Threatened species are breeded in captivity and then released in the natural habitats.

4. Ex-situ centres offer the possibilities of observing wild animals, which is otherwise not possible.

5. It is extremely useful for conducting research and scientific work on different species.

In-situ conservation

In-situ or on site conservation is conservation of wild animals and plants in their natural habitat. The aim of in-situ conservation is to allow the population to maintain or perpetuate itself within the community environment, to which it is adapted. In-situ conservation is the ideal method of conserving wild plant genetic resources. In-situ conservation of plant genetic resources presents a number of advantages as compared to ex-situ conservation.

Advantages of In-Situ Conservation

a. It enables the conservation of a large range of potentially interesting alleles.

b. This method is especially suitable for species, which cannot be established or regenerated outside the natural habitats.

c. This method allows natural evolution to continue because of the existence of variation.

d. It facilitates research on species in their natural habitats.

e. It assures protection of other species that are dependent on the species under consideration.

Methods of In-Situ Conservation:

In-situ conservation is done by providing protection to biodiversity rich areas through a network of protected areas. In India, the protected areas are of the following kinds – national parks, wildlife sanctuaries, biosphere reserves and ecologically fragile and sensitive areas. A protected area network of 85 national parks and 448 wildlife sanctuaries has been created. The results of this network have been significant in restoring viable population of large mammals such as tiger, lion, rhinoceros, crocodiles and elephants.

The main advantages and features of protected areas are as follows:

a. The genetic diversity of all species inhabiting an area can be conserved.

b. Species can be maintained in their natural habitat.

c. In protected areas, human intervention is minimal.

d. Pollution and poaching in the protected area can be checked.

Eco-development programmes involving local communities have been initiated recently for sustained conservation of ecosystems. The economic needs of the local communities are taken care under this programme through provision of alternative sources of income and a steady availability of forest and related products.

Programmes have also been launched for scientific management and wise use of wetlands, mangroves and coral reef ecosystems. Twenty-one wetlands and mangrove areas and four coral reef areas have been identified for intensive conservation and management purposes.

Six significant wetlands of India have been declared as 'Ramsar Sites' under the Ramsar Convention. Under the World Heritage Convention, five natural sites have been declared as 'World Heritage Sites'.

Protected Areas:

The areas of land and/or sea especially for protection and maintenance of biodiversity, and of natural and associated cultural resources. These areas are managed through legal or other effective means, e.g. National Parks and Wildlife Sanctuaries.

The earliest national parks are: The Yellowstone National Park in USA and the Royal National Park near Sydney, Australia. These parks were chosen because of their scenic beauty and recreational values.

Today, many such protected areas throughout the world protect rare species. World Conservation Monitoring Centre (WCMC) has recognized 37,000 protected areas around the world. In India, some important measures are taken. They are as follows:

Approximately 4.7 per cent of the total geographical area of the country has been earmarked for extensive in situ conservation of habitats and ecosystems. A protected area network of 89 National Parks and 492 Wildlife Sanctuaries have been created (MOEF, 2002). The results of this network have been significant in restoring viable population of large mammals, such as tiger, lion, rhinoceros, crocodiles, elephants, etc.

The Jim Corbett National Park, Nainital, Uttaranchal, was the first National Park, in India.

The Indian Council of Forestry Research (ICFRE) has identified 309 forest preservation plots of representative forest types for conservation of viable and representative areas of biodiversity. 187 of these plots are in natural forests and 112 in plantations covering a total area of 8,500 hectares.

Eco-development: A programme entitled 'eco-development' for in situ conservation of biological diversity involving local communities has been initiated in recent years. The concept of 'eco-development' includes the ecological and economic parameters for

sustained conservation of ecosystems by involving the local communities with the maintenance of earmarked regions surrounding protected areas.

The economic needs of the local communities are taken care of under this programme through provision of alternative sources of income and a steady availability of forest and related produce.

The main benefits of protected areas are:

a. To maintain viable populations of all native species and subspecies.

b. To maintain the number and distribution of communities and habitats. Conservation of the genetic diversity of all the existing species.

c. To prevent human caused introductions of alien species.

d. To make it possible for species and habitats and shift in response to environmental changes.

Sanctuaries

A wildlife sanctuary is an area where animal habitats and their surroundings are protected from any sort of disturbance. The capturing, killing and poaching of animals is strictly prohibited in these regions.

They aim at providing a comfortable living to the animals. India has beautiful wildlife sanctuaries, with dense forests, large rivers, high and beautiful mountains. Few of the these in India are mentioned here.

Wildlife Sanctuaries in India

S.No.	Name of Wildlife Sanctuaries	State
1.	Bharatpur Bird Sanctuary	Rajasthan
2.	Chilika Lake Bird Sanctuary	Odisha
3.	Chinnar Wildlife Sanctuary	Kerala
4.	Gir National Park and Wildlife Sanctuary	Gujarat
5.	Govind Wildlife Sanctuary	Uttarakhand
6.	Madumalai Sanctuary	Tamil Nadu
7.	Periyar Wildlife Sanctuary	Kerala

Tourism is not permitted in a wildlife sanctuary. People are not allowed unescorted there. The main objective of establishing a wildlife sanctuary is to educate humans as to how to treat the animals. The animals are taken care of and allowed to live peacefully in their natural habitats.

Importance of Wildlife Sanctuaries

There are a number of reasons for establishing wildlife sanctuaries. Some of the reasons are listed below:

- The wildlife sanctuaries are established to protect the endangered species.
- It is quite difficult to always relocate the animals from their natural habitat, therefore, protecting them in their natural environment is advantageous.
- The endangered species are specially monitored in the wildlife sanctuaries. If they reproduce and grow in number while under protection, few specimens can be kept for breeding in the conservation parks for their survival.
- Biologist activities and researches are permitted in the wildlife sanctuaries so that they can learn about the animals living there.
- A few sanctuaries take in injured and abandoned animals and rehabilitate them to health before releasing them in the forest.
- Wildlife sanctuaries preserve the endangered species and protect them from humans and predators.

National park

What is a National Park

An area, whether within a sanctuary or not, can be notified by the state government to be constituted as a National Park, by reason of its ecological, faunal, floral, geomorphological, or zoological association or importance, needed to for the purpose of protecting & propagating or developing wildlife therein or its environment. No human activity is permitted inside the national park except for the ones permitted by the Chief Wildlife Warden of the state under the conditions given in CHAPTER IV, WPA 1972.

National parks are areas that aim to protect the natural environment. They are also involved in public recreation and enjoyment activities. In a national park, the landscapes and its flora and fauna are present in their natural state.

India is rich in biodiversity. It comprises about 7.6% mammals, 6.2% reptiles, 12.6% birds, and 6.0% flowering plant species under the Indomalayan ecozone. Many eco-regions of our country like Shola forests exhibit high rates of endemism. The forests cover over the ranges from the tropical rainforest, the Western Ghats, and Northeast India to the coniferous forests in the Himalayan region.

The significant terrestrial ecosystem coming along the Indomalayan ecozone consists of temperate, polar, wet, dry regions for different kind of species to live. The species include elephant, tiger, cobra, crocodile, apes, sambar deer, spotted deer, rhinoceros, goats, lions along with different types of flora and faunas.

Indian wildlife has around 99 world-recognized national parks in different parts of the country. All these national parks and the wildlife reserves have been recognized by the IUCN or the International Union for the Conservation of Nature under the second category of protected areas.

List of National Parks in India

National parks provide a haven for wildlife away from civilization. India has currently over 100 national parks distributed across the country, stretching across various biomes.

The Hailey National Park is the first national park in India. It is one of the finest examples of ecological conservation. The other national parks in India include:

1. Bandipur National Park in Karnataka
2. Bandhavgarh National Park in Madhya Pradesh
3. Bhadra Wildlife Sanctuary in Karnataka
4. Chinnar Wildlife Sanctuary in Kerala
5. Corbett National Park in Uttarakhand
6. Dandeli Wildlife Sanctuary in Karnataka
7. Dudhwa National Park in Uttar Pradesh
8. Gir National Park and Sasan Gir Sanctuary in Gujarat
9. Hemis National Park in Jammu & Kashmir
10. Kanha National Park in Madhya Pradesh
11. Kaziranga National Park in Assam
12. Keoladeo Ghana National Park in Bharatpur, Rajasthan
13. Manas National Park in Assam
14. Nagarhole National Park in Karnataka
15. Panna National Park in Madhya Pradesh
16. Periyar National Park in Kerala.
17. Pench National Park in Madhya Pradesh
18. Ranthambore National Park in Rajasthan
19. Sariska National Park in Rajasthan
20. Tadoba Andhari Tiger Reserve in Maharashtra
21. The Great Himalayan National Park in Himachal Pradesh

All these national parks are an abode to a large number of wild animals because of the optimum environmental conditions with proper upbringing and breeding facilities.

Biosphere Reserve Programme:

Biosphere reserves are a special category of protected areas of land and/or coastal environments, wherein people are an integral component of the system.

The biosphere reserves are representative examples of natural biomes and contain unique biological communities.

The concept of Biosphere Reserves was launched in 1975 as a, part of UNESCO's 'Man and Biosphere Programme, dealing with the conservation of ecosystems and the genetic resources contained therein.

Till May 2002, there were 408 biosphere reserves dispersed in 94 countries.

In India, thirteen biodiversity rich areas have been designated as Biosphere Reserves applying the diversity and genetic integrity of plants, animals and microorganisms. (See map and table 14.8).

In India, Biosphere Reserves are also notified as National Parks.

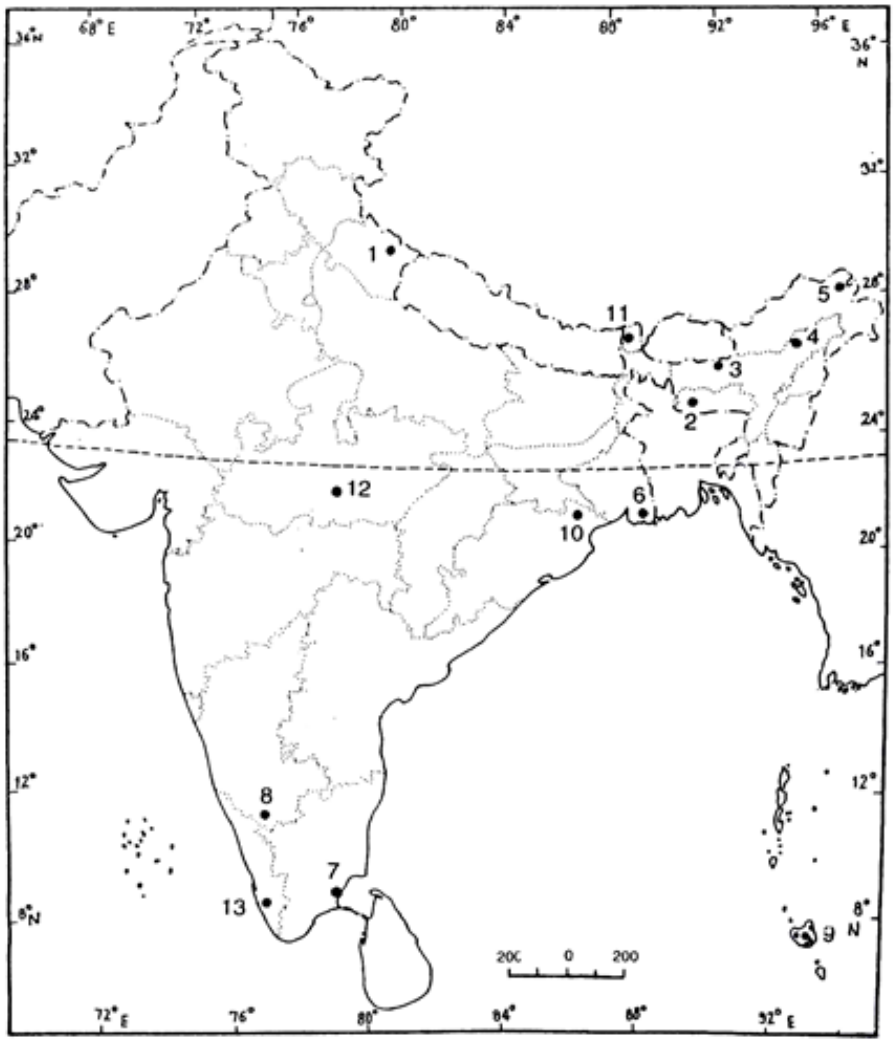


Fig. 14.4. The Biosphere Reserves in India.

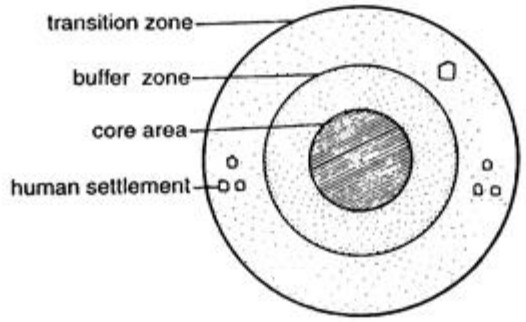


Fig. 14.5. Zonation in a terrestrial biosphere reserve.

Table 14.8. Biosphere Reserves

Name of the site	*Location (State)*
1. Nanda Devi	Part of Chamoli, Pithoragarh, Almora Districts (Uttaranchal)
2. Nokrek	Part of Gora Hills (Meghalaya)
3. Manas	Part of Kokrajhar, Bongaigaon, Barpeta, Nalbari Kamprup and Darang District (Assam)
4. Dibru-Saikhowa	Part of Dibrugarh and Tinsukia District (Assam)
5. Dehang Debang	Part of Siang and Debang Valley in Arunachal Pradesh
6. Sunderbans	Part of delta of Ganga and Brahamputra river system (West Bengal)
7. Gulf of Mannar	Indian part of Gulf of Mannar between India and Sri Lanka (Tamil Nadu)
8. Nilgiri	Part of Wynad, Nagarhole, Bandipur and Madhumalai, Nilambur, Silent Valley and Siruvani Hills (Tamil Nadu)
9. Great Nicobar	Southern most islands of Andaman and Nicobar (A and N islands)
10. Similipal	Part of Mayurbhanj District, (Orissa)
11. Khanghendzonga	Part of Kanchanjanga Hills and Sikkim (Kanchanjanga)
12. Pachmarhi	Parts of Betul, Hoshangabad and Chindwara Districts of Madhya Pradesh
13. Agasthyamalai	Part of Kerala State

Zonatism of a Terrestrial Biosphere Reserve:

A terrestrial biosphere reserve consists of core, buffer and transition zones.

(i) The natural or core zone comprises an undisturbed and legally protected ecosystem.

(ii) The buffer zone surrounds the core area, and is managed to accommodate a greater variety of resource use strategies, and research and educational activities.

(iii) The transition zone, the outermost part of the Biosphere Reserve.

This is an area of active cooperation between reserve management and the local people, wherein activities like settlements, cropping, forestry, recreation and other economic uses continue in harmony with people and conservation goals.

The main functions of biosphere reserves are:

(i) Conservation:

To conserve the ecosystems, a biosphere reserve programme is being implemented, for example, conservation of landscapes, species and genetic resources. It also encourages traditional resource use.

(ii) Eco-Development:

The concept of eco-development integrates the ecological and economic parameters for sustained conservation of ecosystems by involving the local people with the maintenance of earmarked regions. Biosphere reserves are also used to promote economic development which is culturally, socially and ecologically sustainable.

(iii) Scientific Research Programme:

Programmes have also been launched for scientific management and wise use of fragile ecosystem. Specific programmes for management and conservation of wetlands, mangroves and coral reef systems are also being implemented.

Under this programme, 21 wetlands, 15 mangrove areas and 4 coral reef areas have been identified for management. National and sub-national level committees oversee and guide these programmes to ensure strong policy and strategic support.

Sacred Forests and Sacred Lakes:

In India and some other Asian countries, a traditional Strategy for the protection of biodiversity has been in practice in the form of sacred forests or groves. These forest patches of varying dimensions are protected by local people due to their religious sanctity. Generally, they are most undisturbed forests without any human impact.

In India, sacred forests are located in several parts, such as Karnataka, Maharashtra, Kerala, Meghalaya, Uttaranchal, Uttar Pradesh, etc., and serve as refuge for a number of rare and endangered taxa.

Similarly several water bodies are declared sacred by the people, e.g., Khecheopalri lake in Sikkim. Such water bodies protect aquatic flora and fauna.

Ramsar Sites:

Six internationally significant wetlands of India have been declared as Ramsar Sites under the Ramsar Convention. To focus attention on urban wetlands threatened by pollution and other anthropogenic activities, state Governments were requested to identify lakes that could be include the National Lake Conservation Plan (NLCP).

World Heritage Sites:

Under the World Heritage Convention, five natural sites have been declared as 'World Heritage Sites'.

Table 14.9. World Heritage Sites

Site	*Location*
1. Kaziranga National Park	Assam
2. Keoladeo Ghana National Park	Rajasthan
3. Manas Wildlife Sanctuary	Assam
4. Nanda Devi National Park	Uttaranchal
5. Sunderban National Park	West Bengal

Five natural World Heritage Sites are as follows:

a. The Tura Range in Gora Hills of Meghalaya is a gene sanctuary for preserving the rich native diversity of wild Citrus and Musa species.

b. Sanctuaries for rhodendrous and orchids have been established in Sikkim.

c. Project Tiger:

A potential example of an highly endangered species in the Indian Tiger (Pantfiera tigris). It is estimated that India had about 40,000 tigers in 1900, and the number declined to a mere about 1,800 in 1972. Hence project tiger was launched in 1973.

At present these are 25 Tiger Reserves spreading over in 14 states and covering an area of about 33875 sq. km and the tiger population has more than doubled now due to total ban on hunting and trading tiger products at national and international levels.

d. Project Elephant:

This project was launched in 1991-92 to assist states having free ranging population of wild elephants to ensure long term survival of elephants in their natural habitats.

e. Rhinos:

Rhinos have been given special attention in selected sanctuaries and national parks in the North East and North West India.

All these programmes, though focussed on a single species, have a wider impact as they conserve habitats and a variety of other species in those habitats.

Conservation reserves and Community Reserves

Conservation reserves and community reserves in India are terms denoting protected areas of India which typically act as buffer zones to or connectors and migration

corridors between established national parks, wildlife sanctuaries and reserved and protected forests of India.

Such areas are designated as conservation areas if they are uninhabited and completely owned by the Government of India but used for subsistence by communities and community areas if part of the lands is privately owned.

These protected area categories were first introduced in the Wildlife (Protection) Amendment Act of 2002 – the amendment to the Wildlife Protection Act of 1972.

These categories were added because of reduced protection in and around existing or proposed protected areas due to private ownership of land, and land use.

- Conservation Reserve and community Reserves are the outcome of amendments to the wildlife protection act, 2003.
- It provided for the mechanism to provide recognition and legal backing to the community initiated efforts in wildlife protection.
- It provides for a flexible system wherein the wildlife conservation is achieved without compromising the community needs.
- Conservation reserves and community reserves are terms denoting protected areas of the country which typically act as buffer zones to or connectors and migration corridors between established national parks, wildlife sanctuaries and reserved and protected forests.

Community Reserve:

- The amendment act of 2003 provide for the creation of a new type of protected area called a community reserve.
- The state government may notify any community land or private land as a community reserve, provided that the members of that community or individuals concerned are agreeable to offer such areas for protecting the fauna and flora, as well as their traditions, cultures and practices.
- The declaration of such an area is aimed at improving the socio-economic conditions of the people living in such areas as well as conserving wildlife. The reserve is managed through a community reserve management committee.
- The state government may, where the community or individual has volunteered to conserve wildlife and its habitat, declare the area by notification as community reserve.
- No change in land use pattern shall be made within the community reserve, except in accordance with a resolution passed by the management committee and approval of the same by the state government.

There are 214 existing Community Reserves in India covering an area of 1302 km^2, which is 0.04% of the geographical area of the country (National Wildlife Database, Dec. 2020).

State-wise break up of Community Reserves

State & UT	State Area (km^2)	No. of Com R	Area (km^2)	% of State Area
Arunachal Pradesh	83743	9	131.60	0.160
Haryana	44212	5	115.84	0.262
Karnataka	1,91,791	1	3.12	0.002
Kerala	3,88,63	1	1.50	0.004
Manipur	22327	10	103.72	0.234
Meghalaya	22,429	71	64.93	0.610
Nagaland	16,579	114	851.78	3.997
Punjab	50,362	3	29.02	0.058
	TOTAL	**214**	**1302**	**0.04**

What is Conservation Reserves

Conservation reserves and community reserves in India are terms denoting protected areas of India which typically act as buffer zones to or connectors and migration corridors between established national parks, wildlife sanctuaries and reserved and protected forests of India. Such areas are designated as conservation areas if they are uninhabited and completely owned by the Government of India but used for subsistence by communities and community areas if part of the lands are privately owned.

These protected area categories were first introduced in the Wildlife (Protection) Amendment Act of 2002 – the amendment to the Wildlife Protection Act of 1972. These categories were added because of reduced protection in and around existing or proposed protected areas due to private ownership of land, and land use.

Conservation Reserves:

- The amendment act of 2003 provided for the creation of new type of protected area called a conservation reserve.
- It is an area owned by the state government adjacent to the national parks and sanctuaries for protecting the landscape, seascape, and habitat of fauna and flora. It is managed through a conservation reserve management committee.
- The state government may, after having consultations with the local communities, declare any area owned by the government as a conservation reserve.
- Tiruppadaimarathur conservation reserve in tirunelveli, tamilnadu is the first conservation reserve established in the country. It is an effort of the village community to protect the birds nesting in their village and acted for declaration of conservation reserve.

Probable questions:

1. What do you mean by stochastic perturbations? Elaborate its type.
2. What are the uses of Population viability analysis?
3. What is ex situ conservation? What are the advantages of ex situ conservation?
4. What is in situ conservation? What are the advantages of in situ conservation?
5. Write short notes on sanctuary.
6. What is core zone and buffer zone?
7. What is a Conservation reserve? Write short notes on the property of Conservation reserves.

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XIX

Conservation biology of important wild animals: Conservation status, habit & habitat, threats and conservation management of the following animals: Olive Ridley Turtle/Greater one-horned *Rhinoceros*/Ganges river dolphin.

Objective:

In this unit we will discuss about Conservation biology of important wild animals: Conservation status, habit & habitat, threats and conservation management of the following animals: Olive Ridley Turtle/Greater one-horned *Rhinoceros*/Ganges river dolphin.

Olive Ridley Turtle

Introduction:

The olive ridley gets its name from the olive green color of its heart-shaped shell. The species is among the smallest of the world's sea turtles and is found primarily in the tropical regions of the Pacific, Indian, and Atlantic oceans.

Olive ridley turtles are found throughout the world. The numbers of olive ridleys are greatly reduced from historical estimates (for example, 10 million olive ridleys in the Pacific Ocean), due to overexploitation for turtle meat and eggs. By catch in fishing gear and the direct harvest of turtles and eggs are the biggest threats facing olive ridleys.

NOAA Fisheries and our partners are dedicated to protecting and recovering olive ridley turtle populations worldwide. We use a variety of innovative techniques to study, protect, and recover these threatened and endangered populations. We engage our partners as we develop measures and recovery plans that foster the conservation and recovery of olive ridley turtles and their habitats. And we fund research, monitoring, and conservation projects to implement priorities outlined in recovery plans.

Population Status:

Olive ridley turtles are found worldwide and listed under the Endangered Species Act. Their breeding colony populations on the Pacific Coast of Mexico are listed as endangered; all others are listed as threatened. According to the International Union for Conservation of Nature and Natural Resources Red List, there has been between a 30 to 50 percent reduction in global population size. Although some nesting populations have increased in the past few years or are currently stable, the overall reduction in some populations is greater than the overall increase in others.

In the western Atlantic Ocean, although there has been an 80 percent reduction in

certain nesting populations since 1967, Brazil has seen an increase in their nesting population. In the eastern Atlantic Ocean, Gabon currently hosts the largest olive ridley nesting population in the region with 1,000 to 5,000 breeding females per year.

In the Pacific, large nesting populations occur in Mexico and Costa Rica. A single arribada nesting beach remains in La Escobilla, Mexico, where an estimated 450,000 turtles nest, and the Pacific coast of Costa Rica supports an estimated 600,000 nesting olive ridleys between its two major arribada beaches, Nancite and Ostional.

In the Indian Ocean, three arribada beaches occur in Odisha, India (Gahirmatha, Devi River mouth, and Rushikulya) with an estimated +100,000 nests per year. More recently, a new mass nesting site was discovered in the Andaman Islands, India, with more than 5,000 nests reported in a season. Declines in solitary nesting of olive ridleys have been recorded in Bangladesh, Myanmar, Malaysia, and Pakistan. In particular, the number of nests in Terengganu, Malaysia, has declined from thousands of nests to just a few dozen per year.

Appearance:

Olive ridleys look very similar to Kemp's ridley sea turtles. The two species are the smallest of all sea turtles. Olive ridley turtles are an olive/grayish-green with a heart-shaped carapace (top shell) having 5 to 9 pairs scutes. Each of the four flippers of an olive ridley has one or two claws. The size and form of the olive ridley varies from region to region, with the largest animals observed in West Africa.

Behavior and Diet:

Olive ridley turtles, like all sea turtles, are marine reptiles and must come to the surface to breathe. Adult female sea turtles return to land to lay their eggs in the sand—they are remarkable navigators and usually return to a beach in the general area where they hatched.

Arribada nesting is a behavior found only in the genus *Lepidochelys* which includes the Kemp's ridley and olive ridley sea turtles. Although other turtles have been documented nesting in groups, no other turtles (marine or land) have been observed nesting in such mass numbers and synchrony. Solitary nesting occurs extensively throughout this species' range, and nesting has been documented in approximately 40 countries worldwide. Arribada nesting, however, occurs on only a few beaches worldwide.

Olive ridleys often migrate great distances between feeding and breeding grounds. Using satellite tags, scientists have documented both male and female olive ridleys leaving the breeding and nesting grounds off the Pacific coast of Costa Rica and migrating out to the deep waters of the Pacific Ocean.

The olive ridley is omnivorous, meaning it feeds on a wide variety of food items, including algae, lobster, crabs, tunicates, and mollusks. Olive ridleys can dive to depths of 500 feet to forage on benthic invertebrates (those that live on the bottom).

Habitat:

The olive ridley is mainly a pelagic (open ocean) sea turtle, observed by trans-Pacific ships over 2,400 miles from shore, but they are also known to inhabit coastal areas. Olive ridleys are globally distributed in the tropical regions of the Atlantic, Pacific, and Indian oceans. In the Atlantic Ocean, they are found along the coasts of West Africa and South America. In the Eastern Pacific, they occur from Southern California to Northern Chile.

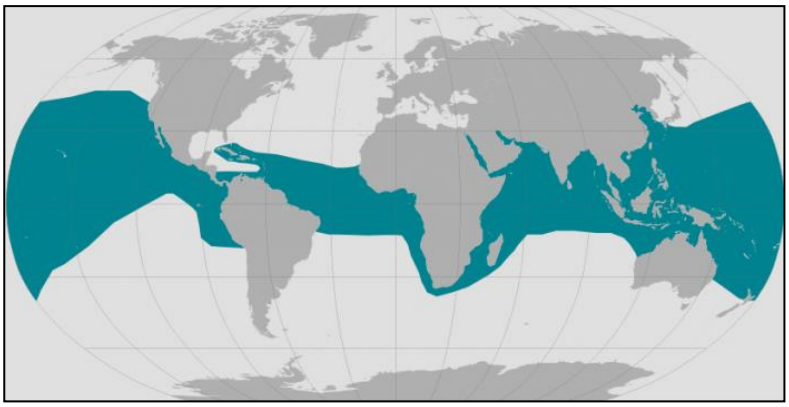


Fig: World map providing approximate representation of the olive ridley's range.

Life span & Reproduction:

No one knows exactly how long olive ridleys live, but like other sea turtles, they are likely long-lived. Olive ridleys reach maturity around 14 years of age. The olive ridley sea turtle has one of the most extraordinary nesting habits in the natural world. Similar to Kemp's ridleys, large groups of turtles gather offshore of nesting beaches. Then, all at once, vast numbers of turtles come ashore and nest in what is known as an "arribada" which means "arrival" in Spanish. During these arribadas, hundreds to thousands of females come ashore to lay their eggs. At many nesting beaches, the nesting density is so high that previously laid egg clutches are dug up by other females while excavating the nest chamber to lay their own eggs.

There are many theories on what triggers an arribada, including offshore winds, lunar cycles, and the release of pheromones by females. However, scientists have yet to conclusively determine why exactly arribadas occur. Not all females nest during an arribada—some are solitary nesters while others employ a mixed nesting strategy. For example, a single female might nest during an arribada, as well as nest alone during the same nesting season.

Females nest every year, one to three times a season, laying clutches of approximately 100 eggs. When finished laying, most sea turtles cover their eggs with sand using their rear flippers to pack it in firmly on top of their clutch. However, since the olive ridley is so small and relatively light, they do not have the power to use their rear flippers in this way—instead, they use their whole bodies, beating the sand down with their lower shells after covering the eggs. The sex of hatchlings is determined by the temperature of

the sand, the eggs hatch and the hatchlings make their way to the water. Hatchlings orient seaward by moving away from the darkest silhouette of the landward dune or vegetation to crawl towards the brightest horizon. On developed beaches, this is toward the open horizon over the ocean.

Threats:

- Bycatch in Fishing Gear

A primary threat to sea turtles is their unintended capture in fishing gear which can result in drowning or cause injuries that lead to death or debilitation (for example, swallowing hooks or flipper entanglement). The term for this unintended capture is by catch. Sea turtle by catch is a worldwide problem. The primary types of gear that result in olive ridley by catch include trawls, long lines, gill-nets, and purse seines.

- Direct Harvest of Turtles and Eggs

The principal cause of the worldwide decline of the olive ridley sea turtle was long-term collection of eggs and mass killing of adult females on nesting beaches. The arribada nesting behavior concentrates females and nests at the same time and in the same place, enabling the taking of an extraordinary number of eggs for human consumption. Historically, egg collection for human consumption was a significant problem, but this threat has been diminished in some countries with bans on the killing of turtles and collection of eggs.

- Loss and Degradation of Nesting Habitat

Coastal development and rising seas from climate change are leading to the loss of nesting beach habitat for olive ridley turtles. Shoreline hardening or armoring (e.g., seawalls) can result in the complete loss of dry sand suitable for successful nesting. Artificial lighting on and near nesting beaches can deter nesting females from coming ashore to nest and can disorient hatchlings trying to find the sea after emerging from their nests.

- Predation of Eggs and Hatchlings

The destruction and consumption of eggs and hatchlings by non-native and native predators (particularly feral pigs, coyotes, coatis, birds, and crabs) is a threat to olive ridley sea turtles.

- Vessel Strikes

Vessel strikes are a threat to sea turtles near ports and waterways along developed coastlines throughout their range. Various types of watercraft can strike sea turtles when they are at or near the surface, resulting in injury or death. High boat traffic areas such as marinas and inlets present a higher risk. Adult sea turtles, in particular nesting females, are more susceptible to vessel strikes when making reproductive migrations and while they are near shore during the nesting season.

- Ocean Pollution/Marine Debris

Increasing pollution of near shore and offshore marine habitats threaten all sea turtles and degrades their habitats. Olive ridley turtles may ingest marine debris such as fishing line, balloons, plastic bags, floating tar or oil, and other materials discarded by humans which they can mistake for food. They may also become entangled in marine debris, including lost or discarded fishing gear, and can be killed or seriously injured.

- Climate Change

For all sea turtles, a warming climate is likely to result in changes in beach morphology and higher sand temperatures, which can be lethal to eggs or alter the ratio of male and female hatchlings produced. Rising seas and storm events cause beach erosion, which may flood nests or wash them away. Changes in the temperature of the marine environment are likely to alter the abundance and distribution of food resources, leading to a shift in the migratory and foraging range and nesting season of olive ridleys.

Conservation & Management:

Since 1977, NOAA Fisheries and the U.S. Fish and Wildlife Service have shared jurisdiction of sea turtles listed under the ESA. A Memorandum of Understanding outlines our specific roles: NOAA Fisheries leads the conservation and recovery of sea turtles in the marine environment, and the U.S. FWS leads conservation and recovery efforts for sea turtles nesting beaches.

We are committed to the protection and conservation of olive ridley turtles by:

- Working with partners to ensure compliance with national, state, and U.S. territory laws to protect sea turtles.
- Cooperating with international partners to implement conservation measures and establish agreements, such as international treaties that protect sea turtles.
- Researching, developing, and implementing changes to fishing gear practices and/or fishing gear modifications (e.g., turtle excluder devices), using large circle hooks in longline fisheries, and implementing spatial or temporal closures to avoid or minimize by catch.
- Protecting and monitoring olive ridley turtles in the marine environment and on nesting beaches.
- Conducting research on threats and developing conservation measures that reduce threats and promote recovery.
- Collecting information on the species biology and ecology to better inform conservation management strategies and to assess progress toward recovery.
- Conducting and supporting education and outreach efforts to the general public by raising awareness on threats to sea turtles, highlighting the importance of sea turtle conservation, and sharing ways people can help sea turtles.

- Working with partners to study and raise awareness about illegal sea turtle trade.

Recovery Planning and Implementation:

Recovery Action

To help identify and guide the protection, conservation, and recovery of sea turtles, the ESA requires NOAA Fisheries and the U.S. FWS to develop and implement recovery plans which provide a blueprint for conservation of the species and measurable criteria to gauge progress toward recovery.

The major recovery actions for olive ridley turtles include:

- Protect sea turtles on nesting beaches and in marine environments.
- Protecting nesting and foraging habitats.
- Reducing by catch in commercial and artisanal fisheries.
- Reducing the effects of entanglement and ingestion of marine debris.
- Reducing vessel strikes in coastal habitats.
- Working with partners internationally to protect turtles in all life-stages.
- Supporting research and conservation projects consistent with Recovery Plan priorities.

The recovery plan to recover and protect the U.S. Pacific populations of the olive ridley turtle was published in 1998.

The highly migratory behavior of sea turtles makes them shared resources among many nations, so conservation efforts for sea turtle populations must extend beyond national boundaries. This necessitates international collaboration and coordination. Learn more about international conservation efforts below.

Implementation

NOAA Fisheries is working to minimize effects from human activities that are detrimental to the recovery of olive ridley turtle populations in the United States and internationally. Together with our partners, we undertake numerous activities to support the goals of the olive ridley turtle recovery plan, with the ultimate goal of species recovery.

Efforts to conserve olive ridley turtles include:

- ✓ Protecting habitat.
- ✓ Reducing bycatch.
- ✓ Rescue, disentanglement, and rehabilitation.
- ✓ Eliminating the killing of turtles and the collection of their eggs.
- ✓ Eliminating the harassment of turtles on nesting beaches and foraging

habitats through education and enforcement.

- ✓ Consulting with federal agencies to ensure their activities are not likely to jeopardize the continued existence of listed species.

Greater one-horned *Rhinoceros*

The greater one-horned rhino (or "Indian rhino") is the largest of the rhino species. Once widespread across the entire northern part of the Indian sub-continent, rhino populations plummeted as they were hunted for sport or killed as agricultural pests. This pushed the species very close to extinction and by the start of the 20th century, around 200 wild greater one-horned rhinos remained.

The recovery of the greater one-horned rhino is among the greatest conservation success stories in Asia. Thanks to strict protection and management from Indian and Nepalese wildlife authorities, the greater one-horned rhino was brought back from the brink. Today populations have increased to around 3,700 rhinos in northeastern India and the Terai grasslands of Nepal.

The greater one-horned rhino is identified by a single black horn about 8-25 inches long and a grey-brown hide with skin folds, which gives it an armor-plated appearance. The species is solitary, except when adult males or rhinos nearing adulthood gather at wallows or to graze. Males have loosely defined home ranges that are not well defended and often overlap. They primarily graze, with a diet consisting almost entirely of grasses as well as leaves, branches of shrubs and trees, fruit, and aquatic plants.

Behaviour:

Greater one-horned rhinos are solitary, except when sub-adults or adult males gather at wallows or to graze. Males have loosely defined territories which are not well defended, and often overlap.

Females become sexually mature at 5-7 years old, while males mature at about 10. Breeding occurs throughout the year, with a gestation period of 15-16 months. The single offspring remains with the mother until the birth of her next calf, usually after 1 to 3 years.

The rhino is a primarily a grazer. Its diet consists almost entirely of grasses, but it also eats leaves, branches, fruit and aquatic plants.

Habit and Habitat:

***Location*:** the Greater one-horned rhino can be found in India and Nepal, particularly in the foothills of the Himalayas. In the past, Greater one-horned rhinos roamed freely in the floodplains and forests alongside the Brahmaputra, Ganges and Indus River valley

***Habitat*:** Greater one-horned rhinos are semi-aquatic and often take up residence in swamps, forests and riversides, and anywhere that is near nutritious mineral licks.

Conservation management

- **Monitoring and protection:**

WWF helps to strengthen security measures and provides critical support for anti-poaching efforts at key sites including Kaziranga National Park and Pobitora and Laokhowa-Burachapori Wildlife Sanctuaries. We also invest in improving rhino monitoring to collect data and measure progress toward achieving rhino conservation goals, assess the reproductive health and growth rate of populations, and make the right decisions to keep rhino numbers growing at a rate of at least 3%.

- **Restoring landscape:**

As rhino populations increase, they need additional space to live and breed. WWF and partners restore rhino habitat in Nepal to increase rhino numbers and improve connectivity between protected areas. Chitwan National Park's population of greater one-horned rhinos is the second largest population of this species in the world, after India's Kaziranga National Park. In Kaziranga National Park, WWF works to secure habitat corridors so that rhinos have access to higher areas outside of the park during annual floods.

- **Working with local community:**

The protected areas of India and Nepal, where rhinos reside, are surrounded by dense human populations. It is vital to ensure that communities that live around rhino reserves are sympathetic to, and benefit from, the rhinos in their midst. WWF supports several projects to improve local livelihoods, like the successful community-run Marmelous juice factory in Khata, Nepal. We help decrease conflict between people and rhinos by encouraging farmers to plant unpalatable species like *mentha* (mint) that generates money for the communities and supports community-based antipoaching operations.

- **Strengthening law enforcement**

WWF partners with national governments to strengthen wildlife laws and their enforcement, and fund antipoaching equipment and operations in protected areas. In response to a poaching spike in Nepal in 2006, WWF increased the number of security posts from eight to 20. We also engaged ex-army and police to patrol vulnerable points outside protected areas. Local youth volunteered to guard individual rhinos through the night. WWF relayed the information collected by these allies to key government departments so they could take action where needed. Nepal's concerted efforts to protect rhinos has resulted in the country achieving four periods of 365 days each of zero rhino poaching since 2011. Today, more than 645 one-horned rhinos live in Nepal—the highest number recorded in the country so far. In 2015, Nepal hosted the first symposium focused on getting to zero poaching. Under Nepal's leadership,

delegates from more than 13 Asian countries shared best practices, tools, and technologies that can be used to respond to the poaching crisis.

- ***Tackling illegal wildlife trade***

WWF and TRAFFIC, the wildlife trade monitoring network, work to stop trafficking of rhino horn by funding antipoaching patrols and supporting intelligence networks in strategic locations to prevent rhinos from entering black markets in Asia. We support the South Asia Wildlife Enforcement Network (SAWEN) so that regional governments can combine information and resources. This includes using early warning systems, investing in effective legislation, and improving enforcement of policies and laws.

Ganges river dolphin

Dolphins are one of the oldest creatures in the world along with some species of turtles, crocodiles and sharks. The Ganges river dolphin was officially discovered in 1801. Ganges river dolphins once lived in the Ganges-Brahmaputra-Meghna and Karnaphuli-Sangu river systems of Nepal, India, and Bangladesh. But the species is extinct from most of its early distribution ranges.

The Ganges river dolphin can only live in freshwater and is essentially blind. They hunt by emitting ultrasonic sounds, which bounces off of fish and other prey, enabling them to "see" an image in their mind. They are frequently found alone or in small groups, and generally a mother and calf travel together. Calves are chocolate brown at birth and then have grey-brown smooth, hairless skin as adults. Females are larger than males and give birth once every two to three years to only one calf.

Habitat:

Water levels in the Ganga are seasonal in nature. Peak flows occur between July and September when the river is fed by monsoon run-off and Himalayan melt-water, while leanest flow occurs from February to March. The flow in the Ganga and its tributaries is regulated, and the natural flow regime has been disrupted, by the construction of dams and barrages. While there were occasional reports of dolphin sightings in some of the smaller tributaries of the Ganga during the 1980s, it is now likely that dolphins have been completely extirpated from these rivers due to insufficient and inconsistent water supplies. The large-scale diversion of river water for irrigation in the dry season causes water flow to diminish especially between Haridwar and Allahabad.

Importance:

The Ganges dolphin is an indicator species for the river ecosystem and is at the apex of the food chain. It is an endemic and rare aquatic mammal found only in the Indian subcontinent and is part of our natural aquatic heritage. Developing a comprehensive program to conserve river dolphins in Ganga is required for the following reasons:

- India is the last stronghold with extant populations in the Ganges-Brahmaputra River Systems. The species is in peril in Nepal and Bangladesh.
- It is an excellent indicator of riverine ecosystem health.
- As a signatory to numerous international conventions, India must fulfill its treaty obligations to aid in the conservation of this species.
- The species has recently been declared the 'National Aquatic Animal' by the Government of India, and is part of our national heritage.
- The Ganges River dolphin is a Flagship Species for river conservation

The following factors make the deliberate exploitation of Ganges dolphin a high-risk endeavor from a conservation viewpoint:

(a) They have intrinsically low rates of population increase;

(b) Most populations are subjected to by catch in fisheries;

(c) There is uncertainty associated with estimates of their life history parameters, absolute abundance, trends in abundance, and total mortality;

(d) The effects of chemical and noise pollution, reduced prey abundance, and habitat degradation are potentially serious but difficult to quantify.

Strategy for the Conservation of Ganges River Dolphins

Successful strategies to facilitate the recovery of depleted populations, reverse trends of population decline and habitat deterioration, and ensure that robust populations with high-quality habitat are secure will need to be multifaceted, adaptable, and tailored to particular local or regional conditions (Reeves *et al*., 2003). The many elements outlined below are integral to a comprehensive conservation strategy for the Ganges River dolphin.

Developing and encouraging alternative fishing techniques

- Dolphin oil is used as bait to attract two target fish species (*Clupisoma garua* and *Eutropiichthys vacha*), which are then netted or hooked. This use of dolphin oil and their body parts creates an incentive for hunting dolphins and a disincentive for fishermen to release any that may become entangled in their nets. Oil extracted from fish offal available at outdoor markets has been tested and found to be an effective substitute for dolphin oil and encouraging its use may result in a reduction of dolphin kills.

Habitat protection and restoration

Protected areas

- Existing protected areas in the Ganga and its tributaries fall far short of what is

needed.

- Few protected areas capable of providing comprehensive protection to the species they support. While a buffer zone aids in protection, animals that are relatively safe from entanglement in fishing gear while inside a reserve with strong enforcement may meet a gauntlet of nets as they move beyond its borders.
- Ganges dolphins require sufficient year round water flow to move, forage, and carry out activities that ensure reproductive success and recruitment into breeding population.

Researching and reducing environmental pollution

- While more research is needed to elucidate the impacts of contaminant exposure on dolphin health, the precautionary principle demands that restrictions on the discharge of untreated effluents into the Ganga river system should be enforced to ensure a reduction in dolphin exposure to toxins.

Enhancing the capacity and governance framework for Ganges dolphin conservation

- Capacity-building refers to enhancement of human capabilities through a combination of education and infrastructure improvement. It is vital that local scientists and activists be able to provide the impetus and expertise for dolphin conservation efforts in their own regions.

Current Status and Distribution:

The Ganges River dolphin ranges into most of the large tributaries in the Ganges Basin: the Ramganga, Yamuna, Gomti, Ghaghara, Rapti, Son, Gandak and Kosi besides the main channel of the Ganga. In the Brahmaputra valley it ranges into the major tributaries such as the Tista, Adadhar, Champamat, Manas, Bhareli, Subhansiri, Dihang, Dibang, Lohit, Disang, Dikho and Kulsi rivers. Downstream it ranges through the larger tributaries between the Hugh and Meghna rivers, as far as the tidal limits at the mouth of the Ganges. They are also reported from the Fenny, Karnaphuli, and Sangu rivers to the southeast of the mouths of the Ganges (Rice 1998). Ganges dolphins have been extirpated from portions of their upstream range in Nepal and India, and populations have been fragmented and reduced in numbers where they still occur .

Conservation status:

This species has been included in Schedule I of the Indian Wildlife (Protection) Act 1972, in Appendix I of the Convention on International Trade in Endangered Species (CITES), in Appendix II of the Convention on Migratory Species (CMS) and categorized as Endangered on the International Union for the Conservation of Nature's (IUCN) Red List.

Probable questions:

1. What are the efforts to conserve olive ridley turtles?
2. Discuss about the behavior strategy of olive ridley turtles.
3. What are the efforts to conserve greater one-horned rhino?
4. What are the efforts to conserve Ganges River Dolphins?
5. What is the Current Status of Ganges River Dolphins?

Suggested reading:

1. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
2. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
3. Odum, E. P. and Barret, G. W. (2005). Fundamentals of Ecology. 5th ed. Thompson Brooks/Cole.
4. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Unit XX

Basic Concept of Wildlife Biology: Wildlife wealth of India; Threatened wildlife and IUCN status - Concept of Extinct, Critically Endangered, Endangered, Vulnerable and rare species; concept of corridor

Objective:

In this unit we will learn about Basic Concept of Wildlife Biology: Wildlife wealth of India; Threatened wildlife and IUCN status - Concept of Extinct, Critically Endangered, Endangered, Vulnerable and rare species; concept of corridor.

Introduction:

IUCN Red List of Threatened Species also called IUCN Red List**,** one of the most well-known objective assessment systems for classifying the status of plants, animals, and other organisms threatened with extinction. The International Union for Conservation of Nature (IUCN) unveiled this assessment system in 1994. It contains explicit criteria andcategories to classify the conservation status of individual species on the basis of their probability of extinction.

The IUCN system uses a set of five quantitative criteria to assess the extinction risk of a given species. In general, these criteria consider:

After a given species has been thoroughly evaluated, it is placed into one of several categories. (The details of each have been condensed to highlight two or three of the category's most salient points below.) In addition, three of the categories (CR, EN, and VU) are contained within the broader notion of "threatened." The IUCN Red List of Threatened Species recognizes several categories of species status:

1. Extinct (EX), a designation applied to species in which the last individual has died or where systematic and time-appropriate surveys have been unable to log even a single individual. Eg. Dodo. West African Black Rhinocerous. Baiji White Dolphin. Tasmanian Tiger.

2. Extinct in the Wild (EW), a category containing those species whose members survive only in captivity or as artificially supported populations far outside their historical geographic range. e.g. *Franklinia aloetamh*a

3. Critically Endangered (CR), a category containing those species that possess an extremely high risk of extinction as a result of rapid population declines of 80 to more than 90 percent over the previous 10 years (or three generations), a current population size of fewer than 50 individuals, or other factors. E.g. Sunda Tiger (*Panthera tigris*

sondaica), Vanquita (*Phocoena sinus*).

4. Endangered (EN), a designation applied to species that possess a very high risk of extinction as a result of rapid population declines of 50 to more than 70 percent over the previous 10 years (or three generations), a current population size of fewer than 250 individuals, or other factors. Eg. Blue whale (*Balaenoptera musculus*), Asiatic elephant (*Elephas maximus indicus*)

5. Vulnerable (VU), a category containing those species that possess a very high risk of extinction as a result of rapid population declines of 30 to more than 50 percent over the previous 10 years (or three generations), a current population size of fewer than 1,000 individuals, or other factors. Eg. Dugong (*Dugong dugon*), Giant panda (*Ailuropoda melanoleuca*), Olive Ridley Turtle (*Lepidochelys olivacea*), Polar bear (*Ursus maritimus*)

6. Near Threatened (NT), a designation applied to species that are close to becoming threatened or may meet the criteria for threatened status in the near future. Eg. Snow leopard (*Panthera uncial*), jaguar (*Panthera onca*).

7. Least Concern (LC), a category containing species that are pervasive and abundant after careful assessment.

8. Data Deficient (DD), a condition applied to species in which the amount of available data related to its risk of extinction is lacking in some way. Consequently, a complete assessment cannot be performed. Thus, unlike the other categories in this list, this category does not describe the conservation status of a species.

9. Not Evaluated (NE), a category used to include any of the nearly 1.9 million species described by science but not assessed by the IUCN.

Rare (R): Taxa with small populations that are not endangered or vulnerable at present but are at risk are included under this category (*Lactuca saligna, Salvia saxicola*). A species may be rare because of restricted geographical range, high habitat specificity and small local population size, or thinly scattered over a more extensive range, or due to a combination of two or more of these characteristics. Rare species have a population of less than 20,000 individuals. Some species are naturally rare and have never occurred in greater numbers, yet they are able to maintain these numbers. Other species become rare through man's action or other natural forces.

All else being equal, a species experiencing an 90 percent decline over 10 years (or three generations), for example, would be classified as critically endangered. Likewise, another species undergoing a 50 percent decline over the same period would be classified as endangered, and one experiencing a 30 percent reduction over the same time frame would be considered vulnerable. It is important to understand, however, that a species cannot be classified by using one criterion alone; it is essential for the scientist doing the assessment to consider all five criteria when determining the status of the species.

Each year thousands of scientists around the world assess or reassess species. The IUCN Red List is subsequently updated with these new data once the assessments have been checked for accuracy. In this way, the information helps to provide a continual spotlight on the status of the world's at-risk plants, animals, and other organisms. As a result, interested parties, such as national governments and conservation organizations, may use the information provided in the IUCN Red List to prioritize their own species-protection efforts.

The IUCN Red List brings into focus the ongoing decline of Earth's biodiversity and the influence humans have on life on the planet. It provides a globally accepted standard with which to measure the conservation status of species over time. By 2019, 96,500 species had been assessed by using the IUCN Red List categories and criteria. Of these, more than 26,500 species of plants, animals, and others fall into the threatened categories (CR, EN, and VU). Today the list appears as an online database available to the public. Scientists can analyze the percentage of species in a given category and how these percentages change over time; they can also analyze the threats and conservation measures that underpin the observed trends.

Wildlife wealth of India

India's rich and abundant animal life is attributed to it's diverse climate and geography. There are 372 species of mammals in India, some of which include the majestic elephant, the Indian bison (*gaur*), rhinoceros, wild Himalayan sheep, *nilgai*, and the swamp deer. The most superb examples of cats in India are the tiger and the lion, but there are other types as well, the clouded leopard, snow leopard and the marbled cat are a few others found. Several species of monkeys reside in India as well.

A few of the 1,228 bird species found in India include peacocks, parrots, kingfishers, parakeets, cranes, *mynahs*, pheasants, ducks, geese and hornbills. Crocodiles and *gharials* are two of the 446 reptile species found. Since crocodiles were threatened with extinction, India has implemented a plan that has successfully increased their numbers. Other reptiles to be found are the cobra, krait, saltwater snake and python.

With the expansion and growth, as well as natural disasters, occurring in India, the fauna is suffering. Possible extinction is a possibility for over 77 mammals, 72 bird species, 17 reptile species, 3 amphibian species, and large amount of butterflies, moths, and beetles, as they are considered to be endangered.

The natural wealth of the Indian subcontinent has remained unique, mysterious and fascinating for nature lovers. In Indian philosophy, life in any form is deemed sacred and it is advocated that compassion for all living creatures is essential. The worship of nature in all its different forms is an essential part of our cultural legacy. The tree has held a vital place in religious tradition, symbolizing the myth of creation. The Agni Purana reveals that the conservation rite becomes a soul-saving deed and that trees are objects of respect.

The rich and fascinating variety of India's wildlife can be seen in the 80 national parks, 440 sanctuaries and 23 tiger reserves established by the Government of India in an attempt to conserve this vital resource. More than 500 species of mammals, 1, 220 species of birds, 1, 600 species of reptiles and amphibians, and 57, 000 species of insects populate the subcontinent. India harbors 60% of the world's wild tiger population, 50% of Asian elephants, 80% of the one-horned rhinoceros and the entire remaining population of the Asiatic lion.

Concept of corridor

A wildlife corridor has been defined as a linear landscape element which serves as a linkage between historically connected habitat/natural areas, and is meant to facilitate movement between these natural areas (McEuen, 1993).

From a landscape ecology viewpoint, a corridor has been defined as a linear habitat that is embedded in a dissimilar matrix which connects two or more larger blocks of habitat and which is proposed for conservation on the grounds that it will enhance or maintain the viability of specific wildlife populations in the habitat blocks (Beier and Noss, 1998).

Benefits of corridor:

McEuen (1993) has summarized the following conservation benefits of corridors:

1. Enhanced immigration, which will support genetic flow, increase genetic diversity and enhance overall meta-population survival in connected patches.
2. Provide opportunity to avoid predation.
3. Accommodation of range shifts due to climate change
4. Provision of a fire escape function.
5. Maintenance of ecological process connectivity.

- **How a corridor can be designed?**

Designing wildlife corridors can be an arduous task. There is no set prescription on design parameters and adopting the right strategy depends on a multitude of factors which include species requirements, geographical setting, land availability, and human interference amongst other things.

Literature in this area is scant and at best posits panoptic best practice guidelines. While there seems to be broad understanding and agreement on useful principles in designing wildlife corridors, there are no universally applicable rules that can be applied without modification to diverse corridor scenarios.

Bond (2003) lists the following broad guidelines with reference to design of wildlife dispersal corridors:

i. The corridor should be as wide as possible. The corridor width may vary with habitat type or target species, but a rule of thumb is about a minimum of 1,000 feet wide (but larger if possible).

ii. Maintain as much natural open space as possible next to any culverts to encourage the use of the culverts.

iii. Maximize land uses adjacent to the corridor that reduce human impacts to the corridor (Beier and Loe, 1992). Isolation effects along corridors can be offset by having surrounding habitat similar to that found within corridors (Perault and Lomolino, 2000).

iv. Do not allow housing or other impacts to project into the corridor to form impediments to movement and increase harmful edge effects.

v. If housing is to be permitted next to the corridor, put conservation easements on adjacent lots to prohibit structures nearest the corridor.

vi. Develop strict lighting restrictions for the houses adjacent to the corridor to prevent light pollution into the corridor. Lights must be directed downward and inward towards the home.

Parameters in designing corridor:

McKenzie and Bio (1995) suggest the following important parameters in designing corridors

I. Habitat:

The extent to which the corridor will be used by dispersers depends on the habitat within the landscape. The habitat within the corridor must be connected by 'high quality' habitat that provides for survival and reproduction. 'Suitable habitat' patches should be continuous and movement is not hindered by presence of gaps (Lovejoy et al., 1986; McEuen, 1993). Studies on several species of rodents have shown that there is a strong tendency to remain within suitable habitat while dispersing.

II. Corridor Shape:

Linear corridor shape was found to be superior than other shapes (Soule and Gilpin, 1991).

III. Corridor Width:

Minimum corridor widths may be estimated using home range data of target species. If the corridor is to contain enough suitable habitat for the target species to permanently occupy the corridor, then the corridor width should be at least one home range wide and contain home ranges that are designed to be rectangular and twice as long as wide (Harrison, 1992).

IV. Corridor Length:

Corridors may be narrower than the minimum width as described above if they are less than one home range in length to facilitate passage to dispersers without foraging (ibid.).

V. Corridor Location:

It is important to align corridors with other habitat suitable for the species (ibid.).

VI. Landscape Context:

Context may be important. In a study of arboreal marsupials, it was found that corridors that included variety of topographic positions—like gullies and ridges—supported a greater abundance of animals and higher species diversity

VII. Human Activities:

Intensity of human activities will determine the effectiveness of the corridor. In this context type and extent of human activities—like livestock grazing, intrusion by dogs, human presence etc.—inside and adjacent to the corridor will have an important bearing (Harrison, 1992). Corridor design can include buffer zones to reduce or mitigate undesirable human activities.

Horn (2003) has designed corridors to connect forest patches in South Australia's green triangle plantations. Horn suggests that varying the width along longer corridors, in particular by creating 'nodes' may also improve their viability and reduce edge effects on target species.

Probable questions:

6. What are the efforts to conserve olive ridley turtles?
7. What are the Conservation management strategies of greater one-horned rhino?
8. What is Red data book?
9. What is IUCN?
10. Describe the IUCN categories.
11. What do you mean by rare animal? Give example.
12. Write note on wildlife wealth of India.
13. What is wild life corridor?
14. Describe the parameters in designing corridor.

Suggested reading:

5. Enger, E. D. and Smith, B. F. (2008). Environmental Science: A study of Interrelationships. 11th ed. McGraw-Hill Higher Education.
6. Kormondy, E. J. (2002). Concepts of Ecology. 4th Indian Reprint, Pearson Education.
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8. Smith, T. M and Smith, R. L. (2006). Elements of Ecology. 6thed. Pearson Education

Disclaimer:

The study materials of this book have been collected from various books,e-books, journals and other e-sources.

POST GRADUATE DEGREE PROGRAMME (CBCS)

in

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

DEVELOPMENTAL BIOLOGY AND CYTOGENETICS

ZCORT-103

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia West
Bengal, India

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Group A : Developmental Biology

Paper	Unit	Content	Credit	Page No.
ZCORT - 103 (Developmental Biology and Cytogenetics)	**I**	**Overview of early embryonic development and morphogenesis.**	**3**	**8-37**
	II	**Potency, induction, germ cell migration, competence, commitment:**		**38-61**
	III	**specification, determination and differentiation, morphogenetic gradient and fate map**		**62-67**
	IV	**Axis specification in Drosophila: role of maternal effect genes, patterning of early embryo by zygotic genes, gap genes, pair– rule genes, segment polarity genes, homeotic selector genes- bithorax and antennapedia complex.**		**68-79**
	V	**Axis specification in vertebrates: Early patterning in vertebrates - Symmetry breaking, Nieuwkoop center. Wnt and cadherin signaling, TGFβ signaling in early developmental process, asymmetric gene expression.**		**80-92**
	VI	**General concepts of organogenesis: Development and patterning of vertebrate limb, homeobox genes in patterning, signaling in patterning of the limb;**		**93-99**
	VII	**Insect imaginal discs– organizing center in patterning of the leg and wing, the homeotic selector genes for segmental identity; insect compound eye.**		**100-110**
	VIII	**Postembryonic development: growth, cell proliferation, growth hormones; aging- genes involved in alteration**		**111-125**

		in timing of senescence		
	IX	**Regeneration– Epimorphic regeneration of reptile (salamander) limb; Morphollaxis regeneration in Hydra.**		**126-135**
	X	**Programmed cell death: apoptosis, autophagy and necrosis.**		**136-145**

Group-B : CYTOGENETICS

Paper	Unit	Contents	Credit	Page No
ZCORT - 103 (Developmental Biology and Cytogenetics)	XI	Organization and Structure of Genomes: Organization and nature of eukaryotic nuclear DNA, Size and complexity of eukaryotic genome;	3	148-159
	XII	Transposable elements, retrotransposons, SINE, LINE, Alu and other repeat elements,		160-170
	XIII	Pseudogenes, segmental duplications; Super coiling of DNA. Giant chromosomes:		171-193
	XIV	Classes of DNA, Virus and Bacterial genomes, Mitochondrial genome.		194-224
	XV	Phases of Cell cycle, Check Points, Regulation of cell cycle; MPF, cyclins and cyclin-dependent kinases.		225-246
	XVI	Cancer-Types and Stages, Carcinogens, Tumor suppressor genes and Proto-oncogenes induction to oncogenes. Concepts of Apoptosis:		247-282
	XVII	Regulators of Apoptosis, Caspases, Pathways of apoptosis; Cell Senescence, Necrosis.		283-296
	XVIII	DNA Replication and Recombination: Topology, Variations and Nature of Replication; Replicon, Replicator, Fidelity and Processivity of replication; "Hayflick limit" and Telomerase activity; Regulation of Replication		297-326
	XIX	DNA damage response; Drugs and Inhibitors targeting replication-antibacterial, antiviral and anticancer; and DNA Repair		327-339
	XX	Extrachromosomal Replicons; Homologous Recombination		340-353

Total counselling time 18 hours

UNIT-I
Overview of early embryonic development and morphogenesis

Introduction to Embryology:

Embryology is the study of structure and development of embryo, including the structure and development of male and female reproductive organs, fertilisation and similar other processes. The Phanerogams (the flowering-plants) are also called spermatophytes (the seed bearing plants). These plants propagate mainly through seeds. The seed is a structure in which the embryo is enclosed. Adjacent to the embryo, foods are stored either inside the endosperm (albuminous) or in cotyledon (exalbuminous) for future use.

The Greek philosopher Aristotle (384-322 B.C.) is the 'Father of the Study of Natural Sciences'. The greater part of his work is not available. With the available data, it seems that he did not recognise the presence of sex in plants. He believed that male and female plants were so blended that they generated on their own and the offspring developed from the superfluous (above what is enough) food of the plant. Later Theophrastus (370-287 B.C), the 'Father of Botany' in third century B.C. referred the pollination of the date palm. After this, the nature of sexuality of plants was forgotten for about 2,000 years and his report was considered as useless. After a long gap, it was Nehemiah Grew who first clearly stated, in 1682, that the stamens are nothing but the male sex organs of a plant. Later, in 1694, R. J. Camerarius suggested that the anthers are the male sex organs of a plant, while the ovaries with the styles were the female sex organs. After many years, J. G. Kolreuter (1761) fully confirmed the idea of Camerarius. Later, Nawaschin (1898) had established the phenomenon of double fertilisation in Fritillaria tenella and Lilium martagon. Later on, the different aspects of embryology were studied by different workers. Most of the facts on development of gametophytes and embryo were discovered later.

During the 20th century, particular interest was developed on experimental embryology which includes the problems on storage and viability of pollen grains, effect of environmental factors on the growth of pollen tube, production of seedless fruits, embryo culture, induction of parthenogenesis etc. Thus embryology maintains an intimate association with genetics and physiology and opens many promising lines of research for the useful and economic development of crops. Embryology played a significant role in consideration of systemic botany. Although Hofmeister and Strasburger pointed out the possibility of utilising embryological characteristics in taxonomy, it was Schnarf (1931) who first prominently brought into focus the role of embryology in taxonomy.

Ontogenetic Development as the Subject Matter of Embryology:

The aim of this article is to familiarize the reader with the basic facts and problems of the science of embryology. The name "embryology" is somewhat misleading. Literally it means the study of embryos. The term "embryo" denotes the juvenile stage of an animal while it is contained in the egg (within the egg envelopes) or in the maternal body.

A young animal, once it has hatched from the egg or has been born, ceases to be an embryo and would escape from the sphere pertaining to the science of embryology, if we were to keep strictly to the exact meaning of the word. Although birth or hatching from the egg is a very important occasion in the life of

the animal, it must be admitted that the processes going on in the animal's body may not be profoundly different before and after the hatching from an egg, especially in some lower animals. It would be artificial to limit studies of the juvenile forms of animal life to the period before the animal is hatched from the egg or is born. It is customary, therefore, to study the life history of an animal as a whole and accordingly to interpret the scope of the science of embryology as the study of the development of animals. The word "development" must be qualified in turn. In the sphere of biology with which we are concerned, the term "development" is used with two different meanings. It is used to denote the processes that are involved in the transformation of the fertilized egg, or some other rudiment derived from a parent organism, into a new adult individual. The term development may, however, also be applied legitimately to the gradual historical transformation of the forms of life, starting with simple forms which might have been the first to appear and leading to the contemporary diversity of organic life on our planet. Development of the first type may be distinguished as individual development or ontogenetic development.

Development of the second type is the historical development of species or phylogenetic development. Phylogenetic development is often referred to as evolutionary development or simply evolution. Accordingly, we will define embryology as the study of the ontogenetic development of organisms. In this article we will be dealing only with the ontogenetic development of multicellular animals, the Metazoa. In multicellular animals, the typical and most widespread form of ontogenetic development is the type occurring in sexual reproduction. In sexual reproduction new individuals are produced by special generative cells or gametes. These cells differ essentially from other cells of the animal, in that they go through the process of maturation or meiosis, as a result of which they lose half of their chromosomes and become haploid, whereas all other cells of the parent individual, the somatic cells, are as a rule, diploid. Once a cell has gone through the process of meiosis, it can no longer function as an integral part of the parent body but is sooner or later extruded to serve in the formation of a new individual. In multicellular animals there exist two types of sex cells – the female cells or ova, and the male cells or spermatozoa.

As a rule the two cells of the opposite sexes must unite in the process of fertilization before development can start. When the two gametes (the ovum and the spermatozoon) unite, they fuse into a single cell, the zygote, which again has a diploid number of chromosomes. The zygote, or fertilized ovum, then proceeds to develop into a new adult animal. Side by side with sexual reproduction there exists in many species of animals a different mode of producing new generations—asexual reproduction. In asexual reproduction the off-spring are not derived from generative cells (gametes) but rather from parts of the parent's body consisting of somatic cells.

The size of the part which is set aside as the rudiment of the new individual may be large or small, but in the Metazoa it always consists of more than one cell. The development of an animal by way of asexual reproduction obviously belongs in the same category as the development from an egg and should be treated as a special form of ontogenetic development. To distinguish between the two forms of ontogenetic development, the term embryogenesis may be used to denote development from the egg, and the term blastogenesis may be used for the development of new individuals by means of asexual reproduction.

Descriptive and Comparative Embryology:

Although the correct understanding of ontogenetic development could be achieved only after the establishment of the cell theory, fragmentary information on the development of animals has been obtained since very ancient times. Aristotle had described the development of the chick in the egg as early

as 340 B.C. Many observations on development of various animals, especially of insects and vertebrates, were made in the seventeenth and eighteenth centuries. However, the data of embryology were first presented in a coherent form by Karl Ernst von Baer (1828). In his book "Ueber Entwicklungsgeschiechte der Tiere, Beobachtung and Reflexion," Baer not only summed up the existing data and supplemented them by his original investigations but also made some important generalizations.

The most important of these is known as Baer's law. The law can be formulated thus; **"More general features that are common to all the members of a group of animals are, in the embryo, developed earlier than the more special features which distinguish the various members of the group."** Thus the features that characterize all vertebrate animals (brain and spinal cord, axial skeleton in the form of a notochord, segmented muscles, aortic arches) are developed earlier than the features distinguishing the various classes of vertebrates (limbs in quadrupeds, hair in mammals, feathers in birds, etc.). The characters distinguishing the families, genera, and species come last in the development of the individual. The early embryo thus has a structure common to all members of a large group of the animal kingdom and may be said to represent the basic plan of organization of that particular group. The groups having a common basic plan of organization are the phyla of the animal kingdom.
Baer's law was formulated at a time when the theory of evolution was not recognized by the majority of biologists. It has been found, however, that the law can be reinterpreted in the light of the evolutionary theory. In its new form the law is known as the biogenetic law of Müller-Haeckel. Müller propounded the law in its new form and supported it by extensive observations on the development of crustaceans (1864). Haeckel (in 1868) gave it the name of the "biogenetic law" and contributed most to its wide application in biology. According to Baer's law, the common features of large groups of animals develop earliest during ontogeny. In the light of the evolutionary theory, however, these features are the ones that are inherited from the common ancestor of the animal group in question; therefore, they have an ancient origin.

The features that distinguish the various animals from one another are those that the animals have acquired later in the course of their evolution. Baer's law states that these features in ontogeny develop at later stages. Briefly, the features of ancient origin develop early in ontogeny; features of newer origin develop late. Hence, the ontogenetic development presents the various features of the animal's organization in the same sequence as they evolved during the phylogenetic development. Ontogeny is a recapitulation of phylogeny. The repetition is obviously not a complete one, and the biogenetic law states that **"ontogeny is a shortened and modified recapitulation of phylogeny."**

The shortening of the process is evident not only from the fact that what had once taken thousands of millions of years (phylogeny) is now performed in a matter of days and weeks (ontogeny), but also from the fact that many stages which occurred in the original phylogenetic development may be omitted in ontogeny. The modifications arise mainly because the embryo at any given time is a living system which has to be in harmony with its surroundings if it is to stay alive. The embryo must be adapted to its surroundings, and these adaptations often necessitate the modification of inherited features of organization. A good example of such adaptation is the placenta in mammals. The placenta is a structure developed by the embryo to establish a connection with the uterine wall of the mother and thus to provide for the nutrition of the embryo. This structure, though developed rather early in the life of the embryo, could not have existed in the adult mammalian ancestors. It is obviously an adaptation to the special conditions in which a mammalian embryo develops.

Even if the repetition of features of their ancestors in the ontogenetic development of contemporary animals is not complete, the fragmentary repetition of certain ancestral characters may still be very useful in elucidating the relationships of animals. As an example of this we may consider the formation of gill clefts or, at least, pharyngeal pouches in the ontogenetic development of all vertebrates. In the aquatic vertebrates, such as Cyclostomata and fishes, the gill clefts serve as respiratory organs. In the adult state of terrestrial vertebrates, the pharyngeal pouches have disappeared completely or have been modified out of all recognition, and the function of respiration has been taken over by other organs—the lungs. Nevertheless, the pharyngeal pouches appear in the embryo.

In amphibians whose larvae are aquatic, the pharyngeal pouches at least temporarily serve for respiration. In reptiles, birds, and mammals, the pharyngeal pouches of the embryo do not serve for respiration at all. Their formation can be explained only as an indication that the terrestrial vertebrates have been derived from aquatic forms with functional gills. The paleontological evidence fully confirms this conclusion. The systematic position of some animals cannot be recognized from adult structure, owing to profound modification acquired as a result of adaptation to very special conditions. Here the knowledge of the development sometimes throws unexpected light on true relationships. The adult ascidian is a sessile animal with no organs of locomotion and a nervous system of a very primitive nature.

The adult animal had been classed as a near relative of molluscs until Kowalevsky (1866) discovered that the larvae of the ascidians possess a well-developed dorsal brain and spinal cord, a definite notochord, and lateral bands of muscles (in short, organs that are typical for the vertebrates). The ascidians are therefore considered as belonging to the same phylum as the vertebrates, the phylum Chordata. In the adult parasitic animal Sacculina, the organization of the animal is very much simplified in relation to the easy life that the parasite enjoys; it is reduced practically to a shapeless sack producing eggs and a system of branched rhizoids, by means of which the parasite is attached to its host, the crab, and absorbs the host's body fluids on which it feeds. It would be impossible to place Sacculina in any group of the animal kingdom if its development were not known. However, the larva of Sacculina is a typical arthropod, bearing a close similarity to the larvae of the lower crustaceans, the Entomostraca.

A rather similar larva is also found in the barnacles (Cirripedia) which, though possessing jointed legs like other arthropods, have lost the segmentation of the body in the adult state. The attachment of the starfish larva, the brachiolaria, to the substrate while it is metamorphosing into the definitive form is an indication that the free-living echinoderms have been derived from sessile forms. This conclusion is again borne out by the evidence of paleontology. Following the principles of Baer's law and the biogenetic law, embryologists have systematically investigated the development of animals belonging to all the major groups of the animal kingdom. As a result of very extensive and painstaking investigations, a magnificent edifice of comparative embryology has been built.

Explaining Development – Theories of Preformation and Epigenesis:

Neither the description of morphological transformations occurring in the embryo nor the comparison of embryos and larvae among themselves and with the adult animals exhausts all the problems presented by the ontogenetic development of animals. The fundamental problem presented by the existence of cyclical ontogenetic changes is the question – Why does ontogeny occur at all? What are the forces which produce the changes? How it is that, starting from a simple spherical cell, the process always ends in producing a

highly complex and specific structure which, though varying in detail, reproduces with astonishing perseverance the same or almost the same adult form?

Attempts at solving this basic problem have been made ever since the human mind recognized the existence of development. For a long time the explanations proposed were purely speculative. Aristotle attempted to give a solution to the problem of ontogeny along the general lines of his philosophical teaching, distinguishing between the substance and the form of things. The form appears in this concept as the creative principle. Aristotle further supposed that the substance for the development of a child is provided by the mother (in the form of nutrition) but that the creative principle is supplied by the father. He thus accounted also for the necessity of fertilization. Although this treatment of the phenomena of development is completely contradictory to what we now know of the material basis of development (the parts played by the ovum and the spermatozoon), still the concept of a creative principle has turned up repeatedly in the teachings of embryologists up to the twentieth century. In the seventeenth and eighteenth centuries, when all biological sciences developed rapidly, together with the physicochemical sciences, there existed a widespread theory explaining the ontogenetic development of animals. This was the theory of preformation. The theory of preformation claimed that if we see that something develops from the egg, and then this something must actually have been there all the time but in an invisible form.

It is common knowledge that in a bud of a tree the leaves, and sometimes also the flowers with all their parts, can be discovered long before the bud starts growing and spreading and thus exposing to view all that before was hidden inside, covered by the superficial scales of the bud. Furthermore, it was known that in a chrysalis of a butterfly the parts of the butterfly's body—the legs, the wings, etc.—can be discovered if the cuticular coat of the chrysalis is carefully removed a few days before the butterfly emerges from the chrysalis. Something of this sort was supposed to exist in the egg. All the parts of the future embryo were imagined to be already in the egg, but they were thought to be transparent, folded together, and very small, so that they could not be seen. When the embryo began to develop, these parts supposedly started to grow, unfold, and stretch themselves, become denser and therefore more readily visible. The embryo, and therefore indirectly also the future animal, was preformed in the egg. Hence the theory is called the theory of preformation. When spermatozoa were discovered in the seminal fluid, the relative significance of the ova and spermatozoa had to be accounted for. It is obvious that a preformed embryo cannot be present in both the egg and the spermatozoon. The preformationists were split therefore into two rival schools, the ovists and the animalculists. (The latter name comes from the word animalcule, as the spermatozoa were then called.)

The ovists asserted that the embryo was preformed in the egg. The spermatozoa then seemed superfluous, and in fact, they were declared parasites living in the spermatic fluid. On the other hand, the animalculists declared that the embryo was preformed in the spermatozoon and that the egg served only to supply nutrition for the developing embryo. A lively discussion arose, which ended in favor of the ovists. The victory of the ovists was due to the discovery of parthenogenetic development in some insects, e.g., the aphids. If the egg could develop without fertilization, it was clear that the embryo could not be preformed in the spermatozoon. The theory of preformation, although very popular in its time, did not satisfy all biologists, and opposing views, denying the existence of a preformed embryo in the egg, were proposed. The most important contribution in this field was the theory of epigenesis, proposed by Caspar Friedrich Wolff (1759). In favor of his theory Wolff adduced his own observations on the formation of the chick embryo. In the earliest stages of the development of the chick he could not find any parts of the future

embryo. Moreover, he found that the egg was by no means devoid of any visible structure; there was a structure present, but it was different from that of the later embryo.

Wolff found that the substance of which the embryo is composed is granular. Presumably the granules must have been the cells or their nuclei. These granules were later arranged into the layers which we now call the germinal layers. Wolff saw that by the formation of local thickenings in some parts of these layers, by thinning out in others, and by the formation of folds and pockets, the layers are transformed into the body of the embryo. He concluded, therefore, that in the early egg there does not exist a preformed embryo but only the material of which the embryo is built. This material does not represent an embryo any more than a heap of bricks represents the house that will be built of them. In both cases there had to be an architect who would use the material for a purpose that he had in mind. In the case of the developing embryo the architect was represented by a vital force, perhaps not essentially different from the "creative principle" postulated by Aristotle.

The Phases of Ontogenetic Development:

The processes leading to the development of a new individual really start before the fertilization of the egg, because the ripening of the egg and the formation of the spermatozoon, which constitute the phase of gametogenesis, create the conditions from which the subsequent embryogenesis takes its start. In both oogenesis and spermatogenesis, meiosis, by discarding half of the chromosomes, singles out the set of genes which are to operate in the development of a particular individual. In both sexes the initial cells giving rise to the gametes are very similar and, as a rule, not essentially different from other cells of the body except that these cells are not involved in any of the differentiations serving to support the life of the parent individual.

In both sexes the first step in the production of gametes is a more or less rapid proliferation of cells by ordinary mitosis. The proliferating cells in the testes are known as the spermatogonia; the proliferating cells in the ovaries are called oogonia. Once proliferation ceases, the cells are called spermatocytes in the male and oocytes in the female. They then enter into a stage of growth and later into a stage of maturation. Although the stage of proliferation is not essentially different in the male and female, the processes of growth and maturation in the two sexes differ to a very great extent. The cytoplasmic differentiations of the spermatozoon enable it to reach the egg by active movement and to fertilize it.

On the other hand, the egg cell accumulates in its cytoplasm substances which are used up during development—either directly, by becoming transformed into the various structures of which the embryo consists, or indirectly, as sources of energy for development. The elaboration in the egg cell of cytoplasmic substances to be used by the embryo and their placing in correct positions are essential parts of what occurs during the first phase of development. The second phase of development is fertilization**.** Fertilization involves a number of rather independent biological and physiological processes. First, the spermatozoa must be brought into proximity with the eggs if fertilization is to occur. This involves adaptations on the part of the parents which insure that they meet during the breeding season, discharge their sex cells simultaneously in cases of external fertilization, or copulate in cases of internal fertilization.

Next the spermatozoa must find the egg and fuse with it. This egg-sperm fusion entails a very finely adjusted mechanism of morphological and physicochemical reactions. The egg is then activated by a sper-

matozoon and starts developing. A further rearrangement of the organ-forming substances in the egg is among the first changes that take place in the egg after fertilization. The third phase of development is the period of cleavage**.** The fertilized egg is still a single cell, since the nucleus and cytoplasm of the spermatozoon fuse with the nucleus and cytoplasm of the egg. If a complex and multicellular organism is to develop from a single cell, the egg, the latter must give rise to a large number of cells. This is achieved by a number of mitotic cell divisions following one another in quick succession. During this period the size of the embryo does not change, the cleavage cells or blastomeres becoming smaller and smaller with each division. No far-reaching changes can be discovered in the substance of the developing embryo during the period of cleavage, as if the preoccupation with the increase of cell numbers excludes the possibility of any other activity.

The whole process of cleavage is dominated by the cytoplasmic organoids of the cells, the centrosomes and achromatic figures. The nuclei multiply but do not interfere with the processes going on in the cytoplasm. The result of cleavage is sometimes a compact heap of cells, but usually the cells are arranged in a hollow spherical body, a blastula, with a layer of cells, the blastoderm, surrounding a cavity, the blastocoele. During this phase the single layer of cells, the blastoderm, gives rise to two or more layers of cells known as the germinal layers. The germinal layers are complex rudiments from which are derived the various organs of the animal's body. In higher animals the body consists of several layers of tissues and organs, such as the skin, the subcutaneous connective tissue, the layer of muscles, the wall of the gut, and so on. All these tissues and organs may be traced back to three layers of cells—the aforementioned germinal layers. Of these the external one, the ectoderm, always gives rise to the skin epidermis and the nervous system. The layer next to the first, the mesoderm, is the source of the muscles, the blood vascular system, the lining of the secondary body cavity (the coelom, in animals in which such a cavity is present), and the sex organs. In many animals, particularly the vertebrates, the excretory system and most of the internal skeleton are also derived from the mesoderm. The third and innermost germinal layer, the endoderm, forms the alimentary canal and the digestive glands. The germinal layers are produced by the disappearance of a part of the blastoderm from the surface and its enclosure by the remainder of the blastoderm. The part that remains on the surface becomes ectoderm; the part disappearing into the interior becomes endoderm and mesoderm.

The disappearance of endoderm and mesoderm from the surface sometimes takes the form of a folding-in of part of the blastoderm, so that the simple spherical body becomes converted into a double-walled cup, as if one side of the wall of an elastic hollow ball had been pushed in by an external force. This in-folding or pushing in of the endoderm and mesoderm is known as invagination, and the resulting embryo is known as a gastrula—whence the term gastrulation. If the gastrula is formed by invagination, the cavity of the double-walled cup is called the archenteron, and the opening leading from this cavity to the exterior is called the blastopore. In animals in which the gastrula is formed in a different way—not by invagination—the cavity (archenteron) and the opening of the cavity to the exterior (blastopore) may still appear later on.

The archenteron, or part of it, eventually gives rise to the cavity of the alimentary system. The fate of the blastopore differs in the three main groups of Metazoa. In Coelenterata it becomes the oral opening. In Protostomia (including Annelida, Mollusca, Arthropoda, and allied groups) it becomes subdivided into two openings, one of which becomes the mouth and the other the anus. In Deuterostomia (including Echinodermata and Chordata) only the anal opening is connected in its development with the blastopore, the mouth being formed later on as an independent perforation of the body wall. The whole of the lining of the alimentary canal does not always consist of endoderm; in all groups of animals the ectoderm may be

invaginated secondarily at the oral or at both oral and anal openings to become a part of the alimentary canal. The parts of the alimentary canal lined by ectoderm are known as the stomodeum (adjoining the mouth) and proctodeum (adjoining the anus).

With the formation of the three germinal layers, the process of subdivision of the embryo into parts with specific destinies commences. This subdivision is continued in the next (fifth) phase of development, the phase of organogenesis (organ formation). The continuous masses of cells of the three germinal layers become split up into smaller groups of cells, each of which is destined to produce a certain organ or part of the animal. Every organ begins its development as a group of cells segregated from the other cells of the embryo. This group of cells we will call the rudiment of the respective organ. The rudiments into which the germinal layers become subdivided are called primary organ rudiments. Some of these are very complex, containing cells destined to produce a whole system of organs, such as the entire nervous system or the alimentary canal. These complex primary organ rudiments later become subdivided into secondary organ rudiments—the rudiments of the subordinated and simpler organs and parts. The formation of the primary organ rudiments follows so closely on the processes of gastrulation that the two processes can hardly be considered separately. With the appearance of primary and secondary organ rudiments, the embryo begins to show some similarity to the adult animal or to the larva if the development includes a larval stage.

The sixth phase of development is the period of growth and histological differentiation. After the organ rudiments are formed they begin to grow and greatly increase their volume. In this way the animal gradually achieves the size of its parents. Sooner or later the cells in each rudiment become histologically differentiated; that is, they acquire the structure and physicochemical properties which enable them to perform their physiological functions. When the cells in all the organs, or at least in the vitally important organs, have become capable of performing their physiological functions, the young animal can embark upon an independent existence—an existence in which it has to procure food from the surrounding environment. In rather rare cases (in the nematodes, for instance) the young animal emerging from the egg is a miniature copy of the adult animal and differs from the latter only in size and the degree of differentiation of the sex organs. In this case the subsequent development consists only of growth and maturation of the gonads. It is more usual, however, for animals emerging from eggs to differ from the adult to a greater extent; not only the gonads but also other organs may not be fully differentiated, or they may even be absent altogether and have to develop later. Sometimes the animal emerging from the egg possesses special organs which are absent in the adult but which are necessary for the special mode of existence of the young animal. In this case the young animal is called a larva. The larva may lead a different mode of life from the adult, and therein lies one of the advantages of having a larval stage in development. The larva undergoes a process of metamorphosis when it is transformed into an animal similar to the adult.

The metamorphosis involves more or less drastic changes in the organization of the larva, depending on the degree of difference between the larva and the adult. During metamorphosis new organs may develop, so that morphogenetic processes become active again after a more or less prolonged period of larval life. A secondary activation of morphogenetic processes may be produced in a different way. Many animals possess considerable plasticity and may be able to repair injuries sustained from the environment or caused experimentally. Lost parts may be regenerated, and this means that the developmental processes may sometimes be repeated in an adult or adolescent organism. Asexual reproduction of animals involves

the development of new parts and organs in animals that have already achieved the adult stage. All morphogenetic processes occurring in the later life of the animal, after the larval stage, or even when the adult stage has been achieved, will be dealt with as constituting a seventh and last phase of development.

Experimental Embryology:

Wolff's observations, however, could not be considered as final in deciding between the alternative theories of preformation and epigenesis. In spite of what he actually observed, it was still conceivable that organs and parts of the body of the future embryo were represented in the egg by discrete particles, qualitatively different among themselves. The granules which he saw might have been different in their properties. Even if the transformation of such qualitatively different parts into the organs of the embryo should have been more complicated than was envisaged by the crude preformistic theory, the principle of preformation might well have held true in spite of the apparent homogeneity of the material of which the embryo was supposedly made. Observation alone could not make further advances toward the solution of this problem, and further progress could be achieved only with the aid of experiment.

One of the experiments which are relevant to the preceding problem is the separation of the two cells into which the egg is divided at the beginning of development. If the theory of preformation is correct, we should expect that one of the two first blastomeres, containing one half of the egg material, should develop into an embryo lacking one half of its organs and parts. If, on the other hand, the substances contained in the egg are but the building material used for the construction of the embryo, then it is conceivable that half of the material might be sufficient for making a complete embryo even if it may have to be on a diminished scale, just as the bricks prepared for the construction of a big house may be used for building two houses of a smaller size. The first embryologist to see this way of solving the problem was Wilhelm Roux (1850-1924). Accordingly, he proceeded to test one of the first two cleavage cells in the common frog for its ability to develop. To achieve his end Roux destroyed one of the two cleavage cells with a red-hot needle (1888). The embryo that was derived from the surviving cleavage cell was found to develop, at least at first, as if it were still forming a half of a complete embryo. In other words, the developing embryo was defective, as it should have been according to the theory of preformation.

It was found later, however, that the technique used by Roux was too crude. The damaged cleavage cell had not been removed, and it was the presence of this damaged cleavage cell, as was later found out, that caused the defects in the surviving embryo. If the two cleavage cells of the egg were separated completely, two whole and, except for their size, normal embryos could develop, one from each of the two cleavage cells. This result was first found by H. Driesch (1891), working on sea urchin eggs, and later by Endres (1895) and Spemann (1901, 1903), working with eggs of newts. Eventually the experiment was repeated by Schmidt (1933) on the frog, the same animal that had served for the experiments of Roux. Schmidt found that if the two cleavage cells were completely separated, each could develop into a whole embryo.

The first experiments on the developing embryo were followed by many others, and soon a new science was born – experimental embryology. Experimental embryology, in contrast to comparative embryology or descriptive embryology, uses experiment as a method of investigation. However, the use of the experimental method in itself does not create a science or a branch of science. New branches of science are created by novel viewpoints and novel problems set before science. It was the problem of what ontogenetic development actually is, what the driving forces behind it are, that necessitated the application

of experiment after the methods of speculation and of pure observation were found to be impotent in solving the problem.

Blastogenesis and Embryogenesis:

In the general survey of ontogenetic development, we have found it useful to consider what tasks have to be performed by the embryo before the final condition (the development of the new adult individual) is achieved. If from this same viewpoint we compare embryogenesis (development of the egg) with blastogenesis (development from a blastema in asexual reproduction), we see at once that the task is very much simpler in the latter case. The process of producing a new individual is simplest in reproduction by fission, when the blastozooid is derived from half the parental organism and in this way is provided with a large proportion of the organs and parts which are necessary for making the new individual self-sufficient. What has to be done is the regeneration of missing parts.

The whole mechanism of regeneration, is brought into play, including the factors determining the regenerating parts. The remnant of the old individual determines the nature, position, and orientation of the newly differentiated organs. The polarity and bi-laterality of the parent organism prevail in the blastozooids. The task of development is more complicated in the case of budding, since all organs and differentiated parts of the blastozooid have to be produced anew. Nevertheless, the initial system, the bud, always has a higher degree of complexity than a fertilized egg or even than a blastula as it occurs in embryogenesis. A typical bud, always consists of two layers of epithelial cells. The young zooid is thus already in possession of the concentric stratification of body layers, a condition which in embryogenesis is achieved only after gastrulation. It is very note-worthy; however, that the layers formed in the blastozooid do not necessarily correspond to the germinal layers developing in embryogenesis. In the case of budding in coelenterates, there is a closer correspondence between the outer and inner layers of the bud and the ectoderm and endoderm of the gastrula. The fate of the two layers is the same, but we have seen that the inner layer may be derived not from the endodermal epithelium of the parent but from a thickening in the ectodermal epithelium. When we turn to the buds of tunicates, we find that the inner vesicle corresponds to the endoderm neither in its origin nor its fate. It has been shown that, although in some tunicates the inner vesicle may be derived from the endoderm of the parent (in the form of an outgrowth from the pharyngeal epithelium), it can also be derived from mesoderm (epicardium, mesodermal septum of the stolon, blood cells) or even ectoderm (lining of the atrial cavity). What is even more important is that the inner vesicle gives rise to parts derived in embryogenesis from any of the three germinal layers. In spite of the diversity of origin of the inner vesicle, its later differentiation shows a considerable degree of uniformity in different tunicates. After the bud has grown to a certain degree—a minimal size, varying of course in different species, is essential — it becomes constricted at least partially from the parent zooid. Then folds start sub-dividing the inner vesicle into sections. (The following description refers to the development of the blastozooid in Botryllus.) First, two folds cut in from what will be the distal part of the new zooid, subdividing the inner vesicle into a median part, which will become the branchial chamber, and two lateral parts, which give rise to the atrial cavity (Fig. 507). (In embryogenesis, the atrial cavity develops as a pair of invaginations of the ectoderm which partially fuse and later have a common opening to the exterior.)

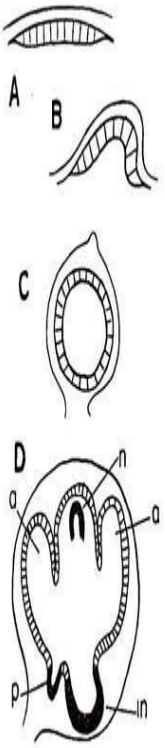

Figure 507. Development of the bud in *Botryllus*. a, Rudiment of atrial cavity; in, rudiment of intestine; n, rudiment of ganglion; p, rudiment of pericardium. (From Berrill, 1951.)

In addition to these three main sub-divisions, further smaller pocket-like evaginations of the vesicle appear. One evagination near the anterior end of the central cavity, gives rise to the nerve center. (In embryrogenesis, the nerve ganglion is a remnant of the neural tube formed by infolding of the ectodermal neural plate, as in Amphioxus and in vertebrates.) Two pockets at the posterior end give rise to the intestinal loop (including the esophagus and the stomach) and to the pericardium, respectively. (In embryogenesis the intestine is, of course, of endodermal origin, and the pericardium develops from mesodermal mesenchyme.) Because the initial state of the system in blastogenesis is not the same as that in embryogenesis, the course of development is different. The morphogenetic processes in budding appear to be simpler and more straightforward than in embryogenesis.

The actively developing part, which is the inner vesicle (the epidermis of the bud is a differentiated tissue all the time, specialized in secreting the cellulose mantle), proceeds directly to the formation of organ rudiments, omitting the stage of germ layer development. In one further respect the morphogenetic processes in budding are simpler than in embryogenesis: The new individual inherits its polarity directly from the parent zooid. The point of attachment of the bud to the maternal body or the stolon always becomes the proximal end of the blastozooid. In the development of gemmules, the task of producing a new individual becomes most complicated and approaches that of the development of the egg.

The special difficulties encountered are:

1. The germination of the gemmule occurs after the death and decomposition of the parent animal; consequently, the polarity of the new individual has to be worked out by itself, and the parent body is no longer there to influence the polarity of the offspring.

2. The complete homogeneity of the contents of the gemmule (in the case of the gemmules of sponges) deprives the new individual of any remnant of morphological organization. The structure of the new

sponge has to be established by the interaction of practically independent cells. The interior of the gemmule consists of only one type of cell, the archeocytes, which are rather large, cells containing platelets of glycoprotein. Even before the germination of the gemmule, some of the archeocytes become activated; they start dividing and in so doing give rise to smaller and smaller cells, very much like the blastomeres which diminish in size as cleavage progresses. The glycoprotein platelets gradually disappear; the nuclei become richer in chromatin, a usual characteristic in actively growing and metabolizing cells.

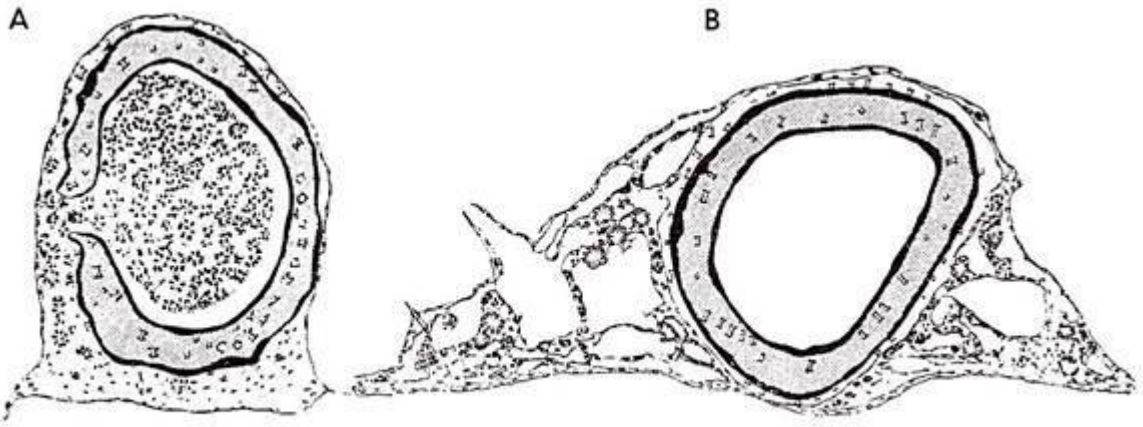

Figure 508. Germination of gemmules. *a*, Contents of the gemmule of *Spongilla*, leaving the shell. *b*, Transformation of the contents of the gemmule of *Ephydatia* into a new sponge. (From Brien, 1932.)

These small and active cells have been referred to as histioblasts—cells producing tissues. When the gemmule germinates, the contents of the gemmule crawl out through the micropyle and form an irregular mass, surrounding the empty shell of the gemmule (Fig. 508a). Both the histioblasts and the remaining glycoprotein-containing archeocytes leave the shell. Outside the shell, the division of archeocytes and their conversion into histioblasts continue (Fig. 509).

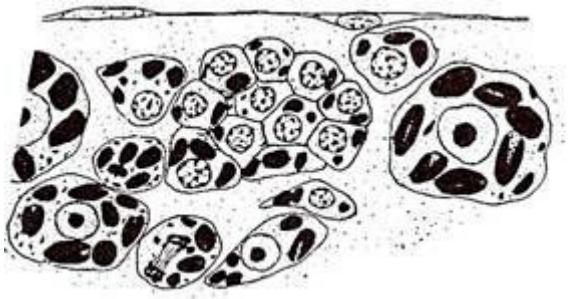

Figure 509. Transformation of archeocytes into histioblasts during development of a new sponge from a gemmule. Archeocytes still with deutoplasmic inclusions; one is in mitosis. (From Brien, 1932.)

The histioblasts now become arranged into an irregular meshwork (Fig. 508b), cavities appear, and some of the histioblasts surrounding these cavities differentiate into choanocytes. Other histioblasts become epidermal cells, scleroblasts, pore cells, or mesenchymal cells arranged in a typical way, so that the mass of cells soon becomes a small sponge, with a system of internal canals with ciliated chambers, ostia, etc. It may be significant that the sponges are capable of reconstituting their structure after complete dis-aggregation. The development of the gemmule proceeds along very much the same lines; the development is direct in the extreme, the individual cells differentiating and taking up their positions in the whole.

A review of different forms of asexual reproduction reveals a principle of general significance. It shows that the complexity of morphogenetic processes is largely determined by the degree of difference that exists between the initial stage of development and the final condition. The initial stage in embryogenesis

is a single cell; therefore a period of cleavage is necessary, which brings the system into a multicellular condition. In asexual reproduction, the initial system is already multicellular, and cleavage falls away. (Something resembling cleavage occurs, in the gemmules of sponges, where the archeocytes accumulate food reserves, similarly to oocytes in the ovary.) In asexual reproduction the initial system may have the cells arranged in more than one layer, and this makes gastrulation dispensable. What remains of the main periods of development are organogenesis, differentiation, and growth. A second very suggestive fact which emerges from a study of asexual reproduction is that, given a normal environment, the organization of the animal's body is entirely determined, in the last instance, by the hereditary constitution of the species-specific cells. The structure of the egg cell with its polarity and the heterogeneous arrangement of cytoplasmic substances is a mechanism which provides for the orderly course of differentiation of the cells in a developing embryo.

This mechanism is, however, dispensable; the same egg product can be attained starting from a different initial constellation, provided that the cells have the same hereditary constitution. In principle, the orderly organization of an animal's body should be attainable by the interaction of an assortment of different types of cells produced on the basis of species-specific hereditary potentialities. In practice, this is possible only in relatively very simple biological systems.

Modern Embryology—Analytical Embryology:

After the middle of the present century embryology had got caught up in the new trend that developed in biological science. Early in the century, the background for this new trend was established mainly by the work of T. H. Morgan and his school.

This work proved that the units of heredity, the genes, are arranged in linear order in the chromosomes of the cells. Analysis shows that chromosomes consist of several chemical components- deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins. In an epoch-making paper published in 1953, Watson and Crick suggested that the deoxyribonucleic acid, as found in the chromosomes, consists of pairs of very elongated molecules twisted spirally around each other in a double helix. Each strand of the helix is made up of a number of units, the mononucleotides, which differ from one another only in the nitrogenous base (i.e., adenine, thymine, guanine, or cytosine) which each contains.

The bases form two pairs, which structurally "fit" together, so that in the intertwined double helix adenine always links with thymine, and guanine with cytosine. Further work made it clear that the arrangement of the bases in the molecule of deoxyribonucleic acid contains a code for the proteins that may be synthesized by a particular species of organism. The code is essentially a series of "triplets"—groups of three bases which correspond to one amino acid in a polypeptide (protein) chain. Thus, a sequence of triplets in the DNA determines a sequence of amino acids in a protein molecule, and the section of the deoxyribonucleic acid molecule containing this sequence is the essential part of what geneticists call a "gene."

The "genetic code," showing which sequences of bases correspond to which of the 20 amino acids constituting most of the proteins in the organic world. Note that several different triplets in the DNA may code for the same amino acid. Between the genetic code in the chromosomal DNA and the cell proteins, there are certain intermediate steps. The "message" contained in the DNA must first be copied in the form of a ribonucleic acid molecule, whose nucleotide sequence is complementary to the nucleotide sequence of

the DNA (except that uridine takes the place of thymine). This is the "transcription" phase. The code is then contained in an RNA molecule ("messenger" RNA). Two further kinds of ribonucleic acid are modeled on the DNA- the ribosomal RNA, which together with certain proteins forms small (± 200 Å in diameter) particles, the ribosomes; and the transfer RNA, which is involved in bringing the correct amino acid to the ribosome, where the amino acids become arranged and joined together in the correct sequence according to the code contained in the messenger RNA. This procedure constitutes the "translation" phase. The importance of these discoveries for embryology derives from the following considerations. It has become evident that all the properties of any organism are determined in the last instance by the sequence of base triplets in the DNA molecules. Furthermore, it is accepted that the sequence of the base triplets directly determines what kinds of proteins can be produced by an organism.

All other manifestations specific to any organism, whether morphological or physiological, depend more or less directly on the assortment of proteins coded for by the hereditary DNA. This new way of looking at the organic world shifts the problem of ontogenetic development directly into the realm of molecular relationships. It also makes possible, in principle, the construction of a complete theory of development. Such a theory would start with the triplet sequences in the DNA and would show first how these sequences are "read out" by transforming them into an array of proteins, placed and distributed in an organized way in space and time, and then would show how the proteins, acting partly on their own and partly through other chemical components, produce the complicated system that is an adult organism. A whole array of new techniques has been mobilized in working toward such a theory of development. Electron microscopy has made great advances after the mid 1950's, when methods were developed for embedding tissues in plastics and for cutting ultrathin sections for the study of the fine structure of cells. Refined methods of chemical analysis, such as chromatography, electrophoresis, ultracentrifugation, and the use of radioactive tracers, have been put at the disposal of embryologists.

With the change in the theoretical background and techniques, a subtle change has permeated the work of embryologists. The aim of investigation is no longer the study of the development of any particular animal, or any group of animals, but the discovery of the basic principles and processes of development. This trend in science may perhaps be called analytical embryology, and this is what "modern" embryology actually is. It must be realized that analytical embryology can proceed only on the basis of knowledge provided by descriptive embryology, because after all, it is the actual course of the transformations that has to be explained by the theory of development, of comparative embryology, because it is necessary to know of how general a significance any particular phenomenon of development is, and of experimental embryology, because it has revealed the causal relationships of many developmental processes.

Gametogenesis:

The embryogenesis (embryonic development) of a sexually reproducing multicellular animal is prefaced by the gametogenis, i.e., the formation and ripening of two highly dissimilar and specialised sex-cells or gametes, namely a large-sized, non-motile, nutrient filled cell the ovum or egg and a small-sized,motile,sex-cell, the spermatozoon or sperm, both of which unite and give origin to a diploid zygote.

Formation of sex-cell or gametes is termed gametogenesis. It is accompanied by a special type of nuclear division, called meiosis. As a result, the nuclei of gametes formed contain only half or haploid number of chromosomes. When male and female sex-cells (sperms and ova) unite at the time of fertilisation, the resulting cell or zygote again has the full or diploid number of chromosomes. The production of male

germ cells, the sperms or spermatozoa occurs in the male gonads, the testes, by a process called spermatogenesis. Each sperm consists of a head, middle piece and tail. It is preferable to call them sperm cells or simply sperms.

The production of female germ cells, the ova takes place in female gonads, the ovaries, and the process is called oogenesis. The word 'egg' is often loosely used for ova or secondary oocytes. It may be reserved for more complex structures such as the hen's egg which may even contain early embryonic stages.

Embryonic Development in Chordates:
The stages of embryonic development differ in various chordates, yet the chief phases are basically similar in all. The differences are related primarily to the amount and distribution of yolk present in an egg. The inert yolk or vitellin furnishes nourishment for the developing embryo. The yolk also influences on the pattern of cleavage, on the morphogenetic movements of the blastomeres during gastrulation and on the type of development, i.e., indirect with larval forms or direct with juvenile stages.

Yolk:
The amount of yolk varies in the eggs of different chordates, it determines the size of the egg and the pattern of early development (cleavage and blastulation, etc.). The eggs are classified according to the distribution of yolk they contain into two main types, namely, isolecithal and telolecithal eggs.

Types of Eggs:

A. Isolecithal or homolecithal eggs have very little yolk which is uniformly distributed evenly in the cytoplasm. Such eggs are found in various chordates, e.g., Amphioxus, tunicates and marsupial and eutherian mammals.

B. Telolecithal eggs contain a considerable amount of yolk, which has a polarised distribution. Due to its gravity, it is concentrated more in vegetal hemisphere than that of animal hemisphere. Such polarised distribution of yolk is found in mesolacithal and macrolecithal eggs.

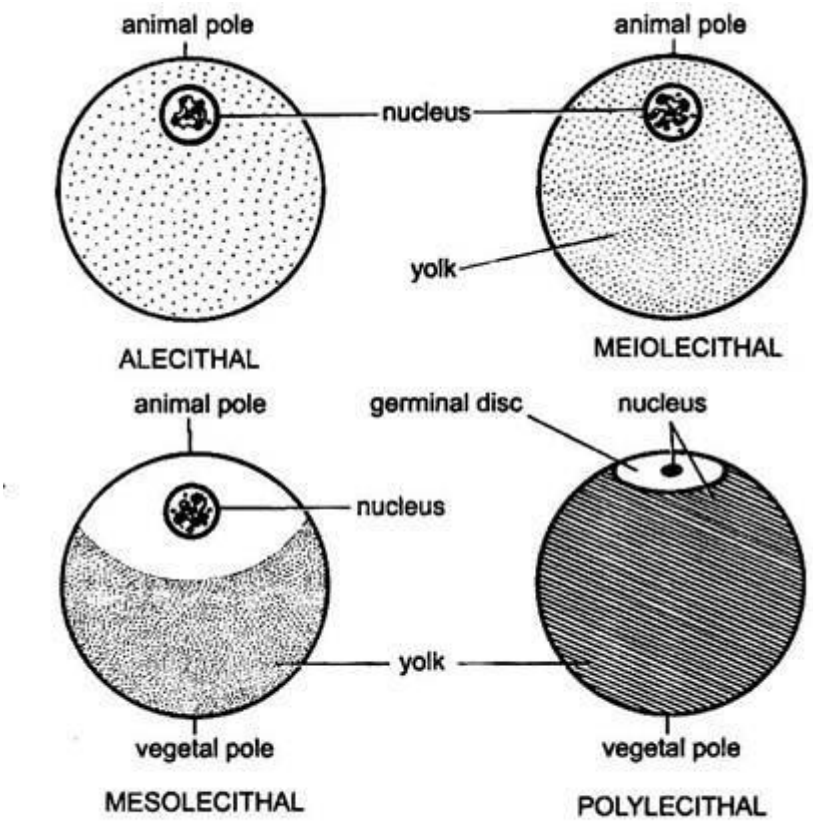

Fig. 34.1. Types of chordate ova. 1 – and 2–Isolecithal; 3 – and 4 –Telolecithal.

In fact, in macrolecithal eggs, the amount of yolk is so massive that it almost occupies the whole space of the egg, except a small space at the animal pole where the nucleus or germinal vesicle lies in the form of cap over the yolk.

The telolecithal eggs may be either moderately telolecithal (e.g., eggs of Amphibia, Petromyzon and Dipnoi) or highly telolecithal, (e.g., cartilaginous and bony fishes, reptiles, birds and egg-laying mammals). All eggs are enclosed in one or two vitelline membranes.

C. Centrolecithal eggs found in insects and some hydrozoa, contain a large amount of yolk concentrated in the centre of the egg surrounded by thin peripheral layer of active cytoplasm.

Classification of Eggs on the Basis of Amount of Yolk:

1. Microlecithal or oligolecithal eggs are small sized, containing a small amount of yolk. Such eggs are found in Amphioxus, tunicates, and marsupial and eutherian mammals, and also in certain invertebrates such as Hydra and sea urchin.

2. Mesolecithal eggs contain moderate amount of yolk, e.g., annelid worms, molluscs, Petromyzontia, Dipnoi and Amphibia.

3. Macrolecithal, megalecithal or polylecithal eggs contain massive amount of yolk such as eggs of insects, Myxine, elasmobranch fishes, reptiles, birds and prototherian mammals.

The egg or ovum is surrounded by a thin plasma membrane and around it is present a vitelline membrane, which is non-cellular and transparent layer of mucoprotein. It is often much thicker and stronger than the underlying fine plasma membrane. It is differently named in various groups of animals such as chorion in fishes and zona pellucida in reptiles and mammals.

Spermatozoa:

A spermatozoon (Gr., sperma = seed + zoon = animal) or male gamete of vertebrates despite its small size is an exceedingly complex cell. It has a head, a middle piece, and a tail, all of these are contained by a continuous plasma membrane, like other living cell.

1. Head:

The head has a nucleus invested by a thin layer of cytoplasm which projects in front as a pointed acrosome, both performing two basic functions of the sperm – genetic and activating, respectively. The nucleus occupies most of the space of the sperm head. It is enveloped by a typical double nuclear membrane, which lacks the nuclear pores except the lower part.

The nucleus contains only its haploid complement of DNA bound by basic proteins. The nucleus has no nucleolus, RNAs and fluid contents. Acrosome lies anterior to the nucleus and its shape and size varies among different species.

It is also bounded by a unit membrane and contains a number of acid hydrolases, such as acid phosphatase, cathepsin, hyaluronidase, etc. In mammals, it contains acrosomin made of hyaluronidase and acrosin (zona lysin).

2. Middle Piece:

It lies behind the nucleus and connected with the head by a narrow neck. Inside the neck, posterior to the nucleus are present two centrioles, both lie at right angles to the other. The anterior or proximal centriole lies in the depression in the posterior surface of nucleus and forms the mitotic spindle in the egg after fertilisation. The distal centriole or posterior centriole forms the microtubules (axoneme) of the sperm tail (flagellum). It acts as basal body for the flagellum. The distal centriole and the proximal part of the axial filament lie in the middle piece of the spermatozoon. The axial filament of the sperm tail has the same organisation as the axial filament of flagella and cilia.

In middle piece the axial filament is surrounded by numerous well developed mitochondria. In mammals, the mitochondria are joined together forming one continuous body twisted spirally around the axial filament. However, in other animals, such as in annelid, Hydroides hexagonus, and in sea urchin, Arbacia punctulata, mitochondria are joined in one or more massive clumps, called mitochondrial bodies forming the bulk of the middle piece. They contain all the respiratory enzymes and are extremely active in oxidative phosphorylation.

Around the periphery of middle piece of the sperm is found a condensed layer of cytoplasm that is composed mainly of the microtubules and is called manchette. It also surrounds the posterior part of head of the sperm. At the posterior end of middle piece occurs a dark ring or fibrous thickenings beneath the plasma membrane, forming the boundary between the middle piece and tail. It is called ring centriole or Jensen’s ring.

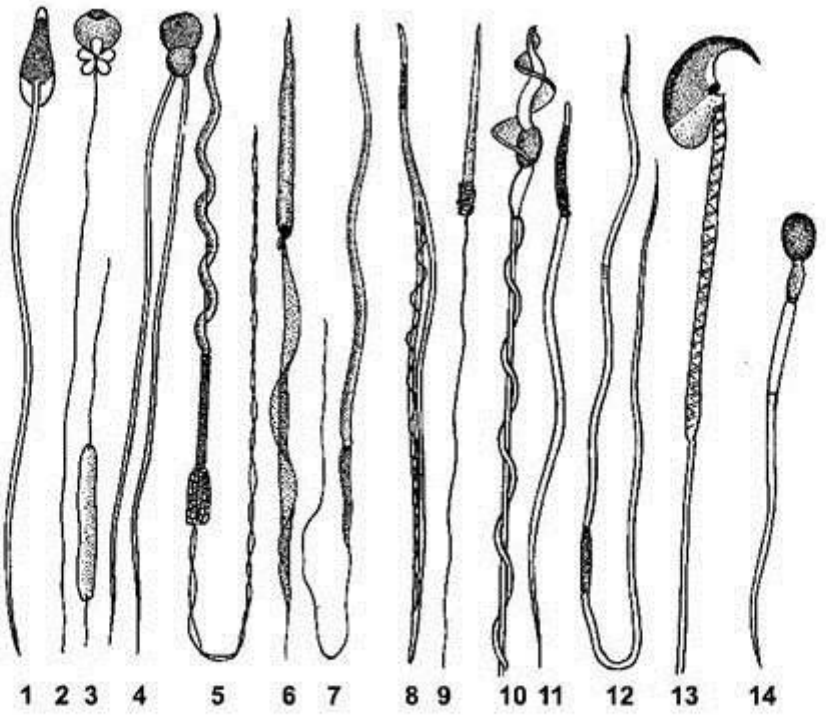

Fig. 34.2. Forms of flagellate spermatozoa. 1. *Arbacia* (sea urchin); 2. *Amphioxus*; 3. *Petromyzon*; 4. *Opeanus* (toad fish); 5. *Raja* (elasmobranch); 6. *Bufo* (toad); 7. *Rana* (frog); 8. *Amphiuma* (salamander); 9. *Anguis* (lizard); 10. *Frigilla* (bird); 11. *Fundulus* (domestic fowl); 12. *Echidna* (monotrematous mammal); 13. *Mus* (eutherian mammal); 14. man.

3. Tail:

The tail is a long vibratile flagellum containing an axial filament along its whole length and projecting behind the cytoplasm of the tail as an end piece. Tail has two main parts- principal piece and end piece. The principal piece constitutes most of the tail length, consists of a central core, comprising the axial filament.

Surrounding this core is a microtubular fibrous tail sheath which some time appears as semicircular ribs oriented perpendicular to the long axis of the filament or as helical coils. In human sperms, out of nine coarse fibres found around axial filament, of the tail two coarse fibres are fused with the surrounding ribs so as to form anterior and posterior columns extending throughout the length of the principal piece. The end piece is merely a short tapering portion of tail containing only the axial filament covered with cytoplasm and plasma membrane.

Spermatozoa are discharged from the body floating in a seminal fluid or semen secreted by the seminiferous tubules and accessory reproductive glands. Spermatozoa are always produced in very large numbers.

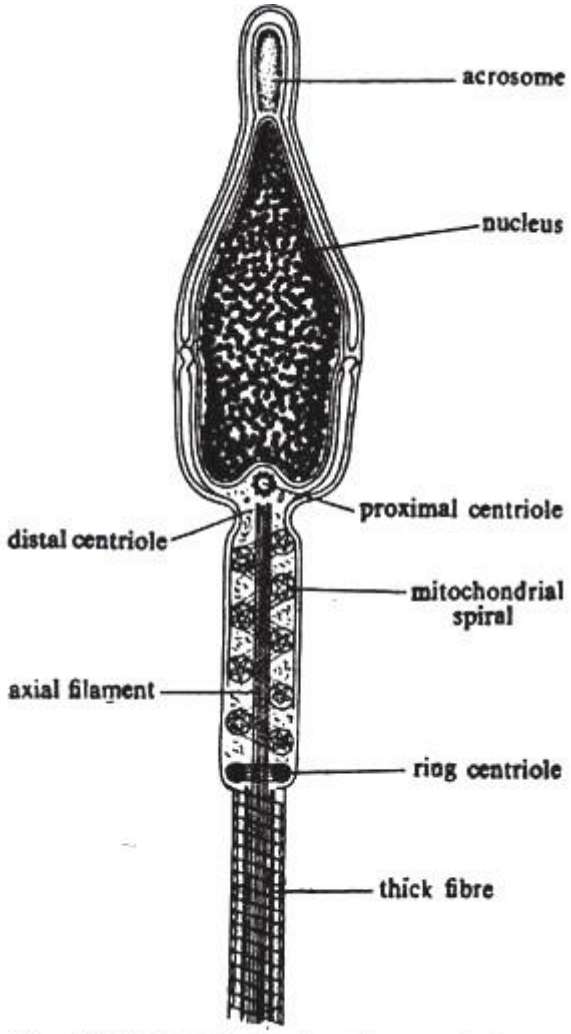

Fig. 5.6. Enlarged view (anterior portion) of a human spermatozoon.

Fertilisation in Chordates:

Fertilisation (L., fertilis = to bear). It is the fusion of two gametes (spermatozoa and ova) and so their nuclei to form a diploid zygote. It activates the egg to form fertilisation membrane outside the egg plasma membrane to start its metabolism and to start its cleavage.

During fertilisation process the jelly coats and egg membranes such as vitelline membrane and plasma membrane secrete the fertilizin and the sperms tip secrete antifertilizin, both interact with each other and sperms, thus, agglutinated. It occurs in the female genital tract.

The membrane of the acrosomal vesicle of the acrosome and the plasma membrane of the sperm breakdown and the severed edges of the two membranes fuse to form an opening through which the contents of the acrosomal vesicle are released.

The inner acrosomal membrane grows into one or many acrosomal tubules which come in contact with the vitelline membrane and plasma membrane. In mammals, the plasma membrane and outer acrosomal membrane break and fuse to give rise to extensive vesiculations and the sperm is possibly phagocytosed by the egg. The acrosome now releases the lytic enzymes or lysins (acrosomin in sea urchins) which help the sperm to penetrate the egg envelopes by liquefying them locally, without affecting the plasma

membrane. In mammals including human females, the sperms first penetrate the multiple layers of follicular cells (granulosa cells) which are held together by an adhesive substance hyaluronic acid.

The acrosome releases the hyaluronidase and proteolytic enzymes for penetrating the follicle cell layers, corona radiata and zona pellucida. Hyaluronidase is supposed to dissolve the cement between cells of corona radiata. Zona lysins or proteolytic acrosomal enzymes are responsible for the passage of the sperms through zona pellucida. The apical part of sperm plasma membrane (originally the inner acrosomal membrane) extends forward to form an acrosomal tubule. It projects through the egg membranes to reach the egg plasma membrane or oolemma. The shape and size of acrosomal tubule varies with species and is entirely absent in mammals.

The tip of acrosomal tubule fuses with egg plasma membrane, while in mammals sperm come in contact with the egg surface by its lateral aspects. After the fusion, the plasma membrane of the egg and tip of acrosomal tubule dissolve at the point of contact. In teleost fishes acrosome is lacking and so the plasma membrane of sperm head fuses directly with the plasma membrane of ovum. After fusion of the both the plasma membranes, the plasma membrane of ovum becomes permeable to sodium, potassium and calcium ions. Calcium is essential for the fertilisation process. pH of egg cytoplasm also increases due to inflow of Na^+ and outflow of H^+ ions. Within seconds after membranes contact, changes occur in egg cortex. In bony fishes and frogs, the cortical granules are broken down after sperms' penetration into the egg cytoplasm and their contents become liquefied and extruded on the surface plasma membrane of the egg. They gradually fill up the perivitelline space in between chorion and egg plasma membrane in bony fishes, and the space between vitelline membrane and egg plasma membrane in frogs.

Thus, fertilisation membrane is formed by the rupture of cortical granules outside the plasma membrane. This is due to the cortical reaction stimulated by the penetrating sperm. Fertilisation membrane blocks the entrance of other living sperms. The vitelline membrane or chorion does not transform into fertilisation membrane. In some mammals (e.g., man, rabbit and hamster) the cortical granules burst open and release their contents in the space between egg plasma membrane and zona pellucida. Cortical granules are not found in urodele amphibians and, hence, no fertilisation membrane formation occurs.

In most species only one sperm enters the egg and this is called monospermic fertilisation. When many sperms penetrate the single ovum (e.g., in polylecithal eggs of some insects, elasmobranchs, urodeles, reptiles and birds, and also in microlecithal eggs of bryozoans), it is called polyspermic fertilisation. In this case, the genetic material of only one sperm is incorporated in the zygote nucleus, and other sperm nuclei are degenerated.

After the penetration of sperm inside the egg cytoplasm, its nucleus moves inward, swells and its chromatin which is very closely packed becomes finely granular. It finally becomes vesicular and is called male pronucleus. Similarly the egg nucleus after second meiotic division undergoes changes and becomes female pronucleus, which swells, increases in volume and becomes vesicular. Later on male and female pronuclei fuse together, that is, the nuclear membrane of both pronuclei are broken at the point of contact and their contents unite into one mass, which is finally bounded by a common nuclear envelope, forming a zygote nucleus. This type of fusion of both pronuclei (male and female) is called amphimixis.

Significance of Fertilization:

1. The male and female nucleus possess haploid (n) number of chromosomes. The fertilisation restores the specific parental diploid chromosome number.

2. Fertilisation brings together the chromosomes and genes from two different parents, resulting into a new genetic recombination.

3. Fertilisation activates the egg to undergo cleavage.

Types of Fertilization:

According to place and nature of fluid media, fertilisation is of two types:

A. External fertilisation,

B. Internal fertilisation.

A. External Fertilization:

When the fertilisation occurs in the aquatic medium outside the body of female, it is called external fertilisation. Aquatic medium may be sea water or freshwater. In marine animals, sexually mature adults shed eggs and sperms freely into the surrounding water. The sperms and eggs are laid in water in astronomical numbers, and also in close proximity.

B. Internal Fertilization:

In terrestrial forms, where eggs are completely enclosed in impermeable envelopes before being laid such as oviparous animals or where they are retained within maternal body throughout development such as ovo-viviparous and viviparous animals (e.g., elasmobranchs and mammals) the sperms are transmitted internally, i.e., in the body of female, by the intromittent organ of male.

In these forms the fertilisation may occur in the lower portion of oviduct (e.g., urodela) or in the upper portion of the oviduct such as salamanders, reptiles, aves and most mammals. In viviparous fishes such as Gambusia affinis and Heterandria formosa, and certain eutherian mammals such as Ericulus, fertilisation occurs in the ovarian haploid follicles.

The results of fertilisation are:

(a) An activation of the egg to undergo its second maturation division for preparing a haploid female nucleus;

(b) An introduction of a centriole by the sperm which divides to form two centrioles, since a centriole is lacking in a mature ovum;

(c) A restoration of a diploid number of chromosomes in the zygote;

(d) A change in the periphery of the egg which precludes the entry of other sperms;

(e) Separation of the vitelline membrane from the egg to allow the zygote to rotate.

Cleavage:

The division of an activated egg (zygote) by a series of mitotic cell divisions into a multitude of cells which become the building units of future organism, is called cleavage or segmentation (Ger., kleiben = to cleave). During cleavage, cells do not grow in size and early cleavage divisions occur synchronously, which is lost during late cleavage.

During cleavage, there is no growth in the resulting blastomeres and the total size and volume of the embryo remains the same. The blastomeres do not move so the general shape of the embryo remains the same except the formation of a cavity, the blastocoel in the interior. During cleavage, chemical conversion of reserve food material (yolk, glycogen and ribonucleotides) into active cytoplasm takes place.

Thus, a steady increase of respiration occurs throughout cleavage. During cleavage, nucleo-cytoplasmic ratio in cells is reduced, which permits the cells to be more metabolically active, because such nuclei have less cytoplasm to control. Thus, the cleavage converts the egg into a compact mass of cells or blastomeres called morula.

Types of Cleavage:

The type of cleavage taking place depends largely on the amount of yolk present.

Following types of cleavages occur:

a. Holoblastic or Total Cleavage:

In this type of cleavage, the entire egg divides by each cleavage furrow.

It is subdivided into two types:

(i) Complete or equal holoblastic cleavage occurs in microlecithal and isolecithal eggs, the entire zygote divides completely to produce a number of almost equal-sized cells, e.g., eutherian mammals, Amphioxus, tunicates.

(ii) Unequal holoblastic cleavage occurs in mesolecithal and telolecithal eggs, the zygote divides completely to form unequal-sized blastomeres, i.e., small-sized cells towards the animal pole which has almost no yolk, larger cells towards the yolky vegetal pole, e.g., cyclostomes, elasmobranchs, Dipnoi and Amphibia.

b. Meroblastic or Incomplete Cleavage:

This occurs in polylecithal eggs in which only the small germinal disc lying at the animal pole consisting of clear cytoplasm and a nucleus, undergoes a series of incomplete divisions forming an area of cells at the animal pole, the large yolky portion beneath the germinal disc remains unsegmented, e.g., toleosts, reptiles, birds and egg-laying mammals. Here the germinal disc is of disc-shape, so the cleavage is also called discoidal.

c. Superficial Cleavage:

This type of incomplete cleavage is found in centrolecithal eggs, e.g., insects and many arthropods. The nucleus lying in the centre of the egg yolk surrounded by an island of cytoplasm undergoes cleavage, and each nuclei is surrounded by small amount of cytoplasm.

They later move towards the periphery in the peripheral cytoplasm. Here their cytoplasm fuses with the peripheral cytoplasm. Later the peripheral cytoplasm becomes subdivided by furrows extending inward from the surface, thus, a layer of peripheral or superficial cells is formed which surrounds the central undivided yolk.

Pattern of Cleavage:

The pattern of cleavage due to organisation of egg may be of following types:

i. Radial Cleavage:

When successive cleavages extend through the egg, at right angles to one another and the resulting blastomeres become symmetrically arranged around the polar axis. Such type of cleavage is called radial cleavage, and is found in echinoderms (e.g., Synapta and Paracentrotus, etc.).

ii. Biradial Cleavage:

When first three cleavage planes are not arranged at right angles to each other, it is called biradial cleavage, e.g., Acoela like Polychoerus and Ctenophora.

iii. Spiral Cleavage:

The rotational movement of cells around the egg axis during cleavage is due to spiral cleavage. The spiral cleavage results due to oblique positions of mitotic spindles in the blastomeres. Thus, it is also called oblique cleavage. In successive cleavages, the rotational movements alternate in clockwise direction or anticlockwise direction. It is found in Turbellaria, Nematoda, Rotifera, Annelida and molluscs except cephalopods.

iv. Bilateral Cleavage:

In this type of cleavage the mitotic spindles and cleavage planes remain bilaterally arranged with reference to a plane of symmetry which coincides with the median plane of the embryo. It is found in Tunicata, Amphioxus, Amphibia and higher mammals.

v. Determinate and Indeterminate Cleavage:

The cleavage in nematodes is of a special type of bilateral cleavage in which definite blastomeres give rise to specific parts of the embryo. This type of cleavage is called determinate or mosaic cleavage. In vertebrates, the plane of cleavage is less rigid, the cleavage pattern has no definite relation to the embryo.

This type of cleavage is called indeterminate or regulative and is found in echinoderms, Balanoglossus, coelenterates and amphibians. A first cleavage blastomere of a sea urchin or an amphibian or a mammal, when isolated can alter its usual destiny and develop into a perfect (but small) embryo. Similarly, when two fertilised eggs, made to adhere like a two-cell stage, they produce a single giant embryo. This is regulative development.

Stages of Embryogeny:

1. Morula:

During early cleavages, the blastomeres tend to assume a spherical shape and their mutual pressure flattens the surfaces of the blastomeres in contact with each other, but their free surfaces remain spherical.

Thus, cleavage process develops a multicellular body with loosely arranged blastomeres with in fertilisation membrane, called morula (Latin word for mulberry) resembling mulberry, e.g., amphibian and coelenterates. In macrolecithal eggs, morula is a cellular flattened disc at the animal pole.

2. Blastula:

As cleavage proceeds the cells increase in number but become smaller. The cells withdraw from the centre and arrange themselves towards the surface to form a true epithelium, which may be single cell thick as in Amphioxus, echinoderms, etc., or many cell thick as in most vertebrates. Due to rearrangement of cells to form the epithelium or blastoderm a fluid-filled space or blastocoel or segmentation cavity is formed. This stage is called blastula and the process of formation is called blastulation.

Types of Blastulae:

i. Coeloblastula:

It is in the form of a hollow sphere formed of a single layer of blastoderm and the blastocoel is filled with mucopolysaccharides. Examples, echinoderms and Amphioxus.

ii. Stereoblastula:

In spirally cleaving eggs of annelids, molluscs, nemerteans and some planarians, blastula is solid, having no blastocoelic cavity. In them micromeres accumulate as cluster of cells over macromeres of vegetal hemisphere.

iii. Periblastula or Superficial Blastula:

In superficially cleaving eggs of insects, the blastocoelic cavity is not found. The central yolk is surrounded by peripherally arranged cells.

iv. Discoblastula:

In large yolky eggs of fishes, reptiles and birds discoblastula is found. It is a small multilayered flat disc separated from the yolk by a narrow subgerminal cavity.

v. Amphiblastula:

It is found in amphibians. The blastula contains micromeres in the animal hemisphere and macromeres in the vegetal hemisphere, and a small fluid-filled eccentric blastocoel in the animal hemisphere.

vi. Blastocyst:

It is found in mammals. Cleavage is regular and a small cavity appears inside the dividing cells, which gradually increases in volume. This is the blastocoel. The cells surrounding the blastocoel are the trophoblast cells or nutritive cells and an inner cell mass of formative cells displaced to one pole of the blastocyst.

3. Gastrula:

A rearrangement of the cells of the blastula occurs in which some cells are differentiated and come to lie inside, while the other cells enclose them, this stage is gastrula and the processes converting the blastula into a gastrula are known as gastrulation. Gastrulation process (morphogenetic movements of cells) converts a simple one-layered blastula into a two-layered (e.g., Amphioxus) or a three-layered (e.g., all vertebrates) gastrula (Gr., gaster = stomach or gut).

The single layer of blastula is called blastoderm, ectoblast or proctoderm. The three layers (ectoderm, mesoderm and endoderm) are called germinal layers. The blastocoel is generally obliterated and the inner layer of cells (endoderm) of the gastrula encloses a new cavity called archenteron which opens on one side to the exterior by a blastopore. During gastrulation embryo acquires antero-posterior polarity and bilateral symmetry.

4. Organogeny:

After gastrulation the continuous masses of cells of the three germ layers split up into smaller groups of cells, called primary organ rudiments, each of which produces a certain organ or part of the animal body. These organ rudiments further develop simple organs and parts and, thus, embryo develops into either larval form or a miniature adult. Thus, the formation of organs from the germ layers is called organogenesis.

Derivatives of Germ Layers:

i. Ectoderm:

The ectoderm forms a neural tube which gives rise to the brain, spinal cord, and nerves. The forebrain forms the retina, and part of the iris. The ectoderm forms the lens, conjunctiva, and a part of the cornea, the membranous labyrinth and the lining of the nose.

In fishes and aquatic amphibians, the sensory parts of the lateral line system arise from the ectoderm. The neural crest cells lying between the outer ectoderm and on both sides of the neural tube give rise to ganglia of the spinal nerves and autonomic nervous system, the neurilemma of peripheral nerves, chromatophores of the skin, some neural crest cells give rise to mesenchyme which produces the visceral arches, and some neural crest cells wander inwards and form the suprarenal gland near the kidneys, but in mammals they form the medulla of adrenal glands.

Supporting part of the central nervous system called neuroglia is derived from the neural tube. The ectoderm forms the epidermis of the skin and many epidermal derivatives, such as skin glands, epidermal scales, nails, claws, hoofs, horns, feathers and hairs. Ectodermal invaginations form the stomodaeum and proctodaeum which meets the archenteron, the ectoderm of the stomodaeum forms the lining of the mouth and lips, glands of buccal cavity, enamel of teeth, covering of tongue, and anterior and intermediate lobes of the pituitary gland (the posterior lobe of the pituitary is formed from the forebrain).

The ectoderm of proctodaeum forms the lining of the cloaca and some anal and cloacal glands. From the dorsal side of the forebrain one or two evaginations take place, the anterior one is an eye-like parietal body which is present in lower forms only, the posterior one is the pineal body found in all vertebrates.

ii. Endoderm:

The archenteron is formed from endoderm, it becomes the lining of the adult alimentary canal, except in the buccal cavity and cloaca. Two outgrowths of the digestive tract form the liver and pancreas, the endoderm forming their epithelial lining only, and also of the gall bladder and bile duct.

From the pharynx, the endoderm pushes out to form several pairs of pharyngeal pouches. In cyclostomes, fishes, and amphibians, the pharyngeal pouches meet the ectoderm to form gill-clefts which open to the exterior. In amniotes, the pharyngeal pouches do not perforate to the exterior, in tetrapoda, the first pair is modified to form the cavity of the middle ear and Eustachian tube.

An evagination of the pharynx along with some pharyngeal pouches forms a thyroid gland. In air-breathing vertebrates the endoderm of pharynx forms the lining of the larynx, trachea, and lungs. Endoderm of some pharyngeal pouches form part of the tonsils, thymus, parathyroid glands and ultimobranchial bodies. In amniotes, the archenteron forms a large bag, the allantois, its lining is endodermal. Endoderm cells of the archenteron grow out in embryos developing from polylecithal eggs to form the lining of the yolk sac to enclose the yolk, the yolk sac disappears in the adult. It must be noted that organs arising from the archenteron have only their lining and epithelial cells formed from endoderm, the supporting tissues of these organs are mesodermal.

iii. Mesoderm:

The mesoderm becomes differentiated into three major parts- a dorsal epimere which is segmented, a median mesomere, and a ventral hypomere. Further development of mesoderm forms mesenchyme which is not a germinal layer but a primitive kind of embryonic connective tissue with branching cells forming a network. Nearly all mesenchyme comes from mesoderm though other germinal layers may also contribute to its formation.

(i) The Epimere is differentiated into sclerotome, dermatome, and myotome. The middle parts of epimeres form mesenchyme which gathers around the neural tube and notochord to form the sclerotome. The mesenchymatous sclerotome forms the vertebral column. The dermatome transforms into mesenchyme which migrates to lie below the ectoderm and gives rise to the dermis of the skin. The remaining portion of the epimere is called myotome, the adjacent myotomes are separated by myocommata which are connective tissue partitions. The myotomes of the two sides grow down between the skin and somatic layer of mesoderm to meet midventrally, they give rise (with some exceptions) to voluntary muscles of the body and body wall.

(ii) Mesomere forms the urogenital organs and their ducts, the terminal parts of these ducts may have ectodermal or endodermal lining.

(iii) Hypomere splits into somatic and splanchnic layers of mesoderm enclosing the coelom. The splanchnic layer forms mesenchyme which gives rise to involuntary muscles and connective tissue of the alimentary canal and of the organs formed as outgrowths of the archenteron. The splanchnic mesoderm forms the heart. The remainder of the splanchnic mesoderm together with the somatic mesoderm forms the lining of the coelom, pericardium and lung pleura or peritoneum. Splanchnic mesoderm also forms the mesenteries and omenta.

(iv) Mesenchyme (Gk., mesos = middle + enchyma = infusion) gives rise to all the connective tissue, blood vessels, lymph vessels, lymph nodes, blood corpuscles, all involuntary muscles, parts of the eye, dentine of teeth, and to cartilage and bones of the entire skeleton, except the vertebral column. It is claimed that voluntary muscles of limbs are formed from mesenchyme and not from myotomes.

TABLE 34.1 GERM LAYERS AND THEIR DERIVATIVES IN VERTEBRATES.

Germ layer	In Embryo	In Adult
1. Ectoderm	1. Somatic ectoderm	Epidermis, cutaneous derivatives, cutaneous sense organs, olfactory organs, lateral line, eye lens, membranous labyrinth, anterior pituitary, lining of stomodaeum and proctodaeum and enamel.
	2. Neural ectoderm	Central and peripheral nervous system, retina and posterior pituitary.
	3. Neural crest	Branchial skeleton, nervous ganglia and sensory nerves, adrenal medulla and chromatophores.
2. Endoderm	Archenteron (enteron)	Lining of alimentary canal except that of stomodaeum, and proctodaeum, lining of glands, liver, pancreas, respiratory organs, tonsils, urinary bladder, thyroid, parathyroid and thymus glands, accessory reproductive glands and auditory tube.
3. Mesoderm	1. Notochord	
	2. Epimere	(a) Dermatome–dermis (b) Sclerotome–vertebral column (c) Myotome–skeletal muscles and appendicular skeleton.
	3. Mesomere	Excretory organs and reproductive tracts.
	4. Hypomere	(a) Somatic or parietal layer (b) Splanchnic layer–visceral layer, heart, blood vessels and cells, gonads, spleen, visceral muscles (c) Coelom–Body cavities.

Morphogenesis (from the Greek morphê shape and genesis creation, literally "the generation of form") is the biological process that causes a cell, tissue or organism to develop its shape. It is one of three fundamental aspects of developmental biology along with the control of tissue growth and patterning of cellular differentiation. The process controls the organized spatial distribution of cells during the embryonic development of an organism. Morphogenesis can take place also in a mature organism, such as in the normal maintenance of tissue by stem cells or in regeneration of tissues after damage. Cancer is an example of highly abnormal and pathological tissue morphogenesis. Morphogenesis also describes the development of unicellular life forms that do not have an embryonic stage in their life cycle. Morphogenesis is essential for the evolution of new forms. Morphogenesis is a mechanical process involving forces that generate mechanical stress, strain, and movement of cells, and can be induced by genetic programs according to the spatial patterning of cells within tissues.

Some of the earliest ideas and mathematical descriptions on how physical processes and constraints affect biological growth, and hence natural patterns such as the spirals of phyllotaxis, were written by D'Arcy Wentworth Thompson in his 1917 book On Growth and Form and Alan Turing in his The Chemical Basis of Morphogenesis (1952). Where Thompson explained animal body shapes as being created by varying rates of growth in different directions, for instance to create the spiral shell of a snail, Turing correctly predicted a mechanism of morphogenesis, the diffusion of two different chemical signals, one activating and one deactivating growth, to set up patterns of development, decades before the formation of such patterns was observed. The fuller understanding of the mechanisms involved in actual organisms required

the discovery of the structure of DNA in 1953, and the development of molecular biology and biochemistry.

Genetic and molecular basis:

Several types of molecules are important in morphogenesis. Morphogens are soluble molecules that can diffuse and carry signals that control cell differentiation via concentration gradients. Morphogens typically act through binding to specific protein receptors. An important class of molecules involved in morphogenesis are transcription factor proteins that determine the fate of cells by interacting with DNA. These can be coded for by master regulatory genes, and either activate or deactivate the transcription of other genes; in turn, these secondary gene products can regulate the expression of still other genes in a regulatory cascade of gene regulatory networks. At the end of this cascade are classes of molecules that control cellular behaviors such as cell migration, or, more generally, their properties, such as cell adhesion or cell contractility. For example, during gastrulation, clumps of stem cells switch off their cell-to-cell adhesion, become migratory, and take up new positions within an embryo where they again activate specific cell adhesion proteins and form new tissues and organs. Developmental signaling pathways implicated in morphogenesis include Wnt, Hedgehog, and ephrins.

Cellular basis:

At a tissue level, ignoring the means of control, morphogenesis arises because of cellular proliferation and motility. Morphogenesis also involves changes in the cellular structure[10] or how cells interact in tissues. These changes can result in tissue elongation, thinning, folding, invasion or separation of one tissue into distinct layers. The latter case is often referred as cell sorting. Cell "sorting out" consists of cells moving so as to sort into clusters that maximize contact between cells of the same type. The ability of cells to do this has been proposed to arise from differential cell adhesion by Malcolm Steinberg through his differential adhesion hypothesis. Tissue separation can also occur via more dramatic cellular differentiation events during which epithelial cells become mesenchymal (Epithelial–mesenchymal transition). Mesenchymal cells typically leave the epithelial tissue as a consequence of changes in cell adhesive and contractile properties. Following epithelial-mesenchymal transition, cells can migrate away from an epithelium and then associate with other similar cells in a new location. In plants, cellular morphogenesis is tightly linked to the chemical composition and the mechanical properties of the cell wall.

Cell-to-cell adhesion:

During embryonic development, cells are restricted to different layers due to differential affinities. One of the ways this can occur is when cells share the same cell-to-cell adhesion molecules. For instance, homotypic cell adhesion can maintain boundaries between groups of cells that have different adhesion molecules. Furthermore, cells can sort based upon differences in adhesion between the cells, so even two populations of cells with different levels of the same adhesion molecule can sort out. In cell culture cells that have the strongest adhesion move to the center of a mixed aggregates of cells. Moreover, cell-cell adhesion is often modulated by cell contractility, which can exert forces on the cell-cell contacts so that two cell populations with equal levels of the same adhesion molecule can sort out. The molecules responsible for adhesion are called cell adhesion molecules (CAMs). Several types of cell adhesion

molecules are known and one major class of these molecules are cadherins. There are dozens of different cadherins that are expressed on different cell types. Cadherins bind to other cadherins in a like-to-like manner: E-cadherin (found on many epithelial cells) binds preferentially to other E-cadherin molecules. Mesenchymal cells usually express other cadherin types such as N-cadherin.

Extracellular matrix:

The extracellular matrix (ECM) is involved in keeping tissues separated, providing structural support or providing a structure for cells to migrate on. Collagen, laminin, and fibronectin are major ECM molecules that are secreted and assembled into sheets, fibers, and gels. Multisubunit transmembrane receptors called integrins are used to bind to the ECM. Integrins bind extracellularly to fibronectin, laminin, or other ECM components, and intracellularly to microfilament-binding proteins α-actinin and talin to link the cytoskeleton with the outside. Integrins also serve as receptors to trigger signal transduction cascades when binding to the ECM. A well-studied example of morphogenesis that involves ECM is mammary gland ductal branching.

Cell contractility:

Tissues can change their shape and separate into distinct layers via cell contractility. Just as in muscle cells, myosin can contract different parts of the cytoplasm to change its shape or structure. Myosin-driven contractility in embryonic tissue morphogenesis is seen during the separation of germ layers in the model organisms Caenorhabditis elegans, Drosophila and zebrafish. There are often periodic pulses of contraction in embryonic morphogenesis. A model called the cell state splitter involves alternating cell contraction and expansion, initiated by a bistable organelle at the apical end of each cell. The organelle consists of microtubules and microfilaments in mechanical opposition. It responds to local mechanical perturbations caused by morphogenetic movements. These then trigger traveling embryonic differentiation waves of contraction or expansion over presumptive tissues that determine cell type and is followed by cell differentiation. The cell state splitter was first proposed to explain neural plate morphogenesis during gastrulation of the axolotl and the model was later generalized to all of morphogenesis.

Branching morphogenesis:

In the development of the lung a bronchus branches into bronchioles forming the respiratory tree. The branching is a result of the tip of each bronchiolar tube bifurcating, and the process of branching morphogenesis forms the bronchi, bronchioles, and ultimately the alveoli.

Branching morphogenesis is also evident in the ductal formation of the mammary gland. Primitive duct formation begins in development, but the branching formation of the duct system begins later in response to estrogen during puberty and is further refined in line with mammary gland development.

Virus morphogenesis

During assembly of the bacteriophage (phage) T4 virion, the morphogenetic proteins encoded by the phage genes interact with each other in a characteristic sequence. Maintaining an appropriate balance in

the amounts of each of these proteins produced during viral infection appears to be critical for normal phage T4 morphogenesis.[28] Phage T4 encoded proteins that determine virion structure includes major structural components, minor structural components and non-structural proteins that catalyze specific steps in the morphogenesis sequence. Phage T4 morphogenesis is divided into three independent pathways: the head, the tail and the long tail fibres as detailed by Yap and Rossman.

Probable Questions:

1. Discuss blastogenesis and embryogenesis.
2. Describe structure of sperm with suitable diagram.
3. Describe different types of blastula.
4. Classify eggs on the basis of yolk content.
5. Differentiate external and internal fertilization.
6. Describe different patterns of cleavage.
7. Describe different types of blastula.

Suggested Readings:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-II

Germ cell migration, Potency, induction, competence, commitment

Meaning of Germ Cells:

Sexual reproduction needs one sperm cell from male and an egg cell from female. These specialised cells are produced from the germ cells. It is believed that these germ cells are set aside from the very beginning and according to Weismann, the germinal material passes without interruption from one generation to the next. The inquest for the search of origin, migration, transformation and fate of the germ cells, is old one, but in recent years many new lights have been thrown by different workers, who have used modern tools and techniques to the search.

Development of Germ Cells:

Development begins with the union of sperm and egg. These reproductive cells originate from the germ cells. The available information shows that series of interesting events happen in the life of germ cells from their origin to transformation. These events are the preparatory phases on which the development of individual depends. These developmental events begin long before the development of the individual. Origin of germ cell is distinct in organisms where chromosome number differs in germ and somatic cells. In the roundworm, Parascaris equorum, the four blastomeres produced by the second cleavage exhibit differences in nuclear behaviour. The blastomeres are designated as A, B, C and P. A large portion of each chromosome in blastomeres A, B and G passes into cytoplasm and breaks up in the Substance of cytoplasm.

This phenomenon is regarded as chromatin diminution. The blastomere P remains unaltered. At the next cleavage division, one of the daughter blastomeres of P shows chromatin diminution, while the other remains unchanged. The same phenomenon occurs at the fourth cleavage division, as a result only one blastomere, P_4, retains the full number of chromosomes. All the germ cells eventually develop from P_4. In recent years, it has been described that in different animals the factor which guides the cell to be germ cell resides in cytoplasm. This area is called the **"area of germ cell determinant".** Any nucleus passing through this area develops, the potency to be the nucleus of germ cell.

Followings are the brief survey of literature on the origin of germ cells:

Waldeyer (1870) regarded that the germ cells originate from the coelomic epithelium around the gonad. Nussbaum (1880) advocated that the germ cells are formed outside the gonad and from the site of origin, the germ cells migrate within the gonad. Weismann (1885, 1892) established that germplasm is segregated completely independent of somatoplasm and results into the differentiation of germ cells.

Boveri (1892) stated that cytoplasm of germ cell plays important part in determining the germplasm. Witschi (1914), Gatenby (1916) stated that in frog, a cytoplasmic substance in the yolk near the vegetal pole and on the roof of the gut is marked as site of germ cell formation. Swift (1914, 1916) stated that in chick, germ cells originate extra-embryonically and are first seen in 16-somite stage. Mintz (1959) established that germ cells originate in the yolk sac of 8-day-old mouse embryo. The above findings indicate clearly that germ cells are segregated at the very onset of development of the individual and germ cells originate at a site far from the reproductive organ. This finding has raised the most interesting question about the migration of germ cells.

Migration of Germ Cells:

Three distinct views are held regarding the migration of germ cells.

These views are:

(a) The germ cells migrate by performing amoeboid movement to the region of developing gonad.

(b) Germ cells, from their site of origin, are carried by the blood stream and after travelling through different organs settle in the reproductive organ.

(c) Various foldings which occur in embryonic development bring about the shifting of germ cells from their site of origin to the gonad.

Fate of Germ Cells:

Two different opinions are forwarded to explain the fate of germ cells:

(a) The germ cells which migrate into the reproductive organs are not destined to be the gametes at all; they degenerate and are replaced by new cells which are formed by the reproductive organs themselves.

(b) The germ cells after migrating within gonad persist and transform into functional gametes. Recent experiments with improved techniques endorse the second view.

Transformation of Germ Cells:

Within the gonad, the primordial germ cells are known as primary gametogonia which multiply rapidly by mitosis. This state is called the phase of multiplication and the resultant cells are then known as secondary gametogonia. The gametogonia enter into the phase of growth which is more pronounced in oogenesis. In male the gametogonia produce sperm cells and are called spermatogonia, while in female, gametogonia giving rise to ova or egg cells are known as oogonia.

Sperm and ovum are responsible for

(a) Bringing together of hereditary factors in the new individual from the parents and

(b) To provide material substance from which the new individual will arise.

The gametogonia in both the sexes transform to fulfil these two purposes. The development of male gametogonium or spermatogonium to sperm is called spermatogenesis and change of female oogonium to ovum is known as oogenesis.

The gametogonia then transform into the specific gametes and in both the cases, the transformation involves:

(a) Reduction of diploid to haploid number of chromosomes and

(b) Considerable preparation in different components of the cell.

It may be mentioned here that germ cells carry the potentiality of becoming both sperm and egg. It is the influence of the cells of the testis or ovary, which determines the final fate of it. Spermatogenesis and oogenesis are discussed below (Fig. 5.2).

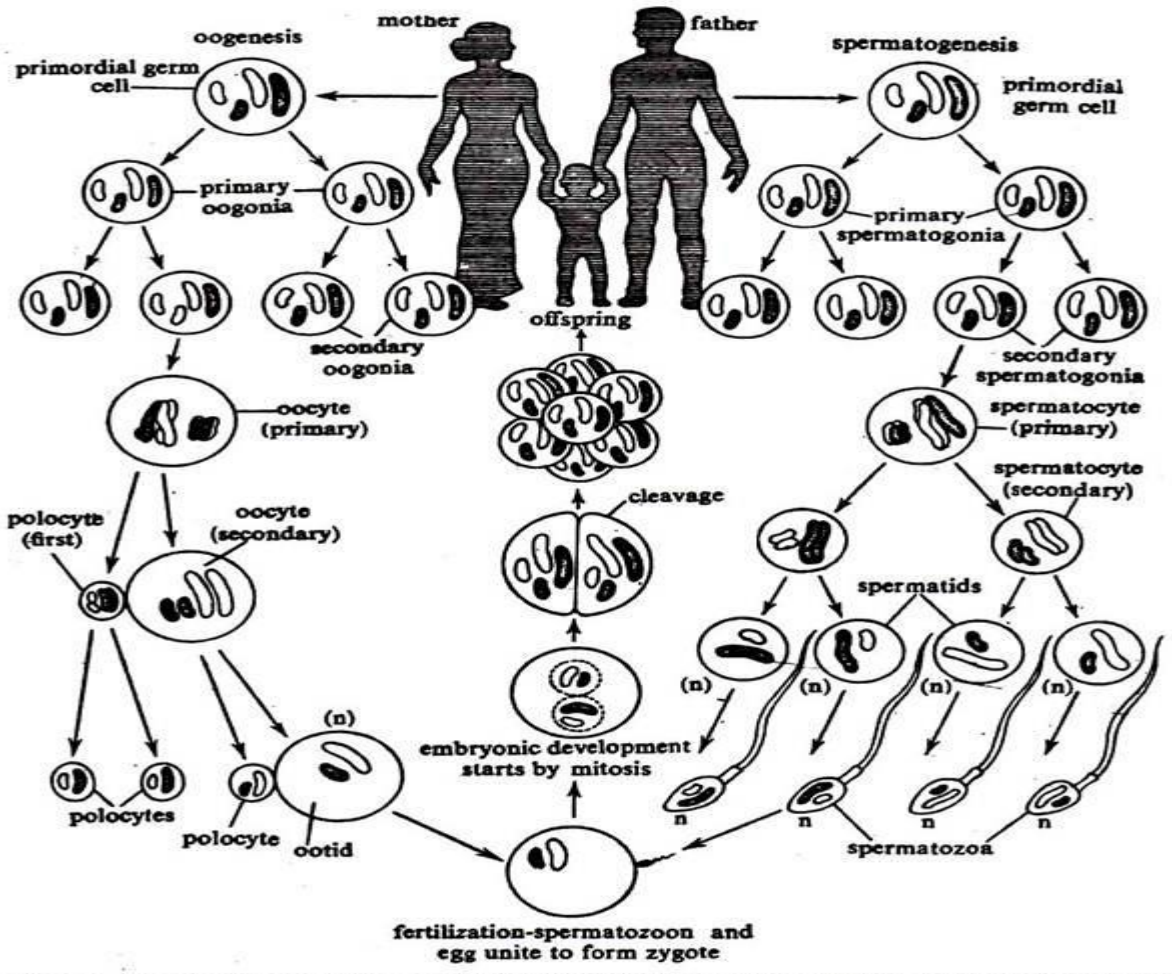

Fig. 5.2. Showing the events in gametogenesis. Note that four spermatozoa of equal size are produced from a spermatocyte while one large egg and three small nonfunctional polocytes are produced from each oocyte.

Spermatogenesis of Germ Cells:

With the differentiation of testes, the primordial germ cells give rise to spermatogonia. Further multiplication results in transformation of gonial cells into primary spermatocytes. The primary spermatocytes undergo the first meiotic division to produce secondary spermatocytes which give spermatids by the second meiotic division. Without further division, each spermatid differentiates into a spermatozoon—the functional male gamete (Fig. 5.3). As a result of meiotic cell-division four spermatids are produced from one primary spermatocyte.

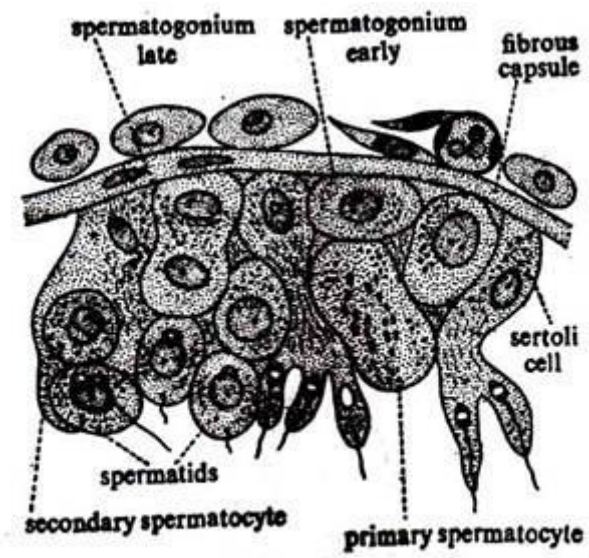

Fig. 5.3. Sectional view of a part of human testis showing spermatozoa in all stages of development in the seminiferous tubule.

Spermateleosis:

The spermatids possess haploid set of chromosomes but are unable to function as male gametes. A spermatid transforms into a spermatozoon which involves a series of cellular changes. This process of differentiation is called spermateleosis. The events in spermateleosis are illustrated diagrammatically in Fig. 5.4.

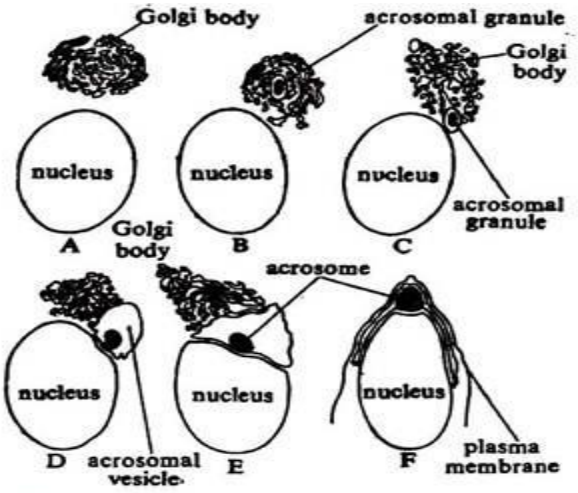

Fig. 5.4. Sequence of development of acrosome from Golgi body in spermatogenesis. A. Golgi body consists of a series of concentric membranes around a collection of small vacuoles. B. The vacuoles start enlarging and enclose a dense body (acrosomal granule). C. The granule becomes enlarged. D–E. The vacuole with its acrosome granule becomes closely applied to the anterior end of the nucleus. F. Showing the formation of the cap of the spermatozoon from the wall of the vacuole.

The acrosome is a derivative of Golgi bodies. Numerous small vesicles appear within Golgi apparatus, each vesicle contains a homogeneous granule. These vesicles coalesce to form a single large vesicle containing numerous granules. This large vesicle ultimately comes to attach with the surface of nucleus. It is known as acrosome vesicle and the granules are called acrosome granules. These granules fuse to form a large acrosomal granule. The nucleus with acrosome vesicle moves towards the peripheral end of the cell. The vesicle spreads over the nucleus, thus the vesicle becomes entirely occupied by the granules. The

centrosome consists of two centrioles after second meiotic division. At the time of formation of acrosome vesicle, the centrioles move towards the plasmalemma and one of the centrioles attaches itself with the membrane. From the point of attachment a fine thread grows out and extends from the cell as a small flagellum, this is the axial filament of the future tail.The centrioles then return to the nucleus and one of them fixes itself to a notch on the nucleus at the opposite pole of acrosome vesicle. During inward journey, the centriole which is in contact with the membrane draws the plasmalemma around the flagellum. Thus the flagellum, which apparently seems to be inside the cell, actually remains outside the cell and is bounded by double plasma membrane.

The proximal centriole continues to remain attached with nucleus, but the distal centriole comes down again to bring the fold of plasma membrane back to the general surface. Here the distal centriole forms a ring and is known as ring centriole. Mitochondrial bodies then associate around the axial filament between proximal and ring centrioles. The axial filament extends outside the cell as a long flagellum and fine fibre's originating from proximal centriole encircle the axial filament, thus establish the structure of the tail of sperm. The nucleus of a spermatid during its transformation into a spermatozoon loses water and becomes elongated to keep the acrosome vesicle in close contact with the plasma-lemma. Only a small portion of cytoplasm is retained around head, middle piece and tail while the major part of cytoplasm is discarded. This transformation from spermatids to spermatozoa, though the structure of sperm varies in different animals, is found to be closely similar.

Structure of Spermatozoa:

Sperm cells may be amoeboid (e.g., crayfish), or flagellated (e.g., vertebrates).

Vertebrate sperm cells may be of different shapes (Fig. 5.5):

(**a) Spheroidal—in teleostean fishes,**

(b) Rod-shaped—in amphibians,

(c) Spirally twisted—in passerine birds,

(d) Spoon-shaped—in man,

(e) Hooked— in mouse, etc.

But in all cases, the sperm is built on same structural plan. The description of sperm cell given below is based on the structure of the sperm of rabbit (Oryctolagus cuniculus) which has been extensively studied by both light and electron microscopes. A spermatozoon of rabbit measures about 60-70 micra long with a head measuring about 8—10 micra.

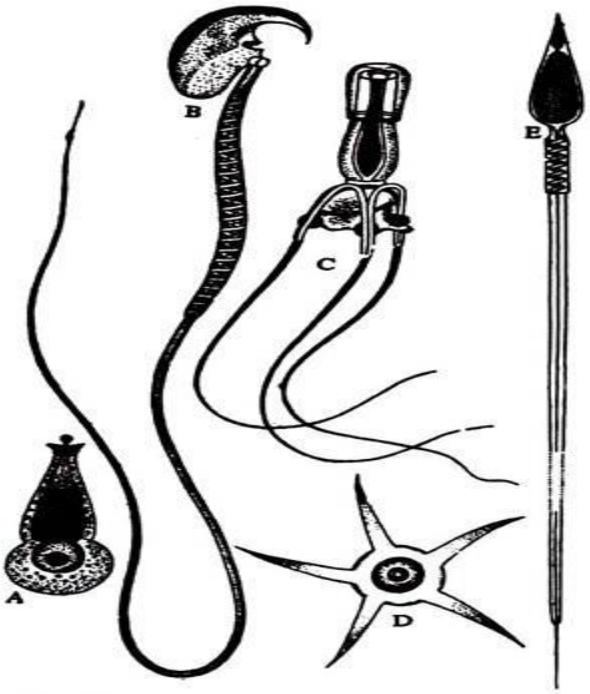

Fig. 5.5. Showing various types of spermatozoa. A. *Ascaris*. B. *Mus*, C. *Nephrops*. D. *Maia*. E. Man.

A typical sperm consists of three distinct parts—head, neck or middle piece and tail or flagellum (Fig. 5.6).

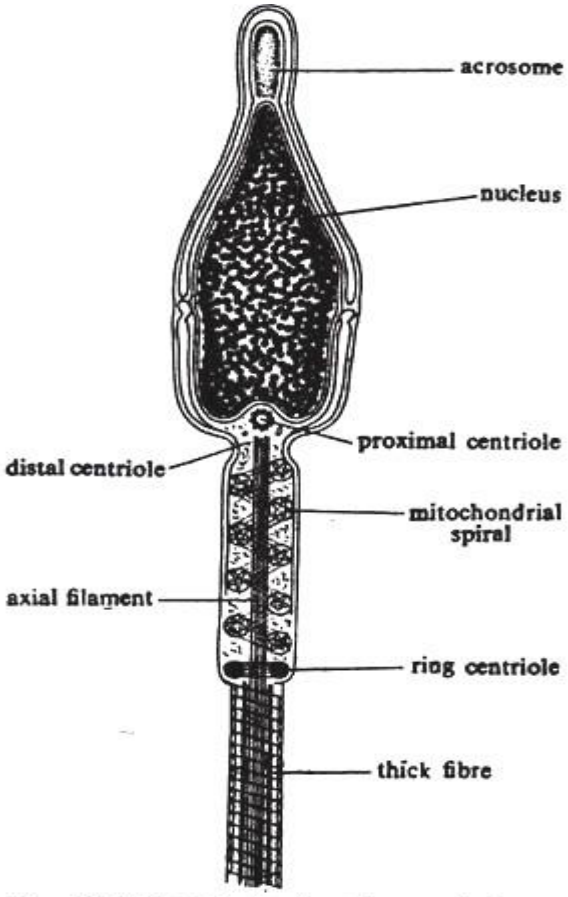

Fig. 5.6. Enlarged view (anterior portion) of a human spermatozoon.

Head:

It is the forward end of sperm and it contains:

(a) Acrosome,

(b) Perforatorium and

(c) Nucleus.

Acrosome:

This is a double-walled sac containing dense granules. It is present at the tip of the nucleus and attached intimately with it. Anteriorly it is convex and flattened posteriorly. It contains enzymes for breaking egg membrane during fertilization. Per-foratorium. This structure is not rabbit and other mammals. But invertebrate sperm (e.g., Sea-urchin) contains perforatorium between acrosome and nucleus. It forms acrosome filaments and plays important role in fertilization.

Nucleus:
It forms the major part of the head and has a groove at its posterior end. It contains densely packed material within its nuclear membrane. The nucleus contains DNA and a protein called histone.

Neck or Middle Piece:

The mid-piece varies greatly in structure in different animals. This piece extends from the proximal centriole near the nucleus to the distal or ring centriole.

It is formed by a firmly coiled spiral of elongated mitochondria around the axial filament and nine fibres from proximal centriole. Due to the presence of mitochondria, it is thought that middle piece is a 'power plant' which provides energy during the locomotion of sperm. Tail The sperm tail is a specialised portion of a spermatozoon which helps in movement. In the spermatozoon of sea- urchin, the tail is made up of fibres which are arranged in a circle of nine with two more extending down the middle. This pattern resembles that of cilia and flagella. In the spermatozoon of rabbit such an array of tail fibres is present which is designated as the axial filament. The fibres in the axial filament are actually the contractile units responsible for the movement of sperm tail. The sperm tail of rabbit has in addition another outer ring of nine more fibres. The fibres of the outer ring are much thicker than the inner fibres. The coarse outer fibres are not present up to the posterior tip, but end one after another at various regions down the tail. But the axial filament remains up to the tip. The axial filament and outer fibres are formed from the proximal centriole which remains attached with the posterior indention of the nucleus.

The pattern of arrangement of fibres (9+9+2) is best developed in mammalian spermatozoa. The said pattern is encountered in other forms, viz. Honeybee (Apis mellifera), Fruitfly (Drosophila melano-gaster), Grasshopper (Gelastorrhinus bicolor), Snail (Helix pomatia), Sparrow (Passer montanus saturatus), Snake (Lampropeltis getulus) and many others.

Characteristics of Sperm Cell:
A sperm cell has the following characteristics:
a. It has a highly specialised structural organisation.

b. The major part is the nucleus and cytoplasm has modified to form special locomotory devices.

c. It is devoid of stored food and protective envelope.

d. Size is much smaller than egg, but is produced in greater number than the egg.

e. Its function is to seek the egg and to stimulate it to develop.

Oogenesis of Germ Cells:

The egg or ovum serves two functions—it carries the maternal chromosomes and at the same time it provides the ground substance on which the future embryo develops.

To be prepared for these double purposes, the egg during its development undergoes complicated modifications which result—

(a) Production of haploid nucleus,

(b) Acquisition of food reserves and

(c) Preliminary organisation of the cytoplasm.

The entire process is called oogenesis. Before entering into the details of the process it must be remembered that the process of oogenesis begins at a stage when the individual itself is in embryonic condition and ends either shortly before or immediately after fertilization.

Stages of Oogenesis:

The process of oogenesis has been worked out in different organisms and the results show close similarity in the process. In the present discussion the oogenesis of amphibians will be followed.

The gametogonium (here known as oogonium) which is destined to form egg is called oocyte. The nucleus of the oocyte swells up to form a vesicle which is called germinal vesicle. It is filled up with a fluid called nuclear sap, which contains sulphydryl protein. Meiosis begins when the individual is in embryonic state, but till the prophase stage of first meiotic division it remains suspended. During the suspended period, considerable amount of preparatory work happens within the oocyte. In the nucleus, chromosomes become dispiralised and appear as long and thin threads. Each chromosome contains at first single filament but later it becomes doubled. These filaments at short intervals contain paired thick, dense swellings called chromomeres. The chromomeres roughly correspond with the expected number of genes. From each chromomere a slender loop is projected outward to unite with the chromomere of other filament. The loop is enclosed by a matrix. The shape and size of the loop and also the matrix vary in different chromosomes. These loops are known as puffs and the chromosomes containing puffs are called lamp-brush chromosomes. Callan and Gall have demonstrated that puffs are constant for each pair of chromo- mere and are always found in the same position on the chromosome. The filament part of the puff is made up of DNA whereas the matrix contains RNA and protein. At the cytoplasm, two kinds of events occur. Establishment of physico-chemical differences occurs in the various parts of the egg cell. This is done by aggregation of different substances within the cytoplasm in graded fashion and mapping out of the different fields of development of the future embryo. Considerable amount of synthesis continues

within the egg for making the reserves of food materials. The most important reserve is the yolk. This inert substance is composed of a phosphoprotein (called vitellin) and lipids (different fats).

Types of Egg:

According to the quantity of yolk, the egg may be:

(a) Alecithal:

Eggs having no yolk, e.g., mammals.

(b) Microlecithal:

Relatively yolk-free eggs are called the microlecithal types. Eggs of coelenterates are of microlecithal types

(c) Megalecithal:

Large yolky eggs are called the megalecithal types. Examples: Eggs of reptiles, birds and monotremes.

(d) Telolecithal:

Eggs are large in size and contain much yolk like megalecithal egg but the yolk pushes the cytoplasm at one end. Examples: Chick.

Factors Controlling the Cytoplasmic Events:

It is believed that the accumulation of different substances which happens within the egg is controlled by external and internal influences.

External Influences:

(a) Immunological studies have shown that there exist similarities between blood protein and the cytoplasmic content of the egg (including yolk).

(b) In the rapid growth phase, vitellin (which is characteristic of yolk) increases rapidly in the blood of the individual. It indicates that external factors are responsible for providing nutritive materials to the egg.

(c) Cells investing the developing oocyte transfer- complex molecules to the developing egg.

These substances are either synthesized by them or transferred from the general circulation. Internal influences. Simple molecules enter within the egg and these are converted into more complex substances by the nucleus and cytoplasm. Callan and Gall have shown that RNA produced at the lateral loop of lamp-brush chromosome comes first to remain free in the nuclear sap and later passes through porous nuclear membrane into the cytoplasm. Considerable amount of protein is synthesized under the instruction of this RNA. Regarding the events of growth during oogenesis, it may be said that the entire event is divisible into a slow phase of growth and a rapid phase of growth. The slow phase continues for a longer period during which synthesis of different substances (including yolk) continues within the egg. Rapid phase begins after the attainment of sexual maturity, when rapid accumulation of different substances occurs. It has been shown that during this phase many substances are contributed to the egg from general circulation. In the oocyte of almost all the vertebrates, the microvilli and pinocytotic vesicles are concerned mainly with the transportation of substances into the oocyte from the follicle cells (Fig. 5.7).

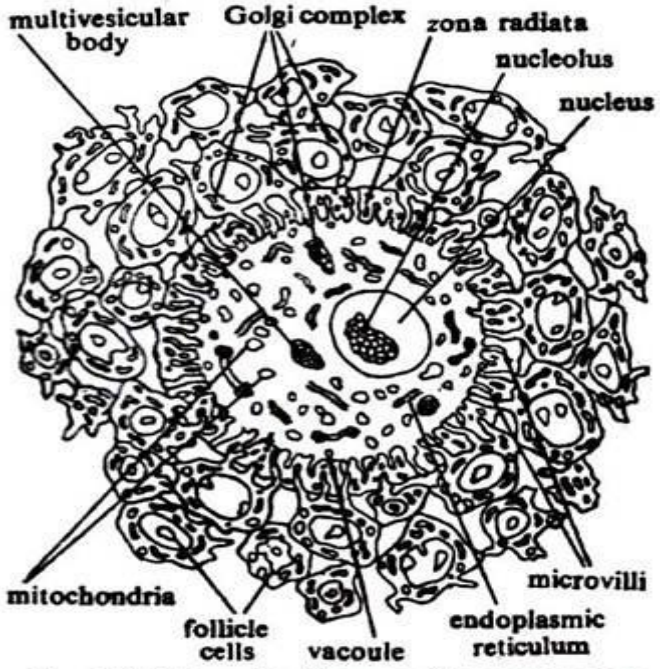

Fig. 5.7. Schematic diagram of a young mammalian oocyte and its associated follicle cells (after Bodemer).

The differentiation of cortical granules (spherical bodies surrounded by a membrane) is also a significant event in egg development. The cortical granules are composed of acid mucopolysaccharides and are arranged in a layer adjacent to the plasma membrane. The cortical granules play vital role in fertilization.

Theories Regarding the Organisation of Ovum:

During organisation of egg, different substances get oriented in specific pattern, which plays important role in establishing the spatial arrangement of the developing embryo. Three different theories are forwarded to explain this establishment of determination.

(a) Quantitative or Gradient Theory:

According to this view, one end of the egg possesses highest metabolic activity and controls the activity of other regions. This activity gradually reduces at the other end.

(b) Qualitative Theory:

According to this concept, there are actually different substances in the egg which control the formation of different parts of the embryo.

(c) Two-Factor Theory:

Quantitative gradient exists within the egg in the beginning but after some time different parts of the egg become qualitatively different. Thus this view is a compromise between the first two theories.

In amphibians, when egg is fully matured the first meiotic division ends—this results into the formation of two haploid cells. But contrary to sperm cell formation the cytoplasmic division is unequal, i. e., a large cell called secondary oocyte and a very small cell called first polocyte are produced. The first polocyte remains attached with the outer surface of the oocyte. At this stage the egg is liberated from the body. After the entry of sperm cells, second division of meiosis ends. At this time the secondary oocyte divides

unequally producing one large cell called egg and a small cell called second polocyte. The first polocyte divides equally.

To sum up the result, it may be said that during oogenesis, single oocyte produces one large cell called egg (or ovum) and three small cells called polocytes. The egg is the functional reproductive cell while the polocytes are abortive in nature.

Structure of Ovum or Egg:

The size of egg varies greatly in different animals. The size is chiefly attributable to the quantity of yolk present. The eggs of reptiles, birds and monotremes have, in addition, a coating of albumen. The albumen subserves both protective and nutritive functions. The egg is surrounded by a plasma membrane which encloses cytoplasmic part called vitellus. Within the vitellus, most prominent is nucleus which is 200-300 times larger than an ordinary cell nucleus. Nucleus contains a single large nucleolus rich in RNA and enveloping chromatin aggregates. Immediately beneath the plasma membrane, there is a thin layer of cytoplasm called cortex.

In many eggs, the cortex contains special granules, called cortical granules which play important role in fertilization. The substance which constitutes the cortex is principally protein which has much influence on the future development. The cytoplasm contains various organelles: mitochondria, Golgi apparatus and reserve food material in the form of yolk. In some forms (e.g., Sea-urchin), the maturation of egg begins after the release from ovary and in others (e.g., Rabbit) maturation begins within ovary. Ovulation (i.e., release of ovum from the ovary) takes place at the metaphase stage of second meiotic division and completion of meiosis occurs immediately with the entry of sperm cell. The egg or ovum contains one or more membranes which differ in different animals.

These egg membranes may be

Primary Membrane:

It is formed by the ovum itself. It includes

(a) vitelline membrane. It is a constant thin membrane which remains closely apposed with the plasma membrane before fertilization.

(b) Chorion. In Stylea, a stiff chorion is found to be originated from the ovum itself.

(c) Zona radiata. Generally a striated and less constant layer which—

(i) In shark, bony fishes, amphibians and reptiles are present as a layer between vitelline membrane and plasma membrane,

(ii) In birds it is difficult to differentiate.

(iii) It is unstriated in mammals here it is known as zona pellucida and it is debatable whether it is primary or secondary in nature.

Secondary Membrane:

It is formed by the cells of the ovary which remain immediately around the ovum. Examples: (i) chitinous shells of different eggs, (ii) membranes which are present immediately outside the frog's egg, (iii) cells of the graafian follicle in mammals. The secondary membranes are not seen in urodeles, reptiles and birds.

Tertiary Membrane:

These are formed by oviducts and-special glands associated with the oviducts.

These are always formed after the discharge of egg from the ovary and are of following kinds:

(i) Albumen or Horny capsule of the elasmobranch eggs. Albumen in birds and shell-membrane and calcareous shell of reptilian and avian eggs. It is to be noted that reptilian eggs contain little, albuminous covering except in snake,

(ii) Tertiary membranes are absent in mammals excepting prototheria where leathery shell is present.

Significance of Egg Membranes:

(a) Primary membranes. Vitelline membrane plays important role in development. After the event of fertilization it prevents the entrance of more sperm cells and at the same time helps the egg to assume bilateral symmetry from a state of radial symmetry in unfertilized state.

(b) Secondary membranes are chiefly protective and are also responsible for processing nutritive materials from the surrounding area.

(c) Tertiary membranes meet one of the two requirements,

(i) Provision of organic food and

(ii) adequate water supply. Eutherian and metatherian mammals have solved both, the problems by the attainment of complete or incomplete viviparity. In other vertebrates, the first requirement is met by the storage of yolk or some other devices, but the second need gives a serious threat to the life of oviparous group.

Amphibians, to avoid the threat of drought, return either to water for egg laying or developed certain devices, viz.,

(i) Eggs deposited in a foam,

(ii) Formation of oviducal jelly and

(iii) Keeping the eggs in urinary bladder.

Reptiles, birds and monotremes have developed waterproof shell for the purpose.

The transformation of the primordial germ cells into gametes occurs in three distinct steps—phase of multiplication, phase of growth and phase of maturation. The phase of multiplication is closely similar in both spermatogenesis and oogenesis, but the other two phases are quite different.

Historical Background of Embryonic Induction:

For the discovery of neural induction, the German embryologist, Hans Spemann and his student, Hilde Mangold (1924) worked a lot and for his work Spemann received Nobel Prize in 1935. These two scientists performed certain heteroblastic transplantations between two species of newt, i.e., Triturus cristatus and Triturus taeniatus and reported that the dorsal lip of their early gastrula has the capacity of induction and organization of presumptive neural ectoderm to form a neural tube and also the capacity of evocation and organization of ectoderm, mesoderm and endoderm to form a complete secondary embryo. They called the dorsal lip of the blastopore the primary organizer since it was first in the sequence of inductions and as it had the capacity to organize the development of a second embryo. Later on, the primary organizer was reported to exist in many animals, e.g. in frogs (Daloq and Pasteels, 1937); in cyclostomes (Yamada, 1938); in bony fishes (Oppenheimer, 1936); in birds (Waddington, 1933) and in rabbit (Waddington, 1934). Primary organizer and neural induction have been reported in certain pre-vertebrate chordates, such as ascidians and Amphioxus (Tung, Wu and Tung, 1932). In 1960 and 1963 Curtis investigated and reported that the organizer of gastrula of Xenopus laevis can be distinguished in the cortex of gray crescent of a fertilized egg. Holtfreter (1945) gave an account of how an enormous variety of entirely unspecific substances-organic acids, steroids, kaolin, methylene blue, sulphhydryl compounds, which had nothing in common except the property of being toxic to sub-ectodermal cells-produced neurulation in explants. Barth and Barth (1968, 69) provided further information about the chemical nature of embryonic induction.

Types of embryonic induction:

Lovtrup (1974) classified different types of embryonic induction into two basic categories-endogenous and exogenous inductions.

1. Endogenous induction:

Certain embryonic cells gradually assume new diversification pattern through the inductors that are produced by them endogenously. Due to these inductors, these cells undergo either self-transformation or self-differentiation. Examples of such induction were reported in Mesenchymal cells of ventral pole of Echinoid and in small sized, yolk-laden cells of dorsal lip of amphibian blastopore.

2. Exogenous induction:

When some external agent or a cell or a tissue is introduced into an embryo, they exert their influence by a process of diversification pattern upon neighbouring cells through contact induction. This phenomenon is called exogenous induction. It may be homotypic or heterotypic depending on the fact that whether the inductor provokes the formation of same or different kind of tissues respectively (Grobstein, 1964). In homotypic induction, a differentiated cell produces an inductor. The inductor not only serves to maintain the state of the cell proper, but also induces adjacent cells to differentiate according to it, after crossing the cell boundaries. Best example of the heterotypic exogenous induction is the formation of a secondary embryonic axis by an implanted presumptive notochord in amphibians.

Experimental evidences to induction:

Spemann and Mangold (1924) transplanted heteroplastically a piece of the dorsal lip of the blastopore of an early gastrula of pigmented newt, Triturus cristatus and grafted it near the ventral or lateral lip of the blastopore of the early gastrula of pigmented newt T. taeniatus. Most of the graft invaginated into the interior and developed into notochord and somite's and induced the host ectoderm to form a neural tube,

leaving a narrow strip of tissue on the surface. With the development of host embryo, an additional whole system of organs was induced at the graft – placement area. Except for the anterior part of the head, almost a complete secondary embryo comprising of the additional organs was formed. Posterior part of the head was present as indicated by a pair of ear rudiments.

Since in this experiment the type of transplantation involved was heteroplastic, it was found that notochord of secondary embryo consisted exclusively of graft cells; the somites consisted partly of graft and partly of host cells (Fig. 1). Few cells, which did not invaginate during gastrulation, were left in the neural tube. The bulk of the neural tube, part of the somites, kidney tubules and the ear rudiments of the secondary embryo consisted of host cells. The graft becomes self-differentiated and at the same time induces the adjoining host tissue to form spinal cord and other structures including somites and kidney tubules. Spemann (1938) described dorsal lip of the early gastrula as a "primary organizer" of the gastrulative process. However, organization of the secondary embryo results from a series of both inductive interactions and self-differentiative changes in the host and donor tissues. Hence, now a days the term "embryonic induction" or "inductive interactions" is preferred. The part, which is the source of induction, is called "inductor".

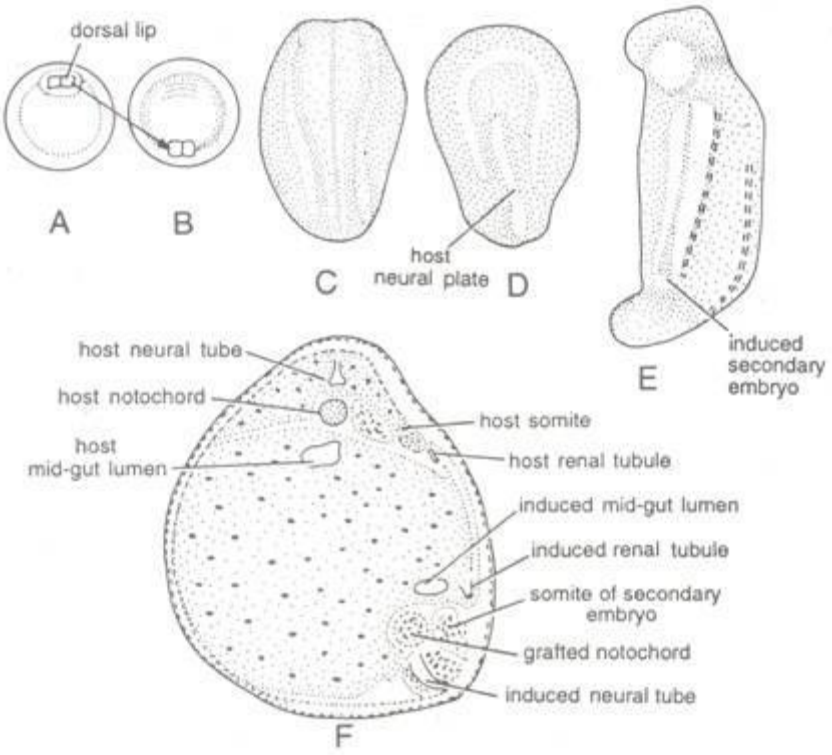

Fig. 1. Induction of secondary embryo in *Triturus* by transplanting a piece of dorsal lip to the future belly region of another gastrula (A–B). C–E, are stages of resulting primary embryo, with a secondary embryo attached to it. F–T.S. of the same embryo.

Characteristics of the organizer:

Organizer has the ability for self-differentiation and organization. It also has the power to induce changes within the cell and to organize surrounding cells, including the induction and early organization of neural tube. Primary organizer determines the main features of axiation and organization of the vertebrate embryo. Induction is a tool-like process, utilized by this center of activity through which it affects changes in surrounding cells and as such influences organization and differentiation. These surrounding cells, changed by the process of induction, may in turn act as secondary inductor centers with abilities to

organize specific sub-areas. Thus, the transformation of the late blastula into an organized condition of the late gastrula appears to be dependent upon a number of separate inductions, all integrated into one coordinated whole by the "formative stimulus" of the primary organizer located in the pre-chordal plate area of the endodermal -mesodermal cells and adjacent chorda-mesodermal material of the early gastrula.

Regional specificity of the organizer:

Vital-staining experiments of Vogt with newt eggs have shown that the material successively forming the dorsal blastoporal lip moves forward as the archenteron roof. Transplants taken from this region are also able to induce a secondary embryo or the belly of a new host i.e. the archenteron roof acts as a primary inductor in essentially the same way as does the dorsal lip tissue proper. The inductions of neural inductor are found to be regionally specific and the regional specificity is imposed on the induced organ by the inductor. Therefore, the inductive capacity of the blastoporal lip varies both regionally and temporally. Most of the dorsal and dorso-lateral blastoporal material is necessary for a graft to induce a more or less complete secondary embryo. Spemann (1931) demonstrated that during gastrulation anterior part of the archenteric roof invaginates over the dorsal lip of the blastopore earlier. Dorsal blastopore lip of the early gastrula contains the archenteric and deuterocephalic organizer and the dorsal blastopore lip of the late gastrula contains the spinocaudal organizer. Inductions produced by the dorsal lip of the blastopore taken from the early and the late gastrula differ in accordance with exception; the first tends to produce head organs and the second tends to produce trunk and tail organs (Fig. 2).

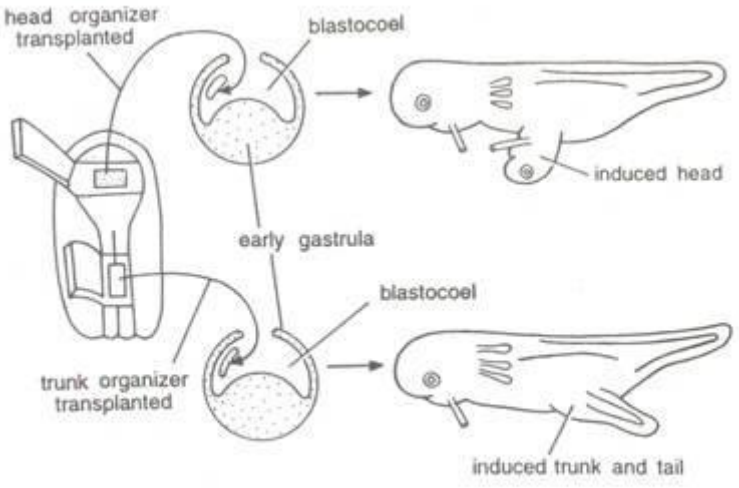

Fig. 2. The separation of the neural inductor into head and trunk organizers.

As invagination continues and the dorsal lip no longer consists of prospective head endo-mesoderm but progressively becomes prospective trunk mesoderm; it acts as a trunk-tail inductor. The most caudal region of the archenteron roof, in fact, specifically induces tail somites and probably other mesodermal tissues. The archenteron roof induces entirely different class of tissues; various neural and meso-ectodermal tissues by its anterior region and various mesodermal tissues by its most posterior region. Therefore, differences in specific induction capacities exist between head and trunk level of archenteron roof and are related to the regional differentiation of the neural tissue into archencephalic (including fore-brain, eye, nasal pit), deuterencephalic (including hind-brain, ear vesicle) and spinocaudal components. Thus, archenteron roof consists of an anterior head inductor including an archencephalic inductor and a deuterencephalic inductor and a trunk or spinocaudal inductor.

Primary induction and gray crescent:

The dorsal lip region of the blastopore at the onset of gastrulation can be traced back to the gray-crescent of the undivided fertilized amphibian egg. It was conceived by some developmental biologists that the crescent material of egg cortex initiated gastrulation and has the capacity of neural induction. A.S.G. Curtis (1963) performed a series of experiments of transplanting parts of the cortex of the fertilized egg of the clawed toad, Xenopus laevis at the beginning of cleavage.

In one experiment, the gray-crescent cortex was excised from the fertilized egg and it was observed that the cell division though proceeded undisturbed, the gastrulation failed to take place (Fig. 3A). In another experiment, the gray crescent cortex of uncleaved fertilized egg was excised and transplanted into a ventral position of a second egg, so that the egg receiving the graft had two gray crescents on opposite sides. As a result, egg cleaved to form a blastula, which underwent two separate gastrulation movements to produce two separate primary nervous systems, notochord and associated somites (Fig. 3D). Similar experiments conducted on the eight-cell stage showed that something had happened during the short – interval represented by the first three cleavages. Gray crescent cortex of the eight-cell stage still retained its inductive capacity when grafted to younger stages (Fig. 3C). Removal of the gray crescent at this stage no longer inhibits subsequent gastrulation and normal development, the missing crescent properties being replaced from adjacent cortical regions (Fig. 3B). According to Curtis, a change in cortical organization spreads across the surface of the egg during the second and third cleavages, starting from the gray crescent; when this change is completed, interactions, probably of a biophysical nature, can take place among various parts of the cortex.

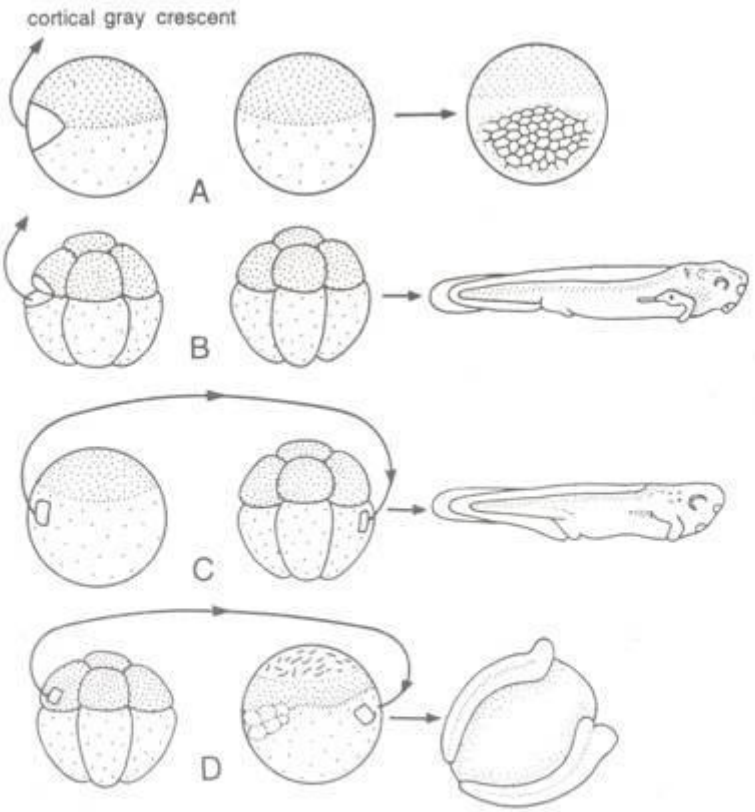

Fig. 3. Experiment of Curtis on *Xenopus*. A–Excision of the cortical gray crescent area at one cell stage : *no gastrulation*. B–Same experiment at the eight-cell stage : *normal embryo*. C–Graft of the gray-crescent cortex of one-cell stage to the ventral part of the eight-cell stage does not result in the induction of a secondary embryonic axis. D–Graft of the gray crescent cortex from the eight-cell stage to the ventral margin of the one-cell stage induces a secondary embryonic axis.

Mechanism of neural induction:

Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or by chemical mediation.

(1) One of the broad possibility is surface interaction of the cells at the inductive interface. The contact of the two cellular layers may provide a device whereby the structural pattern or geometry or behaviour of the ectodermal cell membranes is altered directly by the underlying chorda mesodermal cells.

Thus, the spatial configuration of the latter membranes might induce a change in the spatial configuration of the ectodermal cell membranes, this in turn producing in the interior of the cell changes that determine its development into neural plate. A morphological arrangement of this kind could account for quick and effective transmission of the inductive effect.

(2) Another broad possibility is a chemical mediation of the inductive effect. Therefore, a chemical substance or substances produced and released by inducing chorda mesoderm cells at the archenteron - ectoderm interface may act upon or enter the ectodermal cells to initiate cellular activities leading to neural development. A great deal of evidence favours the idea of an exchange of material between cells and also suggests that a diffusible substance may act as effective inductive stimulus.

Chemical basis of neural induction:

The results of numerous studies to elucidate the mechanism of induction and to identify the chemical substance or substances presumed to be involved have not yielded good results. It was found that many different tissues, embryonic or adult, from a great variety of different species, were capable of inducing nervous tissue in amphibian embryos. Moreover, some foreign tissues were found to be much more potent inductors after they had been killed by heat or alcohol treatment. This fact remains against the concept of a universally present 'masked organizer', released in the primary inductor region. Few inorganic agents as iodine and kaolin, local injury, exposure to saline solutions of excessively high or low pH, cause neural differentiation in ectoderm. These findings establish the early grand concept of master-chemical embryonic organizer of Holtfreter's sublethal cytolysis. It has the concept of reversible cell injury liberating neural inductor. Different chemical substances of either gray crescent or dorsal lip or chordamesoderm are separated by different biochemical methods to find out the molecule which causes the neural induction and then the inductive capacity of each molecule was tested separately. Few experiments show that evocator or inducing substance is a protein. Exhaustive attempts were made by different embryologists to understand the real mechanism of neural induction. Some theories have been put forward to understand the mechanism of neural induction, out of which the most important are as follows:

1. Protein denaturation theory of neural induction:

According to Ranzi (1963) neural induction and notochord formation are related to protein denaturation. Site of notochord formation is amphibian gray crescent, which is a center of high metabolic activity. Such centers of greater metabolic activity correspond to sites of protein denaturation.

2. Gradient theory of neural induction:

Toivonen (1968) and Yamada (1961) stated that two chemically distinct factors are involved in the action of the primary inductor. Out of these two factors, one is neuralizing agent and the other is mesodermalizing agent. These experiments were conducted with denatured bone marrow and liver as the inductors. Regional specificity of the embryonic axis arises from the interaction between two gradients: neutralizing principle has its highest concentration in the dorsal side of the embryo and diminishes laterally, while the mesodermalizing principle is present as an antero-posterior gradient with its peak in the posterior region. Anteriorly the neutralizing principle acts alone to induce forebrain structures, more posteriorly the mesodermalizing principle acts along with the neutralizing one to induce mid-brain and hind-brain structures, while even more posteriorly the high concentration level of the mesodermal gradient produces spino-caudal structures (Fig. 4).

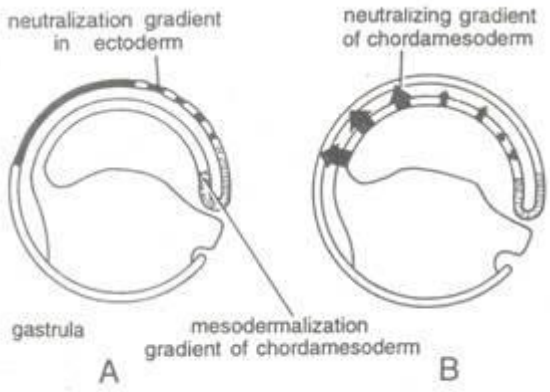

Fig. 4. A– The neutralization gradient in the ectoderm over lying the mesodermalization gradient of the chordamesoderm; B– the neutralization gradient of the chordamesoderm

3. One factor hypothesis of neural induction:

Nieuwkoop (1966) using living notochord as the inductor, postulated that only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structure (Fig. 5) is involved.

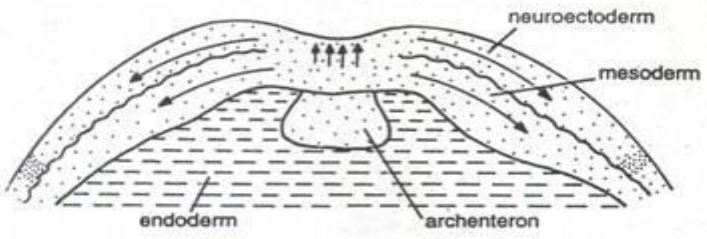

Fig. 5. Medio-lateral spreading of an inductive action within the mesoderm and a similar spreading of the neuralizing action in the overlying ectoderm. Parallel vertical arrows indicate the main site of the neuralizing inductive action (N) in the area of intimate adhesion of two germ layers.

In one experiment, consisting of combining isolated gastrular ectoderm with a piece of notochord and then removing the notochord tissue after varying lengths of time, it was found that only 5 minutes exposure to inductor caused a part of the ectoderm to transform into brain and eye structures.

4. Ionic theory of neural induction:

According to Barth and Barth (1969), the actual process of induction may be initiated by release of ions from bound form, representing a change in the ratio between bound to free ions within the cell of the early gastrula. Induction of nerve and pigment cells in small aggregates of prospective epidermis of the frog gastrula were found to be dependent on the concentration of the sodium ions. Normal induction of nerve and pigment cells by mesoderm in small explants from the dorsal lip and lateral marginal zones of the early gastrula is dependent on the external concentration of sodium. Thus, normal embryonic induction depends on an endogenous source of ions and that an intracellular release of such ions occurs during late gastrulation.

Genic basis of neural induction:

There are evidences that the component tissues of neural inductor become differentiated prior to ectodermal cells. During this process, the rate of transcription of mRNA and differential activation of genes becomes many fold, while the differentiation of ectodermal cells is set in only after mid-gastrulation. According to experiments conducted by Tiedemann (1968), after 2 to 7 days of cultivation of

dorsal blastopore lip of young Triturus gastrula with adjacent ectoderm in a medium containing sufficient quantities of Actinomycin-D to inhibit RNA synthesis, induction could not take place, but some differentiation of muscle and notochord occurred. It shows that mRNA by transcription from the DNA was required, which also requires the presence of Actinomycin-D. Therefore, no neural induction could be detected in this experiment.

Time of neural induction:

Neural induction occurs at the time when the material of chordamesoderm moves from the dorsal lip of blastopore inward and forward (Saxen and Toivonen 1962). The inductive stimuli exhibit a time gradient, which may be crucial with regard to action and reaction events.

Embryonic induction in different chordates:

Although neural induction was first discovered in urodele amphibians, it was found that the dorsal lip of the blastopore and the roof of the archenteron of other vertebrates have the same function. The chordamesoderm in all vertebrates induces the nervous system and sense organs. Neural inductor has been investigated in the following chordates:

(1) In Cyclostomes, especially in lampreys, the property of neural induction lies in the presumptive chorda mesodermal cells of dorsal lip of the blastopore.

Prior to cyclostomes, in Ascidians different blastomeres of eight cell stage have the following presumptive fates-(i) the two anterior animal pole blastomeres produce head epidermis, palps and the brain with its two pigmented sensory structures, (ii) two posterior animal pole blastomeres produce epidermis, (iii) two anterior vegetal blastomeres produce notochord, spinal cord and part of the intestine (iv) two posterior vegetal cells produce mesenchyme, muscles and part of the intestine.

From these experiments, Raverberi (1960) concluded that the formation and differentiation of brain by two anterior animal blastomeres is dependent on the induction of two anterior vegetal blastomeres, which act as neural inductors. It was further concluded that the two anterior vegetal blastomeres gave rise to diverse tissues, namely, endoderm, notochord and spinal cord.

(2) Wu and Tung (1962) proved the existence of the primary organizer and neural induction in Amphioxus. They transplanted pieces of tissues from the inner surface of the dorsal blastopore lip of an early gastrula of Amphioxus into the blastocoel of another embryo in the same stage (Fig. 6) and observed that secondary embryo developed in the ventral region of the host with a notochord and mesoderm produced by the graft and the neural tube from host tissue.

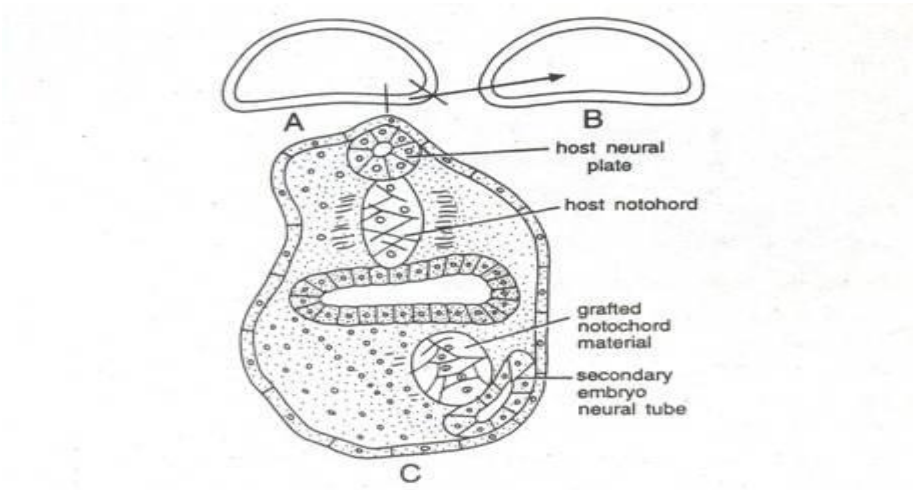

Fig. 6. Neural induction in *Amphioxus*. A–Diagram of operation-cells lying inside dorsal lip of the blastopore transplanted into the blastocoel of an embryo; B–in the same stage of development; C–T.S. of host embryo with the secondary neural tube.

Thus, the chordal tissue of Amphioxus gastrula possesses the power of neural induction, while mesodermal and endodermal tissues have little such inductive power.

(3) In bony fishes, induction of secondary well developed embryos were produced by transplanting the posterior edge of the blastodisc which corresponds to the dorsal lip of the blastopore, into the blastocoel of another embryo (Fig. 7) or by transplanting the chordamesoderm and ectoderm. Neural inductions were also obtained by transplanting the dorsal lip of the blastopore in the sturgeon.

(4) In frogs, the induction of secondary embryo can be produced by the dorsal lip of the blastopore transplanted into the blastocoel of a young gastrula, in very much the same way as in newts and salamandars.

(5) In reptiles archenteron has the same inducing activity as in other vertebrates but there is no experimental proof of occurrence of neural inductor.

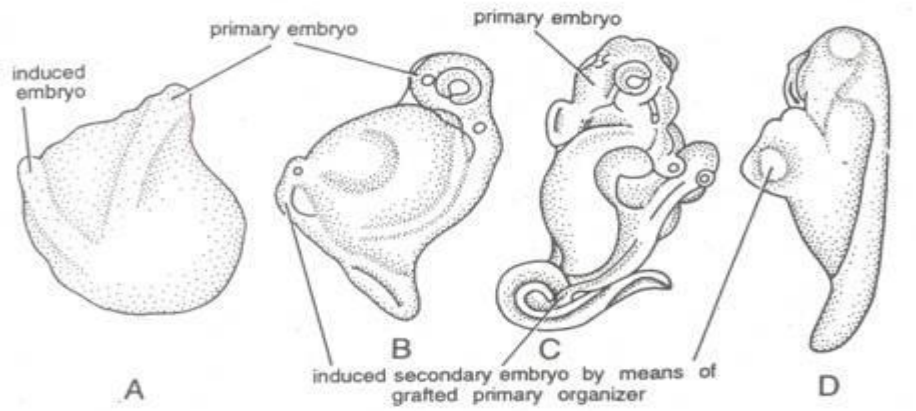

Fig. 7. Induction of secondary embryos by means of grafted primary organizers in the-A-lamprey; B–and C–perch; D–frog.

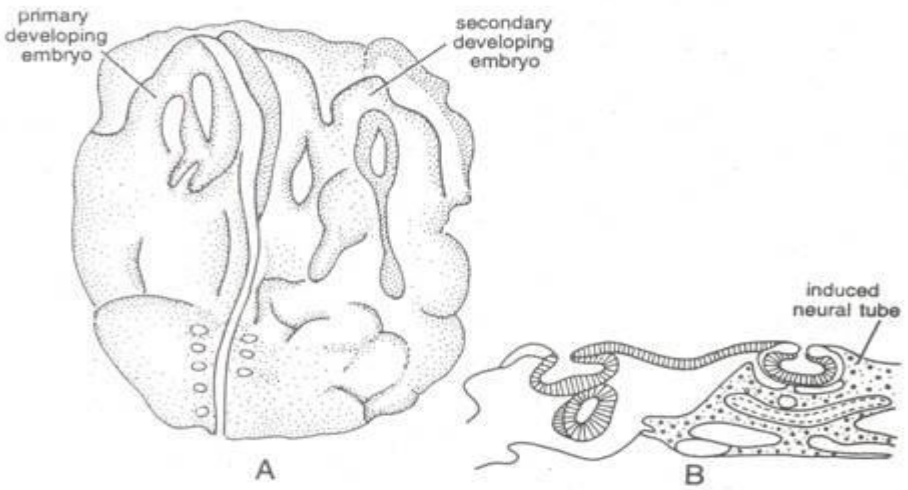

Fig. 8. Induction of a secondary embryo by means of a grafted primitive streak in a bird. A–surface view; the secondary embryo is on the right. B–Section through the same embryo showing the host axial system (right) and the induced neural tube and mesoderm developed from the graft (left).

(6) In birds the existence of primary organizer was established by Waddington and co-workers. Anterior half of the primitive streak was the inducing part similar to the lips of the blastopore in amphibians. In the experiment whole blastoderms were removed from the egg in early gastrulation and cultivated in vitro on the blood plasma clot.

From another embryo, parts of the primitive streak were then inserted between epiblast and hypoblast, inductions of secondary embryos obtained. Primitive streak was found dependent on the underlying hypoblast for its formation (Fig. 8).

(7) A successful neural induction was performed in a rabbit embryo by cultivating the early blastodisc on a plasma clot and implanting the primitive streak of the chick as inductor. Tissues of the mammalian gastrula were found having competence for neural induction. Anterior end of a rabbit embryo, with two pairs of somites, induced a neural plate in a chick embryo when placed under a chick blastoderm.

Other types of embryonic inductions:

Along with gastrulation growth, various organ systems of the embryo begin to differentiate and acquire the power of inducing the differentiation of later formed structures or organs such as eyes, ears, limbs and

lungs, etc. These organs develop organizing property and become the source of induction. Therefore, this series of organizers can be called as secondary, tertiary and quaternary organizers. Progressive development of embryonic organs is dependent on sequential induction. One embryonic tissue interacts with the adjacent one and induces it to develop and this process continues in sequence.

Development of eye:

Chorda mesoderm, the primary organizer induces the formation of fore-brain and optic area in the anterior part of the embryo. The optic area evaginates forming the optic vesicle. By invagination it changes into a double walled cup-like structure, the optic cup which acts as secondary organizer to induce the formation of tertiary organizer to form cornea.

The layer of mesenchyme left in front of the anterior chamber of eye combines with the overlying somatic ectoderm (epidermis) and forms cornea, choroid and sclera (Fig. 9). Thus the whole process of development seems to be a cause of induction and interaction only. Numbers of inductions are secondary or tertiary such as nasal-groove, optic vesicle, lens, cornea and so on involve ectodermal reactions.

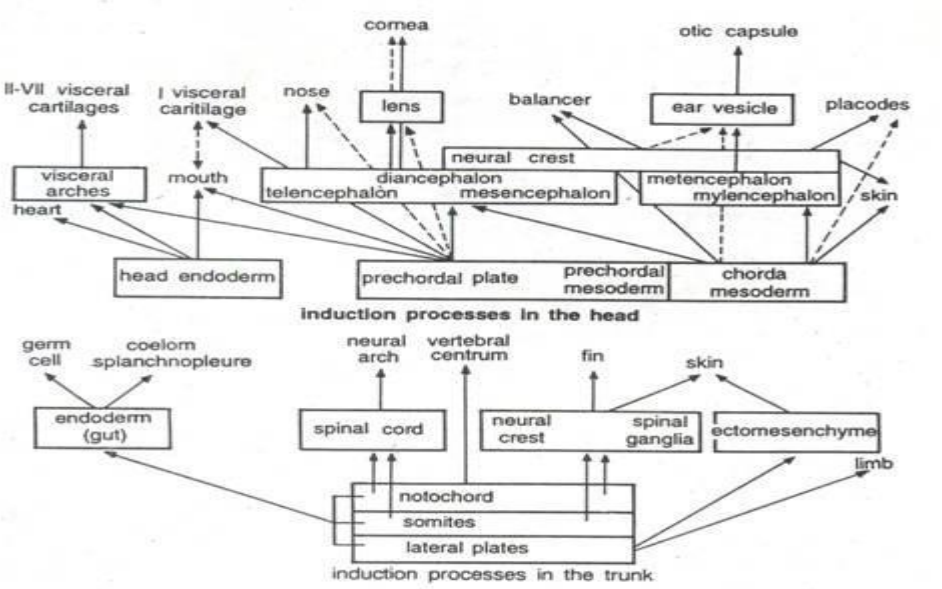

Fig. 9. The induction process during early amphibian development in head and in trunk regions. The inductive action starts from the archenteron roof (inductor of the first degree–primary inductor); the inductors of second, third and following degrees affect the process by their own actions. The different strengths of the inductive action are indicated by thick and broken arrows.

Probable Questions:

1. What are two opinions regarding fate of germ cells.
2. What is Spermateleosis.
3. What are theories regarding the organisation of ovum.
4. Describe primary, secondary and tertiary membranes of ovum.
5. What is exogenous and endogenous induction?
6. What is regional specificity of organizer?
7. What is mechanism of neural induction?
8. What is the role of grey cresent?

Suggested Readings/ References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-III

Specification, determination and differentiation, morphogenetic gradient and fate map

Objective: In this unit we will discuss about Specification, determination and differentiation, morphogenetic gradient and fate map

Meaning of Fate Map:

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study.

A correct interpretation of gastrulation is impossible without a knowledge of the position which the presumptive germinal layers occupy in the blastula. This position may be ascertained in various ways. A chart, showing the fate of each part of an early embryo, in particular, a blastula, is called a fate map. In tracing the fate of various parts of the blastoderm, it is sometimes possible to make use of the peculiarities of the cytoplasm in certain parts of the egg, such as the presence of pigment granules. In the developing amphibian egg, for instance, one may trace in which part of the differentiated embryo the black pigment comes to lie. Originally this pigment is restricted to the animal hemisphere of the egg. However, peculiarities of pigmentation are seldom sufficient to make it possible to reconstruct the fate map in any great detail. Recourse must be had to artificial marking of parts of the blastoderm. A satisfactory method of marking was devised for this purpose by Vogt (1925). The method consists in soaking a piece of agar in a vital dye (Nile blue sulfate, neutral red, Bismarck brown) and then applying the piece of agar to the surface of the embryo in the necessary position. The dye diffuses from the agar, and in a matter of minutes the cells of the embryo to which the agar has been applied take up sufficient dye to produce a stain on the surface of the embryo. This marking-can is done without removing the vitelline membrane, since it is permeable to the vital dyes, and thus the embryo continues to develop normally. The presence of the stain does not change the normal development of the embryo, and the position of the stained cells in the differentiated embryo clearly shows the fate of the stained area. It has been established by trial that the vital dye remains, on the whole, restricted to the cells which had originally taken up the dye and to their descendants.

The diffusion of the stain, if the staining has been done correctly, is negligible and does not interfere with interpreting the results. Several stain marks may be made on the surface of the same embryo, using different colors (red, blue, brown). In this way one experiment may disclose the fate of many parts of the early embryo at the same time. It was later found that cellophane can also be used as a stain carrier instead of agar and that it is actually a more convenient one, as cellophane comes in thin sheets from which it is easy to cut out pieces of the desired size and shape. All vital staining is now done with cellophane as the stain carrier. Independently of vital staining, another method has also been devised for marking cells of a developing embryo. This consists in applying tiny particles of carbon to the 196 surface of the embryo. Carbon particles stick to the surface of the cells and can thus be used as markers enabling the investigator

to follow the movements of the cells and to draw up fate maps. The vital stain marking method was first applied to the reconstruction of the fate map in the amphibian embryo, and the original investigations have subsequently been checked by many embryologists. It is most advantageous, therefore, that the fate map of an amphibian embryo, such as the embryo of a newt (Triturus) or axolotl (Ambystoma), be described first.

Construction of Fate Map:

Fate map of different types of animals have been constructed by the following methods:

i. Observing Living Embryos:

In some invertebrates, the embryos being transparent and having relatively few daughter cells that remain close to one another, it has been possible to look through the microscope and trace the descendants of a particular cell to the organ they subsequently formed.

This type of study was performed by Edwin G. Conklin (1905) in the tunicate, Styela partita, where the different cells contain different pigments. As for example, the muscle-forming cells always have a yellow colour.

ii. Vital Dye Marking:

Most embryos, however, do not have the facilities (transparent, few cells, different colours etc.) as described above in Styela partita. It was in 1929 that Vogt was able to trace the fate of different areas of amphibian eggs by applying vital dyes. These vital dyes stain the cells without killing them.

iii. Radioactive Labelling and Fluorescent Dyes:

A variation of the dye marking technique is to make one area of the embryo radioactive. A donor embryo is taken and grown in a solution containing radioactive thymidine. This thymidine base is subsequently incorporated into the DNA of the dividing embryo. A second embryo, acting as the host embryo, is grown under normal conditions. The region of interest is cut off from the host embryo and is replaced by a radioactive graft from the donor embryo. The cells that are radioactive will be the descendants of the cells of the graft, and are distinguished by autoradiography.

iv. Genetic Marking:

Radioactive and vital dye marking have their own drawbacks such as dilution over many cell divisions and the laborious preparation of slides. One permanent way of cell marking is to create mosaic embryos having different genetic constitutions. The best example of such a marking is to graft quail cells inside a chick embryo. By doing so, fine-structure maps of the chick brain and skeletal system can be made.

Fate Map of Vertebrates:

i. Fate Map of Amphioxus:

The fate map of Amphioxus can be traced at an early stage prior to the onset of cleavage. The presumptive organ forming areas in the un-cleaved egg is given in Fig. 5.32. The future endodermal cells lie at the vegetal pole and would subsequently form the floor or hypoblast of the blastula. The area at the animal pole would form the presumptive ectodermal cells.

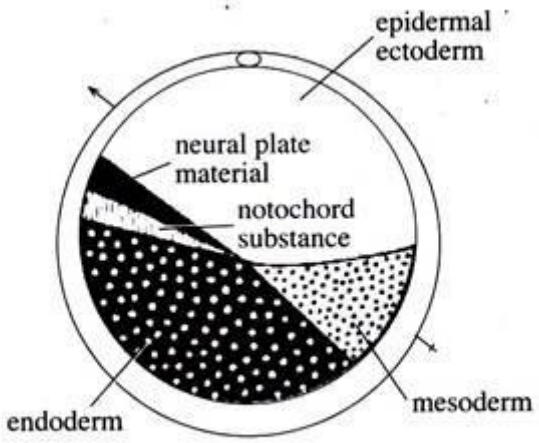

Fig. 5.32 : Presumptive organ forming areas in the uncleaved egg of *Amphioxus*.

The ventral grey crescent area at the future posterior end of the blastula, in between the future ectoderm and endoderm, forms the future mesoderm. Another area, the dorsal crescent, in between the ectoderm and endoderm on the anterior side, gives rise to the notochord and neural cells. The presumptive ectodermal, mesodermal, notochordal and neural cells would subsequently form the epiblast of the blastula.

ii. Fate Map of Frog:

The blastula of Xenopus at the 32 cell stage gives no indication as to how the different regions will develop. However, by following the fate of individual cell, or group of cells, the fate map of the blastula can be made. One way of making the fate map is by staining the various parts of the early embryo with a lipophilic dye such as dil and observe where the labelled regions end up. Another sophisticated way of labelling the blastomeres is by injection of high molecular weight molecules such as rhodamine-labelled dextran, which cannot pass through cell membrane and are, therefore, restricted to the injected cell and its progeny. These cells can be easily detected later, under a UV microscope.

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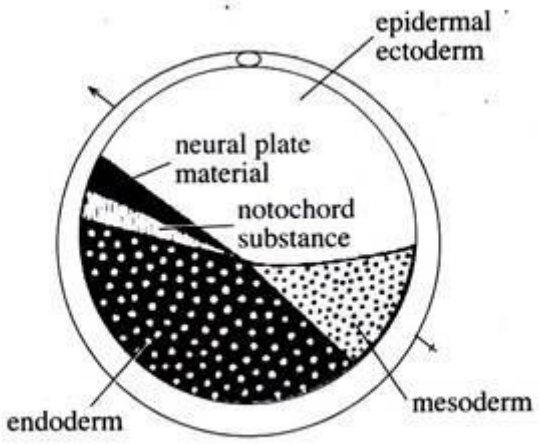

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The fate map of the Xenopus blastula (Fig. 5.33) shows the presence of yolky macromeres at the vegetal pole which gives rise to the endoderm. Depending upon the position of the blastopore, the endodermal area can be divided into the sub-blastoporal and supra-blastoporal endoderm. The cells toward the animal pole gives rise to the ectoderm, which becomes further subdivided into epidermis and the future nervous tissue. The epidermal ectoderm forms at the ventral side of the animal hemisphere, while the neural ectoderm forms at the dorsal side. The mesoderm forms a belt-like region, known as the marginal zone, around the equator of the blastula. The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord. From this ventrally, the- mesoderm is differentiated by the somites (which gives rise to muscle tissue), lateral plate (which contains heart and

kidney mesoderm) and blood islands. In Xenopus, a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone (Fig. 5.33C).

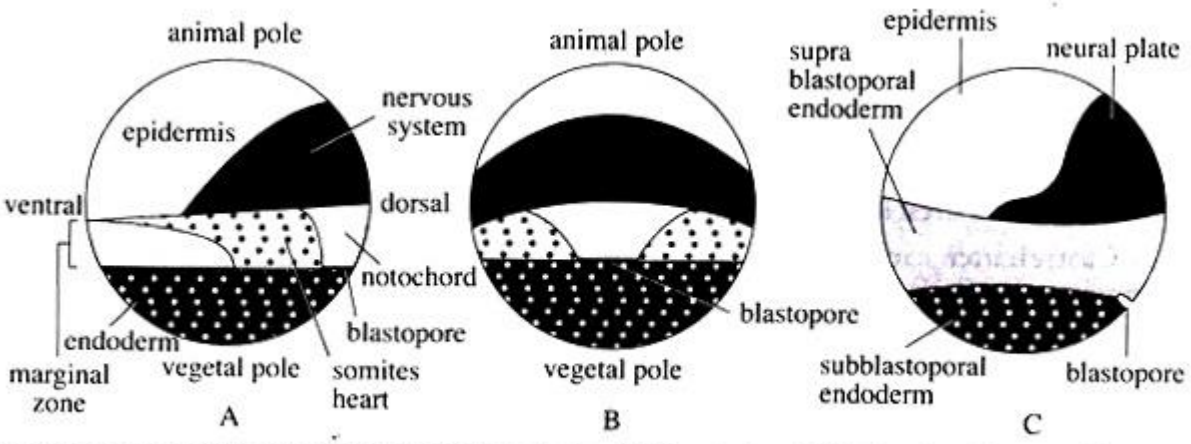


Fig. 5.33 : Fate map of *Xenopus* late blastula (A) Lateral view. (B) Dorsal view. (C) Exterior view showing a thin outer layer of presumptive endoderm overlies the presumptive mesoderm in the marginal zone.

iii. Fate Map of Chick:

Before going through the fate map of chick one should go through the formation of area pellucida and area opaca, and also through the format iod of hypoblast and epiblast. From the study of the above formations, it becomes clear that the hypoblast does not contribute any cells to the formation of the embryo proper, rather they contribute to the formation of a portion of the external membranes. Recent studies with cell adhesion molecules (CAMs), it has become possible to construct the fate map of chick epiblast (Fig. 5.34). All the three germ layers of the embryo proper is formed by the epiblastic cells. The epiblast also forms a considerable amount of extra-embryonic (mesoderm) membrane.

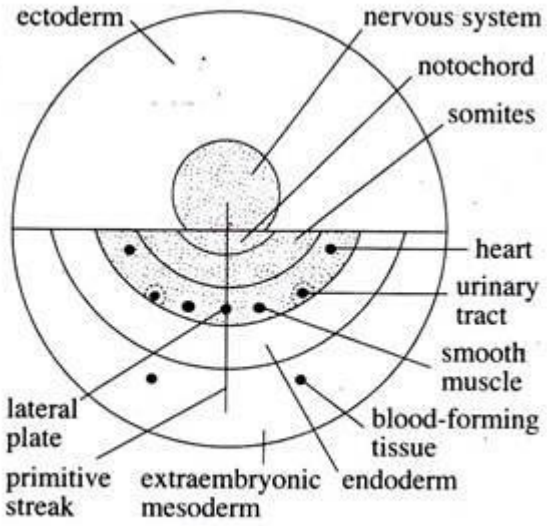


Fig. 5.34 : Fate map of chick embryo.

The fate map of chick (Fig. 5.34) reveals that the cells of the epiblast are organised around the notochord and nervous system. The neural ectoderm is present as a knob-like structure facing towards the anterior side. The cells at the anterior part of the epiblast form the ectoderm, while the cells at the posterior side gives rise to mesoderm (body proper), endoderm and extra-embryonic mesoderm.

Usefulness of Fate Map:

The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis.

Probable Questions:

1. Describe different methods of staining of embryos for fate map preparartion.
2. Describe fate map of frogs and amphioxous.
3. Describe fate maps of chick.

Suggested Readings/ References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-IV

Axis specification in Drosophila: role of maternal effect genes, patterning of early embryo by zygotic genes, gap genes, pair– rule genes, segment polarity genes, homeotic selector genes- bithorax and antennapedia complex

Objective: In this unit we will learn about developmental biology of Drosophila. How anterior posterior and ventral dorsal surfaces are formed bye interaction of different genes.

Introduction: The fruit fly Drosophila melanogaster is a small dipteran insect measuring approx. 3 mm as an adult. The embryonic development of this insect occurs inside an egg which hatches as a larva. Drosophila is easy to breed, hardy, abundant, can withstand diverse conditions. Cellular membranes do not form in Drosophila until after the thirteenth nuclear division. In Drosophila fertilization occurs when sperm enters an egg which is already activated. The Drosophila egg is sausage- shaped which has micropyle, at the future anterior end of the embryo. Sperm enters the egg at micropyle end, which allows entry of one sperm at a time. As the sperm enters the egg fertilization takes place and the zygote undergoes a series of rapid mitotic division (karyokinesis) but without cytokinesis. After 12 nuclear divisions the embryo is called a syncytial blastoderm, it is equivalent to the blastula or blastoderm stage of theother animals.

After thirteenth division blastoderm becomes fully cellular, by the invagination of cell membranes. The cellular blastoderm will give rise to all the future tissues. Gastrulation starts at about 3 hours after fertilization. During gastrulation, endodermal and mesodermal tissues move to their future positions inside the embryo, leaving the ectoderm as the outer layer. The general body plan of Drosophila is same in the embryo, the larva, and the adult possessing a distinct head end, repeating segmental units and a distinct tail. In the repeating segmental units three segments form thorax, while another eight segments form the abdomen. The first thoracic segment has legs; the second thoracic segment has legs and wings and the third thoracic segment has legs and halters (balancing organs).

In early 1990's a powerful "forward genetics" approach was used for identification of most of the genes involved in shaping the larval and adult forms of Drosophila. Flies were randomly mutagenized and screening is done for the mutations that disrupted the normal formation of the body. The genes responsible for the mutant phenotype were cloned and are characterized according to their expression patterns and their functions.

Molecular events critical for Drosophila embryogenesis occur during oogenesis and the single female germ cell- oogonium is the descendant for a single oocyte. The oogonium, before oogenesis begins divides four times with incomplete cytokinesis, to give rise to 16 interconnected cells: 15 nurse cells and the single oocyte precursor. Numerous mRNAs are made in the nurse cells which are transported on microtubules through the cellular interconnections into the enlarging oocyte.

Anterior -Posterior Axis formation in Drosophila:

The follicular epithelium surrounding the developing oocyte is broken by two signals which involve the same gene, gurken organized by the oocyte nucleus. In the oocyte nucleus, gurken gene is localized between the nucleus and the cell membrane and is translated into Gurken protein. The time at which oocyte nucleus is very close to the posterior tip, Gurken signal which results in the "posteriorization" of the follicle cells is received by these follicle cells through a receptor protein encoded by the torpedo gene (Figure 3.1).

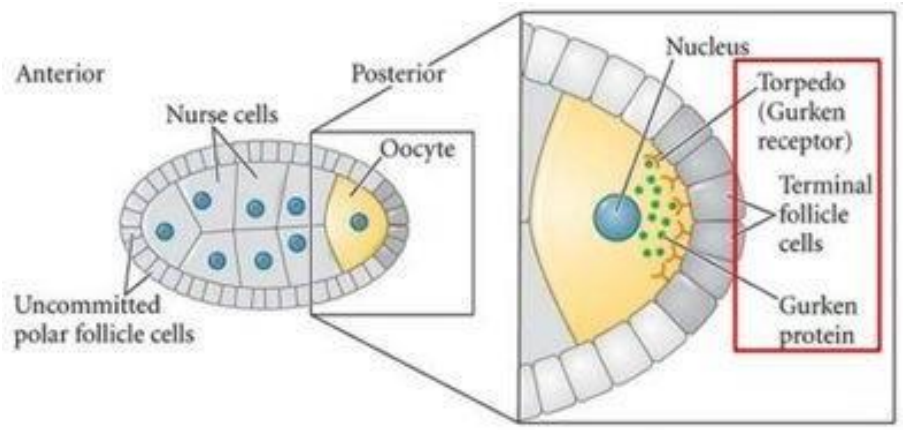


Figure 3.1: Specification of anterior-posterior axis during oogenesis

Anterior -Posterior Body Plan:

Maternal effect genes produce messenger RNAs that are placed in different regions of the egg and encode transcriptional and translational regulatory proteins which after diffusion through the syncytialblastoderm activate or repress the expression of certain zygotic genes.

Maternal genes are expressed by the mother but not by the embryo and during oogenesis they are expressed in the tissues of the ovary. Zygotic genes in contrast are expressed during the development of the embryo in the nuclei of the embryo itself (Figure 4.1). The zygotic genes express in a sequential manner that establishes the body plan along the antero- posterior axis

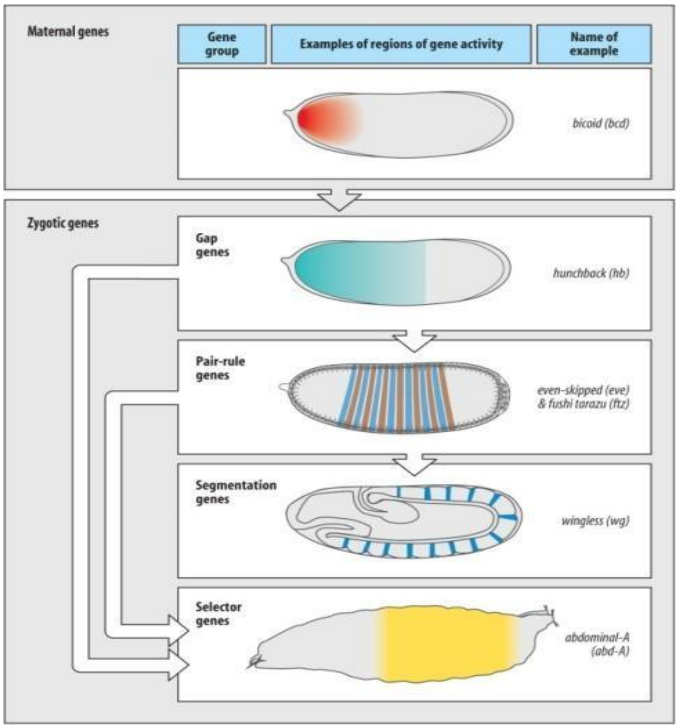


Figure 4.1: Sequential expression of different sets of genes for establishment of the body plan along theantero-posterior axis

Gap genes are the first such zygotic genes to be expressed and encode transcriptional factors. These are called so because mutations in gap genes cause gaps in the segmentation pattern.

Pair – rule genes divide the embryo into periodic units and their transcription is regulated by differing combinations and concentrations of the gap gene proteins. Their transcription results in a striped pattern of seven transverse bands perpendicular to the anterior-posterior axis.

Segment polarity genes are activated by the pair-rule proteins. The mRNA and protein products of the segment polarity genes divide the embryo into 14-segment-wide units and establish the periodicityof the embryo.

Homeotic selector genes which determine the developmental fate of each segment are regulated by the interaction of the protein products of the gap, pair-rule, and segment polarity genes at the same time.

There are three classes of maternal genes which specify the antero-posterior axis (Figure 4.2).

Bicoid gene is anterior class gene and mutations in these genes lead to reduction or loss of head and thoracic structures, even in some cases their replacement with posterior structures.

Mutation in **nanos** which is the gene of the posterior group cause the loss of abdominal regions,results in a smaller than normal larva.

Mutations in the terminal class genes such as **torso** causes loss of both acron and telson.

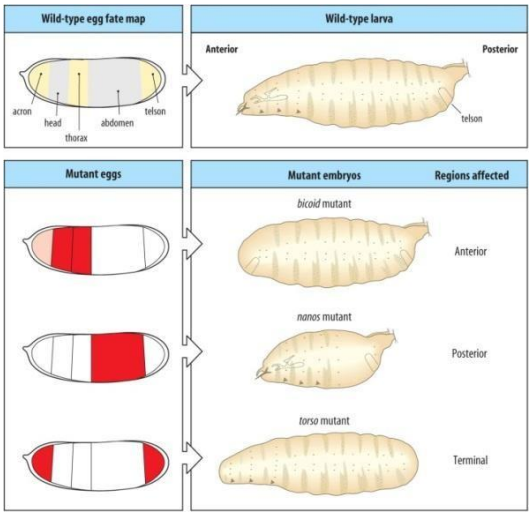


Figure 4.2: Effects of mutations in the maternal gene system

Category	Gene name
Gap genes	Kruppel (kr) hunchback (hb) giant (gt) knirps (kni) hunckebein (hkb) empty spiracles (ems) orthodenticle (otd) buttonhead (btd)
Pair-rule genes	hairy (h) runt (run) even-skipped (eve) fushitarazu (ftz) odd-paired (opa) odd- skipped (odd) sloppy- paired (slp) paired (prd)
Segment polarity genes	engrailed (en) wingless (wg) hedgehog (hh) cubitusinterruptusD (ciD)fused (fu)
Homeotic selector genes	labial (lab) Antennapedia (Antp) sex combs reduced (scr) deformed (dfd) proboscipedia (pb) Ultrabithorax (Ubx)

	abdominal A (abd A) **abdominal B (abd B)**

Table 4.1: Genes affecting segmentation pattern in Drosophila

Nanos mRNA Localization in the Posterior Pole of the Oocyte:

Oskar which is one of the maternal posterior group genes localize nanos mRNA at the extreme posterior pole of the unfertilized egg and specify the posterior germplasm in the egg which gives rise to the germ cells (cells that will give rise to sperm and eggs). Nanos after translation give a concentration gradient of Nanos protein with the highest level at the posterior end of the embryo. Nanos suppress translation of maternal mRNA of hunchback gene by binding to a complex of hunchback mRNA and the protein encoded by the posterior group gene pumilio. Subsequently a clear gradient of zygotically expressed hunchback protein is established which acts as a morphogen for the next stage of patterning (Figure 4.4).

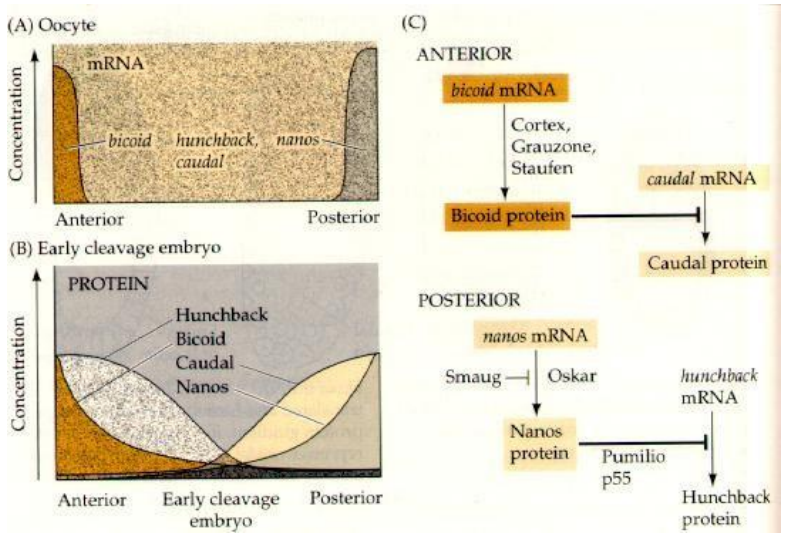


Figure 4.4: Anterior-posterior patterning model by maternal effect genes

Terminal Gene torso specify the Anterior and Posterior Extremities

Torso proteins generate the unsegmented extremities of the anterior-posterior axis: the acron (the terminal portion of the head that includes the brain) and the telson (tail) (Figure 4.5). The torso mRNA which is synthesized by the ovarian cells, deposited in the oocyte and translated after fertilization. Torso must normally be activated only at the ends of the egg because the gain-of-function mutation of torso converts the entire anterior half of the embryo into an acron and the entire posterior half into a telson.

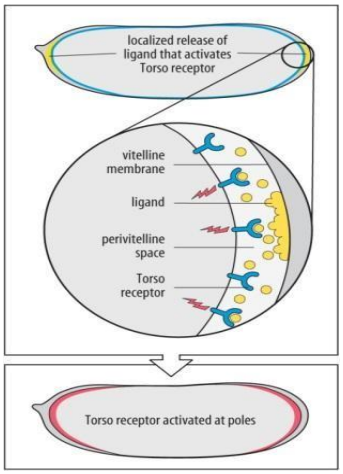


Figure 4.5: Specification of the terminal regions of the embryo by Torso receptor protein Gapgenes

Gap genes define regional differences and are activated or repressed by the maternal effect genes. These genes are expressed in the anterior-posterior domain (Figure 4.6). Examples of gap genes are hunchback, kruppel, and knirps, giant, tailless etc.

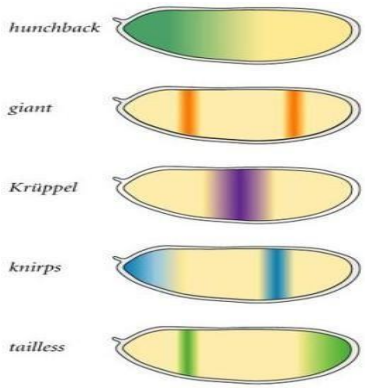


Figure 4.6: Expression of gap genes in the early drosophila embryo

Certain threshold level of of Bicoid enhances the level of Hunchback in the anterior region of the embryo. Hunchback protein is a transcription factor and acts as a morphogen to which other gap genesrespond.

- **Kruppel gene:** High level of Bicoid and low level of Hunchback induces its expression, but high concentration of Hunchback represses expression of kruppel gene. Due to this the expression of kruppel gene is restricted at the centre of the embryo (Figure 4.7).

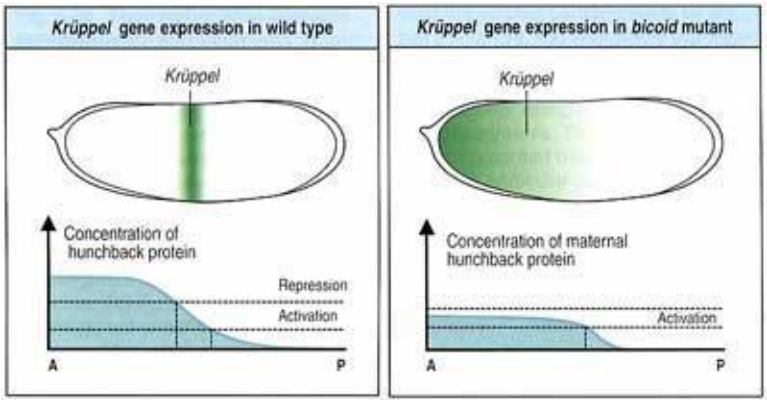


Figure 4.7: Specification of kruppel gene activity by Hunchback protein

- When high concentration of Hunchback is present kruppel is repressed and it is also repressed when Hunchback is present below the threshold concentration.
- In mutants where bicoid is not present, zygotic hunchback gene expression is also absent, so maternal Hunchback is present at the anterior end of the embryo in low level, so expression of kruppel gene is restricted to the anterior end.
-

Pair-Rule Genes

The primary pair-rule genes, hairy, even-skipped, and runt are expressed in seven stripes where each stripe corresponds to every second parasegment. There are pair-rule genes which define odd numbered parasegments (e.g, even-skipped), whereas others define even-numbered parasegments (e.g, fushitarazu). Bicoid and hunchback proteins activates the even-skipped gene, though the boundaries of the stripe are defined by kruppel and giant proteins by repressing even-skipped at posterior and anterior edge of the stripe respectively. In contrast, fushitarazu are not regulated by the gap genes, but they may depend on the prior expression of primary pair-rule genes such as even-skipped and hairy.

Segment Polarity Genes

Segment polarity genes are activated in response to pair-rule gene expression and have two important functions to perform:

- They reinforce the parasegmental periodicity established by the earlier transcription factors.
- Establishing cell-to-cell signaling and cell fates within each parasegment.

Segmentation genes acts in a cellular environment as during pair-rule gene expression the blastoderm becomes cellularized. One of the segmentation genes is engrailed which has a key role in segmentation and is activated by the pair –rule genes. It is activated in cells that have high levels of the even-skipped, fushitarazu, or paired transcription factors. Transcription of engrailed gene marks the anterior compartment of each parasegment and the posterior compartment of a segment. Mutation in engrailed gene cause transformation of posterior compartment in clones of wing cells whereas in the minute clones, the anterior and posterior parts of the segment are not confined andthere is no compartment boundary (Figure 4.8).

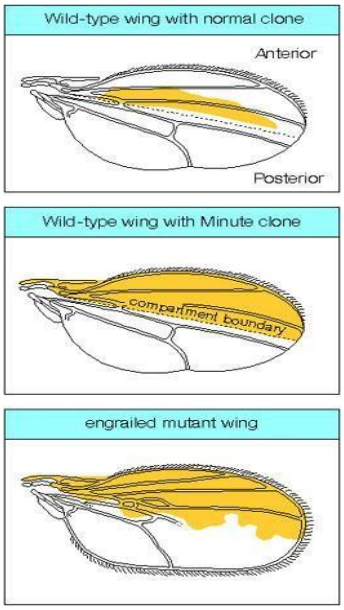


Figure 4.8: Demonstration of the boundary between between anterior and posterior compartments in thewing by marked cell clones

An intercellular signaling circuit sets up between the adjacent cells and delimits the boundary between the parasegments. Three segmentation genes are involved in this circuit. These are wingless, hedgehog and engrailed, which are expressed in restricted domains within the parasegment. The secreted signal hedgehog is expressed in the cells expressing engrailed. Subsequently in the row of cells immediately anterior to the engrailed-expressing cells the expression of the signal protein is activated. And this secreted wingless protein gives a signal that feeds back over the parasegment boundary to maintain hedgehog and engrailed expression. This signal stabilizes and maintains the compartment boundary.

Homeotic Selector Genes:

Specification of each segment is defined by homeotic selector genes. In Drosophila two homeotic gene clusters are present named as **bithorax complex** and **Antennapedia complex** and the chromosome region containing these complexes are referred to as the **homeotic complex (Hom-C)** (Figure 4.9).

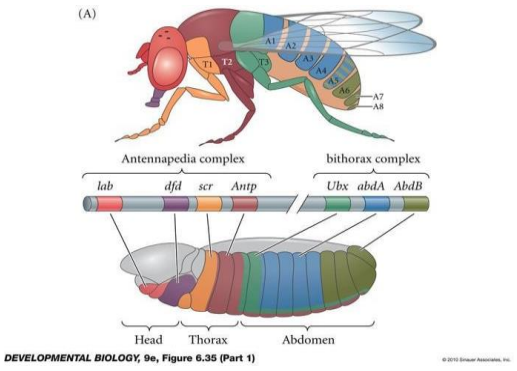


Figure 4.9: Expression of homeotic selector gene in Drosophila

- **Bithorax complex**: these are responsible for diversification of posterior segments. It contains three genes,

- **Ultrabithorax: mutation** in this gene causes transformation of haltares into wings, i.e. fly results in four wings (Figure 4.10).

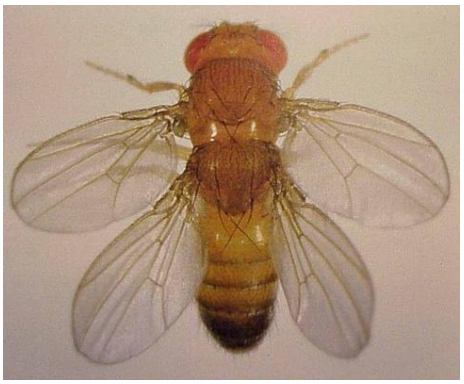


Figure 4.10: Mutations in the ultrabithorax produce a four-winged fly

- **Abdominal A (abd A) and Abdominal B (abd B):** segmental identities of abdominal segment is regulated by these two genes.

- Antennapedia complex: It is a complex of five genes,

- labial (lab)
- deformed (dfd)
- Antennapedia (Antp)
- sex combs reduced (scr)
- proboscipedia (pb)

labial and deformed genes are involved head segment specification, whereas Antp and scr are responsible for thoracic segment specification. Gene pb is active only in the adult flies, due to its mutation transformation of labial palp of mouth into legs occurs.

Dorsal-Ventral Patterning in the Oocyte:

The movement of oocyte nucleus occurs towards anterior dorsal position with increase in volume of oocyte. Message of gurken gene is localized in crescent between the oocyte nucleus and the oocyte cell membrane. The product of gurken gene is Gurken protein which forms an anterior-posterior gradient along the dorsal surface of oocyte. Gurken gene is present only in oocyte, whereas torpedo is active only in the somatic follicule cells. Follicle cells contain Torpedo receptor protein which receives Gurken signals. The Torpedo signal inhibits the expression of pipe gene, because of which Pipe protein is produced only in the ventral follicle cells.

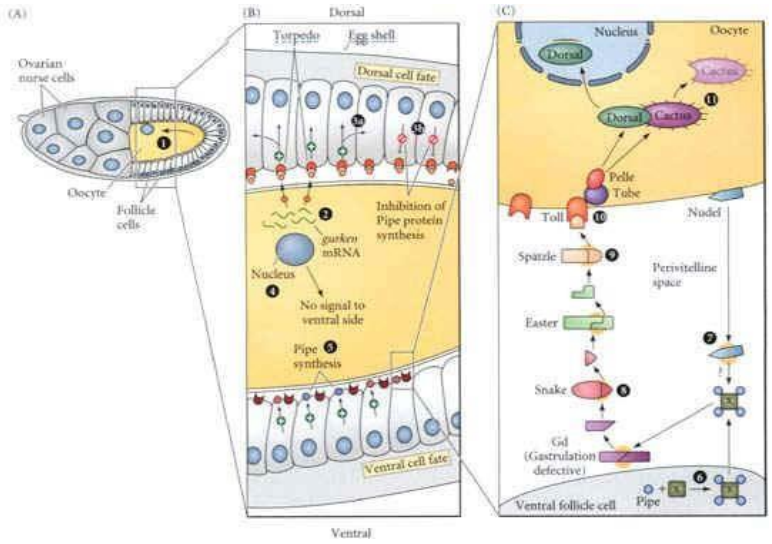


Figure 5.1: Dorsal-ventral patterning in Drosophila

During formation of ventral region of oocyte with the help Pipe protein which is only activated in the ventral cells modifies an unknown factor "X''. Nudal protein and factor "X'' splits Gd (Gastrulation defective) and Snake protein, which in turn activates Spatzle protein. Spatzle protein binds to Toll receptor protein (which is present throughout embryonic cell membrane). The dorsal cell receives Toll signal and separates the Cactus protein from the Dorsal protein due to which Dorsal protein gets translocated into the nuclei which leads to ventralization of cells (Figure 5.1).

Dorsal and Ventral Pattern in the Embryo

Product of the gene dorsal is involved in the Dorsal-ventral patterning of the embryo. Mother gene dorsal produces Dorsal protein which which is placed in the oocyte by nurse cells. Dorsal protein is also known as ventral morphogen. In the syncytial blastoderm of early Drosophila embryo Dorsal protein is present everywhere, but in late embryo Dorsal protein is translocated only in the ventral part. During ventral specification Dorsal enters the nucleus and represses the genes responsible for the dorsal specification, so the embryo becomes specified as ventral cell. But if this does not happen all the cells of embryo becomes specified as dorsal cell. If the dorsal gene or Dorsal protein is absent, all the ventral cells becomes dorsalized.

During ventral patterning the product of dorsal gene is translated in Dorsal protein which remains complexed with Cactus protein in cytoplasm. When the Spatzle binds to Toll protein it gets activated andin turn it activates a protein kinase called Pelle. Pelle remains in bounded form with Tube protein. Onceactivated Pelle phosphorylates Cactus, which is then degraded and in turn Dorsal protein becomes free to enter the nucleus. This process establishes Dorsal protein gradient in the nucleus of embryo. In the ventral cell nuclei the gradient of Dorsal protein is highest.

Effect of Dorsal Protein in Embryo

As the Dorsal protein enters the nuclei, it defines the dorsal-ventral axis on the basis of gene expression. At this stage Dorsal protein also distinguishes the germ layers and specifies the ventral most cells as prospective mesoderm. In the nucleus Dorsal protein acts as transcriptional activator of ventralizing genes (snail, twist, and rhomboid) and a transcriptional repressor of the dorsalizing (decapentapelagic, zerknullt and tolloid) in the ventral region.

- **twist and snail** are activate where the internuclear concentration of Dorsal is highest. These genes are responsible for development of cells as mesoderm and for gastrulation.

- **rhomboid gene** is activated where low level of Dorsal protein is present, and it acts as future neuroectoderm. In more ventral region they are repressed by Snail protein.

- **decapentapelagic, tolloid and zerknullt** are repressed by Dorsal proteins (which is present mainly in the ventral region), so are expressed in the dorsal region of the embryo (where there is no Dorsal protein in the nuclei).

- **Zerknullt** gene is expressed in the dorsal most region of the embryo and forms aminoserosa.

- **decapentapelagic** gene is involved in the specification of dorsal part of embryo where no Dorsal protein is present and also specifies dorsal ectoderm. dpp is a member of TGF-β family of cytokines. Decapentapelagic is homolog of bone morphogenetic protein-4 (BMP-4) present in vertebrates (Figure 7.1).

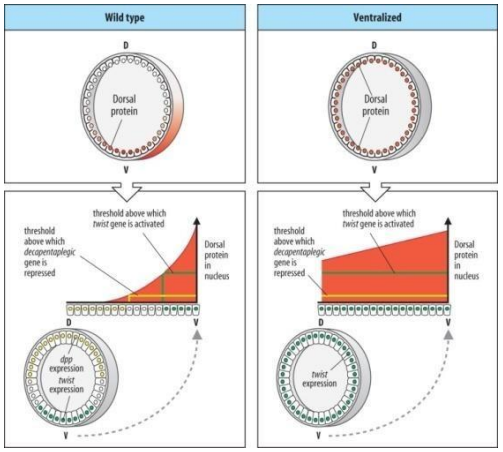


Figure 7.1: Nuclear gradient of Dorsal protein

Mutation in Dorso-Ventral Maternal Gene:

- **Dorsalized embryo:** When Dorsal protein is excluded uniformly from the nuclei, decapentapelagic is no longer repressed and this leads to its expression everywhere. Whereas twist and snail are no longer expressed in dorsalized embryos, as they require high level of internuclear Dorsal protein level.

- **Ventralized embryo:** When Dorsal protein is present is present at high concentration in all nuclei, twist and snail areal so expressed everywhere, whereas decapentapelagic is not present at all.

Probable Questions:

1. Describe the process of pattern formation in Drosophila.
2. What is Homeobox genes and mention its role in development?
3. How the anterior posterior side is determined in Drosophila?
4. How ventral dorsal side is determined in Drosophila?
5. Describe the role of homeotic genes in Drosophila development.
6. Describe the role of gap genes in Drosophila development.
7. Describe the role of pair rules genes in Drosophila development.
8. Describe the role of segment polarity genes in Drosophila development.
9. Describe the role of dorsal proteins in Drosophila development.

Suggested Reading/ References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

Unit- V

Axis specification in vertebrates:

i) Early patterning in vertebrates - Symmetry breaking, Nieuwkoop center. Wnt and cadherin signaling

ii) Left- right asymmetry in vertebrates - Asymmetric gene expression

Objective:

In this Unit we will discuss about the Axis specification in vertebrates: Early patterning in vertebrates - Symmetry breaking, Nieuwkoop center. Wnt and cadherin signalling and also about Left- right asymmetry in vertebrates - Asymmetric gene expression

Early patterning in vertebrates

In vertebrates, the development of the nervous system is triggered by signals from a powerful 'organizing' region of the early embryo during gastrulation. This phenomenon—neural induction—was originally discovered and given conceptual definition by experimental embryologists working with amphibian embryos. Work on the molecular circuitry underlying neural induction, also in the same model system, demonstrated that elimination of ongoing transforming growth factor-β (TGFβ) signalling in the ectoderm is the hallmark of anterior neural-fate acquisition. This observation is the basis of the 'default'model of neural induction. Endogenous neural inducers are secreted proteins that act to inhibit TGFβ ligands in the dorsal ectoderm. In the ventral ectoderm, where the signalling ligands escape the inhibitors, a non-neural fate is induced. Inhibition of the TGFβ pathway has now been demonstrated to be sufficient to directly induce neural fate in mammalian embryos as well as pluripotent mouse and human embryonic stem cells. Hence the molecular process that delineates neural from non-neural ectoderm is conserved across a broad range of organisms in the evolutionary tree. The availability of embryonic stem cells from mouse, primates, and humans will facilitate further understanding of the role of signaling pathways and their downstream mediators in neural induction in vertebrate embryos.

Establishment of the neuroectoderm in vertebrates:

In all vertebrates, the fertilized egg divides to generate a blastocyst (or blastula). Three different territories called embryonic germ layers, ectoderm, mesoderm, and endoderm, emerge in the blastula. In the amphibian embryo, where the dorsal (D) and ventral (V) sides of the embryo are specified during fertilization, each germ layer has a distinct D–V polarity and is fated to generate different tissues as the embryo matures. Subsequently during gastrulation, the primitive ectoderm (called epiblast) covers the outside of the embryo and forms different tissue derivatives depending on position along the embryonic D–V axis. The central nervous system (CNS) derives from the most dorsal region of the ectoderm, which thickens and flattens after gastrulation to form the neural plate. During subsequent stages, the plate rolls into a tube, separates from the overlying epidermis, and goes on to form the brain at the anterior, and spinal cord at the posterior end. In contrast, on the ventral side, most of the remaining ectoderm forms the epidermis. The neural crest forms where the dorsal and ventral boundaries meet at the edge of the neural plate. This progenitor cell population detaches and migrates throughout the embryo to form the peripheral nervous system, cranium, and cartilage of branchial arches. Ectodermal cells at the most anterior edge of the neural–epidermal boundary give rise to placodal areas that will form sensory organs—such as the ear and nose—as well as some cranial sensory ganglia (Figure 1). At the start of gastrulation, cells from any part of the ectoderm can still develop as either epidermis or neural tissue, but by the end of gastrulation commitment has occurred. These events are characteristic of all vertebrates although the timing and geometry vary across phylogeny. Thus, the first step in the establishment of the nervous system invertebrates involves the partition of the ectoderm into epidermal and neuroectodermal primordia during gastrulation.

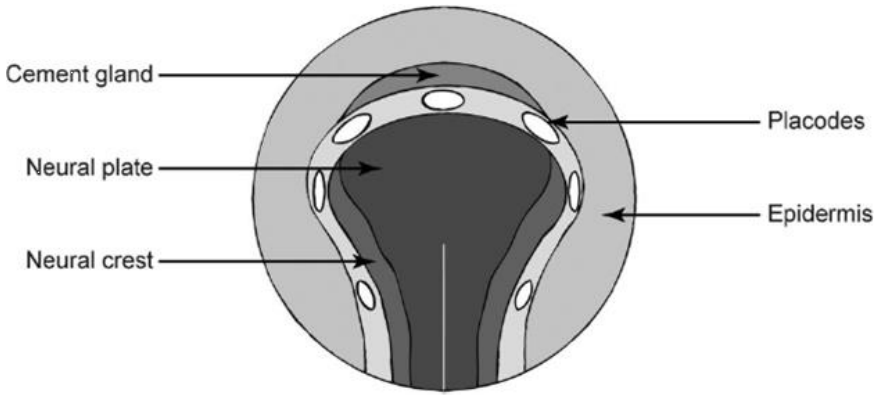


Figure 1: Fate map of the anterior border of the neural plate in Xenopus embryos. Schematic of dorsal–anterior (head-on) view of a Xenopus neurula (the ventral side is up, and the dorsal side is down). Different colors highlight different fates.

Symmetry breakage in the vertebrate embryo

Asymmetric development of the vertebrate embryo has fascinated embryologists for over a century. Much has been learned since the asymmetric Nodal signalling cascade in the left lateral plate mesoderm was detected, and began to be unravelled over the past decade or two. When and how symmetry is initially broken, however, has remained a matter of debate. Two essentially mutually exclusive models prevail. Cilia-driven leftward flow of extracellular fluids occurs in mammalian, fish and amphibian embryos. A great deal of experimental evidence indicates that this flow is indeed required for symmetry breaking. An alternative model has argued, however, that flow simply acts as an amplification step for early asymmetric cues generated by ion flux during the first cleavage divisions. In this review, we critically evaluate the experimental basis of both models. Although a number of open questions persist, the available evidence is best compatible with flow-based symmetry breakage as the archetypical mode of symmetry breakage. Establishment of left–right asymmetry of animal body plans is of the utmost importance for embryonic development and adult health. During vertebrate embryogenesis, the cardiovascular system, the organs of the chest and abdomen, and even the brain, develop morphological and/or functional asymmetries. Developmental defects in laterality

specification and asymmetric morphogenesis are sometimes compatible with embryogenesis, and occasionally, fully mirror-image individuals develop to term. Left–right (L–R) defects are often much less pervasive and usually strike organs at random, resulting in severe visceral misalignment, organ malformations and malfunctions. Asymmetric organ morphogenesis is preceded by an asymmetric signalling cascade, which initiates during neurulation in the left lateral plate mesoderm (LPM). This so-called Nodal cascade consists of the TGFβ-type growth factor Nodal, its secreted feedback repressor Lefty (also known as Antivin) and the homeodomain transcription factor Pitx2. Expression of this cascade is both necessary and sufficient to induce the correct asymmetric placement of organs.

How the Nodal cascade becomes asymmetrically expressed constitutes a conceptual cell- biological problem, because zygotes typically lack recognizable morphological or functional asymmetries that could initiate it. Brown and Wolpert (1990) proposed the concept of an intrinsic biochemical-structural chirality (represented in their model by an "F-molecule"), even though uniformly distributed, such a molecule would operate by undergoing chiral alignment against the A–P and D–V axes of the embryo. Then other molecular interactions feeding off the deduced L–R vector would eventually lead (via an unknown number of steps) to the morphogenetic process of asymmetric organ development. Amongst the animals classified as the bilateria, two cytoskeleton-dependent chirality have been identified, whose mechanism of action fulfill the conceptual nature of the F-molecule hypothesis (though not being single molecules in the initial invocation of the model). Interestingly, although expressed at two different developmental stages, both of these instances result in asymmetric activation of the Nodal pathway. In spirally cleaving snail embryos, asymmetric positioning of the spindle apparatus during cleavage induces Nodal asymmetry, ostensibly by repositioning maternally synthesized factors. In embryos of most vertebrates, including fish, frogs and mammals, chiral rotation of cilia polarized to the posterior pole of cells produces a vectorial leftward flow of extracellular fluids. This flow is necessary and sufficient for Nodal-dependent symmetry breakage, and substantial enough to sweep fluorescent latex microbeads across from one side of the ciliated epithelium to the other. Although both mechanisms lead to asymmetric Nodal activity, they seem to have little else in common, raising the question as

to why and how flow-type symmetry breakage has evolved. We have recently addressed this problem in a hypothesis article, and will not repeat this issue here. One major difference between the two strategies is that one (spiral cleavage in spiralian protostomes) is determined maternally and acts during very early cleavage stages, while the other (leftward flow) operates much later, and depends on zygotic gene expression during neurula stages.

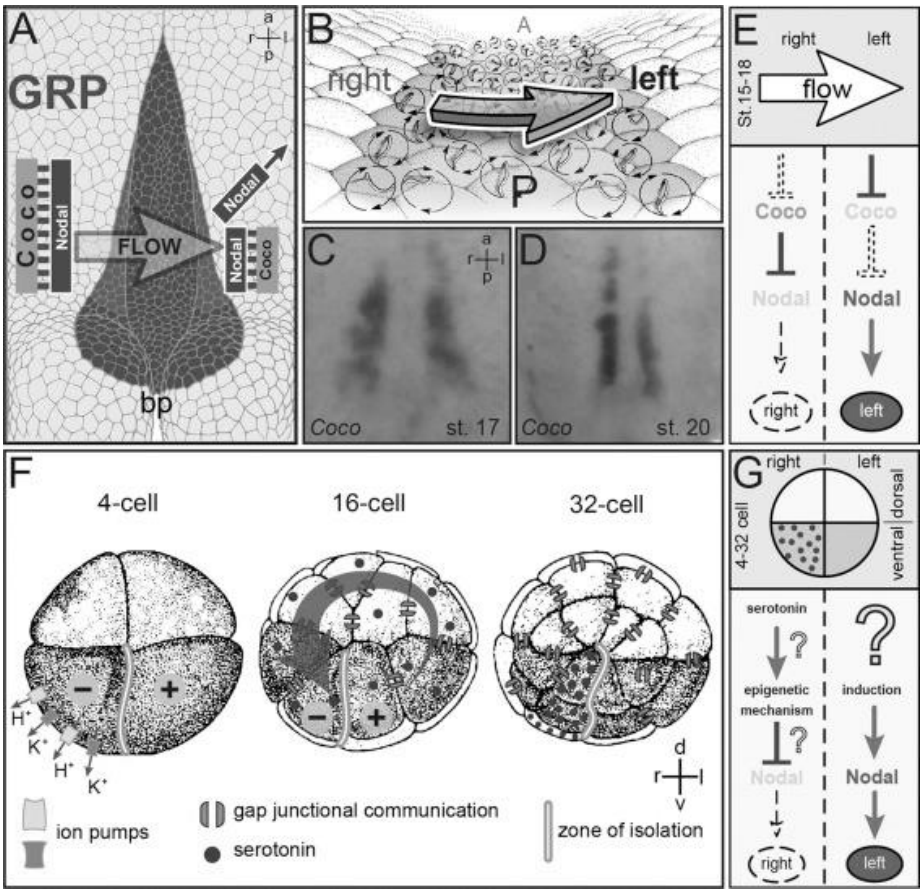


Figure. Prevailing models of symmetry breakage in the frog Xenopus. (A–E) Leftward flow. (A) Schematic representation of a stage 17 archenteron roof in ventral perspective. Flow occurs from the right to the left side of the ciliated gastrocoel roof plate (GRP; red). Nodal and Coco are co-expressed at the lateral GRP margins on both sides (purple). Flow represses Coco, activating Nodal by release of repression. bp, blastopore. (B) GRP at higher magnification. Polarized and flow-producing cilia at the GRP center are bordered by Nodal/Coco-positive cells (purple) which harbor unpolarized, sensory cilia. (C and D) Coco expression during (C) and following (D) leftward flow. Note the decrease in signal intensity on the left at post-flow stage 20 (D). (E) Schematic depiction of events on the left and right

side leading up to asymmetric Nodal cascade induction in the left lateral plate mesoderm (LPM). (F and G) Ion-flux. (F) Asymmetrically expressed ion pumps create a voltage gradient in the 4-cell embryo which initiates the electrogenic transfer of serotonin through gap junctional communication to the ventral-right lineage at the 32-cell stage. Serotonin accumulates in this lineage because the ventral midline is devoid of GJC. (F) Schematic depiction of events on the left and right side leading up to asymmetric Nodal cascadeinduction in the left LPM. Question marks indicate unproven interactions and mechanisms.

Although most of the vertebrates examined so far utilize cilia-generated flow to initiate the asymmetric Nodal cascade, two alternative strategies have also been observed. First, in the chick, large-scale whole-cell repositioning during gastrulation results in a significant asymmetry in the morphology of Hensen′s node, and this appears to play a role in the asymmetric expression of specific intercellular signalling molecules. Second, in amphibians, asymmetric localization of determinants has been proposed to act during early cleavage stages. According to this view, cytoskeletal motor proteins asymmetrically transport a maternal deposit of the ion pump ATP4 (as mRNA and/or translated protein), changing its distribution from a symmetric to an asymmetric one. This asymmetry is hypothesized to generate an intracellular pH and voltage gradient, along which the small charged monoamine, serotonin, transfers via gap junctional communication (GJC) to blastomeres on the right side of the cleavage stage embryo. Almost one day later, when the embryo consists of thousands of cells, this right-sided serotonin asymmetry, by an unknown epigenetic mechanism, is proposed to repress Nodal activity on the right side, thereby initiating the left-asymmetric activation of the Nodal cascade. This mechanism for symmetry breakage will be referred to herein as the “ion-flux” model. Consistent with this model, asymmetries in serotonin, ATP4 and ATP6 were reported in early cleavage stage embryos. In addition, blockage of GJC or mild interference with cytoskeletal dynamics reportedly disrupts L–R development.

When cilia-driven leftward flow was found in the neurula of the Xenopus embryo, as observed previously in mouse, rabbit, zebrafish and medaka, the question arose as to which mechanism is principally instructive for breaking symmetry in the amphibian embryo. Advocates of the ion-flux model have suggested that, throughout the animal kingdom, symmetry breakage occurs very early, i.e. in the zygote or during the first two cell divisions, and that the function of cilia-driven fluid flow must therefore be restricted to a later-stage amplification step. Here, we present our view of the conceptual problems with the ion-flux model, and evaluate the salient experimental support for each of the two opposing models.

Embryological considerations: Spemann′s organizer and left–right asymmetry

A mechanism that breaks symmetry during early cleavage stages is likely to be independentof L–R orientational cues that derive from the gastrula, or Spemann′s organizer. In contrast, a mechanism that operates during or after gastrulation is likely to be strongly influenced by, or even be mandatorily dependent on, the organizer. Experimental analysis of organizer function on L–R asymmetry should thus provide an answer as to when symmetry is broken.

Spemann′s and the left–right organizer: how are they related?

In frogs, symmetry breakage via cilia-driven leftward flow is intrinsically tied to Spemann′s organizer. The ciliated gastrocoel roofplate (GRP), where leftward flow develops during neurulation, is derived from the superficial mesoderm (SM) of the gastrula. The SM constitutes the superficial cell layer of a region that sits above Spemann′s organizer during early gastrulation (Fig. 2A and B). It is thus sandwiched between prospective neuroectoderm and the more vegetally located epithelial layer of the organizer. In the early gastrula, the SM expresses foxj1, the main control gene for motile cilia. In addition, both the organizer and the central part of the GRP contain cells with eventual notochordal fate, reflecting their close relationship. Ciliated, flow-generating epithelia in other vertebrates, despite their common function, display a wide morphological variety. In this review they willbe referred to as left–right organizers (LRO). In rabbit and frog, for example, the LRO develops as a flattened epithelial plate, while it appears as a concavity in the mouse, as a raised dome in medaka, and as a completely enclosed, hollow vesicle in zebrafish.

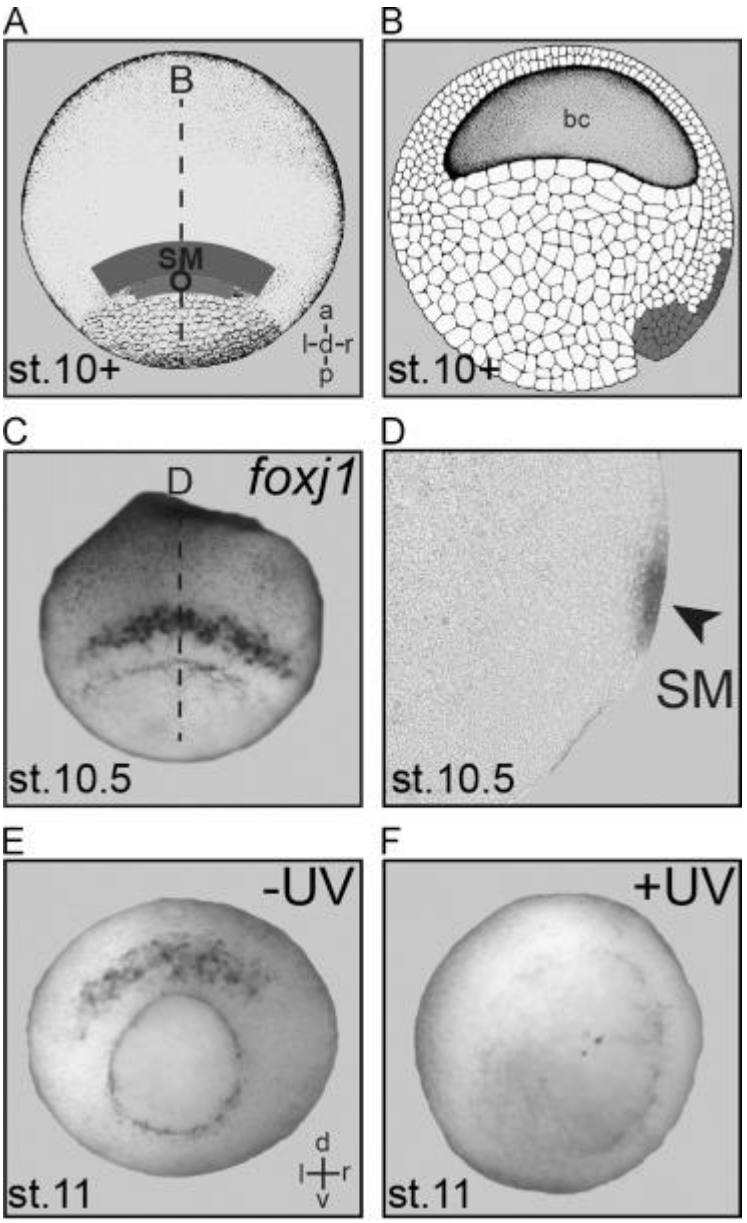


Figure . Structural and functional relationship of Spemann′s organizer and superficialmesoderm. (A) Schematicdepiction of superficial mesoderm (SM; red) and organizer (O; green)in whole-mount stage 10^{+} gastrula embryo shown in dorsal view. (B) Arrangement of organizer and SM in a sagittal section. (C) SM foxj1 expression in a whole-mount gastrula embryo. (D) Sagittal section (plane indicated by dashed line in C) demonstrates foxj1 mRNA in the SM (arrowhead). (E and F) Loss of SM foxj1 expression (E) in UV-ventralized gastrula embryo (F), demonstrating the dependence of SM specification on organizer function.

Nieuwkoop Center: Nieuwkoop Center is the Primary Organizer forming centre seen in the developing egg of some Amphibians. In Xenopus, the Nieuwkoop Center is the dorsal- and vegetal-most region.It gives rise to the Primary Organizer called Spemann-Mangold Organizer which is the regionknown as the dorsal lip of the blastopore (DLB). Spemann and Mangold's experiments found that the DLB dorsalizes surrounding tissue, thus forming the dorsal-ventral axis. In addition to dorsalizing surrounding tissue, the Primary Organizer fates overlying ectoderm as neural

plate tissue; and is determined to be notochord tissue. Dorsalized tissue gives rise to somites and pronephric tubules. Spemann- Mangold Experiment revealed that

1. A transplanted vegetal dorsal cell (from the DLB) induces a new axis.
2. A transplanted vegetal dorsal cell does not itself give rise to new dorsal tissues.
3. A transplanted vegetal dorsal cell restores other cells to correct fates.

The DLB uses induction via secreted diffusible signals. A cell that can be induced is competent; embryonic tissues are only competent during gastrulation. The use of diffusible substances was proven when dorsal lip tissue and ectoderm were cultured together, but separated by a filter with a 0.5µm pore; the ectoderm was induced into neural tissue, despite no cell processes seen to pass through the filter.

The importance of the Nieuwkoop Centre in development is

1. The Nieuwkoop Centre sets up D/V polarity in the blastula and is essential for normal development.
2. The 1st cleavage cuts through the site of sperm entry and the Nieuwkoop Centre.
3. The 2nd cleavage splits the embryo into 4 cells.

Wnt Signalling and Cadherins Compete for beta-Catenin

The concept that canonical Wnt signalling and cadherin-mediated cell adhesion depend on the same pool of □-catenin is based on genetic and overexpression experiments in embryos and cultured cells. Heasman et al. showed in 1994 that overexpression of cadherins in *Xenopus* embryos inhibited dorsal axis formation, which is a clear function of canonical Wnt signalling. Peifer and collaborators showed that armadillo (beta-catenin) mutant embryos of Drosophila, which harbor only one E-cadherin allele, showed a less severe segment polarity phenotype than embryos with two cadherin alleles. Segment polarity is controlled by Wnt signalling. Similarly, cadherin overexpression mimicked the wingless (Wnt) phenotype in Drosophila embryos. These data from the mid 1990s are thus consistent with a model in which there is crosstalk between □-catenin in two different compartments, the adhesion complex at the plasma membrane and a signalling complex in the nucleus. Shortly after these findings in model organisms, Geiger and Ben-Ze'ev and collaborators showed an interplay between cadherin mediated cell adhesion and canonical Wnt signalling in cell

culture experiments. In colon cancer cells, expression of N-cadherin or an interleukin receptor-cadherin hybrid (in which the □-catenin binding region of N-cadherin was maintained) triggered a relocation of b-catenin from the nucleus to the plasma membrane and inhibited LEF1-mediated transcription. Inducible expression of the Fos proto-oncogene in mammary gland epithelial cells resulted in the loss of E-cadherin and cell polarization, the colocalization of b-catenin with LEF1 in the nucleus, and increased Wnt/b-catenin signalling. Moreover, the absence of E-cadherin in E-cadherin -/- embryonic stem (ES) cells led to an accumulation of b-catenin with LEF1 in the nucleus and activation of a Wnt reporter. This could be antagonized by expression of E-cadherin. Behrens and collaborators have recently shown that siRNA-mediated knockdown of E-cadherin augments b-catenin-dependent transcription in colon cancer cells in which the Wnt pathway is active. On the other hand, the same procedure has no effect in nontransformed keratinocytes that do not display Wnt signalling. These data indicate that the mere loss of E-cadherin does not activate Wnt signalling—except in cases in which the b-catenin degradation machinery is compromised. These results are consistent with data from breast cell cancer lines showing that the absenceof E-cadherin alone does not result in activation of Wnt sigalling. Nor does a loss of E- cadherin function in Rip1Tag2 transgenic mice contribute to Wnt/b-catenin sigalling. Weinberg and collaborators recently showed that in rastransformed mammary gland cells (HMLER), shRNA down-regulation of E-cadherin results in translocation of □-catenin from cell–cell junctions to the cytoplasm and nucleus. This type of □-catenin was nonphosphorylated and thus was not targeted for ubiquitination and degradation. However, inthis system, the loss of E-cadherin affected numerous other sigalling pathways that have beenimplicated in metastasis formation. Gottardi and Gumbiner have performed precise studies to determine what controls b-catenin targeting to cadherin adhesion or to TCF transcriptional complexes. They showed that Wnt sigalling generates a monomeric, intramolecularly folded- back form of b-catenin that binds TCF but not cadherins. In contrast, the cadherin binding form of b-catenin builds a dimer with a-catenin. X-ray crystallographic studies have shown that cadherin-binding involves all 12 armadillo repeats of b-catenin, whereas TCF binding requires only the central eight repeats. Thus, it is possible that the carboxyl terminus of Wnt- produced b-catenin folds back over armadillo repeats, affecting binding to cadherin but not TCF. The selective binding of b-catenin induced by Wnt could also involve posttranslational

modifications or the activation of further proteins. Overall, these data suggest that b-catenin's selectivity between adhesion and transcription are not always coupled; in other words, they might be regulated independently. BCL9 protein, the product of a human proto-oncogene, also acts in the switch between cadherin cell adhesion and b-catenin signalling. This story has been worked out through work on BCL9 and its ortholog legless, a Drosophila segment polarity gene. Legless, which was isolated in 2002 by the group of Konrad Basler, is required for Wnt signalling in the fly. It acts by binding directly to b-catenin. Human BCL9 was discovered in a B-cell lymphoma because of a translocation to the immunoglobulin locus, which caused BCL9 overexpression in the tumors. Remarkably, human BCL9 could rescue the segment polarity phenotype of the legless mutation, indicating functional identity. Vertebrates have a second homolog BCL9-2, which also binds to b-catenin like BCL9. BCL9-2 promotes nuclear location of bcatenin, increased b-catenin signalling, and triggers EMT in vertebrate cells and Zebra fish embryos. BCL9-2 cannot colocalize with the E-cadherin/bcatenin/a-catenin complex at the plasmamembrane, but following tyrosine phosphorylation of b-catenin, it is translocated to the nucleus and promotes b-catenin signalling. Thus BCL9 proteins may act in the switch between cadherin-mediated cell adhesion and Wnt signalling.

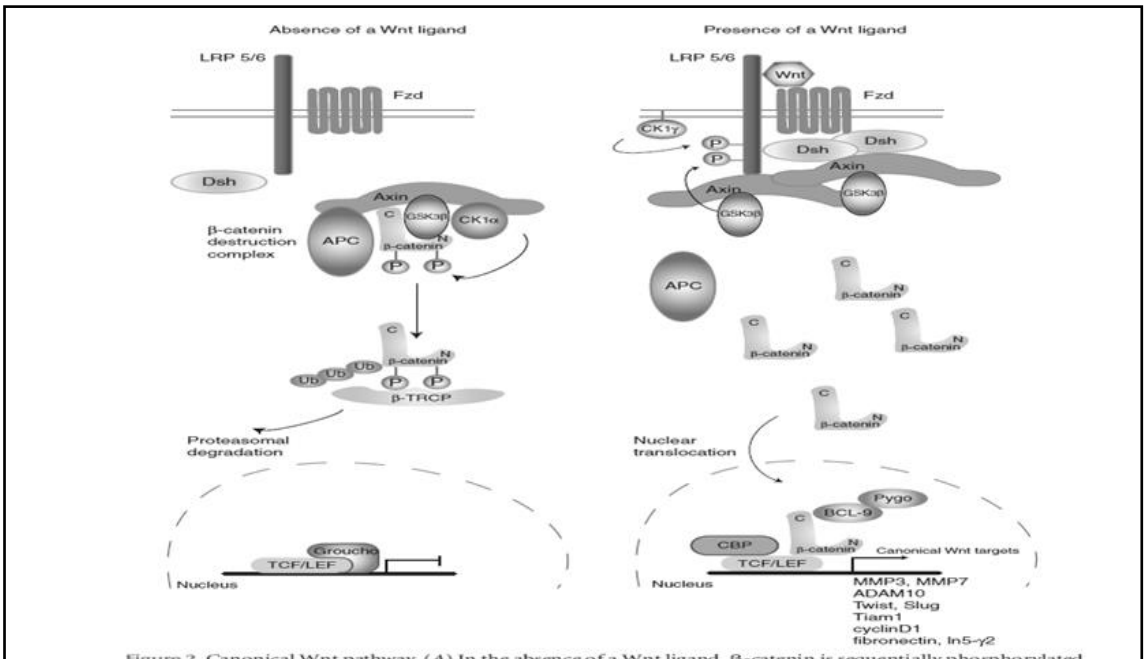


Figure 2. Canonical Wnt pathway. (*A*) In the absence of a Wnt ligand, β-catenin is sequentially phosphorylated in the destruction complex of axin and APC by the kinases CK1α and GSK3β, which results in ubiquitination by β-TRCP and, finally, in the degradation of β-catenin in the proteasom. (*B*) In the presence of a Wnt ligand, a Fzd/LRP5/6 complex is formed and is bound by Dishevelled, leading to the phosphorylation of the LRP5/6 receptor by GSK3β, CK1γ and other kinases. Phosphorylated LRP5/6 recruits axin to the plasma membrane, which leads to decay of the destruction complex, inhibited phosphorylation, and degradation of β-catenin. Stabilized β-catenin is then translocated to the nucleus, a process that can involve BCl9-2. β-Catenin interacts with TCF/LEF, and the recruitment of cofactors like BCL9, Pygopus, or CBP as well as the replacement of Groucho leads to the activation of target genes.

Proteolysis of Cadherins Affects Wnt Sigalling

Competition for b-catenin is one mechanism by which cells can modulate Wnt signals. Another is the cleavage of E- and N-cadherin by proteases, which can lead to the release of b-catenin and to up-regulated b-catenin signalling. This occurs in normal development but also in processes such as wound healing, Ca2þ-influx, and apoptosis. Cadherins are cleaved intracellularly by proteases like caspase 3 or presenilin, or extracellularily by ADAM10. Intracellular cleavage of cadherins releases nearly the entire cytoplasmic domain, whereas extracellular cleavage releases the adhesion domain (Fig. 3). ADAM10 belongs to the family of disintegrins and metalloproteases (ADAM), type I transmembrane proteins that combine cell–cell adhesion and proteinase activity. ADAM10 was recently identified as a b- catenin/TFC target gene. The group of Saftig has shown that cleavage of E- and N-cadherin by ADAM10 in keratinocytes and neuronal cells, respectively, reduced cell adhesion, increased cell migration and led to translocation of b-catenin to the nucleus. Translocated b- catenin activated Wnt/b-catenin target genes like c-Myc and cyclinD1. These are clear examples of an immediate crosstalk between a loss of cadherin-mediated cell adhesion and Wnt/b-catenin signalling. Presenilin1 (PS1) is an integral membraneprotein and a component of the g-secretase complex, which mediates an 1-cleavage of type I membrane proteins such as the amyloid precursor protein (APP). A mutant form of PS1 (PS1 FAD, familial Alzheimer's disease) is known to process APP, leading to increased production of the b- amyloid peptide (Ab42), which is responsible for Alzheimer's disease. Mechanistically, PS1 acts in several ways to influence cell adhesion and transcription. PS1 can bind to E-cadherin and b-catenin and promotes their association to the cytoskeleton, and the overexpression of PS1 results in enhanced cadherin-mediated cell adhesion. Under imbalanced calcium conditions or apoptosis, PS1 does not stabilize cell–cell adhesion but cleaves E-cadherin, which leads to the disassembly of adherens junctions, to the release of a cytoplasmic E- cadherin fragment, and to an increase of soluble b-catenin. It is possible that the release of b- catenin from adherens junctions caused by PS-1 modulates gene expression. It has been shown that the released N-cadherin fragments generated by PS1 favour nuclear localization of b-catenin and promote b-catenin signalling. The released fragments of E-cadherin also translocate to the nucleus and affect transcriptional activity.

Probable Questions:

1. Describe the process of Early patterning in vertebrates with special reference tosymmetry breaking.
2. What is Nieuwkoop center ?
3. Elucidate the mechanism of Wnt and cadherin sigalling in Axis specification invertebrates.

Suggested Readings/ References:

1. Heuberger, J., Birchmeier, W., 2009. Interplay of Cadherin-Mediated Cell Adhesion and Canonical Wnt Signalling. Cold Spring Harbor Perspectives in Biology 2, a002915-a002915.
2. Ozair MZ, Kintner C, Brivanlou AH. 2013. Neural induction and early patterning in vertebrates. Wiley Interdiscip Rev Dev Biol. 2(4):479-98.
3. Blum, M., Schweickert, A., Vick, P., Wright, C., Danilchik, M., 2014. Symmetry breakage in the vertebrate embryo: When does it happen and how does it work?. Developmental Biology 393, 109-123.

UNIT-VI

General concepts of organogenesis: Development and patterning of vertebrate limb, homeobox genes in patterning, signaling in patterning of the limb

Objective: In this unit we will discuss about development and patternng of the vertebrate limb and how homeobox genes play role in this patternng.

Introduction:

For decades, the vertebrate limb has been widely used as a model system to study the cellular basis of pattern formation in higher animals (for reviews, see Tickle, 1991; Maini and Solursh, 1991; Tabin, 1991). The last few years have seen the characterization of some of the molecules involved in these complex processes. Among candidate genes are those encoding homeodomain-containing proteins, originally isolated because of their cognate relationships to Droso~hiEu developmental control genes. In this short review, we would like to discuss the possible roles of homeobox-containing genes during vertebrate limb outgrowth and patterning. Various homeobox genes are expressed at different times and positions during limb development. The best studied are the genes related to either the Drosophila homeotic genes, also called "class I" homeobox genes or "Hox" genes, or to the Drosophila msh gene. The first family contains close to 40 genes (e.g., Boncinelli et al., 1991) which are clustered in four genomic loci, the HOX-1 to HOX-4 complexes (Kappen et ah, 1989; Kessel and Gruss, 1990; Duboule et al., 1990), each composed of about 10 genes. The second family is represented by only a few genes, two of them being extensively analyzed so far-Hoz-7.1 and Hox-8.1 (Robert et al, 1989; 1991; Hill et al., 1989; Monaghan et al, 1991; Davidson et al., 1991). These latter genes are called Hox for historical reasons but are not related to the insects homeotic genes and, consequently, are not members of the so-called Hox family. This observation is important when one considers that different families of homeobox genes have different functions during limb morphogenesis. Indeed it appears that Hox genes may be part of the molecular basis of positional information whereas nl&-related genes (HOP7 and Hoz-8) seem to be involved in those mechanisms responsible for the growth of the structure, through epithelial-mesenchymal interactions. The potential functions of these different gene families, as well as of those of other homeobox genes, will therefore be discussed separately and their possible interactions mentioned at the end.

EXPRESSION OF THE Hox GENES IN DEVELOPING LIMBS:

There are many reports on the expression of Hox genes during limb development. However, only parts of the HOX-1 and HOX-4 complexes (the "posterior" parts, see below) have shown, so far, a coordination in the expression patterns of their gene members. Consequently, we shall discuss here mainly Hox genes for which substantial data are available, in other words those for which some predictions regarding their functions can fairly be done. Hox genes have a biphasic expression profile during limb morphogenesis. They are first expressed widely in mesenchymal cells and become restricted to cartilage differentiating cells at a later stage (Oliver et al., 1989; Doll6 and Duboule, 1989; Dolli! et ab, 1989; Yokouchi et ab, 1991a). At later stages, Hox transcripts are observed in perichondrial areas but not in ossified tissues (e.g., Izpisua-Belmonte et al, 1990; Dolli! et aL, 1991b) in both fore- and hindlimbs, though some subtle differences can be observed (Mackem and Mahon, 1991).

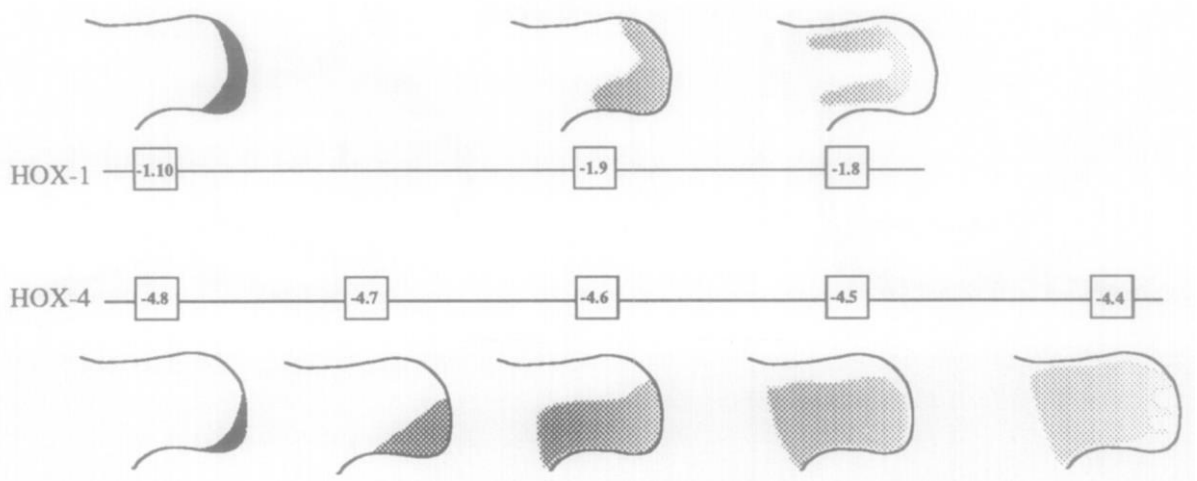
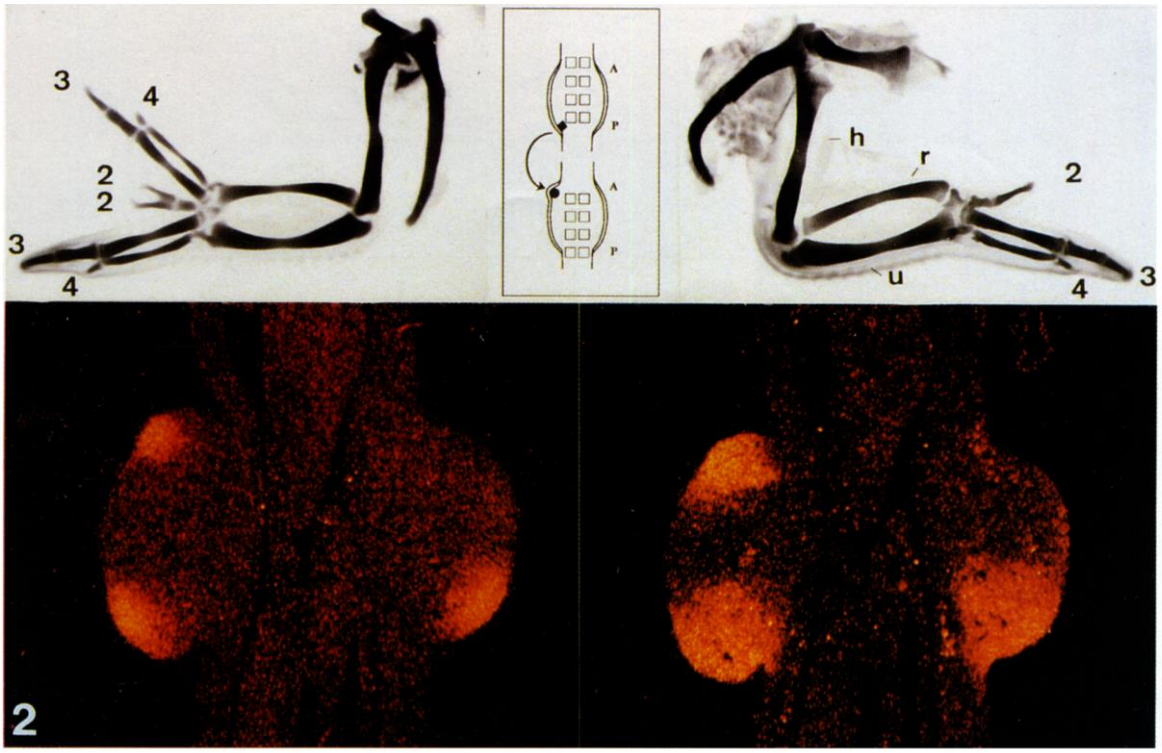


Figure. 1. Schematic representation of the expression domains of the "posterior" Ho%-1 and Hox-4 genes in a chicken wing bud at about stage 25 HH. Only the most 5' parts of the HOX-1 and HOX-4 complexes are shown and the genes are indicated by open boxes. The vertical alignment illustrates the paralogous relationships (e.g., between HOP1.10 and --4.8 or between HOP1.8 and -4.4). HOP4.7 has no counterpart in the HOX-1 complex and the limb expression domain of the Hex-1.7 gene has not yet been published. At this stage, the Hex-4 genes transcripts show a clear restriction for progressively more posterodistal areas whereas the presence of the Hox-1 transcripts is restricted to distal areas exclusively.

Hox gene expression domains in limbs are organized in a way which reflects the clustering of the genes (Dolli et ab, 1989) similar to that described along the trunk axis (Gaunt et (II., 1988; Duboule and Dolle, 1989; Graham et ah, 1989; Gaunt, 1991). The transcript domain of a gene is generally contained within the domain of the gene located 3'. Thus, successively more 5' located genes have progressively smaller expression domains (see Fig. 1). This collinear restriction in transcript localization seems to be "complex-specific" as genes belonging to the HOX-4 complex show a posterodistal restriction (Dolli! et al., 1989) whereas HOP1 genes tend to exhibit a distal restriction (Yokouchi et al., 1991a). Consequently, paralogous genes (such as Hex-4.8 and Hex-1.10; Fig.1) have slightly different expression domains as previously observed for "posterior" Hox genes during trunk development (Gaunt et ah, 1990; Izpisua-Belmonte et ab, 1990; Erlesius et al., 1990). In both cases, however, the expression of these genes in limb complies with the rule of structural colinearity (Doll6 et al., 1989; Yokouchi et al., 1991a), i.e., the spatial restriction of an expression domain is related to the position of the gene within the complex to which it belongs (see Gaunt, 1991, for review). A possible explanation of structural colinearity is provided by the observation that Hox-4 genes are sequentially activated, in time, during early limb bud outgrowth, both in mammals and birds (Dolli! et ab, 1989, 1991b; Izpistia-Belmonte et al., 1991a). This is also probably true for the Hox-1 genes even though not yet clearly demonstrated (Yokouchi et al., 1991a). This phenomenon, the temporal colinearity, is also observed along the crania-caudal axis (Izpistia-Belmonte et al., 1991b). The possible involvement of temporal colinearity in the control of a patterning mechanism has been proposed (Doll6 ef al, 1989, 1991a; Izpisua-Belmonte et al., 1991b) and will be discussed later. In mouse, genes belonging to the HOX-2 complex do not seem to be strongly expressed during limb development (e.g., Bogarad et al, 1989) even though some chicken cognate genes are expressed in early limb buds (Wedden et al., 1989). This apparently controversial result could simply be due to the overall low level of Hex-2 gene transcripts in mesoderm-derived lineages (R. Krumlauf, personal communication). In the case of the Hox-2.2 gene, a regulatory sequence seems to be able to control the expression of a reporter gene in specific parts of the developing transgenic limbs (Schughart et al., 1991). HOC3 genes could have distinct

functions during limb development as illustrated by the proximoanteriorm restriction of the Hox-3.3 protein revealed by using antibodies against the cognate Xenqms protein (Oliver et al., 1988, 1989, 1990). The analyses of more 5 located Hox-3 genes should confirm this interesting specificity. The gene members of the Hox family which show both spatial and temporal coordination in their limb expression domains are related to the Drosophiba Abdominal-B (AbdB) homeotic gene (Izpisua-Belmonte et ah, 1991b). Interestingly, the HOX-1, HOX-3 and HOX-4 complexes contain four or five such genes whereas the HOX-2 complex seems to have only one (Hox-2.5, Graham et al.,1989; Duboule and Doll&, 1989). Other Hox genes which are not related to the Drosophiila AbdB gene, i.e., which are located at more 3' positions in the different complexes, are expressed in limbs (e.g., Hoz-1.6 or Hox-1.4.



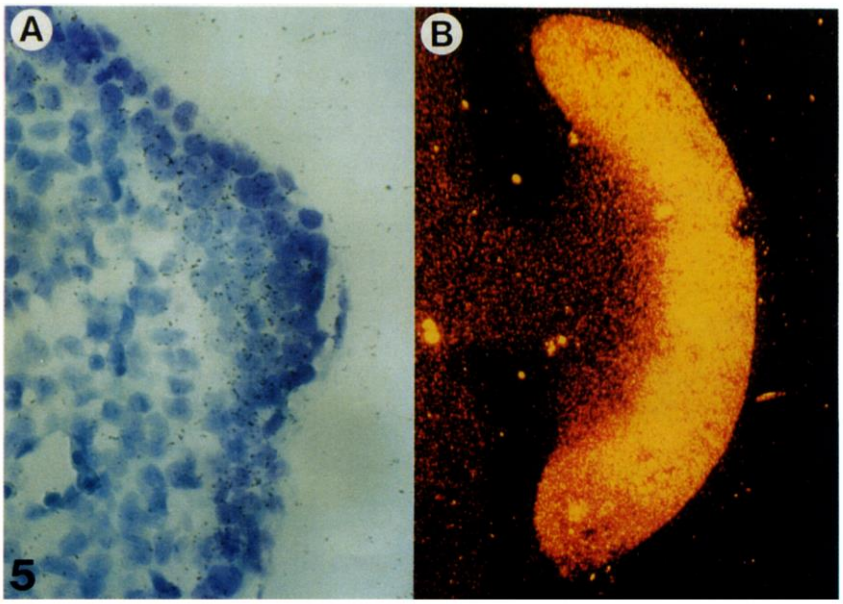


Figure. 2. Hex-4 genes expression domains after graft of a polarizing region. A chicken polarizing region was grafted into a stage 18 host wing. The resulting digit pattern is mirror-image duplicated and shown in the upper left panel. The normal digit pattern is shown in the controlateral wing (upper right). The bottom panels show the expression of the Hoz-4.8 (left) and Hox-4.6 (right) in such a specimen, 24 hr after grafting. The manipulated limb shows a mirror-image duplication of the Ho%-4 genes expression domains (as controlled by the controlateral wings) illustrating the posteriorization of the chicken anterior cells. A, anterior; P, posterior; 2,3,4, digit number (digit 2 is the most anterior digit); h, humerus; r, radius; u, ulna. Figure adapted from Izpisua-Belmonte et ul., 1991a).

Figure. 5. (A) Expression of the mouse D&-l gene in the apical ectodermal ridge. Hybridization grains are found exclusively in the AER and are absent from the underlying mesenchyme. DIz-1 is a vertebrate homolog of a D?osoph.iEag ene, distal-less (dll), involved in the patterning of the insect legs (Cohen et al., 1989). (B) Expression of the Hoc4.8 gene in a stage 24 chicken wing homozygous for the mutation talpid (ta3/ta3).

However, their expression profiles appear to be rather unspecific, i.e., with no particular cell type or spatial restrictions. These genes may be expressed in limbs because of their previous expression in the lateral mesoderm of the limb fields and may thus not have a clear function during limb outgrowth, or a function restricted to very proximal structures (see below). This seems to be confirmed in the case of HOX-1.6, as mouse loss of function mutants for this gene (Lufkin et ab, 1991; Chisaka et al., 1992) do not display obvious limb phenotypes even though Hox- 1.6 transcripts are clearly seen during limb budding (Duboule and Doll&, 1989).

OTHER HOMEOBOX GENES IN LIMB DEVELOPMENT:

Limb development and patterning thus seem to involve genes which exert similar functions in different parts of the vertebrate body which require growth-controlled spatial specification during ontogenesis such as during the formation of the trunk axis (IzpisGa-Belmonte et al., 1992a; Duboule, 1992) or the genital bud (Doll6 et al., 1991a). This reinforces the paradigmatic nature of the developing vertebrate limb and suggests that many other genes encoding homeodomain proteins will be expressed during limb growth and patterning. So

far, we have described genes expressed only in the mesenchymal compartment (the Hex genes) and in both mcsenchymal and ectodermal cells (the w&-related genes). Genes expressed selectively in the ectoderm also exist as examplified by the mouse O/X-1 gene (Price ef (II., 1991). The L)l.r-1 gene has a homeobox only poorly related to the Antp-class (Ho:r) genes and is a member of another, small family of vertebrate genes related to the Dmsophiltr disfal-less (dll) gene (Cohen et ul., 1989; Cohen and Jtirgens, 1989). During development, DZ.z-1 is expressed in different places, notably in the forebrain (Price cdf (11.. 1991), the branchial arches and the AER (Doll6 et (11.. 1992). The labeling in the AER is rather weak but clear and no grains are detected in mesenchymal cells (Fig. 5). This specific Dl.z~l expression domain is particularly interesting when one considers the function of the Drnsoyhiltr till gene in the morphogenesis of the fruitfly limbs (Cohen et ul., 1989; Dolli? c>f ul., 1992). Another example of a vertebrate homeobox gene potentially involved in limb growth and patterning and whose expression is restricted to the ectodermal ridge is that of the En-1 gene (Davis et (II., 1991). This gene is again a member of a small family of genes related to the Dmsophilu gene engrailed (en) and contains a homeobox relatively divergent from those mentioned above. In developing limbs, the Eu-1 gene is expressed in the ectoderm with a ventral specificity. As the dorsoventral polarity of the limb bud seems to be determined by the ectoderm is an interesting candidate in the establishment of this important

assymetry (Davis ef trl., 1991). Thus, vertebrate homeobox genes can be active in either the mesenchymal or the ectodermal cells, or in both. This suggests very different functions for these genes during limb development and reinforces the idea that homeobox genes should be treated as subfamilies rather than being considered as members of a large family of genes containing a homeobox.

GROWTH, PATTERNING, AND POSITIONAL INFORMATION:

Based on expression data and pattern respecification experiments, a hypothesis has been proposed which account for the function of homeobox genes during limb development and which can be summarized as follows: At the time the limb buds out, msh-like and several Hex genes are strongly expressed. However, if the presence of the rnsh-like transcripts is linked to the active growth of the structure, the presence of Her transcripts might only indicate that these genes were expressed in the lateral plate mesoderm of the limb field prior to the budding phase. In fact, the Non genes which are already well expressed when the limbs are budding (such as, e.g., Her-1.4, Galliot et al., 1989) are probably "informative" only for extreme proximal parts of the limbs such as the shoulder and the upper arm in the case of the forelimbs. In contrast, those Har genes which are being turned on at the time of budding and later, will be involved in patterning more distal regions including the digits. The basis for the proposed model is that Hm genes are activated following a temporal sequence which progresses in parrallel with the limb proximodistal morphogenetic sequence. This temporal progression (the fernpor~l colirLea&g; Izpisua-Belmonte et (II., 1991b) could be achieved in a two step mechanism: (1) an "opening" of a given Hex gene (to make it "available") followed by (2) its transcription, provided the correct factors are present in sufhcient amounts (Doll6 ef al., 1989). The 3' to 5' colinear opening of the HOX complexes would occur in the progress zone as only these cells are able to reinitiate HOP4 gene transcription following the correct progression (Izpisua-Belmonte et trl., 1991a; 199213). This opening process would therefore require the presence of the cells near the AER, possibly because of its dependence

on a factor released by the ridge. As cells leave the progress zone, the opening process of their HOX complexes is interrupted and cells are therefore determined to express a given combination of Ho.c genes. As a consequence, this combination (code) would be fixed by the

time these cells spent in the zone. A system of maintenance, perhaps also relying on colinearity, could then be used to conserve the same set of Ho.r genes expressed in the following cell lineages until the appropriate factors disappear (Gyurkovics ef ol., 1991; Sanchez-Herrero, 1991; Simon cf rrl., 1992; Paro, 1990; Gaunt and Singh, 1990).

Hence the opening of the HOX-4 complex can occur only in presence of an AER, in progress zone cells which express &t-related genes, in other words, in areas of active growth (e.g., the genital tubercle), whereas the transcription of the opened genes can be maintained outside these areas. This circumstantial evidence could suggest a function of Hole-7.1 and/or Hoc-X.1 in this opening process. However, we favor the possibility that no causal relationship exists between these two observations and that this coincidence is due to the requirement of a high mitotic activity for an efficient processing of temporal colinearity, on one hand, and to the link between rlzsh-related gene expression and areas of apical growths, on the other hand.

The expression patterns of the Hox genes during early limb development suggest that the polarizing region could contain a factor essential for their (efficient) transcription (Doll6 et ul., 1989). Retinoic acid could be (part of) this factor. Alternatively, RA could help to produce this factor in pattern respecification experiments by transforming anterior cells into polarizing cells (Wanek et ul., 1991). The posterior localization of this activity leads to an asymmetric distribution of HOX proteins. Indeed, cells leaving the progress zone will all have qualitatively and/or quantitatively different combinations (code) of

HOXproteins which will depend on the position of the cell within the limb. In this view, Hex genes could encode positional information (Wolpert and Stein, 1984; Wolpert, 1989) which would be, in fact, the cellular interpretation of a temporal information as the expression and perception of position will be dependent on the time spent in the zone. In this respect, this proposal can be understood as a molecular support of a "progress zone model" (Summerbell et ~1.. 1973; Wolpert et (11.. 1975), including an anterior-posterior component (see Maini and Solursh, 1991). Various combinations of HOX transcription factors could regulate target genes (e.g., molecules involved in cell to cell contacts such as receptors and their ligands, Davidson, 1991) in slightly different ways leading to different arrangements of similar cell types. A coincidence between the Hm expression domains and the branching patterns of cartilage as proposed by Shubin and Alberch (1986) was recently pointed out (Yokouchi et u,l., 1991a). At least, it is clear that cells expressing different Hex genes take part in the formation of different and predictable structures (Izpisua-Bclmonte ef cll., 1991a) and chickens carrying a mutation abolishing the AP wing asymmetry, such as the talpid mutation (Ede and Kelly, 1964; see Ede, 1968 and 1980 for Refs.), also display an homogenization of their Hox-4 expression domains along the AP axis (Izpisua-Belmonte et (11.. 1992c and Fig. 5). This model implies that patterning is a unidirectional process since the opening of the complexes can either go 3' to 5' or be stopped but cannot progress from 5' to 3' (the I;,'i?/bcrltrcstr*cx.ssr; Duboule, 1991, 1992). It also provides an explanation as to why growth and patterning cannot be disconnected during limb development since the opening of the patterning s ystem is controlled by the growth of the system itself.

Hcw4 GENES RESPOND TO VARIOITS POLARIZING ACTIVITIES:

In addition to the posterior margin of the growing limbs, polarizing activity is found in different regions of the developing vertebrate embryo such as the floor plate (Wagner et al., 1990), Hensen's node (Hornbruch and Wolpert, 1986), or the genital tubercle (Dolle et al 1991b). We have analyzed the expression of mouse and chicken Has-4 genes in wings grafted with mouse tissues from various origins (Izpisua-Belmonte rot (II., 1992a; Table 1). This series of experiments led to the following observations: (1) rk r~o1'0 activation of Hw genes is observed each time additional posterior digits are produced. (2) The extent of this activation correlates with the extent of duplication, i.e., a weak duplication (e.g., with the genital bud) will not reactivate the most posterior genes whereas complete duplications (e.g., those produced by grafts of polarizing regions or primitive streak tissue) will reactivate the complete set of Hox genes. (3) The extent of duplication (the strength of polarizing activity) does not depend upon the number and qualities of Ho.r genes expressed in the grafted tissue so that there is no need to express posterior Hw genes to have a strong polarizing activity and the expression of these genes is not enough to confer this property (see the genital tubercle). This confirms that Ho.t proteins do not have per se a polarizing activity. (4) Grafted cells tend to behave like the surrounding cells. For example, mouse anterior primitive streak cells that would never express the posterior Hox-4 genes under normal conditions will express these genes after a certain time spent in a chicken polarizing region. This suggests that each cell expressing a set of Ho.r proteins could, in principle, express the complete family provided it is kept in the proper environment for long enough. This supports the idea that the final state of the system is "posterior" and that "anterior" is an information assigned by default (Duboule, 1991).

Scientists have analyzed the speed at which Hex-4 genes were de NOVO activated in the grafted cells by transplanting mouse limb bud anterior cells into chicken polarizing regions (Izpistia-Belmonte et ul., 1992b). Surprisingly, the complete set of HOT genes are activated, in mouse grafted cells, as early as 16 hr after grafting. This response is much faster than that observed in chicken anterior cells exposed to either

a mouse polarizing region or a bead soaked in retinoic acid (in this last case, 24 hr are required to see HOP4.8, Izpisua-Belmonte et al., 1991b, 1992b). This observation is difficult to explain in the context of a model where the diffusion of an active molecule (morphogen) would be the only manifestation of the polarizing activity. It is, however, easier to reconcile with a model where both long range and short range interactions could participate in this activity. Even though tremendous progress has been made recently in the determination of the molecules involved during vertebrate limb pattern formation, there is still a long way to go before we can understand the process of limb formation. The next steps will be to investigate how different networks of genes are connected to each other, how to translate positional information (a biochemical code) into a cellular response, and how these complex mechanisms are related to the growth of the system.

Probable Questions:

1. How Hox genes contributes in limb development.
2. How Hcw4 genes respond to various polarizing activities?

Suggested Readings/ References:

1. Developmental Biology: Scott F Gilbert.

2. Principles of Development: Louis Wolpert.

3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.

4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-VII

Insect imaginal discs – organizing center in patterning of the leg and wing, the homeotic selector genes for segmental identity; insect compound eye

Introduction:

Arthropods are the most successful group of animals and represent approximately three-quarters of the animal species currently living on Earth. A key factor contributing to their success is their ability to exploit multiple ecological niches, most likely due to body plan modifications and innovations generating vast morphological diversity. Arthropod appendages show great variation in number, shape, and function, allowing multiple purposes from locomotion to feeding or environment sensing. However, besides their morphological disparity, it is likely that all appendages share common generative rules. Appendages have a proximodistal (P-D) axis that forms de novo orthogonal to the main body axes, the anteroposterior (A-P) and the dorsoventral (D-V). Molecular and genetic studies in the fruit fly, Drosophila melanogaster, have provided the basis of appendage development and served

for comparative analysis with other arthropods. Interestingly, many of the signaling pathways and patterning genes identified and characterized in Drosophila have comparable functions during vertebrate appendage development. Thus, vertebrate and invertebrate appendages are built using similar underlying genetic programs, even though they are not homologous structures in the classic sense. These similarities are often referred to as ‘deep homology’.

Primitive insects develop appendages directly from embryonic limb buds that grow as external projections, while more derived insect species with complete metamorphosis, such as Drosophila, develop their appendages from imaginal precursors. The external adult body of Drosophila is formed by two different sets of cells, the imaginal discs and the histoblasts. The imaginal discs are specialized epithelial sheets specified in the embryo that grow and become patterned inside the larva. During the pupal stage, imaginal discs evert and differentiate to form the adult structures. The histoblasts are the precursors of the fly abdomen that, in contrast to the imaginal discs, only proliferate during pupal development. There are 19 imaginal discs in the larvae, 9 appearing in pairs, and the genitalia.

The wing and haltere discs form the corresponding appendages and also the dorsal thorax. In a similar manner, the leg discs develop the appendage proper and the ventral pleura of the thorax. While thoracic imaginal precursors (wing, haltere, and legs) originate from a single embryonic segment, the genital disc primordia is a sexually dimorphic compound primordia derived from three abdominal segments (A8, A9, and A10). In a similar fashion to the genital primordia, the cells from different cephalic segmental identities coalesce together to form the eye-antennal disc. The eye-antennal disc is also a compound structure that gives rise to the olfactory (antenna and maxillary palps) and visual (compound eyes and ocelli) organs plus the head epidermis. We can group the appendages in dorsal or ventral depending on their relative positions within the body and their homology. Therefore, ventral appendages include the legs, antenna, and genitalia, while the wings and halteres are dorsal. In this review, we will focus on the patterning of the thoracic appendages and the antenna. At the molecular level, a small number of signaling pathways are reiteratively used throughout development to specify and pattern the appendages in Drosophila and in vertebrates.

The function of signaling pathways such as hedgehog (Hh), decapentaplegic (Dpp),Wingless (Wg),

Epidermal Growth Factor Receptor (EGFR) and Notch, and the transcription factors Distal-less (Dll), Homothorax (Hth) and members of the Sp family (Btd/Sp1) is fundamental in the formation of appendages. These signaling pathways and transcription factors are linked in regulatory networks, and cooperate to subdivide the developing appendages in different domains of gene expression. The identification and dissection of the cis-regulatory modules (CRMs) that controls the expression of genes required for wing, leg, or antenna development has been fundamental to decipher the regulatory networks that direct appendage formation. Below, we discuss the regulatory logic behind appendage specification and patterning, and the implications in the evolutionary origin of the insect wing.

Allocation of the Thoracic Appendage Primordia:

The development of imaginal discs is a progressive process initiated during embryogenesis with the specification of the imaginal primordia. Each primordium is formed by a characteristic number of cells located in the embryonic ectoderm in precise A-P and D-V location (Figure 1). All imaginal primordia are easily recognized by the restricted expression of the genes escargot (esg) and headcase (hdc), which maintain the imaginal state through the repression of endoreplication. The segmental identity and position along the A-P axis of the different imaginal primordia is provided by the activity of Hox proteins. The Hox genes encode homeodomain containing transcription factors that confer A-P identity along the body of the fly, defining unique developmental programs to each segment. Therefore, the thoracic appendage primordia (legs, wing, and haltere) are restricted to their corresponding thoracic segments through the repression exerted by the abdominal Hox proteins.

The leg and wing/haltere presumptive imaginal discs are recognized in the thoracic segments of a late embryo as a genetically and morphologically distinct group of cells that form the ventral (VP) and dorsal primordia (DP), respectively (Figure 1A). The DP activates the expression of the snail (sna) and vg (vestigial) genes, while the VP expresses Distal-less (Dll) and the Sp family members Sp1 and buttonhead (btd). The first molecular sign of appendage specification is the activation of the homeobox-containing gene Dll. Dll is expressed in the outgrowths of many animals and is essential for appendage formation. In Drosophila, Dll expression is initiated in a group of about 30 cells in each thoracic hemisegment at stage 10 of embryogenesis. Multiple Dll CRMs have been identified that partially reproduce the spatial and temporal expression of Dll. The characterization of allows the description of the developmental fate of Dll expressing cells. At stage 10–11, an early Dll CRM, named Dll-304, is activated in a pattern similar to Dll and its activity decays some hours later [8,23]. These cells are defined as thoracic primordia (TP) because their progeny will contribute to both the VP (leg) and DP (wing/haltere). At this stage, Dll expression is positively regulated by Wg, that is expressed in D-V stripes in the anterior compartment of each thoracic segment. The precise localization of Dll expression along the D-V axis in the thoracic embryo epidermis is also regulated by repression mediated by Decapentaplegic (Dpp) and the Epidermal Growth Factor Receptor (EGFR) pathways in dorsal and ventral cells, respectively (Figure 1B). The regulation exerted by the Wg, Dpp, and EGFR signaling pathways is present in the thoracic and abdominal segments, although Dll expression is restricted to the thorax by repression mediated by the abdominal Hox proteins. The Hox proteins Ultrabithorax (Ubx), Abdominal-A (Abd-A), and Abdominal B (Abd-B) directly bind to the early Dll-304 CRM in a compartment specific manner. Although Dll could be activated in the absence of Antenapedia (Antp), the thoracic Hox protein, it has been recently shown that Antp plays a positive role in enhancing Dll expression levels

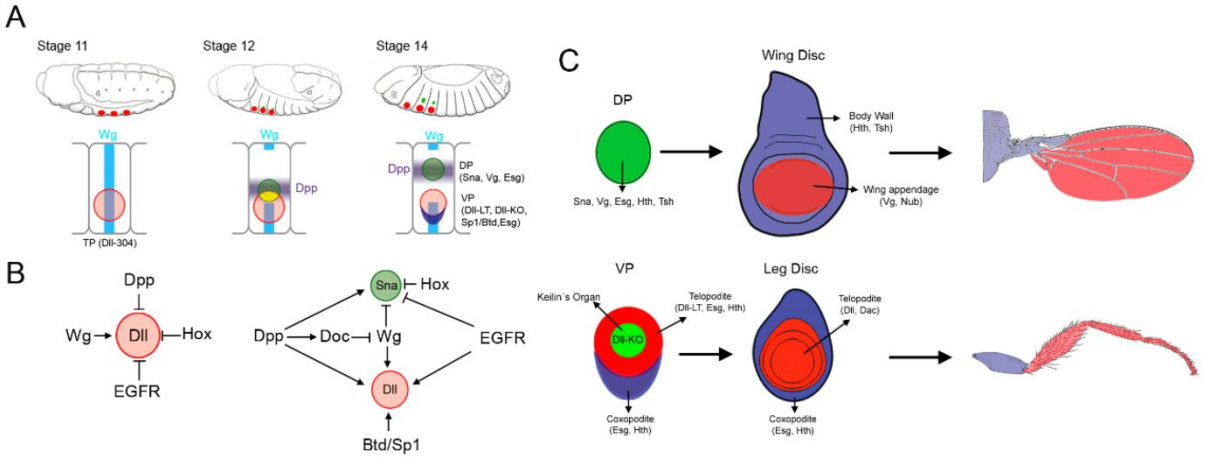


Figure 1. Specification of the thoracic appendages. (A) Cartoon showing three representative stages of Drosophila embryogenesis and the sequential appearance of the thoracic primordia (TP, red circle), the ventral primordia (VP, red circle) and the dorsal primordia (DP, green circle). Below is presented a schematic representation of the second thoracic segment where the expression of TP-, DP-, and VP-specific genes is related to the Wg and Dpp signaling pathways. Note that the DP originates from two populations of cells: one within the TP (drawn in yellow) and another one dorsal to the TP; (B) Genetic inputs that control appendage specification when Dll is first activated (stage 10–11) and during activation of the DP gene sna (stage 12 onwards). (C) Schematic representation of the DP and VP and the imaginal discs and adult structures that they will give rise to (wing and leg, respectively). The subdivision of the wing and leg imaginal discs in the body wall and appendage territories is also shown.

Specification of the Ventral and Dorsal Primordia:

Classic lineage experiments using X-ray somatic recombination and gynandromorphs already suggested that the dorsal and ventral primordia originated at the blastoderm stage in close proximity and presumably derive from an overlapping population of cells. In accordance, more recent lineage-tracing experiments revealed that the progeny of Dll or Dll-304 expressing cells (TP) not only contribute to the entire leg imaginal disc, including the ventral body wall and the appendage, but also to the wing disc. The initial group of Dll expressing cells (TP) is subdivided as embryogenesis progresses in cells that will contribute to the DP (wing and haltere) and the VP (legs). This process requires a correct balance between the D-V positional cues provided by the Dpp, EGFR, and Wg pathways (Figure 1A,B). dpp expression at the dorsal side of the TP field generates a concentration gradient that promotes DP fate at high levels and specifies the VP fate at intermediate levels. Simultaneously, the ventral activation of the EGFR pathway antagonizes Dpp signalling and restricts the activation of the wing promoting genes to the dorsal domain of the TP. Dpp also activates the Dorsocross (Doc) genes in a broad lateral stripe that partially overlaps with the dorsaldomain of the TP. This leads to the repression of wg expression in the lateral ectoderm (Figure 1A,B). In this manner, dorsal TP cells are exposed to high levels of both Doc and Dpp signalling and low Wg and EGFR. These cells will contribute to form the DP, lose Dll expression and activate the DP developmental program. In contrast, ventral TP cells retain high levels of Wg and EGFR, maintain Dll expression, and are fated to form the VP. Contrary to the opposite roles that the Dpp and EGFR pathways have on DP formation, both pathways positively direct VP fates. At this stage, the role of the Wg pathway is restricted to VP, as it is necessary for Dll expression but not for wing formation.

The repression of Wg and the presence of high Dpp levels are a prerequisite for the activation of the DP promoting genes sna, esg, and vg in cells of the lateral ectoderm and dorsal to Dll expressing cells (Figure

1A). The activation of sna and vg is only observed in Dll-negative cells after the separation of the dorsal and ventral primordia. In contrast to Dll, which is expressed in the three thoracic segments, the activation of the DP specific genes (sna, esg, and vg) only occurs in the second and third thoracic segments (T2 and T3). sna and esg act redundantly to specify DP fate while vg is required later for the development of the wing and haltere appendages but not for DP formation. At the end of embryogenesis, the different size of the wing and haltere primordial is already apparent, being the haltere approx. 30% smaller than the wing primordia. The number and size of the DP is regulated by the Hox proteins. Restriction of the DP to the T2 and T3 segments is controlled by the repressor activity of Sex combs reduced (Scr) in T1 and by Ubx, Abd-A, and Abd-B in the abdominal segments. Remarkably, Ubx has two different functions in DP specification: in the abdominal segments represses DP formation, while in the third thoracic segment regulates the size of the haltere. How Ubx exerts these two functions is unknown, but could be possibly explained by the different Ubx levels observed between the T3 and A1 segments. High levels of Ubx in the A1 could repress DP genes while lower levels in T3 would reduce, but not eliminate, their activation. Another possibility is that the different behavior of Ubx would be explained by the use of diverse Hox cofactors in each of these segments.

Dual Developmental Origin of the Drosophila Wing and Its Evolutionary Implications:

The origin of the insect wing is one of the most intriguing evolutionary events in biology,and is still under debate. Two main theories have been proposed to explain the origin of this morphological innovation. The paranotal or tergal hypothesis proposes that wings are created de novo as an extension of the dorsal thorax or tergum. The alternative theory, the gill-exite or pleural hypothesis evolutionarily connects the wings to a preexisting structure, the gill, present in the proximal base of an ancestral leg. Studies in Drosophila and other insects have contributed significantly to decipher the origin and specification of insect appendages. Recently, thanks to the identification and characterization of the sna dorsal primordia CRM (sna-DP), a clear picture of the origin and the genetic inputs that control DP specification was obtained. sna-DP is first activated in a few cells dorsal to Dll expressing cells in T2 and T3 that later label the entire DP. A cell lineage analysis of sna-DP cells demonstrated that this CRM labels the entire wing disc cells including the progenitors of the thorax and appendage. Importantly, cells that have expressed—but no longer actively transcribe—Dll populate half of the DP. These cells can be located at any part of the wing disc, although they are found preferentially in the ventral domain of the disc.

This bias is probably due to the relative location of the DP in relation to the VP in the embryo rather than to a preexisting genetic determination of these cells. In addition, genetic ablation of the TP domain reduces, but does not eliminate the sna-DP cells, suggesting the existence of DP cells that form independently of the TP. Moreover, DP fates could be specified in the absence of Dll or the ventral appendage selector genes buttonhead (btd) and Sp1. All these experiments and the lineage tracing analysis indicate that the DP is derived from two populations of cells: one that derives from early Dll expressing cells (TP), and another that arises close to, but is independent of Dll expressing cells (Figure 1A). Importantly, as discussed above, both populations of cells that conform the DP require the same molecular signals for their specification: high levels of Dpp and low Wg (Figure 1B). This dual developmental origin of the wing described for Drosophila is in accordance with recent evo-devo studies that support the unification of the paranotal and pleural hypotheses.

Proximo-Distal Subdivision of the Ventral Primordia: In contrast to the DP, where distinction between trunk and appendage occurs later in imaginal development (see below), the VP is organized in gene expression domains that subdivide the primordial in different territories (Figure 1C). The

development of the VP requires the function of Dll and of the two paralogous genes btd and Sp1. Btd and Sp1 are members of the highly conserved Sp family of transcription factors required for appendage formation in vertebrates and invertebrates. btd/Sp1 are expressed in the embryonic progenitors of the leg imaginal discs starting around stage 10-11. As it is the case with Dll, btd is activated by Wg and repressed dorsally and in the abdominal segments by Dpp and Ubx, respectively. At stage 11, btd, Sp1 and Dll (Dll-304) are activated in parallel in the TP and are genetically independent of each other. Importantly, as mentioned before, these TP cells could contribute to the DP and to the entire VP. Some hours later, at stage 14, VP and DP are already separated and the activity of the Dll-304 CRM decays. At this time point, Dll expression is controlled by two Dll CRMs with mutually exclusive patterns at the VP, the leg trigger (Dll-LT) and the Keilin organ (Dll-KO) elements (Figure 1C). The progressive refinement of the appendage primordium developmental potential is reflected by the cis-architecture of the Dll gene. At the end of embryogenesis, the VP is genetically subdivided in domains with different cell fates that correspond to the P-D subdivision of the arthropod leg proposed by Robert E. Snodgrass (see below) (Figure 1C). VP cells that express esg but do not activate Dll will form the coxopodite, the most proximal and unsegmented domain of the appendage that forms as an outgrowth of the bodywall, including the coxa. VP cells co-expressing esg and Dll (through the Dll-LT CRM) will form the telopodite or the leg proper, which includes all the distal leg segments that are articulated. Additionally, cells at the center of the VP that are esg negative and Dll positive (Dll-KO) are fated to form a larval mechanosensory structure that shares a common lineage with the leg disc called the Keilin's organ (KO). While Dll-KO cells do not contribute to the leg imaginal disc, the progeny of Dll-LT activating cells form the entire telopodite. Consistently, in Dll mutants the telopodite is lost while the coxopodite is present. Dll-LT is positively regulated by Wg and Dpp and repressed in the center of the VP by members of the achaete–scute complex (ASC).

At the same time, Dll and ASC positively regulate the Dll-KO CRM restricting its activity to the VP. At this stage btd and Sp1 act upstream of Dll and only the elimination of both genes suppresses Dll expression and the activity of the Dll-LT and Dll-KO enhancers. Initially, some studies proposed that the two related homeobox genes Homothorax (Hth) and nuclear Extradenticle (Exd) were markers, along with esg, of coxopodite fate. However, a reevaluation of the expression of hth, esg and Dll and their specific cell fate helped define a high-resolution map of the VP fates formed by three molecularly different domains with distinct developmental potential.

Patterning of the Leg Imaginal Disc:

The third instar leg imaginal disc is roughly circular in shape with the distal-most region of the leg located in the center of the disc, and the most proximal leg segments and the pleura arising from the periphery of the disc. Antp is the selector Hox gene for leg identity that is expressed in the leg imaginal disc but not in the antenna disc. As expected for its selector function, Antp mutant legs are transformed into antenna and Antp ectopic expression in the antenna converts it into leg. The leg P-D axis is formed orthogonally to the previously established A-P and D-V axis, and its formation is intimately connected with the A-P compartment subdivision. Thus, posterior cells express hedgehog (hh) which acts as a short-range signal that activates the expression of dpp and wg in the anterior dorsal and ventral halves of the disc, respectively. Dpp and Wg are both necessary to establish and pattern the D-V and the P-D axes. The D-V axis is organized by the mutually antagonistic repression of Wg and Dpp and interactions between its downstream genes (Figure 2A). Briefly, Wg specifies ventral fates through the activation of H15 and midline (mid) and the repression of dorsal genes such as Doc and optomotor blind (omb). Therefore, in wg

mutants all the ventral structures are lost and replaced by a mirror duplication of dorsal ones and the reverse phenotypes are observed for dpp mutants.

The P-D axis is initiated at the center of the disc where high levels of Wg and Dpp activate downstream genes such as Dll. Dll in turn activates dachshund (dac) in the medial domain of the leg disc, whereas high levels of Wg and Dpp represses its expression in the distal tip. Once activated, Dll and dac expression is maintained in part by autoregulatory mechanisms. Simultaneously, the expression of hth is restricted to the periphery of the disc by a Wg and Dpp dependent repression mechanism. This mechanism could be mediated by the zinc finger proteins encoded by the elbow (el) and no ocelli (noc) genes that act in the leg and the wing discs to repress hth expression, and therefore body wall fates. Consequently, as a result of the A-P compartment subdivision and the restricted expression of Hh, Dpp, and Wg the leg is divided along the P-D axes in proximal, medial, and distal domains defined by the expression of hth, dac, and Dll, respectively (Figure 2A). At this stage Antp prevents hth and Dll coexpression in the leg, suppressing antennal identity. Importantly, the ancestral subdivision of the leg in the coxopodite and telopodite is reflected at the molecular level by the differential response of the leg imaginal disc cells to the Wg and Dpp signaling pathways. Therefore, cells that receive both Wg and Dpp activate the Dll and dac genes and specify the telopodite. In contrast, cells that are unable to respond to these pathways are specified as coxopodite and activate hth (Figure 1C). The distal domain of the leg is further patterned by a P-D gradient of EGFR signaling, mediated by the EGFR ligand Vein (Vn), that regulates the expression in a distal to proximal manner of aristaless (al), Bar (B), and rotund (rn) among others (Figure 2A,B). A complex cross-regulation between these transcription factors ensure the segmental subdivision of the distal domain in five tarsi. Besides providing unique segmental identity along the P-D axis of the leg, this regulatory network is responsible for the segmentally repeated expression in concentric rings of the Notch ligands Delta (Dl) and Serrate (Ser). Notch activation at the distal-end of each presumptive segment is evident at the end of larval development and directs the formation of the joints and the non-autonomous growth of the leg (Figure 2A,B). The ventral appendage selector-like genes btd and Sp1 are co-opted later during imaginal development to control the growth and pattern of the leg, in part through the regulation of Ser expression and therefore Notch signalling. Two types of joints with different morphologies and evolutionary origin could be found in the adult leg. The proximal or true joints are asymmetrical and have associated musculature, whereas the distal joints are symmetrical and not attached to muscles. Notch controls joint morphogenesis through the activation of subsidiary transcription factors required for distal joints such as dysfusion (dysf), proximal joints such as the odd-skipped gen family of for all joints like dAp-2.

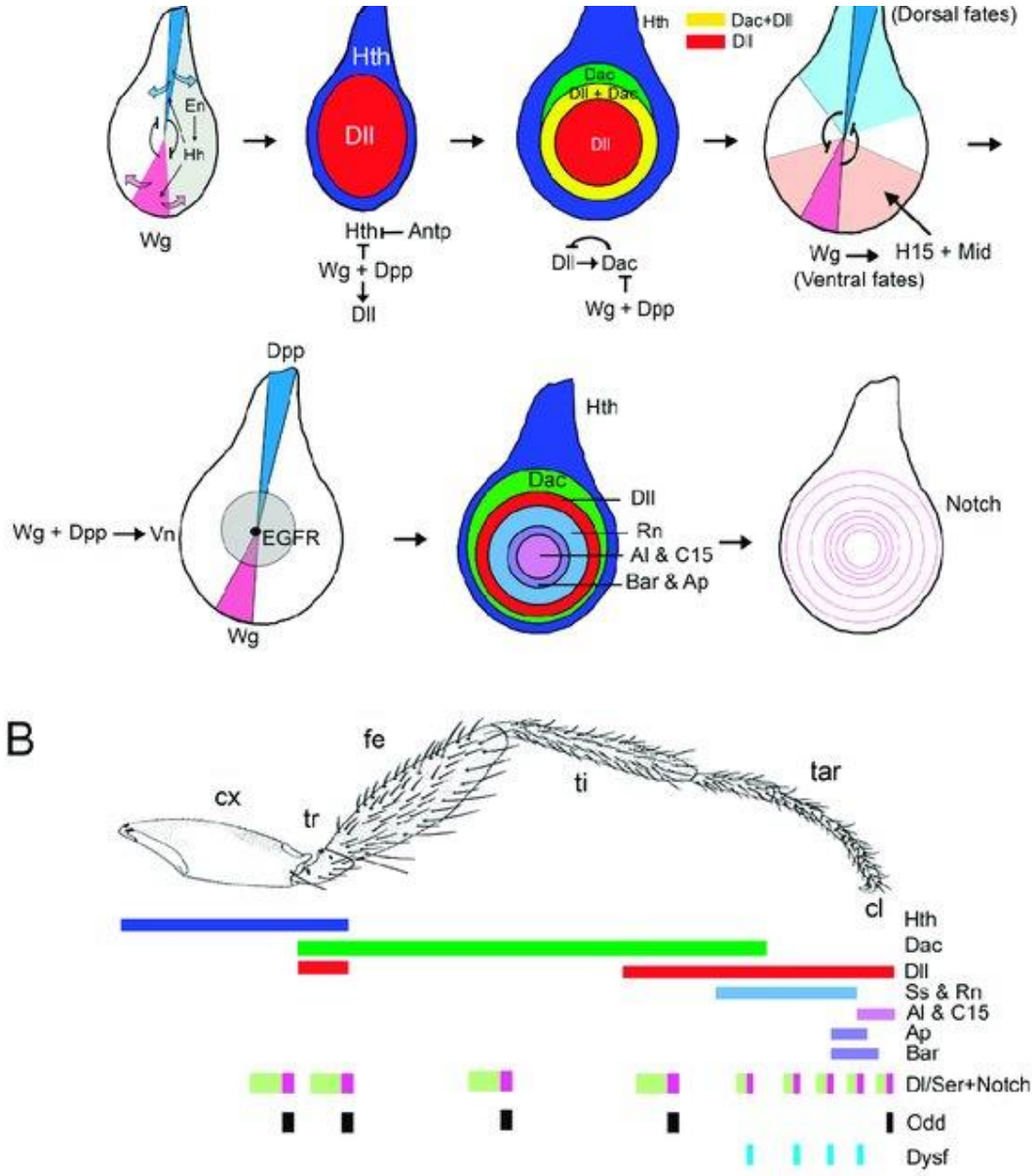


Figure 2. Patterning of the Drosophila leg. (A) Overview of the proximo-distal (P-D) and dorso-ventral (D-V) axes formation during leg development. The restricted expression of dpp and wg in the dorsal and ventral halves of the leg disc is required to initiate and to pattern P-D and D-V axes. The first two leg imaginal discs from the left correspond to early second instar discs, while the rest depict third instar leg discs. Some of the genetic interactions that lead to the leg patterning are indicated.

(B) The 'genetic code' generated by the expression of several transcription factors specify the future segments of the adult leg along the P-D axis. The expression of the Notch ligands Dl and Ser, and the activation of the Notch pathway and its target genes is necessary for the formation of the joints that separate the adjacent segments of the leg.

Patterning of the Wing Imaginal Disc:

The DP contains the progenitors of both the wing and the body wall structures, however in contrast to early P-D specification of the VP, the trunk/appendage distinction is only evident later during larval development (Figures 1C and 3). The mature wing disc is formed by two distinct domains, the body wall and the appendage, which in turn is subdivided in the proximal hinge and the wing blade. The specification of the body wall and the wing appendage is made by the mutually repressive activities of two signaling pathways: the Vn/EGFR acting in the proximal domain of the disc that specifies body wall fates, and Wg at the distal region that will form the wing field (Figure 3).

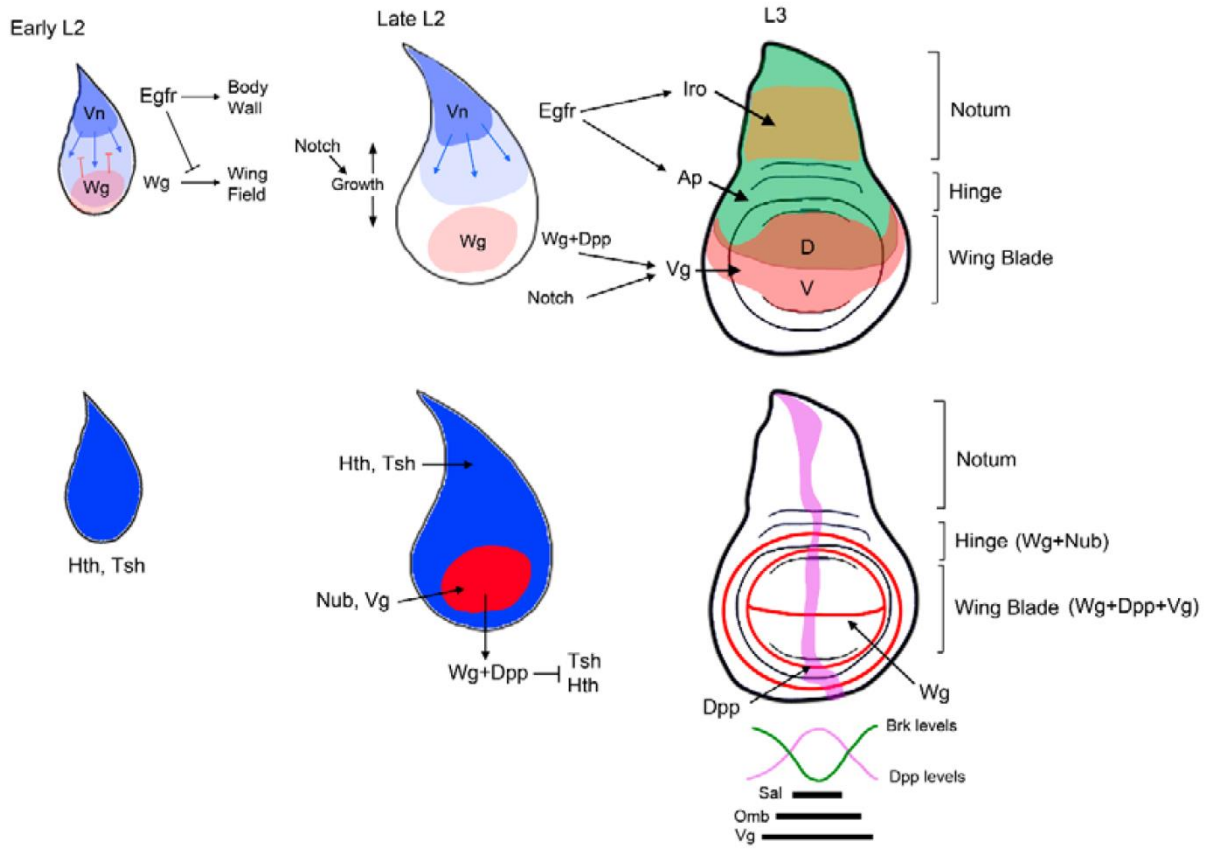


Figure 3. Patterning of the Drosophila wing. Three representative stages of wing disc development are depicted. The signals and genes that direct the specification and patterning of the appendage (wing) versus the body wall are illustrated above. The activity of the EGFR pathway promotes body wall identity while repressing wing fates. Notch induces the growth of the wing disc, which separates the source of the Wg and EGFR pathways and allows the specification of the wing field by Wg and the activation of wing promoting genes. Below are shown the expressions of the Dpp and Wg pathways in a third instar disc and the relative position of the Dpp target genes sal, omb, and brk.

Consequently, in the absence of Wg the wing field is lost and a duplication of proximal structures is observed. In the early primordium, the activity of the EGFR pathway represses the ability to induce wing fates by Wg and promotes notum fates. As the disc grows in size induced by the activity of Notch, the signaling sources of the EGFR and Wg pathways become separated allowing the specification of the wing field by Wg and the activation of wing promoting genes such as nubbin (nub) and vg. The activation of nub and vg is accompanied by the repression of the body wall genes teashirt (tsh) and hth (Figure 3). The different P-D expression of hth/tsh (notum and hinge) and vg (wing blade) is analogous to the leg disc, where hth/tsh are also restricted to the proximal domain to promote body wall structures (Figure 1C). It

has been proposed that as in the leg, Wg and Dpp in the wing disc are required, through the activation of el and noc, to repress hth and tsh expression from the wing territory. However other mechanisms have also been proposed. The EGFR pathway is also required to activate the dorsal selector gene apterous (ap) that subdivide the thorax and the wing field in dorsal (D) and ventral (V) compartments.

This D-V subdivision is absolutely necessary for vg expression and wing outgrowth. Ap regulates the expression of the Notch ligands Dl and Ser that in turn activate the Notch pathway at the D-V boundary. Wg and Notch cooperate to induce the expression of vg in the cells that will become the wing blade. The mutual antagonism between hth and vg subdivide the wing field in the pouch and the hinge. The hinge region of the wing field is specified by the combined action of Wg, Hth, and Tsh. In the proximal region of the disc, the EGFR pathway induces the expression of the notum specifying genes of the Iroquois-complex (Iro-C) that are repressed in the wing field by Dpp secreted in the A-P boundary (Figure 3). Once specified, the notum is further subdivided in medial and lateral domains by the activity of the transcription factor Pannier (Pnr) that promotes medial vs lateral fates.

As in the leg disc, en expression subdivides the wing disc in two populations of cells, the A and P compartments. The A-P boundary acts a source of positional information where Hh secreted from the posterior compartment activates the expression of dpp in A cells. Dpp and Hh are both required to pattern the wing blade along the A-P axis (Figure 3). Dpp diffusion from the A-P boundary restricts the expression of brinker (brk) to the lateral domain of the wing. Dpp and Brk in turn, regulates the expression of omb and the spalt genes (sal) in nested domains. The Hh and Dpp signals act coordinately with the EGFR signaling pathway to position and maintain vein territories in the developing wing.

Patterning of the Eye-Antennal Disc:

The eye-antennal disc (EAD) is formed by different populations of cells located in the cephalic segments that coalesce together. The EAD is a compound disc that gives rise to the eye, the ocelli, the palpus and the antenna plus the head cuticle that surrounds them. Homeotic transformations from antenna to leg have been described for several mutations, including hth, spineless (ss), and Antp that converts every antenna part to its corresponding leg segment (Figure 4B). Therefore, the antenna and leg appendages have been considered serial homologous structures that share a similar developmental program. Thus, variations in this developmental program are responsible for the morphological differences between these two appendages.

Early on larval development (L1) the EAD shows no sign of regional specification and presents uniform expression of several transcription factors including hth and tsh and the eye determinants eyeless (ey) and sine oculis (so) (Figure 4A). The two main developmental fields within the disc, the antenna and the eye, are segregated during larval stages (L2) at the time when specific eye and antenna determinants are restricted to the corresponding EAD domain. The transcription factors Ey, Eyes absent (Eya) and So are selectors for eye development while Hth, Dll, Ss, and Cut have been proposed to select for antennal fates. The antagonistic interactions between the Notch and the EGFR pathways regulate the eye and antennal fate decision (Figure 4A).

Accordingly, the downregulation of the Notch pathway or the activation of the EGFR in the eye field transforms it into an antenna. However, the role of Notch as an eye identity inducer has been debated. In this view, Notch influences eye field specification through its control on cell proliferation, thus modulating the activity of the Dpp and Wg signaling pathways located at opposing sides of the early EA disc. Wg is a negative regulator of eye development while Dpp induces the expression of the eye determinant eya. Once the two morphogenetic fields have been established, the restricted expression of

antenna and eye promoting genes is observed (Figure 4A). For example, Cut and Hth represses the eye specification genes ey and so while the latter represses hth and cut. The patterning logic that controls the P-D axis formation in the antenna is similar to what can be found in the leg. Hh secretion from posterior cells activates the expression of dpp and wg in dorsal and ventral anterior cells, respectively that in turn directs P-D axis formation through the regulation of Dll, Dac, and hth. As in the leg, Dll specifies distal fates while Hth promotes proximal identities.

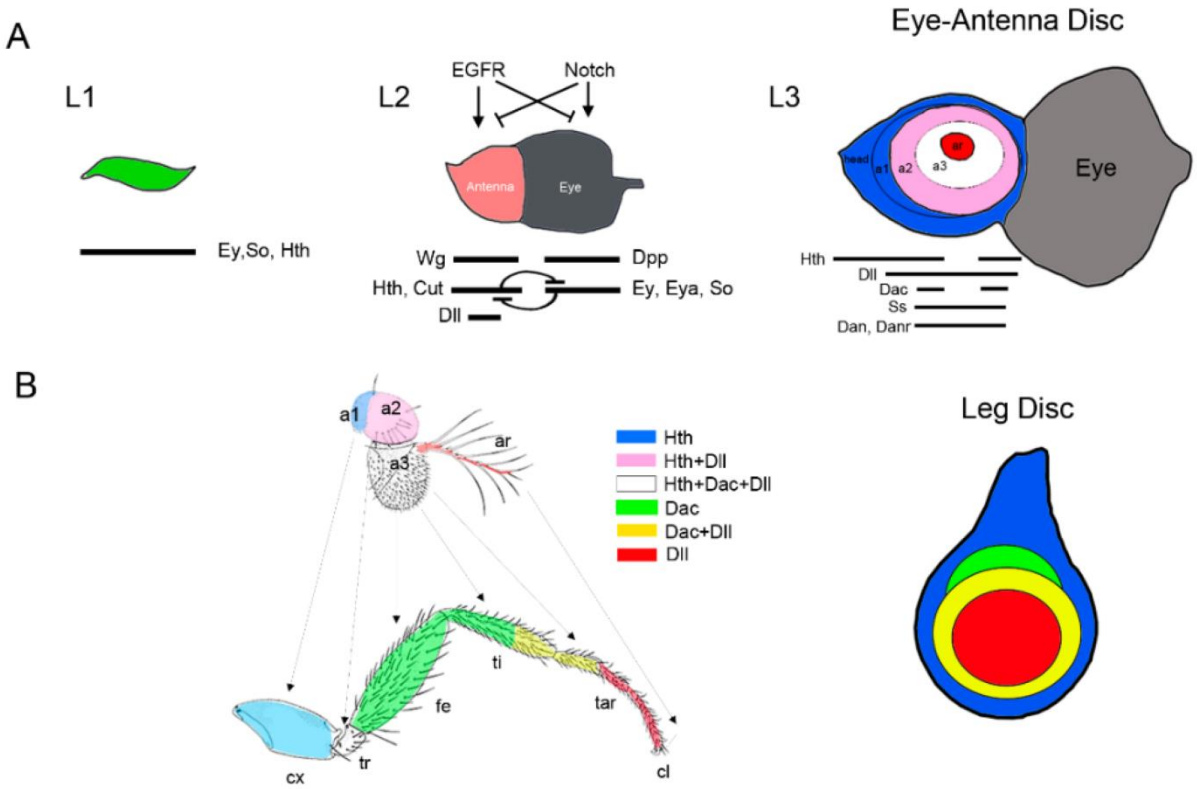


Figure 4. Specification and patterning of the Drosophila antenna. (A) Model of progressive specification of the eye and antenna territories and P-D subdivision of the antenna disc. Schematic representation of three representative stages of EAD development is shown. In each disc the expression and interactions of genes and signaling pathways required for eye and antenna field specification are schematized.
(B) The antenna and leg appendages are homologous structures. Arrows indicate the correspondence between the antenna and leg domains (Postlethwait and Schneiderman, 1971). The expression of the P-D genes Dll, dac, and hth and their overlapping domains are represented by a color code. Compare the relative expression of Dll, dac, and hth in the antenna and the leg imaginal discs. Note that hth and Dll coexpress in a large domain in the antenna while in the leg these genes are expressed in almost exclusive domains.

Conclusion:
Importantly, many of the signaling pathways and transcription factors identified in the fly have similar functions during vertebrate limb development. However, many important questions remain unsolved. First, the target genes and the regulatory networks controlled by
the transcription factors that subdivide the appendages along the different axes are still largely unknown. This includes the analysis of the molecular interactions between patterning genes and signaling pathways. Second, further investigation would be required to unravel how the patterning information is translated into cellular behaviors (cell growth, division, and cytoskeleton organization, among others), which are

ultimately responsible for the characteristic shape and size of the different appendages of the fly. The use of whole genome techniques such as ChIP (Chromatin Immunoprecipitation) or ATAC (assay for transposase accessible chromatin) assays, coupled with whole genome sequencing is essential to identify the target genes and the regulatory landscapes governed by the patterning transcription factors.

Probable Questions:

1. Describe how allocation of the Thoracic Appendage Primordia occurs?
2. How ventral and dorsal primordial are specified?
3. Describe dual development theory of drosophila wing.
4. How patterning of the Leg imaginal Disc occurs in insects?
5. How patterning of the wing imaginal Disc occurs in insects?
6. How patterning of the Eye-Antennal Disc occurs in insects?

Suggested Reading / References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-VIII

Postembryonic development: growth, cell proliferation, growth hormones; aging- genes involved in alteration in timing of senescence

Objective: In this unit we will discuss about postembryonic growth and development. We will also discuss aging- genes which remain involved in alteration in timing of senescence

Aging:

Aging is characterized by a gradual functional decline. In mammals, aging occurs heterogeneously across multiple organ systems, causing a progressive deterioration that eventually results in tissue dysfunction. Consequently, age is a risk factor for many diseases, such as cardiovascular disease, dementia, osteoporosis, osteoarthritis, cancer, type 2 diabetes, idiopathic pulmonary fibrosis and glaucoma.

Although its biological causes remain largely unknown, studies in the past few decades have identified common cellular and molecular traits associated with aging.

Aging hallmarks can be divided into three categories:

(1) primary, or the causes of age-associated damage; (2) antagonistic, or the responses to the damage; and (3) integrative, or the consequences of the responses and culprits of the aging phenotype. Senescence, a cellular response that limits the proliferation of aged or damaged cells, belongs to the antagonistic class. Although senescence plays physiological roles during normal development and it is needed for tissue homeostasis, senescence constitutes a stress response triggered by insults associated with aging such as genomic instability and telomere attrition, which are primary aging hallmarks themselves. There is also an intimate link between senescence and the other antagonistic hallmarks of aging. For example, senescent cells display decreased mitophagy, resulting in an “old,” defective mitochondrial network that may contribute to metabolic dysfunction in age.

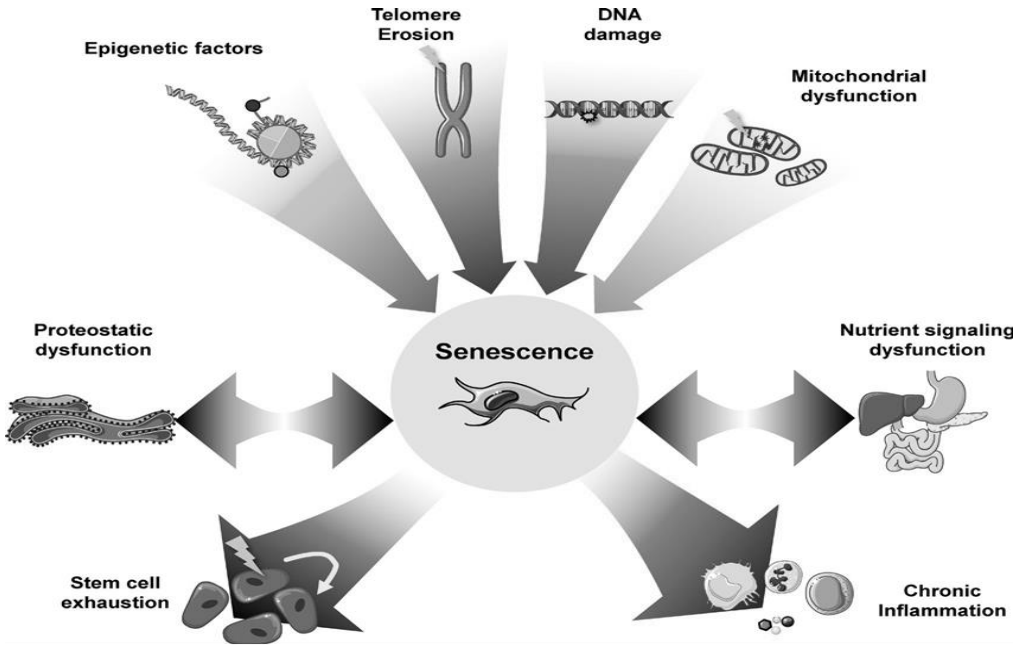


Figure : Senescence as a central hallmark of aging. Telomere damage, epigenetic dysregulation, DNA damage, and mitochondrial dysfunction are primary drivers of damagein aging. Several of these drivers of damage can induce senescence. Senescence can in turn drive the consequential aging hallmarks in response to damage: stem cell exhaustion and chronic inflammation. Other responses to damage, such as proteostatic dysfunction and nutrient sigalling disruption, are also integrally linked with the senescence response.

Senescence also influences the integrative aging hallmarks. Somatic multipotent stem cells facilitate tissue homeostasis; for example, hematopoietic stem cells (HSCs) renew the blood system. Stem cell exhaustion occurs with age, and the consequent decline in stem cellfunctionality and their capacity for renewal leads to tissue deterioration. For example, HSCs display a decreased success rate of transplantation when isolated from elderly patients. This decline correlates with increased numbers of senescent HSCs and diminished immunity, decreased numbers of naive B and T cells, and reduced natural killer cell activity. Somatic stem cell decline is not limited to high-turnover tissues. Neural stem cells (NSCs) experience reduced functionality, with limited neurogenesis capacity with age. This is marked by a twofold reduction in NSC numbers and a decreased proliferation, which correlates with increased expression of senescence markers in the regions where NSCs reside. Mesenchymal stem cells and their descendants, satellite cells, chondrocytes, adipocytes, and osteoclasts, also display a reduced ability to self-renew with age that correlates with increased levels of

senescence markers. This may have an impact in age-associated pathologies such as sarcopenia, cachexia, osteoporosis, and osteoarthritis. Altered intracellular communication is another of the integrative hallmarks of aging. In particular, chronic low-level inflammation is a serious complicating factor for many diseases in which risk increases with age. This detrimental role of inflammation is supported by inflammatory markers such as interleukin-1 (IL-1) and IL-6 acting as prognostic markers for diseases such as type 2 diabetes, atherosclerosis, and breakdown in stem cell function. Inflammatory responses are one of the major extrinsic effects of senescent cells, which suggests that there is a link between senescence and altered intracellular communication. Aging influences a broad range of disease etiologies. Therefore, targeting the underlying aging machinery may provide broad-spectrum protection against many pathologies.

What is senescence?

Senescence is cellular program that induces a stable growth arrest accompanied by distinct phenotypic alterations, including chromatin remodeling, metabolic reprogramming, increased autophagy, and the implementation of a complex proinflammatory secretome. These complex changes to the cell largely serve to implement various aspects of senescence such as growth arrest and the senescence secretome. Despite the many facets of senescence, stable growth arrest is its defining characteristic. A permanent arrest is effective to ensure that damaged or transformed cells do not perpetuate their genomes. This growth arrest is implemented by the activation of p16INK4a/Rb and p53/p21CIP1 tumor suppressor networks.

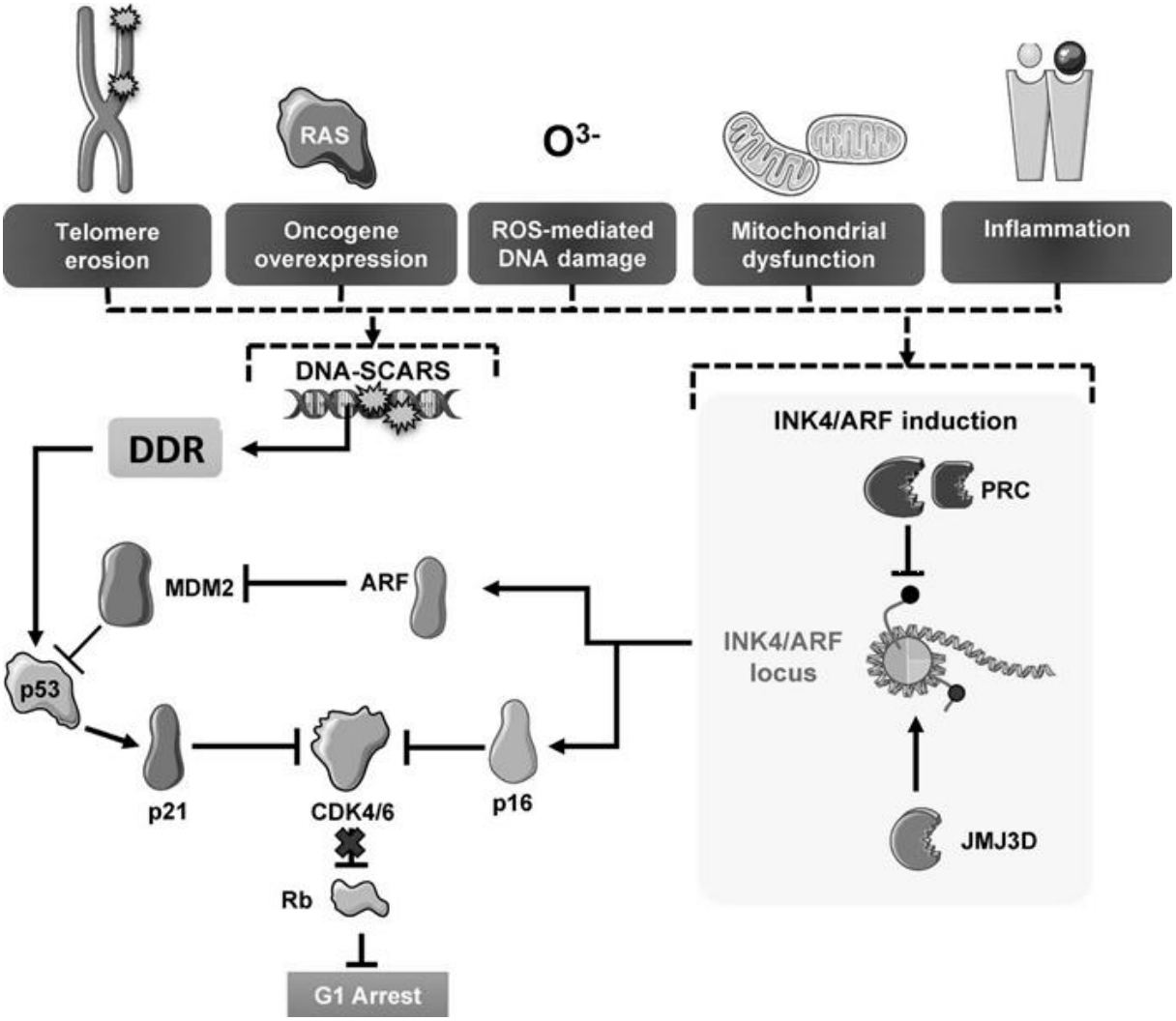


Figure : Pathways regulating senescence- mediated arrest. The senescence growth arrest is regulated through two main pathways, p16INK4a/Rb and p53/p21CIP1, both which convergeon repression of CDK4/6. The INK4A/ ARF locus is normally silenced by Polycomb repressive complexes (PRCs) and becomes activated during senescence. The p53/p21CIP1 pathway is activated downstream of the DNA damage response (DDR) from repair-resistant DNA segments with chromatin alterations reinforcing senescence (DNA-SCA RS).

Historically, senescence was first identified by Hayflick and Moorhead (1961) during serial passage of human fibroblasts. The limit to proliferation that senescence imposes was hypothesized as a barrier to cancer initiation. Senescence is indeed a powerful mechanism of tumor suppression. Senescence has also physiological roles during normal development, acting in concert with apoptosis to facilitate embryonic morphogenesis. In adult tissues, senescence is triggered primarily as a response to damage, allowing for suppression of potentially dysfunctional,

transformed, or aged cells. The aberrant accumulation of senescent cells with age results in potential detrimental effects. In balance, although senescence is a biologically necessary process, it may come at a cost. The early research of Hayflick and Moorhead (1961) hinted at a relationship between senescence and aging, but the consequent discovery that senescent cells accumulate in aged tissues has substantiated the hypothesis thatsenescence itself can drive aging.

Factors driving senescence:

Telomere damage driving senescence in aging

In adult tissues, senescence is engaged in response to different types of damage. One of the insults causing senescence is damage of the telomeres, highly repetitive DNA structures located at the end of chromosomes. Telomeres are protected by a multiprotein complexknown as shelterin. By coating the telomere, shelterin prevents the activation of a DNA damage response, thereby preventing end-to-end chromosome fusions that would result in a telomere crisis. Moreover, cells lacking shelterin components, such as POT1 or TRF2, suffer an aberrant DNA damage response and premature induction of senescence. The end- replication problem is a consequence of the inability of DNA polymerases to synthesize DNAwithout a template, which occurs at telomeres. This results in telomeres that shorten progressively with each cell cycle division. Embryonic tissues circumvent this erosion by expressing telomerase, a ribonucleoprotein complex that serves to concatenate DNA to the ends of chromosomes, thus providing a template for DNA synthesis. Repeated cell division inadult tissues that lack telomerase, however, results in progressive erosion of DNA, reduced shelterin binding, and senescence. As an organism ages, cells accumulate more divisions. This results in increased telomere erosion and senescence. But the extent to which telomere erosion drives senescence during aging and contributes to the aging process itself remains unknown.

Supporting the causative role of telomere erosion in aging, deletion of telomerase in mice eventually results in premature aging. This phenotype can be rescued by transient activation of telomerase reverse transcription expression in mice using a telomerase reverse transcription estrogen receptor construct. Cells isolated from these mice proliferate normally in vitro, and deterioration in multiple tissues is reduced. This evidence correlates with studies showing that fibroblasts or T cells derived from centenarians reset their telomeres, which results in rejuvenation and sustained proliferation. Similarly, stimulation of T cells derived from serially transplanted HSCs results in telomerase expression and rejuvenation. Shortened telomeres are associated with many pathologies such as liver cirrhosis and correlate with an increase in mortality in people older than 60 years. Correlative evidence supports telomere erosion as a major driver of aging decline, yet this is challenged by mammals such as laboratory mice (Mus musculus), whose telomeres do not reach a critical limit during normal aging. Telomere length is also not predictive of aging deterioration in mice, highlighting that alternative factors could also drive aging.

Metabolic dysfunction as a driver of senescence

Several lines of evidence suggest that aging is the result of a complex amalgam of damages such as metabolic and proteostatic dysfunction. Metabolic dysfunction relates to aging at the organismal and molecular level. Multiple studies have demonstrated that caloric restriction can retard the aging decline. Molecularly, pathways finetuning metabolic regulation, such as the mTOR or insulin pathway, have also been linked to increased health span and life span. mTORC1 integrates inputs from nutrient and growth signals to regulate general cellular processes such as protein and lipid synthesis, autophagy, and metabolism. In this regard, mTOR is able to regulate the senescence-associated secretory phenotype (SASP), autophagy, and senescent growth arrest. The connection between autophagy and senescence is complex; although there is an increase in autophagy during senescence that serves to regulate SASP production, inhibition of autophagy can induce senescence through metabolic and proteostatic dysfunction, further emphasizing the intricate connection between metabolic stress and senescence in aging. Sirtuins constitute another molecular link between metabolism and senescence. Sirtuins are ribosyltransferases with a wide

array of functions, such as metabolism regulation and DNA repair. Their role in senescence is antagonistic; SIRT1 deacetylates p53, promotes its degradation and facilitates senescence bypass, whereas SIRT6 deacetylates H3K18 to preventmitotic errors and suppress senescence.

In addition to these forms of damage, general stress is sensed by other mechanisms such as activation of MAPK p38 or induction of p16INK4a. These pathways are up-regulated in response to oxidative stress, DNA damage, telomere attrition, or oncogene activation. Substantiating their role in aging, activation of MAPK p38 or induction of p16INK4a limits the proliferative potential of HSCs and yields proaging phenotypes. Overall, it is likely that the accumulation of senescent cells during aging reflects a gradual increase of different types of damage in different tissues.

Pathways regulating the senescence growth arrest:

Despite the multifaceted nature of senescence, the induction of stable growth arrest is the defining characteristic of senescence. Moreover, stable arrest is paramount to halt the propagation of dysfunctional cells. Two tumor suppressor pathways, p53 and the p16/Rb, are responsible for the implementation of this growth arrest.

p53 and senescence

Senescence inducers such as telomeric attrition and oncogenic or oxidative stress cause DNA damage. DNA damage results in increased deposition of γH2Ax and 53BP1 in chromatin that in turn activates a kinase cascade involving first ATM and ATR and then CHK1 and CHK2, eventually resulting in p53 activation. p53 induces transcription of the cyclin-dependent kinase inhibitor p21CIP1. In turn, p21CIP1 blocks CDK4/6 activity, resulting in hypophosphorylated Rb and cell cycle exit. Although transient increases in p53 levels can enact a quiescent state and activate DNA repair processes, during senescence, there is a sustained induction of p53. This is a result of damage occurring in repair-resistant regions of the genome known as DNA segments with chromatin alterations reinforcing senescence, such as telomeres, that allow for a permanent arrest of the cell cycle by persistent induction of p21cip1. Given the key roles of p53, additional regulatory layers exist. For example, the induction of ARF, a product of the INK4/ARF locus, sequesters the ubiquitin ligase MDM2, contributing to increased levels of p53. Recently, the

interaction between Forkhead box protein O4 (FOXO4) and p53 has been shown to play an important role in modulating p53 localization and transcriptional activity during senescence. Interestingly FOXO transcription factors regulate aging, with FOXO activity in Drosophila melanogaster leading to delayed aging in response to disrupted protein homeostasis and oxidative stress.

The INK4/ARF locus in senescence

Three tumor suppressors reside in the INK4/ARF locus: p16INK4a and ARF, which are both encoded by the CDKN2A gene, and p15INK4b, which is encoded by CDKN2B. Two of these, p15INK4b and p16INK4a, are CDKIs, like p21CIP1, that affect the cell cycle by binding and inhibiting CDK4 and CDK6. In contrast, ARF inhibits MDM2, thereby allowing cross talk with the p53/p21CIP1 pathways. Conversely, p53 can regulate expression of ARF through a negative feedback loop, as demonstrated by elevated ARF expression in p53−/− mouse embryonic fibroblasts.

Given this unusual concentration of three tumor suppressors in barely 35 kb, this locus plays a key regulatory role and is frequently mutated in cancer. Genome-wide association studies have also identified various genomic variants occurring at the INK4/ARF locus as major risk factors for atherosclerosis, stroke, and diabetes, among other pathologies. However, most of these are found in noncoding regions, and the precise mechanism of action is unclear. The INK4/ARF locus behaves as a senescence sensor. In young, normal cells, the INK4/ARF locus is epigenetically silenced through deposition of repressive H3K27me3 marks. H3K27 methylation is controlled by Polycomb repressive complexes (PRC2 and PRC1). Disrupting PRC1 or PRC2 activity by depleting the expression of some of their components, such as BMI1, CBX7, or EZH2, derepresses p16INK4a and induces senescence. There is still debate over how Polycomb is recruited to the INK4/ARF locus. It has been proposed that a long noncoding RNA, ANRIL, divergently transcribed from the INK4/ARF locus, and transcription factors such as those of the homeobox family can contribute to recruiting PRCs. Conversely, during senescence, the H3K27

histone demethylase JMJD3 plays a role inremoving the repressive marks around the INK4/ARF locus, facilitating its induction. INK4/ARF induction can be observed in tissues during natural aging. In particular, p16INK4a is considered an aging biomarker. With exceptions (such as during senescence- induced during development), p16INK4a is also one of the best markers of senescence. An analysis of the pathways regulating p16INK4a shows coincidences with those controlling development. This has been argued to formulate the theory that aging might be driven by gradual functional decay of developmental pathways.

The SASP

Besides growth arrest, the production of a complex mixture of secreted factors, termed the SASP or senescence-messaging secretome, is the most relevant phenotypic program implemented in senescent cells. Senescent cells secrete hundreds of factors that include proinflammatory cytokines, chemokines, growth factors, and proteases.

Regulation of the SASP

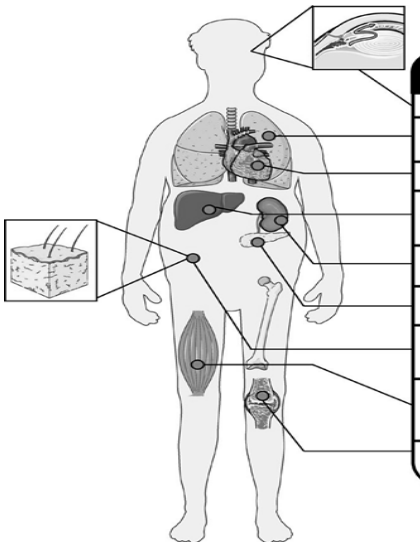
The specific combination of secreted factors is thought to depend on the cell type and the senescent inducer. However, many of the key effectors of the SASP and its regulatory mechanism seemed to be shared. Nuclear factor κB (NF-κB) and CCAAT/enhancer–binding protein beta are the key transcriptional SASP regulators. DNA damage, p38α MAPK, mTOR, mixed lineage leukemia 1, and GATA4 are also able to regulate the SASP. Recently, sensing of cytoplasmic chromatin by the cGAS/STING pathway has been suggested as a trigger for SASP induction. There are additional layers of SASP regulation. For example, mTOR controls IL-1α translation to regulate the SASP. In addition, mTOR indirectly regulates the activity of ZFP36L1, an RNA-binding protein that binds to AU-rich elements in the 5′-end of inflammatory transcripts, targeting them for degradation. There is also a global remodeling of enhancers in senescent cells, and the recruitment of BRD4 to superenhancers adjacent to SASP genes is needed for their induction.

The complex composition of the SASP means that different subsets of the SASP, such as the

proinflammatory and TGF-β secretomes, can be regulated independently. The proinflammatory arm of the SASP is regulated by IL-1 sigalling. IL-1α partially recapitulates the inflammatory SASP in vitro, and inhibiting the NLRP3 inflammasome, which processes IL-1β, can blunt the SASP. Conversely, the juxtacrine Notch sigalling pathway promotes the secretion of a TGF-β–enriched secretome.

Contribution of senescence to age-related diseases

Now that a general causative role for senescence during aging has been established, the next step is to identify how senescence contributes to different age-related pathologies such as glaucoma or osteoarthitis. Thanks to the use of senolytic drugs and genetic models for senescence ablation, we are progressing quickly in that task.



Diseases	Senescence Markers	$p16^{-/-}$ or $p53^{-/-}$ mice	Senolytic	Reference
Glaucoma	✓			Liton et al. 2005
Idiopathic pulmonary fibrosis (IPF)	✓		✓	Yanai et al. 2015 Schafer et al. 2017
Atherosclerosis	✓		✓	Uryga and Bennett, 2016 Childs et al., 2016
Liver cirrhosis / NAFLD	✓	✓	✓	Krizhanovsky et al. 2008 Kim et al., 2013 Ogrodnik et al., 2017 Wiemann et al., 2002
Glomerulosclerosis	✓		✓	Melk et al. 2003, 2004 Baker et al. 2016
Type 2 Diabetes	✓	✓		Chen et al., 2009 Helman et al. 2016
Cachexia	✓	✓	✓	Berry et al., 2017 Xu et al., 2015 Baker et al., 2016
Sarcopenia	✓	✓	✓	Sousa-Victor et al., 2014 Cosgrove et al., 2014 Chang et al., 2016
Osteoarthritis	✓		✓	Price et al. 2002 Kuyinu et al., 2016 Jeon et al., 2017

Figure. Involvement of senescence in disease. Establishment of robust biomarkers of senescence, usage of genetic knockout models and senolytic models are expanding our knowledge on the age-related diseases in which senescence plays a role.

Opposing roles for senescence in cancer

Age is a strong prognostic marker of reduced survival across many cancers. Senescence is a strong tumor suppressor mechanism that limits cancer initiation through both cell-intrinsic and cell-extrinsic mechanisms. However, there is strong evidence suggesting that through the SASP, aged tissues provide a supportive niche for cancer. Senescent cells can contribute to tumor progression by enhancing the proliferative potential of cancer cells or contributing to epithelial to mesenchymal transition. Therefore, the increased numbers of senescent cells present in aged tissues could contribute to the increased incidence of cancer with age. Supporting this, a delayed onset in tumor formation is observed when senescent cells are eliminated. Senolytic therapy also reduces the incidence of metastasis, the leading cause of cancer-related deaths.

Renal dysfunction

Aged individuals often display a reduced glomerular filtration rate and cortical volume that can result in glomerulosclerosis and nephron atrophy, both of which are associated with increased expression of p16INK4a and p53. Senescence has detrimental effects in most renal diseases analyzed. Ablation of senescent cells protects against glomerulosclerosis and improves kidney function in aged mice.

Type 2 diabetes

One of the largest risk factors for the development of type 2 diabetes is age. Several genome- wide association studies of type 2 diabetes have highlighted variants at the INK4/ARF locus, suggesting a possible link between senescence and diabetes. In addition, senescence markers and IL-1β are elevated in β cells from diabetic mice. Surprisingly, although p16INK4a expression drove a decline in β cell regenerative capacity and predisposed mice to mild diabetes, senescent β islets increased insulin secretion, making it unclear how senescence contributes to maintain glucose homeostasis.

IPF:

Fibrosis is a pathological condition whereby tissue accumulates ECM proteins such as collagen, resulting in tissue scarification, usually in response to damage. Senescence appears to have both beneficial and detrimental roles during fibrosis and wound healing. Secretion of MMPs, which occurs as part of the SASP, could help in the resolution of fibrotic plaques. Conversely, fibroblasts and tissues isolated from IPF patients display increased levels of SA- β-Gal staining and p21CIP1, suggesting a link with senescence. The detrimental nature of senescence in IPF was recently demonstrated using senolytics. Elimination of senescent

fibroblasts in a mouse model of lung fibrosis reduced expression of profibrotic SASP components and improved pulmonary function.

Nonalcoholic fatty liver disease

Cirrhosis is the pathological outcome from liver fibrosis and nonalcoholic fatty liver disease, which in turn is a result of hepatic steatosis, the abnormal accumulation of lipids in hepatocytes. Senescence is associated with liver fibrosis and cirrhosis. The risk of developingnonalcoholic fatty liver disease increases with age and is predicted by the presence of senescent hepatocytes. The elimination of senescent cells using INK-ATTC mice reduces liver fat accumulation. The role of senescence in the liver is complex, however, because knocking out p53 or p16INK4a increases liver fibrosis. Moreover, senescent hepatic stellate cells down-regulate collagen and up-regulate MMPs and cytokines that could remodel fibrotic plaques and recruit macrophages.

Cardiovascular disease

The risk of developing atherosclerosis and cardiomyopathy and their respective conditions, coronary heart disease and heart failure, increases with age. In the case of atherosclerosis, the role of senescence has been confirmed using senolytic models. Ablation of senescent cells improved the stability of plaques and reduced both the incidence and progression of plaque formation. Senescent cells were initially identified in atherosclerosis in vascular smooth muscle cells at the site of the plaque. Subsequent studies showed that macrophages were the primary senescent cell present with higher levels of SA-β-Gal staining and SASP production, suggesting their key contribution to coronary heart disease. Cardiomyocyte atrophy is one of the underlying causes of myocardial infarction in the elderly. It is unclear how ablation of senescent cells protects against cardiomyocyte hypertrophy in aged mice and provides resistance to cardiac stress.

Osteoarthritis

Lifelong wear and tear on ligaments is a significant risk factor for the development of arthritis. Failure of chondrocytes to produce cartilage results in degradation of joints and immobilization. Expression of p16INK4a in these cells correlates with severity and progression of the disease. Moreover, when mice were subjected to an acute trauma to model osteoarthritis, senescent cells accumulated in the site of the injury. Clearance of these senescent cells using senolytics resulted in the increased functionality of the remaining chondrocytes with rejuvenation of cartilage soon after.

Decline in immune function with age

One of the primary risk factors for complications in end-of life care is infection. The inability of the body to raise a response to immune offenses is caused by a functional decline in HSCs. The accumulation of senescent HSCs with age contributes to immune decline and senescence bypass allows for stem cell rejuvenation. Interestingly, the removal of these cells restored the functionality of HSCs and increased myeloid, B, and T cell numbers in transplant experiments.

Sarcopenia

Muscle stem cells (MuSCs) undergo a decline in their ability to differentiate and facilitate repair of muscle tissue, which is hypothesized to be the underlying cause of age-dependent muscle wasting or sarcopenia. MuSCs are quiescent unless stimulated to repair muscle. However, with age, they become senescent, up-regulating p16INK4a. The elimination of senescent MuSCs increases the ability of the remaining MuSCs to form muscle cell colonies. Additionally, inhibition of p38 or p16INK4a bypasses MuSC senescence and strengthens muscle in geriatric mice.

Age-related cachexia

Loss of adiposity and loss of muscle mass in aged individuals are primary contributors to age-dependent wastage or cachexia. White adipose tissue isolated from aged mice display SA-β-Gal activity. Removal of senescent cells from mice leads to increased adiposity and prevents mass loss in aged mice. Recently it has been shown that bypass of senescence or senolysis restores adipose beiging and adipogenesis and improves metabolic function in aged mice. This suggests that senescent cells prevent adipocyte differentiation and contribute to an age-dependent loss of adaptive thermogenic capacity and metabolic dysfunction.

Probable questions:

1. What is aging? Describe different hallmarks of aging?
2. What is senescence? How different pathways regulates senescence?
3. Describe the role of p53 and INK4/ARF locus in senescence.
4. How aging and senescence is related to various diseases?

Suggested Readings:

Senescence and aging: Causes, consequences, and therapeutic avenues. A Review. Domhnall McHugh,and Jesús Gil. 2017

UNIT-IX

Regeneration– Epimorphic regeneration of reptile (salamander) limb; Morphollaxis regeneration in Hydra

Objective: In this unit we will discuss about regeneration. We will also discuss regeneration of salamander limb and hydra.

Introduction:

Regeneration is one of the processes in which if an organism is cut into several pieces, each of its parts regrows to the original state. This process is carried out by specialized cells called stem cells. It takes place in organisms that have a very simple structure with very few specialized cells.

The cells divide quickly into a large number of cells. Each cell undergoes changes to form various cell types and tissues. This sequential process of changes is known as development. The tissues form various body parts and organs.

Types of Regeneration :

I. Physiological Regeneration:

There is a constant loss of many kinds of cells due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration

Example:

a. Replacement of R.B.C's : The worn out R.B.C's are deposited in the spleen and new R.B.C's regularly produced fromthe bone marrow cells, since the life span of R.B.C's is only 120days.

b. Replacement of Epidermal Cells of the Skin

The cells from the outer layers of epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells added by the malpighian layer of the skin.

II. Reparative Regeneration

This is the replacement of lost parts or repair of damaged body organs. In this type of regeneration, wound is repaired or closed by the expansion of the adjoining epidermis overthe wound.

Example:

- Regeneration of limbs in salamanders
- Regeneration of lost tail in lizard
- Healing of wound

- Replacement of damaged cells.

Autotomy:

In some animals like starfish, some part of the body is broken off on being threatened by apredator. This phenomenon of self-mutilation of the body is called autotomy

Example:

- Crabs break off their leg on approaching of the enemy
- Holothurians throw off their internal viscera
- Starfish breaks off an arm
-

Regenerative capacity in Animal Group

The capacity of regeneration varies in its extent in various animal groups. Regenerativecapacity is very high among the protozoan, sponges and coelenterates.

Invertebrates

- In **sponges**, the entire body can be reconstructed from isolated body cells. The cells rearrange and reorganize to form bilayered sponge body wall.

- Regeneration was first discovered in hydra by Tremble (1740). Even $1/1000^{th}$ part of the body regenerate into new organisms.

- In hydra and planaria, small fragments of the body can give rise to a whole animal. When a hydra or a planaria is cut into many pieces, each individual part regenerates into a whole individual.

- Many **arthropods** (e.g., spiders, crustaceans, insect larvae, etc) can regenerate limbs only. Regeneration is faster in the young than in the adults. Regenerated part may not always be similar to the part lost. This type of regeneration is called heteromorphosis
- **Echinoderms** (like starfish, brittle star, sea lilly) exhibit autotomy. They can regenerate arms and parts of te body.

Vertebrates

- **Fishes:** Lamprey can regenerate its lost tail. Some fishes have the ability to regenerate parts of its fins.

- **Amphibians**: The regeneration power is well marked in urodel amphibians like salamanders, newts and their axolotl larvae. They can regenerate limbs, tail, external gills, jaws, parts of eye like lens and retina. Tail and limb regeneration is found in the larval stages of frogs and toads.

- **Reptiles:** Lizards exhibit autotomy. When threatened, the lizard detaches its tail near the base to

confuse its predator and later regenerates a new tail. The new tail differs from the old one in its shape, absence of vertebrae and the kind of scales covering it.

- **Birds:** Regeneration is restricted to parts of the beak.

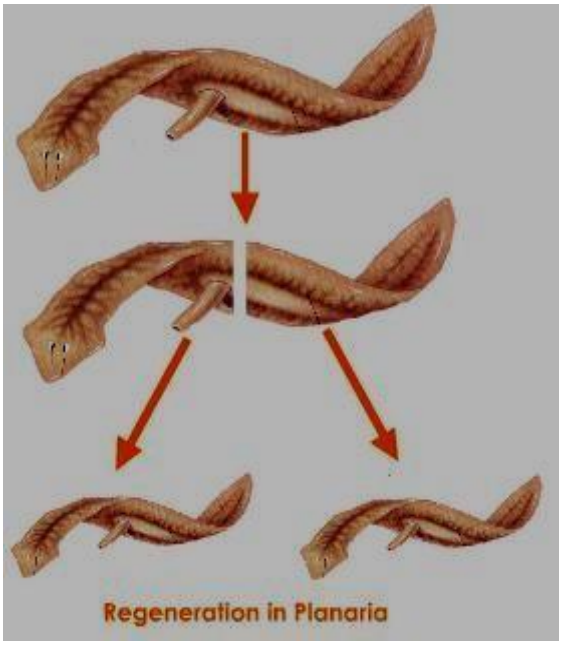
- **Mammals:** Regeneration is restricted to tissues only. External parts are not regenerated. Skin and skeletal tissues possess great power of regeneration. The liver has the maximum capacity of regeneration. If one kidney is damaged or removed, the other enlarges to compensate the lost kidney. This is called as compensatory hypertrophy.
- Regeneration is an usual form of asexual reproduction in several lower groups of animals.

Types of Regeneration based on Cellular Mechanism

Based on cellular mechanisms regeneration can be of two types:

a. Morphallaxis

In this type, regeneration occurs mainly by the remodelling of existing tissues and the re-establishment of boundaries, thus involving very little new growth. As a result, the regenerated individual is much smaller initially. It subsequently increases its size and becomes normal after feeding. This type of regeneration is known as **morphallaxis or morphallactic regeneration**.
Example: Regeneration of hydra from a small fragment of its body.



b. Epimorphosis

In this type, regeneration involves dedifferentiation of adult structures in order to form an undifferentiated mass of cells. They are highly proliferating and accumulate under the epidermis, which has already expanded. Within two days, bulge transforms into a conical hump. This lump of dedifferentiated cells along with the epidermal covering is called regeneration bud or regeneration blastema. The dedifferential cells continue to proliferate and finally redifferentiate to form a rudiment of the limb. The rudiment eventually transforms into a limb. This type of regeneration is

known **as epimorphosis or epimorphic regeneration**.

Example: Limb regeneration in amphibians.

c. Heteromorphosis or heteromorphic regeneration

When a different organ develops from the one that has been removed, the phenomenon is called heteromorphosis. Eg. In shrimp Palinurus, eye is regenerated, If it is removed from the eye stalk. But if the eye is removed along with optic ganglion, instead of eye an antenna like organ is regenerated. **This type of regeneration is exhibited by lower animals.**

d. Super regeneration

The development of superfluous number of organs or parts of the body (eg. Heads, tail limbs) as a result of regeneration is known as super regeneration. When a deep incision is made on the head end of a planaria or earthworm, additional heads will develop. Incisions in the middle part cause the development of both heads and tails.

e. Wolffian regeneration:

It is a special kind of regeneration found in urodles and anurans. In Newt, Triturus, if the lens of the eye is removed, a new lens is formed from uninjured iris. The original lens is developed from epidermal ectoderm but the regenerating lens, formed from iris is neurectodermal in origin. Thus regeneration of a part of an organ from a tissue other than its original embryonic tissue is called Wolffians regeration, named after the discover Wolf(1935).

Mechanism of Regeneration

Regeneration is a complex process which basically involves histological and physiological events.

Regeneration in Hydra

Hydra had been used as the model of regeneration since time immemorial. Once wounded their cells start to regenerate and grow into pre-existing cells.

Hydra grows by continuously losing cells from its base and by budding

↓

A gradient of inhibitor is produced by the head. This prevents the formation of other heads

↓

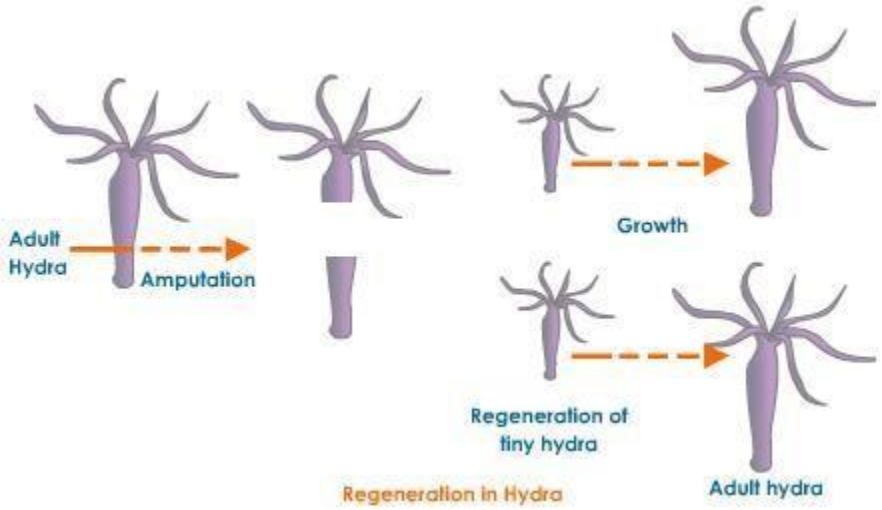
The concentration of inhibitor is reduced

↓

The positional value increases locally

↓

A new head is formed without any new growth



Regeneration of a Limb of a Newt

The mechanism of regeneration in salamander involves the following stages-

- Wound healing: The epidermal cells from the edges of the wound migrate and spread over the exposed surface. This is known as wound healing.
- Blastema formation: A few days later, undifferentiated cells accumulate inside the epidermis, resulting in a bulge. This is known as **regeneration bud or blastema**.
- Redifferentiation and morphogenesis: The blastema develops rudiments of the lost organ, like the digits which grow into new digits.
- Growth: The regenerated limb increases and attains the size of a normal limb.

  In planarians and in Hydra, there are undifferentiated cells called **neoblasts** which multiply and then migrate from the deeper parts of the body to the cut surface.

Epimorphic Regeneration of Salamander Limbs

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper order. In other words, the new cells construct only the missing structures and no more. For example, when a wrist is amputated, the salamander forms a new wrist and not a new elbow. In some way, the salamander limb "knows" where the proximal-distal axis has been severed and is able to regenerate from that point on.

Formation of the apical ectodermal cap and regeneration blastema

Salamanders accomplish this feat by dedifferentiation and respecification. Upon limb amputation, a plasma clot forms, and within 6 to 12 hours, epidermal cells from the remaining stump migrate to cover the wound surface, forming the wound epidermis. This single-layered structure is required for the regeneration of the limb, and it proliferates to formthe apical ectodermal cap. Thus, in contrast to wound healing in mammals, no scar forms,and the dermis does not move with the epidermis to cover the site of amputation. The nerves innervating the limb degenerate for a short distance proximal to the plane of amputation. During the next 4 days, the cells beneath the developing cap undergo a dramatic dedifferentiation: bone cells, cartilage cells, fibroblasts, myocytes, and neural cells lose their differentiated characteristics and become detached from one another. Genes that are expressed in differentiated tissues (such as the MRF4 and myf5 genes expressed in themuscle cells) are downregulated, while there is a dramatic increase in the expression of genes, such as msx1, that are associated with the proliferating progress zone mesenchyme of the embryonic limb. The formerly well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the regeneration blastema. These cells will continue to proliferate, and will eventually redifferentiate to form the new structures of the limb.

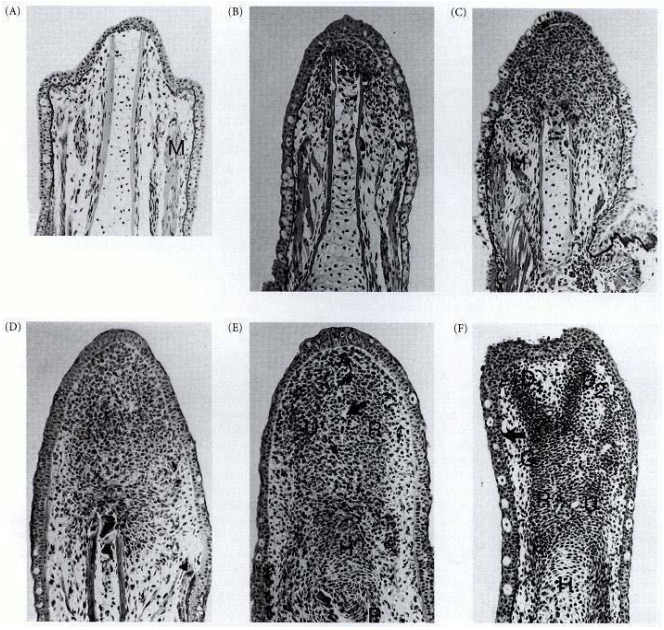


Figure. Regeneration in the larval forelimb of the spotted salamander Ambystoma maculatum. (A) Longitudinal section of the upper arm, 2 days after amputation. The skin and muscle have retracted from the tip of the humerus. (B) At 5 days after amputation, a thin accumulation of blastema cells is seen beneath a thickened epidermis. (C) At 7 days, a large population of mitotically active blastema cells lies distal to the humerus. (D) At 8 days, the blastema elongates by mitotic activity; much dedifferetiation has occured. (E) At 9 days, early redifferentiation can be seen. Chondrogenesis has begun in the proximal part of the

regenerating humerus, H. The letter A marks the apical mesenchyme of the blastema, and U and R are the precartilaginous condensations that will form the ulna and radius, respectively. P represents the stump where the amputation was made. (F) At 10 days after amputation, the precartilaginous condensations for the carpal bones (ankle, C), and the first two digits (D1, D2) can also be seen.

The creation of the blastema depends upon the formation of single, mononucleated cells. It is probable that the macrophages that are released into the wound site secrete metalloproteinases that digest the extracellular matrices holding epithelial cells together. But many of these cells are differentiated and have left the cell cycle. How do they regain the ability to divide? Microscopy and tracer dye studies have shown that when multinucleated myotubes (whose nuclei are removed from the cell cycle) are introduced into a blastema, they give rise to labeled mononucleated cells that proliferate and can differentiate into many tissues of the regenerated limb. It appears that myotube nuclei are forced to enter the cell cycle by a serum factor created by thrombin, the same protease that is involved in forming clots. Thrombin is released when the amputation is made, and when serum is exposed to thrombin, it forms a factor capable of inducing cultured newt myotubes to enter the cell cycle. Mouse myotubes, however, do not respond to this chemical. This difference in responsiveness may relate directly to the difference in regenerative ability between salamanders and mammals.

Proliferation of the blastema cells: the requirement for nerves:

The proliferation of the salamander limb regeneration blastema is dependent on the presence of nerves. Singer (1954) demonstrated that a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis- stimulating factors that increase the proliferation of the blastema cells. There are several candidates for these neural-derived mitotic factors, and each may be important. Glial growth factor (GGF) is known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation. When this peptide is added to a denervated blastema, the mitotically arrested cells are able to divide again. A fibroblast growth factor may also be involved. FGFs infused into denervated blastemas are able to restore mitosis. Another important neural agent necessary for cell cycling is transferrin, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site). When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema. Neural extracts and transferrin are both able to stimulate cell division in denervatedlimbs, and chelation of ferric ions from a neural extract abolishes its mitotic activity.

Pattern formation in the regeneration blastema:

The regeneration blastema resembles in many ways the progress zone of the developing limb. The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved, and cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are very similar. By transplanting regenerating limb blastemas onto developing limb buds, Muneoka and Bryant (1982) showed that the blastema cells could respond to limb bud signals and contribute to the developing limb. At the molecular level, just as Sonic hedgehog is seen in the posterior region of the developing limb progress zone mesenchyme, it is seen in the early posterior regeneration blastema. The initial pattern of Hox gene expression in regenerating limbs is not the same as that in developing limbs. However, the nested pattern of Hoxa and Hoxd gene expression characteristic of limb development is established as the limb regenerates. Retinoic acid appears to play an important role both in the dedifferentiation of the cells to form the regeneration blastema and in the respecification processes as the cells redifferentiate. If regenerating animals are treated with sufficient concentrations of retinoic acid (or other retinoids), their regenerated limbs will have duplications along the proximal- distal axis. This response is dose-dependent and at maximal dosage can result in a complete new limb regenerating (starting at the most proximal bone), regardless of the original level of amputation. Dosages higher than this result in inhibition of regeneration. It appears that the retinoic acid causes the cells to be respecified to a more proximal position .

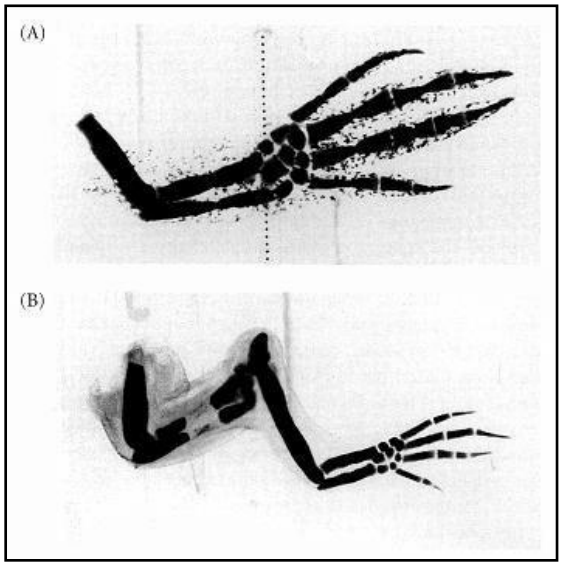


Figure: Effects of vitamin A (a retinoid) on regenerating salamander limbs. (A) Normal regenerated axolotl limb (9×) with humerus, paired radius and ulna, carpals, and digits. Dotted line shows plane of amputation through the carpal area. (B) Regeneration after amputation through the carpal area, but after the regenerating animal had been placed in retinol palmitate (vitamin A) for 15 days. A new humerus, ulna, radius, carpal set, and digit set have emerged (5×).

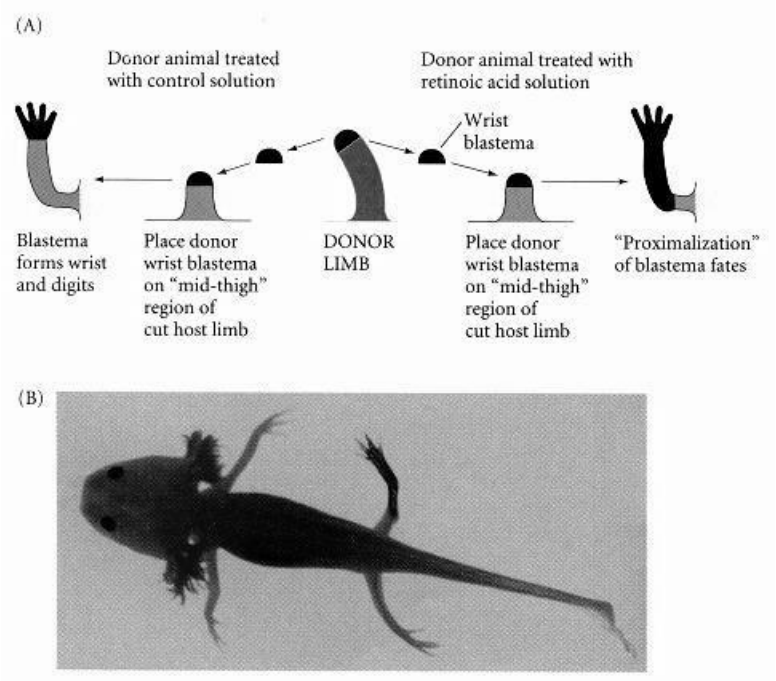


Figure: Proximalization of blastema respecification by retinoic acid. (A) When a wrist blastema from a recently cut axolotl forelimb is placed onto a host hindlimb cut at the mid-thigh level, it will generate only the wrist. The host (whose own leg was removed) will fillthe gap and regenerate up to the wrist. However, if the donor animal is treated with retinoic acid, the wrist blastema will regenerate a complete limb and, when grafted, will fail to cause the host to fill the gap. (B) Wrist blastema from a darkly pigmented axolotl was treated with retinoic acid and placed onto the amputated mid-thigh region of a golden axolotl. The treated blastema regenerated a complete limb.

Retinoic acid is synthesized in the regenerating limb wound epidermis and is seen to form a gradient along the proximal-distal axis of the blastema. This gradient of retinoic acid is thought to activate genes differentially across the blastema, resulting in the specification of pattern in the regenerating limb. One of these retinoic acid-responsive genes is the msx1 gene that is associated with mesenchyme proliferation. Another set of genes that may be respecified by retinoic acid is the Hoxa genes. Gardiner and colleagues (1995) have shown that the expression pattern of certain Hoxa genes in the distal cells of the regeneration blastema is changed by exogenous retinoic acid into an expression pattern characteristic of more proximal cells. It is probable that during normal regeneration, the wound epidermis/apical ectodermal cap secretes retinoic acid, which activates the genes needed for cell proliferation, downregulates the genes that are specific for differentiated cells, andactivates a set of Hox genes that tells the cells where they are in the limb and how much they need to grow. The mechanism by which the Hox genes do this is not known, but changes in cell-cell adhesion and other surface qualities of the cells have been observed. Thus, in salamander limb regeneration, adult cells can go "back to the future," returning to an "embryonic" condition to begin the formation of the limb anew.

Growth Factors

Wound healing is due to accelerated mitosis. This is mediated by proteins called growth factors which act locally.

EGF

Epidermal growth factor stimulates the epithelium to undergo mitosis. EGF is also produced in the salivary glands, which is why an animal's licking heals a wound.

FGF

Fibroblast growth factor stimulates the endothelial cells of the blood vessels to divide and heal the injured blood vessels.

Platelet

Derived growth factor which stimulates the mitosis of fibroblasts at the site of injury to fill in the damaged areas under the blood clot.

Polarity in Regeneration

The body segments of Hydra or planarians exhibit distinct polarity during regeneration. Their anterior end always regenerates into head and posterior into the tail

Probable Questions:

1. Describes different types of regeneration.
2. Describe regeneration in invertebrates.
3. Describe regeneration in vertebrates.
4. Describe Morphallaxis and Epimorphis with examples.
5. Describe mechanism of regeneration in Hydra with suitable diagram.
6. Describe Epimorphic Regeneration of Salamander Limbs

Suggested Reading/ References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

UNIT-X

Programmed cell death: apoptosis, autophagy and necrosis

Objective: In this unit we will discuss about different mechanisms of cell death such as apoptosis, necrosis and autophagy.

Introduction to Apoptosis:

Every normal living cell of animals, plants and even bacteria are mortal. I.e., they must die after some time. Cell death is a finely tuned programme inherent in the cells genetic machinery. This normal cell death which is the part of normal development and maintenance of homeostasis is called apoptosis or programmed cell death (PCD). This phenomenon is very much different from death of a cell due to pathological cause or necrosis. This process is highly regulated and any defect in apoptotic machinery will lead to extended survival of cells which may result in neoplastic cell expansion, leading to genetic instability and accumulation of mutations.

Cellular Events of Apoptosis:

It is a normal physiological response to specific suicide signals or lack of survival signals. During this process at first the nucleus and cytoplasm condense, i.e., chromatin material condenses and migrates to nuclear membrane, the cytoplasm undergoes shrinkage without any damage to plasma membrane. The cell contents are packaged in membrane bound bodies and the cell is broken down into pieces called apoptotic pieces, though still functioning, are engulfed or phagocytosed or digested by macrophages or by neighbouring cells (Fig. 5.33).

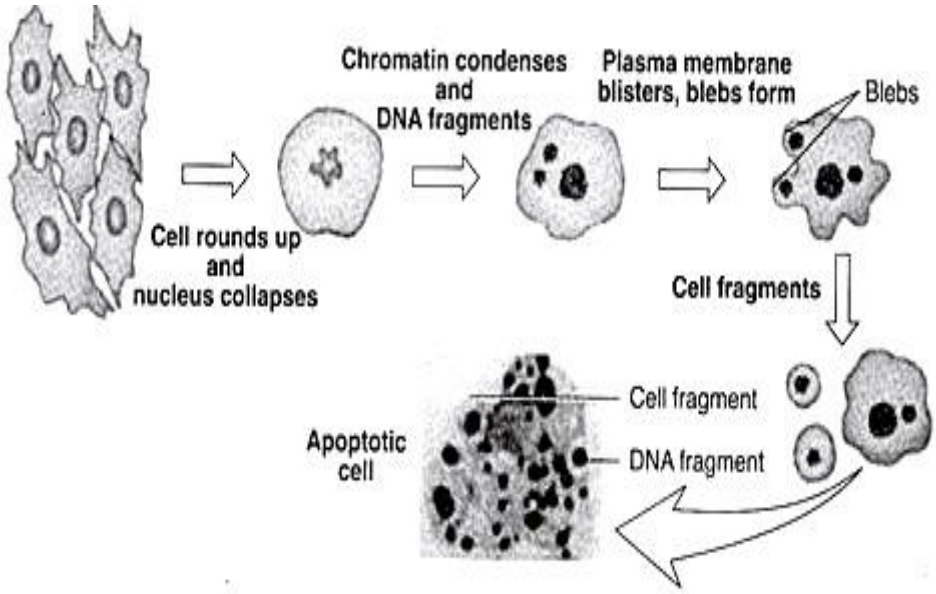


Fig. 5.33A: Sequence of cellular events during apoptosis

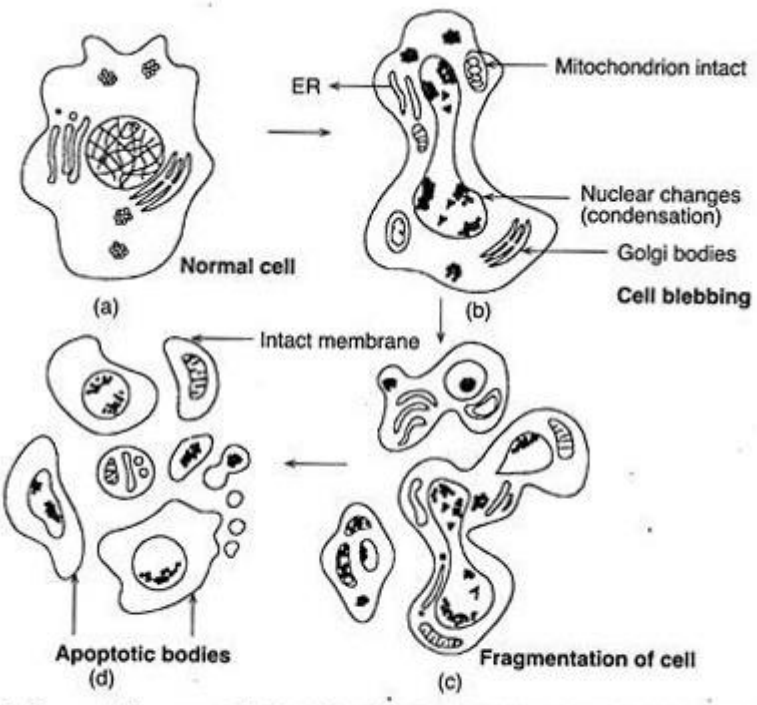


Fig. 5.33B: Cytological changes during apoptosis. Normal cell undergoes shrinkage showing condensation of chromatin and cell blebbing. Thereafter the cell is fragmented to produce apoptotic bodies, but the membranes remain intact (from Rastogi)

Mechanism of Apoptosis:

There are three major pathways for activation of caspase which causes cleavage of substrates leading to apoptosis.

i. Mitochondrial/Cytochrome pathway:

It is mediated through activation of Bcl-2 (gene) which results in production of Apaf-1, caspase-9 and caspase-3 enzyme synthesis which leads to the phenomenon of apoptosis (Fig. 5.34).

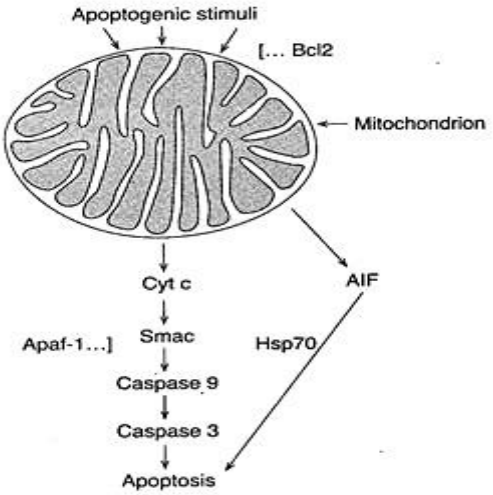


Fig. 5.34: Apoptotic cascade: apoptotic proteins such as Cyt c, Smac, AIF, etc. are released due to several stimuli, triggering caspase activation. Hsp 70 is a negative regulator of Apaf-1. AIF causes condensation of chromatin and cell fragmentation

ii. Tumour-necrosis factor-receptor (TNF) pathway:

In this pathway the ligation of members of the TNF-receptors takes place, activating caspase-8 and then caspase-3 which leads to apoptosis.

iii. Granazyme B pathway:

Granazyme B, a cytosolic T cell product, directly cleaves and activates several caspases, resulting in apoptosis. A number of genes have been identified which play role in the regulation and accomplishment of apoptosis, such as egl-1, Ced-1-10. Studies on these genes indicated that Ced-9 acts upstream of Ced-3 and Ced-4 (Fig. 5.35):

Ced-9 → Ced-3→Ced-4→Cell death

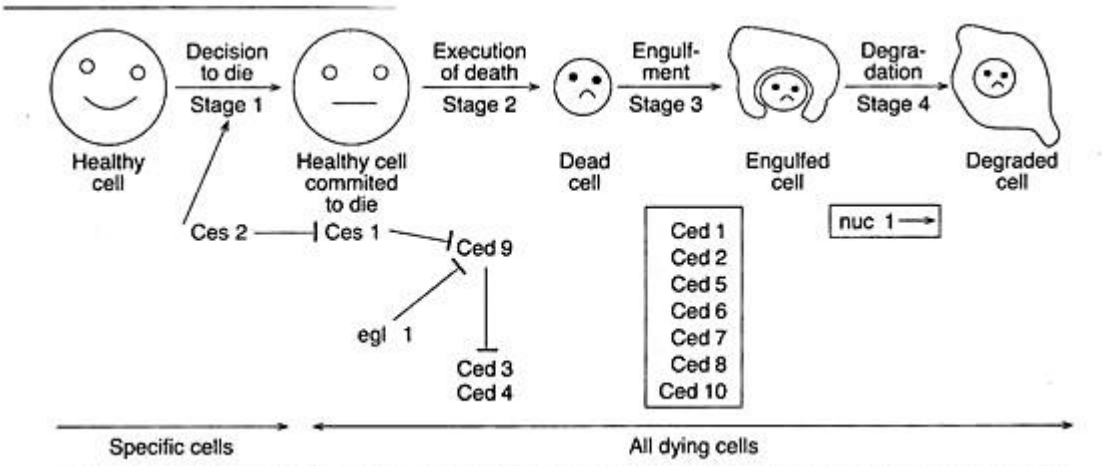


Fig. 5.35: Model for molecular basis of apoptosis in the nematode, Caenorhabditis elegans (after Steller, 1995)

Ced-3 and Ced-4 promote apoptosis, while Ced-9 Is anti-apoptotic and protects cells from apoptosis by antagonizing Ced-3 and Ced-4. The Ced genes are responsible for all programmed cell death. Caspases are cysteine proteases which cleave the substrates at the C-terminal of an aspartic acid residue. Different caspases have different substrate recognition preferences and cleavage of substrates by caspases results in disassembly and consequent death of cell in a highly organized manner. Death receptors are important in **'instructive'** apoptosis where ceil death is brought about by the secretion of death legends which bind to death receptors on the target cell (Fig. 5.36).

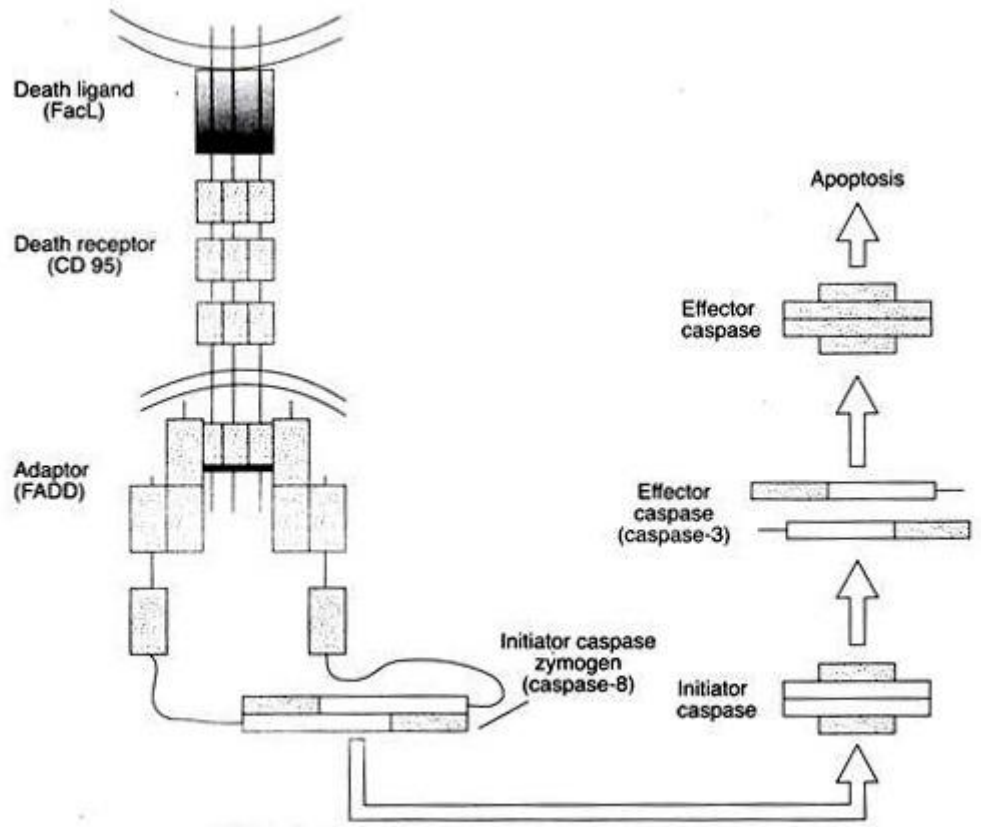


Fig. 5.36: A model showing instructive apoptosis by secretion of death ligands (from Rastogi)

Importance of Apoptosis:

It is a necessary mechanism complementary to proliferation to ensure homeostasis in all tissues. Removal of a number of vestigeal structures (developmental structure, e.g., tail) is caused by programmed cell death. Apoptosis is considered a necessary anticancer mechanism, as defect in this process leads to neoplastic and tumorigenic cell development.

Techniques for the Detection of Apoptosis:

The simplest method for the detection of apoptotic cells is observation of the characteristic bodies in tissue sections at either light or electron microscope level. In vitro, the apoptotic cells can be visualized by means of phase or interference contrast microscopy, where they can be seen to be rounded and lying above the cell monolayer. However, the 'gold standard' lies in the demonstration of 180-200 base oligonucleotide fragments resulting from nuclear damage. These fragment scan be separated by gel electrophoresis and viewed under ultraviolet (UV) light, where they produce a characteristic 'ladder' effect. This method is applied to cell extracts and has the disadvantage that it is not quantitative. Other approaches include the use of cell suspensions stained with fluorescent DNA-binding dyes which are then analysed in a DNA flow cytometer. A novel approach is that of DNA end-labelling, in which nucleotides, tagged with a suitable marker, are bound to the broken ends of DNA fragments in apoptotic cells. This method has the advantage that it enables the enumeration of affected cells.

Major steps of apoptosis:

Cell shrinks

Cell fragments

Cytoskeleton collapses

Nuclear envelope disassembles

Cells release apoptotic bodies

Notably absent from this list is 'send out a signal.' Apoptotic cells do not send out any signal, with one exception: they release apoptotic bodies and 'engulfment proteins' to induce other cells ('phagocytic' cells) to engulf the apoptotic bodies and and break them down in their lysosomes, but this is not much of an immune response.

Proteins important in apoptosis:

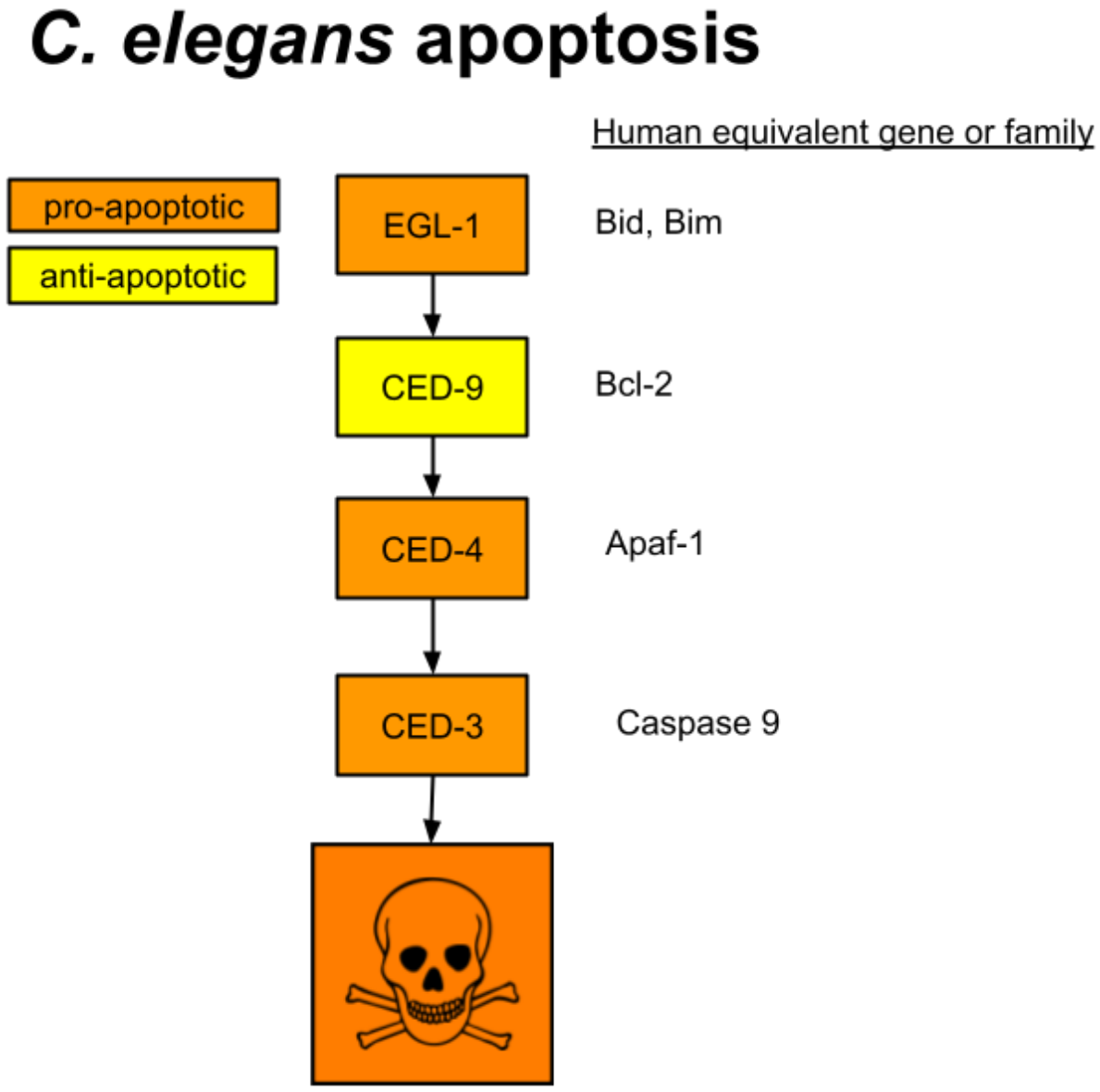
'killer proteins': the caspases (discussed in detail below).

'destruction proteins' that digest DNA, fragment the cell and break down the cytoskeleton

'engulfment proteins' that elicit and promote phagocytosis by other cells

C. elegans has been the major model organism for understanding apoptosis, both by forward and reverse genetics. Forward genetics is observing a phenotype and then determining which gene gives rise to it; reverse genetics is introducing a mutation into a known gene in order to see what phenotype results.

The key pathway in C. elegans apoptosis is shown in figure below:



Here's an explanation of how each of these proteins does its job, from bottom up:

CED-3 pulls the trigger, activating apoptotic proteins that destroy the cell. (In the mammalian equivalent, CED-3 is Caspase 9, which cleaves-thereby-activating Caspase 3, which in turn destroys the cell.)

CED-4 activates CED-3.

CED-9 binds to CED-4, preventing its activation

EGL-1 is transcriptionally activated in response to death signals and catalyzes the release of CED-4 from CED-9.

Note that there is no robustness in this system – it is single points of failure all the way through. If CED-3 is knocked out, no apoptosis can occur. If CED-4 is knocked out, no apoptosis can occur. If CED-9 is knocked out, every cell in the worm will apoptose. If EGL-1 is knocked out, no apoptosis

can occur. Note that the order the arrows point in the above diagram reflects the flow of information in the system. For instance, if EGL-1 and CED-9 are both knocked out, it's the same as if CED-9 alone was knocked out: every cell will apoptose.

In mammals, apoptosis is governed chiefly by caspases (cysteine-aspartic proteases). The entire caspase pathway is post-translationally regulated: the caspases are always present in inactive form (called procaspases, containing a prodomain, which contains a caspase recruitment domain (CARD)) and can be activated by cleavage. This allows a very quick response if cell suicide is needed. In order for apoptosis to occur, the initiator caspases must be cleaved and dimerize. Thus activated, they must then cleave the effector caspases (aka pro-caspases), triggering a 'caspase cascade'. This amplifies the number of activated caspases in the cell. The effector caspase have many targets including the nuclear lamina and cytoskeleton. There are both pro-survival and pro-apoptotic caspases, and they share many common domains. Pro-survival caspases have BH1, 2, 3 and 4; pro-apoptosis caspases have either BH1, 2 and 3 or just BH3. Inhibitor of apoptosis proteins (IAPs) restrain both the initiator and effector caspases. They each have a zinc binding domain that binds directly to caspases, inhibiting their activity. However, there are also mitochondrial proteins called SMAC and DIABLO which inhibit the inhibitors. Upon mitochondrial injury they are released and will bind IAPs, freeing the caspases to go cause apoptosis. Another collection of mitochondrial proteins called Htra2/Omi, apoptosis-inducing factor (AIF) and endonuclease G can also be released and will cleave IAPs. AIF also causes chromosome condensation and DNA fragmentation independent of caspases.

Indeed, the mitochondria are central regulators of apoptosis. Outer mitochondrial membrane proteins Bcl-2, the BH3-only proteins and Bax are involved: Bax can form a pore in the membrane to allow cytochrome c, normally located in the intermembrane space, out into the cytosol. Bax monomers move from the cytoplasm to the outer mitochondrial membrane, where they oligomerize and permit the influx of ions through the membrane. This has also been shown in in vitro experiments where you can show that vesicles made of outer mitochondrial memrbanes are permeabilized in the presence of Bax. It is not currently known why this influx of ions leads to cytochrome c release. Bcl-2 prevents release of cytochrome c, thus blocking apoptosis. Bcl-2 was the first mammalian apoptosis gene to be cloned. In some lymphomas, it gets translocated to a position under a stronger promoter, causing overexpression that prevents the cancer cell from apoptosing. See also bad & bid. Once cytochrome c is released, it binds to Apaf-1 (apoptotic protease activating factor), causing the latter to hydrolyze the ATP to which it is usually bound, thus causing a conformational change that activates Apaf-1 and triggers the caspase cascade. Apaf-1 forms a disc-shaped heptamer called the 'wheel of death' or apoptosome which activates caspases (Wikimedia Commons image by Org1012):

When a trophic factor is present, the receptor activates PI3K, which activates PKB/Akt, which phosphorylates Bad. p-Bad is then retained in the cytosol by 14-3-3, preventing p-Bad from inhibiting Bcl-2. Thus apoptosis is prevented. Trophic factors are an example of a cell extrinsic signal that promotes survival. There are also extrinsic signals that promote death (this is cell murder). Tumor necrosis factor (TNF-alpha) is released by macrophages to trigger cell death by binding to 'death receptors'. Death receptors have a single transmembrane domain. They must trimerize in order to activate FADD (Fas-associated death domain). These serve as adapters for

caspase-8 and -10 and form a death-inducing signaling complex (DISC) which can initiate the caspase cascade. Though this whole process originates independent of mitochondria, it can also activate (?) t-Bid, leading to a mitochondrial apoptosis signal as well. Cells can become murder-resistant by expressing decoy receptors which have only the 'death ligand' binding domain and no active cytosolic domain. This occurs sometimes normally in animal cells but is also a trick that some viruses use – they encode decoy receptor proteins to keep their host cells safe from immune attack. TNF-alpha usually promotes death, but can also promote survival in certain cell types by activating NF-κB. Sometimes cells use decoy receptors to promote an inflammatory response instead of death. p53 is a key regulator of DNA damage response and can promote DNA repair, apoptosis or cell cycle arrest. It does this by binding to promoters of target genes. It is still not clear what determines when p53 will induce cell cycle arrest versus apoptosis.

Experimental methods:

Apoptotic cells exhibit a particular chemical signature. One of these is that an endonuclease cleaves DNA into fragments in the linker regions between nucleosomes and the resulting fragments form a ladder when run on a gel. Another is TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) staining. This involves adding a Tdt enzyme and a BrdU which Tdt will add to the ends of cleaved DNA. After giving it a chance to do this you wash away excess BrdU and then use an antibody against BrdU. Yet another method is that phosphatidylserine (PS) is normally located in the cytosolic leaflet of the plasma membrane; during apoptosis, it flips to the exoplasmic leaflet, where it serves as a signal to request other cells to phagocytose the dying cell. A fluorescently labeled annexin V protein can label PS on the outside of apoptotic cells. Double-stranded DNA cannot get through the plasma membrane of intact cells – and that means healthy cells and apoptotic cells. If it does get out, that is a sign of necrosis. So you can stain with annexin V for exoplasmic PS and with 7-AAD for dsDNA; apoptotic cells are those which are positive for annexin V but negative for 7-AAD.

Cell Necrosis:

The changes in cells undergoing necrosis are essentially a continuation of those described above, in cell injury. However, they are, of course, more profound and are irreversible. It has thus been proposed that there is a 'point of no return', beyond which the process of cell injury cannot be revoked. While moderate mitochondrial swelling, the formation of cell surface 'blebs' and ribosomal disaggregation appear to be reversible, there are certain changes which will inevitably lead to cell necrosis. These include extreme 'blebbing' at the cell surface, together with much greater dilation of the mitochondria ('high amplitude swelling') and the formation of electron dense areas in the mitochondrial matrix. Eventually, there is cell membrane disruption, dissolution of organelles, including the nucleus, and lysosomal degeneration with activation of an inflammatory response. The latter is probably induced by complement-activation by fractions from mitochondria and by leukotrienes formed by lipid peroxidation.

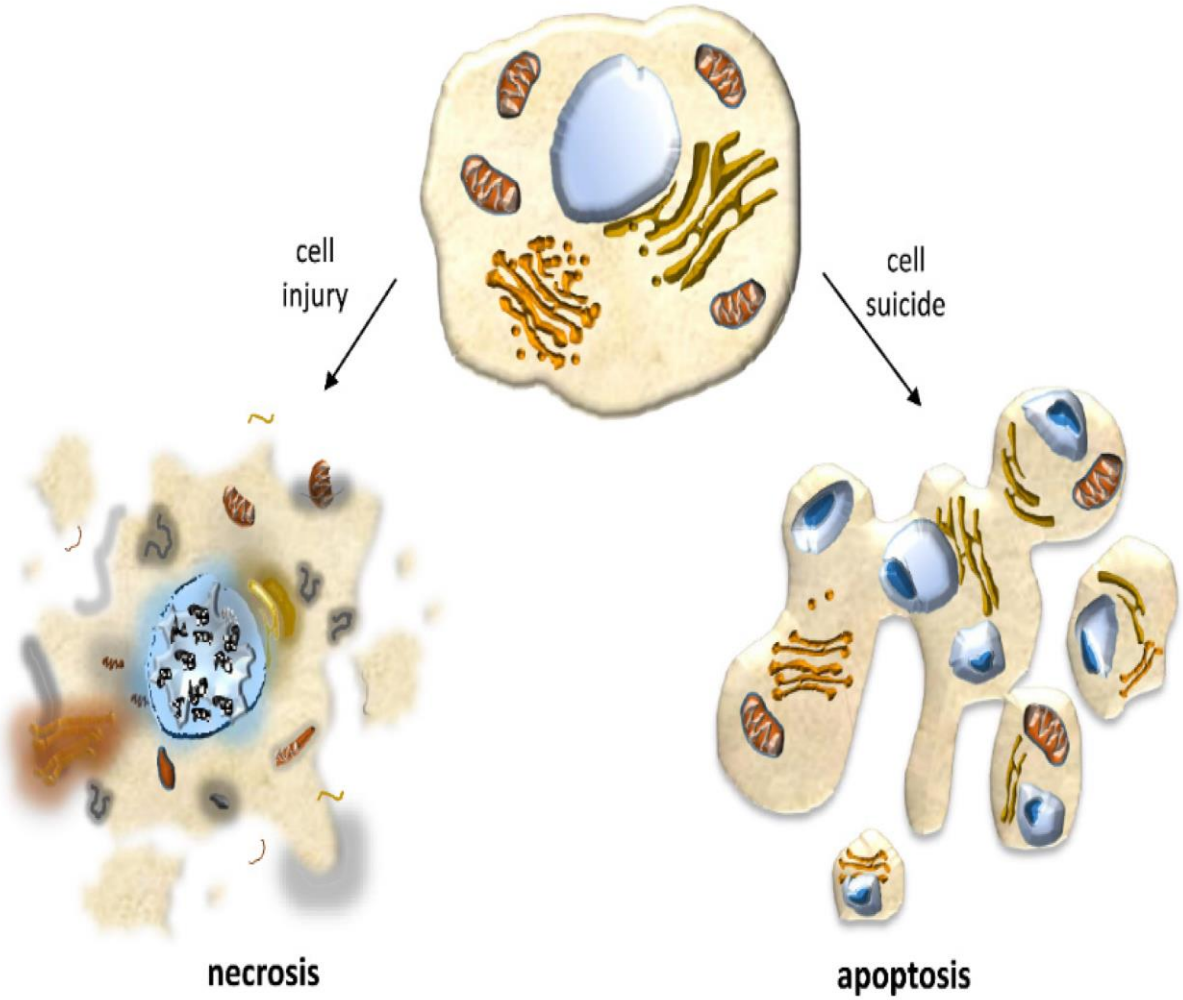
Autophagy:

Autophagy, also called **autophagocytosis**, the degradation of worn, abnormal, or malfunctioning cellular components that takes place within organelles known as lysosomes. Autophagy serves housekeeping functions, enabling the breakdown and recycling of cellular materials, and helps balance energy demands during periods of stress. The term autophagy was introduced in 1963 by Belgian cytologist and biochemist Christian René de Duve, whose work also provided the first evidence for the involvement of lysosomes in the autophagic process.

Three types of autophagy are known: macroautophagy, microautophagy, and chaperone-mediated autophagy. Cells rely primarily on macroautophagy, in which worn or damaged cellular materials in cytosolic regions (the fluid-filled areas surrounding organelles) of cells are engulfed by an autophagosome (vesicles with double membranes that deliver their contents to lysosomes, where the materials are degraded). In microautophagy, cellular components are engulfed directly via invaginations of the lysosomal membrane. Chaperone-mediated autophagy is a selective process, whereby a protein known as hsc70 chaperone recognizes and binds to protein substrates containing a certain amino acid motif. The targeted substrate is carried to the lysosome, where it then translocates across the membrane via a receptor-mediated process. Multiple genes encode the various components of the autophagy machinery required for sequestration, transport, degradation, and recycling of cellular materials. Enzymes encoded by several autophagy genes conjugate (join together), thereby enhancing enzyme activity, particularly during the formation of the autophagosome. A number of nonspecific cellular pathways are also critical to autophagy, including various secretory and endocytic (engulfment) pathways. In addition, the cytoskeleton appears to serve multiple functions in autophagy, key among them the role of microtubules in facilitating autophagosome transport in mammalian cells.

In addition to its housekeeping and stress-response functions, autophagy also contributes to immunity, helping to defend cells against disease-causing organisms and participating in antigen presentation. Autophagy also is involved in programmed cell death, helping eliminate apoptotic cells during embryonic development and aiding death processes in apoptosis-defective cells. Autophagy can also protect against cell death by providing nutrients to cells during periods of starvation. In cancer, autophagy appears to both prevent and, under certain conditions, promote tumour progression. The abnormal accumulation of autophagic vesicles is associated with multiple neurodegenerative conditions, including Parkinson disease and amyotrophic lateral sclerosis, as well as with myopathy (a disease of skeletal muscle tissue).

Apoptosis vs Necrosis:



Loss of membrane integrity	Membrane blebbing, no loss of integrity
Begins with swelling of cytoplasm and mitochondria; ends with total cell lysis	Begins with shrinking of cytoplasm and condensation of nucleus; ends with the cell fragmentation into smaller bodies
Blebs form and the structure of the nucleus changes; no organelles are located in the blebs	The nucleus breaks apart, and DNA breaks into small regular pieces. The organelles are located in the blebs
The cell membrane breaks and releases the cell contents.	The cell membrane breaks into several apoptotic bodies.

Probable Questions:

1. Define apoptosis and necrosis? How these two processes differ?
2. Describe cellular events related to apoptosis?
3. Describe Mitochondrial/Cytochrome pathway of apoptosis.
4. Describe Granzyme B pathway of apoptosis.
5. Describe the techniques used for detection of apoptosis.
6. Describe apoptosis in C. elegans
7. Describe aotophagy

Suggested Reading/ References:

1. Developmental Biology: Scott F Gilbert.
2. Principles of Development: Louis Wolpert.
3. Genetics: A molecular approach. Peter J. Russell. Benjamin Cummings publishers.
4. Principles of Genetics. D.P. Snustad and M.J. Simmons., John Wiley & Sons, Inc.

Group-B : CYTOGENETICS

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	XIII	**Pseudogenes, segmental duplications; Super coiling of DNA. Giant chromosomes:**		**171-193**
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UNIT-XI

Organization and Structure of Genomes: Organization and nature of eukaryotic nuclear DNA, Size and complexity of eukaryotic genome

Objectives:

In general, eukaryotic genomes are larger and more complex when compared to prokaryotic genomes. One obvious belief for this was the sheer number of genes present in complex organisms. However, over the years, scientists have learnt that the size of the genome is not related to the genetic complexity of the organism. For example, salamanders and lilies have 10 fold more DNA than humans, although they are less complex than humans. This difference in size is because of the large amounts of noncoding sequences that are present in the genome.

Introduction:

In eukaryotic organisms — plants, animals and fungi — the major portion of DNA is present in the chromosomes which are well-organised structures and quite different from the prokaryotic counterparts. Besides the chromosomes, mitochondria of both plants and animals and the chloroplasts of green plants also contain DNA. Interesting is the fact that the organization and the nature of the DNA of these cell-organelles are similar to those of bacterial DNA. In both these organelles, the DNA is a covalently closed circular molecule. In eukaryotic cells, the chromosomes are present in a distinct, double-membrane bound structure, the nucleus which occupies on the average about 10% of the cell volume. The membrane is continuous with the endoplasmic reticulum and is provided with pores. The number of chromosome is variable, but fixed for a biological species. Chromosomes change in their physical characteristics during cell division. All these features are absent in the prokaryotic cells.

An individual eukaryotic chromosome contains a single enormously large linear ds-DNA molecule. For example, a diploid human cell containing 46 chromosomes (22 pairs of autosomes and one pair of sex chromosomes) has a total of 6×10^9 base-pairs. The length of the DNA molecules of individual human chromosomes varies from 1.5 cm to 8.7 cm. These large molecules have to be packed into chromosomes, generally measuring a few microns in length and breadth. This is accomplished by binding DNA to proteins. The protein-DNA complex of eukaryotic cells is known as chromatin.

The DNA-binding proteins are distinguished into two main types — the histones and non-histone proteins. The histones are basic proteins, rich in basic amino acids, like lysine and arginine. Histones have large amount of positive charges and can bind tightly the negatively charged DNA molecules. These binding results in the formation of the characteristic structural units called the nucleosomes. The long ds-DNA molecule of each chromosome is folded in a very orderly way around the histones to form the nucleosomes.

The nucleosomes are bead-like structures connected to each other by linker DNA. Each nucleosome consists of a histone core composed of 8 subunits (octamer) of 4 different histones — H_2A, H_2B, H_3 and

H_4 with two molecules of each. The protein core is wrapped by two turns of ds-DNA molecule to form a nucleosome.

The DNA molecule runs as a continuous thread from one nucleosome to another. The intervening portion of DNA between two nucleosomes is the linker. Width of a nucleosome is 11 mm and, on the average, nucleosomes are repeated at intervals of 200 nucleotide pairs of DNA. The length of the linker between two nucleosome is variable.

These features of eukaryotic DNA are shown diagrammatically in Fig. 9.10:

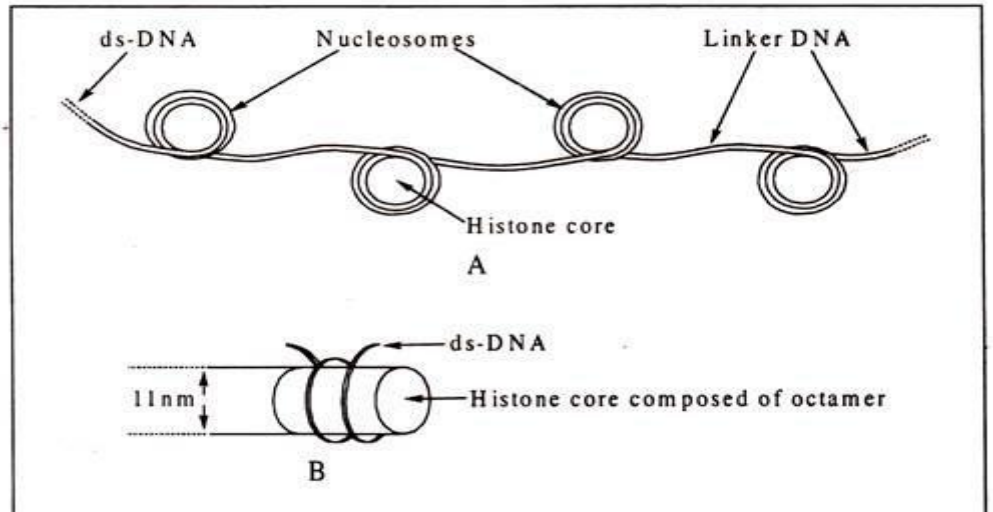


Fig. 9.10 : (A) Nucleosomes connected by linker DNA
(B) A side view of a nucleosome with its histone octamer core wrapped by two turns of a running ds-DNA

The nucleosomes are basic structures from which chromatin is made. They are further organized into closely packed 30 nm fibres of chromatin. These fibres are visible under high resolution electron microscope.

The 30 nm fibres shown in Fig. 9.11, are organized into higher orders of increasing complexity, like 300 nm fibres and 700 nm fibres to produce chromosomes:

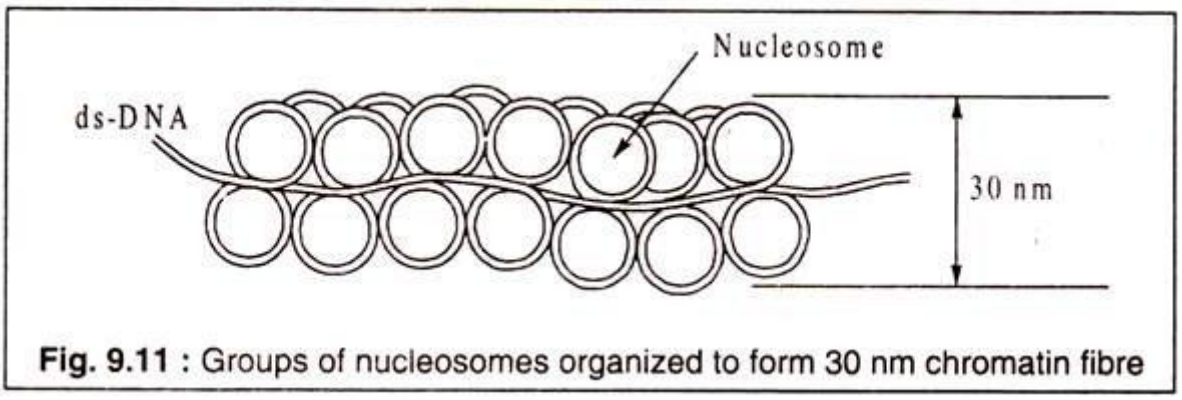


Fig. 9.11 : Groups of nucleosomes organized to form 30 nm chromatin fibre

The eukaryotic chromosomes are characterized by the presence of three types of specialized nucleotide sequences in their DNA. These sequences serve as origin of replication, as centromere which helps the daughter chromosomes to move to opposite poles, and telomere which has a number of repeating sequences functioning as template for RNA-primer in DNA synthesis.

Nucleosome Model:

The nucleosome hypothesis proposed by Roger Kornberg in 1974 was a paradigm shift for understanding eukaryotic gene expression. The assembly of DNA into chromatin involves a range of events, beginning with the formation of the basic unit, the nucleosome, and ultimately giving rise to a complex organization of specific domains within the nucleus. The first step is the assembly of the DNA with a newly synthesized tetramer (H3-H4), are specifically modified (e.g. H4 is acetylated at Lys5 and Lysl2 (H3-H4)), to form a subnucleosomal particle, which is followed by the addition of two H2A-H2B dimers. This produces a nucleosomal core particle consisting of 146 base pairs of DNA bind around the histone octamer. This core particle and the linker DNA together form the nucleosome (Figs 4.38 and 4.39).

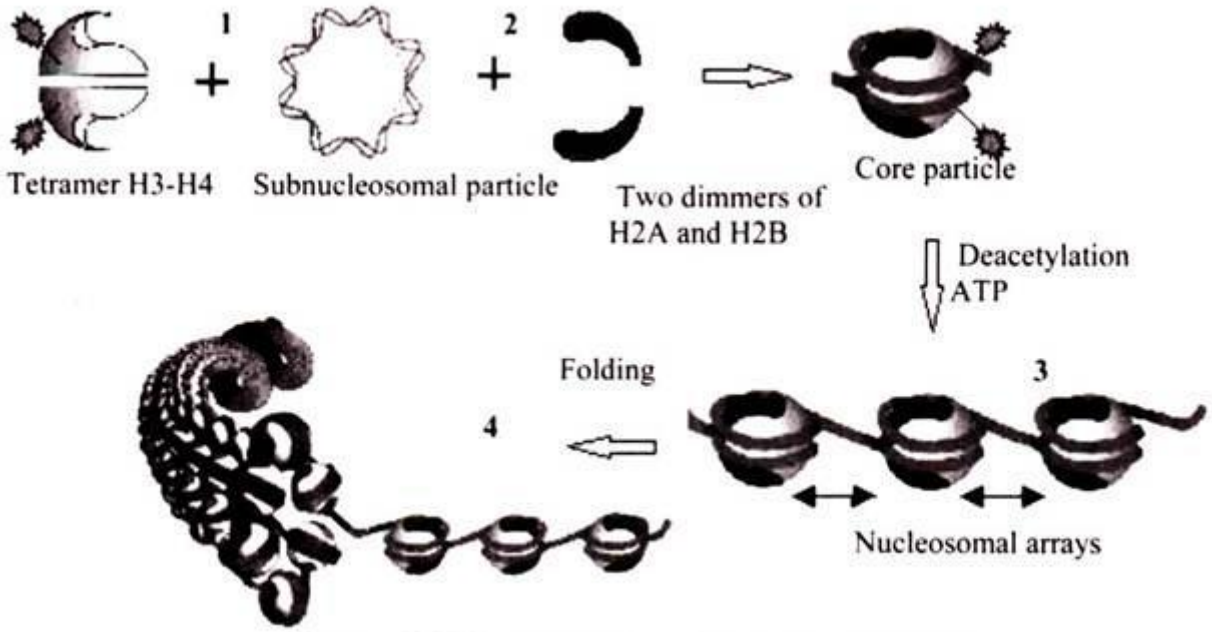


Figure 4.38: The assembly of DNA into chromatin

The next step is the maturation step that requires ATP to establish regular spacing of the nucleosome cores to form the nucleo-filament. During this step the newly incorporated histones are de-acetylated. Next the incorporation of linker histones is accompanied by folding of the nucleo-filament into the 30 nm fibre, the structure of which remains to be elucidated. Two principal models exist- the solenoid model and the zig-zag. Finally, further successive folding events lead to a high level of organization and specific domains in the nucleus.

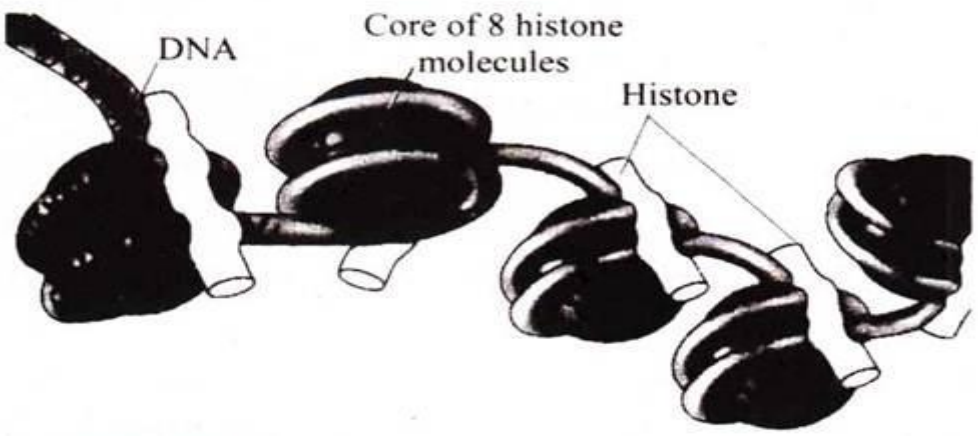


Figure 4.39: Nucleosome model of chromatin assembly

Two molecules of each of the four core histone proteins form the histone octamer via formation of one tetramer of H3 and H4 and two dimers of H2A and H2B. Each of these entities is held together by a so called hand-shake motif of protein structure, forms a "beads on a string" like structure. H1 is involved with the packing of the "beads on a string" substructures into a high order structure.

H1 is present in half the amount of the other four histones. This is because unlike the other histones, H1 does not make up the nucleosome "bead". Instead, it sits on top of the structure, keeping in place the DNA that has wrapped around the nucleosome. Specifically, the H1 protein binds to the "linker DNA" (approximately 80 nucleotides in length) region between the histone beads, helping stabilize the zig-zagged 30 nm chromatin fiber. The nucleosome together with histone HI is called a chromatosome. Chromatosomes are held together by the continuous DNA strand, thus forming linker DNA of 30-50 base pairs in length.

Genome Size and Complexity

A genome is all the genetic information of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes the genes (the coding regions) and the noncoding DNA, aswell as the genetic material of extrachromosomal origins like mitochondria and chloroplasts. Many bacterial species have only one chromosome per cell and, in nearly all cases; each chromosome contains only one copy of each gene. A very few genes, such as those for rRNAs, are repeated several times. Genes and regulatory sequences account for almost all the DNA in bacteria. Moreover, almost every gene is precisely collinear with the amino acid sequence (or RNA sequence) for which it codes. The organization of genes in eukaryotic DNA is structurally and functionally much more complex. The study of eukaryotic chromosome structure, and more recently the sequencing of entire eukaryotic genomes, has yielded many surprises. The genomes of most eukaryotes are larger and more complex than those of prokaryotes.This larger size of eukaryotic genomes is not inherently surprising, since one would expect to find more genes in organisms that are more complex. Many, if not most, eukaryotic genes have a distinctive and puzzling structural feature: their nucleotide sequences contain one or more intervening segments of DNA that do not code for the amino acid sequence of the polypeptide product. These nontranslated inserts interrupt the otherwise colinear relationship between the nucleotide sequence of the gene and the amino acid sequence of the polypeptide it encodes. Such

nontranslated DNA segments in genes are called intervening sequences or introns, and the coding segments are called exons (Figure 1).

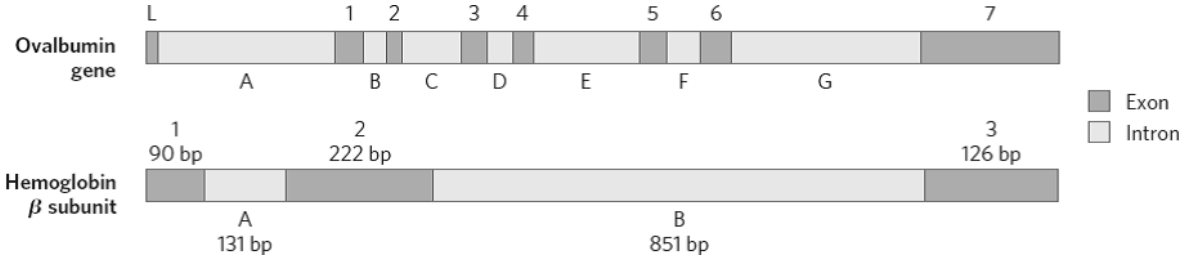


Figure 1: Structure of two eukaryotic genes.

However, the genome size of many eukaryotes does not appear to be related to genetic complexity (Figure 2 and 3). For example, the genomes of salamanders and lilies contain more than ten times the amount of DNA that is in the human genome, yet these organisms are clearly not ten times more complex than humans. This apparent paradox was resolved by the discovery that the genomes of most eukaryotic cells contain not only functional genes but also large amounts of DNA sequences that do not code for proteins. The difference in the sizes of the salamander and human genomes thus reflects larger amounts of noncoding DNA, rather than more genes, in the genome of the salamander. The presence of large amounts of noncoding sequences is a general property of the genomes of complex eukaryotes. Thus the thousand fold greater size of the human genome compared to that of E. coli is not due solely to a larger number of human genes. The human genome is thought to contain 20,000-25,000 genes- only about 5 times more than E.coli has. Much of the complexity of eukaryotic genomes thus results from the abundance of several different types of noncoding sequences, which constitute most of the DNA of higher eukaryotic cells. Thus several kinds of noncoding DNA contribute to the genomic complexity of higher eukaryotes. The lack of precise correlation between the complexity of an organism and the size of its genome was looked on as a bit of a puzzle, the so called **C-value paradox**. In fact the answer is quite simple: space is saved in the genomes of less-complex organisms because the genes are more closely packed together (Figure 2 and 3).

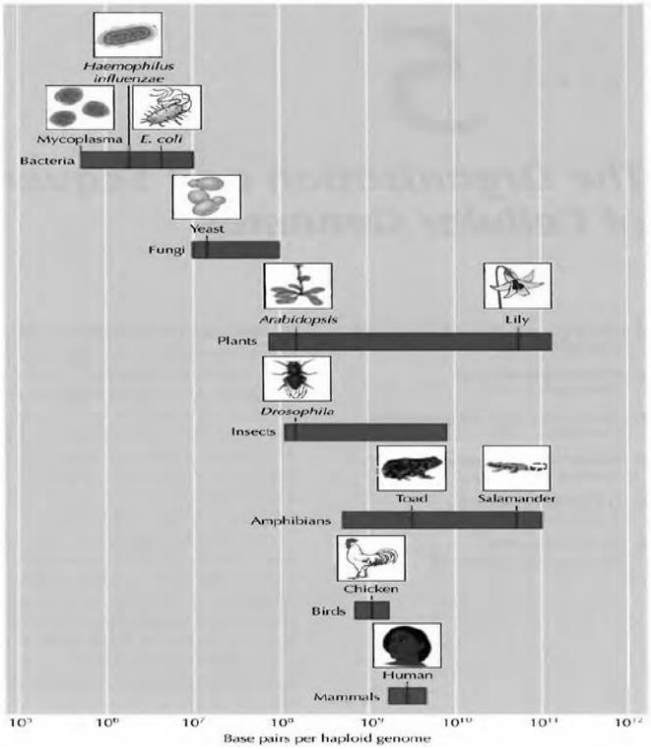


Figure 2: Genome size of representative groups of organisms.

In bacterial genomes, most of the DNA encodes proteins. For example, the genome of E. coli is approximately 4.6 x 10^6 base pairs long and contains about 4000 genes, with nearly 90% of the DNA used as protein-coding sequence. The yeast genome, which consists of 12 x 10^6 base pairs, is about 2.5 times the size of the genome of E. coli, but is still extremely compact. Only 4% of the genes of Saccharomyces cerevisiae contain introns, and these usually have only a single small intron near the start of the coding sequence. Approximately 70% of the yeast genome is used as protein-coding sequence, specifying a total of about 6000 proteins. The relatively simple animal genomes of C. elegans and Drosophila are about 10 times largerthan the yeast genome, but contain only 2- 3 times more genes. Instead, these simple animal genomes contain more introns and more repetitive sequence, so that protein-coding sequences correspond to only about 25% of the C. elegans genome and about 13% of the genome of Drosophila. The genome of the model plant Arabidopsis contains a similar number of genes, with approximately 26% of the genome corresponding to protein-coding sequence. The genomes of higher animals (such as humans) are approximately 20-30 times larger than those of C. elegans and Drosophila. However, a major surprise from deciphering the human genome sequence was the discovery that the human genome contains only 20,000 to 25,000 gene (Figure 2 and 3). It appears that only about 1.2% of the human genome consists of

protein-coding sequence. Approximately, 20% of the genome consists of introns, and more than 60% is composed of various types of repetitive and duplicated DNA sequences, with the remainder corresponding to pseudogenes, to nonrepetitive spacer sequences between genes, and to exon sequences that are present at the 5' and 3' ends of mRNAs but are not translated into protein. The increased size of the genomes of higher eukaryotes is thus due far more to the presence of large amounts of repetitive sequences and introns than to an increased number of genes (Figure 1 and 2).

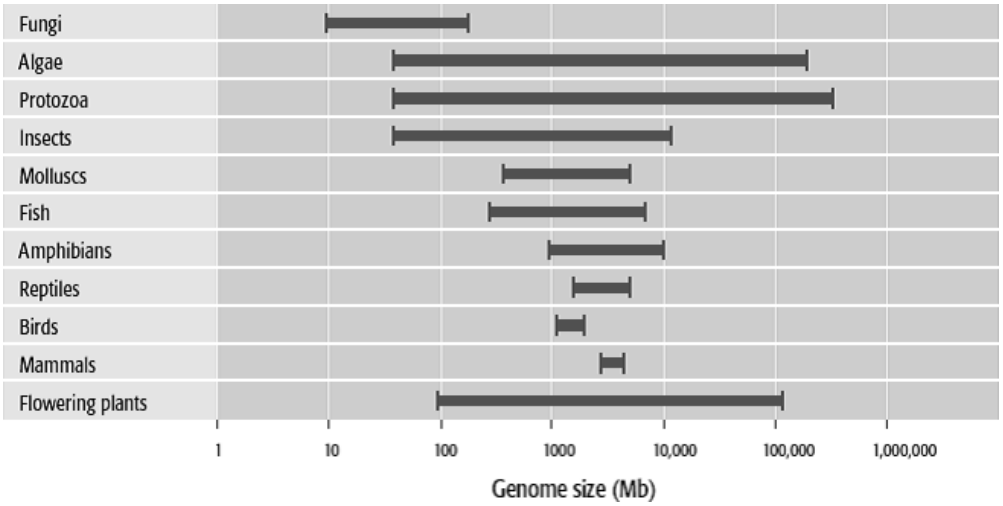


Figure 3: Genome size of eukaryotes.

Complexity of eukaryotic DNA sequences and repetitive DNA sequence

The term **sequence complexity** refers to the number of times a particular base sequence appears throughout the genome. Unique or nonrepetitive sequences are those found once or a few times within the genome. Structural genes are typically unique sequences of DNA. The vast majority of proteins in eukaryotic cells are encoded by genes present in one or a few copies. In the case of humans, unique sequences make up roughly 41% of the entire genome. Apart from unique DNA sequences there are repetitive DNAs, that is, sequences that are similar or identical to sequences elsewhere in the genome. Most large genomes are filled with repetitive sequences; for example, nearly half of the human genome is covered by repeats, many of which have been known about for decades. Although some repeats appear to be non-functional, others have played a part in human evolution at times creating novel functions, but also acting as independent, 'selfish' sequence elements. Repeats arise from a variety of biological mechanisms that result in extra copies of a sequence being produced and inserted into the genome. Repeats come in all shapes and sizes: they can be widely interspersed.

repeats, tandem repeats or nested repeats, they may comprise just two copies or millions of copies, and they can range in size from 1–2 bases (mono-and dinucleotide repeats) to millionsof bases.
One approach that has proven useful in understanding genome complexity has come from renaturation studies. These kinds of experiments were first carried out by Roy Britten and David Kohne in 1968. In a renaturation study, the DNA is broken up into pieces containing several hundred base pairs. The double-stranded DNA is then denatured (separated) into single-stranded pieces by heat treatment. When the temperature is lowered, the pieces of DNA that are complementary can reassociate, or renature, with each other to form double- stranded molecules.

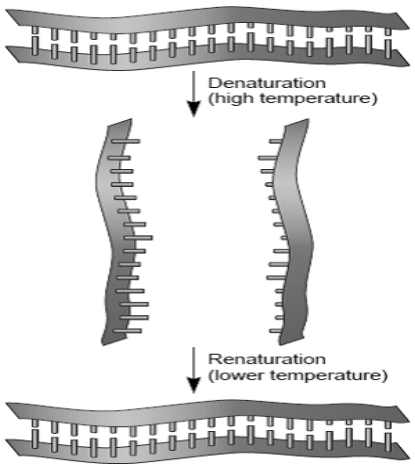


Figure 4: Denaturation and renaturation of DNA strands

The rate of renaturation of complementary DNA strands provides a way to distinguish between unique, moderately repetitive, and highly repetitive sequences. For a given category of DNA sequences, the renaturation rate depends on the concentration of its complementary partner. Highly repetitive DNA sequences renature much faster because many copies of the complementary sequences are present. In contrast, unique sequences, such as those found within most genes, take longer to renature because of the added time it takes for the unique sequences to find each other (Figure 5).

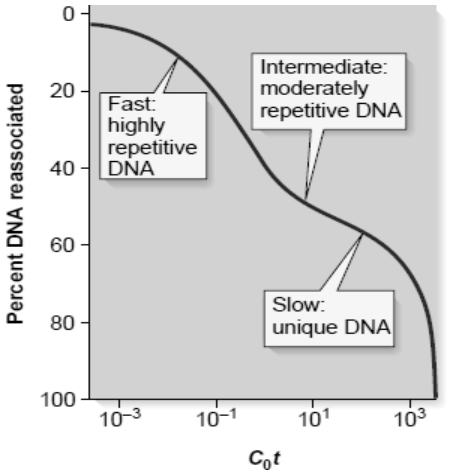


Figure 5: Renaturation of human DNA sequence comple

Highly repeated DNA sequences:

The highly repeated fraction (also called **tandem repeats**) constitutes anywhere from about 1to 10 percent of the total DNA (Figure 6). These sequences are typically short (a few hundred nucleotides at their longest) and present in clusters in which the given sequence repeats itself over and over again without interruption. A sequence arranged in this end-to-end manner is said to be present in tandem. Approximately 3% of the human genome consists of **highly repetitive** sequences, also referred to as **simplesequence DNA** or **simple sequence repeats (SSR)**. These short sequences, generally less than 10 bp long, are sometimes repeated millions of times per cell. The simple sequence DNA has also been called **satellite DNA**, so named because its unusual base composition often causes it to migrate as "satellite" bands (separated from the rest of the DNA) when fragmented cellular DNA samples are centrifuged in a cesium chloride density gradient (Figure 7).

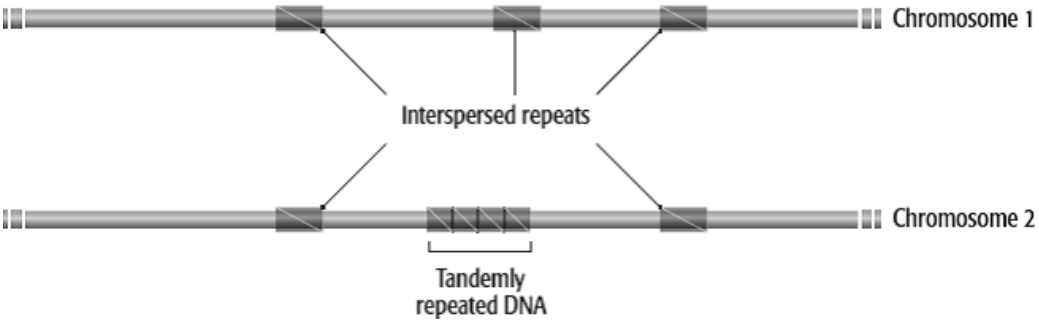


Figure 6: Two different types of repetitive sequence.

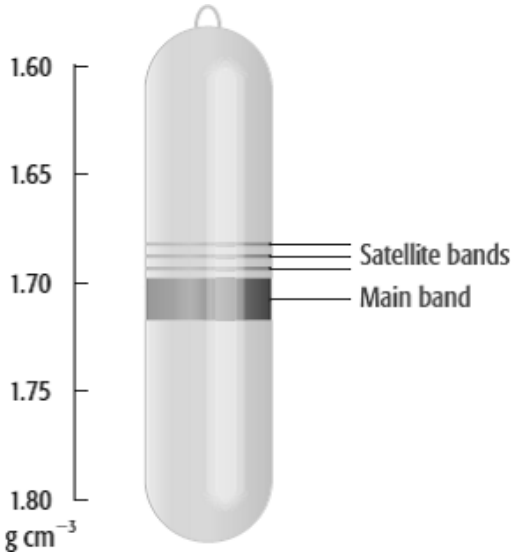


Figure 7: Satellite DNA from human genome

Highly repeated sequences fall into several overlapping categories, including satellite DNAs, minisatellite DNAs, and microsatellite DNAs. The localization of satellite DNAs are found mostly within the centromeres and telomeres of chromosomes.

Satellite DNAs: Satellite DNAs consist of short sequences (about five to a few hundred base pairs in length) that form very large linear arrays, each containing up to several million base pairs of DNA. In many species, the base composition of these DNA segments is sufficiently different from the bulk of the DNA that fragments containing the sequence can be separated into a distinct "satellite" band during density gradient centrifugation (Figure 7). Satellite DNAs tend to evolve very rapidly, causing the sequences of these genomic elements to vary even between closely related species.

Minisatellite DNAs: Minisatellite sequences range from about 10 to 100 base pairs in length and are found in sizeable clusters containing as many as 3000 repeats. Thus, minisatellite sequences occupy considerably shorter stretches of the genome than do satellite sequences. Minisatellites tend to be unstable, and the number of copies of a particular sequence often increases or decreases from one generation to the next, most likely as the result of unequal crossing over. Consequently, the length of a particular minisatellite locus is highly variable in the population, even among members of the same family. Because they are so variable (or polymorphic) in length, minisatellite sequences form the basis for the technique of DNA fingerprinting, which is used to identify individuals in criminal or paternity. **Microsatellite DNAs:** Microsatellites are the shortest sequences (1 to 9 base pairs long) and are typically present in small clusters of about 10 to 40 base pairs in length, which are scattered quite evenly through the genome. DNA replicating enzymes have trouble in copying regions of the genome that contain these small, repetitive sequences, which causes these stretches of DNA to change in length through the generations. Because of their variable lengths within the population, microsatellite DNAs have been used to analyze the relationships between different human populations.

Moderately repeated DNA sequence

The moderately repeated fraction of the genomes of plants and animals can vary from about 20 to more than 80 percent of the total DNA, depending on the organism. This fraction includes sequences that are repeated within the genome anywhere from a few times to tens of thousands of times (Figure 5). Included in the moderately repeated DNA fraction are some sequences that code for known gene products, either RNAs (such as rRNAs) or proteins (including histones), but the bulk of this DNA fraction lacks a coding function. Rather than occurring as clusters of tandem sequences, these noncoding elements are scattered (i.e., interspersed) throughout the genome (Figure 6). Most of these repeated sequences can be grouped into two classes that are referred to as SINEs (short interspersed elements) or LINEs (long interspersed elements). SINEs and LINEs sequences are discussed later.

Nonrepeated DNA sequence

As initially predicted by Mendel, classical studies on the inheritance patterns of visible traits led geneticists to conclude that each gene was present in one copy per single (haploid) set of chromosomes. When denatured eukaryotic DNA is allowed to reanneal, a significant fraction of the fragments are very slow to find partners, so slow in fact that they are presumed to be present in a single copy per genome (Figure 5). This fraction comprises the non-repeated (or single-copy) DNA sequences, which includes the genes that exhibit Mendelian patterns of inheritance. Because they are present in a single copy in the genome, non-repeated sequences localize to a particular site on a particular chromosome. Included within the non-repeated fraction are the DNA sequences that code for virtually all proteins other than histones. Even though these sequences are not present in multiple copies, genes that code for polypeptides are usually members of a family of related genes. This is true for the globins, actins, myosins, collagens, tubulins, integrins, and most other proteins in a eukaryotic cell. Each member of a multigene family is encoded by a different but related sequence.

Probable questions:

1. What is C-Value paradox?
2. What is Satellite DNA?
3. Distinguish between microsatellite and minisatellite DNA?
4. What is moderately repeated and non repeated DNA sequence?
5. The yeast genome is 0.004 times the size of the human genome and yet it contains approximately 0.2 times fewer genes. Give explanation.
6. What are introns and exons?
7. What differences in gene distribution and repetitive DNA content are seen when yeast and human chromosomes are compared?
8. Describe nucleosome model of genome organization with suitable diagram.
9. Describe different types of Histone proteins.

Suggested Readings/References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell and Molecular Biology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genome.

Unit-XII

Transposable elements, retro- transposons, SINE, LINE, Alu and other repeat elements, pseudogenes, segmental duplications; super coiling ofDNA.

Objectives:

Factors contributing to the size and complexity of the genome are: Introns and exons,Pseudogene, repetitive sequences, gene families etc. In addition in eukaryotic cells the length of DNA in the nucleus is far greater than the size of the compartment in which it is contained. To fit into this compartment the DNA is not packaged directly into final structure of chromatin. It contains several hierarchies of organization. The first level of packing is achieved by the winding of DNA around a protein core to produce a "bead-like" structure called a nucleosome. Subsequently, a series of levels of compaction and condensation produce the structural chromosomes in the nucleus. These levels of organization are dynamic. Often the dynamic nature of DNA in its organization pattern produces a coiled-coiled structure which should be properly segregated to maintain its structural and functional property. Supercoiling is introduced into DNA molecules when the double helix is twisted around its own axis in three-dimensional space. Generally, DNA molecules are negatively supercoiled inside cells, although the level of supercoiling is not equal throughout the genome and many supercoils may be constrained by bound proteins. When the ends of a linear DNA molecule are ligated to produce a covalently closed circle, the two strands become intertwined like the links of a chain, and will remain so unless one of the strands is broken.

Transposable Elements and retro transposons:

Definition of Transposons:

Presence of transposable elements was first predicted by Barbara McClintock in maize (corn) in late 1940s. After several careful studies, she found that certain genetic elements were moving from one site to an entirely different site in the chromosome. She called this phenomenon of changing sites of genetic elements as transposition and those genetic elements were called by her as controlling elements. These controlling elements were later on called as transposable elements by Alexander Brink.In late 1960s this phenomenon was also discovered in bacteria. Consequently, the molecular biologists called them as Transposons. A transposon may be defined as: “a DNA sequence that is able to move or insert itself at a new location in the genome.” The phenomenon of

movement of a transposon to a new site in the genome is referred to as transposition. Transposons are found to encode a special protein named as transposase which catalyses the process of transposition. Transposons are particular to different groups of organisms. They constitute a fairly accountable fraction of genome of organisms like fungi, bacteria, plants, animals and humans. Transposons have had a major impact on changing or altering the genetic composition of organisms.

Transposons or transposable genetic elements are often referred to as 'mobile genetic elements' also. They can be categorized on different bases like their mode of transposition or on the basis of the organisms in which they are present.

Types of Transposons:

Different transposons may change their sites by following different transposition mechanisms.

On the basis of their transposition mechanism, transposons may be categorized into following types:

(i) Cut-and-Paste Transposons:

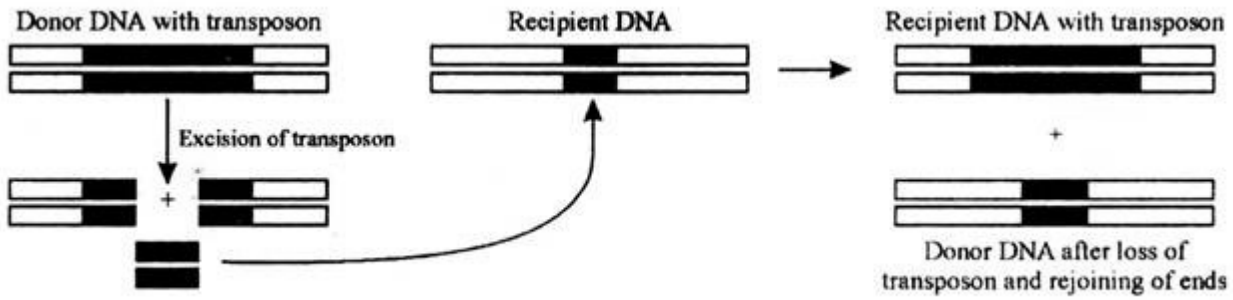


Fig. 1. Cut and Paste Transposons.

They transpose by excision (cutting) of the transposable sequence from one position in the genome and its insertion (pasting) to another position within the genome (Fig. 1).The cut-and-paste transposition involves two transposase subunits. Each transposase submit binds to the specific sequences at the two ends of transposon. These subunits of transposase protein then come together and lead to the excision of transposon.

This excised 'transposon-Transposase Complex' then gets integrated to the target recipient site. In this manner, the transposon is cut from one site and then pasted on other site by a mechanism mediated by transposase protein (Fig. 2).

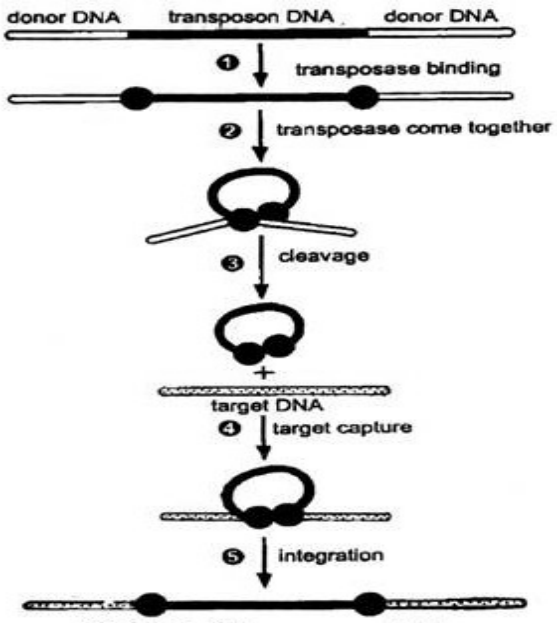


Fig. 2. Role of Transposase protein in cut-and-paste transposition.

Examples of cut-and-paste type of transposons are IS-elements, P-elements in maize, hobo-elements in Drosophila etc.

(ii) Replicative Transposons:

They transpose by a mechanism which involves replication of transposable sequence and this copy of DNA, so formed, is inserted into the target site while the donor site remains unchanged (Fig. 3). Thus, in this type of transposition, there is a gain of one copy of transposon and both-the donor and the recipient DNA molecule are having one-one transposable sequence each, after transposition.

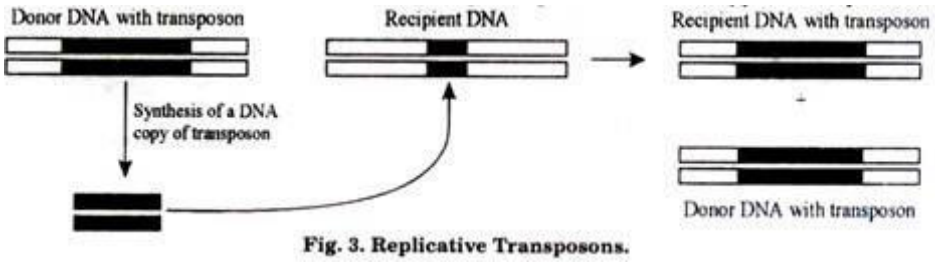


Fig. 3. Replicative Transposons.

Tn3-elements found in bacteria are good examples of such type of transposons.

(iii) Retro Elements:

Their transposition is accomplished through a process which involves the synthesis of DNA by reverse transcription (i.e. RNA to DNA) by using elements RNA as the template (Fig. 4). This type of transposition involves an RNA intermediate, the transposable DNA is transcribed to produce an RNA molecule.

This RNA is then used as a template for producing a complementary DNA by the activity of enzyme reverse transcriptase. This single stranded DNA copy so formed, is then made double stranded and then inserted into the target DNA site. The transposable elements which require reverse transcriptase tor their movement are called retro transposons.

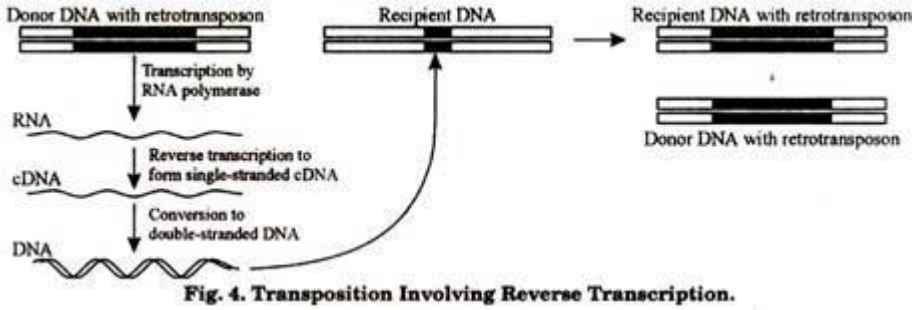


Fig. 4. Transposition Involving Reverse Transcription.

Transposable Elements in Prokaryotes:

Although the presence of transposons was predicted in eukaryotes but first observation at molecular level was done in bacteria, which is a prokaryote.

Bacterial transposable elements are of the following types:

(a) Insertion Sequences or IS Elements:

They are the transposable sequences which can insert at different sites in the bacterial chromosomes.

IS-elements contain ITRs (Inverted Terminal Repeats), these were first observed in E.coli. IS elements are relatively short usually not exceeding 2500 bp. The ITRs present at the ends of IS-elements are an important feature which enables their mobility. The ITRs present in the IS-elements of E.coli usually range between 18-40 bp.

The term 'Inverted Terminal Repeat' (ITR) implies that the sequence at 5 end of one strand is identical to the sequence at 5 end of th e other strand but they run in inverse opposite direction (Fig. 5). In E.coli chromosome, a number of copies of several IS-elements like IS1, IS2, IS3, IS4 and IS5 are present.

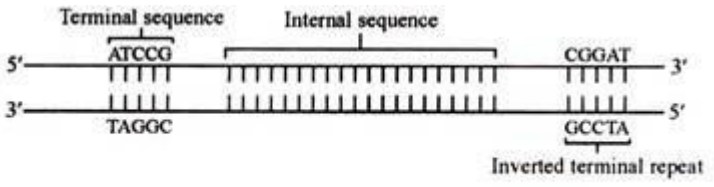


Fig. 5. An Insertion sequence (IS-element) with inverted terminal repeats (ITRs)

(b) Prokaryotic Transposon Element:

These are also called composite transposons and are shown by the symbol Tn. It is made upof two IS elements, one present at each end of a DNA sequence which contains genes whose functions are not related to the transposition process. These transposons have been found to have inverted repeats at the ends. The length of these inverted repeats ranges from a few nucleotides to about 1500 bp.

It can be said that these are the large transposons which are formed by capturing of an immobile DNA sequence within two insertion sequences thus enabling it to move. Examples of such transposons include the members of Tn series like Tn1, Tn5, Tn9, Tn10, etc.

Transposable Elements in Eukaryotes:

(a) Transposons in Maize:

Different types of transposons present in maize are described below:

Ac-Ds system:

This system of transposable elements in maize was analyzed and given by Barbara Mc. Clintock. Here Ac stands for Activator and Ds for Dissociation. Barbara found that Ds andAc genes were sometimes mobile and moved to different chromosomal locations thus resulting in different kernel phenotypes. Ds element is activated by Ac and on activation it serves as the site provider for breakage in chromosome. Ac can move autonomously while Ds can move only in the presence of Ac (Fig. 6). The transposition involving this Ac-Ds system produces altered kernel phenotypes.

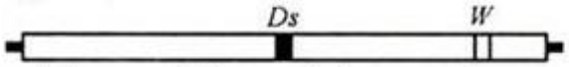
Other transposable elements of maize are:

i. spm (suppressor mutator) system,

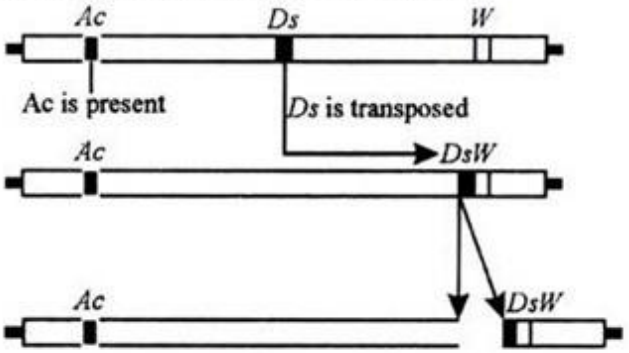
ii. dt (dotted) system,

iii. Mu (Mutator) system, etc.

(a) In absence of *Ac*, *Ds* is not transposable.
Wild type expression of *W* occurs



(b) When *Ac* is present, *Ds* may be transposed



Chromosome breaks and fragment is lost
Expression of W ceases, producing mutant effect.

Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

(b) Transposons in Drosophila:

A number of transposable elements are found in Drosophila which are of different types and account for a quite high fraction of Drosophila genome.

Some of these transposons are given below:

P-elements:

These were discovered during the study of 'hybrid-dysgenesis' which is a sterility causing condition. They are 2.9 kb long and contain 31 bp long inverted terminal repeats High rate of P-element transposition causes hybrid dysgenesis. P-elements encode transposase enzyme which helps in their transposition. These are also useful as vectors for introducing foreign genes into Drosophila.

Copia-elements:

Their transposition causes mutations for eye-colour in Drosophila. They are of size approximately 5-8 kb with direct terminal repeat (DTR) of about 276 bp at each end. Within each of this direct repeats is present short inverted repeat (IR) of about 17 bp length. About 10-80 copia- elements are present in cell-genome (Fig. 7).

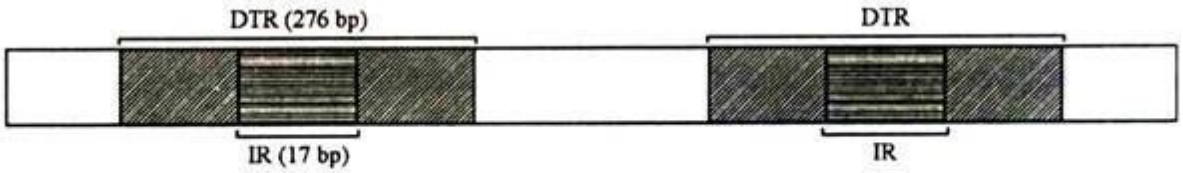


Fig. 7. Organization of a copia transposable element in *D. melanogaster*.

FB Elements:

These are the fold back elements present in Drosophila genome. These have ability to fold back to form a stem and loop structure due to the presence of long inverted terminal repeats. Their transposition results into a changed expression by causing mutation by insertion or by affecting the normal gene expression.

Other important types of transposable elements found in Drosophila are:

i. I elements,

ii. Mariner elements,

iii. Gypsy elements,

iv. Hobo elements, etc.

(a) Transposons in Humans:

Transposons in humans are in the form of repetitive DNA which consists of sequences that are interspersed within the entire human genome. These sequences are transposable and can move to different locations within the genome.

Significance of Transposable Elements:

1. Transposons may change the structural and functional characteristics of genome by changing their position in the genome.

2. Transposable elements cause mutation by insertion, deletion, etc.

3. Transposons make positive contribution in evolution as they have tremendous impact on the alteration of genetic organization of organisms.

4. They are useful as cloning vectors also, in gene cloning. For example, P-elements are frequently used as vector for introducing transgenes into Drosophila.

5. Transposons may also be used as genetic markers while mapping the genomes.

6. Transposon-mediated gene tagging is done for searching and isolation of a particular gene.

LINEs, SINEs and Alu repeats

There are other repetitive DNA sequences which are scattered throughout the genome rather than being clustered as tandem repeats. These interspersed repetitive elements are a major contributor to genome size, accounting for approximately 45% of human genomic DNA (Table 1). The two most prevalent classes of these sequences are called SINEs (short interspersed elements) and LINEs (long interspersed elements). Both SINEs and LINEs are examples of transposable elements, which are capable of moving to different sites in genomic DNA.

Type of sequence	Number of copies	Fraction of genome
Simple-sequence repeats[a]	>1,000,000	~10%
Retrotransposons		
LINEs	850,000	21%
SINEs	1,500,000	13%
Retrovirus-like elements	450,000	8%
DNA transposons	300,000	3%

Table 1: Type of repetitive sequence and their percentage in human genome

LINEs (long interspersed nuclear elements) have been very successful transposons. They have a comparatively long evolutionary history, occurring in other mammals, including mice. As autonomous transposons, they can make all the products needed for retrotransposition, including the essential reverse transcriptase. Human LINEs consist of three distantly related families: LINE-1, LINE-2, and LINE-3, collectively comprising about 20% of the genome. They are located primarily in euchromatic regions and are located preferentially in the dark AT-rich G bands (Giemsa-positive) of metaphase chromosomes. Of the three human LINE families, LINE-1 (or L1) is the only family that continues to have actively transposing members. LINE-1 is the most important human transposable element and accounts for a higher fraction of genomic DNA (17%) than any other class of sequence in the genome.

Full-length LINE-1 elements are more than 6 kb long and encode two proteins: an RNA-binding protein and a protein with both endonuclease and reverse transcriptase activities (Figure 8). The 6.1 kb LINE-1 element has two open reading frames: ORF1, a 1kb open reading frame, encodes p40, an RNA-binding protein that has a nucleic acid chaperone activity; the 4 kb ORF2 specifies a protein with both endonuclease and reverse transcriptase activities. A bidirectional internal promoter lies within the 5□ untranslated region (UTR). At the other end, there is an An/Tn sequence, often described as the 3' poly(A) tail (pA). Unusually, an internal promoter is located within the 5' untranslated region. Full-length copies therefore bring with them their own promoter that can be used after integration in a permissive region of the genome. After translation, the LINE-1 RNA assembles with its own encoded proteins and moves to the nucleus. To integrate into genomic DNA, the LINE-1 endonuclease cuts a DNA duplex on one strand, leaving a free 3' OH group that serves as a primer for reverse transcription from the 3' end of the LINE RNA. The endonuclease's preferred cleavage site is TTTT □A; hence the preference for integrating into AT-rich regions. AT-rich DNA is comparatively gene-poor, and so because LINEs tend to integrate into AT-rich DNA they impose a lower mutational burden, making it easier for their host to accommodate them. During integration, the reverse transcription often fails to proceed to the 5' end, resulting in truncated, nonfunctional insertions. Accordingly, most LINE-derived repeats are short, with an average size of 900 bp for all LINE-1 copies, and only about 1 in 100 copies are full length. The LINE-1 machinery is responsible for most of the reverse transcription in the genome, allowing retrotransposition of the nonautonomous SINEs and also of copies of mRNA, giving rise to processed pseudogenes and retrogenes. Of the 6000 or so full-length LINE-1 sequences, about 80–100 are still capable of transposing, and they

occasionally cause disease by disrupting gene function after insertion into an important conserved sequence.

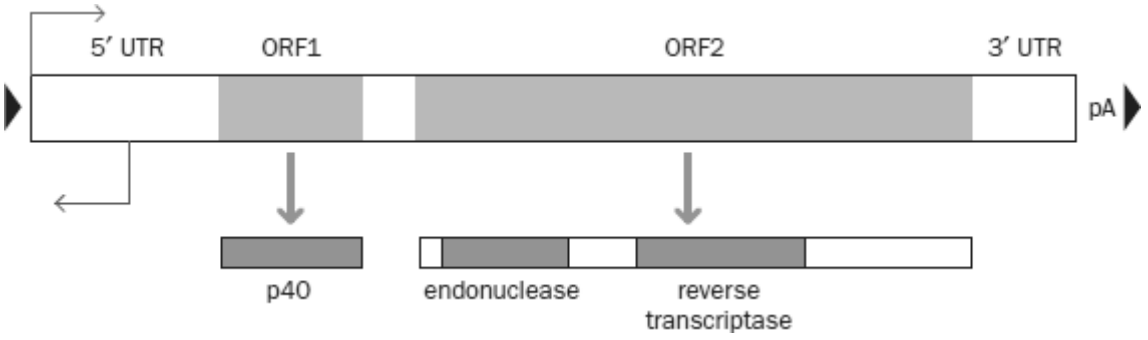


Figure 8: LINE-1 repeats element

SINES and Alu repeats

SINEs (short interspersed nuclear elements) are retrotransposons about 100–400 bp in length. They have been very successful in colonizing mammalian genomes, resulting in various interspersed DNA families, some with extremely high copy numbers. Unlike LINEs, SINEs do not encode any proteins and they cannot transpose independently. However, SINEs and LINEs share sequences at their 3□ end, and SINEs have been shown to be mobilized by neighboring LINEs. By parasitizing on the LINE element transposition machinery, SINEs can attain very high copy numbers. The human **Alu family** is the most prominent SINE family in terms of copy number, and is the most abundant sequence in the human genome, occurring on average more than once every 3 kb. The full-length Alu repeat is about 280 bp long and consists of two tandem repeats, each about 120 bp in length followed by a short An/Tn sequence. The tandem repeats are asymmetric: one contains an internal 32 bp sequence that is lacking in the other (Figure 9). Monomers containing only one of the two tandem repeats, and various truncated versions of dimers and monomers are also common, giving a genome wide average of 230 bp. Like other mammalian SINEs,

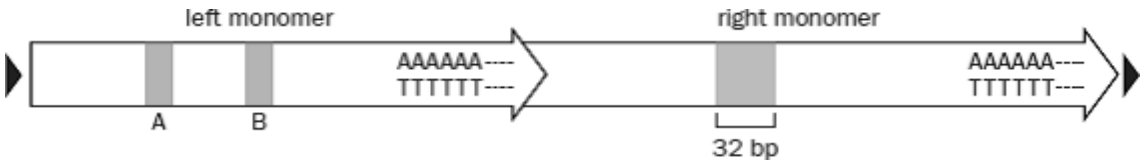


Figure 9: Alu repeats elements

Alu repeats originated from cDNA copies of small RNAs transcribed by RNA polymerase III. Genes transcribed by RNA polymerase III often have internal promoters, and so cDNA copies of transcripts carry with them their own promoter sequences. Both the Alu repeat and, independently, the mouse B1 repeat originated from cDNA copies of 7SL RNA, the short RNA that is a component of the signal recognition particle, using a retrotransposition mechanism. Other SINEs, such as the mouse B2 repeat, are retrotransposed copies of tRNA sequences. Alu repeats have a relatively high GC content and, although dispersed mainly throughout the euchromatic regions of the genome, are preferentially located in the GC-rich and gene-rich R chromosome bands, in striking contrast to the preferential location of LINEs in AT-rich DNA.

Probable Questions:

1. Describe method of cut –paste transposable elements.
2. Describe method of replicative transposable elements.
3. What is retro elements?
4. Describe Ac-Ds system in maize.
5. Briefly discuss copia and P elements.
6. What is SINE and LINE?
7. What are significance of transposable elements.

Suggested Readings / References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell andMolecularBiology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genomes

UNIT-XIII

Pseudogenes, segmental duplications; Super coiling of DNA. Classes of DNA, Giant chromosomes:

Objective: In this unit we will discuss about segmental duplications, pseudogenes. We will also discuss different classes of DNA and also about some giant chromosomes like Lampbrush and Polytene chromosome.

Segmental duplication

The human genome contains numerous blocks of highly homologous duplicated sequence. Segmental duplications (also termed "low-copy repeats") are blocks of DNA that range from 1 to 400 kb in length, occur at more than one site within the genome, and typically share a high level of (>90%) sequence identity. Both in situ hybridization and in silico analyses have shown that ~5% of the human genome is composed of duplicated sequence and many studies have noted a significant association between the location of segmental duplications and regions of chromosomal instability or evolutionary rearrangement. Indeed, segmental duplications have been implicated as the probable mediators of >25 recurrent genomic.Molecular studies have shown that the presence of large, highly homologous flanking repeats predisposes these regions to recurrent rearrangement by nonallelic homologous recombination, resulting in deletion, duplication, or inversion of the intervening sequence.

Large-scale subgenomic duplications can arise as a result of chromosome translocations. Euchromatic regions close to human centromeres and to telomeres (pericentromeric and subtelomeric regions, respectively) are comparatively unstable and are prone to recombination with other chromosomes. As a result, large segments of DNA containing multiple genes have been duplicated. Within the past 40 million years of primate evolution, this process has led to the duplication of about 400 large (several megabases long) DNA segments, accounting for more than 5% of the euchromatic genome. This type of duplication, known as segmental duplication, results in very high (often more than 95%) sequence identity between the DNA copies and can involve both intrachromosomal duplications and also interchromosomal duplications. Segmental duplications are important contributors to copy- number variation and to chromosomal rearrangements leading to disease and rapid gene innovation. Segmental duplication at its simplest involves the tandem duplication of some region within a chromosome (typically because of an aberrant recombination event at meiosis). However, in many cases the duplicated regions are on different chromosomes, implying that either there was originally a tandem duplication followed by

a translocation of one copy to a new site or that the duplication arose by some different mechanism altogether. The extreme case of a segmental duplication is when a whole genome is duplicated, in which case the diploid genome initially becomes tetraploid. As the duplicated copies evolve differences from one another, the genome may gradually become effectively a diploid again, although homologies between the diverged copies leave evidence of the event. Locating and characterizing these segmental duplications is of great interest because these recent genomic changes might have significantly contributed to the species divergence between human and the apes or Old World monkeys and because some genomic rearrangements have been found to be the causes of several genetic diseases in humans.

Pseudogene

Pseudogenes are usually thought of as defective copies of a functional gene to which they show significant sequence homology. They typically arise by some kind of gene duplication event that produces two gene copies. Selection pressure to conserve gene function need only be imposed on one gene copy; the other copy can be allowed to mutate more freely (genetic drift) and can pick up inactivating mutations, producing a pseudogene (Figure 1).

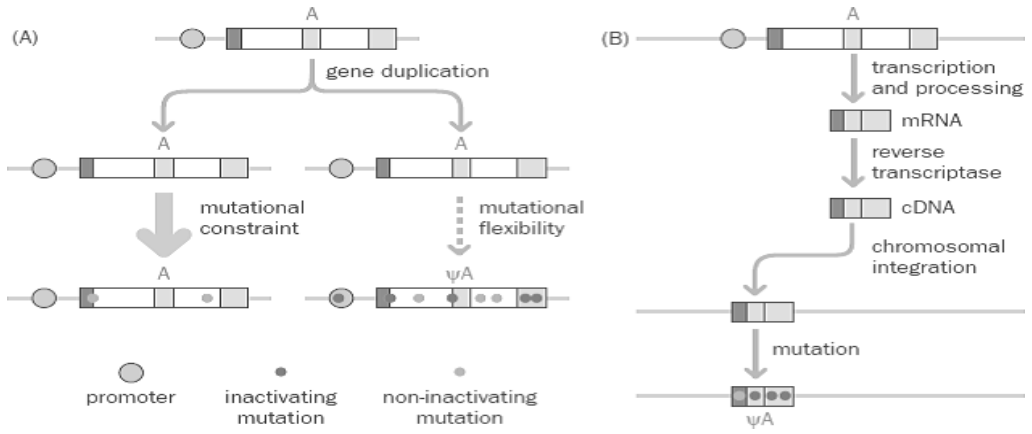


Figure 1. Mechanism of origin of pseudogene (A) Non-processed pseudogene (B) Processedpseudogene

However, some sequences are referred to as pseudogenes even though they have not originated by DNA copying. Humans have rare solitary pseudogenes that are clearly orthologs of functional genes in the great apes and became defective after acquiring harmful mutations in the human lineage. Different gene duplication mechanisms can give rise to multiple functional gene copies and defective pseudogenes. Either the genomic DNA sequence is copied, or a cDNA copy is made

(after reverse transcription of a processed RNA transcript) that integrates into genomic DNA (Figure 10). Gene families frequently have defective gene copies in addition to functional genes (Figure 2). They are represented by thesymbol corresponding to the active gene.

Figure 2: Pseudogene in β-globin gene cluster.

For a protein-coding gene, copying at the genomic DNA level can result in duplication of the promoter and upstream regulatory sequences as well as of all exons and introns. A defective gene that derives from a copy of a genomic DNA sequence is known as a nonprocessed pseudogene (Figure 10). Such pseudogenes usually arise by tandem duplication so that they are located close to functional gene counterparts, but some are dispersed as a result of recombination. Copying at the cDNA level produces a gene copy that typically lacks introns, promoter elements, and upstream regulatory elements. Very occasionally, a processed gene copy can retain some function (a retrogene). However, because they lack important sequences needed for expression, most processed gene copies degenerate into processed pseudogene. Processed pseudogenes are defective copies of a gene that contain only exonic sequences and lack an intronic sequence or upstream promoter sequences. They arise by retrotransposition: cellular reverse transcriptases can use processed gene transcripts such as mRNA to make cDNA that can then integrate into chromosomal DNA (Figure 10). Processedpseudogenes are common in interspersed gene families. Processed pseudogenes lack a promoter sequence and so are typically not expressed. Sometimes, however, the cDNA copy integrates into a chromosomal DNA site that happens, by chance, to be adjacent to a promoter that can drive expression of the processed gene copy. Selection pressure may ensure that the processed gene copy continues to make a functional gene product, in which case it is described as a retrogene. A variety of intronless retrogenes are known to have testis-specific expression patterns and are typically autosomal homologs of an intron-containing X-linked gene. Eukaryotic genomes typically have many pseudogenes. A longstanding rationale for their abundance is that gene duplication is evolutionarily advantageous. New functional gene variants can be created by gene duplication, and pseudogenes have long been viewed as unsuccessful by-products of the duplication mechanisms. Although some prokaryotic genomes seem to have many pseudogenes, pseudogenes are generally rare in prokaryotes because their genomes are generally designed to be compact. The great majority of what are conventionally

recognized as human pseudogenes are copies of protein-coding genes simply because it is relatively easy to identify them (by looking for frameshifting, splice site mutations, and so on). There are more than 8000 different processed pseudogene copies of protein-coding genes in the human genome, plus more than 4000 nonprocessed pseudogenes

DNA Supercoiling

"Supercoiling" means the coiling of a coil. An old fashioned telephone cord, for example, is typically a coiled wire. The path taken by the wire between the base of the phone and the receiver often includes one or more supercoils (Figure 3).

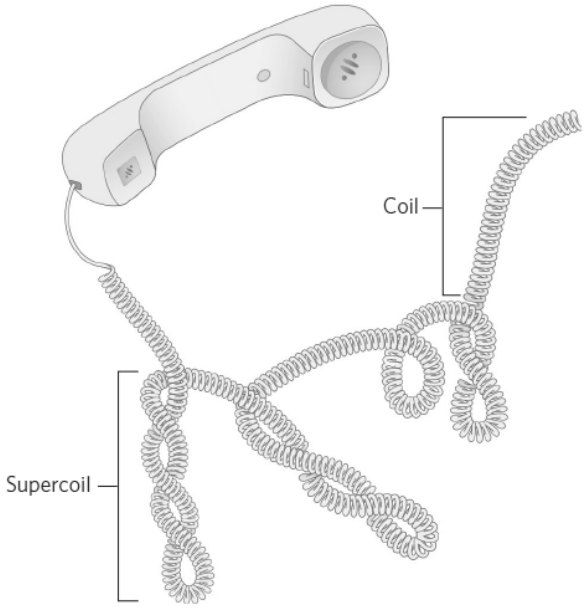


Figure 3: Supercoiling

DNA is coiled in the form of a double helix, with both strands of the DNA coiling around anaxis. The further coiling of that axis upon itself (Figure 4) produces DNA supercoiling. As detailed below, DNA supercoiling is generally a manifestation of structural strain. When there is no net bending of the DNA axis upon itself, the DNA is said to be in a relaxed state.

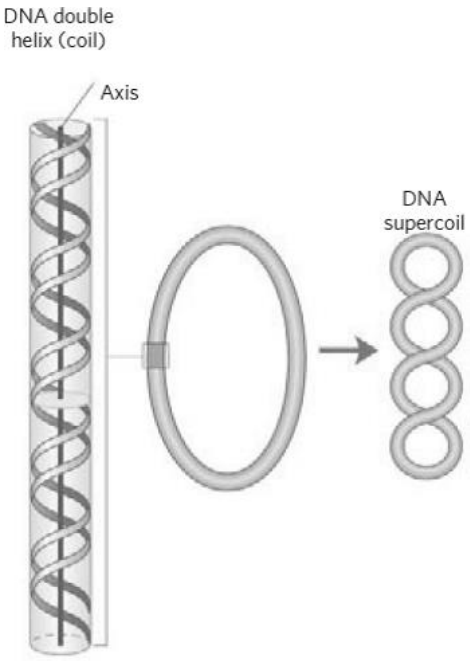


Figure 4: Supercoiling of DNA

As DNA is a flexible structure, its exact molecular parameters are a function of both the surrounding ionic environment and the nature of the DNA-binding proteins with which it is complexed. Because their ends are free, linear DNA molecules can freely rotate to accommodate changes in the number of times the two chains of the double helix twist about each other. But if the two ends are covalently linked to form a circular DNA molecule and if there are no interruptions in the sugar-phosphate backbones of the two strands, then the absolute number of times the chains can twist about each other cannot change. Such a covalently closed, circular DNA is said to be topologically constrained. Even the linear DNA molecules of eukaryotic chromosomes are subject to topological constraints due to their extreme length, entrainment in chromatin, and interaction with other cellular components.

Covalently closed, circular DNA, which is referred to as cccDNA has no interruptions in either polynucleotide chain. The two strands of cccDNA cannot be separated from each other without the breaking of a covalent bond. If we wished to separate the two circular strands without permanently breaking any bonds in the sugar-phosphate backbones, we would haveto pass one strand through the other strand repeatedly. The number of times one strand would have to be passed through the other strand in order for the two strands to be entirely separatedfrom each other is called the linking number. The linking number is the sum of two geometric components called the twist and the writhe. Twist is simply the number of helical turns of one strand about the other,

that is, the number of times one strand completely wraps around the other strand. Consider a cccDNA that is lying flat on a plane. In this flat conformation, the linking number is fully composed of twist. Indeed, the twist can be easily determined by counting the number of times the two strands cross each other (Figure 14). The helical crossovers (twist) in a right-handed helix are definedas positive such that the linking number of DNA will have a positive value. But cccDNA is generally not lying flat on a plane. Rather, it is usually torsionally stressed such that the long axis of the double helix crosses over itself, often repeatedly, in three-dimensional space (Figure 5). This is called writhe.

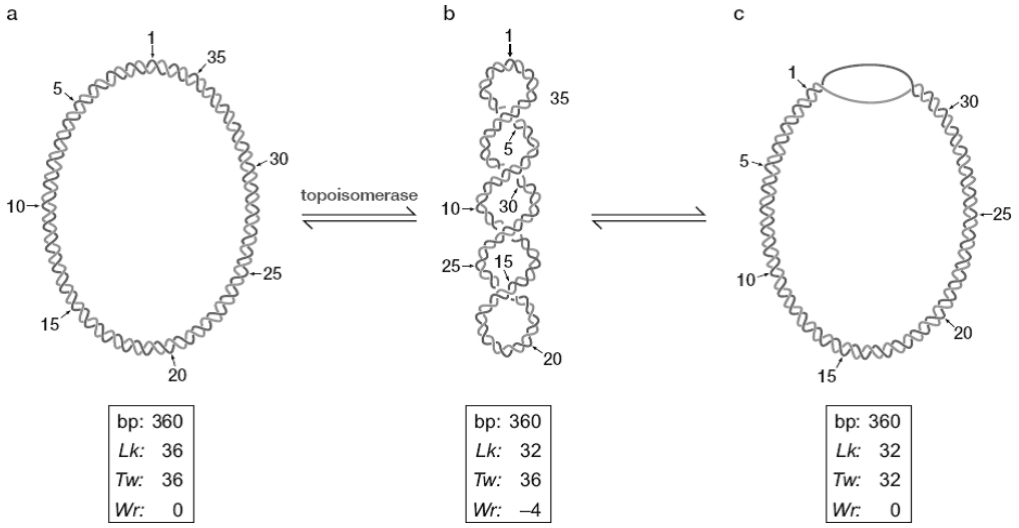


Figure 5: Topological states of covalently closed circular DNA (cccDNA)

To visualize the distortions caused by torsional stress, think of the coiling of a telephone cord that has been overtwisted. Writhe can take two forms. One form is the interwound or plectonemic writhe, in which the long axis is twisted around itself. The other form of writhe is a toroid or spiral in which the long axis is wound in a cylindrical manner, as often occurs when DNA wraps around protein (Figure 15). The writhing number (Wr) is the total number of interwound and/or spiral writhes in cccDNA Interwound writhe and spiral writhe are topologically equivalent to each other and are readily interconvertible geometric properties of cccDNA. Also, twist and writhe are interconvertible. A molecule of cccDNA can readily undergo distortions that convert some of its twist to writhe or some of its writhe to twist without the breakage of any covalent bonds. The only constraint is that the sum of the twist number (Tw) and the writhing number (Wr) must remain equal to the linking number (Lk). This constraint is described by the equation:

$$\mathbf{Lk = Tw + Wr}$$

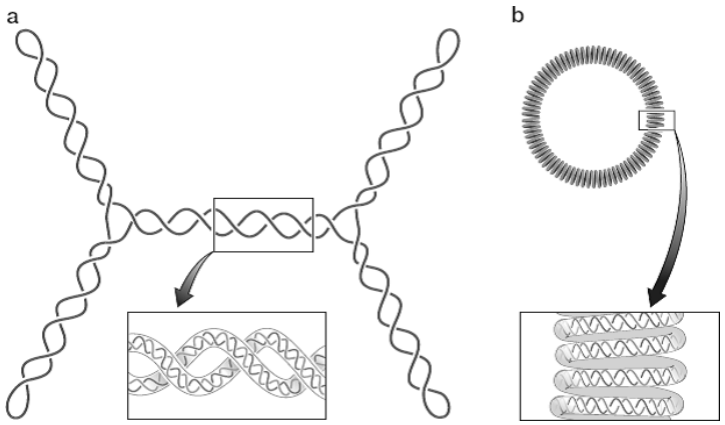


Figure 15: Two forms of Writhe (a) Toroid or spiral and (b) Interwound or Plectonemic

The cccDNA that is free of supercoiling (i.e., relaxed) and whose twist corresponds to that of the B-form of DNA in solution under physiological conditions (~10.5 bp per turn of the helix), the linking number (Lk) of such cccDNA under physiological conditions is assigned the symbol Lk^0. Lk^0 for such a molecule is the number of base pairs divided by 10.5. For a cccDNA of 10,500 base pairs, Lk= +1000. (The sign is positive because the twists of DNA are right-handed). One way to see this is to imagine pulling one strand of the 10,500-bp cccDNA out into a flat circle. If we did this, then the other strand would cross the flat circular strand 1000 times. To remove supercoils from cccDNA (if it is not already relaxed), one procedure is to treat theDNA mildly with the enzyme DNase I, so as to break on average one phosphodiester bond (or a small number of bonds) in each DNA molecule. Once the DNA has been "nicked" in this manner, it is no longer topologically constrained, and the strands can rotate freely, allowing writhe to dissipate (Fig. 6).

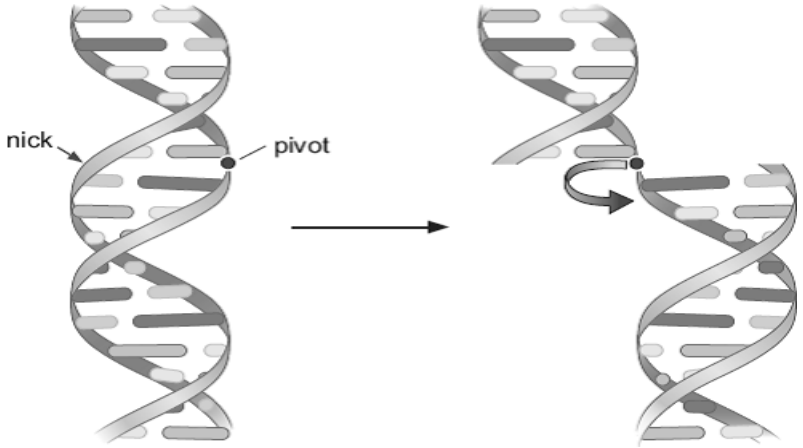


Figure 6: Relaxing DNA with DNase I

If the nick is then repaired, the resulting cccDNA molecules will be relaxed and will have on average an Lk that is equal to Lk^0.

The extent of supercoiling is measured by the difference between Lk and Lk^0, which is called the linking difference. The equation is given below

$$\Delta Lk = Lk - Lk^0$$

If the ΔLk of a cccDNA is significantly different from 0, then the DNA is torsionally strained, and hence it is supercoiled. If Lk < Lk0 and ΔLk < 0, then the DNA is said to be "negatively supercoiled." Conversely, if Lk > Lk^0 and ΔLk > 0, then the DNA is "positively supercoiled." For example, the molecule shown in Figure 15b is negatively supercoiled and has a linking difference of –4 because its Lk (32) is 4 less than that (36) for the relaxed form of the molecule shown in Figure 15a. Because ΔLk and Lk^0 are dependent on the length of the DNA molecule, it is more convenient to refer to a normalized measure of supercoiling. This is the superhelical density, which is assigned the symbol σ and is defined as

$$\sigma = \Delta Lk / Lk0$$

The linking number is an invariant property of DNA that is topologically constrained. It can be changed only by introducing interruptions into the sugar–phosphate backbone. A remarkable class of enzymes known as topoisomerases are able to do just this by introducing transient single-strand or double-strand breaks into the DNA. Topoisomerases are of two general types. Type II topoisomerases change the linking number in steps of two. They make transient double-strand breaks in the DNA through which they pass a segment of uncut duplex DNA before resealing the break. Type II topoisomerases require the energy of ATP hydrolysis for their action. Type I topoisomerases, in contrast, change the linking number of DNA in steps of one. They make transient single-strand breaks in the DNA, allowing the uncut strand to pass through the break before resealing the nick. In contrast to the type II topoisomerases, type I topoisomerases do not require ATP.

Organization and nature of nuclear DNA

Deoxyribose nucleic acid (DNA)

As a chemical, DNA is quite simple. It contains three types of chemical components: (1) phosphate, (2) a sugar called deoxyribose, and (3) four nitrogenous bases-adenine, guanine, cytosine, and thymine. The sugar in DNA is called "deoxyribose" because it has only a hydrogen

atom (H) at the 2′-carbon atom, unlike ribose (a component of RNA), which has a hydroxyl (OH) group at that position. Two of the bases, adenine and guanine, have a double- ring structure characteristic of a type of chemical called a purine. The other two bases, cytosine and thymine, have a single-ring structure of a type called a pyrimidine. The carbon atoms in the bases are assigned numbers for ease of reference. The carbon atoms in the sugar group also are assigned numbers-in this case, the number is followed by a prime (1′, 2′, and so forth). The chemical components of DNA are arranged into groups called nucleotides, each composed of a phosphate group, a deoxyribose sugar molecule, and any one of the four bases. It is convenient to refer to each nucleotide by the first letter of the name of its base: A, G, C, or T. The nucleotide with the adenine base is called deoxyadenosine 5′-monophosphate,where the 5′ refers to the position of the carbon atom in the sugar ring to which the single (mono) phosphate group is attached.

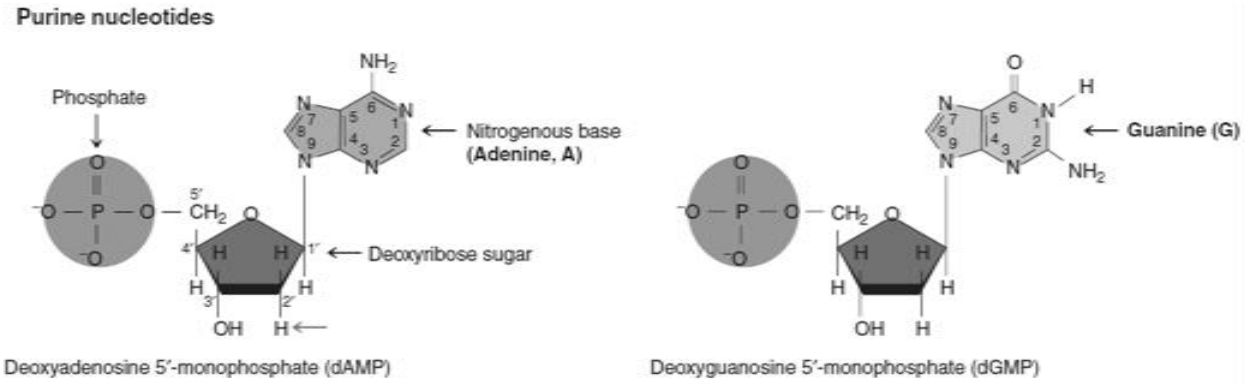


Figure 7: Purine nucleotide of DNA

The three-dimensional structure derived by Watson and Crick is composed of two side-by-side chains (“strands”) of nucleotides twisted into the shape of a double helix(Figure 18). The two nucleotide strands are held together by hydrogen bonds between the bases of each strand, forming a structure like a spiral staircase (Figure 18). The backbone of each strand is formed of alternating phosphate and deoxyribose sugar units that are connected by phosphodiester linkages (Figure 18). As already mentioned, the carbon atoms of the sugar groups are numbered 1′ through 5′. A phosphodiester linkage connects the 5′-carbon atom of one deoxyribose to the 3′-carbon atom of the adjacent deoxyribose. Thus, each sugar–phosphate backbone is said to have a 5′-to-3′ polarity, or direction, and understanding this polarity is essential in understanding how DNA fulfills its roles.

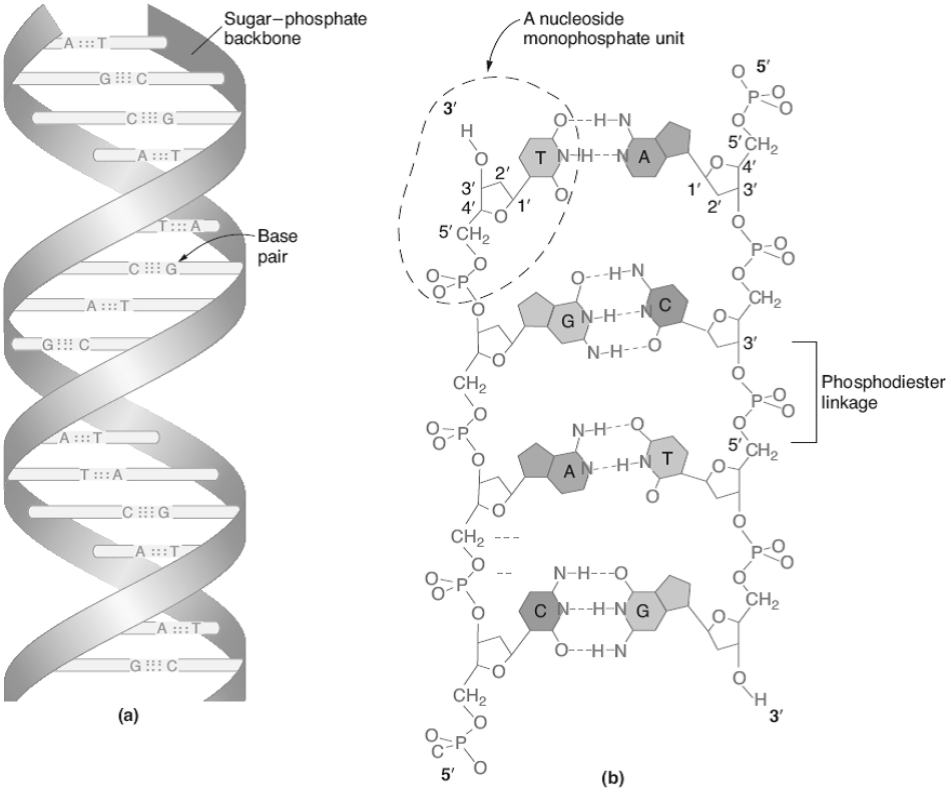


Figure 8: Structure of DNA

In the double-stranded DNA molecule, the two backbones are in opposite, or **antiparallel,** orientation (Figure 18). Each base is attached to the 1′-carbon atom of a deoxyribose sugar in the backbone of each strand and faces inward toward a base on the other strand. Hydrogen bonds between pairs of bases hold the two strands of the DNA molecule together. Two complementary nucleotide strands paired in an antiparallel manner automatically assume a double-helical conformation (Figure 18), mainly through the interaction of the base pairs. The base pairs, which are flat planar structures, stack on top of one another at the center of the double helix. Stacking adds to the stability of the DNA molecule by excluding water molecules from the spaces between the base pairs. The most stable form that results from base stacking is a double helix with two distinct sizes of grooves running in a spiral: the **major groove** and the **minor groove,** which can be seen in both the ribbon and the space-filling models. Most DNA–protein associations are in major grooves (Figure 19). A single strand of nucleotides has no helical structure; the helical shape of DNA depends entirely on the pairing and stacking of the bases in the antiparallel strands. DNA is a right-handed helix; in other words, it has the same structure as that of a screw that would be screwed into place by using a clockwise turning motion.

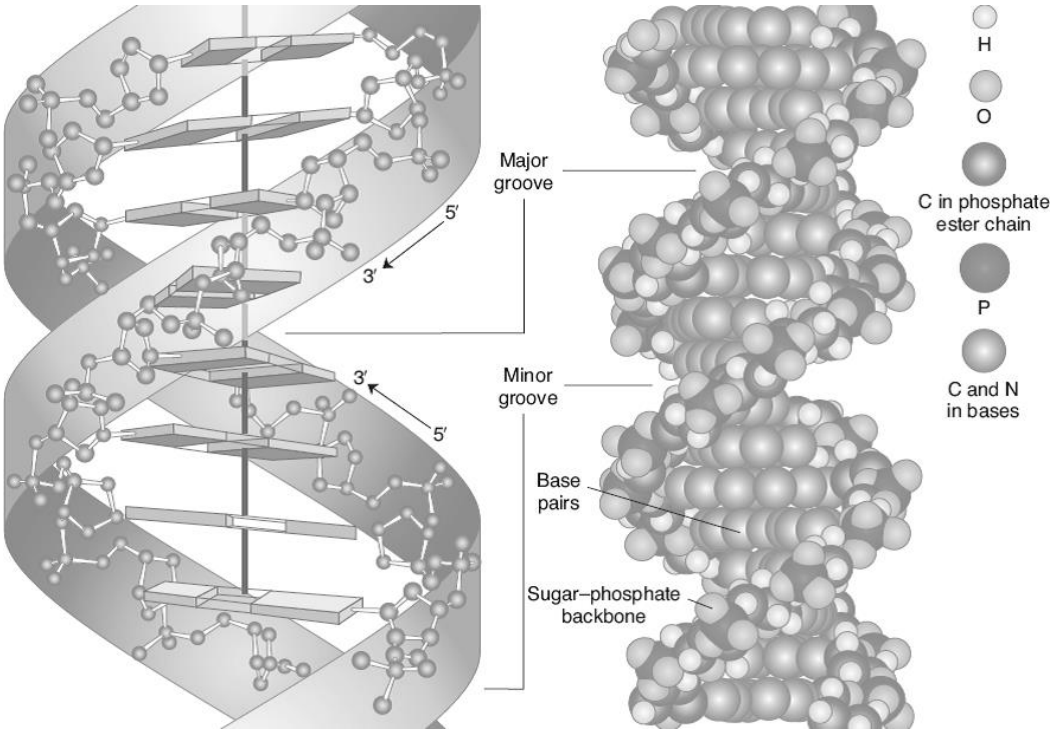


Figure 9: The ribbon diagram of DNA structure

Eukaryotic Chromosome and organization of DNA

Eukaryotic species have one or more sets of chromosomes; each set is composed of several different linear chromosomes. Humans, for example, have two sets of 23 chromosomes each, for a total of 46. A distinguishing feature of eukaryotic cells is that their chromosomes are located within a separate cellular compartment known as the **nucleus.** To fit within the nucleus, the length of DNA must be compacted by a remarkable amount. The term **chromatin** is used to describe the DNA-protein complex found within eukaryotic chromosomes. Chromatin is a dynamic structure that can change its shape and composition during the life of a cell. In this section, we will examine the organization of DNA sequences along the length of eukaryotic chromosomes and the levels of compaction of eukaryotic chromosomes during different stages of the cell cycle. Our discussion of chromatin compaction in this largely focuses on structural features between DNA and DNA-binding proteins that occur in eukaryotic chromosomes. In addition chromatin is a dynamic structure that can alternate between loose and compact conformations in a way that regulates gene expression.

Eukaryotic Chromosome

Individual eukaryotic chromosomes contain enormous amounts of DNA. Each eukaryotic chromosome consists of a single, extremely long molecule of DNA. For all of this DNA to fitinto the nucleus, tremendous packing and folding are required, the extent of which must

change in the course of the cell cycle. The chromosomes are in an elongated, relatively uncondensed state during interphase of the cell cycle, but the term relatively is an important qualification here. Although the DNA of interphase chromosomes is less tightly packed than the DNA of mitotic chromosomes, it is still highly condensed; it's just less condensed. In the course of the cell cycle, the level of DNA packing changes: chromosomes progress from a highly packed state to a state of extreme condensation. DNA packing also changes locally in replication and transcription, when the two nucleotide strands must unwind so that particular base sequences are exposed. Thus, the packing of eukaryotic DNA (its tertiary chromosomal structure) is not static but changes regularly in response to cellular processes.

Chromatin Eukaryotic DNA in the cell is closely associated with proteins. This combination of DNA and protein is called chromatin. The two basic types of chromatin are euchromatin, which undergoes the normal process of condensation and decondensation in the cell cycle, and heterochromatin, which remains in a highly condensed state throughout the cell cycle, even during interphase. Euchromatin constitutes the majority of the chromosomal material and located where most transcription takes place. All chromosomes have heterochromatin at the centromeres and telomeres. Heterochromatin is also present at other specific places on some chromosomes, along the entire inactive X chromosome in female mammals and throughout most of the Y chromosome in males. In addition to remaining condensed throughout the cell cycle, heterochromatin is characterized by a general lack of transcription, the absence of crossing over, and replication late in the S stage.

The most abundant proteins in chromatin are the histones, which are small, positively charged proteins of five major types: H1, H2A, H2B, H3, and H4 (Table 2). All histones have a high percentage of arginine and lysine, positively charged amino acids that give the histones a net positive charge. The positive charges attract the negative charges on the phosphates of DNA; this attraction holds the DNA in contact with the histones. A heterogeneous assortment of nonhistone chromosomal proteins also are found in eukaryotic chromosomes. At times, variant histones, with somewhat different amino acid sequences, are incorporated into chromatin in place of one of the major histone proteins.

Histone Protein	Molecular Weight	Number of Amino Acids
H1	21,130	223
H2A	13,960	129
H2B	13,774	125
H3	15,273	135
H4	11,236	102

Table 2: Characteristics of histone protein

The nucleosome: Chromatin has a highly complex structure with several levels of organization (Figure 10). The simplest level is the double-helical structure of DNA as discussed earlier. At a more complex level, the DNA molecule is associated with proteins andis highly folded to produce a chromosome.

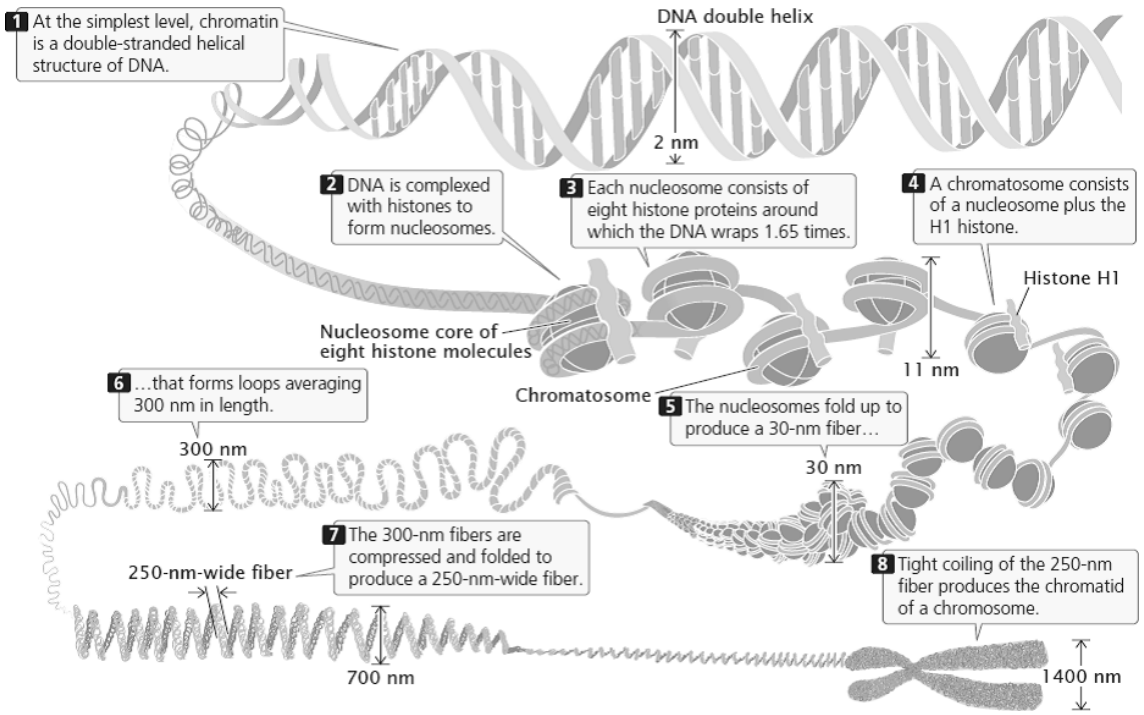


Figure 10: Chromatin and several levels of organization into chromosome

When chromatin is isolated from the nucleus of a cell and viewed with an electron microscope, it frequently looks like beads on a string (Figure 11a). If a small amount of nuclease is added to this structure, the enzyme cleaves the "string" between the "beads,"

leaving individual beads attached to about 200 bp of DNA (Figure 11b). If more nuclease is added, the enzyme chews up all of the DNA between the beads and leaves a core of proteins attached to a fragment of DNA (Figure 21c). Such experiments demonstrated that chromatin is not a random association of proteins and DNA but has a fundamental repeating structure.

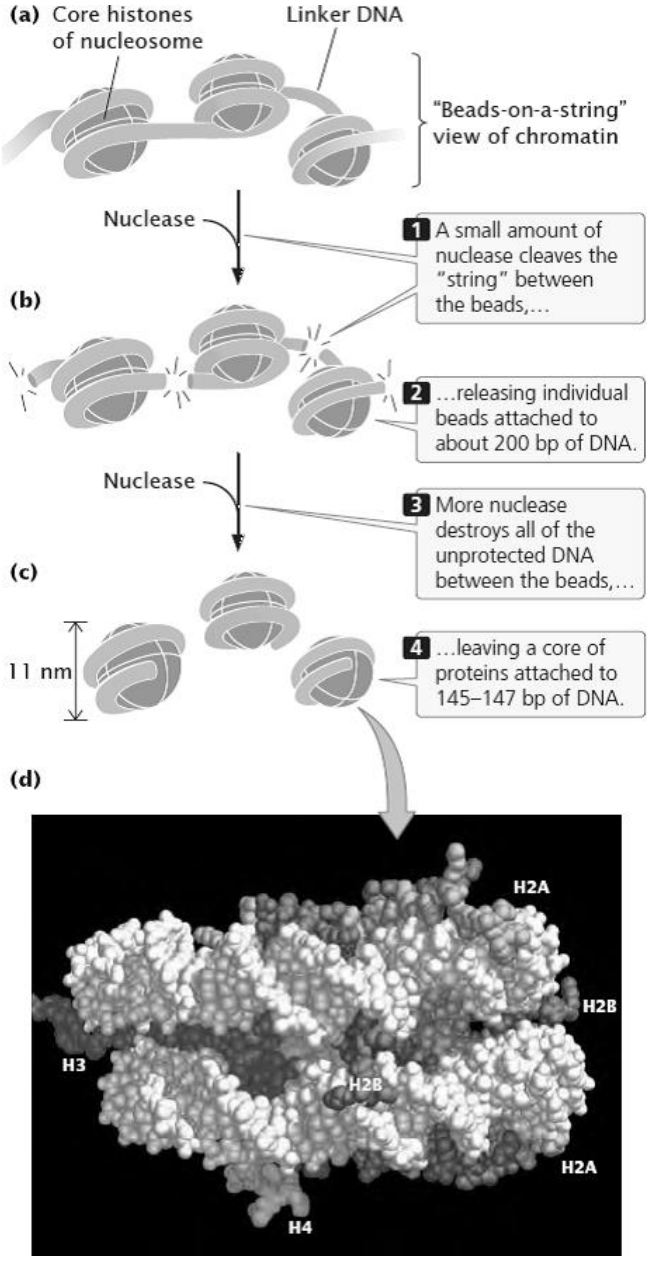


Figure 11: Nucleosome is the fundamental unit of Chromatin

The repeating core of protein and DNA produced by digestion with nuclease enzymes is the simplest level of chromatin structure, the nucleosome (Figure 22). The nucleosome is a core particle consisting of DNA wrapped about two times around an octamer of eight histone

proteins (two copies each of H2A, H2B, H3, and H4), much like thread wound around a spool (Figure 12). The DNA in direct contact with the histone octamer is between 145 and 147 bp in length.

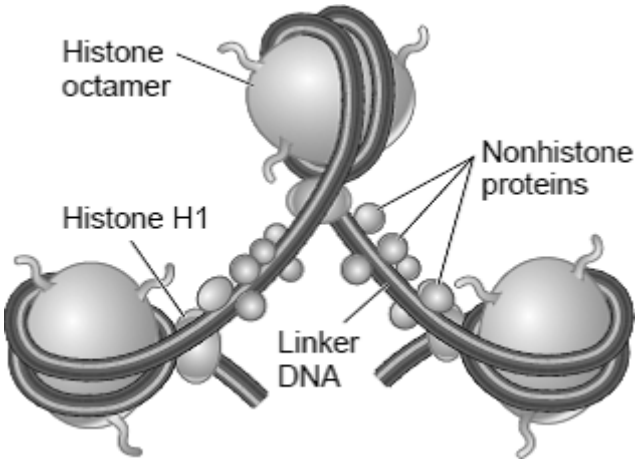


Figure 12: Nucleosome with histone and non-histone protein

Levels of organization of DNA in eukaryotic chromosome

Each of the histone proteins that make up the nucleosome core particle has a flexible "tail," containing from 11 to 37 amino acids, that extends out from the nucleosome. Positively charged amino acids in the tails of the histones interact with the negative charges of the phosphates on the DNA, keeping the DNA and histones tightly associated. The tails of one nucleosome may also interact with neighboring nucleosomes, which facilitates compaction of the nucleosomes themselves. Chemical modifications of the histone tails bring about changes in chromatin structure that are necessary for gene expression. The fifth type of histone, H1, is not a part of the core particle but plays an important role in nucleosome structure. H1 binds to 20 to 22 bp of DNA where the DNA joins and leaves the octamer and helps to lock the DNA into place, acting as a clamp around the nucleosome octamer. Together, the core particle and its associated H1 histone are called the **chromatosome (**Figure 12), the next level of chromatin organization. Each chromatosome encompasses about 167 bp of DNA. Chromatosomes are located at regular intervals along the DNA molecule and are separated from one another by linker DNA, which varies in size among cell types; in most cells, linker DNA comprises from about 30 to 40 bp. Nonhistone chromosomal proteins may be associated with this linker DNA, and a few also appear to bind directly to the core particle.

Higher-order chromatin structure : When chromatin is in a condensed form, adjacent nucleosomes are not separated by space equal to the length of the linker DNA; rather, nucleosomes fold on themselves to form a dense, tightly packed structure (Figure 11) that makes up a fiber with a diameter of about 30 nm (Figure 23). Two different models have been proposed for the 30-nm fiber: a solenoid model, in which a linear array of nucleosomes are coiled, and a helix model, in which nucleosomes are arranged in a zigzag ribbon that twists or supercoils. Recent evidence supports the helix model (Figure 13).

The next-higher level of chromatin structure is a series of loops of 30-nm fibers, each anchored at its base by proteins in the nuclear scaffold (Figure 11). On average, each loop encompasses some 20,000 to 100,000 bp of DNA and is about 300 nm in length, but the individual loops vary considerably. The 300-nm loops are packed and folded to produce a 250-nm-wide fiber. Tight helical coiling of the 250-nm fiber, in turn, produces the structure that appears in metaphase-individual chromatids approximately 700 nm in width.

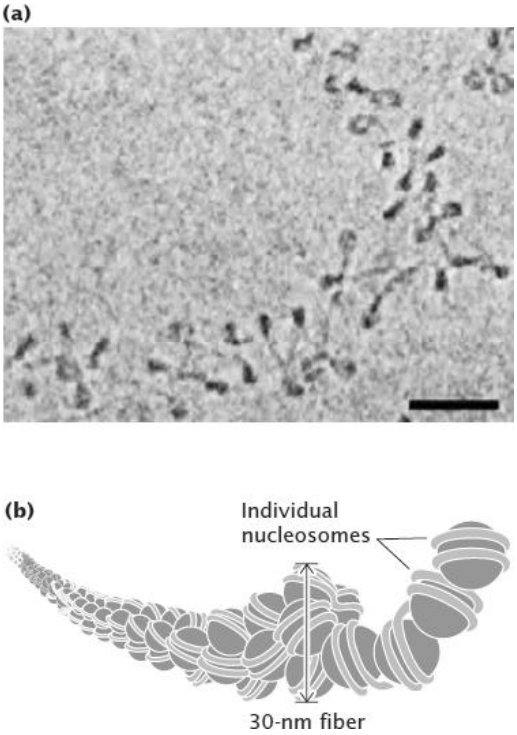


Figure 23: (a) Electron micrograph of nucleosome. (b) The model of 30-nm chromatin fiber.

Non-histone proteins in chromatin organization

In highly condensed chromosomes, such as those found in metaphase, the radial loops are highly compacted and remain anchored to a **scaffold,** which is formed from nonhistone proteins of the nuclear matrix. Experimentally, researchers can delineate the nonhistone proteins of the scaffold

that hold the loops in place. In this condition, the radial loops of DNA are in a very compact configuration. If this chromosome is treated with a high concentration of salt to remove both the core and linker histones, the highly compact configuration is lost, but the bottoms of the elongated loops remain attached to the scaffold composed of nonhistone proteins. Remarkably, the scaffold retains the shape of the original metaphase chromosome when chromosomes are treated with high salt to remove histone proteins, even though the DNA strands have become greatly elongated. These results illustrate that the structure of metaphase chromosomes is determined by the nuclear matrix proteins, which form the scaffold, and by the histones, which are needed to compact the radial loops. Researchers found that cells contain two multiprotein complexes called condensin and cohesin, which play a critical role in chromosomal condensation and sister chromatid alignment, respectively. Condensin and cohesin are two completely distinct complexes, but both contain a category of proteins called SMC proteins. SMC stands for structural maintenance of chromosomes. These proteins use energy from ATP to catalyze changes in chromosome structure. Together with topoisomerases, SMC proteins have been shown to promote major changes in DNA structure. An emerging theme is that SMC proteins actively fold, tether, and manipulate DNA strands. They are dimers that have a V-shaped structure. The monomers, which are connected at a hinge region, have two long coiled arms with a head region that binds ATP (Figure 15). The length of each monomer is about 50 nm, which is equivalent to approximately 150 bp of DNA.

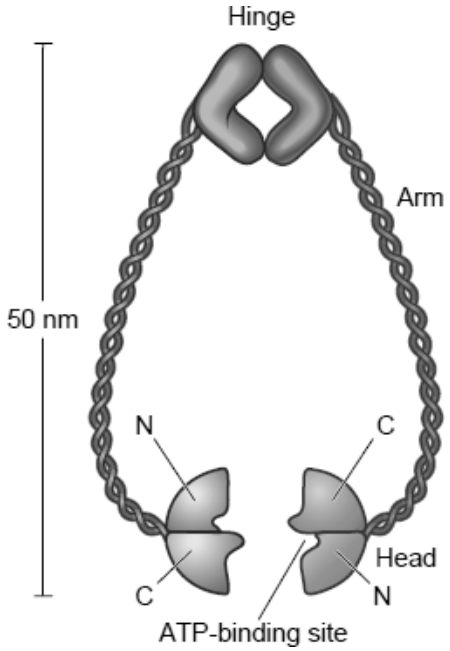


Figure 15: Structure of SMC protein

As their names suggest, condensin and cohesin play different roles in metaphase chromosome structure. Prior to M phase, condensin is found outside the nucleus (Figure 26).However, as M phase begins, condensin is observed to coat the individual chromatids as euchromatin is

converted into heterochromatin. The role of condensin in the compaction process is not well understood. Although + linkage before anaphase. At anaphase, the cohesins bound to the centromere are rapidly degraded by a protease aptly named separase, thereby allowing sister chromatid separation.

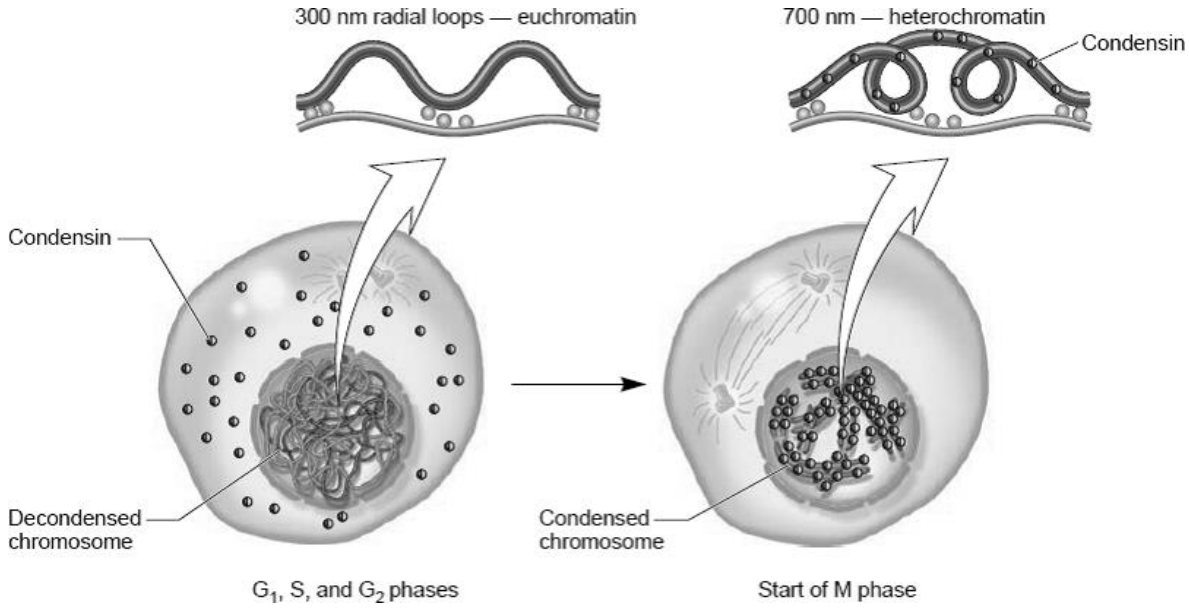


Figure 16: Localization of Condensin during Interphase and start of M phase

Different types of special chromosomes are discussed below:

[I] Lampbrush chromosomes:

These are the largest known chromosomes found in the yolk rich oocytic nuclei of certain vertebrates such as fishes, amphibians, reptiles and birds. They can be seen with naked eye and are characterized by fine lateral loops, arising from the chromomeres, during first prophase (diplotene) of meiosis. These loops give it a brush-like appearance; that is why these are called lampbrush chromosomes first discovered by Flemming in 1882 and were described in shark oocytes by Ruckert (1892). Lampbrush chromosomes of certain urodele oocytes may reach upto 5900µ in length.

It consists of longitudinal axis formed by a single DNA molecule along which several hundred bead-like chromomeres are distributed in a linear fashion. From each chromomere there emerge two symmetrical lateral loops (one for each chromatid), which are able to expand or contract in response to various environmental conditions.

About 5 to 10% of the DNA is in the lateral loops. Loop formation reduces the mass of the corresponding chromomeres, implying a spinning out of chromomere material into the lateral strands. The centromeres

also have the appearance of elongate Feulgen-positive chromomeres but they characteristically lack lateral loops.

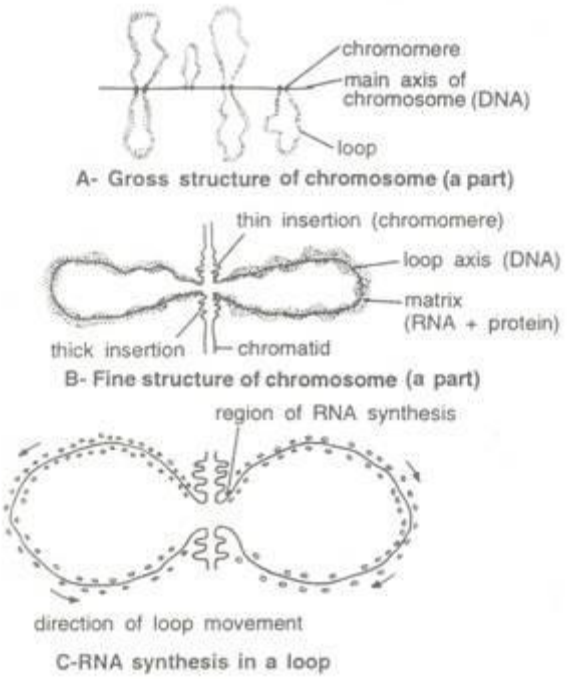


Fig. 11. Lampbrush chromosome.

Lampbrush chromosomes can be dissected in (toto) from oocyte nucleus. Individual chromosomes are liable to stretching. With extreme stretching, chromomeres begin to separate transversely into two halves, so that the paired loops form double stranded bridges. The axis between chromomeres is also double, which can be seen in certain special regions where two elements separate longitudinally and bear single loops (Callan, 1955). These experiments indicate that each chromomere possesses four quadrants separated by both a transverse and a longitudinal line of division (Fig. C). Callan (1963) regards it as that the entire chromatid pair is made up of two continuous strands, which lie parallel to one another in the interchromomere regions, are tightly folded in the chromomeres, and separate as single, unfolded fibres in the loops. Each of the two fibres would correspond to one conventional metaphase chromatid.

There is fundamental similarity in the organization of amphibian lmpbrush chromosomes and dipteran giant polytene chromosomes: in both cases, very long single fibres correspond to single chromatids and are partly but not completely extended. The substructure of the salivary chromosome ‘puffs’ also bear some similarity to that of the lampbrush lateral loops. Lateral loops are formed of DNA, in chromomeres regions DNA is tightly folded and transcriptionally inactive. In lateral loops RNA synthesis is intense. Each loop in turn has an axis formed by a single DNA molecule, which is coated by a matrix of nascent RNA and proteins. The matrix is asymmetrical, being thicker at one end of the loop. RNA synthesis starts at the thinner end and progresses toward the thicker end.

1. Functions of Lampbrush chromosomes:

(a) Synthesis of RNA:

Functions of lampbrush chromosomes involve synthesis of RNA and protein by their loops. RNA is synthesized only at the thin insertion and then carried around the loops to the thick insertion. There it may be either destroyed or released into nucleus.

(b) Formation of yolk material:

There are some probabilities that lampbrush chromosomes help in the formation of certain amount of yolk material for the egg.

[II] Polytene chromosomes:

These are also giant chromosomes but relatively smaller than lampbrush chromosomes, found in the larvae of certain dipterans. Such banded chromosomes occur in the larval salivary glands, midgut epithelium, and rectum and Malpighian tubules of various genera (Drosophila, Sciara, Rhynchosciara, and Chironomus). In these larvae the salivary glands contain salivary cells so large in size that they can easily be seen with the lens power of a dissecting microscope.

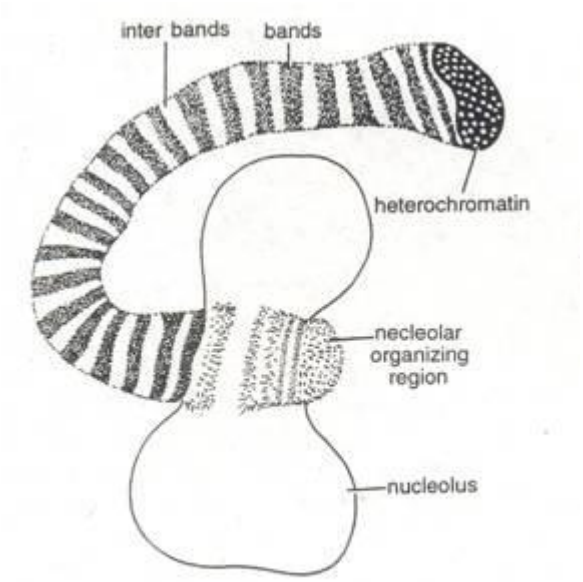


Fig. 12. Structure of a polytene chromosome of *Cecidomyia serotinae* showing nucleolar part.

Nuclei of these cells are much larger than those of ordinary cells being generally about 25µ in diameter, and chromosomes in nuclei are so large that they are 50 to 200 times as large as chromosomes in other body cells of the organism. They were first observed in 1881 by E.G. Balbiani in Chironomus and were studied by Korschelt (1884) and Corney (1884). Heitz and Bauer in 1933 studied these giant chromosomes in Bibio hortulanus larvae, while Painter (1933) described them in salivary glands of Drosophila.

Because of their large size showing numerous strands these are named as polytene chromosomes (name suggested by Kollar) or commonly salivary gland chromosomes. The latter term is a misnomer as these chromosomes may occur in other somatic cells of body besides salivary gland cells.

Ultrastructure of giant polytene (poly=many, tene=strands) chromosomes:

It was first investigated by Beermann and Bahr (1954), who observed numerous fine fibrils in the Balbiani rings of Chironomus and estimated that each chromosome contains 1000 to 2000 separate strands (corresponding to the degree of ploidy). Later Gay (1956) observed strands 200 to 500 A in diameter in sectioned Drosophila salivary chromosomes. The individual fibres in band and interband regions are similar in appearance, but the fibres in the bands exhibit a considerable degree of metaphase-like folding and are much more tightly packed.

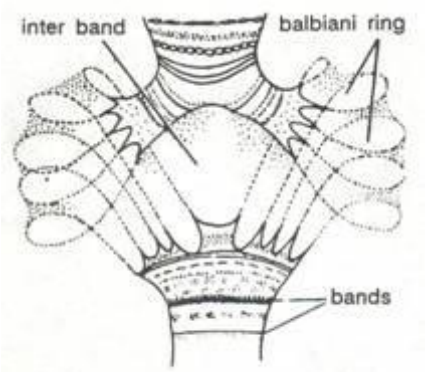


Fig. 13. Balbiani ring of a polytene chromosome.

Polytene chromosomes get their name from the fact that they are formed by many parallel chromatids, often more than a thousand strands, which do not separate from one another following duplication. Along each chromatid strand some regions of chromatin are tightly coiled and other regions are less coiled, with the result that polytene chromosomes appear to consist of light and dark bands when observed under a microscope. During larval development, specific areas on polytene chromosomes become uncoiled, forming localized regions called ‘puffs’. Puffs represent regions of active RNA synthesis (transcription). In the puff individual fibres remain continuous across the puff and they become extended as short lateral loops (Bahr, 1954). DNA is concentrated almost entirely in the bands. Protein and RNA is also found in puffs.

Puffing is due to the uncoiling of chromosome fibres which are usually closely folded or coiled in the dense band regions. These fibres then project in the form of loops.

Puffs and Balbiani rings:

During their initial stages of development these bands or interbands of the chromosomes exhibit swellings or puffs. Their appearance depends on the stage of larval development. It is probable that the metabolic activities, required for the formation of puffs, are related to the secretory function of the salivary glands. The formation of this is controlled by certain specific genes and the puffs are related with the active synthesis of RNA and proteins. This chromosomal RNA differs from the nucleolar and cytoplasmic RNA.

The RNA of puffs is also not similar; it differs from each other in chemical composition. Some regions show larger puffs than others. These larger puffing regions are called Balbiani rings.

These rings are formed by the lateral stretching of loops caused by chromonemata. These loops of chromonemata make up Balbiani rings and give the chromosome a fuzzy outlook. The Balbiani rings are rich in DNA and mRNA, and the formation and function of the Balbiani rings are similar to the puffs.

Functions of giant polytene chromosomes:

(1) Main function of the polytene chromosome is to carry genes which ultimately control physiology of an organism. These genes are formed of DNA molecules.

(2) Shifting of heterochromatin in respect to euchromatin produces giant changes called position effects. These effects cause mutations in animals as well.

(3) Heterochromatic regions contain fewer genes than euchromatic parts. Production of nucleolar material is entirely done by heterochromatin.

(4) Chromosomes also help in protein synthesis indirectly. Nucleolus contains RNA, and this RNA serves as a means of transmission of genetic information to the cytoplasm, leading to the formation of specific protein.

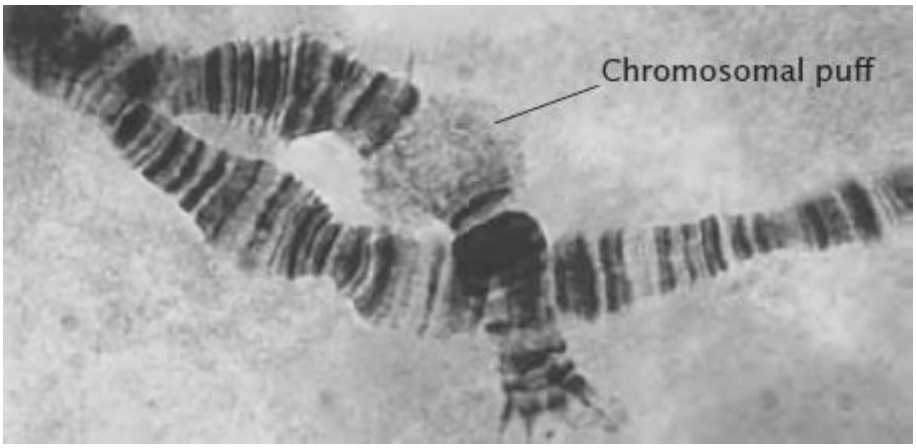


Figure : Polytene Chromosome showing chromosome puff-site of active transcription

If radioactively labelled uridine (a precursor to RNA) is briefly added to a Drosophila larva, radioactivity accumulates in chromosomal puffs, indicating that they are regions of active transcription. Additionally, the appearance of puffs at particular locations on the chromosome can be stimulated by exposure to hormones and other compounds that are known to induce the transcription of genes at those locations. This correlation between the occurrence of transcription and the relaxation of chromatin at a puff site indicates that chromatin structure undergoes dynamic change associated with gene activity.

Probable Questions

1. Explain why a packaging system is needed to fit a DNA molecule into its chromosome in eukaryotes.
2. Describe the role of histones in packaging DNA into eukaryotic chromosomes.
3. Why is negative supercoiling preferred over positive supercoiling?
4. Name the enzyme that negatively supercoils bacterial chromosomes
5. What are catenanes? How are they unlinked?
6. What is a linking number (L)?
7. What are topoisomerases? What is the difference between type I and type II topoisomerases?
8. What differences in gene distribution and repetitive DNA content are seen when yeast and human chromosomes are compared?
9. The human genome contains about 50,000 fewer genes than was predicted by many researchers. Why were these initial predictions so high?
10. Define pseudogene. What is the difference between a non-processed pseudogene and a processed pseudogene?
11. Define chromatin and nucleosome.
12. Name the core histones and the linker histone and describe how they are arranged in a nucleosome.
13. What are the two types of chromatin and how do they differ?
14. What is the linking number, Lk, for a relaxed, closed-circular DNA with 3675 base pairs?

Suggested Readings / References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell andMolecularBiology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genomes

UNIT-XIV

Classes of DNA, Virus and Bacterial genomes, Mitochondrial genome

Objective: In this unit we will learn about different classes of DNA and also learn about different kinds of giant chromosomes. We will also learn about organelle DNA such as mitochondrial DNA and also learn about viral and bacterial DNA.

Introduction: Nucleic acids were first isolated by Friedrich Miescher (1869) from pus cells. They were named nuclein. Hertwig (1884) proposed nuclein to be the carrier of hereditary traits. Because of their acidic nature they were named nucleinic acids and then nucleic acids (Altmann, 1899).

Fisher (1880s) discovered the presence of purine and pyrimidine bases in nucleic acids. Levene (1910) found deoxyribose nucleic acid to contain phosphoric acid as well as deoxyribose sugar. He characterised four types of nucleotides present in DNA. In 1950, Chargaff found that purine and pyrimidine content of DNA was equal. By this time W.T. Astbury had found through X-ray diffraction that DNA is a polynucleotide with nucleotides arranged perpendicular to the long axis of the molecule and separated from one another by a distance of 0.34 nm. In 1953, Wilkins and Franklin got very fine X-ray photographs of DNA. The photographs showed that DNA was a helix with a width of 2.0 nm. One turn of the helix was 3.4 nm with 10 layers of bases stacked in it. Watson and Crick (1953) worked out the first correct double helix model from the X-ray photographs of Wilkins and Franklin. Wilkins, Watson and Crick were awarded Nobel Prize for the same in 1962.

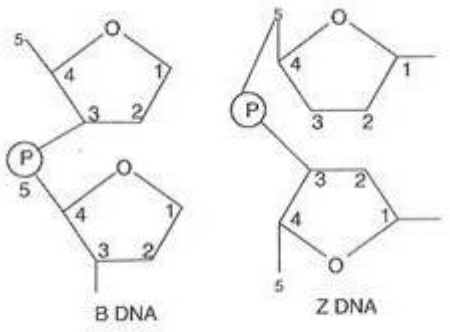
Watson and Crick (1953) built a 3D, molecular model of DNA that satisfied all the details obtained from X-ray photographs. They proposed that DNA consisted of a double helix with two chains having sugar phosphate on the outside and nitrogen bases on the inner side. The nitrogen bases of the two chains formed complementary pairs with purine of one and pyrimidine of the other held together by hydrogen bonds (A-T, C-G). Complementary base pairing between the two polynucleotide chains is considered to be hall mark of their proposition. It is of course based on early finding of Chargaff that A = T and C = G Their second big proposal was that the two chains are antiparallel with 5'→ 3′ orientation of one and У → 5'orientation of the other.

The two chains are twisted helically just as a rope ladder with rigid steps twisted into a spiral. Each turn of the spiral contains 10 nucleotides. This double helix or duplex model of DNA with antiparallel polynucleotide chains having complementary bases has an implicit mechanism of its replication and copying. Here both the polynucleotide chains function as templates forming two double helices, each with one parent chain and one new but complementary strand. The phenomenon is called semi conservative replication. In vitro synthesis of DNA has been carried out by Kornberg in 1959.

Types of DNA:

DNA duplex model proposed by Watson and Crick is right handed spiral and is called B-DNA (Balanced DNA). In the model the base pairs lie at nearly right angles to the axis of helix (Fig. 6.5 D). Another right handed duplex model is A-DNA (Alternate DNA). Here, a single turn of helix has 11 base pairs.

The base pairs lie 20° away from perpendicular to the axis. C-DNA has 9 base pairs per turn of spiral while in D-DNA the number is only 8 base pairs. Both are right handed. Z-DNA (Zigzag DNA) is left-handed double helix with zigzag back-bone, alternate purine and pyrimidine bases, single turn of 45 A length with 12 base pairs and a single groove. B-DNA is more hydrated and most frequently found DNA in living cells. It is physiologically and biologically active form. However, it can get changed into other forms. Right handed DNA is known to change temporarily into the left handed form at least for a short distance. Such changes may cause changes in gene expression.



Orientation of adjacent sugar molecules in B and Z DNA.

Circular and Linear DNA:

In many prokaryotes the two ends of a DNA duplex are covalently linked to form circular DNA. Circular DNA is naked, that is, without association with histone proteins, though polyamines do occur. In linear DNA the two ends are free. It is found in eukaryotic nuclei where it is associated with histone proteins. Linear DNA, without association with histone proteins, also occurs in some prokaryotes, e.g., Myco-plasma. In semi-autonomous cell organelles (mitochondria, plastids) DNA is circular, less commonly linear. It is always naked.

Chargaff's Rules:

Chargaff (1950) made observations on the bases and other components of DNA. These observations or generalizations are called Chargaff's base equivalence rule.

(i) Purine and pyrimidine base pairs are in equal amount, that is, adenine + guanine = thymine + cytosine. [A + G] = [T + C], i.e., [A+G] / [T+C] = 1

(ii) Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equalled by molar concentration of cytosine.

[A] = [T], i.e., [A] / [T] = 1; [G] = [C], i.e., [G] / [C] = 1

(iii) Sugar deoxyribose and phosphate occur in equimolar proportions.

(iv) A-T base pairs are rarely equal to C—G base pairs.

(v) The ratio of [A+T] / [G+C] is variable but constant for a species (Table 6.2). It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

Table 6.2. Base Composition of DNA from Various Sources:

Species	A	G	C	T	A+T/C+G
1. Man	30.4	19.0	19.9	30.1	1.55
2. Calf	29.0	21.2	21.2	28.5	1.35
3. Wheat germ	28.1	21.8	22.7	27.4	1.25
4. Pea	30.8	19.2	18.5	30.5	1.62
5. Euglena	22.6	27.7	25.8	24.4	0.88
6 Escherichia coli	24.7	26.0	25.7	23.6	0.93

Structure of DNA:

DNA or deoxyribonucleic acid is a helically twisted double chain polydeoxyribonucleotide macromolecule which constitutes the genetic material of all organisms with the exception of rhinoviruses. In prokaryotes it occurs in nucleoid and plasmids. This DNA is usually circular. In eukaryotes, most of the DNA is found in chromatin of nucleus. It is linear. Smaller quantities of circular, double stranded DNA are found in mitochondria and plastids (organelle DNA). Small sized DNAs occur in viruses, ϕ x 174 bacteriophage has 5386 nucleotides. Bacteriophage lambda (Phage X) possesses 48502 base pairs (bp) while number of base pairs in Escherichia coli is 4.6×10^6. A single genome (haploid set of 23 chromosomes) has about 3.3×10^9 bp in human beings. Single-stranded DNA occurs as a genetic material in some viruses (e.g., phage ϕ x 174, coliphage fd, M_{13}). DNA is the largest macromolecule with a diameter of 2 nm (20Å or 2×10^{-9}m) and often having 3 length in millimetres.

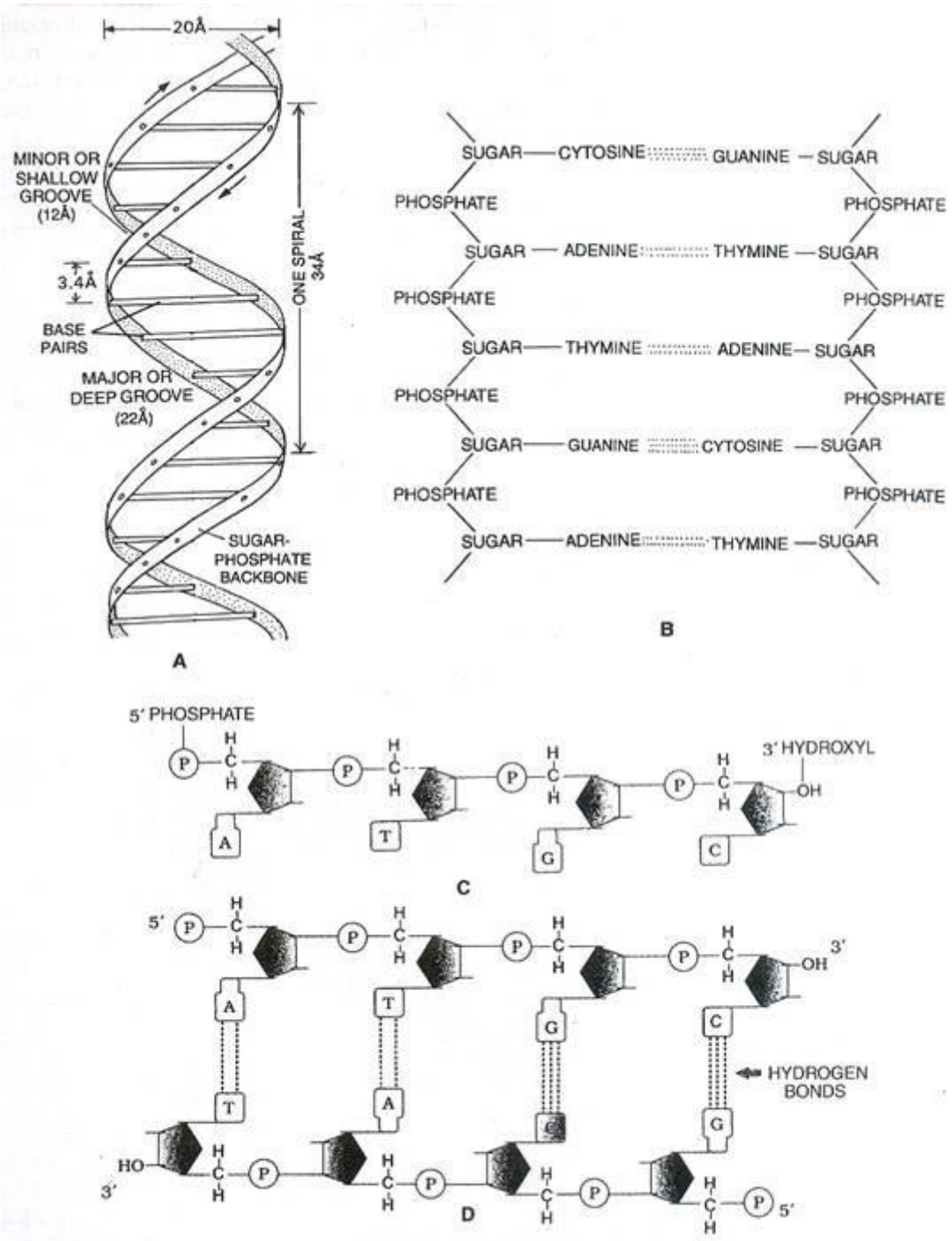


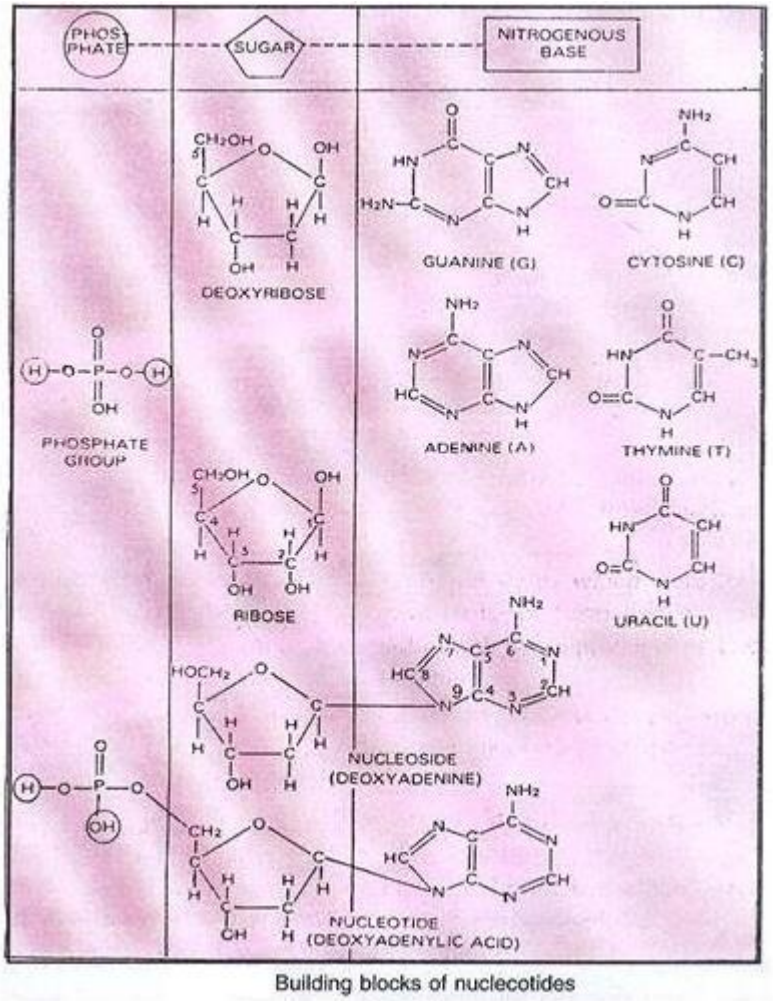
Fig. 6.5. Structure of DNA. A, coiling in double helix or duplex of DNA. B, sequence of nucleotides in a part of the double helix of DNA. C, a single stranded polynucleotide chain. D, a double stranded polynucleotide chain.

It 1s negatively charged due to phosphate groups. It is a long chain polymer of generally several hundred thousand of deoxyribonucleotides. A DNA molecule has two un-branched complementary strands. They are spirally coiled. The two spiral strands of DNA are collectively called DNA duplex (Fig. 6.5).

The two strands are not coiled upon each other but the whole double strand (DNA duplex) is coiled upon itself around a common axis like a rope stair case with solid steps twisted into a spiral. Due to spiral twisting, the DNA duplex comes to have two types of alternate grooves, major (22 Å) and minor (12 Å).

In B-DNA, one turn of the spiral has about 10 nucleotides on each strand of DNA. It occupies a distance of about 3.4 nm (34 Å or 3.4×10^{-9} m) so that adjacent nucleotides or their bases are separated by a space of about 0.34 nm (0.34×10^{-9} m or 3.4 Å).

A deoxyribonucleotide of DNA is formed by cross-linking of three chemicals ortho- phosphoric acid (H_3PO_4), deoxyribose sugar ($C_5H_{10}O_4$) and a nitrogen base. Four types of nitrogen bases occur in DNA. They belong to two groups, purines (9-membered double rings with nitrogen at 1,3,7 and 9 positions) and pyrimidines (six membered rings with nitrogen at 1 and 3 positions). DNA has two types of purines (adenine or A and guanine or G) and two types of pyrimidines (cytosine or C and thymine or T).



Building blocks of nuclecotides

Depending upon the type of nitrogen base, DNA has four kinds of deoxyribonucleotides —deoxy adenosine 5- monophosphate (d AMP), deoxy guaninosine 5-monophosphate (d GMP), deoxy thymidine 5-monophosphate (d TMP) and deoxy cytidine 5- monophosphate (d CMP). The back bone of a DNA chain or strand is built up of alternate deoxyribose sugar and phosphoric acid groups. The phosphate group is connected to carbon 5′ of the sugar residue of its own nucleotide and carbon У of the sugar residue of

the next nucleotide by (3 '—5') phosphodiester bonds. -H of phosphate and -OH of sugar are eliminated as H_20 during each ester formation.

Phosphate group provides acidity to the nucleic acids because at least one of its side group is free to dissociate. Nitrogen bases lie at right angles to the longitudinal axis of DNA chains. They are attached to carbon atom 1 of the sugars by N-glycosidic bonds. Pyrimidine (C or T) is attached to deoxyribose by its N-atom at 1 position while a purine (A or G) does so by N-atom at 9 position. The two DNA chains are antiparallel that is, they run parallel but in opposite directions. In one chain the direction is 5'→ У while in the opposite one it is 3′ →5′ (Fig. 6.5). The two chains are held together by hydrogen bonds between their bases. Adenine (A), a purine of one chain lies exactly opposite thymine (T), a pyramidine of the other chain. Similarly, cytosine (C, a pyrimidine) lies opposite guanine (G a purine). This allows a sort of lock and key arrangement between large sized purine and small sized pyrimidine.

It is strengthened by the appearance of hydrogen bonds between the two. Three hydrogen bonds occur between cytosine and guanine (C = G) at positions 1'-1', 2′- 6′ and 6′-2′. There are two such hydrogen bonds between adenine and thymine (A=T) which are formed at positions 1'-3′ and 6′-4′. Hydrogen bonds occur between hydrogen of one base and oxygen or nitrogen of the other base. Since specific and different nitrogen bases occur on the two DNA chains, the latter are complementary. Thus the sequence of say AAGCTCAG of one chain would have a complementary sequence of TTCGAGTC on the other chain. In other words, the two DNA chains are not identical but complementary to each other. It is because of specific base pairing with a purine lying opposite a pyrimidine. This makes the two chains 2 nm thick. A purine- purine base pair will make it thicker while a pyrimidine- pyrimidine base pair will make it narrower than 2 nm. Further, A and C or G and T do not pair because they fail to form hydrogen bonds between them. 5′ end of each chain bears phosphate radical while the 3′ end possesses a sugar residue (3'-OH).

Different classes of DNA:

The most common form of DNA which has right handed helix and proposed by Watson and Crick is called B-form of DNA or B-DNA. In addition, the DNA may be able to exist in other forms of double helical structure. These are A and C forms of double helix which vary from B- form in spacing between nucleotides and number of nucleotides per turn, rotation per base pair, vertical rise per base pair and helical diameter (Table 5.3).

Table 5.3 : Forms of double stranded DNA helix

Parameters	Forms of DNA				
	A	B	C	D	Z
Conditions	75% rel. Humidity, Na^+ K^+, Cs^+ ions	92% rel. humidity low ions	66% rel. humidity Li^+ ions	—	low high salt conc.
Base pair per turn	11	10	9.33	8	12 (6 dimers)
Rotation per bp	+32.7°	+36.0°	+38.6°	—	-30.0°
Vertical rise per bp	2.56Å	3.38Å	3.32Å	3.03Å	3.71Å
Helical diameter	23Å	20Å	19Å	—	18Å
Pitch of the helix	28.15Å	34Å	31Å	—	45 Å
Tilt of bp Sugar puckering	20.2Å	6.3°	-7.8°	-16.7°	7°

1. The B-Form of DNA (B-DNA):

Structure of B-form of DNA has been proposed by Watson and Crick. It is present in every cell at a very high relative humidity (92%) and low concentration of ions. It has antiparallel double helix, rotating clockwise (right hand) and made up of sugar- phosphate back bone combined with base pairs or purine-pyrimidine. The base pairs are perpendicular to longitudinal axis of the helix. The base pairs tilt to helix by 6.3°. The B-form of DNA is metabolically stable and undergo changes to A, C or D forms depending on sequence of nucleotides and concentration of excess salts.

2. The A-Form of DNA (A-DNA):

The A-form of DNA is found at 75% relative humidity in the presence of Na+, K+ or Cs+ ions. It contains eleven base pairs as compared to ten base pairs of B-DNA which tilt from the axis of helix by 20.2°. Due to this displacement the depth of major groove increases and that of minor groove decreases. The A-form is metastable and quickly turns to the D-form.

3. The C-Form DNA (C-DNA):

The C-form of DNA is found at 66% relative humidity in the presence of lithium (Lit+) ions. As compared to A-and B-DNA, in C-DNA the number of base pairs per turn is less i.e. 28/3 or 9 1/3. The base pairs show pronounced negative tilt by 7.8°.

4. The D-Form of DNA (D-DNA):

The D-form of DNA is found rarely as extreme variants. Total number of base pairs per turn of helix is eight. Therefore, it shows eight-fold symmetry. This form is also called poly (dA-dT) and poly (dG-dC) form. There is pronounced negative tilt of base pairs by 16.7° as compared to C form i.e. the base pairs are displaced backwardly with respect to the axis of DNA helix.

5. The Z-Form of DNA (Z-DNA) or Left Handed DNA:

In 1979, Rich and coworkers at MIT (U.S.A.) obtained Z-DNA by artificially synthesizing d (C-G) 3 molecules in the form of crystals. They proposed a left handed (synistral) double helix model with zig-zag

sugar-phosphate back bone running in antiparallel direction. Therefore, this DNA has been termed as Z-DNA. The Z-DNA has been found in a large number of living organisms including mammals, protozoans and several plant species.

There are several similarities with B-DNA in having:

(i) Double helix,

(ii) Two antiparallel strands, and

(iii) Three hydrogen bonds between G-C pairing.

In addition, the Z-DNA differs from the B-DNA in the following ways:

(a) The Z-DNA has left handed helix, while the B-DNA has right handed helix.

(b) The Z-DNA contains zig-zag sugar phosphate back bone as compared to regular back bone of the B-DNA.

(c) The repeating unit in Z-DNA is a dinucleotide due to alternating orientation of sugar residues, whereas in B-DNA the repeating unit is a mononucleotide, and sugar molecules do not have the alternating orientation.

(d) In the Z-DNA one complete turn contains 12 base pairs of six repeating dinucleotide, while in B-DNA one full turn consists of 10 base pairs i.e. the 10 repeating units.

(e) Due to the presence of high number (12) of base pairs in one turn of Z-DNA, the angle of twist per repeating unit i.e. dinucleotide is 60° as compared to 36° of B-DNA molecule.

(f) In Z-DNA the distance of twist making one turn of 360° is 45Å as against 34Å in B-DNA.

(g) The Z-DNA has fewer diameters (18Å) as compared to the B-DNA (20Å diameter).

6. Single Stranded (ss) DNA:

Almost all the organisms contain double stranded DNA except a few viruses such as bacteriophage φ × 174 which consists of single stranded circular DNA. It becomes double stranded only at the time of replication.

The differences of ssDNA from the dsDNA are as below:

(a) The dsDNA absorbs wavelength 2600 Å of ultra violet light constantly from 0 to 80°C, thereafter rise sharply, whereas in ssDNA absorption of UV light increases steadily from 20° to 90°C.

(b) The dsDNA resists the action of formaline due to closed reactive site, while the ss DNA does not resist it due to exposed reactive sites.

(c) Base pair composition in dsDNA is equal i.e. A=T and G=C, in ssDNA the composition of A, T, G, C is in proportion of 1:1.33:0.98:0.75.

(d) The dsDNA always remains in linear helical form, while the ssDNA remains in circular form; however, it becomes double stranded only during replication (i.e. replicative form).

7. Circular and Super Helical DNA:

Almost in all the prokaryotes and a few viruses, the DNA is organised in the form of closed circle. The two ends of the double helix get covalently sealed to form a closed circle. Thus, a closed circle contains two unbroken complementary strands. Sometimes one or more nicks or breaks may be present on one or both strands, for example DNA of phage PM2 (Fig. 5.7 A).

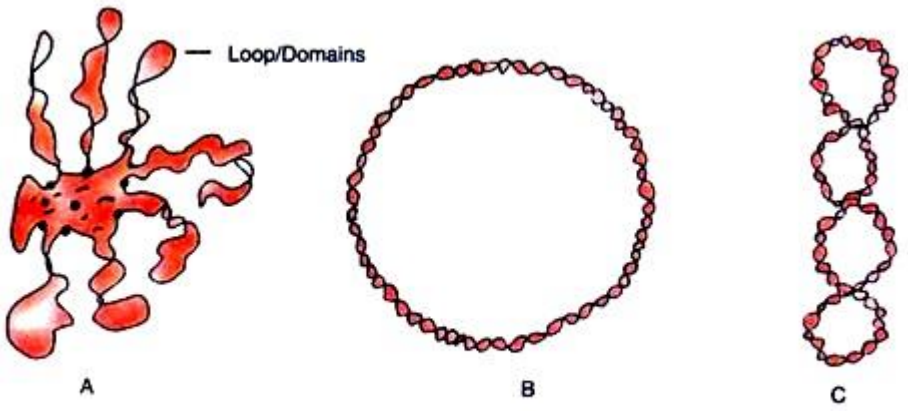


Fig. 5.7 : The forms of DNA. A, Nucleoids of *E.coli*; B, a closed, circular bacterial DNA; C, twisted supercoils of double stranded DNA.

Besides some exceptions, the covalently closed circles are twisted into super helix or super coils (Fig.5.7 B) and is associated with basic proteins but not with histones found complexed with all eukaryotic DNA.

This histone like proteins appear to help the organization of bacterial DNA into a coiled chromatin structure with the result of nucleosome like structure, folding and super coiling of DNA, and association of DNA polymerase with nucleoids. Several histones like DNA binding proteins have been described in bacteria (Table 5.4). These nucleoid-associated proteins include HU proteins, IHF, protein H1, Fir A, H-NS and Fis. In archaeobacteria (e.g. Archaea) the chromosomal DNA exists in protein-associated form. Histone like proteins has been isolated from nucleoprotein complexes in Thermoplasma acidophilurn and Halobacterium salinanim.

Thus, the protein associated DNA and nucleosome like structures are detected in a variety of bacteria. If the helix coils clockwise from the axis the coiling is termed as positive or right handed coiling. In contrast, if the path of coiling is anticlockwise, the coil is called left handed or negative coil.

Table 5.4 : Histone-like proteins of E. coli

Name	*Subunit*	*Molecular Weight*	*Function*
Hu (hupA)	Hu(α)[HLPIIa]	9,000	Can form nucleosome like structure like H_2B
(hupB)	Hu(β)[HLPIIb] linked as heterogenous dimer	9,000	—
HLP I	—	17,000	Affects RNA polymerase transcription
H1(hns)	—	15,000	May modulate in vivo transcription
H	—	28,000	Similar to eucaryotic H_2A

The two ends of a linear DNA helix can be joined to form each strand continuously. However, if one of ends rotates at 360° with respect to the other to produce some unwinding of the double helix, the ends are joined resulting in formation of a twisted circle in opposite sense i.e. opposite to unwinding direction.

Such twisted circle appears as 8 i.e. it has one node or crossing over point. If it is twisted at 720° before joining, the resulting super helix will contain two nodes (Fig. 5.7B).

The enzyme topoisomerases alter the topological form i.e. super coiling of a circular DNA molecule. Type I topoisomerases (e.g. E.coli Top A) relax the negatively super coiled DNA by breaking one of the phosphodiester bonds in dsDNA allowing the 3′-OH end to swivel around the 5′-phosphoryl end, and then resealing the nicked phosphodiester backbone. Type II topoisomerases need energy to unwind the DNA molecules resulting in the introduction of super coils. One of type II isomerases, the DNA gyrase, is apparently responsible for the negatively super coiled state of the bacterial chromosome. Super coiling is essential for efficient replication and transcription of prokaryotic DNA. The bacterial chromosome is believed to contain about 50 negatively super coiled loops or domains. Each domain represents a separate topological unit, the boundaries of which may be defined by the sites on DNA that limit its rotation.

Salient Features of B model of DNA of Watson and Crick:

1. DNA is the largest biomolecule in the cell.

2. DNA is negatively charged and dextrorotatory.

3. Molecular configuration of DNA is 3D.

4. DNA has two polynucleotide chains.

5. The two chains of DNA have antiparallel polarity, 5′ —> 3′ in one and 3′ —> 5′ in other.

6. Backbone of each polynucleotide chain is made of alternate sugar-phosphate groups. The nitrogen bases project inwardly.

7. Nitrogen bases of two polynucleotide chains form complementary pairs, A opposite T and C opposite G.

8. A large sized purine always comes opposite a small sized pyrimidine. This generates uniform distance between two strands of helix.

9. Adenine (A) of one polynucleotide chain is held to thymine (T) of opposite chain by two hydrogen bonds. Cytosine (C) of one chain is similarly held to guanine of the other chain by three hydrogen bonds.

10. The double chain is coiled in a helical fashion. The coiling is right handed. This coiling produces minor and major grooves alternately.

11. The pitch of helix is 3.4 nm (34 A) with roughly 10 base pairs in each turn. The average distance between two adjacent base pairs comes to about 0.34 nm (0.34×10^{-9} m or 3.4 A).
12. Planes of adjacent base pairs are stacked over one another. Alongwith hydrogen bonding, the stacking confers stability to the helical structure.

13. DNA is acidic. For its compaction, it requires basic (histone) proteins. The histone proteins are +vely charged and occupy the major grooves of DNA at an angle of 30° to helix axis.

Sense and Antisense Strands:

Both the strands of DNA do not take part in controlling heredity and metabolism. Only one of them does so. The DNA strand which functions as template for RNA synthesis is known as template strand, minus (-) strand or antisense strand.

Its complementary strand is named nontemplate strand, plus (+) strand, sense and coding strand. The latter name is given because by convention DNA genetic code is written according to its sequence.

(5’) GCATTCGGCTAGTAAC (3′)

DNA Nontemplate, Sense (+) or Coding Strand

(3′) CGTAAGCCGATCATTG (5′)

DNA Template, Antisense, or Noncoding or (-) Strand

(5′) GCAUUCGGCUAGUAAC (3′)

RNA Transcript

RNA is transcribed on 3'→5′ (-) strand (template/antistrand) of DNA in 5 → 3 direction.

The term antisense is also used in wider prospective for any sequence or strand of DNA (or RNA) which is complementary to mRNA.

Denaturation and Renaturation:

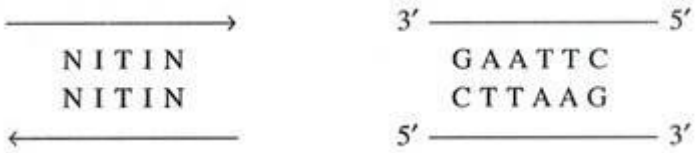
The H-bonds between nitrogen bases of two strands of DNA can break due to high temperature (82-90°C) or low or high pH, so that the two strands separate from each other. It is called denaturation or melting. Since A-T base pair has only 2H bonds, the area rich in A-T base pairs can undergo easy denaturation (melting). These areas are called low melting areas because they denature at comparatively low temperature. The area rich in G- C base pairs (called high melting area) is comparatively more stable and dense because three hydrogen bonds connect the G-C bases.

These areas have high temperature of melting (Tm). On melting the viscosity of DNA decreases. The denatured DNA has the tendency to reassociate, i.e., the DNA strands separated by melting at 82-90°C can reassociate and form duplex on cooling to temperature at 65°C. It is called renaturation or annealing.

Denatured or separated DNA strands absorb more light energy than the intact DNA double strand. The increased absorption of light energy by separated or denatured DNA strands is called hyperchromatic effect. The effect is used in knowing whether DNA is single or double stranded.

Palindromic DNA:

DNA duplex possesses areas where sequence of nucleotides is the same but opposite in the two strands. These sequences are recognised by restriction endonucleases and are used in genetic engineering. Given hereunder sequence of bases in one strand (3′ → 5′) is GAATTC. It is same in other strand when read in 5′ → 3′ direction. It is similar to palindrome words having same words in both forward and backward direction, e.g., NITIN, MALAYALAM.



Repetitive DNA:

It is the DNA having multiple copies of identical sequences of nitrogen bases. The number of copies of the same base sequence varies from a few to millions. DNA having single copy of base sequences is called unique DNA. It is made of functional genes. rRNA genes are, however, repeated several times. Repetitive DNA may occur in tandem or interspersed with unique sequences.

It is of two types, highly repetitive and moderately repetitive. Highly repetitive DNA consists of short sequences of less than 10 base pairs which are repeated millions of times. They occur in preeentromeric

regions, heterochromatic regions of Y-chromosomes and satellite regions. Moderately repetitive dna consists of a few hundred base pairs repeated at least 1000 times. It occurs in telomeres, centromeres and transposons. Tandemly repeated sequences are especially liable to undergo misalignments during chromosome pairing, and thus the size of tandem clusters tends to be highly polymorphic, with wide variations between individuals. Smaller clusters of such sequences can be used to characterize individual genomes in the technique of "DNA-finger-printing".

Satellite DNA:

It is that part of repetitive DNA which has long repetitive nucleotide sequences in tandem that forms a separate fraction on density ultracentrifugation. Depending upon the number of base pairs involved in repeat regions, satellite DNA is of two types, microsatellite sequences (1-6 bp repeat units flanked by conserved sequences) and minisatellite sequences (11-60 bp flanked by conserved restriction sites). The latter are hyper variable and are specific for each individual. They are being used for DNA matching or finger printing as first found out by Jeffreys et al (1985).

Genetic Information:

The arrangement of nitrogen bases of DNA (and its product mRNA) determines the sequence of amino acid groups in polypeptides or proteins formed over ribosomes. One amino acid is specified by the sequence of three adjacent nitrogen bases. The latter is called codon. The segment of DNA which determines the synthesis of complete polypeptide is known as cistron.

In procaryotes, a cistron has a continuous coding sequence from beginning to end. In eucaryotes a cistron contains noncoding regions which do not produce part of gene product. They are called introns. Introns are often variable. The coding parts are known as exons. Cistrons having introns are called split genes.

Coding and Noncoding DNA:

Depending on the ability to form functional or non-functional products, DNA has two types of segments, coding and noncoding. In eukaryotes a greater part of DNA is noncoding since it does not form any functional product. They often possess repeated sequences or repetitive DNA. Most of them have fixed positions. Some can move from one place to another. The mobile sequences are called jumping genes or transposons. In prokaryotes the amount of noncoding or non-functional DNA is small. Coding DNA consists of coding DNA sequences. These are of 2 types — protein coding sequences coding for all proteins except histone and nonprotein coding sequences for tRNA, rRNA and histones.

Functions of DNA:

1. Genetic Information (Genetic Blue Print):

DNA is the genetic material which carries all the hereditary information. The genetic information is coded in the arrangement of its nitrogen bases.

2. Replication:

DNA has unique property of replication or production of carbon copies (Autocatalytic function). This is essential for transfer of genetic information from one cell to its daughters and from one generation to the next.

3. Chromosomes:

DNA occurs inside chromosomes. This is essential for equitable distribution of DNA during cell division.

4. Recombination:

During meiosis, crossing over gives rise to new combination of genes called recombinations.

5. Mutations:

Changes in sequence of nitrogen bases due to addition, deletion or wrong replication give rise to mutations. Mutations are the fountain head of all variations and evolution.

6. Transcription:

DNA gives rise to RNAs through the process of transcription. It is heterocatalytic activity of DNA.

7. Cellular Metabolism:

It controls the metabolic reactions of the cells through the help of specific RNAs, synthesis of specific proteins, enzymes and hormones.

8. Differentiation:

Due to differential functioning of some specific regions of DNA or genes, different parts of the organisms get differentiated in shape, size and functions.

9. Development:

DNA controls development of an organism through working of an internal genetic clock with or without the help of extrinsic information.

10. DNA Finger Printing:

Hypervariable microsatellite DNA sequences of each individual are distinct. They are used in identification of individuals and deciphering their relationships. The mechanism is called DNA finger printing.

11. Gene Therapy:

Defective heredity can be rectified by incorporating correct genes in place of defective ones.

12. Antisense Therapy:

Excess availability of anti-mRNA or antisense RNAs will not allow the pathogenic genes to express themselves. By this technique failure of angioplasty has been checked. A modification of this technique is RNA interference (RNAi).

Viral Genome:

Viruses are a special class of infectious agents that are so small that they can be viewed only under electron microscope. A complete **"viral particle"** or "virion consists of a block of genetic material (DNA or RNA) surrounded by a protein coat and, sometimes by an additional membranous envelope.

The viruses contain neither cytoplasm nor exhibit any growth or metabolic activity. But when their genetic material enters into a suitable host cell, virus-specific protein synthesis replication of the viral chromosome occurs; these processes utilize both cellular (of host) and viral enzymes.

On the basis of the host organisms, viruses are divided into three main groups:

(1) Animal viruses,

(2) Bacterial viruses and

(3) Plant viruses.

Morphological Features of Viruses:

The viral chromosome is enclosed within a protein shell called capsid. The viral chromosome and its protein coat together are called nucleocapsid. Viruses vary considerably in their morphological features (Table 5.4).

TABLE 5.4. Capsid and nucleic acid characteristics of viruses

Morphological group	Virus	Nucleic acid
Helical capsid		
Naked	Coliphage fd	Single-stranded DNA
	Many plant viruses *e.g.* TMV, beet yellow	Single-stranded RNA
*Enveloped**	Influenza	Single-stranded segmented RNA
Icosahedral capsid		
Naked	Adeno associated	Single-stranded DNA
	Coliphage ϕX174	Single-stranded DNA
	Polyoma	Double-stranded DNA
	Adenovirus	Double-stranded DNA
	Coliphage f_2	Single-stranded DNA
	Polio	Single-stranded DNA
	Many plant viruses, *e.g.*, turnip yellow	Single-stranded DNA
	Reovirus	Double-stranded segmented RNA
*Enveloped**	Herpes simplex	Double-stranded DNA
Some components **Helical** and some **Icosahedral** *(Naked)*	Large bacterio-phages, *e.g.*, T_2, T_4, T_6	Double-stranded DNA
Complex virions	Pox viruses, *e.g.*, Vaccinia, Variola-viruses	Double-stranded DNA

*Enveloped viruses are enclosed by a membrane of host origin.

1. Icosahedral virions:

Their capsid is icosahedral, i.e., the virion is a regular polyhedron with 20 triangular faces and 12 corners. Examples are, adenoviruses and bacteriophage φX174.

2. Helical virions:

The nucleic acid of such virions is enclosed in a cylindrical, rod shape capsid that forms a helical structure, e.g., TMV, bacteriophage M13.

3. In some cases, the nucleocapsid is icosahedral while in others, it is helical in some components. Such viruses are enveloped.

4. Complex virions:

These viruses do not have a clearly identifiable capsid. The viral nucleic acid is present in the centre of the shell which is made up of protein molecules. Some of the shells are complex while others are simple. In Herpes, an animal virus that contains DNA as genetic material, the capsid has a diameter of 1000A; it is further surrounded by an envelope making its diameter 1500A. (Fig. 5.19).

The capsid is mode up of protein subunits (capsomers) which form an icosahedron.

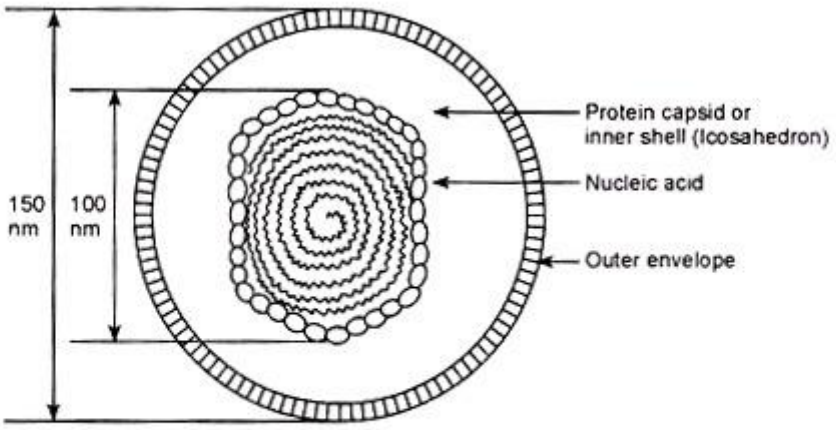


Fig. 5.19. Schematic representation of the general structure of ***Herpes*** virus. It contains DNA and multiplies in animals cells. DNA containing core is embedded in the icosahedral capsid which is made up of protein molecules called capsomers. The capsid is surrounded by an envelope.

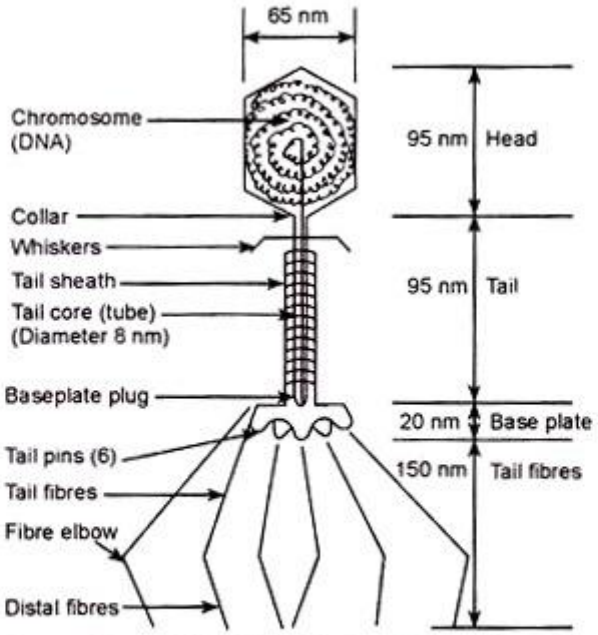


Fig. 5.20. Structure of a T-even phage (T2, T4, T6). The head (capsid) contains DNA that is about 170 kb in length. Fibres bind to specific sites on the host cell. DNA is injected into the cell though the tube.

Bacteriophages have relatively complex structures: they contain a head, a tail, a base plate and several tail fibres (Fig. 5.20). The head is hexagonal (lateral side) and contains the viral DNA. The tail has a core tube surrounded by a sheath. At the tail end, there is a basal plate with 6 spikes from which 6 tail fibres emerge.

At the time of infection, the tail fibres bind to specific receptor sites on the host cell. The base plate is drawn to the cell surface and contraction of tube sheath occurs along with the removal of the base plate plug. The core of the tail penetrates the cell wall which is weakened by some hydrolytic enzymes present in the phage and the viral tail. DNA enters into the host cell through the core tube of the tail.

In the case of tobacco mosaic virus (TMV; multiplying in tobacco plant cells) and some small bacterial viruses (e.g., F2, R17, QB), the protein coat contains a single type of protein. These protein molecules are arranged in either a helical symmetry or a cubical symmetry. The shell of TMV contains about 2150 protein molecules which are identical, each molecule having the molecular weight of-17,000. These molecules are helically arranged around the RNA genome which contains 6,000 nucleotides.

Lytic Viruses:

The viruses which lyse or disrupt the host cell following infection are called lytic viruses. During infection, the nucleic acid is injected into the host cell. The enzymes required for viral DNA replication are then synthesized so that replication of DNA occurs to produce numerous copies of the viral chromosome.

The protein components of the capsid are synthesized in the later stages leading to the formation of heads and tails; the viral DNA is then packed into the heads. In the end, the cell wall ruptures and the progeny phage particles are released (Fig. 5.21).

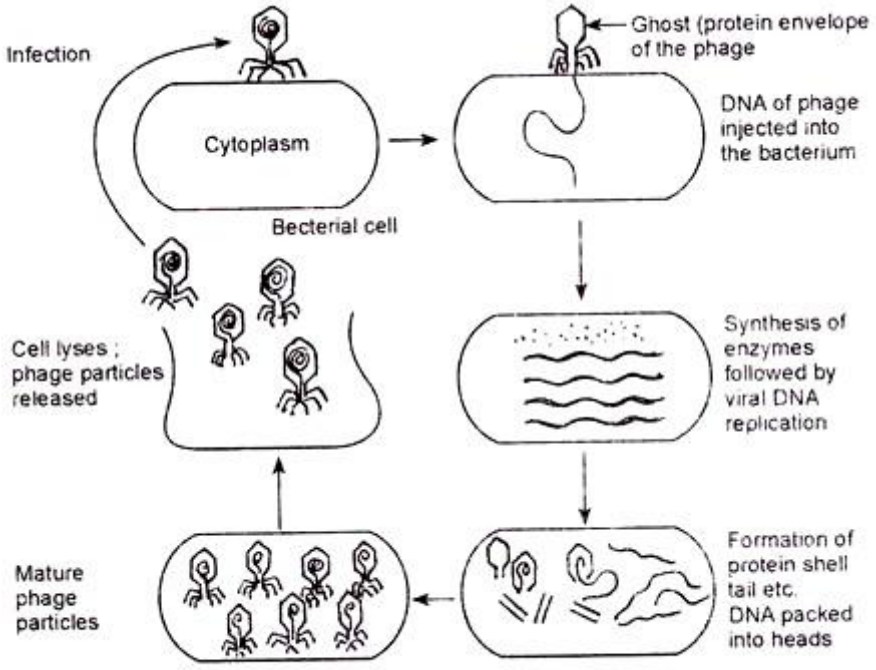


Fig. 5.21. Life cycle of a lytic bacteriophage.

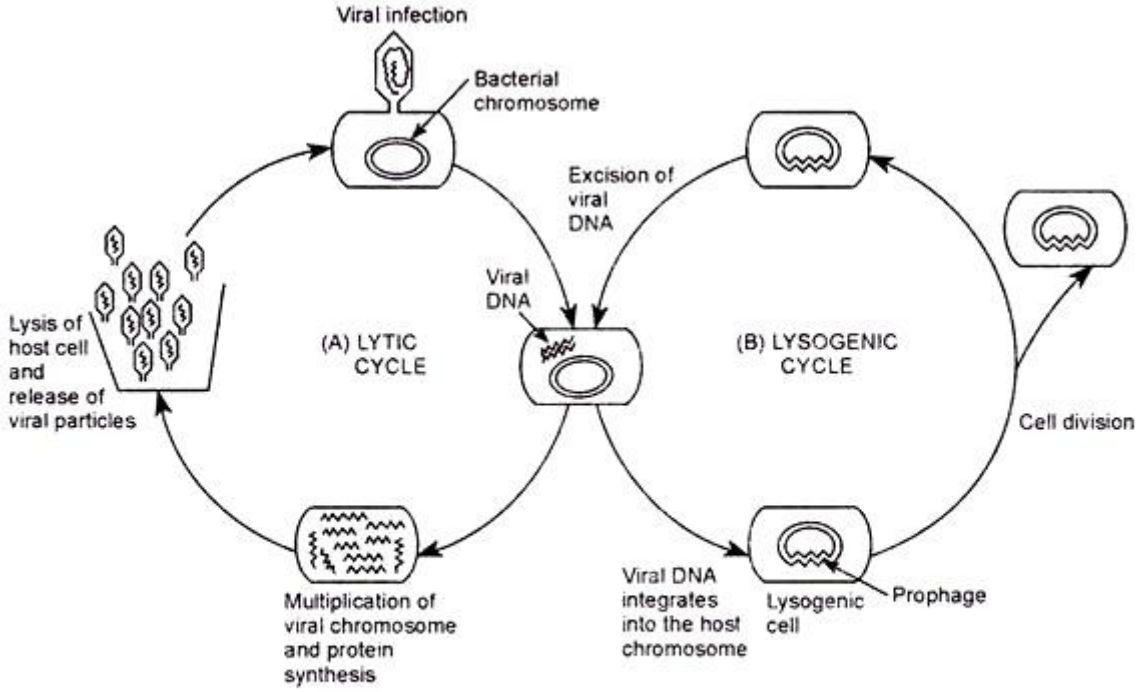


Fig. 5.22. Life cycle of temperate phages **A.** The virulent state of such phages showing their multiplication and the resultant lysis of their host cell; this releases new viral particles. **B.** Viral chromosome integrates into the host chromosome (lysogenic state) and becomes prophage (provirus). After induction, the viral chromosome is excised from the host chromosome and becomes lytic.

Lysogenic Viruses (Temperate Phages):

Lysogeny involves a symbiotic relationship between a temperate phage and its bacterial host. The viral chromosome becomes inserted into the bacterial chromosome, where it remains and replicates along with the latter. The viral DNA integrated into the bacterial genome is called a provirus or prophage (Fig. 5.22). The bacterium containing a prophage is immune to the infection by the same virus.

Viral Chromosomes:

Viruses contain either DNA or RNA as their genetic material. These nucleic acids may be either single or double-stranded (Table 5.5). Small viruses may contain 3 kb (kb =,kilo-bases = 1000 bases), while large viruses could have about 300 kb. in their genome. Thus the number of genes in viral genome may vary from only 3 to hundreds. The retroviruses arc diploid (have two copies of the genome per capsid), while the others are haploid.

a. Double-stranded DNA:

Several viruses possess double-stranded DNA as their genetic material. The base composition of different viruses is modified leading to change in the physical properties of DNA such melting temperature, buoyant density in caesium chloride (CsCl) etc.:

In some of the viruses, such as. T-even coliphages, cytosine (C) is modified into 5-hydroxymethyl-cytosine (HMC). In certain cases, thymine is converted into 5-hydroxy-methyl uracil or 5-di-

hydroxymethyluracil, e.g., in B. subtilis bacteriophges. Certain physical properties of DNA, such as, buoyant density in CsCl or melting temperature are changed due to these substitutions.

TABLE 5.5. Viral nucleic acids and their characteristics

Virus	Nucleic acid	Strands* (DS/SS)	Polarity (+) or (–)	Kiloba-ses/strand	Mol. wt. (10^6 daltons)	Number of segments
Vaccinia virus (a pox virus)	DNA	DS		242	160	
B. subtilis phage SP8	DNA	DS		197	130	
Coliphage T2, T6	DNA	DS		167	110	
Coliphage T4	DNA	DS		170		
Herpes simplex	DNA	DS		151	100	
Coliphage T5	DNA	DS		117	77	
Coliphage λ	DNA	DS		47	31	
Coliphage T7	DNA	DS		38	25	
Adenovirus Type 2	DNA	DS		35	23	
Polyoma virus	DNA	DS (circular)		5	3.5	
Coliphage M 13	DNA	SS (circular)		7.3	2.4	
Coliphage ϕ × 174	DNA	SS (circular)		5.2	1.7	
Phage fd	DNA	SS		6		
Rice dwarf virus	RNA	DS		23	15**	10
Reovirus	RNA	DS		23	15**	10
Influenza virus (a myxovirus)	RNA	SS	(–)	18	6**	8
Rous sarcoma virus (a retrovirus) #	RNA	SS	(+)	10.5	3.5	
Bunya virus	RNA	SS	(–)	9	3**	3
Polio. virus (a picorna virus)	RNA	SS	(+)	7.5	2.5	
Turnip yellow mosaic	RNA	SS	(+)	6	2	
Coliphage R 17	RNA	SS	(+)	4	1.3	
TMV	RNA	SS	(+)	6.4	2	

Some of the viruses contain linear DNA, while others contain circular (cyclic) DNA (Table 5.5). In the case of phage lambda (λ), DNA can exist in both linear and cyclic forms. When isolated from a viral particle, the λ DNA is linear, but when it enters into the host cell, becomes circular. However, it enters into the host cell in its linear form.

The A. chromosome is a double- stranded DNA molecule containing 47,000 nucleotides; it is 17 pm in length. There is single- stranded projection of 12 nucleotides at each 5′-end; these projections are complementary to each other and thus they are called cohesive ends.

These cohesive ends are responsible for the circularization of the chromosome. Circularization of the chromosome protects it from degradation by the host exonucleases. Further, the linear DNA cannot replicate vegetatively; the circularity therefore, provides an advantage in replication as well.

b. Single-stranded DNA:

Single-stranded DNA occurs in very small bacteriophages (Table 5.4). The single-stranded DNA found in the virion is called the positive (+) strand; as a rule only the plus (+) strand is found in the phage particles. However, in adeno-associated viruses, two complementary strands exist in different virions. The single-stranded DNA contains inverted repeating sequences that form hair pins. The hairpin structures have important role in circularization of the linear strands and in replication.

c. Double-stranded RNA:

Double-stranded RNAs are found in several icosahedral viruses of animals and plants. The genomes of such viruses are segmented (Table 5.5). The different segments may be connected short stretches of base pairs. Transcription of each segment occurs separately and the enzyme involved is "Double-stranded RNA transcriptase". Each mRNA, on translation produces a separate polypeptide chain.

d. Single-stranded RNA:

Single-stranded RNA is the genetic material in a number of viruses (Table 5.5). Some viruses contain a single RNA molecule in their genome, while some other viruses contain several segments, e.g., influenza virus has 8 segments. The viruses contain either positive (+) or negative (-) strands of RNA in their capsids.

Positive (+) Strand:

The viral RNA strand that functions as mRNA in the host cell is called the plus (+) strand or positive strand. The RNA genomes of animal viruses have a cap at their 5′-end and a poly (A) sequence at the 3′-end. However, in Picornavirus RNA, there is a special sequence at the 5′-end to which a small protein is covalently attached. The RNA genomes of plant viruses possess a cap at the 5′-end but they do not contain the poly (A) at their 3′-ends; their 3′-end is similar to tRNA. Each retrovirus particle contains two copies of the (+) RNA strand representing its genome; these copies are held together near the 5′-end.

Negative (-) strand:

These RNAs do not contain a cap but terminate into a nucleoside triphosphate at their 5′-ends. These strands do not function as mRNA directly. Instead, they are transcribed by the enzyme "single-stranded RNA transcriptase" present in the virion, to produce the mRNA.

Packaging of Nucleic Acids in the Viruses:

Viral genome (DNA/RNA) is tightly packed into the protein shell (capsid). The density of the nucleic acid in the protein shell is higher than 500 mg/ml, which is much greater than the density of DNA in other

organisms. For example, density of DNA in bacterium is about 10 mg/ml, while in the eukaryotic nucleus, it is about 100 mg/ml. This shows that the nucleic acid is very tightly packaged in the viral particles.

The genetic material of TMV is single-stranded RNA containing 6400 nucleotides, making up a length of 2 pm. This RNA is packaged into the rod-shaped compartment of 0.3 x 0.008 pm. Adenoviruses contain 11 pm long double-stranded DNA consisting of 35,000 bp: this is packaged into an icosahedron type capsid of 0.07 pm diameter. Phage T4 has a very long double-stranded DNA molecule (55 pm) having 170,000 bp. The capsid containing this rather long DNA is an icosahedron with the dimensions of 1.0 x 0.065 pm. Unlike eukaryotic nucleus and bacterial nucleoid, the volume of the capsid is fully packaged with the nucleic acid. Packaging of nucleic acid to form a nucleocapsid occurs in two general ways. In one mechanism, the protein molecules assemble around the nucleic acid, e.g., in TMV. In the other mechanism, the protein coat is formed first and then the nucleic acid is inserted in it. In TMV, a duplex hairpin structure occurs in the RNA.

The assembly of protein monomers begins at this nucleation centre and proceeds in both the directions, reaching the ends. A total of 17 protein units form a circular layer and two such layers together form a unit of capsid. This structure interacts with the RNA which is coiled to form a helix inside the shell. In bacteriophage T4 and λ etc., the protein shell is formed first. The nucleic acid is inserted into the coat from one end and then the tail is joined to the head. In case of circular DNA, it must be first converted into a linear molecule for packaging. The lambda (λ) genome is circular and contains two “cos” sites, cosL and cosR. The free end in λ DNA is produced by enzymatic cleavage at the cosL site. Insertion of DNA occurs from this end and continues till the cosR site enters the capsid; a cleavage then occurs at the cosR site to produce the other end of the λ genome. Some of the viruses, e.g., phage T4 and λ. have terminal redundancy in their genomes. In these viruses, multiple genomes join end-to-end to produce **“concatemeric structure.”** In case of T4, insertion of the viral chromosome starts at a **“random”** point and continues until the required amount of DNA has been inserted into the head. The DNA inserted into the head has a terminal redundancy.

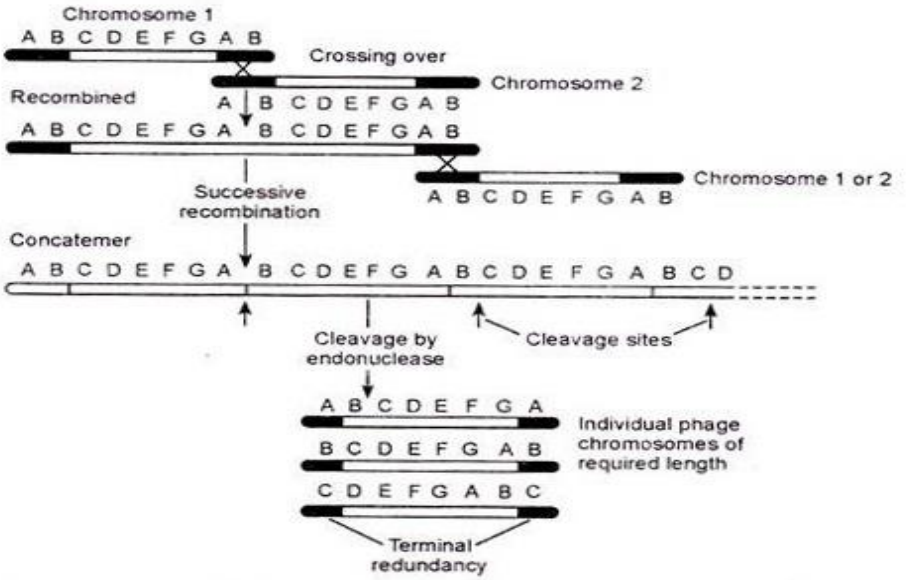


Fig. 5.23. Formation of a tandem series of phage genomes (concatemers) through successive recombination between individual genomes of normal length. Specific endonuclease cuts the concatemeric DNA into required size to be packaged into the capsid. The terminal redundancy may lead to heterozygosity.

One likely origin of the **"concatermeric"** DNA is recombination. Recombination between two chromosomes combines two genomes end-to-end. Then recombination with a third genome produces a concatemer through successive recombination's (Fig. 5.23). Another mechanism suggested for concatemer formation is the rolling circle replication. Specific endonuclease cuts the concatemer at the points that produce the genome of the "required length." The genomic DNA has homologous ends due to the terminal redundancy. Therefore, some chromosomes may be heterozygous for the terminal genes.

Organisation of DNA in bacteria.

The DNA of bacteria, e.g. E. coli, is a covalently closed circular molecule. It forms the bacterial chromosome, though this chromosome is much simpler in structure and in level of organization than the eukaryotic chromosomes of plants and animals. Also, each bacterial cell normally has a single chromosome containing a single circular DNA molecule.

In E.coli, the DNA molecule is 1,300 μm long when fully stretched containing some 4,700 x 10^3 base-pairs which encode about 4,000 genes. In order to pack this long DNA molecule into a cell measuring only about 1 μm x 3 μm, the molecule has to be highly folded and supercoiled. The prokaryotic chromosome — which is also called a nucleoid — consists of a number of loops which are held together by several proteins. For example, E. coli nucleoid has 45 (40-50) loops which radiate from a central protein core. Each loop is supercoiled (Fig. 9.8A). The supercoiled state of the loops of DNA can be removed by treatment with DNase which causes a single-stranded break (nick).

A single nick results in the uncoiling of a single loop without affecting the supercoiling of other loops (Fig. 9.8B). This shows that each loop is isolated from the other, although the ds-DNA molecule runs through all the loops. The association with proteins in the nucleoid core prevents unwinding of other

loops. The E. coli chromosome needs some 45 nicks to remove all the supercoiled loops, thereby producing a closed circular ring.

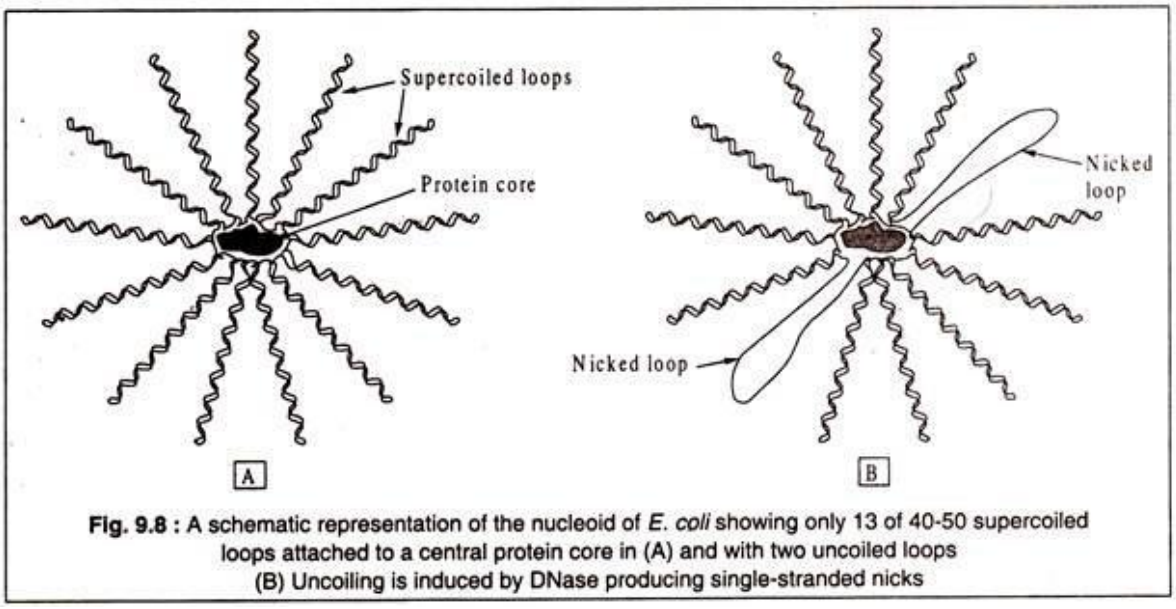


Fig. 9.8 : A schematic representation of the nucleoid of *E. coli* showing only 13 of 40-50 supercoiled loops attached to a central protein core in (A) and with two uncoiled loops (B) Uncoiling is induced by DNase producing single-stranded nicks

A circular DNA molecule without any supercoiling is said to be in a relaxed state. In this state, the standard right-handed DNA-double helix contains about 10 nucleotide pairs per turn of the helix. If now one of the two strands is nicked and rotated through 360° to unwind one complete turn of the helix and the cut-ends are resealed, the circular DNA molecule may respond in either of the two following ways—It may produce a region of unpaired bases, called a bubble; or, alternatively it may twist in a direction opposite to that of unwinding to produce a negatively supercoiled circular DNA molecule.

The three states of circular DNA — relaxed, with bubble and negatively supercoiled — are diagrammatically shown in Fig. 9.9:

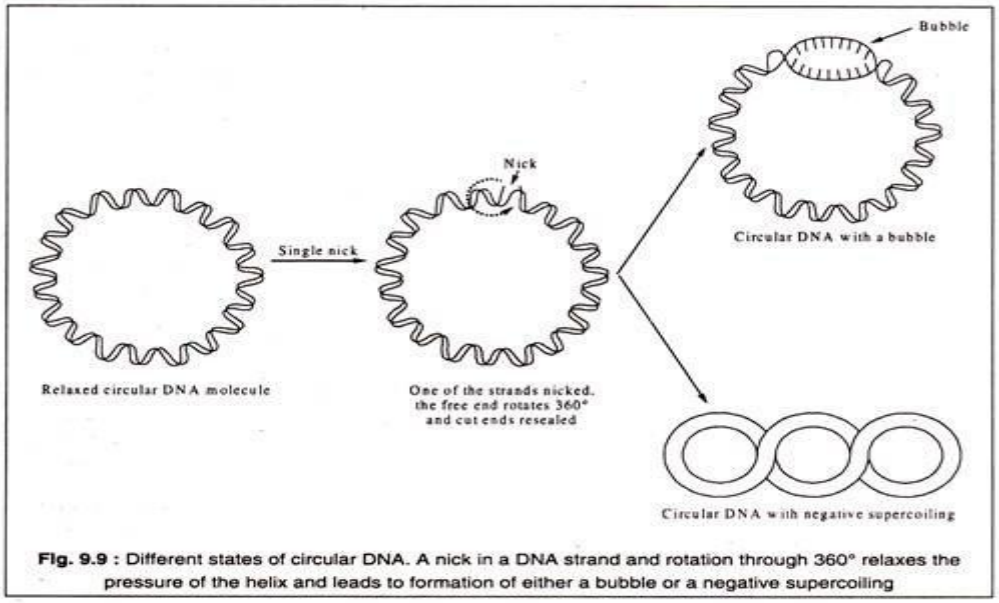


Fig. 9.9 : Different states of circular DNA. A nick in a DNA strand and rotation through 360° relaxes the pressure of the helix and leads to formation of either a bubble or a negative supercoiling

Negative supercoiling of ds-DNA is produced by a class of enzymes known as topoisomerases. The single stranded nick is generated by topoisomerase I producing a break or gap in the phosphodiester bond of the DNA strand. The intact complementary strand is passed through the gap and the nick is then resealed. Another class of topoisomerases, topoisomerase II, is also known as DNA- gyrase. These enzymes induce double-stranded break in the phosphodiester bonds of both strands. This class of enzymes plays a vital role in DNA replication. By inducing breaks in both strands, the enzyme helps to pass an intact double-stranded DNA molecule or a part to pass through another. Thus, when a circular DNA molecule replicates, the two daughter molecules may be interlocked like two rings of a chain. DNA gyrase can separate the two molecules by inducing a double-cut in one and allowing the other to pass through the gap which is then resealed. DNA gyrase has a more important function in normal DNA replication. With the advancement of the replication fork a positive supercoiling develops in the un-replicated portion of the ds-DNA helix. To compensate the tension, DNA gyrase introduces negative supercoiling by nicking.

Organelle Genome:

Introduction:

There are many exceptions to the rule in genetics. One of them is that not all inherited characters are determined by genes located in the nucleus. Few among them are controlled by genes located in cell organelles in the cytoplasm i.e. cytoplasmic genes, and these of course are exceptions to the chromosome theory of inheritance. Since they are extrachromosomal (i.e. outside the chromosomes), such genes are not subject to the normal rules of Mendelian heredity.

Extrachromosomal circular DNA (eccDNA) is ubiquitous in eukaryotic organisms, and has been noted for more than 3 decades. eccDNA occurs in normal tissues and in cultured cells, is heterogeneous in size, consists of chromosomal sequences and reflects plasticity of the genome. Recent findings indicate that this eccDNA can vary in size, sequence complexity, and copy number. However, the best characterized eccDNAs contain sequences homologous to chromosomal DNA. These findings may indicate that eccDNA may arise from genetic rearrangements, for example, from homologous recombination events. Elevated levels of eccDNA are now thought to correlate with genomic instability and exposure to carcinogens. In contrast to the human nuclear genome, which consists of 3.3 billion base pairs of DNA, the human mitochondrial genome is built of a mere 16,569 base pairs. Despite its small size, the mitochondrial genome can be used to establish maternal family ties, thanks to its maternal pattern of inheritance. Mutations in the mitochondrial genome have also been associated with diverse forms of human disease and aging. Extrachromosomal inheritance or cytoplasmic

inheritance is the transmission of genes that occur outside the nucleus or a form of non-Mendelian inheritance in which a trait is transmitted from the parent to offspring through non-chromosomnal cytoplasmic means. Mendelian inheritance patterns involve genes that directly influence the outcome of an organism's traits and obey Mendel's laws. Most genes in eukaryotic species follow a Mendelian pattern of inheritance however, there are many that don't and these genes are present in mitochondria and chloroplast.

Mitochondrial Genome:

Animal mitochondrial genomes are 13-18 kb in size.

Fungal mitochondrial genomes are ~75 kb.

Higher plant mitochondrial genomes are 300-500 kb.

Each mitochondrion has 5-20 copies of the mitochondrial chromosomes. Human cells have a range of numbers of mitochondria: Liver cells have 1000 mitochondria per cell, Skin cells have 100, Egg cells have up to 10 million.

Nuclear DNA is inherited from both parents. However, all the mitochondria for an individual are provided by the egg cell. It means that all mitochondrial DNA is inherited only from one's mother. Nuclear DNA resides in the membrane bound cell nucleus whereas mitochondrial DNA molecules are found in the mitochondria which are scattered throughout the cell cytoplasm. Nuclear DNA is organized into linear strands that make up the 23 pairs of chromosomes in the human genome. There are about 3.3 billion base pairs in the nuclear DNA of a cell. Buried in these linear strands are about 23,000 genes that code for proteins along with other sections that control which genes are expressed in each cell and when.

Mitochondrial DNA is circular in shape and contains only about 16,569 base pairs: There are only 37 genes and a "non-coding" region (also called the D loop) (Fig. 1)that does not code for any gene products (protein, various forms of RNA). Of the 37 genes, 22 code for transfer RNA, two code for ribosomal RNA and 13 code for proteins that are necessary for cellular energy production. The mitochondria are not totally autonomous. Most of the proteins necessary for their function are coded by nuclear DNA.

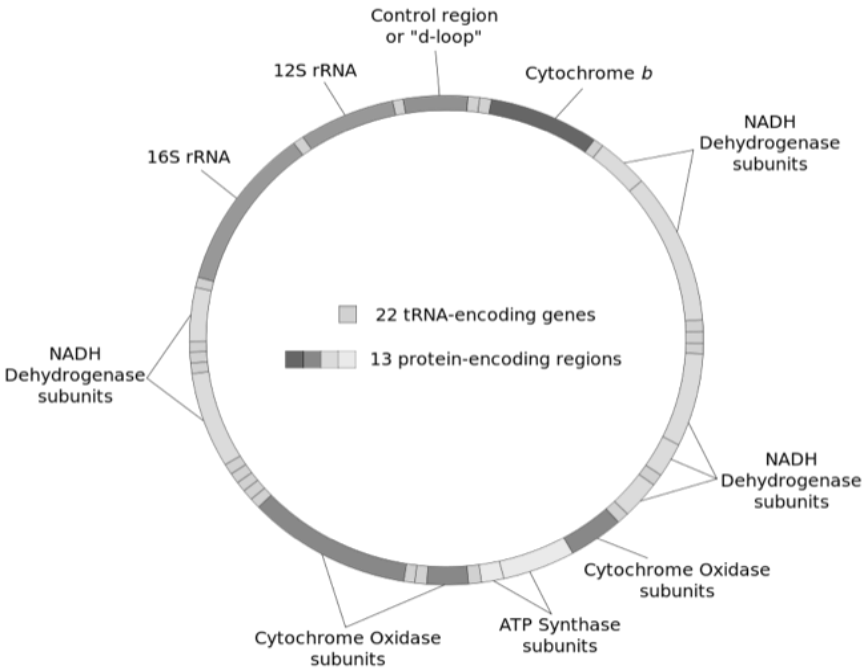


Figure. 1: Human mitochondrial DNA "chromosome" showing the arrangement of regions that code for proteins and RNA molecules. For genetic genealogy, note also the blue "control region" that does not code for any gene products.

Each cell contains only 23 pairs of chromosomal DNA. A cell may have tens to hundreds of mitochondria each with hundreds (or more) of DNA molecules. For example, the average human liver cell contains more than a thousand mitochondria each with hundreds of circular DNA molecules. The chromosomes that comprise nuclear DNA are paired. Each member of a chromosome pair carries the same genes and codes for the same traits (Differences in the nucleotide sequences of the genes are responsible for the variety of traits found in biological organisms). We say that paired chromosomes are diploid. Though mitochondria have multiple copies of their circular DNA molecules, they are not paired and we say mitochondria are haploid. When nuclear chromosomes are replicated during the process of creating new sex cells, cross-over or recombination between the members of the chromosome pairs insures variety and uniqueness to the new organism. No recombination occurs in mitochondrial DNA because there is no pairing. Mitochondrial DNA lacks the repair mechanisms and proofreading capabilities that insure the integrity of the nuclear DNA during replication. This leads to a mutation rate for mitochondria that is about 10 times higher than for nuclear DNA.Unlike nuclear DNA, mitochondrial DNA is densely packed with functional genes with no duplicates for backup in case one gene is disrupted by mutation. Mutations in these coding areas will result in mitochondria deficient in gene products (proteins mostly) necessary to their function.They will most likely be unable to reproduce and pass on their mutated genes. Consequently, despite the high mutation

rate, the functional part of the mitochondrial genomevaries slowly among individuals over time. In contrast, mutations in the control or non- coding region of mtDNA do no harm to the mitochondria nor do they affect the survival of the organism. They accumulate and are passed down through the maternal line. These mutations provide a source of variability that is useful in human identity testing and in investigating evolutionary and genealogical relationships.

The mitochondrial genetic code differs some from the code of nuclear DNA. For example, in mitochondria, the triplet codon AUA codes for the amino acid methionine whereas the same codon in nuclear DNA codes for isoleucine. Similarly the mitochondrial triplet UGA codes for tryptohphan and is NOT a STOP codon as it is in the nuclear DNA. Nuclear DNA has long stretches of non-coding DNA that is interspersed throughout the genome, sometimes even separates nucleotide sequences that code for a specific protein. Introns make up as much as 93% of nuclear DNA. Mitochondrial DNA has no introns. Nuclear DNA produces messengerRNA that codes for only one protein. If there are more than one polypetide chains coded for in a consecutive string of nuclear DNA nucleotides the resulting mRNA is cut apart into separate mRNA's before binding to a ribosome. Nuclear DNA is monocistronic meaning there is a separate strand of mRNA for each protein. Mitochondrial DNA has a scheme more like that of the eukaryotic cells responsible for its origin. If an uninterrupted segment of mitochondrial DNA that consecutively codes for say three proteins, is transcribed to mRNA, that mRNA is NOT cut into three different mRNA's. Rather, the whole mRNA proceeds to bind to a ribosome where it is directs the formation of all three proteins. In contrast to nuclear DNA, mRNA is polycistronic meaning one strand of mRNA may be responsible for the production of more than one type of mRNA.

Key Differences Between Nuclear DNA and Mitochondrial DNA	
Nuclear DNA	Mitochondrial DNA
Inherited from both parents	Inherited from mother only
Linear	Circular
3.2 billion base pairs	16,569 base pairs
20,000 genes	37 genes
23 pairs in each cell	Hundreds to thousands in each cell
Paired or diploid	Not paired or haploid
Varied by recombination	No recombination
Repair and proof reading mechanisms	No repair or proof reading mechanisms
Lower mutation rate	Higher mutation rate
Genetic code differs	Genetic code differs
Has introns	No introns
Monocistronic	Polycistronic

Table 1: Differences between Nuclear DNA and Mitochondrial DNA

Clinical Manifestations of Mitochondrial Mutations

Due to the maternal pattern of mitochondrial inheritance, males with a mitochondrial disease are not considered to be at risk for transmitting the disorder to their offspring. It's important to remember that there are many mitochondria within a cell, each with its own mtDNA and potential mutations. Thus, when discussing mitochondrial mutations, it is necessary to think of mutations present across the entire mitochondrial population rather than in a single mitochondrion. Although mitochondrial populations are considered heteroplasmic, with variations among the many mtDNA genomes, mothers can have mitochondrial populations that are homoplasmic for a given mitochondrial mutation; in this case, the majority of their mitochondrial genome would harbor the mutation. Homoplasmic mitochondrial mutations will be transmitted to all maternal offspring; however, due to the complex interplay between the mitochondrial and nuclear genomes, it is often difficult to predict disease outcomes, even with homoplasmic mitochondrial populations.

Classic Mitochondrial Syndromes:

A list of clinical disorders associated with mitochondrial mutations is provided in Table 2. One of these mitochondria-associated disorders is Leber hereditary optic neuropathy (LHON), which leads to a loss of vision in both eyes and is most commonly associated with a homoplasmic mitochondrial DNA mutation, although heteroplasmic transmission also occurs (Man et al., 2003). While all of the children of a homoplasmic mother will inherit the LHON mutation, not all will develop the disease; in fact, only 50% of the male offspring and 10% of female offspring will

suffer from optic nerve disease. These findings point to the likely involvement of other genes and environmental factors.

Adult

- Neurological: migraine | strokes | epilepsy | dementia | myopathy | peripheral neuropathy | DIPLOPIA | ATAXIA | speech disturbances | sensorineural deafness
- Gastrointestinal: constipation | irritable bowel | DYSPHAGIA
- Cardiac: heart failure | heart block | cardiomyopathy
- Respiratory: respiratory failure | nocturnal hypoventilation | recurrent aspiration | pneumonia
- Endocrinal: diabetes | thyroid disease | parathyroid disease | ovarian failure
- Ophthalmological: optic atrophy | cataract | ophthalmoplegia | PTOSIS

Paediatric

- Neurological: epilepsy | myopathy | psychomotor retardation | ataxia | spasticity | DYSTONIA | sensorineural deafness
- Gastrointestinal: vomiting | failure to thrive | dysphagia
- Cardiac: biventricular hypertrophic cardiomyopathy | rhythm abnormalities
- Respiratory: central hypoventilation | apnoea
- Haematological: anaemia | PANCYTOPAENIA
- Renal: renal tubular defects
- Liver: hepatic failure
- Endocrinal: diabetes | adrenal failure
- Ophthalmological: optic atrophy

Table 2: Mitochondrial gene mutation and clinical disorders

Similarly, a homoplasmic mutation in a mitochondrial genome-encoded ribosomal RNA, called RNR1, causes postlingual deafness (deafness that occurs after three years of age, when a child has already learned to speak). The clinical symptoms of this disease are associated with the administration of a particular type of antibiotic. Therefore, environmental factors also contribute to the phenotypes associated with this mitochondrial mutation. Clinical Syndromes with a High Probability of Mitochondrial DNA Involvement

What are some clues that may suggest a mitochondrial link to disease? Some clinical features include a maternal family history and the involvement of several different tissues. Furthermore, because mitochondria function as the powerhouses of our cells, mitochondrial mutations often lead to more pronounced phenotypes in tissues that have high energy demands, such as brain, retinal, skeletal muscle, and cardiac muscle tissues. A number of clinical syndromes are currently believed to be associated with mitochondrial disease. Possible examples include Pearson syndrome, Leigh syndrome, progressive external ophthalmoplegia, exercise-induced muscle pain,

fatigue, and rhabdomyolysis.

Mitochondrial Mutations That Contribute to Common Disease Phenotypes

Mitochondrial mutations are also likely to contribute to a number of common clinical diseases. One example is diabetes, which is the most prevalent metabolic disease affecting human. It is also likely that mitochondrial mutations may predispose individuals to Alzheimer's disease and Parkinson's disease.

Probable Questions:

1. Differentiate A, B and Z DNA.
2. Describe Chargaff's rule.
3. Describe structure of DNA.
4. What is circular and spiral DNA?
5. Describe denaturation and renaturation of DNA. What is Tm?
6. Describe different types of repetitive sequence.
7. What are the functions of DNA?
8. Describe different types of viral genome.
9. How DNA remain packed in bacteria?
10. Describe characteristics of mitochondrial genome.

Suggested Readings / References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell andMolecularBiology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genomes

Unit -XV

Phases of Cell cycle, Check Points, Regulation of cell cycle; MPF, cyclins and cyclin-dependent kinases.

Objectives: In this Unit we will discuss on Phases of cell cycle. Regulation of cell cycle: Discovery of MPF, cyclins and cyclin dependent kinases, Check points- role of Rb and p53

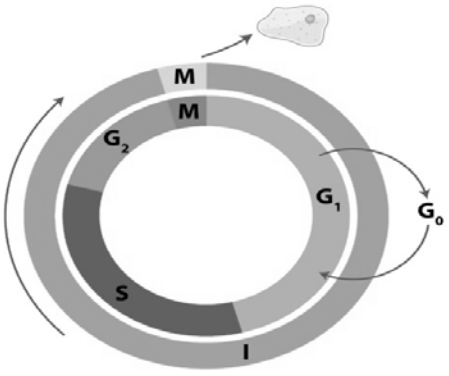
Cell Cycle:

Definition:

The cell cycle is a cycle of stages that cells pass through to allow them to divide and produce new cells. It is sometimes referred to as the "cell division cycle" for that reason. Daughter cells start life, containing only half of the parent cell's cytoplasm and only onecopy of the DNA that is the cell's "blueprint" or "source code" for survival. In order to divideand produce "daughter cells" of their own, the newborn cells must grow and produce more copies of vital cellular machinery – including their DNA.The two main parts of the cell cycle are mitosis and interphase.

Mitosis is the phase of cell division, during which a "parent cell" divides to create two "daughter cells."The longest part of the cell cycle is called "interphase" – the phase of growth and DNA replication between mitotic cell divisions. Both mitosis and interphase are divided into smaller sub-phases which need to be executed in order for cell division, growth, and development to proceed smoothly. Here we will focus on interphase, as the phases of mitosis have been covered in our "Mitosis" article. Interphase consists of at least three distinct stages during which the cell grows, produces new organelles, replicates its DNA, and finally divides. Only after the cell has grown by absorbing nutrients, and copied its DNA and other essential cellular machinery, can this "daughter cell" divide, becoming "parent" to two"daughter cells" of its own.

The graphic below shows a visual representation of the cell cycle. The small section labeled "M" represents mitosis, while interphase is shown subdivided into its major components: the G_1, S, and G_2 phases.



This cell cycle is used by all eukaryotic cells to produce new cells. Prokaryotic cells such as

bacteria use a process called “binary fission.” For some unicellular eukaryotes, the cell cycle is the same as the reproductive cycle. Their “daughter cells” are independent organisms that will go on to reproduce themselves through mitosis. In other organisms, the cell cycle is used for growth and development of a single organism, while other methods are used to reproduce the organism. Animals and some plants, for example, create new offspring through a process of sexual reproduction which involves the creation and combination of special sex cells. But animals and plants still use the cell cycle to produce new cells within their tissues. This allows these multicellular organisms grow and heal throughout their lifespans.

Phases of Cell Cycle:

Mitosis

Let’s start this cell cycle with “birth.”During mitosis, the “parent” cell goes through a complex series of steps to ensure that each “daughter” cell will get the materials it needs to survive, including a copy of each chromosome. Once the materials are properly sorted, the “parent” cell divides down the middle, pinching its membrane in two. Each of the new “daughters” are now independently living cells. But they’re small, and have only one copy of their genetic material. This means they can’t divide to produce their own “daughters” right away. First, they must pass through “interphase” – the phase between divisions, which consists of three distinct phases.

G_1 Phase

In G_1 phase, the newly formed daughter cell grows. The “G” is most often said to stand for “gap,” since these phases appear to an outside observer with a light microscope to be relatively inactive “gaps” in the cell’s activity.However given what we know today, it might be more accurate to say the “G” stands for “growth” – for the “G” phases are flurries of protein and organelle production as well as literal increase in the size of the cell. During the first “growth” or “gap” phase, the cell produces many essential materials such as proteins and ribosomes. Cells that rely on specialized organelles such as chloroplasts and mitochondria make a lot more of those organelles during G_1 as well. The cell’s size may increase as it assimilates more material from its environment into its machinery for life.This allows the cell to increase its energy production and overall metabolism, preparing it for S Phase.

S Phase

During S phase, the cell replicates its DNA. The “S” stands for “synthesis” – referring to the synthesis of new chromosomes from raw materials. This is a very energy-intensive operation, since many nucleotides need to by synthesized. Many eukaryotic cells have dozens of chromosomes – huge masses of DNA – that must be copied. Production of other substances and organelles is slowed greatly during this time as the cell focuses on replicating its entire genome. When the S phase is completed, the cell will have two complete sets of its genetic material. This is crucial for cell division, as it ensures that both daughter cells can receive a copy of the “blueprint” they need to survive and reproduce.

G_2 Phase:

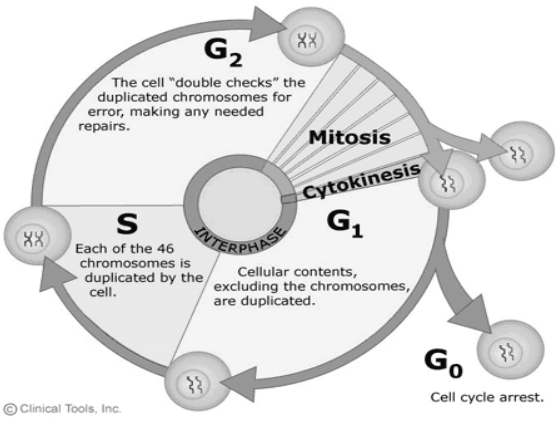
Just like the first "gap" phase of the cell cycle, the G_2 phase is characterized by lots of protein production. During G_2, many cells also check to make sure that both copies of their DNA are correct and intact. If a cell's DNA is found to be damaged, it may fail its "G_2/M checkpoint" – so named because this "checkpoint" happens at the end of the G_2 phase, right between G_2 and "M phase" or "Mitosis."

This "G_2/M checkpoint" is a very important safety measure for multicellular organisms like animals. Cancers, which can result in the death of the entire organism, can occur when cells with damaged DNA reproduce. By checking to see if a cells' DNA has been damaged immediately before replication, animals and some other organisms reduce the risk of cancer.
Interestingly, some organisms can skip G_2 altogether and go straight into mitosis after DNA is synthesized during S phase. Most organisms, however, find it safer to use G_2 and its associated checkpoint! If the G_2/M checkpoint is passed, the cell cycle begins again. The cell divides through mitosis, and new daughter cells begin the cycle that will take them through G_1, S, and G_2 phases to produce new daughter cells of their own.

An Alternative Path: G_0 Phase

After being born through mitosis, some cells are not meant to divide themselves to produce daughter cells.Neurons, for example – animal nerve cells – do not divide. Their "parent cells" are stem cells, and the "daughter" neuron cells are programmed not to go through the cell cycle themselves because uncontrolled neuron growth and cell division could be very dangerous for the organism.

So instead of entering G_1 phase after being "born," neurons enter a phase scientists call "G_0 phase." This is a metabolic state meant only to maintain the daughter cell, not prepare for cell division.Neurons and other non- dividing cell types may spend their whole lives in G_0 phase, performing their function for the overall organism without ever dividing or reproducin



Mitosis:

Prophase

In the phase to follow, called prophase, the duplicated chromosomes from the previous phase condense, meaning they become compacted and more tightly wound. An apparatus known as a mitotic spindle forms on the edges of the dividing cell. The mitotic spindle is made up of proteins called microtubules that gradually lengthen during prophase, which drives the division of the cell by elongating it.

Metaphase

Preceding metaphase is a period called prometaphase, during which the membrane, or nuclear envelope, surrounding the chromosomes breaks down, allowing the condensed chromosomes to come into direct contact with the microtubules of the mitotic spindle. Upon entering metaphase, the pairs of condensed chromosomes line up along the equator of the elongated cell. Because they are condensed, they move more easily without becoming tangled.

Anaphase

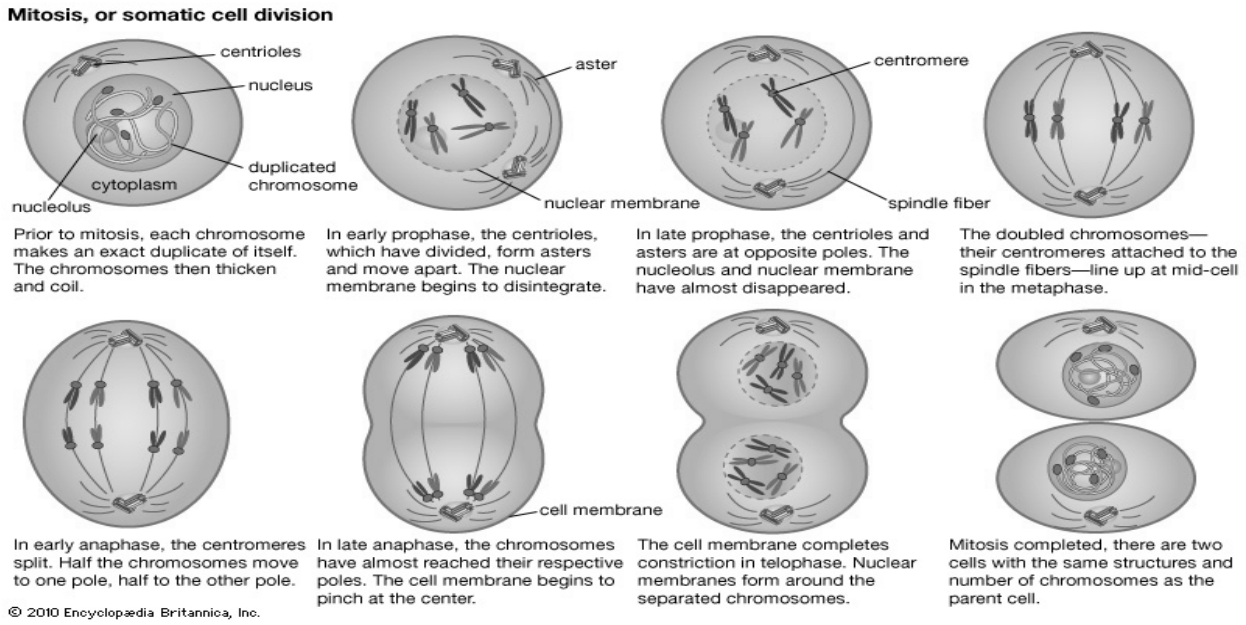
During anaphase, the pairs of chromosomes, also called sister chromatids, are drawn to opposite poles of the elongated cell. Therefore, duplicate copies of the cell's DNA are now oneither side of the cell and are ready to divide completely. At this stage the microtubules get shorter, which begins to allow the cell to separate.

Telophase

The most important characteristic of telophase is that the nuclear envelope, which hadpreviously broken down to allow the microtubules to access and recruit the chromosomes to the equator of the dividing cell, reforms as two new nuclear envelopes around the separated sister chromatids. The complete division of the cell, however, is not complete until cytokinesis takes place. Cytokinesis is the process by which the elongated cell is finally pinched into two brand new cells by a ring of proteins called actin and myosin, the same proteins found in muscle. At this point, the cytoplasm, or fluid in which all cell components are bathed, is equally divided between the two new daughter cells. Each daughter cell is identical, containing it's own nucleus and a complete copy of the organism's DNA.

Cytokinesis

Cytokinesis, or the separating of the two daughter cells, begins during or after the final phases of mitosis. It occurs in animal cells happens when a fiber ring containing a protein called actin, located in the cell's center, contracts, pulling the cell membrane to it, and



subsequently squeezing the two nuclei apart. Because cell walls are so rigid in plant cells, cytokinesis in plant cells occurs when a cell plate takes the place of the metaplate and grows between the two new cells. This plate becomes a membrane that inculcates itself into the cell wall in each of the daughter cells.

Meiosis:

Prophase I

Prophase I, the first step in meiosis I, is similar to prophase in mitosis in that the chromosomes condense and move towards the middle of the cell. The nuclear envelope degrades, which allows the microtubules originating from the centrioles on either side of the cell to attach to the kinetochores in the centromeres of each chromosome. Unlike in mitosis, the chromosomes pair with their homologous partner. This step does not take place inmitosis.

Metaphase I

In metaphase I of meiosis I, the homologous pairs of chromosomes line up on the metaphase plate, near the center of the cell. This step is referred to as a reductional division. The homologous chromosomes that contain the two different alleles for each gene, are lined up to be separated. As seen in the diagram above, while the chromosomes line up on the metaphase plate with their homologous pair, there is no order upon which side the maternal or paternal chromosomes line up. This process is the molecular reason behind the law of segregation.

The law of segregation tells us that each allele has the same chance at being passed on to offspring. In metaphase I of meiosis, the alleles are separated, allowing for this phenomena to happen. In meiosis II, they will be separated into individual gametes. In mitosis, all the chromosomes line up on their centromeres, and the sister chromatids of each chromosome separate into new cells. The homologous pairs do not pair up in mitosis, and each is split in half to leave the new cells with 2 different alleles for each gene. Even if these alleles are the same allele, they came from a maternal and paternal source. In meiosis, the lining up of homologous chromosomes leaves 2 alleles in the final cells, but they are on sister chromatids and are clones of the same source of DNA. Also during metaphase I, the homologous chromosomes can swap parts of themselves thatare the same parts of the chromosome. This is called crossing-over and is responsible for the other law of genetics, the law of independent assortment. This law states that traits are inherited independently of each other. For traits on different chromosomes, this is certainly true all of the time. For traits on the same chromosome, it makes it possible for the maternal and paternal DNA to recombine, allowing traits to be inherited in an almost infinite numberof ways.

Anaphase I

Much like anaphase of mitosis, the chromosomes are now pulled towards the centrioles at each side of the cell. However, the centrosomes holding the sister chromatids together do not dissolve in anaphase I of meiosis, meaning that only homologous chromosomes are separated, not sister chromatids.

Telophase I

In telophase I, the chromosomes are pulled completely apart and new nuclear envelopes form. The plasm membrane is separated by cytokinesis and two new cells are effectively formed.

Results of Meiosis I

Two new cells, each haploid in their DNA, but with 2 copies, are the result of meiosis I. Again, althgough there are 2 alleles for each gene, they are on sister chromatid copies of eachother. These are therefore considered haploid cells. These cells take a short rest before entering the second division of meiosis, meiosis II.

Phases of Meiosis II

Prophase II

Prophase II resembles prophase I. The nuclear envelopes disappears and centrioles are formed. Microtubules extend across the cell to connect to the kinetochores of individual chromatids, connected by centromeres. The chromosomes begin to get pulled toward the metaphase plate.

Metaphase II

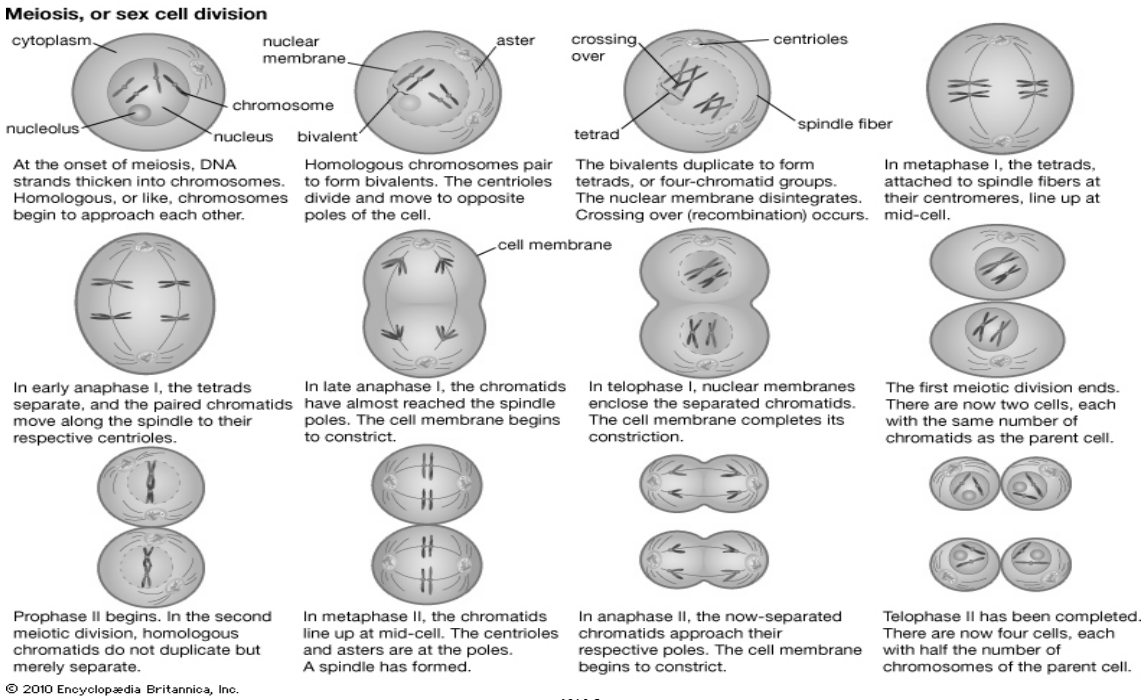
Now resembling mitosis, the chromosomes line up with their centromeres on the metaphase plate. One sister chromatid is on each side of the metaphase plate. At this stage, the centromeres are still attached by the protein cohesin.

Anaphase II

The sister chromatids separate. They are now called sister chromosomes, and are pulled toward the centrioles. This separation marks the final division of the DNA. Unlike the first division, this division is known as an equational division, because each cell ends up with the same quantity of chromosomes as when the division started, but with no copies.

Telophase II

As in the previous telophase I, the cell is now divided in two and the chromosomes are on opposite ends of the cell. Cytokinesis, or plasma division occurs, and new nuclear envelopes are formed around the chromosomes.



Results of Meiosis II

At the end of meiosis II, there are 4 cells, each haploid, and each with only 1 copy of the genome. These cells can now be developed into gametes, eggs in females and sperm in males.

Cell Cycle Regulation:

It's very important for the survival of cells and organisms that the cell cycle be regulated. Organisms need to be able to stop cell division when the cell in question is damaged, or when there isn't enough food to support new growth; they must also be able to start up cell division when growth or wound healing are needed. To accomplish this, cells use a variety of chemical "signal cascades" where multiple links in a chain create complex effects based on simple signals.

In these regulatory cascades, a single protein may change the function of many other proteins, bringing about widespread changes to the functioning or even structure of the cell. This allows these proteins – such as cyclins and cyclin-dependent kinases – to act as "stop points." If the cyclins or cyclin-dependent kinases don't give the go-ahead, the cell cannot progress to subsequent stages of the cell cycle.

Some examples of cell cycle regulators are given below:

Here we'll discuss common examples of how cells regulate their cell cycles, using a complex cascade of signal molecules, protein-activating enzymes, and signal-destroying molecules.

p53:

p53 is a protein that is well-known to scientists for its role in stopping cells with severe DNA damage from reproducing. When DNA is damaged, p53 works with cyclin-dependent protein kinases and other proteins to initiate repair and protection functions – and can also stop the cell from entering mitosis, ensuring that cells with DNA damage do not reproduce.

Cyclins:

Cyclins are a group of proteins that are produced at different points in the cell cycle. There are cyclins unique to most phases of the cell cycle – G_1 cyclins, G_1 /S cyclins that regulate the transition from G_1 into S, S cyclins, and M cyclins that regulate the progress through the stages of mitosis. Most cyclins are found in the cell at very low concentrations during other phases of the cell cycle, but then spike suddenly when they're needed to give the go-ahead to the next stage of the cell cycle. Certain types of DNA damage may prevent these cyclins from appearing to move the cell cycle forward, or may prevent them from activating their cyclin- dependent protein kinases. A few others, such as G_1 cyclins, remain high as a constant "go ahead" signal from G_1 until mitosis.

Cyclin-Dependent Protein Kinases:

The cell's cyclins ultimately do their jobs by interacting with Cyclin-Dependent Protein Kinases i.e., kinases that activate certain enzymes and proteins when they bind to a cyclin. This allows cyclins to function as the "go" signal for many changes in cellular activity that happens throughout the cell cycle. Protein kinases are a special set of enzymes that "activate" other enzymes and proteins by affixing phosphate groups to them. When an enzyme or other protein is "activated" by a kinase, its behavior changes until it returns to its inactivated form. The system by which one protein kinase can change the activities of many other proteins allows simple signals, such as cyclins, to produce complex changes to cellular activity. Signal-dependent protein kinases are used to coordinate many complex cellular activities.

Cyclins and cyclin-dependent kinases (CDKs):

CDKs are important master regulators of the cell cycle. Their role is to phosphorylate proteins on either S or T amino acids and thereby regulate the activity of those proteins. Yeast have just one CDK (Cdk1), while 'metazoans' (animals) like us have nine, of which four are really critical to the cell cycle and will be introduced today.

How are the CDKs themselves regulated?

The levels of these proteins remain pretty constant throughout the cell cycle, yet their levels of activity rise and fall cyclically. CDKs need to hydrolize ATP for energy in order to perform phosphorylation. They have an ATP binding cleft whose ability to bindATP is regulated by two mechanisms. First, CDKs have a 'flexible T loop' which contains a threonine (T) residue which normally blocks the ATP binding cleft, but not when the T is phosphorylated. Second, cyclins bind CDKs and induce a conformational change that also helps to expose the ATP binding cleft. Therefore a fully active CDK is one which is both phosphorylated at the T on the T loop and is bound to a cyclin.The various activities of the cell cycle, then, are determined by the combination of cyclins and CDKs that are active at each stage, as shown in the following table.

cell cycle stage	cyclins	CDKs	Comments
G1	Cyclin D	CDK4&6	Can react to outside signals such as growth factors or mitogens.
G1/S	Cyclins E & A	CDK2	Regulate centrosome duplication; important for reaching START
S	Cyclins E & A	CDK2	Targets are helicases and polymerases
M	Cyclins A & B	CDK1	Regulate G2/M checkpoint. The cyclins are synthesized During S but not active until synthesis is complete. Phosphorylate lots of downstream targets.

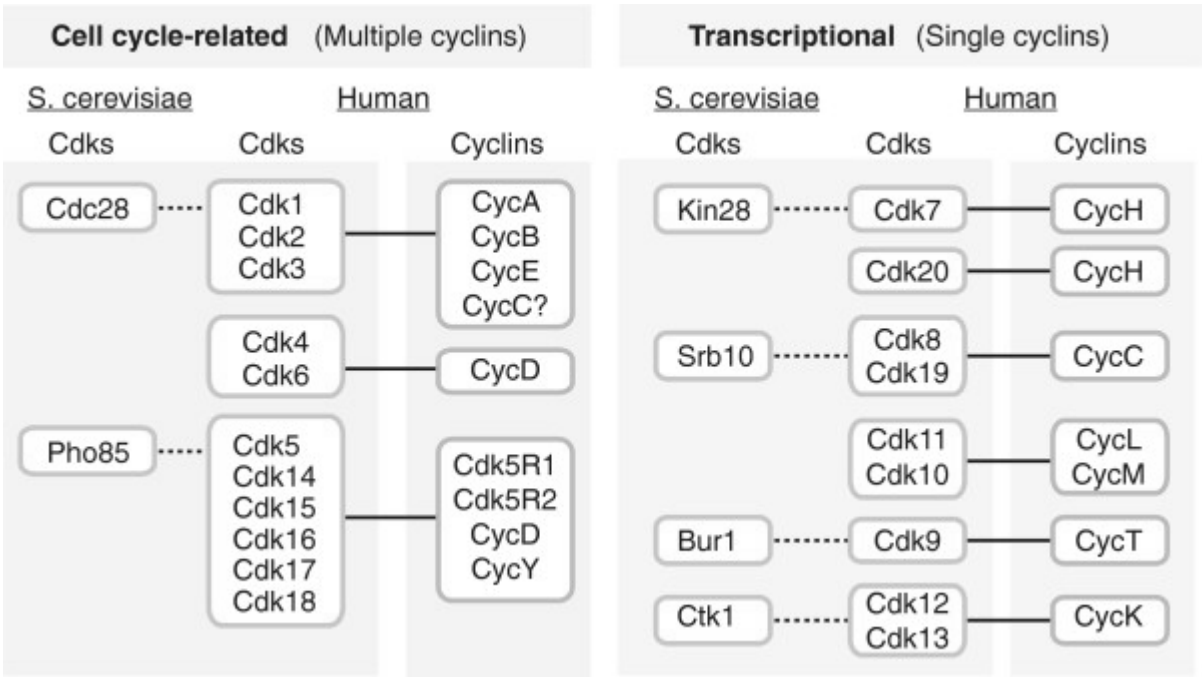
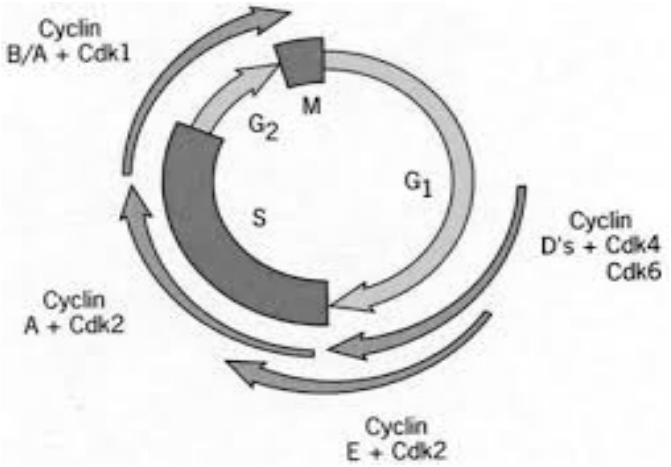
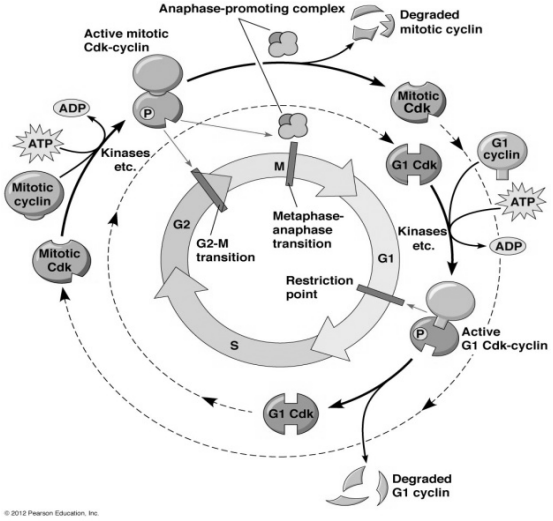


Figure: Comparison of Yeast and Mammalian Cdks and Cyclins.





CYCLIN-DEPENDENT KINASE INHIBITORS

The cyclin-dependent kinase inhibitors are a family of cell cycle regulators. Their primary function seems to be the formation of stable complexes with cyclin-dependent kinase proteins and the subsequent inhibition of the cell cycle. These complexes inactivate the catalytically operative units. Among the most well known and clinically relevant are p21, p27, and p16.

p21

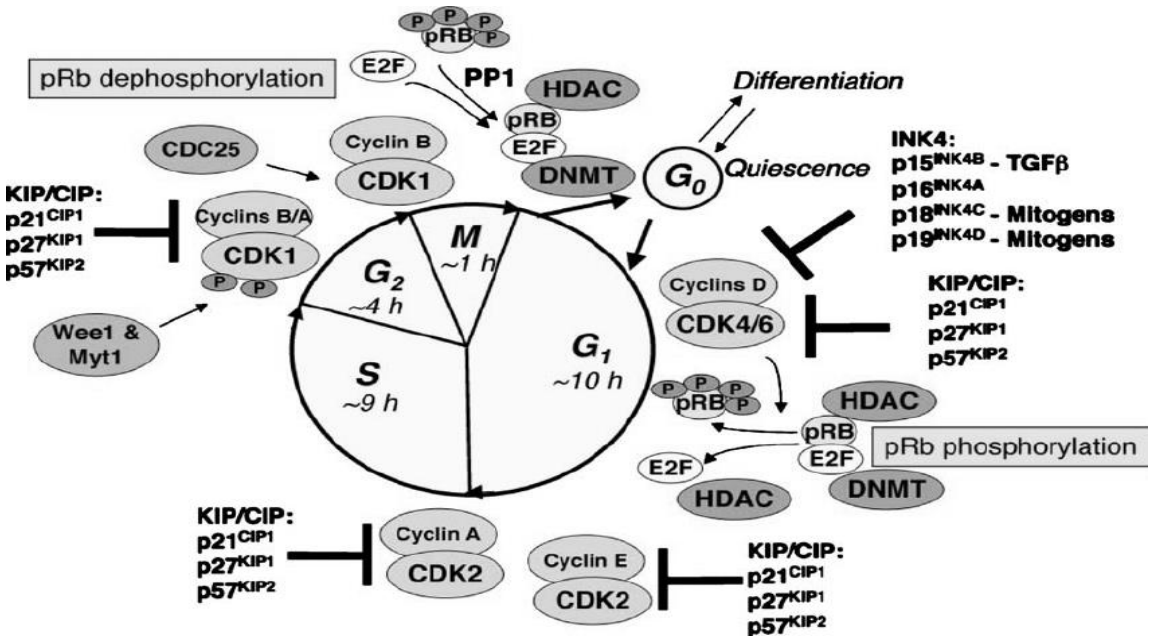
A member of the WAF/CIP/KIP family of cyclin-dependent kinase inhibitors, p21 is probably the best characterized. It acts as a regulator of epithelial carcinogenesis and differentiation and is thought to play an important role in tumor suppression by regulating cell cycle progression, DNA replication, and DNA repair. The protein expression of p21 has been studied in a variety of tumor types, including breast, gastric, ovary, colorectal, and bladder carcinomas. The alteration of protein expression assessed by immunohistochemical methods has been associated with higher tumor grade and worse prognosis in patients with bladder cancer.

p27

The p27 inhibitor is involved in the regulation of the cell cycle at the G_1-S transition, ultimately through the inhibition of pRb phosphorylation. Mutations in the human p27 gene appear to be rare. Loss of p27 expression is associated with colon, breast, prostate, and gastric cancer progression.

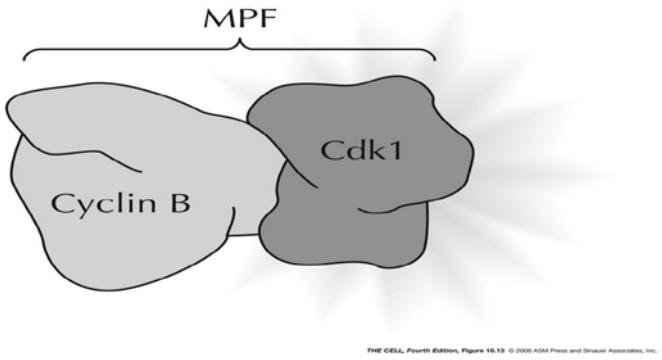
p16

Also known as $p16^{INK4}$ and CDKN2A, p16 is a tumor suppressor protein encoded on the INK4a/ARF locus of chromosome 9p21, which is one of the most frequent sites of genetic loss in human cancer. Numerous studies have found abnormal p16 protein in a variety of tumor types, including melanomas; gliomas; esophageal, pancreatic, lung, and bladder carcinomas; and certain types of lymphomas. In addition, p16 is known to regulate Rb, and immunohistochemical expression of pRb and p16 is inversely correlated in a variety of tumors



Maturation-Promoting Factor:

Maturation-promoting factor (abbreviated **MPF**, also called **mitosis-promoting factor** or **M-Phase-promoting factor**) is the cyclin-Cdk complex that was discovered first in frogeggs. It stimulates the mitotic and meiotic phases of the cell cycle. MPF promotes the entrance into mitosis (the M phase) from the G_2 phase by phosphorylating multiple proteins needed during mitosis. MPF is activated at the end of G_2 by a phosphatase, which removes an inhibitory phosphate group added earlier. One example of a protein kinase at work is the Maturation-Promoting Factor, or MPF. MPF is a protein kinase that is activated by an M cyclin, meaning that it is activated during mitosis. When MPF is activated, it in turn activates several different proteins in the nuclear envelop of its host cell. The changes to these proteins result in the disintegration of the nuclear envelope. This is something that would be very dangerous at other points in the cell cycle, but which is necessary during mitosis so that the chromosomes can be sorted to ensure that each daughter cell receives a copy of each chromosome. If M cyclins do not appear, MPF does not activate, and mitosis cannot go forward. This is a good example of how cyclins and cyclin-dependent kinases work together to coordinate – or stop – the cell cycle.



Discovery:

Before dividing, eukaryotic cells undergo a highly ordered series of events called the cell cycle. These events include S-phase, when the cell's DNA is replicated, and mitosis (M- phase), when the replicated chromosomes are separated. These phases are separated byperiods called gap (G) phases, with the G1 phase preceding S phase and the G2 phase occurring between S phase and mitosis. How the cell controls this essential series of events is one of the fundamental problems of cell biology.

One prominent early approach to studying control of the cell cycle was to introduce a nucleusfrom one phase of the cell cycle into a cytoplasm from another. Techniques for making such nucleo-cytoplasmic hybrid cells, including nuclear transplantation and cell fusion, became available in the 1950s, but it was not until the late 1960s that they were used to study cell cycle activities such as the initiation of DNA synthesis and the condensation of chromosomes. This type of experiment included nuclear transplantation by injection in frog oocytes and eggs, excision and transplantation of cytoplasmic fragments in protozoa, and virus-mediated fusion between tissue culture cells. In all cases the nucleus conformed to the cell cycle stage of the cytoplasm, indicating that cytoplasmic factors control nuclear activitiesduring the cell cycle. Evidence for the existence of factors which might control the initiation of cell cycle events came from other types of

experiments. For example, experiments done with Tetrahymena showed that heat shock synchronized the cell cycles of a population of cells, presumably because of the heat lability of a component that promotes cell division

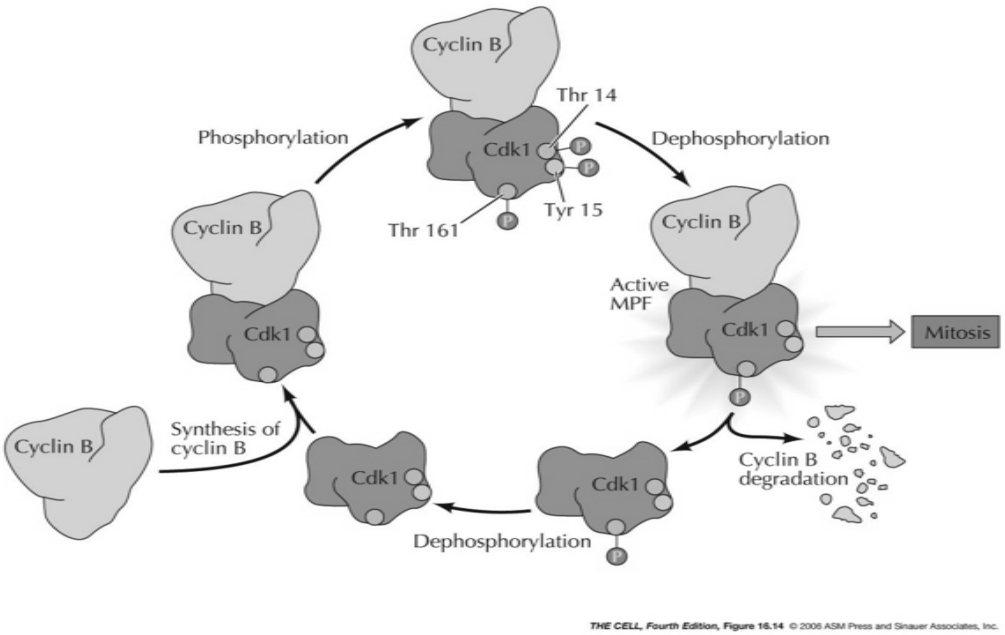
Despite what these experiments revealed about the organization of the cell cycle, none of them provided any clue to the identity of the factors responsible for cytoplasmic control over the nucleus. Nor could any of the experiments be readily adapted to provide an assay with which to identify those factors and study their biochemical mechanisms. That would require an experimental system in which cytoplasm from particular stages of the cell cycle could be isolated and used to cause a transition from one phase of the cell cycle to another. The frog oocyte provided this system and played an essential role in identifying the molecular machinery that drives the cell cycle.

Role in the cell cycle:

During G_1 and S phase, the CDK1 subunit of MPF is inactive due to an inhibitory enzyme, Wee1. Wee1 phosphorylates the Thr-14 residues in yeast and Tyr-15 residues in humans of CDK1, rendering MPF inactive. During the transition of G_2 to M phase, cdk1 is de-phosphorylated by CDC25. The CDK1 subunit is now free and can bind to cyclin B, activate MPF, and make the cell enter mitosis. There is also a positive feedback loop that inactivates wee1.

Activation:

MPF must be activated in order for the cell to transition from G_2 to M phase. There are three amino acid residues responsible for this G_2 to M phase transition. The Threonine-161 (Thr- 161) on CDK1 must be phosphorylated by a Cyclin Activating Kinase (CAK). CAK only phosphorylates Thr-161 when cyclin B is attached to CDK1. In addition, two other residues on the CDK1 subunit must be activated by dephosphorylation. CDC25 removes a phosphate from residues Threonine-14 (Thr-14) and Tyrosine-15 (Tyr-15) and adds a hydroxyl group. Cyclin B/CDK1 activates CDC25 resulting in a positive feedback loop.



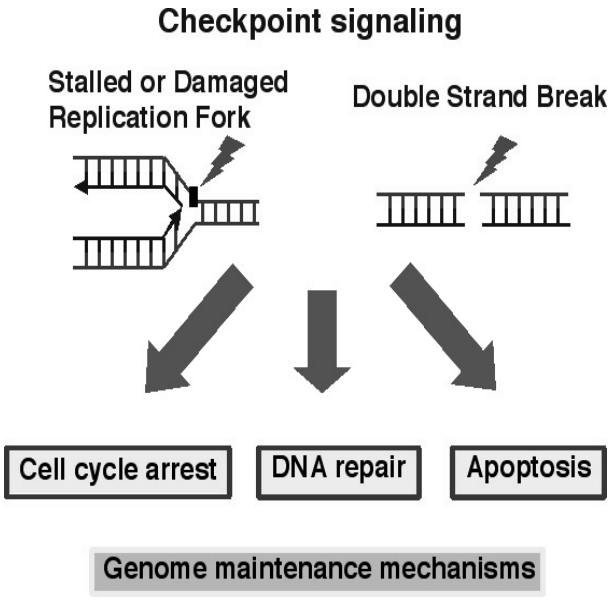
Overview of functions

- Triggers the formation of mitotic spindle through microtubule instability.
- Promotes mitosis i.e. chromatin condensation through phosphorylation of condensins.
- The three lamins present in the nuclear lamina, lamin A, B & C, are phosphorylated by MPF at serine amino residues. This leads to depolymerisation of the nuclear lamina & breakdown of nuclear envelope into small vesicles.
- Causes phosphorylation of GM130, which leads to the fragmentation of the Golgi and the ER

Anaphase-Promoting Complex/Cyclosome

Ingeniously, the protein kinase MPF doesn't just ensure that the nuclear envelope breaks down during mitosis – it also ensures that MPF levels will fall after the nuclear envelope is broken down. It does this by activating the Anaphase-Promoting Complex/Cyclosome, or "APC/C" for short. As its name suggests, the APC/C promotes passage into Anaphase – and one of the ways it does that is by breaking down MPF, a messenger from a previous phase. So MPF actually activates the very proteins that destroy it. The destruction of MPF by the APC/C ensures that the actions MPF promotes – such as the disintegration of the nuclear envelope – do not happen again until the daughter cell makes more MPF after passing through G_1 phase, S phase, and G_2 phase.

Cell Cycle Checkpoint:



The cell cycle proceeds by a defined sequence of events where late events depend upon completion of early events. The aim of the dependency of events is to distribute complete and accurate replicas of the genome to daughter cells. To monitor this dependency, cells are equipped

with the **checkpoints** that are set at various stages of the cell cycle. When cells have DNA damages that have to be repaired, cells activate **DNA damage checkpoint** that arrests cell cycle. According to the cell cycle stages, DNA damage checkpoints are classified into at least 3 checkpoints:

G1/S (G1) checkpoint, intra-S phase checkpoint, and G2/M checkpoint. Upon perturbation of DNA replication by drugs that interfere with DNA synthesis, DNA lesions, or obstacles on DNA, cells activate **DNA replication checkpoint** that arrests cell cycle at G2/M transition until DNA replication is complete. There are more checkpoints such as **Spindle checkpoint** and Morphogenesis checkpoint. The spindle checkpoint arrests cell cycle at M phase until all chromosomes are aligned on spindle. This checkpoint is very important for equal distribution of chromosomes. Morphogenesis checkpoint detects abnormality in cytoskeleton and arrests cell cycle at G2/M transition.

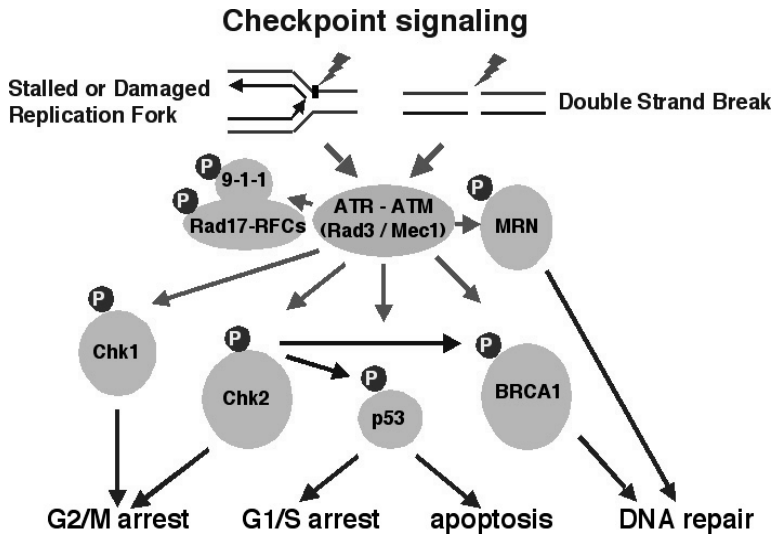
DNA maintenance checkpoint:

Accurate duplication of eukaryotic genome is a challenging task, given that environment of cell growth and division is rarely ideal. Cells are constantly under the stress of intrinsic and extrinsic agents that cause DNA damage or interference with DNA replication. To cope with these assaults, cells are equipped with **DNA maintenance checkpoints** to **arrest cell cycle** and facilitate **DNA repair** pathways. DNA maintenance checkpoints include (a) the **DNA damage checkpoints** that recognize and respond to DNA damage, and (b) the **DNA replication checkpoint** that monitors the fidelity of copying DNA.

DNA damage checkpoint

DNA damage checkpoints ensure the fidelity of genetic information both by arresting cell cycle progression and facilitating DNA repair pathways. Studies on many different species have uncovered a network of proteins that form the DNA damage checkpoints. Central to this network are **protein kinases** of **ATM/ATR** family known as **Tel1/Mec1** in budding yeast and **Tel1/Rad3** in fission yeast. These kinases sense DNA damages and phosphorylate number of proteins that regulate cell cycle progression and DNA repair pathways.

DNA replication checkpoint

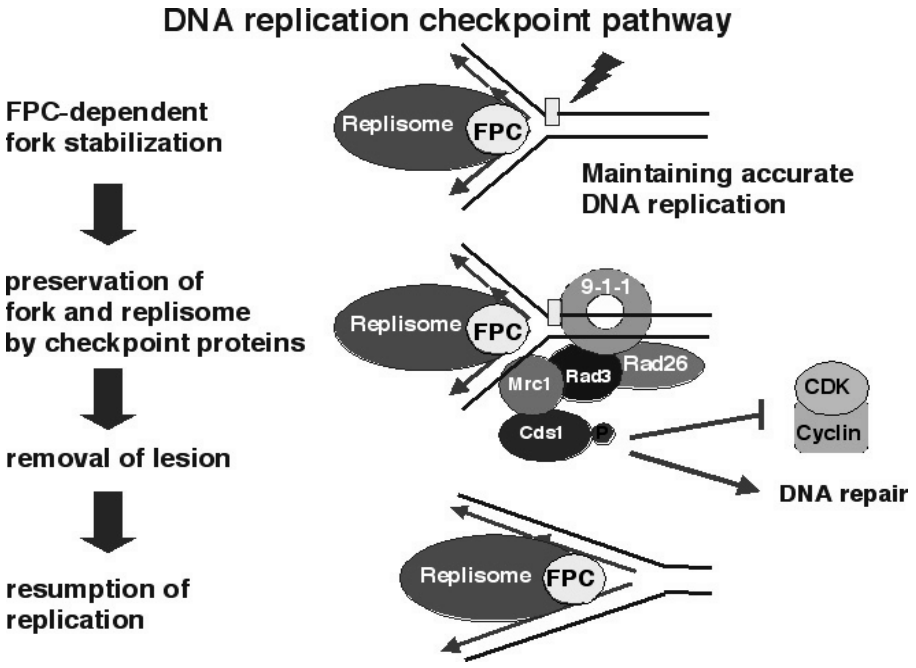


Accurate replication of the millions or billions of DNA base pairs in a eukaryotic genome is a remarkable achievement. This accomplishment is even more astonishing when one considers for DNA synthesis are rarely ideal. Damaged template, protein complexes bound to DNA, and poor supply of dNTPs are among the many obstacles that must be overcome to replicate genome. All of these situations can stall **replication forks**. **Stalled forks** pose grave threatsto **genome integrity** because they can rearrange, break, or collapse through disassembly of the replication complex. The pathways that respond to replication stress are signal transduction pathways that are conserved across evolution.Atop the pathways are also ATM/ATR family kinases. These kinases together with a trimeric checkpoint clamp (termed 9-1-1 complex) and five-subunit checkpoint clamp loader (Rad17-RFC2-RFC3-RFC4-RFC5) senses stalled replication forks and transmit a checkpoint signal. One of major functions of replication checkpoint is to prevent the onset of mitosis by regulating mitotic control proteins such as Cdc25. But perhaps the most important activity of replication checkpoint is to stabilize and protect replication forks. The protein kinase **Cds1** (human **Chk2** homolog; in human, **Chk1**is a functional Cds1 homolog) is a critical effector of the replication checkpoint in the fission yeast Schizosaccharomyces pombe. Cds1 is required to prevent stabilization of replication fork in cells treated with hydroxyurea (HU), a ribonucleotide reductase inhibitor that stalls replication by depleting dNTPs. In the budding yeast Saccharomyces cerevisiae, a failure to activate **Rad53** (Chk2 homolog) is associated with collapse and regression of replication forks and gross chromosomal rearrangements in cells treated with HU.

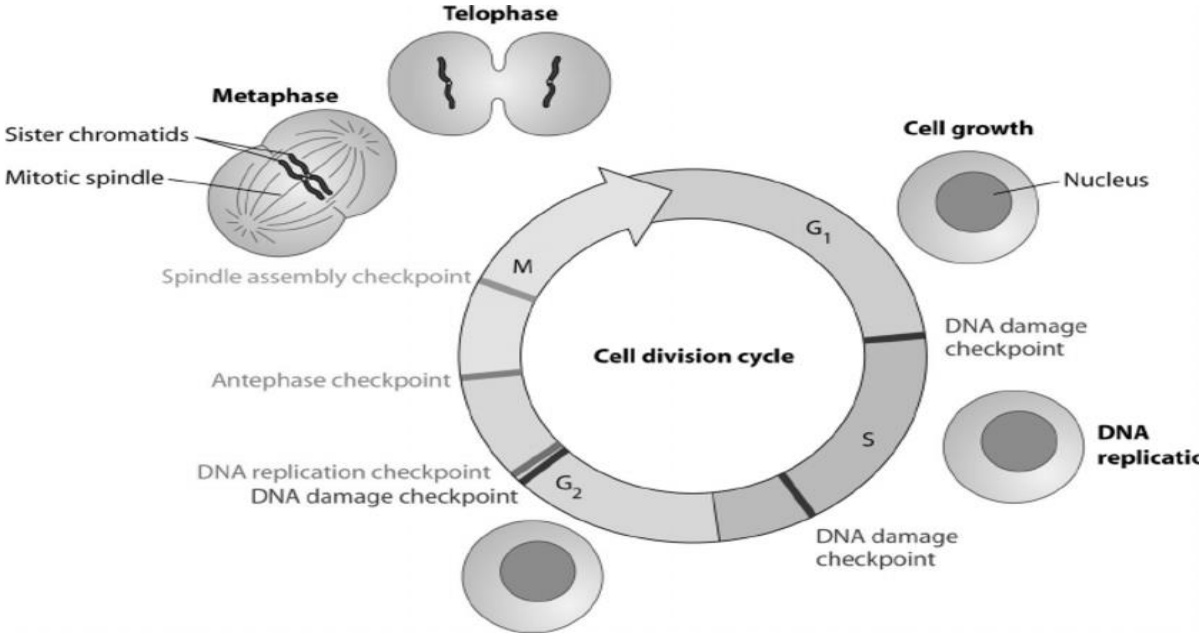
Replication fork protection complex (FPC):

The DNA replication checkpoint stabilizes replication forks that have stalled at DNA adducts and other lesions that block DNA polymerases. In the absence of DNA replication checkpoint, stalled

forks are thought to collapse, creating **strand break** that threatens genome stability and cell viability. Therefore, discovering how cells cope with aberrant replication forks is essential for understanding mechanisms of genome maintenance. The Chk1 and Chk2/Cds1 checkpoint kinases, which are key mediators of DNA damage and DNA replication checkpoints, are thought to be involved in **cancer development**. We found the **Swi1** protein is required for survival of replication fork arrest and effective activation of Chk2 kinase in fission yeast. Swi1 forms tight complex with **Swi3** protein and moves with replication forks. **Swi1- Swi3 complex** is also important for proficient DNA replication even in the absence of agents that cause genotoxic stress, creating **single-strand DNA gaps** at replication forks.



These results led us to propose Swi1-Swi3 define a **replication fork protection complex (FPC)** that stabilizes replication forks in a configuration that is recognized by replication checkpoint sensors. Interestingly, **Tof1** protein (Budding yeast Swi1 homolog) has been reported to have similar functions. Tof1 is also involved in Rad53 (Chk2 homolog) activation and travels with replication fork. Tof1 is needed to restrain fork progression when DNA synthesis is inhibited by HU indicating that Tof1 is required for coordination of DNA synthesis and **replisome** (replication machinery) movement.



Role of Rb and P53 protein in cell cycle:

Rb Inactivation:

Since the 1990s, Rb was known to be inactivated via phosphorylation. Until, the prevailing model was that Cyclin D- Cdk 4/6 progressively phosphorylated it from its unphosphorylated to it hyperphosphorylated state (14+ phosphorylations). However, it was recently shown that Rb only exists in three state: un-phosphorylated, mono-phosphorylated, and hyper- phosphorylated. Each has a unique cellular function.

Before the development of 2D IEF, only hyper-phosphorylated Rb was distinguishable from all other forms, i.e. un-phosphorylated Rb resembled mono-phosphorylated Rb on immunoblots. As Rb was either in its active "hypo-phosphorylated" state or inactive "hyperphosphorylated" state. However, with 2D IEF, it is now known that Rb is un- phosphorylated in G0 cells and mono-phosphorylated in early G1 cells, prior to hyper-phosphorylation after the restriction point in late G1.

Cyclin D - Cdk 4/6 Mono-phosphorylates Rb:

When a cell enters G1, Cyclin D- Cdk4/6 phosphorylates Rb at a single phosphorylation site. No progressive phosphorylation occurs because when HFF cells were exposed to sustained cyclin D-Cdk4/6 activity (and even deregulated activity) in early G1, only mono- phosphorylated Rb was detected. Furthermore, triple knockout, p16 addition, and Cdk 4/6 inhibitor addition experiments confirmed that Cyclin D- Cdk 4/6 is the sole phosphorylator of Rb. Throughout early G1, mono-phosphorylated Rb exists as 14 different isoforms (the 15th phosphorylation site is not conserved in primates in which the experiments were performed). Together, these isoforms represent the "hypo-phosphorylated" active Rb state that was thought to exist. Each isoform has distinct E2F binding preferences which suggest that mono-phosphorylated Rb has a diversity of functions and

can be “active” to varying degrees. It is currently unknown how such specificity is achieved.

Passing a bifurcation point induces hyper-phosphorylation by Cyclin E - Cdk2:

After a cell passes the restriction point, Cyclin E - Cdk 2 hyper-phosphorylates all mono-phosphorylated isoforms. While the exact mechanism is unknown, one hypothesis is that binding to the C-terminus tail opens the pocket subunit, allowing access to all phosphorylation sites. This process is hysteretic and irreversible, and it is thought accumulation of mono-phosphorylated Rb induces the process. The bistable, switch like behavior of Rb can thus be modeled as a bifurcation point. Hyper-phosphorylation of mono- phosphorylated Rb is an irreversible event that allows entry into S phase.

Un-phosphorylated and mono-phosphorylated Rb have unique functional roles:

Presence of un-phosphorylated Rb drives cell cycle exit and maintains senescence. At the end of mitosis, PP1 dephosphorylates hyper-phosphorylated Rb directly to its un-phosphorylated state. Furthermore, when cycling C2C12 myoblast cells differentiated (by being placed into a differentiation medium), only un-phosphorylated Rb was present. Additionally, these cells had a markedly decreased growth rate and concentration of DNA replication factors (suggesting G0 arrest).

This function of un-phosphorylated Rb gives rise to a hypothesis for the lack of cell cycle control in cancerous cells: Deregulation of Cyclin D - Cdk 4/6 phosphorylates un- phosphorylated Rb in senescent cells to mono-phosphorylated Rb, causing them to enter G1. The mechanism of the switch for Cyclin E activation is not known, but one hypothesis is that it is a metabolic sensor. Mono-phosphorylated Rb induces an increase in metabolism, so the accumulation of mono-phosphorylated Rb in previously G0 cells then causes hyper- phosphorylation and mitotic entry. Since any un-phosphorylated Rb is immediately phosphorylated, the cell is then unable to exit the cell cycle, resulting in continuous division. DNA damage to G0 cells activates Cyclin D - Cdk 4/6, resulting in mono-phosphorylation of un-phosphorylated Rb. Then, active mono-phosphorylated Rb causes repression of E2F-targeted genes specifically. Therefore, mono-phosphorylated Rb is thought to play an active role in DNA damage response, so that E2F gene repression occurs until the damage is fixed and the cell can pass the restriction point. As a side note, the discovery that damages causes Cyclin D - Cdk 4/6 activation even in G0 cells should be kept in mind when patients are treated with both DNA damaging chemotherapy and Cyclin D - Cdk 4/6 inhibitors.

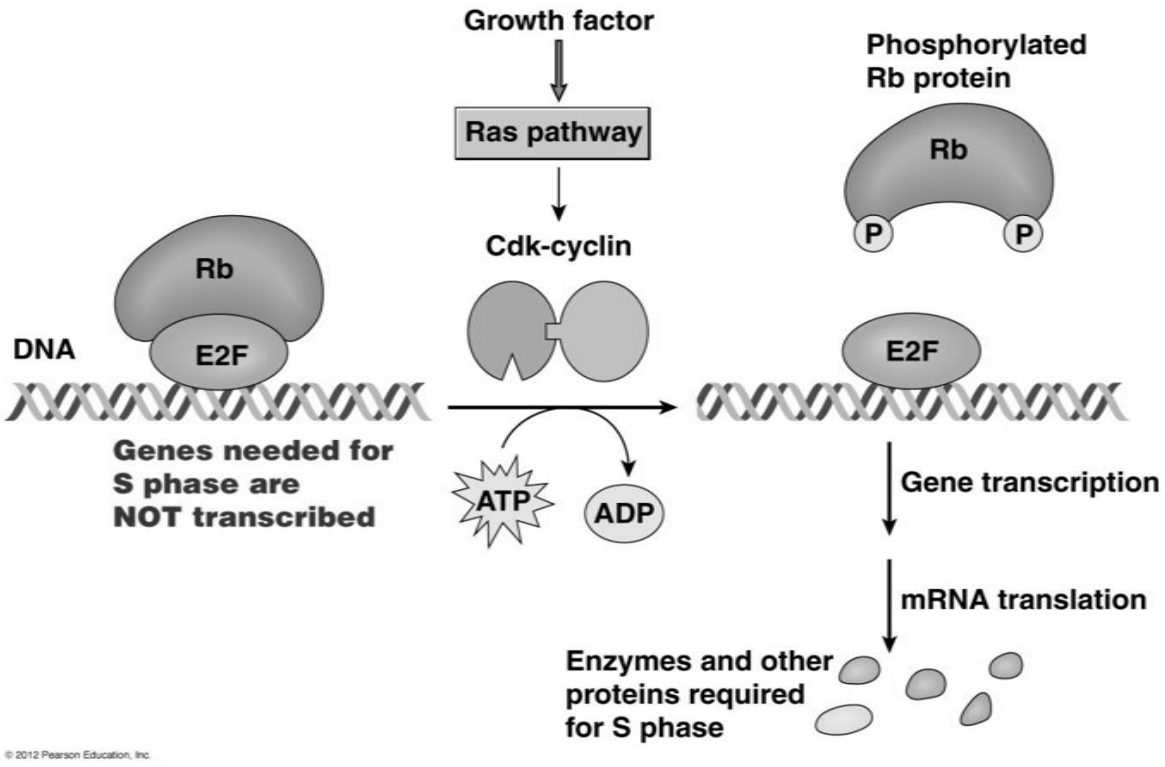
Activation:

During the M-to-G1 transition, pRb is then progressively dephosphorylated by PP1, returningto its growth-suppressive hypophosphorylated state Rb. Rb family proteins are components of the DREAM complex (also named LINC complex), which is composed of LIN9, LIN54,

LIN37, MYBL2, RBL1, RBL2, RBBP4, TFDP1, TFDP2, E2F4 and E2F5. There is a testis-

specific version of the complex, where LIN54, MYBL2 and RBBP4 are replaced by MTL5, MYBL1 and RBBP7, respectively. In Drosophila both DREAM versions also exist, the components being mip130 (lin9 homolog, replaced by aly in testes), mip120 (lin54 homolog, replaced by tomb in testes), and Myb, Caf1p55, DP, Mip40, E2F2, Rbf and Rbf2.

The DREAM complex exists in quiescent cells in association with MuvB (consisting of HDAC1 or HDAC2, LIN52 and L3mbtl1, L3mbtl3 or L3mbtl4) where it represses cell cycle-dependent genes. DREAM dissociates in S phase when LIN9, LIN37, LIN52 and LIN54 form a subcomplex that binds to MYBL2.



Probable questions:

1. Define Cell cycle. Describe different phases of Cell Cycle.
2. How cell cycles are regulated?
3. Write a note on Cyclin Dependent Kinase inhibitors?
4. What are Cell Cycle Checkpoints? How they regulate cell cycle?

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington, MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

5. Cooper, G. and Hausman, R. (n.d.). The cell.

UNIT-XVI

Cancer-Types and Stages, Carcinogens, Tumor suppressor genes and Proto-oncogenes induction to oncogenes

Objective: In this unit we will learn about different aspects of cancer. How this disease is caused, different stages. Role of tumor suppressor genes and oncogenes in cancer causing.

Introduction:

In multicellular organisms, cell division is a normal process. Cells divide for growth, for the development of organs, for healing of wounds and also for the replacement of older and damaged cells. Cell division is a very complex process which is controlled by a regulatory mechanism at both molecular and cellular level. Again, in higher multicellular organism, each and every cell belongs to a particular type of tissue like epithelial tissue, connective tissue muscular tissue etc.

Hence, when a cell of a specific tissue divides, it normally produces its own kinds of cell of the tissue to which it belongs. It never produces the cells of other tissues. Therefore, the process by which cells achieve this specification and specialisation is known as cellular differentiation. Differentiation of cell begins during embryonic gastrulation stage and continues through tissue formation. Actually differentiation has a genetic basis and the process results from the interaction of the nucleus and the cytoplasm. After the cells become well- differentiated, they cannot go back normally to the undifferentiated stage unless disturbed internally or externally.

Therefore, in multicellular organism, the cell division, differentiation and survival of individual cells are carefully regulated to meet the needs of the organism as a whole. When this regulation is lost due to any reason, the cells behave unusually and defy their control mechanism. Then the cells grow and divide in an uncontrolled manner ultimately spreading throughout the body and interfering with the functions of normal tissues and organs. As a whole, this condition leads to cancer. Cancer develops from defects in fundamental regulatory mechanisms of the cell.

Meaning of Cancer:

Cancer is a non-infectious disease. It starts at the molecular level of the cell and, ultimately affects the cellular behaviour. Generally, it can be defined as uncontrolled proliferation of cells without any differentiation.

Types of Cancer:

Cancer is a large class of diverse disease. All types of cancer can result from uncontrolled cell growth and division of any of the different kinds of cells in the body. So there are more than a hundred distinct types of cancer which vary in their behaviour and response to treatment. The uncontrolled cell growth produces a mass of cells which are called tumours or neoplasm tumours may be benign or malignant. A benign tumor remains confined to its original location. They do not invade the surrounding normal tissues. They do not spread to distant body sites.

The most common example of tumour is the skin wart. A benign tumour consists of closely resembles normal cells and may function like normal cells. Generally benign tumours are harmless and can usually be removed surgically. However, these tumours may sometimes become quite harmful if they are located in organs like brain and liver. A malignant tumour does not remain confined to its original location. They are capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems. Malignant tumours become life-threatening if, they spread throughout the body. nly malignant tumours are properly designated as cancers. The cells of malignant tumour are derived from single cell, thus they are monoclonal in character. Malignant tumour is composed of aberrant cells. They behave like embryonic type, undifferentiated, having irregular, large nucleus, and deficient of cytoplasm. Malignant tumours are generally classified into four main types on the basis of cell type from which they arise.

(i) Carcinomas:
It includes approximately 90% of human cancer. This type is principally derived from epithelial cells of ectoderm and endoderm. The solid tumours in nerve tissue and in tissues of body surfaces or their attached glands are example of carcinomas. Cervical, breast, skin and brain carcinomas are developed from malignant tumour.

(ii) Sarcomas:
Sarcomas are solid tumours of connective tissues such as muscle, bone, cartilage and fibrous tissue. This type of malignant tumours are rare in human (about 2% of human cancer).

(iii) Lymphomas:
It is a type of malignancy in which there is excessive production of lymphocytes by the lymph nodes and spleen. It accounts for approximately 8% of human cancers. Hodgkin's disease is an example of human lymphoma.

(iv) Leukemia's:
This type of malignancy arises from the blood forming cell. Leukemia's are commonly known as blood cancer. Leukemia's are neoplastic growth (uncontrolled cell growth at the cost of remaining cells) of leucocytes or WBC. They are characterised by excessive production of WBC of the blood. The name leukemia is derived from Greek leukos (white) + haima (blood) the massive proliferation of leukemia cells can cause a patient's blood to appear milky.

In addition to the types of cancer mentioned above, cancers are further classified according to tissue of origin, for example lung cancer, breast cancer, and the type of cells involved, for example fibro sarcoma arises from fibroblasts, erythromoid leukemia's from precursor of erythrocytes. Although there are many kinds of cancer, the four most common cancers are those of prostrate, breast, lung and colon/rectum.

Development of Cancer:
The development of cancer is a multistep process in which cells gradually become malignant through a progressive series of alternations. This process involves mutation and selection for cells with progressively increasing capacity for cell division, survival, invasion and metastasis (spread of cancer cells through the blood or lymphatic system to other organ sites).

The first step in the process is when a single cell within a tissue of the organ concerned is genetically modified. The modified cell divides rapidly, although surrounding cells do not— and a mass of tumour cells forms. These cells constitute a clone where cells are identical in terms of structure, characteristics and function. Rapid cell proliferation leads to the tumorous outgrowth or adenoma or polyp. This tumour is still benign.

Tumour progression continues as additional mutation occur within cells of tumour population. Some of these mutations give a selective advantage to the cell such as rapid growth and the descendants of a cell bearing such a mutation will consequently become dominant within the tumour population. This process is known as clonal selection. Clonal selection continues throughout tumour development and, consequently, tumour become more and more rapid, growing and increasingly malignant. The tumour cells, by their rapid proliferation, invades the basal lamina that surrounds the tissue. Then tumour cells spread into blood vessels that will distribute them to other sites in the body. This is known as metastasis. If the tumour cells can exit from the blood vessels and grow at distant site, they are considered malignant (Fig. 23.1).

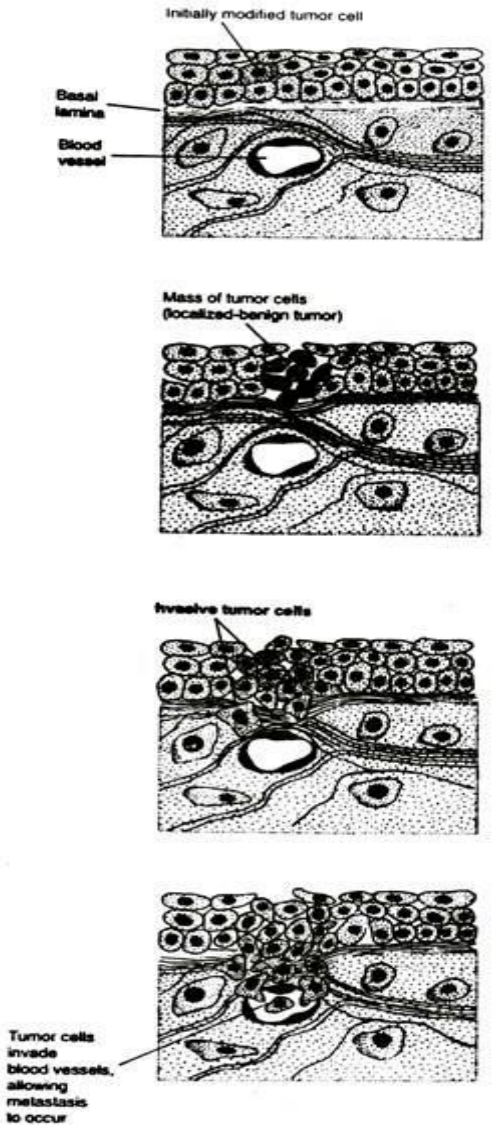


Fig. 23.1: Stages in tumour growth and metastasis.

Characteristics of Cancer Cells:

The uncontrolled growth of cancer cells results from accumulated abnormalities affecting many of the cell regulatory mechanisms. The process of cell change in which a normal cell loses its ability to control its rate of division and thus becomes a tumour cell is called cell transformation.

Cancer cells shows some typical characteristic properties that are absent in normal cells. Sometimes cancer cell properties are just opposite to the properties of normal cell. Cancer cells in vivo differ from their normal counterparts in several respects. Some characteristic properties of cancer cells can also be demonstrated by cell culture in vitro.

(i) Immortalization:

Normal cell culture do not survive indefinitely For example, human cell culture die after about 50 generations. On the other hand, transformed cell cultures can go on indefinitely and remain immortal if the nutrition is provided and overcrowding avoided.

(ii) Loss of Contact Inhibition:

Normal cells growing in tissue culture tend to make cell contacts by adhesion to neighbouring cells. At the points of adhesion some kind of electron-dense plaque is formed in both contacting cells. At the same time there is a slowing down of the amoeboid process which results in contact inhibition of movement. In contrast, cancer cells are unable to form adhesive junctions and do not show this type of contact inhibition. Experimentally, it has been observed that when normal cells have become completely surrounded by other cells, their mobility stops and they form a monolayer. At the same time there is inhibition of growth and the number of cells in the petridish remains practically constant.

On the other hand, cancer cells continue to multiply and pile up forming irregular masses several layers deep. Cancerous cells undergo a change in property of their cell membranes and cell coat such as disappearance of gap junction, loss of coupling changes in glycolipid and glycoprotein and a reduction in gangliosides. In the cell coat fibronectin, a large glycoprotein found in footprints of moving cultured cells is reduced in cancerous cells. These changes enable the cells to dissociate from neighbouring cells and show loss of contact inhibition.

(iii) Reduced Cellular Adhesion:

Most cancer cells are less adhesive than the normal cells due to reduced expression of cell surface adhesive molecules. When normal cells are transformed into cancer cells, then a change of stickiness of their cell membrane results. Normal cells show stickiness or adhesiveness. If normal cells are grown in a liquid nutrient medium kept in a glass vessel, the cells stick to glass wall rather than float in the medium. But when cancer cells are allowed to grow in nutrient medium, they stick to each other less than do normal cells.

Adhesiveness shows considerable specificity. For example, a liver cell tends to stick with another liver cell and not to other types of cell such as kidney cell. Cancerous cells do not show this property. They are able to mix and stick to any type of normal cell. For example, a malignant liver cell can mix and stick to normal kidney cell. Hence this unusual behaviour of cancer cell explains that cancer cells can invade several normal organs.

(iv) Invasiveness:

One of the most important characteristics of cancer cells is their invasiveness. It is the ability to invade other tissues. Malignant cells generally secrete proteases that digest extracellular matrix components, allowing the cancer cells to invade adjacent normal tissues. For example, secretion of collagenase by the cancer cells helps to digest and penetrate through basal laminae to invade the underlying connective tissue.

Cancer cells also secrete growth factors that promote the formation of new blood vessels. This is known as angiogenesis. Angiogenesis is necessary to support the growth of tumour beyond the size of about a million cells at which point new blood vessels are needed to supply oxygen and nutrients to the multiplying tumour cells. Actually the growth factor secreted by the tumour cells stimulates the endothelial cells present in the wall of capillaries. As a result, new outgrowth of the capillaries is formed into the tumour. These outgrowths of capillaries are also helpful for metastasis of malignant cells. Therefore, angiogenic stimulation induces the growth of new blood capillaries which penetrate easily in the tumour tissue and provide the opportunity for the cancer cells to enter the circulatory system. As a result, metastasis process begins.

(v) Failure to Differentiate:

Another general characteristic of most of the cancer cells is that they fail to differentiate. This property is closely related with the abnormal proliferation. Normal cells are fully differentiated. In most fully differentiated cells, cell division ceases. In case of cancer-cells, normal differentiation program is blocked at the early stages of differentiation. The relationship between defective differentiation and rapid proliferation is clearly noted in case of leukemia. All of the different types of blood cells develop from a common pluripotent stem cell in the bone marrow. Some of the descended cells develop erythrocytes but others differentiate to form lymphocytes, granulocytes and macrophages. Cells of each of these types become round as they differentiate but once they become fully differentiated cell division ceases But leukemia cells fail to undergo terminal differentiation. Instead, they become blocked at early stage of maturation at which they retain their capacity for proliferation and continue to divide.

(vi) Auto stimulation of Cell Division:

Cancer cells produce growth factor that stimulates their own cell division. Such abnormal production of a growth factor by the cancer cell leads to continuous auto stimulation of cell division. This is known as autocrine growth stimulation. Hence the cancer cells are less dependent on general growth factor produced within the body physiologically from normal source for inducing growth of all normal cells. It is also noted that the reduced growth factor dependence of cancer cell results from abnormalities in intracellular signalling system.

(vii) Apoptosis:

For every cell, there is a fixed span of life, i.e., time to live and time to die. This cell death is a very orderly process and so it is called Programmed Cell Death or PCD or Apoptosis. Apoptosis is a mechanism of programmed cell death or cell suicide which is essential for the survival of the organism, for the normal development of the organism as the programmed destruction of the organism as the programmed destruction of cells is found during embryo-genesis. It also protects the organism by removing damaged cells which may be due to viral infection or due to exposure to radiations. It also inhibits the tumour development and so any defect in the control of apoptosis may lead to cancer.

There are two methods by which cells may die such as:
1. Death by injury that is through mechanical damage or due to toxic chemicals.

2. By Apoptosis, i.e., through programmed cell death.

(viii) Density-Dependent Inhibition:
One of the primary distinguishing characteristic features between cancer cell and normal cell is that normal cells show density-dependent inhibition of cell division in culture but cancer cells continue to proliferate independent of cell density.

Proliferation of normal cell continues until they reach a finite cell density. Normal cells are very sensitive to cell density. So when they reach a finite density they enter the G_0 state of the cell cycle. But cancer cells continue to divide to high cell density.

(ix) Cellular Characteristics:
Cancer cells can be distinguished from normal cells by microscopic examination. Cancer cells have a high nucleus to cytoplasm ratio, prominent nucleoli, many mitosis, and relatively little specialised structure. Normal cells have a cytoskeleton which consists of microtubules and microfilaments. But the cytoskeleton of cancer cells undergo de-polymerisation and the microtubules disaggregate.

(x) Chromosomal Change:
Normal cell contains normal chromosome number, e.g., normal cells of human beings contain 46 or 23 pairs chromosomes. But in cancer cell the chromosomes can undergo both structural and numerical changes. In human being the parent cell of any cancer has 46 chromosomes Later, after a series of abnormal divisions the cancer cells contain series of chromosome numbers and karyotype. The chromosomes swell up and the number of chromosome sets increase owing to the growth of cancer cells. This condition is known as aneuploidy. Earlier workers have suggested that in different cancer cell populations there are chromosomal stem lines involving a particular spectrum of chromosome structure and number.

An established cancer cell population will have a modal number in most of the cells over quite long periods and it is relatively stable. Generally speaking, no two karyotypes are identical in cancer cell and no typical chromosome group has been found to be involved. Therefore, the occurrence of any aneuploid cells in a particular tissue may have the possibility to become cancerous cell.

(xi) Interaction With Immune System:
A few normal cells may be transformed in pre-cancer cells every day in each of us in response to radiation, certain viruses or chemical carcinogens in the environment. Because they are abnormal cells, some of their surface proteins are different from those of normal body cells. Such proteins act as antigens and stimulate an immune response that generally destroys these abnormal pre-cancer cells.

If the pre-cancer cells are destroyed by the immune system, then how does cancer occur? Further investigation demonstrates that there are some transformed cancer cells whose surface proteins are not so

changed. Hence such cancer cells may remain anti-genetically similar to normal cells. As a result, the immune system cells may fail to distinguish the cancer cell from normal cell. Some workers suggest that sometimes cells of the immune system do recognise cancer cells but are not able to destroy them. In such case, cancer cells can stimulate B cells to produce IgG antibodies that combine with antigens on the surface of the cancer cells. These blocking antibodies may block the T cells so that they are unable to adhere to the surface of the cancer cells and destroy them. For some unknown reason, the blocking antibodies are not able to activate the complement system that would destroy the cancer cells.

Carcinogens:

Many agents including radiation, chemicals and viruses have been found to induce cancer in both experimental animals and humans. Agents which cause cancers are called carcinogens. Radiation (Solar ultraviolet ray, X-ray) and chemical carcinogens act by damaging DNA and inducing somatic mutations. These carcinogens are generally called initiating agent because the induction of mutations in key target genes is supposed to be the initial event leading to cancer development.

Some of the initiating agents that cause human cancers include solar ultraviolet radiation—the major cause of skin cancer. The exposure of the thyroid gland to X-rays greatly increases the incidence of thyroid cancers. Varieties of chemical carcinogen including tobacco smoke (containing benzo(a)pyrene, dimethyl nitrosamine and nickel compound) and aflatoxin produced by some molds are the major identified cause of human cancer. Other carcinogens induce the cancer development by stimulating cell proliferation rather than inducing mutations. Such compounds axe called tumour promoters. The first suggestion that chemicals can cause cancer dates back to 1761, when a doctor noted that people who use snuff suffer from nasal cancer. A few years later a British physician observed a high incidence of cancer of the scrotum among the chimney-sweepers iii their youth. He explained the fact that the chimney soot became dissolved in the natural oil of the scrotum, irritating the skin and, consequently, initiates the development of cancer. On the basis of two separate observations it became evident that certain chemicals (Table 23.1) can cause cancer. Later, as the industrial revolution moved into twentieth century, more and more incidence of cancer were reported among the workers who were continuously exposed to industrial chemicals.

Table 23.1: Gene/Factors

(a) Initiating Stimuli	Function
Tumour Necrosis Factor α receptor family (TNF)	Death signal
Ceramide	gives signal for apoptosis induction.
FAS/Apo–1	Death signal like TNF; For peripheral deletion of T lymphocytes.
Nur 77 (Zinc finger containing steroid receptor)	Death signal in thymocytes.
(b) Inducing Cellular susceptibility	
c-mye	produces myc protein which gives cell susceptibility for apoptosis
Rb–1	Deficiency of Rb–1 gives susceptibility.
	Rb protein may inhibit P 53 mediated apoptosis
E2F1	induces susceptibility
P 53	apoptosis in response to cell injury is dependent on P 53.
(c) Modulating factors	
DAD 1 gene	gives signal for cell death
BCl–2 gene family	Some members inhibit cell death, such as bcl–2, BCl–X. Members which promote death like bax, bid and bad.
(d) Effector mechanisms	
Caspases, ICE, Ich–1	Genes encoding cysteine proteases which are involved in the effector pathway of apoptosis.

In the early 1940s Peyton Rous observed that repeated application of coal tar to rabbit skin causes tumour to develop, but the tumour disappears when application of the coal tar is stopped. It is also noted that when the skin is treated with turpentine, tumour again reappears.

Normally turpentine does not cause cancer itself. Therefore the coal tar and turpentine are playing two different roles. Some carcinogens induce some normal cells to become irreversibly altered to a pre-neoplastic state. This is known as initiation and the carcinogens are known as initiation agents. Here coal tar is an initiating agent. On the other hand, some carcinogens stimulate the pre-neoplastic cells to divide and form tumour. This is known as promotion and the carcinogens are termed promoting agents. Here turpentine behaves as promoting agents. Berenblum observed that painting the skin of a mouse a single time with methylcholanthrene rarely causes the development of tumours. But subsequently application of castor oil (an oil derived from seeds of Croton tiglium) triggers the formation of multiple tumours on the skin which has been exposed previously to methylcholanthren is acting as an initiator whereas castor oil acts as a promoter. Initiation is a quick, irreversible process that causes a permanent change in a cell's DNA. The carcinogenic chemicals that act as initiating agent are capable to bind with DNA. Hence they interfere with the normal function of DNA and induce somatic mutation and, consequently, bring about stable, inheritable changes in the cell's properties.

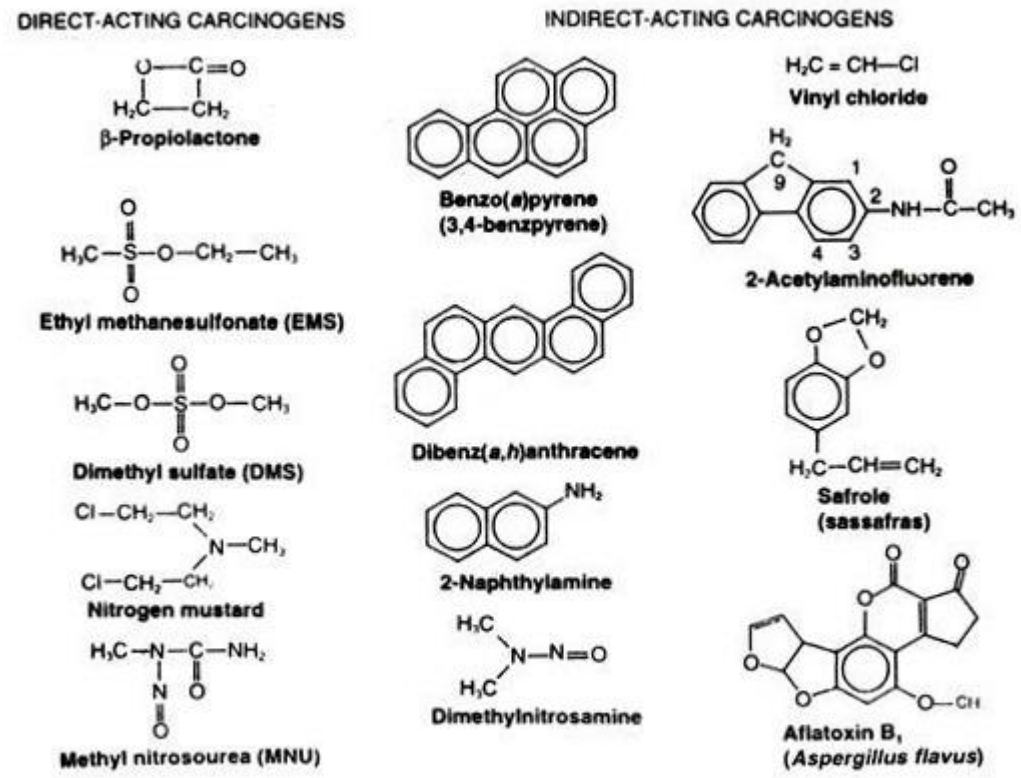


Fig. 23.2: Structure of some direct acting and indirect acting chemical carcinogens.

On the basis of action of chemical carcinogens on DNA, there are two broad categories of carcinogens—direct acting and indirect acting (Fig. 23.2). Direct acting carcinogens are highly electrophilic compounds that react with DNA. Indirect acting carcinogens are converted to ultimate carcinogens by introduction of electrophilic centres. In other words, indirect acting carcinogens must be metabolised before they can react with DNA.

The steps of metabolic activation of benzo(a)pyrene—a polycyclic aromatic hydrocarbon—are shown in Fig. 23.3.:

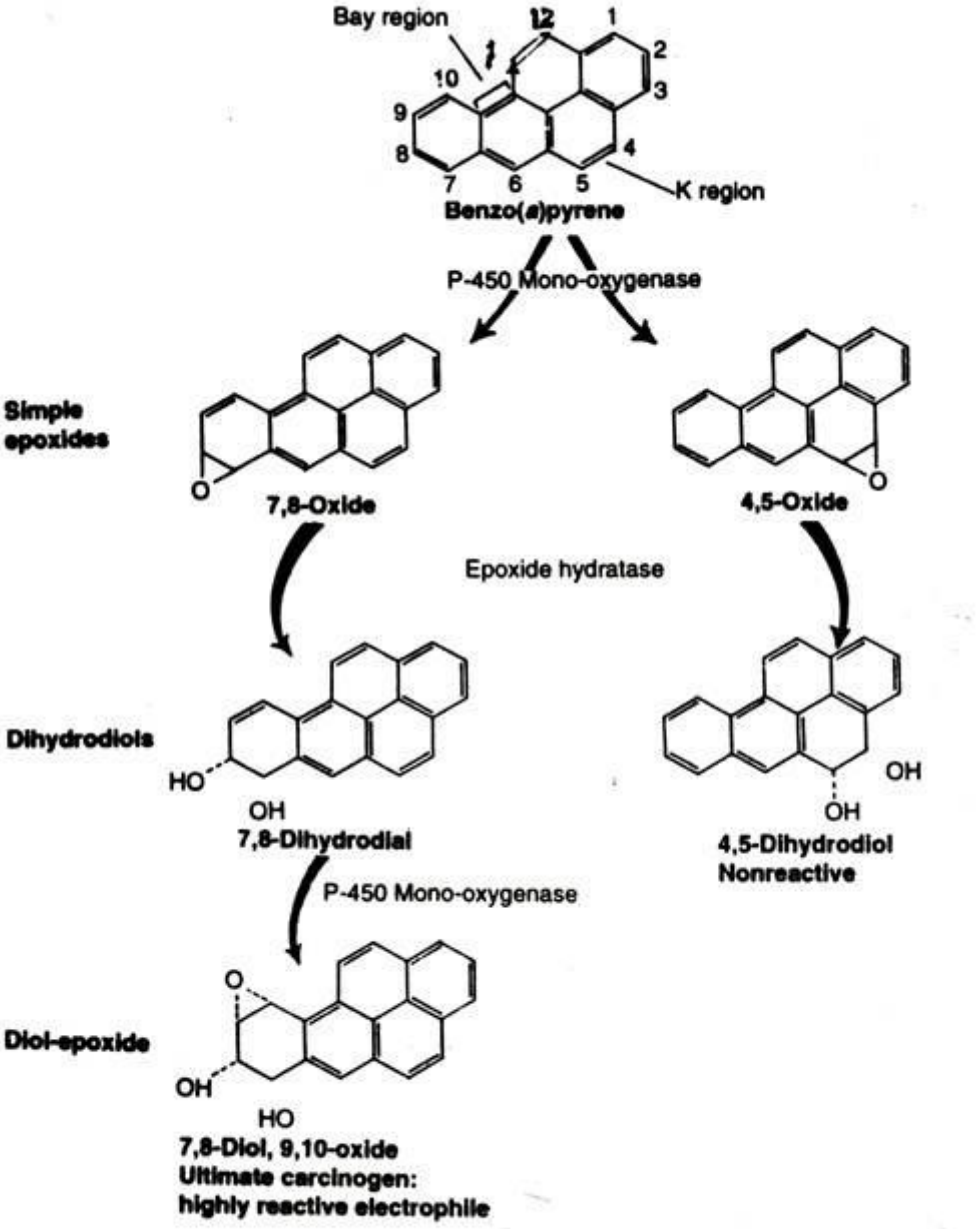


Fig. 23.3: Steps of metabolic activation of benzo(a)pyrene—a powerful carcinogen.

On the other hand, promotion is a gradual, partially reversible process that needs prolonged exposure to promoting agents. If a cell that has already undergone initiation is exposed to a promoting agent, the cell starts to divide and the number of genetically damaged cells goes up. As the damaged cells continue to divide, a gradual selection for cells showing higher growth rate and invasive properties occurs—leading to the formation of malignant tumour. The promotion phase continues for longer period. That is why cancer does not develop just after exposure to a carcinogenic agent. The mechanism of action of promoting agents have come from the studies of phorbol esters which are present in castor oil and act as tumour promoters. Phorbol esters bind to the plasma membrane and activate protein kinase C. Protein kinase C is a component of the phosphoinositide signalling pathway whose activity is normally controlled by the second messenger, diacylglycerol. The activation of protein kinase C leads to phosphorylation of many target proteins and, consequently, activates the transcription factor API which switches on the

transcription of genes involved in stimulating cell proliferation. Therefore, the mode of action of phorbol esters gives an insight into the possible mechanism of action of a promoting agent. Energy that travel through space is known as radiation. Natural source of radiation to which humans are generally exposed are ultraviolet rays, cosmic rays and emission from radioactive elements. We are also exposed to another high- energy radiation like X-ray. Medical, industrial and military activities generally create the high-energy radiation. Sunlight has the ability to cause skin cancer in people who spend long hours in the sunlight. Sunlight contains ultraviolet rays which are also absorbed by normal skin pigmentation. Hence, for this reason, dark-stained or black people usually have lower rates of skin cancer than fair- skinned individual.

Because ultraviolet radiation is very weak to pass through the skin, it does not induce any other type of cancer except skin cancer. It is more or less restricted superficially on skin because skin cancer rarely metastasizes. This type of cancer can be cured by easily removing the affected site surgically. Xeroderma pigmentosum is a type of inherited malignant disease. Individuals with this malignant disease develop extensive skin tumours after exposure to sunlight. Homozygotes for the autosomal recessive mutation responsible for xeroderma pigmentosum are less efficient in the repair of DNA damaged by exposure to ultraviolet light.

X-rays are high energy radiation. They are strong enough to penetrate the skin and reach internal organs. X-rays thus make a serious cancer hazard because they are able to induce gene mutation or DNA damage. Many radioactive elements emit radiation. It also acts as carcinogen and causes cancer. Marie Curie, the co-discoverer of the radioactive elements polonium and radium, died of a form of leukemia that appeared to be caused by her extensive exposure to radioactivity. Another example of radiation-induced cancer occurred in New Jersey in 1920. A group of women was employed by a factory that produced watch which glow in the dark. The luminescent point used to point the watch dial contained radium. The paint was applied with a fine-tipped brush that the employee frequently wetted with their tongue. During this process, minute quantities of radium were ingested through saliva in the digestive system from where they were readily abs, -bed and distributed in the different cells ana tissues thorough circulatory system. Several years later these women suffered from bone cancer caused by radioactive radium that had gradually become concentrated in their bone. The most well-known horrifying examples of radiation-induced cancer occurred in Japan and in Nevada of United States. In 1945 atomic bombs were exploded over Hiroshima and Nagasaki. The massive fallout of radioactive elements increased the incidence of leukemia, lymphomas and cancers of the thyroid, breast,' uterus and gastrointestinal tract.

Similarly, in Nevada, people suffered from cancer due to the radioactive fallout during nuclear bomb testing. It is suggested that radioactive carcinogen is thought to initiate malignant transformation by causing DNA damage. Alternatively, it is also explained that subsequent exposure of radiation damaged cells to promoting agents stimulates the cell to divide abnormally and form tumour. There are many viruses which are capable of causing tumour in animals, human as well as plants (Table 23.2). These viruses are called tumour viruses or oncovirus. Some tumour viruses have RNA genome and are known as DNA tumour viruses. Some tumour viruses have DNA genome and are known as retroviruses. Retrovirus replicates via synthesis of a DNA provirus in the infected cells. In addition, HIV is indirectly responsible for the cancer that develops in AIDS patient as a result of immunodeficiency.

Table 23.2: List of Chemical Carcinogens and Type of Cancer induced by such chemicals

Carcinogen	Type of cancer induced
Acrylonitrile	Colon, lung
4-Aminodiphenyl	Bladder
Aniline derivatives	Bladder
Arsenic compounds	Lung, skin
Asbestos	Lung, mesothelium
Benzene	Leukemia
Cadmium salts	Prostate, lung
Carbon tetrachloride	Liver
Chromium and chromates	Lung, nasal sinuses
Diethylstibestrol (DES)	Uterus, vagina
Lead	Kidney
Mustard gas	Lung, larynx
α-Naphthylamine	Bladder
Nickel	Lung, nose
Organochloride pesticides	Liver
Polychlorinated biphenyls	Liver
Radon	Lung
Soot and tars	Skin, lung, bladder
Vinyl chloride	Liver, lung, brain
Wood and leather dust	Nasal sinuses
Tobacco smoke, which contains the following:	Lung, oral cavity, larynx, esophagus, stomach, pancreas, others
Aminostilbene, arsenic, benz[*a*]anthracene, benz[*a*]pyrene, benzene, benzo[*b*]fluoranthene, benzo[*c*]phenanthrene, benzo[*j*]fluoranthene, cadmium, chrysene, dibenz[*a,c*]anthracene, dibenzo[*a,e*]fluoranthene, dibenz[*a,b*]acridine, dibenz[*a,j*]acridine,dibenzo[*c,g*]carbazone, N-dibutylnitrosamine, 2,3-dimethylchrysene, indeno[*1,2,3-c,d*]pyrene, S-methylchrysene, S-methylfluoranthene, α-naphthylamine, nickel compounds, N-nitrosodimethylamine, N-nitrosomethylethylamine, polonium-210, N-nitrosodiethylamine, N-nitrosonornicotiné, N-nitrosoanabasine, N-nitrosopiperidine	

The herpes viruses are the most complex animal viruses. The genome length of these viruses is 100-200 Kb. Many herpes viruses cause tumour in many animals such as frogs, chickens, monkeys etc. Epstein-Barr virus, a member of herpes virus, can trigger the development of some human malignancies including Burkett's lymphoma in some region of Africa and nasopharyngeal carcinoma in China. It also causes B-cell lymphomas in AIDS patient and other immunosuppressed persons. Cell transformation by herpes viruses is not fully understood because of the complexity of their genome. But it is evident that some viral genes are required to induce transformation of lymphocytes.

Of the DNA tumour viruses, the papoviruses are the best studied DNA tumour viruses from the standpoint of molecular biology and have received particular attention because they have been critically important as models for understanding the molecular basis of cell transformation. The genome size of papoviruses is small (approximately 5 Kb). Simian virus 40 (SV_{40}) and polyomavirus are the important and commonly

known member of papoviruses. Both these viruses are similar in size and general structure. A virus usually multiplies in specific cells derived from animals in which the virus normally grows. Such cells are called permissive cells. Cells which do not allow the viruses to grow are called non-permissive cells. SV_{40} and polyoma viruses, on entering their respective host cells, undergo one of the two types of behaviour—they enter the permissive cell of the host, undergo the lytic phase, and multiply within host cell, ultimately killing them. Since a permissive cell is killed as a consequence of virus replication, it cannot become transformed. Sometimes viruses enter non-permissive cells and are not able to multiply, i.e., virus replication is blocked. In this case, the viral genome sometimes integrates into cellular DNA and expression of specific viral genes results in transformation of the infected cells.

The SV_{40} and polyoma virus genes that trigger cell transformation have been identified, isolated and sequenced by molecular analysis. The genome of SV_{40} and polyomavirus are divided into early and late regions. The early region is expressed immediately after infection and is needed for synthesis of viral DNA. The late region is not expressed until after viral DNA replication has begun. The early region of SV_{40} codes for two proteins which are known as small (17 Kd) and large (94 Kd) T-antigens. In addition to small and large regions, the genome of polyomavirus contains a third early region which is called as middle T region. It codes for a protein of about 55 Kd.

Experimentally, it has been shown that large T of SV_{40} is sufficient to induce transformation and the middle T region of polyoma virus is primarily responsible for transformation. During lytic cycle, the early region proteins are needed to initiate viral DNA replication as well as to stimulate host cell gene expression and DNA synthesis. Since the replication viral DNA is dependent on host cell enzymes, therefore stimulation of gene expression of the host cell is a critical event in the viral life cycle. Most of the cells of adult animal cells become non-dividing. So the enzymes required for cell division are not available within the cell. Therefore they must be stimulated to divide in order to induce the enzymes needed for viral DNA replication. This stimulation of cell division by the early gene products of virus can lead to transformation if the viral DNA becomes stably integrated and expressed in a non permissive cells. The early region proteins of SV_{40} and polyoma virus induce transformation by interacting with host proteins that regulate cell division.

The papilloma viruses are small DNA viruses. The genome length of such viruses is approximately 8 Kd. Some of these viruses induce only benign tumours such as warts. But some others cause malignant carcinomas— particularly cervical and anogenital cancers. Cell transformation by papilloma viruses occurs from the expression of two early region genes E_6 and E_7. The hepatitis B viruses are another group of DNA virus. They have the smallest genomes which is approximately 3 Kb. These viruses mainly infect the liver cells and cause liver damage. But how they induce cell-transformation is not clearly known.

Possibly tumour results from expression of a viral gene. Alternatively, the chronic cell damage of liver simply induce the continuous cell division which, ultimately, causes the cell transformation. The retroviruses, one family of RNA viruses, also cause human cancer. For example, human T-cell lymphotropic virus type-I (HTLV- I), a RNA virus, is the causative agent of T-cell leukemia. A related virus (HTLV-II) cause a rare form of leukemia called hairy T- cell leukemia. HIV (Human

immunodeficiency virus) is the causative agent of AIDS. These viruses, i.e., HTLV-I, HTLV-II, HIV, actually does not cause cancer by directly converting a normal cell into a tumour cell. The AIDS patients become susceptible to high incidence of some malignancies like lymphomas and Kaposi's sarcoma due to immunosuppression of the patient. RNA viruses have an RNA genome which is extended at either end by a long terminal repeat (LTR). The LTR contains many of the signals that allow retrovirus to function (Fig. 23.4). Retroviruses use their genomic RNA as a template to make DNA with the help of reverse transcriptase. This DNA is then integrated into host's DNA as DNA the provirus. The DNA provirus is transcribed to yield genome length RNA provirus directed transcription involves a promoter—a sequence that directs the RNA polymerase to a specific initiation site and an enhancer—a sequence that facilitates transcription.

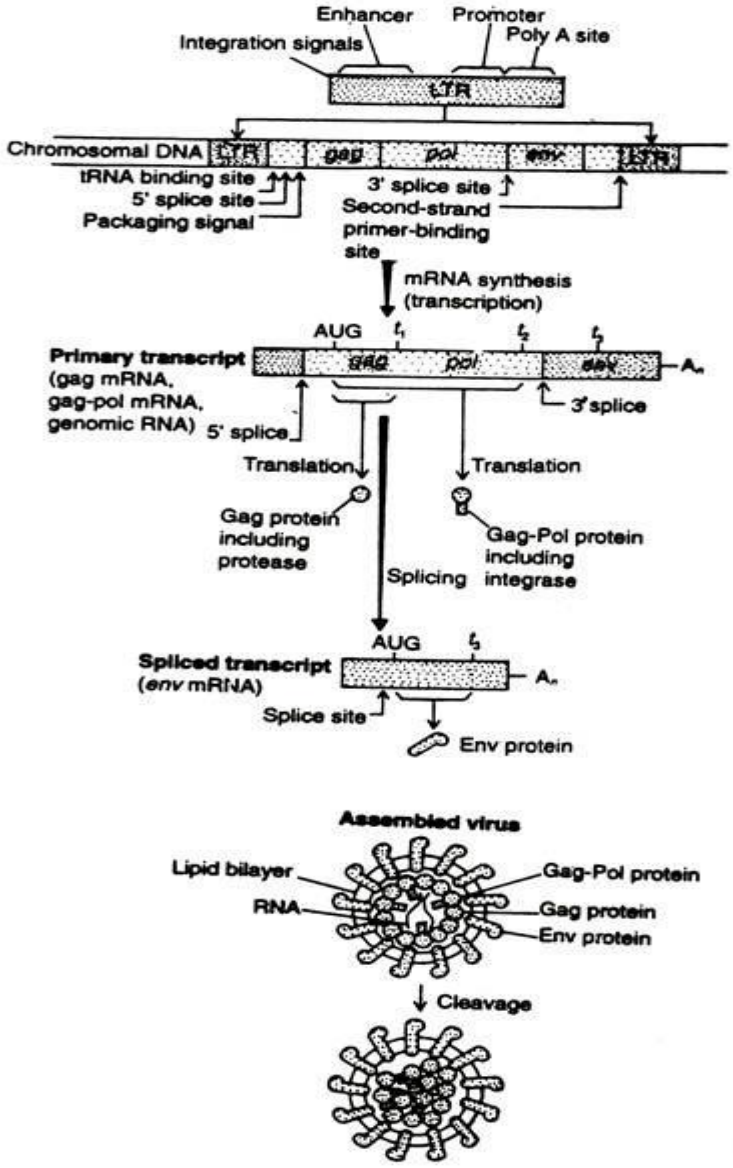


Fig. 23.4: Genetic elements of proviral DNA and the corresponding gene products.

The promoter and enhancer are located in the LTR. The primary transcript serves as the genomic RNA for progeny virus particles and as mRNA for the gag and pol genes. In addition the full length RNA is spliced to yield mRNA for env.

The gag gene encodes the viral protease and structural proteins of the virus particle, pol encodes reverse transcriptase and integrase and env encodes envelope glycoproteins. These three genes are only required for viral replication but play no role in cell transformation.

This type of retrovirus causes tumour only when any mutation results at the time of integration of pro-viral DNA within or adjacent to host's genome. But there are some other retroviruses which contain specific genes which are responsible for the induction of cell transformation and acts as potent carcinogens. The first cancer causing gene is f6und in the retrovirus called Rous Sarcoma virus (Fig. 23.5) that produces sarcomas in chicken. It was later named src gene. Genes like src which are capable of inducing malignant transformation, are referred to as oncogenes. The identification of the first viral oncogene has provided a model for understanding many aspects of cancer development at the molecular level.

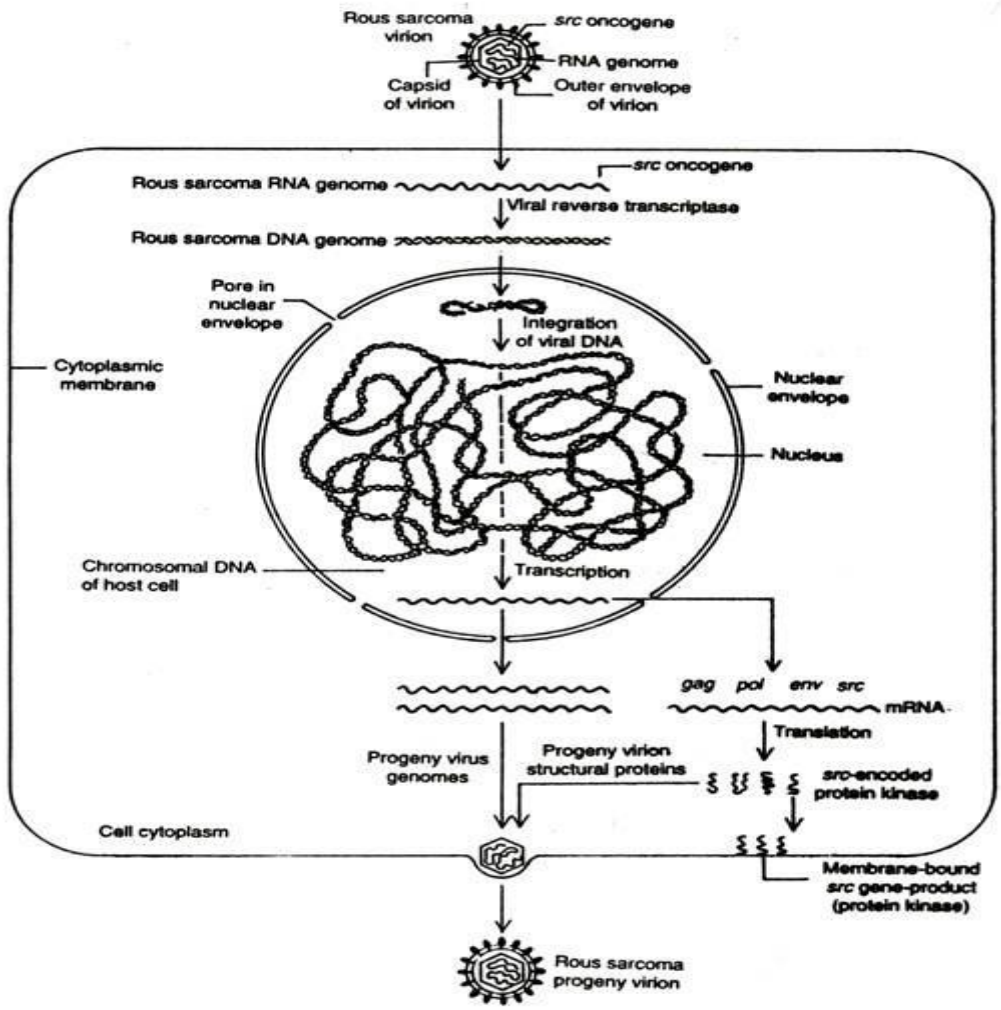


Fig. 23.5: Life cycle of Rous Sarcoma RNA tumour virus.

Proto-oncogene:

All 20 viral oncogenes derive from cellular genes in normal cells. The normal cellular version of the gene is called a proto-oncogene. Retroviruses pick up into their genome sequences from mRNA population that will increase viral production (by increasing cell proliferation). These viruses can transmit genes from one species to another, thus breaking evolutionary barriers (Bishop, 1983).

Another agent that stimulates proliferation, epidermal growth factor (EGF), like Svc, increases phosphorylation of membrane proteins at tyrosines. It is thought that this tyrosine phosphorylation somehow controls cell proliferation. Cancer cells (also called transformed cells) contain ten times more phosphotyrosine than normal cells. About half of the oncogenes code for Tyr protein kinases and in all cases the proteins are integral components of the cell membrane. Most oncogenes seem to be related in one way or another with the same pathway of regulation of cell proliferation by protein growth factors.

EGF stimulates lung development and differentiation. In intact organisms, the production of growth factor induces multiplication at a short distance only, stimulating the cell that secretes it and the nearby cells. This is sometimes called autocrine secretion. When EGF reaches the membrane of a target cell it binds to a specific receptor protein of 170,000 Daltons, and the complex is subsequently internalized. The receptor protein becomes phosphorylated during this process and the phosphorus binds to tyrosine. Phosphorylation of tyrosine is rare in proteins except in cells that have been transformed by retroviruses and become cancerous. Both in EGF and in viral transformation phosphorylation of tyrosine results in increased cell proliferation. Cells start dividing only after many hours of adding the growth factors. Growth inhibitors, called chalones (Gr., to slow down) are numerous, which have been isolated from tissues. These are also numerous and important as growth factors.

Oncogenes:

Oncogene is a type of specific viral gene that is capable of inducing cancer or cell transformation—either in the body of host or in the tissue in culture. After the discovery of src oncogene in RSV, more than 40 different highly oncogenic retroviruses have been isolated (Table 23.3) from a variety of animals like mice, rat, cat, chickens, turkeys, monkeys etc.

All these viruses contain at least one (in some cases two) oncogene like RSV. These oncogene are not needed for viral replication but is responsible for cell transformation. In some cases different viruses contain the same oncogenes. Many of these genes encode protein which, in turn, acts as the key components of signalling pathways that induces cell transformation.

Table 23.3: Examples of Tumour Viruses

Class	Examples	Tumours induced	Organism
DNA viruses:			
Herpesviruses	Lucke virus	Kidney adenocarcinoma	Frogs
	Epstein-Barr virus (EBV)	Burkitt's lymphoma, nasopharyngeal carcinoma	Humans
	Marek's disease virus	Lymphoma	Chickens
Papovaviruses	Shope papilloma virus	Papillomas	Rabbits
	SV-40	Subcutaneous, kidney and lung sarcomas	Hamsters
	Polyoma	Liver, kidney, lung, bone, blood vessels, nervous tissue, connective tissues	Mice
	Human papillomaviruses	Cervical cancer	Humans
Hepatitis B virus		Liver cancer	Duck, Woodchucks, squirrels, human
Adenoviruses	Human adenoviruses	Subcutaneous, intraperitoneal, intracranial	Hamsters
RNA viruses:			
B-type viruses	Bittner mammary tumor virus	Mammary carcinoma	Mice
C-type viruses	Rous sarcoma virus	Sarcomas	Birds, mammals
	Murine leukemia viruses (Gross, Moloney, Friend, Rauscher, and others)	Leukemia	Mice
	Feline leukemia virus	Leukemia	Cats
	Murine sarcoma virus	Sarcoma	Mice
	Feline sarcoma virus	Sarcoma	Cats
	Avian leukemia viruses (avian myeloblastosis and others)	Leukemia	Chickens
	Human T-cell leukemia virus	Leukemias/Lymphomas	Humans
Plant viruses	Wound tumor virus	Roots and stems	Plants

Oncogene in Human Cancer:

Direct evidence for the involvement of cellular oncogenes (the term cellular oncogene is generally used to distinguish this group of cancer- causing genes from viral oncogenes) in human tumour was first derived from gene transfer experiment carried out in the laboratories of Robert Weinberg and Geoffrey Cooper in the early 1980s.

In this process, a DNA segment isolated from tumour cells are artificially introduced into normal cells to see its subsequent changes. DNA isolated from a human bladder carcinoma was found to efficiently induce malignant transformation of recipient mouse cells in culture. This experiment reveals that the human tumour contains a cellular oncogene. The first human oncogene identified in gene transfer experiment was the ras oncogene. The ras oncogenes are not present in normal cells, but they are generated in tumour cells as a consequence of point mutation of the ras proto-oncogene. This results in the change of a single amino acid at critical position of the ras protein molecule encoded by ras gene.

The first such mutation was the substitution of valine for glycine at position 12. A single nucleotide, change which alters codon 12 from GGC (Gly) to GTC(Val) is responsible for the transforming activity. This is detected in bladder carcinoma DNA.

The ras gene encodes membrane-bound guanine-nucleotide binding proteins (G- protein) that plays a central role in the transmission of singles from receptor-bound external growth factor to the cell interior. During this process, GTP is hydrolysed into GDP. Therefore, Ras protein alternates between active (GTP bound) and inactive (GDP bound) states. But oncogenic ras proteins remain in the active GTP bound state and drive unregulated cell proliferation leading to the development of malignancy. In human tumour, point mutation is an important mechanism by which proto-oncogenes are converted into oncogenes. Besides this, the gene rearrangement—resulting mainly from chromosome translocation—sometimes lead to the conversion of proto-oncogene to oncogene. The classical example regarding the conversion of proto-oncogene to oncogene due to translocation of chromosome is the Burkitt's lymphoma. It produces the malignancy of the antibody producing B-lymphocytes. In this case a piece of chromosome(s) 8 carrying c-myc proto-oncogene is trans-located to the immunoglobulin heavy chain locus on chromosome 14 (Fig. 23.6). Since the antibody genes are extremely active in lymphocytes, the transcriptional regulation of the adjacent myc proto-oncogene is disturbed, resulting in an abnormal pattern of synthesis of the myc protein product.

Such abnormal pattern of expression of the c-myc gene—which encodes transcription factor normally induced in response to growth factor stimulation—is sufficient to drive cell proliferation and contribute to tumour development.

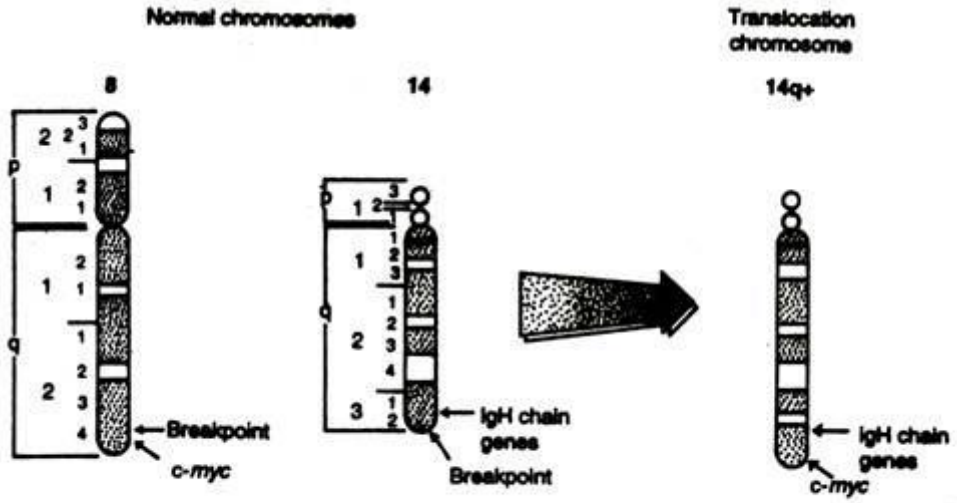


Fig. 23.6: Translocation of a c-myc protooncogene from chromosome 8 to 14.

Translocation of some proto-oncogene often causes the rearrangement of coding sequences which lead to the formation of abnormal gene products. In chronic myelegenous leukemia, the abl proto-oncogene is trans-located from chromosome 9 to chromosome 22 forming Philadelphia chromosome (Fig. 23.7).

The abl proto- oncogene which contains two alternative first exon (1A and IB) is joined to the middle to the bcr gene on chromosome 22. Exon IB is deleted as a result of the translocation. Transcription of the fused gene initiates at the bcr promotor and continues through abl. Splicing then generates a fused bcr/abl mRNA, in which abl exon 1A sequences are also deleted and bcr sequences are joined to abl Exon 2.

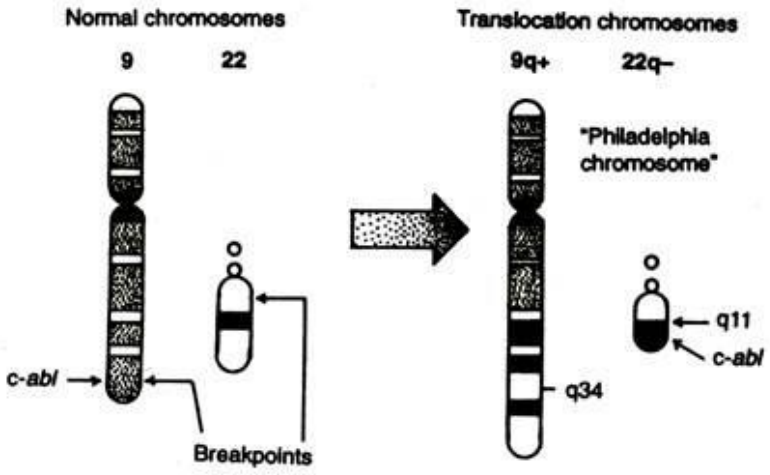


Fig. 23.7: Reciprocal translocation between chromosomes 9 and 22 that produce Philadelphia chromosome.

The bcr/abl mRNA is translated to yield a recombinant bcr/abl fusion protein in which the normal amino terminus of abl proto-oncogene has been replaced by bcr amino acid sequences. The fusion of bcr sequences results in aberrant activity and altered subcellular localisation of the abl protein tyrosine kinase, leading to cell transformation. Gene amplification occurring in the tumour cell is a common process by which proto- oncogenes are converted to oncogene. Gene amplification takes place due to an increase of the number of copies of a gene resulting from the repeated replication of a region of DNA. Therefore, gene amplification leads to the overproduction of a particular protein or enzyme from the amplified gene. A prominent example of oncogene amplification is the involvement of the N-myc gene in neuroblastoma, a tumour of embryonal neuronal cells.

Amplified copies of N-myc gene are frequently present in rapidly growing tumour. Hence it indicates that N-myc amplification is related with the development of neuroblastomas. Amplification of erb B-2 which encodes a receptor protein kinase is similarly associated to the development of breast and ovarian carcinomas.

Table 23.4: Retroviral Oncogenes

Oncogene	Virus	Species
abl	Abelson leukemia	Mouse
akt	AKT8 virus	Mouse
cbl	Cas NS-1	Mouse
crk	CT10 sarcoma	Chicken
erbA	Avian erythroblastosis-ES4	Chicken
erbB	Avian erythroblastosis-ES4	Chicken
ets	Avian erythroblastosis-E26	Chicken
fes	Gardner-Arnstein feline sarcoma	Cat
fgr	Gardner-Rasheed feline sarcoma	Cat
fms	McDonough feline sarcome	Cat
fos	FBJ murine osteogenic sarcome	Mouse
fps	Fujinami sarcome	Chicken
jun	Avian sarcoma-17	Chicken
kit	Hardy-Zuckerman feline sarcome	Cat
maf	Avian sarcoma-AS42	Chicken
mos	Moloney sarcoma	Mouse
mpl	Myeloproliferative leukemia	Mouse
myb	Avian myeloblastosis	Chicken
myc	Avian myelocytomatosis	Chicken
qin	Avian sarcoma 31	Chicken
raf	3611 murine sarcoma	Mouse
rasH	Harvey sarcoma	Rat
rasK	Kirsten sarcoma	Rat
rel	Reticuloendotheliosis	Turkey
ros	UR2 sarcoma	Chicken
sea	Avian erythroblastosis-S13	Chicken
sis	Simian sarcoma	Monkey
ski	Avian SK	Chicken
src	Rous sarcoma	Chicken
yes	Y73 sarcome	Chicken

Subsequent studies have discovered a number of oncogenes (Table 23.4) which are associated with human tumour. Among them chromosomal location of some oncogenes are shown in Fig. 23.8.

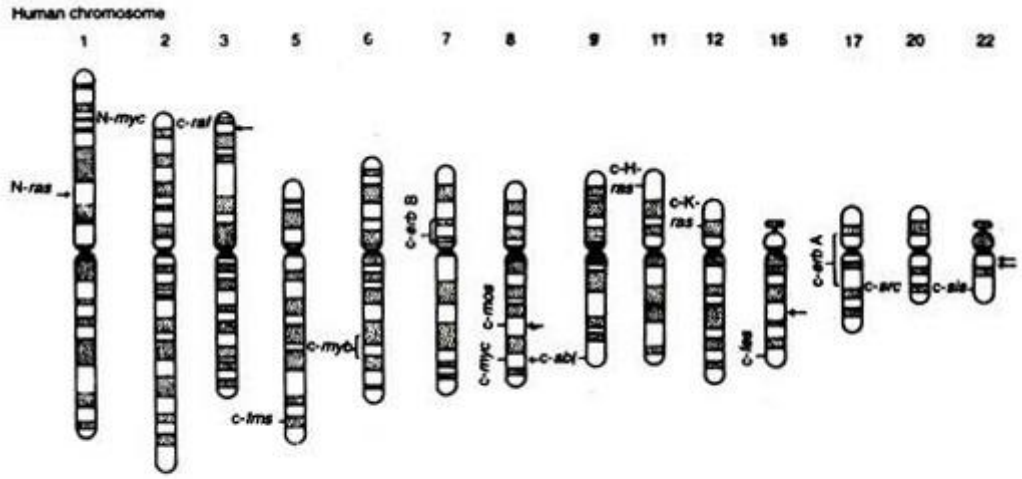


Fig. 23.8: Chromosomal location of some human protooncogenes.

Functions of Oncogene Products:

We have understood that alternation in normal genes, proto-oncogenes, can convert them into oncogenes that code for proteins that are abnormal in structure or are produced in inappropriate amounts. The proteins encoded by the normal genes regulate normal cell proliferation. But the protein encoded by the corresponding oncogene proteins drives the uncontrolled proliferation of the cancer cells.

In addition, some oncogene products involved in other aspects of the behaviour of cancer cells such as defective differentiation and failure to undergo programmed cell death. Besides this, majority of oncogene proteins function as elements of the signalling pathways that regulate cell proliferation in response to growth factor stimulation. These oncogene proteins include polypeptide growth factors, growth factor receptors, elements of intracellular signalling pathway and transcriptional factors (Table 23.5).

Table 23.5: Representative Oncogenes of Human Tumours

Oncogene	Type of cancer	Activation mechanism
abl	Chronic myelogenous leukemia, acute lymphocytic leukemia	Translocation
bcl-2	Follicular B-cell lymphoma	Translocation
E2A/pbx1	Acute lymphocytic leukemia	Translocation
erb B-2	Breast and overian carcinomas	Amplification
gip	Adrenal cortical and ovarian carcinomas	Point mutation
gli	Glioblastoma	Amplification
gsp	Pituitary and thyroid tumors	Point mutation
hox-11	Acute T-cell leukemia	Translocation
lyl	Acute T-cell leukemia	Translocation
c-myc	Burkitt's lymphoma	Translocation
c-myc	Breast and lung carcinomas	Amplification
L-myc	Lung carcinoma	Amplification
N-myc	Neuroblastoma, lung carcinoma	Amplification
PML/RA/Rα	Acute promyelocytic leukemia	Translocation
PRAD1	Parathyroid adenoma	Translocation
PRAD1	Breast carcinoma	Amplification
rasH	Thyroid carcinoma	Point mutation
rasK	Colon, lung, pancreatic, and thyroid carcinomas	Point mutation
rasN	Acute myelogenous and lymphocytic leukemias, thyroid carcinoma	Point mutation
ret	Thyroid carcinoma	DNA rearrangement

If the oncogenes induce uncontrolled cell growth that leads to cancer then it is obvious that the products of these genes would act by stimulating all division in some manner. For example, the product of the v-sis oncogene (the v stands for virus) of simian sarcoma virus is closely related to a polypeptide growth hormone called platelet-derived growth factor (PDGF). This factor produced by platelets promotes wound healing by stimulating growth of cells at wound site.

Simian sarcoma virus with v-sis gene in their genome when injected into the body of woolly monkey, induce sarcoma. They are also able to transform fibroblasts growing in culture to a tumorous state. This type of cellular transformation occurs by a mechanism which is possibly related to the effect of normal PDGF on cells at the wound site. Other oncogenes encode products that are identical to growth hormone as well as hormone receptors. For example, oncogene erb B and fms encode proteins that are closely related to the receptors for epidermal growth factor (EGF) and colony stimulating factor-1 (CSF-1).

CSF-1 is a growth factor that stimulates growth and differentiation of macrophages. The receptor of this growth factor is a trans membrane-protein with growth factor domains on the outside of the cell and protein kinase domains on the inside of the cell (Fig. 23.9).

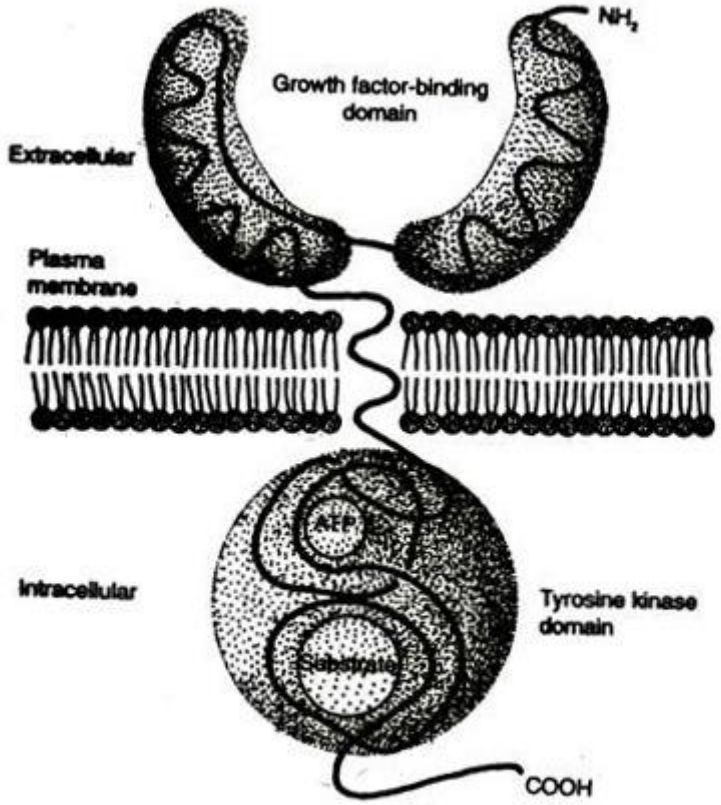


Fig. 23.9: Structure of transmembrane growth factor receptors.

These receptors are key components in trans-membrane signalling pathways. The erb A gene product is an analog of the nuclear receptor for the thyroid hormone T_3. Therefore, all of the gene products are undoubtedly involved in intercellular communication circuit which control cell division during the growth and development of highly differentiated tissue.

Protein tyrosine kinase is a trans-membrane receptor that is capable of transmitting a perfect signal instructing a cell to divide. Alternation in the structure and function of this enzyme will transmit a wrong signal instructing the cell to divide when it normally should not divide—the result will be tumour formation. Following the discovery that the src oncogene codes for a protein kinase, more than 20 other oncogenes have also been found to code for protein tyrosine kinases. These oncogene encoded tyrosine kinases can be subdivided into two main classes such as receptor protein tyrosine kinases and non-receptor protein tyrosine kinases. Receptor protein tyrosine kinases are trans-membrane proteins that contain a growth factor receptor domain which are exposed on the outer surface of the plasma membrane and a tyrosine kinase catalytic domain at the inner surface of the plasma membrane. In a normal receptor of this type, first appropriate growth such as PDGF, EGF, binds with receptors site and activates protein tyrosine kinase domain. Activation of protein tyrosine kinase stimulates cell proliferation through activation of the membrane associated G protein Ras (Fig. 23.10).

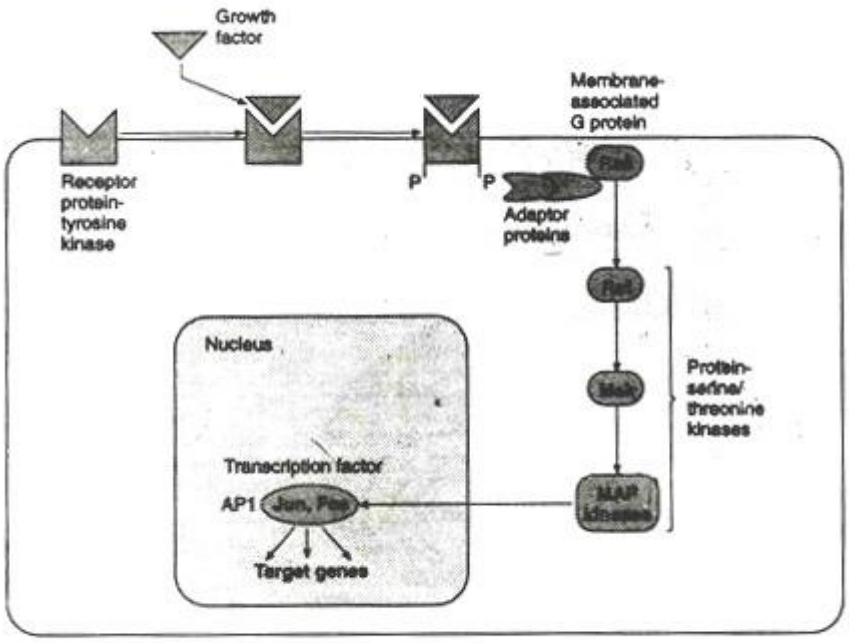


Fig. 23.10: Activation of protein tyrosine kinase that stimulates cell proliferation through activation of the membrane associated G protein Ras.

Activation of Ras triggers the phosphorylation of a series of cytoplasmic protein-serin/theronine kinase, thereby leading to phosphorylation of the nuclear API transcription factor which, in turn, activates genes involved in stimulating cell proliferation. Oncogenes can code for abnormal receptor protein- tyrosine kinases in which the growth factor binding site is disrupted leading to unregulated activity of the protein tyrosine kinase site.

Non-receptor protein tyrosine kinase are usually bound to the membrane's cytoplasm or free in the cytosol. The non-receptor protein tyrosine kinase is encoded by the src gene. ncogene-encoded non-receptor kinases often show excessive unregulated protein-tyrosine kinase activity. Another group of oncogenes code for plasma membrane associated G proteins. In human cancer, ras oncogene shows almost resemblance with cellular ras gene of the host except that ras oncogene is the mutant form in contrast to cellular ras gene. Hence mutant ras G proteins are produced. They retain bound GTP instead of hydrolyzing it to GDP. As a result mutant ras protein in its active form mislead the transmission of signal from external growth factors. Hence the host cells undergo abnormal cell division.

Most of the protein kinase activity showed by mammalian cells catalyses the phosphorylation of the amino acids serine and theonin, not tyrosine. These protein-serine/threonine kinase like protein-tyrosine kinase can be encoded by oncogene. The most important oncogene belonging to this group is the raf oncogene. It codes for a protein serine/threonine kinase that transmits signals from plasma membrane Ras protein to the cell interior. Some oncogenes code for proteins that function within the nucleus, particularly in the regulation of gene transcription. The examples of such oncogenes are the jun and fos oncogene which code for proteins that make up the AP! transcription factor. The AP_1 factor regulates the expression of a

group of genes that are involved in stimulating cell proliferation. The myc oncogene, associated with several kinds of human cancer, also appears to code for a transcription factor.

Proto-Oncogene:

It is well-established that oncogenic virus contains a relatively small number of genes which has facilitated the identification of the viral genes that cause cell to become malignant. The first cancer-causing gene to be identified occurs in Rous sarcoma virus, a small retrovirus that produces sarcomas in chickens.

An unexpected feature of retroviral oncogene is their lack of involvement in virus replication while other viral gene involves efficiently in the same process. Again, the existence of viral oncogene is not an integral part of the virus life cycle. Therefore, the origin and existence of viral oncogene leads to a new line of investigation. Such investigations have led to the surprising discovery that the src gene is not present only in cancer cells. Using nucleic acid hybridisation techniques, it has been shown that DNA sequence that Eire homologous to— but not identical with—the Rous src gene can be detected in the genome of normal cells of a wide variety of organisms including salmon, mice, cows, birds and humans.

The unexpected discovery that cells contain DNA sequences that are closely related to viral oncogenes has been substantiated by studies on a variety of other tumour viruses and, in each case, they resemble genes present in the genome of normal cell. The term proto- oncogene has been introduced to refer to these normal cellular genes that closely resemble oncogenes. The resemblance of viral oncogenes to proto-oncogene suggests that viral oncogenes may have originally been derived from normal cellular genes. According to this concept, the first step in the creation of retro-viral oncogenes took place million years ago when the ancient virus infected cells and became integrated in the host chromosomal DNA adjacent to normal cellular proto-oncogenes.

When the integrated pro-viral DNA was later transcribed to regenerate new viral RNA molecules, the adjacent proto-oncogene sequences might have been transcribed as well. In this way, a viral RNA molecule containing normal proto- oncogene sequences could have been created. Since a proto-oncogene would initially serve no useful purpose for a virus, it would be free to mutate during subsequent cycles of viral infection. Such mutation would eventually convert proto-oncogene into an oncogene. Therefore, the realisation that oncogenic viruses contain genes that cause cell to become malignant raise the question of whether genetic alteration are also involved in non-virus induced cancers. The ability of many carcinogens to act as mutagens provides the reason to believe that genetic changes play a role in non-viral carcinogenesis. Besides this, recent research suggests that cellular oncogenes are derived from normal proto-oncogenes by at least five mechanisms:

(i) Point Mutation:

The simplest mechanism for converting a proto- oncogene into an oncogene, it involves a single base pair substitution or point mutation.

(ii) Local DNA Rearrangement:

The second mechanism for creating oncogenes is based on DNA rearrangements that cause either deletions or base sequence exchanges between proto-oncogene and surrounding genes.

(iii) Insertional Mutagenesis:

The evidence of third mechanism comes from the findings that some cancer-causing retrovirus lack oncogenes and these particular viruses cause cancer by integrating a DNA copy of their genetic information into a host chromosome in a region where a proto-oncogene is located and thus disrupt the structure of the host proto-oncogene and thereby convert it into an oncogene.

(iv) Gene Amplification:

The fourth mechanism for creating oncogenes uses gene amplification to increase the number of copies of a particular protogene. This overproduction of copies of a particular proto- oncogene leads to malignant transformation.

(v) Chromosomal Translocation:

The fifth mechanism for creating an oncogene involves chromosomal translocation. It is a process where a portion of one chromosome is physically broken and joined to another chromosome. As a result, the broken segment containing proto-oncogene is transferred from its normal location to a new location where it is converted as oncogene.

Tumour Suppressor Genes:

We have now seen how the presence of an oncogene can stimulate uncontrolled cell growth and division, thereby fostering the development of malignancy. Cancer can also be induced by the loss of tumour suppressor genes that normally inhibit cell proliferation.

The term tumour suppressor gene implies that the normal function of gene of this type is to restrain cell growth and division. In other words, tumour suppressor genes act as brakes on the process of cell proliferation and inhibits tumour development. In many tumours these genes are lost or inactivated, thereby removing negative regulators of cell proliferation and contributing to the abnormal proliferation of tumour cells. Normally, the function of tumour suppressor gene is just opposite to oncogene. The first evidence of the activity of tumour suppressor gene came from somatic cell function experiment done by Henry Harris etal in 1969. The fusion of tumour cells with normal cell yields hybrids that contain chromosomes from both parents. Such hybrids are usually non-tumerogenic. Suppression of tumourigenicity by cell fusion indicates that genes derived from the normal cell definitely suppress the tumour development.

The first suppressor gene to be identified is involvement in hereditary retinoblastoma, a rare type of eye cancer that develops in children who have a family history of the disease. Such children inherit a chromosomal deletion in a specific region of one copy of chromosome 13. Although the deletion occurs in

all cells, only a few in the retina actually become malignant because the initial deletion in chromosome 13 does not cause cancer by itself; for cancer to develop, a subsequent mutation must also occur in the same region of the homologous chromosome 13.

It has, therefore, been concluded that chromosome 13 contains a gene on homologous chromosome of a normal diploid cell where such gene normally functions to inhibit retinoblastomas. In inherited retinoblastoma one defected copy of gene is genetically transmitted. The loss of this single copy of gene is compensated by the identical second copy of the gene present on the same region of the second copy of chromosome 13. Therefore loss of a single copy of gene is not by itself sufl5cient to trigger tumour development, but retinoblastoma almost always develops in these individuals as a result of a second somatic mutation leading to further loss of the function of the remaining second copy of normal gene.

The gene lost in hereditary retinoblastoma is called RBI. It is a tumour suppressor gene that codes for the nuclear protein p^{RB} that inhibits expression of a group of genes whose products are needed for uncontrolled cell proliferation. In hereditary retinoblastoma a defective or copy of the RBI gene is inherited from the affected person. Hence a lack of p^{RB} resulting from loss of both copies of RBI (one due to deletion and other due to a second somatic mutation) can lead to uncontrolled proliferation which ultimately causes the development of retinoblastoma. In nonhereditary cases, two normal RBI genes are inherited and retinoblastoma develops only if two somatic mutations in adult inactivate both copies of RBI in the same cell.

Table 23.6: Main classes of oncogenes categorised by nature of their Protein Products

Nature of Protein Product	Examples of Oncogenes	Comments
Growth factors	it sis	Platelet-derived growth factor (PDGF)
Protein-tyrosine kinases	*erb B*	Membrane receptor of epidermal growth factor (EGF)
	fms	Membrane receptor for colony-stimulating factor-1 (CSF-1)
	src, yes, fgr	Membrane nonreceptor proteintyrosine kinases
Membrane-associated G proteins	*ras*	Membrane-associated GTP-binding protein
	gsp	G_s(α sub-unit)
	gip	G_1(α sub-unit)
Protein-serine/threonine kinases	*raf, mos*	Cytoplasmic protein-serine/threonine kinases
Transcription factors	*jun, fos*	Components of AP1 transcription factor
	erb A	Thyroid hormone receptor

Following the discovery of the RBI gene several other tumour suppressor genes have been identified (Table 23.6). The second suppressor gene is p^{53} which is frequently inactivated in a wide variety of human cancer including leukemia's, lymphomas, sarcomas, brain tumour and carcinomas of many tissues

including breast, colon and lung. The p^{53} protein is a nuclear transcriptional factor that switches on the activity of genes that arrest cells in the G_1 phase of the cell cycle. Normally, the production of the p^{53} protein is stimulated when DNA is damaged due to exposure to ultraviolet ray or DNA damaging agents.

Hence p^{53} appears to act like a molecular policeman that checks the cell for DNA damage and prevents the cell from proliferation if damage is detected. The loss of p^{53} function allows the survival and reproduction of cells in which DNA damage has led to the production of oncogenes and/or the loss of other tumour suppressor genes. In addition to mediating cell cycle arrest P^{53} is required to apoptosis induced by DJNA damage. Unrepaired DNA damage normally induces apoptosis that eliminates cells which might develop into cancer. Cells lacking p fail to undergo apoptosis.

This failure contributes to the resistance of many tumours to chemotherapy. The failure of function of p is thought to account for the high frequency of p^{53} mutations that lead to inactivation of p^{53}. Like p^{53}, the INK4 is a tumour suppressor gene that prevents lung cancer. Similarly, two other tumour suppressor genes such as APC and DCC prevent colon cancer. When these genes are deleted or mutated, such cancers develop. The product of RBI and INK4 tumour suppressor genes regulate cell cycle progression at the same point. These genes inhibit passage through the restriction point in G_1 by suppressing transcription of a number of genes involved in cell cycle progression and DNA synthesis.

A rare hereditary form of colon cancer, familial adenomatous polyposis, is produced due to inherited mutation of the APC gene. In this type of cancer hundreds of polyps or benign colon adenomas are produced within the colon of an individual. Some of these polyps are transformed into malignancy. Inactivated or mutated form of some additional tumour suppressor genes is also associated with the development of breast, ovarian and pancreatic carcinomas as well as in some rare inherited cancer syndromes such as Wilm's tumour (a childhood kidney tumour). The tumour suppressor gene of Wilm's tumour is WT1 which is frequently inactivated in Wilm's tumour. The product of WT1 gene appears to suppress transcription of a number of growth factor inducible genes.

Prevention and Treatment of Cancer:

There is a general belief among the common people that cancer cannot be cured. Although this is partially true, it depends on several aspects of the patient and the time of detection. In many cases, when it is clinically detected then it is already late and it goes beyond the treatment.

Actually, cancer is a disease that ultimately has to be understood at the molecular and cellular level. In fact many cancers can be cured if they are detected at the early stage of its development. In case of hereditary cancer, regular testing may allow early detection. Therefore, whether cancer is curable or not is a debatable question. With the help of modern and sophisticated technology, cell biologists are always trying to improve the methods for prevention and treatment of cancer. The first step in preventing cancer is to identify the agents that cause cancer. For example, it is already known that tobacco smoke causes cancer. So just to prevent the possibility of this type of lung cancer, it is advisable simply to avoid tobacco smoke. Similarly the discovery of carcinogenic properties of X-ray and sunlight suggests that individuals

should avoid unnecessary medical X-ray and use protective lotions during long time exposure to sunlight. Epidemiological data also allow potential carcinogens to be identified in exposed human population. The epidemiological approach is based on comparison of cancer rates among various groups of people exposed to different environmental conditions. For example, when Japanese individuals move to the United States heir susceptibility to developing stomach and lung cancer changes to reflect the rates for such cancers in the United States. Therefore, the comparison of the frequency of stomach and lung cancer in Japan, in the United States and in Japanese immigrants to the United States suggests that environmental factors play a prominent role in causing cancer. Epidemiological data have played an important role in identifying some of the environmental factors that may cause cancer.

The Ames test is a rapid screening method tor identifying potential carcinogens This method is based on the rationale that most carcinogens act as mutagens, it measures the ability of potential carcinogens to induce mutations in a strain of bacteria that lack the ability to synthesize the amino acids histidine. Each bacterial cell that has mutated to a form in which it no longer needs histidine will grow into a colony that can be counted. The number of colonies indicates the mutagenic potency of the substance to be tested. Chemicals to be tested m the Ames test are first incubated with a liver homogenate because many of the chemicals to which humans are exposed only become carcinogenic after they have undergone biochemical modification in the liver (Fig. 23.11) Cancer can be prevented in several other ways A person can modify his life style in order to reduce the risk of developing cancer. Change of life style sometimes requires minimizing the exposure to carcinogens. Tobacco smoking and extensive meat consumption are the probable causative factors of cancer. If any person method of treatment is most effective when the cancer is detected at the early stage of development and when metastasis has not occurred.This method is not effective when the cancer has already been disseminated throughout the body by the process of metastasis. Therefore, early detection of cancer is very important for its treatment.

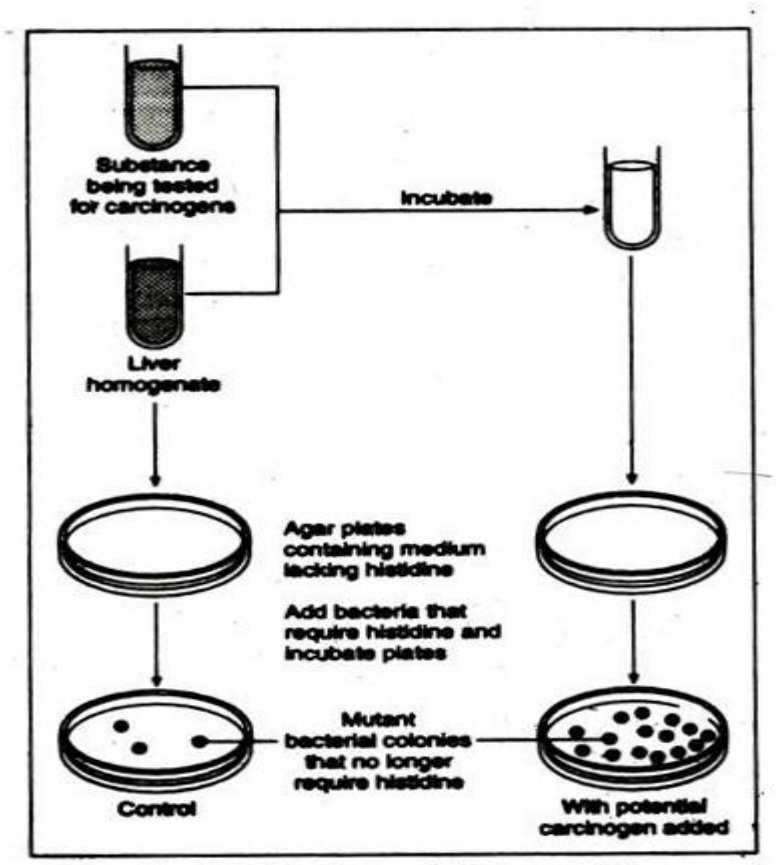


Fig. 23.11: Protocol of Ames Test.

Treatment of cancer-affected part of body with the help of X-radiation is another alternative method of curing cancer. X-ray is very effective for killing the cancer cells that are actively proliferating. The cells that are engaged in DNA synthesis prior to cell division, or are on the way of mitosis, are very sensitive to X-ray. But the main problem of using X- ray for the treatment of cancer is that normal and healthy dividing cells of the body-such as blood-forming cells in the bone marrow are also destroyed along with the cancer cells. Moreover, X-radiation itself is carcinogenic. Hence there is always a chance of developing cancer after X-ray treatment. In spite of such risk posed by X-ray treatment, it is effective for the treatment of certain types of cancers like skin cancer, Hodgkin's disease and specific forms of testicular and bone cancer. Chemotherapy is another approach for treating cancer. This method is based on the use of certain drugs that are designed to kill the proliferating cells as in radiation treatment. This method is also effective when the cancerous cells have already metastasized. The drugs are generally injected in the body and the circulatory system helps the drug to spread throughout the body. Some drugs used m cancer chemotherapy are given in the Table 23.7.

Table 23.7: Tumour Suppressor Genes

Gene	Type of cancer
APC	Colon/rectum carcinoma
BRCA1	Breast and ovarian carcinomas
BRCA2	Breast carcinoma
DCC	Colon/rectum carcinoma
DPC4	Pancreatic carcinoma
INK4	Melanoma, lung carcinoma, brain tumours, leukemias, lymphomas
NF1	Neurofibrosarcoma
NF2	Meningioma
P^{53}	Brain tumours, breast, colon/rectum, esophageal, liver, and lung carcinomas; leukemias and lymphomas
Rb	Retinoblastoma, sarcomas; bladder, breast, and lung carcinomas
VHL	Renal cell carcinoma
WT1	Wilm's tumour

Like radiation, chemotherapeutic drugs also kill the normal and healthy cells along with cancer cells. This type of treatment has also some toxic side-effect-like loss of hair (caused by destruction of hair follicle cells), diarrhea (caused by destruction of cells of the intestinal lining) and susceptibility to infections (caused by destruction of blood cells). Sometimes two or more combination of drugs are also used for the treatment of cancer. Besides its side- effects and other disadvantages, it is true that, for certain types of cancer, chemotherapy is very successful for curing cancer like Burkitt s lymphoma, chorio carcinoma, acute lymphocytic leukemia, Hodgkin's disease, lymphomas, mycosis fungoides, Wilm tumour, Ewing s sarcoma, thabdomyosarcoma, retinoblastoma, and embryonal testicular tumours etc.

Table 23.8: Some drugs used in Cancer Chemotherapy

Class	Examples	Mechanism of Action
1. Antimetabolites	Methotrexate 5-Fluorouracil 6-Mercaptopurine	Inhibit enzymatic pathways for biosynthesis of nucleic acids by substituting for normal substrates
2. Antibiotics (sybstances produced by microorganisms)	Actinomycin D Adriamycin Daunorubicin	Bind to DNA
3. Alkylating agents	Nitrogen mustard Chlorambucil Cyclophosphamide Imidazole carboximides	Crosslink DNA
4. Mitotic inhibitors	Vincristine Vinblastine Taxol	Interfere with mitotic spindle
5. Hormones	Estrogen (for prostate cancer) Cortisone Progesterone Androgens	Inhibit growth of hormone-sensitive cells by interacting with hormone receptors
6. Miscellaneous agents	L-Asparaginase	Hydrolyzes asparagine

Although the use of surgery, radiation and chemotherapy has led to increased survival rates for certain kinds of cancer, many malignancies do not respond well to such treatment. Recent experimentation is attempting to exploit the ability of the immune system to recognize and kill tumour cells. This type of

treatment is known as immunotherapy. The basic principle of immunotherapy is to exploit the ability of the immune system to recognise and kill tumour cells. Tumour cells tend to show cell surface antigens which make them recognisable by the immune system. Initially, some scientists attempted to utilise a person's own lymphocytes to kill cancer cells. For this experiment, lymphocytes were isolated from the blood of cancer patients and grown in culture in presence of Interlaken 2 to stimulate the cancers destroying properties of the cell. The result was the isolation of a population of killer T-cells that were specifically targeted against the patient's tumour.

These cells, called tumour-infiltrating lymphocytes (TILs), were injected back into the patients from whom the blood was drawn. TILs axe more effective in inducing tumour regression. Recently TILs are made even more effective by using recombining DNA technique to insert some genes whose product enhances the additional potency of the TILs. A protein produced by march-panes called tumour necrosis factor (TNF) is effective in promoting the destruction of cancer cells if the TNF gene were inserted into the TILs. Obviously, the genetically engineered TILs would be more effective than normal TILs and would be more powerful to killing the tumour cells. Currently this technique is being tested in the hope of finding ways to promote immune destruction of cancer cells.

Viral Cancers in Animals and Humans:

Viruses cause malignant tumors in animals, as such, tumors have been isolated from many animals such as fish, mice, rat, squirrels, dogs, deer and horses. The polyoma virus has been isolated from mice and the simian virus 40(SV 40) from monkeys.

Although we have no clear evidence that the viruses cause cancers in animals as no infective virus has been isolated from cell cultures, but some evidences are there to show the association of viruses with human cancers.

At present, viruses have been implicated in the genesis of at least eight human cancers:

1. Electron microscopic and immunological studies show that there is association of Epstein-Barr virus (EB virus), a herpesvirus which is one of the best studied human cancer viruses, with Burkitt's lymphoma, a malignant tumour of Jaw and abdomen of children, occurring in certain regions of Africa.

2. EB virus has also been found associated with nasopharyngeal carcinoma found in certain Chinese populations.

3. Some strains of human papillomaviruses have been isolated from malignant tissues (not from normal tissues) from patients suffering from skin and cervical cancer.

4. Hepatitis B virus has been found associated with hepatocellular carcinoma (a type of liver cancer) and can be integrated into the human genome.

5. Hepatitis C virus results in cirrhosis of the liver that may lead to liver cancer.

6. Human herpesvirus-8 has been found associated with the development of Kaposi's sarcoma.

7. Human T-cell lymphotropic virus-1 (HTLV-1) seems able to cause T-cell leukemia.

8. Human T-cell lymphotropic virus-2 (HTLV-2) is found associated with hairy-cell leukemia.

How Viruses Cause Human Cancer?

Although viruses are known to cause cancers in animals since many years, it is still uncertain that they cause cancers in humans. However, it is well established that some kinds of human tumors are strongly associated with infection by specific viruses.

The human T-cell lymphotropic viruses (HTLV-1 and HTLV-2) appear to transform T-cells into tumor cells by producing a regulatory protein that sometimes activates genes related to cell-division as well as virus reproduction. Some oncogenic viruses possess one or more very effective promoters or enhancers.

Whenever, these viruses integrate themselves next to an oncogene in cell-genome, the promoter or enhancer is thought to stimulate its transcription, resulting in a cancer. With the possible exception of HTLV-1, it is not yet known clearly how the viruses associated with human cancers actually aid in cancer development.

DNA Oncogenic Viruses:

Oncogenic viruses are distributed in several families of DNA viruses. These include Herpesviridae, Poxviridae, Papovaviridae and Hepadnaviridae. The Herpesviridae include the Epstein-Barr Virus (EBV) which has been found as the cause of two forms of human cancers — Burkitt's lymphoma and nasopharyngeal carcinoma. EBV has also been implicated with Hodgkin's disease, a cancer of lymphatic system. Other herpes-viruses have been associated with human cancers of lip and cervix.

The Papilloma viruses belonging to the Papovaviridae cause benign tumours as well as cancer in several species including human. In humans, papilloma viruses cause uterine (cervical) cancer. Another member of Papovaviridae, the Simian Virus 40 (SV40) is among the best studied DNA tumour viruses. Natural host of SV 40 is cultured fibroblast cells of monkey. Such a cell culture is called permissive, because it allows viral multiplication and release of progeny viruses by cell lysis. On the other hand, when SV 40 is inoculated into non-permissive cell cultures e.g. the fibroblast cells of mice, the virus cannot multiply, but in a small number cells the viral DNA is, integrated with the host DNA causing their transformation into cancer cells. Due to integration into the host chromosome viral multiplication and cell lysis are absent. The phenomenon is comparable to lysogeny observed in temperate phage infection of bacteria. Integration of some DNA viruses is site-specific i.e. the viral DNA is inserted into a host chromosome at a specific site. But papova-viruses do not have such specificity and can be inserted at random. Hepatitis B virus (HBV) belonging to the Hepadnaviridae causes cancer of liver. Many animal experiments have yielded

results which clearly indicate a connection of HBV and liver cancer. Although direct proof is lacking in case of human beings, a survey revealed that all people with liver cancer had a previous infection of HBV.

Oncogenic DNA Viruses:

1. Papova Viruses:

They induce benign warts and papilloma in natural hosts. Human papilloma virus (HPV) type 6 and 11 are incriminate in pre malignant lesions of the female and male genital tract SV 40 polyoma viruses produce tumours in mice when injected.

2. Herpes Virus:

Herpes simplex type 1 and 2 and Cytomegalovirus transform cultured cells at a very low frequency. They transformed hamster cells, when injected into another hamster, form tumours. In woman, Herpes simplex type 2 is responsible for cervical carcinoma.

No transforming gene is involved Epstein-Barr (EB) virus is associated with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Chinese male population. It is believed that EB virus transforms normal lymphocytes into lymphoblast in immuno-compromised child.

3. Hepatitis B Virus:

It is implicated in hepato-cellular carcinoma. It does not carry any oncogene and is integrated in tumour cells as Hepatitis B virus DNA.

4. Pox Virus:

Yaba virus produces histiocytoma (benign tumour) in natural host (monkey) while shope fibroma virus induces fibroma in rabbit. Molluscum contagiosum virus produces benign growths in humans.

RNA Oncogenic Viruses:

Among the RNA viruses only some members of the family Retro-viridae can cause cancer. Other RNA viruses which replicate by RNA replicase are non-oncogenic. Retroviruses which have a single- stranded RNA genome replicate via a double-stranded DNA produced by an RNA-dependent DNA polymerase (reverse transcriptase) and they insert the DNA copy into the host chromosome as a provirus. Rous Sarcoma Virus (RSV) is of historical importance, because it was the first tumour-inducing virus to be studied. RSV is a retrovirus with a single-stranded RNA genome and its DNA copy is integrated into a specific site of the host chromosome as a provirus.

Research on RSV revealed identification of a cancer-inducing gene (an oncogene) in RSV genome. This gene, called src, is not essential for viral replication, as it does not code for any viral proteins. Later, it was discovered that a copy of the src gene is present in the host chromosome of normal cells and it was not oncogenic. Thus, the viral src gene which is oncogenic is derived from the host. How the non-oncogenic

chromosomal src gene is converted to an oncogene in RSV is not clearly understood. It may occur through a mutation. The entry of a chromosomal gene into the viral genome possibly occurs through a process similar to that which operates in restricted transduction in bacteria.

It is thought that the RSV DNA produced through reverse transcription is inserted next to the chromosomal src gene and during transcription of the RSV RNA genome, src gene might be included. In this way, src gene might enter into the viral genome. RSV causes cancer in chicken. Similar retroviruses are known to cause cancer in other animals including monkey. But definite evidence of retroviruses causing cancer in humans was not available until 1980. In that year Gallo isolated a virus that could transform normal T-lymphocytes into cancerous T-lymphocytes causing a disease, called T-cell leukaemia.

The virus is known as Human T-cell Leukaemia Virus (HTLV). Later research during 1990s has confirmed the role of HTLV in causing human leukaemia. Another HTLV was later discovered causing hairy cell leukaemia in man. The malignant leucocytes develop hairy outgrowths on their surface. The second virus has been designated as HTLV-II. These retroviruses have been shown to transform normal T-cells by a regulatory protein which stimulates uncontrolled cell division. Besides leukaemia, HTLV is also known to cause neurological disorders, like spastic para-paresis. T-cell leukaemia is more or less restricted in several countries, like Japan, West Indies and some parts of West Africa.

i. Avian Sarcoma Leukosis Viral (ALV) Complex:
They are caused by antigenically related viruses which caused lymphoma, leukaemia and sarcoma.

ii. Murine Leukosis Virus:
There are several strains of murine leukaemia and sarcoma viruses which are derived from Mo-Mu Lv during passages in mice and rats.

iii. Mouse Mammary Tumour Viruses (MMTV):
They may be endogenous or exogenous viruses. The endogenous ones have no oncogenic role, whereas the exogenous ones are oncogenic. Mammary cancers develop only in susceptible females after a latent period of 6-12 months.

iv. Sarcoma Leukaemia Viruses of Animals:
Leukaemia, lymphosarcoma in cat is induced by Feline leukaemia virus (Felv); Lymphosarcoma in cattle is due to bone leukaemia virus.

v. Human T-cell Leukaemia Virus (HTLV):
Adult T-cell leukaemia is induced by HTLV- 1 but having cell leukaemia of T-cell type is due to HTLV-II.

Probable Questions:

1. Classify Cancer on the basis of invasiveness and location.
2. Discuss Tumor suppressor gene's role in cancer control
3. Describe the role of oncogenes in cancer development?
4. What are the roles of cancer in cancer development?
5. Describe different DNA virus which causes cancer.
6. Describe different RNA virus which causes cancer.

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UNIT-XVII

Concepts of Apoptosis: Regulators of Apoptosis, Caspases, Pathways of apoptosis; Cell Senescence, Necrosis

Objective: In this unit we will learn about necrosis and apoptosis. We will also learn about regulation of apoptosis, different pathways of apoptosis. Role of caspases and cell senescence.

Introduction:

Two principal modes of cell death are now recognized, namely necrosis and apoptosis. Both may follow cell injury but the nature of the two types of death are quite different.

A. Cell Necrosis:

The changes in cells undergoing necrosis are essentially a continuation of those described above, in cell injury. However, they are, of course, more profound and are irreversible. It has thus been proposed that there is a 'point of no return', beyond which the process of cell injury cannot be revoked.

While moderate mitochondrial swelling, the formation of cell surface 'blebs' and ribosomal disaggregation appear to be reversible, there are certain changes which will inevitably lead to cell necrosis. These include extreme 'blebbing' at the cell surface, together with much greater dilation of the mitochondria ('high amplitude swelling') and the formation of electron dense areas in the mitochondrial matrix.

B. Apoptosis:

Apoptosis or 'programmed cell death' has been describe for over 20 years but only comparatively recently has its fundamental importance been recognized. It is known to be central to many developmental processes where cells have to be lost as part of the organization of tissues or organs. Examples lie in the loss of tadpole tails, the loss of inter-digital webs, the control of B lymphocyte proliferation and the removal of excess cells in nervous system maturation.

In pathological terms, apoptosis is fundamental to the process of atrophy, where cells are lost from mature organs or tissues as a result of endocrine or physical means. For example, hormones may suppress target glands by this route and surgical ligation of the draining ducts of, for example, the pancreas can lead to cell loss by apoptosis. One great 'advantages' of apoptosis versus necrosis is that there is no induction of acute inflammation and thus further tissue damage is not incurred. This is of particular importance in the removal of neutrophils, whose lysosomal enzymes could continue to cause tissue disruption if left unchecked.

Apoptosis also appears to be a major mechanism by which malignant cells are removed and imbalances between apoptotic and mitotic rates could lead to tumour progression or regression. The observation that some chemotherapeutic drugs used for treating cancer can act by inducing apoptosis may be of great therapeutic importance and estimates of apoptotic rates may be of value in assessing the efficacy of therapeutic regimes.

The morphological changes accompanying apoptosis are quite different to those seen in necrosis. First, there is loss of cell-cell adhesion and in vitro the affected cells 'float' above their normal counterparts. The cells also become rounded and smaller, sometimes with lobuation, although some organelles, including mitochondria, remain intact.

This shrinkage results from loss of Na^+ and water. The nuclear chromatin condenses to form 'half-moon'-shaped structures just within the nuclear membrane and just nucleoli appear disorganized. The nucleus may also break up into multiple portions.
There is an increase in transglutaminase activity in the cell, leading to insolubilization of proteins, which form a 'shell' round the inner surface of the cell membrane. There is also a rise in Ca^{2+}, Mg^{2+} -dependent endonuclease, which is responsible for the chromatin condensation and the nuclear fragmentation.
Finally, the apoptotic cells are rapidly recognized by macrophages, which phagocytose them, with the formation of apoptotic bodies within the cytoplasm of the consuming cells, where they may be seen for up to about 9 hours following phagocytosis. The binding of macrophages to apoptotic cells appears to be mediated by the macrophage vitronectin receptor, which is also a cell adhesion molecule. Binding is also facilitated via a lectin-type receptor on the macrophage surface. Apoptosis is a genetically regulated phenomenon and has been studied extensively in the nematode worm Caenorhabditis elegans, where mutations of certain gene loci are related to programmed cell death. Thus, mutations of the loci ced-3 and ced-4 lead to the blockage of the apoptosis known to be necessary during development of this organism.
A further gene, ced-9, appears to have some control over the process and it appears that its gene product may either enable or disenable the apoptotic system. Furthermore, ced-9 has been shown to possess homology with the human oncogene bcl-2. This gene is one of several known to regulate apoptosis in human cells.

Techniques for the Detection of Apoptosis:

The simplest method for the detection of apoptotic cells is observation of the characteristic bodies in tissue sections at either light or electron microscope level. In vitro, the apoptotic cells can be visualized by means of phase or interference contrast microscopy, where they can be seen to be rounded and lying above the cell monolayer.

However, the 'gold standard' lies in the demonstration of 180-200 base oligonucleotide fragments resulting from nuclear damage. These fragment scan be separated by gel electrophoresis and viewed under ultraviolet (UV) light, where they produce a characteristic 'ladder' effect. This method is applied to cell extracts and has the disadvantage that it is not quantitative. Other approaches include the use of cell suspensions stained with fluorescent DNA-binding dyes which are then analysed in a DNA flow cytometer. A novel approach is that of DNA end-labelling, in which nucleotides, tagged with a suitable marker, are bound to the broken ends of DNA fragments in apoptotic cells. This method has the advantage that it enables the enumeration of affected cells.

Introduction to Apoptosis:

Every normal living cell of animals, plants and even bacteria are mortal. I.e., they must die after some time. Cell death is a finely tuned programme inherent in the cells genetic machinery. This normal cell death which is the part of normal development and maintenance of homeostasis is called apoptosis or programmed cell death (PCD). This phenomenon is very much different from death of a cell due to pathological cause or necrosis. This process is highly regulated and any defect in apoptotic machinery will lead to extended survival of cells which may result in neoplastic cell expansion, leading to genetic instability and accumulation of mutations.

Cellular Events of Apoptosis:

It is a normal physiological response to specific suicide signals or lack of survival signals. During this process at first the nucleus and cytoplasm condense, i.e., chromatin material condenses and migrates to nuclear membrane, the cytoplasm undergoes shrinkage without any damage to plasma membrane.

The cell contents are packaged in membrane bound bodies and the cell is broken down into pieces called apoptotic pieces, though still functioning, are engulfed or phagocytosed or digested by macrophages or by neighbouring cells (Fig. 5.33).

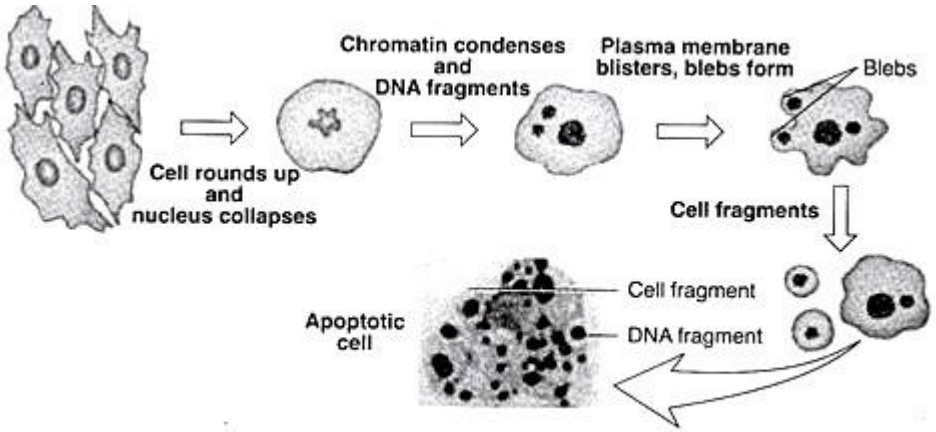

Fig. 5.33A: Sequence of cellular events during apoptosis

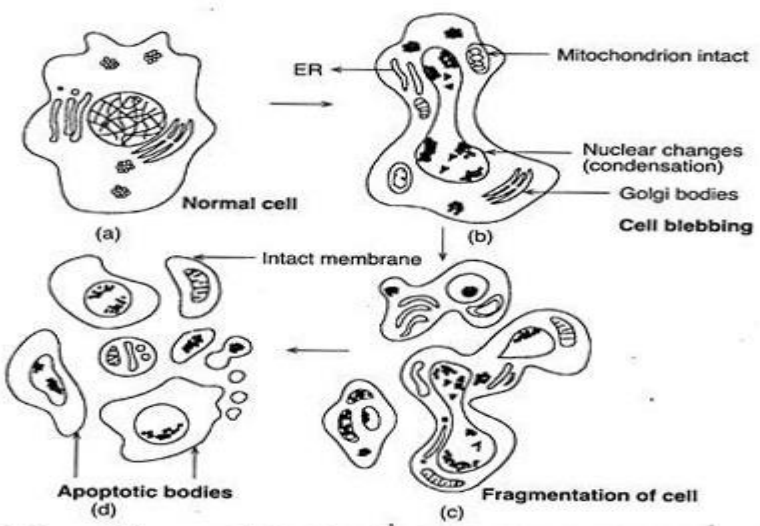

Fig. 5.33B: Cytological changes during apoptosis. Normal cell undergoes shrinkage showing condensation of chromatin and cell blebbing. Thereafter the cell is fragmented to produce apoptotic bodies, but the membranes remain intact (from Rastogi)

Mechanism of Apoptosis:

There are three major pathways for activation of caspase which causes cleavage of substrates leading to apoptosis.

i. Mitochondrial/Cytochrome pathway:

It is mediated through activation of Bcl-2 (gene) which results in production of Apaf-1, caspase-9 and caspase-3 enzyme synthesis which leads to the phenomenon of apoptosis (Fig. 5.34).

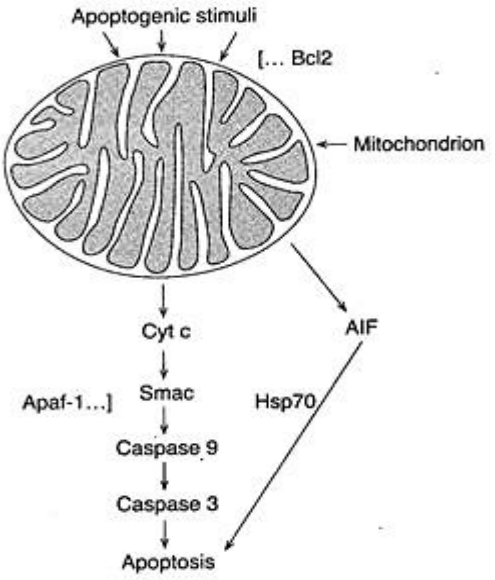

Fig. 5.34: Apoptotic cascade: apoptotic proteins such as Cyt c, Smac, AIF, etc. are released due to several stimuli, triggering caspase activation. Hsp 70 is a negative regulator of Apaf-1. AIF causes condensation of chromatin and cell fragmentation

ii. Tumour-necrosis factor-receptor (TNF) pathway:

In this pathway the ligation of members of the TNF-receptors takes place, activating caspase-8 and then caspase-3 which leads to apoptosis.

iii. Granazyme B pathway:

Granazyme B, a cytosolic T cell product, directly cleaves and activates several caspases, resulting in apoptosis. A number of genes have been identified which play role in the regulation and accomplishment of apoptosis, such as egl-1, Ced-1-10. Studies on these genes indicated that Ced-9 acts upstream of Ced-3 and Ced-4 (Fig. 5.35):

Ced-9 → Ced-3→Ced-4→Cell death

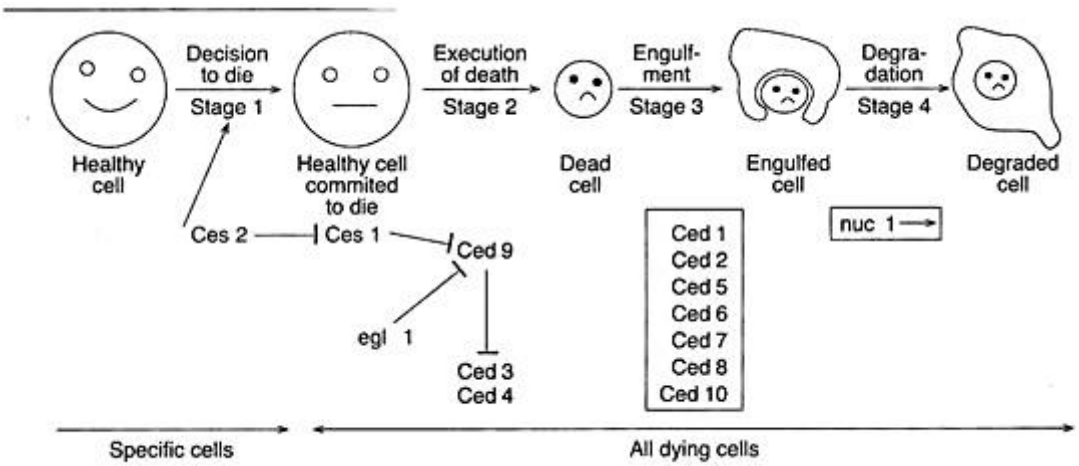

Fig. 5.35: Model for molecular basis of apoptosis in the nematode, Caenorhabditis elegans (after Steller, 1995)

Ced-3 and Ced-4 promote apoptosis, while Ced-9 Is anti-apoptotic and protects cells from apoptosis by antagonizing Ced-3 and Ced-4. The Ced genes are responsible for all programmed cell death.

Caspases are cysteine proteases which cleave the substrates at the C-terminal of an aspartic acid residue. Different caspases have different substrate recognition preferences and cleavage of substrates by caspases results in disassembly and consequent death of cell in a highly organized manner.

Death receptors are important in **'instructive'** apoptosis where ceil death is brought about by the secretion of death legends which bind to death receptors on the target cell (Fig. 5.36).

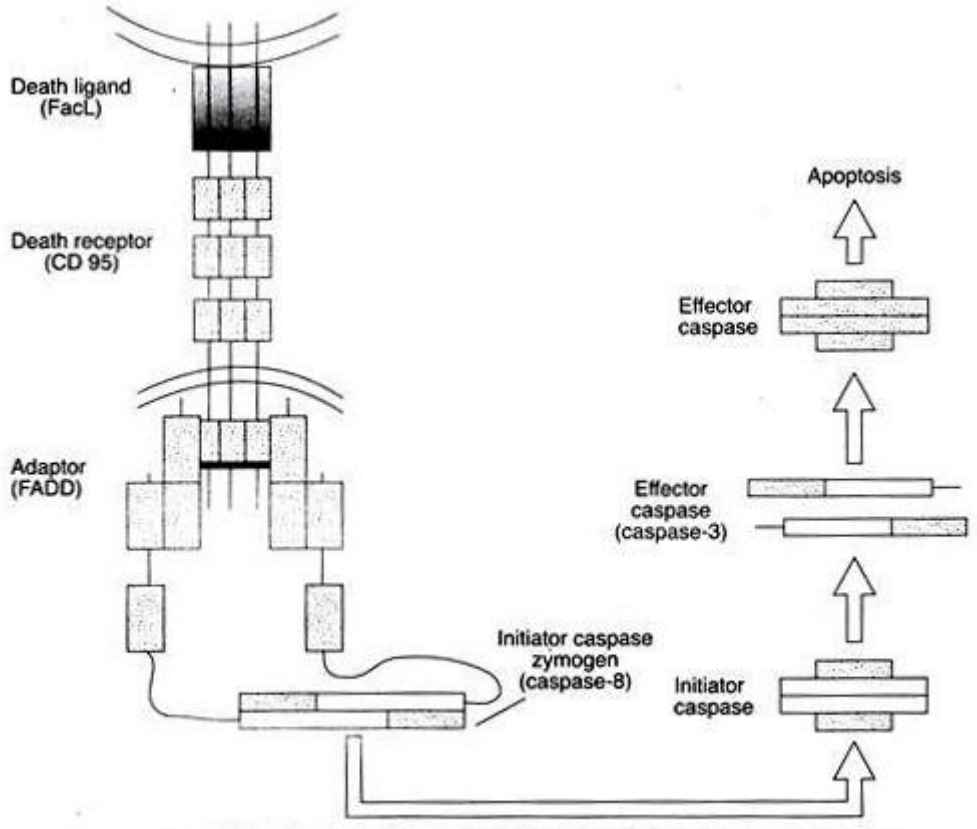

Fig. 5.36: A model showing instructive apoptosis by secretion of death ligands (from Rastogi)

Importance of Apoptosis:

It is a necessary mechanism complementary to proliferation to ensure homeostasis in all tissues. Removal of a number of vestigeal structures (developmental structure, e.g., tail) is caused by programmed cell death. Apoptosis is considered a necessary anticancer mechanism, as defect in this process leads to neoplastic and tumorigenic cell development.

The apoptotic process can be summarized as follows:

i. . Activation of initiating caspases by specific signals.

ii. Activation of executing caspases by the initiating caspases which can cleave inactive caspases at specific sites.

iii. Degradation of essential cellular proteins by the executing caspases with their protease

iv. Death receptors- Fas/CD95, DR4/DR5, DR3, and TNFR (Tumor Necrosis Factor Receptor).

v. Adaptors- FADD (Fas-associated death domain protein) and TRADD (TNFR-associated death domain protein).

vi. Activation- Binding of death ligands (FasL/CD95L, TRAIL/APO-2L, APO-3L and TNF) induces trimerization of their receptors, which then recruit adaptors and subsequently activate the caspases (Fig. 5.14).

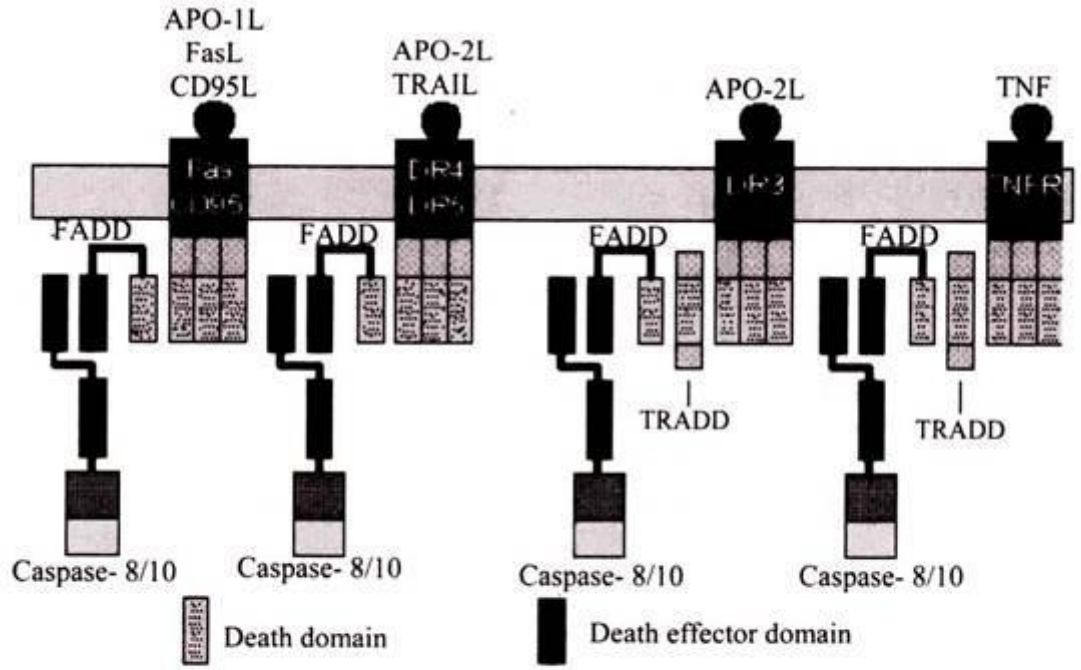

Figure 5.14: Coupling of caspase 8 or 10 to death receptors

Neurotrophic Factors:

Neurotrophins are an important class of neurotrophic factors, but they are by no means the only ones that play important roles. Glial cell line derived neurotrophic factor (GDNF) family has turned out to be crucially important in neuronal development and maintenance as well as the ciliary neurotrophic factor (CNTF) family. Since the first member of the family was discovered by its ability to promote neuronal survival the name of "neurotrophic factor" was acquired. However, all neurotrophic factors also regulate important processes in the non- neuronal cells that are essential for the development and function of the organisms. The multiple functions of neurotrophic factors in different tissues provide an interesting example of how an organism uses the same biologically active factors for several purposes. Many other growth factors, mainly known for their non neuronal activities (e.g. some fibroblast growth factors, insuline-like growth factors) have some trophic effects on selected neuronal populations, but are not called neurotrophic factors.

The family of neurotrophins and their receptors: Neurotrophins are polypeptide molecules that regulate the survival, development and maintenance of specific functions in different populations of nervous cells. This family includes four closely related factors: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophins-4/5 (NT4/5). These four factors share more than 30% sequence homology and are relatively well conserved in all mammals. The most recently discovered neurotrophin is NT-6 whose receptor remains to be defined, although there is evidence for NT-6 binding to proteoglycans on the cell surface and/or to extracellular matrix molecules. Neurotrophins exert their biological activities by activation of two unrelated types of transmembrane receptors. The first type is the low-affinity NGF receptor, also known as low-affinity neurotrophin receptor (LANR, p75NGFR) which binds neurotrophins with varying affinities and does not possess intrinsic tyrosine kinase activity. The second type of receptors has specificity for the binding of individual neurotrophins and is conferred by the Trk family of tyrosine kinases known as TrkA, TrkB, TrkC.

Anti-apoptotic action of Neurotrophins:

Neurons, like any other differentiated cells, require the presence of survival factors to suppress the intrinsic cell death machinery and thereby avoid apoptosis. The regulation of apoptosis by survival factors is therefore critical for normal development an proper functioning of the nervous system. In addition, apoptotic-like death was described in injured neurons following such insults as β-amyloid exposure, excitotoxicity, DNA damage, oroxidative stress. These observations implicated apoptosis as an important element of several major neurological diseases booting the interest in identifying both anti- and proapoptotic signal transduction pathways in neurons. Neurotrophins promote survival and suppress apoptosis in many populations of neurons. It has been suggest that the major pathways

transducing the antiapoptotic effects of neurotrophins are phosphatidylinositol3kinase (PI- 3K) and Erk1/ 2. The nature of the apoptotic stimulus seems to be a critical determinant of pathway preference. Thus, it appears that in most cases of trophic deprivation, PI-3K is the main player, whereas Erk1/ 2 dominates as a major neuroprotective mechanism in damaged cells. In addition to Erk1/2 and PI-3K pathways, there are many more signallingsmall circuits regulated by neurotrophins such as phospholipase Cγ (PLCγ), GTPases ras andrho, protein kinase C, p38, Erk5, Jnk, transcription factor NFκ and tyrosine phosphatase Shp. Some of them including PLCγ, proteinkinase C or protein Kinase p38, a relative of Erk1/2 were implicated in survival responses to such stimuli as NGF, serum Phorbol esters or membrane depolarization. One should also emphasize that although the p75 receptor for neurtrophins is generally considered to be pro-apoptotic, it can activate a protective transcription factor NFκ. The main challenge in the field is to learn how these pathways protect neurons. This knowledge may lead to new strategies for effective neuroprotection in diseases. For example, a substrate for PI-3K is an emerging candidate drug target for neuroprotection since it mediates the suppression of apoptosis.

Neurotrophins regulating neuronal apoptosis:

As we have previously mentioned, neurotrophins mediate the survival, differentiation, growth and apoptosis of neurons by binding to two types of cell surface receptors, the Trk and p75 neurotrophin receptor. These receptors, often present on the same cell, coordinate and modulate the responses of neurons to neurotrophins. The functions of the neurotrophin receptors vary markedly, from sculpting the developing nervous system to regulation of the survival and regeneration of injured neurons. While Trk receptors largely transmit positive Neurotrophins: Responsible for death and survival signals that promote neuronal survival, p75 transmits both positive and negative signals and, in particular, can cause neuronal apoptosis. Since it is known that p75 could mediate neural cell line apoptosis, this has been extended to a large number of primary neural cells in culture. A number of studies indicate that this proapoptotic function is essential for rapid and appropriate apoptosis during developmental cell death. In particular, apoptosis is significantly reduced in certain neuronal populations in mice lacking p75 or its neurotrophin ligands. The proapoptotic function of p75has also been implicated in injury-induced apoptosis. The first study to support this idea, involved the neuron-specific expression of the p75 intracellular domain, which led to the death of the injured facial motor neurons in transgenic mice. Although p75 mechanisms of

apoptosis have been associated to Trk receptors, a number of studies indicate that p75 canalso signal apoptosis in a Trk-independent fashion. For example, p75 activation causedapoptosis when sympathetic neurons were maintained in KCl, when sensory neurons were maintained in ciliary neurotrophic factor, and when Schwann cells were maintained in insulin-like growth factor plus nurteulin, all Trk-independent survival signals. Recent surprising findings have demonstrated that for at least some developing neurons, p75 mediates a constitutive death signal, and that one of the primary ways that Trk receptors mediate neuronal survival is by silencing this constitutive signal. This conclusion derivesfrom studies showing that p75 is essential for apoptosis of some cells following growth factorwithdrawal.

Caspase:

Caspases, Key Apoptotic Proteins:

Virtually all animal cells contain caspases, but they occur as inactive zymogens that can do no harm. There are various triggers that can lead to their activation, which usually occurs through proteolytic processing of the zymogen at conserved aspartic acid residues. Needless to say, their activation and suicidal function is highly regulated. Once activated caspases act as cysteine proteases, using a cysteine side chain for catalysing peptide bond cleavage at aspartyl residues in their substrates. The name "caspase" denotes their function: Cysteine- dependent ASPartyl-specific proteASE. There are many such caspases within an organism, which work together in a proteolytic cascade to activate themselves and one other. Cascades are effective means of amplifying a signal to give a much larger response than could be achieved through a single enzymatic reaction. The high degree of specificity of caspases enables a precisely controlled cascade process, rather than indiscriminate proteolysis. Caspases have several roles within the cascade: as triggers of the cell death process, as regulatory elements within it, and as effectors of cell death itself, the latter usuallybeing activated by caspases acting earlier in the cascade. At the end of this cascade, caspases act on a variety of signal transduction proteins, cytoskeletal and nuclear proteins, chromatin- modifying proteins, DNA repair proteins and endonucleases to target a cell for destruction by disintegrating its contents, including its DNA. Caspases can have roles other than in apoptosis, such as caspase-1 (interleukin-1 beta convertase), which is involved in the inflammatory process. The activation of apoptosis can sometimes lead to caspase-1 activation, providing a link between apoptosis and inflammation, such as during the targeting of infected cells.

Classification:

Caspases involved in apoptosis are classified into two groups, the initiator caspases, such as caspase-9 in mammals or its functional ortholog Dronc in Drosophila, and the effector caspases, such as caspases-3 and -7 in mammals and their homolog DrICE in Drosophila. An initiator caspase invariably contains an extended N-terminal prodomain (>90 amino acids) important for its function, whereas an effector caspase frequently contains 20–30 residues in its prodomain sequence. All caspases are synthesized in cells as catalytically inactive zymogens, and must undergo an activation process.

Caspases and chromatin breakdown

One of the hallmarks of apoptosis is the cleavage of chromosomal DNA into nucleosomal units. The caspases play an important role in this process by activating DNases, inhibiting DNA repair enzymes and breaking down structural proteins in the nucleus.

1) Inactivation of enzymes involved in DNA repair.

The enzyme poly (ADP-ribose) polymerase, or PARP, was one of the first proteins identified as a substrate for caspases. PARP is involved in repair of DNA damage and functions by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks and modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3.

2) Breakdown of structural nuclear proteins.

Lamins are intra-nuclear proteins that maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase 6 results in the chromatin condensation and nuclear fragmentation commonly observed in apoptotic cells.

3) Fragmentation of DNA.

The fragmentation of DNA into nucleosomal units - as seen in DNA laddering assays - is caused by an enzyme known as CAD, or caspase activated DNase. Normally CAD exists as an inactive complex with ICAD (inhibitor of CAD). During apoptosis, ICAD is cleaved by caspases, such as caspase 3, to release CAD. Rapid fragmentation of the nuclear DNA follows.

How is an effector caspase activated? An effector caspase is known to exist constitutively as a homodimer, both before and after the intrachain activation cleavage. However, as a consequence of the intrachain cleavage, the catalytic activity of an effector caspase is increased by several orders of magnitude . The mechanism of activation for a representative effector caspase, caspase-7, is revealed by the conformational changes of the active site following the activation cleavage.

How is an initiator caspase activated? For the initiator caspases, however, the definition of activation carries an entirely different meaning. Although an initiator caspase undergoes an autocatalytic intrachain cleavage, this cleavage appears to have only modest effect on its catalytic activity, the fully processed caspase-9 in isolation is only marginally active, much the same way as the unprocessed caspase-9 zymogen. In sharp contrast, association with the apoptosome leads to an enhancement of three orders of magnitude in the catalytic activity for the processed as well as the unprocessed caspase-9. Thus, for caspase-9, activation has little to do with the intrachain cleavage; rather, it refers to the apopto some-mediated enhancement of the catalytic activity of

caspase-9. At present, we do not understand the molecular mechanism for the activation of any initiator caspase. Nonetheless, two models have been proposed. Based on results using heterologous fusion proteins, an Induced Proximity model was proposed to provide a general explanation for the activation of initiator caspases. It states that the initiator caspases autoprocess themselves when they are brought into close proximity of each other. However, this model merely summarizes what have been observed experimentally in laboratories, and does not reveal the molecular mechanisms for the activation of initiator caspases.

Cellular Senescence

Cellular senescence was formally described more than 40 years ago as a process that limited the proliferation (growth) of normal human cells in culture. Thus, nearly half a century ago, the process now known as cellular senescence was linked to both tumor suppression and aging. Recent data validate the early idea that cellular senescence is important for tumor suppression.

Cellular senescence: a primer

Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells that can divide encounter oncogenic stress. With the possible exception of embryonic stem cells, most division-competent cells, including some tumor cells, can undergo senescence when appropriately stimulated.

What causes cellular senescence?

Senescence inducing stimuli are myriad. It is known now that the limited growth of human cells in culture is due in part to telomere erosion, the gradual loss of DNA at the ends of chromosomes. Telomeric DNA is lost with each S phase because DNA polymerases are unidirectional and cannot prime a new DNA strand, resulting in loss of DNA near the end of a chromosome; additionally, most cells do not express telomerase, the specialized enzyme that can restore telomeric DNA sequences de novo. We also know that eroded telomeres generate a persistent DNA damage response (DDR), which initiates and maintains the senescence growth arrest. In fact, many senescent cells harbor genomic damage at nontelomeric sites, which also generate the persistent DDR sigalling needed for the senescence growth arrest. DNA double strand breaks are especially potent senescence inducers. In addition, compounds such as histone deacetylase inhibitors, which relax chromatin without physically damaging DNA, activate the DDR proteins ataxia telangiectasia mutated (ATM) and the p53 tumor suppressor, and induce a senescence response. Finally, many cells senesce when they experience strong mitogenic signals, such as those delivered by certain oncogenes or highly expressed pro-proliferative genes. Notably, these mitogenicsignals can create DNA damageand a persistent DDR due to misfired replication origins and replication fork collapse. Thus, many senescence-inducing stimuli cause epigenomic disruption or genomic damage.

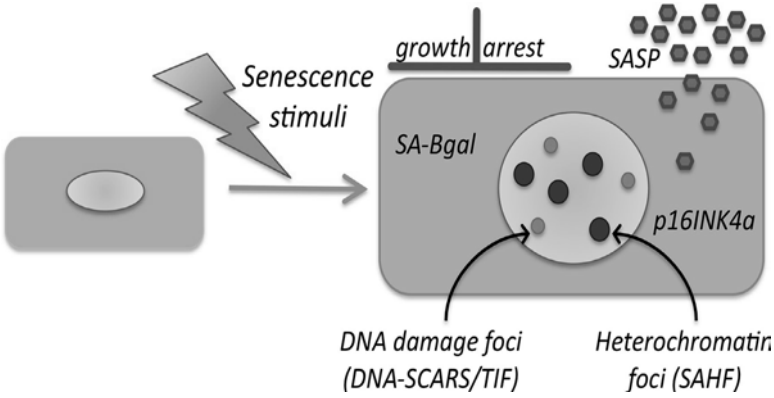

Figure: Hallmarks of senescent cells. Senescent cells differ from other nondividing (quiescent, terminally differentiated) cells in several ways, although no single feature of the senescent phenotype is exclusively specific. Hallmarks of senescent cells include an essentially irreversible growth arrest; expression of SA-Bgal and p16INK4a; robust secretion of numerous growth factors, cytokines, proteases, and other proteins (SASP); and nuclear foci containing DDR proteins (DNA-SCARS/TIF) or heterochromatin (SAHF). The pink circles in the nonsenescent cell (left) and senescent cell (right) represent the nucleus. Senescence can also occur, however, without detectable DDR sigalling. “Culture stress,” the natural and in vivo equivalent of which are unknown, causes a senescence arrest without significant telomere erosion. These stresses could include inappropriate substrata (e.g., tissue culture plastic), serum (most cells experience plasma, not serum, in vivo), and oxidative stress (e.g., culture in atmospheric O2, which is hyperphysiological). Cells also senesce without a DDR upon loss of the Pten tumor suppressor, a phosphatase that counteracts pro proliferative/pro-survival kinases. Additionally, ectopic expression of the cyclin-dependent kinase inhibitors (CDKis) that normally enforce the senescence growth arrest, notably p21WAF1 and p16INK4a, cause senescence without an obvious DDR.

What defines a senescent cell?

a. Senescent cells are not quiescent or terminally differentiated cells, although the distinction is not always straightforward. No marker or hallmark of senescence identified thus far is entirely specific to the senescent state. Further, not all senescent cells express all possible senescence markers. Nonetheless, senescent cells display several phenotypes, which, in aggregate, define the senescent state. Salient features of senescent cells are: a)The senescence growth arrest is essentially permanent and cannot be reversed by known physiological stimuli. However, some senescent cells that do not express the CDKi p16INK4a can resume growth after genetic interventions that inactivate the p53 tumor suppressor. So far, there is no evidence that spontaneous p53 inactivation occurs in senescent cells (whether in culture or in vivo), although such an event is not impossible.

b. Senescent cells increase in size, sometimes enlarging more than twofold relative to the size of nonsenescent counterparts.

c. Senescent cells express a senescence-associated β-galactosidase, which partly reflects the increase in lysosomal mass.

d. Most senescent cells express p16INK4a, which is not commonly expressed by quiescent or terminally differentiated cells. In some cells, p16INK4a, by activating the pRB tumor suppressor, causes formation of senescence-associated heterochromatin foci (SAHF), which silence critical pro-proliferative genes (Narita et al., 2003). p16INK4a, a tumor suppressor, is induced by culture stress and as a late response to telomeric or intrachromosomal DNA damage Moreover, p16INK4a expression increases with age in mice and humans and its activity has been functionally linked to the reduction in progenitor cell number that occurs in multiple tissues during aging.

e. Cells that senesce with persistent DDR sigalling harbor persistent nuclear foci, termed DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). These foci contain activated DDR proteins, including phospho-ATM and phosphorylated ATM/ataxia telangiectasia and Rad3 related (ATR) substrates and are distinguishable from transient damage foci. DNA-SCARS include dysfunctional telomeres or telomere dysfunction–induced foci.

f. Senescent cells with persistent DDR sigalling secrete growth factors, proteases, cytokines, and other factors that have potent autocrine and paracrine activities. As we discuss later, this senescence-associated secretory phenotype SASP) helps explain some of the biological activities of senescent cells.

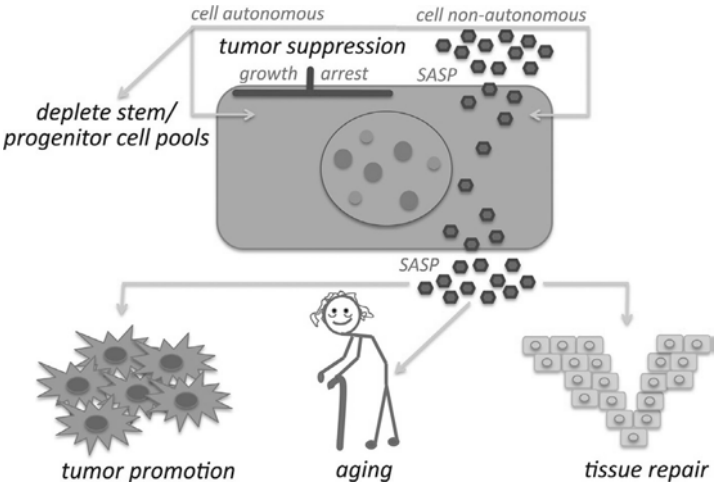

Figure. Biological activities of cellular senescence. Senescent cells arrest growth owing to cell autonomous mechanisms, imposed by the p53 and p16INK4a/pRB tumor suppressor pathways, and cell nonautonomous mechanisms, imposed by some of the proteins that comprise the SASP. The growth arrest is the main feature by which cellular senescence suppresses malignant tumorigenesis but can contribute to the depletion of proliferative (stem/progenitor) cell pools. Additionally, components of the SASP can promote tumor progression, facilitate wound healing, and, possibly, contribute to aging.

Probable Questions:

1. What is the difference between necrosis and apoptosis.

2. Discuss the mechanism of apoptosis.

3. Name some of the assay methods of apoptosis.

4. Discuss about Anti-apoptotic action of Neurotrophins.

5. Write about structure and Function of Telomerase.

6. Write about the role of caspase proteins.

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington,MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

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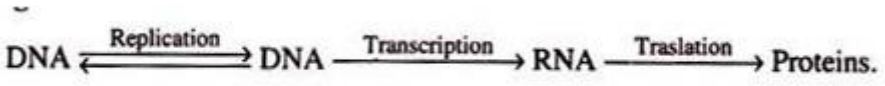
UNIT-XVIII

DNA Replication and Recombination: Topology, Variations and Nature of Replication; Replicon, Replicator, Fidelity and Processivity of replication; "Hayflick limit" and Telomerase activity; Regulation of Replication

Objective: In this unit we will learn about DNA replication process, "Hayflick limit" and also telomerase activity. We will also discuss how replication is regulated.

Central Dogma:

Genetic material is always nucleic acid and it is always DNA except some viruses. DNA is the storehouse of genetic information. This information is in the form of nucleotide sequence called genetic code. This information is copied and transcribed into RNA molecules. This information (genetic code) is for specific sequence of amino acids. The RNA then synthesizes proteins, which are specific sequence of amino acids, by a process called translation. In 1956 Francis Crick called this pathway of flow of genetic information as the Central Dogma.



Both transcription and translation are unidirectional. Proteins never serve as template for RNA synthesis. But sometimes RNA acts as a template for DNA synthesis (reverse transcription), Example is RNA viruses (HIV virus).

DNA Replication:

Genetic information present in double stranded DNA molecule is transmitted from one cell to another cell at the time of mitosis and from parent to progeny by faithful replication of parental DNA molecules. DNA molecule is coiled and twisted and has enormous size. This imposes several restrictions on DNA replication. DNA molecule must be uncoiled and the two strands must be separated for the replication process. One of the most important properties of DNA is that it forms its additional identical copies. The process of forming its replica copy is called replication. Replication is the basis of evolution of all morphologically complex forms of life.

Howard and Pelc (1953) demonstrated that in eukaryotes replication occurs during interphase between mitotic cycles and also during interphase of meiosis. During interphase of cell division the number of DNA molecules doubles which at anaphase is separated into two daughter cells, and thus equal number of chromosomes is maintained. However, replication does not occur during entire anaphase but is confined only to synthesis (vS) phase. There is a post-mitotic gap (G1) between the telophase and S phase. A

second premitotic gap (G2) is between the S phase and prophase. Only S phase involves replication process.

The Gl phase is most variable and in many eukaryotic cells it is completed within 3 to 4 hours or even months depending on physiological conditions. Mostly DNA synthesis is accomplished in 7 to 8 hours. In bacteria growing at log phase, DNA synthesis occurs from the time a cell originates to give rise to two daughter cells. It is noteworthy that bacteria divide only through binary fission. In general, DNA carries out two important functions such as hetero-catalytic function and autocatalytic function. The hetero-catalytic function is protein synthesis directed by DNA, and autocatalytic function is the synthesis of its own DNA into replica copies. However, replication of DNA in prokaryotes differs from that of eukaryotes.

Basic Features of DNA Replication:

All genetically relevant information of any DNA molecule is present in its sequence of bases on two strands. Therefore the main role of replication is to duplicate the base sequence of parent DNA molecule. The two strands have complementary base pairing. Adenine of one strand pairs with thymine of the opposite strand and guanine pairs with cytosine. This specific complementary base pairing provides the mechanism for the replication.

The two strands uncoil and permanently separate from each other. Each strand functions as a template for the new complementary daughter strand. The base sequence of parent or old strand directs the base sequence of new or daughter strand. If there is adenine in the parent or old strand, complementary thymine will be added to the new strand. Similarly, if there is cytosine in the parent strand, complementary guanine will be copied into the new daughter strand. Maintenance of integrity of genetic information is the main feature of replication.

Enzymes Involved In DNA Replication:

Both the prokaryotic and eukaryotic cells contain three types of nuclear enzymes that are essential for DNA replication. These enzymes are nucleases, polymerases and ligases.

(i) Nucleases:

The polynucleotide is held together by phosphodiester bonds. The nucleases hydrolyse the polynucleotide chain into the nucleotides. It attacks either at 3′ OH end or 5′ phosphate end of the chain. The nucleases are of two types (Fig. 5.17-B).

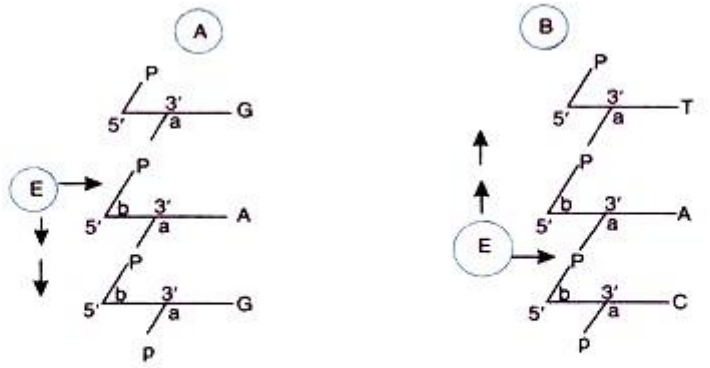


Fig. 5.17 : Exonuclease action on a polynucleotide chain. A, action in 5'→3' direction; B, action in 3'→5' direction; a 3′ OH side of phosphodiester linkage; b, 5' side of phosphodiester linkage.

(a) Exonucleases:

The nuclease that attacks on outer free end of the polynucleotide chain is called exonuclease. It breaks phosphodiester bond either in direction (A) or in 3'→ 5′ direction (B). The enzyme moves in either cases stepwise along the chain and removes nucleotides one by one. Thus, the whole chain is digested.

(b) Endonucleases:

The endonucleases attack within the inner portion of one or the double strands. Therefore, a nick is made on double stranded DNA molecule. However, if the polypeptide chain is single stranded (e.g. in DNA viruses), the attack of endonuclease will render the chain into two pieces. On double stranded DNA the nick contains two free ends that in turn act as template for DNA replication. Apart from this, the nicked double helix is distorted due to rotation of free molecules around its intact strand.

(ii) DNA Polymerases:

DNA polymerases carry out the process of polymerization of nucleotides and formation of polynucleotide chain. This enzyme is called replicase when it replicates the DNA molecules and inherited by daughter cells. In 1959, for the first time A. Romberg discovered an enzyme in E. coli which polymerized the deoxyribonucleotide triphosphate on a DNA template and produced complementary strand of DNA.

This enzyme was called DNA polymerase. Later on it was named as Kornberg polymerase or Romberg enzyme after the name of discoverer, for demonstrating in vitro polymerization of DNA. For the catalysis of polymerization, it requires the four deoxyribonucleotide triphosphates e.g. dATP, dGTP, dTTP and dCTP, a DNA template, a primer for initiation of catalytic activity and Mg^{++} (Fig. 5.18).

In prokaryotes, three types of DNA polymerases e.g. polymerase I (Poly-I), polymerase II(Pol II), and DNA polymerase III (Pol III) are found, whereas in eukaryotes three or four polymerases termed as α, β and γ polymerases and mitochondria (mt) DNA polymerase are present.

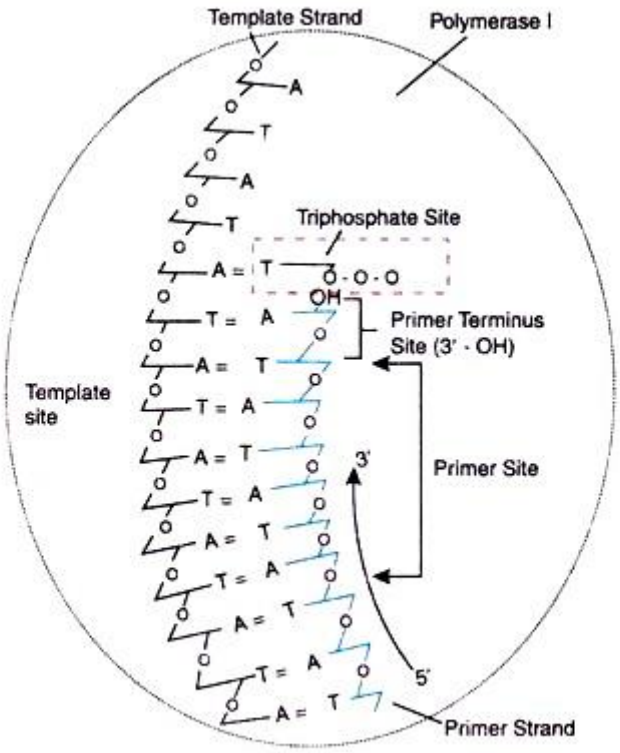


Fig. 5.18 : Diagram of DNA polymerase I of *E. coli.*

The molecular weight of α and γ polymerases are over 100,000 and that of β-polymerase is 30,000-50,000. The α and β polymerases are located in the nucleus. The β-polymerase copies a poly (A) or poly (C) template. The γ-polymerase copies many poly-ribonucleotides such as poly (A), poly (C), etc. The mtDNA polymerase is like γ-polymerase.

(a) Polymerase I (Pol I):

The Kornberg polymerase is known as Pol I. It is a single peptide chain with a molecular weight of 109,000 D. It is the largest single chain of globular protein known so far. One atom of zinc (Zn) per chain is present, therefore, it is metalloenzyine. In E. coli, approximately 400 molecules of Pol I are present.

Early experiments carried out by Kornberg revealed that when artificially synthesized DNA template strands alternating A and T i.e. poly d(AT) were incubated with polymerase and four radio- labelled nucleoside triphosphate, radioactive DN A containing alternating A and T was synthesized.

Though sufficient amount of dGTP and dCTP was present in the solution but these were not synthesized into DNA because the DNA strand contained only poly dAT. This emphasizes that Pol I synthesizes only complimentary copy of the template. Shape of Pol I has been studied through electron microscope. It is roughly spherical of about 65 A diameters (Fig. 5.18) which gets attached regularly to the DNA chain.

Pol I possesses several attachment sites such as:

(i) A template site for attachment to the DNA template,

(ii) A primer site of about 100 nucleotides contemporary to a segment of RNA on which the growth of newly synthesized DNA occur,

(iii) A primer terminus site containing a terminal 3'OH group at the tip, and

(iv) A triphosphate site for matching the incoming nucleoside triphosphates according to complementary nucleotide of DNA template.

Function:

Pol I plays a significant role in polymerization (synthetic) as well as degradation (exonucleolytic) process of nucleotides, Pol I is broken by trypsin into two fragments, a large fragment (MW 75,000) and a small fragment (MW 36,000). The large fragment shows 3′ → 5′ exonuclease activity, and the small fragment shows 5′ → 3′ exonuclease activity. In E.coli the following three types of functions of Pol I have been found.

Polymerization:

Polymerization is a process of synthesis in 5′ → 3′ direction of short segments of DNA chain from deoxyribonucleoside triphosphate monomers to the 3′ -OH end of a DNA strand. It is not the main polymerization enzyme because it cannot synthesize a long chain. It synthesizes only a small segment of DNA. It binds only to a DNA and forms nick in dsDNA. Therefore, it takes part in repair synthesis. In E.coli Pol I polymerize the nucleotides at the rate of 1,000 nucleotides per minute at 37°C. The chief enzyme associated with polymerization is known as polymerase III.

Exonuclease activity:

3' → 5′ exonuclease activity:

Pol I catalyses the breaking of one or two DNA strands in 3' → 5′ direction into the nucleotide components i.e. the nucleotides are set free in 3′ → 5′ direction which is reverse to polymerization direction.

Therefore, it is called 3′ → 5′ exonuclease activity. Pol I correct the errors made during the polymerization, and edits the mismatching nucleotides at the primer terminus before the start of strand synthesis. Therefore, the function of Pol I is termed as repair synthesis.

5′ → 3′ exonuclease activity:

Pol I also breaks the polynucleotide chain in 5′ → 3′ direction with the removal of nucleotide residues. Upon exposure of DNA to the ultraviolet light two adjacent pyrimidines such as thymines are covalently linked forming pyrimidine dimers. These dimers block the replication of DNA. Therefore, removal of

pyrimidine dimers e.g. thymine dimers (T=T) is necessary. Through 5′ → 3′ exonuclease activity, Pol I removes pyrimidine dimers. Secondly, DNA synthesis occurs on RNA primer in the form Okazaki fragments. Through 5′ → 3′ exonuclease activity Pol I remove RNA primer and seal the gap with deoxyribonucleotides. Its onward movement results in removal of ribonucleotides from the front portion followed by of deoxyribonucleotides behind it.

(b) Polymerase II (Pol II):

For several years Pol I was considered to be responsible for replicating in E.coli. but the work done during 1970s made it clear that Pol I is associated only with repair synthesis and the other enzymes, Pol II and Pol III are involved in polymerization process. Pol II is a single polypeptide chain (MW 90,000) that shows polymerization in 5' → 3' direction of a complementary chain. It also shows exonuclease activity in 3' → 5' direction but not in 5' → 3' direction. The polymerization activity of Pol II is much less than Pol I in E.coli cells. About 50 nucleotides per minute are synthesized. E.coli cells contain about 40 Pol II molecules. The 3′ → 5′ exonuclease activity of Pol II shows that it also plays a role in repair synthesis or DNA damaged by U.V. light just like Pol I. In the absence of Pol I, it can elongate the Okazaki fragments. Therefore, Pol II is an alternative to Pol I.

(c) Polymerase III (Pol III):

DNA polymerase III is several times more active than Pol I and Pol II enzymes. It is the dimer of two polypeptide chains with molecular weight 1,40,000 and 40,000 D respectively. Pol III polymerises deoxyribonucleoside triphosphates in direction very efficiently. Therefore, Pol III is the main polymerization enzyme that can polymerize about 15,000 nucleotides per minutes in E. coll. Like Pol II, it cannot polymerize efficiently if the template DNA is too long but can do when ATP and certain protein factors are present. Synthesis of a long template also occurs when an auxiliary protein DNA (copolymerase II) is linked with Pol III and produced Pol III-co Pol II complex. In addition Pol III also shows 3'→ 5′ exonuclease activity like Pol II.

The 5'→ 3′ exonuclease activity is absent. All the polymerases e.g. Pol I, Pol II and Pol III show 3'→ 5′ exonuclease activity, whereas besides Pol I, the other two polymerases (Pol I and Pol II) lack 5'→ 3′ exonuclease activity. However, some workers have shown both 3'→ 5′ and 5'→ 3′ exonuclease activity in Pol III.

(iii) DNA Ligases:

The DNA ligases seal single strand nicks in DNA which has 5'→ 3′ termini. It catalyses the formation of phosphodiester bonds between 3′-OH and 5′-PO_4 group of a nick, and turns into an intact DNA. There are two types of DNA ligases: E. coli DNA ligase and T4 DNA ligase. The E. coli DNA ligase requires nicotinamide adenine dinucleotide (NAD^+) as cofactor, whereas T4 DNA ligase uses ATP as cofactor for joining reaction of the nick (Fig 5.19).

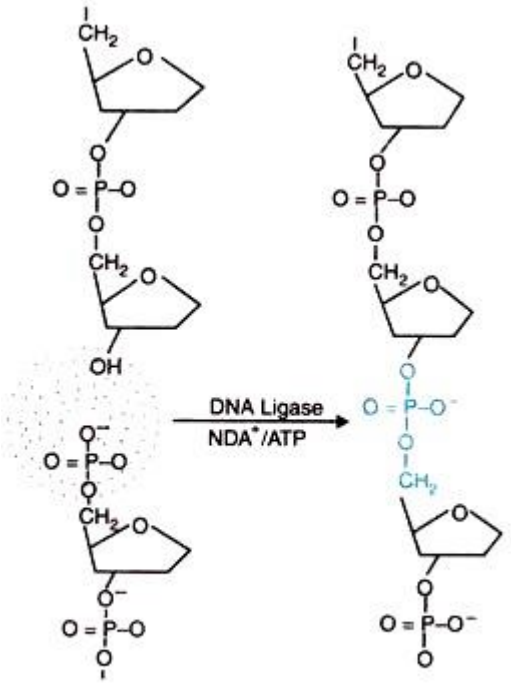


Fig. 5.19 : Action of DNA ligase in the presence of NAD+/ATP.

Mechanism of DNA Replication:

A. Romberg (1992) has nicely discussed the DNA replication. In E. coli DNA replication has been investigated most extensively. It was thought that in eukaryotes probably similar mechanism operates. However, it has been found that in E. coli replication always starts at a very unique site called the origin. In E. coli die replicating apparatus contains an enzyme complex at the point where DNA thread is attached. Through this replicating point DNA thread moves and replication is accomplished. In eukaryotes enzyme moves along the DNA thread. It has earlier been described that E. coli possesses three types of DNA polymerases; each reads DNA template in 3'→ 5' direction and catalyses the synthesis of DNA in 5'→ 3' direction. The polymerases read deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP) as substrate and a DNA template.

To the 3′ end of growing point, the nucleotides are added after interaction of 3′-OH end of deoxyribose with alpha (first) phosphate group of substrate releasing pyrophosphate as below:

$$\text{dNTPs (dATP, dGTP, dCTP, dTTP)} \xrightarrow[\text{DNA template}]{\text{DNApolymerase}} \text{DNA + nPPi}$$

Before the replication begins, DNA double helix must be unwounded to give rise to single strand. Unwinding process occurs very rapidly to form a fork that rotates about 75-l(X) revolutions per second. The unwinding process is facilitated by helicases.

Some important genes and proteins associated with replication are given in Table 5.7:

Table 5.7 : Some important genes and proteins involved in DNA replication (modified after Moat and Foster, 1995)

Proteins	*Genes*	*Map Location*	*Mol. wt.*	*Function*
—	*ori*C	83.5		Origin of replication
Protein I	*dna*T	99		Priming
Protein n	*pri*B	95		"
Protein n'	*pri*A	89		-
Protein n"	*pri* C	10		"
DnaA	*dna*A	82	54,000	Initiation, binds *ori*C
DnaB	*dna*B	91	55,000	Mobile promoter, helicase prepriming
DnaC	*dna*C	99	25,000	Forms dnaB-dnaC complex
Pol III (a)	*dna*E	4	129,000	DNA Pol III holoenzyme, elongation
Primase	*dna*G	67	60,000	Priming, RNA primer synthesis
γ-subunit	*dna*Zx	10	47,500	Synthesis, part of gamma complex
Helix-destabilizing	*ssb*-1	91	20,000	Single strand binding SSB protein
Helix-unwinding	*rep*	85	—	Strand separation
E subunit	*dna*Q	5	27,000	Proof reading
Dna Pol I	*pol*A	85	109,000	Gap filling, primer degradation
Ligase	*lig*	52	75,000	Ligation of single stranded nicks in phosphodiester backbone
Dna gyrase (α)	*gyr*A	48	105,000	Super twisting
(β)	*gyr*B	82	95,000	Super coil relaxation
Dna Pol II	*pol*B	2	120,000	?
Dna helicase I	—	—	180,000	Unwinding
Dna helicase II	—	—	75,000	Unwinding

Overall DNA replication is accomplished in the following stages (Fig 5.20):

(i) Unwinding of Double Helix:

Helicases are responsible for unwinding of double helix. They use energy from ATP to unwind short stretches of helix just ahead the replication fork. After separation of strand it is very necessary to keep them single stranded through single stranded DNA binding proteins (SSB). The SSB is a tetramer with each of four subunits of a molecular weight of 18,500 – 22,000 Dalton. It may bind as a binding sites of 8-10 nucleotides (Fig. 5.20). However there is possibility of leading tension and formation of super coils in helix.

The relieving of tension and promotion of unwinding process are done by the enzyme topoisomerases which transiently break one of two strands in such a way that it remains unchanged. It ties or unties a knot in DNA strand. DNA gyrase is one of the E. coli topoisomerases that removes super coiling of DNA during replication. Thus there is formation of a ssDNA template.

(ii) DNA Replication:

DNA replication is accomplished in several steps. The first step is the RNA-primer synthesis on DNA template near origin of replication. Synthesis of RNA primer is very necessary because during DNA replication there is chance of more error in initial laying down of first few nucleotides to pre-existing DNA template. DNA Pol I and Pol II cannot synthesize DNA without an RNA primer; therefore a special RNA polymerase called primase synthesizes an about 10 nucleotide long short primer.

Before priming, preprimer intermediate is formed with the help of six pre-priming proteins e.g. dnaB, dnaC, n, n', n" and i proteins. For the synthesis of primer, primase needs several accessary proteins which combine with primase. The complex of primase- accessory protein is called primosome. Therefore, DNA Pol III holoenzyme starts synthesis of DNA in 5'→ 3′ direction at the end of RNA primer (Fig. 5.20B). The second step is chain elongation. A new DNA strand starts synthesizing by addition of deoxyribonucieoside triphosphates to the 3′ end of last nucleotide of RNA primer. DNA synthesis occurs in 5'→ 3′ direction catalysed by the replisome. The replisome has two DNA Pol III holoenzyme complexes. It is a very large complex containing DNA Pol III and several proteins. The γ and β-subunits bind the holoenzyme to the DNA template and primer. The a-subunit synthesizes the DNA. One polymerase continuously copies the leading strand (i.e. a strand growing in the direction of replication fork and showing continuous replication). The lagging strand (i.e. a strand growing in opposite direction of replication fork and showing discontinuous replication of strand) loops around replisome continuously. There is formation of Y shaped replicating fork at the point where two strands are separated.

On leading strand DNA synthesis occurs continuously because there is always a free 3′-OH at the replication fork to which a new nucleotide is added. But on the opposite strand called lagging strand, DNA synthesis occurs discontinuously because there is no 3′ -OH at the replication fork to which a new nucleotide can link. On this strand there is free 3′ -OH at the opposite end away from growing point. Therefore, on lagging strand a small (11 bases long) RNA primer must be synthesised by primase to provide free 3′ -OH group. The replication of DNA has two directions, one direction (unidirectional replication) and both the directions (bidirectional replication) from the point of origin. The bidirectional replication is found in most of the bacteria (e.g. E. coli. Bacillus subtilis. Salmonella typhimurium, etc.), whereas unidirectional replication occurs in E. coli bacteriophages (P_2 and 186) and mtDNA of mouse LD cells.

Finally, about 1000-2000 nucleotides long fragment in bacteria and about 100 nucleotides long fragment in eukaryotic cells are synthesized. These fragments are called the Okazaki fragments after the name of a Japanese discoverer, R. Okazaki (Fig. 5.20 B).

(iii) Removal of RNA Primer and Completion of DNA Strand:

When the Okazaki fragments are formed; most of lagging strands become duplicated. The RNA primer is removed by DNA Pol I or RNase H. DNA polymerase I synthesizes a short segment of complementary

DNA to seal the gap. Possibly Pol I remove the primer nucleotide at a time and replace it with suitable complementary deoxyribonucleotide (Fig. 5.20)

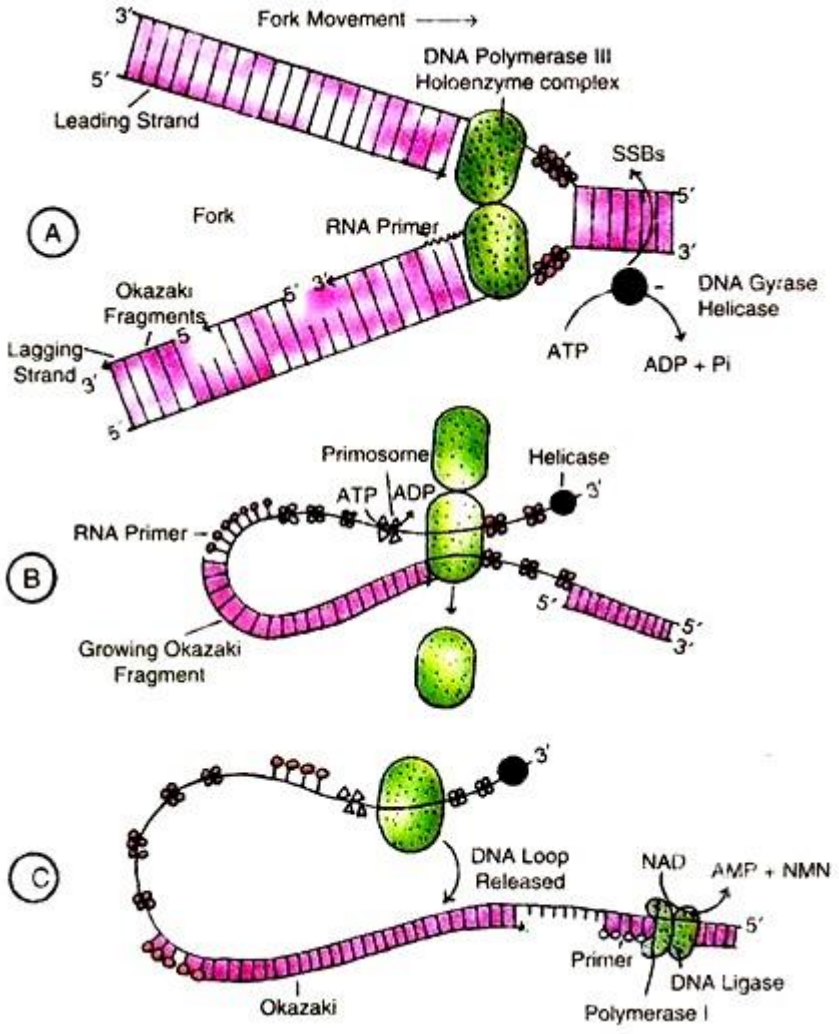


Fig. 5.20 : DNA replication in bacteria (diagrammatic) A. overall process of DNA replication; B. action of replisome, helicases and primosome, and looping of lagging strand around polymerase III; C, complrtion of Okazaki fragments, realease of lagging strand and sealing the gap by DNA ligase.

(iv) Joining of Fragments:

At the end, the fragments are joined by DNA ligase that forms a phosphodiester bond between 3′-OH end of growing strand and 5’ end of an Okazaki fragment (Fig. 5.20 C). Reaction of DNA ligases is given in Fig 5.19. In mutants defective ligase is produced; therefore, joining of Okazaki fragments is greatly improved. E. coli DNA ligase derives energy from NAD. It is first adenylated by AMP moiety of NAD releasing the nicotinamide mononucleotide (NMN). The adenylated ligase reacts with ssDNA having a nick and forms phosphodiester bond.

The complete reaction is as below:

E. coli ligase + NAD → ligase – AMP + NMN

Ligase – AMP + DNA (with break) → phosphodiester + ligase + AMP

AMP + NMN → NAD

Obviously, DNA replication is a very complex process. If any error is made during replication, it leads to mutation. E. coli makes error about 10^{-6} per gene per generation. The DNA Pol I and Pol III act as proof reader of the newly formed DNA. These move along new DNA synthesized, read mistakes formed due to improper base-pairing and correct those through exo-nuclease activity. Despite all these complexity, replication takes place rapidly in bacteria (750-1,000 base pairs per second) and much slower in eukaryotes (50-100 base pairs per second). The replication of the DNA can be schematically divided in three steps: initiation, elongation and termination.

Step # 1. The Initiation Step:

As seen above, no DNA polymerase can perform any de novo synthesis (contrary to RNA polymerases which can initiate by placing a complementary ribonucleoside-5′-triphosphate opposite to the DNA to be transcribed). The primer 3'OH which will serve as start point for the synthesis of DNA must therefore be supplied to the DNA polymerase during the initiation step.

On the other hand, replication starts at a precise point of the DNA called origin of replication. When the DNA is double-stranded, it is first necessary to open out the 2 strands in order to carry out the actual initiation step. We will again examine this mechanism. The 3'OH can be supplied in various ways. In a very large number of cases it is a short RNA fragment either synthesized by a RNA polymerase or a DNA primase (E.coli, B.subtilis, phages M13, ϕX 174, T4, T7, eucaryotic cells, polyoma virus or Simian virus SV40) or vestige of a larger RNA (virus of hepatitis B).

In the case of single-stranded DNA, the primer can also be formed by one end doubling back upon itself (hair-pin structure); this is postulated for the parvoviruses (see fig. 6-31). Lastly, it may be a deoxyribonucleoside-5′-monophosphate bound covalently to a protein; the 2 best known cases are, on the one hand, the adenoviruses, and on the other, a phage of *B.subtilis* called ϕ29.

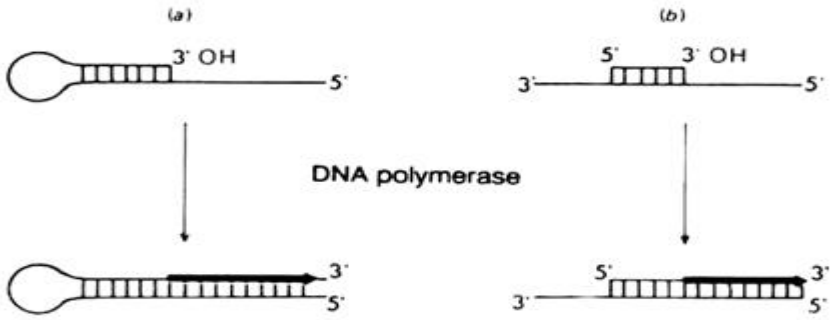


FIG. 6-31. — Polymerization of the DNA from a 3′OH primer
(*a*) Single-stranded DNA with a primer resulting from a pairing of the chain folded back upon itself.
(*b*) Single-stranded DNA with an RNA primer.
The red line represents the chain synthesized by DNA polymerase.

Step # 2. The Elongation Step:

The elongation steps poses more or less problems depending on whether the DNA is single-stranded or double-stranded. The most complex — and most interesting — situation arises when the 2 strands of a double-stranded DNA must be replicated simultaneously. A replication fork is then formed.

We have seen in the foregoing, that the 2 chains of the double helix are of opposite polarities. But we know that polymerization takes place strictly in the direction 5′ → 3′. This implies that the DNA synthesis can take place on one strand continuously in the direction 5′ → 3′, but, in order to copy the other strand, it can take place only in the direction opposite of that of the progression of the fork. To solve this dilemma it was tried to find a mechanism which would allow progression in the direction 3′ → 5′, for example the polymerization of nucleosides 3′ triphosphates. One could never find such precursors, nor a DNA polymerase capable of polymerizing nucleosides 3′ triphosphates synthesized chemically.

The question therefore remained unanswered: how does synthesis take place in the direction 3′ → 5′. The answer was given by experiments carried out on the replication of phage T4, by the Okazaki team; by adding a precursor of DNA synthesis, like thymidine (deoxythymidine) in a highly radioactive form and for a very short time, it is possible to label only the DNA which has just been synthesized. This method shows that the newly synthesized DNA is in the form of nucleotide chains of 1 000 to 2 000 residues (Okazaki fragments). These fragments are then bound to one another to form a continuous chain in the direction 5′ → 3′. The same type of experiment could be reproduced with various phages, bacteria and eucaryotic cells. In eucaryotes however, these fragments are definitely shorter in size (100 to 200 nucleotides). Therefore, the replication fork has, in fact, an asymmetric structure. One of the chains is synthesized in the direction 5′ → 3′ in a continuous manner (leading strand). The “other, although advancing as a whole in the direction 3′ → 5′, consists of fragments synthesized individually in the direction 5′ → 3′; this is a discontinuous synthesis (lagging strand).

Although these results eliminate one difficulty they require a solution to the problem of the primer (initiation of polymerization). Here again, it could be shown that the primer was a short

oligoribonucleotide, synthesized by DNA primase. This primer will then be eliminated, the gap thus created being filled by a DNA polymerase.

The elongation step necessitates the coordinated action of numerous proteins.

Step # 3**. The Termination Step:**

Studies on the synthesis of DNA were first focussed on the elongation step, then, more recently, on the initiation step which in fact, permits the control of the process. Little is therefore known presently on the termination of replication.

DNA Replication is Semi-Conservative:

Watson and Crick model suggested that DNA replication is semi-conservative. It implies that half of the DNA is conserved. Only one new strand is synthesized, the other strand is the original DNA strand (template) that is retained. Each parental DNA strand serves as a template for one new complementary strand. The new strand is hydrogen bonded to its parental template strand and forms double helix. Each of these strands of the double helix contains one original parental strand and one newly formed strand.

Meselson and Stahl Experiment:

Mathew Meselson and Franklin Stahl proved experimentally that parental strands of a helix are distributed equally between the two daughter molecules. They made use of the heavy isotope ^{15}N as a tag to differentially label the parental strands. E. coli was grown in a medium containing ^{15}N labeled NH_4Cl.

In this way both strands of DNA molecules were labeled with radioactive heavy isotope ^{15}N in their purines and pyrimidines. Therefore both strands were heavy or HH DNA. The bacteria were then transferred into a medium containing the common non-radioactive nitrogen ^{14}N, which is a light medium. It was found that after one cell division daughter molecules had one ^{15}N strand the other ^{14}N strand. So this is a hybrid molecule, a heavy light of HL.

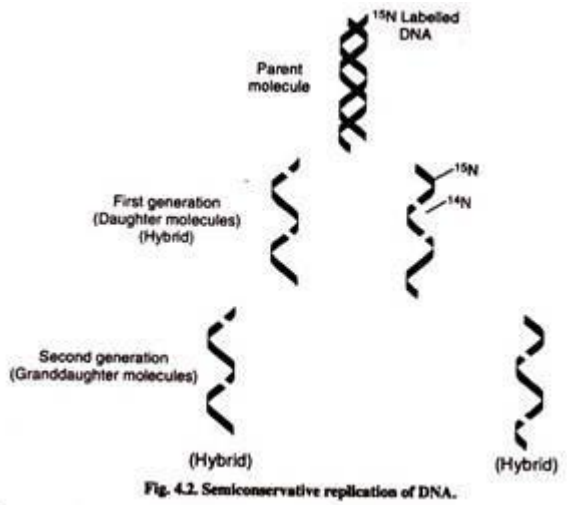


Fig. 4.2. Semiconservative replication of DNA.

After the second cell division, out of four molecules, two DNA molecules contained ^{U}N(LL). The other two were hybrid molecules (HL). This proves that during replication, one parent strand is conserved and the other new strand is synthesized. Thus DNA replication is a semi-conservative process.

DNA Synthesis takes Place in 5′ → 3′ Direction Only:

A new strand of DNA is always synthesized in 5′ → 3′ direction. The free 3′-end enables it to be elongated. Because the two strands are antiparallel, orientation of new growing strand is opposite to the template strand.

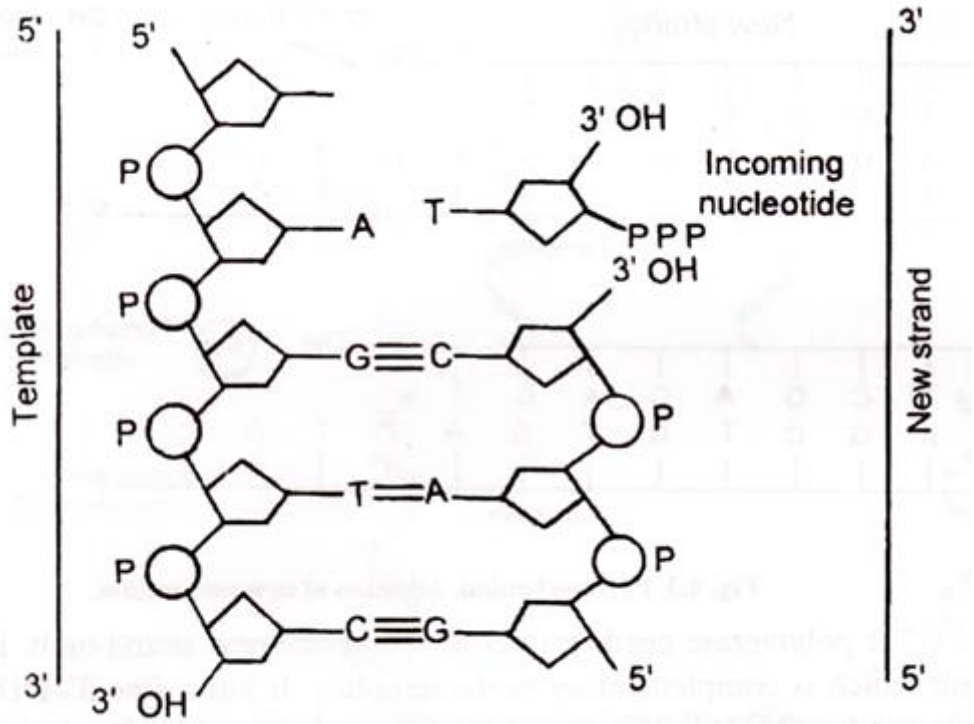


Fig. 4.5.

DNA Replication is Discontinuous in One Strand:

If the synthesis is to proceed in 5′ → 3′ direction, only one strand can be synthesized in correct 5′ → 3′ direction. As the strands are antiparallel, the other strand will have to be synthesized in 3′ 5′ direction. This constraint is overcome in an ingenious way. As the two strands unwind and the replication fork grows, one strand is synthesized in 5′ 3′ direction correctly in a continuous manner. This is called leading strand. It grows in the same direction as the replication fork. Mr. Reiji Okazaki discovered that the other strand is synthesized discontinuously and a little after the leading strand. Therefore this is called lagging strand. The lagging strand also grows in 5′ 3′ direction which is opposite to the direction of replication fork. In lagging strand, the DNA synthesis does not occur continuously but in small fragments, which are called Okazaki fragments. Later these fragments are joined and sealed by the action of DNA ligase enzyme to form a continuous strand.

Okazaki fragments are about 1000-2000 nucleotides long in E. coli and about 100-200 nucleotides long in eukaryotes. Both the strands begin the synthesis starting on a primer segment. Leading strand synthesis starting on a primer proceeds continuously keeping pace with the unwinding of DNA at the replication fork. Each Okazaki fragment is synthesized on a short RNA primer. DNA polymerase III binds to RNA primer and adds deoxyribonucelotides. Lagging strand proceeds in opposite direction from the fork movement.

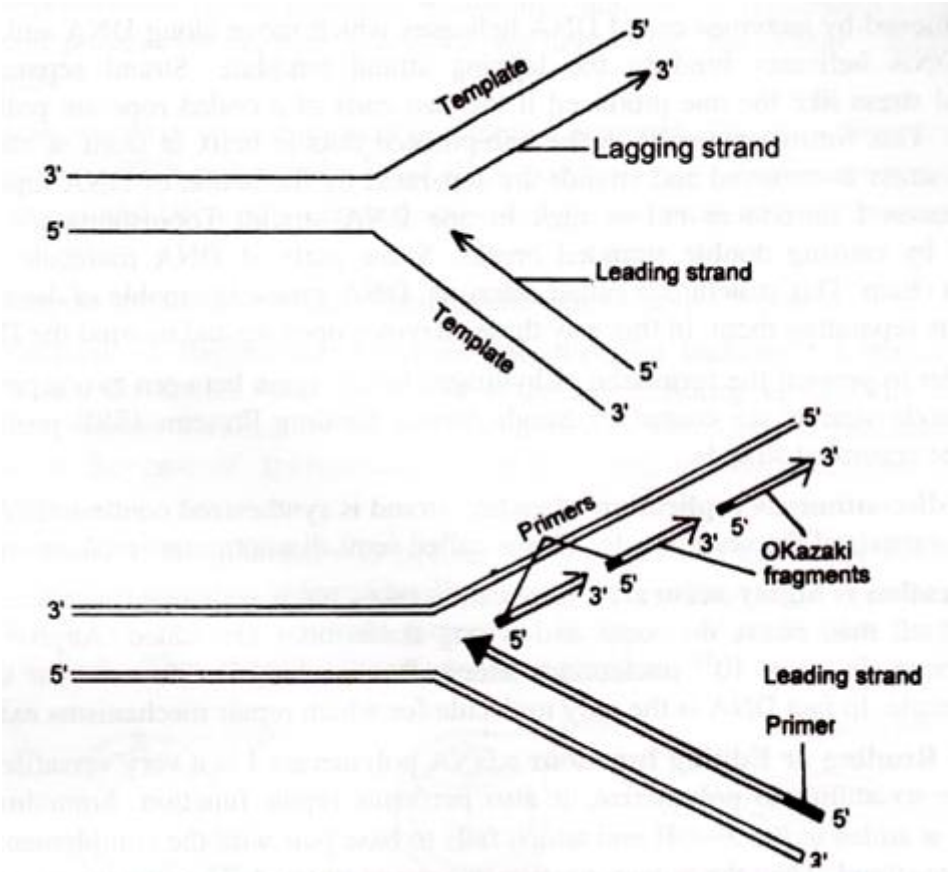


Fig. 4.6. Synthesis of leading and lagging strand on template strands.

The primer ribonucleotides are removed and replaced by deoxyribornucleotides and then joined. The removal of RNA primer is done by exonuclease activity of DNA polymerase I.

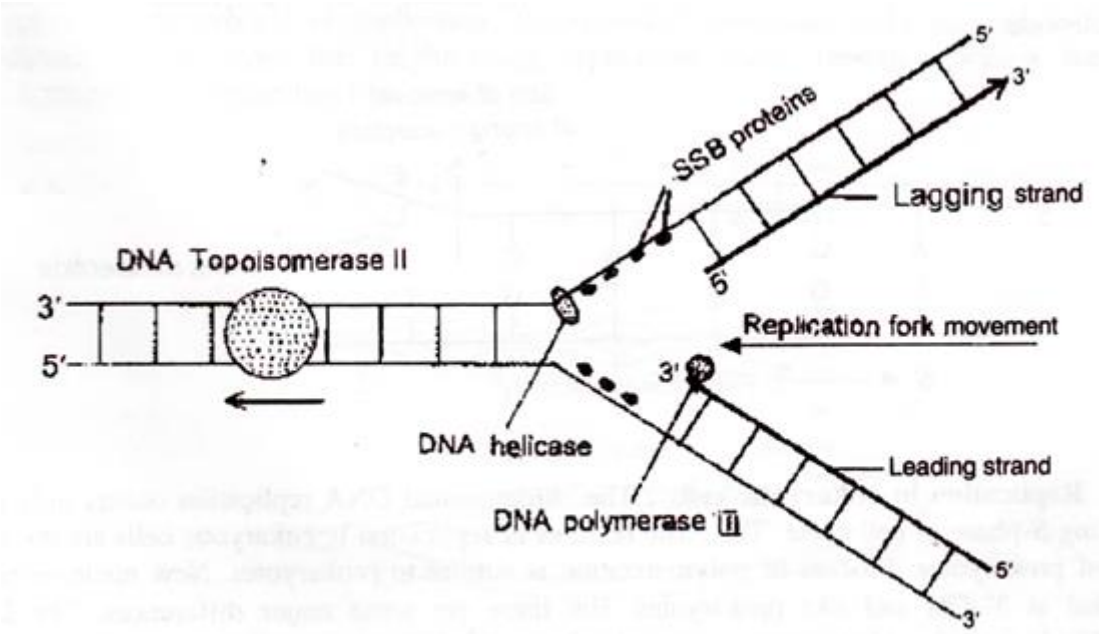


Fig. 4.7. Unwinding of double helix.

Unwinding of Double Helix:

The first step of DNA replication is the unwinding parent double helix molecule so that each strand acts as a template for the new strand. Unwinding mechanism is very complex. Hydrogen bonds between two strands are broken. This is achieved by enzymes called DNA helicases which move along DNA and separate the strands. DNA helicases bind to the lagging strand template. Strand separation create topological stress like the one produced if the two ends of a coiled rope are pulled apart fc- separation. This forms supercoils in the unreplicated double helix in front of the replication fork. This stress is removed and strands are separated by the action of DNA topoisomerase Topoisomerase I introduces cut or nick in one DNA strand.

Topoisonmerase II remove supercoils by causing double stranded breaks. Some parts of DNA molecule have circle linked as a chain. This structure is called catenane. DNA gyrase is capable of decatenating two circles, thus separating them. In this way these enzymes open up and unwind the DNA helix. In order to prevent the formation of hydrogen bonds again between two separated strand- the two single strands are coated by Single Strand Binding Proteins (SSB proteins), which stabilize the separated strands.

Semi-discontinuous Replication:

Leading strand is synthesized continuously but lagging strand is synthesized discontinuously. This is called semi-discontinuous replication.

Replication is Highly Accurate:

Replication takes place with an extraordinary accuracy In spite of all this, errors do occur and wrong nucleotides are added. Approximately one mistake occurs in every 10^{1}" nucleotides added. But

mechanisms do exist for the repair of DNA molecule. In fact DNA is the only molecule for which repair mechanisms exist.

Proof Reading or Editing Functions:

DNA polymerase I is a very versatile enzyme. In addition to its ability to polymerize, it also performs repair function. Sometimes a wrong nucleotide is added at the 3′-OH end which fails to base pair with the complementary base on the template strand. Then the polymerization process is stopped. The enzyme moves backward and removes the wrong base by degrading DNA. Then the polymerization activity resumes and chain growth starts again by adding correct base. It removes only the most recent error. This process is known as 3′ → 5′ exonuclease activity and the enzyme is called proof reading exonuclease. This editing function gives a second chance to DNA polymerase to add correct nucleotide.

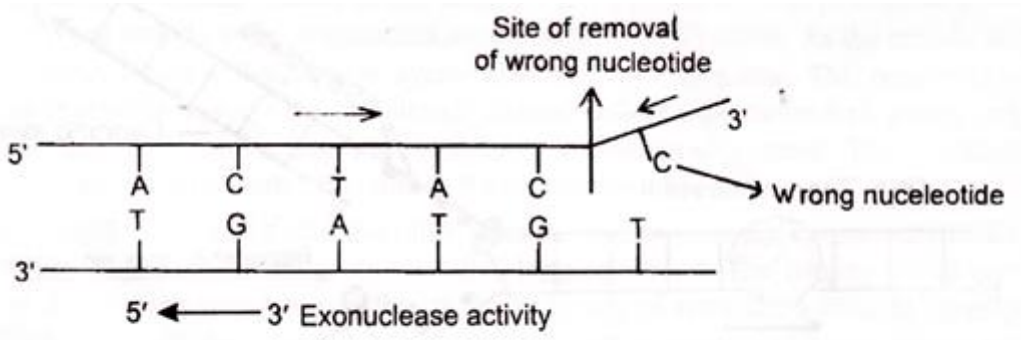


Fig. 4.8.

Replication in Eukaryotic Cells:

The chromosomal DNA replication occurs only once during S-phase of cell cycle. The basic features of replication in eukaryotic cells are the same as of prokaryotes. Process of polymerization is similar to prokaryotes. New nucleotides are added at 3′-OH end like prokaryotes. But there are some major differences. The DNA molecule considerably large as compared to bacterial chromosomes. Eukaryotic chromosomes are linear and has free ends. They are organized into complex nucleoproteins chromatin. Replication in eukaryotes is considerably faster. Replication is completed in three minutes in embryonic cels of drosophilla. Chromosomes of higher organisms have multiple origins of replication and all replication forks proceed bidirectionally. Eukaryotic DNA has repeated units of replication called replicons. Enormous number of replication units require large number of polymerase enzyme molecules. An animal cell has 20000 – 60000 molecules of polymerase ∝. Eukaryotes have several types of polymerase enzymes. The main three enzymes are DNA polymerase ∝ DNA polymerase δ and DNA polymerase ϵ. Replication is initiated by DNA polymerase ∝ and DNA polymerase δ and ϵ bring about rapid polymerization because of their high processivity. Eukaryotic replication also synthesizes end structures or telomeres.

Formation of Replication Forks and Replication Bubbles:

Initiation of replication occurs within the double helix and rarely at the end. Opening up of DNA molecule creates replication bubble. Replication bubble progresses in the form of replication fork in one direction in the case of unidirectional replication and in both directions in bi-directional replications.

Bidirectional Replication:

In circular DNA of bacteria and linear DNA of eukaryotes, DNA replication proceeds bidirectionally starting from a fixed origin of replication. The two replication forks move in opposite directions. Bidirectional replication may have multiple replication forks. They speed up the process of replication.

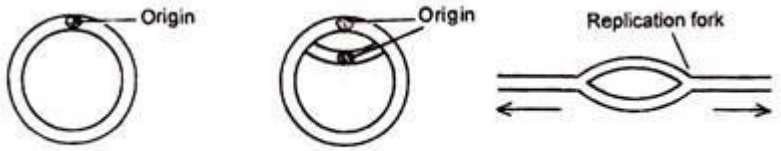


Fig. 4.9. Bidirectional replication.

Eukaryotic chromosomes are very long. In order to speed up the process of replication, a chromosome may have thousands of points of origin of replication (O). They tremendously speed up the process of replication. Bidirectional replication forks proceed in opposite directions and meet the neighbouring replication units, thereby opening the entire chromosome by separating two strands.

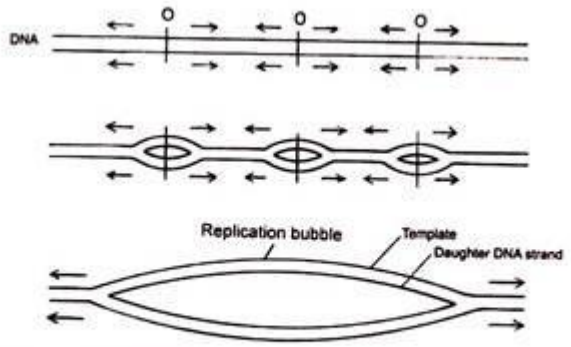


Fig. 4.10. Multiple origins of replication. Replication forks proceed in opposite direction and meet the neighbouring ones, thereby opening the entire chromosome, by separating two strands.

Unwinding and Replication of Circular Double Helix DNA of E. Coli:

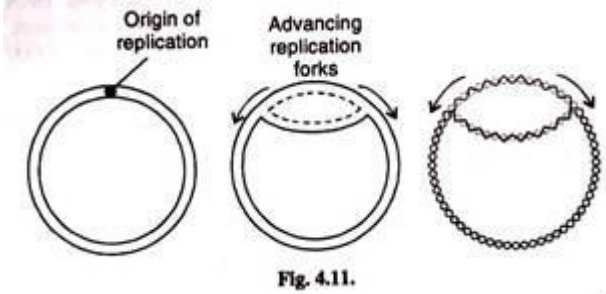


Fig. 4.11.

Most of the bacteria have double stranded circular DNA with no free ends. This poses a problem of unwinding at the time of replication. Replication originates at one point and the two replication forks proceed in opposite directions. The advancing replication forks meet at a point opposite to the point of origin thus opening up the coiled DNA molecule. This is called θ (theta) model of replication.

But the unwinding is a very complex mechanism as the two strands are coiled. The two advancing replication forks make the remaining entire un-replicated portion of DNA overwound. Thus, the un-replicated portion becomes so tightly coiled that the advancing replication forks are not able to advance further. This is because of positive supercoiling of the un-replicated portion. In E. coli an enzyme called DNA gyrase produces negative supercoiling, thus removing the positive supercoiling. This leads to unwinding of the entire double helix circular chromosome of the bacteria.

Newly Synthesized Eukaryotic DNA Immediately Forms Nucleosomes:

Before the replication takes place, the DNA disentangles itself from the nucleosomes. After replication, newly synthesized DNA immediately rejoins the octomers of histone proteins to form nucleosomes. Large amounts of histones are synthesized during .S'-phase of interphase. Old histones are not lost. Old histones are present on both daughter chromosomes. During replication, nucleosomes are broken down into their components and later reassembled into nucleosomes.

Models for DNA Replication:

The pattern of DNA replication in prokaryotes differs from that of eukaryotes. These differences are due to the nature of prokaryotic DNA. For example, the circular DNA of E. coli replicates at a replication point, the origin. In others the mechanism is different.

Some of the models for DNA replication are discussed below:

(i) The Cairns Model for DNA Replication:

J.Caims (1963) was the first to visualize the replicating chromosome of E. coli through auto-radiographic study. This study revealed that the replicating DNA thread got fixed at a specific site called origin, and was moving in one direction and within a replicating fork where the original strands are synthesized (Fig. 5.21).

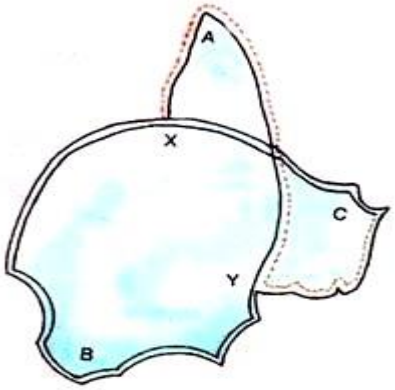


Fig. 5.21 : Diagrammatic presentation of autoradiographic observation of *E.coli*. K12 DNA replication as observed by Cairns (1963). A, B and C are the three section of chromosome arising at two forks, X and Y.

Further studies have shown that in circular DNA (Fig. 5.22A) the two strands denatured at origin site (B). There is bidirectional DNA synthesis i.e. after initiation there appeared two growing points travelling in opposite directions around the circular DNA molecule (C). Growing points proceed with unwinding of DNA double helix.

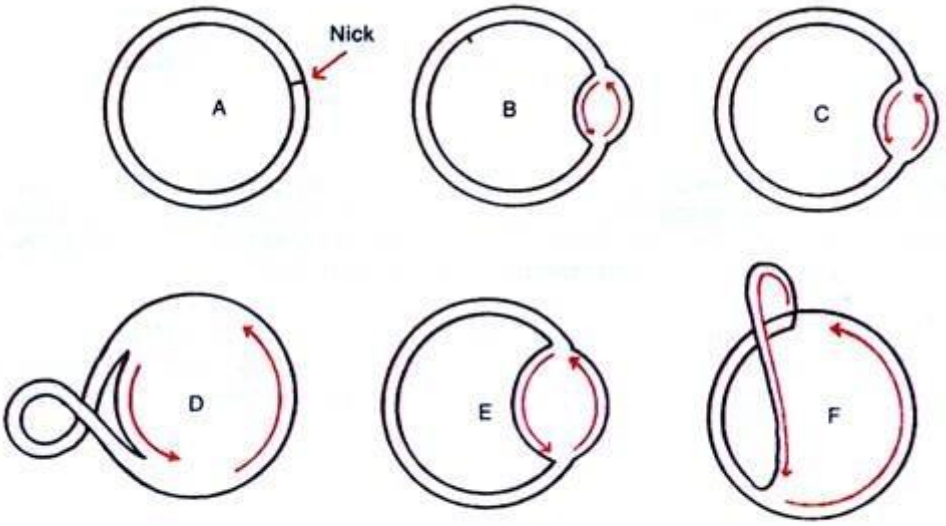


Fig. 5.22 : Cairns model for replication of circular DNA.

The process of unwinding creates a torque that is transmitted to the un-replicated part of the DNA molecule resulting in formation of super helix or super twist (D). Super coil prevents its further replication. A temporary nick is made on one of the strands by a swivelling protein (w) which contracts this effect. The nick allows the parental strand for their free rotation on each other and finally freed. At the end swivelling protein seals the nick to continue the replication process (E). Replication process goes on and the two growing point converge on the terminus (F).

(ii) The Rolling Circle Model:

Gilbert and Dressier (1969) described the rolling circle model to explain reactivation in ssDNA viruses e.g. Ø × 174 and the transfer of E. coli sex factor. (iii) Replication in Eukaryotic Chromosome:

DNA replication in eukaryotic chromosome is not well understood as compared with prokaryotic chromosome. However, the well accepted model for replication of eukaryotic DNA is the bidirectional model (Fig. 5.23). DNA synthesis starts at a midpoint of replication unit which is called initiation point (O-origin) (Fig. 5.23 A-B), and progresses in both the directions until reaches the terminal point (T) (C). The replication fork meets at T point (D) on the entire chromosome. There may be thousands of initiation

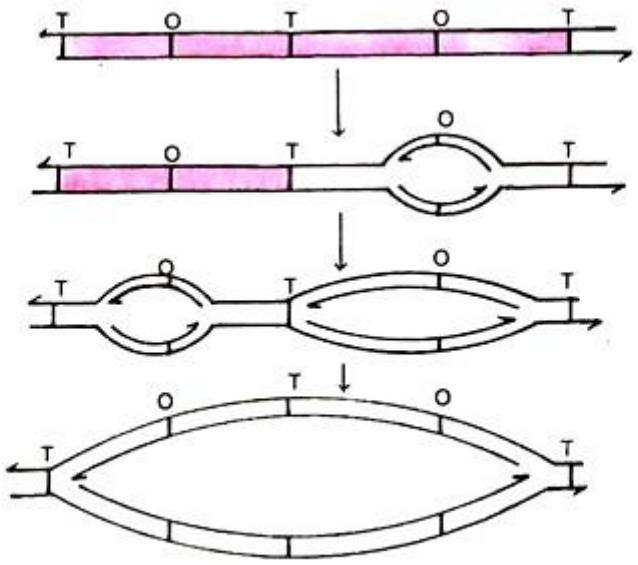


Fig. 5.23 : A bidirectional model for replication of mammalian chromosome. O, origin of replication site; T, termination site.

5. Watson and Crick's Model for DNA Replication:

Each chain of double helix acts as template and is evolved in replication of DNA. Watson and Crick proposed that the hydrogen bonds between the base pairs of two strands are broken and separated from each other. Each purine and pyrimidine base of the strands forms hydrogen bonds with complementary free nucleotides to be involved in polymerization in the cell. The free nucleotides form phosphodiester bonds with deoxyribose residue resulting in formation of a new polynucleotide molecule (Fig. 5.15). This model of Watson and Crick for DNA replication was later on verified experimentally.

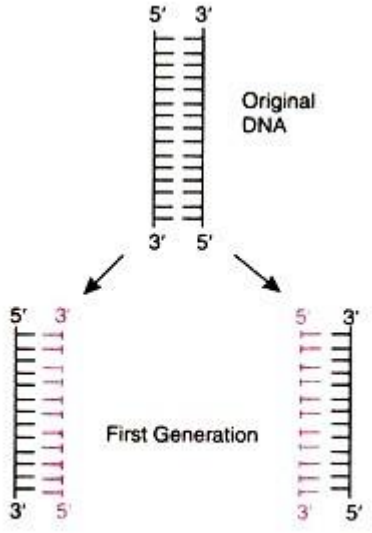


Fig. 5.15 : Semiconservative model for DNA replication as proposed by Watson and Crick.

Experimental Evidence for Watson and Crick's Model for DNA Replication:

M. Meselson and F. Stahl (1958) provided the experimental support for Watson and Crick's model for semiconservative nature of DNA replication which is called Meselson-Stahl experiment. They grew E.

coli cells in medium containing heavy isotopic nitrogen (^{15}N) for several generations. They obtained a population of E. coli that contained totally ^{15}N – labelled DNA. Density was measured by density gradient centrifugation in CsCl containing ethidium bromide. Density of ^{15}N- DNA was heavier (1.722 g/cc) than the normal DNA (1.708 g/cc). Again the cells of E. coli were grown on medium containing less dense isotopic nitrogen (^{14}N) and were allowed to multiply several times.

After the first generation, DNA was extracted which was found to be hybrid of ^{15}N-^{14}N (Fig. 5.16). This strand is ^{15}N and the other ^{14}N. It was neither heavier than ^{15}N nor lighter than ^{14}N. In the cells of first generation 50% ^{14}N – DNA, and 50% hybrid (^{15}N – ^{14}N) DNA was recorded. In the second generation the ratio of normal and hybrid DNA molecules was 3:1. This was the semiconservative nature of DNA replication because in the first generation one of the parental strands is converted into progenies and the other complementary polynucleotide strand is replicated. Thus, the two strands were the hybrids.

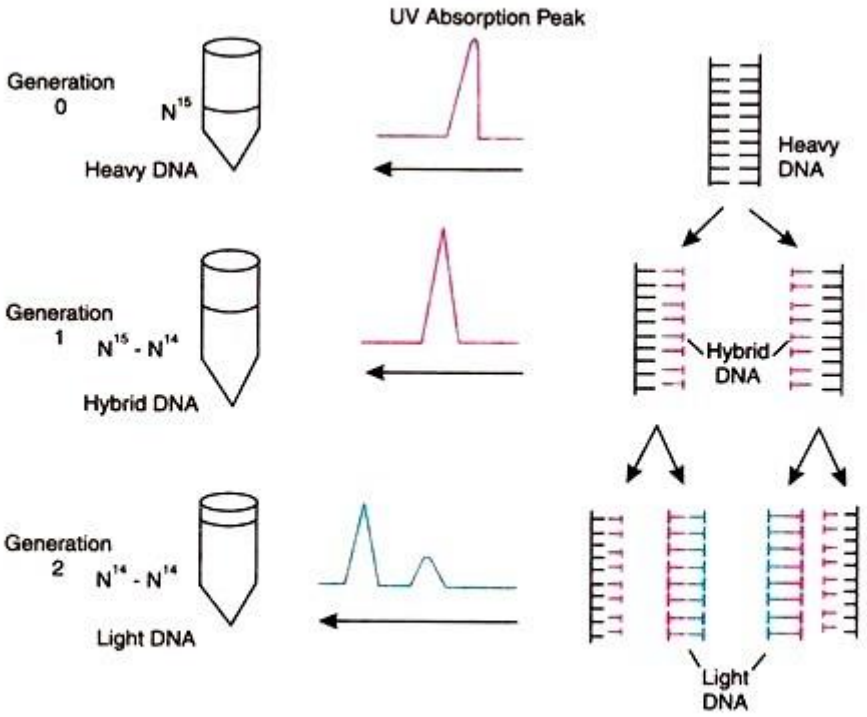


Fig. 5.16 : Meselson and Stahl's experiment demonstrating semiconservative replication of DNA.

One can postulate for conservative mode of DNA replication i.e. both the original DNA strands act as template for a new duplex but is not separated, and results in an old and new double helix in the first generation. No hybrid ^{15}N – ^{14}N-DNA is formed. Therefore, this model could not be supported by Meselson-Stahl experiment. Also, this experiment does not support for dispersive mode of DNA replication in which model there is no pattern of replication. The parental strands break randomly at several points during replication. Each segment will replicate and rejoin randomly. This results in varying amount of old and new DNA molecules in daughter cells. After first generation instead of a single ^{15}N-

^{14}N hybrid, a wide spectrum of DNA densities is detected. Therefore, the dispersive mode of replication is also ruled out through Meselson and Stahl experiment.

Hayflick Limit: The Hayflick Limit is a concept that helps to explain the mechanisms behind cellular aging. The concept states that a normal human cell can only replicate and divide forty to sixty times before it cannot divide anymore, and will break down by programmed cell death or apoptosis

Telomerase

Introduction

Telomerase is the enzyme responsible for maintenance of the length of telomeres by addition of guanine-rich repetitive sequences. Telomerase is a ribonucleoprotein complex. The core enzyme includes telomerase reverse transcriptase and telomerase RNA containing a template site for DNA elongation. The telomerase complex also contains a number of auxiliary components that provide for functioning of telomerase in vivo. Some proteins are necessary for maturation of the telomerase complex and degradation of its components. The existence of the enzyme preventing telomere shortening was predicted long before its discovery by the Russian scientist A. M. Olovnikov. He suggested naming this enzyme telomerase. Telomere length correlates with the cell proliferative potential. A hypothesis by Olovnikov suggests that maintenance of telomere length is responsible for the proliferative potential. Telomeres are DNA–protein structures that are localized at the ends of eukaryotic chromosomes. They protect the linear ends of eukaryotic chromosomes against degradation and fusion, thus maintaining genome stability. The cell replication apparatus is not able to provide for complete replication of chromosome ends (See Fig. below); also, telomeres are subject to the action of nucleases and other destructive factors. As a result, telomeres shorten during each cell division. In most organisms the main mechanism of telomere length maintenance is completion of DNA telomere repeats by telomerase. This enzyme elongates the chromosome ′ 3 -end, whereas the complementary strand is completed by DNA polymerases.

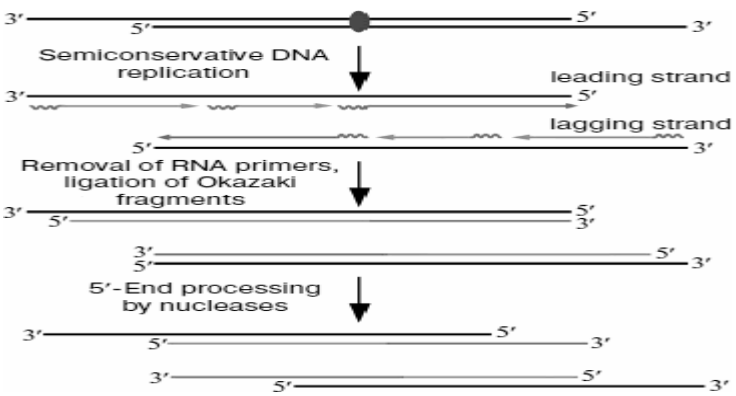


Figure. Telomere shortening due to under-replication and processing in each cell division.

In humans, somatic cells proliferation potential is strictly limited and senescence follows approximately 50-70 cell divisions. No doubt, DNA polymerase is not capable to completely copy DNA at the very ends of chromosomes; therefore, approximately 50 nucleotides are lost during each cell cycle, which results in gradual telomere length shortening. Critically short telomeres cause senescence, following crisis, and cell death. However, in tumor cells the system of telomere length maintenance is activated. Besides catalytic telomere elongation, independent telomerase functions can be also involved in cell cycle regulation. Inhibition of the telomerase catalytic function and resulting cessation of telomere length maintenance will help in restriction of tumor cell replication potential.

On the other hand, formation of temporarily active enzyme via its intracellular activation or due to stimulation of expression of telomerase components will result in telomerase activation and telomere elongation that can be used for correction of degenerative changes. There are data showing that activation of telomerase is associated with the development of cancer, and that it is active in cells exhibiting potential for unlimited division. It is known that telomerase is active in 85% of cancer tumors, while in the other 15% of cases different mechanisms of telomere length maintenance based on recombination are active. It should be noted that telomerase activity is not found in usual somatic tissues.

The main criterion of telomerase efficiency is the number of telomeric repeats at the ends of telomeres. Telomere length reduction is a symptom of many diseases and can be both the result of primary telomerase dysfunction (like those caused by mutations in the main telomerase components, hTERT, hTR, or by disturbance in telomere-organizing systems) and the result of premature telomere loss induced by different factors. Inborn dyskeratosis is of the first type. It was the first identified human genetic disease caused by disturbance in the system of telomere length maintenance. This disease is characterized by skin hyperpigmentation, epithelium keratinization, nail dystrophy, and progressive aplastic anemia. In most cases autosomal diseases are due to mutations in the H/ACA region of human telomerase RNA, while X-chromosome-linked cases emerge due to mutations in protein dyskerin leading to disturbance in telomerase complex assembly.

Telomerase structure and function:

As mentioned above, telomerase is a particular reverse transcriptase working in a complex with special telomerase RNA (See Fig below). Telomerase substrates in its reaction are

deoxynucleotide 5′-triphosphates and the telomere 3′ terminus (in tests in vitro it is DNA-oligonucleotide containing the sequence corresponding to telomeric repeats of chromosomes). The particular property distinguishing telomerase from different RNA- dependent DNA polymerases is the use of a fixed region of special telomerase RNA as template for telomere elongation.

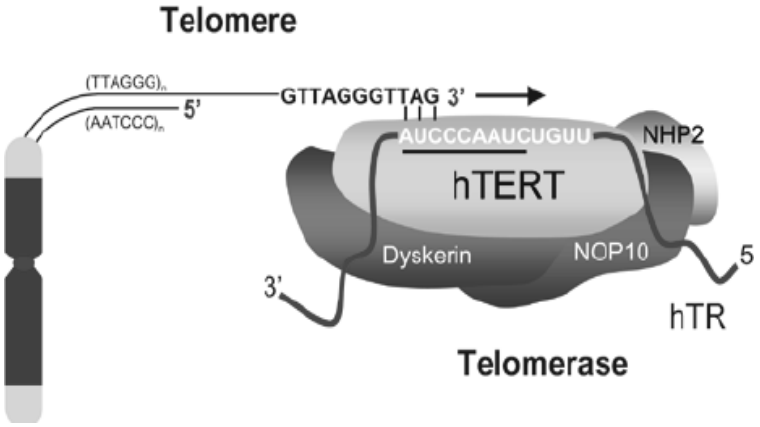


Figure. Structure and function of telomerase. The human telomerase complex consists of a catalytic sub- unit (hTERT), an RNA component (hTR), dyskerin, NOP2, NHP2, and additional associated proteins. Telomerase RNA interacts with telomere not only at this template region, but additionally in the so-called “anchor site”. Telomerase is able to add several telomeric repeats during a single act of attachment to oligonucleotide substrate.

Main Components of Telomerase Complex

Telomerase RNA (TER) contains template region and different functionally important secondary structure elements involved in template region restriction, protein subunit binding, and partially carrying out catalytic and other functions. Telomerase reverse transcriptase (TERT) contains a catalytically important domain resembling that of reverse transcriptase, as well as only telomerase-specific domains necessary for TER and DNA substrate binding and for functional activity of telomerase. TER and TERT form the core enzyme. These components are enough to provide functional activity of telomerase invitro. In vivo functioning requires auxiliary proteins, some of which are included in the holoenzyme. Despite high interest in telomerase and importance of its study in applied aspect, structural data on TERT, TER, and other telomerase proteins have become available relatively recently due to complication of telomerase investigation (very low intracellular enzyme content, difficulties in isolation of its components in soluble form and in sufficient amount, etc.).

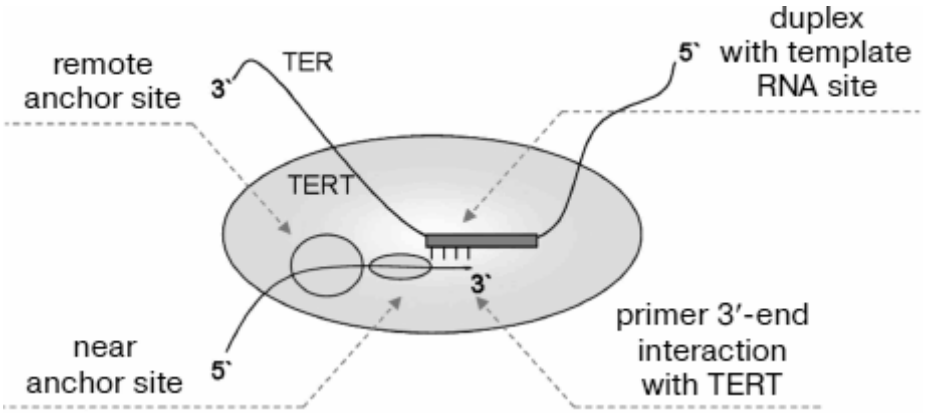


Figure: Interaction of primer with telomerase.

Telomerase binding proteins

Proteins specifically binding telomeric sequence and factors interacting with these proteins form a dynamic ribonucleoprotein structure. This structure has a protective role; it participates in telomere length regulation and is responsible for gene silence at telomeric and telomere-side sites. Also, telomere structures serve as targets for inhibitors that prevent telomerase binding to telomere.

Yeast telomere DNA consists of a 250-350 bp long double-stranded region with $C_{1-3}A/TG_{1-3}$ sequence and a short single-stranded protruding end with TG_{1-3} sequence. Yeast telomeric repeats $((TG)_{0-6}TGGGTGTG(G))_n$ are heterogeneous, unlike the homogeneous repeats of mammalian telomeres $(TTAGGG)_n$. In yeasts the telomere double-stranded region directly binds protein Rap1p, and the single-stranded region binds Cdc13p. Rap1p interacts with complex of Sir (silent information regulators) proteins that are responsible for heterochromatin formation in the subtelomeric region. Protein Rap1p also interacts with telomere proteins Rif1p and Rif2p. These proteins are associated with the telomere during the whole cell.In mammals telomeric DNA is more closely packed in nucleosomes compared to other eukaryotes, and some nucleosomes carry heterochromatin markers. Proteins TRF1 and TRF2 interact with double-stranded telomeric DNA, and protein POT1 and its partner

TPP1 interact with the single-stranded region. Protein TRF2 binds RAP1. Proteins binding single-stranded and double-stranded DNA interact with each other via TIN2 protein interacting with TRF1 and TRF2 as well as with TPP1 protein.

Inhibition of telomerase:

All presently available inhibitors of telomerase activity can be divided into three groups based on their action and chemical properties. They are nucleoside and nucleotide analogs working as substrate inhibitors, various low molecular weight compounds with different mechanisms of action, and oligonucleotide-based inhibitor. Let us consider all three groups.

Nucleos(t)ide analogs are well known inhibitors for DNA-polymerases. Inhibition of DNA polymerization is due to the impossibility of incorporation of the next nucleotide residue because of competitive binding of these substances in the enzyme active site. Since the catalytic subunit of telomerase is RNA-depended DNA-polymerase or a reverse transcriptase and there are antivirus preparations such as those for therapy of HIV- infections blocking reverse transcriptase, the idea to check inhibitory activity of such preparations towards telomerase became apparent. The most widely used inhibitor of HIV reverse transcriptase is azidothymidine (AZT). AZT was the first nucleos(t)ide analog tested for telomerase inhibition. Another class of telomerase inhibitors consists of a group of low molecular weight compounds with structures different from nucleos(t)ides. This group includes substances influencing mainly hTERT. Some of them are used in HIV therapy. Rubromycins and purpuromycin appeared to be powerful inhibitors (50% inhibitory concentrations are as low as 3 μM). It was reported that some quinolines form a family of integrase inhibitors. These inhibitors block telomerase either due to disturbance of protein–nucleic acid interactions supporting telomere structure, or due to blocking the substrate (telomere) binding to enzyme (telomerase). In 2005 it was shown that the natural lactone helenalin is a telomerase inhibitor. The mode of action of this cytostatic agent is not clear. Perhaps helenalin affects telomerase activity through its interaction with nuclear factor κB and regulation of hTERT level. Curiously, polyunsaturated fatty acids inhibit telomerase via direct interaction with the catalytic subunit, and at the same time they switch off expression of the hTERT gene. A unique approach to telomerase inhibition was recently proposed—the use of low molecular weight substances involved in recognition of RNA/DNA heteroduplexes formed upon the interaction of telomerase RNA with chromosome ends.

It was shown that the main catechin of green tea (Epigallocatechin gallate) not only directly inhibits telomerase in a concentration-dependent manner, but also induces apoptosis in cells of tumor of head and neck via inhibiting the telomerase activity. The most interesting potential inhibitor was found upon screening of 16,000 organic compounds. It is an isothiozolin derivative (50% inhibition is achieved at 1 μM concentration) that is a noncompetitive inhibitor relative to substrate and deoxynucleotide triphosphates. It possesses remarkable selectivity. It simultaneously has no effect on DNA polymerase and HIV reverse transcriptase. Glutathione and dithiothreitol enhance its inhibitory activity. This fact suggests telomerase inhibition by affecting cysteine residues in the catalytic subunit. The third class of inhibitors is oligonucleotides. The main components for targeting telomerase by oligonucleotides are hTR and messenger RNA for hTERT.

Fidelity of replication

With replication occurring so rapidly, mistakes can happen during DNA replication, they are extraordinarily rare. In the case of DNA synthesis via DNA polymerase III in *E.coli*, only one mistake per 100 million nucleotides is made. Therefore, DNA synthesis occurs with a high degree of accuracy or **fidelity.** The replication involves several mechanisms to serve the fidelity.First, the hydrogen bonding between G and C or A and T is much more stable than between mismatched pairs. However, this stability accounts for only part of the fidelity, because mismatching due to stability considerations accounts for 1 mistake per 1000 nucleotides. Second, the active site of DNA polymerase preferentially catalyzes the attachment of nucleotides when the correct bases are located in opposite strands. Helix distortions caused by mispairing usually prevent an incorrect nucleotide from properly occupying the active site of DNA polymerase. By comparison, the correct nucleotide occupies the active site with precision and undergoes induced fit, which is necessary for catalysis. The inability of incorrect nucleotides to undergo induced fit decreases the error rate to a range of 1 in 100,000 to 1 million. A third way that DNA polymerase decreases the error rate is by the enzymatic removal of mismatched nucleotides. DNA polymerase can identify a mismatched nucleotide and remove it from the daughter strand. This occurs by exonuclease cleavage of the bonds between adjacent nucleotides at the ´3end of the newly made strand. The ability to remove mismatched bases by this mechanism is called the **proofreading function** of DNA polymerase. Proofreading occurs by the removal of nucleotides in the 3 to 5´ direction at the 3´ exonuclease site. After the

mismatched nucleotide is removed, DNA Polymerase resumes DNA synthesis in the 5′ to 3′ direction (Figure 12).

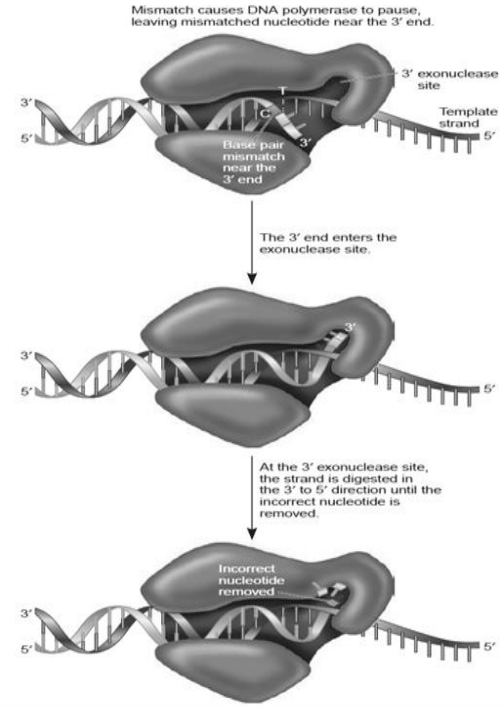


Figure 12. Proof reading function of DNA polymerase

Probable questions:

1. What is the nature of DNA Replication. Explain with examples.
2. Write short notes on a) Replication Fork, b) Okazaki fragments c) Leading strand and Lagging strand
3. How initiation of replication occurs.
4. Discuss enzymology of DNA replication.
5. How termination of replication occurs?
6. Discuss fidelity of Replication.
7.

Suggested Readings:

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3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington,MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

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UNIT-XIX

DNA damage response and DNA Repair

Objective: In this unit we will learn about different types of DNA damages and repair mechanisms. Repair of spontaneous and induced mutations, mechanism of DNA repair and repair by direct reversion.; You will also learn about excision repair and SOS response.

Introduction:

DNA is a highly stable and versatile molecule. Though sometimes the damage is caused to it, it is able to maintain the integrity of information contained in it. The perpetuation of genetic material from generation to generation depends upon keeping the rates of mutation at low level. DNA has many elaborate mechanisms to repair any damage or distortion.

The most frequent sources of damage to DNA are the inaccuracy in DNA replication and chemical changes in DNA. Malfunction of the process of replication can lead to incorporation of wrong bases, which are mismatched with the complementary strand. The damage causing chemicals break the backbone of the strand and chemically alter the bases. Alkylation, oxidation and methylation cause damage to bases. X-rays and gamma radiations cause single or double stranded breaks in DNA.A change in the sequence of bases if replicated and passed on to the next generation becomes permanent and leads to mutation. At the same time mutations are also necessary which provide raw material for evolution. Without evolution, the new species, even human beings would not have arisen. Therefore a balance between mutation and repair is necessary.

Definition of DNA Repair:

One of the main objectives of biological system is to maintain base sequences of DNA from one generation to the other. Changes in DNA sequence arise during replication of DNA damage by chemical mutagens and radiation. During replication if incorrect nucleotides have been added, they are corrected through editing system by DNA Pol I and DNA Pol III.

The other systems also exist for correcting the errors missed by editing function. It is called mismatch repair system. Mismatch repair system edits the errors left by DNA Pol I and DNA III and removes the wrong nucleotides. Proof reading by Pol I and III.

DNA is always damaged and mutated by several chemicals and radiation. Only a few errors accumulate in DNA sequence. The stable errors cause mutation and the rest are eliminated. If errors in DNA sequence are corrected before cell division, no mutation occurs. However, there are some DNA damages which cannot be mutated because the damages are not replicated. Therefore, such damages cause cell death.

Types of Damage:

Damage to DNA includes any deviation from the usual double helix structure.

1. Simple Mutations:

Simplest mutations are switching of one base for another base. In transition one pyrimidine is substituted by another pyrimidine and purine with another purine. Trans-version involves substitution of a pyramidine by a purine and purine by a pyrimidine such as T by G or A and A by C or T. Other simple mutations are detection, insertion of a single nucleotide or a small number of nucleotides. Mutations which change a single nucleotide are called point mutations.

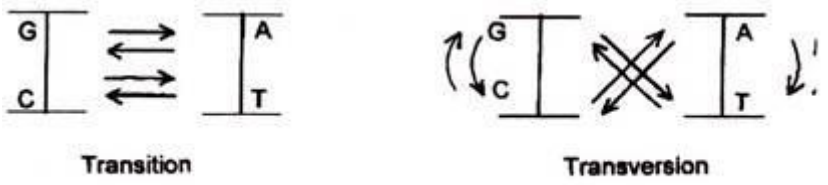


Fig. 5.1.

2. Deamination:

The common alteration of form or damage includes deamination of cytosine (C) to form uracil (u) which base pairs with adenine (A) in next replication instead of guanine (G) with which the original cytosine would have paired.As uracil is not present in DNA, adenine base pairs with thymine (T). Therefore C-G pair is replaced by T-A in next replication cycle. Similarly, hypoxanthine results from adenine deamination.

3. Missing Bases:

Cleavage of N-glycosidic bond between purine and sugar causes loss of purine base from DNA. This is called depurination. This apurinic site becomes non-coding lesion.

4. Chemical Modification of Bases:

Chemical modification of any of the four bases of DNA leads to modified bases. Methyl groups are added to various bases. Guanine forms 7- methylguanine, 3-methylguanine. Adenine forms 3-methyladenine. Cytosine forms 5- Methylcytosine.

Replacement of amino group by a keto group converts 5-methylcytosine to thymine.

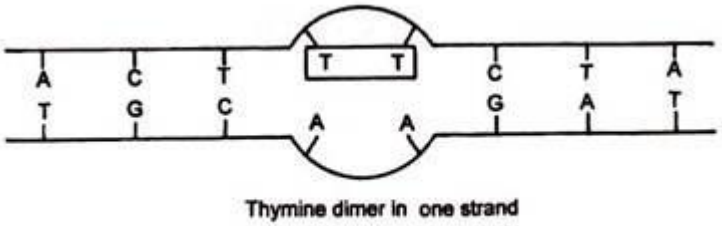


Fig. 5.2.

5. Formation of Pyrimidine Dimers (Thymine Dimers):

Formation of thymine dimers is very common in which a covalent bond (cyclobutyl ring) is formed between adjacent thymine bases. This leads to loss of base pairing with opposite stand. A bacteria may have thousands of dimers immediately after exposure to ultraviolet radiations.

6. Strand Breaks:

Sometimes phosphodiester bonds break in one strand of DNA helix. This is caused by various chemicals like peroxides, radiations and by enzymes like DNases. This leads to breaks in DNA backbone. Single strand breaks are more common than double strand breaks.

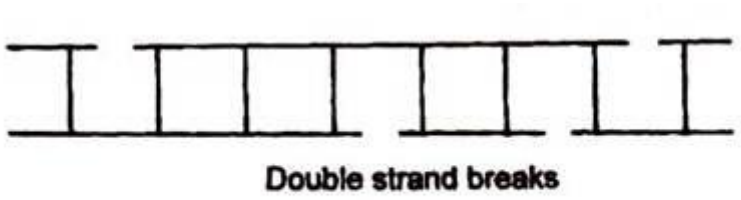


Fig. 5.3.

Sometimes X-rays, electronic beams and other radiations may cause phosphodiester bonds breaks in both strands which may not be directly opposite to each other. This leads to double strand breaks.

Some sites on DNA are more susceptible to damage. These are called hot-stops.

Repair Mechanisms:

A. Photo Reactivation:

We know that exposure of UV-irradiated bacteria immediately afterwards to visible light restores to a considerable degree the viability of the UV-inactivated bacteria. This phenomenon mown as photo reactivation, is based on enzymatic cleavage of the thymine dimers. The enzyme, photolyase, binds to the thymine dimer and catalyses photochemical cleavage of the cyclobutane ring of the dimer to make the thymine's free. The enzyme uses visible light for the reaction. Besides thymine-dimers, other pyrimidine-dimers—like cytosine-cytosine and cytosine-thymine dimer—are also attacked by the enzyme. The enzyme is devoid of any species-specificity. Photolyases have been detected in both prokaryotes and eukaryotes.

The action of photolyase on UV-irradiated DNA containing thymine dimers is schematically represented in Fig. 9.70:

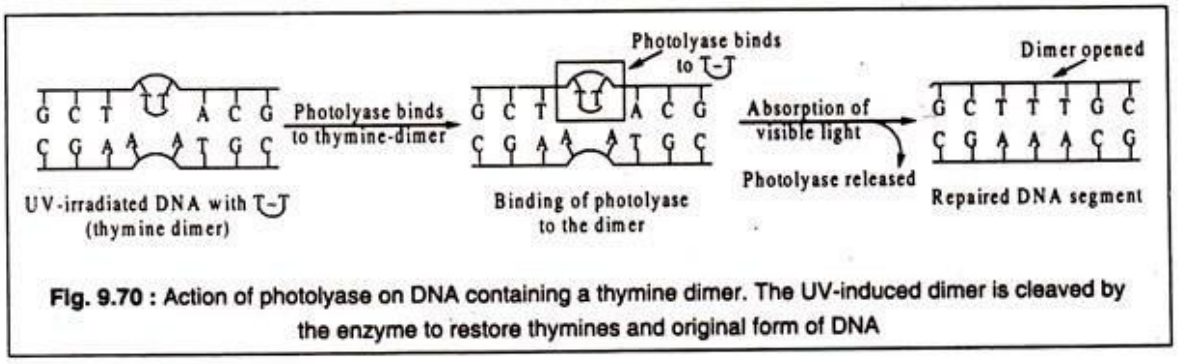


Fig. 9.70 : Action of photolyase on DNA containing a thymine dimer. The UV-induced dimer is cleaved by the enzyme to restore thymines and original form of DNA

It has been observed that UV-irradiated DNA containing 5-bromouracil — which is an analogue of thymine and is incorporated into replicating DNA replacing thymine — is resistant to photo reactivation. Such DNA binds the photolyase enzyme, but the enzyme neither dissociates from the dimer nor can liberate the free thymine molecules.

B. Excision Repair:

Apart from photo reactivation, there are also other mechanisms for repair of damaged DNA. One of these is excision repair which occurs in absence of light i.e. exposure to visible light is not required. It is also known as dark-repair.

The excision repair essentially consists of removal of a segment of DNA containing the damaged portion of one strand of DNA and new synthesis of the removed segment of the DNA strand using the undamaged strand as template. The first step, known as incision step, involves recognition of the damaged segment by an endonuclease. The enzyme cleaves the phosphodiester bond of the sugar- phosphate backbone at the 5′-end about eight nucleotides ahead of the damaged site producing a 3′-OH group free. The next step, known as excision step, involves a cut at a site 4 to 5 nucleotides downstream from the damaged site catalysed by a 5′-3′ exonuclease.

Thereby, a segment of DNA including the damaged site is removed. In the last step, known as the repair step, DNA polymerase I synthesizes a new stretch of DNA strand starting from the 3′-OH end using the intact complimentary strand as template. Finally, the newly synthesized strand is joined with the 5′-end by DNA ligase to complete the repair. The steps are diagrammatically shown in Fig. 9.71.Another variation of excision repair is catalysed by the enzyme glycosylase which cleaves the N-glycosidic bond between a thymine of the thymine dimer to the sugar-phosphate backbone of the DNA strand. At the next step, the phosphodiester bond is cleared by the endonuclease activity of the same enzyme which recognizes a blank deoxyribose without a base attached to it.

In the following step, DNA polymerase I initiates DNA synthesis at the free 3′-OH end displacing the thymine dimer along with a few more adjacent nucleotides as shown in Fig. 9.72. This type of excision repair occurs also in E. coli and several other bacteria like Micrococcus luteus.

Excision repair is observed when UV-treated bacteria are stored in dark for a few hours in a medium which does not support growth before returning them to the normal growth supporting medium (liquid holding recovery). In excision repair mechanism DNA polymerase I seems to play an important role.It has

been shown that E. coli mutants which are deficient in this enzyme show extensive DNA damage following UV-irradiation, presumably because such mutants are unable to repair the damaged DNA by excision repair mechanism. It may be reminded that in normal DNA replication, polymerase III (pol III) catalyses DNA synthesis.

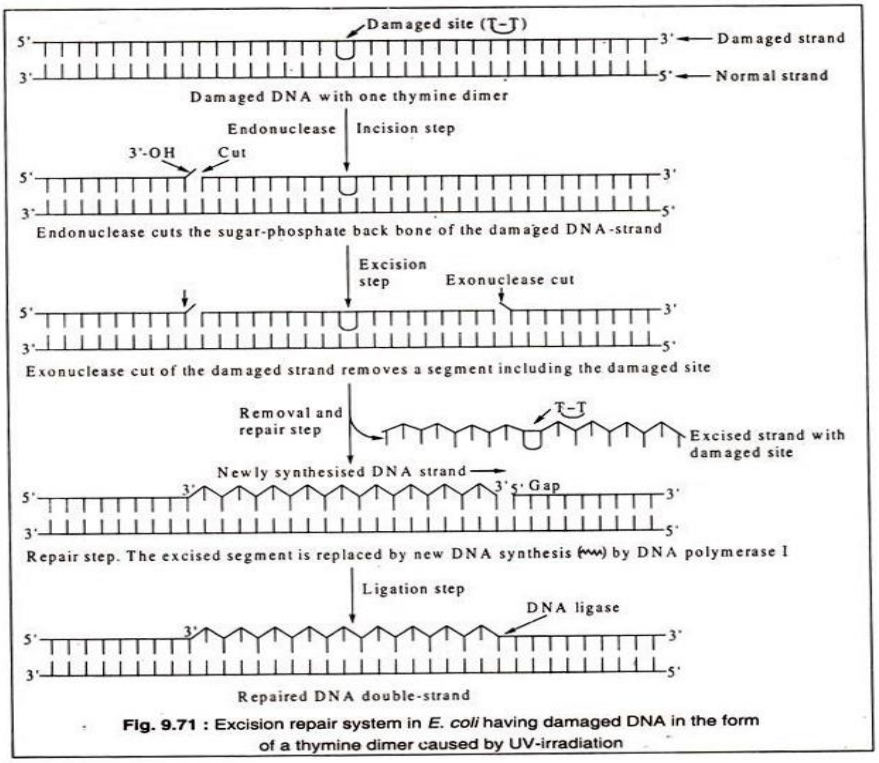


Fig. 9.71 : Excision repair system in *E. coli* having damaged DNA in the form of a thymine dimer caused by UV-irradiation

Excision repair are mainly two types:

(a) Base excision repair:

The lesions containing non-helix distortion (e.g. alkylating bases) are repaired by base excision repair. It involves at least six enzymes called DNA glycosylases.

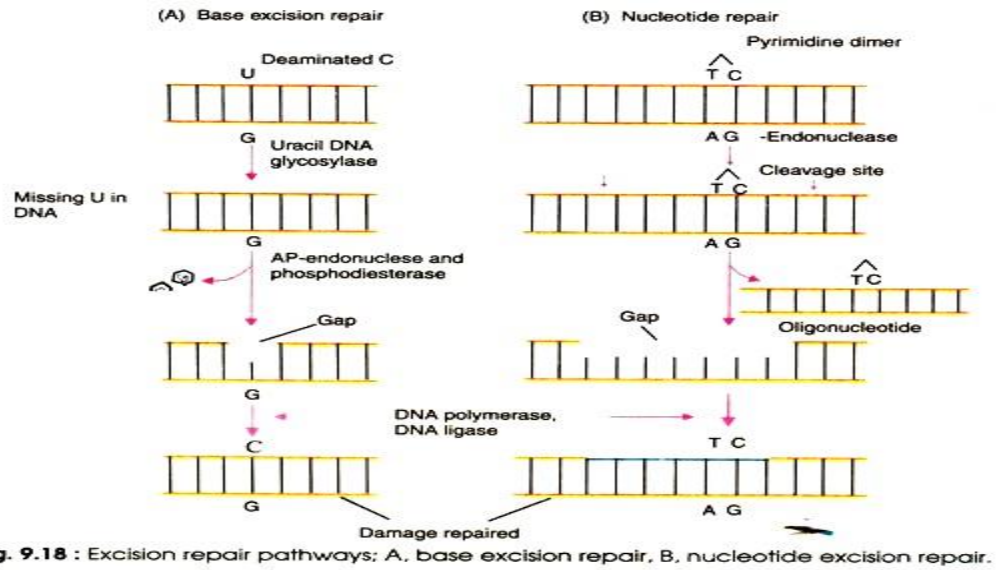


Fig. 9.18 : Excision repair pathways; A, base excision repair, B, nucleotide excision repair.

Each enzyme recognises at least bases and removes from DNA strand. The enzymes remove deaminated cytosine, deaminated adenine, alkylated or oxidised base. Base excision repair pathway starts with a DNA glycosylation. For example, the enzyme uracil DNA glycosylase removes the uracil that has wrongly joined with G which is really deaminated cytosine (Fig. 9.18A).

Then AP- endonuclease (apurinic or apyrimydinic site) and phosphodiesterase removes sugar-phosphate. AP- sites arise as a result of loss of a purine or a pyrimidine. A gap of single nucleotide develops on DNA which acts as template-primer for DNA polymerase to synthesise DNA and fill the gap by DNA ligase.

(b) Nucleotide excision repair:

Any type of damage having a large change in DNA helix causing helical changes in DNA structure is repaired by this pathway. Such damage may arise due to pyrimidine dimers (T-T, T-C and C-C) caused by sun light and covalently joins large hydrocarbon (e.g. the carcinogen benzopyrene).

In E. coli a repair endonuclease recognises the distortion produced by T-T dimer and makes two cuts in the sugar phosphate backbone on each side of the damage. The enzyme DNA helicases removes oligonucleotide from the double helix containing damage. DNA polymerase III and DNA ligase repair the gap produced in DNA helix (Fig. 9.18B).

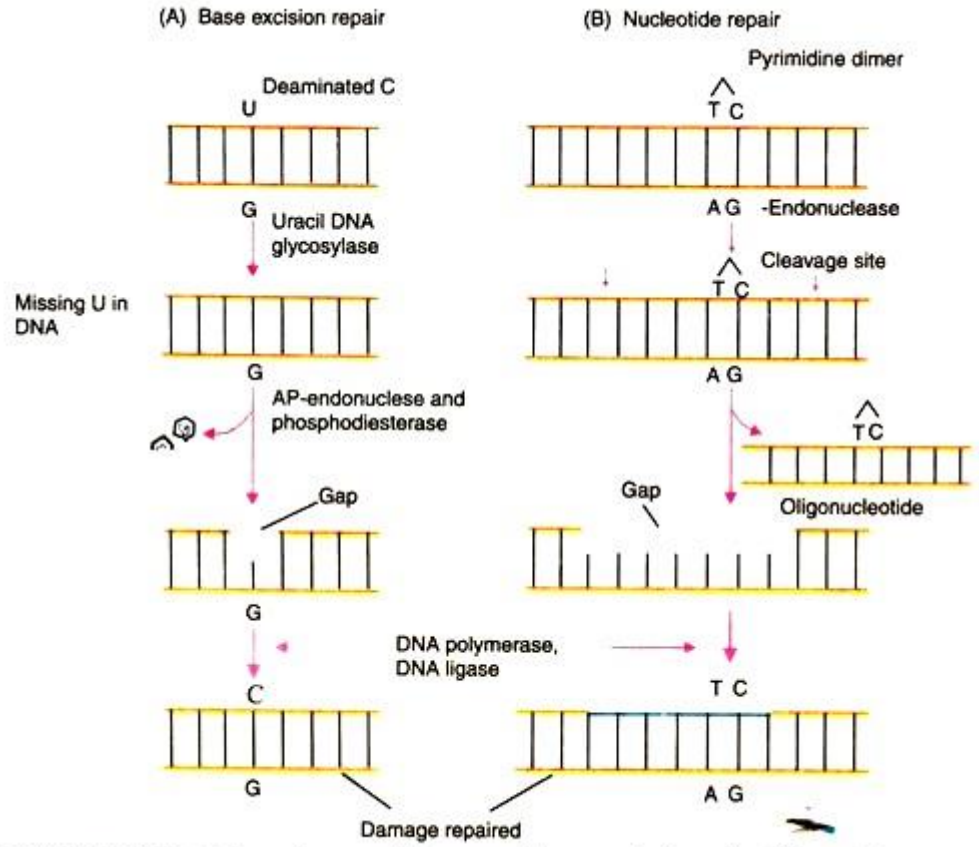


Fig. 9.18 : Excision repair pathways; A, base excision repair, B, nucleotide excision repair.

C. Re-Combinational Repair Mechanism:

This is another mode of repair of damaged DNA. It consists essentially of an exchange of a damaged segment of one DNA molecule by an undamaged segment of another one. As such exchange takes place only after replication of the damaged DNA has taken place it is also known as post- replication repair. When a damaged DNA molecule — e.g. DNA containing thymine-dimers induced by UV-irradiation— begins replication with the help of DNA polymerase III, the enzyme stops synthesis as it reaches a dimer, because of the distortion caused by the dimer in the regular double helix.

As a result, the progress of the replication fork halts temporarily as it reaches a dimer. DNA synthesis is then reinitiated at a new site, few nucleotides past the thymine-dimer site. Thus, a gap is created opposite the dimer site and a few adjoining nucleotides. The newly synthesized daughter strand is produced with several gaps i.e. in short pieces, if several dimers occur in the same template strand (Fig. 9.73).

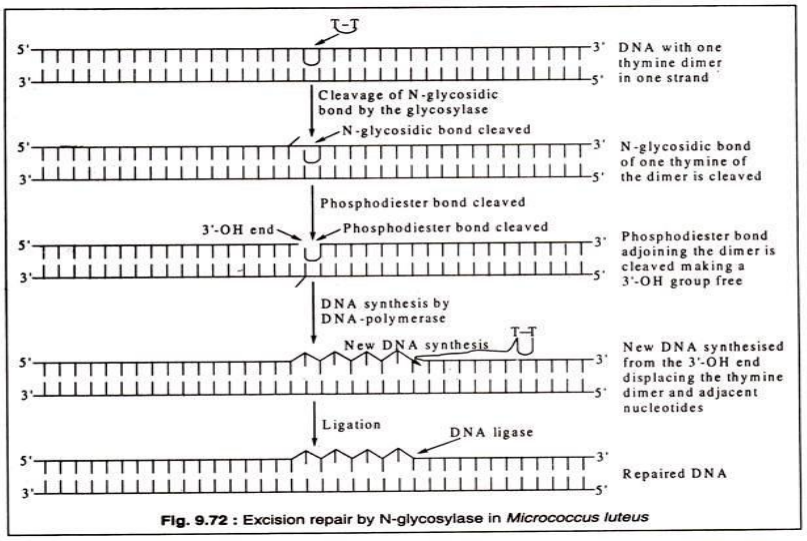


Fig. 9.72 : Excision repair by N-glycosylase in *Micrococcus luteus*

The gaps in the daughter strand are then filled up by exchanging undamaged homologous segments from a sister DNA double helix. The gaps produced in the donor strand are then filled up by new DNA synthesis with the help of DNA polymerase I and sealed by the DNA ligase as shown in Fig. 9.74. Thus, in re-combinational repair system, parts of DNA strand missing in one strand are retrieved from another normal DNA strand of a sister double helix.

The damaged DNA strand will continue to have the damaged site and will replicate to have gaps in the daughter strand which will be repaired by re-combinational repair mechanism. Ultimately, the damaged strand will be outnumbered by normal DNA and will be insignificant. In re-combinational repair mechanism, recA gene plays an important role. It has been observed that recA mutants are extremely sensitive to lethal effects of radiations and chemical mutagens. The recA gene is known to play a very important role in genetic recombination e.g. in conjugation where recA' recipient fails to show genetic recombination. The gene functions also in recombinational repair, where exchange of DNA strands is involved.

D. Repair of DNA by Homologous Recombination:

This type of repair is called for when both strands of DNA molecules are damaged at sites opposite each other. In such a case, the missing segments cannot be replaced from sister strands after replication by the usual recombinational repair mechanism. The lost portions have to be retrieved from another homologous DNA molecule.

In actively growing bacteria, each cell contains generally more than one copy of DNA. So, the lost portions of one damaged molecule can be repaired by crossing-over with a normal DNA molecule involving exchange of homologous segments. This type of DNA repair is known as homologous recombinational repair. Double-stranded breaks of DNA can be induced by exposure to X-rays. Rec A protein plays an important role also in this type of repair.

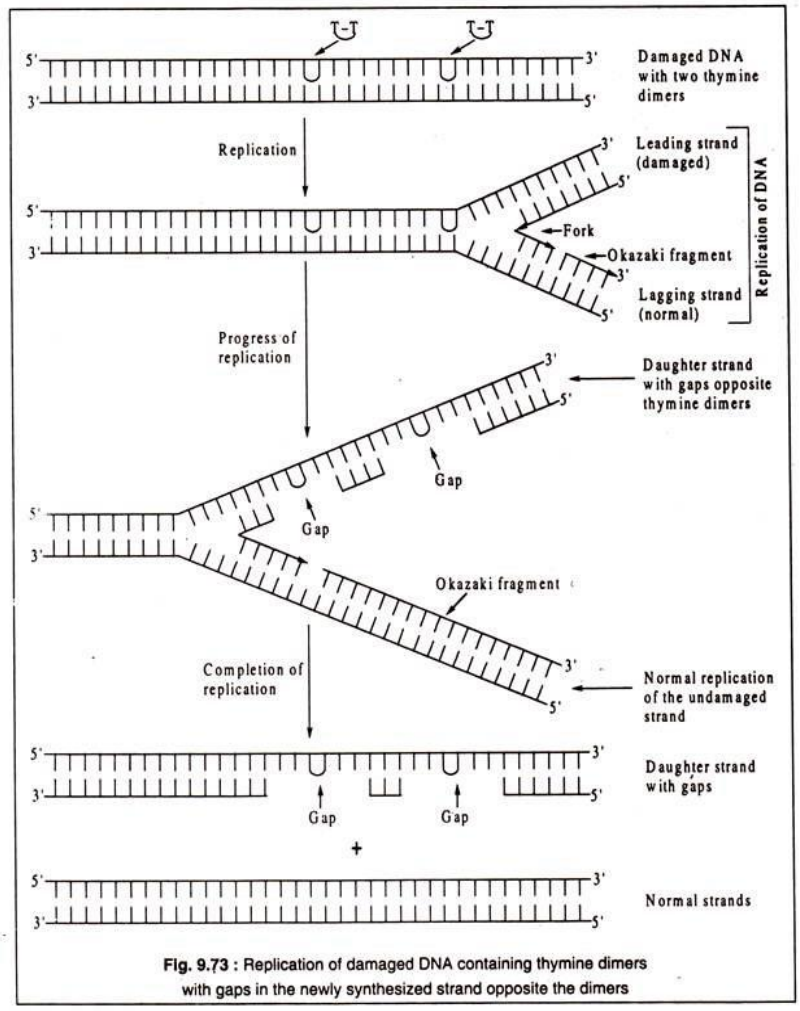


Fig. 9.73 : Replication of damaged DNA containing thymine dimers with gaps in the newly synthesized strand opposite the dimers

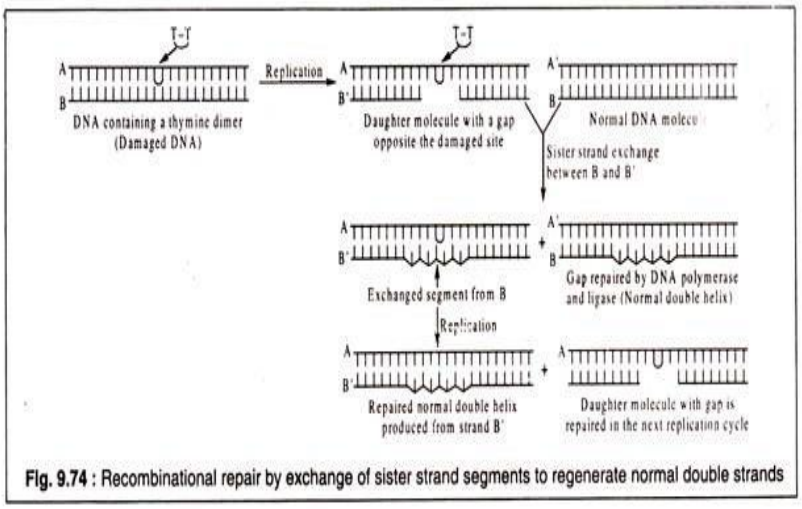


Fig. 9.74 : Recombinational repair by exchange of sister strand segments to regenerate normal double strands

The repair process begins with the production of single-stranded segments at the 3′-OH end of each strand through the action of a nuclease and binding of the Rec A protein to the single strands. The binding of Rec A protein initiates strand exchange between homologous segments of the normal DNA duplex and the damaged one. The crossing-over results in the formation of two DNA molecules, each having an intact strand and a strand with gaps.

The gaps are then filled up by new DNA synthesis catalysed by DNA polymerase and sealing by DNA ligase. The intact strand is used as template. Thus, crossing-over which is normally a mechanism for creating genetic diversity by mixing up genes located on homologous chromosomes, can also function as a means for repairing damaged DNA. Rec A protein plays vital roles in both the processes (Fig. 9.75).

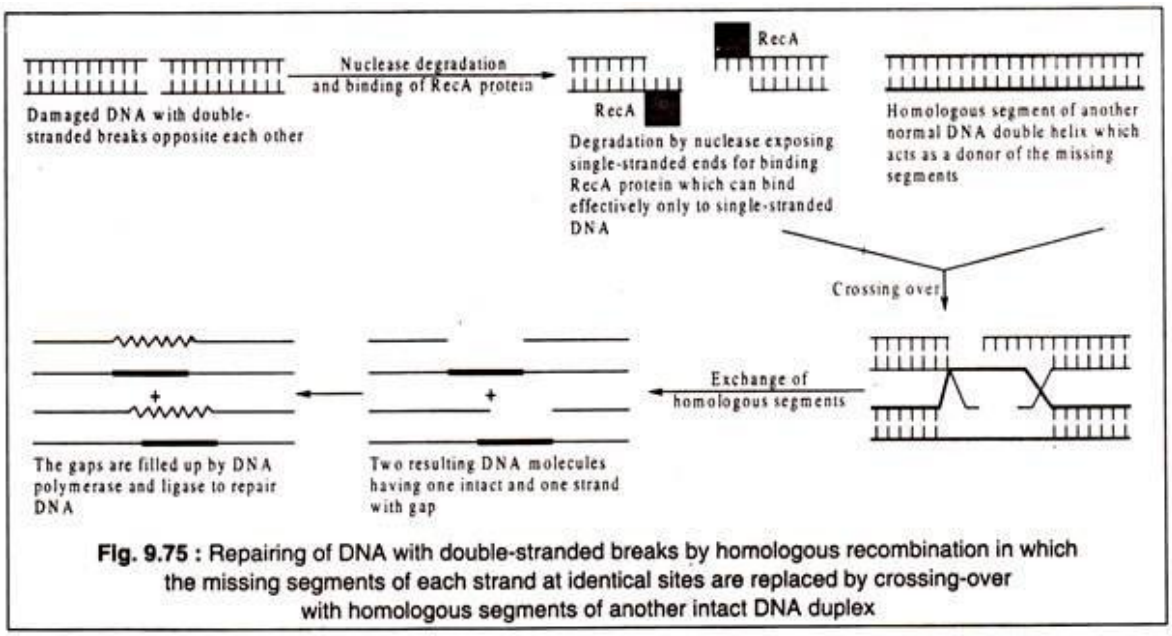


Fig. 9.75 : Repairing of DNA with double-stranded breaks by homologous recombination in which the missing segments of each strand at identical sites are replaced by crossing-over with homologous segments of another intact DNA duplex

E. SOS Repair of Damaged DNA:

The SOS repair mechanism functions in a more complicated way. Damages inflicted on DNA by mutagenic agents induce a complex series of changes which are collectively known as SOS response. The response leads to increased capacity to repair damaged DNA by excision repair and recombinational pathways.

The SOS response is set in action by the interaction of two proteins, — Rec A protein which is a product of the recA gene and Lex A protein, the product of lexA gene. The Rec A protein in addition to having a role in genetic recombination and recombinational repair also has a protease function. The Lex A protein acts as a repressor for a number of genes, known as SOS genes including the recA gene. Under normal conditions i.e. when the SOS response is not necessary, these genes remain repressed by the Lex A repressor.

The initial event in the SOS response is the activation of RecA protease activity induced by DNA damage. The activation of Rec A protease activity occurs within a few minutes of DNA damage. The protease activity catalyses cleavage of the Lex A repressor making it inactive. As a result, the SOS genes can now be expressed to produce the enzymes required for DNA repair. The events are shown in Fig. 9.76:

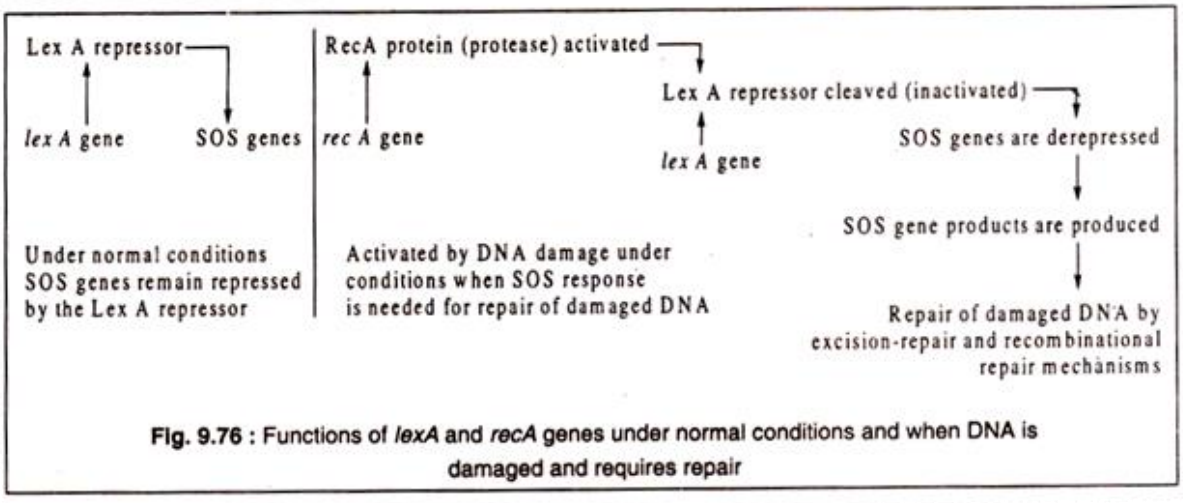


Fig. 9.76 : Functions of *lexA* and *recA* genes under normal conditions and when DNA is damaged and requires repair

The SOS response, as the name suggests, is an emergency measure to repair mutational damage. It makes it possible for the cell to survive under conditions which would have been otherwise lethal. However, the possibility of generating new mutations increases in the repair of DNA molecules. This is because the SOS repair system allows DNA synthesis bypassing the damaged site.

When the DNA polymerase III reaches a damaged site to which Rec A binds, the protein (Rec A) interacts with the epsilon subunit of the DNA polymerase molecule. This subunit is responsible for insertion of the correct base into the growing DNA strand. As a result, chain elongation continues bypassing the damaged site, but the chance of incorporation of a wrong base increases. SOS repair, therefore, enhances the chance of mutation due to mis-pairing of bases. This is known as error-prone bypass repair. A more recent model based on SOS repair of UV-irradiated DNA in bacteriophages has been proposed. UV-irradiation is known to produce dimers of not only thymine, but also of thymine and cytosine and cytosine. During

replication when T-C and C-C are reached, the SOS repair system is halted temporarily and cystosine is deaminated to uracil. Uracil pairs with adenine, bringing about a transition mutation by changing C-G base pair to T-A. This is an error-free bypass repair thought it still causes a mutation. It is called error-free because the template DNA strand is faithfully copied in the newly synthesized strand. The change from C to U occurs in the template strand itself.

F. Methylation-directed very short patch repair:

Very short patch (VSP) repair is accomplished by involving methylation of bases especially cytosine and adenine. In E. coli methylation of adenine and in a sequence of -GATC- is done by the enzyme methylase (a product of dam gene) on both strands of DNA. After replication only A of -GATC- of one strand remains methylated, while the other remains un-methylated until methylase accomplishes methylation (Fig. 9.20 A-B).

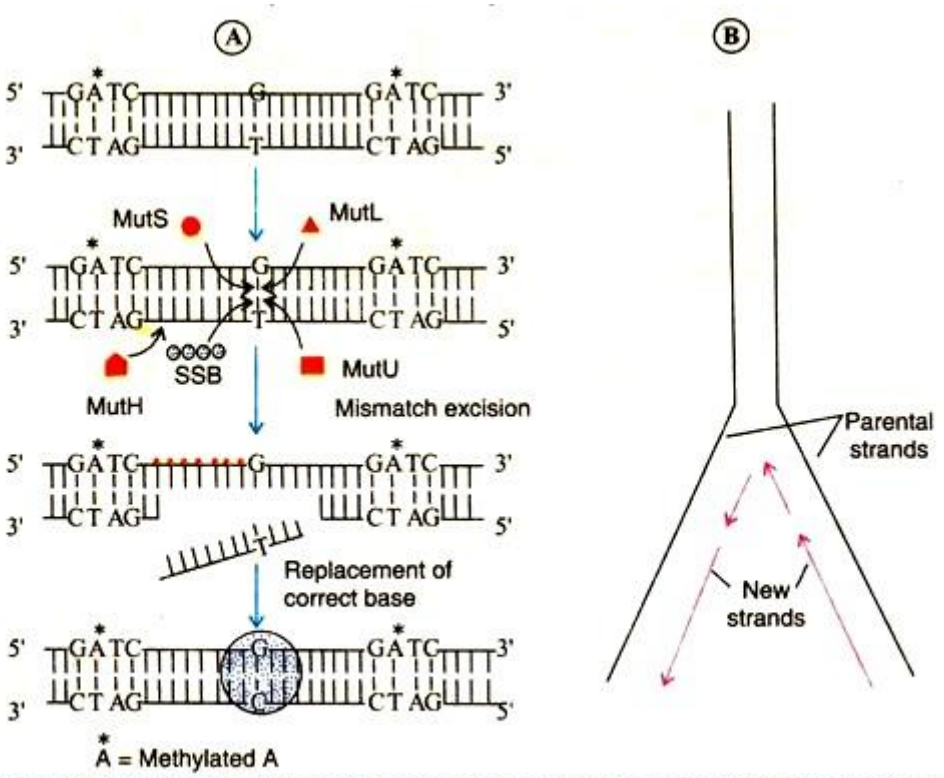


Fig. 9.20 : Mismatch repair. (A) excision of a newly synthesised strand and repair system; (B) arrows shows the region where methylation is not complete and dark region line shows the region where methylation is complete.

In E. coli repairing activity required four proteins viz., Mutl, MutS, MutU (UvrS) and MutH by the genes mutL, mutS, mutU, and mutH, respectively. The mut genes are the loci which increase the frequency of spontaneous mutation. The un-methylated -GATC allows the MutL to recognise the mismatch during transition period. This helps MutS to bind to mismatch. MutU supports in unwinding the single strand and single strand DNA binding (SSB) proteins and maintain the structural topography of single strand. MutH cleaves the newly synthesised DNA strand and the protein MutU separates the mismatch strand (A).However, there is a gradient of methylation along the newly synthesised strand. Least methylation occurs at the replication fork. The parental strand is uniformly methylated. The methylated bases direct the excision mechanisms to the newly synthesised strand containing the incorrect nucleotides (B). During this

transition period, the repair system works and distinguishes the old and new strands and repairs only the new strands.

G. Mismatch Repair:

The rate of mutation varies usually in the range of 10^{-7} to 10^{-11} in bacteria. However, during normal DNA replication an error in inserting a correct base in the new strand occurs at a much higher rate, generally at a frequency the of 10^{-5}. This large difference indicates that bacteria possess an in-built mechanism to rectify most of errors during replication.
Most bacterial DNA polymerases, in addition to having the polymerase activity, have also an exonuclease activity which works in the opposite direction i.e. while polymerization proceeds in the 5′ —> 3′ direction, the exonuclease works in the 3′ —> 5′ direction. Whenever a wrong base is inserted into the polynucleotide chain by mistake, the DNA polymerase stops and goes one step backward and the incorrect base is removed by the exonuclease activity. The polymerase then resumes its normal activity by inserting a correct base. This is known as the proof-reading function of the DNA polymerase. Mutants with an altered epsilon subunit of the DNA polymerase fails to perform the proof-reading function.

Although proof-reading by DNA-polymerase is an efficient way of removing many mismatched bases, a number of such errors may still persist after replication. Such mismatched base-pairs require removal. These are corrected by another repair mechanism, known as mismatch repair.In this repair mechanism, three gene-products (proteins) are involved — Mut S, Mut L and Mut H. The first step of this repair process consists of binding of the Mut S protein to the mismatched base- pair. The second step involves the recognition of a specific sequence of the template which is -GATC- m E. coli in which A (adenine) is methylated in N-6 position. The proteins Mut L and Mut H which bind to the unmethylated -GATC- sequence of the new strand form a complex with Mut S which is bound to the mismatch pair. Thereby the mismatch pair is brought close to the -GATC- sequences. The Mut H protein then nicks the unmethylated DNA strand at the GATC site and the mismatch is removed by an exonuclease. The resulting gap is then filled by DNA polymerase III and DNA ligase (Fig. 9.77):

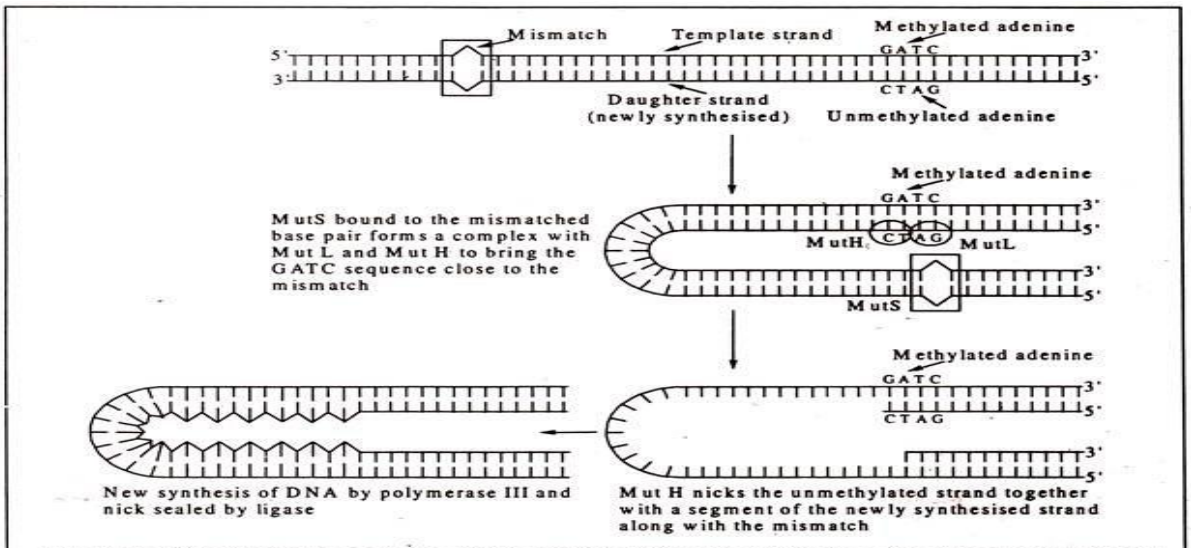


Fig. 9.77 : Mismatch repair mechanism by the proteins Mut S which binds the mismatched base pair and Mut L and Mut H which bind to the GATC site of the unmethylated strand. A segment of DNA including the mismatch is removed and replaced by a newly synthesized segment

Probable Questions:

1. State different types of DNA damage.
2. Describe photoactivation system of DNA repair with suitable diagram.
3. Describe mismatch system of DNA repair with suitable diagram.
4. Describe base excision repair system of DNA repair with suitable diagram.
5. Describe SOS repair system of DNA repair with suitable diagram.
6. What s the difference between base excision repair and nucleotide excision repair system?
7. Describe Re-Combinational Repair Mechanism of DNA with suitable diagram.
8. . Describe homologous recombination DNA repair system with suitable diagram.
9. Describe methylation-directed very short patch repair of DNA with suitable diagram.
10. What are the importance of DNA repair ?

Suggested readings:

1. Molecular Cell Biology by Lodish, Fourth Edition.
2. The Cell – A Molecular Approach by Cooper and Hausman, Fourth Edition
3. Principles of Genetics by Snustad and Simmons, Sixth Edition.
4. Molecular Biology of the Cell – by Bruce Alberts
5. Cell and Molecular Biology by Gerald Karp, 7^{th} Edition.
6. Gene cloning and DNA Analysis by T. A. Brown, Sixth Edition.
7. Genetics . Verma and Agarwal

UNIT-XX

Extrachromosomal Replicons; Homologous Recombination

Objective: In this unit we will learn about different kinds of extrachromosomal replications and homologous recombination.

Extrachromosomal Replication

Extrachromosomal DNA is any DNA that is found outside the nucleus of a cell. It is also referred to as extranuclear DNA or cytoplasmic DNA. Most DNA in an individual genome is found in chromosomes but DNA found outside the nucleus also serves important biological functions. In prokaryotes, extrachromosomal DNA is primarily found in plasmids whereas in eukaryotes extrachromosomal DNA is primarily found in organelles. Mitochondrial DNAis a main source of this extrachromosomal DNA in eukaryotes. Extrachromosomal DNA is often used in research of replication because it is easy to identify and isolate. In addition to DNA found outside the nucleus in cells, infection of viral genomes also provides an example of extrachromosomal DNA. Several models and mechanisms are associated with the replication of extrachromosomal DNA. Some of the replication mechanisms found in plasmid, mitochondria and virus are given below.

Replication of Plasmid DNA

In general, bacterial plasmids replicate independently of the host chromosome, although usually they rely on some host-encoded factors for their replication. They are present in bacterial cells replicating at a specific number of copies per cell, which can range from one or two to several hundreds. Several host- and plasmid-encoded functions are required for plasmid replication. Initiation of plasmid replication is molecule specific and of great importance for the propagation process, copy number, and incompatibility properties of plasmids in both Gram-positive and -negative bacteria. In general, plasmid replicons contain one or several origins (ori) of replication and one or more regulatory elements, located in a DNA fragment no larger than 4 kb. In addition, most plasmid replicons harbor a geneencoding either a protein or an RNA molecule that functions as a primer for DNA replication.The Rep proteins can often act in trans on a specific ori, but in some cases they may only function in cis. However, in all cases examined so far the preprimer RNA acts in cis with the replication initiation sequences. Composite multi-replicon plasmids were also described. One example is R6K, in which three origins are able to function in vivo independently, although the rate of initiation from each origin is different. It was reported that many plasmid origins follow a molecular mechanism similar to oriC, the origin of replication of the E.coli chromosome. However, the major difference is that plasmids require an origin-specific plasmid-encoded protein for the initiation step, generally called Rep proteins. These plasmid- encoded Rep proteins act in place of or in combination with DnaA, the replication initiation protein for chromosomal DNA. Some plasmids require additional host-gene products suchas dam methylases, integration host factor (IHF), and heat shock proteins to replicate. Other plasmids, such as the ColE1-type encode an RNA-specific plasmid molecule and require the host-encoded DNA polymerase I (PolI or PolA), RNA polymerase, and ribonuclease H (RNase H).

Two types of mechanisms basically control the replication of plasmid DNA. One utilizes a series of repeated sequences, designated iterons, located at ori and capable of interacting with the replicator protein. In the other, small complementary RNA molecules (antisense) hybridize with the transcript responsible for the initiation process, either directly or indirectly by encoding the Rep protein. There are three general replication mechanisms for circular plasmids, namely, theta type, strand displacement and rolling circle.

Theta type:

Replication by the theta-type mechanism has been most extensively studied among theprototype circular plasmids of gram-negative bacteria. Replication through the theta mechanism involves melting of the parental strands, synthesis of a primer RNA (pRNA), and initiation of DNA synthesis by covalent extension of the pRNA. DNA synthesis is continuouson one of the strands (leading strand) and discontinuous on the other (lagging strand), although synthesis of the two strands seems to be coupled. Theta-type DNA synthesis can start from one or from several origins, and replication can be either uni- or bidirectional (Figure 13).Under electron microscopy (EM), the replication intermediates are seen as typical (“theta”)-shaped molecules that, when digested with enzymes that cleave within the replicated region, yield Y-shaped molecules (“forks”). With few exceptions, plasmids using the theta mechanism of replication require a plasmid-encoded Rep initiator protein. Some replicons may require the host DNA polymerase I (DNA Pol I) during the early stages of leading-strand synthesis. With some exceptions, initiation of plasmid DNA replication requires a specific plasmid-encoded Rep initiator protein. This is reflected by the presence, at the origin of replication, of specific sequences with which the Rep protein interacts. Additional features found in many origins of theta-replicating plasmids are (i) an adjacent AT-rich region containing sequence repeats, where opening of the strands and assembly of host initiation factors occur, and (ii) one or more sites (dnaA boxes) where the host DnaA initiator protein binds. Multiple Dam methylation sequences, which are present in the origin of replication of the Escherichia coli chromosome, oriC, can also be found at the origin of replication ofplasmids.

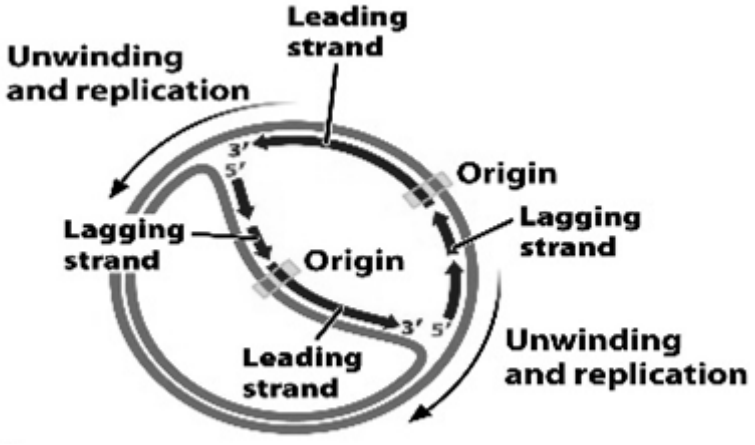


Figure 13: Theta model of replication

Strand displacement replication: Replication of RSF1010 DNA plasmid follow this mechanism and it is independent of the host-encoded DnaA, DnaB, DnaC, and DnaG proteins, whose roles

are played by the combined action of the plasmid-encoded RepA, RepB, and RepC proteins. DNA Pol III-HE and SSB are required for replication. The first stage of this process involves the binding of the RepC protein to the origin also called iterons. It is assumed that the RepA helicase binds to both DNA strands in the AT-rich region, close to the site of interaction of RepC. Subsequent translocation in the 5□□3□ direction of the RepA helicase bound to the L strand melts the duplex, exposing and activating the ssi sites which is palindromic inverted repeat in the origin. Alternatively, the interaction of RepC with the iterons could induce the opening of the duplex near the ssi sites. The exposure of the stem-loop structure in the ssi sites is probably required for the assembly of the RepB-primase to initiate replication. Initiation at either ssi site can occur independently, and replication proceeds continuously, with the RepA helicase facilitating displacement of the nonreplicated parental strand as a D loop. Continuous replication from each ssi signal in opposite directions would originate a double stranded DNA theta-shaped structure in the overlapping region and two D loops beyond this region. The helicase activity of the RepA protein is required during the elongation. The end products of the strand displacement replication mechanism are single strand-displaced circles and double-stranded supercoiled circles. The ssDNA molecules are used to initiate synthesis of the complementary strand, which converts the ssDNA templates into double stranded supercoiled circles. Therefore, double-stranded DNA (dsDNA) molecules, displaced single-stranded circular molecules, and partial double-stranded circles can be formed in this mode of replication (Figure 14).

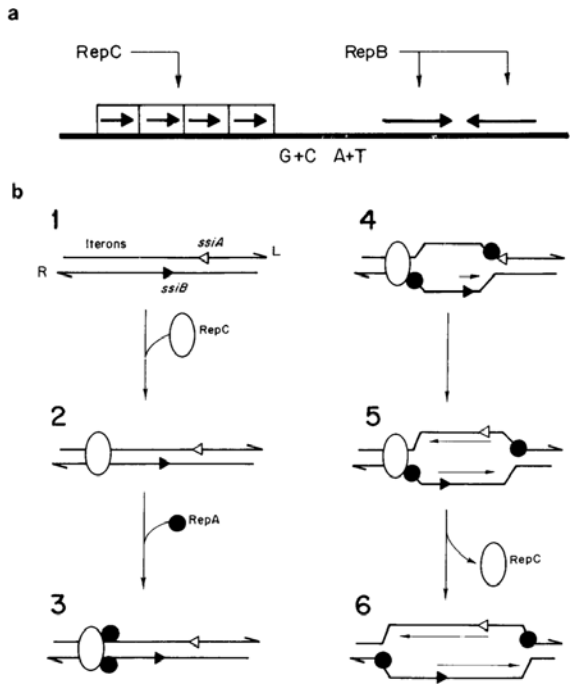


Figure 14: Strand displacement replication

Rolling circle (RC) replication: Replication by the RC mechanism has to be unidirectional, and it is considered to be an asymmetric process because synthesis of the leading strand and synthesis of the lagging strand are uncoupled. One of the most relevant features of RC replication is that the

newly synthesized leading plus strand remains covalently bound to the same parental plus strand. Replication is initiated by the plasmid-encoded Rep protein, which introduces a site-specific nick on the plus strand, at a region termed double stranded origin (dso). The nick leaves a 3□-OH end that is used as a primer for leading-strand synthesis, which most probably involves host replication proteins (at least DNA Pol III, SSB, and a helicase). Elongation from the 3□-OH end, accompanied by the displacement of the parental plus strand, continues until the replisome reaches the reconstituted dso, where a DNA strand transfer reaction(s) takes place to terminate leading strand replication (see below). Thus, the end products of leading-strand replication are a dsDNA molecule constituted by the parental minus strand and the newly synthesized plus strand, and a ssDNA intermediate which corresponds to the parental plus strand. Unlike replication by the strand displacement mechanism, the ssDNA intermediates generated by the RC replication mode correspond to only one of the plasmid DNA strands. Finally, the parental plus strand is converted into dsDNA forms by host proteins initiating at the single-strand origin (sso), which is physically distant from the dso. The last step would be the supercoiling of the replication products bythe host DNA gyrase (Figure 15).

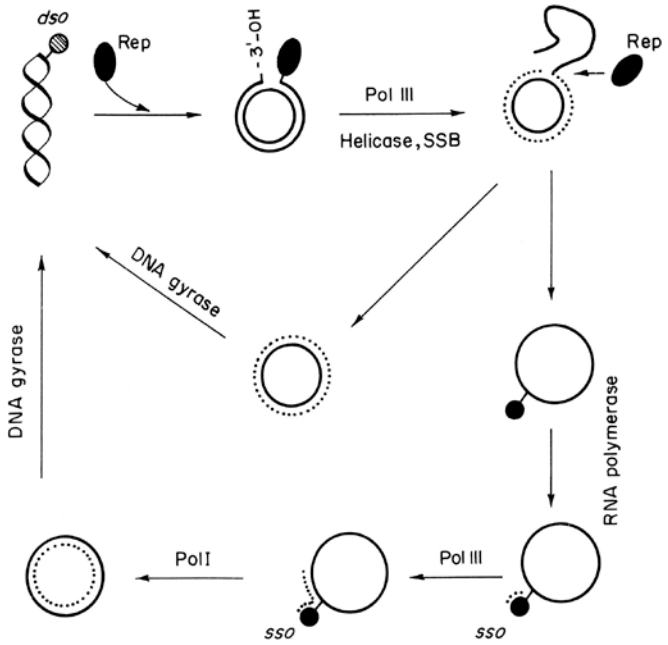


Figure 15: Rolling circle replication

Mitochondrial DNA (mtDNA) replication

Replication of mtDNA may be stochastic because there is no control over which particular copies are replicated, so that in any cycle some mtDNA molecules may replicate more times than others. The mtDNA replication undergoes D-loop formation. Replication starts at a specific origin in the circular duplex DNA. Initially, though, only one of the two parental strands (the H strand in mammalian mitochondrial DNA) is used as a template for synthesisof a new strand. Synthesis proceeds for only a short distance, displacing the original partner (L) strand, which remains single-stranded. The condition of this region gives rise to its name as the displacement loop, or D loop (Figure 16).Replication at the H –

strand origin is initiated when RNA polymerase transcribes a primer. The 3□ ends are generated in the primer by an endonuclease that cleaves the DNA-RNA hybrid at several discrete sites. The endonuclease is specific for the triple structure of DNA- RNA hybrid plus the displaced DNA single strand. The 3' end is then extended into DNA by the DNA polymerase. A single D loop is found as an opening of 500 to 600 bases in mammalian mitochondria. The short strand that maintains the D loop is unstable and turns over; it is frequently degraded and resynthesized to maintain the opening of the duplex at this site. Some mitochondrial DNAs possess several D loops, reflecting the use of multiple origins. The same mechanism is employed in chloroplast DNA, where (in complex plants) there are two D loops. To replicate mammalian mitochondrial DNA, the short strand in the D loop is extended. The displaced region of the original L strand becomes longer, expanding the D loop. This expansion continues until it reaches a point about two-thirds of the way around the circle. Replication of this region exposes an origin in the displaced L strand. Synthesis of an H strand initiates at this site, which is used by a special primase that synthesizes a short RNA. The RNA is then extended by DNA polymerase, proceeding around the displaced single-stranded L template in the opposite direction from L-strand synthesis.

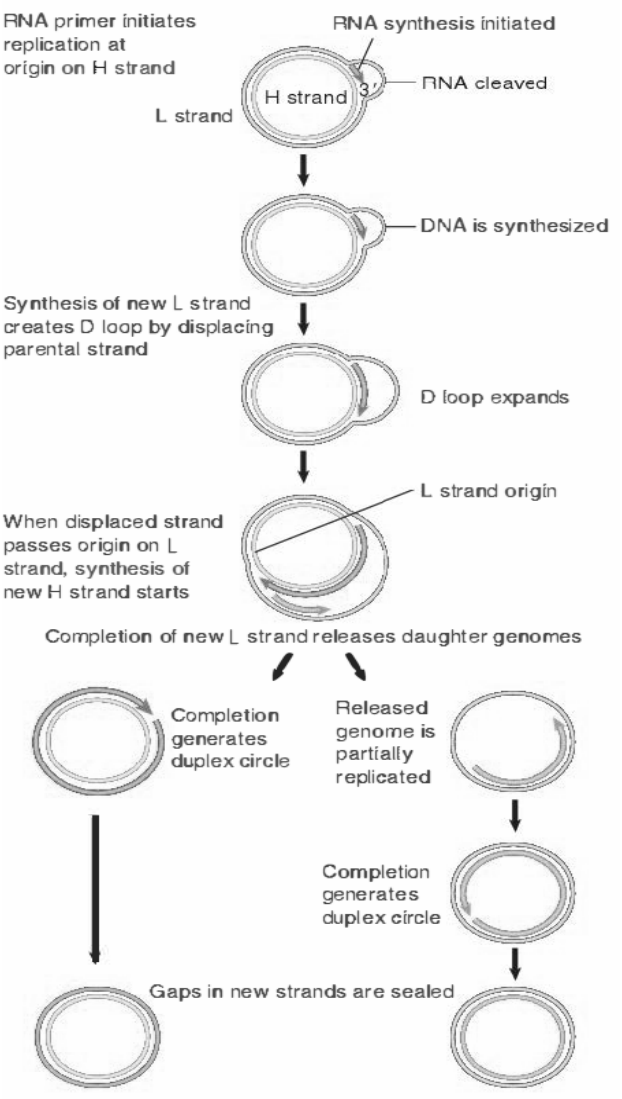


Figure 16: Mitochondrial DNA replication

Virus DNA replication

Replication by rolling circles is common among bacteriophages. Unit genomes can be cleaved from the displaced tail, generating monomers that can be packaged into phageparticles or used for further replication cycles. Phage □X 174 consists of a single-stranded circular DNA known as the plus (+) strand. A complementary strand, called the minus (-) strand, is synthesized. The duplex circle is converted to a covalently closed form, which becomes supercoiled (Figure 17). A protein coded by the phage genome, the A protein, nicks the (+) strand of the duplex DNA at a specific site that defines the origin for replication. After nicking the origin, the A protein remains connected to the 5 ' end that it generates, while the 3 ' end is extended by DNA polymerase (Figure 17).

The nick generates a 3'-OH end and a 5'-phosphate end (covalently attached to the A protein),both of which have roles to play in □X 174 replication. Using the rolling circle, the 3' -OH end of the nick is extended into a new chain. The chain is elongated around the circular (-) strand template until it reaches the starting point and displaces the origin. Now the A protein functions again. It remains connected with the rolling circle as well as to the 5' end of the displaced tail, and is therefore in the vicinity as the growing point returns past the origin. Thus, the same A protein is available again to recognize the origin and nick it, now attaching to the end generated by the new nick. Following this nicking event, the displaced single (+) strand is freed as a circle. The A protein is involved in the circularization. In fact, the joining of the 3' and 5 ' ends of the (+) strand product is accomplished by the A protein as part of the reaction by which it is released at the end of one cycle of replication, and starts another cycle.The displaced (+) strand may follow either of two fates after circularization. First, during the replication phase of viral infection, it may be used as a template to synthesize the complementary (-) strand. The duplex circle may then be used as a rolling circle to generate more progeny. Second, during phage morphogenesis, the displaced (+) strand is packaged into the phage virion.

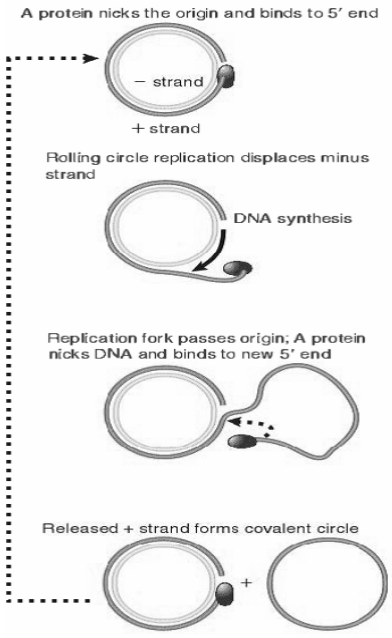


Figure 17. Bacteriophage □X174 replica

Introduction to Genetic Recombination:

Recombination of DNA takes place by mutation, exchange of DNA strands and incorporation of DNA. In this process the genetic information is rearranged between chromosomes that possess similar sequences. Homologous genetic recombination occurs in eukaryotes at the time of gamete formation during long prophase I of meiosis.

Each chromosome has two sister chromatids, each of which contains a duplex DNA. The homologous chromosomes (one maternal and the other paternal) pair with each other, pairing is known as synapsis and involves entire length of homologous chromosomes. Recombination occurs by crossing over. It involves reciprocal exchange of chromosomal segments between non-sister chromatids of a homologous pair involving breakage and subsequent reunion in a new arrangement. Chiasma is formed at the site of crossing over. Enzymes like helicases, endonucleases and ligases are involved.

The genetic recombination causes re-arrangement of genes producing altogether new genotypes and phenotypes. These cause variations which lead to evolution. In humans about 30 homologous recombination events occur during each meiosis. The recombination events are much more in bacteria and even more in fungi. The study of meiosis in lily plants by Herbert Stern and Yasuo Hotta have provided clinching evidence of recombination. Meiocytes of lily flower buds divide synchronously. It has also been discovered that endonuclease, DNA polymerase, ligase and other repair enzymes are present in early prophase.

Mechanics of molecular level of exchange is studied in detail in bacteria and phages. At present we will restrict our discussion to the recombination mechanism where DNA strands recognize each other by complementary strands bounded by base pairs. In bacteria, genetic recombination occurs during conjugation, transformation, transduction post-replication repair, during repair of double strand breaks in DNA, integration of phage DNA with chromosomal DNA and transposons etc. Homologous recombination can lead to gene conversion.

Bacterial Recombination:

Bacteria are haploid, therefore do not undergo meiosis. They possess only one double stranded DNA molecule or chromosome. There are several types of genetic recombination in microorganisms. The most common recombination is the reciprocal exchange between homologous DNA sequences. During genetic recombination usually only a part of the genetic material of a donor cell is transferred to a recipient cell. The DNA of the recipient cell and the donor pair with each other and reciprocally exchange DNA strands by crossing over. This gives rise to a new genetic constitution of the recipient cell with new characters. Subsequent daughter cells that contain only recombined chromosome.

There are following main methods by which recombination of genetic material takes place in bacteria.

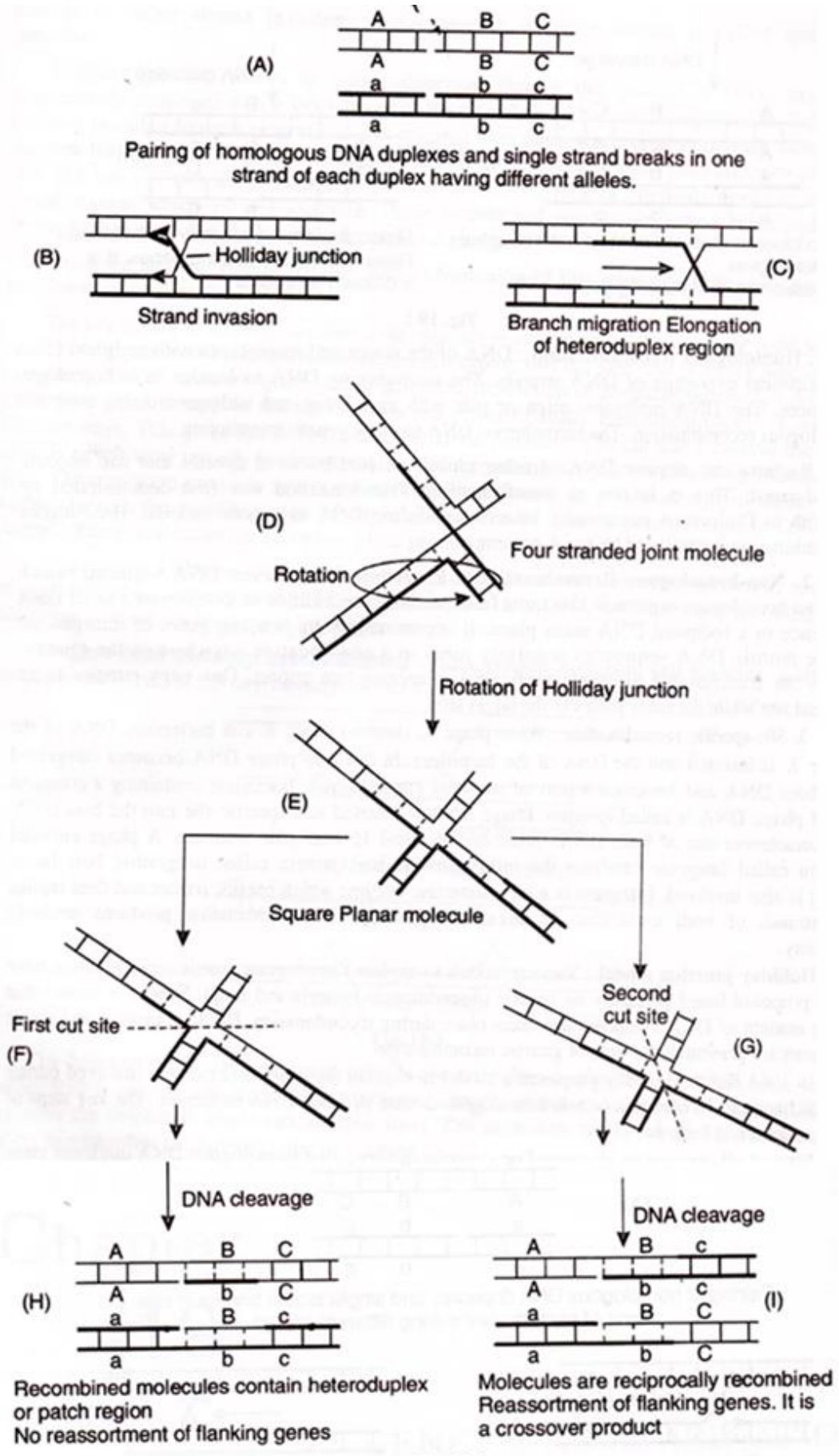

(A)
A B C
A B C
a b c
a b c
Pairing of homologous DNA duplexes and single strand breaks in one strand of each duplex having different alleles.
(B)
Holliday junction
Strand invasion
(C)
Branch migration Elongation of heteroduplex region
(D)
Four stranded joint molecule
Rotation
Rotation of Holliday junction
(E)
Square Planar molecule
First cut site
(F)
Second cut site
(G)
DNA cleavage
DNA cleavage
(H)
A B C
A b C
a B c
a b c
(I)
A B c
A b c
a B C
a b C
Recombined molecules contain heteroduplex or patch region
No reassortment of flanking genes
Molecules are reciprocally recombined
Reassortment of flanking genes. It is a crossover product

Fig. 19.1

1. Homologous Recombination:

DNA of the donor cell recombines with recipient DNA by reciprocal exchange of DNA strands. The recombining DNA molecules have homologous sequences. The DNA molecules align or pair with each other and undergo crossing over and homologous recombination. The recombinant DNA has new genetic constitution.

Bacteria can acquire DNA of other closely related bacterial species and can become transformed. This is known as transformation. Transformation was first demonstrated by Griffith in *Diplococcus pneumoniae* bacteria to confirm DNA as genetic material. Homologous recombination is catalysed by rec A protein.

2. Non-homologous Recombination:

Recombination between DNA segments which have no homologous regions is also quite frequent. Here the addition or insertion of a small DNA sequence in a recipient DNA takes place. It occurs mainly by jumping genes or transposons. These mobile DNA sequences regularly jump to a new location anywhere on the genome. Often the transposable elements replicate to generate two copies. One copy remains at the original site while the other jumps to the target site.

3. Site-specific Recombination:

When phage λ (lambda) infects E. coli bacterium, DNA of the phage λ is inserted into the DNA of the bacterium. In this way phage DNA becomes integrated into host DNA and becomes a part of the host chromosome. Bacterium containing a complete set of phage DNA is called lysogen. Phage DNA is inserted at a specific site into the host DNA.

The attachment site of both DNAs possesses identical 15 base pair sequence. A phage encoded protein called integrase catalyses the integration. A host protein called integration host factor (IGF) is also involved. Integrase is a topoisomerase enzyme which breaks, rotates and then re-joins the strands of both molecules. In eukaryotes site-specific recombination produces antibody diversity.

Holliday Junction Model:

Various models to explain homologous genetic recombination have been proposed based primarily on genetic observation in bacteria and fungi. Now it is known that some amount of DNA synthesis also takes place during recombination. DNA replication and repair enzymes are present at the time of genetic recombination. In 1964 Robin Holliday proposed a model to explain the molecular process involved during the exchange of DNA between two homologous double stranded DNA molecules.

The key steps of this model are as follows:

First of all, pairing or alignment or synapsis between two homologous DNA duplexes takes place. Their sequences are perfectly identical except that they may contain small regions of different genes called alleles.

Then breaks or nick occur at identical sites in one DNA strand of both homologous DNA duplexes precisely at the same point. The broken ends of strands then invade the opposite complementary strands creating short heteroduplex regions (because of different alleles). This process is called strand invasion. This crossover structure formed is called Holiday junction. Holliday junction moves in lateral direction. During this process a DNA strand is progressively exchanged with a DNA strand of the other helix. This lateral migration of Holliday junction is called branch migration. The original base pairs are broken in parental molecules and new base pairs are formed in recombined strands.

If the two molecules have alternate alleles, A/a, B/b and C/c then the exchange of DNA strands during branch migration produces double strand regions which are not identical. These mismatched regions are called heteroduplexes. During branch migration, the heteroduplex region is elongated. Breakage and subsequent reunion lead to formation of this joint molecule composed of four interlocked strands of DNA.

The key feature of Holliday junction is the cleavage or cutting across the crossover point which resolves or separates the recombined molecules. To expose two cut sites, the Holliday junction is rotated by 180° to form a square planar structure. Resolution of Holliday junction occurs by cutting the DNA strands at the site of cross and re-joining them. Resolution occurs in one of the two ways. This gives rise to two classes of DNA products. The cut site 1 cleaves the two strands which were initially broken at the start of recombination process (Invading strands are cut). The resolution produces two non-recombinant molecules as only exchange of alleles has taken place in the middle region of duplex B/b and b/B. Thus a patch of hybrid DNA is formed. These molecules are known as patch products. The cut site 2 cleaves (Non-invading strands are cut) and re-joins two duplexes in such a way that flanking or peripheral genes are exchanged. Here DNA is reciprocally recombined. Crossing over occurs between A and C genes.

Single Strand Exchange Recombination:

According to Mesclson and Radding, a single strand break in one of the two homologous DNA molecules is quite common.

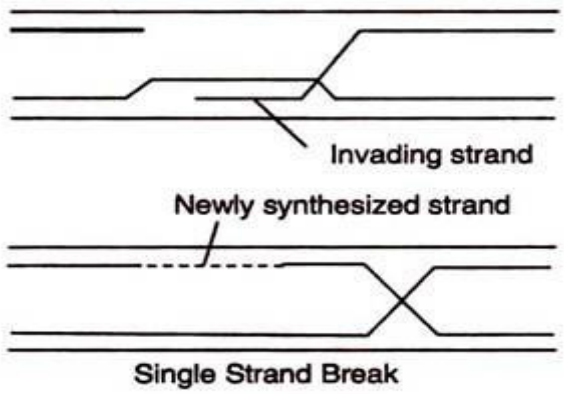
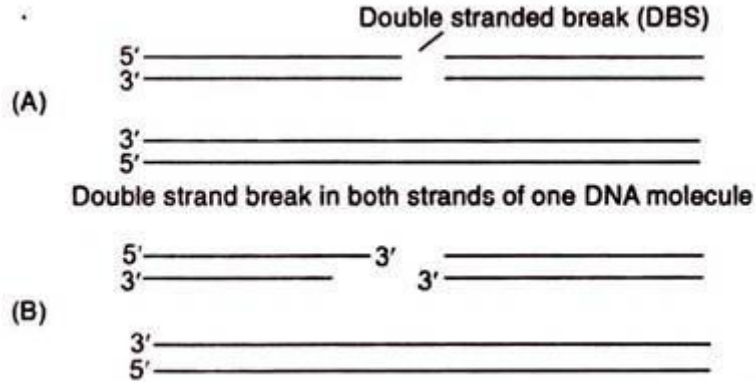


Fig. 19.2.

The free end of the broken strand invades the unbroken double helix and displaces one strand. Rec A protein has the ability to pull out a single strand and displace it. Exonuclease degrades the displaced single strand DNA loop. The unbroken strand of the first molecule serves as a template to synthesize the new strand.

Two Holliday Junctions:

Double stranded breaks in both strands of one DNA molecules occur quite frequently. During DNA repair of double stranded breaks, homologous recombination's occurs. This type of genetic recombination is called double stranded break repair mechanism. These types of breaks may be caused by ionizing radiations and various damaging agents.



Degeneration of broken strands and generation of single strand tails (ss tails) with 3 tends.

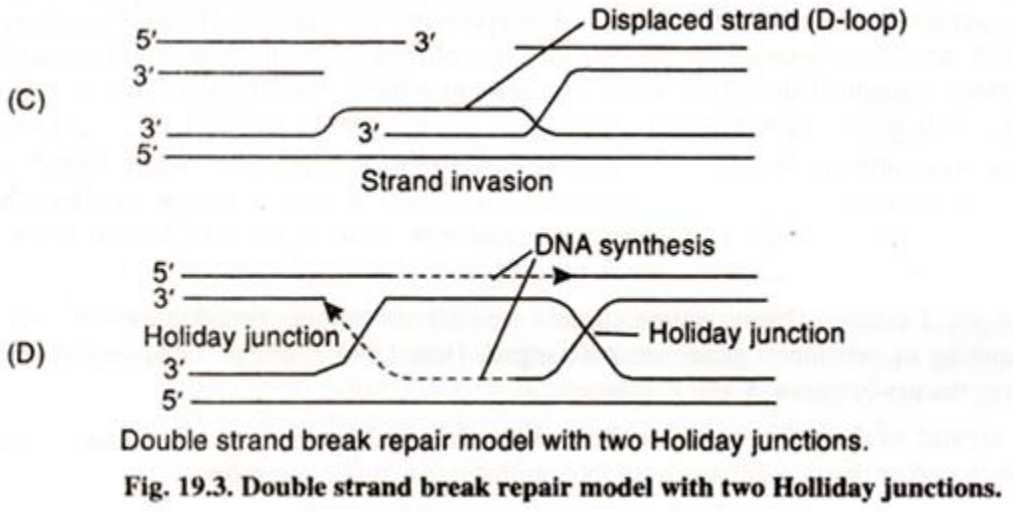


Fig. 19.3. Double strand break repair model with two Holliday junctions.

Enzymes Rec BCD further degrades the broken DNA strands. It generates single stranded tails with 3′ ends. One 3′ end tail invades the other unbroken homologous DNA molecule, displacing one of the strands. The invading 3′ end serves as a primer for new DNA synthesis. The displaced strand serves as a template to fill the gap left in the first DNA. If different alleles are present at the site of break, they are permanently lost as regeneration involves homologous DNA as template which has different alleles. This process is called gene conversion because genes of the broken strand are replaced by genes of the homologous

DNA. In double strand break repair, two Holliday junctions are created. These Holliday junctions move laterally by branch migration and the cleavage resolves and separates the two DNA molecules forming a crossover and a non-crossover structure. This is similar to the resolution in the single Holliday junction.

Enzymes of Homologous Recombination:

There are various proteins that catalyse various steps in the process of homologous recombination in E. coli.

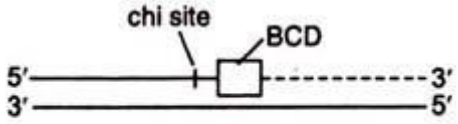


Fig. 19.4.

Enzymes Rec BCD load onto one end of DNA of double stranded break and move along DNA. (Rec-recombination). In the process it unwinds DNA (helical activity) and degrades one or both DNA strands (nuclease activity). Rec BCD is an endonuclease enzyme. It is encoded by three genes, Rec B, Rec C and Rec D. Rec BCD continues its degrading activity until it reaches a chi site (%). At this point activities of Rec BCD are stopped. The chi site has eight nucleotides. 5′ GCTGGTGG 3′. The chi sites promote recombination.

The single strand DNA tails generated by BCD enzymes are coated by Rec A enzyme. Rec A stimulates pairing or synopsis between two homologous DNA molecules Rec A also promotes strand invasion, displacing one strand of unbroken DNA molecule and forming D-loop. The displaced strand invades the broken DNA molecule. The missing portions of DNA strands are synthesized using homologous strand as template and gaps are sealed by ligase enzyme. Ruv AB enzymes recognize and bind to Holliday junction and performs branch migration. Ruv C enzyme cuts DNA strands at Holliday junction and causes separation and resolution of Holliday junction.

Different biological processes like replication, recombination and repair occur in a co-ordinated manner. In this way new DNA can be synthesized, damaged DNA repaired and genetic recombination takes place. Nucleotide sequences can be replaced through heteroduplexes and gene conversion.

Role of Rec A protein in Homologous Genetic Recombination:

In the various homologous genetic recombination models, the central features are similar in all recombination models.

These include breaks or nicks in DNA molecules. Alignment or pairing or synapsis of homologous sequences of two different DNA molecules. Formation of a crossover structure or Holliday junction in which DNA strand from each molecule creates short regions of heteroduplux DNA. Extension of heteroduplex DNA, which is called branched migration Lastly, resolution of crossover junction to yield end products. This is an extremely complex

process involving the action of several different enzymes. The first event of creating breaks or micks in DNA strands and the last event of resolution are undertaken by various enzymes like helicase, nuclease, and ligases. But the event starting from pairing of DNA molecules, formation of Holliday junction branch migration are the central features in recombination process. These events are undertaken by a special protein called Rec A protein. Rec A protein is involved in pairing, exchange of strands and branch migration. It is also known as strand exchange protein. Rec A protein plays a major role in homologous recombination. It is a special protein a completely distinct class of enzymes.

Rec A protein binds quickly to single stranded DNA along the phosphate backbone of DNA helix. DNA is completely covered by Rec A protein. Alongside rec A, a second protein called single strand binding protein (SSB protein) is also involved. Each Rec A molecule has 352 amino acids. There is one rec A monomer every 3-4 nucleotides, of DNA. Then, the ssDNA in duplex is aligned with homologous sequence of the other DNA molecule. Several steps occur in this process. Two types of homologous interactions occur. The first is the formation of paranemic joints in aligned homologous strands. The end second interaction involves formation of plactonemic joints. Rec A protein is a DNA dependent ATPase. ATP hydrolysis is required for branch migration, in which strands are replaced and strand exchange occurs. It exhibits polarity as branch migration proceeds in 5′ -> 3′ direction only.

Exchange of DNA Strands:

Role of strand exchange in post-replication repair is very prominent in E. Coli. Strand exchange plays a very prominent, role in repair of DNA damage. As the advancing replication fork comes across a lesion or damaged site such as thymine dimers, it is bypassed during replication process. The damaged protein may be cleaved which may prove to be lethal.

Repair of this lesion requires conversion of this DNA into double stranded DNA and this is achieved by rec A protein. Rec A protein plays its role in retrieving a portion of the complementary strand from other side of the replication fork to fill the gap. This involves branch migration by Rec A protein. This proves that branch migration is essential activity of the cell.

Probable Questions:

1. What is Theta replication?
2. Discuss mitochondrial DNA replication.
3. What is rolling circle mode of DNA replication.
4. Discuss viral DNA replication.

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland ScienceTaylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington,MA: Jones & Bartlett Learning.

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5. Cooper, G. and Hausman, R. (n.d.). The cell

Disclaimer :

The study materials of this book have been collected from books, various e-books, journals and other e-sources.

POST GRADUATE DEGREE PROGRAMME (CBCS)

in

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

Animal Physiology, Biochemistry and Metabolic Processes

ZCORT-104

SELF LEARNING MATERIAL

DIRECTORATE OFOPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

KALYANI, NADIA,

W.B. INDIA

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HARD CORE THEORY PAPER (ZHT 104)

Animal Physiology, Biochemistry and Metabolic Processes

Group A: Animal Physiology

PAPER	Unit	Content	Credit	Page No.
ZCORT - 104 (Animal Physiology, Biochemistry and Metabolic Processes)	I	Respiratory function of blood: Respiratory pigments – types, distribution and brief chemistry. Structure and function of haemoglobin- i) in adult and ii) during embryonic life.	3	8-28
	II	Transport of oxygen and carbon dioxide in blood and body fluids, Regulation of respiration.		29-50
	III	Physiology of excretion: Formation of urine: Physiology of ultrafiltration, Reabsorption tubular secretion Counter current theory of urine concentration, Regulation of urine formation, Renal regulation of acid- base balance.		51-67
	IV	Synaptic transmission; types of synapses, Pre- and postsynaptic structure and function, Steps in Synaptic Transmission.		68-106
	V	Chemistry and modes of neurotransmitter release, proteins in synaptic transmission: SNARE hypothesis: synapsins, synaptobrevin, synaptotagmin,		107-130

		SNAP and NSF, synaptic plasticity, toxins in synaptic transmission		
	VI	**Cytoskeleton, Extracellular matrix, gap junctions, integrins, cell adhesion molecules and their functions**		**131-145**
	VII	**Intracellular protein trafficking for secretory and non-secretory cells: Protein synthesis, Protein sorting and targeting to organelles; signal sequences, vesicle transport, packaging, storage and release, Targeting of proteins to lysosomes for degradation; Receptor mediated endocytosis.**		**146-170**
	VIII	**Physiology of muscle contraction and proteins associated with muscle contraction: Physiology of muscle contraction, Chemical nature of contractile elements, actin nucleation, actin treadmilling, myosin types, structure, Role of structural and regulatory proteins in muscular contraction (profilin, cofilin, thymosin, troponin, tropomysin, Arp 2/3 complex), ATP and signal molecules in muscular contraction, neuromuscular junction and its functioning.**		**171-201**

Group B: Biochemistry and Metabolic Processes

ZCORT - 104 (Animal Physiology, Biochemistry and Metabolic Processes)	Unit	Content	Credit	Page No.
	IX	Carbohydrate metabolism: Carbohydrates of physiological significance. Glycolysis, Gluconeogenesis.	3	203-223
	X	Hexose monophosphate Shunt, Glycogenesis and glycogenolysis, Control and regulation of carbohydrate metabolism		224-245
	XI	Amino acid metabolism: Amino acid classification, Urea cycle.		246-263
	XII	Proteins: Protein folding and protein stability.		264-276
	XIII	Bioenergetics (anaerobic and aerobic respiration, oxidative and substrate level phosphorylation) basic concept of ETC and ATP synthesis, uncouplers. Spontaneous reaction (concept of -vedel G). Thermodynamic principles and steady-state conditions of living organism.		277-296
	XIV	Biosynthesis of cholesterol, control of cholesterol biosynthesis.		297-311
	XV	Lipoproteins and types of cholesterol transport.		312-318
	XVI	Enzymes: Kinetic analysis of enzyme-catalyzed reaction, Michaelis-Menten Equation, Lineweaver-Burk's plot, Bi-substrate Reactions.		319-337
	XVII	Enzyme inhibitions, Regulation of enzyme activity, Allosteric control of enzyme activity		338-346
	XVIII	Chemistry of free radicals and antioxidants.		347-353
	XIX	Vitamins Role of vitamins as coenzymes.		354-386
	XX	Minerals: Role of minerals in human physiology		387-401

Total Counselling time 18 hours

UNIT-I

Respiratory function of blood: Respiratory pigments – types, distribution and brief chemistry. Structure and function of haemoglobin- i) in adult and ii) during embryonic life

Objectives: To have a precise knowledge about different types of respiratory pigments present in different animals and to know their functions. To study the structure and function of hemoglobin in detail which is the only respiratory pigment in human beings.

Pigments Found in the Blood of Animals

a. Haemoglobin:

A haemoglobin (Hb) molecule is a conjugated protein, because it consists of a simple protein and with a non-protein part. The non-protein part is called prosthetic group. The protein part of the haemoglobin is the globin. The globin part consists of 4 polypeptide chains-two alpha (α) chains and two beta (β) chains. Porphyrins are heterocyclic ring structure containing haem or magnesium. The heterocyclic ring structure is composed of four pyrrole rings which are linket by methine bridges. The globin part helps to prevent oxygen from binding tightly to haem; when globin is present, oxygen binds reversibly to haem and can be released to the tissues. In respiratory organs the haemoglobin combines with O_2 which form Oxyhaemoglobin at normal temperature and pressures. At the low pressure the oxyhaemoglobin dissociates as oxygen and haemoglobin ($HbO_2 \leftrightarrow Hb + O_2$)- Haemoglobin is involved in vertebrates in the transport of respiratory CO_2 (about 10% of the total) as carbamino-haemoglobin in which CO_2 is bound to the globin protein. The molecular weight of a haemoglobin molecule is 64,500 daltons. The oxygenated form of haemoglobin is scarlet and deoxygenated form is bluish-red. The haemoglobin is present in the erythrocytes in almost all vertebrates except a few Antarctic fish. In the invertebrates they are found in the plasma, coelomic fluid and haemoglobin-containing cells. In Annelida, the pigment is found in polychaeta (different kinds of respiratory pigments), in Oligochaeta (e.g., Pheretima, Lumbricus, Tubifex etc.), and in Hirudinea (e.g., Hirudo, Hirudinaria, etc.).

In Mollusca the haemoglobin is found in the plasma of Gastropods (e.g., Planorbis), and in Bivalvia (e.g., Solen, Area, etc.). In Chiton and in some prosobranchs the haemoglobin is present in the muscles of radula as Myoglobin. In Crustacea the pigment is present in small-sized animals (e.g., Artemia, Daphnia, Triops, etc.).

b. Haemocyanin:

A blue-green copper containing respiratory pigments found in some crustaceans, xiphosurans, myriapodes, and in some gastropods, bivalves and cephalopods. Haemocyanin is always found in dissolved condition in plasma. It occurs in two forms— oxidized and reduced forms, and in reduced forms the prism-shaped or needle-shaped crystals are soluble in water.

The oxygen carrying capacity of haemocyanin is lesser than haemoglobin. The mol. weight is variable in different groups of animals. In some crustaceans the mol. weight is about 4,00,000 daltons and in some gastropods it is 13,00,00,000. Haemocyanin binds a molecule of oxygen between a pair of copper atoms linked to amino acid side chains. In oxygenated condition the haemocyanin is bluish- green but it is colourless in deoxygenated state.

c. Chlorocruorin:

It is a green coloured respiratory pigment containing iron found in the plasma of some polychaetes (e.g., Serpulid, spirorbid, sabellid fanworms). It is also found in oxygenated and reduced forms. The metalloprotein of chlorocruorin is similar to haemoglobin except one vinyl group (CH_2 = CH—) is replaced by formyl (HCO—) group. The mol. weight is 30,00,000 daltons and generally it functions as oxygen carrier.

d. Haemerythrin (Haemo-erythrin):

It is an iron containing respiratory pigment found in the blood corpuscles of some invertebrates (e.g., Sipunculans, Priapulids and inarticulate Brachiopods). It is pink or violet coloured in oxygenated state and colourless in deoxygenated state. The mol. weight varies from 40,000 to 108,000 daltons and plays the role of oxygen storage.

A few other less common respiratory pigments are:

(i) Pinnaglobin—a brown coloured manganese containing pigment found in the plasma of Pinna (Lamellibranchs).

(ii) Vanadium—It is a green coloured vanadium containing pigment found in the vanadocytes of some sea squirts (Ascidians).

(iii) Molpadin pigment is present in Holothurian Molpadia, and

(iv) Echinochrome is known in sea urchins of echinoderms.

Hemoglobin:

It is an oxygen/CO_2 carrier protein present in the red blood corpuscles of blood. Hemoglobin is a conjugated chromo-protein having heme as its prosthetic group. Heme is the prosthetic group, not only of hemoglobin but also of myoglobin, cytochromes etc.

Hemoglobin is formed by the combination of heme with globin (protein). Globin is made up of four polypeptide chains (an oligomeric protein). Two of these polypeptides are known as alpha (α) and the other two are known as beta (β). Each alpha chain has 141 amino acids and each beta chain has 146 amino acids, which are arranged in a definite sequence. Its molecular weight is 65,000.

Due to the characteristic folding of its tertiary structure each polypeptide forms a cup like structure with a pocket like area where the prosthetic group, heme is buried. Heme has iron, which is linked to the imidazole nitrogen of the histidine in positions 58 and 87 of the alpha chains. In the beta chain the heme iron is linked with histidine in positions 92 and 63. Altogether there are four heme groups in one hemoglobin molecule. Hemoglobin is the red colouring matter of blood which is present in the red blood cells. It is a conjugated protein consisting of heme and the protein globin. It has a molecular weight of 64,450. It can combine with oxygen and acts as the transport mechanism for oxygen within the blood. It contains 4 gram atoms of iron per mole in the ferrous (Fe^{++}) state.

Structure of Hemoglobin:

The structure of Hemoglobin can be classified under two headings:

a. Structure of Heme, the prosthetic group.

b. Structure of Globin, the protein part— apoprotein.

a. Structure of Heme:

i. It is an iron porphyrin. The porphyrins are cyclic compounds with “tetra pyrrole” structure.

ii. Four pyrrole rings called I to IV are linked through methylene bridges or methylidene bridges.

iii. The outer carbon atoms, which are not linked with the methylidene bridges, are numbered 1 to 8.

iv. The methylidene bridges are designated as α, β, γ, δ, respectively.

v. Iron in the ferrous state is bound to the nitrogen atom of the pyrrole rings.

vi. Iron is also linked internally (5th linkage) to the nitrogen of the imidazole ring of Histidine of the polypeptide chains.

vii. The propionic acid of 6th and 7th position of heme of III and IV pyrroles are also linked to the amino acids Arg and Lys of the polypeptide chain, respectively.

The porphyrins are found in nature in which the various side chains are substituted for the 8 hydrogen atoms as numbered in the porphin nucleus. The arrangement of the A and P substituents in the uroporphyrin shown here is asymmetric (in ring IV the expected order of the acetate and propionate substituents is reversed). This type of asymmetric substitution is classified as a type III porphyrin. A porphyrin with a completely symmetrical arrangement of the substituents is classified as a type I porphyrin. Only types I and III are found in nature and the type III series is more abundant.

b. Structure of Globin:

i. The globin of hemoglobin is a protein which is composed of 4 parallel layers of closely packed polypeptide chains.

ii. Two of the chains (α-chains) have identical amino acid composition of 141 amino acids. The two other chains may be two of the 4 polypeptide chains designated as β, γ, δ, and ε (epsilon). Each is having 146 amino acids.

iii. The total number of amino acids in globin is 574.

iv. α chains have Val-Leu-Ser in N terminal residues and Lys-tyr-Arg in C terminal residues.

v. β chains have Val-His-Leu in N-terminal residues and Lys-tyr-His in C-terminal residues.

vi. γ chains have Gly-His-Phe. N-terminal residues and Arg-Tyr-His in C-terminal residues.

vii. Hemoglobin molecule and its sub-units contain mostly hydrophobic amino acids internally and hydrophilic amino acids on their surfaces. So they form "'Heme pockets".

viii. In **"heme pockets"** α subunits are of size necessary for entry of O_2 molecule but the entry of O_2 molecule in β subunit is blocked by valine residue.

Biosynthesis of Hemoglobin:

i. The biosynthesis of hemoglobin takes place in the bone marrow in the erythroid cell during its development to erythrocyte.

ii. It starts appearing at stage II (early normoblast) and the synthesis is complete when the cell reaches stage IV (late normoblast).

iii. Iron in the ferrous state is incorporated into protoporphyrin to form heme.

iv. The heme gets attached to the newly synthesized globin to form hemoglobin.

v. The iron of heme is coordinated to 2 imidazole nitrogen of histidine at position 38 and 87 in α-chains and 63 & 92 in β-chains.

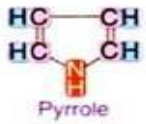
Composition of Hemoglobin:

Heme:

It is an iron-porphyrin compound. It is the prosthetic group embedded in the packet like structure formed by folding of the hemoglobin tertiary structure.

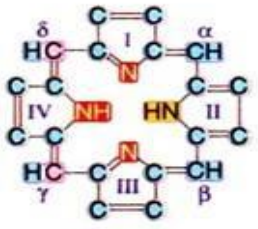
Porphyrins:

Porphyrin is a complex compound with a tetrapyrrole ring structure. Pyrrole is a heterocyclic compound having the following structure.

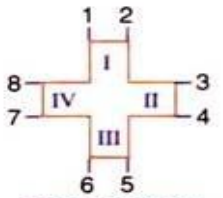


Pyrrole

4 pyrrole rings join together through methylidyne bridges (–CH=) to form a porphin.



Porphin



Fisher's projection formulae for a porphin

This porphin is substituted by different groups at positions numbered from 1-8 to form the porphyrin. Depending upon the groups (methyl, acetyl, propyl, butyl or venyl) present on these positions different types of porphyrins are identified, that will be seen during the synthesis of heme.

Properties of porphyrins:

1. They act both as acids (-COOH) and bases (-NH2).

2. Their isoelectric pH is between 3-4.5.

3. Porphyrins are fluorescent and coloured due to presence of alternating double bonds.

4. Porphyrinogens are colourless.

Chlorophyll (magnesium-containing porphyrin), the photosynthetic pigment of plants and heme (the iron-containing porphyrin) of hemoglobin in animals are synthesized in living cells by a common pathway:

i. The starting materials are 'active succinate' (succinyl-CoA) derived from the citric acid cycle and glycine. Pyridoxal phosphate (B_6-PO_4) is necessary to activate glycine. The product of the condensation reaction is α-amino-β-ketoadipic acid which is catalyzed by the enzyme AmLev synthetase (ALA synthase).

ii. α-amino-β-ketoadipic acid is rapidly decarboxylated by the same enzyme AmLev synthetase producing δ-aminolevulinic acid (AmLev). Synthesis of aminolevulinic acid occurs in the mitochondria. The anemia has been observed in the deficiency of vitamin $B_{(}$, or pantothenic acid.

iii. 2 mols of AmLev condense to form porphobilinogen (the first precursor of pyrrole) which is catalyzed by the enzyme δ-aminolevulinase (AmLev dehydrase).

iv. 3 mols of porphobilinogen condense first to form a tripyrrylmethane which then breaks down into a di-pyrrylmethane and a monopyrrole. The dipyrryl compounds are of two types A and B. The formation of tetrapyrrole occurs by condensation of two dipyrrylmethanes. If two of the (A) components condense, a type I porphyrin results; if one (A) and one (B) condense, a type III results.

v. The uroporphyrinogens I and III are converted to coproporphyrinogens I and III by decarboxylation being catalyzed by uroporphyrinogen decarboxylase.

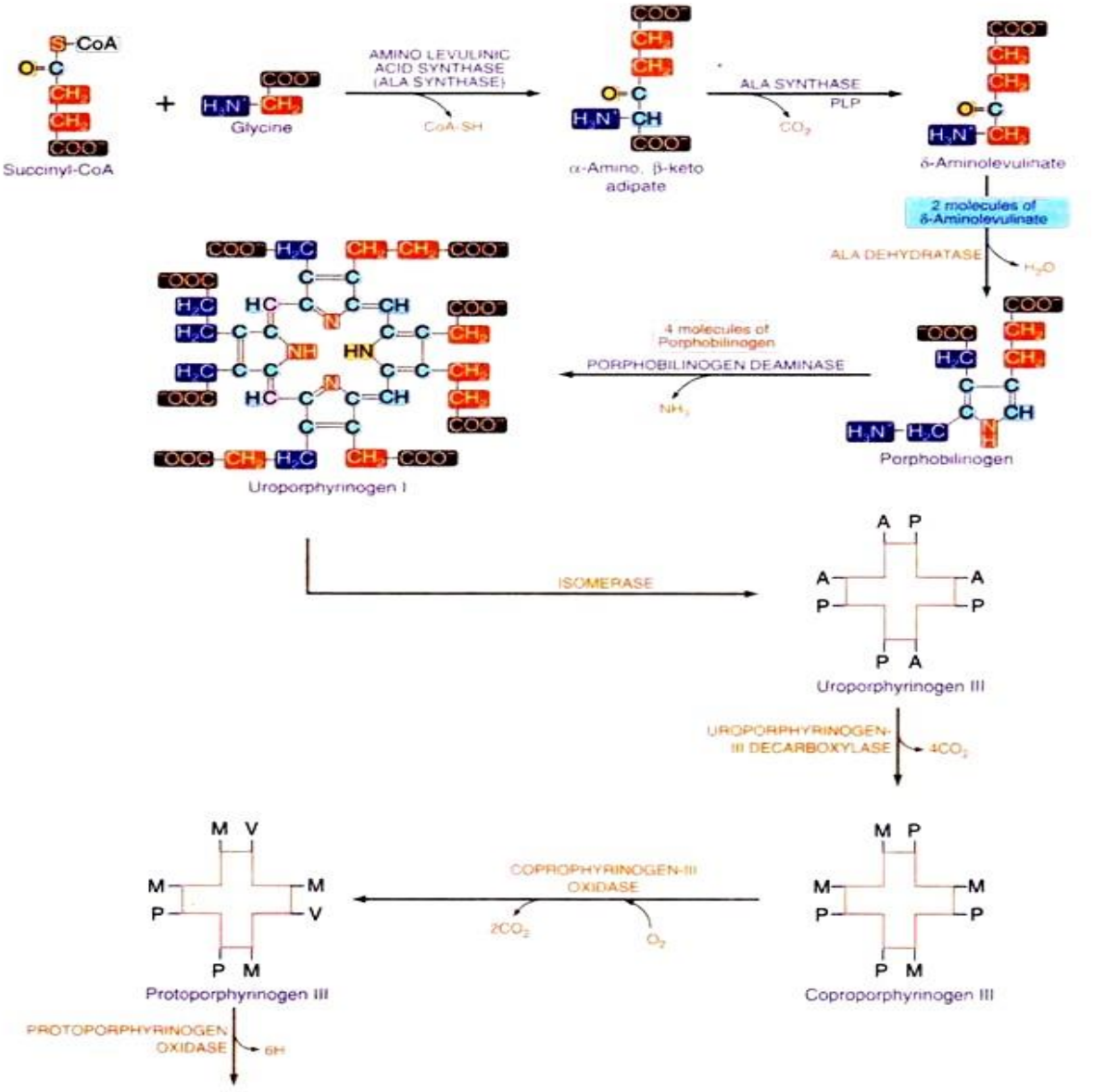
vi. The coproporphyrinogen III then enters the mitochondria where it is converted to protoporphyrinogen III and then to protoporphyrin III. The enzyme coproporphyrinogen oxidase catalyzes the formation of protoporphyrinogen III. The oxidation of protoporphyrinogen to protoporphyrin is catalyzed by the enzyme protoporphyrinogen oxidase.

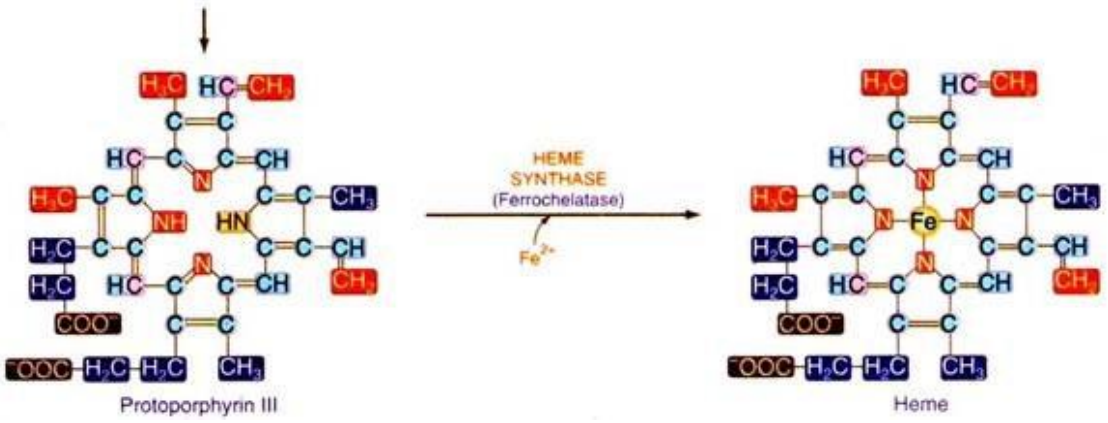
The enzyme coproporphyrinogen oxidase is able to act on type III coproporphyrinogen only for which type I protoporphyrin has not been identified in natural materials. In mammalian liver the reaction of conversion of coproporphyrinogen to protoporphyrin requires molecular oxygen. vii. In the final step of heme synthesis ferrous ion (Fe^{++}) is incorporated into protoporphyrin III which is catalyzed by heme synthetase or ferrochelatase. The reaction takes place readily in the absence of enzymes but becomes rapid in presence of enzymes.

Biosynthesis of heme:

Heme is an iron porphyrin structure, synthesized in the reticuloendothelial cells (bone marrow) of adult human being. Erythropoietin produced in kidney stimulates the formation, maturation and release of erythrocytes by bone marrow. arly stage of erythrocyte cells contain porphyrin, during the course of their development, porphyrin is converted to heme by addition of iron and then to hemoglobin by addition of protein, globin. The type of porphyrin present in heme is protoporphyrin-III (also known as No. IX).

It is synthesized starting from glycine and succinyl-CoA. Given below is the diagrammatic representation of biosynthesis of Heme where 'A' stands for acetyl group, 'P' stands for propyl group, 'M' for methyl group, and 'V' for venyl group.





Regulation of heme synthesis:

The first enzyme in this sequence i.e. ALA synthase is the key regulatory enzyme for heme synthesis which is inhibited by heme the end product of the metabolism. ALA synthase is a regulatory enzyme. Heme acts as an Apo repressor molecule and is a negative regulator for the synthesis of ALA synthase-I (heme inhibits the gene).

Inhibitors of heme synthesis: The following substances inhibit heme synthesis:

i. Succinylacetone (SA) is an inhibitor of heme synthesis that acts on the enzyme aminolevulinic acid dehydratase.

ii. N-methyl mesoporphyrin IX blocks iron insertion into protoporphyrin IX and thus acts as an inhibitor of heme synthesis.

iii. Isonicotinic acid hydrazide (INH) is an inhibitor of 6-aminolevulinic acid synthase.

Disorders related to abnormalities in the synthesis of porphyrins:

Porphyria's:

Porphyria's are a group of diseases in which there is an increased excretion of porphyrins or porphyrin precursors (intermediates of porphyrin synthesis). About 85% of heme synthesis occurs in erythrocyte precursors and 15% in liver. Therefore porphyria's are classified into two types (1) Erythropoietic and (2) Hepatic. Some of them are inherited while others are acquired.

Condition	Enzyme defective	Symptoms
1. Erythropoietic		
a. Congenital erythropoietic porphyria	Non-cooperativity between (a) Uroporphyrinogen-I synthase and (b) Uroporphyrinogen-II cosynthase	Darkening of urine upon standing, abdominal pain, vomiting, constipation, cardiovascular abnormalities
2. Hepatic		
a. Acute intermittent porphyria	Uroporphyrinogen-I synthase	Urine is red due to oxidation of porphobilinogen on exposure to light. Abdominal pain, vomiting, constipation and paralysis
b. Hereditary coproporphyria	Coproporphyrinogen oxidase	Cutaneous hypersensitivity to light
c. Variegate porphyria	Protoporphyrinogen oxidase	No symptoms
d. Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Chin is pink or brown coloured. Hepatic damage and accumulation of iron. Photosensitivity of skin
e. Toxic porphyria	Variable	—
3. Both erythropoietic and hepatic		
a. Protoporphyria	Ferro chelatase is absent, defective or less	

Properties of Hemoglobin:

i. Oxy-hemoglobin:

It forms oxy-hemoglobin in combination with oxygen. When hemoglobin is exposed to air, it takes up two atoms of oxygen for each atom of ferrous ion (Fe^{++}) present. Thus, hemoglobin will take up 4 molecules of oxygen. In low oxygen tension, oxy-hemoglobin gives up O_2 readily. By this way, blood carries O_2 to different parts of the body.

ii. Formation of Carhamino Compound:

It reacts with CO_2 forming carbamino compounds.

$Hb\text{-}NH_2 + CO_2 \rightarrow Hb\text{-}NH.COOH$

iii. Reaction with Carbon Monoxide:

It forms carboxy hemoglobin after reacting with carbon monoxide (CO). Carboxy hemoglobin is stable and prevents the formation of oxy-hemoglobin. So inhalation of even small amounts of carbon monoxide is highly dangerous.

iv. Buffering Action:

One mol of hemoglobin contains 35 histidine residues. Histidine exerts its buffering action through its basic imidazole ring. Hence, hemoglobin plays an important role in regulating the acid-base balance of blood.

v. Formation of Methemoglobin:

Methemoglobin is formed as a result of the oxidation of hemoglobin by the mild oxidizing agent, potassium ferricyanide.

The ferrous ion (Fe^{++}) is oxidized to the ferric ion (Fe^{+++}). Methemoglobin cannot carry oxygen in blood. It is also formed by the action of some drugs. This is found in the blood of some individuals owing to inborn errors of metabolism. This can be reduced to hemoglobin by vitamin C which is used in the treatment of methemoglobinemia.

vi. Sulphemoglobin:

It is formed by the administration of certain drugs. It continues to remain in the blood and cannot be re-converted into hemoglobin.

vii. Cyanomethemoglobin:

It is formed by the addition of cyanide to methemoglobin. It has a bright red colour.

viii. Absorption Spectra:

The different hemoglobin derivatives can be easily identified by this characteristic absorption spectra.

(a) Oxy-hemoglobin:

Two bands—one narrow and the other wide in the green region.

(b) Reduced hemoglobin:

One single broad band in the green region.

(c) Carboxy hemoglobin:

Two bands in the green region.

(d) Methemoglobin:

Three bands – one in red and two in the green regions.

(e) Sulphemoglobin:

Three bands similar to methemoglobin.

In nature, the other metalloporphyrins which are compounds of importance in biologic processes are mentioned:

A. Erythrocruorins:

(a) They are iron porphyrinoproteins occurring in blood and tissue fluids of some invertebrates.

(b) Their function is corresponding to hemoglobin.

B. Myoglobins:

(a) They are the respiratory pigments occurring in the muscle cells of vertebrates and invertebrates.

(b) The purified one has a molecular weight of about 17,000.

(c) They contain only 1 gram atom of iron per mole.

C. Catalases:

(a) They are iron poiphyrin enzymes.

(b) They have been obtained in crystalline form.

(c) Their molecular weight is about 225,000.

(d) They contain 4 gram atoms of iron per mol.

(e) In plants, their activity is minimal.

D. Tryptophan Pyrrolase:

(a) It is an iron porphyrin protein.

(b) It catalyzes the oxidation of tryptophan to formyl kynurenine.

E. Cytochromes:

(a) Cytochromes means the cellular pigments because these pigments are widely distributed not only in the tissues of higher animals and plants but also in yeast and bacteria.

(b) At first, cytochromes a, b and c were identified and they had been shown to exist in oxidized and reduced forms and their fundamental role is in cellular respiration. At present, some thirty cytochromes are known to exist and according to original cytochromes they are designated as a_1, a_2, a_3, c_1, c_2, c_3, c_4, c_5, b_2, b_3, b_4 etc.

(c) They are iron porphyrins and act as electron transfer agents in oxidation-reduction reactions.

(d) The important example is cytochrome C which has been obtained in the purified form.

(e) Cytochrome C has a molecular weight of about 13,000 and contains 0.43% iron.

(f) The iron porphyrin group of cytochrome C is attached to protein more firmly than in the hemoglobin.

(g) Cytochrome C is quite stable to heat and acids.

(h) The reduced form of cytochrome C is not auto-oxidizable.

(i) At physiological pH Ferro cytochrome C does not combine with O_2 or CO as does hemoglobin.

(j) The peptide chain of human heart cytochrome C contains 104 amino acids, Acetyl glycine is the N-terminal amino acid and glutamic acid the C-terminal amino acid. The two cysteine residues are located at positions 14 and 17 in the peptide chain. The linkage of iron in heme occurs through the imidazole nitrogen of a histidine residue at position 18 in the peptide chain.

(k) The degree of difference in primary structure among the 13 cytochrome C might be related to the degree of phytogenetic relationship between the species Eg. The cytochrome C of man as compared to that of rhesus monkey differs by only one amino acid of the 104 amino acids. Human cytochrome C differs from that of the dog in 11 amino acid residues, from that of the horse in 12.

(l) The enzymes that catalyse the reactions of molecular oxygen are known as oxidases. Cytochrome a_3, which is found in heart muscle and other animal tissues is called cytochrome oxidase. These oxidases catalyse many reactions in addition to terminal oxidation at the electron transport chain. They can carry three general types of reactions e.g., oxygen transfer, mixed function oxidation electron transfer.

Transportation Provided by Hemoglobin:

Hemoglobin Transports CO_2 and Protons to the Lungs after releasing O_2 to the Tissues:

i. Hemoglobin can bind CO, directly when oxygen is released and CO, reacts with the amino terminal a-amino groups of the hemoglobin forming a carbamate and releasing protons.The amino terminal is converted from a positive to a negative charge favouring salt bridge formation between the a and P chains.

ii. At the lungs, hemoglobin is oxygenated, being accompanied by expulsion and subsequent expiration of CO_2. CO_2 is absorbed in blood and the carbonic anhydrase in erythrocytes catalyzes the formation of carbonic acid which is rapidly dissociated into bicarbonate and a proton.
A buffering system absorbs these excess protons to avoid the increasing acidity of blood. Hemoglobin binds two protons for every four oxygen molecules. 3. In the lungs, the process is reversed i.e. when oxygen binds to deoxygenated hemoglobin, protons are released and combines with bicarbonate forming carbonic acid which is exhaled.

Thus, the binding of oxygen forces the exhalation of CO_2. This reversible phenomenon is called the Bohr effect. Myoglobin does not exhibit Bohr effect.

2, 3-Biphosphoglycerate (BPG) Stabilizes the T Structure of Hemoglobin:

i. The increased accumulation of 2, 3- biphosphoglycerate is caused by an oxygen shortage in peripheral tissues. BPG is formed from 1, 3-biphosphoglycerate in the glycolytic pathway. One molecule of BPG is bound to central cavity formed by all four subunits of hemoglobin. This cavity is of sufficient size for BPG only when hemoglobin is in the T form. BPG is bound by salt bridges between its oxygen atoms and both chains as well as by Lys EF6 and His H21. Thus, BPG stabilizes the T or deoxygenated form of hemoglobin.

ii. Fetal hemoglobin is more weakly bound to BPG because the H21 residue of the Y chain of HbF is Her rather than His and cannot form a salt bridge with BPG. Hence, BPG has a less profound effect on he stabilization of the T form of HbF and is responsible for HbF to have a higher affinity for oxygen than does HbA.

iii. The trigger for the R to T transition of hemoglobin is movement of the iron in and out of the plane of the porphyrin ring.

De-oxy-hemoglobin S can Form Fibres that Distort Erythrocytes:

i. After the de-oxygenation of hemoglobin S the sticky patch can bind to the complementary patch on another deoxygenated HbS molecule. This binding causes polymerization of de-oxy-hemoglobin S forming long fibrous precipitates. These extend throughout the erythrocyte and mechanically distort it causing lysis and a good number of secondary clinical effects.

ii. De-oxy-hemoglobin A although contains the receptor sites for the sticky patch present on deoxygenated HbS, the binding of sticky hemoglobin S to de-oxy-hemoglobin A cannot extend the polymer. Because de-oxy-hemoglobin A does not have a sticky patch to enhance binding to another hemoglobin molecule. Therefore, the binding of de-oxy-hemoglobin A to the R or the T form of hemoglobin S will reject polymerization.

iii. The polymer forms a twisted helical fiber whose cross section contains 14 HbS molecules. These tubular fibres distort the erythrocyte.

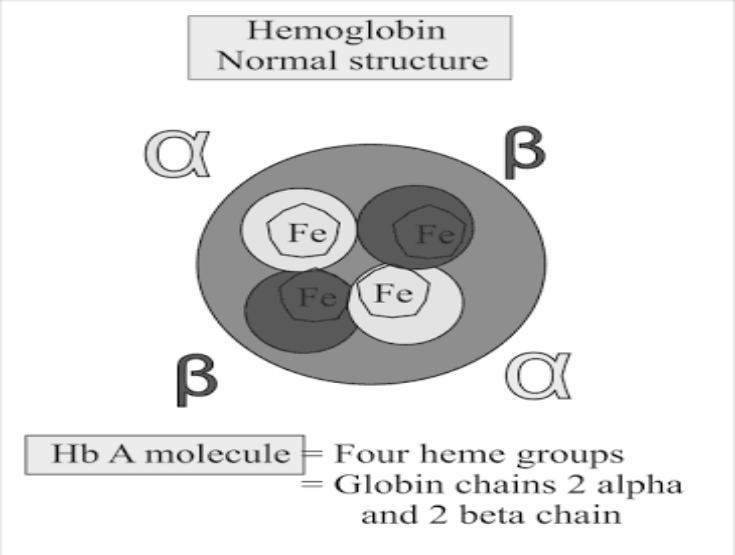
Varieties of Human Hemoglobin:

Normal adult hemoglobin or hemoglobin A has a molecular weight of 64,456 and contains two pairs of peptide chains (α & β) of which α chain contains 141 and β chain contains 146 amino acids. Fetal hemoglobin (F) is present in very small amounts.

All the normal human hemoglobin's possess a common half-molecule, i.e. a pair of peptide chains (a chains); the other half consists of a pair of different types of peptide chains, one type for each hemoglobin. Hemoglobin A_2 has two δ chains and hemoglobin F has two γ chains; both types of chains contain 136 amino acids and thus are of the same length as the β chain. Hemoglobin A is represented as $\alpha_2A\beta_2A$ hemoglobin A_2 as $\alpha_2A\ \delta_2A$ and hemoglobin F as $\alpha_2A\gamma_2A$ for describing abnormal hemoglobin. In early embryonic life, a fourth hemoglobin a_2Ae_2 exists.

Some Amino acid distribution in abnormal hemoglobin

Type of abnormal hemoglobin	Replacement of the position of amino acid	Amino acid present in the normal hemoglobin at that position	Amino acid present in abnormal hemoglobin at that position
C	6	Glutamic acid	Lysine
D (Ibdan)	87	Threonine	Lysine
E	26	Glutamic acid	Lysine
M (Boston)	58	Histidine	Tyrosine
N	95	Lysine	Glutamic acid
S	6	Glutamic acid	Valine



Fetal Hemoglobin:

i. Fetal hemoglobin (F) comprises 50 to 90 per cent of the total hemoglobin in the newborn.

ii. It takes up oxygen more readily at low oxygen tensions and releases carbon dioxide more readily than adult hemoglobin (A).

iii. It is more resistant to denaturation by alkali and is more susceptible to conversion to methemoglobin by nitrites (contaminated water).

iv. Hemoglobin F is gradually replaced by hemoglobin A during the first 6 months of extra uterine life.

v. High concentration of hemoglobin F after two years of age occur in various types of anemia, e.g., sickle cell anemia and thalassemia.

Difference between Adult and Fetal Hemoglobin

HbA	HbF
1. Composed of two α and two β chains.	2. Composed of two α and two γ chains.
3. Life span is a about 120 days.	4. Life span is less than 80 days.

5. Affinity for oxygen less than HbF.	6. Greater affinity for oxygen binding.

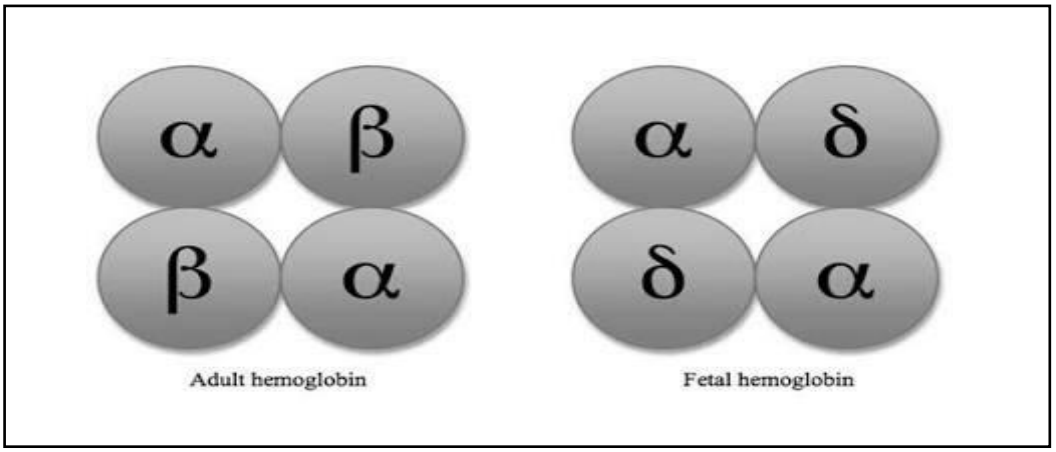


Figure : difference between HbA and HbF.

Abnormal Hemoglobin's:

Over one hundred different types of abnormal hemoglobin's have been described. Some of these are easily differentiated by their electrophoretic mobilities and have given rise to the concept of **"molecular disease"** which explains that a defective gene (mutant) may direct the formation of a molecule similar to a normal molecule but differing from it in shape, composition and electrical charge.

One amino acid of the normal hemoglobin is replaced by another amino acid, i.e. acidic amino acid is replaced by a basic or a neutral amino acid for the formation of abnormal hemoglobin. The abnormal hemoglobin's are named in alphabetic order as C, D, E, F, G, H, K, L, M, N, O, P, Q, S etc.

A. Hemoglobin C:

This occurs in the blood of some Negroes in West Africa. The abnormality is found in the β chain at position 6, the amino acid glutamic acid is replaced by Lysine. It is characterized by the mild anemia with a tendency to infarction.

B. Hemoglobin S:

This appears among the Negroes of Africa. The abnormality occurs in β chain, glutamic acid at position 6 is replaced by valine. Sickle cell anaemia develops and the RBC becomes long and boat-shaped. The blood becomes more viscous which results in reduced blood flow.

C. Hemoglobin F:

HbF is present in fetus and is replaced by adult hemoglobin as the child grows. It is present only in traces in normal adults, it gets hemolysis rapidly producing a severe anemia called "Thalassemia major".

D. Hemoglobin M:

There are two types of HbM-HbM (Boston) and HbM I Wate which are of clinical interest. The abnormality is found in the α chain, the histidine residues in 58 and 87 position are replaced by tyrosine. Abnormal amounts of methemoglobin are found in the blood of persons affected by this condition. This methemoglobin is not reduced to hemoglobin by reducing agents.

E. Hemoglobin D:

This occurs rarely. It exists in two forms – Dα and Dβ. The persons having HbD do not show any clinical signs and symptoms.

Technique for Identification of Hemoglobins:

Finger print technique Ingram developed a technique by which the peptide chains in hemoglobin could be broken down into several smaller peptide fragments by digestion with trypsin. Trypsin splits the peptides only at points where only lysine and arginine occur. A mixture of smaller peptides were obtained. He then separated this mixture using paper electrophoresis technique and paper chromatography. The peptides appeared as spots when ninhydrin was sprayed. Thus peptide maps had been prepared for different hemoglobin's.

Hemoglobin Derivatives:

There are some derivatives of normal Hb that arise due to metabolic changes in the RBC.

The various hemoglobin derivatives are:

1. Oxyhemoglobin (HbO_2):

The main function of hemoglobin is to transport oxygen from the lung to the tissues. In lungs the partial pressure of oxygen is 100 mm of Hg, at this pressure hemoglobin is 95-96% saturated with oxygen. On binding with O_2 in the lungs hemoglobin is converted to oxy-hemoglobin (Hb02). O_2 is bound to heme iron.

$Hb + O_2 \rightarrow HbO_2$

2. Reduced Hemoglobin (HHb):

Oxy-hemoglobin moves to the tissue where the partial pressure of O_2 is 26 mm of Hg due to which oxygen is released into the tissues and in turn H^+ binds to Hb and forms reduced hemoglobin.

$HbO_2 + H^+ \rightarrow HHb + O_2$

3. Carbaminohemoglobin:

Hemoglobin also binds to CO_2 in the tissues. CO_2 is bound to the α-amino group at the N-terminal end of each of the four polypeptide chains of hemoglobin to form carbaminohemoglobin. As one CO_2 binds O_2 is released.

4. Methemoglobin:

In RBC the iron of hemoglobin is normally in ferrous (Fe^{2+}) form, but it is readily oxidized to the ferric (Fe^{3+}) form by hydrogen peroxide formed by RBC cell metabolism, to yield met-hemoglobin. Ferric iron is incapable of binding O_2 therefore the functions of hemoglobin are disturbed. Normally 1.7 to 2.4 % of total hemoglobin will be in the form of met-hemoglobin. Increase in the percent of met-hemoglobin is prevented by the peroxidase action of a naturally occurring peptide known as glutathione present in the RBC. Met-hemoglobin is dark brown in colour.

The percent of met-hemoglobin can increase if the person consumes drugs like ferricyanide, nitrite, quinines, hydroxylamine's, acetanilide and sulfonamide. Higher levels of met-hemoglobin is observed clinically in factory workers who inhale (or contact through skin) aromatic nitro and amino compounds and in patients taking large amounts of acetanilide and sulfonamides. The symptoms are cyanosis (blue skin) and dyspnoea (labored breathing).

Importance of methemoglobin:

Met-hemoglobin can be used to overcome cyanide poisoning. By injecting met-hemoglobin it combines with cyanide to form cyanomethemoglobin preventing cyanide poisoning.

5. Carboxyhemoglobin:

Oxy-hemoglobin can bind to carbon monoxide (CO). Even normal, non- oxygenated hemoglobin can bind with CO to form carboxyhemoglobin. [Hb + CO → HbCO]. CO has got an affinity of 200 times more than that of O_2 towards Hb. Hemoglobin can bind more readily to CO than to O_2. Even if there is a little amount of CO in air, it can displace oxyHb to form carboxyHb. Due to this there will be tissue hypoxia because the oxygen binding capacity is reduced and there is also reduced O_2 releasing capacity i.e. it cannot release O_2 though it may be bounded to O_2.

City dwellers have at least 1% of carboxyhemoglobin which can increase to 8% depending upon the pollution. Over traffic can increase carboxyHb to 40% which leads to death. Clinically such patients show cherry red colour of skin. CO poisoning can be treated if high amount of O_2 is provided continuously at high pressure, then at such high concentrations and pressure HbCO is dissociated forming HbO_2 + CO. When treatment continues for 2 hours CO is expelled out.

Types of Hemoglobin:

There are three types of hemoglobin's that are normally found in human beings, they are:

1. HbA;

Found in normal adult human beings – contains 2α and 2β chains.

2. HbA_2:

Found in some human beings and is considered normal — contains 2α and 2β chains.

3. HbF:

Foetal hemoglobin — found in growing foetus — contains 2α and 2γ chains.

Each chain is synthesized by the information obtained from the gene for hemoglobin, α chain is synthesized from a genes of hemoglobin, β chain from β genes of hemoglobin likewise y and 8 from their respective genes. There are 2 pairs of a genes but only one pair each of β, γ and δ genes. Abnormal hemoglobin's arise due to mutation in the gene for the hemoglobin synthesis. There are about 300 abnormal hemoglobin's. Some of them are those which have defect in α genes and some are with defective β chains.

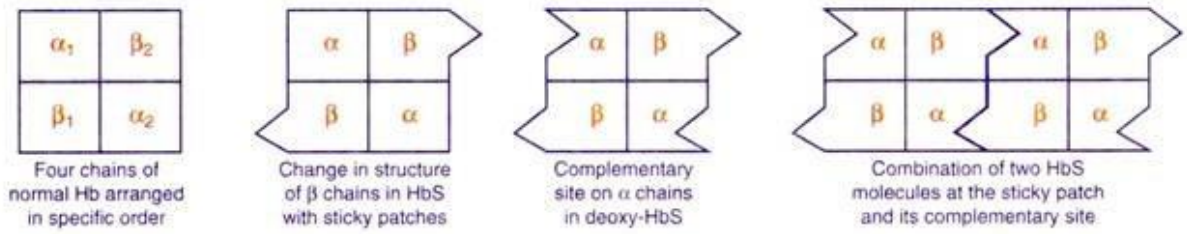
Name of Hb	Abnormality present at position	Actual amino acid present in normal Hb	Replaced amino acids in abnormal Hb
Hemoglobin (Hb) with abnormal α chain			
HbI	16	Lysine	Glutamic acid
HbG	23	Glutamic acid	Valine
HbM_B	58	Histidine	Tyrosine
Hemoglobin (Hb) with abnormal β chain			
HbS	6	Glutamic acid	Valine
HbC	6	Glutamic acid	Lysine
HbM_S	63	Histidine	Tyrosine

Biochemistry of Abnormality in the Hemoglobin:

HbS or Sickle Cell Hemoglobin:

Sickle cell hemoglobin (HbS) arises due to the defect in β chain in which glutamic acid present at the 6^{th} position is replaced by valine. Valine is also present naturally at position one. These two valine residues form hydrophobic interaction producing a sticky patch on HbS. Due to this replacement there is a sticky patch on HbS which appears on the oxy HbS. There is a complementary site to this sticky patch on deoxy HbS and also on deoxy HBA.

The mechanism of biconcave RBC converting to sickle shape is given here under:



When hemoglobin molecules combine together in chains they form precipitates of HbS. The precipitate formed in the RBC sinks down and the biconcave shape of RBC is converted to sickle shape.

The life span of RBC is reduced to less than half (about 30 days). HbS is very unstable, due to which there is excessive hemolysis. This results in anemia called sickle cell anemia. The physiological changes observed in sickle cell anemia are – physical exertion, weakness, short of breath, leukemia and heart murmurs.

HbM or Methemoglobin:

The defect lies both in α and β chains. This is due to replacement of histidine residue in 58^{th} position in α chain and 63^{rd} position in β chain. Due to this replacement, the iron (Fe) present in the ferrous state is oxidized to ferric state. This ferric iron cannot bind oxygen. Therefore the oxygen carrying capacity is disrupted leading to anemia and hypoxia (low O_2 to tissues).

Thalassemia's:

The defect in thalassemia's is the decreased rate of synthesis of one of the polypeptide chains of the globin molecule. One of the chains is synthesized in less amounts than the other due to the defect in DNA.

There are two types of thalassemia:

1. β-thalassemia:

β-thalassemia occurs due to the decreased synthesis rate of P-chain of globin. Due to the deficiency of P-chain the a-chains either combine among themselves forming a-4-globin or it can combine with γ or δ chains, thereby forming more of HbA2 and HbF. This results in the impairment of the transport of O_2 by Hb resulting in hypoxia. There are very low levels of Hb i.e. 2-3 g/100ml (hypochromic cells). The life span of such RBC is greatly reduced. The symptoms include anemia, growth retardation, wasting and fever.

2. α-thalassemia:

α-thalassemia occurs due to the decreased rate of synthesis of α-chain of globin. But this is rarely seen due to the presence of two pairs of genes for a chain in the Hb gene. Due to lack of a chain, the β chain may combine either with δ, γ or among itself forming β_4, or $\beta_2\delta_2$ or $\beta_2\gamma_2$.

Probable questions:

1. Enlist the different types of respiratory pigments found throughout the animal kingdom.
2. How is fetal hemoglobin different from that of adult hemoglobin?

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

UNIT-II
Transport of oxygen and carbon dioxide in blood and body fluids, Regulation of respiration

Mechanism of Breathing:

It means the inflow (inspiration) and outflow (expiration) of air between atmosphere and the alveoli of the lungs. It is affected by the expansion and contraction of lungs. There are mainly two processes by which the lungs are expanded or contracted.

(i) The downward and upward movement of the diaphragm which increases and decreases the diameter of the thoracic cavity (chest cavity).

(ii) The elevation and depression of the ribs, which lengthens and shortens the thoracic cavity.

1. Inspiration:

It is a process by which fresh air enters the lungs. The diaphragm, intercostal muscles and abdominal muscles play an important role.

(i) Diaphragm:

The diaphragm becomes flat and gets lowered by the contraction of its muscle fibres thereby increasing the volume of the thoracic cavity in length.

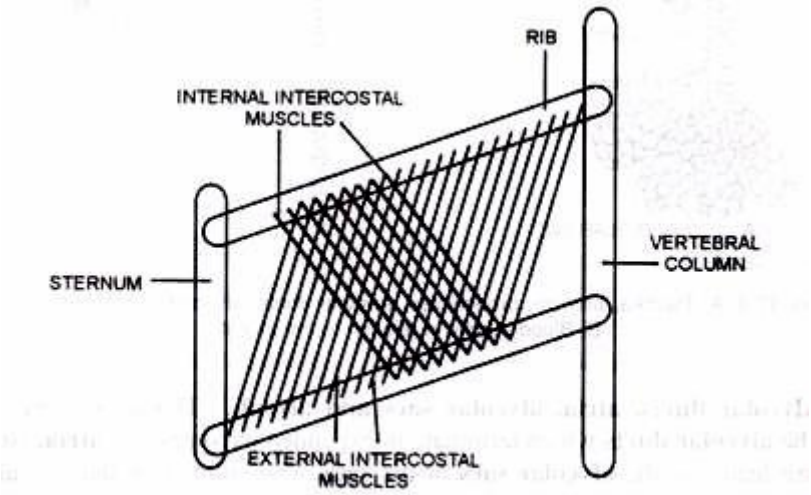


Fig. 17.5. Diagrammatic representation of the position of the intercostal muscles.

(ii) External intercostal muscles:

They occur between the ribs. These muscles contract and pull the ribs and sternum upward and outward thus increasing the volume of the thoracic cavity

(iii) Abdominal Muscles:

These muscles relax and allow compression of abdominal organs by the diaphragm. The abdominal muscles play a passive role in inspiration. The muscles of the diaphragm and external intercostal muscles are principal muscles of inspiration.

Movement of Fresh Air into the Lungs:

Thus overall volume of the thoracic cavity increases and as a result there is a decrease of the air pressure in the lungs. The greater pressure outside the body now causes air to flow rapidly into external nares (nostrils) and through nasal cavities into internal nares.

Thereafter the sequence of air flow is like this:

External nares → Nasal cavities → Internal nares → Pharynx → Glottis → Larynx → trachea → Bronchi → bronchioles → alveolar ducts → alveoli.

From the alveoli oxygen passes into the blood of the capillaries and carbon dioxide diffuses out from the blood to the lumen of the alveoli.

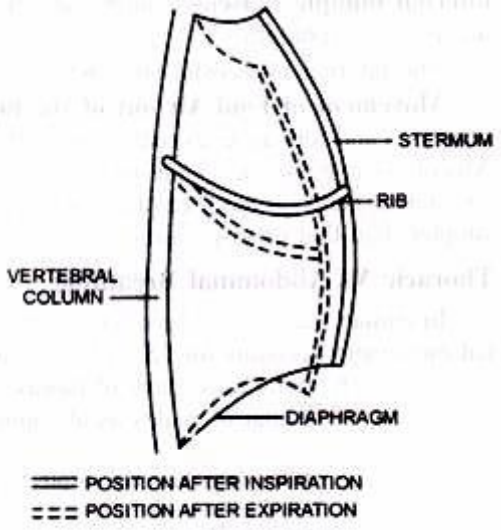


Fig. 17.6. Side view of thorax to show movements during breathing (only one rib shown).

2. Expiration:

It is a process by which the foul air (carbon dioxide) is expelled out from the lungs. Expiration is a passive process which occurs as follows.

(i) Diaphragm:

The muscle fibres of the diaphragm relax making it convex, decreasing volume of the thoracic cavity.

(ii) Internal Intercostal Muscles:

These muscles contract so that they pull the ribs downward and inward decreasing the size of me thoracic cavity.

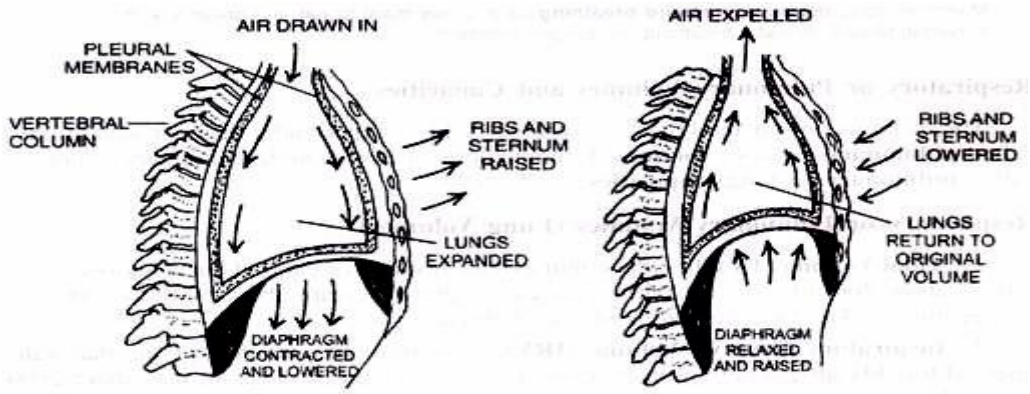


Fig. 17.7. Mechanism of preathing showing inspiration and expiration.

(iii) Abdominal Muscles:

Contraction of the abdominal muscles such as external and internal oblique muscles compresses the abdomen and pushes its contents (viscera) towards the diaphragm. The internal intercostal and abdominal muscles are muscles of expiration.

Movement of Foul Air out of the lungs:

Thus overall volume of the thoracic cavity decreases and foul air goes outside from the cavities of the alveoli in the following manner:

Alveoli → alveolar ducts → bronchioles → bronchi → trachea → larynx → glottis → pharynx → internal nares → nasal cavities → external nares → outside. The process of expiration is simpler than that of inspiration.

Thoracic Vs. Abdominal Breathing:

In human males, lateral movement of thorax constitutes 25% of breathing while abdominal movement accounts for 75% of breathing. In pregnant women, almost the entire breathing is through lateral movement of thorax. Therefore, breathing of women is often regarded as thoracic while that of males as abdominal.

Advantages of Nasal Breathing:

Breathing through nose is healthier because it is a natural process. The air which is inhaled contains dust, bacteria, etc., get filtered in the nose. Thus the air which goes into lungs is cleaner. The conchae of the nose also filter and warm up the air.

Respiratory or Pulmonary Volumes and Capacities:

The quantities of air the lungs can receive, hold or expel under different conditions are called pulmonary (= lung) volumes. Combinations of two or more pulmonary volumes are called pulmonary (= lung) capacities.

Respiratory or Pulmonary Volumes (Lung Volumes):

1. Tidal Volume (TV):

It is the volume of air inspired or expired during normal breath. This is about 500 mL, i.e., a healthy man can inspire or expire about 6000 to 8000 mL of air per minute. The lowest value is of tidal volume.

2. Inspiratory Reserve Volume (IRV):

It is the extra amount of air that can be inspired forcibly after a normal inspiration. Thus it is forced inspiration. It is about 2500 to 3000 ml. of air

3. Expiratory Reserve Volume (ERV):

It is the extra amount of air that can be expired forcibly after a normal expiration. Thus it is forced expiration. It is about 1000 to 1100 ml.

4. Residual Volume (RV):

It is the volume of air which remains still in the lung after the most forceful expiration. It is about 1100 mL to 1200 ml.

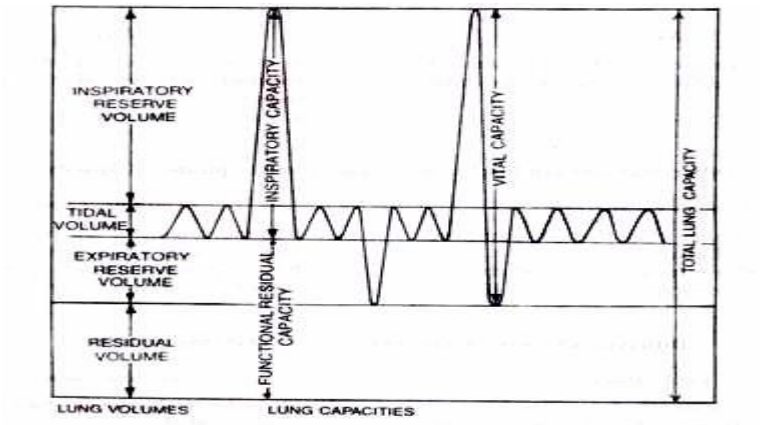


Fig. 17.8. Diagram showing Pulmonary volumes and Pulmonary capacities.

Respiratory or Pulmonary Capacities (Lung Capacities):

1. Inspiratory Capacity (IC):

It is the total volume of air a person can inspire after a normal expiration. It includes tidal volume and inspiratory reserve volume (TV + IRV).

2. Expiratory Capacity (EC):

It is the total volume of air a person can expire after a normal inspiration. This includes tidal volume and expiratory reserve volume (TV + ERV).

3. Functional Residual Capacity (FRC):

Volume of air that will remain in the lungs after a normal expiration is called functional residual capacity. This includes residual volume and the expiratory reserve volume (RV + ERV).

4. Vital Capacity (VC):

The maximum volume of air a person can breathe in after a forced expiration or the maximum volume of air a person can breathe out after a forced inspiration is called vital capacity. This includes tidal volume, inspiratory reserve volume and expiratory reserve volume (TV + IRV + ERV). In fact total lung capacity minus residual volume is called vital capacity. VC varies from 3400 mL to 4800 ml. depending upon age, sex and height of the individual. The vital capacity is higher in athletes, mountain dwellers than in plain dwellers, in men than women and in the young ones than in the old persons.

5. Total Lung Capacity (TLC):

It is the total volume of air present in the lungs and the respiratory passage after a maximum inspiration. It includes vital capacity and the residual volume (VC + RV). All pulmonary volumes and capacities are about 20 to 25 per cent less in women than in men and they are greater in tall persons and athletes than in small and asthenic (slight build) people.

Respiratory Quotient (RQ):

Respiratory quotient is the ratio of the volume of carbon dioxide produced to the volume or oxygen consumed over a period of time in respiration.

RQ = Volume of CO_2 evolved/Volume of O_2 absorbed

Respiratory quotient varies with different foods utilized in respiration. For glucose, RQ (RQ $6CO_2/6O_2$ – 1), for fats it is about 0.7, for proteins it is about 0.9 and for organic acids it is about 1.3 or 1.4. In anaerobic respiration, there is no consumption of oxygen. Carbon dioxide is produced in most of the cases. Therefore R.Q. is infinity. The respiratory quotient indicates the type of food oxidized in the body of the animal during respiration.

Table 17.1 : Partial Pressure of Respiratory Gases in mm Hg

Gas	Inspired air	Alveolar air	Deoxygenated blood	Oxygenated blood	Expired air	Tissues
Oxygen	159	104	40	95	116	40
Carbon dioxide	0.3	40	45	40	32	45

Exchange of Gases:

(A) Exchange of gases between alveoli and blood (Fig. 17.9 & 17.11):

The exchange of gases (i.e., oxygen and carbon dioxide) between lung alveoli and pulmonary capillaries is called external respiration

It occurs as follows:

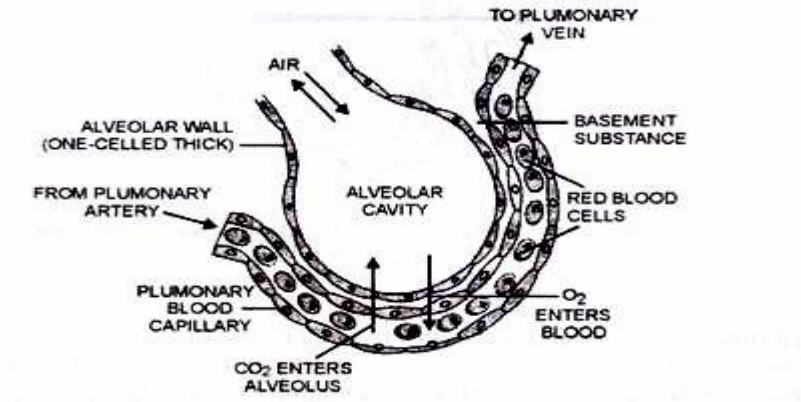


Fig. 17.9. A diagram of a section of an alveolus with a pulmonary blood capillary.

The wall of the alveoli is very thin and has rich network of blood capillaries. Due to this, the alveolar wall seems to be a sheet of flowing blood and is called respiratory membrane (= alveolar-capillary membrane).

The respiratory membrane (Fig. 17.10) consists mainly of:

(a) Alveolar epithelium,

(b) Epithelial basement membrane,

(c) A thin interstitial space

(d) Capillary basement membrane and

(e) Capillary endothelium.

All these layers form a membrane of 0.2 mm thickness. The respiratory membrane has a limit of gaseous exchange between alveoli and pulmonary blood. It is called diffusing capacity. The diffusing capacity is

defined as the volume of gas that diffuses through the membrane per minute for a pressure difference of 1 mm Hg. It is further dependent on the solubility of the diffusing gases. In other words at the particular pressure difference, the diffusion of carbon dioxide is 20 times faster than oxygen and that of oxygen is two times faster than nitrogen. The partial pressure of oxygen (PO_2) in the alveoli is higher (104 mm Hg) than that in the deoxygenated blood in the capillaries of the pulmonary arteries (95 mm Hg.). As the gases diffuse from a higher to a lower concentration, the movement of oxygen is from the alveoli to the blood. The reverse is the case in relation to carbon dioxide.

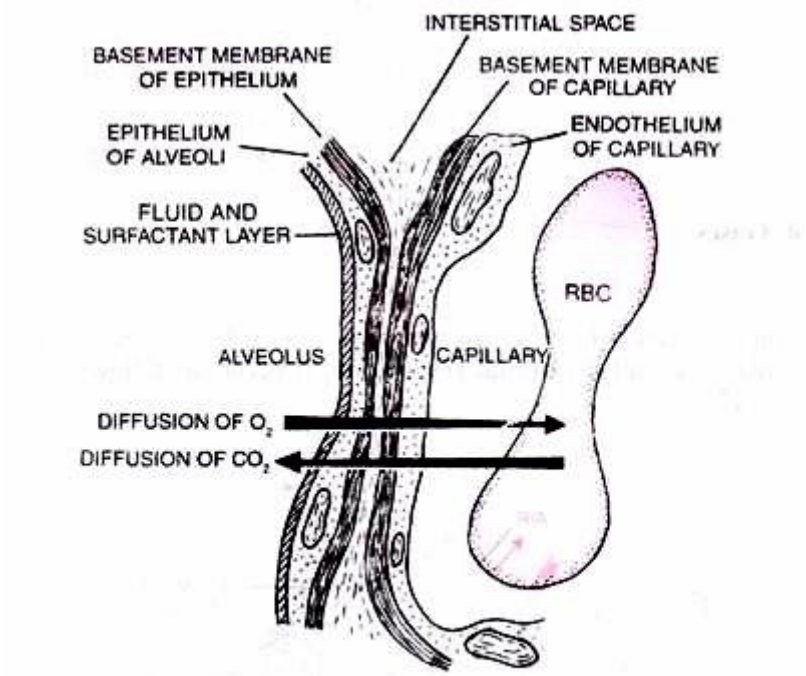


Fig. 17.10. Diagram showing Ultrastructure of Alveolar Respiratory membrane and Red blood corpuscle (RBC).

The partial pressure of carbon dioxide (PCO_2) is higher in deoxygenated blood (45 mm Hg) than in alveoli (40 mm Hg), therefore, carbon dioxide passes from the blood to the alveoli. The partial pressure of nitrogen (PN_2) is the same (537 mm Hg) in the alveoli as it is in the blood. This condition is maintained because nitrogen as a gas is not used up by the body.

(B) Exchange of gases between blood and tissue cells (Fig. 17.11):

The exchange of gases (i.e., oxygen and carbon dioxide) between tissue blood capillaries and tissue cells is called internal respiration. The partial pressure of oxygen is higher (95mm Hg) than that of the body cells (40 mm Hg) and the partial pressure of carbon dioxide is lesser (40 mm Hg) than that of the body cells (45 mm Hg).

Therefore, oxygen diffuses from the capillary blood to the body cells through tissue fluid and carbon dioxide diffuses from the body cells of the capillary blood via tissue fluid. Now the blood becomes deoxygenated. The latter is carried to the heart and hence to the lungs.

Transport of Gases (Fig. 17.11):

Blood transports oxygen and carbon dioxide.

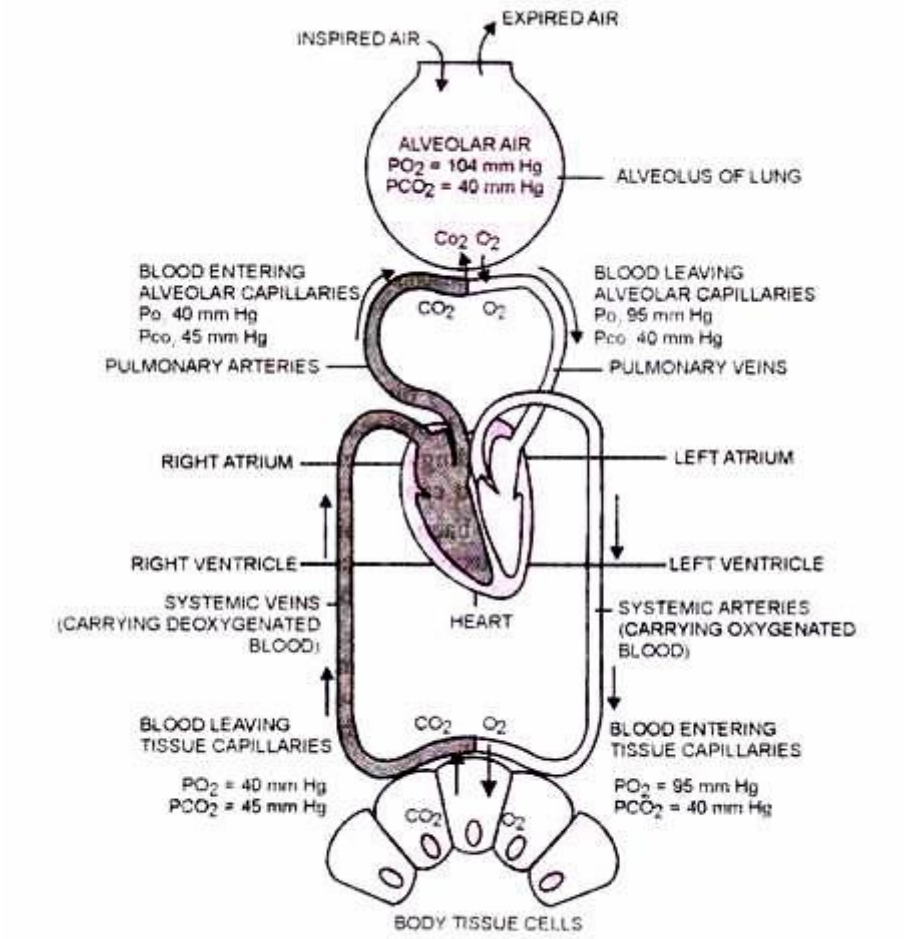


Fig. 17.11. Diagrammatic representation of exchange of gases (i) between the alveolus and pulmonary blood capillary and (ii) between the blood capillary and body tissue cells.

(A) Transport of Oxygen in the Blood:

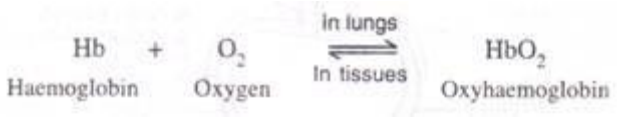
Blood carries oxygen from the lungs to the heart and from the heart to various body parts.

Oxygen is transported in the following manners:

(i) As dissolved gas. About 3 per cent of oxygen in the blood is dissolved in the plasma which carries oxygen to the body cells.

(ii) As oxyhaemoglobin. About 97% of oxygen is carried in combination with haemoglobin of the erythrocytes.

Haemoglobin (Hb) consists of a protein portion called globin and a pigment portion called heme. The heme portion contains four atoms of iron, each capable of combining with a molecule of oxygen. Four molecules of oxygen bind one molecule of haemoglobin. Oxygen and haemoglobin combine in an easily reversible reaction to form oxyhaemoglobin.



Under the high partial pressure, oxygen easily binds with haemoglobin in the pulmonary (lung) blood capillaries. When this oxygenated blood reaches the different tissues, the partial pressure of oxygen declines and the bonds holding oxygen to haemoglobin become unstable. As a result, oxygen is released from the blood capillaries.

A normal person has about 15 grams of haemoglobin per 100 ml of blood. 1 gram of haemoglobin binds about 1.34 ml of O_2. Thus on an average 100 ml of blood carries about 20 ml (19.4 ml exactly) of O_2 Hence under normal conditions, about 5 ml of oxygen is transported to tissues by 100 ml. of blood. During exercise or under strenuous conditions, the muscle cells consume more oxygen. The partial pressure of oxygen in the tissue falls, as a result of which, the blood at the tissue level has only 4.4 ml of oxygen/100 ml of blood. Thus about 15 ml. of oxygen is transported by haemoglobin during exercise.

Oxygen-haemoglobin Dissociation curve (=Oxygen Dissociation Curve):

The amount of oxygen that can bind with haemoglobin is determined by oxygen tension. This is expressed as a partial pressure of oxygen (PO_2). The percentage of haemoglobin that is bound with O_2 is called percentage saturation of haemoglobin.
The relationship between the partial pressure of oxygen (PO_2) and percentage saturation of the haemoglobin with oxygen (O_2) is graphically illustrated by a curve called oxygen haemoglobin dissociation curve (also called oxygen dissociation curve).

Normal Oxygen Haemoglobin Dissociation Curve:

Under normal conditions, the oxygen haemoglobin dissociation curve is sigmoid shaped or 'S' shaped (Fig. 17.12). The lower part of the curve indicates dissociation of oxygen from haemoglobin. The upper part of the curve indicates the acceptance of oxygen by haemoglobin. When the partial pressure of oxygen is 25 mm Hg the haemoglobin gets saturated to about 50%. It means the blood contains 50% oxygen. The partial

pressure at which the haemoglobin saturation is 50% is called P_{50}. At 40 mm Hg of partial pressure of oxygen, the saturation is 75%. It becomes 95% when the partial pressure of oxygen is 100 mm Hg.

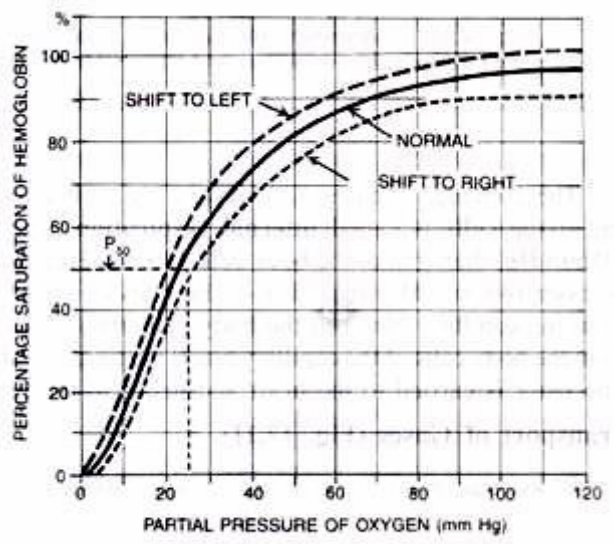


Fig. 17.12. Oxygen-haemoglobin dissociation curve .

Factors Affecting Oxygen Haemoglobin Dissociation Curve:

The oxygen haemoglobin dissociation curve is shifted either to right or left by various factors.

Shift to Right:

Shift to right indicates dissociation of oxygen from haemoglobin.

The oxygen-haemoglobin curve is shifted to right in the following conditions:

(1) Decrease in partial pressure of oxygen.

(2) Increase in partial pressure of carbon dioxide (Bohr effect).

(3) Increase in hydrogen ion concentration and decrease in pH (acidity).

(4) Increased body temperature.

(5) Excess of 2, 3 diphosphoglycerate (DPG). DPG is a by-product in glycolysis. It is present in RBCs.

Shift to Left:

Shift to left indicates acceptance (association) of oxygen by haemoglobin.

The oxygen haemoglobin dissociation curve is shifted to left in the following conditions:

(1) In the foetal blood, because, foetal haemoglobin has more affinity for oxygen than the adult haemoglobin.

(2) In the low temperature and high pH.

Bohr Effect:

An increase in carbon dioxide in the blood causes oxygen to be displaced from the haemoglobin. This is Bohr effect. This is an important factor increasing oxygen transport. It is named after the Danish physiologist Christian Bohr (1855-1911). The presence of carbon dioxide decreases the affinity of haemoglobin for oxygen and increases release of oxygen to the tissues. The pH of the blood falls as its CO_2 content increases so that when the PCO_2 rises the curve shifts to the right and the P_{50} rises. As stated in the oxygen haemoglobin dissociation curve, the partial pressure at which the haemoglobin saturation is 50% is called P_{50}.

Factors Influencing Bohr Effect:

All the factors, which shift the oxygen haemoglobin dissociation curve to the right (mentioned above) increase the Bohr effect.

(B) Transport of Carbon dioxide:

In the oxidation of food, carbon dioxide, water and energy are produced. Carbon dioxide in gaseous form diffuses out of the cells into the capillaries, where it is transported in three ways.

(i) Transport of CO_2 in Dissolved Form:

Because of its high solubility, about 7 percent carbon dioxide gets dissolved in the blood plasma and is carried in solution to the lungs. Thus as compared to O_2, a much larger volume of CO_2 is transported in dissolved form. This is about 7% of all the CO_2 transported by blood from tissues to the lungs.

(ii) Transport of CO_2 as Bio-carbonate:

The three main fractions are:

a. A small amount of carbonic acid.

b. The “carbamino-bound” CO_2 which is transported in combination with proteins (mainly hemoglobin).

c. That carried as bicarbonate in combination with cations of sodium or potassium.

The largest fraction of carbon dioxide (about 70%) is converted to bicarbonate ions (HCO_3^-) and transported in plasma. When carbon dioxide diffuses into the RBCs, it combines with water, forming carbonic acid (H_2CO_3). H_2CO_3 is unstable and quickly dissociates into bicarbonate ions and hydrogen ions:

$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \xrightleftharpoons[\text{anhydrase}]{\text{Carbonic}} \underset{\text{Carbonic acid}}{H_2CO_3} \xrightleftharpoons[\text{anhydrase}]{\text{Carbonic}} \underset{\text{Bicarbonate ion}}{HCO_3^-} + \underset{\text{Hydrogen ion}}{H^+}$$

Although this reaction also occurs in plasma, it is thousands of times faster in erythrocytes because they (and not plasma) contain carbonic anhydrase, an enzyme that reversibly catalyzes the conversion of carbon dioxide and water to carbonic acid. Hydrogen ions released during the reaction bind to hemoglobin, triggering the Bohr effect; thus, no oxygen release is enhanced by carbon dioxide loading (as HCO_3~). Because of the buffering effect of hemoglobin, the liberated hydrogen ions cause little change in pH under resting conditions. Hence, blood becomes only slightly more acidic (the pH declines from 7.4 to 7.34) as it passes through the tissues.

Chloride Shift (= Hamburger's Phenomenon):

Since the rise in the HCO_3^- content of red cells is much greater than that in plasma as the blood passes through the capillaries, about 70% of the HCO_3 formed in the red cells enters the plasma.

The excess HCO_3 leaves the red cells in exchange for Cl^- (Fig. 17.13). This exchange is called the chloride shift. Because of it, the Cl^- content of the red cells in venous blood is, therefore, significantly greater than in arterial blood. The chloride shift occurs rapidly and is essentially complete in 1 second. Consequently, the red cells take up water and increase in size.

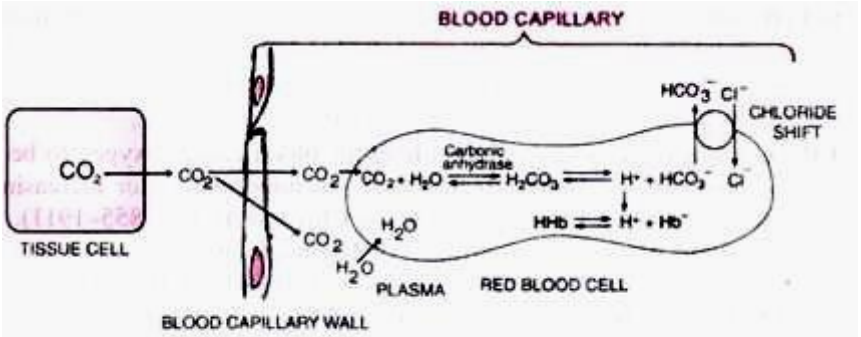


Fig. 17.13. Transport of CO_2 between tissue cell and tissue blood capillary. It is also showing chloride shift.

(iii) Transport of CO_2 as Carbaminohaemoglobin. About 23 per cent CO_2 is carried by haemoglobin as carbaminohaemoglobin. In addition to reacting with water, carbon dioxide also reacts directly with amine radicals (NH_2) of haemoglobin to form an unstable compound carbaminohaemoglobin (Hb CO_2). This is reversible reaction.

CO_2 + Hb (Haemoglobin) ⇋ $HbCO_2$ (Carbominohaemoglobin).
Every 100 mL of deoxygenated blood delivers approximately 4 mL of CO_2 to the alveoli.

Release of Carbon Dioxide in the Alveoli of Lung:

The pulmonary arteries carry deoxygenated blood to the lungs. This blood contains carbon dioxide as dissolved in blood plasma, as bicarbonate ions and as carbaminohaemoglobin.

(i) CO_2 is less soluble in arterial blood than in venous blood. Therefore, some CO_2 diffuses from the blood plasma of the lung capillaries into the lung alveoli.

(ii) For the release of CO_2 from the bio-carbonate, a series of reverse reactions takes place. When the haemoglobin of the lung blood capillaries takes up O_2, the H^+ is released from it.
Then, the Cl^- and HCO_3^- ions are released from KC1 in blood, and $NaHCO_3$ in the RBC respectively. After this HCO_3^- reacts with H^+ to form $H_2CO_3^-$ As a result H_2CO_3 splits into carbon dioxide and water in the presence of carbonic anhydrase enzyme and CO_2 is released into the alveoli of the lungs.

(iii) High PO_2 in the lung blood capillaries due to oxygenation of haemoglobin favours separation of CO_2 from carbaminohaemoglobin.

Haldane Effect:

It was proposed by J.S. Haldane, a Scotish physiologist, 1860-1936. Binding of oxygen with haemoglobin tends to displace carbon dioxide from the blood. This is called Haldane effect. It is far more important in promoting carbon dioxide than is the Bohr effect which promotes oxygen transport. The Haldane effect encourages CO_2 exchange in both the tissues and lungs.
It is quantitatively far more important in promoting CO_2 transport than the Bohr effect in promoting O_2 transport. Thus, Haldane effect and Bohr effect complement each other. In the tissues addition of CO_2 to the blood facilitates unloading of O_2 by Bohr effect. In turn, O_2 unloading favours uptake of CO_2 by Haldane effect.

Effect of CO_2 on Blood pH:

a. CO_2 evolved from the tissues forms carbonic acid. Most of the carbonic acid formed is promptly converted to bicarbonate as shown in the equation below (B^+ represents, principally, Na^+ or K^+).

$$\mathbf{H_2CO_3 \rightleftharpoons H^+ + HCO_3^- + B^+ \rightleftharpoons BHCO_3^- + H^+}$$

b. At the pH of blood (7.4), a ratio of 20: 1 must exist between the bicarbonate and carbonic acid. This ratio is calculated from the Henderson-Hasselbalch equation. Any change in H^+ activity is met by an adjust-

ment in the reaction. Any alteration in the ratio disturbs the acid-base balance of the blood in the direction of acidemia or alkalemia.

Cellular Respiration:

As the name indicates it occurs inside the cells. It takes place in all types of living cells. Respiratory substrates are those organic substances which can be catabolized to liberate energy inside the living cells. The most common respiratory substrate is glucose. Fats are used as respiratory substrates by a number of organisms because they contain more energy as compared to carbohydrates.

However, fats are not directly used in respiration. Instead they are first broken to intermediates common to glucose oxidation, viz., acetyl CoA, glyceraldehyde phosphate. Proteins are used rarely in respiration. Proteins are hydrolysed to form amino acids from which organic acids are produced through deamination. Organic acids enter Krebs cycle, e.g., aspartic acid, glutamic acid. At other times, proteins are employed as reparatory substrates under starvation conditions only when carbohydrates and fats become unavailable. As stated earlier respiration is of two main types: anaerobic and aerobic. In anaerobic respiration food is oxidised without using molecular oxygen. Less energy is produced in anaerobic respiration. In aerobic respiration organic food is completely oxidised with the help of oxygen into carbon dioxide and water. 686 Kcal of energy is also liberated per mole of glucose.

Aerobic respiration consists of four steps:

(i) Glycolysis:

It is a first step which is common to both anaerobic and aerobic modes of respiration. It occurs in cytoplasm and does not require oxygen. Glycolysis consumes ATP molecules. No carbon dioxide is released in glycolysis. Water and ATP molecules are released,

(ii) Krebs Cycle:

It is the second step in respiration. It operates inside mitochondria and uses oxygen and, therefore, occurs only in aerobic respiration. It does not consume ATP but liberates ATP molecules. Water and carbon dioxide are produced during Krebs cycle,

(iii) Electron Transport Chain (ETC):

It is a series of coenzymes and cytochromes that take part in the passage of electrons from a chemical to its ultimate acceptor. The enzymes involved in electron transport chain are components of the inner mitochondrial membrane. Thus ETC occurs in mitochondria. Oxygen is the ultimate acceptor of electrons.

It becomes reactive and combines with protons to form metabolic water $2H^+ + O^{2-} \rightarrow 2H_2O$]. (iv) Oxidative Phosphorylation. It is the synthesis of energy rich ATP molecules with the help of energy liberated during

oxidation of reduced co-enzymes (NADH, $FADH_2$) produced in respiration. The enzyme required for this synthesis is called ATP synthase. ATP synthase is located in F_1 or head piece of F_1 or elementary particles. The particles are present in the inner mitochondrial membrane. The net gain from complete oxidation of a molecule of glucose in muscle and nerve cells is 36 ATP molecules. However, in aerobic prokaryotes, heart, liver and kidneys, 38 ATP molecules are produced per glucose molecule oxidised.

ENVIRONMENTAL INFLUENCES

a. Availability of oxygen:

Insufficient oxygen in atmosphere (in high altitude and polluted area), causes inadequate oxygenation of blood in the lungs, leads to **Hypoxia**. In this condition the oxygen binding capacity of hemoglobin is influenced by the partial pressure of oxygen in the environment. The partial pressure of oxygen falls to 60 mmHg or below. causes problems with blood flow in the tissues, leads to breathing problem.

The respiratory function of hemocyanin containing blood of Libiniaemarginataand Ocypodeguadratawas studied, exposed to hypoxic condition. During progressive hypoxia convection initially increases on both sides of the gill in *L.emarginata* , while in *O.quadrata* cardiac output decreases. Blood pH increases with decreasing ambient Po_2 below 60 torr in *L. emarginata* inducing a greater hemocyanin O_2 affinity.

b. CO Poisoning:

CO binds with Hb hundred times tighter than oxygen and disrupts the oxygen carrying capacity of blood. The ranges of CO –4-6ppm at a resting level.

In urban areas 7-13ppm and for smokers-20-40ppm. The level of 40ppm is equivalent to a reduction in hemoglobin levels of 10 g/L. It removes the allosteric shift of the oxygen dissociation curve and shifting the foot of the curve to the left. So the Hb is less likely to release its oxygens at the tissues. So the poor supply of oxygen occurs.

c. Higher concentration of CO_2:

In polluted areas, there are excess carbon dioxide. Increase in carbon di oxide, increases alveolar Pco_2, rises about 60 to 75 mmHg. Due to greater binding affinity of carbon dioxaide with Hb leads to rapid and deep breathing, called air hunger leads to dyspnea.

If partial pressure of carbondioxaide increases 80-100 mmHg, the person becomes lethargic and even semi comatose. Above 120 mmHg rising partial pressure depresses the respiration and causes respiratory death.

d. Temperature:

Decreased temperature causes the oxygen hemoglobin dissociation curve shift to leftward, tends to lower oxygen supply in the tissues. Exposure of slightly increasing temperature on hermit crab associated problem of dehydration. Changes in enthalpy (>39 kj/mol) changes the hemocyanin concentration, which affect the oxygen affinity.

e. Other factors:

Some inflammatory agents (air born viruses, bacteria, moulds) infects the membrane of pulmonary and walls of alveoli, raptures the red blood cells to leak out of blood into the alveoli leads to infection spreads. This infection causes reduction in the area of respiratory membrane and decreased ventilation perfusion ratio. The both causes low blood oxygen and high concentration of carbon dioxide, which causes respiratory problems.

Artificial Respiration:

Conditions when artificial respiration is required. It is required when persons have stopped breathing because of (i) drowning, (ii) electric shock, (iii) accidents, (iv) gas poisoning, or (v) anesthesia.

Methods of Artificial Respiration:

Two methods of artificial respiration are (i) manual methods and (ii) mechanical methods.

1. Manual Method:

Manual method of artificial respiration can be applied quickly without waiting for the availability of any mechanical aids. The mouth to mouth breathing is very common.

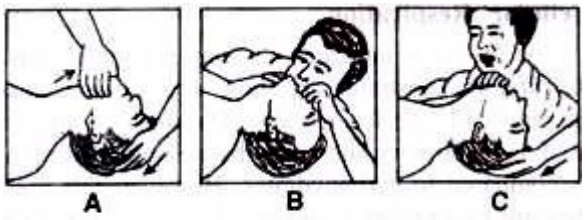


Fig. 17.14. Showing artificial respiration (mouth to mouth breathing).

2. Mechanical Methods:

During the respiratory failure due to paralysis of respiratory muscles or some other cause, the manual method of respiration is not useful because in these conditions, the resuscitation should be given for a longer period.

This can be done only by means of mechanical methods which are of two types:

(i) Drinker's method:

The machine used in this method is called iron lung or Drinker's respiration or tank respiration invented by Philips Drinker in 1929. By using the tank respirator, the patient can survive for a longer time, even up to the period of one year till the natural respiratory functions are restored

(ii) Ventilation Method:

A rubber tube is introduced into the trachea of the patient through the mouth. When air is pumped, inflation of lungs occurs, when it is stopped expiration occurs, and the cycle is repeated. The apparatus used for this is called ventilator.

Exercise and Respiration:

On the basis of severity, the exercise is classified into three types:

1. Severe Exercise:

It includes strenuous muscular activity but the severity can be maintained only for short duration. Fast running for a distance of 100 or 4(X) metres is the best example of this type of exercise. Complete exhaustion occurs at the end of severe exercise.

2. Moderate Exercise:

This type of exercise can be performed for a longer period. The examples of this type of exercise are fast walking and slow running. Exhaustion does not occur at the end of moderate exercise.

3. Mild Exercise:

This is very simple form of exercise like slow walking. So exhaustion does not occur at the end of mild exercise. After a period of severe muscular exercise the amount of oxygen consumed is enormously more. The oxygen required is more than quantity available to muscles.

This much of oxygen is utilized for reversal of the following metabolic processes:

(i) Reformation of glucose from lactic acid accumulated during exercise,

(ii) Re-synthesis of ATP and creatine phosphate, and

(iii) Restoration of amount of oxygen dissociated from haemoglobin and myoglobin.

Regulation of Respiration (= Regulation of Breathing):

Respiration is under both nervous and chemical regulation.

1. Neural Regulation:

Normal quiet breathing occurs involuntarily. Adult human beings breathe 12 to 14 times per minute, but human infants breathe about 44 times per minute. In each breathe in human beings, inspiration accounts for about two and expiration for about three seconds.

The respiratory centre is composed of groups of neurons located in the medulla oblongata and pons varolii. Hence respiratory centre is divided into the medullary respiratory centres and pons respiratory centres.

Medullary Respiratory Centres:

(i) Dorsal Respiratory Group (DRG):

It is located in dorsal portion of the medulla oblongata. The dorsal respiratory group mainly causes inspiration.

(ii) Ventral Respiratory Group (VRG):

It is located in the ventrolateral part of the medulla oblongata. The ventral respiratory group can cause either inspiration or expiration, depending upon which neurons in the group are stimulated.

Pons Respiratory Centres:

(i) Pneumotaxic centre:

It is located in the dorsal part of pons varolii. The function of the pneumotaxic centre is primarily to limit inspiration.

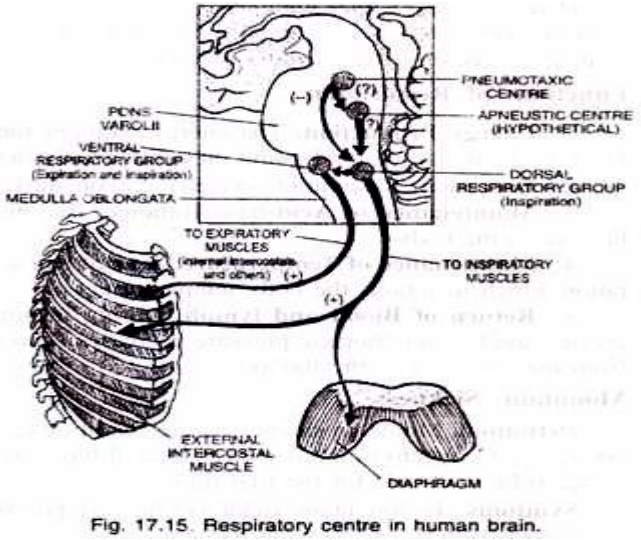


Fig. 17.15. Respiratory centre in human brain.

(ii) Apneustic Centre:

There is another strange centre called the apneustic centre, located in the lower part of the pons varolii. The function of this centre is not well understood but it is thought that it operates in association with the pneumotaxic centre to control the depth of inspiration. Apneustic centre is considered hypothetical.

2. Chemical Regulation:

The largest number of chemoreceptors is located in the carotid bodies. However, a sizeable number of chemoreceptors are in the aortic bodies. The carotid bodies are located bilaterally in the bifurcation of the common carotid arteries and their afferent nerve fibres pass through glossopharyngeal cranial nerves and hence to the dorsal respiratory area of the medulla oblongata. The aortic bodies are located along the arch of the aorta and their afferent nerve fibres pass through the vagi (sing, vagus), cranial nerves and hence to the dorsal respiratory area.

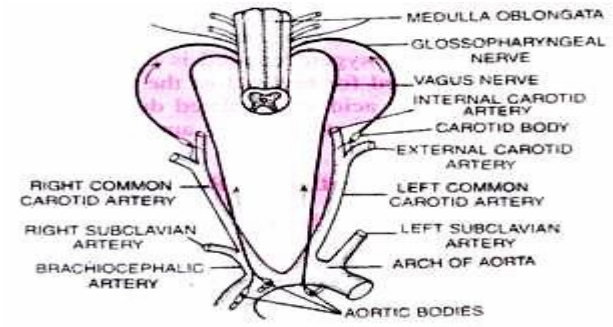


Fig. 17.16. Respiratory regulation by the carotid and aortic bodies.

Excess carbon dioxide or hydrogen ions mainly stimulate the respiratory centre of the brain and increase the inspiratory and expiratory signals to the respiratory muscles. Increased CO_2 lowers the pH resulting acidosis. However, oxygen does not have a significant direct effect on the respiratory centre of the brain

in controlling respiration. Thus carotid and aortic bodies send chemical signals to the respiratory centre in the medulla oblongata.

Functions of Respiration:

1. Energy Production:

The energy required for daily metabolic activities is derived from the oxidation of food going on continuously in the body.

2. Excretion:

Respiration excretes carbon dioxide, water, etc.

3. Maintenance of Acid-base Balance:

Elimination of CO_2 maintains the acid-base balance in the body.

4. Maintenance of Temperature:

A large amount of heat is expelled out during expiration which maintains the body temperature.

5. Return of Blood and lymph:

During inspiration the intra-abdominal pressure increases and the intrathoracic pressure decreases. This results the return of blood and lymph from the abdomen to the thorax.

Functions of respiratory system can be broadly classified into:

a. Respiratory

b. Non-respiratory.

a. Respiratory Function:

It is to provide adequate volume of oxygen to the tissues. Normal person at rest needs about 250 ml of oxygen per minute. Atmospheric air enters the lungs during inspiration. Oxygen from the air diffuses through the alveoli into pulmonary capillary blood. The oxygenated blood reaches the left ventricle and from there it gets pumped to reach all parts of the body. About 200 ml of carbon dioxide is produced in the body every minute because of tissue metabolism. From the tissues, carbon dioxide enters the blood to reach the lungs for the process of excretion. When deoxygenated blood reaches the lungs, carbon dioxide gets diffused from the pulmonary capillaries into the alveoli. The air from the alveoli is expelled out from the lungs by the process of expiration.

b. Non-Respiratory Functions:

1. Regulation of acid-base (pH) balance.

2. Mast cells present in the lungs produce heparin, which acts as an anticoagulant.

3. Macrophages in the alveoli have phagocytic function.

4. Converting enzyme present in the lungs play a role in converting angiotensin I to angiotensin II, which is a powerful vasoconstrictor.

5. The passage of the air through the larynx is essential for vocalization and has role in communication by speech.

6. Plays a minor role in body temperature regulation.

Mountain Sickness:

Mountain sickness is the condition characterized by the ill effect of hypoxia (shortage of oxygen) in the tissues at high altitude. This is commonly developed in persons going to high altitude for the first time.

Symptoms: In mountain sickness, the symptoms occur mostly in digestive system, respiratory system and nervous system.

1. Digestive System:

Loss of appetite, nausea and vomiting occur because of expansion of gases in the gastrointestinal tract.

2. Respiratory System:

Breathlessness occurs because of pulmonary oedema. Pulmonary oedema develops because of the response of the pulmonary blood vessels to hypoxia.

3. Neural System:

The symptoms are headache, depression, disorientation, irritability, lack of sleep, weakness and fatigue. These symptoms are developed because of cerebral oedema.

Treatment: The symptoms of mountain sickness disappear by breathing oxygen.

Probable Questions-

1. Explain briefly how oxygen binds with hemoglobijn and transported to the tissues.

2. What is oxygen-hemoglobin dissociation curve? What will happen to the curve if the pH of blood is increased temperature is increased? Justify your answer.

3. What is Bohr's effect? How is the concentration of carbon dioxide related to this phenomenon?

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.

2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.

3. Ganong's review of medical physiology.

UNIT-III

Physiology of excretion: Formation of urine: Physiology of ultrafiltration, Reabsorption tubular secretion Counter current theory of urine concentration, regulation of urine formation, renal regulation of acid-base balance

Objective: In this unit we will learn about physiology of excretion. How urine is formed through several steps in the nephron and how urine formation is regulated in different physiological conditions. We will also learn about how acid-base balance is regulated.

Definition of Excretion:

It is the elimination of metabolic waste products from the animal body to regulate the composition of the body fluids and tissues. The terms excretion and defecation should not be confused. Defecation is the removal of wastes and undigested food, collectively called faeces, through the anus.

Modes of Excretion:

Depending upon the excretory product, animals show five types of nitrogenous excretion in which ammonotelism, ureotelism and uricotelism are major types and aminotelism and guanotelism are minor types. Nitrogenous waste substances such as ammonia, urea or uric acid are produced during protein metabolism according to the species.

Small amount of nitrogenous waste substances are also produced during the metabolism of nucleic acids. Ammonia is the most toxic, followed by urea and uric acid. The latter is the least toxic.

1. Ammonotelism:

Many aquatic animals like protozoans, (e.g., Amoeba, Paramecium), sponges (e.g., Sycon), cnidarians or coelenterates (e.g., Hydra), liver fluke, tape worms, Ascaris, Nereis, Earthworm, Leech, most aquatic arthropods (e.g., Prawn), most aquatic molluscs (e.g., Pila) bony fishes (e.g., Labeo), Amphibian tadpole (e.g., tadpole of frog), tailed amphibians (e.g., Salamanders), and crocodiles excrete ammonia. Animals which excrete ammonia are called ammonotelic and excretion of ammonia is termed as the ammonotelism.

2. Ureotelism:

Excretion of urea is known as ureotelism and the animals which excrete urea are called ureotelic. Ureotelic animals include Ascaris, earthworm (both are ammonotelic and ureotelic), cartilaginous fishes like sharks and sting rays, semi-aquatic amphibians such as frogs and toads, aquatic or semi aquatic reptiles like turtles, terrapins and alligators, and man and all other mammals. Urea is less toxic and less soluble in water than ammonia. Hence, it can stay for some time in the body. Sharks retain large quantity of urea in their blood,

therefore, blood osmotic pressure approaches that of sea water, which minimizes water loss from their body.

3. Uricotelism:

Excretion of uric acid is known as uricotelism and the animals which excrete uric acid are called uricotelic. Animals which live in dry conditions have to conserve water in their bodies. Therefore, they synthesize crystals of uric acid from their ammonia. Uric acid crystals are non-toxic and almost insoluble in water.

Hence, these can be retained in the body for a considerable time. Uricotelic animals include most insects, (e.g., cockroach) some land crustaceans (e.g., Oniscus commonly known as "wood louse"), land snails (e.g., Helix commonly known as "land snail"), land reptiles (e.g., lizards and snakes) and birds. The concentration of uric acid is so high in guano (waste matter dropped by sea birds, used as fertilizer) that uric acid is commercially extracted from guano which is collected from uninhabited marine or littoral (part of country which is along the coast) islands. Primates including man also excrete some uric acid which is formed in their body by the breakdown of nucleic acids.

4. Aminotelism:

Certain invertebrates like some molluscs (Unio, Limnaea, etc.) and some echinoderms (e.g., Asterias) excrete excess amino acids as such. These animals are called aminotelic and their mode of excretion is called aminotelism.

5. Guanotelism:

Spiders excrete guanine and are said to be guanotelic and their mode of excretion is called guanotelism.

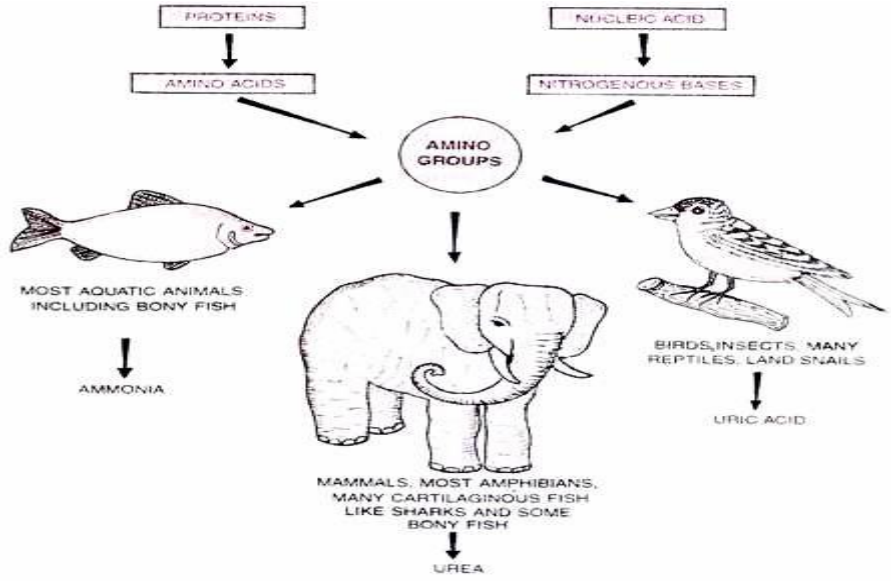


Fig. 19.2. Major nitrogenous wastes of different animal groups.

Dual Excretion:

Some animals perform two modes of excretion. That is called dual excretion. Some important examples of dual excretion are mentioned here. Earthworms excrete ammonia when sufficient water is available while they excrete urea instead of ammonia in drier surroundings.

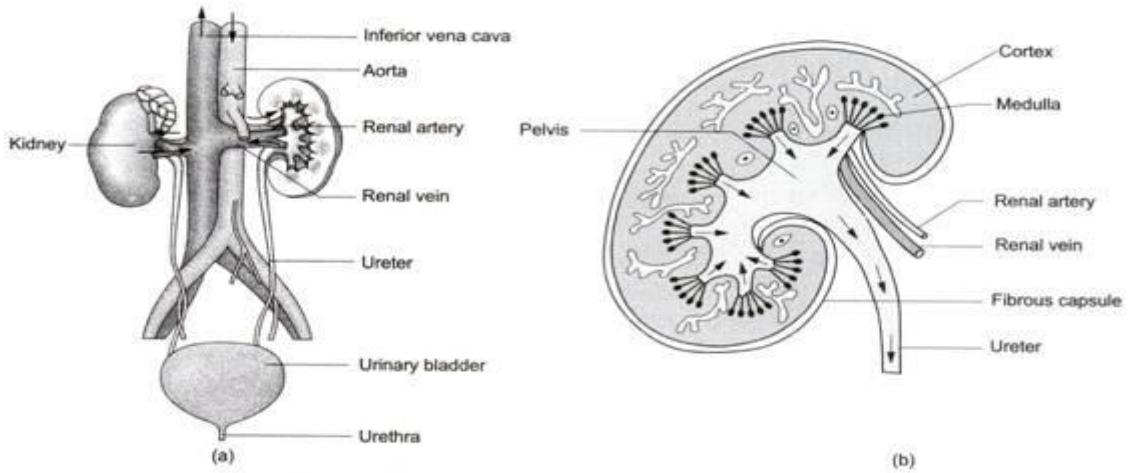
When lung fishes and Xenopus (African toad) live in water they are normally ammonotelic but they become ureotelic when they lie immobile in moist air or mud during summer. Amphibian tadpoles (larvae) are aquatic and ammonotelic but they become ureotelic during their metamorphosis. Crocodiles spend most of their time in water and creases. Although man is ureotelic yet he excretes a small amount of uric acid in his urine. But it is too little amount as compared to total urinary nitrogen. However, in some patients the concentration of uric acid is raised in their body fluids and subsequently uric acid is deposited in joints, cartilages and kidneys causing gout and kidney failure. In gouty arthritis, crystals of uric acid are deposited in the joints causing a severe pain.

Excretory System in Man:

Our excretory system consists of kidneys, blood vessels that join them, ureters, urinary bladder and urethra. They help produce and excrete urine.

There are two bean-shaped kidneys that lie in the abdominal cavity, one on either side of the vertebral column. The kidneys are reddish brown. Each of them is about 10 cm long and weighs about 150 g. Although they weigh less, they receive a lot of blood for filtration.

A volume of blood nearly equivalent to that in the whole body passes through the kidneys every four or five minutes. The kidneys produce urine to filter out the waste products, like urea and uric acid, from the blood.



(a) Excretory organs of man (b) Internal structure of a kidney

Urine leaves each kidney through a tube called ureters. The ureters from both the kidneys are corrected to the urinary bladder that collects and stores urine. Ureters carry urine from the kidneys into the urinary bladder. The urethra is a canal that carries urine from the bladder and expels it outside the body.

Internal Structure of a Kidney:

Each kidney is enclosed in a thin, fibrous covering called the capsule. A renal artery brings blood into the kidney, along with nitrogenous waste materials. After filtration in the kidney, the purified blood leaves the kidney through a renal vein.

Two distinct regions can be seen in the section of a kidney:

(1) An outer, dark, granular cortex and (2) an inner, lighter medulla. The hollow space from where the ureter leaves the kidney is called the pelvis. Each kidney is made up of numerous (about one million) coiled excretory tubules, known as nephrons, and collecting ducts associated with tiny blood vessels. A nephron is the structural and functional unit of a kidney, having three functions— filtration, reabsorption and secretion.

A cluster of thin-walled blood capillaries remains associated with the cup-shaped end of each nephron tubule. These capillaries bring blood from the body to the nephron for filtration. The network of capillaries spreads over the nephron tubules also. These capillaries finally carry purified blood to the body.

Structure and Function of a Nephron:

A nephron consists of a long coiled tubule and the Malpighian corpuscle. The tubule of the nephron is differentiated into the proximal convoluted tubule, Henle's loop and the distal convoluted tubule. The distal tubule opens into the collecting duct.

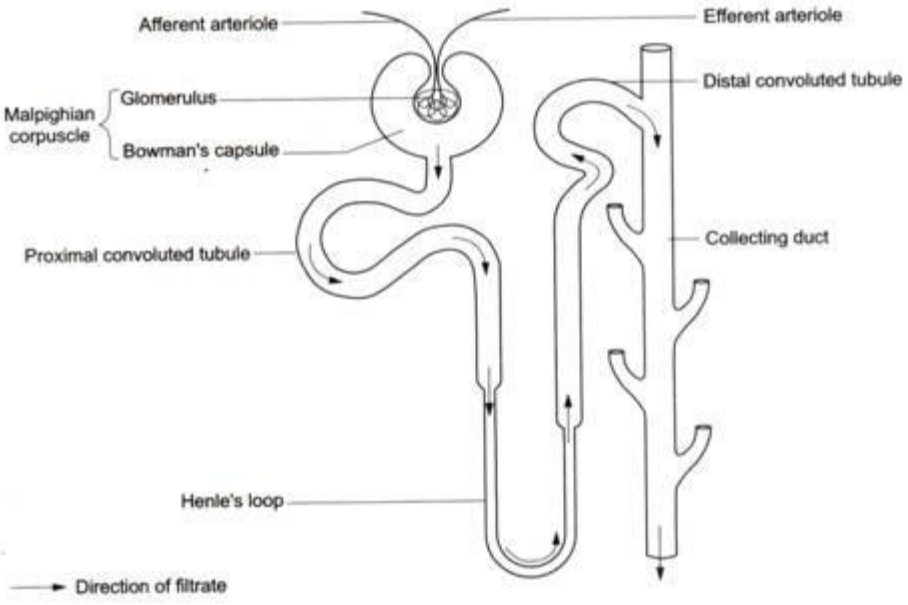
At the proximal end of the nephron is the Malpighian corpuscle, which consists of Bowman's capsule and the glomerulus. Bowman's capsule is a double-walled cuplike structure which surrounds the dense network of blood capillaries called the glomerulus. It is a functional basic unit of kidney. Each kidney is provided with about one million nephrons containing the glomerulus and the tubule. The glomerulus is a network of afferent and efferent capillaries.

Each glomerulus is surrounded by a double-walled epithelial sac known as Bowman 's Capsule which leads to the tubule which is divided into three parts—proximal convoluted tubule, loop of Henle, and the distal convoluted tubule. The Proximal Convoluted Tubule (PCT) is about 45 mm long and 50 mm in diameter. This lies in the cortex along with glomerulus. Its lumen is continuous with that of the Bowman's Capsule. It consists of cells with scalloped outline and brush border. The brush border is formed by numerous microvilli which increases the surface enormously for absorption.

The loop of Henle consists of three parts—the descending limb, a thin segment, and an ascending limb. The proximal convoluted tubule opens into the descending limb which is continued into the thin segment from where the ascending limb arises. The whole loop of Henle is lined by a single layer of flattened epithelial cells.

The ascending limb of the loop of Henle continues into the distal convoluted tubule (DCT) which finally opens into a collecting tubule or duct which carries the urine to the renal pelvis from where it is carried to the bladder by the ureter. The distal convoluted tubule commences near the pole of the glomerulus and establishes a close proximity to the afferent arteriole of its parent glomerulus. The DCT contains cuboidal epithelium.

Nephrons are mainly of two types—cortical and juxtamedullary. The loop of Henle of the juxtamedullary is long and dips deep into the substance of the medulla. But the loop of Henle of cortical is short and only a very small part of it dips into the medullary tissue and the greater part remains embedded in the cortical substances. Moreover, the glomeruli of the juxtamedullary lie very close to the medulla while those of cortical lie close to the surface of the kidney. The juxtamedullary nephrons constitute 20 per cent of nephrons, while the cortical nephrons constitute 80 per cent of the total nephrons. These two types of nephrons have the same common function.



Different parts of a nephron

Blood Supply of the Kidneys:

The short renal artery arising from the abdominal aorta supplies the blood to the kidney. The renal artery after entering the kidney divides into a number of arterioles—the afferent arterioles which further branch into capillaries and enter into each glomerulus.

The capillaries then join to form another arteriole—the efferent arteriole which opens into another set of capillaries called peritubular capillaries surrounding the proximal tubule, the loop of Henle, and the distal tubule. Ultimately, the capillary set opens into a venule which joins with other venules to form the renal vein. The renal vein then opens into the inferior vena cava.

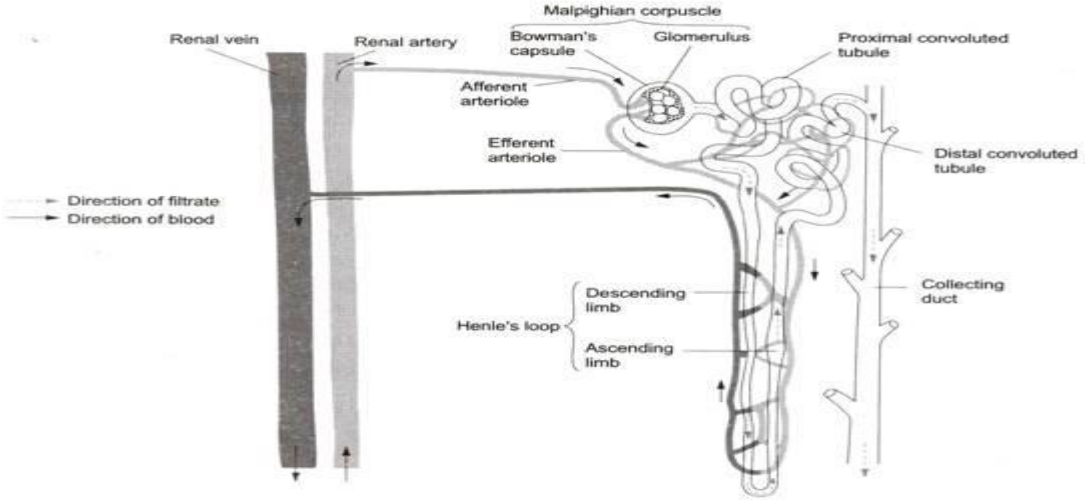
Blood Flow to Kidney through the Nephron:

The blood flows through both the kidneys of an adult weighing 70 kg at the rate of about 1200 ml/mt. The portion of the total cardiac output (about 560 ml/ mt.) which passes through the kidneys is called the renal fraction. This is about 560/1200 ml per minute, i.e., about 21 per cent.

There are two sets of capillaries—the glomerulus and the peritubular. These two capillaries are separated from each other by the efferent arteriole which contributes sufficient resistance to blood flow. The glomerular capillary bed provides a high pressure of about 70 mm Hg, while the peritubular bed provides a low pressure about 13 mm Hg. The pressures in the artery and vein are 100 mm of Hg. and 8 mm of Hg respectively. The high pressure in the glomerulus exerts the filtering of fluids continually into the Bowman's Capsule. The low pressure in the peritubular capillary system, on the other hand, functions in the same way as the usual venous ends of the tissue capillaries with the fluid being absorbed continually into the capillaries.

Control of excretion:

The urine that is formed continually collects in the urinary bladder. As the bladder expands, its pressure creates an urge to pass urine through the urethra. As the bladder is muscular, the urge to urinate is under voluntary nervous control.



Blood supply to a nephron

Urine Formation in Kidney:

The regulatory activities of kidneys form urine as a by-product. Urine formation involves three main steps—the glomerular filtration, the tubular reabsorption, and the tubular secretion.

a. Glomerular Filtration (Ultrafiltration):

Glomerulus filters out substances of low molecular weight from the blood with the retention of substances of high molecular weight, especially the proteins. Therefore, proteins are retained in the glomeruli and are not normally found in urine. If protein is detected in the urine, it indicates the kidney damage or other disease which effect the glomerular membrane.

In normal adult, two million nephrons filter one litre of blood each minute to give about 1200 ml of glomerular filtrate (primary urine) at Bowman's Capsule. Therefore, the Glomerular Filtration Rate (GFR) in adult is about 120 ml per minute. The hydrostatic pressure of the blood in the glomerular capillaires (P_g) is the main force for driving the fluid (Water and solute) out of the glomerulus.

The pressure is opposed by two forces:

(i) The hydrostatic pressure of the Bowman's Capsule fluid (P_{BC}).

(ii) The osmotic pressure of the plasma proteins (P_{pp}).

Therefore, the effective filtration pressure (P_{ef}) is calculated by the following relation:

$$P_{ef} = P_g - (P_{PP} + P_{BC})$$

$$\therefore P_{ef} = 74 - (30 + 20) \text{ mm of Hg}$$

$$\therefore P_{ef} = 24 \text{ mm of Hg.}$$

Thus, by substituting the normal values of the various forces, it has been found that the calculated effective (net) filtration pressure (P_{ef}) is 24 mm Hg.

A fall in blood pressure may reduce the P_{ef} which results in less amount of urine. When the aortic systolic pressure is reduced to 70 mm Hg, the hydrostatic pressure of the blood in glomerular capillaries is reduced to 50 mm. Hg. This reduces the P_{ef} to Zero [50 – 50] and thus filtration will be ceased. Under such circumstances, urine will not be formed (anuria) until the blood pressure is maintained.

b. Tubular Reabsorption:

The rate of formation of the primary urine is 120 ml/minute, while the rate of urine passing to the bladder under the same condition is 1-2 ml/ minute. Therefore, it indicates that about 99 per cent of the glomerular filtrate is reabsorbed during its passage through the different segments of the renal tubule. Although, the glomerular filtrate contains nearly the same concentration of glucose as in plasma, the urine contains nil or very little glucose. Hence, glucose is also practically completely reabsorbed in the tubules when the blood sugar level is normal. The capacity of reabsorption depends on the renal threshold of that substance.

The reabsorption of different solids takes place at different sites in the renal tubules. Amino acids, glucose, and small amounts of protein that pass through the glomerulus are reabsorbed in the first part of the proximal tubule. Sodium, chloride, and bicarbonate are reabsorbed uniformly along the entire length of the proximal tubule and also in the distal tubule. Potassium is reabsorbed in the proximal and secreted in the distal tubule.

The glomerular filtrate produces about 170 litres in a day; whereas the tubules reabsorb about 168.5 litres of water, 170 gm of glucose, 100 gm of NaCl, 360 gm of $NaHCO_3$, and small amounts of phosphate, sulphate, amino acids, urea, uric acid, etc. and excrete about 60 gm of NaCl, urea and other waste products in about 1.5 litres of urine. Most of these solids are reabsorbed by active transport mechanism, while some (e.g., urea) are reabsorbed by passive transport mechanism. In diseases, the reabsorption mechanism is altered developing glycosuria, phosphaturia, and amino aciduria.

c. Tubular Secretion:

Although, most of the substances are reabsorbed by the tubular cells, some substances are actively transported or actively excreted into the tubular lumen. The secreted substance by the tubular epithelium in man are creatinine and potassium. The tubular epithelium also removes a number of foreign substances that are introduced into the body for therapeutic and diagnostic purposes. These foreign substances are penicillin, p-Aminosalicylic acid, phenosulphonphthalein (PSP), p-Aminohippuric acid, and diodrast. The hydrogen ions and ammonia formed in the distal tubular cells are also actively excreted into tubular lumen and thus pass to urine.

Hormonal regulation:

The function of kidney is regulated by three important hormones. These hormones are aldosterone (from adrenal cortex), parathormone (from parathyroid), and vasopressin (from hypophyseal posterior lobe). Aldosterone restricts the excretion of Na^+ and stimulates the excretion of K^+. Parathormone stimulates excretion of phosphate. Vasopressin, the antidiuretic hormone, is held responsible mainly for the reabsorption of water. In the absence of this hormone, a large amount of very dilute urine is excreted

Hormones of the Kidney:

a. Not only the kidney performs excretory functions but it acts as an endocrine organ. It liberates many hormones which affect other organs and tissues and some hormones which locally act within the kidney itself. It also destroys several hormones which are liberated from other endocrine organs.

b. The juxtaglomerular cells of the renal cortex produce the proteolytic enzyme rennin and secrete it into the blood. Rennin acts on a_2-globulin which is normally present in blood plasma, although it is produced in the liver.

Rennin splits off a polypeptide fragment called angiotensin I which is decapeptide containing 10 amino acids. Another enzyme of the lung acts on angiotensin I to split off 2 amino acids and thus form the octapeptide angiotensin II. Angiotensin increases the force of the heartbeat and constricts the arterioles. It raises blood pressure and causes contraction of smooth muscle. It is destroyed by the enzyme angiotensinases present in normal kidneys, plasma and other tissues. Recent studies suggest that rennin angiotensin system is important in the maintenance of normal blood pressure.

c. Prostaglandins are the other hormones of the kidney. They cause relaxation of smooth muscles. They cause vasodilatation and a decrease in blood pressure. They also increase renal blood flow. Kininogen which is produced by the kidney has an antihypertensive effect.

d. The two hormones erythropoietin and erythrogenin have an effect on bone marrow to stimulate production of red cells. Kidney plays an important role in the release of erythropoietin and thus in control of red cell production. Hypoxia stimulates production of erythropoietin.

Hormonal regulation of tubular reabsorption and tubular section

Four hormones affect the extent of sodium, chloride, and water reabsorption as well as potassium secretion by the renal tubules. The most important hormonal regulation of electrolytes reabsorption and secretion and angiotensin 2 and aldosterone. The major hormone that regulates water reabsorption is antidiuretic hormone.

Renin-angiotensin–aldosterone system

When blood volume and blood pressure decrease, the juxtaglomerular cells secrete the enzyme renin into the blood. Renin clips off a 10-amino-acid peptide called angiotensin1 from angiotensinogen, which is synthesized by hepatocytes. By clipping off two more amino acid, angiotensin converting enzyme converts angiotensin I to angiotensin II, which is the active form of the hormone.

Antidiuretic hormone (ADH) and its mechanism of action

Antidiuretic hormone or vasopressin released by the posterior lobe of the pituitary plays a role in water reabsorption at the collecting duct. When more ADH is present, more water is reabsorbed and the decreased amount of urine formed. Blood borne ADH binds with its receptor. Present on the basolateral membrane of the cell in the distal or collecting tubule. This binding activates the cAMP signaling. ADH regulates the water channel in the membrane. water enters the tubular cell from the tubular lumen through the inserted water channel. water exits the cell through a different, always open water channel permeability positioned at the basolateral border, and then enters the blood, in this way being reabsorbed.

The Countercurrent Multiplier System

Water cannot be actively transported across the tubule wall, and osmosis of water cannot occur if the tubular fluid and surrounding interstitial fluid are isotonic to each other. In order for water to be reabsorbed by osmosis, the surrounding interstitial fluid must be hypertonic. The osmotic pressure of the interstitial fluid in the renal medulla is, in fact, raised to over four times that of plasma by the juxtamedullary nephrons. This results partly from the fact that the tubule bends; the geometry of the loop of Henle permits interaction between the descending and ascending limbs. Since the ascending limb is the active partner in this interaction, its properties will be described before those of the descending limb.

Countercurrent Multiplication

Countercurrent flow (flow in opposite directions) in the ascending and descending limbs and the close proximity of the two limbs allow for interaction between them. Since the concentration of the tubular fluid in the descending limb reflects the concentration of surrounding interstitial fluid, and since the concentration of this fluid is raised by the active extrusion of salt from the ascending limb, a positive feedback mechanism is created. The more salt the ascending limb extrudes, the more

concentrated will be the fluid that is delivered to it from the descending limb. This positive feedback mechanism multiplies the concentration of interstitial fluid and descending limb fluid, and is thus called the countercurrent multiplier system. First, assume that the loop of Henle is filled with fluid with a concentration of 300 mOsm/L, the same as that leaving the proximal tubule. Next, the active ion pump of the thick ascending limb on the loop of Henle reduces the concentration inside the tubule and raises the interstitial concentration; this pump establishes a 200- mOsm/L concentration gradient between the tubular fluid and the interstitial fluid (step 2). The limit to the gradient is about 200 mOsm/L because paracellular diffusion of ions back into the tubule eventually counterbalances transport of ions out of the lumen when the 200- mOsm/L concentration gradient is achieved. Step 3 is that the tubular fluid in the descending limb of the loop of Henle and the interstitial fluid quickly reach osmotic equilibrium because of osmosis of water out of the descending limb. The interstitial osmolarity is maintained at 400 mOsm/L because of continued transport of ions out of the thick ascending loop of Henle. Thus, by itself, the active transport of sodium chloride out of the thick ascending limb is capable of establishing only a 200-mOsm/L concentration gradient, much less than that achieved by the countercurrent system. Step 4 is additional flow of fluid into the loop of Henle from the proximal tubule, which causes the hyperosmotic fluid previously formed in the descending limb to flow into the ascending limb. Once this fluid is in the ascending limb, additional ions are pumped into the interstitium, with water remaining in the tubular fluid, until a 200-mOsm/L osmotic gradient is established, with the interstitial fluid osmolarity rising to 500 mOsm/L (step 5). Then, once again, the fluid in the descending limb reaches equilibrium with the hyperosmotic medullary interstitial fluid (step 6), and as the hyperosmotic tubular fluid from the descending limb of the loop of Henle flows into the ascending limb, still more solute is continuously pumped out of the tubules and deposited into the medullary interstitium. These steps are repeated over and over, with the net effect of adding more and more solute to the medulla in excess of water; with sufficient time, this process gradually traps solutes in the medulla and multiplies the concentration gradient established by the active pumping of ions out of the thick ascending loop of Henle, eventually raising the interstitial fluid osmolarity to 1200 to 1400 mOsm/L as shown in step 7. Thus, the repetitive reabsorption of sodium chloride by the thick ascending loop of Henle and continued inflow of new sodium chloride from the proximal tubule into the loop of Henle is called the countercurrent multiplier. The sodium chloride reabsorbed from the ascending loop of Henle keeps adding to the newly arrived sodium chloride, thus “multiplying” its concentration in the medullary interstitium.

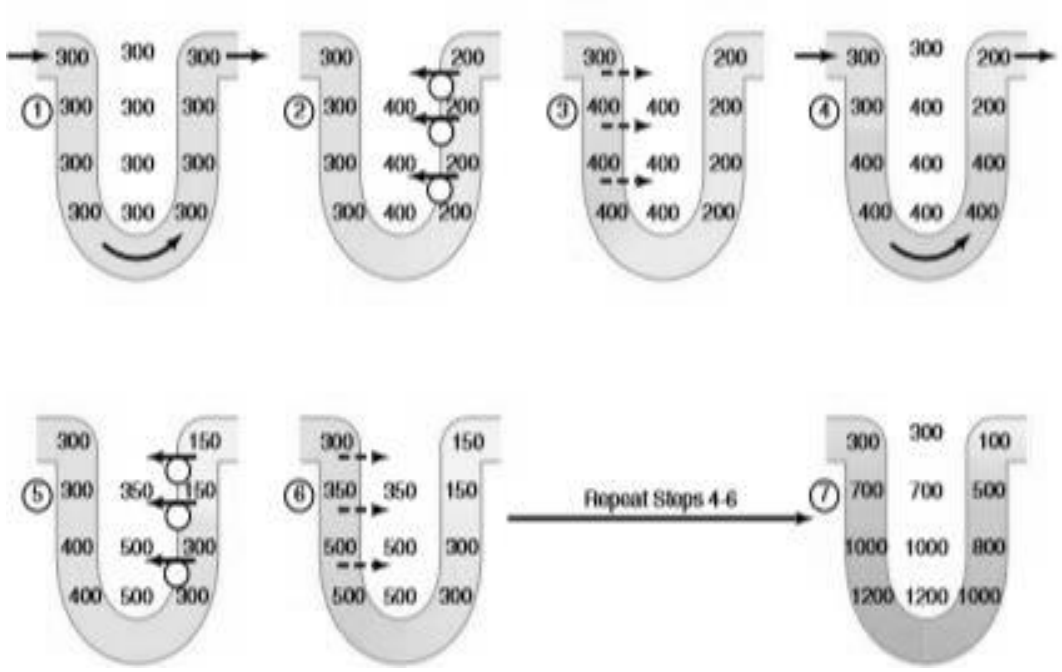


Figure : Countercurrent multiplier system in the loop of Henle for producing a hyperosmotic renal medulla.

Role of Lungs in Excretion:

Human lungs regularly remove about 18 L of CO_2 per hour and about 400 ml of water per day in normal resting condition. Water loss through the lungs is small in hot humid climate and large in cold dry climate. Thus CO_2 and water (both are metabolic wastes produced during oxidation of food in the cells) are removed via lungs.

Role of Skin in Excretion:

In many aquatic animals ammonia is mainly excreted out into the surrounding water by diffusion through the skin. Human skin has two types of glands: sudoriferous (sweat) glands and sebaceous (oil) glands.

(i) Sudoriferous glands (Sweat glands) secrete an aqueous fluid called sweat. Sweat contains water (99.5%), NaCl, urea, lactic acid, amino acids and glucose. Sweat does not contain uric acid.

The volume of sweat varies from negligible to 14L a day, depending upon activity and temperature. When sweat evaporates, it provides cooling to the body. Normal pH of sweat is 4.5. Sweat production is also influenced by atmospheric temperature.

(ii) Sebaceous glands (Oil glands) secrete an oily or wax-like secretion called sebum. It keeps the skin oily. Sebum removes some lipids like waxes, sterols, other hydrocarbons and fatty acids from the body.

Role of Liver in Excretion:

Urea is formed in the liver which is eliminated through kidneys. Liver cells also degrade the haemoglobin of worn out red blood corpuscles into bile pigments (bilirubin and bi-liver- din). Liver cells also excrete cholesterol, certain products of steroid hormones, some vitamins and many drugs. Liver secretes these substances in the bile. The bile carries these substances to the intestine and are passed out with faeces.

Role of Intestine in Excretion:

The epithelial cells of the intestine (colon) excrete certain salts such as iron and calcium. These salts are eliminated with the faeces.

Role of Salivary glands in Excretion:

Salivary glands excrete substances like mercury, potassium iodide, lead and thiocyanate. In aquatic animals like fish, gills remove carbon dioxide. Gills of many bony fish also excrete salt.

Kidney Failure and the Survival Kit—Haemodialysis:

The kidneys may be damaged due to infection, injury, diabetes, and extremes of blood pressure. A damaged kidney cannot function efficiently to remove urea, ions, water, etc., from the blood. This malfunctioning results in the accumulation of toxic wastes like urea (uremia), which can lead to death.

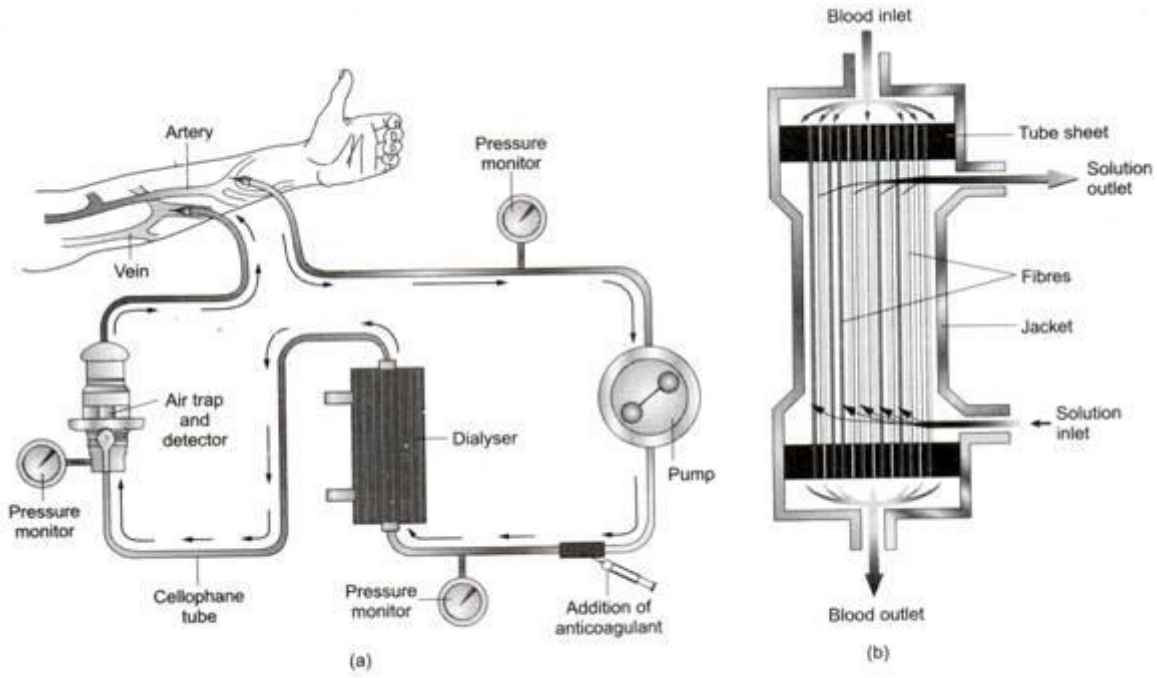
One of the ways to treat kidney failure is to use a ‘dialysis machine’ that acts as an artificial kidney. It has a long tube like structure made of Cellophane suspended in a tank (dialyser) of a fresh dialysis fluid (dialysis). The Cellophane tube is partially permeable and therefore allows solutes to diffuse through. The dialysis fluid has the same concentration as normal tissue fluid, but nitrogenous wastes and excess salts are absent. During dialysis, the blood of the patient is withdrawn from an artery and cooled at 0°C. It is maintained in a liquid state by adding an anticoagulant and by other special treatments. It is pumped through the dialysis machine. Here, the nitrogenous waste products from the blood diffuse into the dialysis fluid. The purified blood is then warmed to the body temperature and pumped back into the patient’s body through a vein. The dialyser is specific for each patient to avoid infections. Dialysis through an artificial kidney has to be carried out at frequent intervals. This process of purification of blood is called haemodialysis. A dialysis machine works like a kidney except that no selective reabsorption takes place in the former.

An artificial kidney:

(1) Helps remove harmful wastes, extra salts and water;

(2) Controls blood pressure; and

(3) Maintains the balance of sodium and potassium salts in a patient whose kidneys have failed.



(a) Haemodialysis (b) Dialyser

Utility of Artificial Kidney:

The artificial kidneys can clear 100 to 200 ml of blood urea per minute which signifies that it can function about twice as rapidly as two normal kidneys together whose urea clearance is only 70 ml per minute. However, the artificial kidney can be used for not more than 12 hours once in three to four days because of danger from excess heparin and infection to the subject.

Congenital Tubular Function Defects in Kidney:

a. Diabetes Insipidus:

(i) This disease is developed due to the non- production of ADHr. The individual passes large volume of urine (5-20 litres in 24 hours). The individual has to drink large amount of water to make up the loss.

(ii) The reabsorption of water in the distal tubules does not take place in the absence of ADH.

b. Vitamin D Resistant Rickets:

(i) The tubular reabsorption of phosphate does not take place under this condition.

(ii) Excessive loss of phosphate in urine leads to the development of a type of rickets which does not respond to usual doses of Vitamin D.

c. Renal Glycosuria:

In this condition, the tubular reabsorption of glucose is affected. Although the blood sugar is within normal level but glucose is excreted in urine due to defective reabsorption by the tubules.

d. Idiopathic Hypercalcinuria:

Calcium is not reabsorbed by the renal tubules in this condition. Hence, large amounts of calcium are excreted in the urine. Renal calculi may be developed owing to the presence of large amounts of calcium in urine.

e. Salt losing Nephritis:

(i) Large amounts of sodium and chloride ions are excreted in urine in this condition due to the defect in the tubular reabsorption of these ions resulting in severe dehydration, hyponatremia and hypo-chloremia.

(ii) Blood urea is increased due to the reduced glomerular filtration rate.

(iii) This condition does not respond to aldosterone administration but responds to parenteral administration of sodium chloride solution.

f. Renal Tubular Acidosis:

(i) In this condition, the urine becomes alkaline or neutral due to the defect in the sodium and hydrogen ion exchange mechanism in the distal tubules. There is a loss of sodium in the urine.

(ii) The acidosis is accompanied by excessive mobilization and urinary excretion of calcium and potassium.

(iii) These abnormalities led to clinical manifestation of dehydration, hypokalemia, defective mineralisation of bones and nephrocalcinosis.

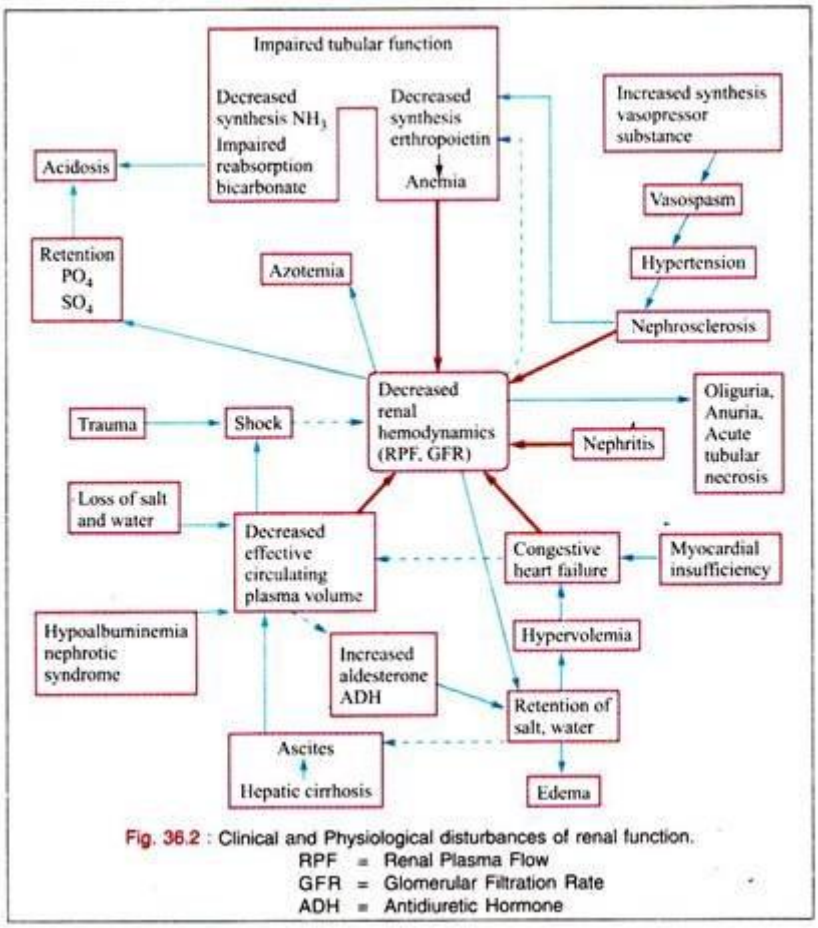


Fig. 36.2 : Clinical and Physiological disturbances of renal function.
RPF = Renal Plasma Flow
GFR = Glomerular Filtration Rate
ADH = Antidiuretic Hormone

g. Fanconi Syndrome:

(i) A number of defects in tubular reabsorption exist in this condition. The defects are renal amino acid in renal glycosuria, hyperphosphaturia, metabolic aciduria, with increased urinary excretion of Na, Ca and K.

(ii) In some individuals, cystinosis prevails due to the abnormality of cystine metabolism in which cystine crystals are deposited in macrophages in the liver, kidney, spleen, bone marrow, lymph nodes and cornea.

h. Hartnup Syndrome (Hard Syndrome):

(i) In this condition, a number of amino acids are not reabsorbed owing to the defect in tubular reabsorption mechanism.

(ii) Disturbances in tryptophan metabolism is suggested by the presence of increased amounts of tryptophan, indican and indole acetic acid in urine.

(iii) The clinical symptoms are of niacin deficiency—a pellagra like skin lesions and mental deficiency.

i. Nephrogenic Diabetes Insipidus (Water-Losing Nephritis):

This condition is due to congenital defect in water reabsorption in the distal tubules and may, therefore, resemble true diabetes insipidus.

Probable Questions

1. Write a short note on juxta-glomerular apparatus.
2. How does ADH and aldosterone regulate the salt water balance in our body?
3. How does the counter-current mechanism help in the formation of hypertonic urine? Elaborate.

Suggested readings / References-

1. Animal physiology- Mohan P. Arora.
2. Textbook of medical physiology / Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

UNIT-IV

Nervous System. Structure of Nervous System. Evolution of nervous system.

Objective: In this unit we will learn about nervous system, how the nerve impulse is propagated and structure and function of neurons. We will also learn about evolution of nervous system in various phylogenetic group and also discuss different parts and functions of nervous system.

Nervous System: Organized group of cells specialized for the conduction of electrochemical stimuli from sensory receptors through a network to the site at which a response occurs. All living organisms are able to detect changes within themselves and in their environments. Changes in the external environment include those of light, temperature, sound, motion, and odour, while changes in the internal environment include those in the position of the head and limbs as well as in the internal organs. Once detected, these internal and external changes must be analyzed and acted upon in order to survive. As life on Earth evolved and the environment became more complex, the survival of organisms depended upon how well they could respond to changes in their surroundings. One factor necessary for survival was a speedy reaction or response. Since communication from one cell to another by chemical means was too slow to be adequate for survival, a system evolved that allowed for faster reaction. That system was the nervous system, which is based upon the almost instantaneous transmission of electrical impulses from one region of the body to another along specialized nerve cells called neurons.

Nervous systems are of two general types, diffuse and centralized. In the diffuse type of system, found in lower invertebrates, there is no brain, and neurons are distributed throughout the organism in a netlike pattern. In the centralized systems of higher invertebrates and vertebrates, a portion of the nervous system has a dominant role in coordinating information and directing responses. This centralization reaches its culmination in vertebrates, which have a well-developed brain and spinal cord. Impulses are carried to and from the brain and spinal cord by nerve fibres that make up the peripheral nervous system.

Form and function of nervous systems

Stimulus-response coordination:

The simplest type of response is a direct one-to-one stimulus-response reaction. A change in the environment is the stimulus; the reaction of the organism to it is the response. In single-celled organisms, the response is the result of a property of the cell fluid called irritability. In simple organisms, such as algae, protozoans, and fungi, a response in which the organism moves toward or away from the stimulus is called taxis. In larger and more complicated organisms—those in which response involves the synchronization and integration of events in

different parts of the body—a control mechanism, or controller, is located between the stimulus and the response. In multicellular organisms, this controller consists of two basic mechanisms by which integration is achieved—chemical regulation and nervous regulation. In chemical regulation, substances called hormones are produced by well-defined groups of cells and are either diffused or carried by the blood to other areas of the body where they act on target cells and influence metabolism or induce synthesis of other substances. The changes resulting from hormonal action are expressed in the organism as influences on, or alterations in, form, growth, reproduction, and behaviour.

Plants respond to a variety of external stimuli by utilizing hormones as controllers in a stimulus-response system. Directional responses of movement are known as tropisms and are positive when the movement is toward the stimulus and negative when it is away from the stimulus. When a seed germinates, the growing stem turns upward toward the light, and the roots turn downward away from the light. Thus, the stem shows positive phototropism and negative geotropism, while the roots show negative phototropism and positive geotropism. In this example, light and gravity are the stimuli, and directional growth is the response. The controllers are certain hormones synthesized by cells in the tips of the plant stems. These hormones, known as auxins, diffuse through the tissues beneath the stem tip and concentrate toward the shaded side, causing elongation of these cells and, thus, a bending of the tip toward the light. The end result is the maintenance of the plant in an optimal condition with respect to light.

In animals, in addition to chemical regulation via the endocrine system, there is another integrative system called the nervous system. A nervous system can be defined as an organized group of cells, called neurons, specialized for the conduction of an impulse—an excited state—from a sensory receptor through a nerve

network to an effector, the site at which the response occurs. Organisms that possess a nervous system are capable of much more complex behaviour than are organisms that do not. The nervous system, specialized for the conduction of impulses, allows rapid responses to environmental stimuli. Many responses mediated by the nervous system are directed toward preserving the status quo, or homeostasis, of the animal. Stimuli that tend to displace or disrupt some part of the organism call forth a response that results in reduction of the adverse effects and a return to a more normal condition. Organisms with a nervous system are also capable of a second group of functions that initiate a variety of behaviour patterns. Animals may go through periods of exploratory or appetitive behaviour, nest building, and migration. Although these activities are beneficial to the survival of the species, they are not always performed by the individual in response to an individual need or stimulus. Finally, learned behaviour can be superimposed on both the homeostatic and initiating functions of the nervous system.

Intracellular systems

All living cells have the property of irritability, or responsiveness to environmental stimuli, which can affect the cell in different ways, producing, for example, electrical, chemical, or mechanical changes. These changes are expressed as a response, which may be the release of secretory products by gland cells, the contraction of muscle cells, the bending of a plant-stem cell, or the beating of whiplike “hairs,” or cilia, by ciliated cells. The responsiveness of a single cell can be illustrated by the behaviour of the relatively simple amoeba. Unlike some other protozoans, an amoeba lacks highly developed structures that function in the reception of stimuli and in the production or conduction of a response. The amoeba behaves as though it had a nervous system, however, because the general responsiveness of its cytoplasm serves the functions of a nervous system. An excitation produced by a stimulus is conducted to other parts of the cell and evokes a response by the animal. An amoeba will move to a region of a certain level of light. It will be attracted by chemicals given off by foods and exhibit a feeding response. It will also withdraw from a region with noxious chemicals and exhibit an avoidance reaction upon contacting other objects.

Organelle systems

In more-complex protozoans, specialized cellular structures, or organelles, serve as receptors of stimulus and as effectors of response. Receptors include stiff sensory bristles in ciliates and the light-sensitive eyespots of flagellates. Effectors include cilia (slender, hairlike projections from the cell surface), flagella (elongated, whiplike cilia), and other organelles associated with drawing in food or with

locomotion. Protozoans also have subcellular cytoplasmic filaments that, like muscle tissue, are contractile. The vigorous contraction of the protozoan *Vorticella*, for example, is the result of contraction of a threadlike structure called a myoneme in the stalk. Although protozoans clearly have specialized receptors and effectors, it is not certain that there are special conducting systems between the two. In a ciliate such as *Paramecium*, the beating of the cilia—which propels it along—is not random, but coordinated. Beating of the cilia begins at one end of the organism and moves in regularly spaced waves to the other end, suggesting that coordinating influences are conducted longitudinally. A system of fibrils connecting the bodies in which the cilia are rooted may provide conducting paths for the waves, but coordination of the cilia may also take place without such a system. Each cilium may respond to a stimulus carried over the cell surface from an adjacent cilium—in which case, coordination would be the result of a chain reaction from cilium to cilium.

The best evidence that formed structures are responsible for coordination comes from another ciliate, *Euplotes*, which has a specialized band of ciliary rows (membranelles) and widely separated tufts of cilia (cirri). By means of the coordinated action of these structures, *Euplotes* is capable of several complicated movements in addition to swimming (e.g., turning sharply, moving backward, spinning). The five cirri at the rear of the organism are connected to the anterior end in an area known as the motorium. The fibres of the motorium apparently provide coordination between the cirri and the membranelles. The membranelles, cirri, and motorium constitute a neuromotor system.

Nervous systems

The basic pattern of stimulus-response coordination in animals is an organization of receptor, adjustor, and effector units. External stimuli are received by the receptor cells, which, in most cases, are neurons. (In a few instances, a receptor is a non-nervous sensory epithelial cell, such as a hair cell of the inner ear or a taste cell, which stimulates adjacent neurons.) The stimulus is modified, or transduced, into an electrical impulse in the receptor neuron. This incoming excitation, or afferent impulse, then passes along an extension, or axon, of the receptor to an adjustor, called an interneuron. (All neurons are capable of conducting an impulse, which is a brief change in the electrical charge on the cell membrane. Such an impulse can be transmitted, without loss in strength, many times along an axon until the message, or input, reaches another neuron, which in turn is excited.) The interneuron-adjustor selects, interprets, or modifies the input from the receptor and sends an outgoing, or efferent, impulse to an efferent neuron, such as a

motor neuron. The efferent neuron, in turn, makes contact with an effector such as a muscle or gland, which produces a response.

In the simplest arrangement, the receptor-adjustor-effector units form a functional group known as the reflex arc. Sensory cells carry afferent impulses to a central interneuron, which makes contact with a motor neuron. The motor neuron carries efferent impulses to the effector, which produces the response. Three types of neurons are involved in this reflex arc, but a two-neuron arc, in which the receptor makes contact directly with the motor neuron, also occurs. In a two-neuron arc, simple reflexes are prompt, short-lived, and automatic and involve only a part of the body. Examples of simple reflexes are the contraction of a muscle in response to stretch, the blink of the eye when the cornea is touched, and salivation at the sight of food. Reflexes of this type are usually involved in maintaining homeostasis.

The differences between simple and complex nervous systems lie not in the basic units but in their arrangement. In higher nervous systems, there are more interneurons concentrated in the central nervous system (brain and spinal cord) that mediate the impulses between afferent and efferent neurons. Sensory impulses from particular receptors travel through specific neuronal pathways to the central nervous system. Within the central nervous system, though, the impulse can travel through multiple pathways formed by numerous neurons. Theoretically, the impulse can be distributed to any of the efferent motor neurons and produce a response in any of the effectors. It is also possible for many kinds of stimuli to produce the same response.

As a result of the integrative action of the interneuron, the behaviour of the organism is more than the simple sum of its reflexes; it is an integrated whole that exhibits coordination between many individual reflexes. Reflexes can occur in a complicated sequence producing elaborate behaviour patterns. Behaviour in such cases is characterized not by inherited, stereotyped responses but by flexibility and adaptability to circumstances. Many automatic, unconditioned reflexes can be modified by or adapted to new stimuli. The experiments of Russian physiologist Ivan Petrovich Pavlov, for example, showed that if an animal salivates at the sight of food while another stimulus, such as the sound of a bell, occurs simultaneously, the sound alone can induce salivation after several trials. This response, known as a conditioned reflex, is a form of learning. The behaviour of the animal is no longer limited by fixed, inherited reflex arcs but can be modified by experience and exposure to an unlimited number of stimuli. The most evolved nervous systems are capable of even higher associative functions such as thinking and memory. The complex manipulation

of the signals necessary for these functions depends to a great extent on the number and intricacy of the arrangement of interneurons.

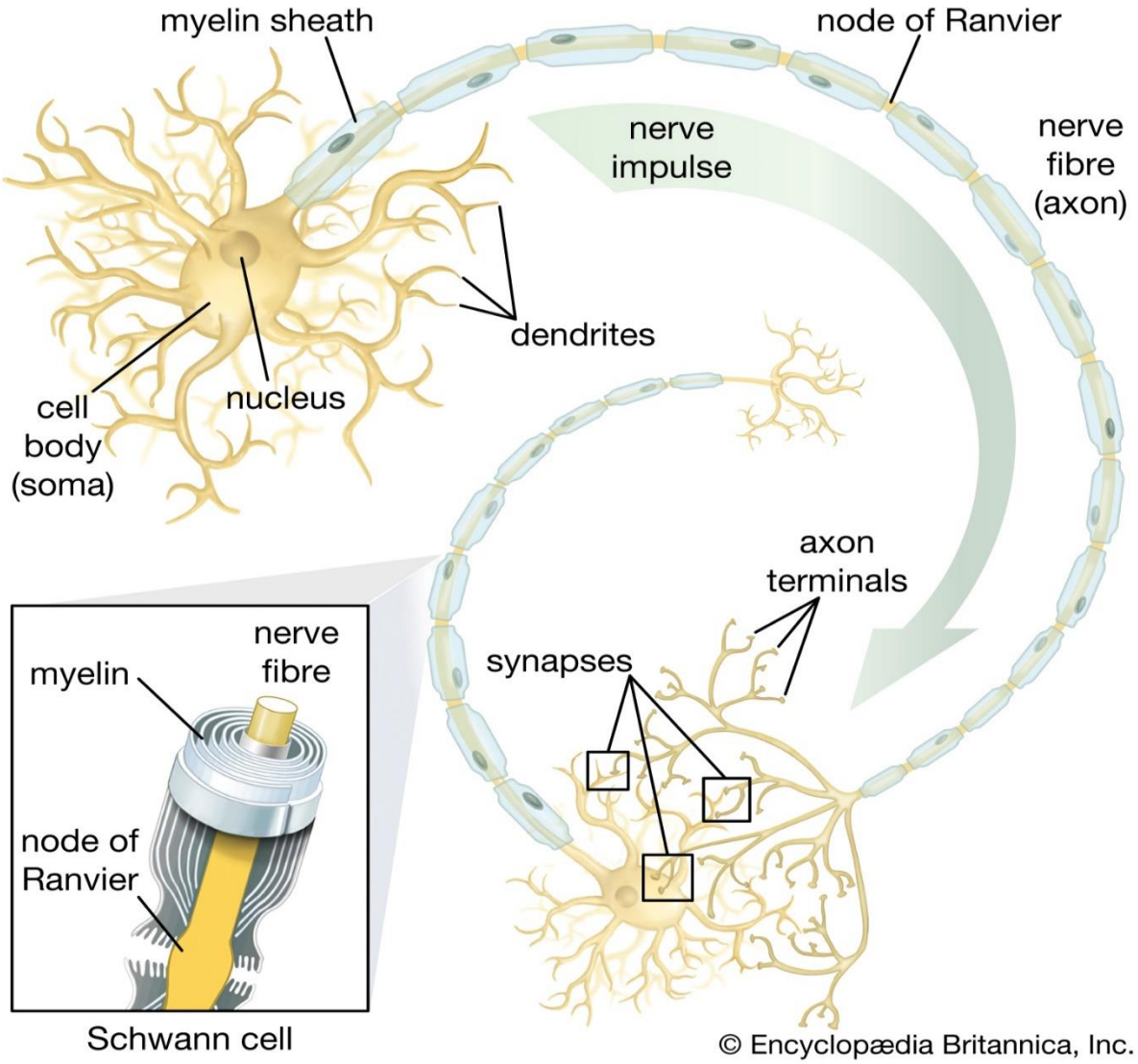
The nerve cell :

The watershed of all studies of the nervous system was an observation made in 1889 by Spanish scientist Santiago Ramón y Cajal, who reported that the nervous system is composed of individual units that are structurally independent of one another and whose internal contents do not come into direct contact. According to his hypothesis, now known as the neuron theory, each nerve cell communicates with others through contiguity rather than continuity. That is, communication between adjacent but separate cells must take place across the space and barriers separating them. It has since been proved that Cajal's theory is not universally true, but his central idea—that communication in the nervous system is largely communication between independent nerve cells—has remained an accurate guiding principle for all further study.

There are two basic cell types within the nervous system: neurons and neuroglial cells.

The neuron:

In the human brain there are an estimated 85 billion to 200 billion neurons. Each neuron has its own identity, expressed by its interactions with other neurons and by its secretions; each also has its own function, depending on its intrinsic properties and location as well as its inputs from other select groups of neurons, its capacity to integrate those inputs, and its ability to transmit the information to another select group of neurons. With few exceptions, most neurons consist of three distinct regions, as shown in the diagram: (1) the cell body, or soma; (2) the nerve fibre, or axon; and (3) the receiving processes, or dendrites.



Soma:

a. Plasma membrane

The neuron is bound by a plasma membrane, a structure so thin that its fine detail can be revealed only by high-resolution electron microscopy. About half of the membrane is the lipid bilayer, two sheets of mainly phospholipids with a space between. One end of a phospholipid molecule is hydrophilic, or water attaching, and the other end is hydrophobic, or water repelling. The bilayer structure results when the hydrophilic ends of the phospholipid molecules in each sheet turn toward the watery mediums of both the cell interior and the extracellular environment, while the hydrophobic ends of the molecules turn in toward

the space between the sheets. These lipid layers are not rigid structures; the loosely bonded phospholipid molecules can move laterally across the surfaces of the membrane, and the interior is in a highly liquid state.

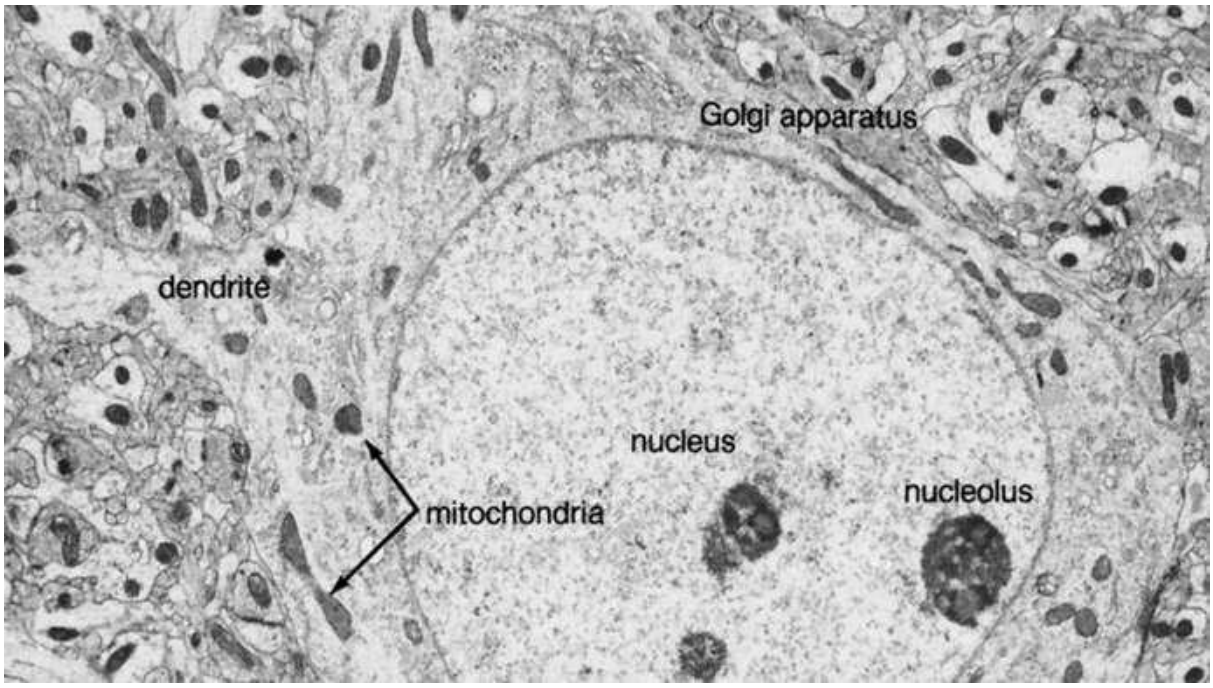


Fig: neuron from visual cortex of a rat.The centre of the field is occupied by the cell body, or soma, of the neuron. Most of the cell body is occupied by the nucleus, which contains a nucleolus. The double membrane of the nucleus is surrounded by cytoplasm, containing elements of the Golgi apparatus lying at the base of the apical dendrite. Mitochondria can be seen dispersed in the cytoplasm, which also contains the rough endoplasmic reticulum. Another dendrite is seen to the side, and the axon hillock is shown at the initial segment ofthe emerging axon. A synapse impinges onto the neuron close to the axon hillock.

Embedded within the lipid bilayer are proteins, which also float in the liquid environment of the membrane. These include glycoproteins containing polysaccharide chains, which function, along with other carbohydrates, as adhesion sites and recognition sites for attachment and chemical interaction with other neurons. The proteins provide another basic and crucial function: those which penetrate the membrane can exist in more than one conformational state, or molecular shape, forming channels that allow ions to pass between the extracellular fluid and the cytoplasm, or internal contents of the cell. In other conformational states, they can block the passage of ions. This action is the fundamental mechanism that determines the

excitability and pattern of electrical activity of the neuron. A complex system of proteinaceous intracellular filaments is linked to the membrane proteins. This cytoskeleton includes thin neurofilaments containing actin, thick neurofilaments similar to myosin, and microtubules composed of tubulin. The filaments are probably involved with movement and translocation of the membrane proteins, while microtubules may anchor the proteins to the cytoplasm.

Nucleus:

Each neuron contains a nucleus defining the location of the soma. The nucleus is surrounded by a double membrane, called the nuclear envelope, that fuses at intervals to form pores allowing molecular communication with the cytoplasm. Within the nucleus are the chromosomes, the genetic material of the cell, through which the nucleus controls the synthesis of proteins and the growth and differentiation of the cell into its final form. Proteins synthesized in the neuron include enzymes, receptors, hormones, and structural proteins for the cytoskeleton.

Organelles:

The endoplasmic reticulum (ER) is a widely spread membrane system within the neuron that is continuous with the nuclear envelope. It consists of series of tubules, flattened sacs called cisternae, and membrane-bound spheres called vesicles. There are two types of ER. The rough endoplasmic reticulum (RER) has rows of knobs called ribosomes on its surface. Ribosomes synthesize proteins that, for the most part, are transported out of the cell. The RER is found only in the soma. The smooth endoplasmic reticulum (SER) consists of a network of tubules in the soma that connects the RER with the Golgi apparatus. The tubules can also enter the axon at its initial segment and extend to the axon terminals. The Golgi apparatus is a complex of flattened cisternae arranged in closely packed rows. Located close to and around the nucleus, it receives proteins synthesized in the RER and transferred to it via the SER. At the Golgi apparatus, the proteins are attached to carbohydrates. The glycoproteins so formed are packaged into vesicles that leave the complex to be incorporated into the cell membrane.

Axon:

The axon arises from the soma at a region called the axon hillock, or initial segment. This is the region where the plasma membrane generates nerve impulses; the axon conducts these impulses away from the

soma or dendrites toward other neurons. Large axons acquire an insulating myelin sheath and are known as myelinated, or medullated, fibres. Myelin is composed of 80 percent lipid and 20 percent protein; cholesterol is one of the major lipids, along with variable amounts of cerebrosides and phospholipids. Concentric layers of these lipids separated by thin layers of protein give rise to a high-resistance, low-capacitance electrical insulator interrupted at intervals by gaps called nodes of Ranvier, where the nerve membrane is exposed to the external environment. In the central nervous system the myelin sheath is formed from glial cells called oligodendrocytes, and in peripheral nerves it is formed from Schwann cells

While the axon mainly conducts nerve impulses from the soma to the terminal, the terminal itself secretes chemical substances called neurotransmitters. The synthesis of these substances can occur in the terminal itself, but the synthesizing enzymes are formed by ribosomes in the soma and must be transported down the axon to the terminal. This process is known as axoplasmic flow; it occurs in both directions along the axon and may be facilitated by microtubules. The presynaptic terminal is unmyelinated and is separated from the neuron or muscle cell onto which it impinges by a gap called the synaptic cleft, across which neurotransmitters diffuse when released from the vesicles. In nerve-muscle junctions the synaptic cleft contains a structure called the basal lamina, which holds an enzyme that destroys neurotransmitters and thus regulates the amount that reaches the postsynaptic receptors on the receiving cell. Most knowledge of postsynaptic neurotransmitter receptors comes from studies of the receptor on muscle cells. This receptor, called the end plate, is a glycoprotein composed of five subunits. Other neurotransmitter receptors do not have the same structure, but they are all proteins and probably have subunits with a central channel that is activated by the neurotransmitter. While the chemically mediated synapse described above forms the majority of synapses in vertebrate nervous systems, there are other types of synapses in vertebrate brains and, in especially great numbers, in invertebrate and fish nervous systems. At these synapses there is no synaptic gap; instead, there are gap junctions, direct channels between neurons that establish a continuity between the cytoplasm of adjacent cells and a structural symmetry between the pre- and postsynaptic sites. Rapid neuronal communication at these junctions is probably electrical in nature. (For further discussion, *see below* Transmission at the synapse.)

Dendrites:

Besides the axon, neurons have other branches called dendrites that are usually shorter than axons and are unmyelinated. Dendrites are thought to form receiving surfaces for synaptic input from other neurons. In

many dendrites these surfaces are provided by specialized structures called dendritic spines, which, by providing discrete regions for the reception of nerve impulses, isolate changes in electrical current from the main dendritic trunk. The traditional view of dendritic function presumes that only axons conduct nerve impulses and only dendrites receive them, but dendrites can form synapses with dendrites and axons and even somata can receive impulses. Indeed, some neurons have no axon; in these cases nervous transmission is carried out by the dendrites.

The neuroglia

Neurons form a minority of the cells in the nervous system. Exceeding them in number by at least 10 to 1 are neuroglial cells, which exist in the nervous systems of invertebrates as well as vertebrates. Neuroglia can be distinguished from neurons by their lack of axons and by the presence of only one type of process. In addition, they do not form synapses, and they retain the ability to divide throughout their life span. While neurons and neuroglia lie in close apposition to one another, there are no direct junctional specializations, such as gap junctions, between the two types. Gap junctions do exist between neuroglial cells.

Types of neuroglia

Apart from conventional histological and electron-microscopic techniques, immunologic techniques are used to identify different neuroglial cell types. By staining the cells with antibodies that bind to specific protein constituents of different neuroglia, neurologists have been able to discern two (in some opinions, three) main groups of neuroglia: (1) astrocytes, subdivided into fibrous and protoplasmic types; (2) oligodendrocytes, subdivided into interfascicular and perineuronal types; and sometimes (3) microglia.

Fibrous astrocytes are prevalent among myelinated nerve fibres in the white matter of the central nervous system. Organelles seen in the somata of neurons are also seen in astrocytes, but they appear to be much sparser. These cells are characterized by the presence of numerous fibrils in their cytoplasm. The main processes exit the cell in a radial direction (hence the name *astrocyte*, meaning “star-shaped cell”), forming expansions and end feet at the surfaces of vascular capillaries. Unlike fibrous astrocytes, protoplasmic astrocytes occur in the gray matter of the central nervous system. They have fewer fibrils within their cytoplasm, and cytoplasmic organelles are sparse, so that the somata are shaped by surrounding neurons and fibres. The processes of protoplasmic astrocytes also make contact with capillaries.

Oligodendrocytes have few cytoplasmic fibrils but a well-developed Golgi apparatus. They can be distinguished from astrocytes by the greater density of both the cytoplasm and the nucleus, the absence of fibrils and of glycogen in the cytoplasm, and large numbers of microtubules in the processes. Interfascicular oligodendrocytes are aligned in rows between the nerve fibres of the white matter of the central nervous system. In gray matter, perineuronal oligodendrocytes are located in close proximity to the somata of neurons. In the peripheral nervous system, neuroglia that are equivalent to oligodendrocytes are called Schwann cells. Microglial cells are small cells with dark cytoplasm and a dark nucleus. It is uncertain whether they are merely damaged neuroglial cells or occur as a separate group in living tissue.

Neuroglial functions:

The term neuroglia means “nerve glue,” and these cells were originally thought to be structural supports for neurons. This is still thought to be plausible, but other functions of the neuroglia are now generally accepted. Oligodendrocytes and Schwann cells produce the myelin sheath around neuronal axons. Some constituent of the axonal surface stimulates Schwann cell proliferation; the type of axon determines whether there is loose or tight myelination of the axon. In tight myelination a glial cell wraps itself like a rolled sheet around a length of axon until the fibre is covered by several layers. Between segments of myelin wrapping are exposed sections called nodes of Ranvier, which are important in the transmission of nerve impulses. Myelinated nerve fibres are found only in vertebrates, leading biologists to conclude that they are an adaptation to transmission over relatively long distances. Another well-defined role of neuroglial cells is the repair of the central nervous system following injury. Astrocytes divide after injury to the nervous system and occupy the spaces left by injured neurons. The role of oligodendrocytes after injury is unclear, but they may proliferate and form myelin sheaths.

When neurons of the peripheral nervous system are severed, they undergo a process of degeneration followed by regeneration; fibres regenerate in such a way that they return to their original target sites. Schwann cells that remain after nerve degeneration apparently determine the route. This route direction is also performed by astrocytes during development of the central nervous system. In the developing cerebral cortex and cerebellum of primates, astrocytes project long processes to certain locations, and neurons migrate along these processes to arrive at their final locations. Thus, neuronal organization is brought about to some extent by the neuroglia. Astrocytes are also thought to have high-affinity uptake systems for neurotransmitters such as glutamate and gamma-aminobutyric acid (GABA). This function is important in the modulation of synaptic transmission. Uptake systems tend to terminate neurotransmitter action at the synapses and may also act as storage systems for neurotransmitters when they are needed. For instance, when motor nerves are severed, nerve terminals degenerate and their original sites are occupied by Schwann cells. The synthesis of neurotransmitters by neurons apparently also requires the presence of neuroglial cells in the vicinity.

Finally, the environment surrounding neurons in the brain consists of a network of very narrow extracellular clefts. In 1907 Italian biologist Emilio Lugaro suggested that neuroglial cells exchange substances with the extracellular fluid and in this way exert control on the neuronal environment. It has since been shown that glucose, amino acids, and ions—all of which influence neuronal function—are exchanged between the extracellular space and neuroglial cells. After high levels of neuronal activity, for instance, neuroglial cells can take up and spatially buffer potassium ions and thus maintain normal neuronal function.

Transmission of information in the nervous system:

In the nervous system of animals at all levels of the evolutionary scale, the signals containing information about a particular stimulus are electrical in nature. In the past the nerve fibre and its contents were compared to metal wire, while the membrane was compared to insulation around the wire. This comparison was erroneous for a number of reasons. First, the charge carriers in nerves are ions, not electrons, and the density of ions in the axon is much less than that of electrons in a metal wire. Second, the membrane of an axon is not a perfect insulator, so that the movement of current along the axon is not complete. Finally, nerve fibres are smaller than most wires, so that the currents they can carry are limited in amplitude.

The ionic basis of electrical signals:

Ions are atoms or groups of atoms that gain an electrical charge by losing or acquiring electrons. For example, in the reaction that forms salt from sodium and chlorine, each sodium atom donates an electron, which is negatively charged, to a chlorine atom. The result is sodium chloride (NaCl), composed of one positively charged sodium ion (Na^+) and one negatively charged chloride ion (Cl^-). A positively charged ion is called a cation; a negatively charged ion, an anion. The electrical events that constitute signaling in the nervous system depend upon the distribution of such ions on either side of the nerve membrane. Underlying these distributions and their change are crucial physical-chemical principles.

Diffusion of ions across a membrane:

Uncharged molecules:

Molecules in solution move randomly; the energy for their movement is derived from thermal energy. When a permeable membrane (a membrane that allows molecules to cross it) divides a heavily concentrated solution from a less-concentrated solution, there occurs a diffusion of molecules through the membrane and down their concentration gradient—that is, from the fluid with the higher concentration to that with the lower concentration. The number of molecules moving per unit of time is called the flow rate, or flux rate. Diffusion continues until the concentrations on both sides of the membrane are equal. A condition of no net flux is then established with an equal, random diffusion of molecules in both directions. This is called the equilibrium state. A membrane with pores allowing passage of molecules of only a particular size is called a semipermeable membrane. The semipermeable membrane imposes a condition of restricted diffusion in which the flux rate of the diffusing material is controlled by the permeability of the membrane, which in turn is dictated by the size of the pores and is given a unit of measure called the permeability coefficient.

Water:

The water molecule, like other molecules, diffuses down its concentration gradient. If a rigid vessel contains water on one side of a semipermeable membrane and an impermeant substance (a substance that cannot cross the membrane) on the other side, the water tends to cross the membrane, diluting the substance and increasing the hydrostatic pressure on the other side, as shown in the diagram. The pressure then will tend to push water back across the membrane in opposition to the net flux. When the pressure built up equals the diffusion of water in the opposite direction, no net flux occurs and equilibrium is established. The migration of water (or any solvent) across a membrane is called osmosis, and the pressure necessary to establish equilibrium is called osmotic pressure. Water moves from a region of low osmotic pressure to a region of high osmotic pressure.

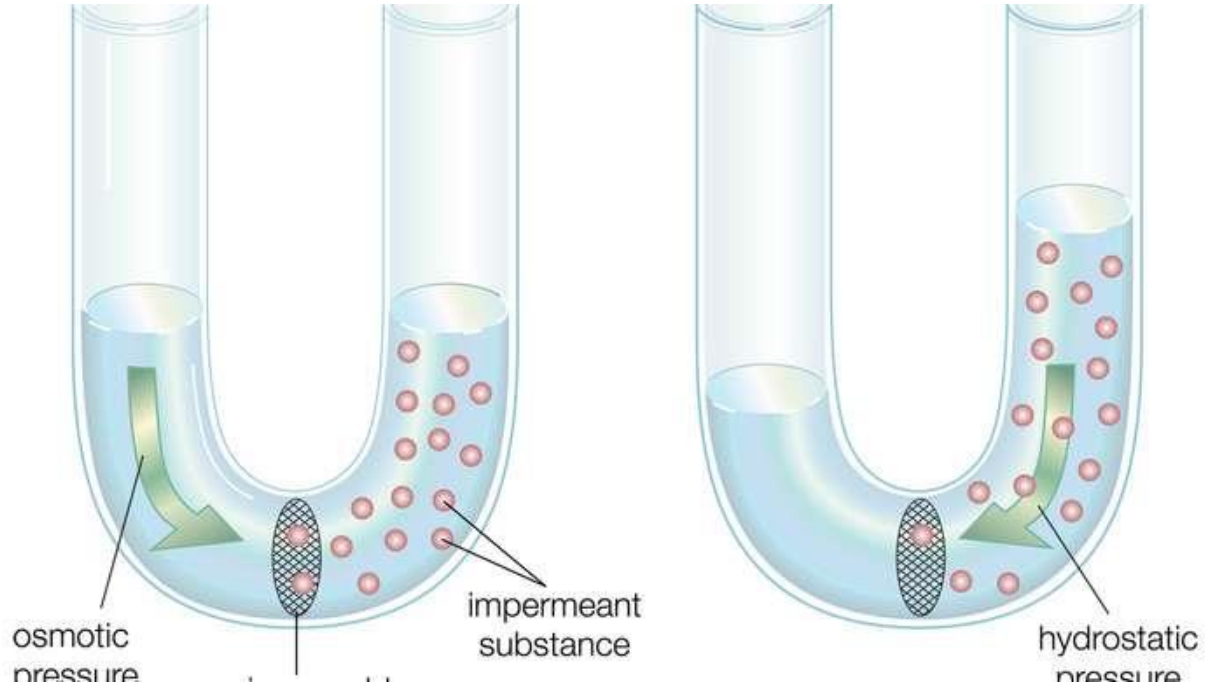


Fig: diffusion of water across a semipermeable membrane. (A) Water diffuses down its concentration gradient from side 1 to side 2 of a rigid container to dilute the impermeant substance. (B) The net flux of the water increases the hydrostatic pressure on side 2, tending to force the water back to side 1.

The above example refers to water in a container with rigid walls. The neuron, however, has somewhat flexible walls, so that as water enters it, the cell tends to increase in volume, or swell. There is a direct relation between osmotic pressure across the plasma membrane and the final volume of a cell at equilibrium, so that if the osmotic pressure of the cell exterior is halved, the equilibrium volume of the cell will be twice its original volume.

Ions:

When potassium chloride (KCl) is placed into solution, the elements separate into potassium cations (K^+) and chloride anions (Cl^-). Ions follow much the same principles of diffusion as uncharged molecules. For example, if a highly concentrated solution of KCl is separated from a lower concentration by a semipermeable membrane—one that is permeable to cations only—then K^+ from the higher concentration diffuses across the membrane, following its concentration gradient to the region of lower concentration.

Cl^-, being blocked by the membrane, remains behind. At this point the diffusion of ions creates conditions quite different from the diffusion of uncharged molecules and water molecules. The movement of cations toward the less-concentrated solution creates a separation of electrical charge across the membrane—that is, a greater number of positively charged ions will have moved to the side with the less-concentrated solution of KCl, and the side of the membrane with the higher concentration will have a more negative charge. This separation of charge—actually a difference in electrical potential—is called the potential difference, and it is the starting point of all electrical events in nervous systems. When present in the plasma membrane of the neuron, the potential difference transforms the neuron into an electrolytic cell that is capable, upon stimulation, of generating and transmitting electrical impulses.

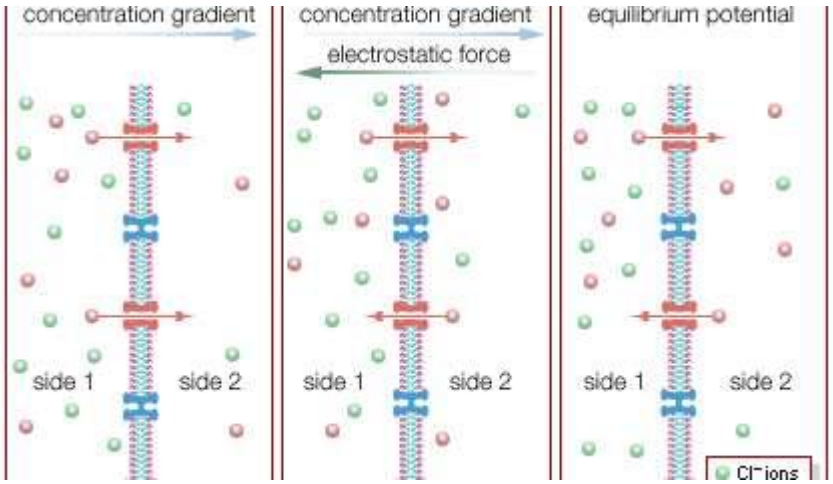


Fig: ion diffusion across a semipermeable membrane. Diffusion of ions across a semipermeable membrane. (A) A high concentration of KCl is placed on side 1, opposite a semipermeable membrane from a low concentration. The membrane allows only K^+ to diffuse, thereby establishing an electrical potential difference across the membrane. (B) The separation of charge creates an electrostatic voltage force, which draws some K^+ back to side 1. (C) At equilibrium, there is no net flux of K^+ in either direction. Side 1, with the higher concentration of KCl, has a negative charge compared with side 2.

Complicating the ionic diffusion process is the phenomenon that opposite charges attract. This means that, in the example above, some of the K^+ diffusing across the membrane is electrostatically drawn back up its concentration gradient toward the Cl^-. This creates a situation in which two tendencies oppose each other: (1) the diffusing tendency of the cation down its concentration gradient; and (2) the electrostatic voltage force tending to draw the cation back. These two forces eventually reach a state of no net flux, when the number of cations that they draw in each direction across the membrane is equal. The system is then in

electrochemical equilibrium. At equilibrium, one side of the membrane may still have a more negative charge than the other. The potential difference is then called the equilibrium potential. (It is also called the Nernst potential, after Walther Nernst, a German physical chemist who, in the late 19th century, developed equations for calculating the electrical potential at which there is no longer a net flux of a specific ion across a membrane.)

The law of electroneutrality states that in any single ionic solution a sum of negative electrical charges attracts an equal sum of positive electrical charges. If a solution of KCl is divided into two parts by a membrane that is permeable to both ions, then the equal concentration of KCl across the membrane preserves chemical equilibrium between the two sides, while the equal concentrations of K^+ and Cl^- on each side preserve electroneutrality on each side as well. This equilibrium can be upset by the addition to side 1 of a large number of K^+ and an equal charge of impermeant anions (that is, negatively charged ions other than Cl^- that cannot permeate the membrane). In this case electroneutrality on side 1 is preserved, since the sum of positive charges added to that side is equaled by the sum of added negative charges. However, chemical equilibrium between side 1 and side 2 is not preserved, since side 1 now has a greater concentration of ions than side 2. Therefore, K^+ diffuses down its concentration gradient, crossing the membrane to side 2 while drawing Cl^- with it to preserve electroneutrality. Diffusion continues until a new state of electrochemical equilibrium is reached; this occurs when the ratio of K^+ concentration (on side 2 to that on side 1) is equal to the ratio of Cl^- concentration (on side 1 to that on side 2). Stated mathematically, equilibrium is reached when

$$\frac{[K^+]_{side\,2}}{[K^+]_{side\,1}} = \frac{[Cl^-]_{side\,1}}{[Cl^-]_{side\,2}}.$$

This is known as the Donnan equilibrium, after Frederick George Donnan, a British chemist who, in 1911, first measured the changes brought about by adding an impermeant substance to one side of a divided solution at equilibrium. In the new state of equilibrium, both sides are electrically neutral, since the impermeant anions added to side 1 are equaled by the added K^+, and the K^+ that has diffused to side 2 is balanced by the Cl^- electrostatically drawn along with it. But the entire solution is not at osmotic equilibrium, because the larger amount of ions on side 1 tends to draw water from side 2. Osmotic equilibrium can be established by the addition of ions to side 2. Indeed, in the neuron, osmotic equilibrium

is maintained partly because large amounts of K^+ and impermeant anions inside the cell are balanced by large amounts of salt outside the cell.

The neuronal membrane:

The principles outlined above can be applied to the neuron and its ionic contents. The plasma membrane of the neuron is semipermeable, being highly permeable to K^+ and slightly permeable to Cl^- and Na^+. In the extracellular fluid, electroneutrality is preserved by a balance between a high concentration of Na^+ on the one hand and a high concentration of Cl^-, as well as small quantities of impermeant anions such as bicarbonate, phosphate, and sulfate, on the other. In the cytoplasm, where K^+ concentration is high, the concentration of Cl^- is much below that necessary to balance the sum of the positive charges. Electroneutrality is maintained there by negatively charged impermeant proteins and phosphates. Osmotic balance is maintained between the extracellular fluid and the cytoplasm by movement of water through the plasma membrane when the total concentration of particles on one side is not equal to that on the other.

These three characteristics of the neuron—semipermeability of the membrane, osmotic balance, and electroneutrality on each side—create an equilibrium electrical potential at which the inside of the membrane is more negative than the outside. In most neurons this potential, called the membrane potential, is between −60 and −75 millivolts (mV; or thousandths of a volt; the minus sign indicates that the inner surface is negative). When the inside of the plasma membrane has a negative charge compared to the outside, the neuron is said to be polarized. Any change in membrane potential tending to make the inside even more negative is called hyperpolarization, while any change tending to make it less negative is called depolarization.

As stated above, the Nernst potential is the potential difference that exists across a membrane when a particular ion, having reached equilibrium between the tendency to diffuse down its concentration gradient and the tendency to be drawn back by other ions, is in a state of no net flux. The plasma membrane of the neuron is highly permeable to K^+, and in fact the recorded membrane potential of most neurons (−60 to −75 mV) is close to that predicted by the Nernst equation for K^+. However, it is not exactly the same, because K^+ is not the only ion affecting the membrane potential. The membrane is also slightly permeable to Na^+ and Cl^-. The permeability to Na^+ may be low, but the high concentration of this cation outside the cell and the slightly negative electric charge inside the cell tend to drive Na^+ inward. This in turn causes the inside of the cell to depolarize, placing K^+ out of equilibrium. As a consequence, K^+ leaves the cell

until an equilibrium state is reached in which the leak inward of Na^+ is equaled by the leak outward of K^+ and there is no net flux of ions. There is also a tendency for Cl^- to permeate the membrane, since that ion is at higher concentration outside the neuron than inside. Therefore, for an equilibrium state to be produced, the sum of all three net currents must equal zero.

Given the concentrations of all three ions on each side of the membrane and the relative permeability of the membrane to each ion, researchers can calculate the combined effect of K^+, Na^+, and Cl^- on the membrane potential by using the so-called constant-field equation. This equation, by including relative permeability as an important factor, takes into account the phenomenon that the more permeable a membrane is to a particular ion, the greater is the influence of that ion on the membrane potential. The permeance of Na^+, for example, is only a fraction of that of K^+, and the permeance of Cl^- is lower yet; therefore, while the membrane potential is highly sensitive to changes in the concentration of K^+, it is less affected by changes in Na^+ and almost unaffected by changes in Cl^-.

Evolution and development of the nervous system:

The study of the evolutionary development of the nervous system traditionally concentrated on the structural differences that exist at various levels of the phylogenetic scale, but certain functional characteristics, including biochemical and biophysical processes laid down early in evolution and amazingly well conserved to the present, can no longer be ignored. Two basic aspects of the evolution of the nervous system must be considered: first, how primitive systems serve newer functions, and, second, how the formation of new systems serves newer functional requirements.

Early theories on the evolutionary origin of the nervous system argued for a three-stage process: first, the development of non-nervous "independent effectors," such as muscle cells; second, the appearance of non-nervous receptors responding to certain modalities in a receptor-effector mechanism; and finally, the formation of a "protoneuron," from which primitive nerves and ganglia evolved. This model is no longer considered valid. In primitive systems there appear to be many examples of non-nervous electrical conduction. For instance, large areas of epithelium covering the swimming bells in the hydrozoan order Siphonophora (which contains certain families of jellyfish) contain neither nerve nor muscle, yet depolarizing potentials between cells of the epithelium have been recorded. Similar examples from other systems in related orders suggest that the evolution of the nervous system may have begun with non-

nervous epithelial tissue. The conduction of electrical potentials from one epithelial cell to the next may well have been via tight junctions, in which the plasma membranes of adjacent cells fuse to form cellular sheets. Tight junctions have low electrical resistance and high permeability to molecules. They also occur in large numbers in embryos, suggesting that the electrical potentials of cells joined in this manner serve as a driving force for the movement of ions and even nutritive substances from one cell to the next. These phenomena suggest that electrically mediated junctional transmission is older than chemically mediated synaptic transmission, which would require that some epithelial cells secrete chemical substances.

Many investigators suspect that neurons originated from endothelial secretory cells that could secrete chemical substances, respond to stimulation, and conduct impulses. Specialization may then have brought about an outer receptor surface and an inner conducting fibre. In fact, neurosecretory cells can propagate action potentials, and many neurons secrete chemical substances, called neurohormones, that influence the growth and regeneration of cells at other sites of the body. Some researchers suggest that neurons may have first appeared as neurosecretory growth-regulating cells in which elongated processes were later adapted to rapid conduction and chemical transmission by release of neurotransmitters at their endings. There is an amazing consistency in neurotransmitters present in different organisms of a given phylum, although different phyla may show striking differences. Thus, in vertebrates, including fishes, amphibians, reptiles, birds, and mammals, the motor neurons (neurons whose fibres innervate striated muscle) are always cholinergic (that is, they secrete the neurotransmitter acetylcholine). In arthropods, on the other hand, they are not cholinergic, although the sensory neurons do secrete acetylcholine. The number of known neurotransmitters in the animal kingdom is small, and their presence in more primitive organisms as well as in nervous systems of later vertebrates shows a striking conservation of these substances throughout evolution.

If later organisms evolved from single-celled ancestors, then there must have been some system for the transmission of information from one evolutionary stage to the next. These conditions have been defined as: (1) a stable means for encoding, transmitting, and decoding characteristics from one generation to the next, (2) the possibility of alterations in the code taking place by mutation or sexual recombination, and (3) a means of selecting only those characteristics for transmission that are favourable for survival. As mentioned in the section Stimulus-response coordination, protozoans (single-celled organisms) move toward places that are favourable for survival, such as areas with optimal conditions of light and temperature. As the metazoans (multicelled organisms) developed, entire groups of cells probably tended to move toward favourable conditions, and when the number of cells became very large, a system of

internal communication—in effect, a nervous system—developed. Two general types developed: the diffuse nervous system and the centralized nervous system.

Diffuse nervous systems:

The diffuse nervous system is the most primitive nervous system. In diffuse systems nerve cells are distributed throughout the organism, usually beneath the outer epidermal layer. Large concentrations of nerve cells—as in the brain—are not found in these systems, though there may be ganglia, or small local concentrations of neurons. Diffuse systems are found in cnidarians (hydroids, jellyfish, sea anemones, corals) and in ctenophores, or comb jellies. However, the primitive nervous systems of these organisms do not preclude prolonged and coordinated responses and integrated behaviour to the simplest stimuli. An example is the movement of the sea anemone *Calliactis* onto the shell of the hermit crab *Pagurus* in response to a factor present in the outer layer of the empty mollusk shell occupied by the crab. This movement requires integration of the highest order.

Most cnidarians, such as those of the genus *Hydra*, have what is called a nerve net—a meshlike system of individual and separate nerve cells and fibres dispersed over the organism. Species of *Hydra* have two nets, one located between the epidermis and the musculature and the second associated with the gastrodermis. Connections occur at various points between the two nets, individual neurons making contact but not fusing, thereby forming structures similar to the chemically mediated synapses of vertebrates. Several specializations occur within various species. In *Hydra* the neurons are slightly more concentrated in a ring near the pedal disk and the hypostome (the “mouth”), but in jellyfish of a related genus the nerve fibres form a thick ring at the margin of the bell to form “through” conduction pathways. The nervous systems of cnidarians correspond to their radially symmetrical bodies, in which similar parts are arranged symmetrically around a hollow gut cavity called the coelenteron. In some species nerve fibres course along the radial canals, where there may be arranged sensory bodies, called rhopalia, which contain ganglionic concentrations of neurons. In the sea anemone *Metridium* some of the nerve fibres are 7 to 8 mm (0.3 inch) long and form a system for fast conduction of nerve impulses. Such specializations may have allowed the evolution of different functions. Rapid coordination of swimming movements requires a fast-conducting pathway, while feeding relies on the nerve net. Integrative activity is likely to occur at the sensory ganglia, which may represent the first forms of a centralized nervous system.

The terminals forming synapselike structures in nerve nets contain synaptic vesicles that are thought to be packed with neurotransmitters and neuroactive peptides. Peptides present in *Hydra* nervous systems also exist in mammalian systems as neuromodulators, neurohormones, or even possible neurotransmitters. Transmission in the nerve net is relatively slow compared with that in other nervous systems (0.04 metre per second in radial fibres of *Calliactis* compared with 100 metres per second in some fibres of the dog). Many repetitive stimuli may be required to elicit responses at these synapses. Long refractory periods are also characteristic of nerve nets, having durations about 150 to 300 times those seen at mammalian nerve fibres. Finally, pacemaker systems are present in animals with nerve nets. In the sea anemone *Metridium* these systems are expressed in a series of spontaneous rhythmic movements that occur in the absence of any detectable stimulus. It is not known whether the movements originate from a "command" neuron or group of neurons or whether they arise without neuronal stimulation. It has been postulated that pacemaking cells were present in epithelial conducting systems known not to be nervous but that eventually evolved into neuronal tissue.

Centralized nervous systems:

The development of the nerve net allowed an organism to engage in several different behaviours, including feeding and swimming. The development in the net of rapidly conducting bundles of fibres and of pacemaker systems allowed rapid withdrawal and rhythmic swimming activities, respectively, in some cnidarians. However, it is at the level of the flatworms (phylum Platyhelminthes) that there appears a longitudinal nerve cord and an anterior collection of nerve cells that can be called a brain. Furthermore, there are well-defined sensory and motor pathways as well as coordinating interneurons. Although nerve nets and pacemaker activity are still present in the flatworms, the presence of ganglia or a brain concentrated at the cephalic (head) end of the organisms represents a simple beginning to the complex centralized systems that develop at higher levels of the phylogenetic tree.

Simple bilateral systems:

The flatworms were the first invertebrates to exhibit bilateral symmetry and also the first to develop a central nervous system with a brain. The nervous system of a free-living flatworm such as *Planaria* consists of a brain, longitudinal nerve cords, and peripheral nerve plexuses (interlacing networks of peripheral nerves; from Latin *plectere*, "to braid"). The brain, located in the anterior portion of the animal, is composed of two cephalic ganglia joined by a broad connection called a commissure.

Longitudinal nerve cords, usually three to five pairs, extend posteriorly from the brain; they are connected by transverse commissures, and smaller, lateral nerves extend from the cords. The lateral nerves give rise to the peripheral nerve plexuses. The submuscular nerve plexus—consisting of sensory cells, ganglion cells, and their processes—is situated in the loose tissue (mesenchyme) below the subepidermal musculature. Another subepidermal plexus is located at the bases of the epithelial cells above the muscular layer.

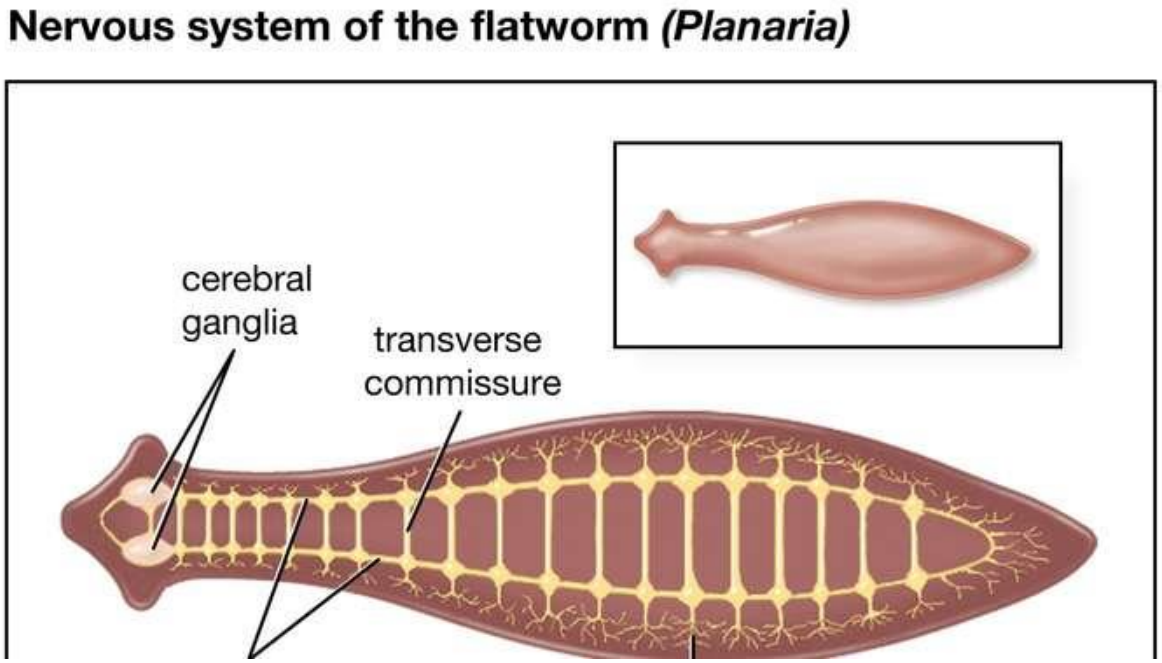


Fig: planarian nervous system : In the flatworm *Planaria*, the brain consists of two cerebral ganglia (clusters of nerve cells) from which nerve cords extend the length of the body.

Planaria are richly supplied with sensory receptors. Single sensory cells in the nerve plexuses are widely scattered over the organism. Sensory organs also are present and include ciliated pits and grooves, auricles, the frontal organ, statocyst, and eyes. The ciliated pits and grooves contain chemical receptors, or chemoreceptors, which permit the animal to detect food. The statocyst is responsible for balance and such reactions as rising to the surface of the water or sinking. The eyes, or ocelli, may occur as a pair situated anteriorly or may be scattered abundantly over the head region depending on the species. Short optic nerves connect the eyes with the brain. Seven types of nerve cell bodies and two types of neuroglia have been described in *Planaria*. Removal of the brain results in the abolition of such functions as food finding and recognition and severe deficits in locomotion. However, the nerve cords by themselves can mediate a

certain amount of locomotion as well as righting and avoidance reactions. Nematodes (phylum Aschelminthes) have a high degree of centralization, with three-quarters of all nerve cells concentrated in a group of anteriorly placed ganglia and no peripheral plexuses or nets. They usually have eight longitudinal cords, commissures between dorsal and ventral cords, six cephalic nerves, a few special ganglia and nerves in the tail, and two sympathetic systems (one anterior and one posterior).

Moderately cephalized systems:

Basic similarities in the nervous systems of the annelid worms, molluscs, and arthropods include an anteriorly situated brain, connectives running from the brain around the esophagus and joining paired longitudinal cords, and ventral nerve cords with ganglia along their length. The trend toward greater centralization and cephalization of nervous functions is continued within these groups, reaching its peak in the higher molluscs and arthropods.

Annelids:

The brain of most annelids (phylum Annelida; segmented worms, including the leeches and terrestrial earthworms) is relatively simple in structure. The earthworm brain is a bilobed mass lying above the pharynx in the third body segment. Sensory nerves leave the brain and run forward into the prostomium (extreme anterior end) and first segment. The brain of the active, predatory polychaetes (a class of marine worms) is more complicated. In some the brain can be divided into a forebrain, a midbrain, and a hindbrain; a single pair of circumesophageal or circumpharyngeal connectives leave the brain, surround the anterior gut, and connect with the ventral nerve cord.

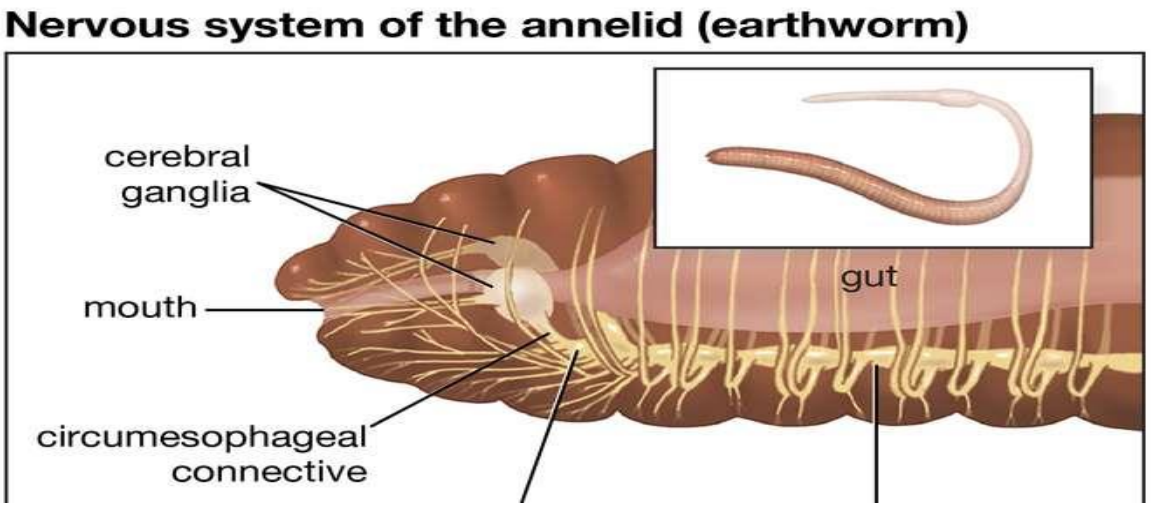


Fig: Annelid nervous system : In most annelids (segmented worms) such as the earthworm, two cerebral ganglia (bundles of nerve cells) form a primitive bilobed brain, from which sensory and motor nerve fibres lead to other areas of the body.

The most primitive annelids have a pair of ventral nerve cords joined by transverse connectives; the most advanced forms have the cords fused to form a single cord. A ganglionic swelling of the cord is found in each body segment, with the most anterior ganglion, the subpharyngeal ganglion, being the most prominent. Two to five pairs of lateral nerves leave each ganglion to innervate the body wall of that segment. A subepidermal nerve plexus occurs over the whole body. Another plexus, called the enteric, stomodaeal, or sympathetic system, is found in the wall of the gut.

Giant axons, usually few in number, travel the length of the cord. They may belong to one cell or be composed of many neurons. These axons are capable of very rapid conduction of impulses to the segmental muscles; their main function is to permit the worm to contract very rapidly as a defence against predators. The usual slow crawling movements of worms are mediated by a series of reflex arcs. During crawling, the contraction of muscles in one segment stimulates stretch receptors in the muscle. Impulses are carried over sensory nerves to the cord, causing motor neurons to send impulses to the longitudinal muscles, which then contract. The longitudinal pull activates stretch receptors in the following segment, and a wave of contraction moves along the worm. Studies of the nervous systems of annelids show certain behavioral capabilities, including perception, motor coordination, and learning. Because the neuronal organization behind these capabilities can be deduced, they may give an indication of the mechanisms underlying similar patterns of activity and behaviours at other levels of the phylogenetic scale.

Two rhythmic movements generated by the leech, the heartbeat and swimming rhythm, have been extensively studied. The coordinated heartbeat rhythm is produced by heart excitor motor neurons, which show rhythmic activity in which bursts of action potentials alternate with bursts of inhibitory synaptic potentials derived from rhythmically firing inhibitory interneurons. The heartbeat appears to be produced by a central rhythm generator. The swimming movement, on the other hand, is generated by a neuronal network requiring many more cells. These neuronal oscillators may form the basis for neuronal generators of rhythmic movements in other animals at higher levels of the phylogenetic scale.

Simple mollusks:

The nervous systems of the more primitive molluscs (snails, slugs, and bivalves, such as clams and mussels) conform to the basic annelid plan but are modified to conform with the unusual anatomy of these animals. In snails a pair of cerebral ganglia constitutes the brain, which overlies the esophagus. Nerves leave the brain anteriorly to supply the eyes, tentacles, and a pair of buccal ganglia. These last ganglia, also called the stomatogastric head ganglia, innervate the pharynx, salivary glands, and a plexus on the esophagus and stomach. Other nerve cords—the pedal cords—leave the cerebral ganglia ventrally and terminate in a pair of pedal ganglia, which innervate the foot muscles. Another pair of nerve cords—the visceral cords—leave the brain and run posteriorly to the visceral ganglia. The pleural ganglion, supplying the mantle, or fleshy lining of the shell, and the parietal ganglion, innervating the lateral body wall and mantle, are located along the visceral nerves. Intestinal ganglia connected with the pleural ganglia innervate the gills, osphradium (a chemical sense organ), and mantle. Sense organs of snails include eyes, tentacles, statocysts, and osphradia.

In the bivalves, a cerebropleural ganglion is situated on either side of the esophagus. An upper pair of nerve cords leaves these ganglia and runs posteriorly to the visceroparietal, or visceral, ganglia. The visceral ganglia supply the mantle, adductor muscles (which close the shell), and internal organs. A second pair of nerve cords travels ventrally to the pedal ganglia. Most of the sense organs are found at the edge of the mantle. In the scallop, for example, the eyes are set in a row. They are well developed and consist of a cornea, a lens, and a retina, in which the photoreceptor cells are not placed superficially (an arrangement much like that in the vertebrate retina).

Elementary forms of learning and memory have been studied at a cellular level by analysis of the neuronal activity of the marine snail (*Aplysia californica*). This simple mollusk withdraws its gill and siphon in

response to a mild tactile stimulus. The neural circuit for this reflex consists of a sensory component from the siphon that forms single-synapse junctions with motor neurons that cause the gill to withdraw. The sensory cells also project onto interneurons whose outputs converge onto the same motor neurons. In response to a stimulus, the sensory neurons generate large excitatory postsynaptic potentials at both interneurons and motor neurons, causing the generation of action potentials in the motor neurons that in turn cause the gill to withdraw. When the stimulus is repeated many times, the postsynaptic potentials become reduced in size and the response becomes weaker. Finally, the postsynaptic potentials become so small that action potentials are no longer generated and the gill no longer responds. This reduced behavioral response is known as habituation. Habituation may be caused by the closing of calcium channels, which decreases calcium influx into the presynaptic terminals and, therefore, decreases neurotransmitter release. Other evidence suggests that habituation results from fewer neurons in the network being activated. Another behavioral paradigm, sensitization, has also been examined in *Aplysia*. In sensitization the reflex activity increases in strength with added stimulation. The mechanism underlying this response is presynaptic facilitation, which is thought to be caused by an increase in the second messenger cAMP in the terminals of the sensory neurons.

These two examples—habituation and sensitization—show that important features of a more complex nervous systems can be studied in organisms at lower stages of evolution. First is what can be called the plasticity of the nervous system, the phenomenon of changes occurring in the strength of synaptic responses. Changes in synaptic efficacy may underlie certain mechanisms for short- and long-term memory—even in more complex animals such as humans. Changes in the structure of the synapse may be a long-term effect of plasticity. For example, the numbers of active zones at nerve terminals are reduced with long-term habituation but increased with long-term sensitization. Finally, the molecular mechanisms underlying these changes may be the same or at least similar at all levels of the phylogenetic tree. Habituation of the escape response has been seen in polychaete worms, cockroaches, and crayfish.

Complexly compartmentalized systems

The highest degree of development of the invertebrate nervous system is attained by the cephalopods (squids, cuttlefishes, and octopuses) among the molluscs and by the insects and spiders among the arthropods. Although the basic plan of these nervous systems is similar to that of the annelids, there are several advances. First, there is a high degree of cephalization, with nervous functions concentrated in the head region of the animal. In addition, ganglia are fused and farther forward,

and nerve cells, less abundant in the peripheral nervous system, are situated in the brain or ganglia so that the nerve cords consist only of nerve fibres. Finally, control and coordination of specific functions, such as locomotion and feeding, are compartmentalized in particular parts of the nervous system.

Complex mollusks:

The complex nervous system of the cephalopods is correlated with the active movement and predatory habits of these organisms. Most of the ganglia typical of molluscs are concentrated or fused in a brain that encircles the esophagus. Nerves extend from the brain to ganglia at the base of the arms or tentacles and from the ganglia the length of the arms. A pair of large pallial nerves connects the brain with a pair of stellate ganglia on the inner surface of the mantle. The stomatogastric ganglia supply nerves to the digestive tract.

A great variety of functions are centralized in the brain and compartmentalized to specific brain regions. These activities may be local, simple, and uncoordinated with other regions or may be extensive, complex, and coordinated, involving large groups of muscles. The highest centres of the cephalopod brain are the associative areas, which are thought to be involved with discrimination between objects, learning, and memory. The giant-fibre system—also seen in earthworms and insects—is very well developed in the squid. The diameter of giant fibres is many times greater than the diameter of most other nerve fibres. Giant neurons in the brain send fibres to the retractor muscles of the head and the funnel or to the stellate ganglion. Fibres from the stellate ganglion fuse to form giant fibres that innervate the mantle. Because of their large size, these fibres are capable of rapid conduction, which, in turn, permits extremely rapid movement. The eyes of cephalopods are especially well developed and bear close resemblance to the vertebrate eye. The eye fits into a socket of cartilaginous plates separate from the cartilages that protect the brain, and external muscles permit its movement. A transparent cornea covers the surface and can be focused for both near and far objects. There is a pupil formed by an iris diaphragm, which can regulate the amount of light reaching the retina. The retina contains light-sensitive cells. The axons of the photoreceptors, or rod cells, form the optic nerves, which terminate in the extremely large optic lobes of the brain.

The cephalopods are strikingly different in many respects from other molluscan classes. The nervous system as described above is more highly developed and, consequently, the behavioral repertoire much more complex. First, the animals are predators; they move, they use their eyes in search of food, they use

receptors in their arms for detection of tactile or chemical stimuli, and they have exceptionally fast muscle action. Second, they have an enormous flexibility of response, discriminating between palatable and unpalatable prey and "learning" to attack or not to attack. They can also change colour to blend into their environment if needed. The molluscs as a whole provide an important link in the developing complexity of the nervous system. Indeed, the presence in their systems of vertebrate as well as natural molluscan neuroactive peptides may give some clue to the true place of these animals in the phylogenetic scale.

Arthropods:

The other complex compartmentalized nervous system is found in arthropods. The arthropodan brain consists of three main regions: the protocerebrum, deutocerebrum, and tritocerebrum. The anterior protocerebrum, which receives the nerves of the eyes and other organs, contains centres, or neuropils, such as the optic centres and bodies known as corpora pedunculata. The neuropils function as integrative systems for the anterior sense organs, especially the eyes, and in control of movement; they also are the centres for the initiation of complex behaviour. The deutocerebrum contains the association centres for the first antennae. The posterior tritocerebrum contains association neuropils for the second antennae (of crustaceans) and gives rise to nerves that innervate the mouthparts and the anterior digestive canal. The latter constitute the stomatogastric system, which regulates the intake of food and the movement of the gut necessary for digestion. This system bears a resemblance to the vertebrate autonomic system.

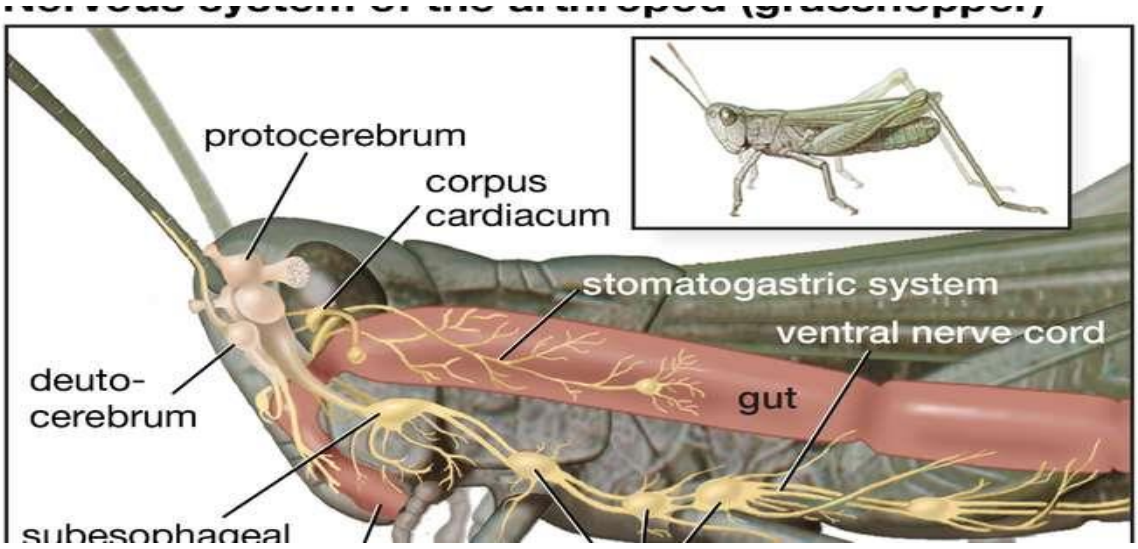


Fig: Arthropod nervous system: In arthropods such as the grasshopper, the brain is divided into specialized areas, including the protocerebrum (controlling vision) and the deutocerebrum (processing antenna signals). The stomatogastric system regulates digestion.

The ventral nerve cord, connected to the brain by the circumesophageal connectives, is composed of a double row of ganglia connected longitudinally by connectives and transversely by commissures. Different groups of arthropods exhibit different degrees of fusion of the ganglia. In insects the first ganglion, the subesophageal, is formed by fusion of three pairs of ganglia; it sends nerves to the mouthparts and to the salivary glands. The segmental ganglia in the thorax and abdomen provide nerves to the appendages, dorsal muscles, sense organs, and heart. Insects have 3 pairs of thoracic ganglia and up to 10 abdominal ganglia.

The most common sensory receptors in arthropods are the cuticular hairs, many of which are mechanoreceptors, sensitive to touch, vibration, water currents, or sound waves; some hairs are chemoreceptors, which detect odours or chemicals in the water. Hairs situated near the joints are stimulated by body movements and thus provide a sense of the position of the joint or appendage during locomotion or flight. Many sensory cells and organs are concentrated in the antennae, and a statocyst is found at the base of each antenna. Spiders have several pairs of simple eyes with cup-shaped retinas. Crustaceans and insects, however, have a pair of well-developed compound eyes, each consisting of a large number of visual units called ommatidia. Each ommatidium contains six to eight sensory receptors arranged under a cornea and refractile cone and is surrounded by pigment cells, which adjust the intensity of light. Each ommatidium can act as a separate eye and is capable of responding to its own visual field. Such an arrangement seems particularly well suited for detecting movement across a wide visual field.

In spite of the small size of insects, some of their nerve cells and axons are larger in diameter than any neuron in the human nervous system. The number of insect neurons is relatively small, so that each neuron must be capable of dealing with a maximum amount of information. Evasive behaviour requires mechanisms of this sort—as exemplified by the jumping muscle of the grasshopper, which is supplied by only a few motor axons, while those muscles of a human required for a similar purpose have tens of thousands of axons. Another example is the ear of a noctuid moth. Each ear is essentially a tympanic membrane forming the outer wall of an air-filled cavity in the thorax. A five-tissue strand, the acoustic sensillum, runs from the centre of the tympanic membrane across the tympanic cavity to a nearby skeletal support. This sensillum has two acoustic sensory receptors, called A cells. From the central end of each A

cell, an axon passes within the sensillum to the skeletal support and then in the tympanic nerve to the thoracic ganglia of the moth.

The A cells encode the intensity of ultrasound by the frequency with which they fire action potentials. Each ear is capable of responding differently to different stimuli, so that differences between the two ears in the duration of the action potentials and response times could allow for binaural detection of the source of a sound. The impulses are conducted to the pterothoracic ganglion, where they must influence muscles used for avoidance of predators. The important point here is that two A cells provide enough information to allow the moth to take evasive behaviour. Clearly, simplicity of the neural circuitry is required for speedy response; while some information may be sacrificed, escape will not be compromised. In fact, the quick evasion of predators has probably influenced the evolution of the giant-fibre systems of worms and squid as well as crustaceans and insects. These giant fibres conduct impulses at much higher conduction velocities than do smaller axons, while the information-handling capacity of many small axons acting together is far greater than that of giant axons. Under these circumstances it is clear that different systems evolved in the invertebrate nervous system in response to different qualities of stimuli in the environment to which the organism had to react—one responsible for survival and the other for information. Neurosecretory cells, which have been identified in all the major invertebrate groups, reach their highest degree of development in the arthropods. The principal system of insects consists of neurosecretory cells in the protocerebrum of the brain. The axons of these cells form nerves that innervate structures called corpora cardiaca, situated just posterior to the brain. A corpus cardiacum is an organ in which neurosecretory products are stored in the nerve terminals for later release into the vascular system. The neurosecretory cells of the protocerebrum manufacture a hormone called ecdysiotropin, or brain hormone. Sensory nerve impulses reaching the brain regulate the release of the hormone from the nerve endings into the blood within the corpora cardiaca. The hormone then stimulates a non-neural endocrine gland, the ecdysial gland, located in the thorax. The ecdysial glands in turn release the hormone ecdysone, which initiates molting during larval development and also stimulates differentiation into adult tissues. Another hormone, however, the juvenile hormone, keeps tissue in a juvenile or larval form. This hormone is released by the corpora allata, another pair of non-neural endocrine glands, located behind the corpora cardiaca. The successive life stages in insect development are, therefore, determined by the varying levels of ecdysone and juvenile hormone.

The vertebrate nervous system:

The nervous system of vertebrates has two main divisions: the central nervous system, consisting of the brain and spinal cord, and the peripheral nervous system, which in humans includes 12 pairs of cranial nerves, 31 pairs of spinal nerves, and the autonomic, or involuntary, nervous system. Anatomic structures such as the nervous system are described according to their position. In four-legged animals the upper (back) surface is called dorsal and the lower (belly) surface ventral. The terms *anterior*, *cranial*, *cephalic*, and *rostral* refer to the head end of the body, *posterior* and *caudal* to the tail end. In humans, since they stand erect, the situation is more complicated: dorsal becomes equivalent to posterior, and ventral is the same as anterior; cranial is often called superior, and caudal inferior. Objects near the middle plane of the body are medial and those farther away are lateral. *Proximal* refers to structures nearest the central bulk of a structure and *distal* to ones away from it. In referring to another structure, if it is located on the same side of the body, it is known as ipsilateral; if it is on the opposite side, it is contralateral.

Neurons are often gathered into localized masses. In the peripheral nervous system these accumulations are called ganglia; in the central nervous system they are called nuclei. Portions of the central nervous system in which unmyelinated neurons and neuroglia predominate are called gray matter; areas in which myelinated neurons dominate are called white matter. Efferent, or motor, nerve fibres carry impulses away from the central nervous system; afferent, or sensory, fibres carry impulses toward the central nervous system. Visceral fibres innervate the viscera such as the heart and intestines, and somatic fibres innervate the body-wall structures such as skin and muscle. In the central nervous system the nerve fibres are organized in bundles called tracts, or fasciculi. Ascending tracts carry impulses along the spinal cord toward the brain, and descending tracts carry them from the brain or higher regions in the spinal cord to lower regions. The tracts are often named according to their origin and termination; for example, the corticospinal tract consists of fibres running from the cerebral cortex in the brain to the spinal cord.

The primitive condition:

The vertebrates constitute an advanced subdivision of the phylum Chordata. All chordates at some time in their life have a rodlike bar called the notochord running the length of the body. Lower chordates (acorn worms, tunicates, and amphioxus), which lack a vertebral column, illustrate the most primitive features of the chordate nervous system. In these animals the nerve cord is a rather uniform-appearing dorsally placed

tube with a hollow cavity, which corresponds roughly to the spinal cord of the vertebrates, suggesting that the spinal cord is the most primitive component of the central nervous system.

In amphioxus and in lower vertebrates such as lampreys, the sensory fibres and motor fibres leave the cord in dorsal and ventral roots to supply the adjacent body segments called myotomes. The dorsal and ventral roots remain separate nerves and arise at alternate positions along the cord. In lower fishes there is still alternation of dorsal and ventral roots, but the roots unite in a single spinal nerve. In higher vertebrates the two roots unite in a single spinal nerve and leave the cord at the same level, one above the other. Each spinal nerve supplies a single myotome. When appendages (fins, wings, arms, and legs) develop from several myotomes, the nerves continue to supply their original segments, and branches of the spinal nerves become interwoven to form plexuses. The brain of vertebrates developed by the accumulation of nerve cells at the cephalic end of the nerve cord. At first this diffuse collection of nerve cells regulated the reflex activity of spinal motor neurons. These cells are comparable to the reticular formation occupying the brainstem of higher vertebrates. The brainstem, thus, is the oldest portion of the brain.

Encephalization:

Early in the evolution of vertebrates, a special sensory system became associated with each major part of the brain: the olfactory organs with the forebrain, the eye with the midbrain, and the ear and related organs with the hindbrain. Each of the three sections, furthermore, developed dorsal outgrowths of gray matter forming, respectively, the cerebrum, the midbrain roof, or tectum, and the cerebellum. With these developments the three-part brainstem was then transformed into a brain of five regions: telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon. The addition of these nerve centres to the primitive brainstem allowed greater coordination and association between the sensory and motor fibres.

In tracing the development of the parts of the brain in the different vertebrate classes, some general features are apparent. There is a correlation between the size of a particular part of the brain and its importance in the functions of an animal. Some neural structures (e.g., the olfactory bulb) have considerable size and importance in more-primitive animals but are less conspicuous in most recent animals. Progressing from primitive to recent animals, there is a gradual cephalic shift of function from the lower brainstem to the higher cerebral cortex.

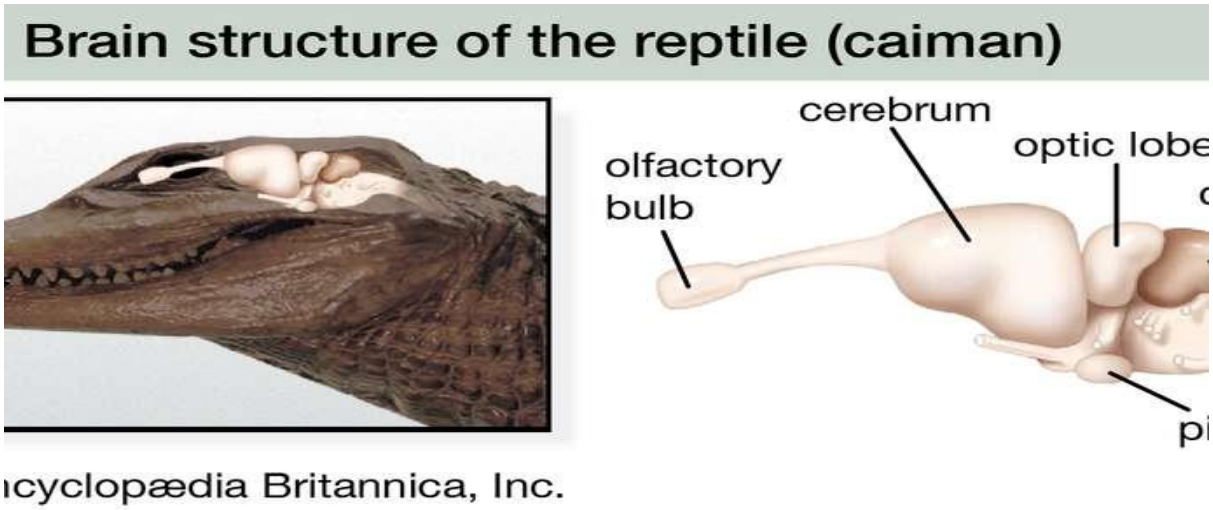


Fig: Reptilian brain structure: In the brain of reptiles such as the caiman (related to the alligator and crocodile), the olfactory bulb (regulating the sense of smell) remains a large and important structure.

The hindbrain is comparable to an enlarged, anterior section of the spinal cord. In the gray matter are dorsal sensory and ventral motor columns similar to those present in the cord. The longitudinal continuity of these columns is preserved in the earlier vertebrates, but in more recent vertebrates the columns break up into discrete nuclei that serve some of the cranial nerves. The hindbrain exerts partial control over the spinal motor neurons through the reticular formation. Fish and tailed amphibians, in addition, have a pair of giant cells called the cells of Mauthner, which exert some control over the local spinal-cord reflexes responsible for the rhythmic swimming undulations and the flip-tail escape response characteristic of these animals. The hindbrain is the area of reception of one of the main sensory systems, the acoustico-lateralis system, which consists of the ear (hearing and equilibrium) and the lateral-line organs (vibration and pressure). The latter, situated in rows along the head and body, are retained in fish but disappear in the land vertebrates.

The cerebellum originated as a specialized part of the acoustico-lateralis area. The oldest part of the cerebellum—the archicerebellum—is concerned with equilibrium and connected with the inner ear and the lateral-line system. The anterior lobe of the cerebellum represents the paleocerebellum, an area that regulates equilibrium and muscle tone. It constitutes the main mass of the cerebellum in fish, reptiles, and birds. In mammals the development of the cerebral cortex and its connections with the cerebellum are correlated with the appearance of the large cerebellar hemispheres. This new part of the cerebellum, or neocerebellum, coordinates skilled movements initiated at cortical levels. In mammals a great mass of fibres connects the brainstem to the cerebellum. This region forms the pons, which, together with the

cerebellum, constitutes the metencephalon. The caudal part of the hindbrain remains as the medulla oblongata (myelencephalon).

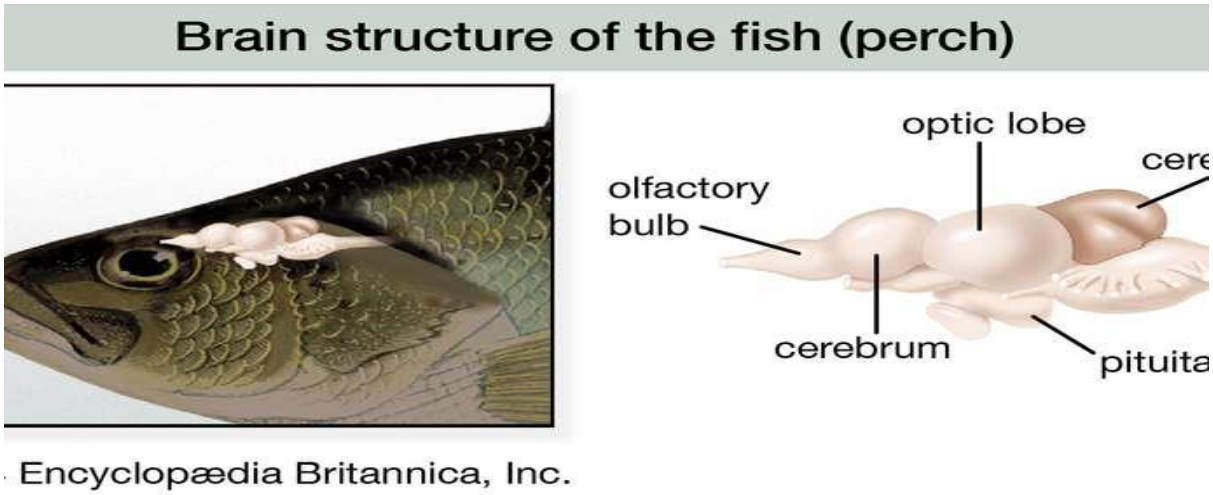


Fig: Brain structure of the fish: The cerebellum is a prominent structure in the brain of fish, indicating the importance of such functions as sensing pressure, maintaining balance, and regulating muscle movement.

The midbrain (mesencephalon) and the diencephalon constitute the anterior portion of the brainstem. Sensory and motor nuclei for cranial nerves extend from the hindbrain to the midbrain. The roof of the midbrain, or tectum, developed as the primary visual centre. The optic lobes, especially prominent in fish and birds, are a part of this area. In fish and amphibians the tectum is the major centre of the nervous system and wields the greatest influence on body activity. While this area is still significant in reptiles and birds, it is supplanted in importance by the cerebral hemispheres. In mammals most of the optic sensations are relayed to the cerebral cortex. With development of the cerebral cortex, the thalamus becomes less significant as an association area and more important as a relay centre for sensory impulses. Centres for visceral sensations and visceral motor responses become established in the hypothalamus.

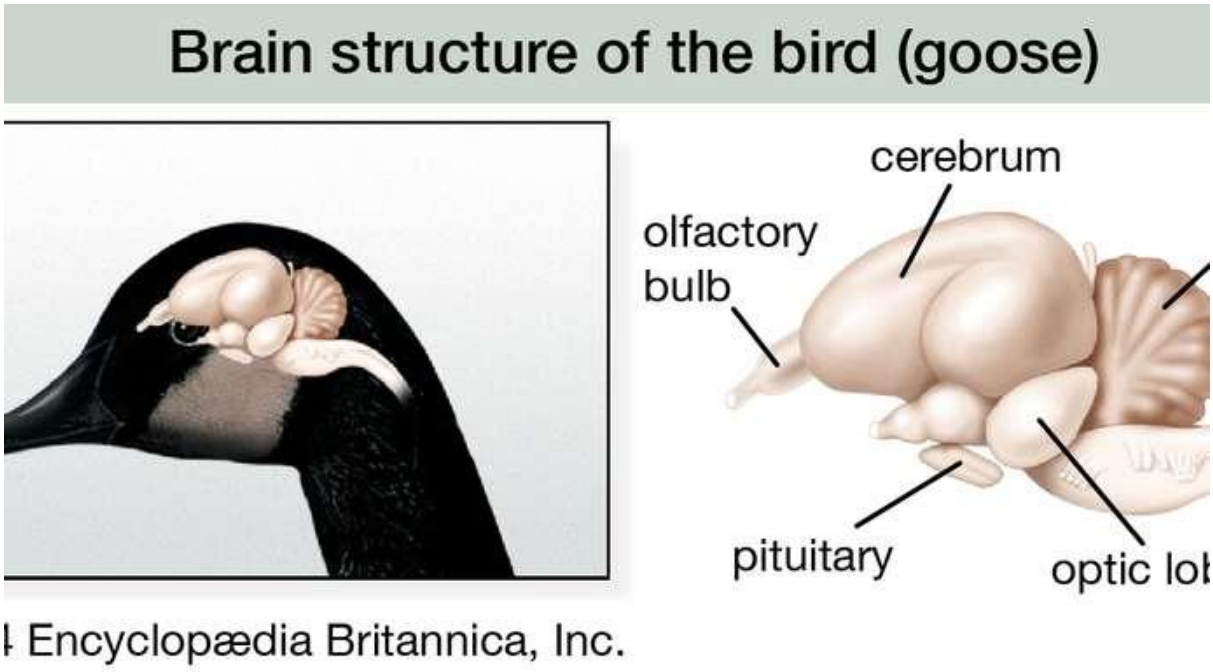


Fig: Brain structure of the bird: In the brain of a bird, the optic lobe remains an important fuctional centre, but it is surpassed in size and importance by the lobes of the cerebrum.

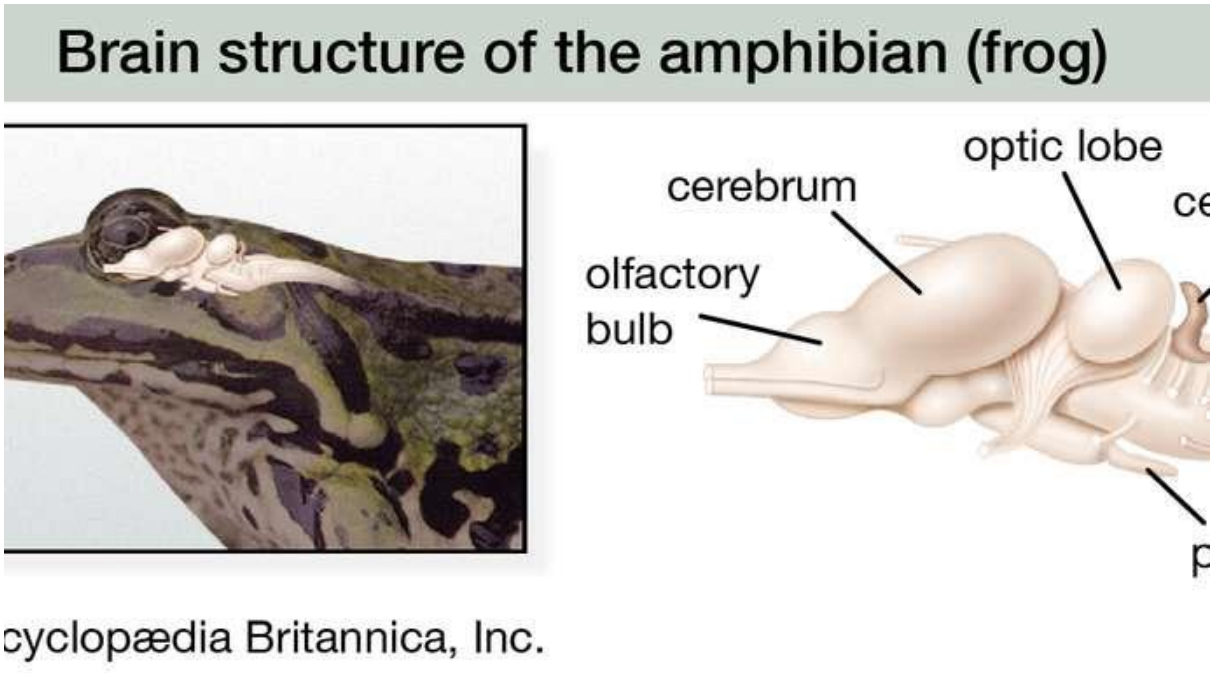


Fig: amphibian brain structure: In amphibians such as the frog, the midbrain, containing the optic lobe, is the main functional area of the brain.

Dominance of the cerebrum:

Ascending the vertebrate scale, the cerebral hemispheres become more and more important as association centres. The cerebral hemispheres begin their development as paired outgrowths of the forebrain and serve as centres of olfactory reception. In the older vertebrates the forebrain is divided into the olfactory bulb—where the olfactory nerve fibres end—and the cerebral hemisphere. The hemisphere at this time, referred to as the paleopallium, is merely an olfactory lobe serving as an association area for olfactory impulses. The olfactory lobes are prominent in animals such as amphibians, but in birds and primates in which the sense of smell is less important the lobes are reduced in extent. In amphibians the hemispheres consist of three parts: the paleopallium (olfactory lobe), the archipallium, and the basal nuclei. All three areas receive olfactory stimuli and discharge impulses to the brainstem. The archipallium is a correlation centre and a forerunner of the mammalian hippocampus. The basal nuclei are equivalent to the corpus striatum and function as association areas with connections to the thalamus. In primitive reptiles the basal nuclei have moved to the inner part of the hemisphere, whereas the other areas of gray matter have moved toward the surface. In advanced reptiles a new association centre, the neopallium, appears between paleopallium and archipallium. In birds there is nothing corresponding to the neopallium, but the basal ganglia (that is, the corpus striatum) are enormously expanded. In mammals the neopallium becomes greatly enlarged, exceeding all the other parts of the brain in size. This region assumes more and more of the higher types of neural activity in correlation, association, and learning. At first the neopallium expands to envelop the other brain structures. The archipallium becomes folded into a small area on the median part of the hemispheres, where it remains as the hippocampus. The paleopallium (olfactory lobes) constitutes a small ventral region on the hemisphere, the pyriform lobe. The corpus striatum (old basal nuclei) becomes a central part of the hemisphere. Further expansion of the neopallium in primates and humans causes extensive folding and results in a very convoluted surface of the brain.

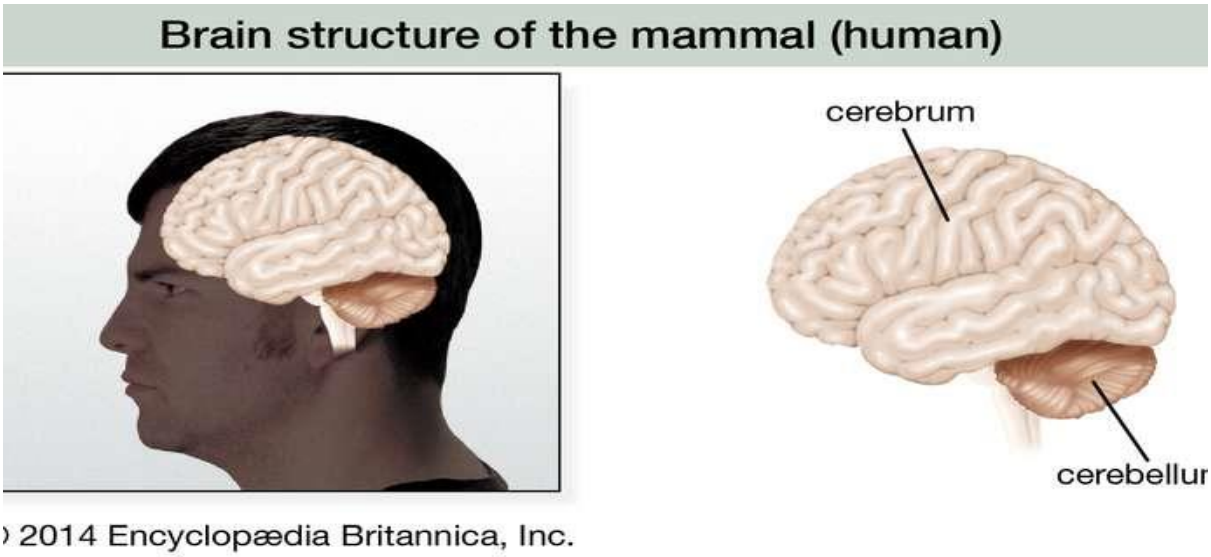


Fig: cerebrum and cerebellum : In the brains of primates such as humans, the cerebrum has grown into the largest region of the brain and has a characteristic lobed appearance.

Probable questions:

1. Describe the structure of a neuron.
2. How nervous system has evolved in amphibia and birds?
3. Why mammals have mostly advanced cerebrum?
4. Describe nervous system evolution in different kinds of mollusks.

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

UNIT-V

Synaptic transmission; types of synapses, Pre and Postsynaptic structure and function, Steps in Synaptic Transmission, chemistry and modes of neurotransmitter release, proteins in synaptic transmission: SNARE hypothesis: synapsins, synaptobrevin, synaptotagmin, SNAP and NSF, synaptic plasticity, toxins in synaptic transmission

Transmission in the neuron:

The discussion above demonstrates that the electrical potential existing in neurons is based on the distribution of ions across the plasma membrane and that this distribution comes about through permeation of the membrane. In fact, ions are almost always hydrated in the form of ion-water complexes, which have great difficulty in penetrating the hydrophobic lipid bilayer of the plasma membrane. Permeation actually occurs through protein structures embedded in the lipid bilayer and spanning the membrane from cytoplasm to extracellular fluid. These structures, sometimes pumping ions from one side to the other and sometimes merely providing channels through which diffusing ions can flow past the lipid molecules, maintain the ionic distribution that keeps the membrane polarized, and they also allow the abrupt changes in distribution that create nerve impulses. The protein structures are described in detail in the section Ion transport. Following is a discussion of the electrical events, created by movement of ions, that lead to nervous transmission in the neuron.

Resting potential:

The electrical potential across the nerve membrane can be measured by placing one microelectrode within the neuron (usually in the soma) and a second microelectrode in the extracellular fluid. The microelectrode consists of a sharp-tipped glass capillary tube filled with conducting solution. Upon penetration of the neuron, the potential at the tip of the electrode becomes electrically negative in relation to the outside of the electrode. As described above and shown in the graph, the value of this negative charge is usually between −60 and −75 mV. This is the membrane potential of the neuron at rest (i.e., when it is not generating a nerve impulse), and for this reason it is called the resting potential.

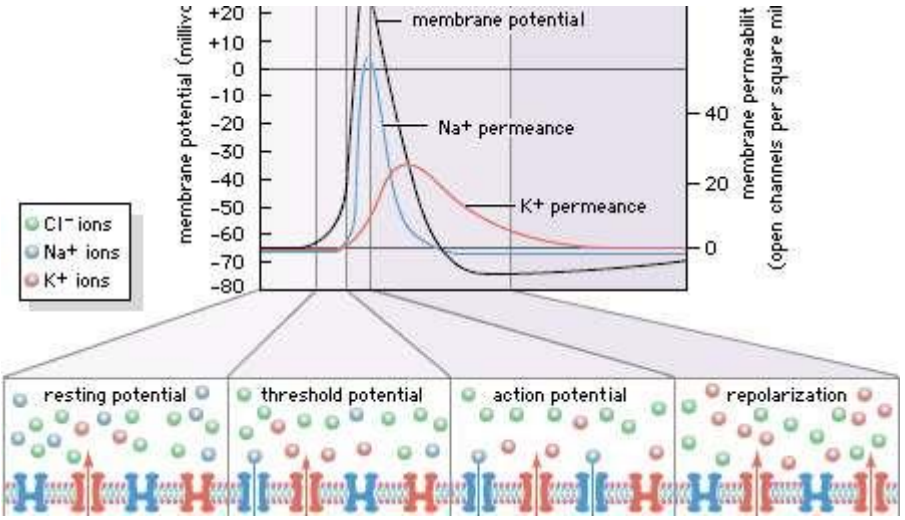


Fig: ion permeance and action potential. Changes in ion permeance underlying the action potential Electrical potential is graded at left in millivolts, ion permeance at right in open channels per square millimetre. At the resting potential, the membrane potential is close to E_K, the equilibrium potential of K^+. When sodium channels open, the membrane depolarizes. When depolarization reaches the threshold potential, it triggers an action potential. Generation of the action potential brings the membrane potential close to E_{Na}, the equilibrium potential of Na^+. When sodium channels close (lowering Na^+ permeance) and potassium channels open (raising K^+ permeance), the membrane repolarizes.

The resting potential is maintained by the sodium-potassium pump, which steadily discharges more positive charge (i.e., Na^+) from the cell than it allows in, and by the relatively high permeance of K^+, which leaks out of the cell through its membrane channels faster than Na^+ leaks in.

Localized potential:

When a physical stimulus, such as touch, taste, or colour, acts on a sensory receptor cell specifically designed to respond to that stimulus, then the energy of the stimulus (e.g., mechanical, chemical, light) is transduced, or transformed, into an electrical response. This response is called the receptor potential, a type of local potential that, when it reaches high enough amplitude, generates the nerve impulse. (Another type of local potential is the postsynaptic potential, which originates in chemical receptors at the synaptic cleft. Sensory receptors transduce stimuli into electrical responses by activating ion channels in their membranes. For example, in the stretch receptors of neurons attached to muscle cells, the stretching

action of the muscle is thought to put a mechanical stress on protein filaments of the cytoskeleton, which in turn alter the shape of ion channels, inducing them to open and allowing cations to diffuse into the cell. Receptor cells sensitive to chemical and light energy, on the other hand, activate ion channels through the second-messenger system. In this system, stimulated receptor molecules on the surface of the cell membrane catalyze a series of enzymatic reactions within the cytoplasm; these reactions in turn release energy, which activates the ion channels.

By permitting a flux of Na^+ into the cell, the opening of ion channels slightly depolarizes the membrane. The extent to which the membrane is depolarized depends upon the extent to which the sodium channels are activated, and this in turn depends upon the strength and duration of the original stimulus at the receptor. If depolarization reaches what is called the threshold potential, it triggers the nerve impulse, or action potential *see below*. If it does not reach that amplitude, then the neuron remains at rest, and the local potential, through a process called passive spread, diffuses along the nerve fibre and back out through the membrane. When it is of the postsynaptic type, the local potential usually begins in the dendrites and spreads toward the soma and axon. It is at the initial segment of the axon where, if the local potential is of threshold amplitude, the nerve impulse is generated.

Action potential:

Depolarization:

Because it varies in amplitude, the local potential is said to be graded. The greater the influx of positive charge—and, consequently, depolarization of the membrane—the higher the grade. Beginning at the resting potential of a neuron (for instance, −75 mV), a local potential can be of any grade up to the threshold potential (for instance, −58 mV). At the threshold, voltage-dependent sodium channels become fully activated, and Na^+ pours into the cell. Almost instantly the membrane actually reverses polarity, and the inside acquires a positive charge in relation to the outside. This reverse polarity constitutes the nerve impulse. It is called the action potential because the positive charge then flows through the cytoplasm, activating sodium channels along the entire length of the nerve fibre. This series of activations, by propagating the action potential along the fibre with virtually no reduction in amplitude, gives the nerve impulse its regenerative property.

Researchers call the nerve impulse an "all-or-none" reaction since there are no gradations between threshold potential and fully activated potential. The neuron is either at rest with a polarized membrane, or it is conducting a nerve impulse at reverse polarization. The reverse polarity of active neurons is measured at about +30 mV. This is close to the Nernst potential for Na^+—that is, the membrane potential at which electrochemical equilibrium would be established if the membrane were completely permeable to Na^+.

Repolarization:

As instantaneous as the opening of sodium channels at threshold potential is their closing at the peak of action potential. This is called sodium inactivation, and it is caused by gates within the channel that are sensitive to depolarization. Following sodium inactivation is the opening of potassium channels, which allows the diffusion of K^+ out of the cell. The combined effect of sodium inactivation, which blocks the influx of cations, and potassium activation, which causes the efflux of other cations, is the immediate return of the cell membrane to a polarized state, with the inside negative in relation to the outside. After repolarization there is a period during which a second action potential cannot be initiated, no matter how large a stimulus current is applied to the neuron. This is called the absolute refractory period, and it is followed by a relative refractory period, during which another action potential can be generated, but only by a greater stimulus current than that originally needed. This period is followed by the return of the neuronal properties to the threshold levels originally required for the initiation of action potentials.

Conduction:

The sequence of sodium activation–sodium inactivation–potassium activation creates a nerve impulse that is brief in duration, lasting only a few milliseconds, and that travels down the nerve fibre like a wave, the membrane depolarizing in front of the current and repolarizing behind. Because nerve impulses are not graded in amplitude, it is not the size of the action potential that is important in processing information within the nervous system; rather, it is the number and frequency with which the impulses are fired. As stated above, the action potential is propagated along the axon without any decrease in amplitude with distance. However, the velocity of conduction along the nerve fibre is dependent upon several factors. The first factor is the outside diameter of the nerve fibre. The fastest conduction velocity occurs in the largest diameter nerve fibres. This phenomenon has formed the basis for classifying mammalian nerve fibres into groups in order of decreasing diameter and decreasing conduction velocity. Another factor is the

temperature of the nerve fibre. Conduction velocity increases at high temperatures and decreases at low temperatures. Indeed, nerve conduction can be blocked by the local application of cold to a nerve fibre. Conduction velocity is also affected by myelination of the nerve fibre. Since ions cannot cross the lipid content of the myelin sheath, they spread passively down the nerve fibre until reaching the unmyelinated nodes of Ranvier. The nodes of Ranvier are packed with a high concentration of ion channels, which, upon stimulation, propagate the nerve impulse to the next node. In this manner the action potential jumps quickly from node to node along the fibre in a process called saltatory conduction (from Latin *saltare*, "to jump").

Transmission at the synapse:

Once an action potential has been generated at the axon hillock, it is conducted along the length of the axon until it reaches the terminals, the fingerlike extensions of the neuron that are next to other neurons and muscle cells. At this point there exist two methods for transmitting the action potential from one cell to the other. In electrical transmission, the ionic current flows directly through channels that couple the cells. In chemical transmission, a chemical substance called the neurotransmitter passes from one cell to the other, stimulating the second cell to generate its own action potential.

Electrical transmission:

This method of transmitting nerve impulses, while far less common than chemical transmission, occurs in the nervous systems of invertebrates and lower vertebrates, as well as in the central nervous systems of some mammals. Transmission takes place through gap junctions, which are protein channels that link the cellular contents of adjacent neurons. Direct diffusion of ions through these junctions allows the action potential to be transmitted with little or no delay or distortion, in effect synchronizing the response of an entire group of neurons. The channels often allow ions to diffuse in both directions, but some gated channels restrict transmission to only one direction.

Chemical transmission:

There are two classic preparations for the study of chemical transmission at the synapse. One is the vertebrate neuromuscular junction, and the other is the giant synapse of the squid *Loligo*. These sites have the advantage of being readily accessible for recording by electrodes—especially the squid synapse,

which is large enough that electrodes can be inserted directly into the presynaptic terminal and postsynaptic fibre. In addition, only a single synapse is involved at these sites, whereas a single neuron of the central nervous system may have many synapses with many other neurons, each with a different neurotransmitter.

Neurotransmitter release:

Two factors are essential for the release of the neurotransmitter from the presynaptic terminal: (1) depolarization of the terminal and (2) the presence of calcium ions (Ca^{2+}) in the extracellular fluid. The membrane of the presynaptic terminal contains voltage-dependent calcium channels that open when the membrane is depolarized by a nerve impulse, allowing Ca^{2+} to diffuse into the terminal along its concentration gradient. (*See* the figure.) Following the entrance of Ca^{2+} is the release of neurotransmitter.

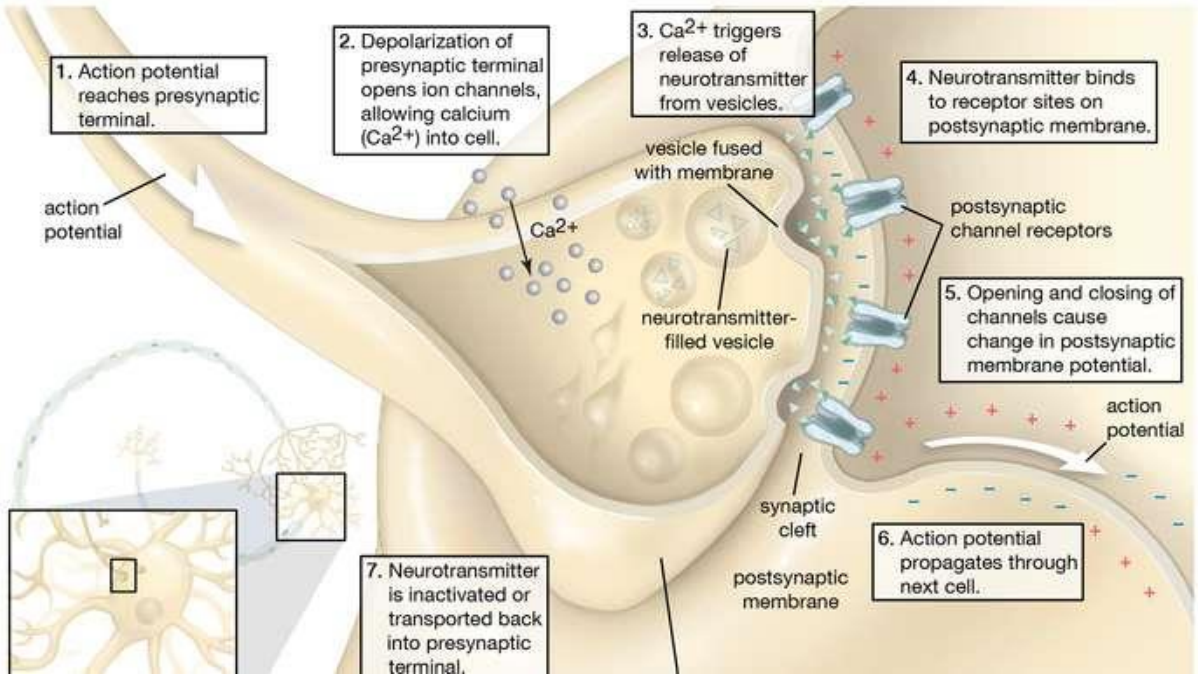


Fig: synapse of neuron

Chemical transmission of a nerve impulse at the synapse. The arrival of the nerve impulse at the presynaptic terminal stimulates the release of neurotransmitter into the synaptic gap. The binding of the neurotransmitter to receptors on the postsynaptic membrane stimulates the regeneration of the action potential in the postsynaptic neuron.

It is uncertain what happens in the time between Ca^{2+} entry and transmitter release. Ca^{2+} is known to be sequestered by certain organelles within the terminal, including the endoplasmic reticulum. The ions may attach to the membranes of synaptic vesicles, in some way facilitating their fusion with the nerve terminal

membrane. They may also be removed from the terminal by exchange with extracellular Na^+—a mechanism known to occur at some neuronal membranes. What is certain is that when the concentration of Ca^{2+} is increased within the terminal, the probability of transmitter release is also increased.

Neurotransmitters are packed into small, membrane-bound synaptic vesicles. Each vesicle contains thousands of neurotransmitter molecules, and there are thousands of vesicles in each axon terminal. Once stimulated by Ca^{2+}, the vesicles move through the cytoplasm and fuse their membranes with the plasma membrane of the terminal. The transmitter molecules are then expelled from the vesicles into the synaptic cleft. This expulsion process is called exocytosis. Vesicle membranes are then recovered from the plasma membrane through endocytosis. In this process the membranes are surrounded by a protein coat at the lateral margins of the synapse and are then transferred to cisternae, which form in the terminal during nerve stimulation. There the vesicles lose their coats, are probably refilled with neurotransmitter, and pinch off from the cisternae to become synaptic vesicles once more.

Because the neurotransmitter chemicals are packed into separate, almost identically sized vesicles, their release into the synaptic cleft is said to be quantal—that is, they are expelled in parcels, each vesicle adding its contents incrementally to the contents released from other parcels. This quantal release of neurotransmitter has a critical influence on the electrical potential created in the postsynaptic membrane.

Postsynaptic potential:

After neurotransmitter is released from the presynaptic terminal, it diffuses across the synaptic cleft and binds to receptor proteins on the postsynaptic membrane. Some receptors are ion channels that open or close when their molecular configuration is altered by the binding action of the neurotransmitter. Others are membrane proteins that, upon activation, catalyze second-messenger reactions within the postsynaptic cell; these reactions in turn open or close the ion channels. Whether acting upon ion channels directly or indirectly, the neurotransmitter molecules cause a sudden change in the permeability of the membrane to specific ions. Exactly which ions now permeate the membrane vary according to the neurotransmitters and their receptors (*see* the section Neurotransmitters and neuromodulators), but the net result of a change in ion diffusion is a change in electrical potential across the membrane. This change is called the postsynaptic potential, or PSP. (In reference to the neuromuscular synapse, it is called the end-plate potential, or EPP.)

The most common potential change is depolarization, caused by a net influx of cations (usually Na^+). Because this infusion of positive charge brings the membrane potential toward the threshold at which the nerve impulse is generated, it is called an excitatory postsynaptic potential (EPSP). Other neurotransmitters stimulate a net efflux of positive charge (usually in the form of K^+ diffusing out of the cell), leaving the inside of the membrane more negative. Because this hyperpolarization draws the membrane potential farther from the threshold, making it more difficult to generate a nerve impulse, it is called an inhibitory postsynaptic potential (IPSP). The interaction of competing EPSPs and IPSPs at the hundreds or even thousands of synapses on a single neuron determines whether the nerve impulse arriving at the presynaptic terminals will be regenerated in the postsynaptic membrane. The PSP is a type of local potential, having properties similar to the electrical potential set up at sensory receptor neurons (*see* the section Transmission in the neuron: Localized potential). Like the receptor potential, the PSP is a graded response, varying in amplitude according to the duration and amount of stimulation by neurotransmitters. At the neuromuscular junction, brief depolarizations measuring no more than one millivolt can be observed in the postsynaptic muscle membrane, even when it is at rest. These tiny electrical events, called miniature end-plate potentials (MEPPs), or miniature postsynaptic potentials (MPSPs), are caused by the random release of single quanta of neurotransmitter from a resting presynaptic terminal. The EPP is actually made up of multiple MEPPs, which arise when an activated terminal releases hundreds of neurotransmitter quanta. A series of EPPs, or a number of them stimulated simultaneously at many synapses, can then bring the cell to the threshold of the action potential. This combined action of EPPs is called summation.

In contrast to electrical transmission, which takes place with almost no delay, chemical transmission exhibits synaptic delay. Recordings from squid synapses and neuromuscular junctions of the frog reveal a delay of 0.5 to 4.0 milliseconds between the onset of action potential at the nerve terminal and action potential at the postsynaptic site. This delay may be accounted for by three factors. First, diffusion of the neurotransmitter across the synaptic cleft takes approximately 0.05 millisecond. Second, the response of the postsynaptic receptor takes about 0.15 millisecond. This leaves 0.30 to 3.80 milliseconds for other processes. A third process, called mobilization of the transmitter, is traditionally postulated as taking up the remaining time, but evidence suggests that the time is occupied at least partially by the opening of calcium channels to allow the entry of Ca^{2+} into the presynaptic terminal.

Inactivation:

A series of nerve impulses arriving in rapid succession at the axon terminal is accurately reproduced as a series in the postsynaptic cell because the quanta of neurotransmitter released by each impulse are inactivated as soon as they stimulate the receptor proteins. Neurotransmitter inactivation is carried out by a combination of three processes. First, the neurotransmitter molecules simply diffuse out of the narrow synaptic cleft. Second, they are taken back into the presynaptic terminal by transmitter-sensitive transport molecules. Third, they are metabolized into inactive compounds by enzymes in the synaptic cleft.

Ion transport:

As is stated above, the lipid bilayer of the neuronal membrane tends to repel electrically charged, hydrated ions, making virtually impossible the movement across the membrane that is necessary for the generation of nerve impulses. The transmembrane movement of ions is actually carried out by molecular mechanism—specifically, by protein molecules embedded in the lipid layers. One mechanism, the sodium-potassium pump, maintains the resting potential, and another, the various ion channels, helps create the action potential.

Active transport: the sodium-potassium pump:

Since the plasma membrane of the neuron is highly permeable to K^+ and slightly permeable to Na^+, and since neither of these ions is in a state of equilibrium (Na^+ being at higher concentration outside the cell than inside and K^+ at higher concentration inside the cell), then a natural occurrence should be the diffusion of both ions down their electrochemical gradients—K^+ out of the cell and Na^+ into the cell. However, the concentrations of these ions are maintained at constant disequilibrium, indicating that there is a compensatory mechanism moving Na^+ outward against its concentration gradient and K^+ inward. This mechanism is the sodium-potassium pump. Actually a large protein molecule that traverses the plasma membrane of the neuron, the pump presents receptor areas to both the cytoplasm and the extracellular environment. That part of the molecule facing the cytoplasm has a high affinity for Na^+ and a low affinity for K^+, while that part facing the outside has a high affinity for K^+ and a low affinity for Na^+. Stimulated by the action of the ions on its receptors, the pump transports them in opposite directions against their concentration gradients.

If equal amounts of Na^+ and K^+ were transported across the membrane by the pump, the net charge transfer would be zero; there would be no net flow of current and no effect on the membrane potential. In fact, in many neurons three sodium ions are transported for every potassium ion; sometimes the ratio is three sodium ions for every two potassium ions, and in a few neurons it is two sodium ions for one potassium ion. This inequality of ionic transfer produces a net efflux of positive charge, maintaining a polarized membrane with the inner surface slightly negative in relation to the outer surface. Because it creates this potential difference across the membrane, the sodium-potassium pump is said to be electrogenic. The sodium-potassium pump carries out a form of active transport—that is, its pumping of ions against their gradients requires the addition of energy from an outside source. That source is adenosine triphosphate (ATP), the principal energy-carrying molecule of the cell. ATP is formed by an inorganic phosphate molecule held in high-energy linkage with a molecule of adenosine diphosphate (ADP). When an enzyme in the pump, called sodium-potassium-ATPase, splits the phosphate from the ADP, the energy released powers the transport action of the pump.

Passive transport: membrane channels:

The sodium-potassium pump sets the membrane potential of the neuron by keeping the concentrations of Na^+ and K^+ at constant disequilibrium. The sudden shift from a resting to an active state, when the neuron generates a nerve impulse, is caused by a sudden movement of ions across the membrane—specifically, a flux of Na^+ into the cell. Given the relative impermeability of the plasma membrane to Na^+, this influx itself implies a sudden change in permeability. Beginning in the 19th century, researchers puzzled over the mechanism by which this change could occur. The idea arose that there must exist pores, or channels, through which the ions could diffuse, passing the barrier posed by the lipid bilayer. However, for years only the gross currents accompanying ionic movement could be measured, and it was only by inference that the presence of membrane channels could be postulated.

The breakthrough came in the 1970s and ’80s with the development of the patch-clamp technique, which enabled researchers to directly measure currents flowing across single ion channels in the membrane. The patch-clamp technique electrically isolates a small patch of neuron or muscle cell membrane by applying the tip of a micropipette filled with conducting solution to the membrane and forming a tight seal with it. As single channels in the patch undergo various transitional states between fully open and fully closed, the times of opening and closing are recorded and the amplitudes and duration of the currents are measured.

Since the pioneering studies, the electrical and biochemical properties of certain channels have been characterized. Known as “voltage dependent” when activated by changes in the membrane potential and “neurotransmitter sensitive” when activated by neurotransmitter substances, these channels are protein structures that span the membrane from the extracellular space to the cytoplasm. They are thought to be cylindrical, with a hollow, water-filled pore wider than the ion passing through it except at one region called the selectivity filter. This filter makes each channel specific to one type of ion.

Sodium channels:

Voltage-sensitive sodium channels have been characterized with respect to their subunit structure and their amino acid sequences. The principal protein component is a glycoprotein containing 1,820 amino acids. Four similar transmembrane domains, of about 300 amino acids each, surround a central aqueous pore through which the ions pass. The selectivity filter is a constriction of the channel ringed by negatively charged carbonyl oxygens, which repel anions but attract cations. Also within the channel are thought to be two types of charged particles forming the gates that control the diffusion of Na^+. One gate closes at polarization and opens at depolarization; the other closes at depolarization.

It is thought that the resting, activated, and inactivated states of the sodium channel are due to voltage-dependent conformational changes in the glycoprotein component. These changes result from effects of the electrical field on the charges and dipoles of the amino acids within the protein. With a large electrical field applied to it, the protein has been observed to change its conformation from a stable, closed resting state to a stable, open state in which the net charge or the location of the charge on the protein is changed.

Potassium channels:

There are several types of voltage-dependent potassium channels, each having its own physiological and pharmacological properties. A single neuron may contain more than one type of potassium channel. The best-known flow of K^+ is the outward current following depolarization of the membrane. This occurs through the delayed rectifier channel (I_{DR}), which, activated by the influx of Na^+, counteracts the effect of that cation by allowing the discharge of K^+. By repolarizing the membrane in this way, the I_{DR} channel restricts the duration of the nerve impulse and participates in the regulation of repetitive firing of the neuron. Another outward K^+ current, occurring with little delay after depolarization, is the A

current. I_A channels are opened by depolarization following hyperpolarization. By increasing the interval between action potentials, they help a neuron to fire repetitively at low frequencies.

Another type of potassium channel, the $I_{K(Ca)}$ channel, is activated by high concentrations of intracellular Ca^{2+}. The opening of these channels results in hyperpolarization of the membrane, so that they appear to slow the repetitive firing of nerve impulses. The I_M channel is opened by depolarization but is deactivated only by the neurotransmitter acetylcholine. This property may serve to regulate the sensitivity of neurons to synaptic input.

A final type of potassium channel is the anomalous, or inward, rectifier channel (I_{IR}). This channel closes with depolarization and opens with hyperpolarization. By allowing an unusual inward diffusion of K^+, the I_{IR} channel prolongs depolarization of the neuron and helps produce long-lasting nerve impulses.

Calcium channels:

As with potassium channels, there is more than one type of calcium channel. The inward calcium current is slower than the sodium current. There are at least two types of current in certain neurons of the central nervous system—a long-lasting current activated at positive potential and a transient current activated at more negative potential. There are two corresponding types of calcium channels: a large conductance channel that gives rise to a long-lasting current at positive membrane potentials and a low conductance channel that gives rise to a transient current at more negative potentials. In some neurons a third channel current occurs that is transient and can only be activated at high negative potential.

Anion channels:

There may be channels that pass anions such as Cl^-, but their existence is difficult to prove. Single-channel recordings of cultured tissue have shown selective Cl^- channels that are voltage dependent and of high conductance. Channels with lower conductance have been demonstrated in reconstituted artificial membranes as well as in neurons.

Neurotransmitters and neuromodulators:

The traditional models for the study of neurotransmitter release are either the neuromuscular junction of the frog, crayfish, and rat or the giant synapse of the squid. These synapses are relatively simple in their structure, with a single axon terminal forming an identifiable synapse at the postsynaptic membrane of a muscle fibre or neuron. Recordings can be obtained from these single-synaptic junctions in response to the release of a single neurotransmitter. At neurons of the central nervous system, on the other hand, the situation is more complex. Each central neuron has several synapses with other neurons at various locations, such as on the dendrites, soma, and initial segment of the axon. Several neurotransmitters, therefore—some excitatory and others inhibitory—may be involved in the final integrated response of a central neuron, making their identities difficult to determine.

Further complicating neurotransmitter action is the presence not only of multiple transmitter substances but also of neuromodulators. Neuromodulators are substances that do not directly activate ion-channel receptors but that, acting together with neurotransmitters, enhance the excitatory or inhibitory responses of the receptors. It is often impossible to determine, in the presence of many substances, which are transmitters and which are modulators. Such is the case with many of the neuropeptides.

In addition to the multiplicity of transmitters and modulators there is a multiplicity of receptors. Some receptors directly open ion channels, while others activate the second-messenger system, any of a number of reactions that take place in the cytoplasm or plasma membrane and indirectly act upon the ion channels. One second-messenger system involves the activation by receptor proteins of linking proteins, which move across the membrane, bind to channel proteins, and open the channels. Another system is the cyclic adenosine monophosphate (cAMP) system. In this chain reaction, receptor proteins activate linking proteins, which then activate the enzymes that synthesize cAMP. The cAMP molecules activate other enzymes that, in turn, activate ion channels. Whether they activate channels directly or through a second-messenger system, neurotransmitters are considered to be primary messengers. Described below are the principal proved or suggested neurotransmitters of the mammalian nervous system and their corresponding receptors.

Acetylcholine:

Although early studies of acetylcholine were undertaken at neuromuscular junctions, where it is especially concentrated, the concept leading to the identification of the substance as a neurotransmitter of the central nervous system is a landmark in neuroscience. The concept is called Dale's principle after Sir Henry Dale, a British physiologist who, in 1935, stated that a neurotransmitter released at one axon terminal of a neuron can be presumed to be released at other axon terminals of the same neuron. (Dale's principle refers only to the presynaptic neuron, as the responses of different postsynaptic receptors to a single neurotransmitter can vary in the same or different neurons.) The first application of Dale's principle was at the mammalian spinal cord, from which motor neurons send their axons to striated muscles, where the terminals are observed to release acetylcholine. According to Dale's principle, all the branches of a single motor neuron axon should release acetylcholine—including the terminals in the spinal cord. In fact, it was found that some collateral branches leave the motor axons and reenter the gray matter of the spinal cord, where they synapse onto spinal interneurons. The neurotransmitter released at these terminals is acetylcholine. High concentrations of the acetylcholine-synthesizing enzyme, choline acetyltransferase, and the enzyme for its breakdown, acetylcholinesterase, are also found in motor neuron regions of the spinal cord.

Acetylcholine receptors (also called cholinergic receptors) appear in clusters on muscle-cell membranes opposite the active zones of presynaptic terminals. Their density at these receptor regions is between 7,000 and 30,000 sites per square micrometre (micron; millionth of a metre). The number drops drastically even a few nanometres (billionths of a metre) away from the receptor region, so that sensitivity to acetylcholine is about 50 to 100 times less one millimetre from the receptor region than it is at the receptor site itself. Cholinergic receptors also exist on the presynaptic terminals of neurons that release acetylcholine as well as on terminals that release other neurotransmitters. These receptors are called autoreceptors, and they probably regulate the release of neurotransmitter at the terminal. There are two main categories of cholinergic receptor, nicotinic and muscarinic. The nicotinic receptor is a channel protein that, upon binding by acetylcholine, opens to allow diffusion of cations. The muscarinic receptor, on the other hand, is a membrane protein; upon stimulation by neurotransmitter, it causes the opening of ion channels indirectly, through a second messenger. For this reason, the action of a muscarinic synapse is relatively slow. Muscarinic receptors predominate at higher levels of the central nervous system, while nicotinic receptors, which are much faster acting, are more prevalent at neurons of the spinal cord and at neuromuscular junctions in skeletal muscle.

The nicotinic receptor channel is a glycoprotein composed of five subunits (*see* the figure). Two alpha- (α-) subunits contain the two acetylcholine-binding sites associated with the channel. Three other subunits—a beta- (β-) subunit, a gamma- (γ-) subunit, and a delta- (δ-) subunit—complete the protein. High-resolution electron microscopy with optical image reconstruction, as well as freeze-fracture electron microscopy, reveal a highly symmetrical structure, looking from the top somewhat like a life belt, with the presumed channel in the centre. About one-third of the protein protrudes from the plasma membrane, while the rest is embedded in the membrane or protruding into the cell.

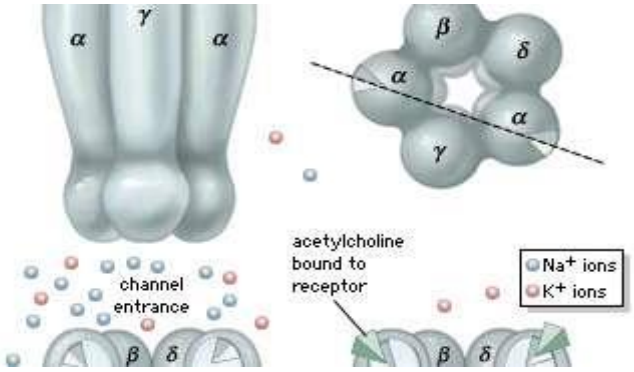


Fig: nicotinic receptor : The nicotinic receptor, composed of two α-subunits and β-, γ-, and δ-subunits arranged symmetrically around a central channel, binds acetylcholine, which causes the channel to open and allows diffusion of sodium (Na^+) and potassium (K^+) ions into the cell interior.

Patch-clamp techniques give information on single channel currents and, therefore, on the conductance and kinetics of the cholinergic receptor channel. At the neuromuscular junction, approximately 20,000 univalent ions carry the charge across a single activated channel, and a quantum of acetylcholine activates about 1,500 channels. The time constant for the decay of the MEPP is the same as that for channel closing. The time constant for channel closing is voltage dependent, with depolarization shortening the duration of open channels and hyperpolarization lengthening the duration.

Studies show that nicotinic acetylcholine-activated channels allow cations to permeate the membrane with no specificity—that is, all cations can diffuse through the channels indiscriminately. Because the resting membrane is already near the equilibrium potential of K^+, this means that much more Na^+ and Ca^{2+} diffuse into the cell than K^+ out, causing depolarization and excitation of the neuron or muscle cell. However, in certain molluscan neurons, nicotinic acetylcholine receptors can also activate Cl^- channels, causing hyperpolarization of the postsynaptic membrane and inhibition of excitability. With respect to muscarinic

receptors, the situation is not clear. Second messengers may be involved, and potassium channels may be activated.

Epinephrine and norepinephrine:

These related hormones, also called adrenaline (epinephrine) and noradrenaline (norepinephrine), act to increase the heart rate, blood pressure, and levels of sugar and fat in the blood. They are secreted into the bloodstream by the adrenal glands in response to stress, but they are also synthesized and released as neurotransmitters by axon terminals in the central nervous system and in sympathetic fibres of the autonomic nervous system. Receptors sensitive to norepinephrine and epinephrine are called adrenergic receptors. They are divided into two types, α and β. These are further classified into subtypes α_1, α_2, β_1, and β_2.

Both types of adrenergic receptors produce changes in the postsynaptic membrane potential by acting upon ion channels specific to K^+ and Ca^{2+}. They differ in the mechanisms that, upon stimulation by neurotransmitter, they employ to activate those channels. Stimulated β_1 receptors bind to linking proteins that in turn bind to calcium channels, changing their shape and altering their permeability to the cation. More important, the linking proteins stimulate the synthesis of cAMP, which, through another series of reactions, opens potassium channels. The efflux of K^+ tends to hyperpolarize the postsynaptic membrane, inhibiting the generation of a nerve impulse. The β_2 receptor has been found on glial cells.

The α_2 receptor activates potassium channels in both the postsynaptic and presynaptic membranes, probably via linking proteins and the synthesis of cAMP. The α_1 receptor acts on calcium channels through a series of reactions linked to the lipid molecules of the plasma membrane. Both epinephrine and norepinephrine are terminated by uptake back into the presynaptic terminals, where they are enzymatically degraded or inactivated.

Dopamine:

Dopamine is a precursor of norepinephrine that acts as a neurotransmitter at certain synapses of the brain. Disorders at these synapses have been implicated in schizophrenia and Parkinson disease. There are two types of dopaminergic receptors, called the D_1 and the D_2. The former catalyzes the synthesis of cAMP, and the latter inhibits its synthesis. These reactions then regulate calcium and potassium channels in the

postsynaptic membrane. Dopaminergic receptors also exist on the presynaptic membrane. The neurotransmitter is terminated by uptake into the presynaptic terminal.

Serotonin (5-hydroxytryptamine):

Although the brain has only a small percentage of the serotonin found in the human body, there appears to be a strong relationship between the levels of this neurotransmitter at some regions of the brain and certain behavioral patterns, including sleep, sexual urge, and mood. At synapses of the peripheral nervous system, serotonin seems to prime muscle cells for an excitatory response to other neurotransmitters. Serotonin receptors, or 5HT receptors, activate calcium and potassium channels through linking proteins and the cAMP second-messenger systems. After acting on the postsynaptic receptors, the neurotransmitter is taken up by the presynaptic terminal and enzymatically degraded.

Amino acids:

Several amino acids exist in the central nervous system in extremely high concentrations, but their ubiquity makes their identification as true neurotransmitters difficult. Furthermore, because some of them are essential components of metabolic reactions, their presence within a neuron does not prove that they function as neurotransmitters. Nevertheless, there is enough evidence that some amino acids act as either excitatory or inhibitory transmitters. The excitatory amino acids include glutamic acid (or glutamate) and aspartic acid (or aspartate), and the inhibitory amino acids include gamma-aminobutyric acid (GABA) and glycine. Glutamate is the most abundant amino acid in the brain. Unlike acetylcholine, glutamate does not vary greatly in concentration from one region to the next. However, the dorsal gray matter of the spinal cord, which contains terminals of incoming dorsal roots, has large concentrations of glutamate. Aspartate, on the other hand, is believed to be concentrated in the interneurons of the ventral gray matter.

At postsynaptic receptor sites glutamate depolarizes the membrane by opening nonspecific cation channels, which allow a net influx of Na^+ and Ca^{2+}. Of the excitatory amino acid receptors, the N-methyl-D-aspartic acid (NMDA) receptor has been thoroughly characterized. Patch-clamp studies show that this receptor is influenced by the presence of magnesium ions (Mg^{2+}). In the absence of Mg^{2+}, activated NMDA receptors open nonspecific cationic channels with no variation when the voltage is changed. With Mg^{2+} added to the extracellular medium, though, the frequency of channel openings is reduced when the membrane is hyperpolarized. Both glutamate and aspartate are probably inactivated by uptake systems at

the presynaptic terminals and at glial cells surrounding some of the synaptic junctions. GABA and glycine cause hyperpolarization of the postsynaptic membrane. GABA is widely distributed in the brain, being especially prevalent at higher levels of the central nervous system. It is produced from glutamate by the enzyme glutamic acid decarboxylase (GAD). Consequently, the concentrations of GABA and GAD parallel each other in the nervous system.

At postsynaptic receptor sites GABA opens chloride channels, causing in most cells a hyperpolarization of the membrane as Cl^- diffuses inward to reach its equilibrium potential. However, GABA inhibits presynaptic nerve fibres as well. At certain synaptic junctions the release of neurotransmitter is modulated by the binding to presynaptic receptors of neurotransmitter released from other neurons. An example of this is at the axon terminals of incoming dorsal roots in the dorsal gray matter. Projecting onto these terminals are other terminals that release GABA. Although GABA causes an increased Cl^- conductance at these terminals, the result is depolarization, not hyperpolarization, of the membrane. This is because the resting membrane potential of the receiving nerve terminal is much more negative than the Cl^- equilibrium potential. This means that as Cl^- flows into the terminal to reach equilibrium, the membrane is actually depolarized. The effect at the terminal is a decrease in neurotransmitter release. Unlike GABA, glycine is found mostly at lower levels of the central nervous system, including the spinal cord, medulla oblongata, and pons. It is a major inhibitor released by interneurons to suppress motoneuronal activity. Like GABA, glycine acts by increasing Cl^- conductance at the postsynaptic membrane, although it acts at a clearly different receptor.

It appears that at least two molecules of glycine and GABA must bind to their respective receptors to activate a chloride channel. The action of both neurotransmitters is terminated by uptake back into the presynaptic terminal or into surrounding glial cells.

Neuroactive peptides:

Neuroactive peptides are sequences of amino acids, usually longer than amino acid neurotransmitters yet shorter than hormones or proteins. Unlike the classic neurotransmitters described above, which are formed by enzymes near the presynaptic terminals, neuroactive peptides are assembled by ribosomes attached to the endoplasmic reticulum. From there they are transferred to the Golgi apparatus, where they are packed into secretory vesicles and transported to the terminals. Some peptides are secreted by neuroendocrine cells of the hypothalamus or pituitary gland. Because they are released into the capillary system of the

bloodstream and act at distant sites of the body, these are called neurohormones. Other peptides are released into the synaptic cleft between neurons of the central nervous system (including the hypothalamus). Many of these neuropeptides fulfill some criteria of neurotransmitters, evoking excitatory or inhibitory responses in postsynaptic ion channels, yet it is still uncertain to what extent they act as true neurotransmitters or as neuromodulators.

Distinguishing neuropeptides from the classic neurotransmitters is the longevity of their action. While acetylcholine, for example, acts upon synaptic receptors for only a few milliseconds, neuropeptides have a course of action lasting from several seconds to several days. Also, neuropeptides are released in much lower concentrations than are transmitter substances, although the peptides have a much higher potency. The list of neuropeptides is not yet complete. Among those peptides known to affect synaptic transmission are substance P, neurotensin, somatostatin, vasoactive intestinal peptide, cholecystokinin, and the opioid peptides. The best-studied are the opioid peptides, so called because opiate drugs, such as morphine, are known to bind to their receptors and mimic their painkilling and mood-altering actions. All opoid peptides belong to three genetically distinct families: the β-endorphins, the enkephalins, and the dynorphins.

It has long been known that opioids and opiate drugs have varied and powerful effects on pain, mood, sleep, sedation, and the cough reflex—apart from effects on the gastrointestinal tract and the cardiovascular system. It is not surprising, therefore, that there are multiple receptors for these substances. There may in fact be as many as eight different types of opioid receptors, but the four best-described are designated mu (μ), kappa (κ), delta (δ), and sigma (σ). The μ receptors, which readily bind morphine, are thought to mediate euphoria, respiratory depression, and physical addiction and to block pain pathways in the brain. The κ receptors bind preferentially to dynorphin and are thought to mediate analgesia and sedation at the spinal cord. The δ receptors, located primarily in the limbic portions of the brain, bind enkephalin. They may be responsible for dysphoria (extreme depression), hallucination, and respiratory and vasomotor stimulation. The σ receptors, found in the hippocampus, may be involved in alterations of affective behaviour, but their functions are unclear.

The opioid receptors mediate their effects mainly by inhibiting regeneration of the nerve impulse at the postsynaptic membrane. They accomplish this by opening potassium channels or closing calcium channels, causing a net outflow of positive charge that keeps the postsynaptic membrane from reaching threshold potential. As with other neuropeptides, it is not known whether all the opioid receptors are activated by the opioids alone or by a combination of opioid and other transmitter substances. For this

reason it is uncertain whether the opioid peptides are true neurotransmitters or are neuromodulators. The presence of peptides within certain structures of the central nervous system is well established; more important, peptides are often found in the same neurons with classic neurotransmitters or with other peptides. For example, substance P can be found in the same neurons of the brainstem as serotonin. In the sympathetic system, norepinephrine is found with somatostatin in some neurons and with enkephalin in others.

Because some neuropeptides and neurotransmitters are stored in the same vesicles and secreted together in response to stimulation, a form of interaction between the substances appears likely. The interaction may take place at presynaptic terminals, altering the release of neurotransmitter, or it may take place postsynaptically, altering the effect of neurotransmitter. At the neuromuscular junction of the lobster, for example, the neurotransmitters serotonin and octopamine and the neuropeptide proctolin can act presynaptically to alter the amounts of GABA or glutamate released from the nerve terminals. In a similar manner, at some regions of the central nervous system opioid peptides inhibit the release of norepinephrine, acetylcholine, dopamine, and substance P.

The discovery of more than one type of neuroactive substance in one set of axon terminals has disproved an assumption implied by Dale's principle—that a single neuron synthesizes and secretes a single neurotransmitter. Also called into doubt is another assumption—that a single neuron secretes a single set of neurotransmitters at all of its synapses. Researchers are finding evidence that different synapses of the same neuron act somewhat independently. This may mean that different areas of a single neuron synthesize different neuroactive substances. Such a phenomenon would be another example of the metabolic and functional complexity of the nervous system.

Synaptic vesicle cycle :

The synaptic vesicles functions in the synaptic knob and also is recycled for further use.

1. Trafficking to the synapse : Synaptic vesicle components are initially trafficked to the synapse using members of the kinesin motor family. In *C. elegans*, the major motor for synaptic vesicle is UNC-104. There is also evidence that other proteins such as UNC-16/Sunday Driver regulate the use of motor for transport of synaptic vesicles. Transport vesicles used to traffic synaptic vesicle components probably differ in composition from mature synaptic vesicles though these differences have not been well defined. Another question that has not been well resolved is how

motors release cargo st the synapses. Finally, though there is substantial evidence that active zone components are also trafficked to synapses on vesicles, the nature of the motors that performs this transport remains unclear.

2. Transmitter loading : Once at synaptic sites, vesicles are loaded with neurotransmitter. Loading of transmitter is an active process requiring a neurotransmitter transporter and a vacuolar-type proton pump ATPase that provides a pH and electrochemical gradient. These transporters are selective for different classes of transmitters. Interestingly, the identity of many of these transporters was determined through the molecular characterization of *C. elegans* mutants. Characterizations of *unc*-17 and *unc-47*, which encode the vesicular acetylecholine transporter and vesicular GABA transporter, defined the founding members of these two families of transporters. To what extent loading is regulated to modulate transmitter release is not known.

3. Docking: Next, loaded vesicles must dock near release sites. Docking is a step of the cycle that we know little about. Though many proteins on vesicles and at release sites have been identified, none of the identified protein interactions that occur between vesicle proteins and proteins at release sites can account for docking. Mutants in *rab-3* and *unc-18* alter vesicle docking or vesicle organization at release sites, but they do not completely disrupt docking. Perhaps surprisingly, the SNAREs, which are thought to mediate fusion, do not appear to be involved in the docking process.

4. Priming: When vesicles initially dock they are not fusion competent. Vesicles first need to be primed so that they are able to fuse rapidly in response to calcium influx. This priming step is thought to involve the formation of partially assembled SNARE complexes. SNARE proteins must assemble into trans-SNARE complexes so that they can provide the force that is necessary for vesicle fusion. The four α-helix domains (1 each from synaptobrevin and syntaxin, and 2 from SNAP-25) come together to form a coiled-coil motif. The rate-limiting step in the assembly process is the association of the syntaxin SNARE domain, since it is usually found in a “closed” state where it is incapable of interacting with other SNARE proteins. When syntaxin is in an open state, trans-SNARE complex formation begins with the association of the four SNARE domains at their N-termini. The SNARE domains proceed in forming a coiled-coil motif in the direction of the C-termini of their respective domains. The proteins UNC—13 and Rim participate in this event. UNC-13 is thought to stimulate the change of the t-SNARE syntaxin from a closed conformation to an open conformation, which

stimulates the assembly of v-SNARE/t-SNARE complexes. Rim also appears to regulate the priming, but it is not essential for the step.

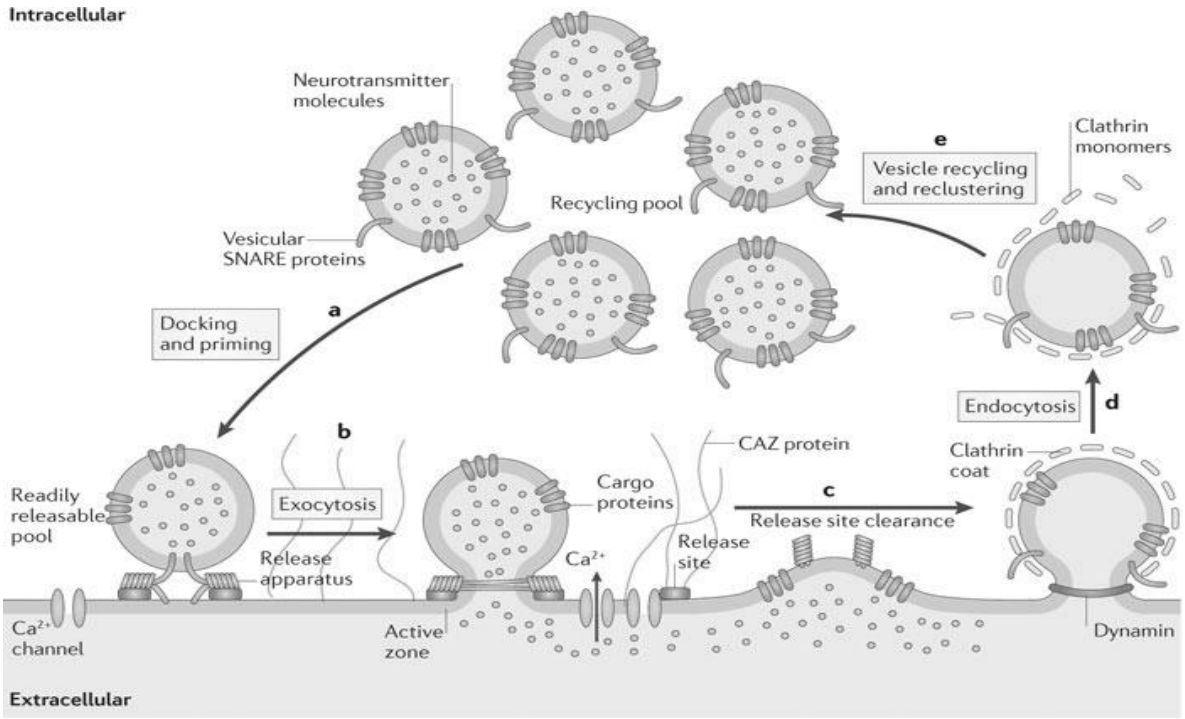


Figure: Synaptic vesicle cycle (source: Nature review)

5. Fusion : Primed vesicles fuse very quickly in response to calcium elevations in the cytoplasm. This fusion event is thought to be medicated directly by the SNAREs and driven by the energy provided from SNARE assembly. The calcium-sensing trigger for this event is the calcium-binding synaptic vesicle protein synaptotagmin. The ability of SNAREs to mediate fusion in a calcium-dependent manner recently has been reconstituted *in vitro*. Consistent with SNAREs being essential for the fusion process, v-SNARE and t-SNARE mutants of *C. elegans* are lethal and even viable hypomorphic mutants exhibit almost no evoked release. Similarly, mutants are *Drosophila* and knockouts in mouse indicate that these SNAREs play a critical role in synaptic exocytosis. One model hypothesizes that the force required to bring two membranes together during fusion comes from the conformational change in trans- SNARE complexes to form cis-SNARE complexes. The current hypothesis that describes this process is referred to as SNARE "zippering". Several models to explain the subsequent step – the formation of stalk and fusion pore – have been proposed. However, the exact nature of these processes remains debated. In accordance with the "zipper" hypothesis as the SNARE complex forms, the tightening helix bundle puts torsional force on the

transmembrane (TM) domains of synaptobrevin and syntaxin. This causes the TM domains to tilt within the separate membranes as the proteins coil more tightly. The unstable configuration of the TM domains eventually causes the two membranes to fuse and the SNARE proteins come together within the same membrane, which is referred to as a "cis"-SNARE complex. As a result of the lipid rearrangement, a fusion pore opens and allows the chemical contents of the vesicle to leak into the outside environment. However, our understanding of fusion is farfrom complete. One major issue in the field is defining the contribution of incomplete fusion events (also referred to as kiss-and-run) where a fusion pore forms briefly to allow exit of transmitter without complete fusion of the vesicle and plasma membranes. There is substantial evidence that kiss and run occurs at least at some synapses. Studies examining the rates of endocytosis suggest that both very rapid and slow endocytosis occur at synapses. Analysis of SNAP-25 and synaptobrevin mouse knockout mutant revealed that although evoked release was completely disrupted, that some spontaneous fusion events continued. Similarly, *C. elegans* null mutants lacking the v-SNARE synaptobrevin and the t-SNARE SNAP-25 still are capable of some movements, in contrast to completely paralysis seen in t-SNARE syntaxin mutants. The energy input that is required for SNARE-mediated fusion to take place comes from SNARE-complex disassembly. The suspected energy source is N-ethylmaleimide-sensitive factor (NSF), an ATPase that is involved with membrane fusion. NSF homohexamers, along with the NSF cofactor α-SNAP, bind and dissociate the SNARE complex by coupling the process with ATP hydrolysis. This process allows for reuptake of synaptobrevin for further use in vesicles, whereas the other SNARE proteins remain associated with the cell membrane. The dissociated SNARE proteins have a higher energy state than the more stable cis-SNARE complex. It is believed that the energy that drives fusion is derived from the transition to a lower energy cis-SNARE complex. The ATP hydrolysis-coupled dissociation of SNARE complexes is an energy investment that can be compared to "cocking the gun" sothat, once vesicle fusion is triggered, the process takes place spontaneously and at optimum velocity. A comparable process takes place in muscles, in which the myosin heads must first hydrolyze ATP in order to adapt the necessary conformation for interaction with actin and the subsequent power stroke to occur.

6. Endocytosis : Synaptic vesicle proteins that have been incorporated into the plasma membrane after fusion are retrieved by endcytosis. A large cohort of proteins have been identified which participate in these events including endophilin, synaptojanin, synaptotagmin, dynamin, clathrin, AP180, as well as others. The span of presynaptic membrane containing the primed vesicles and

dense collection of SNARE proteins is referred to as active zone. Voltage-gated calcium channels are highly concentrated around active zones and open in response to membrane depolarization at the synapse. The influx of calcium is sensed by synaptotagmin 1, which in turn dislodges complexin protein and allows the vesicle to fuse with the presynaptic membrane to release neurotransmitter. It has also been shown that the voltage-gated calcium channels directly interact with the t-SNAREs syntaxin 1A and SNAP-25, as well as with synaptotagmin 1. The interactions are able to inhibit calcium channel activity as well as tightly aggregate the molecules around the release site. Rate of endocytosis vary widely in different preparations and also vary depending on the stimulus intensity. A variety of evidence suggests that different pathways may be utilized even at the same synapse. One of the major outstanding questions remains whether synaptic vesicle membranes are selectively endocytosed as synaptic vesicle entities oras precursors with other membrane proteins which must then traffic through as endosomal compartment to yield mature vesicles.

7. Neurotransmitter clearance : After transmitter is released and binds to receptor on the postsynaptic membrane, it must be cleared to permit subsequent signalling. Some transmitters like dopamine are transported back into the neuron using plasma membrane transporters. Other transmitter including acetylcholine and some neuropeptides are broken down in the synaptic cleft. Acetyl cholinesterase does this job at the neuromuscular junction.

Probable Questions-

1. Explain the structure of a typical synapse with proper diagram.
2. What is the role of SNARE proteins in the release of neurotransmitter from the synapse?
3. Describe how neurotransmitter is released in a calcium-dependent manner.

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong’s review of medical physiology

UNIT-VI

Cytoskeleton, Extracellular matrix, gap junctions, integrins, cell adhesion molecules and their functions

Objective: In this unit we will learn about cytoskeleton, Extracellular matrix, gap junctions, integrins, cell adhesion molecules and their functions.

Introduction

If you were building a building, what kinds of connections might you want to put between the rooms? In some cases, you'd want people to be able to walk from one room to another, in which case you'd put in a door. In other cases, you'd want to hold two adjacent walls firmly together, in which case you might put in some strong bolts. And in still other cases, you might need to ensure that the walls were sealed very tightly together – for instance, to prevent water from dripping between them.

As it turns out, cells face the same questions when they're arranged in a tissue next to other cells. Should they put in doors that connect them directly to their neighbors? Do they need to spot-weld themselves to their neighbors to make a strong layer, or perhaps even form tight seals to prevent water from passing through the tissue? Junctions serving all of these functions can be found in cells of different types, and here, we'll look at each of them in turn.

Plasmodesmata

Plant cells, surrounded as they are by cell walls, don't contact one another through wide stretches of plasma membrane the way animal cells can. However, they do have specialized junctions called **plasmodesmata** (singular, **plasmodesma**), places where a hole is punched in the cell wall to allow direct cytoplasmic exchange between two cells.

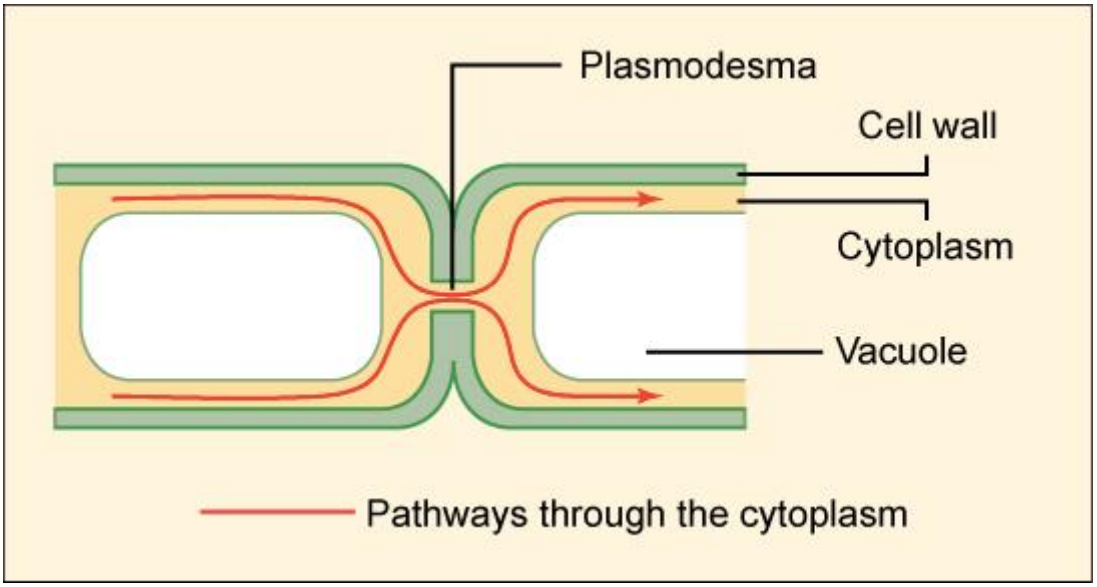


Image of two cells connected by a plasmodesma, showing how materials can travel from the cytoplasm of one cell to the next via the plasmodesma.

Plasmodesmata are lined with plasma membrane that is continuous with the membranes of the two cells. Each plasmodesma has a thread of cytoplasm extending through it, containing an even thinner thread of endoplasmic reticulum (not shown in the diagram above).

Molecules below a certain size (the size exclusion limit) move freely through the plasmodesmal channel by passive diffusion. The size exclusion limit varies among plants, and even among cell types within a plant. Plasmodesmata may selectively dilate (expand) to allow the passage of certain large molecules, such as proteins. although this process is poorly understood.

Gap junctions:

Functionally, **gap junctions** in animal cells are a lot like plasmodesmata in plant cells: they are channels between neighboring cells that allow for the transport of ions, water, and other substances. Structurally, however, gap junctions and plasmodesmata are quite different.

In vertebrates, gap junctions develop when a set of six membrane proteins called **connexins** form an elongated, donut-like structure called a **connexon**. When the pores, or "doughnut holes," of connexons in

adjacent animal cells align, a channel forms between the cells. (Invertebrates also form gap junctions in a similar way, but use a different set of proteins called innexins).

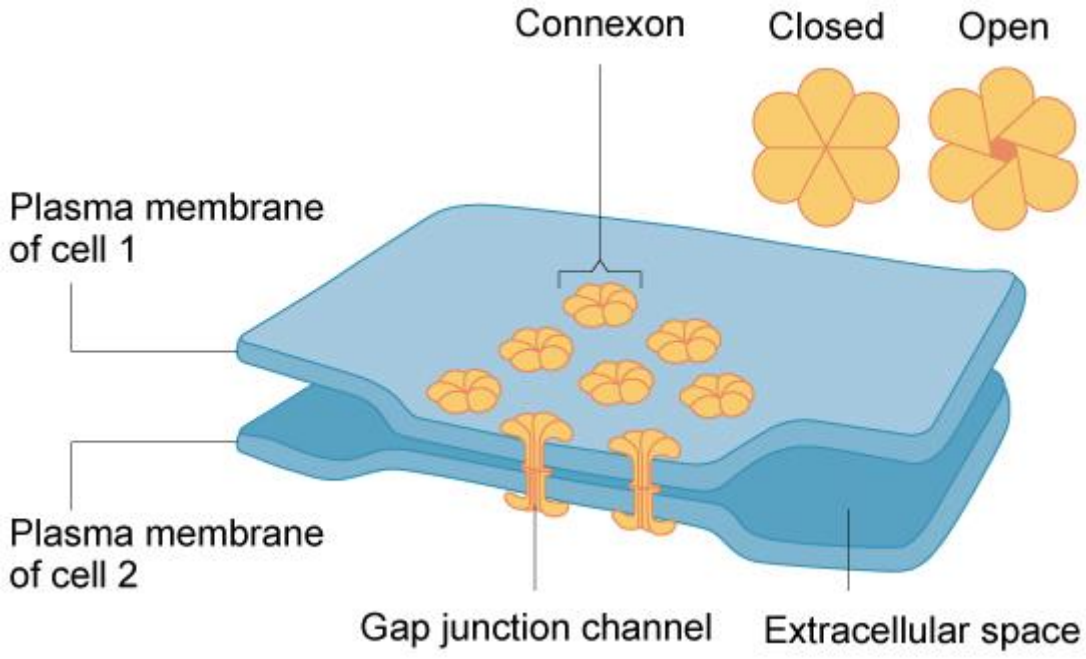


Image of the plasma membranes of two cells held together by gap junctions. Where two connexons from the different cells meet, they can form a channel leading from one cell into the next.

Gap junctions are particularly important in cardiac muscle: the electrical signal to contract spreads rapidly between heart muscle cells as ions pass through gap junctions, allowing the cells to contract in tandem.

Tight junctions

Not all junctions between cells produce cytoplasmic connections. Instead, **tight junctions** create a watertight seal between two adjacent animal cells. At the site of a tight junction, cells are held tightly against each other by many individual groups of tight junction proteins called **claudins**, each of which interacts with a partner group on the opposite cell membrane. The groups are arranged into strands that form a branching network, with larger numbers of strands making for a tighter seal.

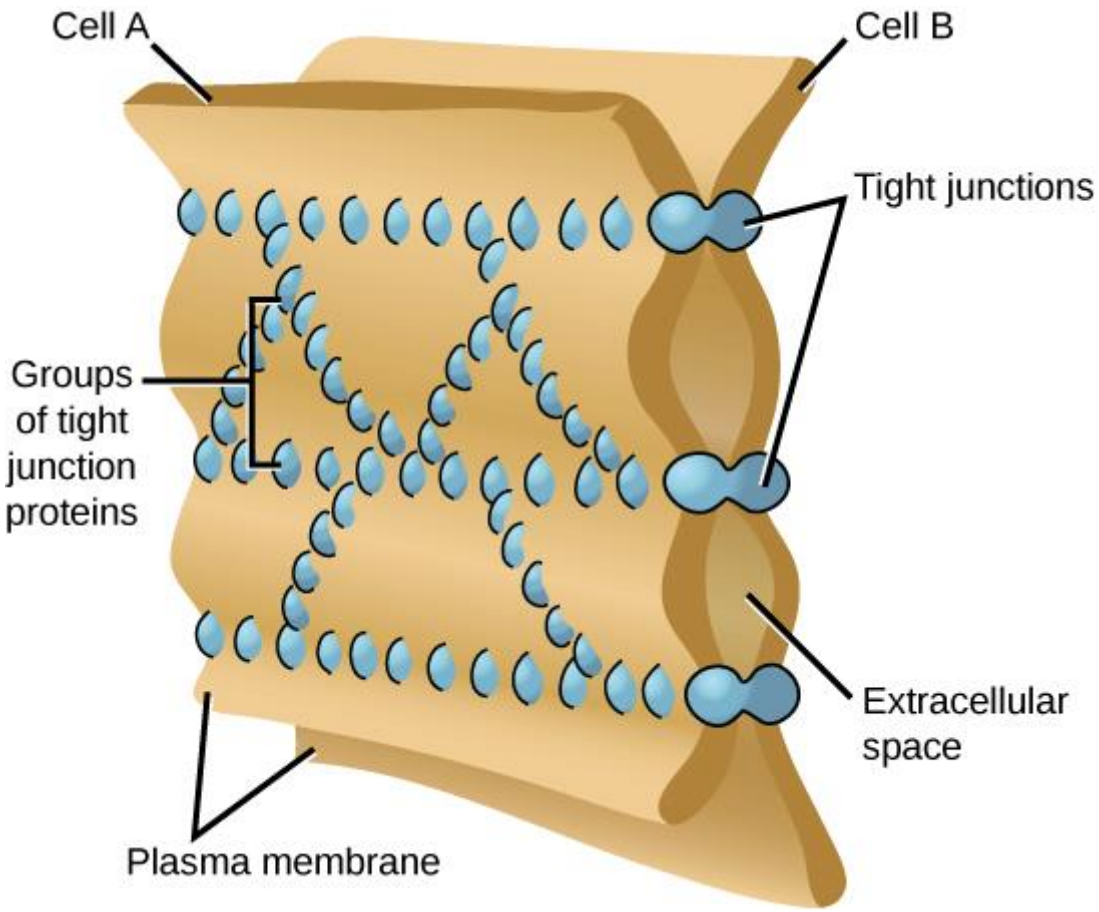


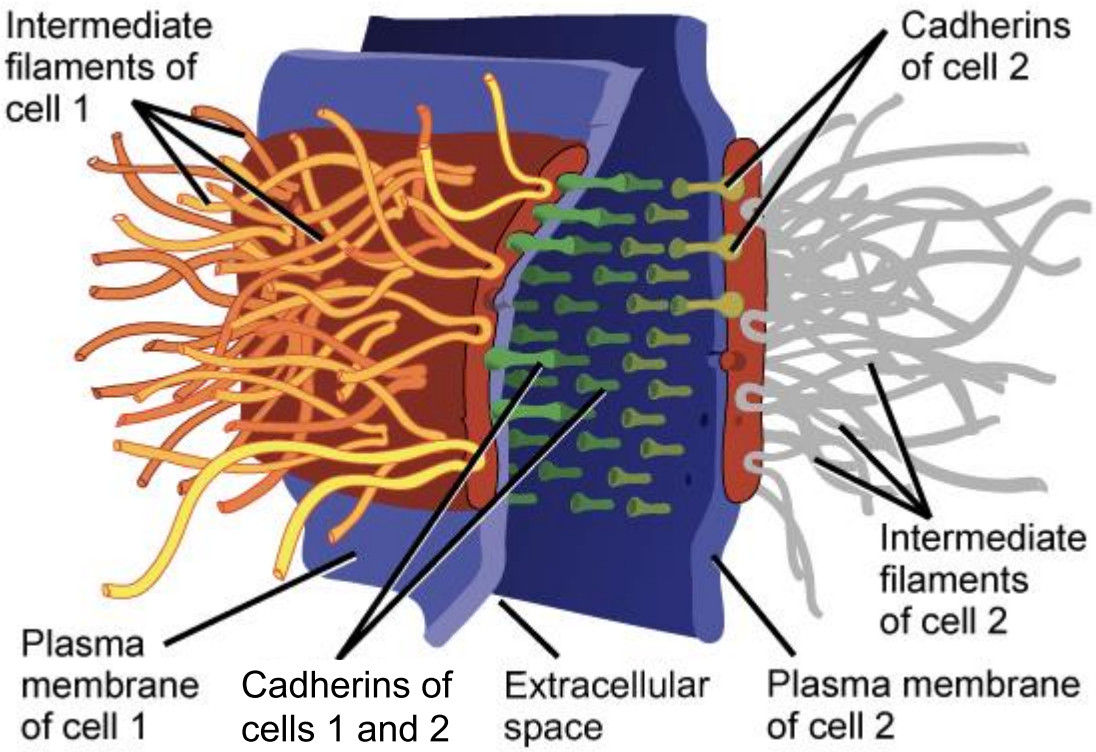
Image of the membranes of two cells held together by tight junctions. The tight junctions are like rivets, and they are arranged in multiple strands that form lines and triangles.

The purpose of tight junctions is to keep liquid from escaping between cells, allowing a layer of cells (for instance, those lining an organ) to act as an impermeable barrier. For example, the tight junctions between the epithelial cells lining your bladder prevent urine from leaking out into the extracellular space.

Desmosomes

Animal cells may also contain junctions called **desmosomes**, which act like spot welds between adjacent epithelial cells. A desmosome involves a complex of proteins. Some of these proteins extend across the membrane, while others anchor the junction within the cell. **Cadherins**, specialized adhesion proteins, are found on the membranes of both cells and interact in the space between them, holding the membranes

together. Inside the cell, the cadherins attach to a structure called the cytoplasmic plaque (red in the image at right), which connects to the intermediate filaments and helps anchor the junction.



Desmosomes pin adjacent cells together, ensuring that cells in organs and tissues that stretch, such as skin and cardiac muscle, remain connected in an unbroken sheet.

The following points highlight the two main types of junctions.

The types are: 1. Cell Junctions 2. Adherens Junctions

Type 1. Cell Junctions:

Commonly, there is a uniform intercellular space or gap of 200-300 A separating closely adjacent cells. Sometimes however, many cells in tissues are linked to each other and to the extracellular matrix at many specialized contact sites called cell junctions that permit or restrict the passage of ions and macromolecules between cells. Cell junctions are very minute structures and are not visible by light microscopy.

Cell junctions fall into three function groups:

1. Occluding or tight junction;

2. Anchoring junction;

3. Communicating junction.

The anchoring and communicating junctions can be again classified into several subtypes. So the classification of all types of cell junctions is shown in details in the chart above.

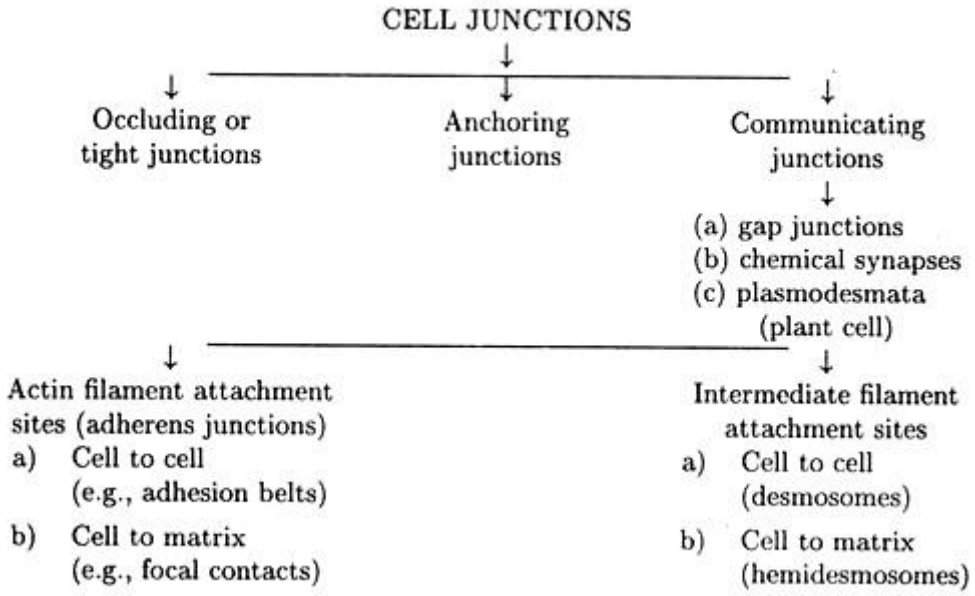
(i) Occluding or Tight Junctions:

All epithelial cells of the mammalian body have at least one important function in common: they act as selective permeability barriers that inhibit even small molecules or fluid from leaking from one side to other side. This function is successfully well maintained due to presence of many tight junctions also called the zonulae occludens.

Tight junctions are formed through fusion of plasma membranes of two adjacent cells at a series of points of contact without leaving any intercellular space. When thin sections through a tight junction are seen in the electron microscope, the plasma membranes of the adjacent cells appear to touch each other at intervals and even' to fuse. Tight junctions alternate with a region where plasma membranes are separated by intercellular space. In epithelial cells of the small intestine, tight junctions are usually located just below the apical microvillar surface. Tight junctions are composed of a belt-like band of anastomosing sealing fine standards that completely encircles the cell. But Freeze-fracture electron microscope gives a different view of the tight junction. The tight junction appears to consist, of an interlocking network of ridges on the cytoplasmic face of the plasma membrane of each of the two contacting cells. These ridges are made of trans membrane protein particles 3-4 nm in diameter.

The contact points of tight junction is formed by two rows of these particles of which one row is donated by one cell and other row is provided by the adjacent cell. The protein particles on the two cells are joined very tightly with each other to exclude the intercellular space at the point of contact. As a result, two membranes are fused at point of contact where it creates an impenetrable seal [Fig. 4.20(a) and (b)].

Fig. 4.20



Tight junctions are physiologically very important. The epithelial lining of the small intestine absorbs all nutrients from lumen of gut and does not allow to flow back the same again into the lumen due to presence of the tight junctions. They release the absorbed nutrients from the other side into the blood via extracellular fluid. Another function attributed to these junctions is their role in maintaining cell polarity by affording a physical barrier to the movement of integral proteins laterally.

(ii) Anchoring Junctions:

Anchoring junction is a type of cell junction by which a group of cells are joined together into strong structural units by connecting elements of their cytoskeleton. This type of junction is situated below the zone of tight junction and widely distributed in different tissues. They are most abundant in tissues that are subjected to severe mechanical stress, such as skin epithelium, the neck of the uterus, cardiac muscle etc.

They are found in two structurally and functionally different forms:

(a) Adherens junctions—are connection site for actin filaments (a type of contractile filament found in muscle cells),

(b) Desmosomes and hemi-desmosomes are connection sites for intermediate filaments.

All of these cell junctions are made of two classes of proteins:

(i) Intracellular attachment proteins that join the junctional complex to specific elements of the cytoskeleton (Fig. 4.21).

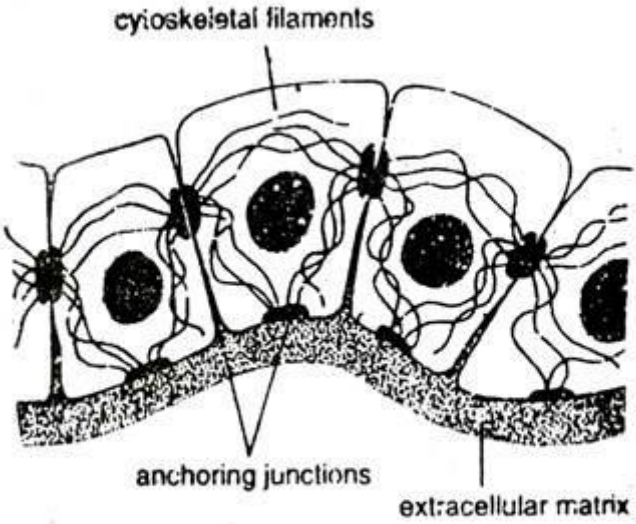


Fig. 4.21: Highly schematised drawing of anchoring junctions.

(ii) Trans-membrane linker glycoproteins whose intracellular part bind to one or more intracellular attachment proteins and the extracellular part bind with the same of the neighbouring cell or with the extracellular matrix (Fig. 4.22).

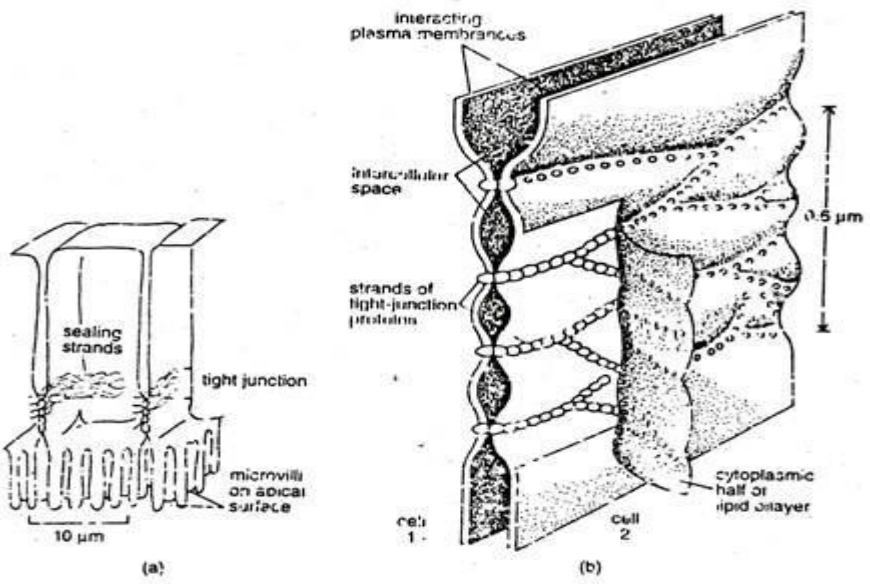


Fig. 4.20: Showing schematic drawing of (a) tight junction, and (b) a current model of a tight junction.

(iii) Communicating Junctions:

It is a type of cell junction that mediates the passage of chemical or electrical signals from one interacting cell to its partner.

Communicating junctions are of following types:

(a) Gap Junction:

These are communicating junctions composed of clusters of channel proteins that allow molecules of less than 1,500 Daltons to pass directly from the inside of one cell to the inside of the other. Almost all animal cells that come close to each other are separated by a gap of about 15 nm. But they are connected at several points by means of gap junction. Electron microscopic and X-ray diffraction observation reveal that both membranes contain cylinders of six dumbbell-shaped connection sub-units which are constructed from trans membrane protein. Two such cylinders join in the gap between the cells to form a channel about 1.5-2.0 nm in diameter that connects the cytoplasm of the two cells [Fig. 4.25(a) and (b)]. Each sub-unit of connection contains a single major protein of about 30,000 Daltons. The protein is made of 280 amino acid residues and crosses the lipid bilayer as four-helix.

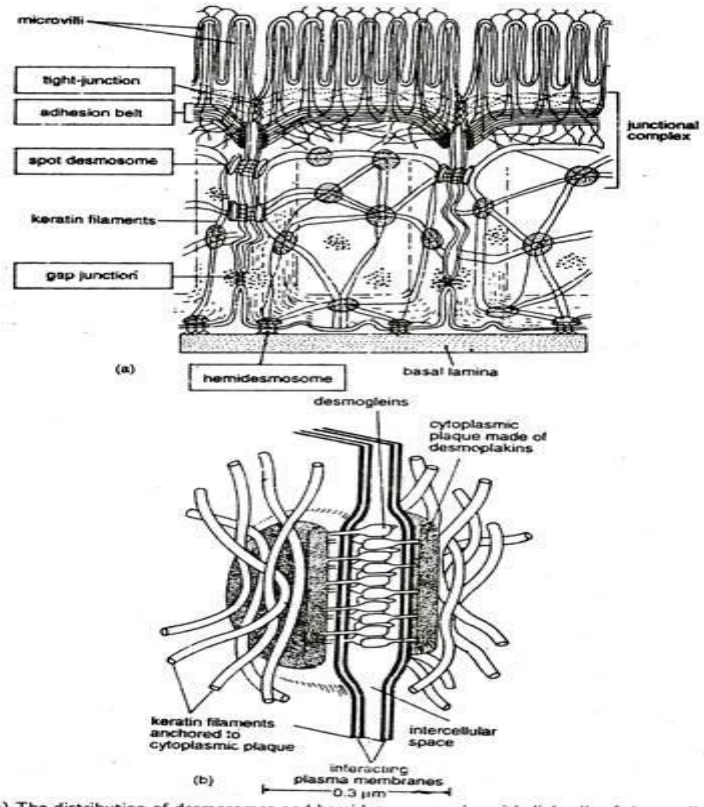


Fig. 4.24: (a) The distribution of desmosomes and hemidesmosomes in epithelial cells of the small intestine; (b) A highly schematised drawing of a desmosome.

Model of a gap junction derived from electron-micrographic analysis shows that one rotation of the six connexion sub-units about a central axis mediates the transition from an open to closed state. This closes the channels that connect the cell with its neighbours and prevents the exchange of small molecules between two cells. The channels close in presence of Ca^{2+} ion when its concentration rises markedly in the cytosol. Even slight increases in the level of cytosol Ca^{2+} ions or decreases in cytosolic pH can decrease the permeability of gap junctions.

Gap junctions allow many small molecules to pass from one cell to other cell. For example, AMP, ADP or ATP, inorganic ions, sugar, amino acids, nucleotides and vitamins can pass through gap junction, but not their macromolecules protein, nucleic acid and polysaccharide etc. Another important compound passed from cell to cell through gap junction is cyclic AMP which acts as an intracellular messenger and regulates a number of metabolic activities.

(b) Chemical Synapse:

Neurological impulses are transmitted from neurons to target cell by the synapse. There are two types of synapse—chemical and electrical which differ in both structure, and function, In chemical synapse, a narrow region, the synaptic cleft separates the plasma membranes of the presynaptic and postsynaptic cells.

The axon terminal of the presynaptical cell is filled with a particular neurotransmitter substance such as epinephrine or acetylcholine. The postsynaptic cells may be a dendrite, the cell body, the axon of another neuron or muscle or gland cell. When the postsynaptic cell is a muscle cell, the synapse is called neuromuscular junction or motor end plate. When a nerve impulse reaches the axon terminal, some of the synaptic vesicles fuse with the membrane and are exocytosed and discharge its neurotransmitter contents into the cleft. The transmitter diffuses across the cleft and, after a lag period of about 0.5 millisecond binds to receptors on the postsynaptic cells.

The receptors fall into two categories: channel-linked receptors and non-channel linked receptors. The channel linked receptors, upon binding neurotransmitter, promptly change their conformation to create an open channel for specific ions to cross the membrane. Therefore, they alter the membrane permeability. In case of non-channel receptors, the neurotransmitter-binding site is functionally coupled to an enzyme which catalyzes the production of an intracellular messenger, such as cyclic AMP, in presence of neurotransmitter. The intracellular messenger, in turn, causes changes in the postsynaptic cell, including modifications of the ion channels in its membrane.

Neurons communicating by an electric synapse are connected by gap junctions across which electric impulse can pass directly from the presynaptic cell to the postsynaptic one. Electric synapse allow an action potential to be generated in the postsynaptic cell with greater certainty than chemical synapses and without a lag period.

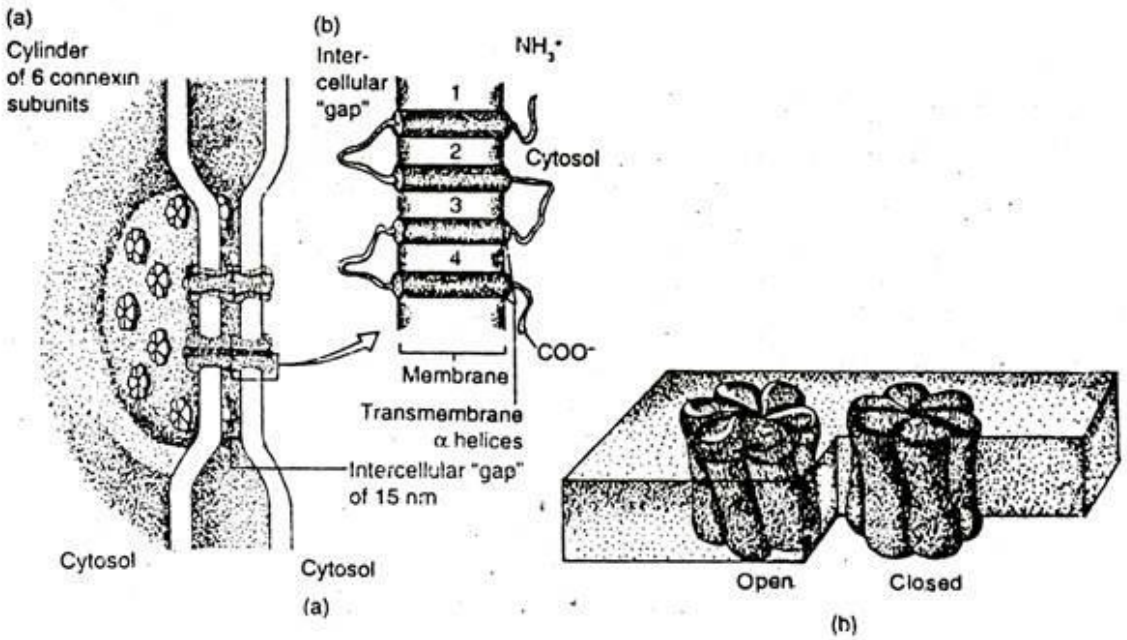


Fig. 4.25: (a) Model of a gap junction, based on electron microscopic and x-ray diffraction analyses; (b) Model of a gap junction derived from electron-micrographic analysis.

(c) Plasmodesmata:

Except few types of specialized cells, every living cell in a higher plant is interconnected to its living neighbors by fine cytoplasmic channels—each of which is called plasmodesma—that passes through the intervening cell walls.

Like gap junctions, plasmodesmata provide intercellular channels for molecules of about 1,000 molecular weight, including a variety of metabolic and signalling compounds. Depending on the plant type, the density of plasmodesmata varies from 1 to 10 per /μm^2, and even the smallest meristematic cells have more than 1,000 interconnections with their neighbors.

Electron micrographs of plasmodesmata show that it is a roughly cylindrical, membrane-lined channel with a diameter of 20 to 60 nm and it traverses cell walls up to 90 nm thick. Running from cell to cell through the centre of most plasmodesmata is a narrower cylindrical structure which is called desmotubule. Electron micrographs of desmotubule show that is continuous with elements of endoplasmic reticulum membrane of each of the connected cells.

Between the outside of the desmotubule and the inner face of the cylindrical plasma membrane is an annulus of cytosol. It often appears to be constricted at each end of the plasmodesmata. These constrictions

may regulate the flux of molecules through the annuals that joins the two cytosols. Many evidences indicate that plasmodesmata are, in fact, needed in cell-cell communication. Fluorescent water-soluble dye microinjected into plant cells spread to the cytoplasm of adjacent cells but not into the cell wall. Similarly, if pulses of electrical current are injected, through an electrode into one cell, a measuring electrode in an adjacent cell will detect the same pulses.

Many normal metabolic products such as sucrose, spread from cell to cell. As with gap junctions, movement of molecules through plasmodesmata is reversibly inhibited by an increase in cytosolic Ca^{2+}. Certain plant viruses and viriods can enlarge plasmodesmata in order to use this route to pass from cell to cell.

Type 2. Adherens Junctions:

Adherens junctions connect bundles of actin filaments from cell to cell or from cell to extracellular matrix.

(i) Cell to Cell Adherens Junctions:

They are generally found at the interface between lateral plasma membranes of adjacent columnar epithelial cells, just below the region of the tight junctions. In the junctional zone, the intercellular space is filled with fine filaments. They are connected with actin filaments and form a continuous band that girdles the inner surface of the plasma membrane of the connecting cells. This band is known as adhesion belt or zonula adherens and is' made of a web of 6 nm actin microfilaments. The actin bundles attach to plasma membranes through a complex of intracellular attachment proteins containing vinculin. It is thought that the contractile actin filament bundles play an important role in animal morphogenesis. They help in rolling up of the epithelial sheet into tube or other related structures (Fig. 4.23).

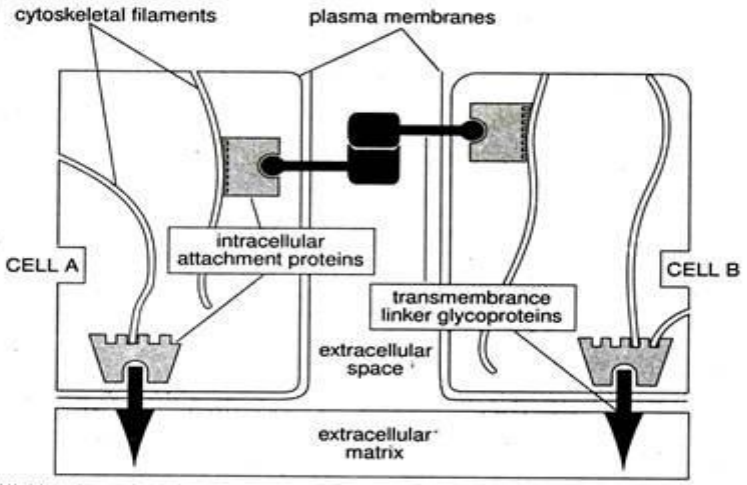


Fig. 4.22: Highly schematised drawing showing the two classes of proteins that constitute an anchoring junction.

(ii) Cell to Matrix Adherens Junctions:

The bundle of actin filaments within each cell comes out partly as trans membrane linker through some discrete sites of plasma membrane at the intracellular space and adhere the cell tightly with the extracellular matrix. In the junctional zone the specialised regions of plasma membrane are called focal contacts or adhesion plaques.

(iii) Desmosomes and Hemi Desmosomes:

Desmosomes are button-like points of intercellular contact that rivet cells together. They are connected with intermediate filaments (a type of cytoplasmic filament 8-12 nm in diameter). The particular type of intermediate filaments joined to the desmosome depends on the cell type. They are keratin filaments in most epithelial cell, desmin filaments in heart muscle cells and vimentin filaments found in some of the cells covering the surface of the brain.

The structure of desmosome is very complex. On the cytoplasmic surface of each interacting plasma membrane there is a dense disc-shaped plaque (0.5 µm. in diameter) composed of mixture of intracellular attachment proteins called desmoplakins. Each plaque is connected with a thick network of intermediate filaments which pass along the surface of the plaque. Trans membrane linker glycoproteins called desmogleins bind to the plaque and interact through their extracellular part to hold the adjacent membrane [Fig. 4.24 (a) and (b)].

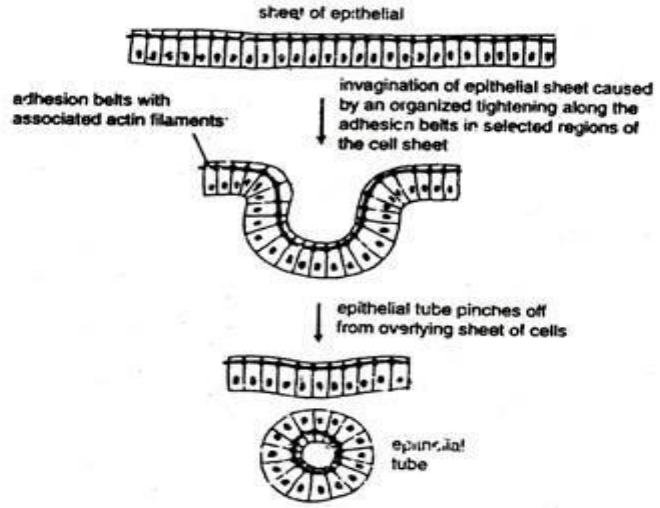


Fig. 4.23: The folding of an epithelial sheet to form an epithelial tube (as in the formation of the neural tube).

Hemi desmosomes or half-desmosomes are more or less morphologically similar to desmosomes but they are distinct from each other. Instead of joining adjacent cell membranes, hemi desmosome bind the basal surface of the cell to the underlying basal lamina. Both desmosomes and hemi desmosomes act as rivets to distribute tensile or shearing forces through an epithelium and its underlying connective tissue.

Probable Questions-

1. Describe different structure and function of plasmodesmata with suitable diagram
.
2. Describe different structure and function of Tight Junction with suitable diagram.
3. Describe different structure and function of Gap Junction with suitable diagram.
4. Describe different structure and function of Desmosomes with suitable diagram

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

UNIT-VII

Intracellular protein trafficking for secretory and non-secretory cells: Protein synthesis, Protein sorting and targeting to organelles; signal sequences, vesicle transport, packaging, storage and release, Targeting of proteins to lysosomes for degradation; Receptor mediated endocytosis

Objective: The secretory and endocytic pathways of eukaryotic organelles consist of multiple compartments, each with a unique set of proteins and lipids. Specific transport mechanisms are required to direct molecules to defined locations and to ensure that the identity, and hence function, of individual compartments are maintained. The localization of proteins to specific membranes is complex and involves multiple interactions. The topic summarizes the general principles of protein sorting in the secretory and endocytic pathways and highlight the dynamic nature of these processes. The molecular mechanisms involved in this transport along the secretory and endocytic pathways are discussed along with the signals responsible for targeting proteins to different intracellular locations.

Introduction to Protein Targeting:

A typical mammalian cell may contain numerous kinds of proteins and numerous individual protein molecules. The eukaryotic cell is a multi-compartmental structure. Its many organelles each requires different proteins. Except a few of them which are synthesized in mitochondria and chloroplasts all other proteins necessary for the cell and the ones to be secreted by the cell are synthesized in the cytosol on free ribosomes and on ribosomes bound to the endoplasmic reticulum. Most proteins are coded by the nuclear genome and synthesized in the cytoplasm. The proteins are present in the ER, mitochondria, chloroplasts, Golgi, peroxisomes, nucleus, in the cytosol and in the membranes of all these organelles. They are selectively transported into their appropriate organelles inside the cell and across the plasma membrane to be secreted outside the cell.

Some of them are carried into membrane bound vesicles which bud off from one organelle and transported in definite pathways. Different destinations of different proteins require sophisticated system for labelling and sorting newly synthesized proteins and ensuring that they reach their proper places. This transportation of proteins to their final destinations is called protein targeting. Proteins destined for cytoplasm and those to be incorporated into mitochondria, chloroplasts and nuclei are synthesized on free ribosomes in the

cytoplasm. Proteins destined for cellular membranes, lysosomes and extracellular transport, use a special distribution system. The main structures in this system are the rough endoplasmic reticulum (RER) and Golgi complex. The RER is a network of interconnected membrane enclosed vesicles or vacuoles. The endoplasmic reticulum is coated with polyribosomes to give it a rough appearance. The Golgi complex is also a stack of membrane bound sacs but they are not interconnected. The Golgi complex acts as a switching center for proteins to various destinations. Proteins to be directed to their destinations via Golgi complex are synthesized by ribosomes associated with endoplasmic reticulum.

Signal Sequence:

Protein sorting requires proper address labels which are in the form of peptide signal sequences. A signal sequence that directs the protein to its target is present in the form of 13-35 amino acids in the newly synthesized protein itself. It is the first to be synthesized and is mostly present at the amino N-terminal, sometimes at the carboxyl C- terminal. It is known as signal sequence or leader sequence. Some proteins are further sorted to a sub-compartment within the target organelle. For this purpose, a second signal sequence is present behind the first signal sequence which is cleaved. Proteins carried inside the membrane bound vesicles are called cargo proteins. An embedded or integrated protein is carried in the membrane of the vesicle, while secretory protein is carried within the lumen of the vesicle. The vesicle buds off from the donor surface and fuses with the target surface releasing its contents into the target organelle and the membrane protein is incorporated into the membrane of the target organelle. The process is repeated during the passage of protein from ER to Golgi to lysosomes and from Golgi to plasma membrane.

Function of Signal	Example of Signal Sequence
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into peroxisomes	-Ser-Lys-Leu-

Positively charged amino acids are shown in *red* and negatively charged amino acids in *blue*. Important hydrophobic amino acids are shown in *green*.
$^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

Table 1 : Some typical signal sequence

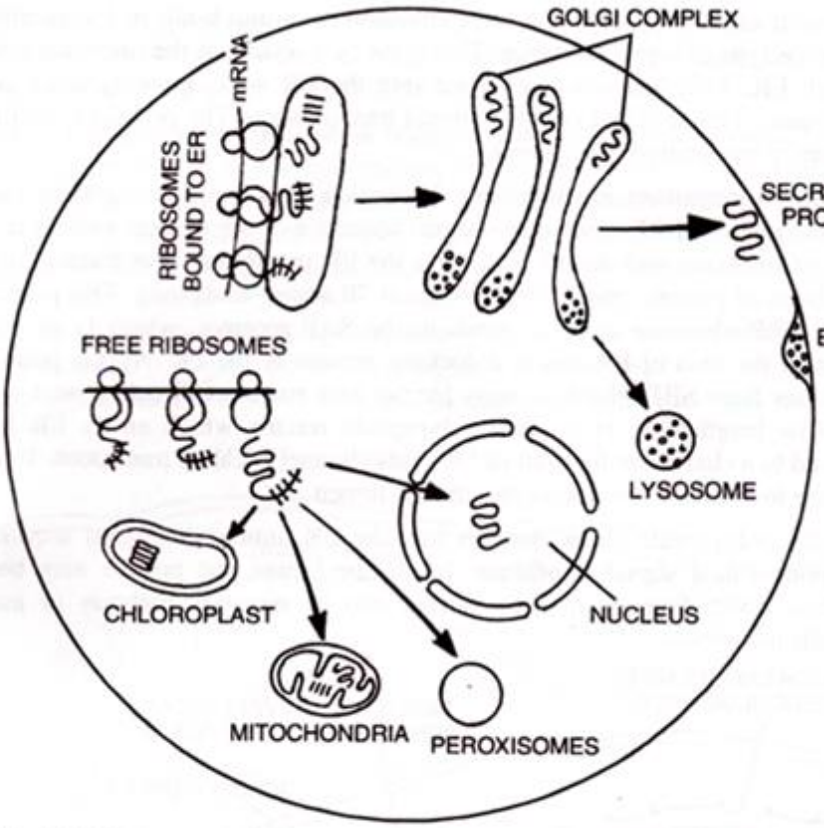


Fig. 21.1. Nascent proteins targeting to different organelles of the cell and cell secretion.

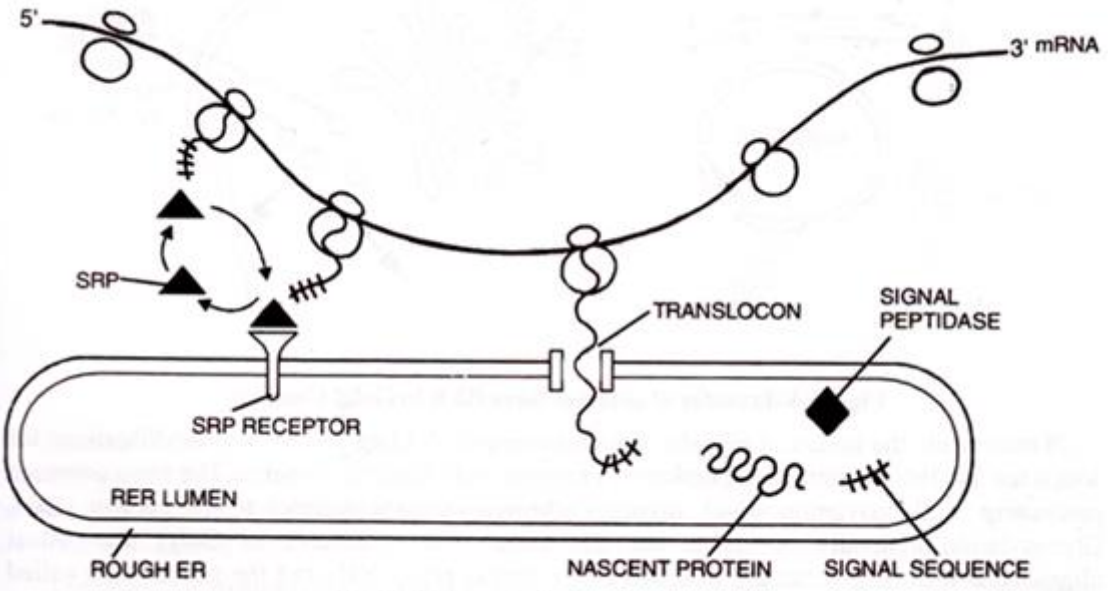


Fig. 21.2. Transport of proteins into ER

Transport of Proteins into ER:

A short N-terminus signal sequence at the beginning of the growing nascent protein chain' determines whether a ribosome synthesizing the proteins binds to ER or not. The protein synthesis always begins on free ribosomes. As the signal sequence emerges out of the ribosome, the large ribosomal sub-unit binds to ER membrane. This is decided by the type of signal sequence. This is the first sorting as the ribosome binds to ER, forming rough ER. Translocation takes place into the ER while growing chain is still bound to the ribosome. This is called co-translational translocation. The process is facilitated by the signal sequence recognition mechanism.

Signal Sequence Recognition Mechanism:

It consists of a signal recognition particle (SRP) present in the cytosol. SRP binds to the signal sequence of the nascent protein as soon as it emerges out of ribosome and directs it towards the ER membrane. The binding of SRP stops further synthesis of protein chain when it is about 70 amino acids long.

This prevents it from folding. The SRP-ribosome complex binds to the SAP receptor, which is an integral membrane protein in the wall of ER and is a docking protein of the ER. At this point GTP hydrolysis hydrolyses frees SRP which is ready for the next round of directing next nascent protein of ER. Now lengthening of nascent polypeptide restarts which enters ER lumen. Ribosome is aligned to a channel in the wall of ER. This channel is called translocon. It allows the elongating chain to enter the translocon into the ER lumen. As the growing polypeptide chain emerges into the ER lumen, the signal sequence is cleaved by a peptide called signal peptidase. Inside the lumen, the protein may become folded into its final active form or may be carried into its secretary pathway or may be embedded in the ER membrane.

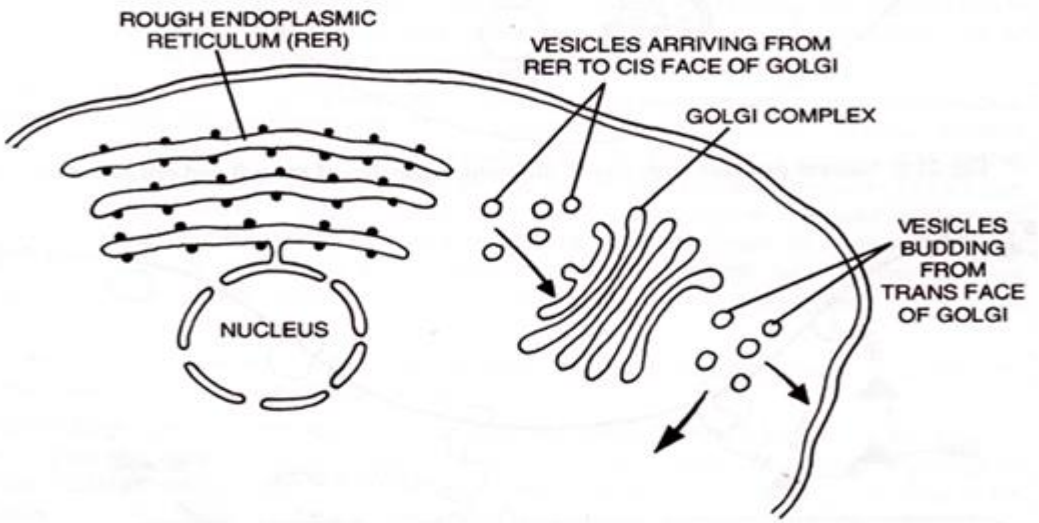


Fig. 21.3. Transfer of proteins form RER to Golgi Complex

Once inside the lumen of ER, the protein undergoes folding and several modifications for which the ER lumen contains a number of enzymes and chapreone proteins. The most common processing is glycosylation which involves addition of carbohydrates to the protein chain. Glycosylation generally occurs in the ER lumen but sometimes in Golgi also. Most oligosachharides or glycons are attached to the amino group NH_3 and the proteins are called N-linked glycoprotiens e.g. oligosachharide attached to aspargine. A preformed oligosachharide is added to the proteins. This structure is Man 9 $(Glc\ NAC)_2$ called high mannose structure. This contains mannose, glucose and N-acetylglucosamine). All nascent proteins start the sorting pathway by addition of the same pre-formed oligosachharide in plants and animals. Almost all proteins that enter the secretary pathway are glycosylated. In ER lumen, after glycosylation, many proteins are folded and stabilized by disulphide proteins bonds (-S-S-). This reaction is catalyzed by an enzyme, protein disulphide isomerase (PDI). Most of human proteins are stabilized by disulphide bonds.

Role of Golgi Complex in Protein Transportation:

The role of Golgi complex is to act as a switching center for proteins to various destinations. Both ER and Golgi apparatus are flattened cisternae. Transport of proteins from one compartment (donor) to the next one (target) is carried out in transport vesicles. The vesicles contain cargo proteins in their lumen and integral membrane proteins in their membranes.

The vesicles bud off from ER and fuse with the cis-compartment or receiving compartment of Golgi. In this process cargo proteins are delivered into the lumen of Golgi and membrane proteins become part of the membrane of the target vesicles. The proteins are glycosylated, folded, modified and sorted in ER. This process of glycosylation, modification and sorting of proteins continues in successive Golgi cisternae.

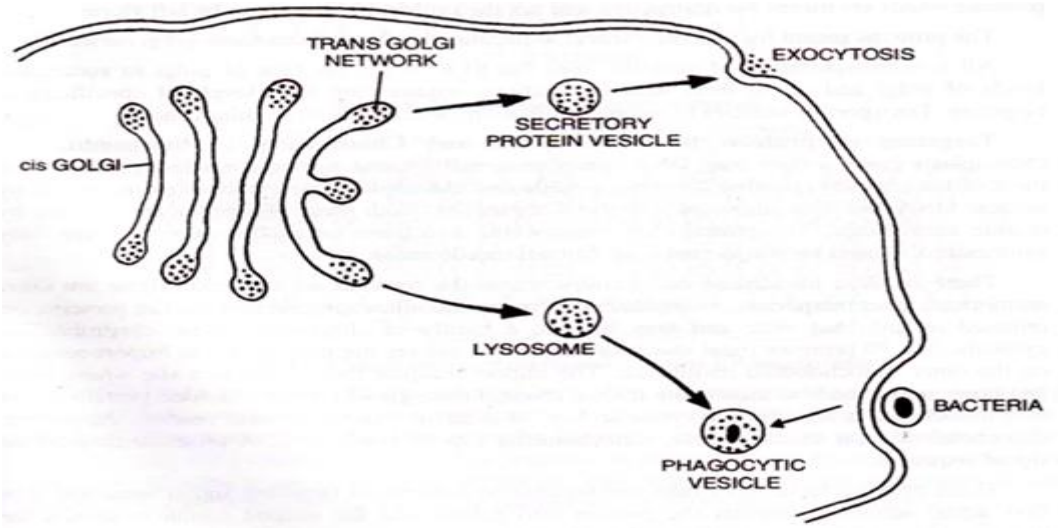


Fig. 21.4. Transport of proteins from Golgi to Lysosomes

Starting from the cis-compartment to medial compartment and lastly to trans-Golgi network proteins are exported to the end target. In trans-golgi network (TGN) proteins are further sorted to be delivered to lysosomes, for secretion outside the cell and to plasma membrane according to signals present in the nascent proteins.

Transport of Proteins from Golgi to Lysosomes:

The lysosomal enzymes and lysosomal membrane proteins are synthesized in rough ER and transported to Golgi cisternae and ultimately to lysosomes. The sorting signal that directs the lysosomal enzymes from the trans- Golgi network (TGN) to lysosomes is mannose 6-phosphate (M6P). The attachment of M6P to lysosomal enzymes prevents their further modification.

Separation of M6P bearing lysosomal enzymes from other proteins takes place in TGN. The wall of TGN contains M6P receptors. These M6P receptors bind to lysosomal proteins. The vesicles containing these receptor bearing proteins bud off from TGN. These vesicles are called lysosomes. Later these vesicles fuse with vesicles which have arisen by pinacocytosis and phagocysis to form secondary lysosomes. Low pH of Lysosomes triggers the dissociation of enzymes from the receptors. The M6P receptors are recycled back to trans-golgi network in vesicles. Lysosomes contain hydrolyzing proteolytic enzyme, which digests proteins meant for degradation. A protein named ubiquitin marks the proteins meant for destruction. Ubiquitin is present in all eukaryotic cells. This mechanism degrades only those proteins which are meant for destruction and not the proteins which are to be left alone. The proteins meant for secretion travel to plasma membrane from trans-golgi network.

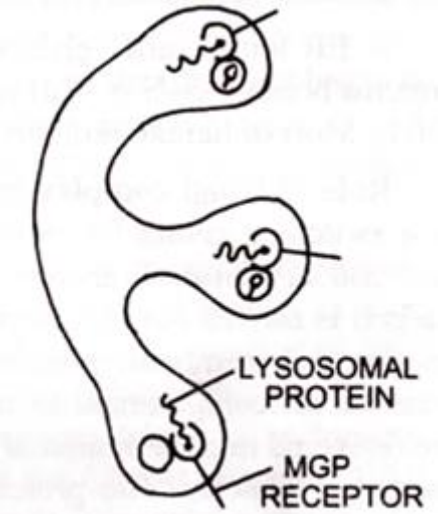


Fig. 5. Trans-Gologi Network

All this transportation of vesicles from the RER to the cis face of golgi to successive levels of golgi and on to their final destinations requires the high levels of specificity in targeting. Transport of vesicles to wrong destinations would lead to cellular chaos.

Translocation of Peptides.

The synthesis of peptides in the ER through signed sequence has been described. There are several types of processes for the transfer of peptides into and through membranes in the ER, mitochondria etc. Generally, peptides with cleared or un-cleared signal sequence are transported into these membranes.

There are differences in the composition of the signal recognition particle (SRP), receptor of the SRP and in the maturation process of the peptides. When the signal sequences are in the interior of the peptide molecule and not at the N terminal portion of the peptide, they act as stop-transfer signals. In other words, peptide molecules will not pass-through ER. The transport of peptides takes place along with its translation, so it is called co-translation process. The transport of the peptide may take place either after cleavage of the terminal signal sequence or the peptides may enter into the two domains, i.e., in the cytoplasm and in the lumen of ER with the cleavage of signal sequence. The signal sequence remains bound to the membrane.

Cleavage of Signal Sequence:

Cytochrome b_5, Calcium transferring ATPase and some plasma membrane proteins are transported through the membrane without any cleavage of the signal sequence (Fig. 3.3).

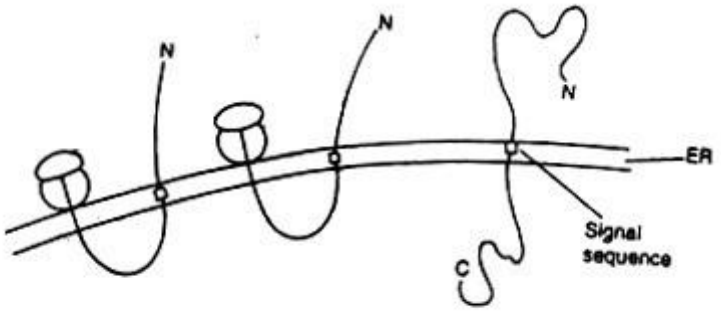


Fig. 3.3: Co-translational transport of protein without any cleavage of signal sequence.

The uptake of proteins into mitochondria and chloroplasts takes place in a special way. Most of the proteins of these organelles are synthesised on the ribosomes of the cytoplasm and then transported to the organelles.

Co-translational transport, i.e., simultaneous synthesis and uptake of protein/peptide into the membrane, do not occur in these organelles like that of endoplasmic reticulum membrane. In case of these organelles, proteins are first synthesised on the cytoplasm and then these are bound to the outer membrane by a membrane receptor for transportation. These proteins are then distributed to the outer membrane, inner membrane and the matrix of the mitochondria. At first, proteins are synthesised in the cytoplasm, then a 40 KD protein is attached with the signal sequence present at the N terminal portion. This 40 KD protein helps to identify the receptor protein present in the inner membrane of the mitochondria. Finally, with heads protruding out of proteins, they are distributed to the different areas of the mitochondria with the cleavage of signal sequence.

Intracellular protein traffic for secretory and non-secretory cells:

Before a eukaryotic cell divides, it must duplicate its membrane-enclosed organelles. Even in cells that are not dividing, proteins are being produced continually. These newly synthesized proteins must be accurately delivered to their appropriate organelle-some for eventual secretion from the cell and some to replace organelle proteins that have been degraded. For some organelles, including mitochondria, chloroplasts, peroxisomes, and the interior of the nucleus, proteins are delivered directly from the cytosol. For others, including the Golgi apparatus, lysosomes, endosomes, and the inner nuclear membrane, proteins and lipids are delivered indirectly via the ER, which is itself a major site of lipid and protein synthesis. Proteins enter the ER directly from the cytosol: some are retained there, but most are transported by vesicles to the Golgi apparatus and then onward to the plasma membrane or other organelles. Peroxisomes acquire some of their membrane proteins from

the ER, but the bulk of their enzymes enter directly from the cytosol. Proteins made in the cytosol are dispatched to different locations in the cell according to specific address labels contained in their amino acid sequence. Once at the correct address, the protein enters either the membrane or the interior lumen of its designated organelle.

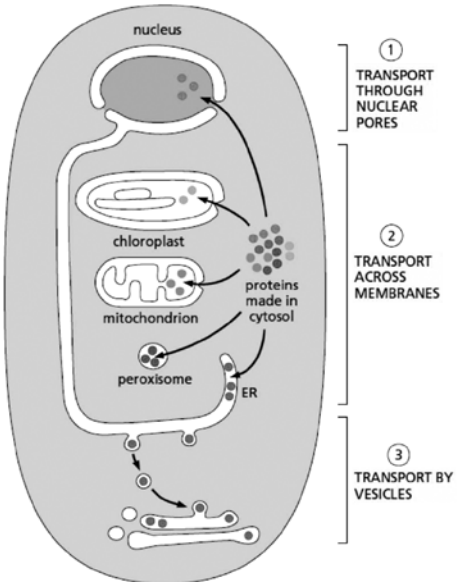
Protein synthesis and sorting signal : The synthesis of virtually all proteins in the cell begins on ribosomes in the cytosol. The exceptions are the few mitochondrial and chloroplast proteins that are synthesized on ribosomes inside these organelles; most mitochondrial and chloroplast proteins, however, are made in the cytosol and subsequently imported. The fate of any protein molecule synthesized in the cytosol depends on its amino acid sequence, which can contain a sorting signal that directs the protein to the organelle in which it is required. Proteins that lack such signals remain as permanent residents in the cytosol; those that possess a sorting signal move from the cytosol to the appropriate organelle. Different sorting signals direct proteins into the nucleus, mitochondria, chloroplasts (in plants), peroxisomes, and the ER.

This task is accomplished in different ways by different organelles.

A. Proteins moving from the cytosol into the nucleus are transported through the nuclear pores, which penetrate both the inner and outer nuclear membranes. The pores function as selective gates that actively transport specific macromolecules but also allow free diffusion of smaller molecules (mechanism 1 in Figure 1).

B. Proteins moving from the cytosol into the ER, mitochondria, or chloroplasts are transported across the organelle membrane by *protein translocators* located in the membrane. Unlike transport through nuclear pores, the transported protein must usually unfold in order to snake across the membrane through the translocator (mechanism 2 in Figure 1).

C. Proteins moving onward from the ER-and from one compartment of the endomembrane system to another-are transported by a mechanism that is fundamentally different. These proteins are ferried by *transport vesicles*, which pinch off from the membrane of one compartment and then fuse with the membrane of a second compartment (mechanism 3 in Figure below). In this process, transport vesicles deliver soluble cargo proteins, as well as the proteins and lipids that are part of the vesicle membrane.



- **Figure : Mechanism of protein import in organ**

Proteins Transport in Nucleus

The nuclear envelope, which encloses the nuclear DNA and defines the nuclear compartment, is formed from two concentric membranes. The inner nuclear membrane contains some proteins that act as binding sites for the chromosomes and others that provide anchorage for the *nuclear lamina*, a finely woven meshwork of protein filaments that lines the inner face of this membrane and provides structural support for the nuclear envelope. The composition of the *outer nuclear membrane* closely resembles the membrane of the ER, with which it is continuous. The nuclear envelope in all eukaryotic cells is perforated by nuclear pores that form the gates through which molecules enter or leave the nucleus. A nuclear pore is a large, elaborate structure composed of a complex of about 30 different proteins. Many of the proteins that line the nuclear pore contain extensive, unstructured regions in which the polypeptide chains are largely disordered. These disordered segments form a soft, tangled meshwork-like a kelp forest-that fills the center of the channel, preventing the passage of large molecules but allowing small, water-soluble molecules to pass freely and nonselectively between the nucleus and the cytosol (Figure below).

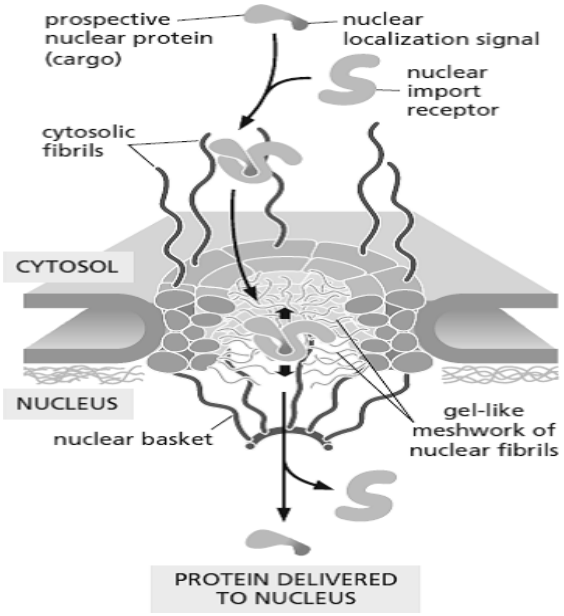


Figure : Protein transport in nucleus

Selected larger molecules and macromolecular complexes also need to pass through nuclear pores. RNA molecules, which are synthesized in the nucleus, and ribosomal subunits, which are assembled there, must be exported to the cytosol. And newly mad proteins that are destined for the nucleus must be imported from the cytosol. To gain entry to a pore, these large molecules and macromolecular complexes must display an appropriate sorting signal. The signal sequence that directs a protein from the cytosol into the nucleus, called a *nuclear localization signal*, typically consists of one or two short sequences containing several positively charged lysines or arginines (Table 1). The nuclear localization signal on proteins destined for the nucleus is recognized by cytosolic proteins called *nuclear import receptors*. These receptors help direct a newly synthesized protein to a nuclear pore by interacting with the tentacle-like fibrils that extend from the rim of the pore into the cytosol Once there, the nuclear import receptor penetrates the pore by grabbing onto short, repeated amino acid sequences within the tangle of nuclear pore proteins that fill the center of the pore. When the nuclear pore is empty, these repeated sequences bind to one another, forming a loosely packed gel. Nuclear import receptors interrupt these interactions, and they open a local passageway through the meshwork. The import receptors simply bump along from one repeat sequence to the next, until they enter the nucleus and deliver their cargo (Figure below). The empty receptor then returns to the cytosol via the nuclear pore for reuse.

Protein transport in Mitochondria and Chloroplast:

Both mitochondria and chloroplasts are surrounded by inner and outer membranes, and both organelles specialize in the synthesis of ATP. Chloroplasts also contain a third membrane system, the thylakoid membrane. Although both organelles contain their own genomes and make some of their own proteins, most mitochondrial and chloroplast proteins are encoded by genes in the nucleus and are imported from the cytosol. These proteins usually have a signal sequence at their N-terminus that allows them to enter their specific organelle. Proteins destined for either organelle are translocated simultaneously across both the inner and outer membranes at specialized sites where the two membranes contact each other. Each protein is unfolded as it is transported, and its signal sequence is removed after translocation is complete. Chaperone proteins inside the organelles help to pull the protein across the two membranes and to fold it once it is inside. Subsequent transport to a particular site within the organelle, such as the inner or outer membrane or the thylakoid membrane in chloroplasts, usually requires further sorting signals in the protein, which are often only exposed after the first signal sequence has been removed. The insertion of transmembrane proteins into the inner membrane, for example, is guided by signal sequences in the protein that start and stop the transfer process across the membrane, as observed in transmembrane proteins in the ER membrane (Figure below).

The growth and maintenance of mitochondria and chloroplasts also require the incorporation of new lipids into the organelle membranes. Most of their membrane phospholipids are thought to be imported from the ER, which is the main site of lipid synthesis in the cell. Phospholipids are transported to these organelles by lipid-carrying proteins that extract a phospholipid molecule from one membrane and deliver it into another. Such transport may occur at specific junctions where mitochondrial and ER membranes are held in close proximity. Thanks to these lipid-carrying proteins, the different cell membranes are able to maintain different lipid compositions.

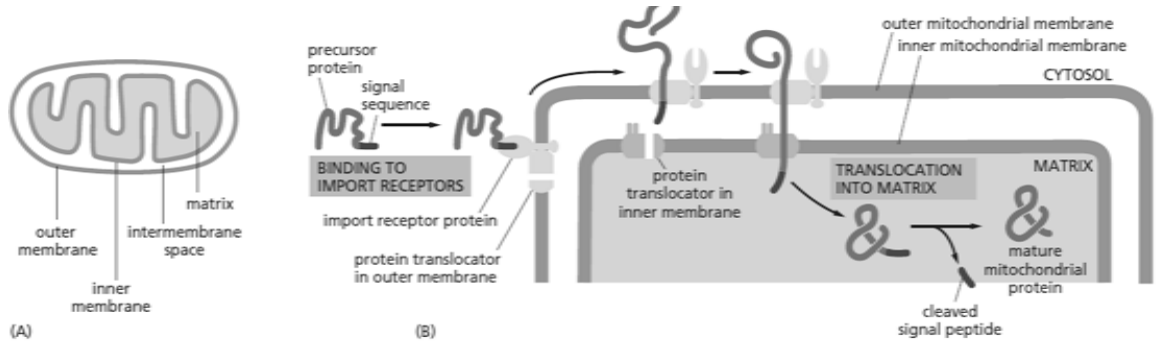


Figure: Protein transport in Mitochondria and Chloroplast

Protein Transport in Peroxisome

Peroxisomes generally contain one or more enzymes that produce hydrogen peroxide, hence their name. These organelles are present in all eukaryotic cells, where they break down a variety of molecules, including toxins, alcohol, and fatty acids. They also synthesize certain phospholipids, including those that are abundant in the myelin sheath that insulates nerve cell axons. Peroxisomes acquire the bulk of their proteins via selective transport from the cytosol. A short sequence of only three amino acids serves as an import signal for many peroxisomal proteins. This sequence is recognized by receptor proteins in the cytosol, at least one of which escorts its cargo protein all the way into the peroxisome before returning to the cytosol. Like the membranes of mitochondria and chloroplasts, the peroxisomal membrane contains a protein translocator that aids in the transport. Unlike the mechanism that operates in mitochondria and chloroplasts, however, proteins do not need to unfold to enter the peroxisome-and the transport mechanism is still mysterious.

Although most peroxisomal proteins-including those embedded in the peroxisomal membrane-come from the cytosol, a few membrane proteins arrive via vesicles that bud from the ER membrane. The vesicles either fuse with preexisting peroxisomes or import peroxisomal proteins from the cytosol to grow into mature peroxisomes. The most severe peroxisomal disease, called Zellweger syndrome, is caused by mutations that block peroxisomal protein import.

Protein Transport in Endoplasmic reticulum (ER)

The endoplasmic reticulum serves as an entry point for proteins destined for other organelles, as well as for the ER itself. Proteins destined for the Golgi apparatus, endosomes, and lysosomes, as well as proteins destined for the cell surface, all first enter the ER from the cytosol. Once inside the ER lumen, or embedded in the ER membrane, individual proteins will not re-enter the cytosol during their onward journey. They will instead be ferried by transport vesicles from organelle to organelle within the endomembrane system, or to the plasma membrane.

Two kinds of proteins are transferred from the cytosol to the ER: (1) water soluble proteins are completely translocated across the ER membrane and are released into the ER lumen; (2) prospective transmembrane proteins are only partly translocated across the ER membrane and become embedded in it. The water-soluble proteins are destined either for secretion (by release at the cell surface) or for the lumen of an organelle of the endomembrane system. The transmembrane proteins are destined to reside in the membrane of one of these organelles or in the plasma

membrane. All of these proteins are initially directed to the ER by an *ER signal sequence*, a segment of eight or more hydrophobic amino acids, which is also involved in the process of translocation across the membrane.

Unlike the proteins that enter the nucleus, mitochondria, chloroplasts, or peroxisomes, most of the proteins that enter the ER begin to be threaded across the ER membrane before the polypeptide chain has been completely synthesized. This requires that the ribosome synthesizing the protein be attached to the ER membrane. There are, therefore, two separate populations of ribosomes in the cytosol. *Membrane-bound ribosomes* are attached to the cytosolic side of the ER membrane (and outer nuclear membrane) and are making proteins that are being translocated into the ER. *Free ribosomes* are unattached to any membrane and are making all of the other proteins encoded by the nuclear DNA. When a ribosome happens to be making a protein with an ER signal sequence, the signal sequence directs the ribosome to the ER membrane. Two protein components help guide ER signal sequences to the ER membrane: (1) a *signal- recognition particle* (*SRP*), present in the cytosol, binds to both the ribosome and the ER signal sequence when it emerges from the ribosome, and (2) an *SRP receptor*, embedded in the ER membrane, recognizes the SRP. Binding of an SRP to a ribosome that displays an ER signal sequence slows protein synthesis by that ribosome until the SRP engages with an SRP receptor on the ER. Once bound, the SRP is released, the receptor passes the ribosome to a protein translocator in the ER membrane, and protein synthesis recommences. The polypeptide is then threaded across the ER membrane through a *channel* in the translocator (Figure 4). Thus the SRP and SRP receptor function as molecular matchmakers, uniting ribosomes that are synthesizing proteins with an ER signal sequence and available translocation channels in the ER membrane (Figure below).

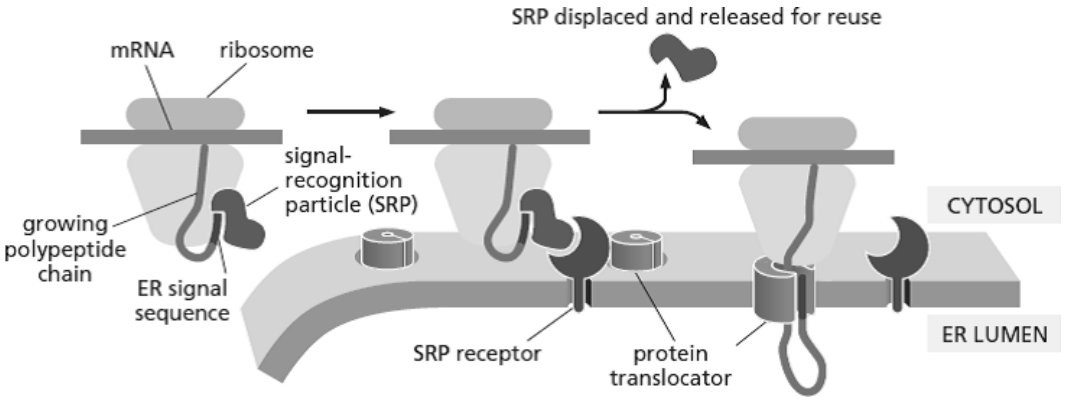


Figure 4: Protein transport in ER

Some proteins remain embedded in the ER membrane as transmembrane proteins. Such proteins has some parts of the polypeptide chain that must be translocated completely across the lipid bilayer, whereas other parts remain fixed in the membrane. In the simplest case, that of a transmembrane protein with a single membrane-spanning segment, the N-terminal signal sequence initiates translocation-as it does for a soluble protein. But the transfer process is halted by an additional sequence of hydrophobic amino acids, a *stop-transfer sequence*, further along the polypeptide chain. At this point, the translocation channel releases the growing polypeptide chain sideways into the lipid bilayer. The N-terminal signal sequence is cleaved off, whereas the stop-transfer sequence remains in the bilayer, where it forms an α- helical membrane-spanning segment that anchors the protein in the membrane. As a result, the protein ends up as a single-pass transmembrane protein inserted in the membrane with a defined orientation-the N-terminus on the lumenal side of the lipid bilayer and the C-terminus on the cytosolic side (Figure below).

Some transmembrane proteins, an internal, rather than an N-terminal, signal sequence is used to start the protein transfer; this internal signal sequence, called a *start-transfer sequence*, is never removed from the polypeptide. This arrangement occurs in some transmembrane proteins in which the polypeptide chain passes back and forth across the lipid bilayer. In these cases, hydrophobic signal sequences are thought to work in pairs: an internal start-transfer sequence serves to initiate translocation, which continues until a stop-transfer sequence is reached; the two hydrophobic sequences are then released into the bilayer, where they remain as membrane-spanning α helices (Figure below). In complex multipass proteins, in which many hydrophobic α helices span the bilayer, additional pairs of start- and stop-transfer sequences come into play: one sequence reinitiates translocation further down the polypeptide chain, and the other stops translocation and causes polypeptide release, and so on for subsequent starts and stops. Thus, multipass membrane proteins are stitched into the lipid bilayer as they are being synthesized, by a mechanism resembling the workings of a sewing machine (Figure below).

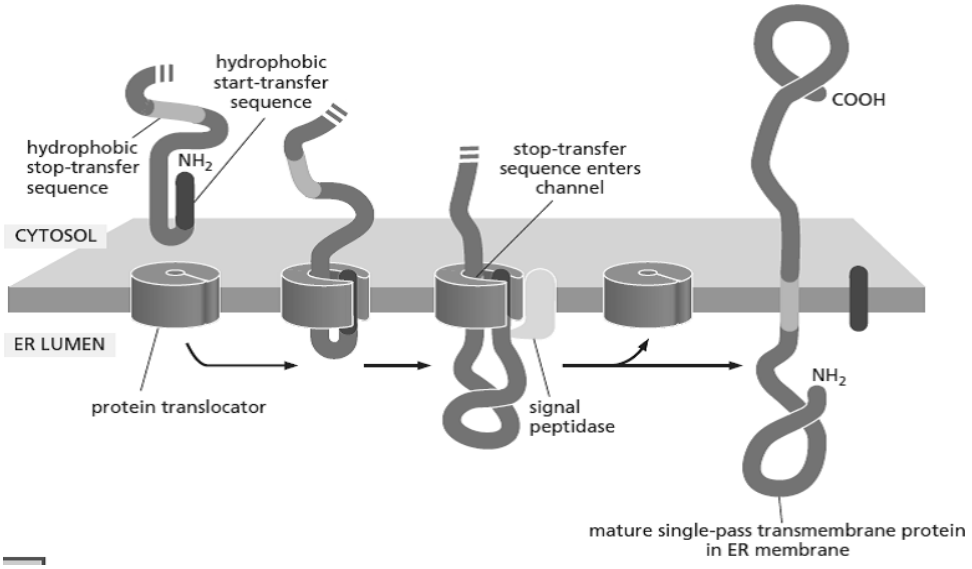


Figure : Transmembrane protein transport in ER

Vesicular Protein Transport:

Entry into the ER lumen or membrane is usually only the first step on a pathway to another destination. That destination, initially at least, is generally the Golgi apparatus; there, proteins and lipids are modified and sorted for shipment to other sites. Transport from the ER to the Golgi apparatus and from the Golgi apparatus to other compartments of the endomembrane system is carried out by the continual budding and fusion of transport vesicles. This vesicular transport extends outward from the ER to the plasma membrane, and inward from the plasma membrane to lysosomes, and thus provides routes of communication between the interior of the cell and its surroundings. As proteins and lipids are transported outward along these pathways, many of them undergo various types of chemical modification, such as the addition of carbohydrate side chains.

Vesicular transport between membrane-enclosed compartments of the endomembrane system is highly organized. A major outward *secretory pathway* starts with the synthesis of proteins on the ER membrane and their entry into the ER, and it leads through the Golgi apparatus to the cell surface; at the Golgi apparatus, a side branch leads off through endosomes to lysosomes. A major inward *endocytic pathway*, which is responsible for the ingestion and degradation of extracellular molecules, moves materials from the plasma membrane, through endosomes, to lysosomes (Figure below). To function optimally, each transport vesicle that buds off from a compartment must take with it only the proteins appropriate to its destination and must fuse only with the appropriate target

membrane. A vesicle carrying cargo from the Golgi apparatus to the plasma membrane, for example, must exclude proteins that are to stay in the Golgi apparatus, and it must fuse only with the plasma membrane and not with any other organelle. While participating in this constant flow of membrane components, each organelle must maintain its own distinct identity, that is, its own distinctive protein and lipid composition. All of these recognition events depend on proteins displayed on the surface of the transport vesicle.

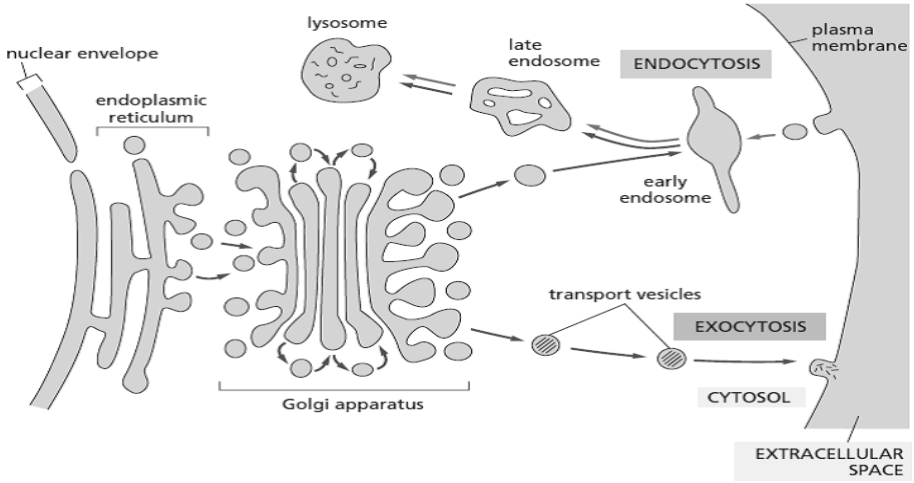


Figure : Transport in Endomembrane system

Protein Packaging via Coated Vesicle :

Vesicles that bud from membranes usually have a distinctive protein coat on their cytosolic surface and are therefore called coated vesicles. After budding from its parent organelle, the vesicle sheds its coat, allowing its membrane to interact directly with the membrane to which it will fuse. Cells produce several kinds of coated vesicles, each with a distinctive protein coat. The coat serves at least two functions: it helps shape the membrane into a bud and captures molecules for onward transport. The best- studied vesicles are those that have an outer coat made of the protein clathrin. These *clathrin- coated vesicles* bud from both the Golgi apparatus on the outward secretory pathway and from the plasma membrane on the inward endocytic pathway. At the plasma membrane, for example, each vesicle starts off as a *clathrin-coated pit*. Clathrin molecules assemble into a basketlike network on the cytosolic surface of the membrane, and it is this assembly process that starts shaping the membrane into a vesicle (Figure below). A small GTP-binding protein called *dynamin* assembles as a ring around the neck of each deeply invaginated coated pit. Together with

other proteins recruited to the neck of the vesicle, the dynamin causes the ring to constrict, thereby pinching off the vesicle from its parent membrane. Other kinds of transport vesicles, with different coat proteins, are also involved in vesicular transport. They form in a similar way and carry their own characteristic sets of molecules between the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane.

The mechanism of selecting its particular cargo in transport vesicle is best understood for clathrin-coated vesicles. Clathrin itself plays no part in choosing specific molecules for transport. This is the function of a second class of coat proteins called *adaptins*, which both secure the clathrin coat to the vesicle membrane and help select cargo molecules for transport. Molecules for onward transport carry specific *transport signals* that are recognized by *cargo receptors* in the Golgi or plasma membrane. Adaptins help capture specific cargo molecules by trapping the cargo receptors that bind them. In this way, a selected set of cargo molecules, bound to their specific receptors, is incorporated into the lumen of each newly formed clathrin-coated vesicle (Figure below). There are different types of adaptins: the adaptins that bind cargo receptors in the plasma membrane, for example, are not the same as those that bind cargo receptors in the Golgi apparatus, reflecting the differences in the cargo molecules to be transported from each of these sources.

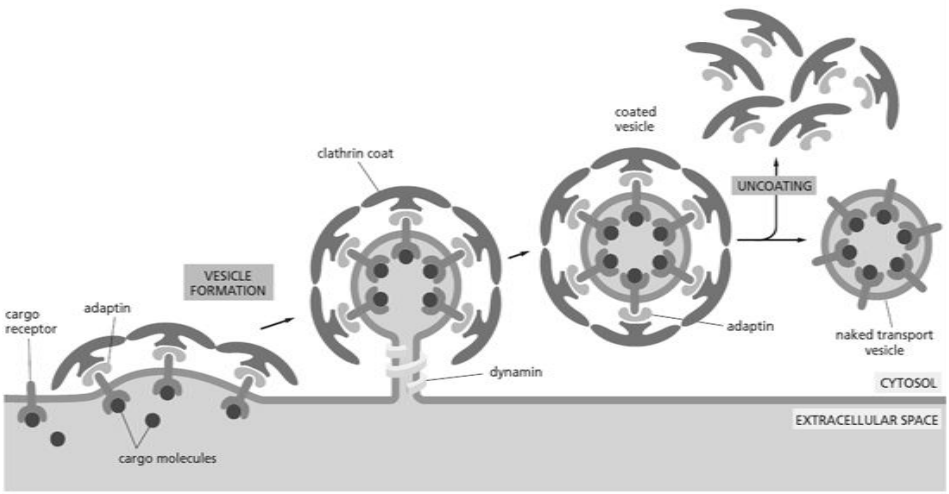


Figure: Clathrin coated vesicle transport system

Another class of coated vesicles, called *COP-coated vesicles* (COP being shorthand for "coat protein"), is involved in transporting molecules between the ER and the Golgi apparatus and from one part of the Golgi apparatus to another (Table 2).

Type of Coated Vesicle	Coat Proteins	Origin	Destination
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COP-coated	COP proteins	ER Golgi cisterna Golgi apparatus	Golgi apparatus Golgi cisterna ER

Table 2: Type of Coated vesicles

Coated vesicle recognition, docking and unloading of cargo into destination membrane :

Once a transport vesicle has reached its target, it must recognize and dock with its specific organelle and finally fuse with the target membrane to unload the vesicle's cargo. The impressive specificity of vesicular transport suggests that each type of transport vesicle in the cell displays molecular markers on its surface that identify the vesicle according to its origin and cargo. These markers must be recognized by complementary receptors on the appropriate target membrane, including the plasma membrane. The identification process depends on a diverse family of monomeric GTPases called Rab proteins. Specific Rab proteins on the surface of each type of vesicle are recognized by corresponding *tethering proteins* on the cytosolic surface of the target membrane. Each organelle and each type of transport vesicle carries a unique combination of Rab proteins, which serve as molecular markers for each membrane type. The coding system of matching Rab and tethering proteins helps to ensure that transport vesicles fuse only with the correct membrane (Figure below).

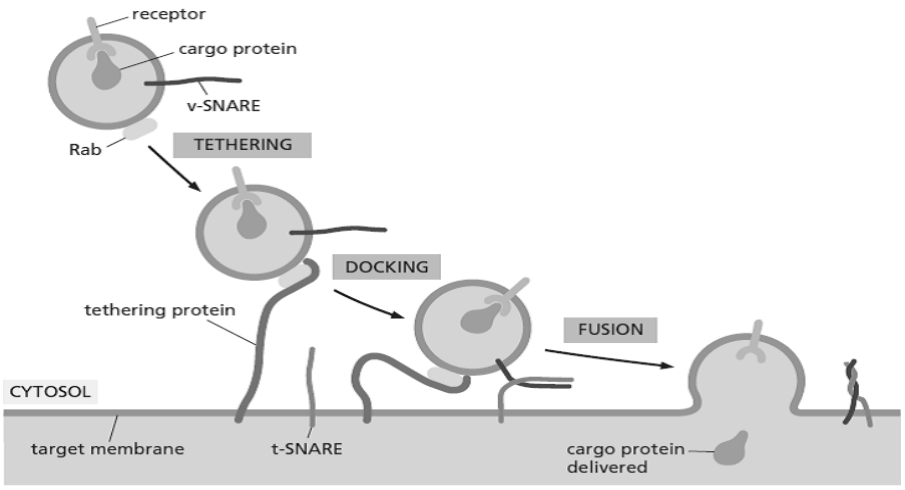


Figure 8: Transport of vesicle into destination membrane

Additional recognition is provided by a family of transmembrane proteins called SNAREs. Once the tethering protein has captured a vesicle by grabbing hold of its Rab protein, SNAREs on the vesicle (called v-SNAREs) interact with complementary SNAREs on the target membrane (called t-SNAREs), firmly docking the vesicle in place (Figure 8). The same SNAREs involved in docking also play a central role in catalyzing the membrane fusion required for a transport vesicle to deliver its cargo. Fusion not only delivers the soluble contents of the vesicle into the interior of the target organelle, but it also adds the vesicle membrane to the membrane of the organelle (Figure 8). After vesicle docking, the fusion of a vesicle with its target membrane sometimes requires a special stimulatory signal. Whereas docking requires only that the two membranes come close enough for the SNAREs protruding from the two lipid bilayers to interact, fusion requires a much closer approach: the two bilayers must come within 1.5 nm of each other so that their lipids can intermix. For this close approach, water must be displaced from the hydrophilic surfaces of the membranes-a process that is energetically highly unfavorable and thus prevents membranes from fusing randomly. All membrane fusions in cells must therefore be catalyzed by specialized proteins that assemble to form a fusion complex, which provides the means to cross this energy barrier. The SNARE proteins themselves catalyze the fusion process: once fusion is triggered, the v-SNAREs and t-SNAREs wrap around each other, thereby acting like a winch that pulls the two lipid bilayers into close proximity.

Protein Storage and Release in Secretory Pathway:

Secretory Pathway: Vesicular traffic extends to and from the plasma membrane. Newly made proteins, lipids, and carbohydrates are delivered from the ER, via the Golgi apparatus, to the cell surface by transport vesicles that fuse with the plasma membrane in the process of *exocytosis*. Each molecule that travels along this route passes through a fixed sequence of membrane-enclosed compartments and is often chemically modified en route.

The outward path of proteins travels from the ER, where they are made and modified, through the Golgi apparatus, where they are further modified and sorted, to the plasma membrane. As a protein passes from one compartment to another, it is monitored to check that it has folded properly and assembled with its appropriate partners, so that only correctly built proteins make it to the cell surface. Incorrect assemblies, which are often in the majority, are degraded inside the cell.

Most proteins that enter the ER are chemically modified there. *Disulfide bonds* are formed by the oxidation of pairs of cysteine side chains, a reaction catalyzed by an enzyme that resides in the ER lumen. Many of the proteins that enter the ER lumen or ER membrane are converted to glycoproteins in the ER by the covalent attachment of short branched oligosaccharide side chains composed of multiple sugars.

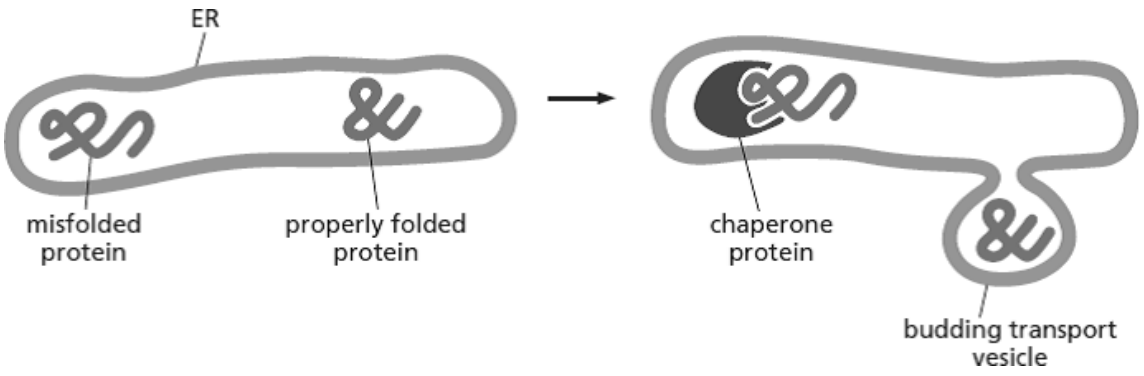


Figure: Chaperone protein in ER

Some proteins made in the ER are destined to function there. They are retained in the ER (and are returned to the ER whenever they escape to the Golgi apparatus) by a C-terminal sequence of four amino acids called an *ER retention signal*. This retention signal is recognized by a membrane-bound receptor protein in the ER and Golgi apparatus. Most proteins that enter the ER, however, are destined for other locations; they are packaged into transport vesicles that bud from the ER and fuse

with the Golgi apparatus Exit from the ER is highly selective. Proteins that fail to fold correctly, and dimeric or multimeric proteins that do not assemble properly, are actively retained in the ER by binding to *chaperone proteins* that reside there. The chaperones hold these proteins in the ER until proper folding or assembly occurs. Chaperones prevent misfolded proteins from aggregating, which helps steer proteins along a path toward proper folding (Figure 9); if proper folding and assembly still fail, the proteins are exported to the cytosol, where they are degraded. If the misfolded proteins accumulate in the ER, the buildup is large enough, it triggers a complex program called the unfolded protein response (UPR). This program prompts the cell to produce more ER, including more chaperones and other proteins concerned with quality control (Figure above). The UPR allows a cell to adjust the size of its ER according to the load of proteins entering the secretory pathway. In some cases, however, even an expanded ER cannot cope, and the UPR directs the cell to self-destruct by undergoing apoptosis.

Golgi Apparatus in Secretory Pathway: The Golgi apparatus consists of a collection of flattened, membrane-enclosed sacs called cisternae, which are piled like stacks of pita bread. Each stack contains 3–20 cisternae (Figure below). Each Golgi stack has two distinct faces: an entry, or *cis*, face and an exit, or *trans,* face. The *cis* face is adjacent to the ER, while the *trans* face points toward the plasma membrane. The outermost cisterna at each face is connected to a network of interconnected membranous tubes and vesicles. Soluble proteins and membrane enter the *cis Golgi network* via transport vesicles derived from the ER. The proteins travel through the cisternae in sequence by means of transport vesicles that bud from one cisterna and fuse with the next. Proteins exit from the *trans Golgi network* in transport vesicles destined for either the cell surface or another organelle of the endomembrane system. Both the *cis* and *trans* Golgi networks are thought to be important for protein sorting: proteins entering the *cis* Golgi network can either move onward through the Golgi stack or, if they contain an ER retention signal, be returned to the ER; proteins exiting from the *trans* Golgi network are sorted according to whether they are destined for lysosomes (via endosomes) or for the cell surface.

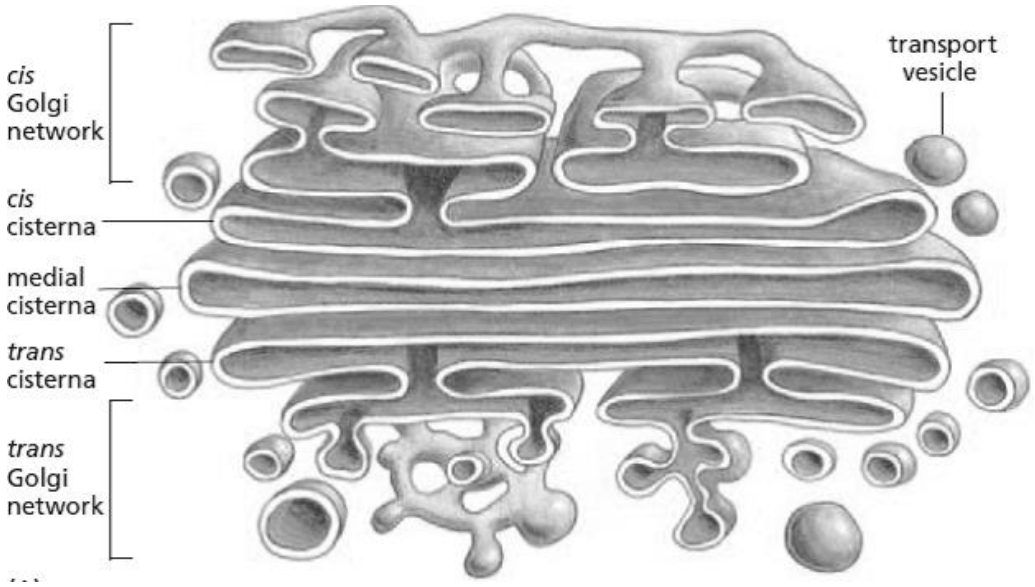


Figure : Golgi Apparatus

Exocytosis of Protein : In all eukaryotic cells, a steady stream of vesicles buds from the *trans* Golgi network and fuses with the plasma membrane in the process of exocytosis. This *constitutive exocytosis pathway* supplies the plasma membrane with newly made lipids and proteins, enabling the plasma membrane to expand prior to cell division and refreshing old lipids and proteins in nonproliferating cells. The constitutive pathway also carries soluble proteins to the cell surface to be released to the outside, a process called secretion. Some of these proteins remain attached to the cell surface; some are incorporated into the extracellular matrix; still others diffuse into the extracellular fluid to nourish or signal other cells. Entry into the constitutive pathway does not

require a particular signal sequence like those that direct proteins to endosomes or back to the ER. In addition to the constitutive exocytosis pathway, which operates continually in all eukaryotic cells, there is a *regulated exocytosis pathway*, which operates only in cells that are specialized for secretion. Each specialized *secretory cell* produces large quantities of a particular product-such as a hormone, mucus, or digestive enzymes-which is stored in secretory vesicles for later release. These vesicles, which are part of the endomembrane system, bud off from the *trans* Golgi network and accumulate near the plasma membrane. There they wait for the extracellular signal that will stimulate them to fuse with the plasma membrane and release their contents to the cell exterior by exocytosis (Figure below).

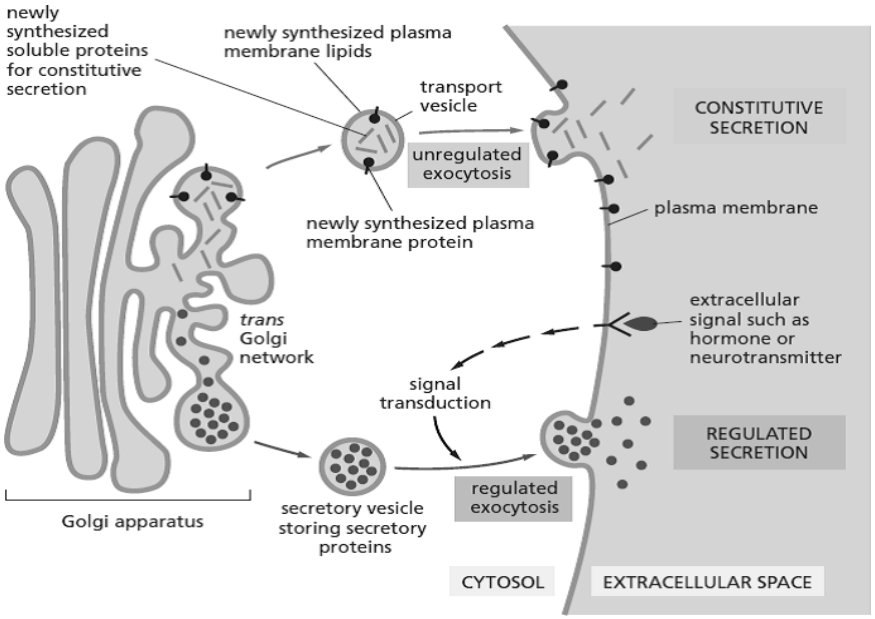


Figure : Secretory pathway in secretory vesicle

Proteins destined for regulated secretion are sorted and packaged in the *trans* Golgi network. Proteins that travel by this pathway have special surface properties that cause them to aggregate with one another under the ionic conditions (acidic pH and high Ca2+) that prevail in the *trans* Golgi network. The aggregated proteins are packaged into secretory vesicles, which pinch off from the network and await a signal instructing them to fuse with the plasma membrane. Proteins secreted by the constitutive pathway, on the other hand, do not aggregate and are therefore carried automatically to the plasma membrane by the transport vesicles of the constitutive pathway. Selective aggregation has another function: it allows secretory proteins to be packaged into secretory vesicles at concentrations much higher than the concentration of the unaggregated protein in the Golgi lumen. This increase in concentration can reach 200-fold, enabling secretory cells to release large amounts

of the protein promptly when triggered to do so. When a secretory vesicle or transport vesicle fuses with the plasma membrane and discharges its contents by exocytosis, its membrane becomes part of the plasma membrane. Although this should greatly increase the surface area of the plasma membrane, it does so only transiently because membrane components are removed from other regions of the surface by endocytosis almost as fast as they are added by exocytosis. This removal returns both the lipids and the proteins of the vesicle membrane to the Golgi network, where they can be used again.

Probable Questions :

1. How are particular proteins targeted to particular subcellular compartments?
2. What are the major morphological differences between the RER and SER? What are the major differences in their functions?
3. Describe the steps that occur between the time a ribosome attaches to a messenger RNA encoding a secretory protein and the time the protein leaves the RER.
4. How are newly synthesized integral proteins inserted into a membrane?
5. Contrast the roles of COPI and COPII coated vesicles in protein trafficking.
6. How does protein transport occur in Nucleus, Mitochondria, ER and Golgi Apparatus?
7. What is Signal sequence? Give an account on the characteristic feature of Signal sequence of protein transported to mitochondria, nucleus and ER.
8. How do retrieval signals ensure that proteins are kept as residents of a particular membrane compartment?
9. Describe the steps that ensure that a lysosomal enzyme will be targeted to a lysosome rather than a secretory vesicle.
10. Describe the molecular structure of clathrin and the relationship between its structure and function.
11. What is the fate of a protein with no sorting signal?

Suggested Readings/ References :

1. Gerald Karp-Cell and Molecular Biology
2. Geoffrey M. Cooper, Robert E. Hausman-The Cell: A Molecular Approach
3. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter-Molecular Biology of Cell.
4. Harvey F Lodish-Molecular Cell Biology

UNIT-VIII

Physiology of muscle contraction and proteins associated with muscle contraction: Physiology of muscle contraction, Chemical nature of contractile elements, actin nucleation, actin treadmilling, myosin types, structure. ATP and signal molecules in muscular contraction, neuromuscular junction and its functioning

Objective: In this unit we will learn about molecular mechanism of muscle contraction. How different contractile elements interact with each other for muscle contraction.

Types of Muscles:

In humans muscles constitute about 40 to 50 percent of the total body weight.

These muscles are broadly classified into three categories:

1. Skeletal or striped or striated or voluntary muscles

2. Smooth or un-striped or non-striated or visceral or involuntary muscles

3. Cardiac muscles

1. Skeletal Muscles:

These muscles are found in the limbs, body wall, tongue, pharynx and beginning of oesophagus. These muscles are under the control of animal's will. These muscles are normally attached to the skeleton. The major component of muscles is water.

Potassium is the most abundant mineral element in muscles. Other minerals such as sodium, calcium, phosphorus and magnesium are present only in traces. Muscles store glycogen. They have oxygen carrying pigment myoglobin or "muscle haemoglobin". Muscles also contain ATP, phosphocreatine, creatine, urea, etc.

2. Visceral Muscles (Smooth Muscles):

These are found in the posterior part of oesophagus, stomach, intestine, lungs, urinogenital tract, urinary bladder, blood vessels, iris of eyes, dermis of skin and arrector pili muscle of hair. Smooth muscles never connect with skeleton. These muscle fibres or cells are elongated and spindle shaped. Each fibre contains a single oval nucleus surrounded by the cytoplasm (sarcoplasm). In the cytoplasm the myofibrils are

arranged longitudinally. There is no sarcolemma, however, the fibre is enclosed by plasma membrane. Action of these muscles is controlled by autonomic nervous system and hence they are not under the control of the animal's will.

3. Cardiac Muscles:

The cardiac muscles are found in the wall of the heart and in the wall of large veins (e.g., pulmonary veins and superior vena cava) where these veins enter the heart. These fibres show the characters of both un-striped and striped muscle fibres.

Each fibre is a long and cylindrical structure which lacks a definite sarcolemma. The fibres are uninucleate and the nuclei lie near the centre. The fibres have some lateral branches, known as oblique bridges to form a contractile network. The myofibrils have transverse faint dark and light bands, which alternate with each other. In this way cardiac muscle fibres are also striped, but having dark intercalated discs at intervals.

The intercalated discs are specialized regions of cell membrane of two adjacent fibres. The intercalated discs function as boosters of contraction wave and permit the wave of muscle contraction to be transmitted from one cardiac fibre to another.

Detailed Structure of Skeletal Muscle:

To understand the structure and mechanism of contraction, each skeletal muscle is made of a number of muscle bundles or fascicles. Each muscle bundle contains a number of muscle fibres (muscle cells). Each muscle fibre is surrounded by the plasma membrane called sarcolemma enclosing the sarcoplasm.

Muscle fibre is a syncitium as the sarcoplasm contains many nuclei. The endoplasmic reticulum of the muscle fibre is called sarcoplasmic reticulum which is store house of calcium ions. There are present large number of parallelly arranged filaments called myofilaments or myofibrils. A myofibril has dark and light bands. The dark bands are also called A-bands (Anisotropic bands). The light bands are also called I-bands (Isotropic bands). At the centre of A- band, a comparatively less dark zone called H-Zone (Hensen zone, named after Hensen who first described) is present. In the centre of the H-zone is the M-line: The letter 'M' is from the German word Mittleschiebe (mittle = middle). Each I-band has at its centre a dark membrane called Z-line. The letter 'Z' is from the German word Zwischenschiebe (zwischen = between, schiebe = disc).

The Z-line is also called Z-disc, or Krause's membrane or Dobie's line. The part of the myofibril between two successive Z-lines is called sarcomere. Therefore, the sarcomere comprises A-band and half of each adjacent I-band. The sarcomere is the functional unit of myofibril. In fact each sarcomere is a bundle of thick and thin myofilaments. The thick myofilaments have diameters of about 150A, whereas the thin myofilaments have diameters of about 70A.

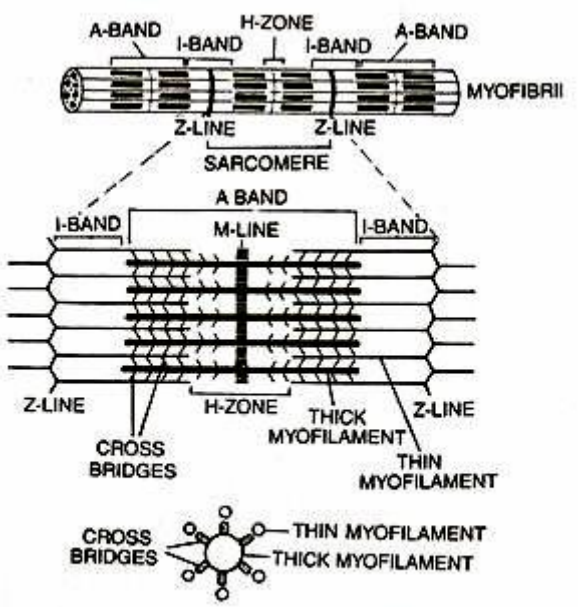


Fig. 20.1. Structure of skeletal muscle (only detail of myofril is shown).

Structure of Contractile Proteins:

The thick myofilaments are formed by myosin protein. The thin myofilaments are formed by three types of proteins called actin, tropomyosin and troponin. These four proteins are called contractile proteins.

(i) Thick Myofilaments:

The thick filament consists mainly of myosin protein. Myosin contributes 55% of muscle protein by weight. The myosin molecule is composed of six polypeptide chains, two identical heavy chains and four light chains. The two heavy chains wrap spirally around each other to form a double helix. However, one end of each of these chains is folded into a globular protein mass called myosin head. Thus, there are two free heads lying side by side at one end of the double helix myosin molecule.

The elongated part of the coiled helix is called the tail. The four light chains are also parts of the myosin heads, two to each head. These light chains help control the function of the head during the process of muscle contraction. Myosin is split by enzyme trypsin into two fragments, called light meromyosin (LMM) and heavy meromyosin (HMM). LMM lacks adenosine triphosphatase (ATPase) activity and does not combine with actin. HMM consists of two globular sub-fragments and one rod shaped fibrous sub fragment. Each globular sub fragment contains an ATP binding site and actin binding site (Fig. 20.2B). It can form a cross bridge with the active site present on the actin.

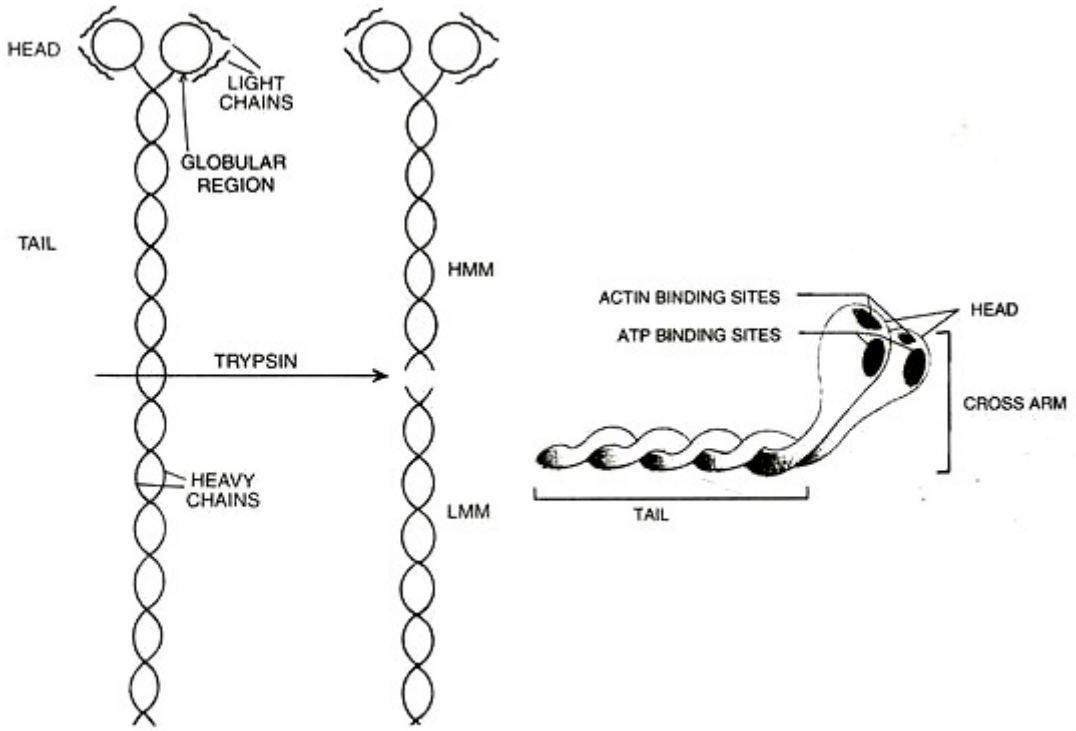


Fig. 20.2. A, Diagram of a myosin molecule and enzymatic cleavage of myosin.
HMM = Heavy meromyosin, LHH = Light meromyosin. B, Myosin Monomer (meromyosin).

(ii) Thin Myofilaments:

The thin filament is composed of three different proteins— actin, tropomyosin and troponin (Fig. 20.3).

(a) Actin:

Actin is a globulin protein and has low molecular weight. It occurs in two forms, the monomeric G-actin and the polymeric F-actin. G-actin (G = globular) polymerizes to the fibrous form F-actin (F = fibrous) in the presence of Mg^{++}.

(b) Tropomyosin:

Tropomyosin is a double stranded a-helical rod. It is fibrous molecule that attaches to F-actin in the groove between its filaments. In the resting state, the tropomyosin molecules are believed to lie on top of the active sites of the actin strands so that attraction cannot occur between the actin and myosin to cause contraction.

(c) Troponin:

Troponin is a complex of 3 polypeptides. Troponin T (TpT) binds to tropomyosin as well as to the other two troponin components. Troponin I (Tpl) inhibits the F-actin-myosin interaction and also binds to other components of troponin. Troponin C (TpC) is a calcium-binding polypeptide. The strong affinity of the troponin for calcium ions is believed to initiate the contraction process.

i. Tendons:
A muscle may be attached to a single bone or different bones by one end or by both the ends, either directly by epimysium or by way of inelastic connective tissue cords, the tendons.

ii. Fascia:
The term fascia is applied to a sheet or broad band of fibrous connective tissue beneath the skin or around muscles and other organs of the body.

iii. Motor Unit:
A neuron that transmits a stimulus to muscle tissue is called motor neuron. A motor unit consists of a single motor neuron (nerve cell) and the muscle fibres it innervates. The portion of the muscle plasma membrane (sarcolemma) that lies beneath the nerve endings (axon terminals) is called the motor end plate. The axon terminals and the motor end plate together constitute the neuromuscular junction or neuromotor junction.

iv. Single Muscle Twitch:
A muscle fibre contracts only once if it is stimulated by a single nerve impulse or by a single electric shock of adequate strength. This single isolated contraction of the muscle fibre is called single muscle twitch. Immediately after a twitch, the muscle fibre relaxes.

v. Threshold Stimulus:
For contraction, muscle fibre always requires a specific minimum strength or intensity of the stimulus or nerve impulse. This is called threshold stimulus. If the stimulus or the nerve impulse is below this intensity, the muscle fails to contract.

vi. AII-or-None Principle (= Bowditch's Law):
According to the 'all-or-none law', when a fibre contracts, it contracts maximally.

vii. Tetanus:
The continued state of contraction is called tetanus.

viii. Muscle Tonus (= Muscle Tone):
The state of sustained partial contraction is called muscle tonus or muscle tone. It is a sort of mild tetanus. It is essential to maintain posture and form of the body.

ix. Proteins of Muscles:
Myosin actin tropomyosin, troponin, myoglobin.The major component of muscle is water. Potassium is the most abundant mineral element in muscle. Other minerals such as sodium, calcium, phosphorus and

magnesium are present only in traces. Muscles store glycogen. They have oxygen carrying pigment myoglobin or 'muscles haemoglobin'. Muscles also contain ATP, phosphocreatine, creatine, and urea. Etc.

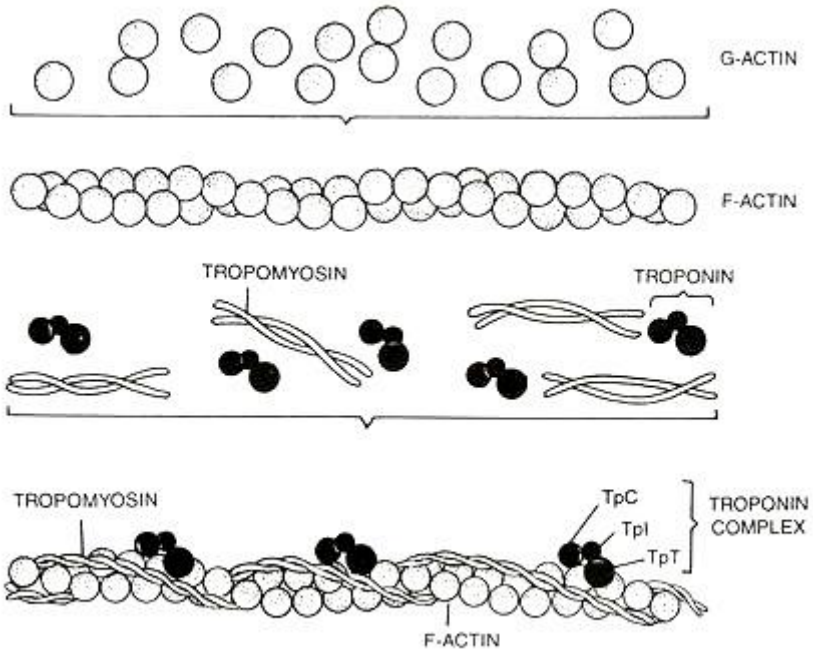


Fig. 20.3. Representation of the thin myofilament.

Myosin Filaments:

A myosin myofilament consists of two distinct region, a long rod-shaped tail called myosin rod and two globular intertwined myosin head. The globular head appear at interval along the myosin myofilament, projecting from the sides of the filament. The myosin head can attach to the neighboring acting filament where actin and myosin filaments overlaps.

Characteristics of Myosin filaments:

1. Found only in A-band of sarcomere.

2. Thicker (0.01 mn) but longer (4.5 mn)than actin filaments.

3. Cross bridges present, hence have rough surface.

4. Fewer than actin filaments.

5. Free at both the ends.

6. Consist of 2 proteins: myosin and meromysin.

7. Do not slide during muscle contraction.

Actin Filaments:

An actin myofilament is made up of actin molecule, tropomyosin and troponin complex. Troponin is composed of three sub-units (troponin I, T and C). Tropomyosin form two helical strand which are wrapped around actin molecules (G-actins) longitudinally in thin twisted stranded form. Each G-actin is attached with an ATP molecule. The whole assembly of actin molecules is known as F-actin (Fibrous actin). Tropomyosin switches ON or OFF the muscle contraction mechanism. Troponin complex is a globular protein which binds to tropomyosin and calcium ions.

Characteristics of Actin myofilaments:

1. Found in both A and I -bands.

2. Thinner (0.005 mn) but shorter (2 -2.6 mn) than myosin filaments.

3. Cross bridges absent, hence have smooth surface.

4. More numerous than myosin filaments, six of them surround each myosin filament.

5. Free at one end and are joined to Z -line by other end.

6. Consist of 3 proteins: actin, tropomyosin and tropinin.

7. Slide into H-zone during muscle contraction.

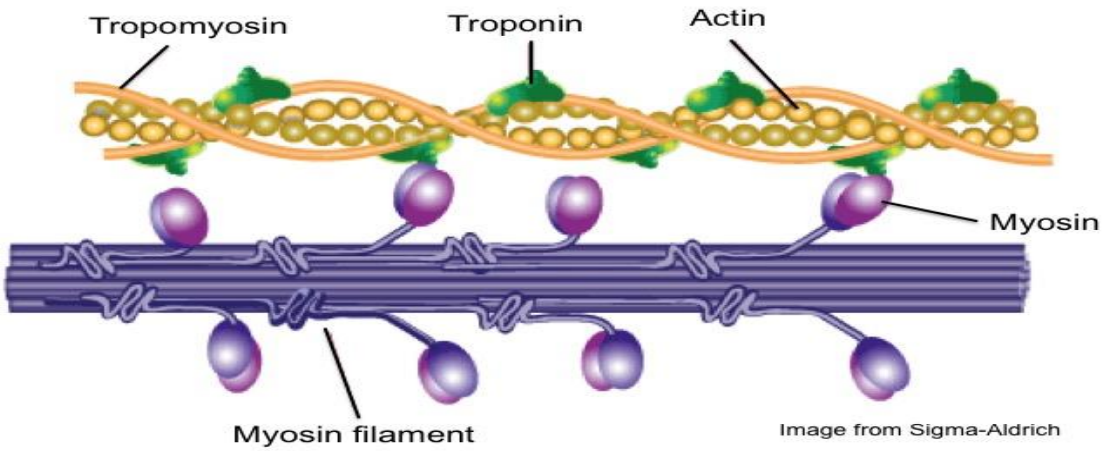


Figure: Actin and Myosin myofilament

Functions:

Striated muscles are under the control of animal s will. Calcium is an essential element for the contraction of muscles. In the presence of calcium ions and energy from ATP, actin and myosin interact forming actomyosin which causes contraction of muscles. During muscle contraction conversion of pyruvic acid to lactic acid proceeds anaerobically. Lactic acid is transported by blood to liver where it is converted to glycogen. Chemical energy is changed into mechanical energy during muscle contraction. The contraction of muscles of shortest duration is seen in eye lids. Shivering in cold is a method for production of heat by muscle contraction.

Functional Classification of Body Muscles:

According to the type of movement they bring about, the skeletal muscles are of the following types:

1. Flexor:

This muscle bends one part of a limb on another at a joint, e.g., biceps. It brings the fore arm towards the upper arm.

2. Extensor:

This muscle extends or straightens a limb, e.g., triceps. It extends the fore arm.

3. Adductor:

This muscle brings a limb towards the mid line of the body, e.g., latissimus dorsi. It presses the entire arm against the side.

4. Abductor:

This muscle pulls a limb away from the mid-line of the body, e.g., deltoideus. It draws the entire arm to the side.

5. Pronator:

This muscle turns the palm downward or to the posterior, e.g., pronatorteres.

6. Supinator:

This muscle turns the palm upward or to the anterior, e.g., supinator.

7. Elevator:

This muscle raises a part of the body, e.g., masseter. It lifts up the lower jaw to close the mouth.

8. Depressor:

This muscle lowers a part of the body, e.g., depressor mandibulae. It lowers down the lower jaw to open the mouth.

9. Rotator:

This muscle rotates a part of the body, e.g., pyriformis. It raises and rotates the thigh.

10. Sphincter:

This muscle decreases the size of an opening, e.g., pyloric sphincter between stomach and duodenum.

11. Dilator:

This muscle enlarges the size of an opening.

Antagonistic Muscles:

Muscles which act in opposition to other muscles are called the antagonistic muscles. The biceps, for example bends or flexes the arm and is called a flexor. Its antagonist, the triceps straightens or extends the arm and is termed as extensor. Similar pairs of opposing flexors and extensors are found at the wrist, knee, ankle and other joints. When a flexor contracts, the opposing extensor must relax to permit the bone to move.

This requires proper coordination of nerve impulses going to the two sets of muscles. Other antagonistic pairs of muscles are adductors and abductors, which move parts of the body toward or away from the central axis of the body; elevators and depressors, which raise and lower parts of the body; pronators which rotate body parts downward and backward and supinators, which rotate them upward and forward; and sphincters and dilators which decrease and enlarge the size of an opening.

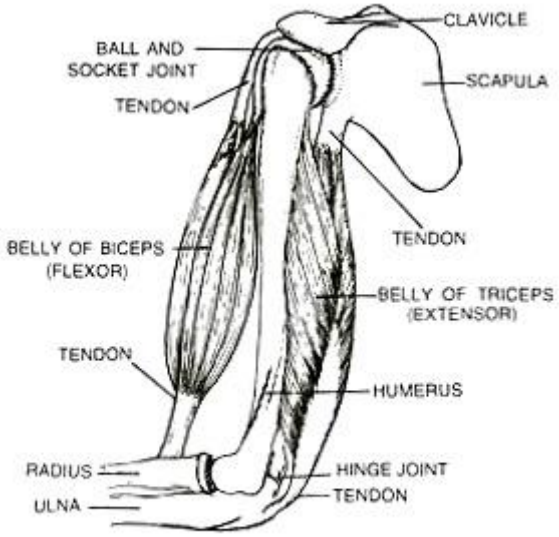


Fig. 20.5. Diagram showing the antagonistic arrangement of the biceps and triceps muscles.

Mechanism of Muscle Contraction

Sliding Filament Theory :

Two groups of workers proposed the sliding filament theory.

The essential features of this theory are as follows:

1. During muscle contraction, the thin myofilaments slide inward towards the H-zone.

2. The sarcomere shortens, but the lengths of thin and thick myofilaments do not change.

3. The cross bridges of the thick myofilaments connect with portions of actin of the thin myofilaments. The myosin cross bridges move on the surface of the thin myofilaments and the thin and thick myofilaments slide past each other.

4. As the thin myofilaments move past the thick myofilaments, the H zone narrows and even disappears when the thin myofilaments meet at the centre of the sarcomere. Thus the length of the sarcomere decreases during contraction. Size of I band also decreases.

5. The lengths of the thick and thin myofilaments do not change during muscle contraction.

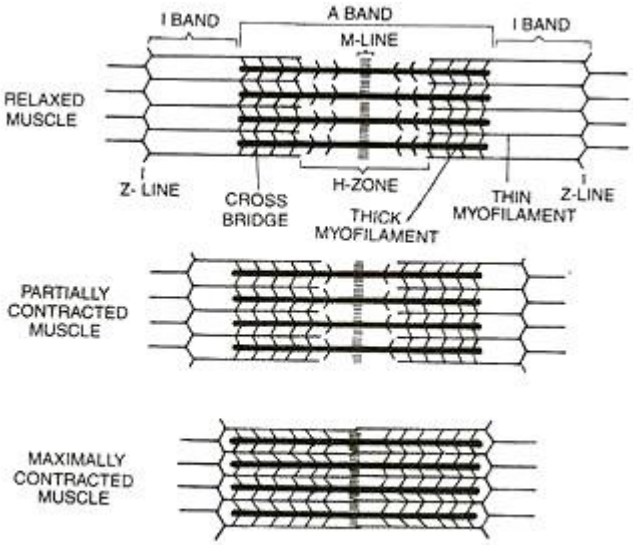


Fig. 20.6. Sliding filament theory of muscle contraction.

In a resting muscle fibre, the outside of sarcolemma is positively charged with respect to the inside. This potential difference across a membrane is called resting potential. A membrane with a resting potential is said to be polarised. It is maintained by sodium and potassium ions.

Sodium ions predominate on the outside of the sarcolemma and potassium ions predominate on the inside. Sodium ions are pumped out and potassium ions enter inside, both by active transport. The process of moving ions against concentration is called sodium pump (= sodium-potassium exchange pump).

Electrical and Biochemical Events in Muscle Contraction:

These events have been worked out by Albert Szent Gyorgyi and others.

These events are summarized as follows:

1. As a nerve impulse reaches the terminal end of the axon, small sacs called synaptic vesicles fuse with the axon membrane and release a chemical transmitter, acetylcholine.

Acetylcholine diffuses across the synaptic cleft (the space between the axon membrane and the motor end plate) and binds to receptor sites of the motor end plate. When depolarization of the motor end plate reaches a certain level, it creates an action potential. After this, an enzyme cholinesterase present along with receptor sites for acetylcholine, breaks down acetylcholine into acetate and choline. A portion of the choline diffuses back to the axon and is reused to synthesize more acetylcholine for transmission of subsequent impulses.

2. An action potential (impulse) passes from the motor end plate over the sarcolemma (muscle plasma membrane) and then into the T-tubules and sarcoplasmic reticulum and stimulates the sarcoplasmic reticulum to release calcium ions into the sarcoplasm (cytoplasm of the muscle fibre).

3. Calcium plays a key regulatory role in muscle contraction. The calcium ions bind to troponin causing a change in its shape and position. This in turn alters shape and the position of tropomyosin, to which troponin binds. This shift exposes the active sites on the F-actin molecules. Myosin cross-bridges are then able to bind to these active sites.

4. The heads of myosin molecules project laterally from thick myofilaments towards the surrounding thin myofilaments. These heads of myosin are called cross bridges. The head of each myosin molecule contains an enzyme myosin ATPase. In the presence of myosin ATPase, Ca^{++} and Mg^{++} ions, ATP breaks down into ADP and inorganic phosphate, releasing energy in the head.

$$ATP - Myosin\ ATPase/Ca^{++}, Mg^{++} \rightarrow ADP + Pi + Energy$$

5. Energy from ATP causes energized myosin cross bridges to bind to actin.

6. The energized cross-bridges move, causing thin myofilaments to slide along the thick myofilaments. This movement is like the movement of the oars of a boat. Rowing with oars pushes a boat across the water, or the water along the sides of the boat in somewhat the same way that thick and thin myofilaments slide along one another. As stated in the sliding filament theory, there is no shortening of thin and thick myofilaments. However, the sarcomere shortens because of the sliding of the thin myofilaments produced by cross-bridge movements. The H-zones and I-bands shorten, but the width of the A-band remains constant.

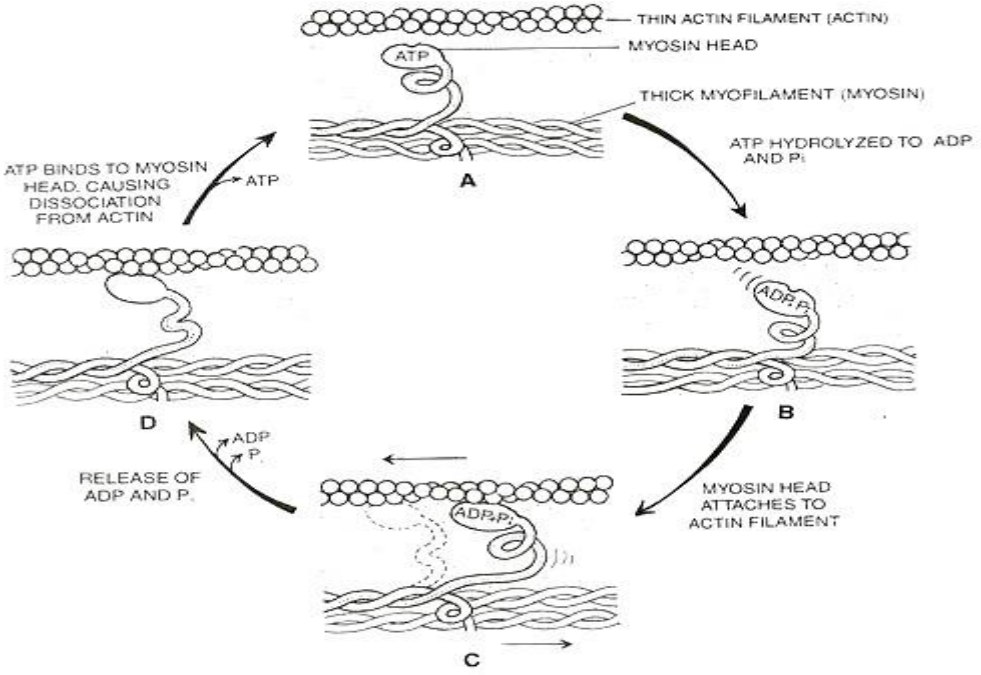


Fig. 20.7. Stages in cross bridge formation, rotation of head and breaking of cross bridge.

Cori's Cycle:

It was proposed by Cori and Cori, who got Nobel prize with Houssay in 1947. This cycle occurs in the muscles and liver. During glycolysis lactic acid is produced from pyruvic acid in the muscles. This lactic acid is carried in the blood to the liver where 1/5th of lactic acid is oxidised to water and carbon dioxide and 4/5th of lactic acid is converted into glycogen. The glycogen releases glucose into the blood which is reconverted to glycogen in the muscles. The cycle is repeated.

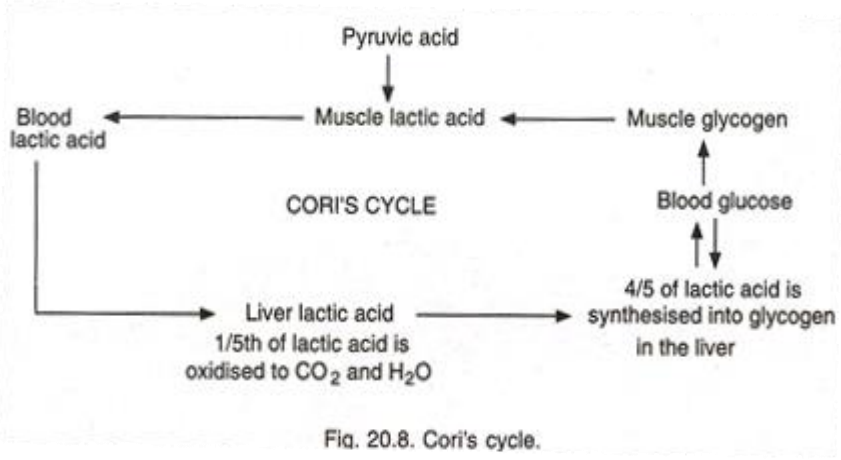


Fig. 20.8. Cori's cycle.

Oxygen Debt:

During strenuous exercise, the muscle does not get sufficient oxygen to meet its energy needs immediately. So it contracts anaerobically and accumulates lactic acid produced by anaerobic glycolysis. During recovery, the oxygen consumption of muscle exceeds. The extra oxygen consumed during recovery is called oxygen debt of the muscle.

It is used in oxidising the accumulated lactic acid aerobically and in restoring the depleted creatine phosphate and ATP in the muscle fibre. A small part of oxygen debt also goes to myoglobin which binds and stores oxygen for future use. For extra oxygen, deep and rapid breathing occurs carrying more oxygen into the lungs and eventually to the tissues.

Role of Biomolecules in Muscle Contraction:

A number of biomolecules are involved in the muscular contraction.

1. Muscle proteins such as myosin, actin, tropomyosin and troponin play a significant role during muscle contraction as described above. Myoglobin is similar to that of haemoglobin, that is, to carry oxygen.

2. Carbohydrates (e.g., glycogen) and lipids (e.g., neutral fats) are stored as food and supply energy.

3. High energy phosphates such as ATP and phosphocreatine provide energy.

4. Inorganic substances. They are of following two types:

(i) Cations:
Potassium is the principal mineral of the muscle. The other cations are sodium, calcium and magnesium,

(ii) Anions:
Chloride and phosphate are the anions present in the muscle. Role of potassium, sodium, calcium, magnesium, chloride and phosphate have already been described in the mechanism of muscle contraction.

5. Enzymes catalyze all biological reactions that are involved in muscular contraction. Myosin ATPase is the important enzyme taking part in the muscle contraction.

Muscle Relaxation:

After muscle contraction, the calcium ions are quickly returned to the sarcoplasmic reticulum by active transport, a process that requires ATP. Troponin and tropomysin molecules move to their previous position and block the active sites on the thin myofilaments. The myosin cross-bridges separate from actin. When myosin cannot attach to actin, the muscle relaxes.

Isotonic and Isometric Contraction:

The force produced by a whole muscle when it contracts is termed muscle tension and the force exerted on a muscle by a weight is called the load.

For example, when we pick up a book, the book is the load and the force produced by the muscles in our arm is the tension. Thus load and tension are opposing forces. When the tension remains the same whereas the change occurs in the length of the muscle fibres, it is called isotonic (same tension) contraction. The muscle shortens during this type of contraction. Example of isotonic contraction is the simple bending of arm. When the length of muscle fibres remains the same and the tension is increased, it is termed as isometric (same length) contraction. The muscle does not shorten during this type of contraction. Example of isometric contraction is pulling any heavy object.

Differences between Isotonic Contraction and Isometric Contraction

i. Summation:
If a second stimulus is given before complete relaxation of muscle's response to the firs stimulus, the force produced by the second contraction will be stronger than the first; similarly, the third will be stronger than the second. This phenomenon is called summation.

ii. Rigor mortis:

The rigidity of muscles that occurs after death is called rigor mortis. Cellular metabolism comes to halt. Rigor mortis disappears some fifteen to twenty five hours after death as proteins are degraded.

iii. Energy:

Muscle fibres contain two organic phosphates, namely adenosine triphosphate (ATP) and phosphocreatine. They store energy in the muscles.

Like other cells of the body, muscle cells synthesize ATP as follows:

$$ADP + P + E \text{ (energy)} \rightarrow ATP$$

Whenever required ATP gives energy

$$ADP + P + E \text{ (energy)} \longrightarrow ATP$$

Whenever required ATP gives energy

$$ATP \xrightarrow[Ca^{++}, Mg^{++}]{\text{Myosin ATPase}} ADP + Pi + E$$

Creatine is produced in the liver. In a resting muscle some of the ATP produced reacts with creatine to form phosphocreatine and ADP. When a muscle is active some of the energy from phosphocreatine is transferred back to ATP where the energy can be used to power contraction.

$$ATP + Creatine \underset{\text{Active muscle}}{\overset{\text{Resting muscle}}{\rightleftharpoons}} Phosphocreatine + ADP$$

Muscle Fatigue:

The reduction in the force of contraction of a muscle after prolonged stimulation is called muscle fatigue.

Cause:

A muscle is able to contract for a short time in the absence of oxygen. But it gets fatigued sooner because in the absence of oxygen, the metabolic products of glycolysis mainly lactic acid accumulate. The accumulation of lactic acid leads to muscle fatigue. Pain is experienced in the fatigued muscle. The site of fatigue is the junction between nerve and muscle. A muscle gets fatigued sooner after a strenuous exercise than after a mild exercise.

Remedy:

Fatigued muscle needs extra oxygen to dispose off excess lactic acid. After a strenuous exercise, faster breathing should be continued for some time to supply extra oxygen for oxidizing excess lactic acid. This results in the disappearance of fatigue.

a. Hypertrophy:
Increase in the size of muscle cells is called hypertrophy.

b. Atrophy:
Reduction in the size of individual muscle cells is called atrophy.

Red and White Muscle Fibres:

Birds and mammals have in their skeletal muscles two kinds of striated muscle fibres, red or slow muscle fib

Structure of Smooth Muscle:

Smooth muscle cells are spindle-shaped (2 to 10 m diameter and 50 to 400 m in length), have single nucleus and are capable of cell division. They lack cross striations and hence the name smooth muscle.

They contain actin and myosin filament and contract by sliding filament mechanism. The actin filaments take their origin from dense bodies in the cytoplasm which are functionally similar to Z lines in skeletal muscle. Actin filaments contain actin and tropomyosin, but troponin is absent. Sarcoplasmic reticulum is present but poorly developed. T-tubules are absent, and hence, triads are absent.

Contraction of smooth muscle occurs by sliding mechanism. Ca2+ ions are responsible for excitation-contraction coupling. Calcium binds to calmodulin, a calcium binding protein present in the cytoplasm. This calcium-calmodulin complex binds to another cytosolic protein, myosin light-chain kinase, thereby activating the enzyme for cross-bridge formation. In smooth muscle cross-bridge cycling is controlled by calcium-regulated enzyme that phosphorylates myosin, instead of actin filaments that regulates cross-bridge cycling in skeletal muscle.

Types of Smooth Muscle:

1. Single-Unit Smooth Muscle:

It is also called as visceral smooth muscle, since they are present in the walls of hollow viscera such as gastrointestinal tract, uterus, urinary bladder and respiratory tract.

Features:
i. Has a low resistance bridge (gap-junctions) between individual muscle cells and function in a syncytial fashion.

ii. Have own rhythmic contractility myogenic tone that is independent of nerve supply.

iii. Contraction is also stimulated by stretching.

iv. Contractile activity is also controlled by hormones and local tissue factors.

2. Multi-Unit Smooth Muscle:

It is made up of multiple individual units without interconnecting bridges, i.e. non-syncytial in nature. These are located in most blood vessels, epididymis, vas deferens, iris, ciliary body and piloerector muscles.

Features:

i. Multiple individual units of muscle fibers each innervated by a single nerve ending.

ii. No spontaneous contractions, i.e. no pacemaker activity.

iii. Gap junctions are absent; hence the excitation remains localized within the motor unit.

iv. Does not respond to stretching.

Properties of Smooth Muscle:

1. RMP:

It ranges from -50 to -70 mV. The peculiarity of this RMP is it is highly unstable.

2. Sinusoidal Wave:

The instability of RMP can be recorded from the longitudinal muscles of stomach and intestine. This is known basic electrical rhythm (BER).

3. Action Potential (AP):

Three types of AP occur in visceral smooth muscle:

a. Spike potential

b. Spike potential initiated by slow wave rhythm

c. Action potential with plateau

d. Tonic contraction of the muscle without any AP.

Characteristics of Smooth Muscles:

Some of the characteristics of smooth muscles are:

i. It is Nonstriated

ii. Actin and myosin not organized into sarcomeres

iii. Gap junctions

iv. Electrical syncytium

v. Calmodulin to bind calcium

vi. Low ATPase activity (slow muscle)

vii. Limited sarcoplasmic reticulum

viii. Lack T-tubules

ix. Autonomic nerve supply

Increase cytosolic Ca^{2+} → Ca^{2+} binds to calmodulin → Ca^{2+} – calmodulin complex binds to myosin light-chain kinase → Myosin light-chain kinase uses ATP to phosphorylate myosin cross-bridges → Phosphorylated cross-bridges bind to actin filaments → Cross-bridge cycling produces tension and shortening of smooth muscle.

Factors Influencing Smooth Muscle Contractile Activity:

i. Spontaneous electrical activity in plasma membrane of smooth muscle cell

ii. Neurotransmitters released by autonomic nervous system

iii. Hormones

iv. Stretch

v. Changes in chemical composition of ECF surrounding the cell (paracrine agents, acidity, oxygen, osmolarity and ion concentration).

Red Muscle Fibres:

1. These muscle fibres are dark red which is due to the presence of red haemoprotein called myoglobin. Myoglobin binds and stores oxygen as oxymyogiobin in the red fibres. Oxymyogiobin releases oxygen for utilization during muscle contraction.

2. Mitochondria are more in number.

3. Red muscles have less sarcoplasmic reticulum.

4. They carry out considerable aerobic oxidation without accumulating much lactic acid. Thus red muscle fibres can contract for a longer period without fatigue.

5. These muscle fibres have slow rate of contraction for long periods.

Example: Extensor muscles of the human.

White Muscle Fibres:

1. These muscle fibres are lighter in colour as they do not have myoglobin.

2. Mitochondria are less in number.

3. White muscles have more sarcoplasmic reticulum.

4. They depend mainly on anaerobic oxidation (glycolysis) for energy production and accumulate lactic acid in considerable amounts during strenuous work and soon get fatigued.

5. These muscle fibres have a fast rate of contraction for short periods.

Example: Eye ball muscles.

Vertebrates possess three types of muscle tissue:

(1) Smooth muscle, the contractile elements of most of the digestive system and most visceral organs;

(2) Cardiac muscle, found only in the heart; and

(3) Striated (or skeletal) muscle, responsible for most of the gross movements of the body, especially the skeleton.

Each of these tissues is composed of cells called muscle fibers, which contain cytoplasmic filaments of actin, myosin, and other proteins responsible for the contractile nature of muscle. The filaments are arranged differently in each of the tissue types. The arrangement is most highly organized in striated muscle, and it is this type of muscle tissue that has been most extensively studied.

The organization of striated muscle is shown diagrammatically in Figure 24-10. During embryonic development, the muscle fiber is formed by the end-to- end fusion of many ceils into a continuous tube-like structure.

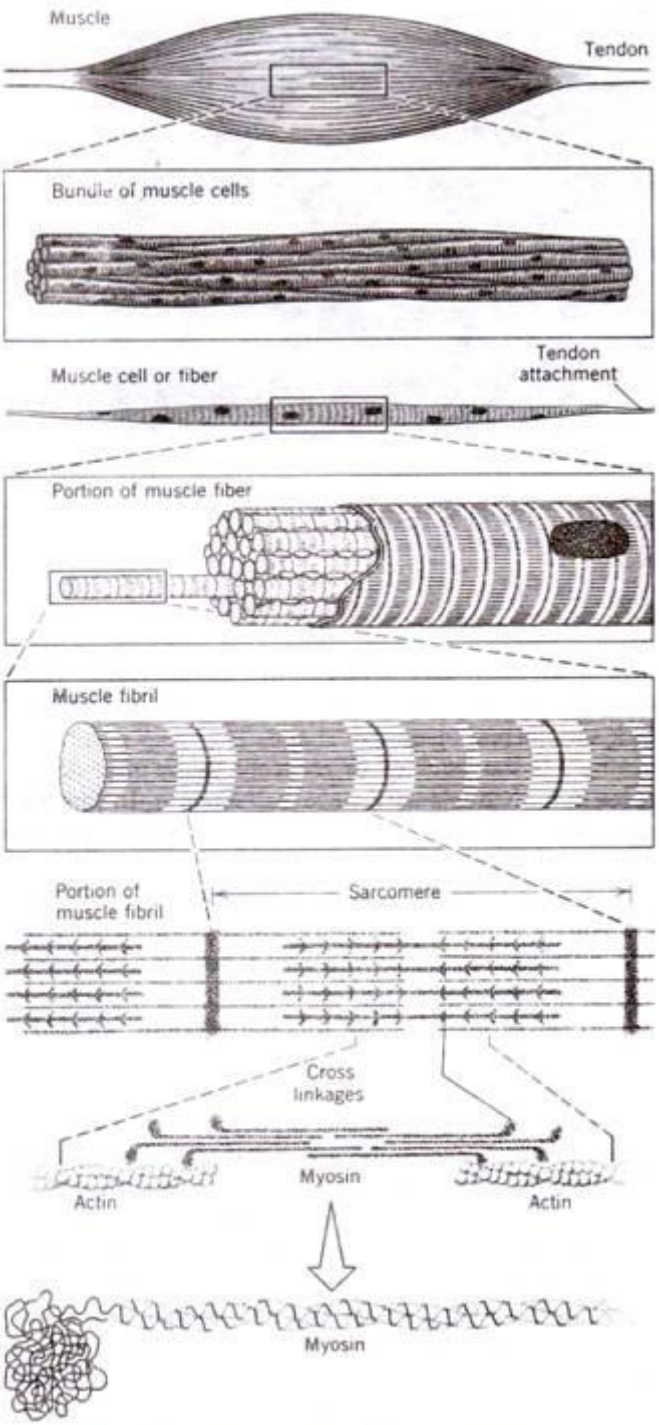


FIGURE 24–10 Cellular, subcellular, and molecular organization of striated muscle tissue.

This explains why striated muscle fibers are such long cells and are multinucleate. The plasma membrane of the muscle fiber is called the sarcolemma; in addition to its exceptional length, the sarcolemma is characterized by numerous pore like invaginations that extend into the sarcoplasm (cytoplasm) at right angles to the long axis of the cell.

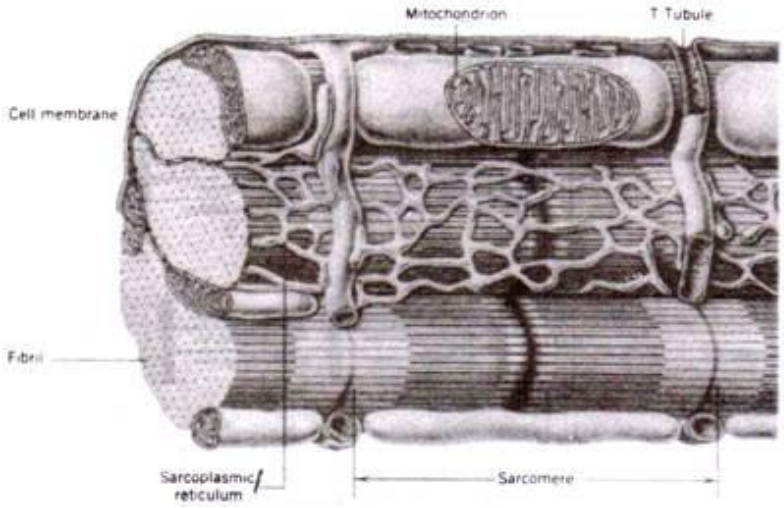


FIGURE 24–11 The sarcoplasmic reticulum and T system of striated muscle cells.

These transverse or T system extensions of the sarcolemma (Fig. 24-11) make contact with most of the internal myofibrils—the contractile units of the cell. Each myofibril contains a large number of thick (15-nm diameter) and thin (7-nm diameter) filaments called myofilaments that run parallel to each other and to the long axis of the cell. Seen in cross section (24-12), the filaments are arranged in a repeating geometric pattern. The thick filaments are equidistant from each other with each surrounded by six thin filaments in a hexagonal array (Fig. 24-12b).

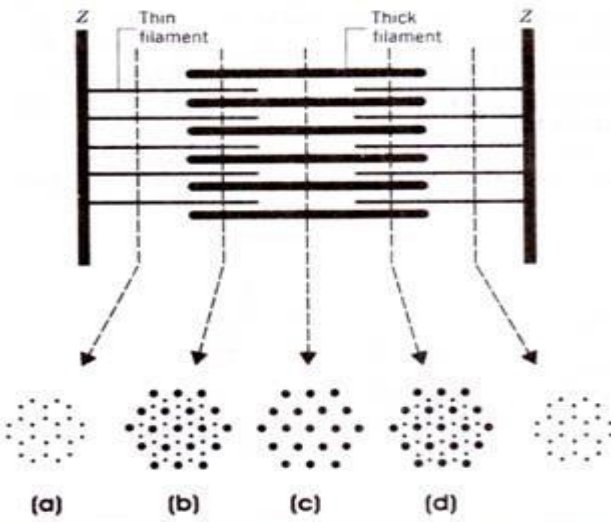


FIGURE 24–12 Cross sections through various positions of a sarcomere. The thin (actin) filaments are arranged parallel to the thick (myosin) filaments and form an hexagonal array in regions of overlap (i.e., regions *b* and *d*).

During the contraction of the cell, the thick and thin filaments slide past one another. When examined microscopically in longitudinal section, each myofibril reveals areas of different density (Fig. 24-13). The alternating light and dark areas are respectively called the I (i.e., isotropic) and A (i.e., anisotropic) bands. At the center of each I band there is a dark line called a Z line, and those portions of a myofibril that extend from one Z line to the next are termed sarcomeres.

One end of each thin filament is anchored in the Z line and the other end projects toward the center of the sarcomere. The thick filaments are sandwiched between the thin filaments but are not attached to the Z lines. Within each sarcomere, the A bands extend from one end of a stack of thick filaments to the other. As seen in Figures 24-12 and 24-13, there is a region within each A band that is devoid of actin filaments; this region appears somewhat less dense than the remainder of the A band and is called an H zone. Finally, at the center of the H zone is the somewhat darker M line. As contraction occurs, the thick and thin filaments slide past each other, so that the H zone disappears and the I band becomes much narrower (Fig. 24-14). The Z lines are drawn closer together and the myofibril as a whole becomes thicker.

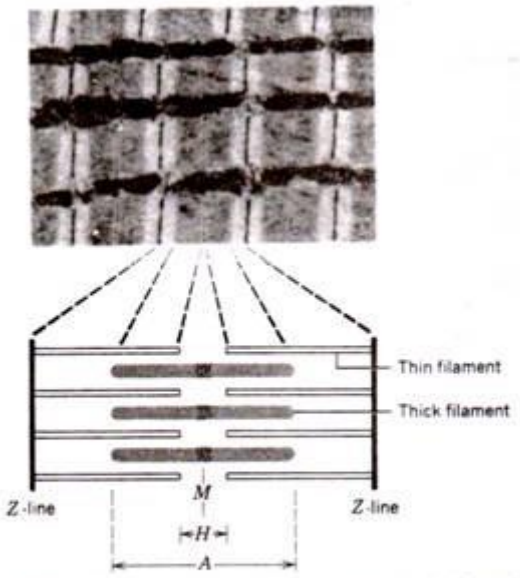


FIGURE 24–13 Relationship between the light and dark zones seen in electron photomicrographs of striated muscle tissue and the arrangements of thick and thin filaments. (Photomicrograph courtesy of R. Chao.)

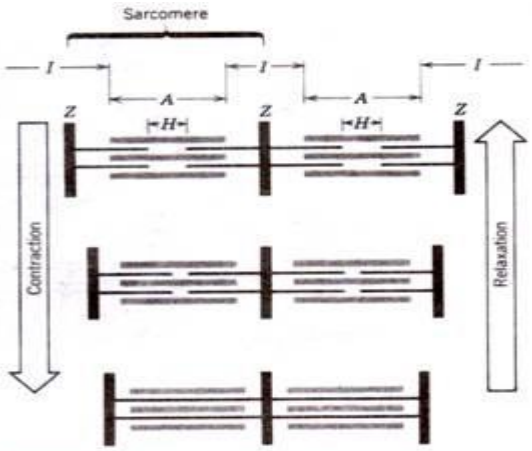


FIGURE 24–14 Changes in the relative positions of the thick and thin filaments in neighboring sarcomeres during contraction and relaxation.

The myosin monomers that comprise the thick filaments are fibrous proteins containing a "head" and "tail" portion (Fig. 24-15). Each myosin molecule is composed of two heavy polypeptide chains of MW 200,000 and four light chains of MW 20,000. Myosin can be cleaved into three pieces using proteolytic enzymes. Treatment with trypsin releases part of the tail (i.e., light meromyosin, LMM) from the remainder of the molecule.

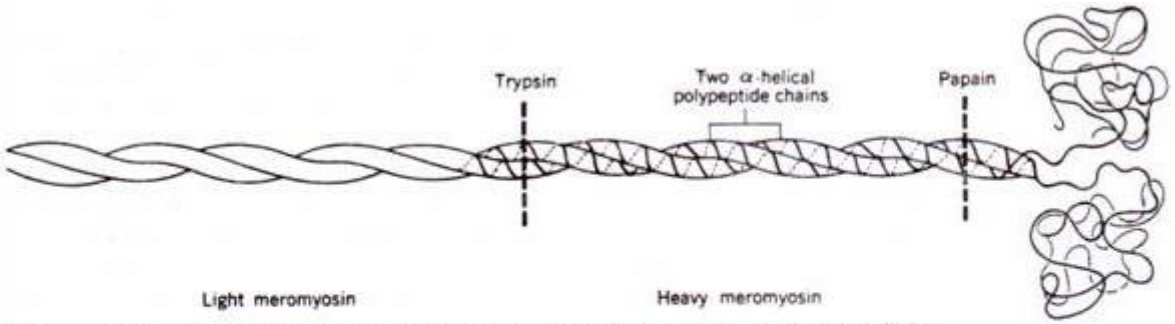


FIGURE 24–15 The myosin molecule. The enzyme trypsin cleaves the molecule into two pieces called light meromyosin and heavy meromyosin. The enzyme papain severs the head region (i.e., the region containing ATPase activity) from the tail. (Redrawn with permission. Copyright © 1979 by Worth Publishing, Inc.; A. L. Lehninger, *Biochemistry*, p. 512.)

Light meromyosin has no ATPase activity and cannot combine with actin. The remaining portion of the molecule, called heavy meromyosin (HMM), contains ATPase activity and binds actin. Treatment of HMM with the enzyme papain severs the head (the portion containing the ATPase activity) from the tail.

In thick filaments, the myosin molecules are arranged with their tails parallel to each other and their heads projecting away from the long axis of the filament at intervals (Fig. 24-16). No heads are present in the center of the filament, this region coinciding with the H zone. The heads of the myosin molecules form cross-bridges with adjacent actin filaments. Two functional domains can be identified in each head. One

domain contains the two cross-bridges that link the myosin to actin, and the other domain provides a flexible connection or hinge between the cross-bridges and the remainder of the myosin molecule.

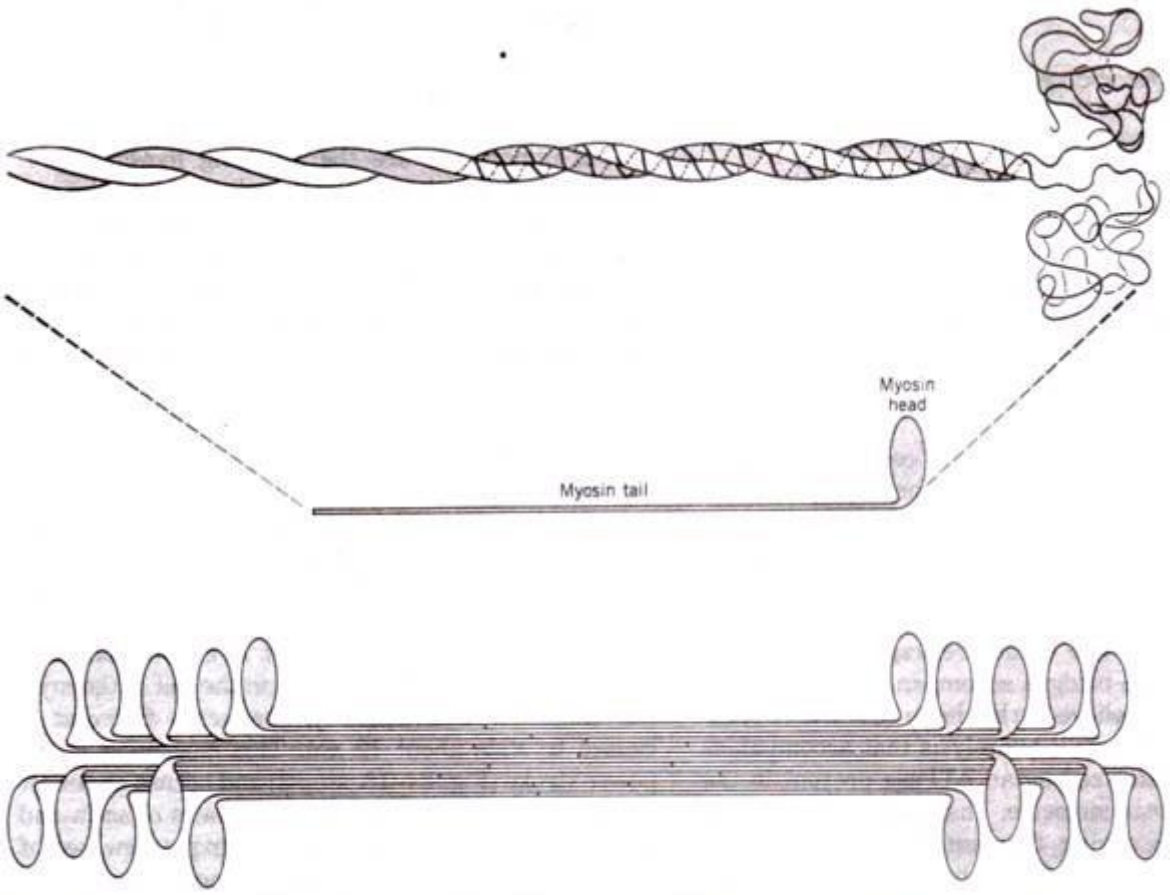


FIGURE 24–16 The thick filament. In thick filaments, the myosin molecules are arranged with their tails parallel to each other and their heads projecting away from the long axis of the filament.

Thin filaments are composed primarily of actin, but also present are small amounts of tropomyosin, troponin, α actinin, and β actinin (see Fig. 24-17). In the ionic environment of the cell, the actin exist in the fibrous (i.e., F-actin) form consisting of two chains of actin monomers coiled about each other. When extracted from muscle tissue and dialyzed, actin becomes globular (i.e., G-actin) as the monomers separate. Each actin monomer is a single polypeptide of MW 43,000. The proteins tropomyosin and troponin are arranged at intervals along the actin filament and serve as regulatory agents in contraction. Actinin may function in polymerization of the actin monomers.

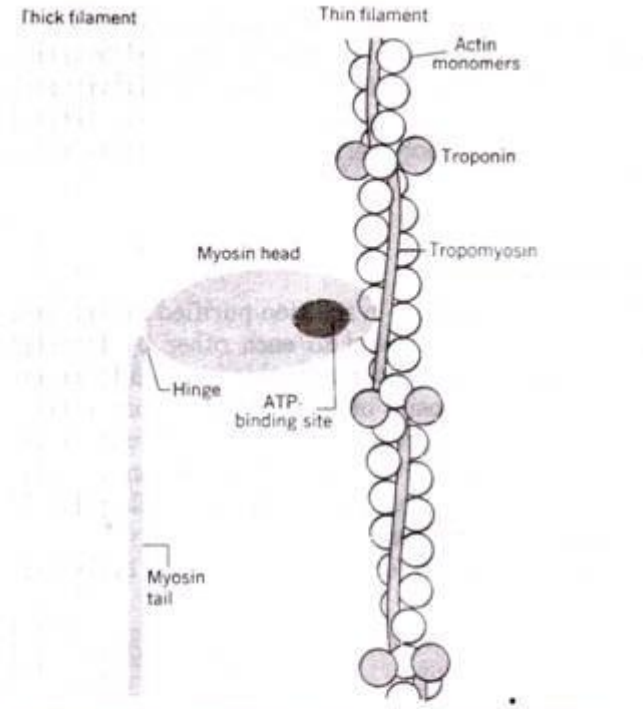


FIGURE 24–17 Association of the thick and thin filaments in striated muscle. The head of the myosin molecule is oriented at an angle to the long axis of the filament and forms a cross-bridge with the thin filament. The thin filaments are composed of two helical rows of actin monomers. The regulatory proteins tropomyosin and troponin occur in regular association with the actin filament. (Redrawn with permission. Copyright © 1979 by Worth Publishing, Inc.; A. L. Lehninger, *Biochemestry*, p. 397.)

Muscle Contraction:

When mixed together in solution purified myosin and actin molecules will bind to each other by forming cross-bridges. Experimentally, this is noted by an increase in the viscosity of the solution. Cross-bridges between actin and myosin filaments also exist in intact muscle fibers. It is the oar-like action of these cross-bridges accompanied by filament sliding that is the basis of contraction.

It has been known for some time that the cyclic contraction and relaxation of muscle cells is intimately tied to the metabolism of ATP. For example, when ATP and Mg2 + are added to an actin/myosin mixture, the solution's viscosity rapidly diminishes and the ATP is hydrolyzed. This is interpreted as an ATP-elicited disengagement of the cross-bridges between actin and myosin.

The cyclic breakage and reformation of these cross-bridges accompanied by their oar like movement is believed to be the in situ source of filament sliding. The ATP hydrolysis that accompanies this action is catalyzed by an ATPase present in the head of the myosin molecule. The exact role played by ATP

hydrolysis during filament sliding has been a subject of controversy for many years and still remains elusive.

A full cycle from relaxation through contraction and back to relaxation involves four phases:

(1) Triggering (or initiating) contraction,

(2) The "power stroke" (i.e., contraction per se),

(3) Relaxation, and

(4) The generation of energy for the process.

Triggering (Initiating) Contraction:

Prior to contraction the following conditions exist:

(1) The cross- bridges between the myosin heads and the actin filaments are in a weakly interacting state, with each myosin head oriented at right angles to the filament's long axis;

(2) The ATP and Mg^{2+} concentrations of the sarcoplasm are high, whereas the concentration of Ca^{2+} is low (Fig. 24-18, step 1); and

(3) The myosin heads contain tightly bound ADP and phosphate molecules. Strong cross-bridge interaction between myosin and actin is prevented by the regulatory proteins troponin and tropomyosin.

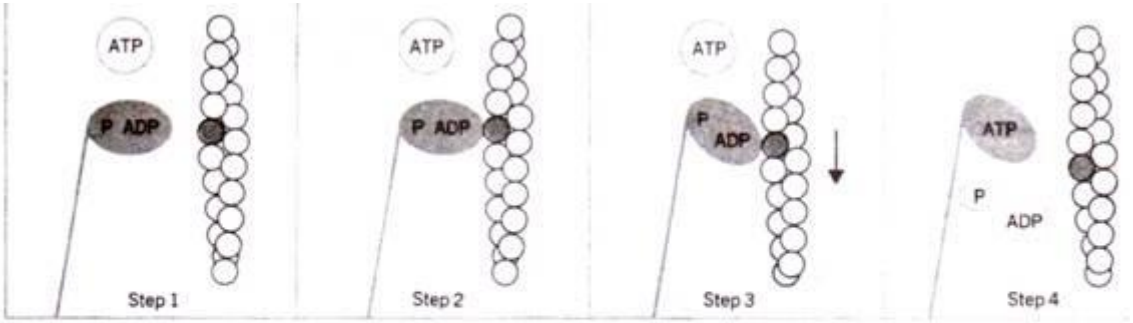


FIGURE 24–18 Steps in the contraction process. For easy reference, the specific actin monomer involved in the round of contraction is shown in color (see text for additional details). (Courtesy of K. C. Holmes. Copyright © 1973 by Nature: *Nature New Biol.* **241**, 226.)

Normally, contraction of a muscle cell is initiated when a nerve impulse causes depolarization of the sarcolemma at the myoneural junction (i.e., the junction between a muscle and nerve cell). The depolarization quickly spreads across the sarcolemma and down through the complex system of invaginations forming the sarcoplasmic reticulum or T system.

Depolarization is accompanied by a change in the permeability of the sarcoplasmic reticulum membranes to ions. Of special importance are the resulting movements of calcium ions, which are rapidly released into the sarcoplasm from storage vesicles elaborated by the sarcoplasmic reticulum. Calcium ions bind to troponin causing a conformational change in the complex and a shift in the position of tropomyosin. As a result, the binding sites on actin become available for further and stronger interaction with the myosin.

The Power Stroke:

As just noted, calcium ions entering the sarcoplasm bind to troponin, causing the troponin and tropomyosin to undergo a conformational change. As a result, a binding site on a G-actin monomer is exposed and reacts with the actin binding site of the myosin head (Fig. 24-18, step 2).

The myosin head immediately undergoes a conformational change, altering to 45° its angle to the tail of the myosin molecule. Consequently, the actin filament is caused to slide about 12 nm. This movement is the power stroke (Fig. 24-18, step 3) and occurs at almost the same moment for thousands of pairs of actin and myosin molecules, thereby positioning a new set of myosin heads and actin binding sites near one another. The process is repeated again and again, causing each actin filament to move a total distance of about 250 nm. The transition between the 90° and 45° conformation is accompanied first by the release of the bound phosphate from the myosin head and then by the release of ADP (Fig. 24-18, step 3). It is to be noted that no ATP is consumed during the power stroke. ATP binds to the myosin head, thereby occupying the site just vacated by ADP (Fig. 24-18, step 4). Binding of the ATP is accompanied by a transient detachment of the cross-bridges and transition back to the 90° conformation. With the myosin head once again oriented at right angles to the filament's long axis, the ATP is hydrolyzed (Fig. 24-18, step 1). Thus, the filament system is primed for another round of sliding activity as the cycle is ready to be repeated.

Relaxation:

The cessation of nervous stimulation allows the sarcolemma of the muscle cell to reestablish its polarity and regain its normal permeability. As the membrane repolarizes, calcium ions that entered the sarcoplasm are actively transported back into vesicles of the sarcoplasmic reticulum. The transport of Ca^{2+} is effected by an enzyme of the sarcoplasmic reticulum (i.e., a Ca^{2+}-dependent ATPase); this enzyme constitutes a large proportion of the sarcoplasmic reticulum protein.

With the removal of Ca^{2+} from the sarcoplasm, the conformation of troponin changes so that the binding site on the actin is again shielded from the myosin head. The thick and thin filaments slide back to their

former positions, their movement being passive (i.e., resulting from the natural elasticity of the muscle tissue or the effects of gravity) or as a result of the contraction of antagonistic muscles (muscles pulling in the opposite direction).

ATP Consumption during Muscle Activity:

Large amounts of ATP are consumed during muscle activity. At least two ATP molecules are consumed for each interaction between a myosin molecule and actin (i.e., there are two ATP-binding sites per myosin molecule), and one ATP is required for each calcium ion returned to the sarcoplasmic reticulum (i.e., two-thirds of ATP consumption is attributed to contraction and one- third to relaxation).

The ultimate source of ATP for the muscle fiber is the phosphorylation of ADP during cellular glycolysis and respiration. However, the immediate source is the phosphorylation of ADP using phosphocreatine (Fig. 24-19), a substance uniquely present in high concentration in muscle cells. Phosphate is rapidly transferred to ADP through the action of creatine kinase, which maintains an almost constant level of ATP in the muscle fiber during normal levels of contractile activity. (Experimentally it is possible to deplete the phosphocreatine supply by causing repeated contractions while blocking cellular glycolysis and respiration.)

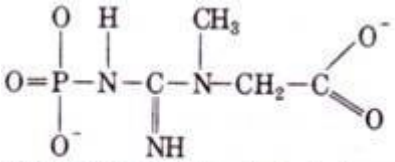


FIGURE 24–19 Chemical structure of phosphocreatine.

The restoration of the depleted phosphocreatine is brought about first by the synthesis of ATP during glycolysis and/or oxidative respiration and then through the action of creatine kinase, which now catalyzes the transfer of phosphate in the opposite direction (Fig. 24-20).

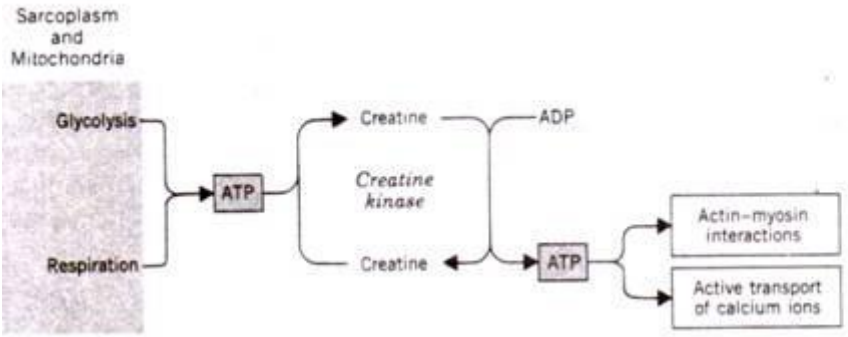


FIGURE 24–20 Action of *phosphocreatine kinase*.

There are two types of striated muscle tissue—red and white—and the relative amounts of ATP contributed in each tissue by glycolysis and respiration vary with each type. The red (or slow) fibers are rich in mi-

tochondria and cytochromes and employ oxidative phosphorylation for ATP production. Their demand for oxygen is satisfied in part by the presence in these cells of large quantities of the oxygen-storing protein myoglobin. Fatty acids are a major substrate of these cells and are converted to acetyl-CoA. Acetyl-CoA enters the Krebs cycle, producing CO_2 and the reducing power for oxidative phosphorylation. White fibers contain few mitochondria and little myoglobin. In these fibers, glycolysis is the primary source of ATP. Red fibers are found in greater number in muscles with slow, rhythmic contraction properties such as the flight muscles of birds. They are slow contracting because they have low myosin-ATPase activity. They resist fatigue because ATP production via oxidative phosphorylation is able to keep pace with ATP hydrolysis. White fibers predominate in quickly contracting muscles, such as the jumping muscles of frogs. These fibers are fast contracting because they have high myosin-ATPase activity. They fatigue quickly because ATP production via glycolysis does not keep pace with ATP breakdown. The proportion of red to white fibers in the leg muscles of humans appears to be determined genetically and is believed to account for individual differences between long distance and sprint runners.

During vigorous activity, blood supplying oxygen to and removing carbon dioxide from the muscles may be inadequate. Oxygen depletion of muscle promotes glycolysis and leads to the accumulation of lactic acid. On relaxation, oxygen reaches the muscle in adequate amounts and the lactic acid is removed by oxidation. Following a lengthy period of muscle exertion one's breathing rate may remain above normal for several minutes even though exercise is stopped. The extra oxygen consumed during this period is used to oxidize the accumulated lactic acid and is called the oxygen debt. Excess lactic acid is transported to the liver, where it is "recycled" first into glucose and then into glycogen.

Mechanism of Muscle contraction: When the nerve impulse from brain and spinal cord are carried along motor neuron to neuromuscular junction, Ca++ ions are released in the terminal axon. Increases calcium ion concentration stimulates the release of neurotransmitter (Acetylcholine) in the synaptic cleft. The neurotransmitter binds to the receptor on the sarcolemma and depolarization and generate action potential across muscle fiber for muscle contraction. The action potential propagates over entire muscle fiber and move to the adjacent fibers along transverse tubules. The action potential in transverse tubules causes the release of calcium ion from sarcoplasmic reticulum, which stimulate for muscle contraction.

1. Blocking of myosin head:

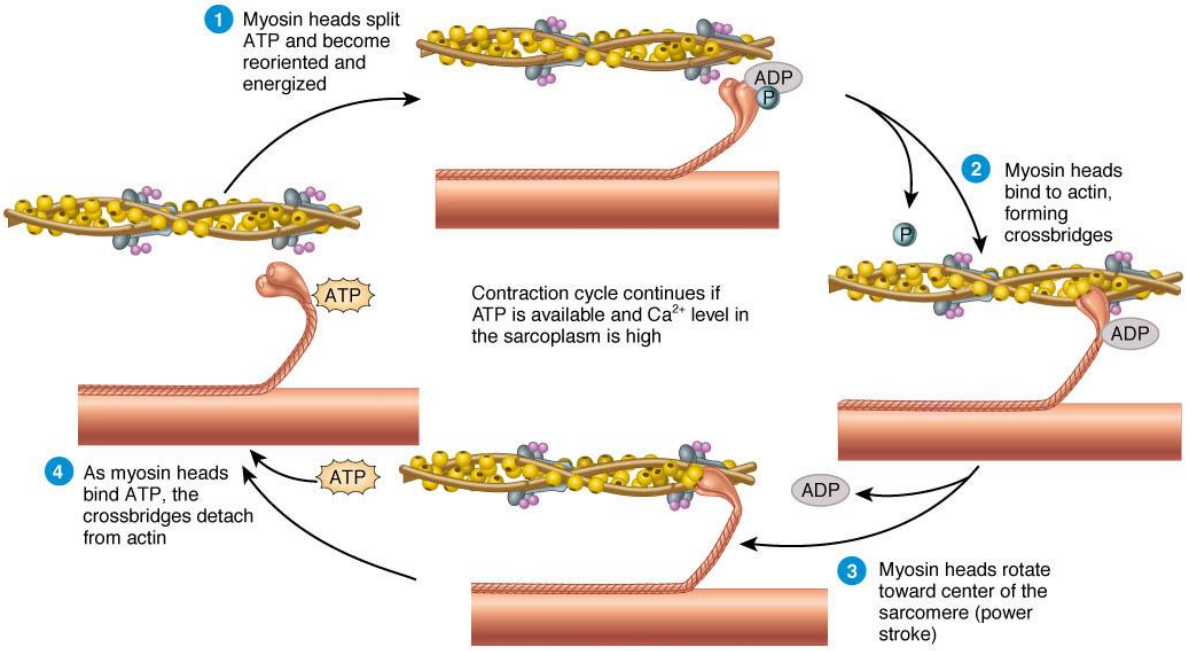
Actin and myosin overlaps each other forming cross bridge. The cross bridge is active only when myosin head attached like hook to the actin filament. When muscle is at rest, the overlapping of actin filament to the myosin head is blocked by tropomyosin. The actin myofilament is said to be in OFF position.

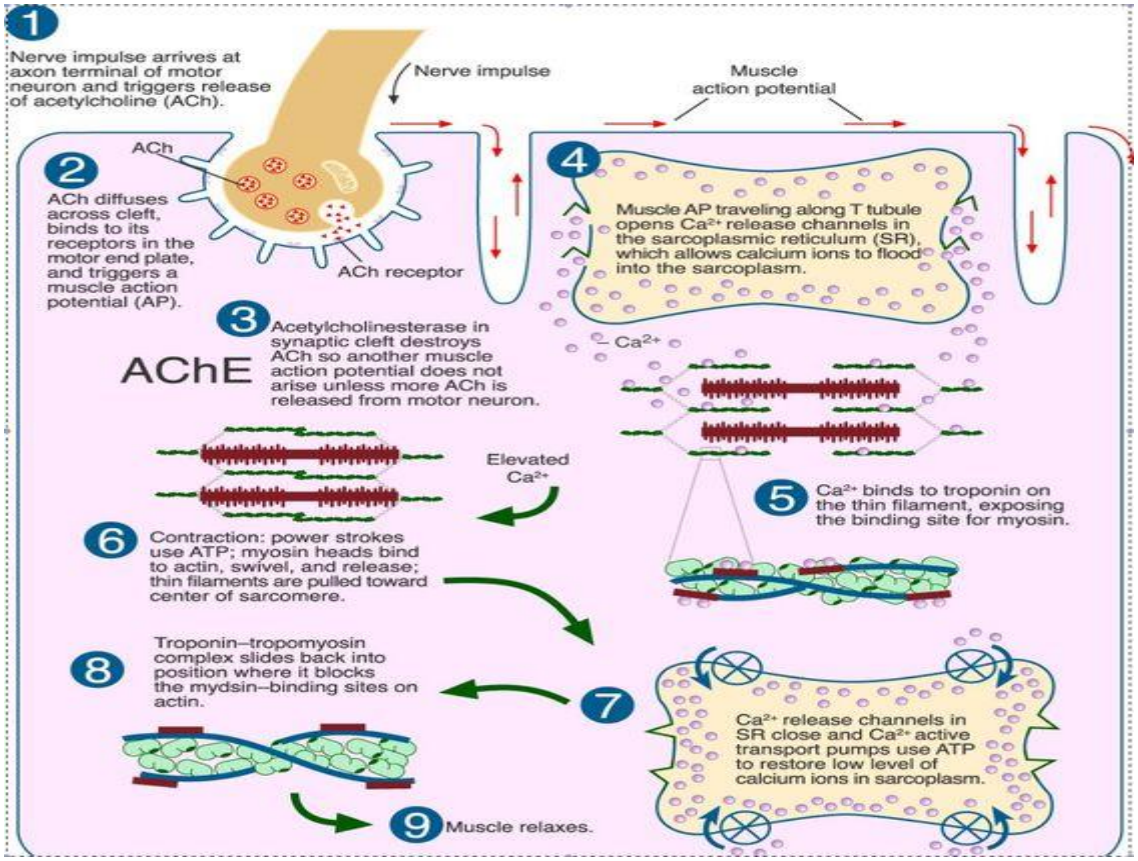
2. Release of calcium ions:

Nerve impulse causing depolarization and action potential in the sarcolemma trigger the release of calcium ions from sarcoplasmic reticulum. The calcium ion then binds with the troponin complex on the actin myofilament causing displacement of troponin complex and tropomyosin from its blocking site exposing myosin binding site. As soon as the myosin binding site is exposed, myosin head cross bridge with actin filament. Now, the actin myofilament is said to be in ON position. Probable Questions-

3. Active Cross-bridge formation:

When myosin head attached like hooks to the neighboring actin filament, active cross bridge is formed. The cross bridge between actin and myosin filament acts as an enzyme (Myosin ATPase). The enzyme Myosin ATpase hydrolyses ATP stored into ADP and inorganic phosphate and release energy. This released energy is used for movement of myosin head toward actin filament. The myosin head tilts and pull actin filament along so that myosin and actin filament slide each other. The opposite end of actin myofilament within a sarcomere move toward each other, resulting in muscle contraction. After sliding the cross bridge detached and the actin and myosin filament come back to original position. The active cross bridge form and reform for 50-100 time within a second using ATP in rapid fashion. Therefore, muscle fiber consists of numerous mitochondria. In muscle contraction, sarcomere can contracts by 30-60% of its length.





Probable Questions:

1. Explain with the help of a diagram the structure of a sarcomere.

2. What effect does the deficiency of calcium ion has on muscle contraction?

3. Describe with the help of diagram the sliding filament theory of muscle contraction.

Suggested Readings / References-

1. Animal physiology- Mohan P. Arora.

2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.

3. Ganong's review of medical physiology

Group B: Biochemistry and Metabolic Processes

ZCORT - 104 (Animal Physiology, Biochemistry and Metabolic Processes)	Unit	Content	Credit	Page No.
	IX	Carbohydrate metabolism: Carbohydrates of physiological significance. Glycolysis, Gluconeogenesis.	3	203-223
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	XI	Amino acid metabolism: Amino acid classification, Urea cycle.		246-263
	XII	Proteins: Protein folding and protein stability.		264-276
	XIII	Bioenergetics (anaerobic and aerobic respiration, oxidative and substrate level phosphorylation) basic concept of ETC and ATP synthesis, uncouplers. Spontaneous reaction (concept of -vedel G). Thermodynamic principles and steady-state conditions of living organism.		277-296
	XIV	Biosynthesis of cholesterol, control of cholesterol biosynthesis.		297-311
	XV	Lipoproteins and types of cholesterol transport.		312-318
	XVI	Enzymes: Kinetic analysis of enzyme-catalyzed reaction, Michaelis-Menten Equation, Lineweaver-Burk's plot, Bi-substrate Reactions.		319-337
	XVII	Enzyme inhibitions, Regulation of enzyme activity, Allosteric control of enzyme activity		338-346
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UNIT-IX

Carbohydrate metabolism: Carbohydrates of physiologic significance. Glycolysis, Hexose monophosphate Shunt.

Objective: Objective of this unit is to learn about different biochemical pathways such as glycolysis and HMP shunt which is involved in the carbohydrate metabolism and also how these pathways are controlled.

Meaning of Carbohydrates:

Carbohydrates are a group of organic compounds consisting of C, H, O usually in the ratio of 1: 2: 1 and include such well known compounds as sugars, starch, cellulose etc. Previously, the carbohydrates were regarded as hydrates of carbon and corresponded to general formula $(C.H_2O)$ n.

But the group name 'carbohydrates' was sometimes found misleading because:

(i) Some organic compounds e.g., formaldehyde (HCHO), acetic acid (CH_3COOH), lactic acid ($C_3H_6O_1$) inositol ($C_6H_{12}O_6$) etc. correspond to the general formula but are not carbohydrates.

(ii) Some carbohydrates e.g., rhamnose ($C_6H_{12}O_5$), rhamnohexose ($C_7H_{14}O_6$), digitoxose ($C_6H_{12}O_4$) do not correspond to the above general formula.

(iii) Besides containing C, H and O, some carbohydrates also contain nitrogen and sulphur.

Classification of the Carbohydrates:

Depending upon their complexity and behaviour on hydrolysis, the carbohydrates are classified into following 3 categories (Fig. 13.1).

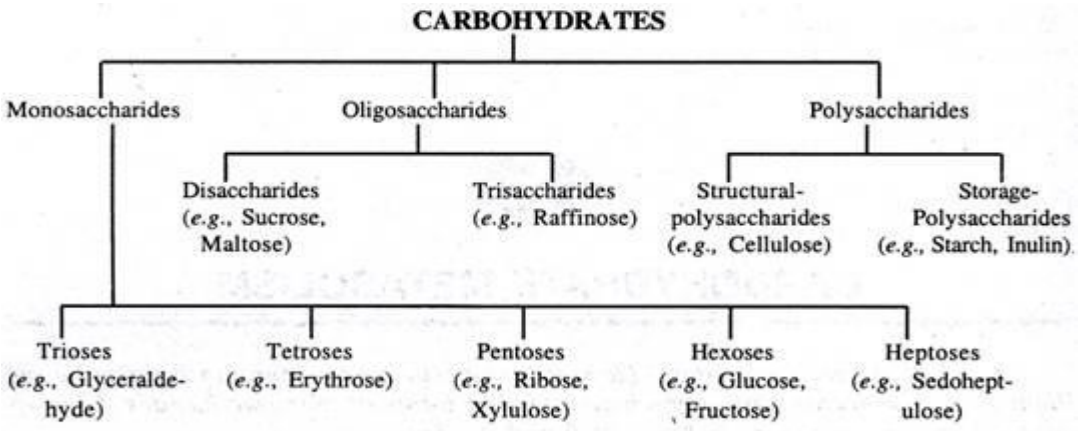


Fig. 13.1. Classification of the Carbohydrates

1. Monosaccharides:

i. These are simplest of carbohydrates and are known as sugars.

ii. These are the building units of complex carbohydrates.

iii. These cannot be hydrolysed.

iv. These are sweet-tasting, crystalline and soluble in water.

v. They have a potential aldehyde or keto group and hence, are reducing in nature.

vi. Aldehyde group or the reducing centre always lies at C No. 1 of the monosaccharide molecule. Such sugars are known as aldoses or aldose sugars.

vii. Monosaccharide's having keto group are known as ketoses or ketose sugars. In such sugars the keto group or the reducing centre always lies at C No. 2.

Monosaccharides are simple sugars, having 3 to 7 carbon atoms. They can be bonded together to form polysaccharides. Cells also use simple sugars to store energy and construct other kinds of organic molecules. The names of most sugars end with the letters 'ose'. Glucose and other kinds of sugars (fructose, and galactose) may be linear molecules ($C_6H_{12}O_6$) but in aqueous solution they take ring form.
There are two isomers of the ring form of glucose. They differ in the location of the OH group on the number 1 carbon atom. The number 1 carbon atom of the linear form of glucose is attached to the oxygen on the number 5 carbon atom.

(b) Disaccharides:

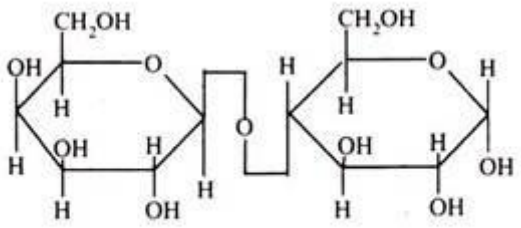
Disaccharides are composed of 2 monosaccharides joined together by a condensation reaction.

There are three common disaccharides:

i. Maltose (or malt sugar) consists of glucose monomers. Amylase enzyme digests starch molecules to produce Maltose.

ii. Sucrose (or cane sugar) composed of glucose and fructose. Plants synthesize sucrose to transport to non-photosynthetic parts of the plant, because it is less reactive than glucose.

iii. Lactose (or milk sugar) is made up of galactose and glucose. It is found only in mammalian milk.



Lactose

Depending upon the number of the C atoms, the monosaccharide's are further classified as follows:

(i) Triose Sugars, $C_3H_6O_3$ (e.g., glyceraldehyde, dihydroxyaeetone)

(ii) Tetrose Sugars, $C_4H_8O_4$ (e.g., erythrose)

(iii) Pentose Sugars, $C_5H_{10}O_5$ (e.g., ribose, ribulose, xylose, xylulose, arabinose).

(iv) Hexose Sugars, $C_6H_{12}O_6$ (e.g., glucose, fructose, galactose mannose).

(v) Heptose Sugars, $C_7H_{14}O_7$ (e.g., sedoheptulose).

2. Oligosaccharides:

i. These consist of more than one but fewer number of monosaccharide molecules joined together by glycosidic bonds.

ii. On hydrolysis, they yield the monosaccharide units which may be similar or dissimilar.

iii. These are also sweet tasting, crystalline, soluble sugars.

iv. These may or may not have a free -OH group at the reducing centre and accordingly may or may not be reducing.

Depending upon the number of the monosaccharide molecules which constitute them, the oligosaccharides are grouped in following categories:

(i) Disaccharides. $C_{12}H_{22}O_{11}$ (e.g., sucrose, maltose, lactose etc.)

(ii) Trisaccharides, $C_{18}H_{32}O_{16}$ (e.g., raffinose, gentianose etc.)

3. Polysaccharides:

Monosaccharides may be bonded together to form long chain compounds called polysaccharides. The monomeric building blocks used to generate polysaccharides can be varied; in all cases, however, the predominant monosaccharide found in polysaccharides is D-glucose. Polysaccharides that are composed of a single monosaccharide building block are termed as homopolysaccharides, while polysaccharides composed of more than one type of monosaccharide, they are termed as heteropolysaccharides.

For examples, starch and glycogen are composed of glucose monomers bonded together, producing long chains. They serve the function as stored food, starch in plants and glycogen in animal, in the liver and muscles. Glycogen is poly (1-4) glucose with 9% (1-6) branches (Fig. 3.5). Starch is a long (100s) polymer of glucose molecules, where all the sugars are oriented in the same direction. Unbranched starch is called amylose, while branched starch is known as amylopectin. Amylose is simply poly-(1-4) glucose units in a straight chain. In fact the chain is floppy, and it tends to coil up into a helix. Amylopectin is poly (1-4) glucose with about 4% (1-6) branches. This gives it a more open molecular structure than amylose.

As it has more ends, it can be broken more quickly than amylose by amylase enzymes. Amylopectin is a form of starch that is very similar to glycogen except for a much lower degree of branching (about every 20-30 residues). Another example of polysaccharide is cellulose. Cellulose is a long (100's) polymer of glucose molecules. However, the orientation of the sugars is little different. In Cellulose, every other sugar molecule is "upside-down". Glycogen is different from both, starch and cellulose in that the glucose chain is branched or "forked" (Fig. 3.6).

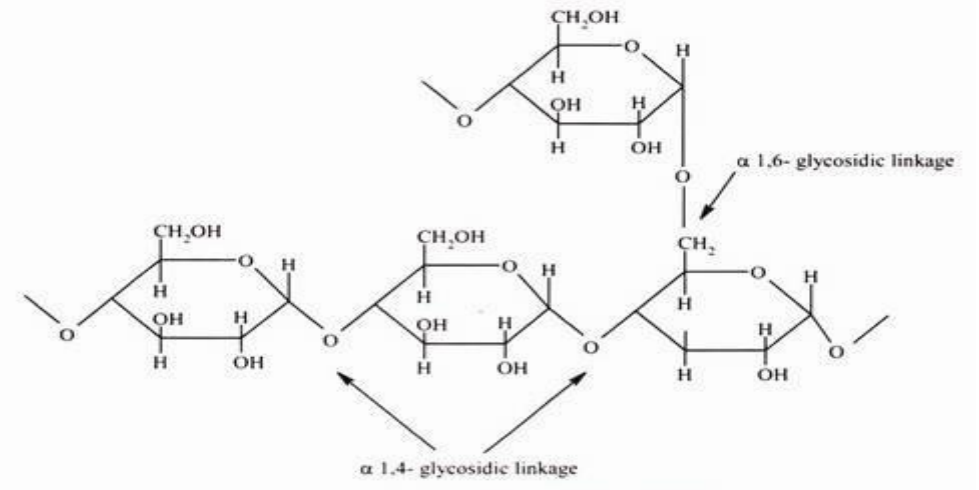


Figure 3.5: Structure of Glycogen

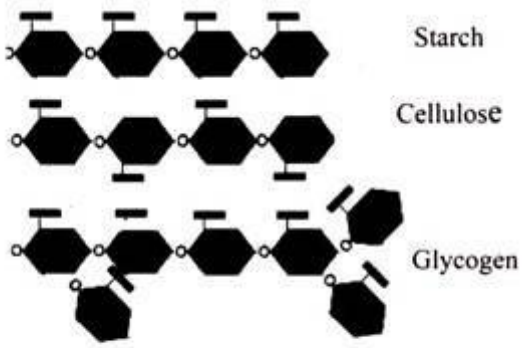


Figure 3.6: Orientation of monomers in polysaccharides

Characteristics of Polysaccharides:

i. These consist of a large number of (often thousands) monosaccharide units to form branched or un-branched chains.

ii. These can be hydrolysed to yield monosaccharide units which are usually similar.

iii. These are usually amorphous, tasteless, non-sugars and insoluble in water.

Polysaccharides can be grouped into two categories:

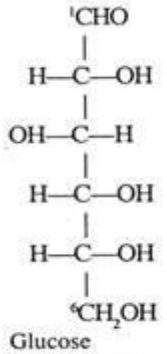
(i) Structural Polysaccharides (e.g., cellulose, hemi-cellulose, pectic substances, chitin, gum, mucilage etc.)

(ii) Storage Polysaccharides (e.g., starch, inulin, glycogen etc.)

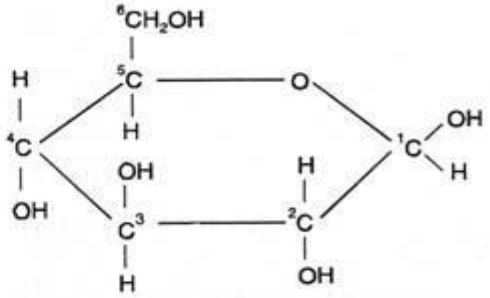
Structural Features:

Open Chain and Ring Forms of Monosaccharides:

Many monosaccharide's e.g ribose, glucose, fructose etc. exist both in open straight chain and ring form. If the ring is 5-membered it is called as furanose sugar. Sugar with a 6-membered ring is called as pyranose sugar e.g.,

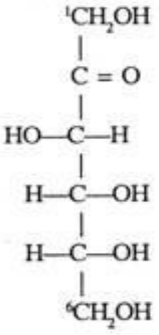


Glucose
(Open Chain Structure)
or Fischer's projection formula)*

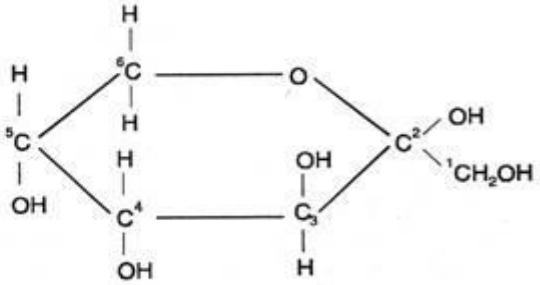


Glucopyranose
(Six membered ring structure of Glucose)

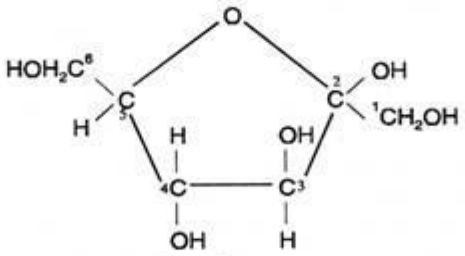
Similarly,



Fructose
(Open Chain Structure)



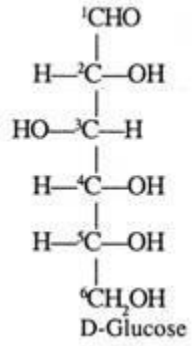
Fructopyranose
(6-membered ring structure of Fructose)



Fructofuranose
(5-membered ring-structure of Fructose)

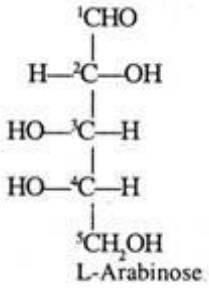
(The names pyranose and furanose are derived from six and five membered cyclic ethers called pyran and furan respectively with which these sugars bear a formal resemblance).

If the highest numbered asymmetric carbon atom of the sugar molecules contains—OH group on right-hand side in open chain structure, the sugar is known as D-Sugar and if on left side it is known as L-Sugar. For example, in open chain structure of glucose carbon number 2, 3, 4 and 5 are asymmetric. The highest numbered asymmetric C-atom is therefore, 5. As shown below, it bears -OH group on right-hand side and hence, glucose is called a D-sugar and written as D-Glucose or D-Glucopyranose (in case of 6-membered ring structure).



D-Glucose

In arabinose (a pentose sugar), the highest numbered asymmetric carbon atom is 4 which bears—OH group on left-hand side, therefore, this is a L-Sugar and called as L-Arabinose.



L-Arabinose

(The carbon atom whose 4 valancies are satisfied by four different groups or atoms is called as asymmetric carbon atom).

i. Almost all the sugars in plants are D-Sugars.

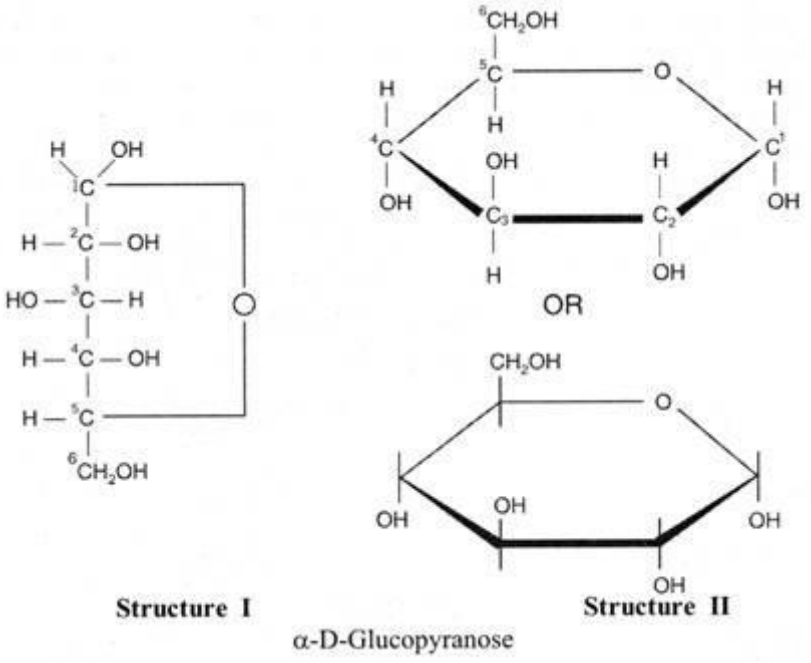
ii. Symbols D and L should not be confused with the optical activity. If the compound is optically active and rotates the plane polarised light to right, it is called as dextrorotary and is denoted by small italic letter d or + sign. But, if it rotates the plane to left side it is called as laevorotatory and is denoted by I or – sign. For example, D-Glucose is dextrorotatory and D- Fructose leavorotatory and are written as:-

D (+) Glucose or D (d) Glucose

D (-) Fructose or D (l) Fructose

iii. The ring form of sugars is in fact a cyclic hemi-acetal or hemiketal structure formed by combination of the carbonyl group (i.e., aldehyde group at C_1 in aldose sugars and keto group at C_2 in ketose sugars) and one of the hydroxyl groups (usually at the highest numbered asymmetric C atom) of the sugar molecule in open chain structure.

The hemi acetal or hemiketal bond formation creates a new asymmetric centre at C_1 in aldose sugars and C_2 in ketose sugars which is now called as anomeric carbon atom. Taking for example a-D-Glucopyranose, the ring form of the sugars is usually depicted by following two structural formulas:



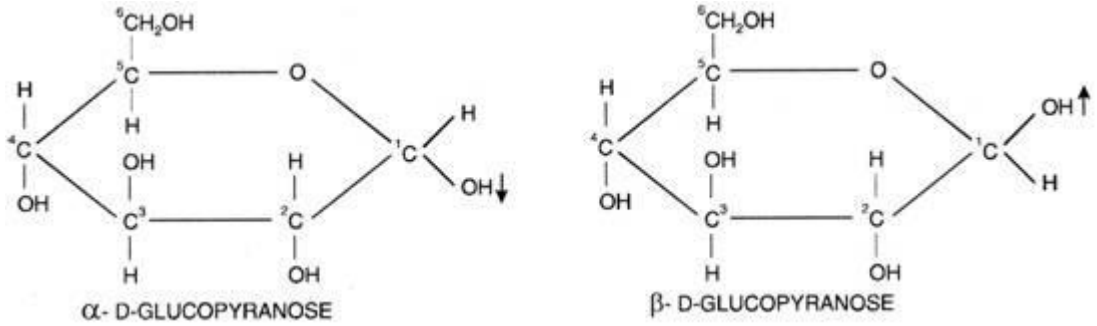
Although structure I indicates the basic features of a-D-Glucopyranose, it provides little insight as to the actual shape of the molecule and the spatial relationship of the various functional groups to one another. The latter features are clearly represented by structure II which is also called as Haworth's formula.

iv. In Haworth's perspective formula, the plane of the pyranose ring is considered to be perpendicular to that of the page on which it is written with the substituents (-H, -OH and -CH_2OH groups) either above or below the plane of the ring. The thin bonds of the ring are behind the plane of the paper while the thick bonds in front of it.

(As a rule, the substituents written to the right side of the C chain in structure I are shown below the plane of the ring in Haworth's representation while those on left side above the plane of ring. However, opposite rule applies to that C atom whose—OH group is involved in the formation of cyclic hemiacetal. Thus, the CH_2OH group is written in an above position and the H atom on the same carbon written below despite the fact that it is on the left side in structure I.

In practice, however, the carbon atoms of the ring and the H atoms are omitted and often the thick bonds of the ring are not shown. The formula may also be inverted or twisted in oligo and polysaccharides to show the linkages between various monosaccharide molecules more clearly.)

If in the Haworth representation of the sugars the—OH group at the reducing centre (i.e., C No. 1 in aldose sugars and C No. 2 in ketose sugars) is present below the plane of the ring, the sugar is said to be in a-form. And if it is present above the plane of the ring, the sugar is said to be β-form. For example,



When α and β-forms change into one another the phenomenon is called as mutarotation.

There is an equilibrium between these two forms of ring compound. For example, the equilibrium in the mutarotation of a synthetic sample of D-Glucopyranose corresponds to a mixture of 36% of the a-form and 64% of the β-form. α and β-forms of a given sugar are anomers of each other. (The proportion of the open chain and ring forms of sugars in solution differs with different sugars. For most of the aldohexoses and aldopentoses the ring form is predominant one. Fructose (a ketose sugar) and most of the aldopentoses exist predominantly in the furanose form while glucose and other aldohexoses exist mainly in pyranose form. Ketopentoses such as ribulose usually prefer open chain form).

Glycolysis:

Glycolysis (Gk. glykys = sweet, lysis = splitting), also called glycolytic pathway or Embden-Meyerhof-Parnas (EMP) pathway, is the sequence of reactions that metabolises one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP.

Glycolysis is almost an universal central pathway of glucose catabolism, and the complete pathway of glycolysis was elucidated by 1940, largely through the pioneering contributions of G. Embden, O. Meyerhof, J. Parnas, C. Neuberg, O. Warburg, G. Cori, and C. Cori. However, glycolysis occurs in all major groups of microorganisms and functions in the presence or absence of oxygen. It is located in the cytoplasmic matrix of the cells of an organism. The whole process of glycolysis (i.e., the breakdown of the 6-carbon glucose molecule into two molecules of the 3-carbon pyruvate) occurs in ten steps (Fig. 24.1).

The first five-steps constitute the preparatory phase while the rest live-steps represent the payoff phase (oxidation phase).

In preparatory phase there is phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate at the expense of two molecules of ATP. Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH is the feature of payoff phase.

The whole of glycolysis can be represented by the following simple equation:

Glucose + 2ADP + 2Pi + $2NAD^+$ = 2 pyruvate + 2ATP + 2NADH + $2H^+$

The four stages involved in the aerobic respiration process are: (1) Glycolysis (or EMP-Pathway of Glycolytic Breakdown) (2) Pyruvate Oxidation or Conversion of Pyruvic Acid to Acetyl Coenzyme A (3) TCA Cycle or Krebs' Cycle and (4) Terminal Oxidation.

Stage I of Respiration: Glycolysis (or EMP-Pathway of Glycolytic Breakdown):

Although glycolysis occurs in all organisms except primitive bacteria, it has been studied in greatest detail in yeast and animal muscle cells. The same sequence of reactions is found in both types of cells. The chemical reactions of glycolysis were discovered mainly by the efforts of G.G. Embden (1874-1933), Otto Meyerhof (1884 -1951) and J.K. Parnas (1884 -1949), and hence this pathway is also generally referred to as EMP-pathway. Within a period of about 10 years following 1914 the essential features of the EMP pathway were established. It is common to the majority of living organisms, both plants and animals.

It occurs chiefly in the soluble part of the cytoplasm (i.e., cytosol), rather than being associated with mitochondria or other organelles. This is first stage of respiration, which includes a sequence of chemical reactions through which glucose is incompletely oxidized to yield the 3-carbon compound pyruvic acid (Glycolysis = lysis or splitting of glucose).

The essential feature of glycolysis is the breakdown of 6-carbon sugar glucose into two 3-carbon fragments (Pyruvic acid molecules). Glycolysis takes place in ten consecutive steps, each catalyzed by a different enzyme complex (Fig. 7.2). Biochemical steps 1, 3 & 10 are irreversible in glycolysis.

Figure 7.2 summarises steps involved in glycolysis.

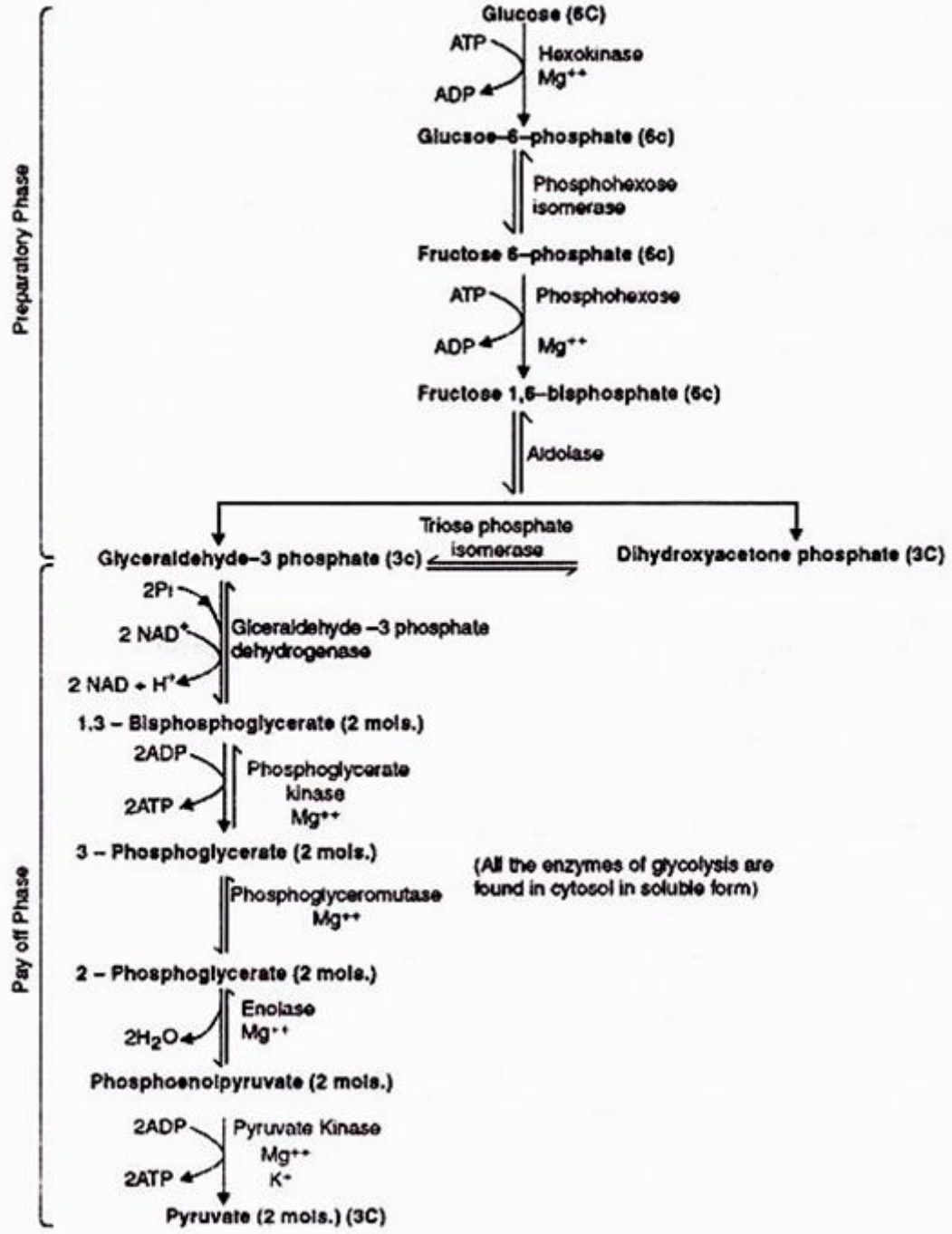
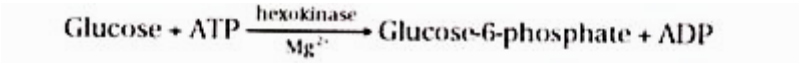


Fig. 7.2 Glycolysis or EMP pathway.

(i) First Half of Glycolysis (Preparatory Phase):

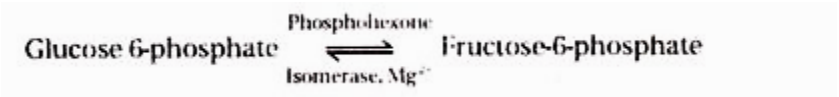
Step 1 (1st Phosphorylation):

In order to obtain energy in the form of ATP (Adenosine triphosphate) from the breakdown of glucose, it is first necessary to "spark off" the reaction sequence by putting ATP into it. During step 1 the primary alcohol group (- CH_2OH) at position C-6 of glucose reacts with the terminal phosphate group of ATP, forming glucose-6-phosphate and ADP (Adenosine diphosphate). The reaction is catalysed by enzyme hexokinase and activator Mg^{++} ions.



Step 2 (Isomerization):

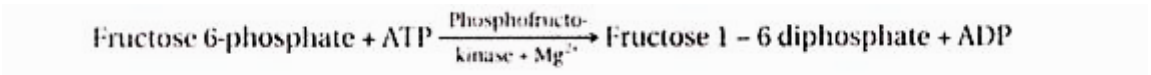
During second step of reactions the glucose-6-phosphate is rearranged (isomerized) to form fructose-6-phosphate. In this reaction, the aldehyde group (- CHO) at C-1 is reduced to a primary alcohol group (- CH_2OH) as a result of simultaneous oxidation of the secondary alcohol group (> CHOH) at C-2 to a keto group (> CO). This isomeric change is catalysed by the enzyme phosphohexoisomerase.



Fructose 6-phosphate can also be produced directly by phosphorylation of fructose with the help of enzyme fructokinase.

Step 3 (2nd Phosphorylation):

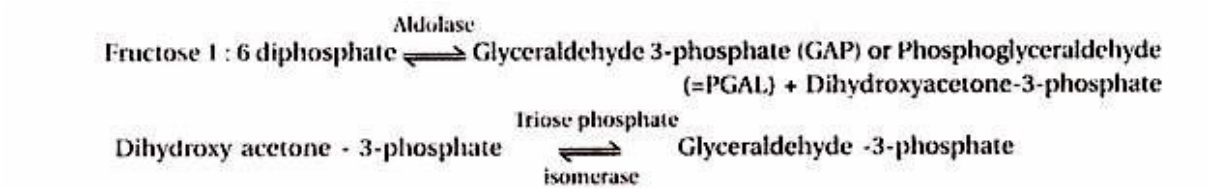
The formation of a primary alcohol group at C-1 makes possible step 3, which is a repetition of the phosphorylation effected in step 1. Fructose -6-phosphate is again phosphorylated in the presence of one ATP molecule. The reaction is catalysed by an enzyme kinase and Mg^{++} cofactor, forming fructose-1-6-diphosphate (phosphate attached at C-1 position) and ADP. This reaction is also reversible.



Step 4 (Splitting of hexose molecule):

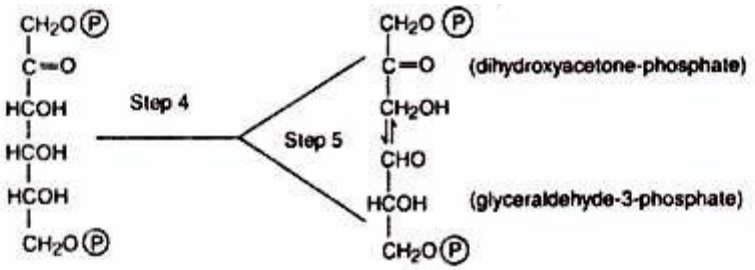
We have seen that the first three steps of glycolysis have thus converted, at the expense of two molecules of ATP, a molecule of glucose into one of fructose- 1-6-diphosphate, with a phosphate group at each end. Fructose-1-6-di-phosphate is now split into two smaller fragments. In this step the 6-carbon sugar, fructose-1-6-di-phosphate, is split between C-3 and C-4 to form two 3-carbon fragments, one an aldehyde (glyceraldelyde-3-phosphate) and the other a ketone (dihydroxyacetone-phosphate). This reaction is carried out under the influence of an enzyme aldolase.

The EMP pathway is common to a great many microorganisms as well as higher forms. The enzyme fructose diphosphate aldolase (also called as fructose biphosphate aldolase) is one of the most critical steps in the pathway. In the absence of this enzyme, glucose or other hexose sugars must be metabolized via one of several alternative pathways.



Step 5:

These two fragments, which may collectively be called triose phosphates, are readily inter-convertible by an isomerization under the effect of an enzyme complex Phospho-triose- isomerase. Mostly dihydroxyacetone- phosphate is converted to glyceraldehyde-3-phosphate.



(ii) Second Half of Glycolysis (Pay off Phase):

In the second half of glycolysis, comprising steps 6 to 10, there is a net gain of ATP, achieved by the oxidation of glyceraldehyde-3-phosphate, one of the triose phosphates formed in step 4. Because the two triose phosphates are inter-convertible, both follow the same pathway and steps 6 to 10 are therefore repeated twice to complete the breakdown of one molecule of glucose.

Step 6 (Dehydrogenation and Phosphorylation):

From the point of view of energy production step 6 is very important one. During this step glyceraldehyde-3-phosphate is simultaneously oxidized and phosphorylated, and the energy liberated by the oxidation of the aldehyde group is conserved to form the high-energy compound 1, 3-diphosphoglycerate, i.e., it has a high free energy of hydrolysis.

The electrons removed from the aldehyde group during its oxidation are accepted by the coenzyme NAD^+ (Nicotinamide adenine dinucleotide), which is thereby reduced to give $NADH_2$ ($NADH + H^+$). The NAD^+ is bound to the enzyme glyceraldehyde-3-phosphate dehydrogenase which catalyzes the overall reaction.

In this reaction one inorganic H_3PO_4 group combines at C-1 of 3-phosphogiyceraldehyde (GAP) by non-enzymatic condensation to from 1-3-diphosphogly-ceraldehyde, and then two hydrogen atoms are removed by NAD^+ coenzyme 1 under the effect of enzyme glyceraldehyde-3-phosphate dehydrogenase to yield NADH and 1, 3-diphospholgyceric acid (1, 3-PGA). In this dehydrogenation energy is carried along with H atoms into $NADH_2$.

Net Products of Glycolysis:

In glycolysis two molecules of ATP are consumed during double phosphorylation of glucose to form fructose 1: 6 diphosphate. In return four molecules of ATP are produced by substrate level phosphorylation (conversion of 1: 3 diphosphoglycerate to 3-phosphoglycerate and phosphoenol pyruvate to pyruvate). Two molecules of NADH2 are formed at the time of oxidation of glyceraldehyde 3-phosphate to 1: 3 diphosphoglycerate.

The net reaction is as follows:

$$\text{Glucose} + 2NAD^+ + 2\,ADP + 2H_3PO_4 \longrightarrow 2\,\text{Pyruvate} + 2\,NADH + 2H^+ + 2ATP$$

Each NADH is equivalent to 3ATP, so that the net gain in glycolysis is 8ATP.

Total number of ATP formed by glycolysis under anaerobic conditions up to pyruvic acid:

From one molecule of glucose, two molecules of glyceraldehyde-3-phosphate are formed. After that, in the reactions of glycolysis, each product is 2 molecules.

Reactions	ATP formed
1. Glyceraldehyde-3-phosphate → 1, 3-bisphosphoglycerate	6
2. 1, 3-bisphosphoglycerate → 3-phosphoglycerate	2
3. Phosphoenolpyruvate → Enol pyruvate	2
Total	10

Reactions	ATP formed
ATP consumed in :	
1. Glucose → glucose-6-phosphate	1
2. Fructose-6-phosphate → Fructose-1, 6-bisphosphate	1
Net ATP Synthesized	8

But in anaerobic conditions, the total number of ATP will be only 2 up to lactic acid. Because the reduced NAD (NADH) in glyceraldehyde-3-phosphate dehydrogenase is utilized in lactate dehydrogenase, NADH is not oxidized in mitochondria. The total calories in this case is 2 x 7,600= 15,200 calories.

Regulation of Glycolysis:

a. The enzymes involved in the utilization of glucose are activated on the more availability of glucose whereas the enzymes responsible for gluconeogenesis are inhibited at the same time. The activity of the key enzymes glucokinase, phosphofructokinase-1 and pyruvate kinase is increased by glucose.

b. The hormone insulin stimulates the synthesis of the key enzymes responsible for glycolysis and antagonizes the effects of glucocorticoids and glucagon-stimulated cAMP in enhancing the key enzymes responsible for gluconeogenesis.

c. The hormones epinephrine and glucagon increase cAMP level to activate cAMP- dependent protein kinase which phosphorylates and inactivates the key enzyme pyruvate kinase and thus, inhibit glycolysis.

d. Phosphofructokinase-1 is involved in “feedback” control. This enzyme is activated by AMP and inhibited by ATP and citrate. When ATP is utilized in energy requiring processes, the concentration of AMP is highly increased.

e. In hypoxia, the concentration of ATP in the cell is decreased with the increase in the concentration of AMP which explains clearly the inner significance of increase of glycolysis in absence of oxygen.

Effect of Hormones in Glycolysis:

a. Insulin stimulates hexokinase and glucokinase which catalyze the conversion of glucose to glucose-6-phosphate.

b. Insulin stimulates phosphofructokinase which catalyzes the conversion of fructose-6-phosphate to fructose-1, 6-bisphosphate.

c. Glucagon stimulates liver glucose-6- phosphatase which is involved in the conversion of glucose-6-phosphate to glucose and also fructose-1, 6-bisphosphatase involved in the conversion of fructose-1, 6-bisphosphate to fructose-6 phosphate.

Inhibitors:

a. lodoacetate is the inhibitor of glyceraldehyde-3-phosphate dehydrogenase involved in the conversion of glyceraldehyde-3-phosphate to 1, 3-bisphosphoglycerate.

b. Arsenitc inhibits the synthesis of ATP by accomplishing uncoupling of oxidation and phosphorylation in the conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate.

c. Fluoride inhibits enolase involved in the conversion of 2-phosphoglycerate to phosphoenolpyruvate.

Reaction chart (Fig 17.12) of the pathway of glycolysis ℗ PO_3^{2-}; Pi, $H0P0_3^{2-}$; (-), inhibition carbon atoms 1-3 of fructose bisphosphate form dihydroxyacetone phosphate, whereas carbons 4-6 form glyceraldehyde-3-phosphate. The term bis, as in bisphosphate, indicates that the phosphate groups are separated, whereas diphosphate, as in adenosine diphosphate, indicates that they are joined.

Pentose Phosphate Pathway or Hexose Monophosphate Pathway (HMP Pathway):

Pentose phosphate pathway or hexose monophosphate pathway (HMP pathway) is the other common pathway to break down glucose to pyruvate and operates in both aerobic and anaerobic conditions.This pathway produces NADPH, which carries chemical energy in the form of reducing power and is used almost universally as the reductant in anabolic (energy utilization) pathways (e.g., fatty acid biosynthesis, cholesterol biosynthesis, nucleotide biosynthesis) and detoxification pathways (e.g., reduction of oxidized glutathione, cytochrome P450 monooxygenases).

Also, the pentose phosphate pathway generates pentose sugar ribose and its derivatives, which are necessary for the biosynthesis of nucleic acids (DNA and RNA) as well as ATP, NADH, FAD, and coenzyme A. In this way, though the pentose phosphate pathway may be a source of energy in many microorganisms, it is more often of greater importance in various biosynthetic pathways. Pentose phosphate pathway (Fig. 24.2.) consists of two phases: the oxidative phase and the non-oxidative phase. In oxidative phase, there is generation of NADPH when glucose 6-phosphate is oxidised to ribose 5-phosphate.

In non-oxidative phase, the pathway catalyzes the inter conversion of three-, four-, five-, six-, and seven-carbon sugars in a series of non-oxidative reactions that can result in the synthesis of five-carbon sugars for nucleotide biosynthesis or the conversion of excessive five-carbon sugars into intermediates of glycolysis. All the reactions of non-oxidative phase take place in the cytoplasm of the cell.

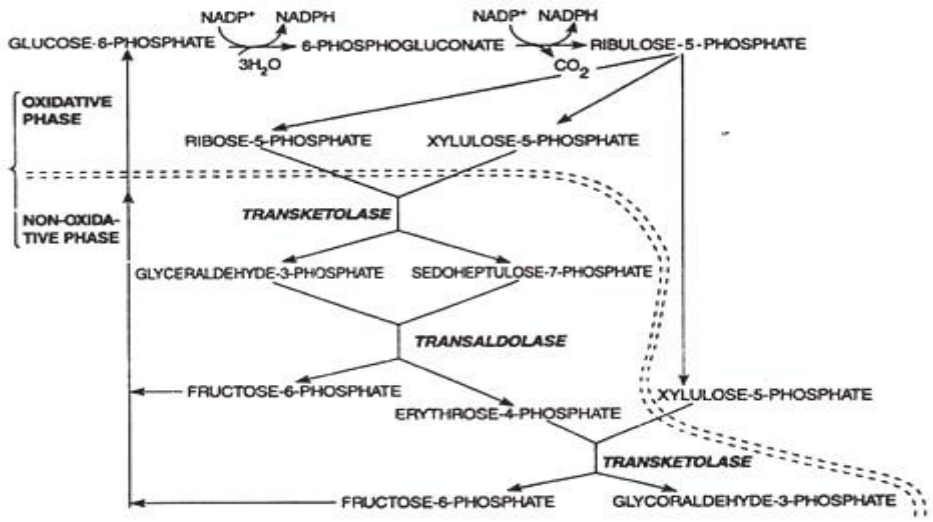


FIG. 24.2. Pentose phosphate pathway (or hexose monophosphate pathway).

Characteristics of Hexose Monophosphate Shunt:

a. This is an alternate aerobic pathway for the oxidation of glucose in the liver, lactating mammary gland and adipose tissue in addition to the Embden-Meyerhof pathway for glycolysis.

b. The enzymes of this pathway are present in the extra-mitochondrial portion of the cell. This pathway is active in liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis and lactating mammary gland.

c. In this pathway, 3 molecules of glucose- 6-phosphate yield 3 molecules of CO_2 and 3 molecules of 5 carbon residues (pentose sugars). The latter are converted ultimately to 2 molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3- phosphate.

d. NADP, instead of NAD, is used as a hydrogen acceptor in this pathway.

Oxidative Phase:

The oxidative phase of the pentose phosphate pathway initiates with the conversion of glucose 6-phosphate to 6-Phosphogluconate. $NADP^+$ is the electron acceptor yielding NADPH during this reaction. 6-Phosphogluconate, a six-carbon sugar, is then oxidatively decarboxylated to yield ribulose 5-phosphate, a five-carbon sugar. $NADP^+$ is again the electron acceptor yielding NADPH.

In the final step of oxidative phase, there is isomerisation of ribulose 5-phosphatc to ribose 5-phosphate by phosphopentose isomerase and the conversion of ribulose 5-phosphate into its epimer xylulose 5-phosphate by phosphopentose epimerase for the transketolase reaction in non-oxidative phase.

Nonoxidative Phase:

In the non-oxidative phase, enzyme transketolase catalyzes the transfer of a two carbon fragment of xylulose 5-phosphate to ribose 5-phosphate forming the seven-carbon sedoheptulose 7-phosphate and three-carbon glyceraldehyde 3-phosphate.

Enzyme transaldolase then catalyses the transfer of a three-carbon fragment from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate resulting in six-carbon fructose 6-phosphate and four carbon erythrose 4-phosphate. Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate. Two molecules of glyceraldehyde 3-phosphate formed by two interations of these reactions can be converted into a molecule of fructose 1, 6-bisphosphate.

The overall result of pentose phosphate pathway is that 3 glucose 6-phosphates are converted to two fructose 6-phosphates, glyceraldehyde 3-phosphate, and three CO_2 molecules, as shown in the following equation:

3 glucose 6-phosphate + 6 $NADP^+$ + $3H_2O$ → 2 fructose 6-phosphate + glyceraldehyde 3-phosphate + $3CO_2$ + 6 NADPH + $6H^+$

Fructose 6-phosphate and glyceraldehyde 3-phosphate intermediates are used in two ways. The fructose 6-phosphate can be converted back to glucose 6-phosphate, while glyceraldehyde 3-phosphate is converted to pyruvate by glycolysis-enzymes.

The glyceraldehyde 3-phosphate also may be returned to pentose phosphate pathway through glucose 6-phosphate formation. This results in the complete degradation of glucose 6-phosphate to CO_2 and the production of great deal of NADPH.

This pathway of glucose metabolism takes place in liver, mammary gland, testis, adrenal cortex and leucocytes. Glucose-6-phosphate derived from different sources is dehydrogenated by glucose-6-phosphate dehydrogenase into 6-phospho-gluconolactone which, through several steps described in the Figure 10.10, is ultimately converted into sedoheptulose-7-phosphate and enters again into the main glycolytic pathway at fructose-6-phosphate and glyceraldehyde-3-phosphate. The formation of sedoheptulose-7-phosphate is catalysed by transketolase whereas the breakdown is catalysed by the enzyme transaldolase. The transketolase and transaldolase reactions are important in this path which is responsible for conversion of aldehydes to ketones and vice versa, as well as lower sugars to higher sugars and vice versa.

The physiological benefits in the cycle are as follows:

i. Synthesis of pentose to be required for the synthesis of nucleotides.

ii. Pentoses can enter into the glycolytic path and it itself may be oxidized in pentose phosphate pathway (PPP).

iii. Hexose (glucose-fructose) may be burnt in this PPP or may supply pentoses.

iv. $NADPH_2$ formed in the PPP, is utilized in the fat and steroid synthesis.

v. Energy formed in this path is 36 ATP per molecule of glucose if all the NADPH, are oxidized in the mitochondria to NADP.

vi. The oxidation of glucose (Fig. 10.13) in this path is independent of TCA cycle components.

vi. Components of PPP may enter into the path of formation of glucuronic acid an ascorbic acid (vitamin C).

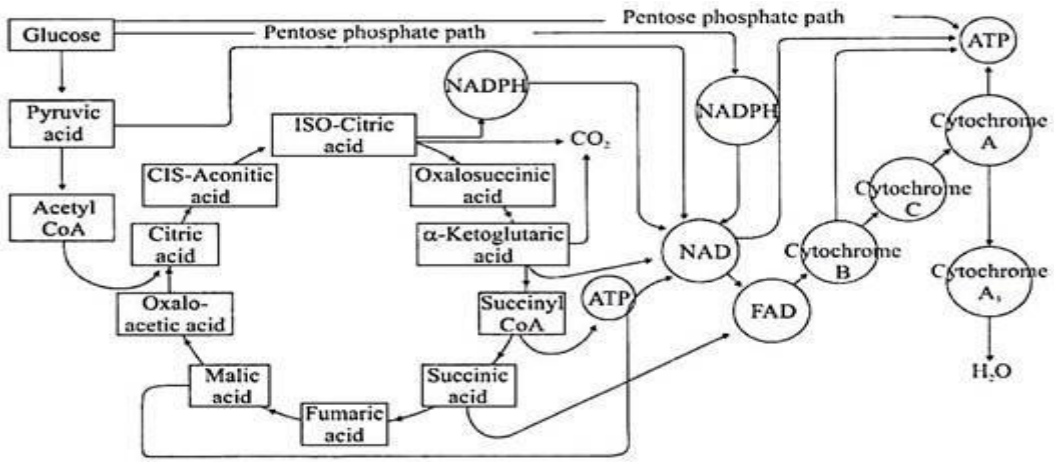


Fig. 10.13 Chain of oxidation of glucose (schematic representation).

Regulation of HMP Shunt:

a. The first reaction of this pathway catalysed by glucose-6-phosphate dehydrogenase is the "rate limiting" step. This is mainly regulated by the cytoplasmic levels of $NADP^+$ and NADPH.

b. The high carbohydrate diets enhance the activities of both dehydrogenases and the rate of the pathway. But reverse occurs in case of starvation and diabetes mellitus.

c. The increased fatty acid synthesis and steroid synthesis re-oxidize NADPH to $NADP^+$ for which cytoplasmic ratio of NADP7 NADPH is increased enabling to enhance the shunt pathway.

d. The hormone insulin stimulates the synthesis of both the dehydrogenases and thus enhances the activity of the pathway.

e. Thyroid hormones also stimulate the activity of glucose-6-phosphate dehydrogenase and thus enhances the shunt pathway.

Probable Questions:

1. What is the significance of the preparatory phase and pay off phase of glycolysis?

2. State the significance of glycolysis.

3. Mention the steps of glycolysis where ATP is produced or consumed and calculate the total ATP production in glycolysis.

Suggested Readings/ References :

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2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

UNIT-X

Glycogenesis, Gluconeogenesis. and Glycogenolysis, Control and regulation of Carbohydrate metabolism

Objective: In this unit we will focus on different metabolic pathway of carbohydrate such as Glycogenesis, Gluconeogenesis. and Glycogenolysis. We will also discuss about control and regulation of carbohydrate metabolism.

Chemistry of Glycogen:

Glycogen is called animal starch; because it is in this form that glucose remains stored in the liver and muscles. Glycogen is branched polysaccharides (amylopectin type) consisting of hundreds of glucose units linked together by glucosidic linkages, i.e., α-1, 4′ linkage and 1, 6′ linkage which are formed by specific enzymes—uridine diphosphate glucose (UDPG)—pyrophosphorylase, glycogen synthetase and amylo-(1, 4′ —1, 6′)- transglucosidase respectively.

Glycogen is soluble in water and makes an opalescent solution and gives red colour with iodine. Glycogen liberates more energy than the corresponding weight of glucose. It does not diffuse into the intracellular fluid, as it exerts no osmotic pressure. It may be easily broken down into glucose by enzymes present in the liver.

Amount and Distribution of Glycogen:

In a normal adult about 700 gm of glycogen is present in the body, about 300 gm in liver and 400 gm in muscles. Liver and muscles are the chief storehouses. All growing tissues can store glycogen. Consequently, they are present in large amounts in the placenta in its early stage, the foetal muscles, and the yeast, etc. In the foetal muscles it may be as much as 40% of the total dried solids. Oyster is very rich in glycogen and is a good source for its manufacture.

Glycogen in any tissue is not a static quantity. It is being constantly used up and re-synthesised. So that at any time the glycogen of the tissue should be considered as a balance between the constant production and loss. Liver glycogen is most mobile. It is the first to be formed and is also the first to be mobilized. Muscle glycogen is much slower to move. There are remarkable differences between the metabolism of liver glycogen and muscle glycogen.

Mobilization of Glycogen:

Glycogen is formed both in the liver and muscles. When blood sugar tends to fall, liver glycogen is converted into glucose and mobilized in the blood stream (Fig. 10.9). Thus blood sugar is maintained. In muscular exercise, starvation, exposure to cold and such other conditions, in which extra energy is demanded, liver glycogen is mobilized. This action is helped by certain hormones such as adrenaline (epinephrine), glucagon, thyroxine, growth or somatotrophic hormone (STH) of anterior pituitary, etc. Stimulation of the sympathetic has same function. It is antagonised by insulin. Insulin helps glycogenesis in liver and prevents glycogenolysis.

The former process is the breakdown of glycogen to glucose whereas the latter is the process of breakdown of glycogen or glucose to pyruvic acid (anaerobic) which is further oxidized to CO_2 and H_2O (aerobic) through TCA cycle. In both the processes, glycogen is converted to glucose-6-phosphate and in the process of glycogenolysis glucose-6-phosphate is splitted into glucose and Pi by phosphatase whereas in the process of glycolysis glucose-6-phosphate is converted further into fructose-6-phosphate by phosphohexose isomerase.

Glycogen is broken down to glucose-1-phosphate, catalysed by the enzyme phosphorylase-a. (active form). Phosphorylase exists in an inactive form, phosphorylase. b. Cyclic AMP (CAMP or 3′-5′-AMP) donates a phosphate group and converts it into an active form, phosphorylase- a. An enzyme, adenyl cyclase, helps in the formation of cyclic AMP from ATP which is accelerated by glucagon and adrenaline (epinephrine). The glucose-l-phosphate is converted into glucose-6-phosphate, catalyzed by the enzyme phosphoglucomutase. The enzyme phosphohexo isomerase converts glucose-6-phosphate to fructose-6-phosphate.

In the process of glycogenesis, glucose is phosphorylated to glucose-6-phosphate by hexokinase (glucokinase) in presence of a phosphate donor, ATP a common to the first reaction in the glycolytic path of glucose metabolism. Glucose-6 phosphate is transformed into glucose-1-phosphate, catalysed by the enzyme phosphoglucomutase. In the next step glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (activated glucose units as UDPG) and inorganic pyrophosphate (PPi). This reaction is catalysed by enzyme UDPG – pyrophosphorylase. An enzyme UDPG – glycogen transglucosylase (glycogen synthetase) helps in the addition of glucose residue present in its activated form (UDPG) to a pre-existing glycogen chain at non-reducing outer end of the molecule (glycogen) so that glycogen tree is elongated successively due to the 1, 4′ linkage formation. Thus uridine diphosphate (UDP) is liberated and re-synthesised with the help of ATP – UDP + ATP ↔ ADP + UTP. A second enzyme,

called branching enzyme [amylo-(1, 4′-1,6′) – transglucosidase] transfers a part of the 1, 4′-chain to adjacent chain to form α-1, 6′ linkage and helps in glycogen synthesis by forming a branch point (1, 6′ linkage) in the molecule.

Pathway of Formation of Pyruvic Acid:

Fructose-6-phosphate accepts another phosphate group from ATP and is transformed into fructose-1-6-diphosphate. This reaction is influenced by the enzyme 6-phosphofructokinase. Another enzyme aldolase breaks down the above hexose diphosphate into dihydroxyacetone phosphate and glyceral dehyde-3-phosphate each containing 3 carbon atoms (triosephosphate).

Enzyme triosephosphate isomerase keeps the above two triosephosphates in equilibrium. Glyceraldehyde-3-phosphate is then dehydrogenated by triosephosphate dehydrogenase into 1-3-diphosphoglycerate, the hydrogen being accepted by NAD. Phosphorylation also takes place at this stage and requires inorganic phosphate (Pi). Diphosphoglycerate then donates one high energy phosphate to ADP to convert it into ATP and it itself is transformed into 3-phosphoglycerate. This reaction is catalysed by the enzyme, ATP phosphoglyceric transphosphorylase (phosphoglyceric acid kinase). Phosphoglyceromutase then transforms 3-phosphoglycerate to 2-phosphoglycerate and enolase converts it into 2-phosphoenolpyruvate. Phosphoenolpyruvate is spontaneously converted to pyruvate which is oxidised further to CO_2 and H_2O through TCA cycle if aerobic condition exists or otherwise reduced to lactic acid. ATP-phosphopyruvic transphosphorylase (pyruvic acid kinase) then transfers one energy-rich (~) phosphate bond from phosphoenolpyruvate to ADP to form ATP and pyruvic acid. Other monosaccharides (galactose, fructose, and mannose) gain their entrance in the glycolytic pathway as indicated in the Figure 10.10.

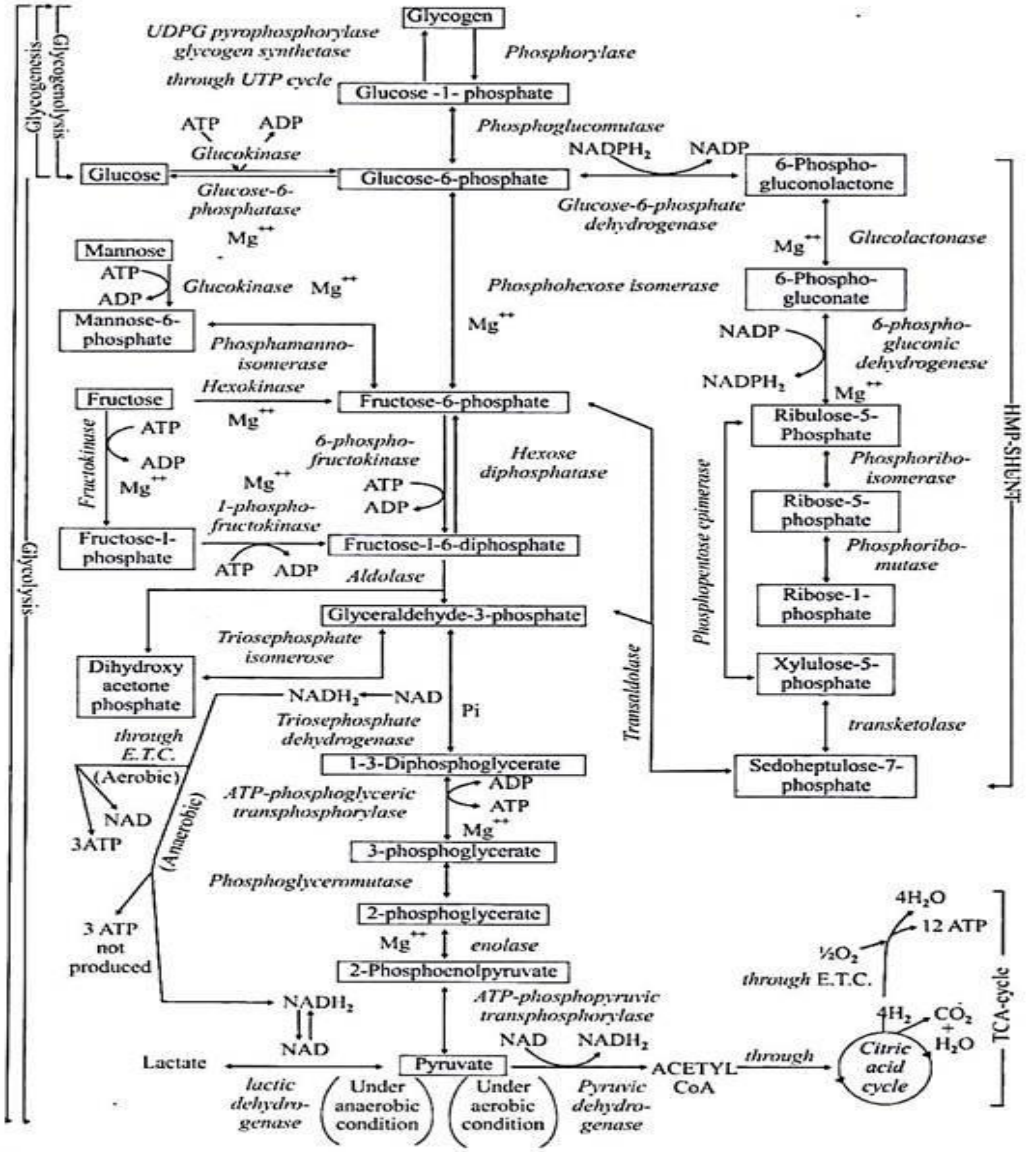


Fig. 10.10 Schematic representation of paths of glycogenolysis, glycogenesis and glycolysis, oxidation of glucose through hexose monophosphate (HMP) shunt (PPP = pentose phosphate pathway) and oxidation of acetyl CoA through TCA cycle.

Citric Acid Cycle or Krebs Cycle:

The citric acid cycle is one of the most important biochemical mechanisms of oxidation of the activated metabolites and it is perhaps the major terminal pathway of biological oxidation in all animal tissues. The activated metabolites, which are few in number derived from carbohydrate, protein, and fat, are oxidised by the electron-transport chain and most of the utilisable energy (ATP) are produced for the organism.

The activated metabolites, which are derived from different foodstuffs (Fig. 10.11), are given below:

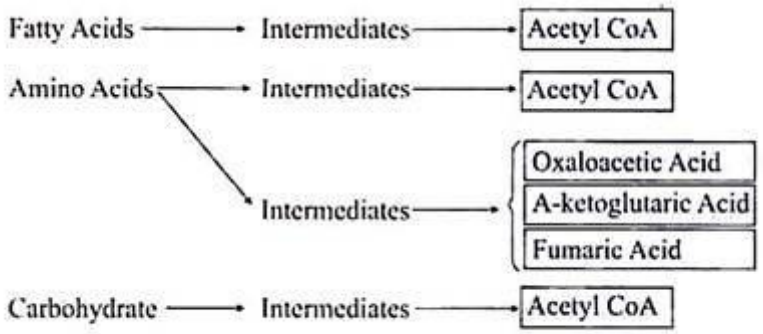


Fig. 10.11 Substances in boxes are activated compounds oxidised in TCA cycle.

The components, included in this cycle, are interrelated by oxidation and reduction, and other reactions which produce $2CO_2$, H_2O and energy ATP. In case of carbohydrate, the pyruvic acid which is formed by glycolytic path of oxidation enters this cycle by first being transformed into acetyl CoA.

This cycle is known as Krebs (citric acid) cycle after the English biochemist H.A. Krebs who first formulated and proposed the mechanism. Citric acid being one of the member of the cycle and some of the members contains these carboxylised groups so this cycle is also known as citric acid cycle and tricarboxylic acid (TCA) cycle.

Acetyl CoA or Active Acetate Formation:

In presence of six factors, i.e., Mg^{++}, NAD, thiamine pyrophosphate, lipoic acid, FAD and coenzyme A, the pyruvic oxidase along with enzyme complex converts pyruvate to active acetate as a result of oxidative decarboxylation and as a result the $NADH_2$ is formed which is reconverted to NAD by electron-transport chain. It enters into the TCA cycle.

A condensing enzyme, citrate synthetase, helps in the condensation of acetyl CoA with oxalo-acetate to form citrate. Citrate first by a process of dehydration is converted into cis-aconitate which again by a process of rehydration is transformed into iso-citrate. The enzyme aconitase catalyses the reaction at both the two steps, so-citrate in presence of the enzyme iso-citrate dehydrogenase is then dehydrogenated to oxalosuccinate. NAD or NADP acts as a hydrogen-acceptor and is converted into $NADH_2$ or $NADPH_2$.

An enzyme oxalosuccinate decarboxylase in presence of Mn^{++} removes CO_2 from oxalosuccinate which is thus converted into α-ketoglutarate. A process of oxidative decarboxylation, similar to that in the conversion of pyruvate to acetyl CoA, transforms a-ketoglutarate to succinyl CoA catalysed by α-ketoglutaric oxidase which also requires coenzyme A, lipoic acid and NAD acting as hydrogen-acceptor.

Succinyl CoA, while it being converted into succinate, provides energy for synthesis of GTP (guanosine-5′-triphosphate) from GDP (guanosine-5′-diphosphate). So GTP in turn supplies energy for synthesis of ATP from ADP while it is reconverted to GDP. Thus succinyl CoA supplies ultimately energy for the synthesis of ATP.

[The enzyme thiophorase present in tissues, other than liver, can help in the conversion of succinyl CoA → succinate.] Enzyme succinate dehydrogenase converts succinate to fumarate, the hydrogen is transferred directly to flavoprotein (FAD) converting it into $FADH_2$. Fumarase helps in the addition of water to fumarate, malate is formed in this process. Oxaloacetate is regenerated from malate under the influence of malate dehydrogenase, NAD again is the hydrogen-acceptor (Figure 10.12).

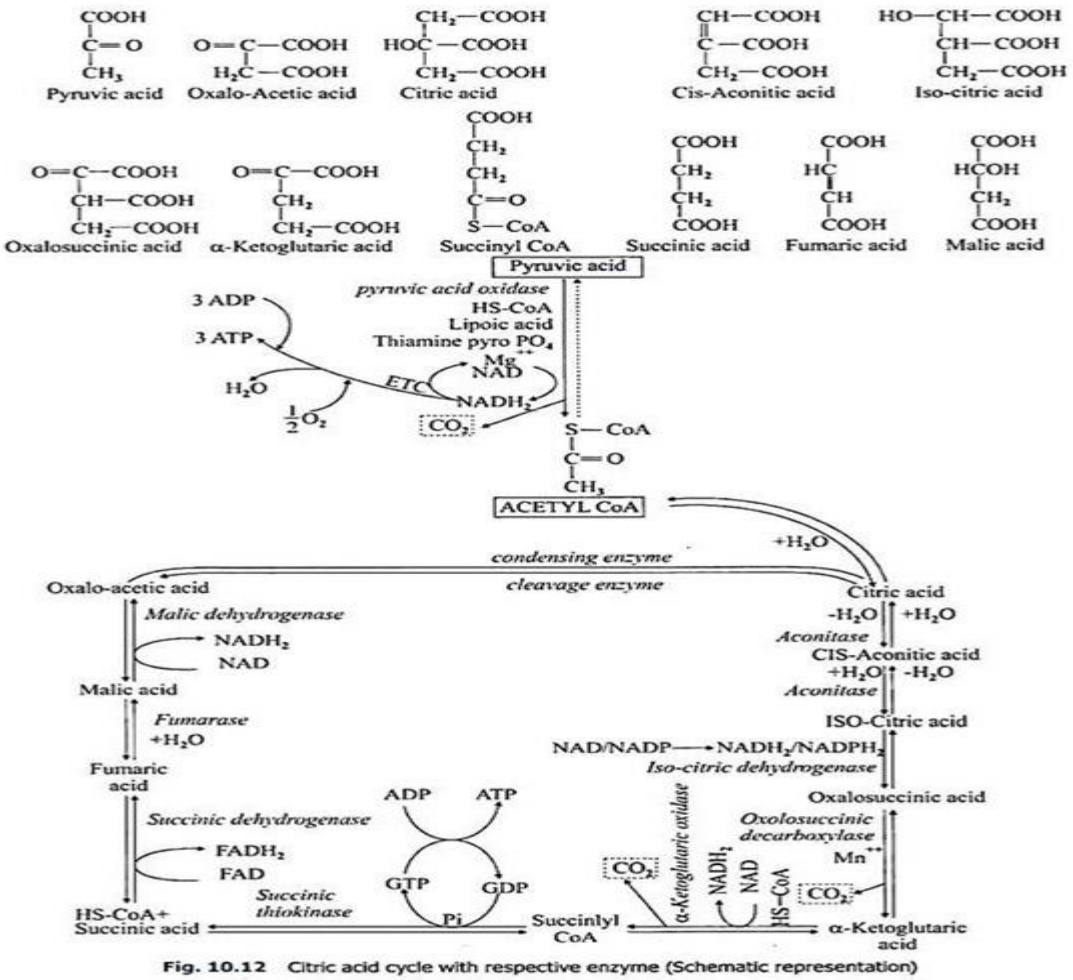


Fig. 10.12 Citric acid cycle with respective enzyme (Schematic representation)

Metabolism of Glycogen:

I. Metabolism of Glycogen in Liver:

Sources of Liver Glycogen:

Glycogenesis (formation of glycogen) in the liver can take place from the following:

i. From Carbohydrates and the Related Substances:

For instance, glucose, galactose, fructose, mannose, lactic acid (from muscles or otherwise), pyruvic acid, methyl glyoxal, etc. Lactic acid of muscles is carried through blood stream to the liver where it is converted into glycogen very readily. It is believed that pentose does not form glycogen.

ii. From Proteins:

The antiketogenic amino acids (e.g., glycine, alanine, aspartic acid and glutamic acid, etc.) can readily form glucose through TCA cycle or reversible glycolytic path or both, as the case may be, as seen in diabetes mellitus. In diabetes mellitus the G: N ratio increased indicating the glucose is formed from protein (neoglucogenesis). It is reasonable to believe that this glucose may be available for glycogen formation.

iii. From Fats:

The glycerol part of fats is converted into glucose from which glycogen may be derived.

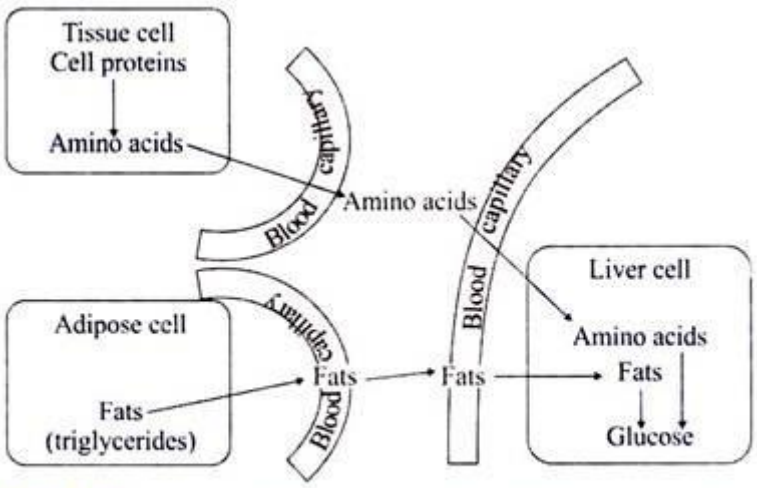


Fig. 10.14 Neoglucogenesis in liver from mobilized tissue proteins and fats (simplified) (Schematic representation).

Functions of Liver Glycogen:

i. Liver glycogen is a ready source of glucose supply in the blood.

ii. It helps in the de-toxicating mechanism in the liver.

iii. It protects the liver from the toxic effects of arsenic, carbon tetrachloride, etc.

iv. If the liver glycogen level is high, ketone body formation and rate of deamination of amino acids are depressed.

II. Metabolism of Glycogen in Muscles:

Muscle contains about 0.5%-1.0% of glycogen as opposed to 5% in the liver. But due to greater amount of muscles in the body, the total quantity is higher and is about 400 gm or approximately equal depending on the total muscle mass of the body.

1. Glycogenesis in Muscles:

Source:

Muscle glycogen can be derived from the following sources:

i. From Glucose:

Which is obviously taken from the blood stream?

ii. From Lactic Acid:

Which is produced in the muscle during muscular contraction? The major part (4/5ths) of the lactic acid produced during exercise is reconverted into glycogen. A small part of it (1/5th) is oxidised into carbon dioxide and water through TCA cycle.

The conversion of lactic acid into glycogen in the muscle is comparatively much slower than in the liver. So that during heavy muscular exercise, a large amount of lactic acid is produced in the muscles. A good part of it diffuses into the blood stream and is brought to liver where it is readily reconverted into glycogen. Probably glycogen is not produced in the muscles from proteins and carbohydrates.

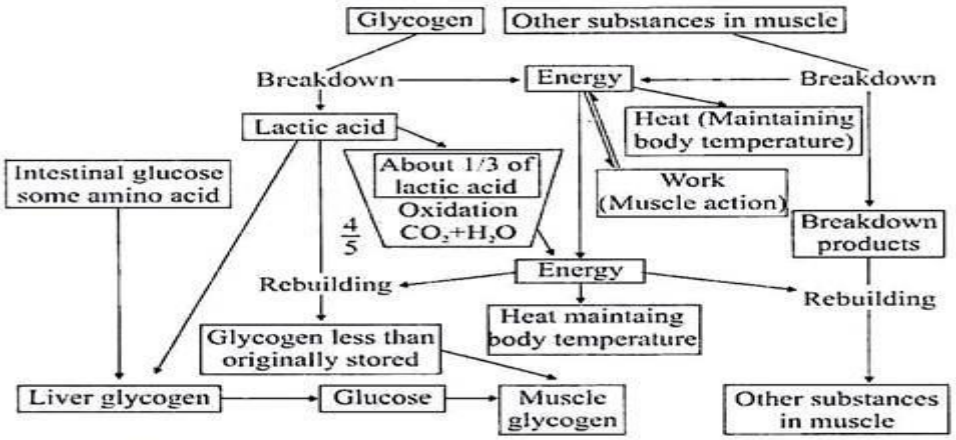


Fig. 10.15 Metabolism during muscle activity (Schematic representation).

2. Glycolysis in Muscles:

Glycolysis is the process of breakdown of glycogen or glucose in muscles and other tissues into pyruvic and lactic acids (Embdert-Meyerhof glycolytic pathway). Glycogen leaves the liver in the form of glucose, but it leaves the muscles in the form of pyruvic and lactic acids. The difference is probably due to the fact that, the enzyme systems and the chemical reaction in the liver and muscles are not the same.

Lactic acid that emerges from the muscles is carried to liver through blood stream where it is reconverted into glycogen. This glycogen is again mobilised in the form of glucose which enters into the blood stream. Muscles take up this glucose from the blood stream and recover its lost glycogen. This cyclic process of circulation of carbohydrate in different forms in different tissues is known as Cori cycle through which muscle and liver glycogens become readily interchangeable (Fig. 10.16).

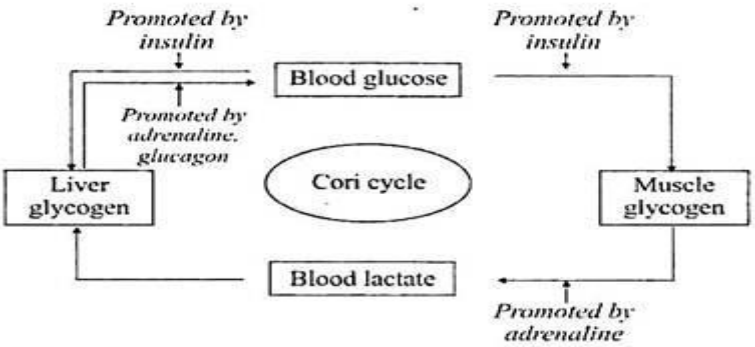


Fig. 10.16 Schematic representation showing circulation of carbohydrate in different forms in different tissues (Cori cycle) and effects of adrenaline, insulin and glucagon upon it.

Glycogen, in other tissues excepting liver, exhibits the same pattern of breakdown as in the muscles.

Table 10.1 Difference in glycogen metabolism in Liver and muscle

Liver	Muscle
1. *Glycogenesis*–from carbohydrates, muscle lactic acid and other related compounds, as well as from proteins and fats.	1. *Glycogenesis*-from blood glucose and muscle lactic acid. Not from proteins and fats.
2. Liver glycogen is most mobile, i.e., first formed and first mobilised.	2. Muscle glycogen is less mobile. The process is comparatively slower.
3. Glycogen breakdown is mostly up to glucose and the glucose then enters the blood stream (*glycogenolysis*).	3. Glycogen breakdown is up to pyruvic and lactic acids (*glycolysis*)-1/5th of lactate is broken down into CO_2 and H_2O, and 4/5ths reconverted into glycogen in liver
4. Liver glycogen is utilised for maintaining blood sugar. From this glucose, muscle glycogen is replenished.	4. Muscle glycogen is not available for maintaining blood sugar. It comes out as lactic acid from which liver glycogen is formed.

Glycogenesis:

a. Glucose is first phosphorylated to glucose- 6-phosphate by the enzyme hexokinase and glucokinase in presence of the coenzyme ATP and the activator Mg^{++}. ATP is converted to ADP. Hexokinase has high affinity for glucose. Glucokinase is to remove glucose from the blood following a meal.

b. Glucose-6-phosphate is then converted to glucose-1-phosphate. The reaction is catalyzed by the enzyme phosphoglucomutase with Mg^{++} and the reaction is reversible. Glucose-1, 6-diphosphate is formed as an intermediate due to the phosphorylation of the enzyme.

c. Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide (uridine diphosphate glucose (UDPG). The reaction is catalyzed by the enzyme UDPG pyrophosphotylase with the release of inorganic pyrophosphate.

d. The C_1 of the activated glucose of UDPG forms a glycosidic bond with the C_4 of the terminal glucose residue of glycogen liberating UDP by the enzyme glycogen synthetase (glucosyl transferase). The glycogen primer is formed on a protein primer known as glycogenin which is a protein.

e. By the successive 1, 4 linkages by glucose units to the preexisting glycogen chain, the branches of the glycogen 'tree' become elongated.

When the chain has been lengthened to between 6 and 11 glucose residues, a second enzyme, the branching enzyme (amylo 1, 4 → 1, 6 trans-glucosidase), acts on the glycogen. This enzyme transfers a part of the -1, 4-chain (minimum length of 6 glucose residues) to a neighbouring chain to form α-1, 6-linkage which establishes a branch point in the molecule.

Storage of Carbohydrate in postabsorptive normal adult (70 kg.)		
Liver glycogen	4.0 % =	72 grams
Muscle glycogen	40.7% =	245 gms.
Extra cellular glucose	0.1 % =	10 gms.
Total		327 gms
Liver weight	1800 gms.	
Muscle mass	35 kg.	
Total Volume	10 L	

Regulation of Glycogenesis:

Glycogen synthase (glycogen synthetase) is the key enzyme which regulates the process of glycogenesis. This enzyme exists in "active" as well as "inactive" forms which are inter-convertible. Glycogen also exerts an inhibition on its own formation and insulin also stimulates glycogen synthesis in muscle by promoting dephosphorylation and activation of inactive glycogen synthase. The inhibition of glycogenesis enhances net glycogenolysis. Hexokinase stimulates the oxidation of glucose in the muscle whereas glucokinase stimulates the oxidation of glucose in the liver.

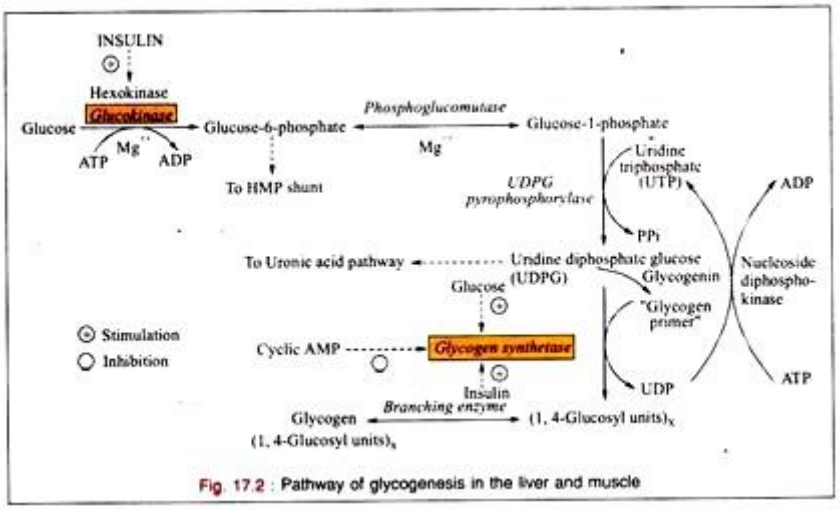


Fig. 17.2 : Pathway of glycogenesis in the liver and muscle

Neoglucogenesis:

a. The formation of glucose from non-carbohydrate substances such as lactic acid, amino acids and glycerol is called gluconeogenesis or neoglucogenesis.

b. When the carbohydrate is insufficient in the diet, gluconeogenesis meets the needs of the body for glucose.

c. A continued supply of glucose is necessary as a source of energy and glucose is the only fuel which supplies energy to skeletal muscle under anaerobic conditions.

d. There is always a certain basal requirement for glucose even when fat is supplied to the caloric requirement of the organism.

e. In mammals, the liver and kidney are the principal organs responsible for gluconeogenesis. Gluconeogenesis is essentially a reversal of glycolysis. Therefore, the glycolytic activity of liver and kidney is low when there is active gluconeogenesis.

Metabolic Pathways in Gluconeogenesis:

a. The metabolic pathways in connection with gluconeogenesis are the modifications or the Embden-Meyerhof pathways and the citric acid cycle.

b. They are concerned with the conversion of glucogenic amino acids, lactate, glycerol, propionate (in ruminants) to glucoses or glycogen.

c. The energy barriers obstruct a simple reversal of glycolysis:
(i) Between pyruvate and phosphoenolpyruvate,

(ii) Between fructose-1,6-bisphosphate and fructose 6- phosphate,

(iii) Between glucoses-phosphate and glucose,

(iv) Between glucose- 1-phosphate and glycogen.

These barriers are overcome by the following reactions:

(i) The enzyme Pyruvate carboxylase present in mitochondria converts pyruvate to oxaloacetate in presence of ATP, biotin and CO_2. A second enzyme phosphoenolpyruvate carboxykinase present in the extra-mitochondrial part of the cell converts oxaloacetate to phosphoenolpyruvate in presence of GTP. Lactate with the help of these two enzymes and lactate dehydrogenase is converted to phosphoenolpyruvate.

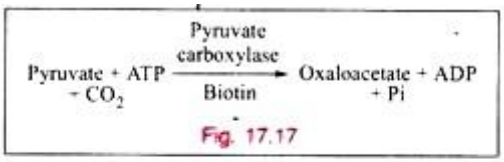


Fig. 17.17

In the extramitochondrial part of the cell :

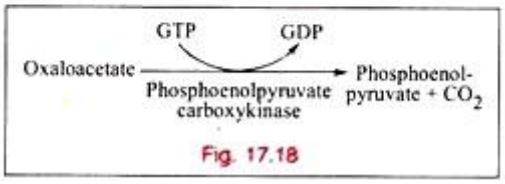


Fig. 17.18

But, oxaloacetate does not diffuse readily from mitochondria. Alternative means are applied to convert oxaloacetate to malate which is readily diffused from mitochondria. Malate is then converted to oxaloacetate in the extra-mitochondrial portion of the cell.

(ii) The conversion of fructose-1, 6- bisphosphate to fructose-6-phosphate catalyzed by another enzyme fructose-1. 6-bisphosphatase. This enzyme is present in liver, kidney and striated muscle but absent from adipose tissue, heart muscle and smooth muscle.

(iii) Glucose-6-phosphate is converted to glucose by glucose-6-phosphatase which is present in intestine, liver and kidney but absent from muscle and adipose tissue.

(iv) The conversion of glucose-1-phosphate to glycogen is through UDPG and glycogen synthetase.

Glucogenic amino acids after transamination or deamination form either pyruvic acid or members of the citric acid cycle. Therefore, the glucogenic amino acids and lactate can be converted to glucose or glycogen. Propionate in ruminants enters the main glucogenic pathway via the citric acid cycle being converted to succinyl-CoA.

Conversion of Propionate to Succinyl-CoA:

a. Propionate is first activated by thiokinase with ATP and CoA to form propionyl-CoA.

b. Propionyl-CoA undergoes CO_2 fixation reaction to form D-methyl-malonyl-CoA, catalyzed by propionyl-CoA carboxylase and biotin is required as a coenzyme.

c. D-methyl-malonyl-CoA is converted to L-methyl-malonyl-CoA by methyl-malonyl- CoA racemase.

d. L-methyl-malonyl-CoA is isomerized to succinyl-CoA by methyl-malonyl-CoA isomerase which requires vitamin B_{12} as a coenzyme.

Conversion of Glycerol:

a. Glycerol is first converted to glycerol-3- phosphate by glycerokinase with ATP in liver and kidney.

b. Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by glycerol- 3-phosphate dehydrogenase in presence of NAD^+. Dihydroxyacetone phosphate is then converted to glucose.

Conversion of Lactate to Glucose:

a. Lactic acid is the major end product in muscle in anaerobic glycolysis. Muscle tissue is incapable of resynthesizing glucose from lactate. The conversion takes place entirely in the liver.

b. Muscle lactate is transported to the liver by the blood. In the liver, it is converted to glucose and glycogen by the enzymes concerned in gluconeogenesis.

c. Liver glycogen is converted to glucose which is carried back to muscle by blood.

This conversion of muscle lactate to glucose in liver and its re-entry into muscle is called “Cori Cycle”.

Conversion of Amino Acids to Glucose:

a. The glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination which is given in Fig. 17.19.

b. These intermediates are converted to malate and finally converted to glucose by the enzymes involved in gluconeogenesis.

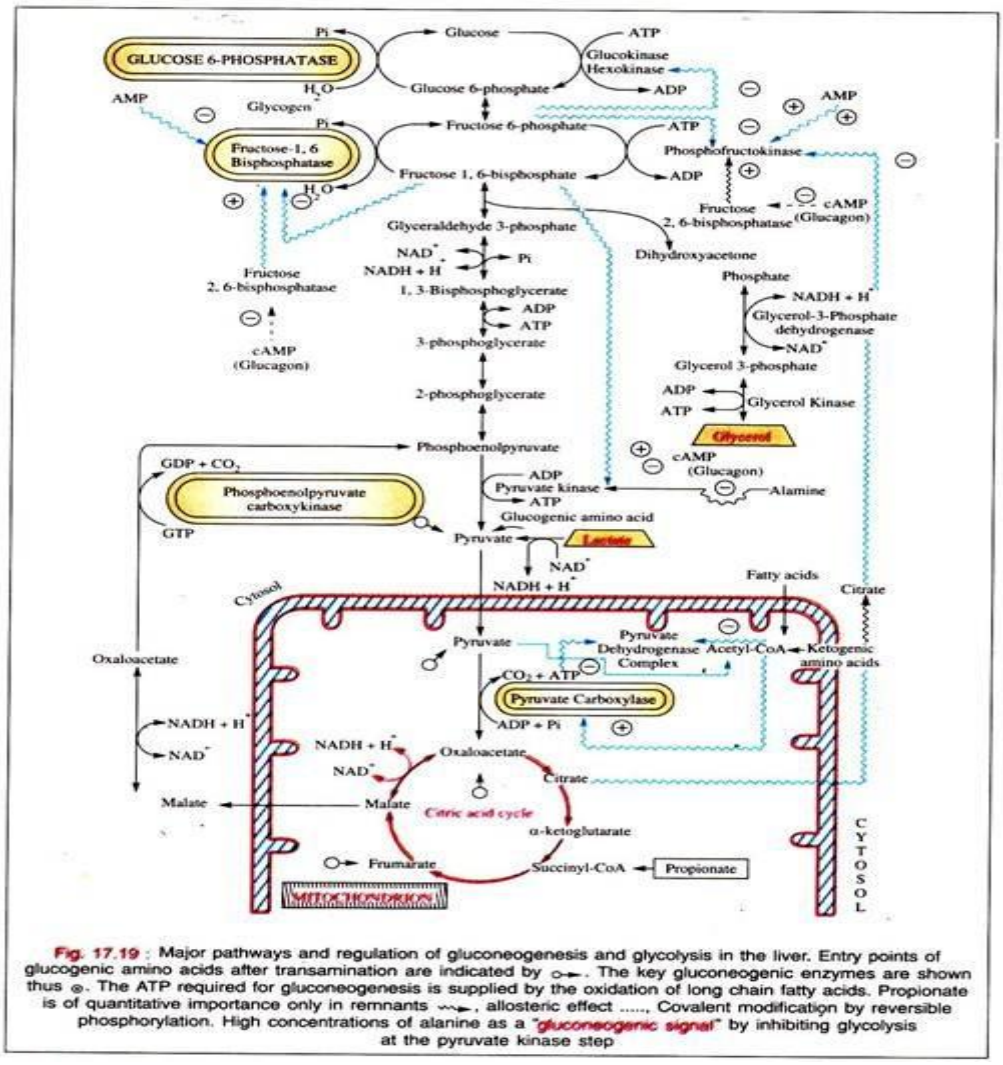


Fig. 17.19 : Major pathways and regulation of gluconeogenesis and glycolysis in the liver. Entry points of glucogenic amino acids after transamination are indicated by ○→. The key gluconeogenic enzymes are shown thus ⊛. The ATP required for gluconeogenesis is supplied by the oxidation of long chain fatty acids. Propionate is of quantitative importance only in remnants ⇝, allosteric effect ....., Covalent modification by reversible phosphorylation. High concentrations of alanine as a "*gluconeogenic signal*" by inhibiting glycolysis at the pyruvate kinase step

Conversion of Fatty Acids to Glucose:

Fatty acids are metabolized to acetyl-CoA by β-oxidation. Acetyl-CoA enters the citric acid cycle and then converted to malate. Malate is diffused from the mitochondria to the extra-mitochondrial portion of the cell where it is finally converted to glucose by the enzymes involved in gluconeogenesis.

Acetyl-CoA is not permeable to pass from the mitochondria to the cytosol through the mitochondrial membrane. But citrate is permeable through mitochondrial membrane to pass to the cytosol where it is splitted to acetyl-CoA and oxaloacetate.

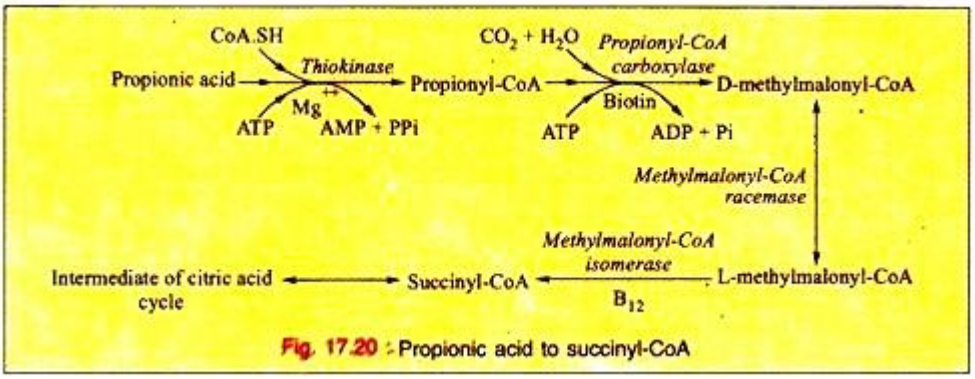


Fig. 17.20 : Propionic acid to succinyl-CoA

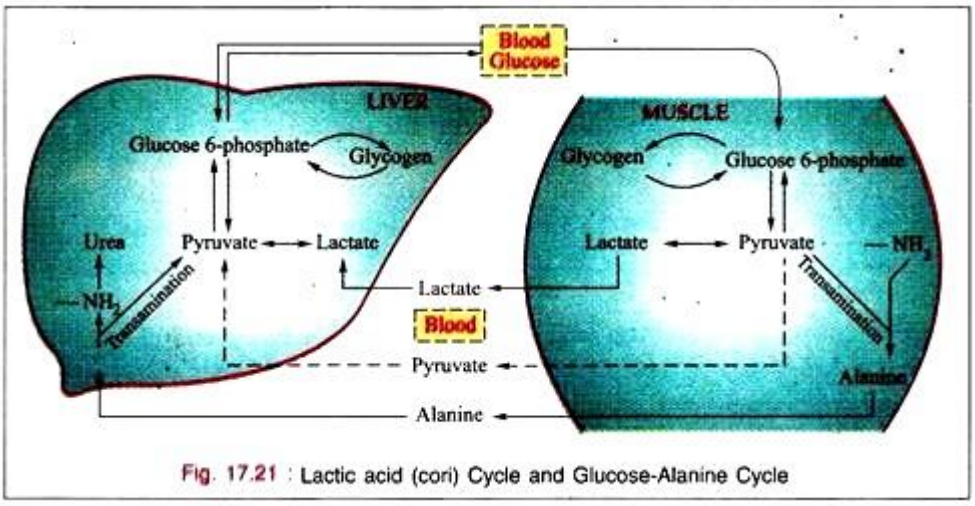


Fig. 17.21 : Lactic acid (cori) Cycle and Glucose-Alanine Cycle

Role of fructose 2, 6-Bisphosphate in the Regulation of Glycolysis and Gluconeogenesis in Liver:

a. Fructose 2, 6-bisphosphate is the most potent positive allosteric effector of phosphofructokinase-I and inhibitor of fructose-l, 6-bi-phosphatase in liver.

b. It relieves inhibition of phosphofructokinase-I by ATP and increases affinity for fructose 6-phosphate.

c. It inhibits fructose-1, 6-bisphosphatase by increasing the K_m for fructose 1, 6- bi-phosphate.

d. It is formed by phosphorylation of fructose 6-phosphate by phosphofructokinase-2 which is also responsible for its breakdown since it contains fructose-2, 6- bisphosphatase activity. This bi-functional enzyme is under the allosteric control of fructose 6-phosphate.

e. When glucose is short, glucagon stimulates the production of cAMP which inactivates phosphofructokinase-2 and activates fructose-2, 6-bisphosphatase by phosphorylation.

f. In the superfluity of glucose, the concentration of fructose 2, 6-bisphosphate increases stimulating glycolysis by activating phosphofructokinase-I and inhibiting fructose-1, 6-bisphosphatase.

g. In glucose shortage, gluconeogenesis is stimulated by a decrease in the concentration of fructose 2, 6-bisphosphate which deactivates phosphofructokinase-I and de-inhibits fructose-1, 6-bisphosphatase. This mechanism also shows that glucagon stimulation of glycogenolysis in liver results in glucose release rather than glycolysis.

h. Recently, it has been indicated that glucose 1, 6-bisphosphate plays a similar role in some extra-hepatic tissues.

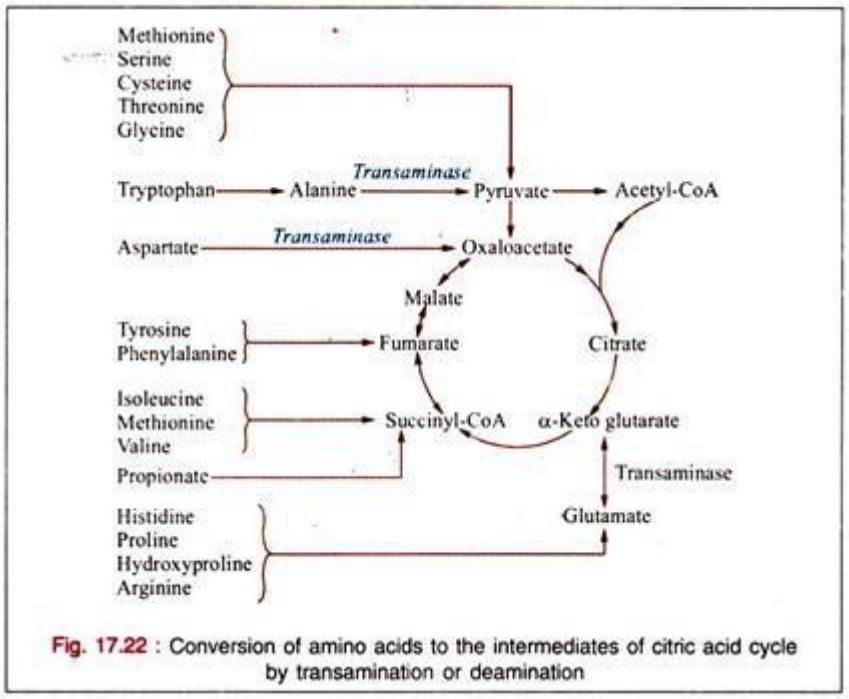


Fig. 17.22 : Conversion of amino acids to the intermediates of citric acid cycle by transamination or deamination

Substrate (Futile) Cycle:

(a) The control points in glycolysis and glycogen metabolism involve a cycle of phosphorylation and dephosphorylation catalyzed by the enzymes like glucokinase and glucose-6-phosphatase; phosphofructokinase-1 and fructose-1, 6- bisphosphatase; pyruvate kinase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase; glycogen synthase and phosphorylase.

If these would be allowed to cycle unchecked, ultimately there would be hydrolysis of ATP. But this cannot happen due to the different control mechanisms which can signify that one limb of the cycle is inhibited as the other is stimulated as per the requirements of the tissue and of the body,

(b) In the phosphofructokinase and fructose- 1, 6-bisphosphatase, the allosteric modifier, fructose 2, 6-bisphosphate, causes a large change in the net effect of metabolites in either direction of the cycle. This fine effect of metabolic control occurs only at the expense of some loss of ATP.

Regulation of Gluconeogenesis:

a. The key enzymes of gluconeogenesis are pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1, 6-bisphosphatase and glucose-6-phosphatase.

b. The hormones glucagon and glucocorticoids which are secreted during starvation stimulate glucose-6-phosphatase to enhance gluconeogenesis.

c. High carbohydrate diets increase the insulin/glucagon ratio and thus reduce the activities of the key enzymes of gluconeogenesis to minimize gluconeogenesis.

d. ATP and citrate are the activators of fructose-1, 6-bisphosphatase and, hence, gluconeogenesis is increased. But the high level of AMP in liver cells inhibits fructose-1, 6-bisphosphatase and thus reduces gluconeogenesis.

e. In glucose shortage, the more secreted glucagon stimulates gluconeogenesis by decreasing the concentration of fructose-2, 6-bisphosphate which in turn inhibits phosphofructokinase-1 and activates the enzyme fructose-1, 6-bisphosphatase.

f. During starvation the excessive liberated glucagon stimulates the enzyme phosphoenolpyruvate carboxykinase and thus increases gluconeogenesis.

g. The enzyme pyruvate carboxylase is allosterically activated by acelyt-CoA. During starvation increased fatty acid oxidation provides more acetyl-CoA which promotes gluconeogenesis.

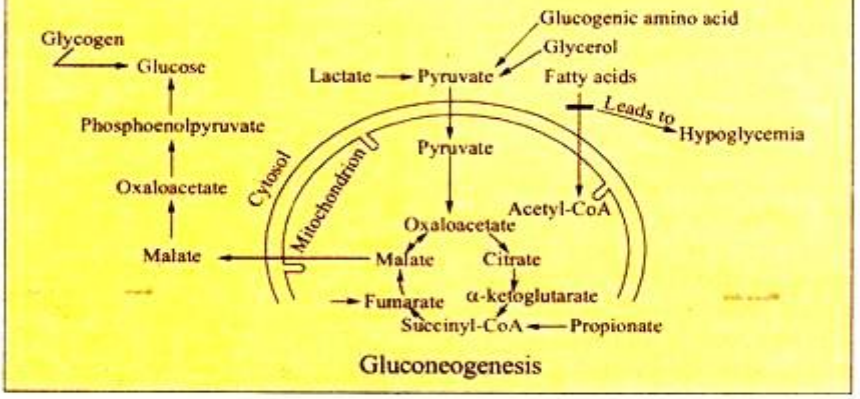
h. Increased ADP allosterically inhibits pyruvate carboxylase and thus reduces gluconeogenesis.

i. The hormones glucagon, epinephrine, and glucocorticoids stimulate the synthesis of pyruvate carboxylase and thus enhance gluconeogenesis. But the hormone insulin depresses the enzyme pyruvate carboxylase and thus reduces gluconeogenesis.

CLINICAL ORIENTATION

- The need for glucose by the body is met by gluconeogenesis when carbohydrate supply is insufficient from the diet. The supply of glucose as a source of energy should be continuous for the following purposes :
  - (a) As a source of energy for the nervous system and the erythrocytes. Failure of gluconeogenesis becomes fatal. Brain dysfunction leads to coma and death when blood glucose concentration reaches below a critical level.
  - (b) As a source of glyceride-glycerol in adipose tissue.
  - (c) Required for maintaining the level of intermediates of the citric acid cycle in many tissues.
  - (d) Acts as a precursor of milk sugar (lactose) in the mammary gland and is taken up actively by fetus.

SCHEMATIC COMPENDIUM



Significance of Gluconeogenesis:

1. Glucose is universal building molecule and provides energy to all cells. Mainly brain cell and nervous tissue as well as erythrocytes, testes, renal medulla require glucose as sole source of energy.

2. Glycogen stored in adipose tissue and in skeletal muscle are converted to glucose by glycogenolysis. However the stored glycogen may not be sufficient during heavy exercise, diabetic conditions, etc. so

during shortage glucose is synthesized from carbohydrate or non-carbohydrate precursor by the process called gluconeogenesis.

3. Gluconeogenesis meets the needs of the body for glucose when sufficient carbohydrate is not available from the diet or glycogen reserves.

4. Glycogen stored in adipose tissue and in skeletal muscle is converted to glucose by glycogenolysis. However the stored glycogen may not be sufficient during heavy exercise, diabetic conditions, or during fasting etc. so during shortage, glucose is synthesized by gluconeogenesis process.

5. A continual supply of glucose is necessary as a source of energy especially for the nervous system and erythrocytes.

6. Gluconeogenesis mechanism is used to clear the products of the metabolism of other tissues from the blood, eg: Lactate, produced by muscle and erythrocytes and glycerol, which is continuously produced by adipose tissue.

Glycogenolysis:

Glycogenolysis, process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting. Glycogenolysis occurs primarily in the liver and is stimulated by the hormones glucagon and epinephrine (adrenaline). When blood glucose levels fall, as during fasting, there is an increase in glucagon secretion from the pancreas. That increase is accompanied by a concomitant decrease in insulin secretion, because the actions of insulin, which are aimed at increasing the storage of glucose in the form of glycogen in cells, oppose the actions of glucagon. Following secretion, glucagon travels to the liver, where it stimulates glycogenolysis.

The vast majority of glucose that is released from glycogen comes from glucose-1-phosphate, which is formed when the enzyme glycogen phosphorylase catalyzes the breakdown of the glycogen polymer. In the liver, kidneys, and intestines, glucose-1-phosphate is converted (reversibly) to glucose-6-phosphate by the enzyme phosphoglucomutase. Those tissues also house the enzyme glucose-6- phosphatase, which converts glucose-6-phosphate into free glucose that is secreted into the blood, thereby restoring blood glucose levels to normal. Glucose-6-phosphate is also taken up by muscle cells, where it enters glycolysis (the set of reactions that breaks down glucose to capture and store energy in the form of adenosine triphosphate, or ATP). Small amounts of free glucose also are produced during

glycogenolysis through the activity of glycogen debranching enzyme, which completes the breakdown of glycogen by accessing branching points in the polymer. Epinephrine, similar to glucagon, stimulates glycogenolysis in the liver, resulting in the raising of the level of blood glucose. However, that process is generally initiated by the fight-or-flight response, as opposed to the physiological drop in blood glucose levels that stimulates glucagon secretion.

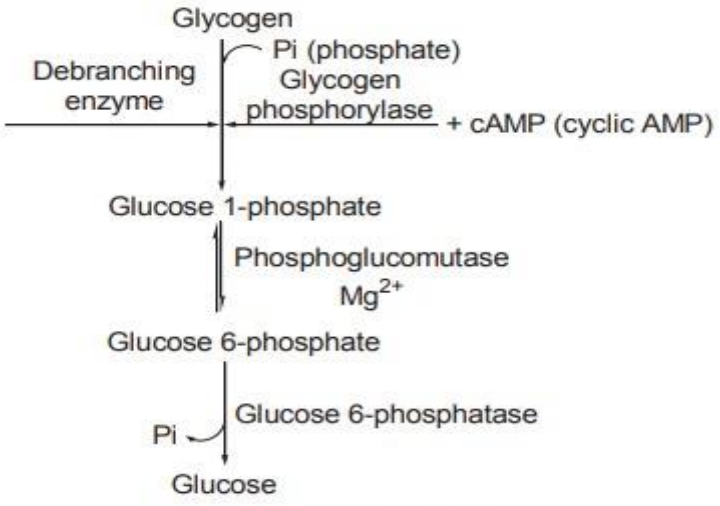


Fig.3.4 Glycogenolysis

Various rare inherited diseases of glycogen storage produce abnormalities in glycogenolysis. For example, glycogen storage disease type V (McArdle disease) results in a lack of glycogen phosphorylase, which impairs glycogen breakdown and prevents muscles from meeting the energy demands of exercise. Glycogen storage disease type III (Cori, or Forbes, disease) is caused by mutations in a gene involved in the production of glycogen debranching enzyme. The disease results in cellular accumulation of abnormal, incompletely broken down glycogen molecules, leading to tissue damage, particularly in the liver and muscles.

Regulation of Glycogenolysis

Covalent modification by hormones: Norepinephrine and epinephrine (in liver and muscle) or glucagon in the liver only activate glycogen phosphorylase, while insulin inhibits it.

Allosteric regulation

G-6-P and ATP inhibit glycogen phosphorylase because their elevated levels indicate that the cell isn't in need of more energy and there is no need to breakdown glycogen.

AMP stimulates glycogen phosphorylase in muscles.

Calcium indirectly activates phosphorylase in muscle and liver.

Probable Questions :

1. State how glucose is produced from glycogen (or Schematically represent the steps of glycogenolysis)

2. What is gluconeogenesis? Schematically represent how glucose is produced from glycerol.

3. State the interrelationship between different carbohydrate metabolism pathways and mention their significance in brief.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

UNIT-XI

Amino acid metabolism: Amino acid classification, Urea cycle

Objective: Objective of this unit is to learn about classification of Amino acids, Urea cycle.

Concept of Amino Acids:

Amino acids are the building blocks of proteins. Among the thousands of amino acids available in nature, proteins contain only 20 different kinds of amino acids, all of them are L-alpha-amino acids. The same 20 standard amino acids make proteins in all the living cells, may it either be a virus, bacteria, yeast, plant or human cell. These 20 amino acids combine in different sequences and numbers to form various kinds of proteins. The number of proteins that can be had from these 20 amino acids can be calculated from 20 factorial, i.e., 20 x 19 x 18 x 17 x 16 x …… x 2 x 1 = 2.4 x 10^{18}. In human beings alone there are more than 100 000 different types of proteins.

The general formulae for an amino acid can be written as 'R-CH-NH_2—COOH'. Depending upon the 'R' group present in the amino acid it is named accordingly. The 20 amino acids found in the proteins are known as primary or standard amino acids. In addition to these, some other amino acids are also found in proteins like 4-hydroxyproline, 5-hydroxylysine, 6-IV-methyllysine, gamma carboxyglutamic acid and desmosine, all of these are derivatives of standard amino acids.

Classification of Amino Acids:

I. Depending upon the Charge:

Amino acids can be broadly classified into three major groups:

(1) Neutral

(2) Acidic and

(3) Basic.

1. Neutral amino acids:

Those amino acids that do not contain any charge on the 'R' group.

They are further classified into the following categories:

(a) Aliphatic:

Those amino acids whose 'R' group contains a chain of carbon atoms—Gly, Ala, Ser, Thr, Val, Leu, lie, Asn, Gin.

(b) Aromatic:

Those amino acids whose 'R' group has a benzene ring—Phe, Tyr, Trp.

(c) Heterocyclic:

The "R" group has a heterocylic ring, i.e., any of the ring structures which contain different atoms—Pro, His.

(d) Sulphur containing:

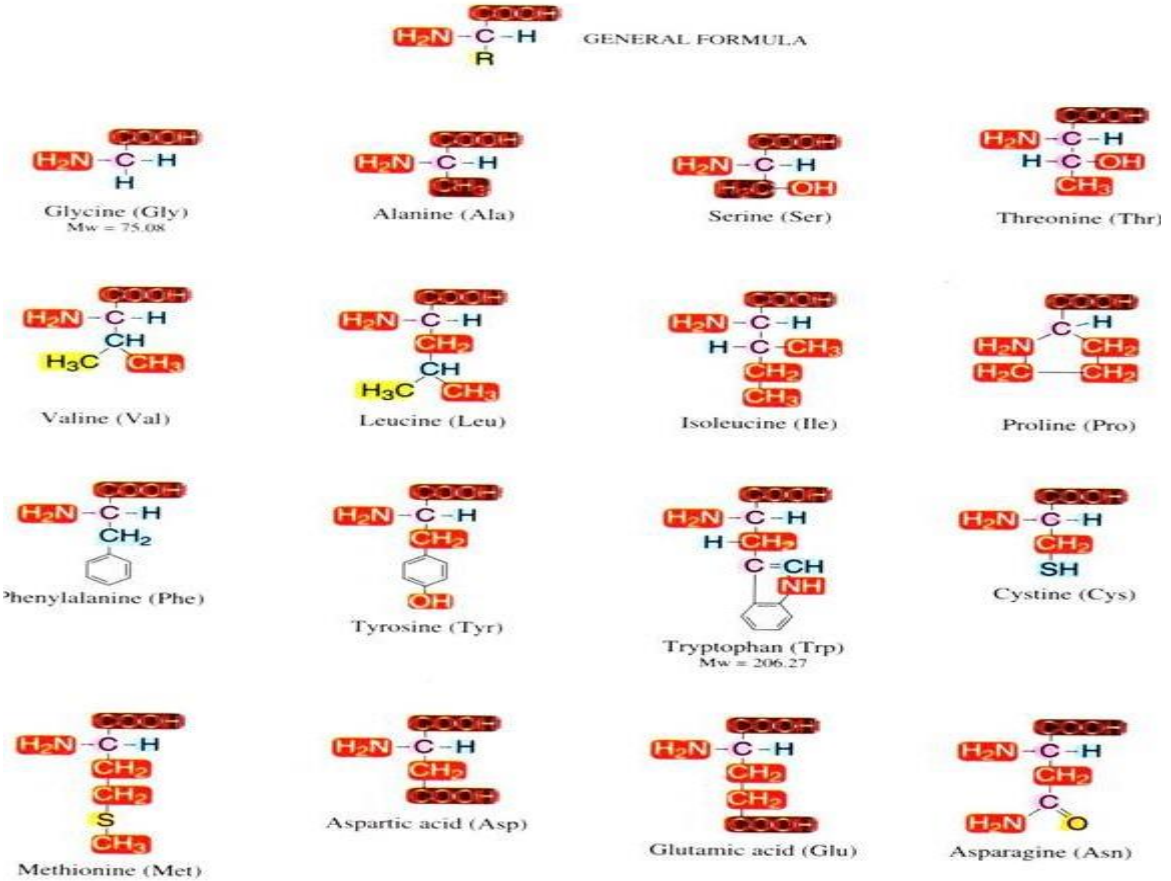
Those amino acids which contain a sulphur atom-Cys, Met.

2. Acidic amino acids:

Those amino acids that contain a negative charge or an acidic group-Asp, Glu.

3. Basic amino acids:

Those amino acids that contain a positive charge or a basic group-Arg, Lys and His.



II. Depending upon the Solubility in Water:

The amino acids can also be grouped into two different categories, depending upon their solubility in water. They are—

1. Hydrophobic amino acids:

Amino acids insoluble in water are known as hydrophobic amino acids. They are—Ala, Val, Leu, lie, Pro, Met, Phe, Trp.

2. Hydrophilic amino acids:

Amino acids soluble in water are known as hydrophilic amino acids. They are—Gly, Ser, Thr, Cys, Tyr, Asp, Asn, Glu, Gin, Lys, Arg, His.

III. Depending upon their Nutritional Requirements:

The amino acids are classified into two groups**. They are:**

1. Essential amino acids:

Are those which cannot be synthesized by the human body and hence they should be taken through the diet. There are 10 essential amino acids. Among these amino acids, arginine and histidine are known as semi-essential amino acids.

M – Methionine
A – Arginine
T – Threonine
T – Tryptophan
V – Valine
I – Isoleucine
L – Leucine
P – Phenylalanine
H – Histidine
Ly – Lysine

2. Non-essential amino acids:

These acids are those that can be synthesized in the human body and are not required in the diet. These include gly, ala, ser, pro, tyr, cys, asp, asn, glu, gin.

Glucogenic and Ketogenic Amino Acids:

Those amino acids which on oxidation give intermediate compounds, resembling those of carbohydrate metabolism and which may be converted to glucose are termed as glucogenic amino acids. Ex. Ala, Arg, Asp, Asn, Cys, Gly, Glu, Gin, His, Pro, Met, Ser, Tyr, Val, Lys. Those amino acids which form acetate or acetoacetate intermediates found during fatty acid metabolism are termed as ketogenic amino acids i.e.

they can give rise to ketone bodies. Ex. Leu. The amino acids lie, Lys, Phe, Tyr and Trp can give rise to both glucose and ketone bodies hence they are both glucogenic and ketogenic amino acids.

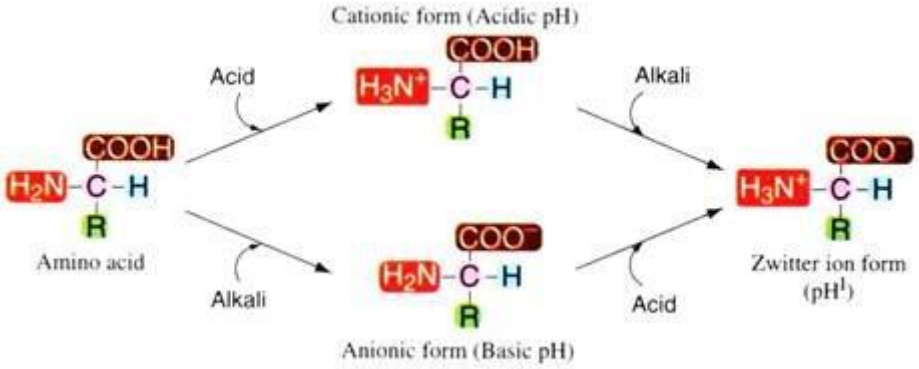
Physical Characters of Amino Acids:

1. Zwitter ions:

Amino acids have an acidic group (—COOH group), i.e., a proton, donor. They also have a basic group (—NH_2 group), i.e., a proton, acceptor. A compound capable of both donating and accepting protons and thus able to act either as an acid or a base is known as amphoteric molecule. Amino acids have both anions and cations in solution and such compounds are called zwitter ions.

2. Isoelectric pH (pH^1):

The pH at which the positive charge on the amino acid (or any other molecule) is equal to the negative charges, is known as isoelectric pH. At this pH the net charge will be zero and hence it does not move either to positive (anode) or to negative (cathode) electrode, when subjected to an electric field. At pH^1 all the molecules exists in zwitter ion form.

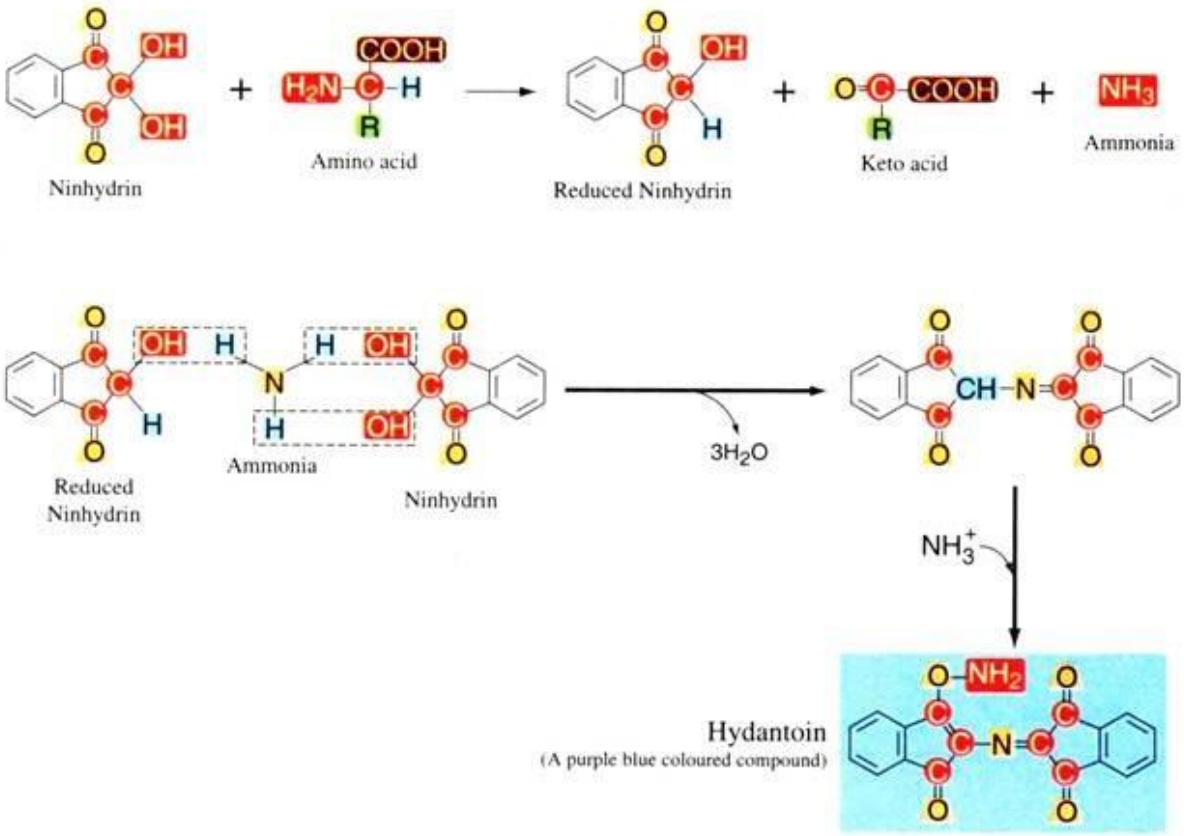


Chemical Properties:

1. Reactions due to amino group:

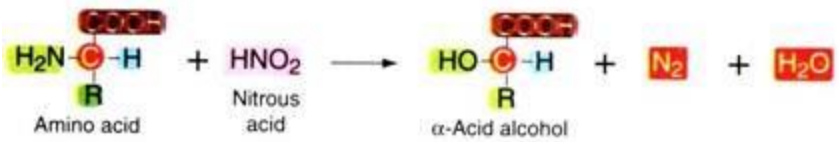
(a) Ninhydrin test:

This test identifies or detects amino acids. If amino acids are heated with ninhydrin, they form a purple blue coloured compound, which is measured colorimetrically.



(b) Reaction with nitrous acid:

It is a method by which amino acids are measured depending upon the amount of nitrogen released.

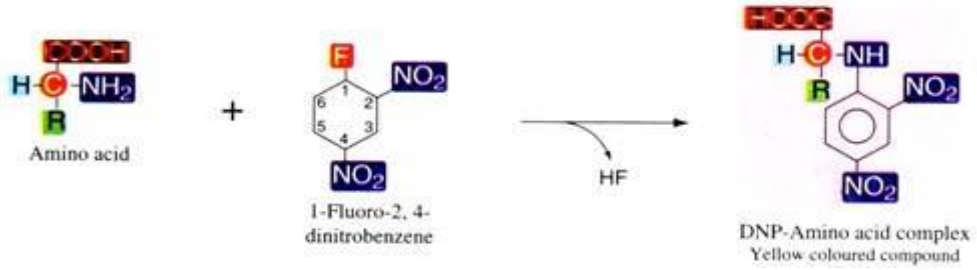


(c) Reaction with carbonyl compounds (RCHO):

The amino group in the amino acids reacts with carbonyl compounds forming a Schiff's base.

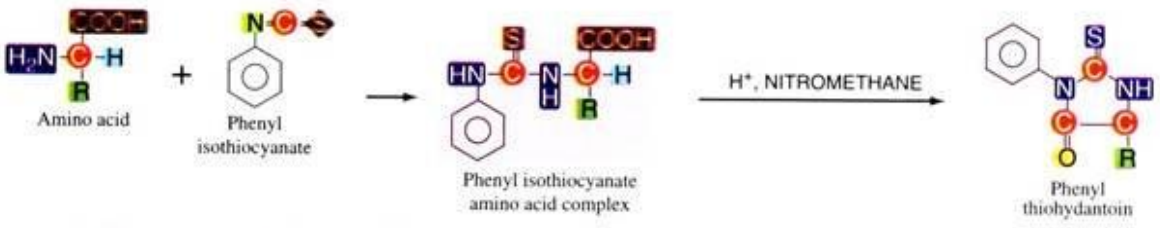
(d) Reaction with Sanger's reagent:

Amino acids react with Sanger's reagent, i.e., 1-fluoro-2, 4-dinitrobenzene, forming a yellow coloured complex. This reagent is used to detect the IV-terminal amino acid in the proteins.



(e) Edmann's reaction:

Edmann's reagent is phenyl isothiocyanate, which is also used to detect the N-terminal amino acid in a protein. It forms a purple coloured derivative.

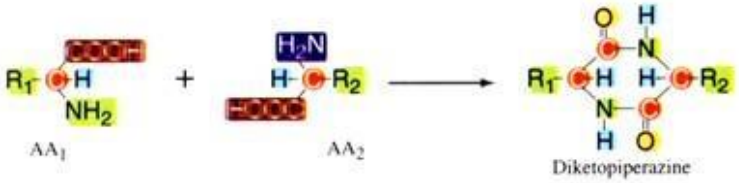


(f) Reaction with dansyl chloride:

Dansyl chloride, i.e., 1-dimethyl-amino-naphthalene-5-sulphonyl chloride forms a fluorescent derivate of the N-terminal amino acid of proteins. This is yet another reagent available for the detection of N-terminal amino acid.

(g) Condensation of two amino acids to form diketopiperazine:

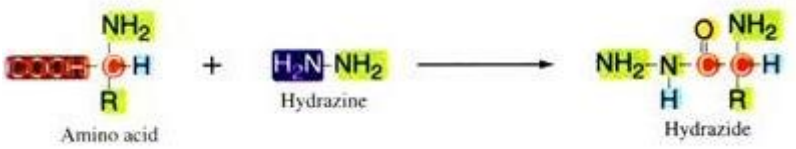
Two amino acids react with amino groups of each amino acid and the carboxylic groups of the other amino acid forming a diketopiperazine.



2. Reaction due to carboxylic:

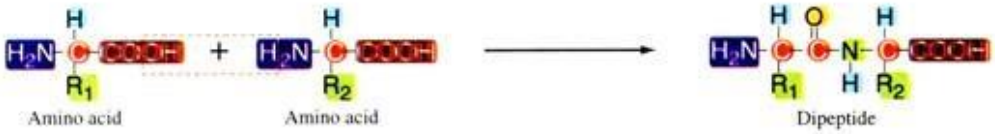
(a) Reaction with hydrazine:

Hydrazine is used to detect the C-terminal amino acid in proteins. It forms a complex with the amino acid by reacting with the carboxylic group.



3. Reaction due to both amino and carboxylic group:

Due to the presence of both amino (basic) and carboxylic (acid) groups in amino acids, the amino group of one amino acid reacts with the carboxylic group of another amino acid to form a peptide bond.



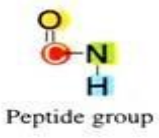
Polymerization of amino acids in a similar manner gives a polypeptide chain.

Peptide bond:

The bond linking two amino acids is known as a peptide bond. It is formed due to reaction between an amino group of one amino acid and carboxylic group of another amino acid.

Peptide group:

The group forming the peptide bond is known as peptide group. It has a double bond character and hence is very rigid in nature.



Peptide group

Polypeptide or peptide:

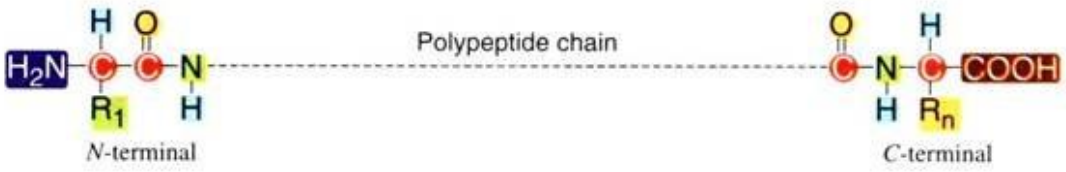
A chain made up of two or more amino acids, linked by a peptide bond is known as a polypeptide or just a peptide.

Difference between a peptide and a protein:

A peptide is that which has less than 50 amino acids or whose molecular weight is less than 5000 Daltons. A protein is that which has more than 50 amino acids or whose molecular weight is more than 5000 Daltons. This differentiation is based upon the immunological property of the two units. Peptides are non-immunogenic, whereas proteins are immunogenic.

N-terminal and C-terminal of a protein:

The end of a protein or polypeptide where the amino group is free in known as N-terminal end and that amino acid whose amino group is free is known as N-terminal amino acid. Sanger's, Edmann's and Dansyl chloride are the reagents used to determine the N-terminal amino acids.



The end of the protein or polypeptide whose carboxylic group is free is known as C-terminal end and that amino acid whose carboxylic group is free in the protein is known as C-terminal amino acid. Hydrazine is used to detect the C-terminal amino acid. While representing a protein on paper, the N-terminal amino acid is written first (on the left) and the C-terminal amino acid is the last one (written at the right side of the paper).

Peptides of physiological importance:

(a) Glutathione:

It is a tripeptide made up of Glu, Cys and Gly. It is found in RBC and other tissues and functions to prevent oxidation of —SH groups of many enzymes.

(b) Bradykinin and kallidin:

These are small polypeptides containing 9 and 10 amino acids respectively. They are formed by partial hydrolysis of plasma protein due to snake poisoning (venom). They are powerful vasodepressors and inhibitors of heart function. Others are tyrocidin, gramicidin, glucagon, insulin, oxytocin, etc.

Some of the major properties of amino acids are as follows:

1. Amino acids are colourless crystalline water soluble but largely insoluble in organic solvents. Most α-amino acid have melting points near 300°C whereas their non-ionic derivative usually melt around 100°C.

2. Glutamic acid was the first amino acid formed during the amino acid synthesis. From this all other amino acids are formed by reductive deamination and transamination.

3. Stereoisomerism:

Glycine is the simplest AA where the R-group is a hydrogen atom. Except glycine, all amino acids are asymmetrical (optically active) and exist in two stereo-isomeric forms i.e. L- isomer and D-isomer. Only the a-amino acids are found in proteins, while D-amino acids are rare in nature. Recently 2 free D-amino acids found in mammals, i.e. D-serine in the forebrain and D-aspertate in brain and in PNS.

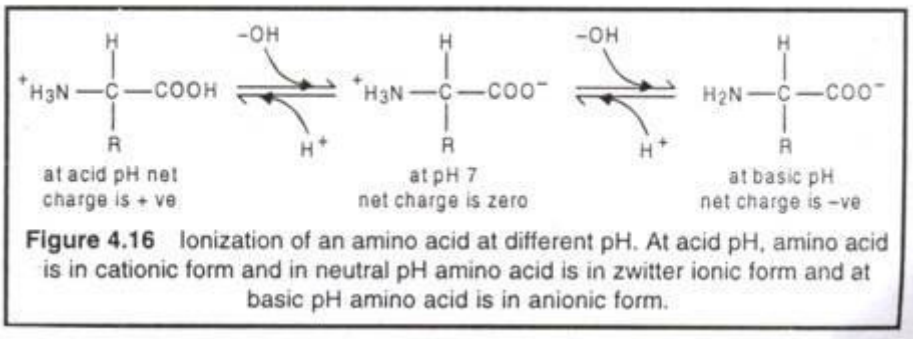


Figure 4.16 Ionization of an amino acid at different pH. At acid pH, amino acid is in cationic form and in neutral pH amino acid is in zwitter ionic form and at basic pH amino acid is in anionic form.

4. Ionization:

Amino acids have two ionizable groups i.e. a-amino and a-carboxyl groups. In addition to these, some amino acids have additional ionizable side-chain group (Asp, Glu, Arg, Lys,). In neutral solution (pH=7.0), amino acids are predominantly zwitterions or dipolar ions, where the α- amino group is protonated ($-NH_3^+$) and the a-carboxyl group is dissociated ($-COO^-$).

Therefore, a- amino acids are ampholytes (amphoteric electrolytes) i.e. can act as both acid (protein donor) and base (proton acceptor). In acid solution (e.g., pH1), only the a-amino group is ionized. In alkaline solution (e.g., pH1), only the a-carboxyl group is ionized. The pH at which an amino acid bears no net electric charge i.e. exist in form of Zwitter ion is called pi or isoelectric pH. pI can be calculated as the midpoint between the pK_1 and pK_2 values.

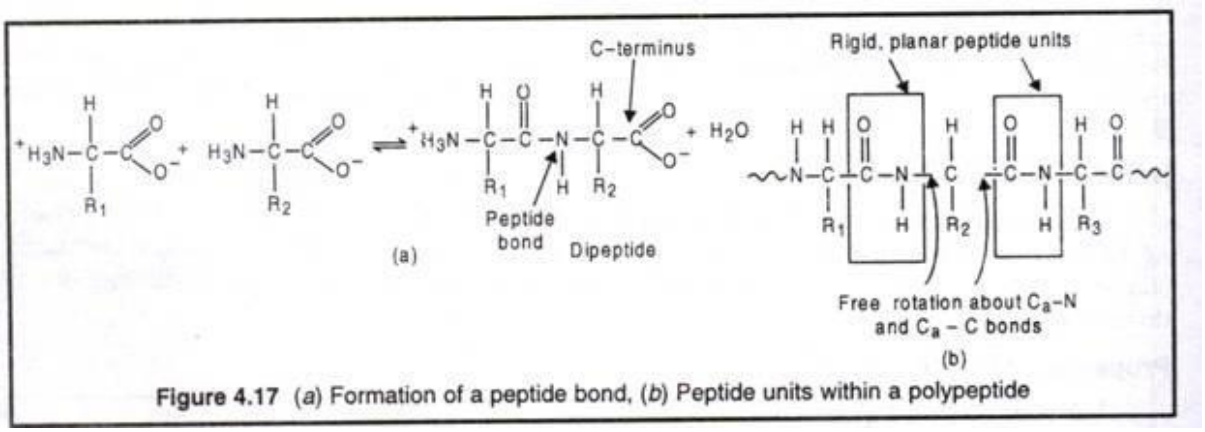


Figure 4.17 (*a*) Formation of a peptide bond, (*b*) Peptide units within a polypeptide

5. pK value :

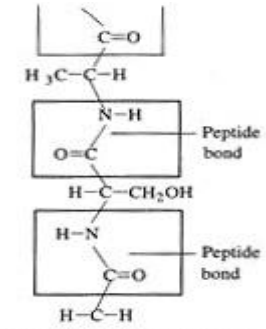
All amino acids possess at least two weakly acidic functional groups. However, – COOH is several thousand times stronger acid than – NH^{3+}. The pK value is the pH at which these acidic groups are half dissociated. The pK ot- COOH group and – NH^{3+} group are respectively called pK_1 and pK_2. In all the 20 standard amino acids, the value of pK_1 varies from 1.8-2.9 and the pK_2 value varies from 8, 84 -10.78. Aromatic amino acids like tryptophan, tyrosine, histidine and phenylalanine absorb UV-light.

6. Peptide bond Formation:

Amino acids condense to produce peptides. Generally condensation reaction occurs between a primer amino acid or peptide and another amino acid. Molecule of water is eliminated. The bond thus formed — NHCO— is actually amide bond which is popularly known as peptide bond or linkage.

Chain containing two amino acids linked by a peptide bond is called dipeptide. There are three amino acids in a tripep- tide, a few in oligopeptide and numerous in a polypeptide. The term peptide is more commonly used instead of oligopeptide. Like an amino acid a peptide has carboxylic group at one end and amino

group at the other end. Occasionally the peptide is acidic or basic due to the presence of specific amino acids.



A part of peptide showing peptide bonds linking amino acids.

Some of the most important functions of amino acids are as follows:

1. Building blocks of proteins

Only L-amino acids are polymerized to form proteins, though both D-amino acids and non-L-amino acids found in nature.

2. Biological buffers

Amino acids being amphoteric, act as buffers in solutions, resisting changes in pH. They do so by donating H^+ ions as pH increases and accepting H^+ as pH decreases.

3. Nitrogen storage

Asparagine and glutamine are amide derivatives of as-artic acid and glutamic acid. They serve as storage of nitrogen.

4. Proline forms bands or kinks in polypeptide chains.

5. Cysteine links chains together by forming disulfide bonds.

6. Histidine found in the active site of enzymes where it causes making and breaking of bonds.

7. The aromatic rings of Phe, Tyr and Trp help in electron transfer.

8. Formation of glucose: Some amino acids form glucose by losing amino group.

9. Formation other compounds

(i) Tyrosine produces the hormones thyroxin and adrenaline and the skin pigment melanin,

(ii) Glycine forms heme.

(iii) Tryptophan produces vitamin, nicotinamide and plant hormone Indole Acetic acid (IAA). The coenzyme A, a vitamin pantothenic acid, coenzyme glutathione and alkaloids are some other compounds formed by amino acids.

10. Antibiotics: The non-protein amino acids are useful compounds of antibiotics e.g. Azaserine, Valinomycin etc.

11. Genetic defects: Inborn errors in the metabolism of amino acids cause several disorders e.g. Phenylketonuria.

12. L-amino acids and their derivatives help in nerve transmission, cell growth and biosynthesis of porphynine, purines, pyrimidines and urea.

Urea cycle :

Living organism excrete the excess nitrogen resulting from the metabolic breakdown of the amino acids in one of three ways. Urea is formed from ammonia, co2 and aspartate in a cyclic pathway referred to as the urea cycle. Because the urea cycle is discovered by Krebs and Henseleit, it is often referred to as Krebs-Henseleit cycle.

Why is Urea Cycle important to us?

Through urea nitrogenous waste of the body are excreted. Urea is less toxic than ammonia and can be effectively excreted by kidneys. The urea cycle mediates the removal of ammonia as urea in the amount of 10 to 20 g per day in the healthy adult. In the absence of a fully functional urea cycle, hyperammonemic encephalopathy and irreversible brain injury in occur. Hyperammonemia also may affect brain volume control; cell swelling is sometimes observed, perhaps because of the marked increase of brain glutamine. Hyperammonemia also affects neurotransmitter metabolism. Major effects on the handling of GABA and serotonin have been observed.

The Synthesis of Urea:

Urea synthesis which occurs in the hepatocytes in liver, consists of five sequential enzymatic reactions. The first two reactions occur in the mitochondria and the remaining reactions takes place in the cytosol. Urea cycle begins with the formation of carbamoyl phosphate in the mitochondria. The substrate for this reaction, catalyzed by carbamoyl phosphate synthetase I, are NH_4^+ and HCO_3^- . Because two molecules of ATP are required in carbamoyl phosphate synthesis, this reaction is essentially irreversible. Carbamoyl phosphate subsequently reacts with ornithine to form citrulline. Citrulline passes into the cytosol. Next three steps that occur in cytosol involves :

1. Formation of argininosuccinate by ATP dependent by ATP dependent reaction of citrulline with aspartate.

2. Formation of arginine from argininosuccinate. This reaction release fumarate, which enters the citric acid cycle.

3. Formation of urea and regeneration of ornithine

Steps of Urea Cycle:

1. Formation of Carbamoyl Phosphate:

Condensation of ammonium ion with bicarbonate ion resulting in the formation of carbamoyl phosphate by the help of the enzyme carbamoyl phosphate synthase-I present in the liver mitochondria. It requires Mg^{2+} and a dicarboxylic acid i.e. N-acetyl glutamate. This step requires 2 ATPs.

2. Synthesis of Citrulline:

Carbamoyl phosphate formed in the first step combines with ornithine resulting in the synthesis of citrulline aided by the enzyme citrulline synthase or ornithine transcarbamoylase. Citrulline is easily permeable to the mitochondrial membrane and hence it diffuses into the cytosol.

3. Synthesis of Argininosuccinate:

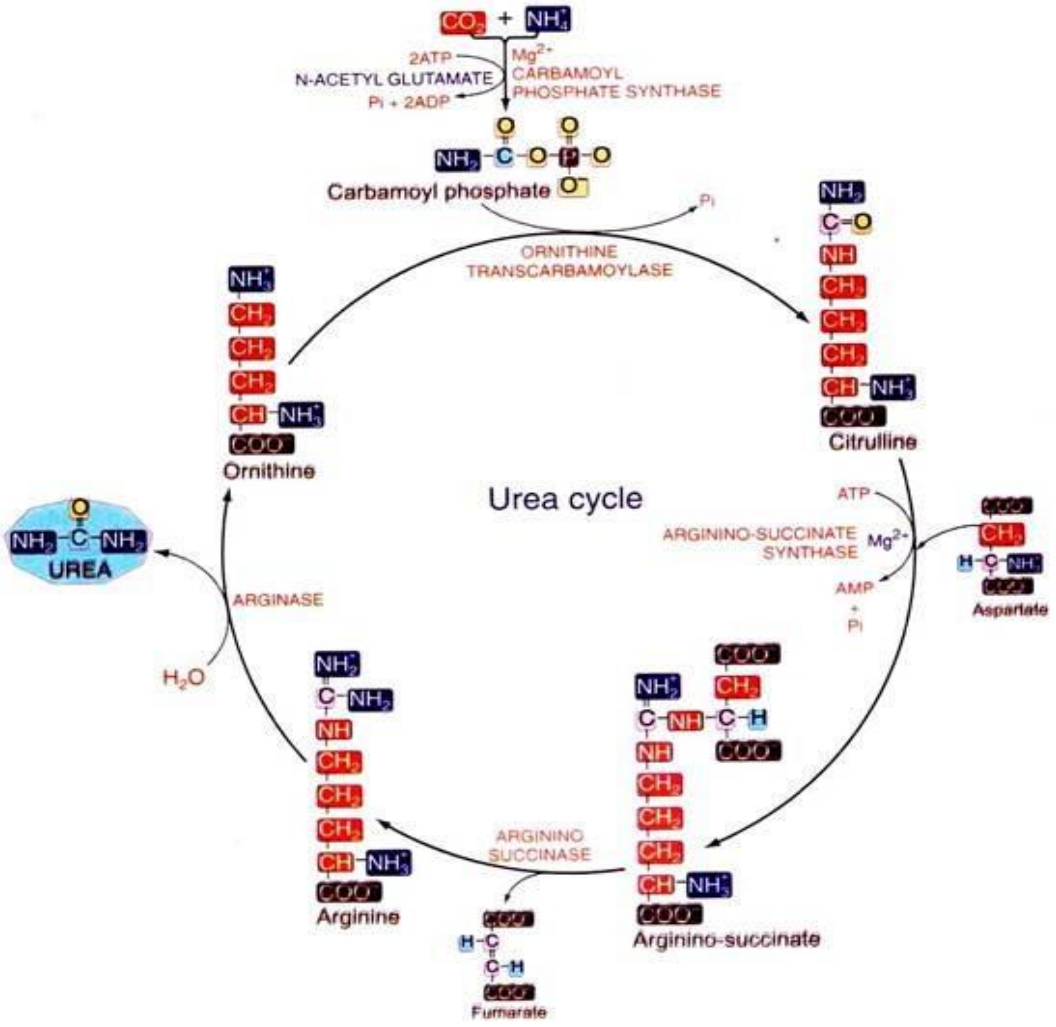
In the cytosol, citrulline combines with the amino acid aspartate forming argininosuccinate catalysed by the enzyme argininosuccinate synthase. It requires ATP which is hydrolysed to AMP resulting in utilization of two high energy bonds. Mg^{2+} acts as cofactor.

4. Cleavage of Argininosuccinate:

The enzyme argininosuccinase acts reversibly to cleave argininosuccinate into Arginine and fumarate. Fumarate enters the TCA cycle (the linkage between TCA and urea cycle is known as Krebs bi-cycle).

5. Cleavage of Arginine:

Arginine is lysed into ornithine and urea under the influence of the enzyme arginase. Hence arginine is known as semi-essential amino acid i.e. though it is synthesized in the body it is not available for protein synthesis. Ornithine is regenerated in this step and the urea cycle completes by the formation of urea. Ornithine and lysine are potent inhibitors of the enzyme arginase. Arginase is also present in testis, renal tubules, mammary gland and skin in minute quantities. The intermediate amino acids formed in the urea cycle i.e. ornithine, citrulline and argininosuccinate are known as non-protein amino acids.



The overall equation of urea formation is:

NH_4 + CO_2 + Asparate + 3ATP → Urea + Fumarate + 2ADP + 2P_i + AMP + PP_i

The urea cycle brings two amino groups and HCO3 together to form urea. Thus toxic, insoluble ammonia is converted into non-toxic, water soluble, excretable urea. Hence, urea cycle disposes two waste products i.e. NH_4 and HCO_3. This fact suggests that urea cycle participates in the regulation of blood pH, which depends on the HCO_3/H_2CO_3. Though 3 ATPs are utilized, the ultimate cost of making a molecule of urea is 4 ATPs (one ATP is converted into AMP). The rate limiting steps of urea cycle are 1, 2, & 5.

B. Metabolic Disorders of Urea Cycle:

Since urea cycle converts toxic ammonia to urea, disorders of this cycle lead to ammonia intoxication. This ammonia intoxication is more when there is block at step 1 or 2. Common symptoms of the disorders of urea cycle are vomiting in infancy, avoidance of high protein diet, intermittent ataxia, irritability, lethargy and mental retardation.

1. Hyperammonemia type-I:

Due to the deficiency of carbamoyl phosphate synthase-I. It is a familial disorder.

2. Hyperammonemia type-II:

Due to the deficiency of ornithine transcarbamoylase. It is X-linked. Clinical finding is, the elevation of glutamine in the blood, CSF and urine.

3. Citrullinemia:

More citrulline is excreted in the urine i.e. upto 1 to 2 gm/day, due to the defect in the enzyme argininosuccinate synthase.

4. Arginino-succinic aciduria:

It is a rare recessive disease. Higher level of arginino-succinic acid in plasma and CSF. Usually present in the early age. Feeding arginine and benzoate promotes nitrogen excretion in these patients. This is due to lack of the enzyme argininosuccinase.

5. Hyper-argininemia:

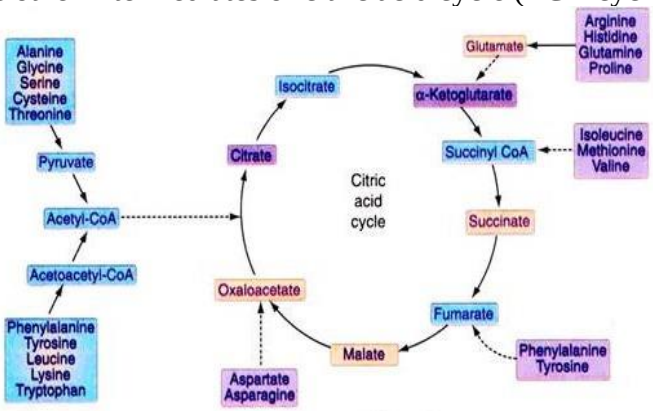
High level of arginine due to lack of arginase enzyme.

Regulation of urea cycle:

The urea cycle operates only o eliminate excess nitrogen. On high-protein diets the carbon skeletons of the amino acids are oxidized for energy for stored as fat and glycogen, but the amino nitrogen must be excreted. To facilitate this process, enzyme of the urea cycle are control at the gene level. with long term changes in the quantity of dietary protein, Changes of 20-fold or greater in concentration of cycle enzyme are observed. When dietary proteins increase significantly, enzyme concentrations rise. On return to balanced diet, enzyme levels decline. Under conditions of starvation, enzyme levels rise as protein are degraded and amino acid carbon skeletons are used to provide energy, thus increasing the quantity of nitrogen that must be excreted. Short-term regulation of the cycle occurs principally at CPS-I, which is relatively inactive in absence of its Allosteric activator n-acetyleglutamate. This metabolite is synthesized from glutamate and acetyl CoA by N-acetylglutamate synthase. Increased urea synthesis is required when amino acid breakdown rates increase, generating excess nitrogen that must be excreted. Increased in these breakdown rates are signaled by an increase in glutamate concentration through transmission reactions. This situation, in turn, cause an increase in N acetyl-glutamate synthesis, stimulating carbamoyl phosphate synthetase and thus entire urea cycle.

Oxidation of Carbon Skeleton of Amino Acids:

Once ammonia is released from the amino acids the remnant carbon back bone undergoes various oxidative reactions to yield one or the other intermediates of citric acid cycle (TCA cycle) as shown below:



Amino acids entering TCA cycle

Probable Questions:

1. With the help of a schematic diagram describe the synthesis of urea in the liver.

2. What is the rate limiting step in urea cycle?

3. How is urea cycle regulated by n-acetylglutamate?

4. Write a short note on optical isomerism of amino acids.

5. Schematically represent the steps of urea cycle.

6. Classify amino acids on the basis of hydrophilicity.

7. Classify amino acids on the basis of their acidic/ basic/ neutral nature.

8. Classify amino acids on the basis of their polarity.

9. Discuss ketogenic and glucogenic amino acids.

10. Discuss structure of an ideal amino acids.

11. Discuss about chemical reactions of amino acids.

12. Discuss about physical properties of amino acids

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistr

UNIT-XII

Proteins: Protein folding and protein stability

Objective: In this unit we will learn about structural hierarchy of proteins and the folding of protein molecules which are essential for their function.

Protein Structure: Protein molecules can have four different levels of organization, known as their primary, secondary, 'tertiary and quaternary structures. The primary structure possessed by all proteins is their specific sequence of amino acids in the polypeptide chains.
The primary structure of a segment of a polypeptide chain or of a protein is the amino-acid sequence of the polypeptide chain(s), without regard to spatial arrangement (apart from configuration at the alpha-carbon atom).

The "R" in the amino acid generic structure stands for the term "radical" and represents one of twenty or so different possibilities. So with 20 different "R" groups, there are twenty different amino acids in nature. All amino acids have attached to the same carbon both an amino group (NH_2) and a carboxyl group (COOH). The hydrogen from the carboxyl group actually exists in a cloud around the amino group and itself, thus creating the zwitterion. This renders the amino acid active and proteins formed from them capable of great activity. Buffers and enzymes are examples of active proteins. The peptide bond is formed when the amino group of one amino acid and the carboxyl group of another amino acid unite with the loss of one water molecule per bond.

The commonly occurring amino acids are of 20 different kinds (a protein may contain a chain of 100 to 1000 amino acids; 26 alphabets in English making several thousand words, 20 amino acids form different proteins) which contain the same dipolar ion group $H_3N^+.CH.COO^-$. They all have in common a central carbon atom to which are attached a hydrogen atom, an amino group (NH_2) and a carboxyl group (COOH). The central carbon atom is called the C_{alpha} -atom and is a chiral centre. All amino acids found in proteins encoded by the genome have the L-configuration at this chiral centre. This configuration can be remembered as the CORN law (Fig. 8.6). Imagine looking along the H-C_{alpha} bond with the H atom closest to you.

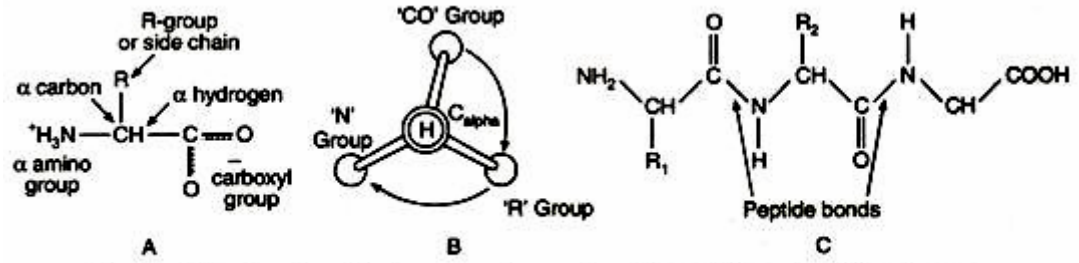


Fig. 8.6. (A) Carboxylic and amino group of an amino acid, (B) CORN orientation of groups, (C) Peptide bonds.

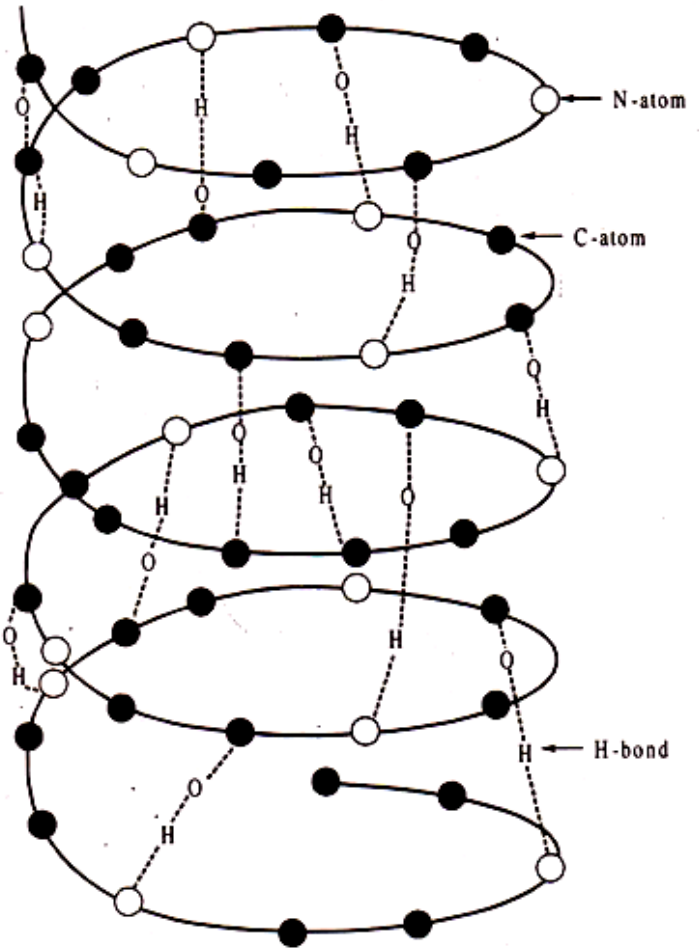
When read clockwise, the groups attached to the C_{alpha} spell the word CORN. There are 20 side chains found in proteins encoded by the genetic machinery of the cell. The side chains confer important properties on a protein such as the ability to bind ligands and catalyse biochemical reactions. They also direct the folding of the nascent polypeptide and stabilise its final conformation.

Amino acids in proteins (or polypeptides) are joined together by peptide bonds. The sequence of R-groups along the chain is called the primary structure. Proteins can occur as primary, secondary, tertiary or quaternary structures.

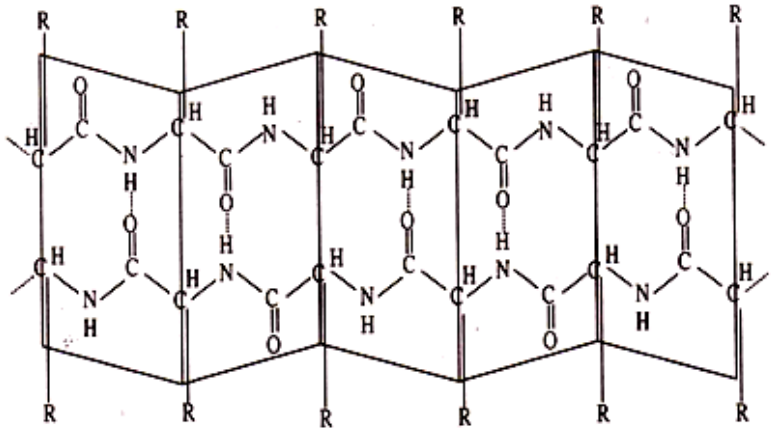
Secondary Structure:

The secondary structure of a segment of polypeptide chain is the local spatial arrangement of its main-chain atoms without regard to the conformation of its side chains or to its relationship with other segments. There are three common secondary structures in proteins, namely alpha helices, beta sheets and turns. The alpha-helix and beta-structure conformations for polypeptide chains are generally the most thermodynamically stable of the regular secondary structures. However, particular amino acid sequences of a primary structure in a protein may support regular conformations of the polypeptide chain other than alpha-helical or beta-structure.

Secondary structure of polypeptides results from twisting and folding of the molecules in localized portions of the polypeptide chain. Generally, two types of folding occur. One type is formation of a clockwise spiral, called α-helix structure which is held in proper form by hydrogen bonds between the -CO-NH- groups of the constituent amino acids brought close to each other due to spinalization (Fig. 8.7A). Another type of secondary structure, known as pleated-sheet, is produced by hydrogen bonding's between parallelly running segments of a polypeptide chain (Fig. 8.7B).



(A) Diagrammatic representation of an a-helix structure of a polypeptide chain



(B) Pleated sheet structure of polypeptide molecules

Fig. 8.7 : Secondary structures of polypeptide chains. (A) The α-helix structure. (B) Pleated sheet structure. Both structures are stabilized by H-bonds formed between –CO– and –NH groups of amino acids which are brought close to each other either due to spiralization as in α-helix or when portions of a polypeptide chains run parallel to each other

Tertiary Structure:

The tertiary structure of a protein molecule, or of a subunit of a protein molecule, is the arrangement of all its atoms in space, without regard to its relationship with neighboring molecules or subunits.

Tertiary structure of a polypeptide gives the molecule a three-dimensional shape. The polypeptide with its secondary structure further folds into a characteristic shape due to interactions of the side chains i.e. the R group of amino acids.

Interactions between the side chains may be strong due to formation of covalent bonds, like the formation of disulphide bridges between two cysteine residues, or may be weak, like the H-bonds, hydrophobic interaction or ionic bonds.

The bonds stabilizing the tertiary structure are shown in Fig. 8.8:

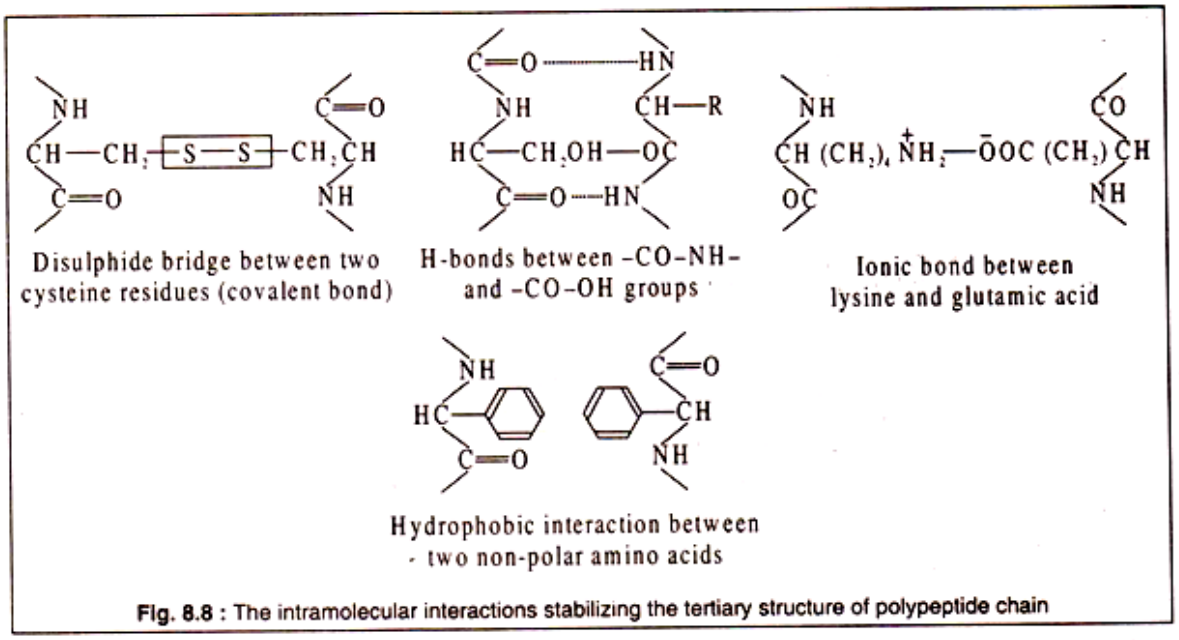


Fig. 8.8 : The intramolecular interactions stabilizing the tertiary structure of polypeptide chain

Quaternary Structure:

The quaternary structure of a protein molecule is the arrangement of its subunits in space and the ensemble of its inter-subunit contacts and interactions, without regard to the internal geometry of the subunits. The subunits in a quaternary structure must be in non-covalent association. Hemoglobin contains four polypeptide chains (alph a_2b_2) held together non-covalently in a specific conformation as required for its function.

Although some proteins consist of a single polypeptide chain, many others — particularly globular proteins — having molecular weights of 60,000 Daltons or more, consist of more than one polypeptide chains. Such proteins are oligomeric and the constituent polypeptides are called protomers.

The association of protomers to form a super molecule of an oligomeric protein is known as the quaternary structure. The forces that keep the protomers together are basically the same as the intermolecular bonds which contribute to the formation of tertiary structure of individual polypeptides.

The association of two polypeptides to form a quaternary structure is shown in Fig. 8.10:

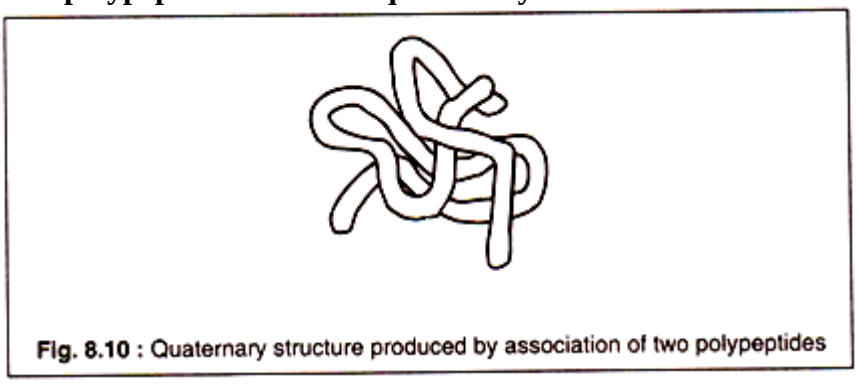


Fig. 8.10 : Quaternary structure produced by association of two polypeptides

Soluble proteins subjected to abnormal conditions, such as temperature, acidity or alkalinity, high salt concentrations, solvents etc. undergo denaturation. During this process, the protein molecules lose their characteristic structures, particularly the tertiary and quaternary structures. Denatured proteins are generally biologically inactive particularly their catalytic properties are lost. Many proteins in biological systems combine with various other organic molecules to form the conjugated proteins. Thus, when a protein combines with carbohydrates, a glycoprotein is formed. Similarly, conjugation with nucleic acids and lipids results in a nucleoprotein and lipoprotein, respectively. Combination with a chromogenic prosthetic group yields a chromo-protein such as haemoglobin.

Polypeptides Fold Rapidly by a Stepwise Process:

In living cells, proteins are assembled from amino acids at a very high rate. For example, E. coli cells can make a complete, biologically active protein molecule containing 100 amino acid residues in about 5 seconds at 37°C. How does such a polypeptide chain arrive at its native conformation? Let's assume conservatively that each of the amino acid residues could take up 10 different conformations on average, giving 10100 different conformations for the polypeptide. Let's also assume that the protein folds itself spontaneously by a random process in which it tries out all possible conformations around every single bond in its backbone until it finds its native, biologically active form. If each conformation were sampled in the shortest possible time (~10-13 second, or the time required for a single molecular vibration), it would take about 1077 years to sample all possible conformations. Thus protein folding cannot be a completely random, trial-and-error process. There must be shortcuts. This problem was first pointed out by Cyrus Levinthal in 1968 and is sometimes called Levinthal's paradox. The folding pathway of a large polypeptide chain is unquestionably complicated, and not all the principles that guide the process have been worked out. However, extensive study has led to the development of several plausible models. In one, the folding process is envisioned as hierarchical. Local secondary structures form first. Certain amino acid sequences

fold readily into α helices or β sheets, guided by constraints. This is followed by longer-range interactions between, say, two α helices that come together to form stable supersecondary structures. The process continues until complete domains form and the entire polypeptide is folded. In an alternative model, folding is initiated by a spontaneous collapse of the polypeptide into a compact state, mediated by hydrophobic interactions among nonpolar residues. The state resulting from this "hydrophobic collapse" may have a high content of secondary structure, but many amino acid side chains are not entirely fixed. The collapsed state is often referred to as a molten globule. Most proteins probably fold by a process that incorporates features of both models. Instead of following a single pathway, a population of peptide molecules may take a variety of routes to the same end point, with the number of different partly folded conformational species decreasing as folding nears completion (Figure 5).

Figure 5 : A simulated folding pathway. The folding pathway of a 36-residue segment of the protein villin (an actin-binding protein found principally in the microvilli lining the intestine) was simulated by computer. The process started with the randomly coiled peptide and 3,000 surrounding water molecules in a virtual "water box." The molecular motions of the peptide and the effects of the water molecules were taken into account in mapping the most likely paths to the final structure among the countless alternatives. The simulated folding took place in a theoretical time span of 1 ms; however, the calculation required half a billion integration steps on two Cray supercomputers, each running for two months.

Some Proteins Undergo Assisted Folding :

Proteins that facilitate the folding of other proteins are called molecular chaperones. The term "chaperone" was first used by Ron Laskey and his colleagues to describe a protein (nucleoplasmin) that is required for the assembly of nucleosomes from histones and DNA. Nucleoplasmin binds to histones and mediates their assembly into nucleosomes, but nucleoplasmin itself is not incorporated into the final nucleosome structure. Chaperones thus act as catalysts that facilitate assembly without being part of the assembled complex. Subsequent studies have extended the concept to include proteins that mediate a variety of other

assembly processes, particularly protein folding. Not all proteins fold spontaneously as they are synthesized in the cell. Folding for many proteins is facilitated by the action of specialized proteins. Molecular chaperones are proteins. that interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments in which folding can occur. Two classes of molecular chaperones have been well studied. Both are found in organisms ranging from bacteria to humans. The first class, a family of proteins called Hsp70, generally have a molecular weight near 70,000 and are more abundant in cells stressed by elevated temperatures (hence, heats hock proteins of Mr 70,000, or Hsp70). Its mode of action is depicted in Figure 7. Hsp70 proteins bind to regions of unfolded polypeptides that are rich in hydrophobic residues, preventing in appropriate aggregation. These chaperones thus "protect" proteins that have been denatured by heat and peptides that are being synthesized (and are not yet folded). Hsp70 proteins also block the folding of certain proteins that must remain unfolded until they have been translocated across membranes. Some chaperones also facilitate the quaternary assembly of oligomeric proteins. The Hsp70 proteins bind to and release polypeptides in a cycle that also involves several other proteins (including a class called Hsp40) and ATP hydrolysis (Figure below).

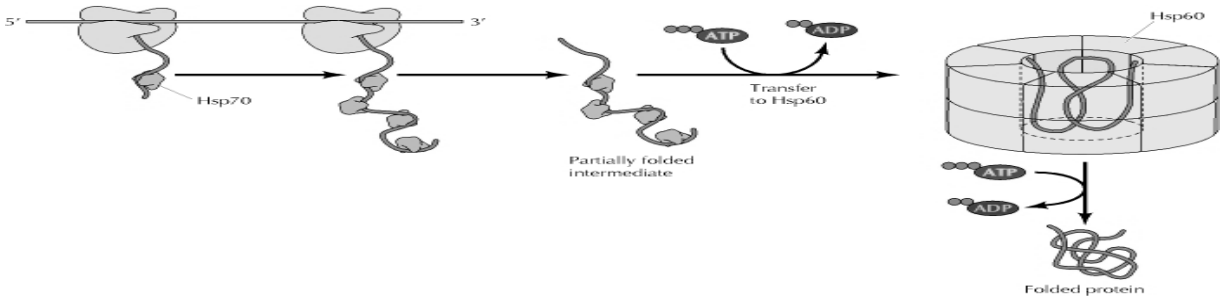


Figure : Sequential actions of Hsp70 and Hsp60 chaperones

Chaperones of the Hsp70 family bind to and stabilize unfolded polypeptide chains during translation. The unfolded polypeptide is then transferred to chaperones of the Hsp60 family, within which protein folding takes place. ATP hydrolysis is required for release of the unfolded polypeptide from Hsp70 as well as for folding within Hsp60. The second class of chaperones is called chaperonins. These are elaborate protein complexes required for the folding of a number of cellular proteins that do not fold spontaneously. In E. coli an estimated 10% to15% of cellular proteins require the resident chaperonin system called

GroEL/GroES, for folding under normal conditions (up to 30% require this assistance when the cells are heat stressed). These proteins first became known when they were found to be necessary for the growth of certain bacterial viruses (hence the designation “Gro”). Unfolded proteins are bound within pockets in the GroEL complex, and the pockets are capped transiently by the GroES “lid”. GroEL undergo substantial conformational changes, coupled to ATP hydrolysis and the binding and release of GroES, which promote folding of the bound polypeptide. Although the structure of the GroEL/GroES chaperonin is known, many details of its mechanism of action remain unresolved. Finally, the folding pathways of a number of proteins require two enzymes that catalyze isomerization reactions. Protein disulfide isomerase (PDI) is a widely distributed enzyme that catalyzes the interchange or shuffling of disulfide bonds until the bonds of the native conformation are formed (Figure below). Among its functions, PDI catalyzes the elimination of folding intermediates with inappropriate disulfide cross-links.

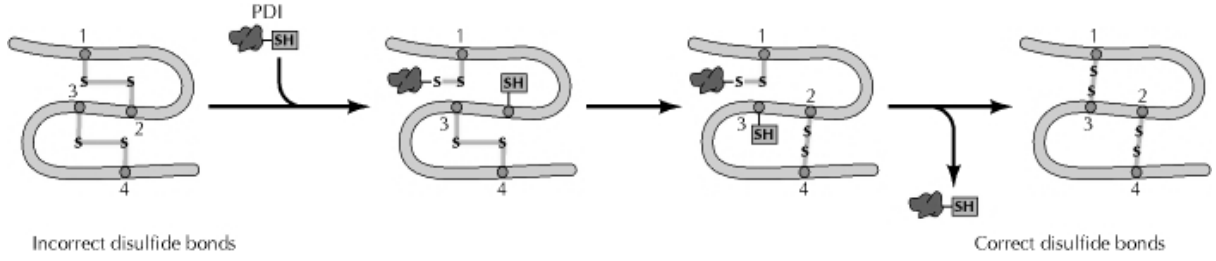


Figure. : The action of protein disulfide isomerase

Protein disulfide isomerase (PDI) catalyzes the breakage and rejoining of disulfide bonds, resulting in exchanges between paired disulfides in a polypeptide chain. The enzyme forms a disulfide bond with a cysteine residue of the polypeptide and then exchanges its paired disulfide with another cysteine residue. In this example, PDI catalyzes the conversion of two incorrect disulfide bonds (1-2 and 3-4) to the correct pairing (1-3 and 2-4). Peptide prolyl cis-trans isomerase (PPI) catalyzes the interconversion of the cis and trans isomers of Pro peptide bonds (Figure below), which can be a slow step in the folding of proteins that contain some Pro residue peptide bonds in the cis conformation.

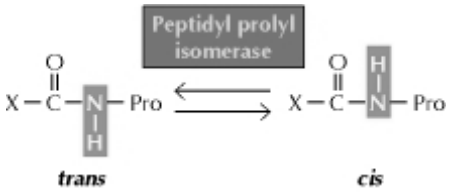


Figure : The action of peptidyl prolyl isomerase:Peptidyl prolyl isomerase catalyzes the isomerization of peptide bonds that involve proline between the *cis* and *trans* conformations.

Protein Misfolding and Degenerative Diseases:

For many proteins, the most prominent structural motif of the functional protein in its native conformation is known as the alpha helix, a right-handed spiral coil. When a protein becomes toxic, an extensive conformational change occurs and it acquires a motif known as the beta sheet. Note that the beta sheet conformation also exists in many functional native proteins, such as the immunoglobulins, but the transition from alpha helix to beta sheet is characteristic of amyloid deposits. The abnormal conformational transition from alpha helix to beta sheet exposes hydrophobic amino acid residues and promotes protein aggregation. Most of the time, only the native conformation is produced in the cell. But as millions and millions of copies of each protein are made during our lifetimes, sometimes a random event occurs and one of these molecules follows the wrong path, changing into a toxic configuration. This kind of conformational change is most likely to occur in proteins that have repetitive amino acid motifs, such as polyglutamine; such is the case in Huntington's disease. Remarkably, the toxic configuration is often able to interact with other native copies of the same protein and catalyze their transition into the toxic state. Because of this ability, they are known as infective conformations. The newly made toxic proteins repeat the cycle in a self-sustaining loop, amplifying the toxicity and thus leading to a catastrophic effect that eventually kills the cell or impairs its function. A prime example of proteins that catalyze their own conformational change into the toxic form is the prion proteins, under normal circumstances, the cell has mechanisms to prevent proteins from folding incorrectly, as well as to get rid of misfolded proteins. Proteins that have problems achieving their native configuration are helped by chaperones to fold properly, using energy from ATP. Chaperones can avoid the conformational change to beta sheet structure and the aggregation of these altered proteins; thus they seem fundamental to the prevention of protein misfolding. Despite chaperone actions, some proteins still misfold, but there is a remedy: The misfolded proteins can be detected by quality-control mechanisms in the cell that tags them to be sent to the cytoplasm, where they will be degraded (Figure below).

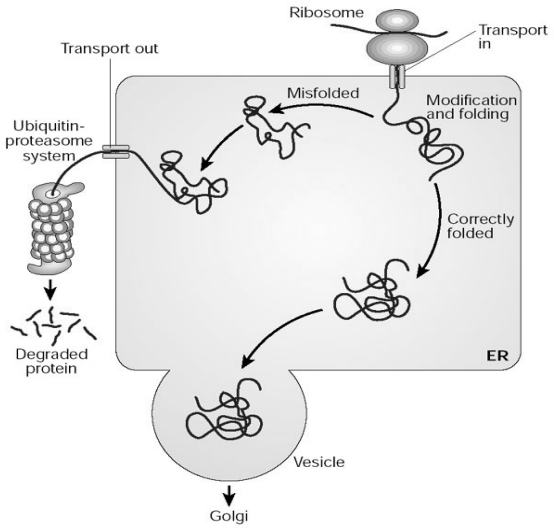


Figure : A schematic diagram shows how a newly synthesized protein is transported from a ribosome into the lumen of the endoplasmic reticulum (ER) where modification and folding of the nascent polypeptide occurs. A newly translated protein, represented by a purple squiggly line, is exiting the ribosome and entering the ER through the pore. After the protein enters the ER lumen, it can follow two pathways to different destinations in the cell, depending on whether it is folded correctly or misfolded. A correctly folded protein is shown entering a budding vesicle at the bottom edge of the ER, where it will be transported to the Golgi apparatus. A misfolded protein is exported from the ER to the cytosol where it is degraded by the ubiquitin-proteasome system.

Misfolded Proteins and Neurodegenerative Diseases:

Accumulation of misfolded proteins can cause disease, and unfortunately some of these diseases, known as amyloid diseases, are very common. The most prevalent one is Alzheimer's disease, which affects about 10 percent of the adult population over sixty-five years old in North America. Parkinson's disease and Huntington's disease have similar amyloid origins. These diseases can be sporadic (occurring without any family history) or familial (inherited). Regardless of the type, the risk of getting any of these diseases increases dramatically with age. The mechanistic explanation for this correlation is that as we age (or as a result of mutations), the delicate balance of the synthesis, folding, and degradation of proteins is perturbed,

resulting in the production and accumulation of misfolded proteins that form aggregates (Figure 10). Figure below :

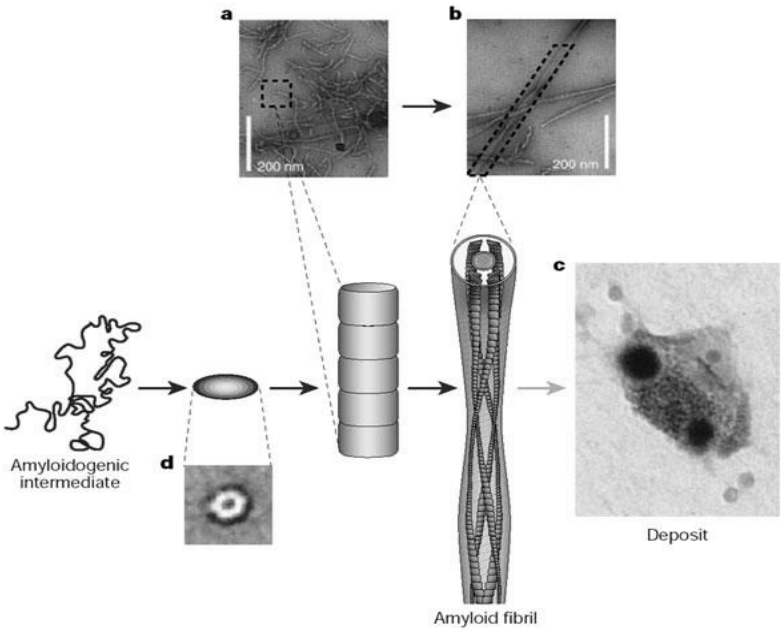


Figure-: Unfolded or misfolded amyloid proteins associate with each other in different stages, starting with the formation of a small, soluble aggregate, which is shown on the far left side of this diagram as a purple squiggly line that is labeled "amyloidogenic intermediate." A black arrow points to the right to indicate that the soluble aggregates can form a circular early aggregate, which is shown as a blue oval shape. Another black arrow points to the right to indicate that the early aggregates can combine to form a protofilament, which is shown as five vertically stacked cylinders. Another black arrow points to the right to indicate that protofilaments can combine to form a mature amyloid fibril, which is shown as four protofilaments that are twisted around each other with a green-colored border around them. A green arrow points to the right to indicate that mature fibrils can combine to create plaques or other structures, which is shown as an electron micrograph of Lewy body structures that are associated with Parkinson's disease. A series of panels extend from the illustrations to additional electron micrographs of the corresponding structures to show how they appear in a cell. Panel A is an electron micrograph of a protofibril that extends from the illustration of the protofibril; it shows grey filamentous structures that are approximately 10 nm in diameter. Panel B is an electron micrograph of a mature amyloid fibril that extends from the illustration of the same structure; they look much like the protofibrils shown in panel A, but the mature amyloid fibrils are thicker.

Among the environmental factors known to increase the risk of suffering degenerative diseases is exposure to substances that affect the mitochondria, increasing the amount of oxidative damage to proteins. However, it is clear that no single environmental factor determines the onset of these disorders. In addition, there are genetic factors. For example, in the simplest forms of familial Parkinson's disease, mutations are associated with dominant forms of the disease. This means that an individual with a single copy of a defective gene will develop the disease, yet two copies of the defective gene are required for recessive forms of the disease to develop. In the case of Alzheimer's disease, and for other less common neurodegenerative diseases, the genetics can be even more complicated, since different mutations of the same gene and combinations of these mutations may differently affect disease risk.

Misfolding in Non-neurological Diseases : Protein aggregation diseases are not exclusive to the central nervous system; they can also appear in peripheral tissues. In general, the genes and protein products involved in these kinds of diseases are called amyloidogenic. Such diseases include type 2 diabetes, inherited cataracts, some forms of atherosclerosis, hemodialysis-related disorders, and short-chain amyloidosis, among many others. All these diseases have in common the expression of a protein outside its normal context, leading to an irreversible change into a sticky conformation rich in beta sheets that make the protein molecules interact with each other. The general pattern that emerges in all these diseases is an abnormal tendency of proteins to aggregate as a result of misfolding. The aggregation can be caused by chance; by protein hyperphosphorylation (a condition where multiple phosphate groups are added to the protein), by prion self-catalytic conformational conversion, or by mutations that make the protein unstable. Aggregation can also be caused by an unregulated or pathological increase in the intracellular concentration of some of these proteins. Such imbalances in protein concentration can be a consequence of mutations such as duplications of the amyloidogenic gene or changes in the protein's amino acid sequence. Imbalances can also be caused by deficiencies in the proteasome, the cellular machinery involved in the degradation of aging proteins. Inhibition of autophagy (a process by which cells engulf themselves) also promotes amyloid aggregation. In addition, some evidence suggests that the severity of these diseases correlates with an increase in oxidative stress, mitochondrial dysfunction, alteration of cytoplasmic membrane permeability, and abnormal calcium concentration.

Probable Questions:

1. Discuss protein folding.

2. Discuss interrelation between protein misfolding and degenerative diseases.

3. Discuss Misfolded Proteins and Neurodegenerative Diseases

Suggested Readings:

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition,

W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw-Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry.

5. Lin, M. T. & Beal, M.F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443, 787–795 (2006) doi:10.1038/nature05292.

UNIT-XIII

Bioenergetics (anaerobic and aerobic respiration, oxidative and substrate level phosphorylation) basic concept of ETC and ATP synthesis, uncouplers. Spontaneous reaction. Thermodynamic principles and steady-state conditions of living organism.

Objective: In this Unit we will learn about bioenergetics, concept of entropy, enthalpy, Gibbs free energy and thermodynamic principles. We will also learn about oxidative phosphorylation and Electron transport chain for the production of cellular ATP.

Bioenergetics:

Energy is defined as a measure of a system's capacity to do work. The various forms of energy inter convertible by suitable means, include potential, kinetic, electrical, heat, chemical, nuclear and radiant energy. Inter-conversions or transformations between these forms of energy can only occur in presence of matter. Energy can exist in the absence of matter only in the form of radiant energy. "The study of energy transformations in living organisms is called as bioenergetics".

The matter has mass and occupies space, while energy has neither mass nor it occupies space. However, energy can transform or act on matter. We can observe energy only by observing its effect on matter. The derived SI unit of energy is joule. Symbol of energy is E.

The energetic of cellular processes may be related to chemical equilibrium and oxidation- reduction potential (redox potential) of chemical reactions. Whether at the level of molecules, cells, tissues, organs, whole organisms or ecosystems, the flow of energy is essential for maintenance of life. Therefore, bioenergetics is sometimes also defined as "field of study concerned with flow of energy (or energy transductions) through living organisms and the nature and function of the chemical processes underlying these transductions." Nearly all living organisms derive their energy, directly or indirectly from the radiant energy of sunlight. The latter arises from thermonuclear fusion reactions carried out in the sun. The thermonuclear fusion reactions carried out in the sun convert four protons ($4H^+$) with atomic mass of $1.0079 \times 4 = 4.0316$ into one helium (He) atom with atomic mass of 4.0026. The remaining mass of 4.0316 – 4.0026 i.e., 0.0290 gm atoms is simultaneously transformed into energy in the form of electromagnetic radiations.

A small part of this radiant energy is in the form of visible light that reaches earth after travelling a long distance of about 160 million km. This conversion of mass into energy is in conformity with Einstein's

equation: $E = mc^2$ (where E is energy; m = mass; c = speed of light). In recent past, the main purpose of bioenergetics has been to unfold or explain intricacies of energy transductions in photosynthesis and respiration and to understand how this energy is used to carry out energy requiring reactions such as ATP synthesis and accumulation of ions across membranes against electrochemical gradients.

The photosynthesizing cells absorb light energy and convert it into chemical energy by driving electrons from water to carbon dioxide and forming energy-rich products such as glucose, starch and sucrose and releasing oxygen into the atmosphere during the process.

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} \underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 + 6H_2O$$

The energy stored in photosynthetic products such as glucose (a carbohydrate) is released during cellular oxidation of these products by passing electrons to atmospheric oxygen into H_2O and CO_2 in respiration. Part of this released energy is conserved in the form of ATP molecules; the rest is lost as heat.

$$C_6H_{12}O_6 + 6O_2 + 6H_2O \longrightarrow 6CO_2 + 12H_2O + \underset{\substack{(2868\ \text{k.J})\\ \text{or}\\ (686\ \text{k. cal.})}}{\text{Energy}}$$

Energy rich ATP molecules are now utilized to drive off various metabolic processes of the cells, thus fulfilling their energy need. In-fact, ATP is the major carrier of chemical energy in all the cells. Almost all energy transductions in the cells can be linked or traced to flow of electrons from one molecule to another in a 'down hill' manner from higher to lower electrochemical potential. As will be discussed later, energy transformations in living cells/organisms, like all other natural processes, are governed by laws of thermodynamics.

Thermodynamics:

Thermodynamics is the science that deals with flow of heat and other forms of energy into or out of a system. The science of thermodynamics arose during 19th century out of efforts to understand working of steam engines and why heat is evolved when boring cannon barrels.

However, the principles of thermodynamics are now universal and apply to all forms of energy and are widely used in every field of science including biology. Thermodynamics, provides an indispensable quantitative framework for understanding energy transformations in/living organisms i.e., bioenergetics.

In thermodynamics, the term system means that region of space or quantity of matter on which one has focused his interest and attention, or in other words "everything within a defined region of space". A system may be a chlorophyll molecule, a cell, a photosynthesizing leaf, a beaker of sugar or salt solution or the

Milky Way Galaxy. The system is separated from its surroundings by a boundary which may be real or definite but very often imaginary. The system and its surroundings constitute the universe (Fig. 26.1).

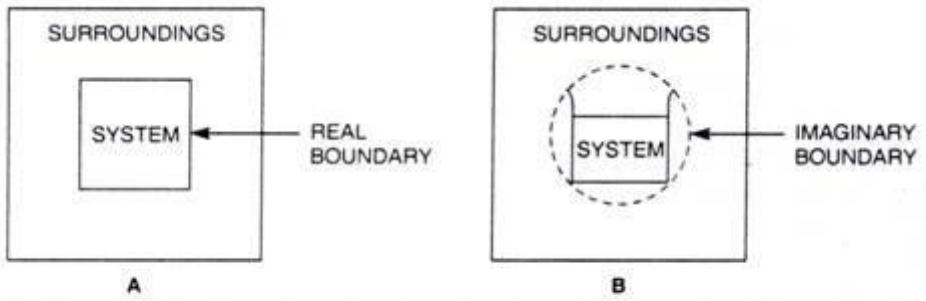


Fig. 26.1. Illustration of a thermodynamic system A. with real boundary: B. with imaginary boundary. The system and the surroundings constitute the universe.

Thermodynamics is often concerned with the energy transfer and/or interactions which take place across the boundary.

Depending upon the nature of boundary, there are three types of thermodynamic systems (Fig. 26.2):

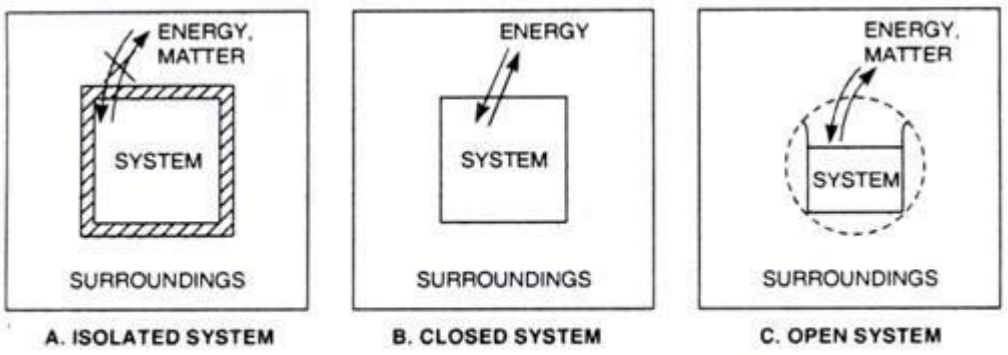


Fig. 26.2. Illustrations of three types of thermodynamic systems A. with sealed & insulated boundary; B. with sealed boundary; C. with imaginary boundary.

(i) Isolated System:

If the boundary is sealed, no matter will pass through it. And, if the boundary is insulated, no energy (e.g., heat) can pass through it. When the boundary is both sealed and insulated, neither matter nor energy will pass through it or in other words there will be no interaction between system and its surroundings. Such a system where neither matter nor energy can pass through it is called as an isolated system (Fig. 26.2A). Example of such a system is hot water contained in a thermos flask.

(ii) Closed System:

In such a system (Fig. 26.2 B), the boundary is sealed but not insulated so that only energy (in the form of heat, work and radiation) and not matter can be transferred to and from its surroundings. A specific quantity

of hot water contained in a sealed vessel is an example of closed system where no water vapours (matter) can escape from the system. It can only transfer heat (energy) through the walls of the vessel into the surroundings.

(iii) Open System:

In such a system (Fig. 26.2 C), the boundary is neither sealed nor insulated, so that both energy and matter can be transferred across it. For example, hot water contained in a beaker is an open system where both energy (heat) and matter (water vapours) are transferred across the imaginary boundary into the surrounding. A living organism is also an open system which exchanges both matter and energy with its surroundings. The living organisms exist in dynamic steady state and never at equilibrium with their surroundings. To maintain this steady state, they require continuous input of energy. When cells can no longer generate energy, the organism dies and begins to decay towards equilibrium with its surroundings. Thermodynamics is the study of energy changes, that is, the conversion of energy from one form into another. Such changes obey the first two laws of thermodynamics.

The First Law of Thermodynamics:

The first law is concerned with the conversion of energy within a "system," where a system is defined as a body (e.g., a cell or an organism) and its surroundings.

This law, which applies to both biological and non-biological systems, states the following: Energy cannot be created or destroyed but can be converted from one form into another: during such a conversion, the total amount of the energy of the system remains constant. This law applies to all levels of organization in the living world; it applies to organisms, cells, organelles, and to the individual chemical reactions that characterize metabolism. In practice, it is difficult to measure the energy possessed by cells (i.e., to limit the "system" to an individual cell), because energy may escape into the environment surrounding the cell during the measurement.

Similarly, energy may be acquired by the cell from its environment; for example, a photosynthesizing cell absorbs energy from its environment in the form of light. A cell's acquisition of energy from its environment (or its loss to the environment) should not be confused with the destruction or creation of energy, which according to the first law of thermodynamics does not occur. From a biological viewpoint, the first law of thermodynamics indicates that at any given moment a cell possesses a specific quantity of energy.

This energy takes several forms; it includes:

(1) Potential energy (e.g., the energy of the bonds that link atoms together in a molecule or the pressure-volume relationships within the cell as a whole or within membrane- enclosed intracellular components);

(2) Electrical energy (e.g., the distribution of different amounts of electrical charge across cellular membranes); and

(3) Thermal energy (e.g., the temperature-dependent constant and random motions of molecules and atoms).

According to the first law, these forms of energy may be inter-converted; for example, some of the cell's potential energy can be converted into electrical or thermal energy, but the cell cannot create or destroy energy. When a cell breaks down a polysaccharide to ultimately form CO_2 and H_2O, some of the potential energy present in the carbohydrate is conserved as potential energy by phosphorylating ADP, thereby forming ATP.
The ATP so produced represents a new energy source (and also one that is of greater immediate utility for the cell). However, not all of the energy of the original carbohydrate is conserved as potential energy; some of it becomes thermal energy and is transferred to the surroundings as heat. It is important to recognize that none of the energy is destroyed and it should be possible to account for all of the energy originally present in the polysaccharide in other forms within the system (i.e., in the ATP that is produced and in the heat that is released).

The Second Law of Thermodynamics:

The first law of thermodynamics tells us that the total energy of an isolated system consisting of a cell (or organism) and its surroundings is the same before and after a series of events or chemical reactions has taken place. What the first law does not tell us is the direction in which the reactions proceed.

This problem can be illustrated using a simple example. Suppose we place a small cube of ice in a liter of hot water, seal the combination in an insulated container (e.g., a vacuum bottle), and allow the system (i.e., the ice and the water) to reach an equilibrium. In such a system, we would not be surprised to find that the ice melts and that this is accompanied by a decrease in the temperature of the water. When we later examine the system, we find that we are left only with water (no ice) and that the water is at a reduced temperature.

The flow of heat, which is thermal energy, from the hot water to thrice thereby causing the ice to melt is spontaneous and the energy that is "lost" by the water is "gained" by the melting ice so that the total energy of the system remains the same.

We certainly would not expect ice to form spontaneously in a sealed system that contains warm water, even though such an eventuality is not prohibited by the first law. Consequently, the important lessons to be learned from this illustration are that energy changes have direction and may be spontaneous.

To anticipate the spontaneity of a reaction and predict its direction, one must take into account a function called entropy. Entropy is a measure of the degree of randomness or disorder of a system, the entropy increasing with increasing disorder. Accordingly, the second law of thermodynamics states: In all processes involving energy changes within a system, the entropy of the system increases until an equilibrium is attained. In the illustration that was presented above, the highly ordered distribution of energy (i.e., large amounts of energy in the hot water and smaller amounts of energy in the ice) was lost as the ice melted to form water. In the resulting warm water, the energy was more randomly and uniformly distributed among the water molecules.

The units of entropy are J/mole (or cal/mole), indicating that entropy is measured in terms of the amount of substance present. When equal numbers of moles of a solid, liquid, and gas are compared at the same temperature, the solid has less entropy than the liquid and the liquid has less entropy than the gas (the gaseous state is the state of greatest disorder). Entropy can be thought of as the energy of a system that is of no value for performing work (i.e., it is not "useful" energy). For example, the catabolism of sucrose or other sugars by a cell is accompanied by the formation of energy-rich ATP. Although superficially it may appear as though useful energy has increased in the form of the ATP gained by the cell, the total amount of useful energy has actually decreased and the amount of unavailable energy increased. It is true that some of the potential energy of the sugar has been converted to potential energy in the form of ATP, but some has also been converted to thermal energy, which tends to raise the temperature of the cell and therefore its entropy.

Suggestions that cells can decrease entropy by carrying out photosynthesis are misleading. Although it is true that during photosynthesis cells convert molecules with very little potential energy (CO_2 and H_2O) into larger molecules with considerably more potential energy (sugars) and that there is an accompanying decrease in the entropy of the cell, energy in the form of light was absorbed from the cell's environment. Because the light energy consumed during photosynthesis is a part of the whole system (i.e., the cell and its surroundings), it is clear that there has actually been an overall decrease in useful energy and an increase in entropy (see Fig. 9-4).

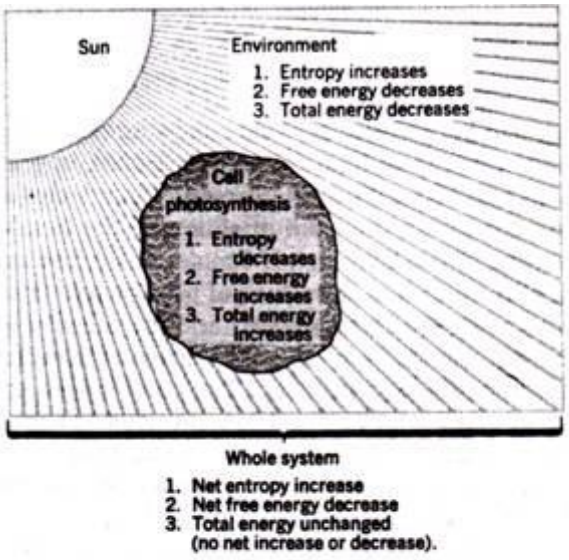


FIGURE 9–4 Relationships between the changes in entropy and free energy within a whole system and within its parts.

The entropy change during a reaction may be quite small. For example, when sucrose undergoes hydrolysis to form the sugars glucose and fructose, much of the potential energy of the original sucrose is present in the resulting glucose and fructose molecules. Changes in entropy are extremely difficult to calculate, but the difficulty can be circumvented by employing two other thermodynamic functions: enthalpy or heat content (denoted H) and free energy (denoted G). The change in a system's enthalpy (ΔH) is a measure of the total change in energy that has taken place, whereas the change in free energy (ΔG) is the change in the amount of energy available to do work. Changes in entropy (ΔS), enthalpy, and free energy are related by the equation in which T is the absolute temperature of the system.

$$\Delta G = \Delta H - T\Delta S \quad \ldots(9\text{-}1)$$

The change in free energy can also be defined as the total amount of free energy in the products of a reaction minus the total amount of free energy in the reactants, that is,

$$\Delta G = G\,(\text{products}) - G(\text{reactants}) \quad \ldots(9\text{-}2)$$

A reaction that has a negative ΔG value (i.e., the sum of the free energy of the products is less than that of the reactants) will occur spontaneously, a reaction for which the ΔG is zero is at equilibrium; and a reaction that has a positive AG value will not occur spontaneously and proceeds only when energy is supplied from some outside source.

ΔG, ΔG^0, and $\Delta G^{0'}$ Values

The hydrolysis of sucrose

Sucrose + H_2O → glucose + fructose

has a negative AG value, and therefore when sucrose is added to water, there is the spontaneous conversion of some of the sucrose molecules to glucose and fructose. However, the reverse reaction

glucose + fructose →sucrose + H_2O

has an equal but positive ΔG value and therefore does not occur without an input of energy. Hence, special attention must be paid to the direction in which the reaction is written (i.e., the direction of the arrow) and the sign of the ΔG value. If 5 moles of sucrose are mixed with water, the formation of glucose and fructose will take place spontaneously and the ΔG may be determined; this value is, of course, greater than if 4 or 2 miles of sucrose are used.

Thus, ΔG values are dependent on the amounts and concentrations of reactants and products. More uniform standards of reference that have been established by convention are the standard free energy changes, ΔG^0 and $\Delta G^{0'}$ values. ΔG^0 represents the change in free energy that takes place when the reactants and products are maintained at 1.0 molar concentrations (strictly speaking, 1.0 molar) during the course of the reaction and the reaction proceeds under standard conditions of temperature (25°C) and pressure (1 atmosphere) and at pH 0.0. The $\Delta G^{0'}$ value is a much more practical term for use with biological systems in which reactions take place in an aqueous environment and at a pH that usually is either equal or close to 7.0. The $\Delta G^{0'}$ value is defined as the standard free energy change that takes place at pH 7.0 when the reactants and products are maintained at 1.0 molar concentration (Table 9-2).

TABLE 9–2 FREE ENERGY: SYMBOLS, DEFINITIONS, AND EXPLANATIONS

Symbol	Definition	Explanation
G	Free energy (also called Gibbs free energy)	The maximum energy that can be derived from a particular molecule capable of doing work under conditions of constant temperature and pressure.
ΔG	Change in free energy	The change in free energy that takes place during a chemical reaction (equal to the free energy of the products of the reaction minus the free energy of the reactants.)
ΔG^0	*Standard* free energy change[a]	An expression more frequently used in physical chemistry than in biochemistry. The change in free energy that takes place under the following standard conditions: reactants and products are maintained at 1.0 molal concentrations; the temperature is 25°C; the pressure is 1 atm; and the pH is 0.0.
ΔG^0	*Standard* free energy change[a]	Expression used in biochemistry and physiology. Change in free energy under the following standard conditions: reactants and products are maintained at 1.0 molar concentrations; the temperature is 25°C; the pressure is 1 atm; and the pH is 7.0.
$\Delta G'$	*Standard* free energy change[a]	Expression rarely used anymore same as ΔG^0 but not necessarily at pH 0.0. The pH value must be specified.
$\Delta G‡$	Free energy of activation	A measure of the activation energy of a chemical reaction; does not provide information about the direction or equilibrium of the reaction. (See Chapter 8.)

The changes in standard free energy are independent of the route that leads from the initial reactants to the final products. For example, glucose can be converted to carbon dioxide and water either by combustion in the presence of oxygen or through the actions of cellular enzymes. Changes in standard free energy are the same, regardless of the method that is used; thus, the value of the standard free energy change provides no information about the reaction sequence by which the change has taken place. By the same token, the values obtained for changes in standard free energy tell us nothing about the rate at which the changes have taken place.

The ΔG^{0}‘ can be calculated from the equilibrium constant, K'_{eq}, of a reaction using the relationship

$$\Delta G^{0}\text{‘}=-RT\ \mathrm{In}K'_{eq} \quad (9\text{-}3)$$

$$= -2.303\ R\ T\ \log_{10} K'_{eq} \quad (9\text{-}4)$$

Where R is the gas constant (8.314 J/mole/degree), T is the absolute temperature (in degrees Kelvin), -and K'_{eq} is the equilibrium constant. Table 9-3 lists a number of ΔG^{0}‘ values for common reactions.
For the reaction

$$A+B\rightarrow C+D$$

The equilibrium constant is defined as $K'_{eq} = [C][D]$ at equilibrium (9-5)

Where [A] and [J3] are the concentrations of the reactants and [C] and [D] are the concentrations of the products. If the equilibrium constant is 1.0, then the ΔG^{0}‘ value equals zero. If the equilibrium constant is greater than 1.0, then the ΔG^{0}‘ value is negative (e.g., -11.41 kJ/mole for a K'_{eq} value of 100), and the reaction is said to be exergonic (i.e., “energy releasing”) because it proceeds spontaneously in the direction written when starting with unimolar concentrations of reactants and products.
When the K'_{eq} value is less than 1.0, the ΔG^{0}’ value is positive (e.g., 5.71 kJ/mole for a K'_{eq} of 0.1), and the reaction is said to be endergonic (i.e., “energy consuming”) because it does not proceed spontaneously in the direction written when starting with unimolar concentrations of reactants and products.
Calculations of AG^{0}‘ values are usually based on experimental measurements of isolated reactions, that is, with reactions that take place independently of other reactions and that are not associated with cells. ΔG^{0} and ΔG^{0}‘ values do not provide information about the free energy changes of reactions as they might take place in cells or under conditions in which the concentrations of reactants and prod acts, pH, etc., may change. This may be dramatically illustrated by considering the following example. At pH 7.0 and 25°C, the equilibrium constant for the reaction dihydroxyacetone phosphate → glyceraldehyde-3-phosphate is 0.0475. Therefore, using equation 9-3,

$$\Delta G^{0}\text{’} = -2.303\ RT\ \log_{10} K'_{eq}$$

$$= -2.303\ (8.314\ \text{J/mole/degree})\ (298)\ \log_{10}(0.0475)$$

$$= +7.55\ \text{kJ/mole}$$

The positive value indicates that this reaction does not proceed spontaneously in the direction written. However, in cells, this reaction is but one of a series of reactions in a metabolic pathway called glycolysis. Other reactions of glycolysis that occur prior to this one and that have negative $\Delta G^{0\text{‘}}$ values produce additional substrate (i.e., dihydroxyacetone phosphate) and reactions with negative $\Delta G^{0\text{‘}}$ values that occur after this step remove the product glyceraldehyde-3-phosphate.

As a result, the reaction proceeds in the direction written under the conditions specified above, even though the $\Delta G^{0\text{‘}}$ value is positive. This example illustrates the important point that the $\Delta G^{0\text{‘}}$ value for a specific biological reaction cannot be used to predict reliably whether or not that particular reaction is actually taking place within the cell.

TABLE 9–3 STANDARD FREE ENERGY CHANGES OF COMMON BIOCHEMICAL REACTIONS AT pH 7.0 AND 25°C

Reaction	$\Delta G^{0\text{‘}}$ kJ/mole	$\Delta G^{0\text{‘}}$ kcal/mole
Hydrolysis:		
Acid anhydrides:		
Acetic anhydride + $H_2O \rightarrow$ 2 acetate	−91.2	−21.8
Pyrophosphate + $H_2O \rightarrow$ 2 phosphate	−33.4	−8.0
Esters:		
Ethyl acetate + $H_2O \rightarrow$ ethanol + acetate	−19.7	−4.7
Glucose-6-phosphate + $H_2O \rightarrow$ glucose + phosphate	−13.8	−3.3
Amides:		
Glutamine + $H_2O \rightarrow$ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + $H_2O \rightarrow$ 2 glycine	−9.2	−2.2
Glycosides:		
Sucrose + $H_2O \rightarrow$ glucose + fructose	−29.3	−7.0
Maltose + $H_2O \rightarrow$ 2 glucose	−16.7	−4.0
Esterification:		
Glucose + phosphate $\rightarrow$ glucose-6-phosphate + H_2O	+13.8	+3.3
Rearrangement:		
Glucose-1-phosphate $\rightarrow$ glucose-6-phosphate	−7.11	−1.7
Fructose-6-phosphate $\rightarrow$ glucose-6-phosphate	−1.67	−0.4
Elimination:		
Malate $\rightarrow$ fumarate + H_2O	+3.14	+0.75
Oxidation:		
Glucose + 6 $O_2 \rightarrow$ 6 CO_2 + 6 H_2O	−2870	−686
Palmitic acid + 23 $O_2 \rightarrow$ 16 CO_2 + 16 H_2O	−9782	−2338

:

Mitochondrial Electron Transport Chain:

The mitochondrial electron transport chain is composed of three main membrane-associated electron carriers flavoproteins (FMN, FAD), cytochromes, and quinones (coenzyme Q, also known as ubiquinone because it is a ubiquitous quinone in biological systems). All these electron carriers reside within the inner membrane of the mitochondria and operate together to transfer electrons from donors, like NADH and $FADH_2$, to acceptors, such as O_2. The, electrons flow from carriers with more negative reduction potentials to those with more positive reduction potentials and eventually combine with O_2 and H to form water.

However, the mitochondrial electron transport system is arranged into four enzyme complexes of carriers, each capable of transporting electrons part of the way to O_2 (Fig. 24.5). Coenzyme Q and cytochrome c connect the complexes with each other. The four enzyme complexes of carriers are: NADH-Q oxidoreductase, succinate-Q-reductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase. These complexes are the enzyme complex and each of them consists of different prosthetic groups (Table 24.2).

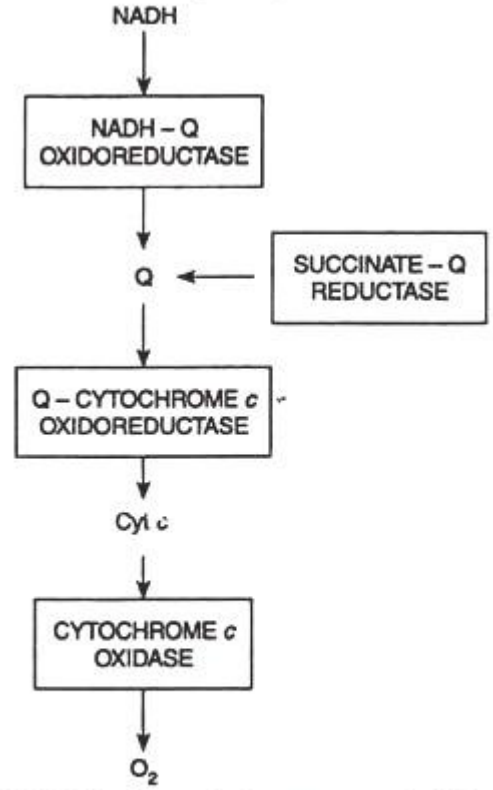


FIG. 24.5. Sequential arrangement of four complexes of electron carriers in mitochondrial electron transport chain.

TABLE 24.2. Four enzyme complexes of mitochondrial electron transport chain

	Enzyme complex	*Mass (KDa)*	*Number of subunits*[1]	*Prosthetic groups*
Complex I :	NADH-Q oxidoreductase (NADH dehydrogenase)	880	42(14)	FMN, FeS
Complex II:	Succinate-Q reductase (succinate dehydrogenase)	140	4	FAD, FeS
Complex III:	Q-cytochrome *c* oxidoreductase	250	10	Cyt b_H (Heme b_H), Cyt b_L (Heme b_L), FeS, Cyt c_L (Heme c_L)
Complex IV :	Cytochrome *c* oxidase	160	10 (3-4)	Cyt *a* (Heme *a*), Cyt a_3 (Heme a_3), Cu_A, Cu_B

The process of mitochondrial electron transport chain is summarized in Figure 24.6, which shows the flow of electrons and protons through the four enzyme complexes of the transport chain.

The whole process of mitochondrial electron transport can be represented in brief in the following manner:

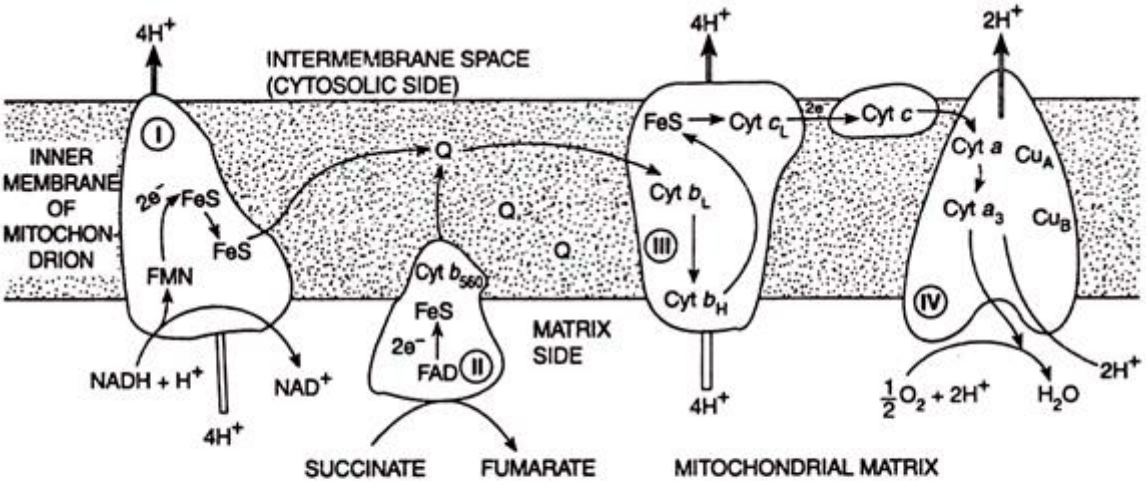


FIG. 24.6. Summary of mitochondrial electron transport chain showing the flow of electrons and protons (H^+) through the four enzyme complexes of the transport chain. Electrons reach quinone (Q) through complexs I and II. Q serves as a mobile carrier of electrons and passes them to complex III, which then passes them to cytochrome *c*, another mobile connecting link. Complex IV then transfers electrons from reduced cytochrome *c* to O_2. Electron flow through complexes I, III and IV is accompanied by proton flow from the mitochondrial matrix to the intermembrane space (cytosolic side)

1. Electrons donated by NADH enter the chain at complex I (NADH-Q-oxidoreductase) and pass through a flavoprotein (FMN) to a series of iron-sulphur-proteins (FeS) and then to ubiquinone (Q).

2. Electrons donated by succinate enter the chain at Complex II (succinate-Q-reductase) and pass through a flavoprotein (FAD) and FeS centres and then to ubiquinone (Q).

3. Ubiquinone (Q) serves as a mobile carrier of electrons received from complexes I and II and passes them to complex III (Q-cytochrome c oxidoreductase).

4. Complex III called Q-cytochrome c oxidoreductase or cytochrome bc_1 complex passes the electrons through its prosthetic groups Cyt b_L (Heme b_L), Cyt b_H (heme b_H), FeS, and Cyt c_L (Heme c_L) to cytochrome c.

5. Cytochrome c (Cyt c), a mobile connecting link between complex III and IV, passes electrons to complex IV (cytochrome c oxidase). The latter carries electrons through its prosthetic groups Cyt a (Heme a), Cyt a_3 (Heme a_3) Cu_A and Cu_B and transfers them to molecular oxygen, reducing it to H_2O.

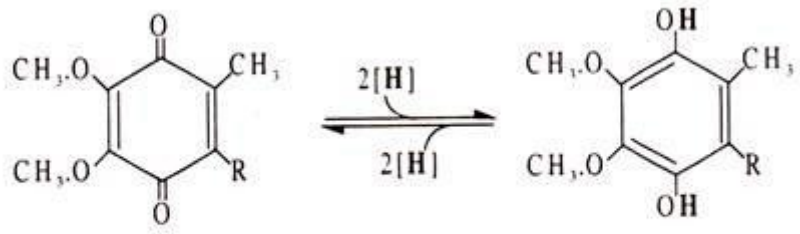
6. Electron flow through complexes I, III and IV is accompanied by proton flow from the mitochondrial matrix (which becomes negatively charged) to inter membrane space or cytosolic side (which becomes positively charged). The number of protons (H^+) moved across the membrane at each site per pair of

electrons transported is still somewhat uncertain; the current consensus is that at least 10 protons move outward during NADH oxidation.
Catabolism of energy-giving substrates (mostly glucose) up to the stage of pyruvic acid through EMP or EDP yields comparatively small amount of energy in the form of ATP by substrate-level phosphorylation. The anaerobic organisms have to be satisfied with this small amount of energy.

But the aerobic organisms can extract much larger quantity of energy from oxidation of the same amount of substrate through the TCA cycle and the electron transport system in which the H-atoms — transferred to specific acceptors, like NAD and FAD in the TCA cycle — are fully oxidized in course of electron transport. The ultimate acceptor of H^+ and electrons is oxygen in oxygen-respiring organisms. In course of the electron transport through several carriers, ATP is generated by oxidative phosphorylation. As it will be seen, from every mole of glucose oxidized, 38 moles of ATP are formed, in contrast to only two moles in EMP and one mole in EDP.

The ETS is a sequence of carrier molecules which are capable of alternately acting as an electron (or hydrogen) acceptor and electron donor, i.e. undergoing alternate reduction and oxidation. The components of ETS are located in the inner membrane of the mitochondria in eukaryotes and in the cytoplasmic membranes in prokaryotes. There are three classes of carrier molecules. The first of these are flavoproteins which contain FMN (flavin mononucleotide) as coenzyme. An example of this class is $NADH_2$ dehydrogenase which contains, in addition to FMN, a non-haem iron. The function of this enzyme is to transfer hydrogen from $NADH_2$ to ubiquinone.

Ubiquinone or coenzyme Q constitutes another class of carrier molecule which can reversibly accept hydrogen atoms. It is a benzoquinone derivative with a long isoprenoid chain (R).



Ubiquinone (Coenzyme Q) Ubihydroquinone

The third and the most important class of electron carrier molecules are constituted by the cytochromes. There are several different cytochromes, but all of them are not present in all organisms, but some members of this class are present in all aerobic organisms.

All cytochromes are composed of an iron-porphyrin prosthetic group known as haemin ring (also present in haemoglobin) attached to a protein. The haemin group is common to all cytochromes which differ from each other in their protein component. The central iron atom of the haemin ring undergoes reversible valency change with reduction or oxidation.

The terminal cytochrome of the ETS is cytochrome oxidase. It reacts with oxygen and transfers two electrons to oxygen to form doubly negatively charged oxygen atom which combines with $2H^+$ to form a molecule of water.

In the respiratory chain hydrogen or electrons move from a negative redox-potential to a positive one. For example, NAD/$NADH_2$ has a redox-potential of – 0.32 volts while oxygen has a redox- potential of + 0.81 volts. During the passage from the negative to the positive potential, there is a fall in the free-energy which can be trapped for phosphorylation of ADP to form ATP. This mode of ATP formation is known as oxidative phosphorylation.

The passage of hydrogen and electrons through the ETS is shown in a simplified manner in Fig. 8.49 and in details in Fig. 8.50:

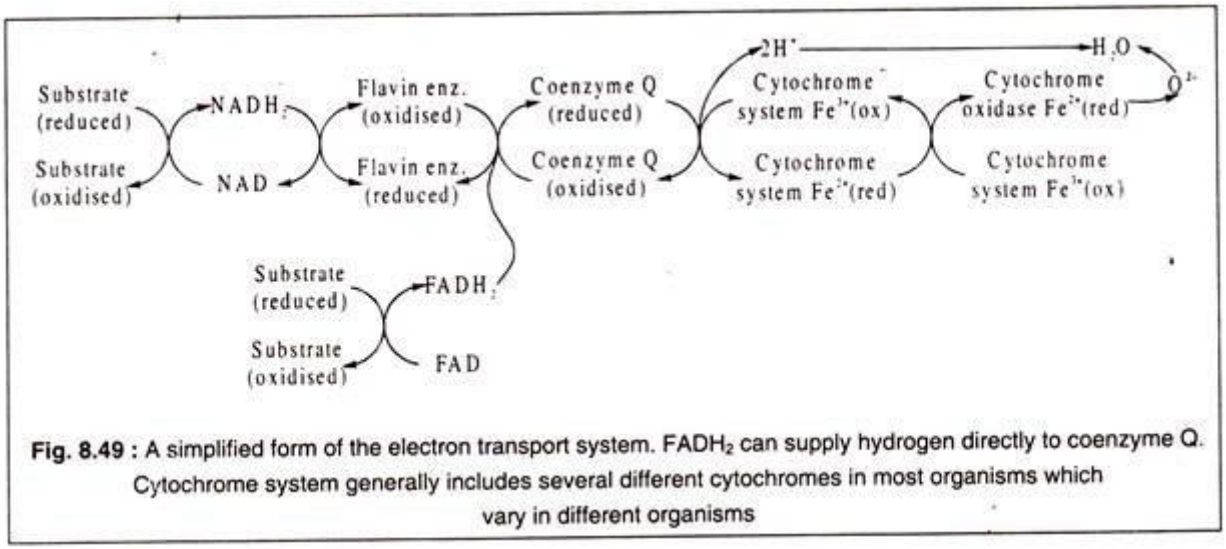


Fig. 8.49 : A simplified form of the electron transport system. $FADH_2$ can supply hydrogen directly to coenzyme Q. Cytochrome system generally includes several different cytochromes in most organisms which vary in different organisms

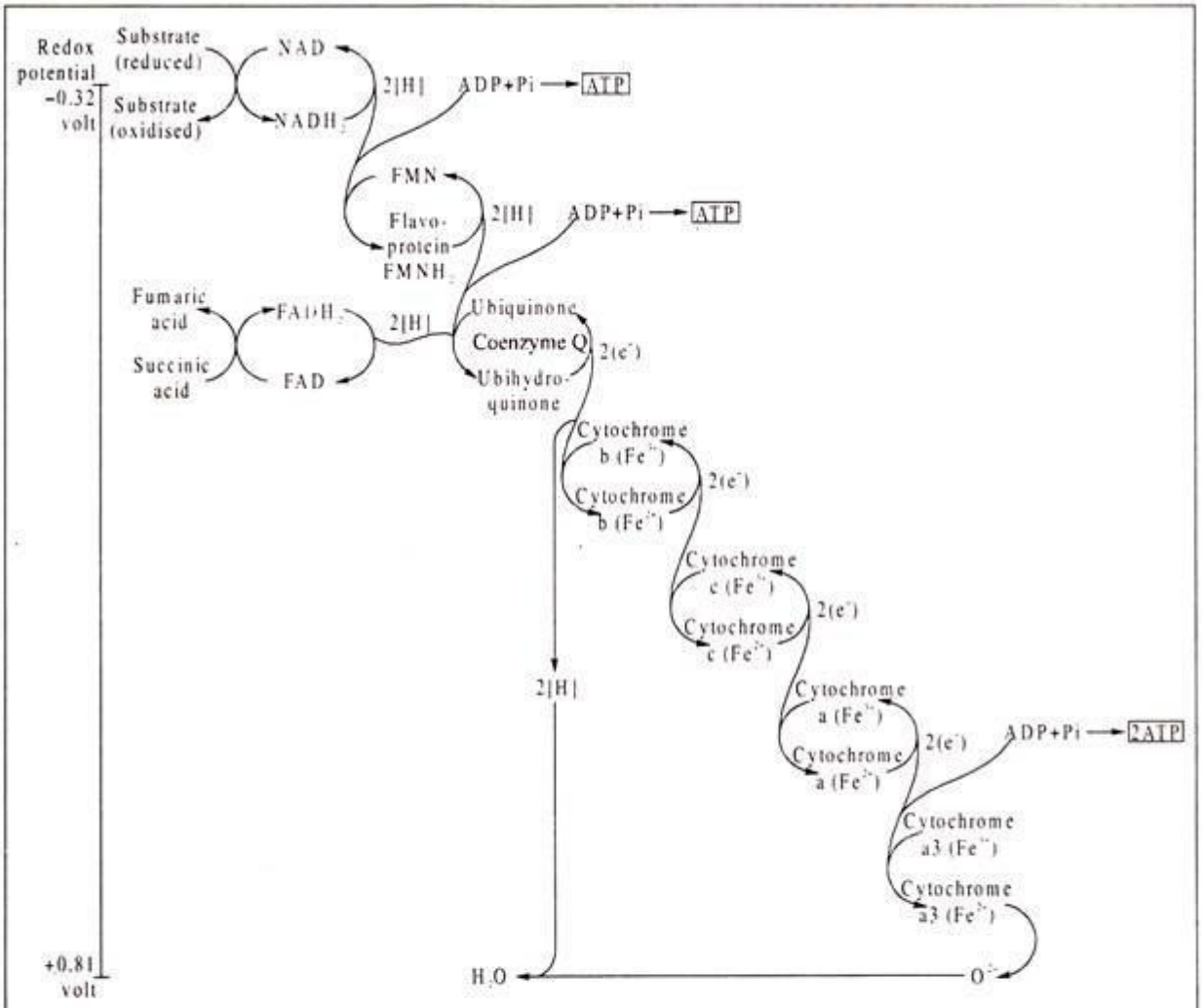


Fig. 8.50 : Electron transport system in aerobic respiration — a generalized representation. The last cytochrome is cytochrome oxidase which transfers two electrons to oxygen. The oxygen atom with two electrons combines with two H^+ ions released by ubiquinone (Co Q) to produce one molecule of H_2O. The other cytochromes, like b, c, a etc., may vary from organism to organism. $FADH_2$ produced by succinic acid dehydrogenase reaction can feed hydrogen directly to coenzyme Q which is oxidized through the cytochrome system. ATP can be generated in three steps as shown in the figure, where the fall in the free energy is sufficient to phosphorylate ADP i.e. ΔG is –7 Kcal or more

From Fig. 8.50 it may be observed that electron transport chain begins by transfer of high energy protons and electrons which have a redox potential of – 0.32 volt to gradually diminishing redox- potential. The downhill flow of electrons releases free energy which is trapped in ATP molecules. The production of ATP by oxidative phosphorylation of ADP can be explained by the chemiosmotic mechanism. This theory postulates that the transport of electrons by the carrier molecules which are located in the membrane creates a gradient of H^+-ions across the membrane. This is possible because the membrane is impermeable to H^+-ions.

The proton-gradient is formed by active transport of H^+-ions by the proton pumps outwards. In case of mitochondria, H^+-ion concentration increases in the space between the outer and inner membrane compared to the H^+-ion concentration in the matrix. In case of bacteria, the H^+-ion concentration in the surrounding medium becomes higher compared to that of the cytoplasm. The proton gradient created by active expulsion of H^+-ions by proton pumps also creates an electric charge gradient, because of accumulation of excess of positively charged H^+-ions on one side of the membrane. The resulting electrochemical gradient has potential energy which is known as the proton motive force (pmf).

Protons expelled by the proton pumps can cross the membrane only through some special proton channels where an enzyme ATP synthase is located. When the protons pass through these channels the potential energy is released and it is utilized by the enzyme for synthesis of ATP from ADP and inorganic phosphoric acid. Both eukaryotic organisms and prokaryotes use the chemiosmotic mechanism for ATP synthesis.

ATP formation by this mechanism in bacterial cells is shown diagrammatically in Fig. 8.51:

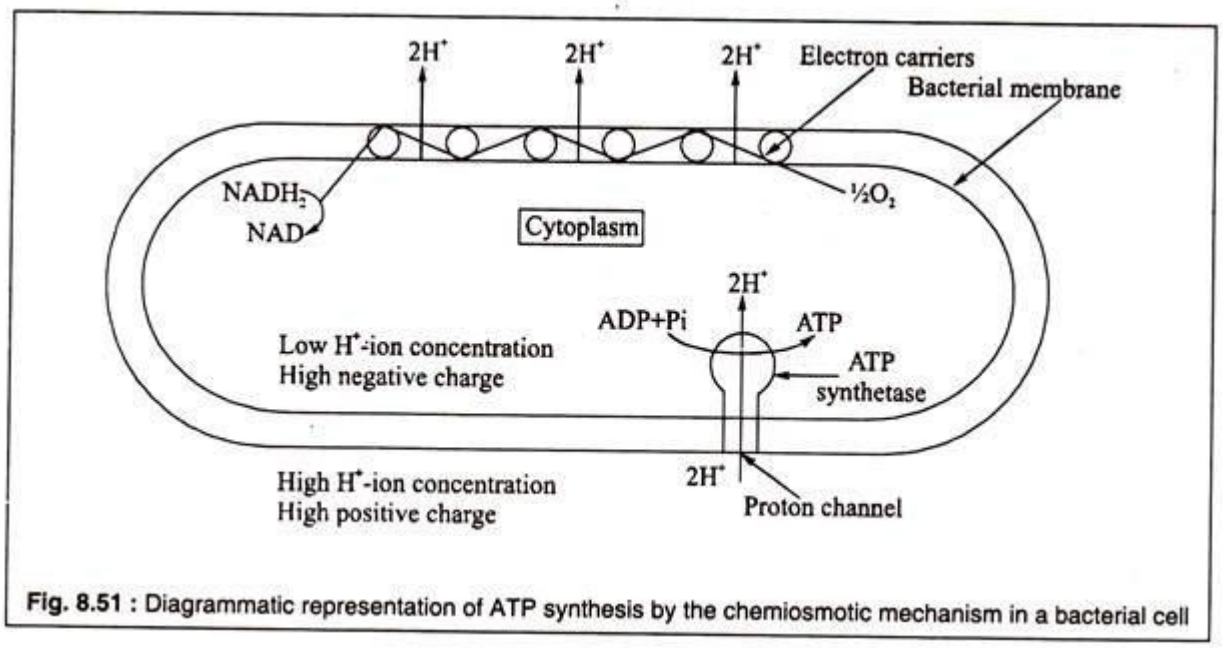


Fig. 8.51 : Diagrammatic representation of ATP synthesis by the chemiosmotic mechanism in a bacterial cell

ATP yield in aerobic respiration:

The total yield of ATP when glucose is fully oxidized to CO_2 and H_2O amounts to 38 moles per mole of glucose. It may now be examined in which steps this amount of ATP is formed (Table 8.3). For calculating ATP yield, it should be noted that $NADH_2$ can generate 3 ATP through ETS and $FADH_2$ can produce 2 ATP (Fig. 8.50).

Table 8.3 : ATP formation in aerobic respiration

Pathway	Step	ATP yield	Mode of ATP formation
EMP (Glucose → Pyruvic acid)	(i) 1.3-diphosphoglyceric acid → 3-phosphoglyceric acid	2 ATP	Substrate-level phosphorylation
	(ii) Phosphoenol pyruvic acid → Pyruvic acid	2 ATP	
[In EMP, 2ATP are consumed and 4ATP are produced, so the net gain is 2ATP per glucose molecule].		Net ATP yield is 2 ATP	
EMP	(iii) Glycerin aldehyde phosphate → 1,3-diphosphoglyceric acid NAD → $NADH_2$ for each half of glucose i.e. $2NADH_2$	6 ATP	Oxidative phosphorylation
Pyruvic acid decarboxylase reaction	(iv) Pyruvic acid → Acetyl CoA NAD → $NADH_2$ for each half of glucose i.e. $2NADH_2$	6 ATP	Oxidative phosphorylation
TCA-cycle [As acetyl-CoA which is fed into the TCA cycle originates from half-molecule of glucose, so the number of GTP or $NADH_2$ molecules have to be doubled for calculating energy yield]	(v) Succinyl CoA → Succinic acid GDP+Pi→GTP·ATP	2 ATP	Substrate-level phosphorylation
	(vi) Isocitric acid→α-Ketoglutaric acid NAD→$NADH_2$	6 ATP	Oxidative phosphorylation
	(vii) α-Ketoglutaric acid→Succinyl CoA NAD→$NADH_2$	6 ATP	"
	(viii) Malic acid→Oxalacetic acid NAD→$NADH_2$	6 ATP	"
	(ix) Succinic acid→Fumaric acid FAD→$FADH_2$	4 ATP	"
Total yield 38 ATP			

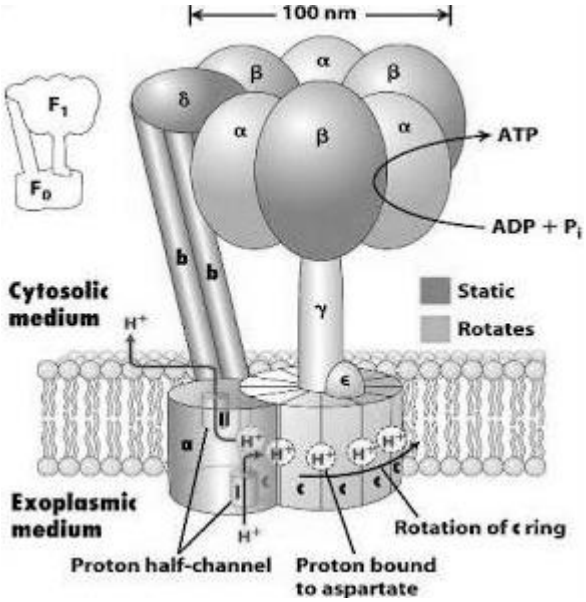
So, the overall reaction of glucose oxidation in aerobic respiration can be written as:

$$C_6H_{12}O_6 + 6O_2 + 38\ ADP + 38\ Pi \rightarrow 6CO_2 + 6\ H_2O + 38\ ATP$$

When 1 mole of glucose is oxidized under non-biological conditions, 674 K calories of energy is liberated as heat. Now, it can be calculated how much of this energy is preserved in the form of ATP. Taking an average value of energy liberated by ATP hydrolysis producing ADP + Pi as -Δ K calories (ATP ADP + Pi, ΔG = -7 K cal), it is observed that a little less than 40% of the total energy released by glucose oxidation is conserved as ATP in aerobic respiration. Thus the efficiency of biological oxidation is nearly 40%. The rest of the energy is given out in the form of heat.

Oxidative phosphorylation :

It is the synthesis of ATP from ADP and inorganic phosphate, which occurs with the help of energy obtained from oxidation of reduced coenzymes formed in cellular respiration.In 1961, Mitchell proposed a mechanism for the coupling of electron transfer through mitochondria to ATP synthesis. He suggested that, as electrons are passed down the electron transport chain, protons are pumped across the membrane. This results in a pH and electrical gradient. The protons move back into the matrix through a pore created by ATP synthase allowing the enzyme to produce ATP at the expense of this gradient. This has been called the "chemiosmotic theory".



The ATP synthase, which is embedded in cristae of the inner mitochondrial membrane, includes two major subunits:

1. F_1: the catalytic subunit, made of 5 polypeptides with stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$.

2. F_0 : a complex of integral membrane proteins that mediated proton transport.

The F_1F_0 complex couples ATP synthesis to H^+ transport into the mitochondrial matrix. Transport of at least $3H^+$ per ATP synthesized is required. The phosphorus/oxygen ratio (P/O) that is ATP made per oxygen atom reduced is about 3 for NADH and 2 for $FADH_2$.

ATP produced in the mitochondria must exit to the cytosol to be used by transport pumps, kinases, etc. ADP and Pi, arising from ATP hydrolysis in the cytosol, must re-enter the mitochondria to be converted again to ATP. Two carrier proteins in the inner mitochondrial membrane are required for this metabolic cycle. The outer membrane is considered to be not a permeability barrier. The large VDAC channels in the outer membrane are assumed to allow passage of adenine nucleotides and Pi. The Adenine Nucleotide Translocase (ADP/ATP carrier) is an antiporter that catalyzes exchange of ADP for ATP across the inner mitochondrial membrane.

Total ATP production from TCA cycle:

Isocitrate to Oxalosuccinate (1 molecule of NADH) →	3 molecules of ATP
α-ketogluterate to Succinyl CoA (1 molecule of NADH) →	3 molecules of ATP
Succinyl CoA to Succinate (substrate level phosphorylation)→	1 molecule of ATP
Succinate to Fumerate (1 molecule of $FADH_2$) →	2 molecules of ATP
Malate to Oxaloacetate (1 molecule of NADH) →	3 molecules of ATP
Total →	12 molecules of ATP

Total ATP production in complete oxidation of one molecule of glucose :

One molecule of glucose forms 2 molecules of pyruvic acid by glycolysis

From glycolysis (2 molecules of NADH 2 molecule of ATP) → 8 molecules of ATP

From oxidation of pyruvate (1 molecule of NADH) →	6 molecules of ATP (3 x 2)
From TCA cycle →	24 molecules of ATP (12x2)
Total à	**38 molecules of ATP**

Probable questions :

1. Define Bioenergetics. State the laws of thermodynamics.

2. Describe the role of energy Phosphates in Bioenergetics & energy capture.

3. Write a note on Role of High-energy Phosphates as the "Energy Currency" of the Cell.

4. Briefly describe the oxidative decarboxylation of pyruvic acids in cell.

5. What is Krebs Cycle. Describe the significance of Krebs cycle.

6. What is Electron Transport Chain? Describe the different complex of ETC.

7. What is Oxidative Phosphorylation. Describe the structure of F_0F_1 particle.

8. How many ATP are produced in complete oxidation of one molecule of glucose?

Suggested Readings and References :

1. http://www.biologydiscussion.com/biochemistry/bioenergetics/essay-on-bioenergetics-biochemistry/42121

2. Lehninger Principles of Biochemistry. Nelson and Cox. Seventh edition.

3. Fundamentals of Biochemistry. J L Jain, Nitin Jain, Sunjay Jain. S. Chand Publications.

4. Biochemistry, by Jeremy M. Berg and John L. Tymoczko. Seventh Edition

UNIT-XIV

Biosynthesis of cholesterol, control of cholesterol biosynthesis,

Objective: In this unit we will learn about cholesterol, its distribution, function and steps of biosynthesis. We will also learn about how cholesterols are transported through the blood by lipoproteins.

Cholesterol and its structure:

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. It is therefore of critical importance that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol, as well as cholesterol synthesized de novo by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins sent to the peripheral tissues. In humans, the balance between

cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life- threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-cerebro and peripheral vascular disease. Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings called the "steroid nucleus", and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17. Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbon 5 and carbon 6.

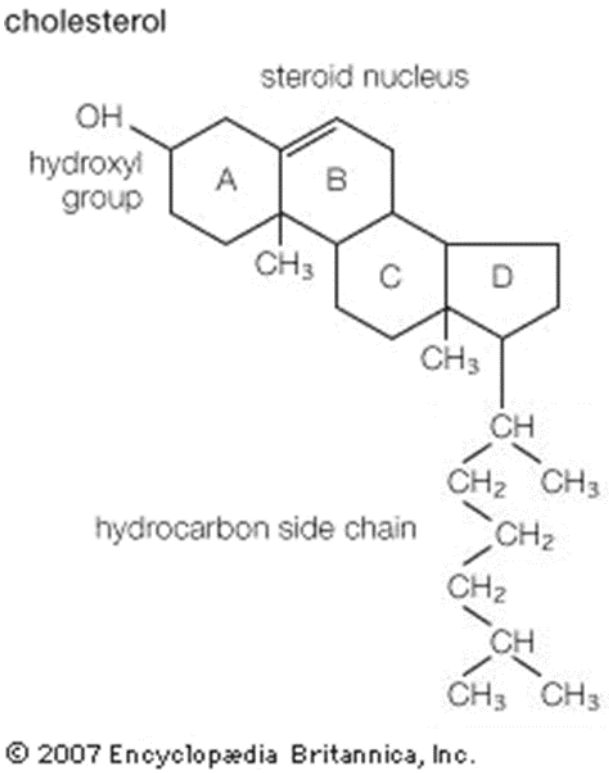


Figure: Structure of Cholesterol

Distribution of Cholesterol:

The broad facts about the distribution of cholesterol are as follows:

i. It is present in all cells – both in the cell membrane and cytoplasm. It is a part of the 'element constant'.

ii. All body fluids contain cholesterol excepting cerebrospinal fluid (CSF) in which the amount is negligible.

iii. It may exist in the Free State as well as in the form of esters, but these two forms are not equally distributed everywhere. In bile it is present only in the free form.

iv. Brain contains (17%) it and is mainly in free form and suprarenals contain the largest amount.

v. Blood cholesterol. Normal blood cholesterol varies between 150-200 mgm per 100 ml which is equally distributed between plasma and corpuscles. But in the corpuscles it is present chiefly in the free form, while in the plasma the major part remains as esters.

vi. The free cholesterol content of a tissue is characteristic and normally remains very constant but the cholesterol esters may vary in amount.

vii. The distribution of cholesterol in different types of muscles is directly proportional to their degree of activity. The cardiac and smooth muscles have richer cholesterol content than the voluntary muscle. Moreover, the amount of cholesterol in any tissue is roughly proportional to their degree of activity.

viii. Cholesterol and phospholipids always remain together. Moreover, there appears to be a definite ratio between phospholipids and cholesterol for each tissue.

Absorption of Cholesterol:

Absorption of cholesterol from the intestine follows the same principles as other lipids. Ingested cholesterol requires fat for its absorption from the lumen of the intestine into the lymphatics. These are esterified with fatty acids in the intestinal epithelium during their passage into the lymphatics. It is a complex monohydric secondary alcohol being a very important member of the sterol class. With fatty acids if forms waxes. It is a stable white, crystalline substance, insoluble in water but readily soluble in chloroform, ether, alcohol and other fat-solvents. The crystals have a rhombic or rectangular shape, with one corner broken, off.

Effects of Cholesterol Feeding:

i. Ingestion of cholesterol-rich food, for some length of time, increases the level of cholesterol in blood and such increase is not observed when such sample of diet taken once. The rise of blood cholesterol is accompanied with the rise in bile cholesterol. There is also rise in blood cholesterol when cholesterol-rich diet is not ingested which indicates that this rise is due to increased absorption of cholesterol derived from bile.

ii. Cholesterol feeding is one of the most certain means of producing excess deposition of fats, as well as cholesterol esters in the liver (antilipotropic action).

iii. Arteriosclerosis or atherosclerosis has been observed in animals and man fed with diet containing high cholesterol but it has not been proved in man.

Biosynthesis of Cholesterol:

Liver is the principal organ for its synthesis. Other tissues such as adrenal cortex, intestine, skin, ovary, kidney, testis also can synthesize cholesterol. The microsomal and cytosol fraction of the cell is responsible for cholesterol synthesis. It is interesting to note that the brain of the newborn can synthesize cholesterol while the adult brain cannot synthesize cholesterol.

The biosynthesis of cholesterol is divided into five stages:

a. Mevalonate, a six carbon compound, is synthesized from acetyl- CoA.

b. Isoprenoid units are formed from mevalonate by loss of CO_2.
c. Six isoprenoid units condense to form squalene ($C_{30}H_{50}$).
d. Squalene cyclizes to form lanosterol, the parent steroid. |

e. Cholesterol ($C_{27}H_{46}O$) is formed from lanosterol after several steps with the loss of three methyl groups:

(1) Cholesterol synthesis is extra-mitochondrial and follows two pathways:

(i) Two molecules of acetyl-CoA condense to form acetoacetyl-CoA by cytosolie thiolase enzyme,

(ii) In liver, acetoacetate formed in the mitochondria diffuses into the eytosol and is activated to acetoacetyl-CoA by acetoacetyl-CoA synthase requiring ATP and CoA. Acetoacetyl-CoA then combines with a molecule of acetyl-CoA to form HMG-CoA by HMG-CoA synthase.

(2) HMG-CoA is converted to mevalonate in a two stage reduction by NADPH catalyzed by a microsomal enzyme HMG-CoA reductase which catalyzes then rate-limiting steps in the pathway of cholesterol synthesis and is the site of action of the most effective class of cholesterol lowering drugs, the HMG-CoA reductase inhibitors (Statins).

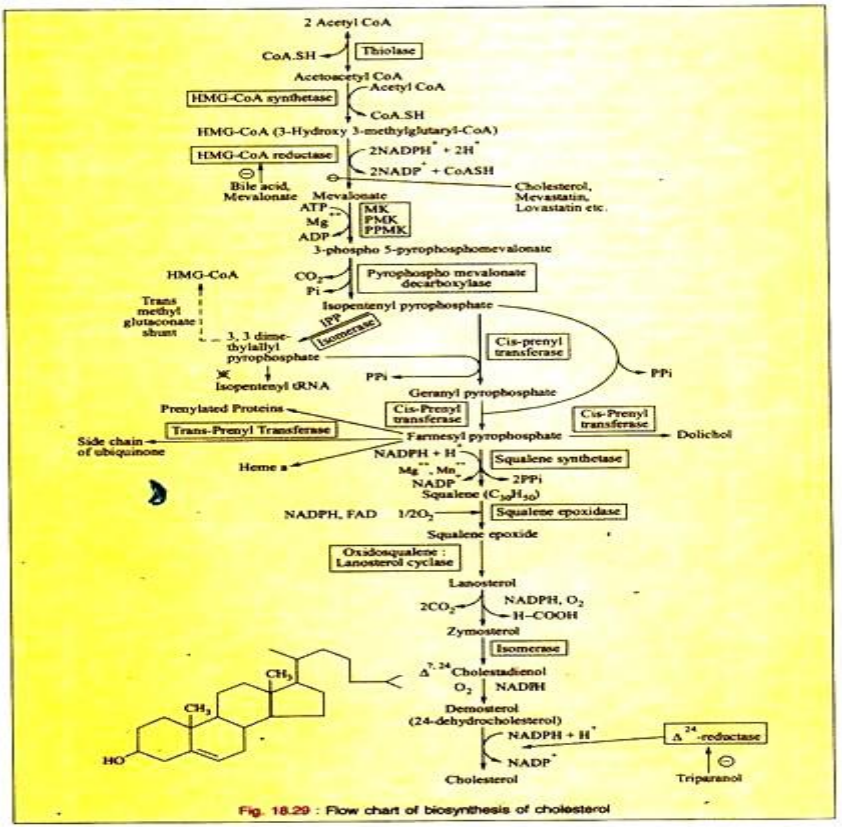


Fig. 18.29 : Flow chart of biosynthesis of cholesterol

(3) Mevalonate is phosphorylated by ATP to form several intermediates which by decarboxylation produces isopentenyl pyrophosphate.

(4) Farnesyl pyrophosphate is formed by the condensation of three molecules of isopentenyl pyrophosphate.

(5) Geranyl pyrophosphate is formed by the condensation of dimethylallyl pyrophosphate with another molecule of isopentenyl pyrophosphate. This, with a further condensation with isopentenyl pyrophosphate, forms farnesyl pyrophosphate. Two molecules of farnesyl pyrophosphate condense to form squalene by squalene synthetase with the help of NADPH+ H^+.

(6) Squalene is then converted to squalene epoxide by squalene epoxidase. Squalene epoxide is converted to lanosterol by oxidosqualene: lanosterol cyclase.

(7) The methyl group is oxidized to CO_2 from 14-des-methyl lanosterol and two more methyl groups are likely removed to produce zymosterol which on isomerisation forms $\Delta^{7,24}$-Cho- lestadienol. This $\Delta^{7,24}$-cholestadienol by the help of NADPH and O_2 yields demosterol which, on reduction, produces cholesterol.

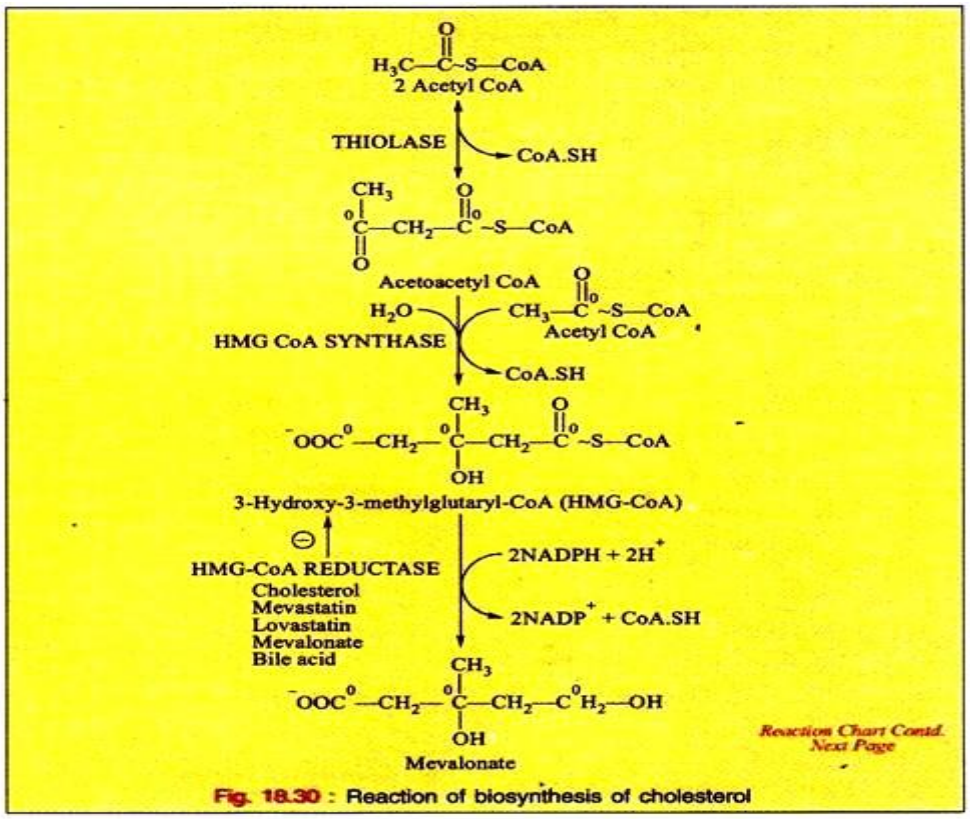


Fig. 18.30 : Reaction of biosynthesis of cholesterol

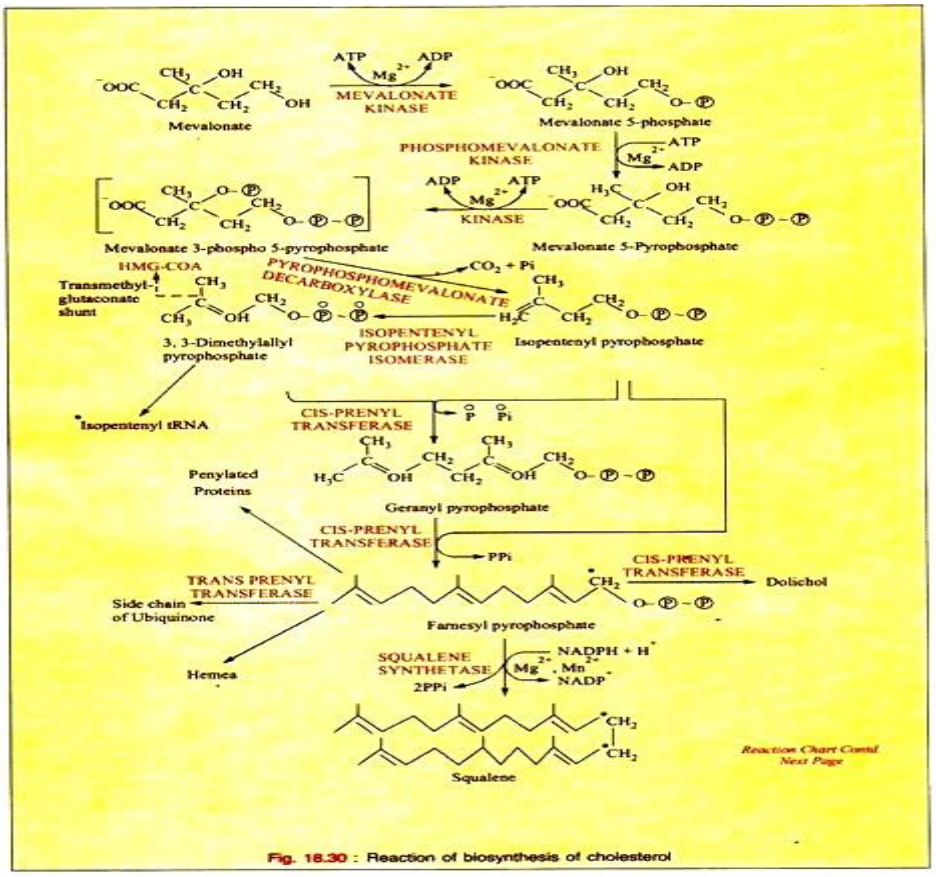


Fig. 18.30 : Reaction of biosynthesis of cholesterol

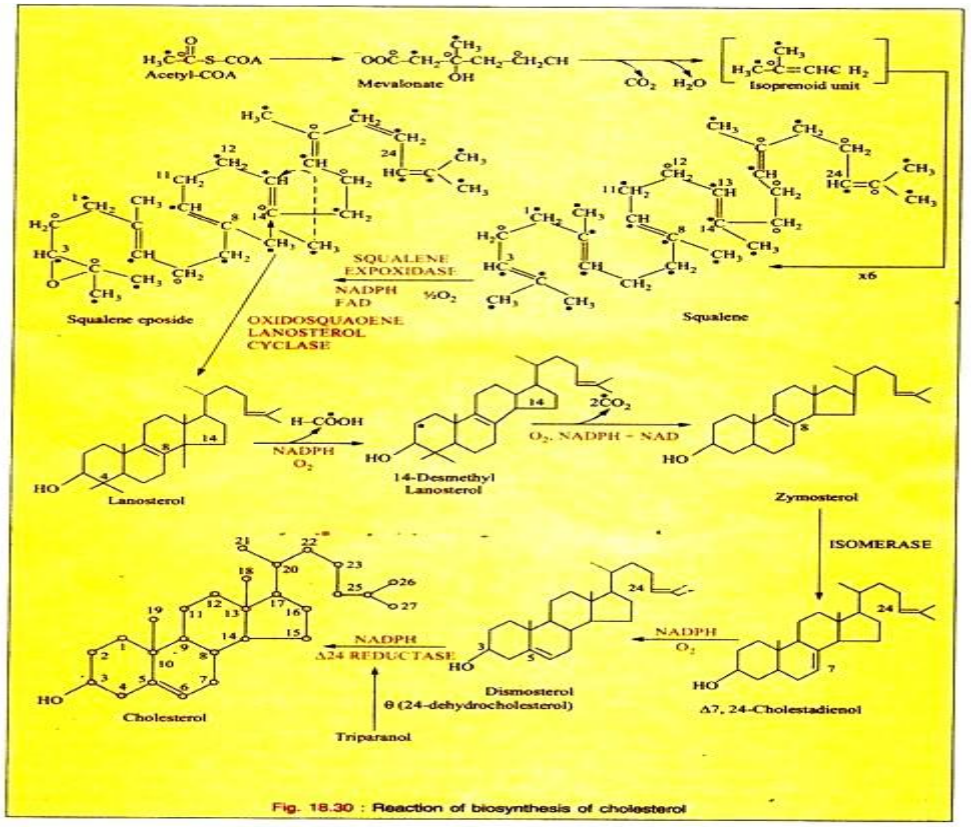


Fig. 18.30 : Reaction of biosynthesis of cholesterol

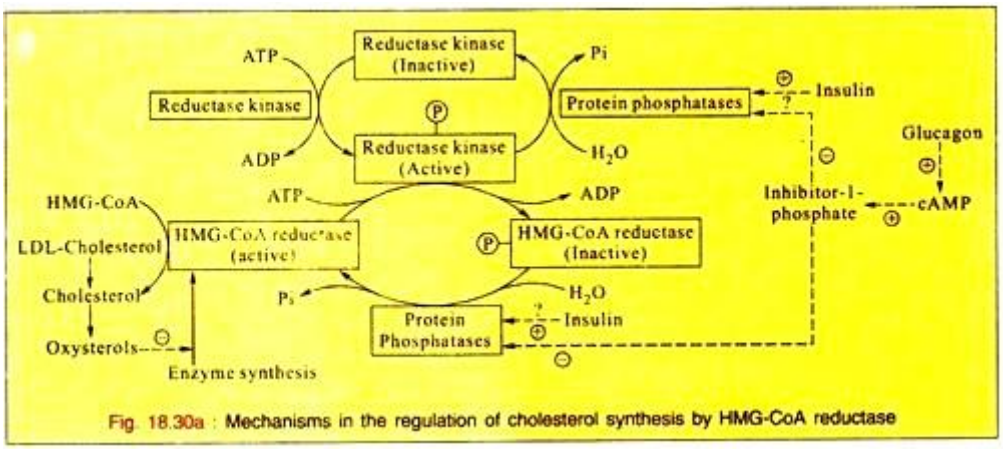


Fig. 18.30a : Mechanisms in the regulation of cholesterol synthesis by HMG-CoA reductase

(8) The intermediates from squalene to cholesterol are attached to a special carrier protein known as the squalene and sterol carrier protein. It seems likely that it is in the form of cholesterol sterol carrier protein that cholesterol is converted to steroid hormones and bile acids and participates in the formation of membranes and of lipoproteins.

(9) Farnesyl pyrophosphate is the branch point for the synthesis of the other polyisoprenoids, dolichol and ubiquinone. The activity of HMG-CoA reductase is decreased during fasting. The activity is not reduced in diabetes mellitus. But the activity of HMG-CoA reductase is inhibited by cholesterol feeding.

Administration of insulin or thyroid hormone increases the activity of HMG-CoA reductase; whereas the administration of glucagon and glucocorticoids reduces the activity of the enzyme. More recent experiments have shown that cholesterol synthesis is inhibited by cAMP, indicating that one or more reactions in the synthetic pathway may be controlled by a cAMP-dependent protein kinase. Plasma cholesterol in humans is made lower by reducing the amount of cholesterol in the diet. An increase of 100 mg in dietary cholesterol causes a rise of 5 mg cholesterol per 100 ml of serum. Hypercholesterolemia has been observed in uncontrolled diabetes mellitus, impairment of liver, obstructive jaundice, glomerulonephritis, hypothyroidism and nephrosis. Hypocholesterolemia has been found in anemia, hyperthyroidism, hepatic diseases, infection, carcinoma and acute pancreatitis.

Polyunsaturated fatty acids can lower cholesterol level. Because, the cholesteryl esters of polyunsaturated fatty acids are more rapidly metabolized by the liver and other tissues which may enhance their rate of turnover and excretion. Recently, it has been found that saturated fatty acids cause higher rates of secretion of VLDL by the perfused liver than do unsaturated free fatty acids. Cholesterol level is also decreased on the minimum intake of animal fat. The cholesterol level in tissues is increased due to uptake of cholesterol-containing lipoproteins by receptors, e.g., the LDL receptor, uptake of free cholesterol from cholesterol-

rich lipoproteins to the cell membrane, cholesterol synthesis, and hydrolysis of cholesteryl esters by the enzyme cholesteryl ester hydrolase. The level is also decreased due to efflux of cholesterol from the membrane to lipoproteins, particularly to HDL, or nascent HDL, esterification of cholesterol by ACAT (acyl- CoA: cholesterol acyltransferase), and utilization of cholesterol for synthesis of other steroids such as hormones or bile acids in the liver.

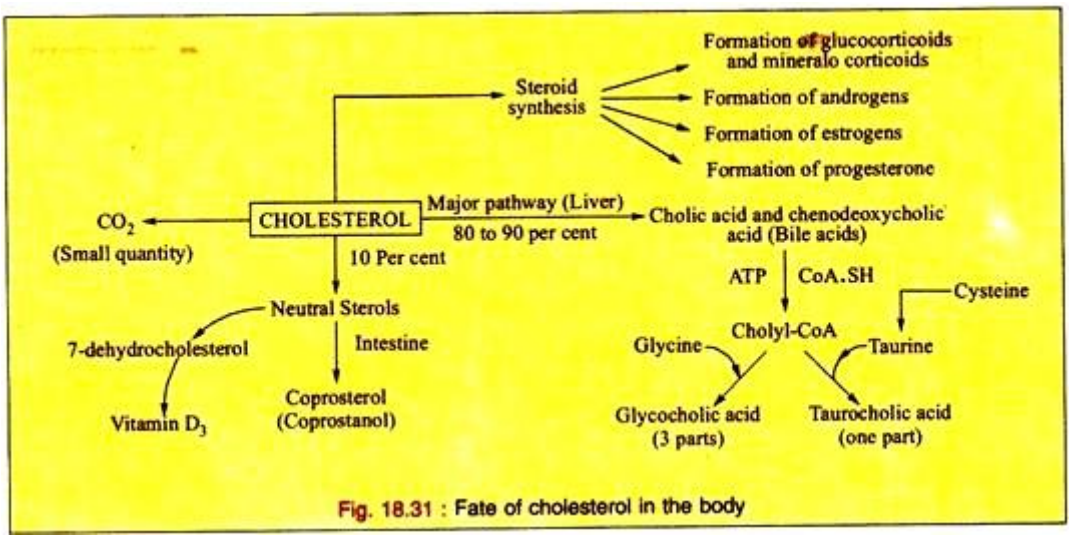


Fig. 18.31 : Fate of cholesterol in the body

Role of HMG-CoA Reductase in Cholesterol Metabolism:

a. Marked decrease in the activity of HMG-CoA reductase has been observed in fasting animals which shows the reduced synthesis of cholesterol during fasting.

b. HMG-CoA reductase in liver is inhibited by mevalonate and by cholesterol.

c. Cholesterol is considered to act by repression of transcription of the HMG-CoA reductase gene, cholesterol synthesis is also inhibited by LDL-cholesterol taken up via LDL receptors. A diurnal variation takes place in both cholesterol synthesis and reductase activity.

d. HMG-CoA reductase activity is increased by the administration of insulin or thyroid hormone, whereas its activity is decreased by glucagon or glucocorticoids.

e. HMG-CoA reductase exists in both active and inactive forms.

Regulation of cholesterol synthesis

Cholesterol biosynthesis is controlled by the rate limiting enzyme HMG CoA reductase at the beginning of the pathway. HMC CoA reductase is found in association with endoplasmic reticulum and is subjected to different metabolic controls.

1. **Feedback control :** The end product cholesterol controls its own synthesis by a feedback mechanism. Increase in the cellular concentration of cholesterol reduces the synthesis of the enzyme HMG CoA reductase. This is achieved by decreasing the transcription of the gene responsible for the production of HMC CoA reductase. Feedback regulation has been investigated with regard to LDL-cholesterol taken up by the cells, and the same mechanism is believed to operate whenever cellular cholesterol level is elevated.

2. **Hormonal regulation :** The enzyme HMG CoA reductase exists in two interconvertible forms. The dephosphorylated form of HMC CoA reductase is more active while the phosphorylated form is less active. The hormones exert their influence through cAMP by a series of reactions which are comparable with the control of the enzyme glycogen synthase. The net effect is that glucagon and glucocorticoids favor the formation of inactive HMC CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, insulin and thyroxine increase cholesterol production by enhancing the formation of active HMC CoA reductase (dephosphorylated form).

3. **Inhibition by drugs :** The drugs compactin and lovastatin (mevinolin) are fungal products. They are used to decrease the serum cholesterol level in patients with hypercholesterolemia. Compactin and lovastatin are competitive inhibitors of the enzyme HMG CoA reductase and, therefore, reduce cholesterol synthesis. About 50 to 60% decrease in serum cholesterol level has been reported by a combined use of these two drugs.

4. **HMG CoA reductase activity is inhibited by** bile acids. Fasting also reduces the activity of this enzyme.

Degradation of Cholesterol

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50%) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D, coprostanol and cholesterol. The latter two are the fecal sterols, besides cholesterol.

l. Synthesis of bile acids

The bile acids possess 24 carbon atoms, 2 or 3 hydroxyl groups in the steroid nucleus and a side

chain ending in carboxyl group. The bile acids are amphipathic in nature since they possess both polar and non-polar groups. They serve as emulsifying agents in the intestine and actively participate in the digestion and absorption of lipids.

The synthesis of primary bile acids takes place in the liver and involves a series of reactions. The step catalyzed by 7 a-hydroxylase is inhibited by bile acids and this is the rate limiting reaction. Cholic acid and chenodeoxycholic acid are the primary bile acids and the former is found in the largest amount in bile. On conjugation with glycine or taurine, conjugated bile acids (glycocholic acid, taurocholic acid etc.) are formed which are more efficient in their function as surfactants. In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as bile salts.

2. Synthesis of steroid hormones from cholesterol

Cholesterol is the precursor for the synthesis of all the five classes of steroid hormones (a) Glucocorticoids (e.g. cortisol) (b) Mineralocorticoids (e.g. aldosterone) (c) Progestins (e.g. progesterone).

3. Synthesis of vitamin D7-Dehydrocholesterol, an intermediate in the synthesis of cholesterol, is converted to cholecalciferol (vitamin O3) by ultraviolet rays in the skin.

Effect of Drugs on Cholesterol:

a. Sitosterol is hypocholesterolemic agent which blocks the esterification of cholesterol in the gastrointestinal tract and thus reduces cholesterol absorption.

b. Drugs like choloxin and neomycin cause the increased fecal excretion of cholesterol and bile acids.

c. Clofibrate acts by inhibiting the secretion of VLDL by the liver or by inhibiting hepatic cholesterol synthesis.

d. The hypocholesterolemic drugs include nicotinic acid and estrogens.

e. Significant reductions of plasma cholesterol can be effected by the use of cholestyramine resin.

f. The fungal inhibitors of HMG-CoA reductase, mevastatin and lovastatin, reduce LDL cholesterol levels by up-regulation of the LDL receptors.

g. Probucol causes to increase LDL catabolism, but its antioxidant properties are more important in preventing accumulation of oxidized LDL in arterial walls. Oxidized LDL is a prime cause of atherosclerosis.

Test for Cholesterol:

a. Salkowski's Test:

A little cholesterol is dissolved in 2 ml of chloroform. An equal volume of conc. H_2SO_4 added to it. Shaked gently, upper layer of chloroform turns red and the sulphuric acid layer assumes a yellow colour with green fluorescence.

b. Libermann-Burchard Reaction:

A crystal of cholesterol is dissolved in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops conc. H_2SO_4 are added. Mixed well. A red rose colour develops which quickly changes through blue to green.

Storage, Destruction and Control of Cholesterol:

A good deal of cholesterol, derived from the disintegration of blood cells, is stored in the cells of reticulo-endothelial system and retained in the body. Body utilizes cholesterol very economically. The metabolism of cholesterol is under the influence of hormones. Injection of adrenocorticotrophic hormone (ACTH) reduces cholesterol content of adrenal cortex.

In hypothyroidism blood cholesterol rises whereas there is fall of blood cholesterol in hyperthyroidism. A variation in serum cholesterol has also been noted during pregnancy. In diabetes mellitus and atherosclerosis, blood cholesterol is often found to be high. Liver, tissue cells, muscles, organs like heart and brain, etc. play an important part in cholesterol metabolism.

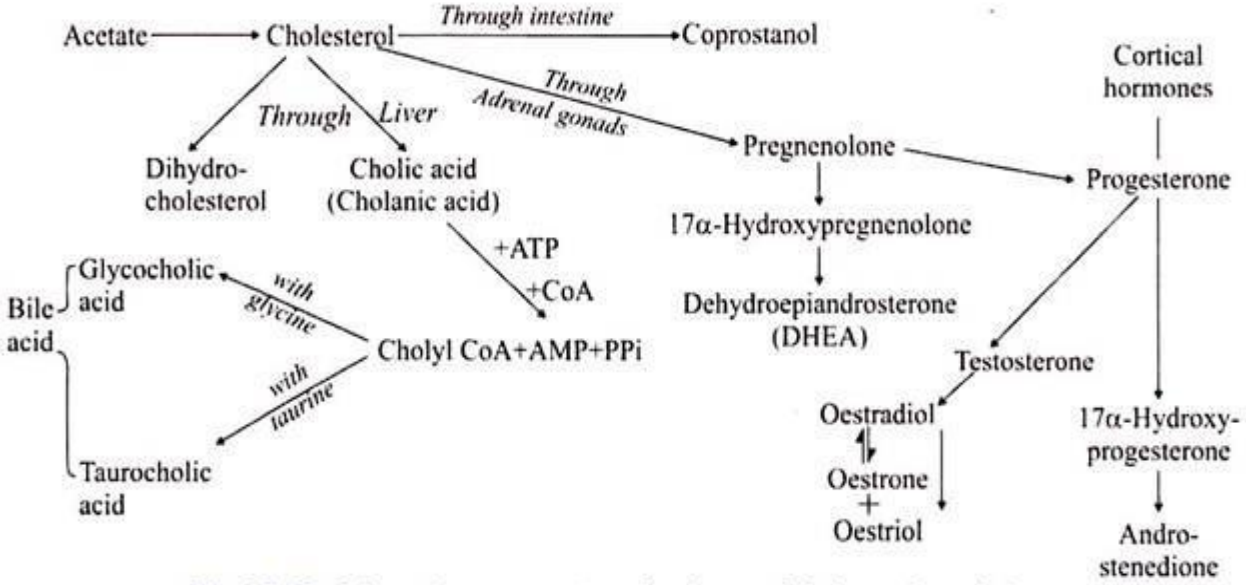


Fig. 10.29 Schematic representation of pathways of cholesterol metabolism.

Functions of Cholesterol:

i. Essential Constituent of all Cells:

It is a part of the 'element constant' of the cells. Its presence in constant amount in cell indicates that it is intimately concerned with the function of the cells.

ii. Controls Cell Permeability:

Being a constant constituent of cell membrane it is believed to be related to the permeability of the tissue cells.

iii. Prevents Haemolysis:

In some unknown way, it protects the red cells from being easily haemolysed. Low blood cholesterol is associated with haemolysis

iv. Defensive Action:

Cholesterol is intimately related to the defensive mechanism of the body. During acute infections blood cholesterol falls and tends to rise during recovery.

v. Fat Transport:

A large part of fat is transported through blood as cholesterol esters.

vi. Formation of Cholic Acid (Bile Salts):

It has been shown that cholesterol is the mother substance from which cholic acid is synthesised. Cholic acid is a constituent of bile salts.

vii. Antilipotropic Action:
Cholesterol feeding increases the deposition of fat in the liver. This effect is due to the formation of cholesterol esters and depression of phospholipid formation in the liver.

viii. Controls Cell Division:
Rapidly growing tissues are very rich in cholesterol, such as, the granulation tissues of healing ulcer and rapidly growing tumours, etc.

ix. Antagonistic to Phospholipids:
Its physio-chemical properties are antagonistic to phospholipids; hence, they are always found together.

x. It is a parent substance of all the steroid hormones of sex glands, adrenal cortex.

Interrelation with Other Sterols:

Cholesterol is very closely related to many other sterols of immense physiological importance, for instance:
(a) The active principle of adrenal cortex,

(b) The male sex hormones,

(c) The female sex hormones,

(d) Vitamin D and ergosterol, and

(e) Cholic acid, etc.

Although cholesterol has not been proved to be the mother substance for the formation of other sterols in the body except cholic acid but there are some indications to believe that cholesterol may serve as precursor for the synthesis of other sterols since it has been possible to synthesise sex hormones, both male and female, in the laboratory from cholesterol.

Excretion of Cholesterol:

Excretion takes place in the following ways:
i. In the Bile:
It is found as free cholesterol but a good part of it is reabsorbed from the intestine.

ii. In the Stool:

A part of the bile cholesterol undergoes bacterial putrefaction in the intestine and is excreted as coprosterol. But a small amount of free cholesterol is also found in the stool.

iii. In the Urine:

It appears in the urine only in traces, but appears in larger amounts in different diseases namely in hypercholesteremia where blood cholesterol is high.

iv. In the Skin:

Sebum secretion of the sebaceous glands of the skin contains large quantities of cholesterol and thus drying of the skin is prevented by sebum for its cholesterol constituent.

Blood Cholesterol in Pregnancy:

During pregnancy blood cholesterol rises and rapidly comes back to normal after confinement. It may be that this rise is due to hyperactivity of the adrenal cortex, produced during pregnancy and the exact function is not known. It may also be that this high cholesterol ensures the supply of the mother substance from which large amounts of sex hormones are manufactured during pregnancy.

Atherosclerosis:

A very common disease of advanced age, indicated by deposition of cholesterol in the damaged arterial wall. This leads to cerebral thrombosis, myocardial infarctions, etc. It has been established that abnormal lipid metabolism produces atherosclerosis. Several other factors make the system more prone to the disease. One of the principle factors is an injury to arterial wall resulting from any conditions. A high blood cholesterol level and genetic inheritance might be other contributory factors. Treatment includes administration of thyroid hormones or oestrogen low doses. If dietary saturated fat is substituted by unsaturated one, the blood cholesterol level is lowered and atherosclerosis is prevented.

Probable Questions:

1. Draw the chemical structure of cholesterol. Describe the process of cholesterol biosynthesis in our body.
2. State the different modes of regulation of the cholesterol biosynthesis in our body.
3. What are the tests for cholesterol?
4. Discuss the effects of drugs on cholesterol.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

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5. Donald Voet and Judith G. Voet Biochemistry.

UNIT-XV

Lipoproteins and types of cholesterol transport

Objective: In this unit we will learn about lipoproteins, their types, transport mechanism and functions.

Introduction: **Lipoprotein**, any member of a group of substances containing both lipid (fat) and protein. They occur in both soluble complexes—as in egg yolk and mammalian blood plasma—and insoluble ones, as in cell membranes. Lipoproteins in blood plasma have been intensively studied because they are the mode of transport for cholesterol through the bloodstream and lymphatic fluid. Cholesterol is insoluble in the blood, and so it must be bound to lipoproteins in order to be transported. Two types of lipoprotein are involved in this function: low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs). LDLs transport cholesterol from its site of synthesis in the liver to the body's cells, where the cholesterol is separated from the LDL and is then used by the cells for various purposes. HDLs probably transport excess or unused cholesterol from the body's tissues back to the liver, where the cholesterol is broken down to bile acids and is then excreted. About 70 percent of all cholesterol in the blood is carried by LDL particles, and most of the remainder is carried by HDLs. LDL-bound cholesterol is primarily responsible for the atherosclerotic build up of fatty deposits on the blood vessel walls, while HDL particles may actually reduce or retard such atherosclerotic build ups and are thus beneficial to health.

Body cells extract cholesterol from the blood by means of tiny coated pits (receptors) on their surfaces; these receptors bind with the LDL particles (and their attached cholesterol) and draw them from the blood into the cell. There are limits to how much cholesterol a body cell can take in, however, and a cell's capture of LDL particles inhibits the making of more LDL receptors on that cell's surface, thus lowering its future intake of cholesterol. Fewer receptors on the body cells means that less cholesterol is ingested by the cells and that more remains in the bloodstream, thus increasing the risk of cholesterol accumulating in the interior walls of blood vessels. Several hereditary genetic disorders, called hyperlipoproteinemias, involve excessive concentrations of lipoproteins in the blood. Other such diseases, called hypolipoproteinemias, involve abnormally reduced lipoprotein levels in the blood.

Five groups of lipoproteins present in plasma exhibit important role in the transport and metabolism of lipids.

These are:

a. Chylomicrons:

Derived from intestinal absorption of triacylglycerol.

b. Very Low Density Lipoproteins (VLDL or Pre-β-lipoproteins):

Mainly derived from the liver for the export of triacylglycerol and also formed from dietary lipids.

c. Low Density Lipoproteins (LDL or β-lipoproteins):

These represent a final stage in the catabolism of VLDL and chylomicrons.

d. High Density Lipoproteins (HDL or α-lipoproteins):

These are involved in VLDL, chylomicrons and cholesterol metabolism.

e. Free Fatty Acids:

These are not classified with the other plasma lipoproteins as their structure is different. These consist of long chain fatty acids attached to serum albumin.

Apo lipoproteins:

The lipoproteins contain one or more proteins or polypeptides known as apoprotein.

Electrophoretic Separation of Lipoprotein and their Normal Concentration in Human Plasma:

a. α-Lipoproteins:

These occupy the α- globulin region after electrophoresis. These contain 45% proteins, 8% triacylglycerol, 20% cholesterol, 27% phospholipids. Normal concentration: 300 mg%

b. β-Lipoproteins:

These occupy the β- globulin region after electrophoresis. They contain 46% cholesterol, 23% phospholipids, 10% triacylglycerol. Their concentration increases in atherosclerosis and coronary thrombosis. Normal concentration: 300 mg%.

c. Pre-β-lipoproteins:

These occupy the region in between a- and P-lipoproteins. These contain low protein, but contain 50% triacylglycerol, fair amount of cholesterol and phospholipids. Their concentration is also increased in atherosclerosis and coronary thrombosis etc. Normal concentration: 150 mg%.

Formation of Chylomicrons and VLDL:

a. Both chylomicrons and VLDL are found in chyle formed by the lymphatic system draining the intestine. VLDL is the vehicle of transport of triacylglycerol from the liver to the extra hepatic tissues.

b. Apoprotein B which is essential for their formation is synthesized by ribosomes in the rough endoplasmic reticulum and is incorporated into lipoproteins in the smooth endoplasmic reticulum which is the main site of synthesis of triacylglycerol, phospholipids and cholesterol. Carbohydrate is added to the lipoproteins found in the Golgi apparatus.

c. Both are released from the intestine or hepatic cell by reverse pinocytosis.

d. Chylomicrons pass into the spaces between the intestinal cells making their way into the lacteals draining the intestine. VLDL are secreted by hepatic parenchymal cells into the space of Disc and then into the hepatic sinusoids.

Catabolism of Chylomicrons and VLDL:

A. Role of lipoprotein lipase clearing factor lipase:

a. It is present in the walls of blood capillaries and also found in the extracts of heart, lung, spleen, lactating mammary gland.

b. Its concentration in the normal blood is less. It is released from the tissues into the circulation following injection of heparin.

c. Phospholipids and apoprotein C-1 1 are required as cofactors for its activity.

d. It hydrolyzes triacylglycerol to mono-acylglycerol through diacylglycerol. The mono-acylglycerol is finally hydrolyzed by mono-acylglycerol hydrolase.

B. Role of liver:

Chylomicron remnants (about half the diameter of parent chylomicrons) are taken by the liver in vivo and by the perfused liver, in which the cholesteryl esters are hydrolyzed and the triacylglycerol, fatty acids are metabolized.

Metabolism of LDL:

a. It is formed from VLDL and chylomicrons.

b. It is removed from the circulation by the liver. The half-time of disappearance of apoprotein B in LDL from the circulation is about 2½ — days.

c. Fibroblasts and lymphocytes may degrade LDL in extra hepatic tissues.

Metabolism of HDL:

a. It is synthesized and secreted from liver and intestine.

b. Nascent HDL from intestine does not contain apoprotein C but only apoprotein A. Nascent HDL formed by the liver contains apoprotein and free cholesterol. These lipoproteins are similar to the particles found in the plasma of patients with a deficiency of the plasma enzyme lecithin: Cholesterol acyl transferase (LCAT) and in the plasma of patients with obstructive jaundice.

c. The liver and the intestine are the final sites of degradation of HDL Apo proteins.

Plasma Lipoprotein Abnormalities:

a. In cases of abnormal hyperlipemia, the concentration of serum VLDL is increased and the concentration of serum HDL may be decreased, increased or normal.

b. In cases of hyperlipemia with marked hypercholesterolemia, serum LDL is increased.

c. Serum LDL has been found to be increased in diabetes mellitus, hypothyroidism, obstructive jaundice, the nephrotic syndrome and in glycogen storage diseases.

d. The concentration of serum LDL and of total serum cholesterol is significantly increased in atherosclerosis.

e. Studies of serum lipoproteins are still of limited clinical value in myocardial infarction, cerebral thrombosis etc.

f. LDL and also VLDL are possible risk factors in studies related to increased susceptibility to ischemic heart disease.

Role of Liver in Lipid Metabolism:

a. The liver has active enzyme systems for synthesizing triacylglycerol’s, phospholipids, cholesterol, plasma lipoproteins and for converting fatty acids to ketone bodies.

b. The fatty acids used in the synthesis of liver triacylglycerol are derived from two sources:
(a) From acetyl-CoA derived from carbohydrate,

(b) Uptake of free fatty acids from the circulation.

c. It is the site for the synthesis of bile acids from cholesterol.

d. It is the major site for the oxidation of fatty acids.

e. Feeding of diets high in carbohydrate containing sucrose or fructose, high levels of circulating free fatty acids, ingestion of ethanol and the presence of high level of insulin enhance the synthesis of triacylglycerol and the secretion of VLDL by the liver.

f. It has the enzyme systems for lengthening and shortening of fatty acids and for saturating and de-saturating fatty acids.

g. This organ is chiefly concerned in removal of phospholipids, cholesterol and lipoproteins from the plasma.

h. Hepatic glucokinase increases with the availability of carbohydrate in the diet. This increases glucose incorporation into the liver and hence glycolysis and fatty acid synthesis. The fatty acids are carried to the adipose tissue as triacylglycerol in VLDL.

Starvation diminishes glucokinase and leads to diminished fatty acid synthesis in the liver. The hypoglycemia stimulates growth hormone production which, in turn, stimulates lipolysis. The free fatty acids so liberated from adipose tissue can influence carbohydrate metabolism in the liver where they are broken down with the formation of acetyl-CoA. The lipolytic effect of growth hormone is also necessary for its protein anabolic action.

Role of Fatty Liver in Lipid Metabolism:

Lipid (mainly as triacylglycerol) can accumulate in the liver for the following reasons causing fatty liver:

a. The increased levels of plasma free fatty acids resulting from mobilization of fat from adipose tissue.

b. The hydrolysis of lipoprotein or chylomicron triacylglycerol by lipoprotein lipase in extra hepatic tissues.

c. Increasing amounts of free fatty acids are taken up by the liver and esterified.

d. The production of plasma lipoprotein does not keep pace with the influx of free fatty acids allowing triacylglycerol to accumulate.

e. During starvation and the feeding of high-fat diets, the quantity of triacylglycerol present in the liver is significantly increased.

f. Uncontrolled diabetes mellitus and toxaemia of pregnancy cause fatty appearance and enlargement of the liver.

g. The metabolic block in the synthesis of lipoproteins from lipid and apoprotein.

h. The deficiency of lipotropic factor causes triacylglycerol to accumulate even though only a normal rate of fatty acid synthesis and uptake of free fatty acids take place.

i. Carbon tetrachloride, chloroform, phosphorus, lead, arsenic and ethionine (α- amino-γ-ethyl-mercaptobutyric acid) cause fatty liver. These substances inhibit hepatic protein synthesis. Orotic acid blocks apo-B synthesis.

j. Alcoholism also leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis.

k. Protein deficiency, essential fatty acid and vitamin deficiencies (e.g., vitamin E, pyridoxine, pantothenic acid) cause fatty liver. The deficiency of essential fatty acids depresses the synthesis of phospholipids and, therefore, cholesterol is involved in esterification causing fatty livers.

Lipotropic Factor:

The substances that prevent the accumulation of fat in the liver are known as lipotropic factor. The phenomenon is said to be lipotropism. Choline, methionine and betaine and β- propiothetin act as lipotropic agents in curing fatty livers due to choline deficiency. Diets poor in protein (containing methionine) or lecithin (containing choline) tend to favour the production of fatty liver. Choline is synthesized using labile methyl groups donated by methionine in the process of trans-methylation. Vitamin B_{12} and folic acid which are important in hematopoiesis are also able to produce lipotropic effect. Vitamin B_{12} is concerned in the biosynthesis of labile methyl groups and folic acid in trans-methylation reactions. Inositol exerts a limited lipotropic effect in fat free diets. Casein and certain other proteins possess lipotropic activity

Probable Questions :

1. Give a short note on transport of cholesterol.
2. Describe role of VLDL and LDL in cholesterol transport.
3. Describe the role of liver in lipid metabolism.
4. Describe the role of fatty liver in lipid metabolism.
5. What are the lipotropic factors?

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

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5. Donald Voet and Judith G. Voet Biochemistry.

UNIT-XVI

Enzymes: Kinetic analysis of enzyme-catalyzed reaction, Michaelis-Menten Equation, Lineweaver-Burk's plot, Bi-substrate Reactions.

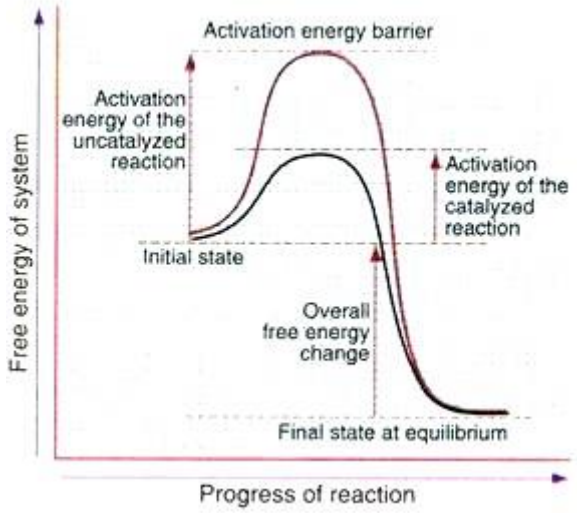
Objectives: In this unit we will learn about enzymes and how they react with their substrates. There are various mechanisms by which enzymes react with their substrates.

Meaning of Enzymes:

Enzymes are proteinaceous (and even nucleic acids) biocatalyst which alter (generally enhance) the rate of a reaction.

Free energy of activation and effect of catalysis:

A chemical reaction like substrate to product, will take place when a certain number of substrate molecules at any instant, possess enough energy to attain an activated condition called the "transition state" in which the probability of making or breaking a chemical bond to form the product is very high. **"Free energy of activation"** is the amount of energy required to bring all the molecules in one gram mole of a substrate at a given temperature to the transition state.



In presence of a catalyst, the substrate combines with it to produce a transient state having a lower energy of activation than that of the substrate alone. This accelerates the reaction. Once the product is formed, the enzyme (catalyst) is free to combine with another molecule of the substrate and repeat the process. Though there is a change in the free energy of activation in presence of an enzyme, the overall free energy change of the reaction remains the same whether the reaction is catalyzed by an enzyme or not.

Classification of Enzymes:

Classification of enzymes are based upon:

(1) The reaction catalyzed,

(2) The presence or absence at a given time,

(3) The regulation of action,

(4) The place of action and

(5) Their clinical importance.

1. Classification Based upon the Reaction Catalysed:

Enzymes are broadly divided into six groups based on the type of reaction catalysed.

They are:

(1) Oxidoreductases

(2) Transferases

(3) Hydrolases

(4) Lyases

(5) Isomerases and

(6) Ligases.

(a) Oxidoreductases:

Enzymes which bring about oxidation and reduction reactions.

Ex. Pyruvate + NADH—lactate dehydrogenase → Lactate + NAD^+

Glutamic acid + NAD—glutamate dehydrogenase → α-ketoglutarate + NH_3 + NADH

(b) Transferases:

Enzymes which catalyze transfer of groups from one substrate to another, other than hydrogen. Ex. Transaminase catalyses transfer of amino group from amino acid to a keto acid to form a new keto acid and a new amino acid.

Ex. (α-Ketoglutarate + Alanine—alanine aminotransferase → Glutamate + Pyruvate

Aspartate + α-Ketoglutarate —aspartate aminotransferase Oxaloacetate + Glutamate

(c) Hydrolases:

Those enzymes which catalyse the breakage of bonds with addition of water (hydrolysis). All the digestive enzymes are hydrolases. Ex. Pepsin, trypsin, amylase, maltase.

(d) Lyases:

Those enzymes which catalyse the breakage of a compound into two substances by mechanism other than addition of water. The resulting product always has a double bond.

Ex. Fructose-1-6-diphosphate—ALDOLASE → Glyceraldehyde-3-phosphate + DHAP

(e) Isomerases:

Those enzymes which catalyze the inter-conversion of optical and geometric isomers.

Ex. Glyceraldehyde-3-phosphate—ISOMERASE → Dihydroxyacetone phosphate

(f) Ligases:

These enzymes catalyze union of two compounds. This is always an energy requiring process (active process).

Ex. Pyruvate + CO_2 + ATP—pyruvate carboxylase Oxaloacetate + ADP + Pi

2. Classification Based upon the Presence or Absence at a Given Time:

Two types are identified:

(a) Inducible enzymes:

Those enzymes that are synthesized by the cell whenever they are required. Synthesis of these enzymes usually requires an inducer. Ex. Invertase, HMG-CoA reductase, p-galactosidase and enzymes involved in urea cycle.

(b) Constitutive enzymes:

Enzymes which are constantly present in normal amounts in the body, irrespective of inducers. Ex. Enzymes of glycolysis.

3. Classification Based upon the Regulation of Enzyme Action:

They are of two types:

(a) Regulatory enzymes:

The action of these enzymes is regulated depending upon the status of the cell. The action of regulatory enzymes is either increased or decreased by a modulator at a site other than the active site called the "allosteric site".

Ex. Phosphofructokinase (PFK) and glutamate dehydrogenase.

(b) Non-regulatory enzymes:

The action of these enzymes is not regulated.

Ex. Succinate dehydrogenase.

4. Classification Based upon the Place of Action:

Depending upon the two sites of action, they are divided into—

(a) Intracellular enzymes:

Enzymes that are produced by the cell and act inside the same cell are known as intracellular enzymes. Ex. All the enzymes of glycolysis and TCA cycle.

(b) Extracellular enzymes:

Enzymes produced by a cell but act outside that cell independent of it. Ex, All the digestive enzymes viz. trypsin, pancreatic lipase etc.

5. Classification Based upon their Clinical Importance:

(a) Functional plasma enzymes:

Enzymes present in the plasma in considerably high concentration and are functional in the plasma due to the presence of their substrate it plasma. Ex. Serum lipase, blood clotting enzymes.

(b) Non-functional plasma enzymes:

Enzymes present in the plasma in negligible concentration and have no function in the plasma due to the absence of their substrate in it. Non-functional plasma enzymes are of diagnostic importance.

Enzymes are named in 4 digits by the enzyme nomenclature commission, wherein the;

1^{st} digit refers to main classification
2^{nd} digit refers to sub-classification
3^{rd} digit refers to sub-sub classification
4^{th} digit refers to that particular enzyme

Ex. 2.7.3.2 is adenosine triphosphate-creatine phosphotransferase (creatine kinase).

Mechanism of Enzyme Action:

An enzyme (or protein) should be in its native conformation to be biologically active. The three dimensional conformation of enzymes have a particular site where the substrate binds and is acted upon, this site is called the active site.

The active site is earmarked into two specific areas:

(1) Binding site—where the substrate binds and

(2) Catalytic site—where the enzyme catalysis takes place.

The amino acids present at the active site are tyrosine, histidine, cysteine, glutamic acid, aspartic acid, lysine and serine. In aldolase, lysine is present at the active site. In carboxypeptidase, two tyrosine residues are present at the active site. Ribonuclease has two histidines at the active site. Michaelis and Menten established the theory of combination of enzyme with substrate to form the enzyme-substrate complex. According to this, the enzyme combines with the substrate on which it acts to form an enzyme-substrate complex. Then, this enzyme is liberated and the substrate is broken down into the product of the reaction.

E [Enzyme] + S [Substrate] → ES [Enzyme-Substrate complex] → E + Product

The ES complex is also called as ‘Michaelis Menten complex’.

Enzymes accelerate the rate of chemical reaction by four major mechanisms viz.

1. Proximity and Orientation:

The enzyme binds to the substrate in such a way that the susceptible bond is in close proximity to the catalytic group and also precisely oriented to it resulting in the catalysis.

2. Strain and Distortion or Induced Fit Model:

Binding of the substrate induces a conformational change in the enzyme molecule which strains the shape of the active site and also distorts the bounded substrate, thus bringing about the catalysis. The binding of the substrate to the enzyme will bring about a change in the tertiary or quaternary structure of enzyme molecule, which destabilizes the enzyme. In order to attain stability, the enzyme distorts the substrate thereby forming the reaction product.

3. General Acid-Base Catalysis:

The active site of the enzyme has amino acids that are good proton donors or proton acceptors, this result in acid-base catalysis of the substrate.

4. Covalent Catalysis:

Some enzymes react with their substrates to form very unstable, covalently joined enzyme-substrate complexes, which undergo further reaction to form the products.

Factors Affecting Enzyme Action:

The factors influencing the rate of the enzyme catalyzed reaction are:

1. Temperature

2. pH

3. Substrate concentration

4. Enzyme concentration

5. Concentration of products

6. Light

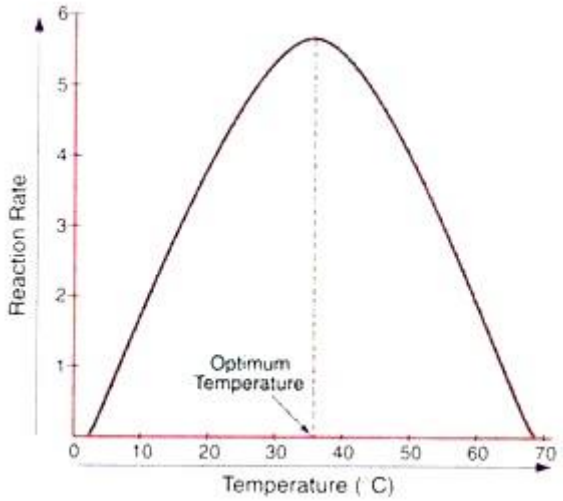
7. Ions

1. Effect of Temperature:

When all the other parameters are kept constant (i.e. at their optimum level), then the rate of enzyme reaction increases slowly with increase of temperature till it reaches a maximum. Further increase in temperature denatures the protein resulting in decrease in the enzyme action and a further increase in temperature may totally destroy the protein.

Optimum temperature:

The temperature, at which the enzyme activity is maximum, is termed as the optimum temperature. Most of the enzymes are totally inactive at 0° C to 4° C, their activity starts at 10° C and slowly increases reaching its maximum capacity at its optimum temperature. Majority of the enzymes in the human body have their optimum temperatures between 37° C and 40° C. Beyond this temperature the enzymes become less active and may loose their activity completely at higher temperatures. In fever, rise in temperature increases the metabolic activity due to increase in enzymatic action. Decrease in the temperature leads to hypothermia which is seen in organ transplantation and open heart surgery.



However, life exists in very cold regions and also in hot springs, indicating that the same enzyme that exists in human cell, for instance the enzymes of glycolysis and TCA cycle have their optimum temperatures at extremes of temperatures. Thus refrigeration bacteria exists with the optimum temperature of its enzymes being near 4°C. Likewise bacteria surviving in hot springs have the enzymes with their optimum temperatures nearing hundred(s) degree Celsius ex. the optimum temperature of Taq polymerase is 72°C.

Vant Hoff's coefficient:

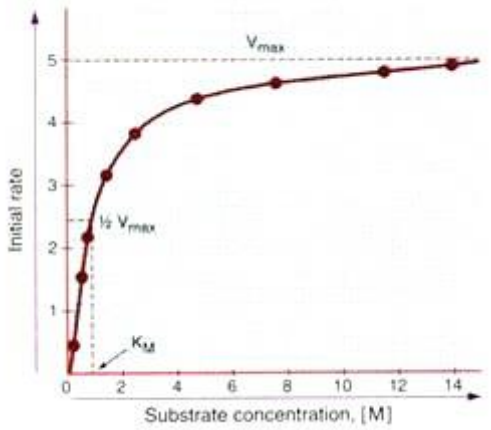
It is the coefficient which explains that for every 10°C rise in temperature the enzyme activity increases 2 fold till the optimum temperature is reached.

2. Effect of pH:

When all the other parameters are kept constant, the velocity of an enzyme catalysed reaction increases till it reaches the optimum pH and then decreases with further increase/decrease in pH. The activity is maximum for most of the enzymes at the biological pH of 7.4. Optimum pH for pepsin is 1.5, acid phosphatase is 4.5 and for alkaline phosphatase it is 9.8.

3. Effect of Substrate Concentration:

When all the other parameters are kept constant including the enzyme concentration, then, as the substrate concentration increases the rate of reaction increases steadily, till the enzyme is saturated with the substrate. At this stage the reaction rate does not increase and remains constant. When a graph is plotted with velocity versus substrate concentration it gives a hyperbolic curve.



This is because, as the concentration of substrate is increased, the substrate molecules combine with all available enzyme molecules at their active sites till no more active sites are available. Thus at this stage, substrate only replenishes the sites when the products are liberated and cannot increase the rate of reaction.

Michaelis Menten constant:

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

$V \rightarrow$ Velocity at a given concentration of the substrate

$V_{max} \rightarrow$ Maximum velocity with excess of substrate

$[S] \rightarrow$ Concentration of the substrate at velocity V

$K_m \rightarrow$ Michaelis-Menten constant for the enzyme

When the velocity is half the maximum velocity then,

$V = \frac{1}{2} V_{max}$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

Dividing by V_{max} we get

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

Definition:

K_m is defined as the substrate concentration at which the velocity of the enzyme catalysed reaction is half the maximum velocity.

i. A high K_m value indicates weak binding between the enzyme and the substrate.

ii. Low K_m indicates strong binding.

Limitations of Michaelis-Menten equation:

i. This equation enables the calculation of approximate value of the maximum velocity and not the accurate value.

ii. It holds good for enzymes which have active site only and not the allosteric site.

iii. It calculates the K_m for mono-substrate reactions and not for multi-substrate reactions.

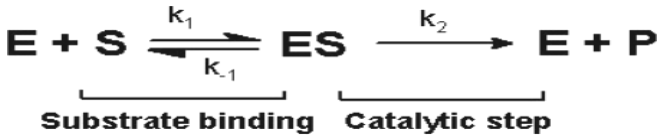
iv. It is used to know the velocity of non-regulatory enzymes but not of regulatory enzymes.

In order to overcome the above limitations a Line weaver-Burke plot is drawn so as to establish a relation between the reciprocals of substrate concentration and velocity.

Kinetics of Enzyme Catalyzed Reaction :

A particularly useful model for the kinetics of enzyme-catalyzed reaction was devised in 1913 by Leonor Michaelis and Maud Menten when they studied the reaction in which the invertase hydrolyzes sucrose, a disaccharide, into monosaccharides, glucose and fructose.

Michaelis-Menten described the reaction velocity and substrate concentration. To explain their results in the conversion of sucrose to glucose and fructose by the enzyme invertase, Michaelis and Menten proposed the following scheme of reactions:



The Michaelis-Menten kinetic model of a single-substrate reaction is shown that there is an initial bimolecular reaction between the enzyme E and substrate S to form the enzyme–substrate complex ES. In this reaction k_1 is the rate constant for the formation of enzyme-substrate complex and for the unimolecular reaction $ES \rightarrow E + P$ rate-determining enzymatic step that allows the mechanism to be modeled as a single kinetic step of rate constant k_2.

According to the Michaelis-Menten approach when the rate (also called velocity) of an enzyme catalyzed reaction is measured at varying substrate concentration, the rate depends on the substrate concentrations [S]. At a relatively low concentration of substrate, initial velocity (V) increases almost linearly with an increase in substrate concentration and it does not increase any further by increasing the concentration of substrate.

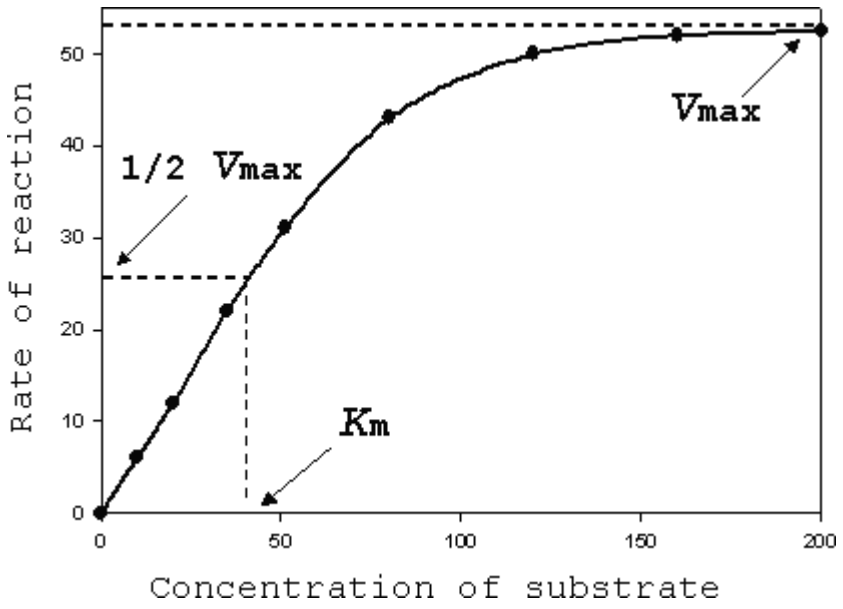


Figure: The hyperbolic relationship between initial velocity (V) and substrate concentration (S) of an Enzyme catalyzed reaction

Concentration of substrate at which reaction velocity reaches half its maximum velocity is called **Michaelis constant (K_m).** Lower value of K_m describes the greater affinity of the enzyme for the substrate.

Michaelis-Menten put forward a mathematical equation to establish the mathematical relationship among the quantities [E], [S], V_{max} and K_m.

$$v = \frac{V_{max}[\mathrm{S}]}{K_m + [\mathrm{S}]}$$

1. When [S] << K_m, then V ∝[S]
2. When [S]>> K_m, then V = [S]
3. When [S]=K_m, then V = 1/2V_{max}

1. Since at low concentration of the substrate, velocity is proportional to the substrate concentration [S], the enzyme catalyzed reaction is first order reaction. At high [S], velocity becomes virtually independent of [S] and approaches a maximum limit. Since rate is no longer dependent on [S] at high concentration, the enzyme catalyzed reaction obey zero order kinetics.
2. Michaelis-Menten model is based on the assumptions. The following assumptions are made in driving the Michaelis-Menten rate equation:
3. The concentration of substrate[S] is much greater than enzyme[E].
4. The rate of formation of ES is equal to that of the breakdown of ES.
   (steady state assumption)
5. Very little accumulation of P, so the formation of enzyme-substrate complex from E+P is negligible.

Steady-state assumption: The interpretation of the Michaelis-Menten model were refined by an assumption termed the steady state assumption. The steady state hypothesis states that the concentration of enzyme-substrate complex remains constant through much of the reaction. When the enzyme substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so called steady state level. The more normal situation where $k_2 > k_{-1}$ is sometimes called Briggs- Haldane kinetics. The Michaelis–Menten equation still holds under these more general conditions, as may be derived from the **steady-state assumption**. During the initial-rate period, the reaction rate *v* is roughly constant, indicating that [ES] is similarly constant.

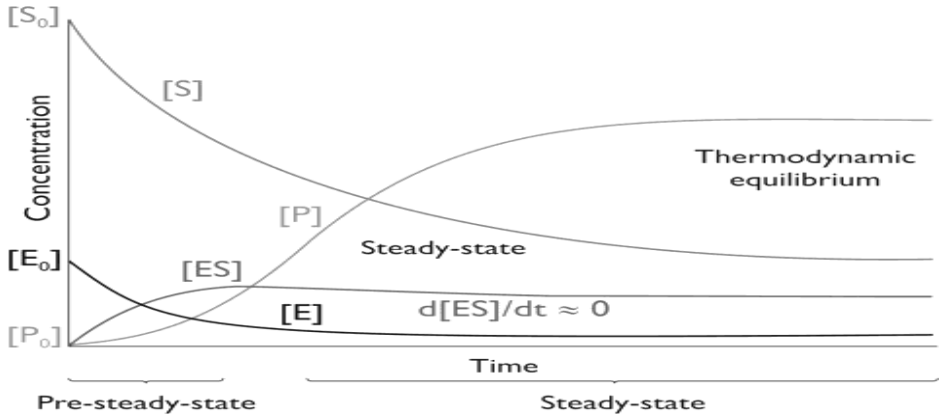


Figure: change in concentrations over time for enzyme E, substrate S, complex ES and product

$$\frac{d}{dt}[\mathrm{ES}] = k_1[\mathrm{E}][\mathrm{S}] - k_2[\mathrm{ES}] - k_{-1}[\mathrm{ES}] \approx 0$$

Therefore, the concentration [ES] is given by the formula

$$[\mathrm{ES}] \approx \frac{[\mathrm{E}]_{tot}[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

where the Michaelis constant K_m is defined

$$K_m \stackrel{\text{def}}{=} \frac{k_2 + k_{-1}}{k_1} \approx \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

([E] is the concentration of *free* enzyme). Taken together, the general formula for the reaction rate *v* is again the Michaelis-Menten equation:

$$v = k_2[\mathrm{ES}] = \frac{k_2[\mathrm{E}]_{tot}[\mathrm{S}]}{[\mathrm{S}] + K_m} = \frac{V_{max}[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

Turn over number (K_{cat}): The number of substrate molecules converted into product by an enzyme molecule per unit time when the enzyme is fully saturated with substrate . The term K_{cat} represents the kinetic efficiency of the enzyme. Its unit is sec^{-1}.

At saturating [s], $V=V_{max}=K_2\ [E_t]$ Thus,

$K_2 = V_{max}/ E_t = K_{cat}$

The specificity constant k_{cat} / K_m is a measure of how efficiently an enzyme converts a substrate into product. Using the definition of the Michaelis constant K_m, the Michaelis- Menten equation may be written in the form

$$v = k_2[\mathrm{ES}] = \frac{k_2}{K_m}[\mathrm{E}][\mathrm{S}]$$

where [E] is the concentration of free enzyme. Thus, the specificity constant is an effective bimolecular rate constant for free enzyme to react with free substrate to form product. The specificity constant is limited by the frequency with which the substrate and enzyme encounter each other in solution, roughly 10^{10} M^{-1} s^{-1} at 25°C. Remarkably, this maximum does not depend on the size of either the substrate or the enzyme. The ratio of the specificity constants for two substrates is a quantitative comparison of how efficient the enzyme is in converting those substrates. The slope of the Michaelis-Menten equation at low substrate concentration [S] (when [S] << K_m) also yields the specificity constant.

Kinetics of bi or multi-reactant system :

So, far we have considered enzyme catalyzed reactions with single substrate. This situation is not common. Usually, enzymes catalyze reactions are those in which two (or more) substrates take part. Consider the case of An enzyme catalyzing a reaction involving two substrates A and B, and yielding the products P and Q:

$A+B \rightleftharpoons P+Q$

Such a reaction is termed as bisubstrate reaction. In general, bisubstrate reactions proceed by one of the two possible routes:

1. Both A and B are to the enzyme and then reaction occurs to give P+Q:

$E+A+B \rightleftharpoons E+P+Q$

Reaction of this type are defined as sequential or single-displacement reactions.

Sequential reactions can be either of two distinct classes :

1. If there is no obligatory order of addition of substrates or release of products, it is called random sequential.

b. If substrates add in an obligatory order, the mechanism is called ordered sequential.

A B P Q

↓ ↓ ↑ ↑

E EA ~~EAB~~ ~~EP~~Q EQ ~~E f~~ig: ~~ordered~~ sequential

In order sequential reactions, one substrate is obligated to bind to the enzyme before a second substrate. In random sequential mechanisms, there is no preference.

In practice, there is usually some degree of order in binding.

2. The other general possibility is that one substrate, A, binds to the enzyme and reacts with it to yield a chemically modified form of the enzyme (E') plus the product, P. The second substrate, B, then reacts with E', regenerating E and forming the other product, Q. Reactions that fit this model are called ping-pong or double-displacement reactions.

Line weaver-Burke plot:

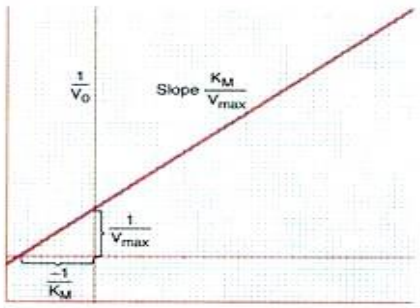
Inverting the Michaelis-Menten equation, we get

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\text{or,} \quad \frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

$$\text{or,} \quad \frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

This equation is similar to the mathematical equation i.e., y = ax + b, which gives a straight line plot. Here y = 1/V; a = K_m/V_{max}; x = 1/S; b = $1/V_{max}$.



By this equation we can calculate accurately:

i. The velocity of any enzyme catalyzed reaction.

ii. The rate of reaction where more than one substrate is present.

iii. The velocity of all the enzymes.

Regulatory enzymes give a sigmoid curve and non-regulatory enzymes give a hyperbolic curve.

4. Effect of Enzyme Concentration:

As the enzyme concentration increases, the rate of reaction increases steadily in presence of an excess amount of substrate, the other factors being kept constant. A linear curve is produced.

5. Effect of Products:

When the product is more in the reaction mixture, then the rate of reaction decreases due to feedback inhibition.

6. Effect of Light:

The speed of activity of various enzymes changes in different wavelength of light ex. blue light enhances the activity of salivary amylase whereas, U.V. light decreases the velocity.

7. Effect of Ions:

Presence or absence of particular ions enhances or reduces the activity of enzymes ex. Pepsinogen is converted to pepsin in presence of H^+ ions. Kinases act in presence of Mg^{+2} ions.

Enzyme Specificity:

Enzymes are very specific in their reaction. They either act on one particular substrate or catalyze one particular reaction.

Accordingly enzyme specificity is of two types:

1. Reaction Specificity:

These enzymes are specific for the type of reaction they catalyze, irrespective of the substrate on which they act. Thus different enzymes bring about different reactions on the same substrate i.e. enzymes are specific for one particular reaction no matter which substrate it may be ex. amino acids are acted upon both by amino acid oxidase which oxidizes the amino acids to keto acids and decarboxylase that removes carbon dioxide from them.

2. Substrate Specificity:

These enzymes are specific for the substrate upon which they act. This is further classified as follows.

(a) Absolute specificity:

These enzymes are highly specific and act on one particular substrate only and no other substrate. Ex. Urease, catalase, aspartase.

(b) Relative specificity:

These enzymes act on one particular bond. Ex. D-amino acid oxidase.

(c) Group specificity:

These enzymes act on only one particular group.

i. Pepsin:

Is a proteolytic enzyme that acts on peptide bonds contributed by aromatic amino acids like tyrosine, tryptophan and phenylalanine.

ii. Trypsin:

Is specific for basic amino acids. Hence it cleaves peptide bonds contributed by lysine and arginine.

iii. Amino peptidase:

Acts on peptide bond near the free amino end.

iv. Carboxypeptidase:

Specific for free carboxylic group.

v. Amylase:

Specific for α-1 → 4 glycosidic linkages.

(d) Stereo specificity:

These enzymes act on one particular stereo isomer.

i. Succinate dehydrogenase:

Is specific for the stereo isomer fumarate i.e. cis form of double bond.

ii. Cellidase:

Is specific for β glycosidic linkage.

iii. L-amino acid oxidases:

Act on L-amino acids only and not on D-amino acids

Coenzymes:

They are non-protein, heat stable, low molecular weight dialyzable organic compounds that are required for the action of enzymes. Generally vitamins act as coenzymes ex. biotin, pyridoxine etc. Enzyme along with a co-enzyme is known as 'holoenzyme' and that without a co-enzyme is an 'apoenzyme'. Apoenzyme (protein) + Co-enzyme (non-protein) → Holoenzyme (active enzyme protein).

Holoenzyme may contain an organic or inorganic compound (metal ions) or both. If organic substances are present with enzymes then they are known as 'co-enzymes' and if inorganic substances are acting with the enzymes then they are called as 'co-factors' (Mg, Mn, Zn, Co, Se, etc.).

The role of co-enzymes is:

(i) They act as co-substrate or second substrate ex. Pyruvate + NADH → Lactate + NAD^+. NADH acts as a coenzyme or second substrate,
(ii) They help in transferring of groups either hydrogen or groups other than hydrogen, and

(iii) Specific activity of a co-enzyme is the number of units of co-enzyme present in one milligram of enzyme protein.

Enzyme unit or activity:

One unit of enzyme activity is the amount of enzyme that converts 1.0 (J.M of the substrate per minute into the products at 25°C.

Specific activity of an enzyme:

It is defined as the number of enzyme units per milligram of the protein.

Enzyme turnover number:

The number of substrate molecules transformed per minute (unit time) by a single enzyme is known as enzyme turnover number. Carbonic anhydrase has the highest turnover number of 36,000,000.

First and second order reaction:

A reaction in which there is only one substrate is termed as 1^{st} order reaction. A reaction in which two substrates are involved to form a product is termed as 2^{nd} order reaction, also known as bi-substrate reaction. This involves either single displacement (i.e. both substrates binding to two active sites in the enzyme at the same time) or double displacement (ping-pong displacement, wherein only one substrate binds to the enzyme active site at a given time, once this is released the other substrate binds).

Zymogen:

The inactive form of an enzyme is known as zymogen or pro-enzyme. Pepsinogen and trypsinogen are the zymogens of pepsin and trypsin respectively.

Ribozyme:

Ribonucleic acids that catalyze a reaction similar to that of enzymes are known as ribozymes. These ribozymes help in the processing of the newly transcribed RNA ex. small nuclear RNA (SnRNA) and hetero-nuclear RNA (hnRNA).

Probable Questions:

1. State how the rate of enzyme activity is influenced by several factors.
2. What are the assumptions of the Michaelis-Menten equation? Derive the Michaelis- Menten equation.
3. What is Lineweaver Burk plot? Explain graphically.

Suggested Readings/References

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5. Donald Voet and Judith G. Voet Biochemistry

UNIT-XVII

Enzyme inhibitions, Regulation of enzyme activity, Allosteric control of enzyme activity

Objective: In this unit we will discuss different types of enzyme inhibitions and will also discuss about allosteric control of enzyme activity

Enzyme Inhibition:

Alteration in the enzyme activity by specific substances other than non-specific substances like pH, temperature etc. is called enzyme inhibition.

There are two types of enzyme inhibitions:

(a) Irreversible and

(b) reversible.

1. Irreversible Enzyme Inhibition:

The activity of the enzyme is inhibited by covalent binding of the inhibitor at the active site. The enzyme inhibitor bond cannot be dissociated, so it is permanent and irreversible i.e. it cannot be reversed.

i. Aldolase is inhibited permanently by iodoacetate.

ii. Di-isopropylflurophosphate (DFP), a component of nerve gas, inhibits most of the digestive enzymes permanently in human beings. Hence it is very poisonous.

iii. Para chloromercuric benzoate (PCMB) inhibits the enzymes hexokinase and urease irreversibly.

iv. Organic reagents like alkaloid reagents inhibit enzymes irreversibly.

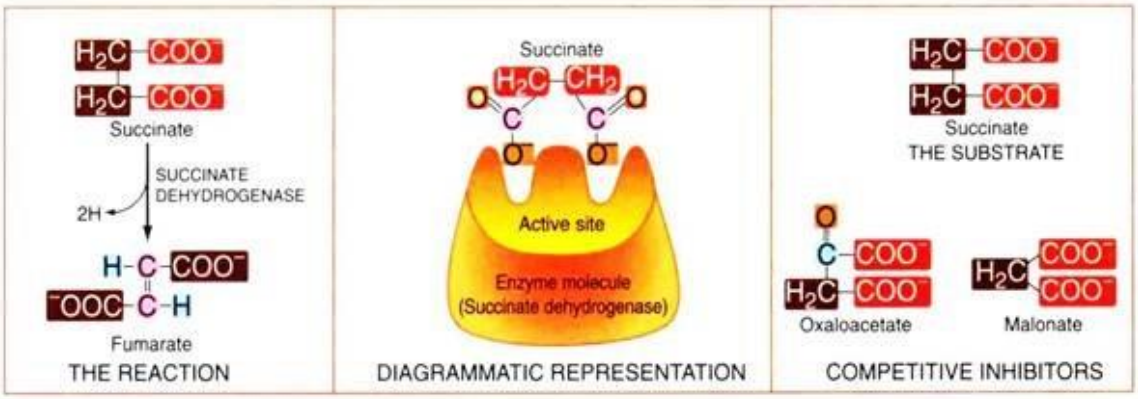
2. Reversible Enzyme Inhibition:

The inhibitors bind reversibly to the enzyme and so it is not permanent. The inhibition can be reversed by various mechanisms.

(a) Competitive enzyme inhibition:

It is a type of reversible inhibition in which there is competition between substrate and inhibitor for the active site of an enzyme because of the structural similarity. All non-regulatory enzymes show competitive inhibition. Clinically competitive enzyme inhibition is of great importance since most of the drugs act by competitive inhibition.

(i) The enzyme succinate dehydrogenase's (SDH) substrate is succinic acid and the competitive inhibitors are oxalic acid, mallonic acid and glutaric acid. Among these, mallonic acid is the most potent competitive inhibitor of SDH.



(ii) Folic acid, a vitamin for human beings has para-amino benzoic acid (PABA) as one of its components. Whereas it is not a vitamin for microorganisms i.e., they cannot utilize preformed folic acid from external source, instead they synthesize their own folic acid from aba. Sulpha drugs contain para-amino sulphonate which is structurally similar to PABA and hence competes for the enzyme active site of folic acid synthesis in microorganisms. If excess dose of sulpha drug is given, it results in inhibition of folic acid synthesis thus acting as an antibiotic. Human beings are not affected, because they do not synthesize folic acid.

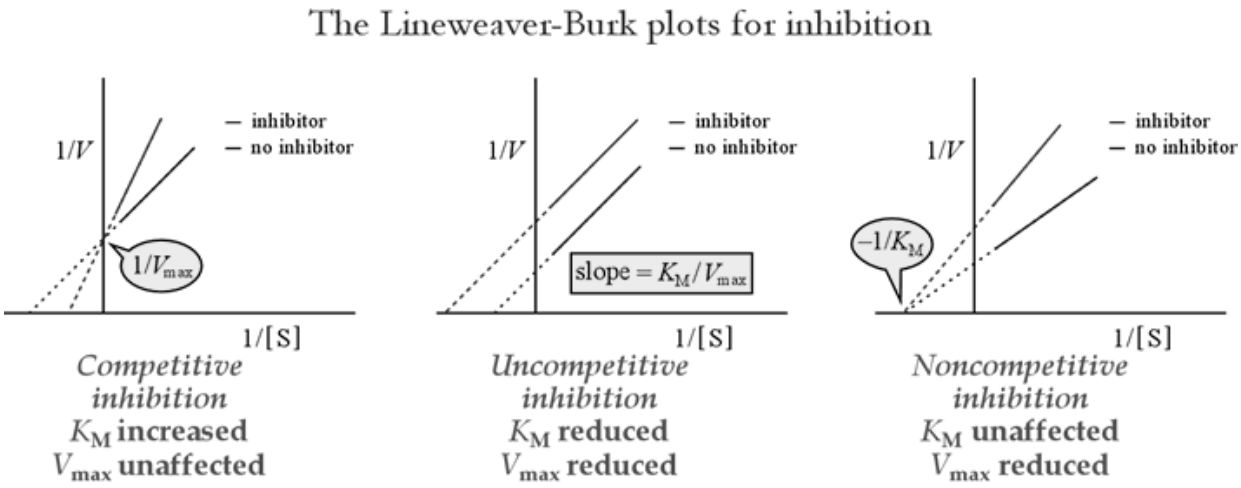
(iii) Methanol is acted upon by the enzyme alcohol dehydrogenase forming formaldehyde which is highly poisonous. If ethanol if given to methanol intoxicated patients then ethanol competitively binds to alcohol dehydrogenase thereby preventing formation of formaldehyde.

(iv) Allopurinol is the competitive inhibitor of the enzyme xanthine oxidase whose substrate is hypoxanthine. Allopurinol prevents the formation of uric acid by competitive inhibition because it has structural similarity to hypoxanthine. This principle is used in the treatment of gout i.e. abnormal accumulation of uric acid crystals in the joint causing inflammation.

(v) Glaucoma is a condition in which there is accumulation of fluid in the lens resulting in enlargement of eye. This can be treated with 'acetazolamide' which inhibits the enzyme carbonic anhydrase competitively. This prevents water formation and subsequent release of more water through the urine.

(b) Non-competitive enzyme inhibition:

It is shown by regulatory enzymes, also called allosteric enzymes.



Allosteric enzymes:

These are the enzymes that contain a site other than the active site which is called 'allosteric site'. The action of some enzymes is regulated by 'effectors' which can bind reversibly to the enzyme molecule at specific sites other than the substrate binding site called the modulator site or the allosteric site. There is no competition between substrate and inhibitor for the active site since the inhibitor or modulator binds at the modulator site or allosteric site. If the binding of the effector causes inhibition of the enzyme action then it is called a negative effector and the process is called 'allosteric inhibition'. If the enzyme reaction is activated by a modulator then it is called a positive modulator or effector and the process is called 'allosteric activation'. Ex. Phosphofructo kinase (PFK) is an allosteric enzyme of the glycolytic pathway. The positive modulators of this enzyme are AMP and ADP. The negative modulators of PFK are ATP and citrate.

ALLOSTERIC MODULATION OF ENZYMES :

Sometimes, the activity of an enzyme may be either enhanced or inhibited due to its conformational changes brought abouit by non-covalent binding of some specific low molecular weight ligands, to specific sites of the enzyme other than the active site. Such

modification of enzyme activity is called allosteric modulation or allosterism.

Positive modulation

Positive allosteric modulation occurs when the binding of one ligand enhances the attraction between substrate molecules and other binding sites. An example is the binding of oxygen molecules to hemoglobin, where oxygen is effectively both the substrate and the effector .

Negative modulation

Negative allosteric modulation occurs when the binding of one ligand decreases the affinity for substrate at other active sites. For example, when 2,3-BPG binds to an allosteric site on hemoglobin, the affinity for oxygen of all subunits decreases.

ALLOSTERIC MODULATORS :

The substances which bind at the allosteric site of the enzyme and regulate their activity are called allosteric modulators or effectors. These are generally small molecular weight by products of that particular reaction pathway. The binding site of allosteric modulators on enzyme is called allosteric site and the enzyme thus being regulated is called allosteric enzyme. Example- 1) Citrate enhances the activity of Acetyl CoA carboxylase which produces malonyl CoA from acetyl CoA (positive regulation).

2) Palmitoyl CoA suppresses the activity of Acetyl CoA carboxylase (negative regulation).

The activation state of an enzyme is often referred to as **R**, or the relaxed state, where the enzyme is on, and its activity is turned up. In the **T**, or the tense state, the enzyme is off, and its activity is turned down.

One molecule may bind the allosteric site and make the enzyme change from the T to R state, while a different molecule can bind the same enzyme and change it from the R to T state. The state of the enzyme will also affect its function. An example of this can be found in respiration, where a specific enzyme, phosphofructokinase-1, is activated by adenosine diphosphate (ADP), but inactivated by adenosine triphosphate (ATP).

Antienzymes:

These are substances (generally proteinacious in nature) that inhibit most of the digestive enzymes, ex. certain roundworms and hookworms survive in the intestine by secreting anti enzymes. Uncooked rice contains certain proteins that act as antienzymes.

Reversible covalent modification:

Enzyme activity can be regulated by reversible covalent modification.

It is regulated by cyclic inter-conversion of enzyme into two forms:

(i) Modified form and (ii) Unmodified form.

The inter-conversion is brought about by a 'converting enzyme'. The process of activation and inactivation of the enzyme is generally brought about by covalent phosphorylation or de-phosphorylation of the target enzyme. For example hormones like epinephrine, glucagon etc. bind to the hormone receptor site on the cell membrane and activate the enzyme adenyl cyclase, which in turn converts ATP to cyclic AMP (cAMP). This cAMP converts inactive protein kinase to active protein kinase ('a' form). This protein kinase phosphorylates many enzymes in the cell, some of which become active and yet some others become inactive. The inactive phosphorylase ('b' form) gets converted to active phosphorylase ('a' form) upon phosphorylation and affects the breakdown of glycogen to glucose. On the other hand glycogen synthase becomes inactive upon phosphorylation thereby inhibiting the formation of glycogen.

Diagnostic Importance of Enzymes:

Enzymes were classified into two groups based upon their clinical importance as 'functional plasma enzymes' i.e., those enzymes present in the plasma in considerably high amounts and are functional in the plasma due to the presence of their substrate in it. Ex. serum lipase, blood clotting enzymes, and 'non-functional plasma enzymes' i.e., those enzymes that are present in the plasma in negligible amounts and have no function in the plasma due to the absence of their substrate in it. Non-functional plasma enzymes are of diagnostic importance. The non-functional plasma enzymes are present in higher concentration in tissues and very low concentration in the plasma i.e. in trace amounts, but their concentration in plasma increases immediately following tissue injury or destruction. If there is tissue damage leading to cell rupture then the enzymes present in that tissue leaks into the blood leading to the increase in the concentration of these enzymes in the plasma. Increase in the level of non-functional plasma enzymes in the blood, indicates the disorder to the tissue where they exist. Different enzymes

exist in different tissues in varying levels. Damage to a specific tissue releases a particular enzyme. Therefore estimation of enzymes in the plasma has a diagnostic importance.

The non-functional plasma enzymes include lactate dehydrogenase (LDH), creatine phosphokinase (CPK), alanine amino transferase (ALT) or serum glutamate pyruvate transaminase (SGPT), aspartate transaminase (AST) or serum glutamate oxaloacetate transaminase (SGOT), sorbitol dehydrogenase, alkaline phosphatase, acid phosphatase, amylase, pancreatic lipase etc. However functional plasma enzymes are already in higher concentration in the plasma, hence their decrease in the concentration in the plasma indicates malfunction of the organ where they are synthesized ex. blood clotting enzymes are synthesized in the liver; hence decrease in their concentration indicates liver dysfunction. Anyway an immediate assessment of the liver function cannot be made by this assessment because by the time the enzyme concentration in the plasma decreases (may take 4 to 5 days), the liver must have regained its normal vitality.

Some clinically important enzymes

Enzymes and their Concentration	Concentration increases in
Lactate dehydrogenase (LDH) – 60-12 IU/litre	Myocardial infarction, myopathy or muscle disorder. Also in leukemias, acute hepatitis, carcinomatitis.
Transaminases— (a) Aspartyl transaminase (AST) or Serum glutamyl oxaloacetate transaminase (SGOT) – 5-20 IU/litre	Myocardial infarction
(b) Alanine transaminase (ALT) or Serum glutamyl pyruvate transaminase (SGPT) – 5-15 IU/litre	Liver disorders
Creatine phosphokinase (CPK) – 10-60 IU/litre	Myocardial infarction, myopathy
Alkaline phosphatase – 4-17 King Amstrong (KA) units/100 ml	Bone disorders, obstructive jaundice, hyperparathyroidism
Acid phosphatase	Prostrate carcinoma
Isocitrate dehydrogenase	Brain tumor and meningitis, liver diseases
Amylase	Pancreatitis, parotitis (inflammation of parotid gland) intestinal obstruction, diabetes
Lipase	Pancreatitis or carcinoma of pancreas
Gamma glutamyl transpeptidase (g-GT)	Liver damage (indicator of alcoholism)

Diagnosis of Myocardial Infarction using Enzyme Assay:

There are three main enzymes that are used in the diagnosis of myocardial infarction (1) Lactate dehydrogenase (LDH) (2) Creatine phosphokinase (CPK)—marker enzyme and (3) Transaminase (AST or SGOT).

(1) Lactate dehydrogenase (LDH):

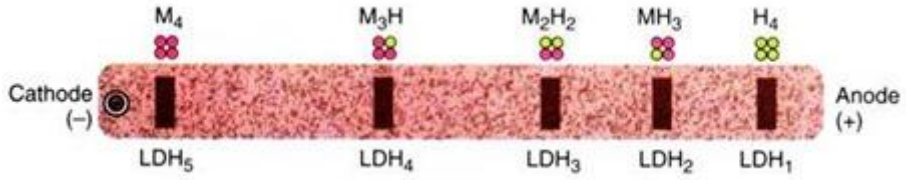
LDH catalyses the inter conversion of pyruvate to lactate, a very important reaction of anaerobic glycolysis. Glycolysis occurs in each and every cell, in some cells it is always anaerobic (RBC) whereas in others it is aerobic sometimes and anaerobic at some other time (muscle tissue, liver, kidney etc.). In other words LDH is present in each and every cell of the body. Therefore damage to any of the tissues of the body results in release of LDH into the plasma. Hence it becomes a difficult task to trace out the organ from which it has leaked.

However LDH exists in five isoenzyme forms i.e. multiple forms of the same enzyme (These enzymes bring about the same reaction but exhibit different physical characters like molecular weight, charge, electrophoretic mobility, K_m and isoelectric pH). The polypeptides in LDH are designated as 'H chain' and 'M chain'.

All the isoenzyme forms of LDH are tetramer i.e. has four polypeptides in the following combinations:

(a) H_4 or LDH_1—Heart
(b) H_3M or LDH_2—RBC
(c) H_2M_2 or LDH_3—Brain and lungs
(d) HM_3 or LDH_4—Kidney
(e) M_4 or LDH_5—Liver and skeletal muscle

All these isomers have been successfully separated on Sodium Dodecyl Sulphate Polyacryl Amide Gel Electrophoresis (SDS-PAGE) and their banding pattern from the plasma is established as under—



LDH_1 or H_4 is predominantly present in the cardiac muscle, whereas the isoenzyme form LDH_5 or M_4 is more abundant in the skeletal muscle. These two enzymes have different Km values and Km is indirectly proportional to affinity (K_m a 1/affinity).

The skeletal muscle enzyme M_4 has low K_m value for pyruvate and hence greater affinity for pyruvate resulting in high rate of conversion of pyruvate to lactate. The cardiac isoenzyme LDH_1 or H_4 has high K_m value for pyruvate hence lesser affinity for pyruvate, therefore low rate of conversion of pyruvate to lactate. Thus the concentration of H_4 or LDH_1 isoenzyme form of lactate dehydrogenase increases in the plasma during myocardial infarction. The peak levels of LDH are maintained in the plasma for 6 days following the attack, after which it starts receding in its concentration.

(2) Creatine phosphokinase (CPK):

This is known as the marker enzyme for the diagnosis of myocardial infarction or heart attack, because this is the first enzyme to increase within a short time in the blood plasma following a heart attack. CPK is an enzyme that catalyzes the conversion of creatine to creatine phosphate, a high energy compound that works to supply energy during muscle contraction. Therefore this enzyme is present only in a few tissues like the cardiac muscle, skeletal muscle and the brain. CPK also exists in various isoenzyme forms. It has two polypeptides 'B' & 'M' that form dimers in the following combinations to give rise to three isoenzymes of CPK.

MB — Predominant in cardiac muscle

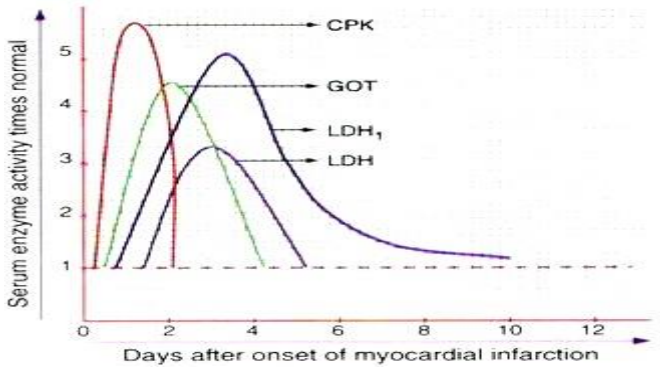
BB — Predominant in brain

MM — Predominant in skeletal muscle

Thus estimation of the isoenzyme MB is indicative of heart attack. CPK maintains a higher concentration in the plasma for 1-2 days. The concentration of CPK after the first attack is 10 times more than the normal and if another attack occurs within a day or two the concentration further increases to 100 fold and a third attack within a short span of time raises the level of CPK to 300 fold which is lethal concentration.

(3) Transaminases:

Among the two transaminases, aspartyl transaminase (AST or SGOT) increases in the plasma following an attack and the higher levels are seen in 4 to 5 days following an attack.



Probable questions:

1. Describe the different modes of reversible inhibition of enzymatic reactions.

2. What do you mean by allosteric modulation of enzymes?

Suggested Readings/References

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

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5. Donald Voet and Judith G. Voet Biochemistry

UNIT-XVIII

Chemistry of free radicals and antioxidants.

Objectives: In this unit we will discuss about chemistry of different free radicals which acts as antioxidants.

Characteristics of Free Radicals:

ROS and RNS are the terms collectively describing free radicals and other non-radical reactive derivatives called oxidants. A molecule with one or more unpaired electron in its outer shell is called a free radical. Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, by cleavage of a radical to give another radical and via redox reaction. These radicals are hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide (NO_2), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$).

Also, hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($1O_2$), hypochlorous acid (HOCI), nitrous acid (HNO_2), per-oxy-nitrite ($ONOO^-$), di-nitrogen trioxide (N_2O_3), lipid peroxide(LOOH), are not free radicals and generally called oxidants, but can easily lead to free radical reactions in living organisms. Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation.

Generation of Free Radicals:

Formation of ROS and RNS can occur in the cells by two ways: enzymatic and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the respiratory chain, the phagocytosis, the prostaglandin synthesis and the cytochrome P_{450} system.

The superoxide anion radical ($O_2^{\bullet-}$) is generated via several cellular oxidase systems such as N ADPH oxidase, xanthine oxidase, peroxidases and then it participates in several reactions yielding various ROS and RNS which are hydrogen peroxide, hydroxyl radical ($OH^{\bullet}$), per-oxy-nitrite (ONOO-), hypochlorous acid (HOCI), etc. Non-radical H_2O_2 is produced by the action of several oxidase enzymes, including amino acid oxidase and xanthine oxidase. Hydroxyl

radical ($OH^{\bullet}$), the most reactive free radical in vivo, is formed by the reaction of $O_2^{\bullet-}$ with H_2O_2 in the presence of Fe^{2+} or Cu^{++} (catalyst). This reaction is known as the Fenton reaction.

$$2Fe^{3+} + \text{Ascorbate} \rightarrow 2Fe^{2+} + \text{De-hydro-ascorbate}$$
$$2Fe^{2+} + 2H_2O_z \rightarrow 2Fe^{3+} + 2OH^{\bullet} + 20H^{+}$$

The neutrophil-derived enzyme, myeloperoxidase, which oxidizes chloride ions in the presence of H_2O_2, produces hypochlorous acid (HOCI). Nitric oxide radical ($NO^{\bullet}$) is formed in biological tissues from the oxidation of L-arginine to citrulline by nitric oxide synthase. Free radicals can be produced from non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The non-enzymatic process can also occur during oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria. ROS and RNS are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, aging.

Exogenous ROS/RNS result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation.

Beneficial Activities of Free Radicals:

At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and can act as weapons for the host defence system. Indeed, phagocytes (neutrophils, macrophages, monocytes) release free radicals to destroy invading pathogenic microbes as part of the body's defence mechanism against disease. The importance of ROS production by the immune system is clearly exemplified by patients with granulomatous disease due to defective membrane-bound NADPH oxidase system which makes them unable to produce the superoxide anion radical ($O_2^{\bullet-}$), thereby resulting in multiple and persistent infection. Other beneficial effects of ROS and RNS involve their physiological roles in the function of a number of cellular signalling systems.

Their production by non-phagocytic NADPH oxidase isoforms plays a key role in the regulation of intracellular signalling cascades in various types of non-phagocytic cells including fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes, and thyroid tissue. For example, nitric oxide (NO) is an intercellular messenger for modulating blood flow, thrombosis, and neural activity.

In brief, ROS/RNS at low or moderate levels are vital to human health cytic NADPH oxidase isoforms plays a key role in the regulation of intracellular signaling cascades in various types

of non-phagocytic cells including fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes, and thyroid tissue.

Harmful Activities of Free Radicals and Pathogenesis:

An oxidative stress, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA) due to generation of excess free radicals and oxidants. Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. In other words, oxidative stress results from an imbalance between formation and neutralization of ROS/RNS. For example, hydroxyl radical and per-oxy-nitrite in excess can damage cell membranes and lipoproteins by a process called lipid peroxidation.

This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Proteins may also be damaged by ROS/RNS, leading to structural changes and loss of enzyme activity. Oxidative damage to DNA leads to the formation of different oxidative DNA lesions, which can cause mutations. The body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants. If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases as well as the aging process and some acute pathologies like stroke, atherosclerosis, cancer, cardiovascular disease etc.

Formation of Free Radicals:

Free radicals are basically formed as accidental and deliberate by-products and phagocytosis from oxygen, H_2O_2, etc. Some of them are superoxide (O_2^-), hydroxyl ($OH^{\cdot}$), etc. Independent on the identity of the cellular O_2-activator, the initial reduction product is always a super-oxide radical.

SOD, which is present in all cells, destabilizes O_2^- by a diffusion limited dis-mutation of two $O_2^{-\bullet}$ to H_2O_2 and O_2 (Figure 31.1). Due to cellular compartmentation of SOD, O_2 radicals are not expected to be totally metabolized to H_2O_2.

This is also the case with H_2O_2 which is a substrate for both catalase and glutathione-peroxidase (GSH- POD). H_2O_2 which escapes detoxification by the latter enzymes may become subject to the transfer of a further electron thereby forming $OH^{\bullet}$ radicals; these are the most reactive metabolites known in biological systems.

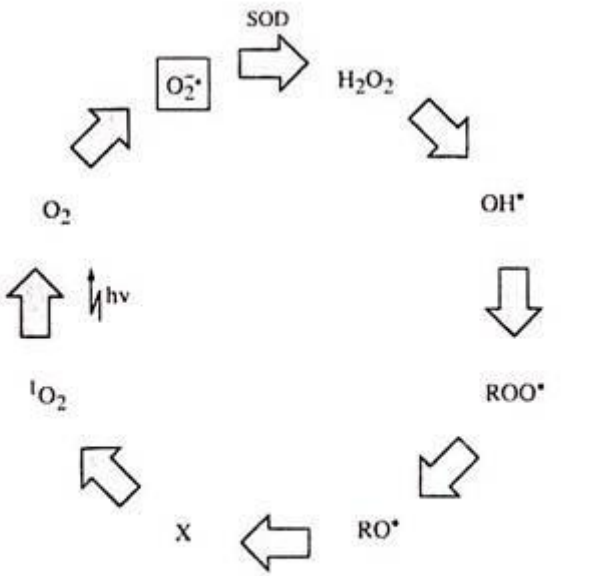

Fig. 31.1: Generation sequence of reactive oxygen species following univalent reduction of oxygen.

Due to its extremely high reaction potential, diffusion of $OH^\bullet$ from its site of generation can be excluded. Consequently respective reaction products are expected to be formed in close proximity to $OH^\bullet$ generation sites. The most frequent reaction product stems from an interaction of $OH^\bullet$ with membrane-phospholipids. The first step in this reaction sequence is a hydrogen-abstract from the divinyl-methane structure of polyunsaturated fatty acid and the subsequent addition of an O_2-molecule, resulting in the formation of peroxylradical.

This peroxyl-radical can enter a chain reaction forming an alkoxylradical by a bimolecular reaction and O_2 in an excited state (singlet state). Vitamin E in cooperation with Vitamin C is able to terminate self-sustaining chain reaction either by a direct interaction with $OH^\bullet$ or by the chemical reduction of propagating organic radical intermediates.

The excited state of singlet oxygen ($^1O_2$) is unstable and will lead to photo-emission by mono- or dimol reactions. Alternatively, the energy can be transmitted directly to other molecules such as carbonyl groups, which are in turn transferred into an excited state (singlet or triplet state). Presence of transition metals is essential for free radical formation in most cases. Antioxidants present in foods counter the action of oxygen free radicals which are produced under a number of circumstances. They are also formed as a part of body's normal metabolic process. Synthetic (xenobiotic) chemicals, radiation, x rays, pollution and even stress can produce these damaging entities.

The chemicals known to produce free radicals include chlorinated hydrocarbons, aromatic hydrocarbons, industrial acids, solvents, most pesticides and herbicides, preservatives in foods, printing pigments and inks and other industrial chemicals, fragrances and perfume vehicles, cosmetic vehicles and cosmetics, pollutants in air and water, many if not all pharmacological

agents used in medicine and anesthetics, which have a profound effect in producing radicals in the central nervous system. Even the transitional metal catalysts iron and copper, which are ubiquitous, have a most powerful generating effect on chain initiating radicals. Chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage in particular to the immune and nervous systems.

Under conditions of continuing and excessive emotional stress, higher levels of the hormones adrenaline and nor adrenaline are secreted by the adrenal glands. As a natural part of their metabolic processing, these stress hormones are oxidized to simpler molecules and in doing so become free radicals. It is possible, through this increased production of hormone radicals that stress increased biological degenerative processes occur resulting in wide-spread molecular, cell and tissue damage. In the living organism, free radical chain reactions are produced normally in the mitochondrial respiratory chain, liver mixed function oxidases, by bactericidal leucocytes, through xanthene oxidase activity, by atmospheric pollutants, and from transitional metal catalysts, drugs and xenobiotic chemicals.

Superoxide is formed by the reaction of oxygen with an electron.

$$O_2 + e^- \rightarrow O_2^{-\bullet}$$

Superoxide on reaction with hydrogen peroxide yields hydroxyl radical with the liberation of oxygen.

$$O_2^{-\bullet} + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2$$

Oxygen on reaction with 2 electrons yields hydrogen peroxide.

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

Hydrogen peroxide is itself uncharged but is called reactive oxygen species (ROS) due to its highly reactive nature. Two superoxide ions may also react together to yield hydrogen peroxide and oxygen. This is known as dis-mutation reaction.

$$2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

Hydrogen peroxide in the presence of transition metals like Fe^{2+} forms hydroxyl radical which is known as Haber Weiss reaction.

General Effects of Free Radicals:

Free radicals decompose enzymes, sequester proteins, scavenge different metabolic compounds. But they are hard to study due to their short life span. Their life-span may range from a few nanoseconds to one or two seconds only.

However during the period of time they are present, they can do untold damage to biological systems including DNA, macromolecules such as proteins, lipids and intracellular organelles such as mitochondria, Golgi apparatus and lysosomes. They are also induced by several external agents. A classic example is carbon tetrachloride (CCI_4). It is believed that CCI_4 causes hepatic toxicity by free radical attack. Lipids are most susceptible to free radical attack. They cause extensive lipid peroxidation. Lipids are present in the cell membrane in the form of poly unsaturated fatty acids or PUFA. Lipid peroxidation triggers a chain of degenerating events. It directly leads to decomposition of membrane. Indirectly, they lead to formation of other highly reactive harmful aldehydes. Peroxidation is also believed to be responsible for atherosclerosis. It has less effect directly on proteins. But formation of lens crystalline proteins in the eye by peroxidation is believed to be caused by peroxidation of lipid leading to cataract. It has a direct and more expansive effect on DNA which may be the cause of cancer. Its effect on DNA may be considered to be causing indirect effect on the protein constituents of the cell.

Defenses against Free Radical Attack:

Defenses against the free radical attack may be basically categorized into two:

1. Prevent the production of free radicals.

2. Intercept or scavenge the produced free radicals.

One way of preventing the production of free radicals is to sequester transition mental ions required for their formation. Hydrogen peroxide, lipid peroxides formed during lipid peroxidation may be removed by catalase and glutathione peroxidase (GSH) enzymes found in peroxisomes and cytosol respectively leading to prevention of further damage.

Probable Questions:

1. How free radicals are generated?
2. What are the benefits of free radicals?
3. What are the harmful activities of free radicals?

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.
2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.
3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

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5. Donald Voet and Judith G. Voet Biochemistry

UNIT-XIX

Vitamins and minerals: Role of vitamins as coenzymes

Objective: In this unit will discuss about different types of Vitamins regarding their structure, function and deficiency.

Introductions: Vitamins are organic compounds that are required in small amount (µg or mg/day) for normal growth metabolism and proper functioning of the body. Vitamins are also known as accessory food factors or food hormones. Animal can't synthesize vitamins, and therefore depends upon plants and microbes for their supply. Any deficiency of vitamins for a longer period or presence of anti-vitamins in the diet causes deficiency disease or avitaminosis. However, treatment with antibiotics often results in deficiency of B-Complex vitamins because the intestinal bacteria that synthesize them get killed by such drugs. For this reason, doctors recommend vitamin B-complex during antibiotic therapy. Excess intake of vitamins may cause some pathological conditions called hyper-vitaminosis. However, it is rare and occurs in case of fat soluble vitamins which can be stored in considerably high amounts.

Types of Vitamins:

On the basis of solubility, vitamins are of two types:

(a) Fat soluble vitamins: Vitamins A, D, E & K.

(b) Water soluble vitamins: Vitamins C and B-complex (B_1, B_2, B_6 etc.)

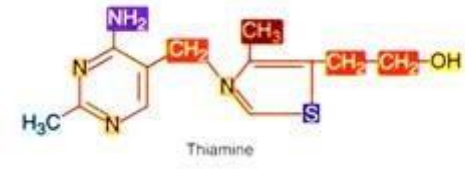
1. Water Soluble Vitamins:

Thiamine (Vitamin—B_1) :

Vitamin B_1 is also called as Anti-Beri Beri vitamin and And-Neuritic vitamin.

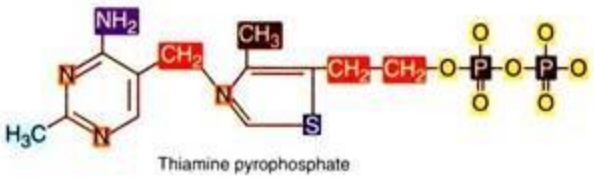
Chemistry:

Thiamine is present as a crystal of thiamine hydrochloride. Chemically thiamine contains a pyrimidine ring and a thiazole ring linked by a methylene bridge. Both the rings are substituted.



Thiamine

The alcohol group on the thiazole ring is esterified with two phosphoric acid molecules to form thiamine pyrophosphate (TPP). Thiamine pyrophosphate is the active co-enzyme form of thiamine.



Thiamine pyrophosphate

Source:

The richest sources of thiamine are rice bran, wheat bran, whole grains, nuts, germinating seeds, pulses, beans, lentils, yeast, liver, eggs, fish, meat and milk.

Daily requirement:

This vitamin cannot be stored in the body, as it is excreted in the urine, hence it should be provided daily in the diet. However the skeletal muscle can retain this vitamin for a short duration of time. Brain cannot retain any of the thiamine. The daily requirement of thiamine depends upon the calorie requirement and the carbohydrate intake. For 3000 Kcal of energy 1.5 mg of thiamine is required per day. However—

i. For normal adult males the daily requirement is from 1.5 to 2.0 mg/day.

ii. For adult females the daily requirement is from 1.0 to 1.2 mg/day.

Assay methods:

Vitamin B_1 is oxidized to thiochrome which can be assayed by fluorescence. Other biological and microbiological procedures are also available for its assay.

Metabolism:

Vitamin-B_1 is freely absorbed by small intestine and drained into the liver. In the liver it is phosphorylated to thiamine pyrophosphate (TPP). This TPP coenzyme is present in all the

tissues. There is no storage of this vitamin, hence regularly needed in the diet. About 10% of vitamin-B_1 taken in the diet is normally excreted in the urine.

Physiological functions and biochemical role:

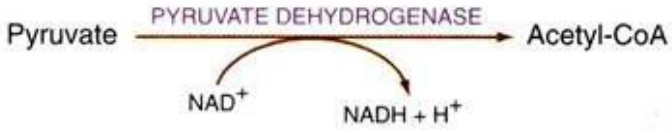
1. Utilization of carbohydrates in the body.

2. Maintenance of good appetite.

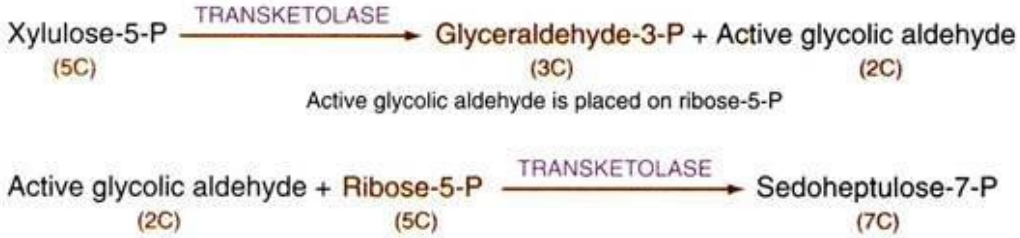
3. Normal brain metabolism.

Thiamine in its active coenzyme form i.e. as thiamine pyrophosphate and Mg function as coenzyme for (1) Oxidative decarboxylation reactions and (2) Transketolation reactions. Ex.

For oxidative decarboxylation's:



For Transketolations:



Deficiency diseases:

Thiamine deficiency results in impaired utilization of carbohydrates due to which pyruvate and lactate accumulates in the cells. But this impairment is not uniform; the brain cells are more affected than the skeletal muscle. Blood brain barrier becomes permeable to pyruvate in rats. Erythrocyte transketolase levels are lower then normal.

The symptoms of thiamine deficiency are loss of appetite (anorexia). The clinical condition of thiamine deficiency is known as 'Beri-Beri', characterized by polyneuritis (various defects of nervous system), edema, cardio-vascular changes, weakness, muscular atrophy, headache, insomnia, gastro-intestinal disorders etc. Wernicke's encephalopathy or acute thiamine deficiency is seen in alcoholics.

Beri-Beri is of four types viz.:

(a) Dry Beri-Beri:

In this nervous symptoms or polyneuritis predominates.

(b) Wet Beri-Beri:

In this the symptoms are associated with edema and serous effusions.

(c) Acute pernicious Beri-Beri:

In this the symptoms of heart are involved.

(d) Mixed Beri-Beri:

All the above symptoms.

Another type of Beri-Beri is infantile Beri-Beri seen in breast fed children, whose mothers' milk is deficient in thiamine.

Antagonists:

Pyrithiamine, oxythiamine and 2-n-butyl thiamine are the antagonists of thiamine. Foxes develop a type of paralysis called 'chastek paralysis' when they eat raw fish. It is caused by the presence of enzyme "thiaminase" in raw fish which destroys thiamine.

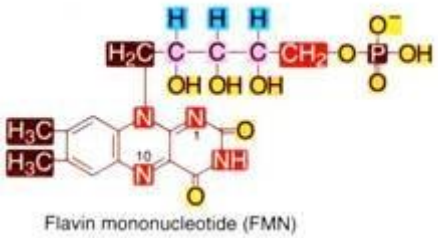
Riboflavin:

Chemistry:

Riboflavin is made up of substituted isoalloxazine ring. To this ring ribityl alcohol is attached.

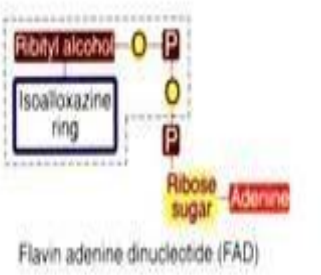
Riboflavin

The ribityl alcohol of the riboflavin is phosphorylated to form riboflavin phosphate or flavin mononucleotide (FMN).



Flavin mononucleotide (FMN)

Riboflavin is also linked to an adenosine nucleotide through a pyrophosphate linkage to form flavin adenine dinucleotide (FAD).



FMN and FAD are the two active coenzyme forms of riboflavin.

Source:

Riboflavin is rich in animal sources like milk, liver, kidney, heart, egg yolk and in sprouts.

Daily requirement:

The daily requirement of riboflavin depends upon the calorie requirement of an individual. For 1000 cal of energy 0.5 mg of riboflavin is recommended. On an average 1.5-2.0 mg/day of riboflavin is recommended.

Physiological functions of riboflavin:

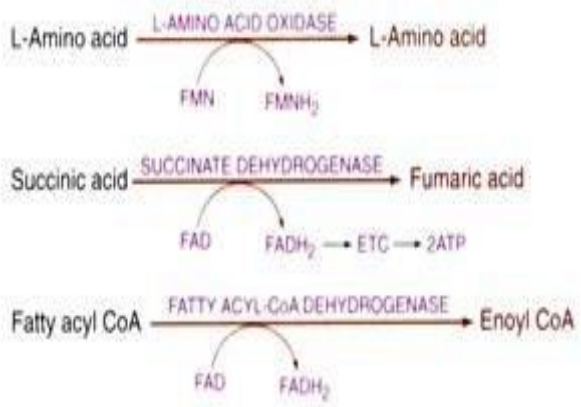
1. It is required for the regulatory functions of some hormones concerned with carbohydrate metabolism.

2. It stimulates the optic nerve in presence of light.

Coenzyme activities:

FAD and FMN act as coenzyme for some enzymes called flavor proteins. They help in the oxidation-reduction reactions of cell metabolism. The hydrogen's are transported by reversible reduction of the coenzyme by two hydrogen atoms added to the nitrogen (N) at positions 1 and 10.

The enzyme reactions catalyzed are as follows:



Deficiency diseases:

(1) Characteristic lesions of the lips.

(2) Fissures at the angles of the mouth (cheilosis).

(3) Dermatitis of the face.

(4) Magenta tongue.

(5) Certain functional and organic disorders of the eye.

Antagonists:

Dichlororiboflavin and isoriboflavin

Niacin:

Chemistry:

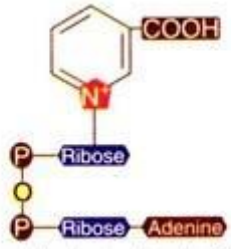
The chemical name of niacin is nicotinic acid. It contains a pyridine ring with a carboxylic acid.

Nicotinic acid
(NIACIN)

Nicotinic acid is converted to its amide form called niacin amide or nicotinamide.

Nicotinamide
(NIACINAMIDE)

Nicotinamide combines with a ribose phosphate and later on gets esterified to an adenine nucleotide to form the 'Nicotinamide adenine dinucleotide (NAD^+)



Nicotinamide adenine dinucleotide (NAD)

The ribose sugar of adenine may be phosphorylated to form 'Nicotinamide adenine dinucleotide phosphate ($NADP^+$)'. NAD^+ and $NADP^+$ are the two coenzyme forms of niacin.

Source:

Liver, fish, beans and peanuts. Niacin can also be formed in the human body form the amino acid – tryptophan. 60 mg of tryptophan can give rise to 1 mg of niacin.

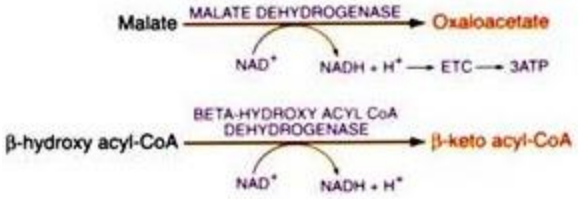
Daily requirement:

15-20 mg/day. Niacin requirements depend upon the quality and quantity of protein in the diet, as niacin can be formed from tryptophan.

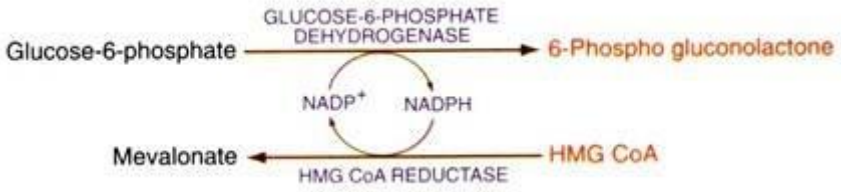
Functions:

Niacin in the form of NAD^+ and $NADP^+$ acts as coenzyme for a number of oxidoreductases. They act as electron acceptors during the enzymatic removal of hydrogen atoms. NAD acts as coenzyme for dehydrogenases in oxidation of various food stuffs. $NADPH^+$ acts as coenzyme for reductases in the synthesis of fatty acids and cholesterol.

Functions of NAD^+ as coenzyme:



Functions of NADP as coenzyme (its formation and utilization):



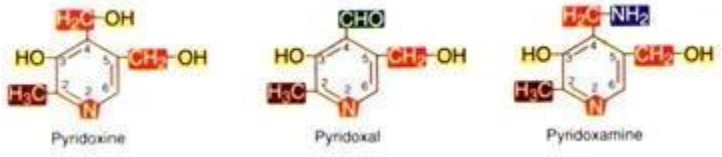
Deficiency diseases:

The deficiency disease of niacin is pellagra. It is characterized by "3 Ds" i.e. Dermatitis, Diarrhoea and Dementia (headache, forgetfulness, depression, anxiety, etc.). If not treated it results in 4th 'D' i.e. Death.

Pyridoxine: Vitamin – B_6:

Chemistry:

The chemical name of pyridoxine is 2-methyl-3-hydroxy-4, 5-di-hydroxymethyl pyridine. The alcohol on 4th position may be oxidized to an aldehyde to form pyridoxal or it may be substituted by an amino group to form pyridoxamine. Hence vitamin B_6 exists in three forms i.e., pyridoxine, pyridoxal and pyridoxamine.



The – OH group at the 5th position may be phosphorylated to give pyridoxal phosphate and pyridoxamine phosphate. These are the two active coenzyme forms of this vitamin.

Source:

Yeast, rice polishing, milk, meat, eggs, leafy vegetables and liver. Intestinal bacteria can also synthesize this vitamin.

Daily requirement:

1.6-2.0 mg/day.

Functions:

1. Pyridoxine is essential for the growth of infants.

2. Pyridoxal phosphate acts as coenzyme for—

(a) Transamination reactions—takes up amino group to form pyridoxamine phosphate.

(b) Decarboxylation—in the formation of dopamine (GABA).

(c) Dehydration.

(d) Desulphuration.

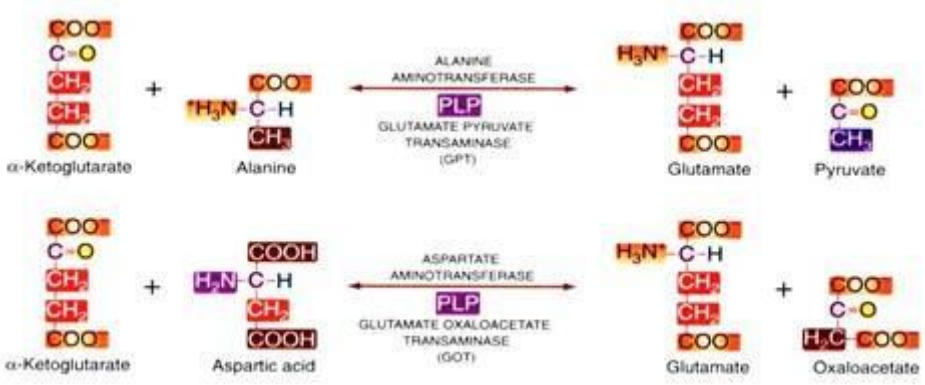
(e) Deamination.

(f) Trans-sulphuration.

(g) Kynureninase enzyme in niacin synthesis.

(h) Synthesis of serotonin, catecholamine's and heme.

A Schiff's base is formed as an intermediate in the reactions catalysed by pyridoxal phosphate.



Deficiency diseases:

Vitamin B_6 deficiency is rare. However irritability, depression, peripheral neuropathy, hypochromic microcytic anemia, cystathionuria, etc. may be seen.

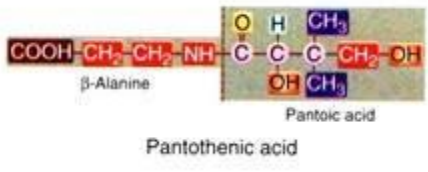
Antagonists:

Isonicodnic acid hydrazide (INH) and hydrazoline. Infants when treated with INH for tuberculosis suffer form convulsions because of structural resemblance of INH with vitamin B_6.

Pantothenic Acid:

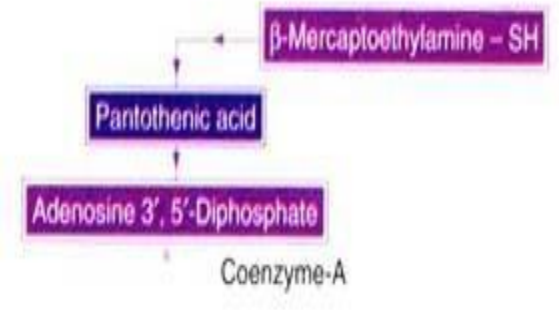
Chemistry:

Pantothenic acid contains β-alanine and pantoic acid joined by a peptide bond.



Pantothenic acid

The active coenzyme form of pantothenic acid is coenzyme-A. Coenzyme-A is formed by combination of pantothenic acid with adenine, ribose, phosphoric acid (Adenine nucleotide) and β-mercaptoethylamine.



Source:

Pantothenic in Greek means 'from everywhere'. It is so named because it is present in all the common food sources. Its richest source is honey. The intestinal flora also supplies considerable amount of this vitamin.

Food source:

Cereals, nuts, oil seeds, egg, liver, wheat grain, legumes, rice polishing, milk, meat and fish are good sources of this vitamin. Vegetables like potato have relatively less amount of this vitamin.

Functions:

1. Pantothenic acid is required for the growth of infants and children.

2. Coenzyme-A is required for the activation of acetate to acetyl-CoA.

3. Co-A is required to form succinyl-CoA for the citric acid cycle to operate normally.

4. Coenzyme-A is required for the activation of fatty acids for their oxidation.

5. Coenzyme-A is also required for fatty acid and cholesterol synthesis.

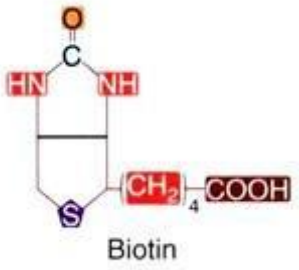
Deficiency diseases:

As this vitamin is widely distributed, the deficiency rarely occurs. However nausea, vomiting, G.I tract disorders, irritability, inadequate growth, anemia, fatty liver, etc. may occur due to its deficiency. Impaired growth and reproduction, loss of hair, sensitivity to insulin, burning feet syndrome are other deficiency manifestations of this vitamin.

Biotin:

Chemistry:

It is a heterocyclic compound containing sulphur and a valeric acid side chain. It is attached to lysine residue of the enzyme through the valeric acid to form the active coenzyme i.e., biocytin.



Biotin

Source:

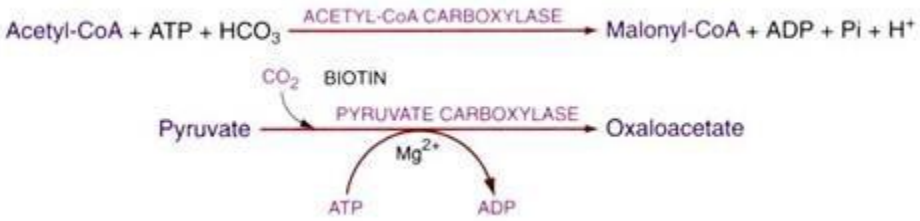
Egg, liver, fish, meat, beans, germinating seeds, intestinal flora, and peanut. Richest source is → honey. Poor source is → fruits and vegetables.

Daily requirement:

Only if the intestinal flora is disturbed, the requirement of biotin ranges form 100-300 (µg/day).

Functions:

Biotin is involved in CO_2 transfer and CO_2 fixation. Ex.



Deficiency diseases:

Only if raw egg white which contains avidin is taken, it binds and inhibits the absorption of biotin that leads to deficiency. Signs include—alopecia, greying of hair and nervous symptoms.

Folic Acid:

Chemistry:

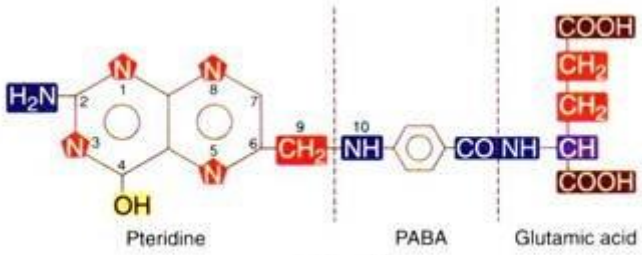
There are three components in folic acid viz:

(1) Pteridine

(2) Para amino benzoic acid (PABA) and

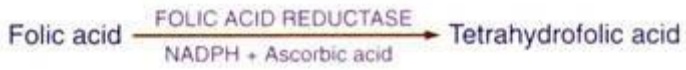
(3) Glutamic acid.

Pteridine nucleus comprises of pyrimidine and pyrazine rings. Further pteridine and PABA are collectively called pteroic acid. All the components are together known as pteroyl glutamic acid (PGA) i.e. folic acid.



Folic acid

Folic acid is readily absorbed by the intestine and not stored but undergoes reduction in the liver by the enzyme folic acid reductase that reduces folic acid to tetrahydrofolic acid (THF). This is the active coenzyme form of folic acid. This reaction requires $NADPH^{+}$ and ascorbic acid.



Functions:

The main function of folic acid is the transfer of one carbon units (moiety or fragments).

The various one carbon moieties transferred by THF are:

1. —CH_3 = Methyl group
2. —CH_2OH = Hydroxy methyl group
3. —CH_2– = Methylene
4. —CHO = Formyl

5. —CH = Methylidine or methenyl

6. —COOH = Carboxyl

7. —C — O = Carbonyl

8. —CH=NH = Formimino

These one carbon moieties get attached either to the 'N' at position 5 or to the 'N' at position 10. They can get attached both to 5^{th} nitrogen and 10^{th} nitrogen. If the one carbon (1-C) moiety is attached to the 5 nitrogen of folic acid then it is known as N – (1-C)-THF. If the one carbon moiety is attached to the 10^{th} nitrogen then it is N^{10}-(1-C)-THF. If the 1-carbon moiety is linked to both 5 nitrogen then it is called N^5-N^{10}-(1-C)-THF.

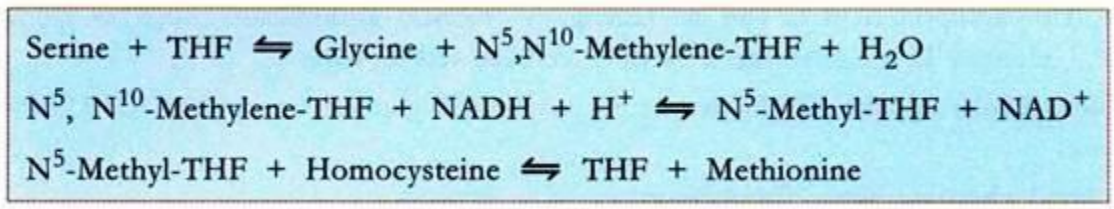
Some examples:

1. N^5-Methyl tetrahydrofolate
2. N^5, N^{10}-Methylene tetrahydrofolate
3. N^5-Formyl tetrahydrofolate

The N and N^{10} forms are inter-convertible. The one carbon moieties are derived from several sources and utilized to form several compounds.

One carbon moiety donors	One carbon moiety acceptors
Formimino group of formiminoglutamic acid (formed from histidine)	Positions 2 and 5 of purine ring
Methyl group of methionine	Glycine to form serine
Methyl group of betaine	Homocysteine to form methionine
Methyl group of methionine	Uracil to form thymine
Beta carbon of serine	Ethanolamine to form choline

Action of THF:



Source:

The microorganisms of the intestinal tract can synthesize folic acid in considerable amounts. These microorganisms need PABA to synthesize this vitamin. Therefore if PABA is supplied in sufficient quantities the requirement of folic acid can be fulfilled. During antibiotic therapy the micro flora die leading to deficiency of folic acid hence the exogenous food sources of folic acid like liver, eggs and leafy vegetables are required in the diet.

Daily requirement:

As adult human liver can store to about 5-20 mg of folic acid the daily requirement of folic acid is about 300-400 µg, which can be easily supplied by the intestinal flora.

Deficiency disease:

During treatment with sulphonamide drugs there will be deficiency of folic acid as the sulphonamide drugs competitively inhibit the synthesis of folic acid by the microorganisms. This competition is as a result of structural similarity between the drug and PABA. The deficiency results in poor synthesis of the pyrimidine thymine which in turn affects DNA synthesis, hence affects cell division. The signs of deficiency are megaloblastic anemia, glossitis and G.I tract disturbances and leucopenia.

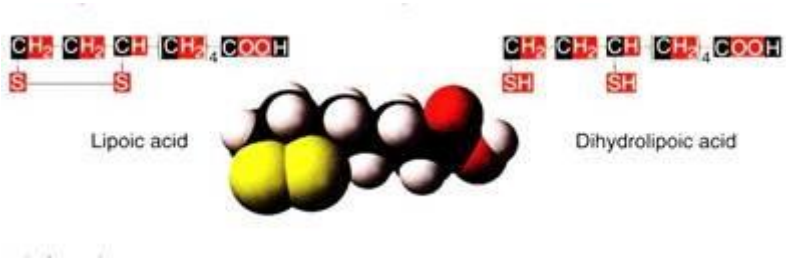
Antagonists:

Aminopterin, amethopterin, trimethoprim and methotrexate.

Lipoic Acid:

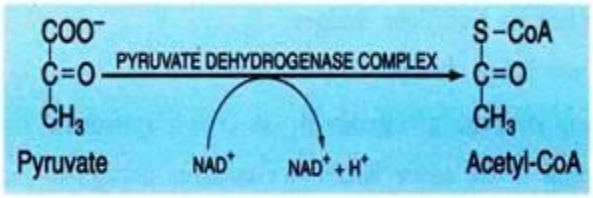
Chemistry:

It is a sulfur containing fatty acid named 6, 8-dithiooctonoic acid. Dihydrolipoic acid is the reduced form of lipoic acid and is the active coenzyme form.



Biochemical functions:

1. It is an essential cofactor for many enzyme complexes in aerobic metabolism, specifically the pyruvate dehydrogenase complex.



2. Other enzymes that use lipoic acid are 2-oxoglutarate dehydrogenase (OGDH) complex, branched chain oxoacid dehydrogenase (BCDH) complex, glycine cleavage complexes (CtCY) and acetoin dehydrogenase (ADH) complex. Lipoate participates in transfer of acyl or

methylamine groups in 2-oxoacid dehydrogenase (2-OADH) and glycine cleavage complexes (GCV), respectively.

3. Lipoic acid is an effective antioxidant and thus it prevents the symptoms of vitamin C and vitamin E deficiency. Dihydrolipoic acid is able to regenerate (reduce) antioxidants, such as glutathione, vitamin C and vitamin E, maintaining a healthy cellular redox state.

4. It increases the cellular uptake of glucose by recruiting the glucose transporter 'GLUT4' to the cell membrane.

5. It acts as a good chelating agent for mercury.

Clinical uses:

i. Intravenous administration of alpha lipoic acid (ALA) to people with acute and severe liver damage results in recovery of full liver function. Hence it is used successfully for the treatment of chronic liver disease (viral hepatitis, autoimmune hepatitis, etc.).

ii. Use of ALA with various oral antioxidants results in the long term survival of patients with metastatic pancreatic cancer.

iii. Alpha lipoic acid has the ability to modify gene expression by stabilizing NF kappa B transcription factor, hence ALA is used for the treatment of various cancers for which no effective treatments exist.

iv. Intravenous ALA completely reverses the signs and symptoms of B-cell lymphoma. Its enhancement of glucose uptake by cells favours its use in diabetes.

v. The use of carnitine and lipoic acid results in improved memory performance and delayed structural mitochondrial decay. As a result, it may be helpful for people with Alzheimer's disease or Parkinson's disease.

vi. It is used as a chelating agent in treatment of mercury intoxication. It is particularly suited to this purpose as it can penetrate both the blood-brain barrier and the cell membrane. Other chelators such as dimercaptosuccinic acid (DMSA) and 2, 3-dimercapto-1-propanesulfonic acid (DMPS) are unable to cross the brain-blood barrier and to remove mercury from the brain.

Food sources:

Lipoic acid is found in a variety of foods, notably kidney, heart and liver meats as well as spinach, broccoli and potatoes. Dietary requirement: 60 mg/kg bw/day.

Vitamin-B_{12} (Cobalamin):

Chemistry:

1. It has four pyrrole rings — I, II, III and IV.

2. Three rings are linked by methylene bridges.

3. I^{st} and IV^{th} rings are linked directly.

4. The tetrapyrrole ring structure of vitamin B_{19} is known as 'corrin' ring system.

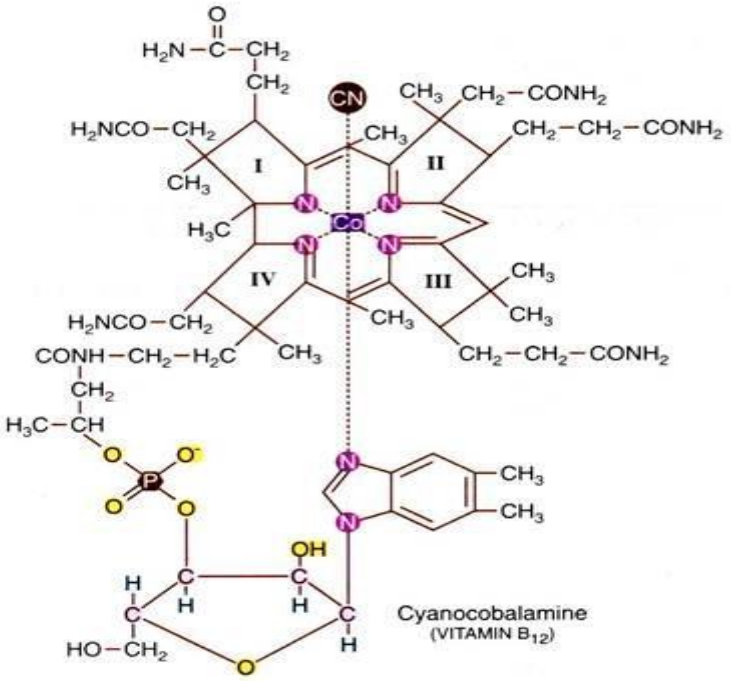
5. Cobalt atom is present in the centre linked by coordinate linkages to the nitrogen's of the pyrrole rings.

6. The cobalt is also linked to cyanide. Hence it is called cyanocobalamine.

7. Cobalt is even linked to a ribonucleotide having the base 5,6-dimethylbenzimidazole.

8. The side chains of the corrin ring are much longer.

9. The side chain of the IV^{th} ring is linked to the ribonucleodde.

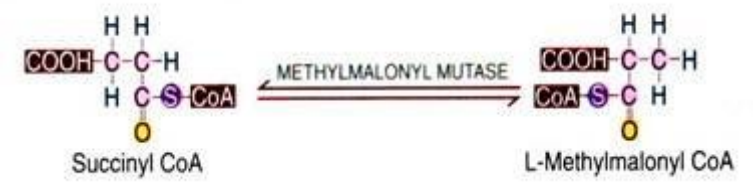


Metabolism:

Vitamin B_{12} is known as the 'extrinsic factor of castle'. Absorption of vitamin B_{12} requires an 'intrinsic factor of castle' secreted by the gastric glands. It is a glycoprotein and helps in the absorption of vitamin B_{12} from the intestine along with a releasing factor. B_{12} is transported in the plasma bound to specific carrier proteins called transcorrin-I and transcorrin-II. The active coenzyme form of vitamin B_{12} is known as cob-amide coenzyme.

Functions:

The coenzyme activity of cyanocobalamine is that of 1, 2-hydrogen shift and methyl group transfer.



Folic acid takes up the methyl group from the methyl donor and donates it to cobalamine and folic acid itself goes to folate pool to accept another methyl group. This is known as folate cycle.

1, Vitamin B_{12} is essential for the normal maturation and development of erythrocytes.
2. It is necessary for the synthesis of DNA.

3. It is required for the conversion of methyl malonyl-CoA to succinyl-CoA.

4. Methylation of homocysteine to methionine.

5. Inter conversion of glutamic acid and P-methyl aspartic acid.

Source:

Neither plants nor animals can synthesize vitamin B_{12}. Only certain microorganisms can synthesize it. However as B_{12} is stored in the organs of various animals the sources of B_{12} are liver, egg, meat and fish.

Daily requirement:

5 µg/day.

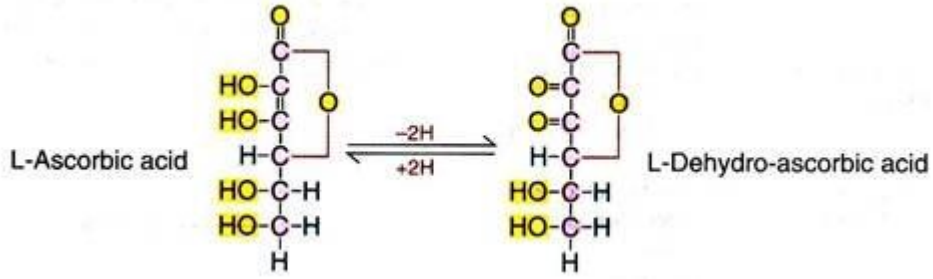
Deficiency diseases:

Megaloblastic anemia (pernicious anemia), demyelinadon, neurological lesions and infertility.

Vitamin-C (Ascorbic Acid):

Chemistry:

It is a strong reducing substance. The structure resembles a hexose sugar. It is easily oxidized to dehydroascorbic acid.



Functions:

It is concerned with the metabolism of connective tissue, particularly of collagen.

1. It maintains the redox potential of the cell.

2. Proline is converted to hydroxyproline in presence of vitamin C. Hydroxyproline is an important constituent of collagen.

3. It helps in the absorption of Iron from the intestine.

4. High doses of vitamin-C in the diet, reduces the duration and severity of common cold.

Source:

The citrus fruits (lemons and oranges) are the richest sources. Other sources are fresh green vegetables, cabbage, lettuce, guavas, berries, melons and tomatoes.

Daily requirement:

30 mg for infants and 70 mg for adults.

Deficiency diseases:

Scurvy is the deficiency disease of vitamin C. The main defect is poor deposition of intercellular cement substance (i.e. collagen). The capillaries are fragile and so there is a tendency to hemorrhage. Wound healing is delayed due to deficiency in the formation of collagen. Gums are swollen and decay easily. There is poor dentine formation and tooth loss. Weak bones leading to fracture.

Fat Soluble Vitamins:

Vitamins insoluble in water but soluble in fats or fat solvents are known as fat soluble vitamins. The fat soluble vitamins are vitamin A, D, E and K.

Vitamin–A:

Chemistry:

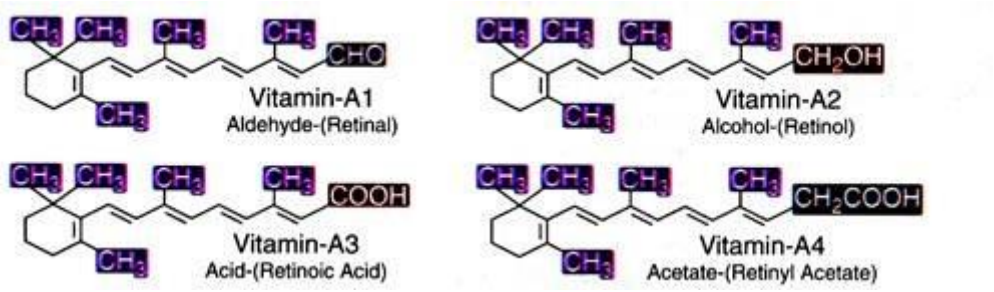
Vitamin A functions in the human body in four different forms-

1. Vitamin A1 known as Retinal—the aldehydic form

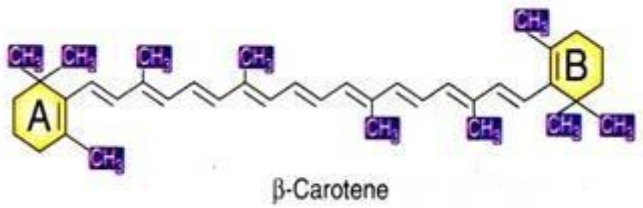
2. Vitamin A2 known as Retinol—the alcoholic form

3. Vitamin A3 known as Retinoic acid—the acid form

4. Vitamin A4 known as Retinyl acetate—the acetate form



Vitamin A, as such, is neither synthesized by the animals nor by the plants. Instead, plants synthesize the pro-vitamins of vitamin A known as carotenoids that are converted to the active vitamin in the animal body. There are many carotenoids that includes α, β, γ carotenes and others. Among the various carotenoids; β-carotene is the most potent precursor of vitamin A. β-Carotene is made up of two β-ionone rings connected through an eighteen member hydrocarbon chain, substituted by methyl groups at a few points.



β-Carotene

β-Carotene:

The pro-vitamins (β-carotene) are converted into vitamin A in the intestinal wall in animals but in man this transformation takes place in liver. There is an enzyme called β-carotene 15, 15′-oxygenase in the liver of man and intestinal wall of other animals that cleaves β-carotene at the central position releasing two molecules of active vitamin A. This reaction takes place in presence of α-tocopherol (vitamin E) and it is a dioxygenase reaction in which molecular oxygen reacts with the 2 central carbon atoms of beta- carotene followed by cleavage of its central double bond to yield 2 molecules of vitamin A aldehyde (retinal). Vitamin A alcohol is then produced by reduction of the aldehyde in a NADH-dependent reaction catalysed by retinene reductase.

Absorption and transport:

The dietary sources of vitamin A to the humans and animals are via the conversion of beta-carotenes (plant sources) to vitamin A and hydrolysis of retinyl esters (animal sources) to retinol in the intestine. Retinol is absorbed in the cell membrane of the intestine, re-esterified inside the cell of the intestine and finally absorbed via the lymph. A significant amount of vitamin A is also absorbed directly into the blood circulation along with the other dietary fats that form chylomicrons. Vitamin A is stored in the liver as palmitate esters.

Vitamin A is transported from the storage organ (liver) to the organs of utilization (eye, skin etc.) being bound to retinol-binding protein (RBP) which has a molecular weight of about 20,000 Da. It is also transported being bound to pre-albumin in the blood.

Biochemical Functions:

1. It is required for the normal vision and general growth of the body.

2. It accelerates the development of the nervous system and bones.

3. It maintains the structural integrity of the cell membrane and membranes of lysosomes and mitochondria. Thus it keeps the skin, kidney and other organs intact thereby preventing their degeneration.

4. It enhances the carbohydrate metabolism especially gluconeogenesis from lactate, acetate and glycerol.

5. It is also involved in mucopolysaccharide biosynthesis.

6. It enhances protein synthesis by activating aminoacyl-tRNA synthetizes.

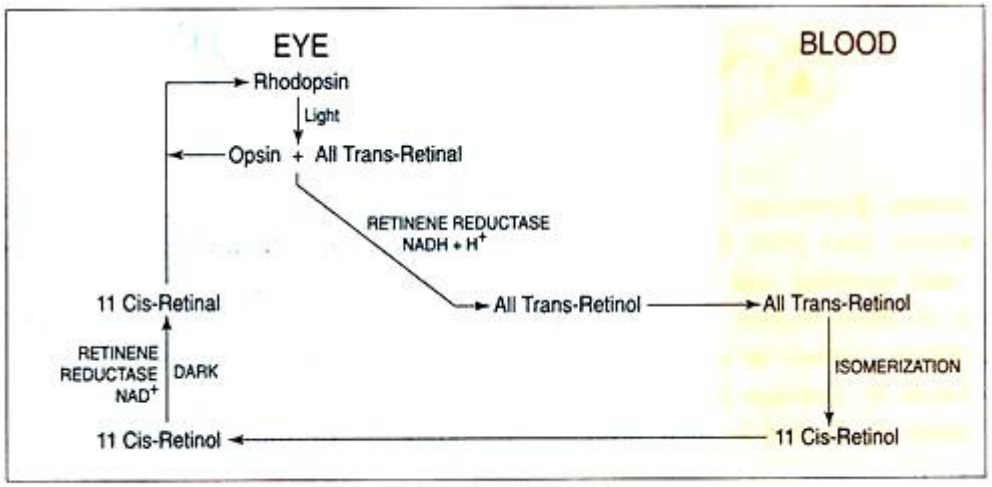
7. It accelerates the transcription and translation process in the cell.

8. It is also required for DNA metabolism.

Vitamin – A and vision (Rhodopsin cycle):

There are two types of cells in the retina of the eye viz. rods (for dim light vision) and cones (for bright light vision). The rod cells contain rhodopsin (retinal pigment or visual purple). When light strikes the retina, rhodopsin is split into its protein component; opsin and the non-protein; retinene (all trans-retinal). In the eye, the trans-retinal is reduced to trans retinol by the enzyme retinene reductase and NADH. The trans-retinol is inactive in the synthesis of rhodopsin and hence it passes into the blood, where it isomerizes into cis-retinol.

In the dark or dim light the active cis-retinol enters the retina from the blood where it is oxidized to cis-retinal by the reverse action of retinene reductase and NAD. Now the cis-retinal combines with the protein opsin to give back rhodopsin and thus the cycle is repeated thereby helping in the normal vision of the eye.



Rhodopsin cycle

Deficiency diseases:

1. Nyctalopia—night blindness

2. Xeropthalmia—complete blindness

3. Colour blindness

4. Keratomalacia—Dryness of eye, skin and keratinization of respiratory, intestinal, urinary tracts, salivary glands and the genital system.

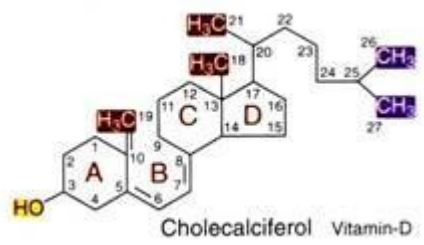
Hypervitaminosis:

Nausea, vomiting and headache are the common symptoms of vitamin A excess.

Vitamin–D:

Chemistry:

Vitamin-D is chemically known as calciferol. It is commonly known as antiricketic vitamin. It is a fat-soluble vitamin and exists in two forms—(1) vitamin D_2 (ergocalciferol) and (2) vitamin D_3 (cholecalciferol). Cholecaliferol is the most prominent form of vitamin D in humans. Vitamin D is similar to the classic steroid hormones. It is derived from cholesterol.



Daily requirement and source:

The daily requirement of vitamin D is 100 units. Lactating women need 400 units/day. The rich dietary sources of vitamin D are cod liver oil, fish liver oil, egg yolk, milk and animal liver. Vitamin D_3 can be produced photo chemically by the action of sunlight or ultraviolet light from the sterol precursor, 7-dehydrocholesterol which is present in the epidermis or skin. Vitamin D_3 can be endogenously produced and as long as an individual has access on a regular basis to sunlight there is no dietary requirement.

Absorption:

The dietary vitamin D is absorbed from the upper part of small intestine and requires bile salts. Some of it is also absorbed along with other dietary fats through the chylomicrons. It is also absorbed by the skin if cod liver oil is massaged over the skin. Vitamin D formed by the ultraviolet radiation is also absorbed through the skin.

Metabolism:

Cholesterol is dehydrated at the 7^{th} position to 7-dehydrocholesterol. In the skin, photo conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) takes place. Cholecalciferol is metabolised with the help of the enzyme D_3-25-hydroxylase in the liver to 25-hydroxycholecalciferol (25(OH)D_3), which is the major form of vitamin D circulating in the blood compartment. This is further metabolized in the kidney by the enzyme 25 (OH) D_3-l-

hydroxylase (mediated by parathormone) to 1, 25- Dihydroxycholecalciferol, this is the active form of vitamin D.

Plasma vitamin D binding protein (DBP) carries vitamin D_3 and all of its metabolites to their various target organs through the blood. The production of 1, $25(OH)_2D_3$ is modulated according to the calcium and other endocrine needs of the body. The chief regulatory factors are $1,25(OH)_2D_3$ itself, parathyroid hormone (PTH), and the serum concentrations of calcium and phosphate. Probably the most important determinant of the 1-hydroxylase is the vitamin D status of the individual. When circulating concentrations of $1,25(OH)_2D_3$ are low, production of $1,25(OH)_2D_3$ by the kidney is high, and when circulating concentrations of $l,25(OH)_2D_3$ are high, the output of $1,25(OH)_2D_3$ by the kidney is sharply reduced.

Biochemical functions:

Vitamin D has three different sites of action i.e. intestine, bones and kidneys. The primary biochemical action of vitamin D is to regulate blood calcium.

This is brought about by the following mechanisms:

1. Vitamin D increases the absorption of calcium and phosphorus from the intestine by decreasing the pH.

2. It increases the biosynthesis of calcium binding protein in the intestinal mucosal cells that helps in the transport of calcium through the intestine.

3. It reduces the excretion of calcium and phosphorus from the kidneys with the help of parathyroid hormone, thereby increasing the blood calcium levels.

4. When the serum calcium reduces, it promotes the mobilization of calcium from bones and releases it into the blood.

5. It aids in mineralization of bones (collagen).

6. It increases the citrate level of blood, bone, kidney and heart tissues and also excretion of citric acid.

7. It stimulates the activity of phytase which catalyses the hydrolysis of phytic acid in the intestine.

Deficiency diseases:

Human clinical disorders related to vitamin D can be considered as those arising because of:

i. Altered availability of vitamin D

ii. Altered conversion of vitamin D_3 to $25(OH)D_3$ and then to $1,25(OH)_2D_3$.

iii. Variations in end organ responsiveness to $1,25(OH)_2D_3$ or possibly $25(OH)_2D_3$.

There are two major clinical disorders related to vitamin D deficiency, they are:

(1) Rickets:

Bowed legs.

(2) Osteomalacia:

Soft bones with tendency to break easily.

Hypervitaminosis:

Nausea, vomition and headache are the common symptoms of vitamin D excess.

Vitamin-E:

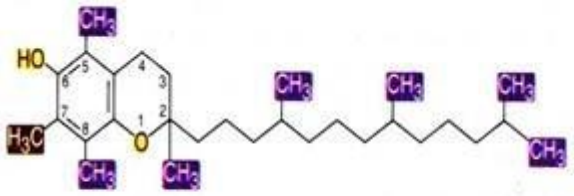
Introduction:

Vitamin E is the anti-sterility factor. It is necessary for fertility of male and the birth process in the female, therefore it is called tocopherol (Greek word Tokos — child birth, Pheros — to bear and ol = alcohol).

Chemistry:

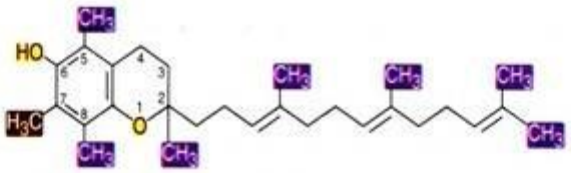
Vitamin E is chemically known as a-tocopherol. It is an oily substance, heat stable, readily oxidized and acts as powerful antioxidant. Owing to its antioxidant property it protects other vitamins like vitamin A from oxidation.

There are many derivatives of this vitamin viz. α, β, γ and δ due to the presence of different substituents on the aromatic ring at positions 5, 6, 7, and 8. α-tocopherol is 5,7,8- trimethyl derivative and has the highest vitamin activity. Vitamin E has a characteristic double ring structure called the chomane ring.



Vitamin–E (α-Tocoferol)

The tocotrienols share the same ring structure, but have an unsaturated tail.



Tocotrienol structure

Position of methyl groups on aromatic ring	Tocopherol structure	Tocotrienol structure
5, 7, 8	alpha-Tocopherol	alpha-Tocotrienol
5, 8	beta-Tocopherol	beta-Tocotrienol
7, 8	tau-Tocopherol	tau-Tocotrienol
8	delta-Tocopherol	delta-Tocotrienol

Absorption and storage:

Vitamin E is readily absorbed along with fat from the GI tract. It is metabolized to unidentified substances. It is absorbed to a great extent from salt solution. Bile is essential for vitamin E absorption, since bile contains bile salts which emulsify the fat by lowering the surface tension and theory favouring its absorption. It is stored in the liver, muscle and body fat stores.

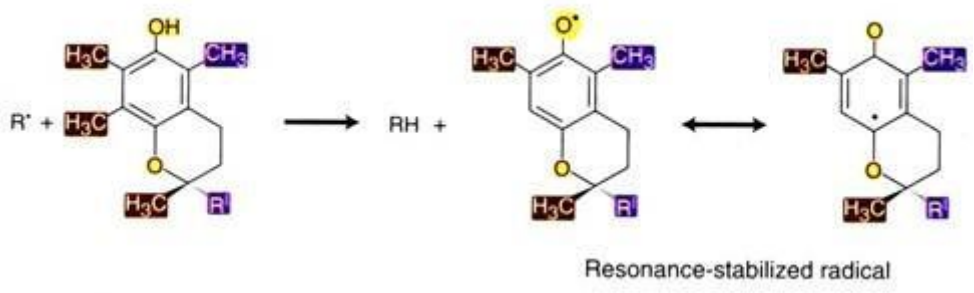
Sources:

Meat, liver, fish, chicken, vegetable oils, particularly wheat germ oil, corn oil, cotton seed oil and safflower oil are rich sources. Others are green leafy vegetables like spinach and lettuce and egg yolk.

Biochemical role:

It has antioxidant activity. Tocopherols (vitamin E) can interrupt free radical chain reactions by capturing the free radical. This imparts to them their antioxidant properties. The free hydroxyl group on the aromatic ring is responsible for the antioxidant properties. The hydrogen

from this group is donated to the free radical, resulting in a relatively stable free radical form of the vitamin.



Polyunsaturated fatty acids (constituent of cell membranes) are easily attacked by molecular oxygen resulting in the formation of peroxides. The tocopherols prevent this. Vitamin E and other antioxidants such as vitamin C, selenium, sulphur containing amino acids (cystine and methionine), ubiquinone, vitamin A and carotenes prevent lung tissue damage from atmospheric ozone and nitrogen dioxide. Tocopherols prevent oxidation of vitamin A. It prevents enzymes in muscles, nerves and gonads from destruction. It also prevents the development of cerebral disorder. It is involved in heme synthesis.

Thus the physiological role of vitamin E can be summarized as under:

1. It prevents peroxidation of polyunsaturated fatty acids in tissues and membranes.

2. It prevents haemolysis of erythrocytes by oxidizing agents like H_2O_2 and dialuric acid.
3. It prevents the degeneration of cellular and subcellular membranes rich in polyunsaturated fatty acids (PUFA).

4. It prevents poisoning of liver cells. The liver is exposed to carbon-tetrachloride, chloroform and other toxic chemicals.

5. It prevents demyelination of nerve fibres and prevents distortion of the axis of the nerves in the spinal cord.

6. Respiration in the mitochondria is depended upon the availability of vitamin E and the activators present in microsomal supernatant extraction of the cells also need this vitamin.

7. Requirement of vitamin E is dependent on the amount of PUFA in diet and selenium status in the body. It spares the activity of selenium present in traces in the body.

8. It prevents hepatic necrosis produced by diet deficient in sulphur containing amino acids.

9. Its action as an antioxidant prevents rancidity.

10. It is important for reproductive physiology. In male rats that are deprived of vitamin E, the seminiferous epithelium undergoes irreversible degeneration and permanent sterility occurs. In females the deficiency of vitamin E does not affect the ovary. Ovulation, conception and implantation take place normally, but foetus dies in the uterus a few weeks after conception and undergoes resorption.

Deficiency diseases:

1. It causes discolouration of the enamel of the teeth due to oxidation of unsaturated fatty acids present in these structures to peroxide.

2. Anaemia is caused in monkeys due to lack of hemophysins in bone marrow, rather than by destruction of RBC.

3. Increased fragility of RBC.

4. Thrombocytosis and edema.

5. Permanent sterility in males and death of foetus in uterus after few weeks of implantation in females.

6. Necrosis of hepatic cells.

Daily requirement:

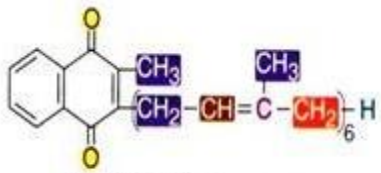
Adults-10 mg/day. If PUFA in the diet is 1 gm./day then the requirement of vitamin E is as high as 35 gms/day. Pregnant or lactating women require greater amount of vitamin E.

Hypervitaminosis:

Leads to nausea.

Vitamin-K:

It is chemically known as menaquinone (i.e. K_3) whereas K_1 and K_2 are napthaquinones (present in plants). Menaquinone is an anti-hemorrhagic vitamin i.e. it prevents hemorrhages by activating the



Vitamin–K (Menaquinone)

Sources:

Green leafy vegetables like cabbage, spinach etc. predominantly contain vitamin K, and are the best sources. Cauliflower, soya-bean, wheat-germ etc.. have proved to be good sources of this vitamin. Tops of carrots contain considerable amounts. Animal products contain very little although milk and eggs contain small amounts. Vitamin K_2 is produced by most bacteria present in the human intestine if it is not supplied in the diet.

Biochemical role:

Vitamin K is necessary for proper formation of prothrombin (the blood plasma protein), the inactive precursor of thrombin which is an enzyme that converts the protein fibrinogen (of blood plasma) into fibrin, the insoluble fibrous protein that holds blood clots together.

1. Vitamin K increases the activity of many clotting factors.

2. It initiates the biosynthesis of the enzyme proconvertin of the liver cells which catalyses the formation of prothrombin (precursor of thrombin protein).

3. It takes part in election transport chain.

4. It acts as a coenzyme for carboxylation of glutamate to y-carboxyglutamate.

Deficiency conditions:

Increased clotting time and decreased blood prothrombin levels are seen in vitamin K deficiency. There is continuous bleeding specially during delivery of the foetus.

Daily requirement:

The average diet contains adequate amount of the vitamin being synthesized by the bacteria present in the intestine. Hence deficiency symptoms are not seen in healthy individuals except in new born infant fed on mother's milk when the mother's diet has low vitamin K content.

Hypervitaminosis:

Very large doses of vitamin K are toxic.

Daily requirement and source:

The daily requirement of vitamin D is 100 units. Lactating women need 400 units/day. The rich dietary sources of vitamin D are cod liver oil, fish liver oil, egg yolk, milk and animal liver. Vitamin D_3 can be produced photo chemically by the action of sunlight or ultraviolet light from the sterol precursor, 7-dehydrocholesterol which is present in the epidermis or skin. Vitamin

D_3 can be endogenously produced and as long as an individual has access on a regular basis to sunlight there is no dietary requirement.

Absorption:

The dietary vitamin D is absorbed from the upper part of small intestine and requires bile salts. Some of it is also absorbed along with other dietary fats through the chylomicrons. It is also absorbed by the skin if cod liver oil is massaged over the skin. Vitamin D formed by the ultraviolet radiation is also absorbed through the skin.

Metabolism:

Cholesterol is dehydrated at the 7^{th} position to 7-dehydrocholesterol. In the skin, photo conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) takes place. Cholecalciferol is metabolized with the help of the enzyme D_3-25-hydroxylase in the liver to 25-hydroxycholecalciferol ($25(OH)D_3$), which is the major form of vitamin D circulating in the blood compartment. This is further metabolized in the kidney by the enzyme 25 (OH) D_3-l-hydroxylase (mediated by parathormone) to 1, 25- Dihydroxycholecalciferol, this is the active form of vitamin D. Plasma vitamin D binding protein (DBP) carries vitamin D_3 and all of its metabolites to their various target organs through the blood. The production of 1, $25(OH)_2D_3$ is modulated according to the calcium and other endocrine needs of the body. The chief regulatory factors are $1,25(OH)_2D_3$ itself, parathyroid hormone (PTH), and the serum concentrations of calcium and phosphate.

Probably the most important determinant of the 1-hydroxylase is the vitamin D status of the individual. When circulating concentrations of $1,25(OH)_2D_3$ are low, production of $1,25(OH)_2D_3$ by the kidney is high, and when circulating concentrations of $l,25(OH)_2D_3$ are high, the output of $1,25(OH)_2D_3$ by the kidney is sharply reduced.

Biochemical functions:

Vitamin D has three different sites of action i.e. intestine, bones and kidneys. The primary biochemical action of vitamin D is to regulate blood calcium.

This is brought about by the following mechanisms:

1. Vitamin D increases the absorption of calcium and phosphorus from the intestine by decreasing the pH.

2. It increases the biosynthesis of calcium binding protein in the intestinal mucosal cells that helps in the transport of calcium through the intestine.

3. It reduces the excretion of calcium and phosphorus from the kidneys with the help of parathyroid hormone, thereby increasing the blood calcium levels.

4. When the serum calcium reduces, it promotes the mobilization of calcium from bones and releases it into the blood.

5. It aids in mineralization of bones (collagen).

6. It increases the citrate level of blood, bone, kidney and heart tissues and also excretion of citric acid.

7. It stimulates the activity of phytase which catalyzes the hydrolysis of phytic acid in the intestine.

Deficiency diseases:

Human clinical disorders related to vitamin D can be considered as those arising because of:

i. Altered availability of vitamin D

ii. Altered conversion of vitamin D_3 to $25(OH)D_3$ and then to $1,25(OH)_2D_3$.

iii. Variations in end organ responsiveness to $1,25(OH)_2D_3$ or possibly $25(OH)_2D_3$.

There are two major clinical disorders related to vitamin D deficiency, they are:

(1) Rickets:

Bowed legs.

(2) Osteomalacia:

Soft bones with tendency to break easily.

Hypervitaminosis:

Nausea, vomiting and headache are the common symptoms of vitamin D excess.

Vitaminoids:

These are the organic compounds having similar biological properties like vitamins but required in larger amounts.

They are of following types:

(a) Fat soluble vitaminoids:

Essential Fatty acids and ubiquinone's.

(b) Water soluble vitaminoids:

Biotin (Vit-B), Choline (Vit-B_4), Inositol (Vit-B_8), bioflavonoids, N- Lipoic acid etc.

History:

Lunin (1881) first discovered them and Funk (1912) coined the term 'vitamins'. Later, Drummond (1920) proposed the term vitamin by dropping the final 'e'

Pseudovitamin, Antivitamin and Provitamin:

Pseudovitamins (false vitamins) are certain organic compounds that are structurally similar to some vitamins but don't show the physiological actions of the vitamin. For example, methylcobalmine is a pseudovitamin of cyanocobalamine (Vit – B_{12}). Antivitamin is a compound which resembles structurally to a vitamin but inhibits or antagonize the function of the vitamin. For example, pyrithiamine, galactoflavin and avidin are antivitamins of thiamine, riboflavin and biotin respectively. Provitamin is the precursor of a vitamin which when taken through diet is converted into the vitamin in the body, e.g. (3-carotene (provitamin (A), ergosterol (Provitamin D) etc.

Vitamin deficiency:

Vitamins are very essential for the body although vitamins are not needed by the body in large quantity but required for proper growth and development of body. There are two types of vitamins, viz., fat soluble vitamins such as vitamins, A, D, E and K and water soluble vitamins such as B complex group and vitamin C.

Deficiency of vitamins causes diseases which are as follows:

Table 9.1

	Vitamin	Sources	Functions (essential for)	Deficiency diseases
1	Vitamin A	Oil, fish, liver egg, milk, butter and carrots	Eye and lungs	Night blindness
2	Vitamin D	Animal fat, milk ghee, butter	Bones and teeth formation	Rickets
3	Vitamin E	Vegetable, milk, egg yolk and vegetable oils	Sex glands	Hemolysis & sterility
4	Vitamin K	Liver, spinach cauliflower green tomatoes	Blood clotting	Haemorrhage
5	Vitamin B_1	Cereals, wheat, carrot, milk	Nervous system	Beri-beri
6	Vitamin B_2	Cereals, milk, egg, liver	Eyes, skin, blood	Slow growth, sore eyes
7	Vitamin B_4	Meat, fish, cereals, peanuts	Gum and tongue	Inflammation of the tongue and lateral margins of tongue and gums become swollen and red
8	Vitamin C	Lemon, grapes, tomatoes, oranges, apples and vegetables	Gums and wound healing	Scurvy

Probable Questions:

1. Describe different types of Beri beri.
2. What are the effects of deficiency of Vit A, B, C, K, D
3. What are the functions of Vitamin A?
4. Discuss the physiological role of vitamin E.
5. What are the functions of Vitamin D ?
6. Discuss about vitaminoids.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.
2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.
3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,
4. P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.
5. Donald Voet and Judith G. Voet Biochemistry

UNIT-XX

Minerals: Role of minerals in human physiology

Objective: In this unit will discuss about different types of Minerals regarding their structure, function and deficiency.

Introduction : It is now known that the following 17 elements are essential for majority of the plants: C, H, O, N, P, K, Ca, S, Mg, Fe, Mn, Zn, B, Cu, Ni, CI and Mo (molybdenum.) Besides these, Al, Si, Se, Na, Co, V (vanadium) and Ga (gallium) may be essential for some plants.

Subject-Matter of Essential Mineral Elements:

Most of the minerals present in the soil can enter plants. With the help of sensitive techniques, concentration as low as 10^{-8} g/ml can be detected. More than sixty minerals present in soil have been recorded in plants. Some plants accumulate heavy and toxic materials, e.g., Astragalus (selenium), Phacelia sericea (gold), plants growing near nuclear sites (radioactive strontium). All the elements found in plants are not essential for plants. Similarly, all elements essential for animal growth are not required by plants. The most important of them are iodine and sodium. An essential element is the one which has a specific structural or physiological role and without which plants cannot complete their life cycle.

Criteria of Essential Mineral Element:

Amon and Stout (1939) proposed criteria for knowing the essentiality of an element. They are:
1. It is indispensable for the growth of plants.

2. The element is directly involved in the metabolism of plants. It becomes a component of either a structural or functional molecule. The element may additionally have a corrective effect on mineral balance and other soil conditions.

3. A plant is unable to complete its vegetative or reproductive phase in the absence of the element.

4. The element cannot be replaced by any other element.

5. The absence or deficiency of the element produces disorders. These disorders are a direct result of the lack or deficiency of the element.

6. The element alone can correct the disorders produced by its absence or deficiency.

17 elements have been found to be essential. They are C, H, O, N, P, K, S, Mg, Ca, Fe, B, Mn, Cu, Zn, Mo, Cl and Ni. Others are called nonessential elements. However, some of the nonessential elements have been found to be required in metabolic activities of certain plants. They include cobalt, silicon, sodium, vanadium, aluminum, etc. Silicon is required by most grasses and cereals.

Its deficiency produces leaf necrosis and stunted growth in Rice. Sodium seems to be involved in membrane permeability though its essentiality has not been proved. These elements are called functional elements or non- essential functional or beneficial elements.

Types of Essential Mineral Element:

Essential elements occur in different proportions (Table 12.1) in the plants. They are differentiated into two categories, macro-elements and microelements. The terms major, minor and trace elements are generally reserved for elements involved in animal nutrition.

Table 12.1 Sach's Formulae for Solution Culture in 1 litre of distilled water (Sachs, 1860)

Constituents	*Normal*	*—P*	*—Ca*	*—K*	*—N*	*—Mg*	*—S*	*—Fe*
	gm	gm	gm	gm	gm	gm	gm	gm
KNO_3	0.70	0.70	0.70	—	—	0.70	0.70	0.70
$CaSO_4.2H_2O$	0.25	0.25	—	0.25	0.25	0.25	—	0.25
$Ca(H_2PO_4)_2.2H_2O$	0.25	—	—	0.25	0.25	0.25	0.25	0.25
$MgSO_4.7H_2O$	0.25	0.25	0.25	0.25	0.25	—	—	0.25
NaCl	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
$FeCl_3.6H_2O$	0.005	0.005	0.05	0.005	0.005	0.005	0.005	—
$Ca(NO_3)_2$	—	0.16	—	—	—	—	—	—
K_2SO_4	—	—	0.20	—	—	0.17	—	—
$Na_2HPO_4.12H_2O$	—	—	0.71	—	—	—	—	—
$NaNO_3$	—	—	—	0.59	—	—	—	—
KCl	—	—	—	—	0.52	—	—	—
$CaCl_2$	—	—	—	—	—	—	0.16	—
$MgCl_2$	—	—	—	—	—	—	0.21	—

Table 12.4. Average concentration of various elements in plants
(as calculated on the basis of dry matter by Strout, 1961)

Macronutrient	*Concentration* µg	*Micronutrient*	*Concentration* µg
Carbon	450,000	Iron	100
Oxygen	450,000	Chlorine	100
Hydrogen	60,000	Manganese	50
Nitrogen	15,000	Boron	20
Potassium	10,000	Zinc	20
Calcium	5,000	Copper	6
Phosphorus	2,060	Nickel	3
Magnesium	2,000	Molybdenum	0.1
Sulphur	1,000		

Macro-elements (Macronutrients):

They are those essential elements which are present in easily detectable quantities, 1-10 mg per gram of dry matter. Macro-elements are usually involved in the synthesis of organic molecules and development of osmotic potential.

They are nine in number— C, H, 0, N, P, K, S, Mg and Ca. 96% of the dry matter is formed of carbon, hydrogen and oxygen. On fresh weight basis, oxygen is the most abundant element in plant body as well as other organisms. On dry weight basis, it is almost as abundant as carbon. Of the nonessential functional or beneficial elements, silicon and sodium often occur in the range of macro-elements. Iron occurs in the concentration of less than 1 mg/gm. However, its essentiality was discovered along with other macro-elements long before microelements were found to be required. Therefore, it is often regarded to be macro-element.

Subject-Matter of Essential Mineral Elements:

Most of the minerals present in the soil can enter plants. With the help of sensitive techniques, concentration as low as 10^{-8} g/ml can be detected. More than sixty minerals present in soil have been recorded in plants. Some plants accumulate heavy and toxic materials, e.g., Astragalus (selenium), Phacelia sericea (gold), plants growing near nuclear sites (radioactive strontium). All the elements found in plants are not essential for plants. Similarly, all elements essential for animal growth are not required by plants. The most important of them are iodine and sodium. An essential element is the one which has a specific structural or physiological role and without which plants cannot complete their life cycle.

Mineral and Non-Mineral Elements:

Essential elements derived from soil are termed as mineral elements. Essential elements got from air or water are known as non-mineral elements, e.g., C, H, O. They are building blocks of macromolecules that form the bulk of plant body.

In aquatic habitats as well as in soil solution, mineral elements occur dissolved in water. Carbon is mostly got from air as CO_2. Hydrogen is obtained from water. Oxygen is a component of water.

It is also available from air. Nitrogen is present in abundance in the air as a non-mineral element but plants usually obtain it from soil as nitrate or ammonium ion. The two are formed by the process of fixation of atmospheric nitrogen though a small quantity can also come from weathering of rocks. Chlorine is not as a mineral from soil but can also be obtained from the atmosphere. Similarly, some sulphur is also absorbed from the atmosphere where it is present as a pollutant in the gaseous state of sulphur dioxide.

Mineral Elements:

P, K, S, Mg, Ca, Fe, Zn, Mn, B, Cu, Mo, Cl and N.

Non-Mineral Elements:

C, O, H and N.

Role of Essential Elements and Deficiency Symptoms

Element	*Obtained as*	*Region of plant in which required*	*Constituent of*	*Major Functions*	*Deficiency Symptoms*
1. Nitrogen	NO_3^-, NH_4^+ rarely NH_2^-	Everywhere but more in meristems	Amino acids, proteins, enzymes, some coenzymes, nucleotides, ATP nucleic acids, vitamins, hormones, cytochromes, chlorophyll.	Cell division, growth, metabolic activities, photosynthesis	Chlorosis starting from older leaves, stunted growth due to decreased protein synthesis, smaller cells and inhibition or slow divisions, premature leaf fall, lateral buds and tillering suppressed, late flowering, purple colouration of stem, petiole and under surface of leaf in some plants, wrinkling of cereal grains, reduced yield.
2. Phosphorus	$H_2PO_4^{2-}$, PO_4^{3-},	Meristems, developing fruits and seeds, withdrawn from older tissues. Stored as phytin or phytic acid.	Nucleotides, ATP nucleic acids, nucleoproteins, phospholipids, NAD^+, $NADP^+$ and some other coenzymes.	Energy transfer, cell division, membranes, phosphorylation reactions	Stunted growth, leaves dull green or with purple and red spots of anthocyanins, chlorosis (appears late) with necrosis first in older leaves or premature abscission, delayed flowering, premature fall of flower buds, poor vascular tissues and delayed seed germination.
3. Potassium	K^+	Leaves, meristems, buds and root tips.	Not constituent of any organic substance, cofactor or activator of several enzymes, determines osmotic potential	Maintenance of cell turgidity, opening and closing of stomata, balancing other ions, cation-anion balance, increases hardiness, essential for photosynthesis, respiration, protein synthesis and synthesis of various other types, membrane permeability.	Mottled interveinal chlorosis appearing first in older leaves, marginal or apical yellowing or scorch and curling, die back, bushy habit, shorter internodes, loss of apical dominance, cereals may show lodging, loss of cambial activity, plastid disintegration and increase in rate of respiration.
4. Calcium	Ca^{2+}	Meristematic and differentiating tissues. Excess in older leaves.	Calcium pectate of middle lamella, activator of enzymes connected with chromosome formation and many aspects of metabolism.	Selective permeability of cell membrane, organisation of mitotic spindle, meristematic activity, metabolism, prevention of mineral and organic acid toxicity, second messenger for some hormonal signals.	Stunted growth, degeneration of meristems, especially root apex, chlorosis, necrosis and curling appearing first in young leaves, premature flower abscission, blossom end rot of Tomato.

Element	*Obtained as*	*Region of plant in which required*	*Constituent of*	*Major Functions*	*Deficiency Symptoms*
5. Magnesium	Mg^{2+}	Leaves, growing areas of root and stem, seeds, withdrawn from ageing leaves.	Chlorophyll, magnesium pectate, activator of enzymes connected with phosphate transfer in respiration, photosynthesis, DNA and and RNA synthesis, fat and carbohydrate metabolism, binding of ribosomes.	Formation of chlorophylls, carotenoids and nucleic acids, growth, metabolism and nodule formation in legumes.	Marginal curling, interveinal chlorosis with purple anthocyanin pigmentation appearing first in older leaves, veins green, chlorotic areas may turn necrotic, premature leaf abscission, reduced growth, underdeveloped phloem and pith.
6. Sulphur	SO_4^{2-}, also as SO_2 from air	Young leaves and meristems, withdrawn from senescent organs.	Two amino acids (methionine and cysteine) and hence their proteins, vitamins (thiamine, biotin), CoA, lipoic acid and ferredoxin, also component of allyl oils of Onion, Garlic and Crucifers.	Chlorophyll formation, growth metabolism and nodule formation in legumes.	Similar to those of nitrogen deficiency both being consituents of proteins. Chlorosis more commonly appearing first in young leaves accumulation of anthocyanins, stunted growth, leaf curl, less juice content in Citrus, reduced nodulation in legumes, smaller chlorotic leaves in Tea (tea yellow).
7. Iron (Micro)	Fe^{3+} (mostly) Fe^{2+} (mostly acidic soils)	Everywhere, more along veins. Excess stored as ferritin.	Cytochromes, ferredoxin, nitrogenase, activator of catalase and some other enzymes.	Electron transport in photosynthesis and respiration ($Fe^{2+} \rightleftharpoons Fe^{3+}$), development of chloroplasts, chlorophyll and other pigments, protein synthesis.	Interveinal chlorosis appearing first in young leaves, veins initially green, necrosis later on, growth reduced.
8. Manganese (Micro)	Mn^{2+}	Leaves and seeds	Activates enzymes of respiration,photosynthesis and nitrogen meta-bolism performing oxidation, reduction, decarboxylation, component of oxygen evolving complex.	Metabolism and photolytic evolution of oxygen.	Interveinal chlorosis, grey specks and streaks, legume cotyledons with brownish spots (marsh spot disease), stunted growth, flowers often sterile.
9. Molybdenium (Micro)	$HMoO_4^-$ MoO_4^{2-}	Everywhere, Mo^{4+} more common in roots	Nitrogenase, nitrate reductase, activator of dehydrogenases.	Nitrogen metabolism (nitrogen fixation, nitrate reduction), ascorbic acid synthesis. Oxidation–reduction reaction.	Mottled chlorosis with marginal necrosis and infolding, lamina or upper half of lamina falls down (whiptail disease), loosening of inflorescence in Cauliflower, slight growth retardation.
10. Boron (Micro)	BO_3^- or $B_4O_7^{2-}$	Leaves and seeds	Connection with any enzyme system not known.	Carbohydrate transport through phloem, uptake and utilization of calcium, pollen germination, root nodulation, synthesis of pectins, proteins and nucleic acids, membrane functions, cell elongation and cell differentiation.	Death of root and shoot tips, loss of apical dominance, stunted growth, small size of fruits, rossetting of leaves, stem brittle but stout, black necrosis, disintegration of softer parts–top sickness, brown heart (*e.g.*, Turnip), internal cork (*e.g.*, Apple), heart rot of Sugar Beet, browning of Cauliflower, decreased nodulation in legumes.
11. Copper (Micro)	Cu^{2+}	Everywhere	Component or activator of plastocyanin, cytochrome oxidase, RuBP carboxylase and many other enzymes.	Electron transfer ($Cu^+ \rightleftharpoons Cu^{2+}$) maintenance of carbohydrate/nitrogen balance, chlorophyll synthesis.	Die back, skin splitting or exanthema, exuding gummy substance, apical necrosis of of young leaves extending towards base along the margins, (leaf tip disease = reclamation disease), blackening of Potato tubers.
12. Zinc (Micro)	Zn^{2+}	Enverywhere	Component or activator or several enzymes including carbonic anhydrase, dehydrogenases and carboxylases.	IAA, RNA and protein synthesis, evolution and utilization of carbon dioxide	Leaf malformations like little leaf, leaf rosettes, interveinal chlorosis and several types of leaf distortions, white bud, stunted growth.
13. Chlorine (Micro)	Cl^-	Everywhere	Component of oxygen liberation complex of photolysis, amylase, anion-cation balance, osmotic potential alongwith K^+, Na^+.	Photoproduction of oxygen, cell division, normal production of fruits.	Bronze colour in leaves, leaf wilting, chlorosis and necrosis, stunting of roots and swollen root tips, flower abscission, reduced fruiting.
14. Nickel (Micro)	Ni^{2+}	Leaves and root.	Urease, also required for hydrogenase.	Metabolism of urea and ureides.	Leaf tip necrosis.

(A) The Macronutrients (Major Elements):

1. Nitrogen:

Specific Role (function):

i. It is important constituent of proteins, nucleic acids, porphyrins, alkaloids, some vitamins, coenzymes etc.

ii. Porphyrins are important part of chlorophylls and cytochromes.

Thus it plays very important role in metabolism, growth, reproduction and heredity.

Deficiency Symptoms:

i. Nitrogen deficiency causes yellowing i.e. chlorosis of leaves. Older leaves are affected first. (It is because nitrogen is very mobile in the plant and is readily transported from older to the rapidly developing younger leaves under conditions of nitrogen deficiency). ii. In many plants e.g., tomato, the stem, petiole and the leaf veins become coloured due to the formation of anthocyanin pigments. Plant growth is stunted (because protein content, cell division, and cell-enlargement are decreased).

2. Phosphorous:

Specific Role:

i. It is important constituent of nucleic acids, phospholipids, coenzyme NAD, NADP and ATP etc.

ii. Phospholipids along with proteins may be important constituents of cell membranes.

iii. Through nucleic acids and ATP it plays an important role in protein synthesis.

iv. Through coenzymes NAD, NADP, and ATP it plays an important role in oxidation-reduction and energy transfer reactions of cell metabolism e.g., photosynthesis, respiration, fat metabolism etc.

Deficiency Symptoms:

i. Phosphorous deficiency may cause premature leaf fall.

ii. Dead necrotic areas may be developed on leaves or fruits.

iii. Leaves may turn dark to blue-green in colour.

3. Sulphur:

Specific Role:

i. It is important constituent of some amino acids (cysteine and methionine) which with other amino acids form the proteins.

ii. Disulphide linkages help to stabilize the protein structure.

iii. It is also constituent of vitamins biotin, thiamine, and coenzyme-A.

iv. Sulfhydryl groups are necessary for the activity of many enzymes.

Deficiency symptoms:

i. Sulphur deficiency causes yellowing (i.e., chlorosis) of the leaves. The younger leaves are affected first.

ii. Tips and margins of the leaf roll inward.

iii. Stem becomes hard due to the development of sclerenchyma.

4. Calcium:

Specific Role:

i. It is important constituent of the middle lamella in cell wall.

ii. It is essential in the formation of cell membranes.

iii. It acts as second messenger in metabolic regulation.

iv. It helps to stabilize the structure of the chromosomes.

v. It may be an activator for many enzymes.

Deficiency Symptoms:

i. Calcium deficiency causes disintegration of growing meristematic regions of the root, stem and leaves.

ii. Chlorosis occurs along the margins of the younger leaves.

iii. Malformation of younger leaves also takes place.

5. Magnesium:

Specific Role:

i. It is very important constituent of chlorophylls.

ii. It acts as activator for many enzymes in phosphate transfer reactions particularly in carbohydrate metabolism and nucleic acids synthesis.

iii. It plays important role in binding ribosomal particles during protein synthesis.

Deficiency Symptoms:

i. Magnesium deficiency causes interveinal chlorosis of the leaves. The older leaves are affected first. (It is because magnesium is quite mobile in the plant and is readily transported from older to the rapidly growing younger leaves under conditions of magnesium deficiency).

ii. Dead necrotic patches appear on the leaves.

6. Potassium:

Specific Role:

i. Although potassium is not a constituent of important organic compound in the cells, it is essential for the process of respiration and photosynthesis.

ii. It probably acts as an activator of many enzymes involved in carbohydrate metabolism and protein synthesis.

iii. It is major contributor to osmotic potential of plant cells.

iv. It serves to balance the charge of both diffusible and non-diffusible ions.

v. Plays important role in stomatal movements.

Deficiency Symptoms:

i. Mottled chlorosis of leaves occurs.

ii. Necrotic areas develop at the tip and margins of the leaf which curve downward.

iii. Plant growth remains stunted with marked shortening of internodes.

(B) The Micronutrients (Minor or Trace Elements):

1. Iron:

Specific Role:

i. It is important constituent of iron porphyrin-proteins like cytochromes peroxidases, catalases etc.

ii. It is essential for the synthesis of chlorophyll.

iii. It is very important constituent of ferredoxin which plays important role in biological nitrogen fixation and primary photochemical reaction in photosynthesis.

Deficiency Symptoms:

i. Iron deficiency causes rapid chlorosis of the leaves which is usually interveinal. The younger leaves are affected first. (It is because iron is sparingly mobile in the plant and is not easily withdrawn from the older leaves to the rapidly growing younger leaves under conditions of iron deficiency). (In soil solution, iron is less mobile due to its precipitation chiefly as insoluble hydrous oxides (Fe_2O_3, $3H_2O$) which interferes with its absorption by the plants. Because of this, the plants may show iron deficiency symptoms even though there is plenty of iron present in the soil especially in neutral or alkaline calcareous soil.

To avoid this problem in nutrient solution, suitable chelating agent (or chelator) such as citric acid or tartaric acid is added which forms soluble complex with the metal ion making the latter easily available to plant. These days, EDTA (ethylenediamine tetraacetic acid) is commonly used for this purpose).

2. Manganese:

Specific Role:

i. It is an activator of many respiratory enzymes.

ii. It is necessary for the evolution of oxygen during photosynthesis.

Deficiency Symptoms:

i. Manganese deficiency causes chlorotic and necrotic spots in the interveinal areas of the leaf.

3. Copper:

Specific Role:

i. It is constituent of several oxidizing enzymes.

ii. Its higher concentrations are toxic to plants.

Deficiency Symptoms:

i. Copper deficiency causes necrosis of the tip of the young leaves.

ii. It also causes die-back of citrus and other fruit trees and reclamation disease of cereals and leguminous plants.

4. Zinc:

Specific Role:

i. It is involved in the biosynthesis of the growth hormone auxin, Indole -3-Acetic acid (IAA).

ii. It acts as activator of many enzymes like carbonic anhydrase, alcohol-dehydrogenase etc.

Deficiency Symptoms:

i. Zinc deficiency causes chlorosis of the older leaves which starts from tips and the margins.

ii. It causes mottle leaf disease in apple, citrus, walnut and other fruit trees.

5. Boron:

Specific Role:

i. Specific role of boron in the metabolism of plants is not clear.

ii. It probably facilitates the translocation of sugars.

Deficiency Symptoms:

i. Boron deficiency causes death of the shoot tip.

ii. Flower formation is suppressed.

iii. Root growth is stunted.

iv. Leaves become coppery in texture.

6. Molybdenum:

Specific Role:

i. It is associated with the prosthetic group of the enzymes nitrate reductase and nitrogenase and thus plays important role in nitrogen metabolism.

Deficiency Symptoms:

i. Molybdenum deficiency causes chlorotic interveinal mottling of the older leaves.

ii. Flower formation is inhibited.

iii. Causes whip tail disease in cauliflower plants.

7. Chlorine:

Specific Role:

i. In the form of chloride ions it is involved in photolysis of water and oxygen evolution in photosynthesis.

ii. It is required for cell division in leaves and roots.

iii. It is an important osmotically active solute.

Deficiency symptoms:

i. Wilting of leaf tips occurs which is followed by general chlorosis and necrosis.

ii. Leaves show reduced growth and ultimately bronzing (bronze colour) occurs.

iii. Roots become stunted in length but thickened near the tips.

8. Nickel:

Specific Role:

i. It is cofactor of the enzyme urease in higher plants

Deficiency symptoms:

i. Due to accumulation of urea in leaves, necrosis of leaf tips occurs.

Sodium and silicon are beneficial to many plants and they are emerging as strong candidates for inclusion in list of essential elements in future

Mineral deficiency:

The metals, non-metals and their salts are called minerals, because they are mined from the soil, ground or earth's crust. Minerals are needed in smaller quantity for the growth and development of body, minerals do not supply any energy to the body. Our body can use minerals in the compound form and not as pure elements. Humans get most of the minerals from plant sources.

The following table shows the uses of some minerals in our body:

Table 9.2.

Mineral	Daily requirement	Major source	Deficiency Disease	Functions
Sodium	2.5 g	Common salt, fish, meat, eggs, milk	Muscle cramps	Associated with contraction of muscles transmission of nerve impulses in nerve fibre, controls water balance in the body.
Potassium	5-7 g	milk, banana, vegetables and meat	Weakness of muscles, paralysis	Involves in chemical activities inside cell. Contraction of muscles.
Calcium	1 gram	milk, egg, cheese, green vegetables, fish	Rickets (soft bones)	Essential for hardening the bones and teeth, coagulation of blood, muscle contraction
Phosphorus	1 gram	milk, cheese, green leafy vegetables, bajra, liver, nuts, beans and grains	bad teeth and bones	building strong bones and teeth.
Iron	25 mg (boys) 35 mg (girls)	Liver, kidney egg yolk, bajra, apple, banana, green vegetables	anaemia	Essential for the formation of haemoglobin in red blood cells, for tissue oxidation.
Iodine	20 g	Salt water, fish, iodized common salt sea food	Goitre	Essential for the formation of thyroxine.

Deficiency Symptoms of Essential Mineral Elements:

Deficiency symptoms are externally visible pathological conditions (morphological and physiological deformities or abnormalities) which are produced due to absence or deficiency of some essential nutritive substance. Deficiency symptoms are also called hunger signs.

They appear when the availability of the essential nutrients falls below the critical concentration. Critical concentration is that limited concentration of the essential element below which growth of the plant is reduced. As each essential element has one or more specific structural and functional roles, its deficient supply results in appearance of abnormal signs called deficiency symptoms. The symptoms disappear when the deficient element is supplied. Deficiency symptoms appear first in young leaves and young tissues for elements which are relatively immobile inside the plant, e.g., Ca, S. They appear first in old leaves and tissues for those elements which are mobilised from senescing regions for supply to young tissues.

Knowledge of deficiency symptoms in relation to particular elements help the farmer and the farm scientist to recognise the same when they are present in a field or area. Immediate corrective measure can then be taken by supplying the missing or deficient nutrient to the soil or directly spraying it over the crop. Deficiency symptoms are first studied by means of pot

and culture experiments. Rapidly growing plants which develop characteristic symptoms are used in culture experiments. They are called test (= indicator) plants. They are then grown in soil under test in small pots. The results are compared to know the deficiency elements. Similar tests are performed with selected crops. Excess of minerals are often toxic. The toxicity is studied first in culture experiments and then field or soil trials.

Common Deficiency Symptoms:

(1) Chlorosis:
Non-development or loss of chlorophyll. N, K, Mg, S, Fe, Mn, Zn, Mo.

(2) Stunted Growth:
N, K, Ca, S, Zn, B, Mo, Cl.

(3) Purple Colouration of Shoot Axis or Leaves:
N, P, Mg, S, Mo.

(4) Necrosis:
Death of tissues. Ca, Mg, Cu, K.

(5) Premature Fall of Leaves and Buds:
P, Mg, Cu.

(6) Inhibition of Cell Division:
N, K, S, Mo.

(7) Wrinkling of Cereal Grains:
N, S, Mo.

(8) Dormancy of Lateral Buds:
N, S, Mo.

(9) Late Flowering:
N, S, Mo.

(10) Die Back:
Killing of shoot apex (stem tip and young leaves). K, Cu.

(11) Wilting. Loss of turgor. Cl.

(12) Death of Root and Shoot Tips. B.

(13) Bushy Habit of Shoot. K.

(14) Scorched Leaf Tips. K.

(15) Interveinal Chlorosis. Fe.

(16) Whiptail Disease of Leaves. Mo.

Toxicity of Micronutrients:

In higher doses, micronutrients become toxic. Any tissue concentration which reduces dry weight of tissue by 10% is called toxic concentration. Critical toxic concentration is different for different micronutrients as well as different plants. For example, Mn^{2+} is toxic beyond 600 µg g^{-1} for Soya-bean and beyond 5300 µg g^{-1} for Sunflower. Toxic effects may be due to direct excess of the micronutrient or its interference in the absorption and functioning of other nutrients.

Manganese toxicity (brown spots surrounded by chlorotic veins) is due to:

(i) Reduction in uptake of iron and magnesium,

(ii) Inhibition of binding of Magnesium to specific enzymes,

(iii) Inhibition of Calcium translocation into shoot apex.

Therefore, excess of Manganese causes deficiency of Iron, Magnesium and Calcium. The toxicity symptoms of Mn are actually combined deficiency symptoms of Fe, Mg and Ca

Probable questions:

1. discuss deficiency symptoms of any three microelements.
2. discuss deficiency symptoms of any three macroelements.
3. Discuss about Criteria of Essential Mineral Element.
4. Discuss about Toxicity of Micronutrients.

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The study materials of this book have been collected from books, various e-books, journals and other e-sources.