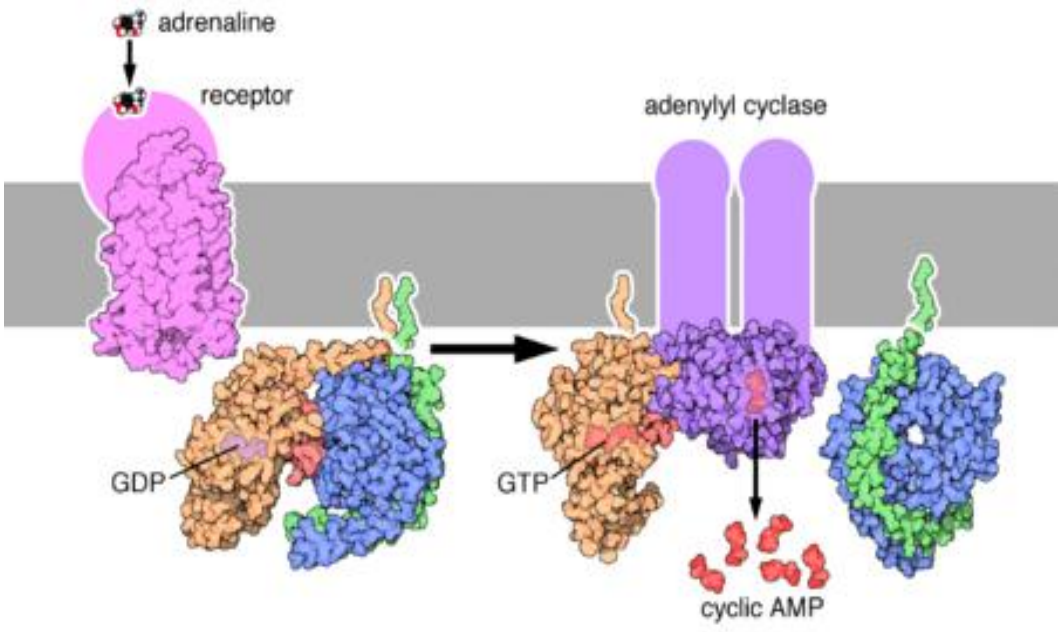
STATE UNIVERSITY OF MEDICINE AND PHARMACY *NICOLAE TESTEMITANU*
BIOCHEMISTRY AND CLINICAL BIOCHEMISTRY DEPARTMENT

LUDMILA GAVRILIUC

BIOCHEMISTRY

Lectures for students of Medical Departments



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2011
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INTRODUCTION

Biochemistry can be defined as the *science concerned with the chemical basis of life.* The **cell** is the structural unit of living systems. Thus, biochemistry can also be described as the *science concerned with the chemical constituents of living cells and with reactions and processes they undergo.* By this definition, biochemistry encompasses large areas of **cell biology,** of **molecular biology,** and of **molecular genetics.**

The major objective of biochemistry is the complete understanding, at the molecular level, of all of the chemical process associated with living cells. To achieve this objective, biochemists have sought to isolate the numerous molecules found in cells, determine their structures, and analyze how they function. Many techniques have been used for these purposes.

Knowledge of biochemistry is essential to all life sciences. The biochemistry of the nucleic acids lies at the heart of *genetics*; in turn, the use of genetic approaches has been critical for elucidating many areas of biochemistry. *Physiology*, the study of body function, overlaps with biochemistry almost completely. *Immunology* employs numerous biochemical techniques, and many immunologic approaches have found wide use by biochemists. *Pharmacology* and *pharmacy* rest on a sound knowledge of biochemistry and physiology; in particular, most drugs are metabolized by enzyme-catalyzed reactions. Poisons act on biochemical reactions or processes; this is the subject matter of *toxicology*. Many workers in *microbiology* employ biochemical approaches almost exclusively.

Biochemical approaches are being used increasingly to study basic aspects of *pathology* (the study of disease), such as inflammation, cell injury, and cancer. Biochemistry and medicine are intimately related. Health depends on a harmonious balance of biochemical reactions occurring in the body, and disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes. These relationships are not surprising, because life as we know it depends on *biochemical reactions* and *processes.* Biochemical approaches are often fundamental in illuminating the causes of diseases and in designing appropriate therapies. The judicious use of various biochemical laboratory tests is an integral component of diagnosis and monitoring of treatment.

This book can be very useful to the students of Medical Departments: General Medicine, Stomatology, and Pharmacy. Biochemistry integrates and summarizes the essentials of medical biochemistry for students in the health-related professions.

Anything more than an extremely superficial comprehension of life – in all its diverse maknifestations – demands the knowledge of biochemistry.

The major objective of Biochemistry is the complete – in all its diverse manifestations – demands a knowledge of Biochemistry.

Medical students who acquire a sound knowledge of Biochemistry will be in a position to confront, in practice and research, the 2 central concerns of the health sciences:

1. the understanding and maintenance of health;
2. the understanding and effective treatment of disease.

LECTURE 1

Subject: INTRODUCTION IN BIOCHEMISTRY. AMINO ACIDS

Biochemistry or Biological chemistry is the branch of knowledge that deals with the structure of chemical compounds that make up part of living matter, their transformations and physico-chemical processes that constitute the basis of vital activity. Biochemistry is a part of Biology; it encompasses the areas that require physico-chemical and chemical approaches, methods, and techniques. The specificity of Biochemistry is easily seen from its name, which suggests the chemical basis of this discipline as well as the important role played by functional (biological) studies of the chemical processes that occur.

Historically, Biochemistry is intimately related to Organic Chemistry, which deals with the chemical properties of compounds that make part of living matter, and to Physiology, which deals with the functions of living organisms. The terms "*physiological chemistry"* and "*biochemistry"* as equivalent concepts were therefore not accidental.

Biochemistry owes its formation to numerous contiguous disciplines and remains closely related to them in the study of animate nature. Nonetheless, it is an original and independent branch of knowledge whose major objectives are the investigation of the structural and functional interrelationships and conversions of chemical compounds in the living organism, the routes of energy transformation in the living systems, the regulatory mechanisms of chemical conversions and physico-chemical processes in cells, tissues, and organs, the molecular mechanisms of the transfer of genetic information in living organisms, etc.

Proteins. Introduction to protein chemistry

Proteins (from the Greek ***proteios,*** primary) are the major cell components of any living organism. Proteins play the most important role in all biological processes.

Proteins are distributed among subcellular structures not in a uniform manner: in highest amounts they occur in the cell sap (hyaloplasm, cytosol). The protein level in organelles is rather determined by the size and number of the organelles in a cell. Proteins are high-molecular nitrogen-containing organic compounds with complex structural organization, polymers composed of amino acids linked into chains by covalent peptide bonds. Protein monomers are α-amino acids of the L-series (levorotatory).

High molecular mass is a very important characteristic of proteins. Depending on the chain length all polypeptides are conventionally classified into peptides (containing from 2 to 10 amino acids), polypeptides (from 10 to 40 amino acids), and proteins (over 40 amino acids). The molecular mass for peptides is close to 1000, for polypeptides to 4000, and that for proteins from 4000-5000 to a few 10^6.

For example:

glucagon – 4 000
insulin – 6 000
trypsin – 23 800
glutamate dehydrogenase – 1 000 000.

Amino acids as structural monomers for proteins

Amino acids, or aminocarboxylic acids, are organic carboxylic acids in which at least one hydrogen atom of the hydrocarbon chain is replaced by an amino group. L-Amino acids are classified into α-, β-, γ-types, depending on the position of the carbon bearing an $–NH_2$ group with respect to –COOH group. About 200 various amino acids have been identified in different species of the animate nature. About 60 different amino acids and their derivatives are found in the human organism; still, not all of them serve as constituents for proteins.

Amino acids are 20 which are as constituents of proteins. Amino acids are divided into two groups: ***proteogenic,*** or ***proteinogenic,*** amino acids, which are normally components of proteins, and ***nonproteogenic (nonprotein)*** amino acids, usually not incorporated into proteins.

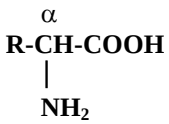
Among the proteogenic amino acids, there are ***major amino acids*** (in total number of 20) and rare amino acids. Actually, the rare protein amino acids (for, example, hydroxyproline, hydroxylysine, aminocitric acid, etc.) are derivatives of the 20 major amino acids.

Other amino acids do not participate in the protein synthesis; they occur in the cells either in a free state (as metabolites), or are part of nonprotein compounds.

γ-Aminobutyric acid occurs in a free state and functions as an inhibitory neurotransmitter; β-alanine is a component of a vitamin, pantothenic acid.

Structure and classifications of proteogenic amino acids

All protein amino acids are L-amino acids bearing an NH_2 group on the carbon at α-position:



Three classifications of amino acids are currently adopted:

1. *Structural classification*, based on the structural features of side-chain radicals of amino acids.
2. *Electrochemical classification*, based on the acid-basic properties of amino acids.
3. *Biological classification*, based on the functional priority of amino acids for the organism.

According to their electro-chemical (or acid-basic) properties, amino acids are divided into three groups: acidic, basic and neutral, depending on the physico-chemical properties of the side-chain radical **R.**

Acidic amino acids are those having additional carboxylic groups in the side-chain radical, which provide for enhanced acidic properties in this group of amino acids: aspartic, glu-tamic.

Basic amino acids include lysine, arginine, histidine, i.e. amino acids carrying an additional basic group (amino) contributing to their enhanced bacic properties.

Neutral amino acids are the remaining acids. Their side-chain radicals exhibit neither acidic, nor basic properties.

Depending on the side-chain radical polarity, amino acids of the two former groups (acidic and basic) belong to polar; and amino acids of the third group (neutral) to nonpolar, or hydrophobic, acids.

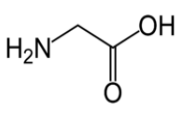
According to their biological or physiological importance, amino acids are also subdivided into three groups: *essential, half-essential,* and *nonessential.*

Essential amino acids cannot be synthesized in the organism from other compounds; therefore they must be supplied to the organism as taken with the food. For the human organism, eight amino acids are absolutely essential. These are acids: valine, leucine, isoleucine, threonine, lysine, methionine, phenylalanine, and tryptophan.

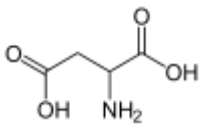
Half-essential amino acids are formed within the organism, but not in sufficient amounts; therefore, they must partly be supplied in food. For the human organism, such amino acids are arginine, tyrosine, and histidine.

Nonessential amino acids are synthetized by the organism in adequate amounts from essential amino acids or other compounds. The remaining amino acids are included among nonessential amino acids. They are: glycine, alanine, serine, cysteine, cystine, aspartic acid, glutamic acid, and imino acids proline, hydroxyproline.

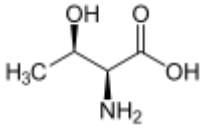
Structural classification, based on the structural features of side-chain radicals



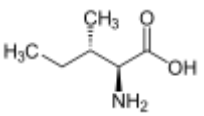
glycine



aspartic acid



threonine



isoleucine

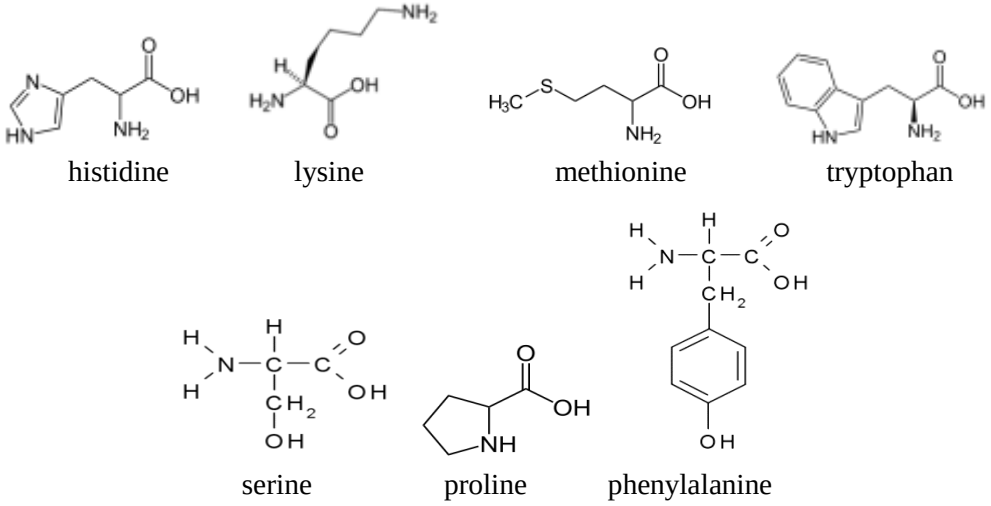


Figure 1. **Structure of amino acids**

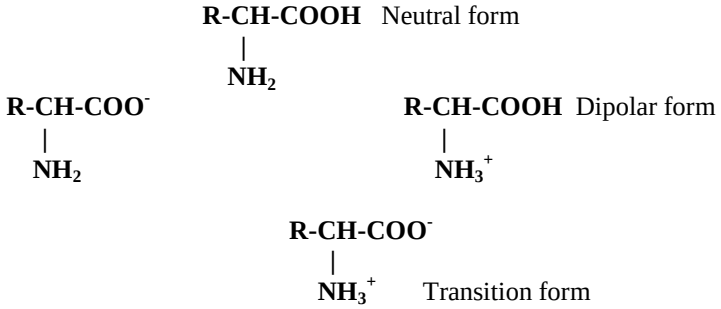
The amino acids provide material for the synthesis of such important constituents as proteins which are basically indipensable for the perpetuation of life.

Physical and chemical properties of amino acids

1. Acid-basic properties of amino acids

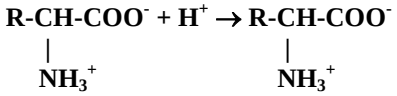
Viewed from the chemical standpoint, amino acids are amphoteric electrolytes, i.e. they exhibit properties of both an acid and a base. The *acidic groups* of amino acids are: carboxylic group (-$COOH \rightarrow COO^- + H^+$) and protonated α-amino group (-$NH_3^+ \rightarrow NH_2 + H^+$). *Basic groups* of amino acids are: dissociated carboxyl group (-$COO^- + H^+ \rightarrow COOH$) and α-amino group (-$NH_2 + H^+ \rightarrow NH_3^+$).

Amino acids in aqueous solutions have been shown to occur as a dipolar species, or zwitterion.

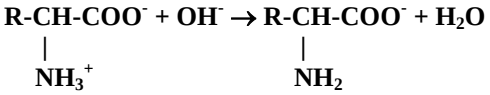


2. Effect of the medium pH on ionization of amino acids

The change of medium pH from acidic to basic affects the charge on dissolved amino acids. In acidic medium (pH<7), all amino acids carry a positive charge (i.e. they occur as cations), since an excess in environmental protons suppresses the dissociation of the carboxyl group:



In acidic medium under applied electric field, amino acid molecules are carried over to the cathode (-). In basic medium (pH>7) with an excess of OH^- ions, amino acids occur as negatively charged species (anions), the NH_3^+ group being subject to dissociation:



In this case, under applied electric field amino acid molecules move towards the anode (+). Therefore, amino acids, depending on the medium pH, can carry net *zero, positive,* or *negative* charges.

The state in which the net charge on an amino acid is equal to zero is referred to as ***isoelectric.*** The value of pH at which such a state is attained, when the amino acid molecules are at rest under applied electric field without preferential displacement neither to anode, nor to cathode, is named the ***isoelectric point*** and is denoted pH. The isoelectric point is a very accurate indicator of acid-basic properties for various functional groups in an amino acid and is an important feature characteristic of the amino acid. The isoelectric points for nonpolar (hydrophobic) amino acids are close to the neutral value of pH (from 5,5 for phenylalanine to 6,3 for proline); for acidic amino acids, it has lower values (e.g. 3,2 for glutamic acid and 2,8 for aspartic acid). The isoelectric point for the major amino acids, histidine and, especially, lysine and arginine, is significantly greater than 7.

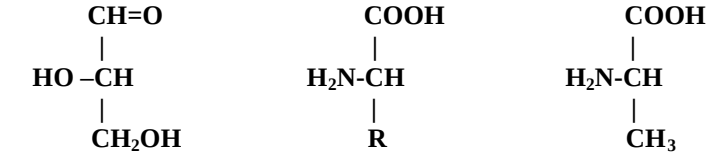
3. Stereoisomerism of amino acids

All proteogenic amino acids, excepting glycine, possess at least one asymmetric carbon atom (**C***) and exhibit optical activity, most of them being levorotatory. They exist as spatial isomers, or stereoisomers. The stereoisomers are referred to the *L*- or *D*-series according to the arrangement of substituents around the asymmetric carbon atom.

All the protein-constituting amino acids belong to the *L*-isomeric series.

Formerly it was believed that *D*-amino acids do not occur in the animate nature. *D*-amino acids have been found in the bacteria and antibiotics generated by microorganisms.

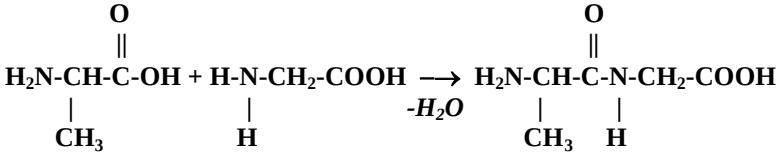
In all the amino acids (except glycine) the carbon atom attached to nitrogen and alpha (α) to the carboxyl group is asymmetric. It has been found that in general the configuration of the asymmetric group of the naturally occurring amino acids is the same as the configuration of this group in L-glyceric aldehyde to which the configuration of the L-series of sugars is referred.



L-glyceric aldehyde *L-amino acid* *L-alanine*

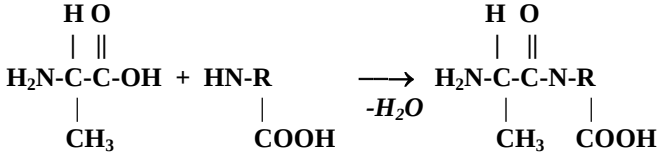
Nomenclature for peptides and polypeptides

Peptide is defined as a linear peptide chain that is composed of amino acid residues linked through peptide bonds. The high stability of the structure is provided by the covalent peptide bonds that are formed by α-amino group of one amino acid and the α-carboxyl group of the neighbouring amino acid:



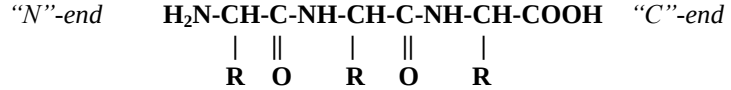
***ala**nine* ***gly**cine* *alanylglycine (**ala-gly**)*

Peptide linkage formed by condensation of the carboxyl of one amino acid and the amino group of another with the elimination of water. The peptide bond formed with the involvement of the imino group of proline (**R**) or hydroxyproline takes a different configuration:



ala**nine* ***pro**line* ***ala-pro

The peptide bond is a repeating unit of the polypeptide chain. Open chains contain a free α-amino group at one end (**N-end**) of the chain and a free α-carboxyl group at the other end (**C-end**):



Nomenclature for peptides and polypeptides

The name of a peptide is composed of the names of constituent amino acids. Two amino acids give a dipeptide, three amino acids give a tripeptide, etc. In naming a polypeptide, all of the amino acids are enumerated in consecutive order, always starting with the N-terminal amino acid and finishing with the C-terminal amino acid. Each amino acid is given the *ending-**yl*** in place of the most common *ending-**ine*** the C-terminal amino acid retains its name unchanged. For example, the tripeptide **ala-ser-met** is named *alan**yl**-ser**yl**-methion**ine***.

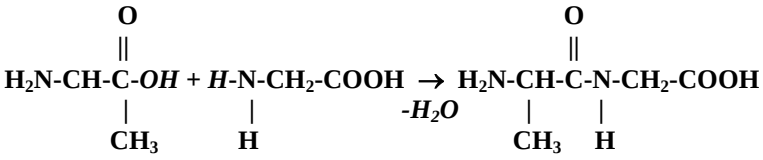
LECTURE 2

Subject: PROTEINS: STRUCTURE AND LEVELS OF STRUCTURAL ORGANIZATION OF PROTEINS

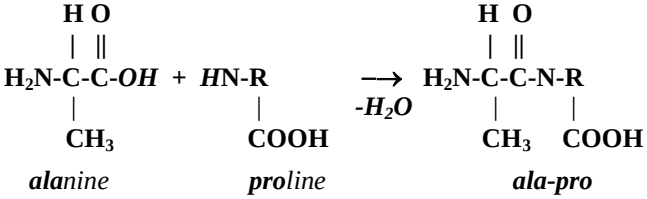
Four levels of structural organization of proteins are recognized: *primary, secondary, tertiary* and *quarternary.* Each level has its proper specificity.

Primary structure of protein

The primary structure of protein is defined as a linear polypeptide chain that is composed of amino acid residues linked through peptide bonds. The primary structure is the simplest level of structural organization of any protein molecule. The high stability of the structure is provided by the covalent peptide bonds that are formed by the neighbouring amino acid:



The peptide bond formed with the involvement of the imino group of proline (**R**) or hydroxyproline takes a different configurations:



Methods for determination of protein primary structure are: acidic, basic and enzymic *hydrolysis, ion-exchange chromatography* coupled to amino acid analyzer, *sequencing* (as performed on sequencers).

Secondary structure of protein

The secondary structure refers to the way the peptide is folded into an ordered structure owing to hydrogen bonding between peptide groups of the same chain or juxtaposed polypeptide chains. By configuration the secondary structures are classified into helical structures (α-helix) and pleated sheets (β-structure and cross-β-form).

α-Helix. This type of secondary structure, which resembles a regular helix, is formed owing to hydrogen bonds between peptide groups within the same polypeptide chain. The α-helical structural model, which takes into account all known properties of the peptide bonds, was proposed by Pauling and Cori.

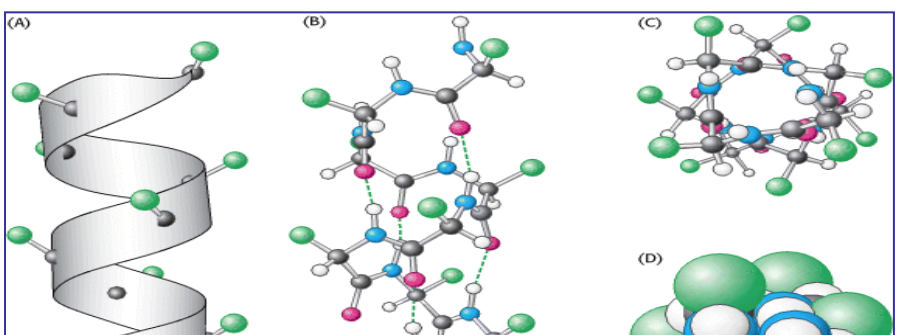


Figure 2. **Secondary structure of protein (α-structure)**

The main features of the α-helix are:

1. Helical configuration of the polypeptide chain of screw-type (rotation-translational) symmetry.
2. Formation of hydrogen bonds between the peptide groups of every first and fourth amino acid residues.
3. Regularity of the turns along the helix length.
4. Equivalence of all amino acid residues in the α-helix, irrespective of the structure of their side-chain radicals.
5. Nonparticipation of the amino acid side-chain radicals in hydrogen bonding, i.e. in the formation of α-helix.

The α-helix resembles a slightly distended heating coil. The regularity of hydrogen bonds between every first and fourth peptide groups determines the regularity of the polypeptide chain turns. The axial pitch of α-helix is 0.54 nm; it contains 3.6 amino acid residues.

β-Structure. This variety of the polypeptide chain secondary structure adopts a slightly bent configuration and is formed by means of interpeptide hydrogen bonds within the same polypeptide chain or with involvement of hydrogen bonds between juxtaposed polypeptide chains. β-structure is also referred to as a *pleated sheet structure*. Several types of β-structure are known. Finite pleated lengths of a single peptide chain are called cross-β-forms (short β-structures). In the cross-β-form, hydrogen bonds are formed between the peptide groups of polypeptide chain loops. Another type of β-structure is characteristic of the *whole polypeptide chain*, which is held is a stretched state by hydrogen bonds between juxtaposed parallel polypeptide chains.

In shape, this structure resembles the bellows of an accordion. β-Structures can be formed by *parallel chains* (with N-termini of the polypeptide chains pointing in the same direction), or by antiparallel chains (with the N-termini pointing in the opposite direction). The side-chain radicals of one sheet are located between the side-chain radicals of the other sheet.

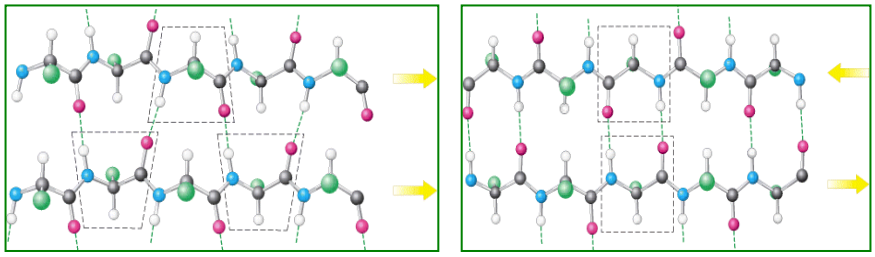


Figure 3. **Secondary structure of protein (β-structure)**

Owing to the reorganizable hydrogen bonds in proteins the α-structure can be reversibly converted to the β-structure. The regular interpeptide hydrogen bonds by means of which a polypeptide chain retains its α-helical conformation become replaced by interchain hydrogen bonds between the stretched fragments of neighbouring uncoiled polypeptide chains. Such a transition has been established in the hair protein, keratin.

Other types of bonds contribute, but little to the secondary structure formation, with the exception of disulphide bonds formed at the sites along the polypeptide chain where cysteine residues are present. Short peptides, owing to the disulphide bonds, become closed to rings.

Numerous proteins possess both α-helical regions and β-structures. Proteins exhibit a varied extent of helicity. A high percentage of α-helical structures is observed in paramyosin, myoglobin, and hemoglobin. In contrast, a sizeable portion of the polypeptide chain in trypsin and ribonuclease is

pleated in sheet β-structures. The proteins of supporting tissues, viz. keratin (hair and wool protein), collagen (skin and tendon protein) and fibroin (natural silk protein) all possess a β-configuration of their polypeptide chains.

Methods for determination of protein secondary structure are: *spectropolarimetry* (measurement of rotation angle for linearly polarized light on spectrophotometers), *isotope exchange method*, *UV spectrophotometry* (measurement of protein UV absorption at 190 nm on spectrophotometers), *IR spectroscopy* (measurement of infrared absorption spectra of proteins using infrared spectrophotometers).

Tertiary structure of proteins

The tertiary structure of protein is referred to as a specific mode of spatial arrangement of the polypeptide chain. In regard to their tertiary structure, the proteins are chiefly divided into *globular* and *fibrous* (or *fibrillar*) species. Globular proteins have most commonly an ellipsoid shape, while fibrous proteins are elongated (rodor spindle-like).

Bonds contributing to stabilization of tertiary protein structure. Bonds that are formed between the side-chain radicals of amino acids play a role in the stabilization of the tertiary structure. These bonds may be classified into strong (covalent) and weak (polar, van-der-Waals) bonds.

Covalent bonds comprise disulphide bonds (-S-S-) between the side chains of cysteine residues

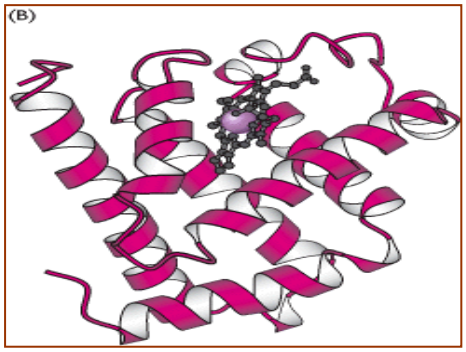


Figure 4. **Tertiary structure of protein (B, myoglobin)**

located in different parts of polypeptide chains; isopeptide (or pseudopeptide) bonds between the amino groups of the side chains of lysine or arginine and the –COOH side-chain groups of aspartic or glutamic acids. This explains the name (peptide-like) conferred on the bonds of this kind. Of rare occurrence is the *ester bond* formed with the involvement of a –COOH group of dicarboxylic amino acids (aspartic and glutamic) and the –OH group of hydroxyamino acids (serine and threonine).

Polar bonds comprise hydrogen and ionic bonds. Hydrogen bonds commonly arise between $–NH_2$, -OH, or –SH side-chain groups of one amino acid and carboxylic group of the other amino acid. Ionic, or electrostatic, bonds are formed between charged side-chain groups $–NH_3^+$ (in lysine, arginine, histidine and $–COO^-$ (in aspartic and glutamic acids) brought into a closer contact.

Nonpolar, or ***van-der-Waals,*** bonds are formed between hydrocarbon radicals of amino acids. The hydrophobic radicals of alanine, valine, isoleucine, methionine, and phenylalanine interact in aqueous medium. Weak van-der-waals forces favour the formation of a hy-drophobic core composed of nonpolar radicals in the interior of a protein globule. The contribution of van-der-waals forces to the architecture of polypeptide chains increases with the number of nonpolar amino acids.

The multiplicity of bonds between the side-chain radicals of constituent amino acids determines the spatial configuration of a protein molecule. The conformation of the polypeptide chain tertiary structure is determined by the properties of the side-chain radicals of constituent amino acids (which exert no sizeable influence on the formation of primary and secondary structures) and those of the microenvironment, i.e. medium. In folding, the protein polypeptide chain tends to adopt an energetically favorable configuration corresponding to a minimum of free energy.

In protein molecules with tertiary structure, there occur regions formed as α-helices, β-structures (pleated sheets) and random coils. It is only a proper spatial arrangement that renders the protein active; otherwise a disorganization of its spatial structure produces alterations is its properties and may lead to a loss of biological activity.

Methods for determination of tertiary protein structure are: *electron microscopy*, *X-ray structural analysis*.

Quarternary structure of proteins

Proteins composed of a single polypeptide chain possess only a tertiary structure. They include myoglobin, a muscle tissue protein involved in oxygen binding, as well as a number of enzymes (lisozyme, pepsin, trypsin, etc.). However, certain proteins are built of several polypeptide chains, each chain of which has a tertiary structure.

For such proteins, the notion of a *quarternary structure* has been suggested. The quarternary structure presents itself as an aggregation of two or more polypeptide chains with a tertiary structure organized into a single functional protein molecule. A protein with a quarternary structure is referred to as an *oligomer* and its polypeptide chain with a tertiary structure are referred to as *protomers*, or *subunits.*

At the quarternary level of organization, proteins retain the main configuration of their tertiary structure (globular or fibrous). For example, hemoglobin, a protein exhibiting a quarternary structure, is composed of four subunits. Each of the subunits is a globular protein, and hemoglobin also has an overall globular configuration.

Quarternary structure of hemoglobin (Hb: 2α and 2β subunits)

Stabilization of protein quarternary structure

All proteins exhibiting a quarternary structure are amenable to isolation as individual macromolecules resisting dissociation into the constituent subunits. Numerous ionic, hydrogen and, occasionally, disulphide bonds are formed between the subunit polar groups providing thereby for the persistence of the involved subunits as an organized tightly bound complex.

Methods for determination of protein IV structure are: *electron microscopy, X-ray structural analysis , and electrophoresis.*

Specific features of structural organization in certain fibrous proteins. The structural organization of fibrous proteins reveals a number of specific features as compared with that of globular proteins. These features may be exemplified by *keratin*, *fibroin,* and *collagen.* Keratins exist in α- and β-conformations. α-Keratin and fibroin both possess a secondary pleated sheet structure; still, in keratin, the polypeptide chains are parallel and in fibroin, antiparallel. The fibrous protein is typified by collagen, a major protein in the human organism (about $^{1}/_{3}$ of total protein mass). Collagen is responsible for the strength and low flexibility of connective tissue and is found in bones, tendons, skin, teeth, etc. In collagen, glycine accounts for one third, and proline or hydroxyproline, for about one fourth of the total of amino acid residues. Polypeptide chain of collagen resembles a broken line. It contains about 1000 amino acids and has a molecular mass of the order of 10,000. The polypeptide chain is built of repeating units, each unit being composed of tree amino acids (triplet) **glycine-proline-hydroxyproline**. Proline, hydroxyproline, and glycine (antihelical amino acids) hinder the formation of α-helices. Therefore, three chains form a kind of twisted helices similar to three threads wound around a cylinder. The three helical α-chains form a repeating collagen structure referred to as *tropocollagen.* Tropocollagen, by its organization, is a tertiary structure for collagen and a subunit for collagen fibrils. The folding of tropocollagen subunits into a collagenic quaternary structure is accomplished stepwise. Stabilization of collagenic structures is effected through the agency of interchain ionic, hydrogen, and van-der-Waals bonds, with a minor participation of covalent bonds.

LECTURE 3

Subject: PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS

The physico-chemical properties of a protein are determined by its amino acid composition and spatial organization. Proteins exhibit acid-basic, buffering, colloidal, and osmotic properties.

Proteins as amphoteric macromolecules

Proteins are amphoteric polyelectrolytes, i.e. they combine, similar to amino acids, acidic and basic properties. However, the nature of the constituent groups imparting amphoteric properties to proteins differs to a significant extent from that of amino acids. The acid-basic properties of amino acids are primary due to occurrence of α-amino and α-carboxyl groups (i.e. acid-base pairs) in them. The amphoterism of proteins is due to the acid-base groups of side-chain radicals of protein-constituting amino acids. It stands to reason that each molecule (polypeptide chain) of a native protein possesses a minimum of one terminal α-amino and one terminal α-carboxyl group (providing the protein displays a tertiary structure only). For a protein with a quaternary structure, the number of terminal -NH_2 and –COOH groups is equal to the number of subunits, or protomers. However, the small number of these groups does not suffice to explain the amphoterism of protein macromolecules. Since the majority of the polar groups are located on the surface of globular proteins, precisely these groups provide for the acid-basic properties and the charge of a protein molecule. Acidic amino acids (aspartic, glutamic) are responsible for acidic, and basic amino acids (lysine, arginine, and histidine), for basic properties of proteins. Acidic properties of a protein become more pronounced, as the number of its constituent acidic amino acids increases; accordingly, basic properties manifest themselves to a greater extent with an increased number of basic amino acids. Poorly dissociating SH-groups of cysteine and the phenolic group of tyrosine (formally treated as weak acids) practically do not affect the amphoterism of proteins.

Buffering properties

Proteins, while exhibiting buffering properties, do not, nevertheless, possess any high buffering capacity at physiological values of pH. An exception to the rule are proteins containing a large number of histidine residues, since it is only the side chain of histidine that exhibits buffering properties within a pH range close to the physiological pH. Such proteins are quite scarce, though. Hemoglobin is actually the only protein with a histidine content reaching 8% and is, therefore, a powerful intracellular buffer in the erythrocytes which is capable of maintaining the blood pH value at a constant level.

The charge on a protein molecule is dependent on the contents of acidic and basic amino groups in the side-chain radicals of these amino acids. The dissociation of –COOH groups of acidic amino acids causes a negative charge to develop on the protein surface, while the side-chain radicals of basic amino acids carry a positive charge (owing to the addition oh H^+ ions to the basic groups). In a native protein molecule the charge distribution is asymmetric, depending on the way the polypeptide chain is folded in space. Acidic amino acids being in preponderance over basic amino acids in a protein, the protein molecules acquire a net negative charge, i.e. become polyanions, and vice versa, if basic amino acids prevail, the protein molecules develop a positive charge and behave as polycations.

Undoubtedly, the net charge on the protein molecule is dependent upon the pH of the medium: in an acidic medium the charge is positive, and in a basic medium, negative. The value of the pH at which a protein exhibits a net zero charge is referred to as the isoelectric point for the given protein. At this point the protein is incapable of migrating under an applied electric field. The knowledge of the isoelectric point is of almost importance for understanding the stability of proteins in solution, since in the isoelectric state, proteins are the least stable. Uncharged protein particles may coalesce and precipitate from solution.

Colloidal and osmotic properties of proteins

The behavior of proteins in solution displays a number of specific features. Common colloidal solutions are stable only in the presence of a stabilizer whose molecules are adsorbed at the "*solute-solvent"* interface, preventing thereby the colloid coagulation.

Aqueous solutions of proteins are stable and equilibrated; they do not precipitate with time (do not coagulate) and do not require the presence of a stabilizer. Protein solutions are homogeneous and may be referred to as true solutions. However, the high molecular mass of proteins imparts a number of properties typical of colloidal systems to their solutions:

- characteristic optical properties (opalescence of solutions and ability to scatter the visible light);
- low diffusion rate;
- inability to pass across semipermeable membranes;
- high viscosity;
- propensity to gelation.

Optical properties of proteins

Protein solutions, especially concentrated ones, exhibit a characteristic opalescence. With a protein solution irradiated sideways, the light beam becomes visible as a luminescent cone; this phenomenon is known as the *Tyndal effect*. This light-scattering effect is explained by light diffraction due to protein particles suspended in solution. It is commonly recognized that protein in the cellular protoplasm occurs as a colloidal solution, or sol. The ability of proteins and other biological molecules (nucleic acids, polysaccharides, etc.) to scatter the light is made use of in the microscopic studies of cellular structures: in the visible microscope, colloidal particles are apparent against the dark background as light spots in the cytoplasm.

The light-scattering properties of proteins and other high-molecular compounds are also used in the quantitative determination by the *nephelometric method:* the light intensities scattered by suspended particles in the sample and reference solutions are compared.

Low diffusion rate. Proteins are characterized by limited diffusion rates as compared with common molecules and ions capable of moving at the rates higher over those for proteins by a few orders of magnitude. The diffusion rate for proteins is influenced by the molecular shape of protein particles rather than by their molecular mass. Globular proteins in aqueous solutions are more mobile than fibrous proteins. The intracellular distribution of proteins is effected via diffusion. Since protein diffusion proceeds at a slow rate, it happens to be a rate-limiting factor for other processes dependent on the biological function of diffusing protein in respective regions of the cell.

Osmotic properties of proteins

Proteins, owing to their high molecular mass, are incapable of diffusing across semipermeable membranes, whereas low-molecular compounds pass easily through such membranes. This property of proteins is made use of in practice for purifying protein solutions from low-molecular contaminants. This process is referred to as *dialysis*.

Biological membranes are likewise impermeable to proteins, therefore the osmotic pressure produced by protein is dependent both on the protein concentration inside and outside the cell. The osmotic pressure due to protein is also referred to as the *oncotic* (produced by swellling) *pressure.*

High viscosity of protein solutions

High viscosity is characteristic not only of protein solutions, but also of high-molecular compound solutions in general. As the protein concentration in solution becomes greater, the viscosity of the solution increases due to a concomitant increase in colligative forces between the protein molecules. The viscosity is dependent on the shape of molecules. Solutions of fibrous proteins are always more viscous than globular protein solutions. Temperature and the presence of electrolytes are the factors that strongly affect the protein solution viscosity. As the temperature rises, the protein solution viscosity decreases. The viscosity of a protein solution may increase to such an extent that the solution loses fluidity and converts to a gel-like state.

Aptitude of proteins for gelation

Interaction of protein macromolecules in solution may lead to the formation of structural networks with water molecules trapped inside them. Such structurized systems are referred to as *gels* or *jellies.* It is recognized that the protein of cell protoplasm can be converted to a gelatinous state. A demonstrative example is the body of a medusa which may be regarded as a living jelly with water content in it up to 90%.

Hydration of proteins and factors affecting protein solubility

Proteins are hydrophilic materials. A dry protein, when submerged in water, starts swellling immediately as any high-molecular hydrophilic compound; with time, the protein molecules gradually start passing into solution. In the course of swelling, water molecules penetrate into the interior of protein structure of polypeptide chains becomes loose. Further water intake leads to the detachment of

individual protein molecules from the protein bulk and to their passage into a state of dissolution. Still, the dissolution is not necessary sequent upon swellling; certain proteins, for example, collagen, persist in the swollen state even after having taken up, a large amount of water.

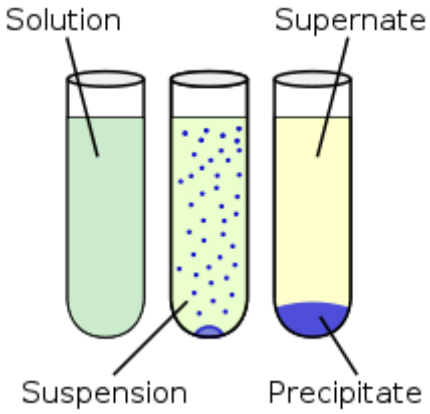
Factors affecting protein solubility

Solubility of proteins varies over a wide range. The solubility is determined by amino acid composition (polar amino acids impart a greater solubility to proteins over nonpolar amino acids), specificity of structural organization (globular proteins are, as a rule, more soluble than fibrous proteins), and the properties of a solvent.

The charge on protein molecules and the hydration shell around them impart stability to the protein solutions. Each macromolecule of an individual protein carries a net charge of the same sign, which prevents agglutinations of the protein molecules in solution and their precipitation. Any factor contributing to the conservation of charge and hydration shell favors the solubility of proteins and their stability in solution. There is a close relationship between the charge on a protein (or the number of polar amino acids in it) and its hydration properties: the higher the number of polar amino acids in the protein, the greater the amount of water it is capable of binding (per 1g of dry weight). A protein contains a large number of cationic and anionic groups; nonetheless, its solubility in water is rather poor. The intermolecular salt bridges formed make protein particles coagulate and precipitate from solution.

What environmental factors affect the solubility of proteins and their stability in solution?

Neutral salts, acids, alkalis, organic solvents (ethanol, acetone), alkaloids, heavy metal salts (mercury, copper, zinc, etc.). The process of protein precipitation by a neutral salt solution is called *salting-out*. A specific feature in the behaviour of proteins obtained by salting-out is the retention of their native biological properties after the salt has been removed. The salting-out mechanism is operable in that the anions and cations of the added solution destroy the protein stability. It is probable also that simultaneously neutralization of the protein charge by salt ions takes place, which facilitates precipitation. The salting-out is widely used for separation and purification of proteins.



Chemical Precipitation

Denaturation (denativation) and renaturation (renativation) of proteins

Certain agents destroy the higher levels of structural organization of protein molecules (secondary, tertiary, and quarternary) with the retention, however, of the primary structure, and the protein thus loses its native physico-chemical, and, what is important, biological property. This phenomenon is referred to as *denaturation (denativation).* It is characteristic of the protein molecules that possess a complex spatial organization. Synthetic and natural peptides are incapable of denaturation.

Denaturation is accomplished through breakdown of the bonds that stabilize the quarternary, tertiary, and occasionally, secondary structures. The polipeptide chain unfolds and remains in solution in the unfolded state, or as a random coil. Macromolecules become stripped of the hydration shells, and the protein separates from solution as a precipitate. However the precipitated denatured protein differs markedly from the precipitated form of the same protein produced by salting-out, since in the former case native properties of the protein are lost and in the latter, retained. This implies that the mechanisms operable in denaturation and salting-out are different. The salting-out leaves the protein native structure unaffected, whereas the denaturation destroys it.

The factors producing denaturation are differentiated into physical and chemical ones. *Physical factors* are: temperature, pressure, mechanical action, and ultrasonic and ionizing irradiation. Heat denaturation of proteins has been studied more thoroughly than other processes and has been recognized as a characteristic feature of proteins. It was since long known that proteins, when heated, curdle (coagulate) to form a precipitate. Most proteins are thermolabile, or heat-modifiable; still, there are proteins known to be very resistant to heat. For example, such are: trypsin, lysozyme, chymotripsin and certain biomembrane proteins.

Chemical factors eliciting denaturation are acids and alkalis, organic solvents (ethanol, acetone), detergents (cleansing agents), certain amides (urea), alkaloids, and heavy metal salts (mercury, copper, zinc, etc.). The mechanism of denaturation by chemical agents is dependent on their physico-chemical properties. Acids and alkalis are widely used as protein precipitants, or coagulators. In the stomach of humans and animals, the natural denaturating agent, hydrochloric acid, is secreted, which denatures proteins and facilitates their enzymic degradation.

Denaturation was for a long time believed to be an irreversible process. In certain occasions, however, the denatured protein is capable of restoring its biological activity after the denaturation agent has been removed. The process of recovering by the protein of its physico-chemical and biological properties is called renaturation, or renativation. If the denaturated protein (after removal of denaturants) is again self-organized to its initial structure, its activity also becomes restored.

Methods for purification of proteins are: mechanical grinding (homogenization), differential centrifugation, treatment with agents disjoining intercellular contacts, extraction, salting-out, acid-base treatment, dialysis, ultrafiltration; ion-exchange, adsorption, gel, affinity chromatography; electrophoresis, isoelectrical focusing in pH gradient, etc.

LECTURE 4

Subject: **ENZYMES: CLASSIFICATION, NOMENCLATURE AND CHEMICAL STRUCTURE OF ENZYMES, COENZYMES**

Enzymes are biological catalysts of protein nature. The term ***"enzyme"*** (from the Greek *en, inzyme,* leaven) was first coined in the early 17th century by the Holland physician *van Helmont* for substances affecting fermentation. The enzyme is a synonym of the term "ferment" (from the Latin *fermentum,* leaven). The enzymes are peculiar to the animate nature only and are used as specific regulators of metabolism. No chemical process in the living organisms can be effected without involvement of enzymes.

At the present time, the ***enzymology*** is an extremely important, rapidly growing branch of biochemistry, and its achievements are widely used in practical medicine, pharmaceutics, food industry, and other related industries.

Common and distinct features in enzymes and nonenzymic catalysts

Enzymes and nonbiological catalysts, in obeying the general laws of catalysis, share the following common features:

1. They catalyze energetically feasible reactions only.
2. They never alter the reaction route.
3. They go not affect the equilibrium of a reversible reaction, but rather accelerate its onset.
4. They are never consumed during the reaction. Therefore, a cellular enzyme functions until it becomes impaired for one or another reason.

However, enzymes exhibit a number of features that distinguish them from nonbiological catalysts. These distinctions are due to structural specificities of the enzymes which are complex protein molecules.

1. The rate of enzymic catalysis is much superior to that of nonenzymic catalysis. It follows there from that enzymes lower the activation energy of reactions to a greater extent as compared with nonbiological catalysts.

2. Enzymes exhibit a high specificity. There ae enzymes that act selectively on only one stereo isomer of a compound. The high specificity of enzymes enables them to direct metabolic processes to strictly defined channels.

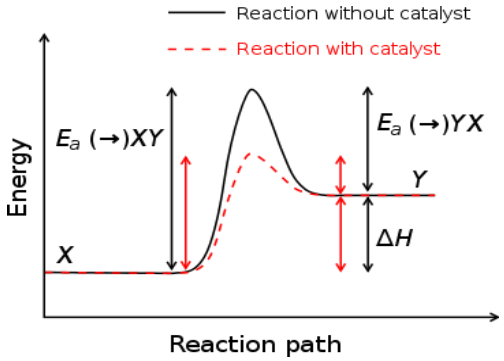
3. Enzymes catalyze chemical reactions under "*mild'* conditions, i.e. at normal pressure, low temperature (about +37^{o}C), and pH close to that of the neutral medium (pH=7±1). This behaviour differentiates them from other catalysts active at high pressure, extreme pH values, and high temperature.

Enzymes, because of their proteinic nature, are susceptible to temperature variations (i.e. are thermolabile) and to the change of medium pH.

4. Enzymes are catalysts with controllable activity, the behaviour never encountered in nonbiological catalysts. This unique property in enzymes allows changing the rate of metabolism in the organism depending on the environmental conditions, i.e. adapting the metabolic activity to the action of various factors.

5. The rate of an enzymic reaction is proportional to the amount of enzyme, while no strictly defined relationship of this kind is found in nonbiological catalysts. Therefore, the short supply of an enzyme in the living organism signifies a lower rate of metabolism and, on the contrary, the additional production of an enzyme is one of the adaptive routes for the organism cells.

The highest energy position (peak position) represents the transition state. With the catalyst, the energy required to enter transition state decreases, thereby decreasing the energy required to initiate the reaction.



The relationship between activation energy (E_a) and enthalpy of formation (ΔH) with and without a catalyst

Classification and nomenclature of enzymes

At present, it is believed that the cell contains about 10^4 enzyme molecules capable of catalyzing over 2000 various reactions. There are 1800 enzymes known to date. About 150 enzymes have been isolated in the crystalline form.

There are 2 classifications of enzymes:

1. The trivial (working) classification

The trivial name for an enzyme was made up of the ending *–ase* added to the name of the substrate subject to the action of the enzyme in question: for example, ***saccha*rose + *ase* = *saccharase***

2. The systematic classification

The systematic name for an enzyme is constructed in a more complicated manner. It is made up of the names of substrates of the chemical reaction catalyzed by the enzyme, *the name of the type of the catalyzed chemical reactions*, and the ending *–ase*. For example, the systematic name for the enzyme *lactate dehydrogenase* is written as:

L-lactate:	***NAD^+***	***-Oxidoreductase***
substrate 1	substrate 2	type of chemical reaction

The systematic names are given to the explore enzymes only. All the enzymes are classified into 6 groups; of these, each is assigned a definite number:

1. oxidoreductases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases (or synthetases).

The name of the group indicates the type of the chemical reaction catalyzed by enzymes. Therefore, there are six major types of enzymic reactions. The groups are divided into subgroups; the latter are further subdivided into subsubgroups. According to the numerical classification system, each enzyme receives a four-part number whose numerals are separated by a dot:

LACTATE DEHYDROGENASE E.C. 1. 1. 1. 27

Enzymatic classification _____↑

group number _____ ↑
subgroup number _____ ↑
subsubgroup number _____ ↑
ordinal number in subsubgroup _____ ↑

All new enzymes are classified only in accordance with the recommendations of the Committee on Enzyme Nomenclature of the International Union of Biochemistry.

Oxidoreductases are enzymes that catalyze redox reactions. The substrate subject to oxidation with oxidoreductases is regarded as a hydrogen donor. For this reason, the enzymes in this group are called dehydrogenases, or, less commonly, reductases. They play a decisive role in the energy metabolism.

Transferases are enzymes that catalyze reactions of transfer of various moieties from one substrate (donor) to another (acceptor). The enzymes that catalyze the transfer of methyl groups are called *methyl transferases*; those that catalyze the amino group transfer are called *amino transferases*, etc.

Hydrolases are enzymes that catalyze the substrate bond cleavage by adding water. The trivial names for hydrolases are made up by adding the ending *–ase* to the name of substrate. Systematic names must, by convertion, contain the term *hydrolase* (for example, amyl*ase*, *saccharase*).

Lyases are enzymes that catalyze bond-cleaving reactions in a substrate without oxidation or addition of water (for example, *pyruvate decarboxylase*).

Isomerases are enzymes that catalyze structural rearrangements within a single molecule (for example, *triose isomerase*).

Ligases *(synthetases)* are enzymes that catalyze the addition of two molecules using the energy of phosphate bond. ATP or other nucleoside phosphates serve as energy sources in the synthetase-catalyzed reactions (for example, *DNA-ligase*).

Structural and functional ogranization of enzymes

The enzymes exhibit all features characteristic of the protein structural organization. They possess four levels of organization: primary, secondary, tertiary, and quaternary. The enzymes with quaternary structure are composed of protomers (subunits) and constitute a preponderant type. Similarly to other functional proteins, they are classified into simple enzyme proteins and conjugated (or compound) enzyme proteins. A conjugated enzyme is composed of a protein portion, *apoenzyme*, and a nonprotein portion, *cofactor*. The cofactors in enzymes are metal ions and coenzymes. Taken singly, the apoenzyme and cofactor exhibit a low (if any) activity as catalysts, but united, they make up a molecule of the active enzymes, or *holoenzyme*.

Functional organization of enzyme

In the three-dimensional structure of a simple, as well as a conjugated, enzyme, there are distinguished a number of regions responsible for certain specific functions. A portion of the enzyme molecule constitutes the *active center (centre*), i.e. the site in the enzyme spatial structure where the binding with a *substrate (**S**)* takes place (substrate is a compound that undergoes conversion by the action of enzyme).

Alongside of the active center, an enzyme has a *regulatory*, or *allosteric, center* spatially remote from the active center in the enzyme molecule. The name allosteric center (from the Greek *allos*, other, or foreign) implies that the molecules bound to this center are structurally (sterically) dissimilar from the substrate but exert influence on the binding and conversion of substrate at the active center by changing the substrate configuration.

The enzyme molecule can have more than one allosteric center. Compounds capable of binding to an allosteric center are called *allosteric effectors.* They exert influence, through the allosteric center, on the function of the active center in a facilitating or an inhibitory manner. Accordingly, allosteric effectors are referred to as positive (activators) or negative (inhibitors).

Structure of the active center

There are distinguished, in the active center, a *contact site* (or anchor site) for binding a substrate, and a *catalytic site* at which the conversion of the bound substrate takes place. Usually, the enzyme active center is made up of 12 to 16 amino acid residues of a polypeptide chain; occasionally, their number may be larger.

Functional groups of enzyme active center

In simple enzymes, the role of functional groups at the contact and catalytic sites is assigned to the side-chain radicals of amino acids only. In conjugated enzymes, the leading part in these processes is played by cofactors.

The following functional enzyme groups take part in catalysis: -COOH, -NH_2, -OH, -SH and other.

Cofactors and their role in enzyme function

Cofactors are either bound to the enzyme active center, or susceptible to easy cleavage by dialysis. The term ***prosthetic*** group is applied to tightly-bound cofactors, similar to the accepted definition of the nonprotein portion in nonenzymic protein. Still, such a definition appears to be somewhat arbitrary, since the cofactor (usually coenzyme) can be tightly bound to the active center of one enzyme and none, to that of another enzyme. Vitamins are parent materials for coenzymes, therefore, their dietary deficiency affects the synthesis of these coenzymes and leads, as a consequence, to an impaired function of the corresponding conjugated enzymes.

Thiamine coenzyme is derived from thiamine (vitamin B_1).

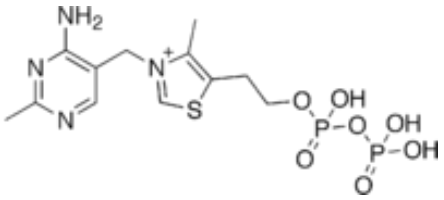


Figure 5. **Structure of TPP (or TDP)**

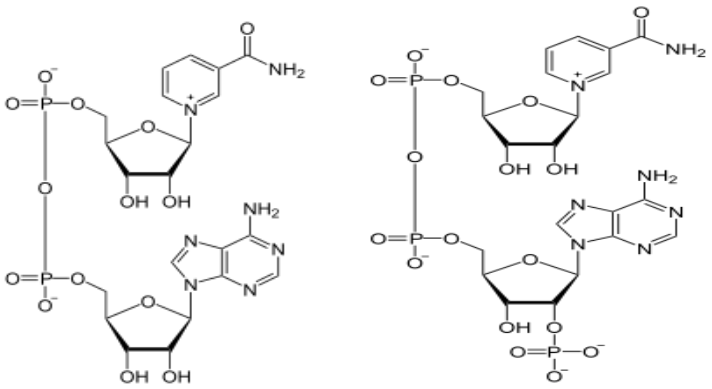


Figure 6. **Nicotinamide coenzymes (structures of NAD and NADP)**

Niacin (vitamin B_5, nicotinic acid) serves as a source for generating nicotinamide coenzymes. The latter species include nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

One of the mononucleotides of these coenzymes contains nicotinamide, the other one is represented by adenilic acid.

Pyridoxine coenzyme. Pyridoxine coenzyme is derived from pyridoxine (vitamin B_6).

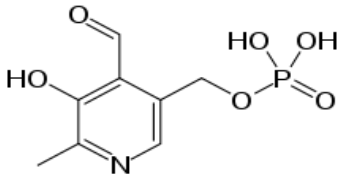


Figure 7. **Coenzyme of vitamin B_6, pyridoxal phosphate**

Flavin coenzymes. These derived from riboflavin (vitamin B_2) structurally related to the isoalloxazine derivatives. Coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are synthesized from riboflavin.

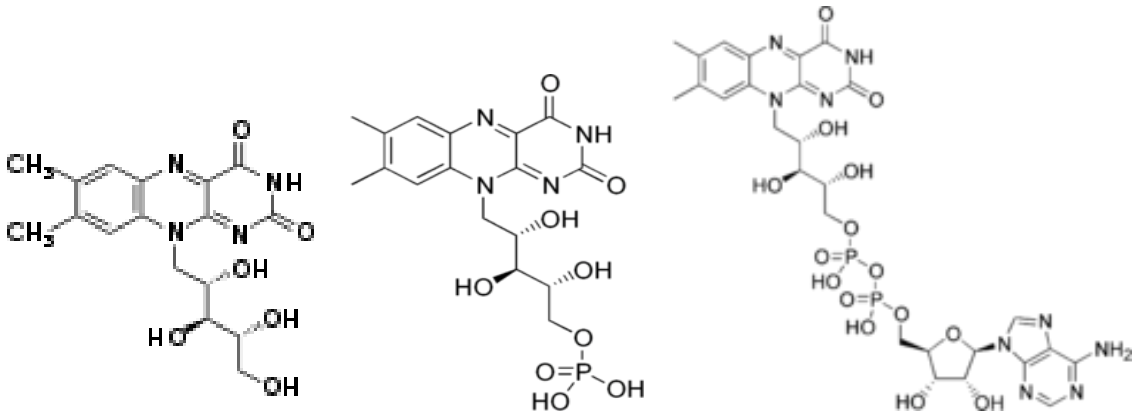


Figure 8. **Chemical structures of vitamin B_2 and its coenzymes, FMN and FAD**

Metalloporphyrin coenzymes include the hemes that participate as coenzymes in redox-reactions catalyzed by oxidoreductases (cytochromes, catalase, peroxidase, etc.).

Metal ions are likewise capable of acting as cofactors. Metalloenzymes constitute a very widespread group of enzymes accounting for 25% of all the enzymes. The role of the metal in these enzymes may be quite different. Metalloenzymes are divided into two groups:

1. Enzymes in which the metal ion acts as an activator (these enzymes exhibit catalytic activity in the absence of metal as well).
2. Enzymes in which the metal ion acts as a cofactor (in the absence of metal these enzymes are inactive).

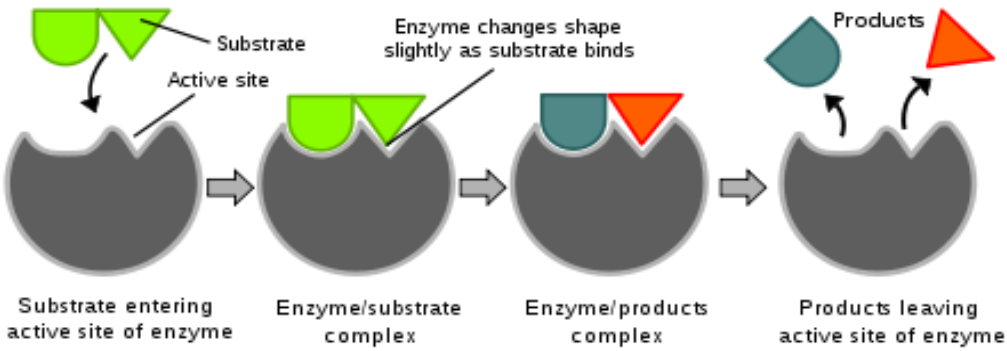
LECTURE 5

Subject: MECHANISM OF ENZYMIC ACTION. REGULATION OF ENZYME ACTIVITY

The process of enzyme catalysis may conventionally be differentiated into three steps, each exhibiting its specificity.

1. Diffusion of a substrate to an enzyme resulting in a stereospecific binding of the former to the active site of enzyme (formation of an enzyme-substrate complex **ES**).
2. Conversion of the primary enzyme-substrate complex into one or more activated enzyme-substrate complexes (denoted **ES*** in the scheme).
3. Detachment of the reaction products from the active center of enzyme and their diffusion into the environment (complex **EP** dissociates into **E** and **P**).

$$\mathbf{E + S \leftrightarrow ES \rightarrow ES^* \rightarrow EP \rightarrow E + P}$$



Diagrams to show the induced fit hypothesis of enzyme action

Specificity of enzyme action

Enzymes exhibit a varying degree of specificity towards substrates. Viewed from this standpoint, the enzymes are subdivided into the following major types which are discussed in the order of their decreasing specificity.

1. ***Absolute substrate specificity:*** the enzyme catalyzes conversion of a single substrate only. For example, *urease* is active only in the conversion of urea.

2. ***Absolute group substrate specificity:*** the enzyme catalyzes conversion of a related group of substrates. For example, *alcohol dehydrogenase* catalyzes conversion not only of ethanol, but other alcohols too, though at different rates.

3. ***Relative substrate specificity:*** the enzyme catalyzes conversion of substrates belonging to different groups of chemical compounds. For example, the enzyme *cytochrome* P_{450} *(hydroxylase*) participates in the hydroxylation of a large number (about 700) of compounds. Enzymes with relative substrate specificity constitute the least specific enzymic system involved in the conversion of naturally occurring materials, drugs, and toxins.

4. ***Relative group substrate specificity:*** the enzyme exhibits a specific activity towards individual bonds within a group of substrates. For example, digestive enzymes (*pepsin, trypsin*) are specific towards the peptide bonds formed between certain amino acid residues in various proteins.

5. ***Stereochemical substrate specificity:*** the enzyme catalyzes conversion of only one among all possible substrate stereoisomers. This is an extreme case of enzymic specificity. For example, *D-alanine oxidase* catalyzes the conversion of only *D*-alanine amino acid, and never of its stereoisomer, *L*-alanine amino acid.

Kinetics of enzymic reactions

The kinetics of enzymic activity is a branch of enzymology concerned with the studies of enzyme-catalyzed reaction rates as affected by the chemical nature of substrate and enzyme, by the conditions for their interaction, as well as by environmental factors. Otherwise stated, the enzyme kinetics allows one to gain insight into the molecular mechanistic nature of the factors affecting enzymic catalysis rates.

The rate of an enzymic reaction is defined by the amount of a material (or materials) converted per unit time. The rates of such reactions are dependent on the environmental factors (temperature, pH of medium, influence of native or foreign materials, etc.).

The principles of enzymic reaction kinetics have been laid down in the works of *Michaelis* and *Menten*. The Michaelis–Menten equation relates the initial reaction rate v_0 to the substrate concentration [S]. The corresponding graph is a rectangular hyperbolic function; the maximum rate is described as v_{max} (asymptote).

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

The Michaelis–Menten equation describes the rates of irreversible reactions. A steady state solution for a chemical equilibrium modeled with Michaelis–Menten kinetics can be obtained with the Goldbeter–Koshland equation.

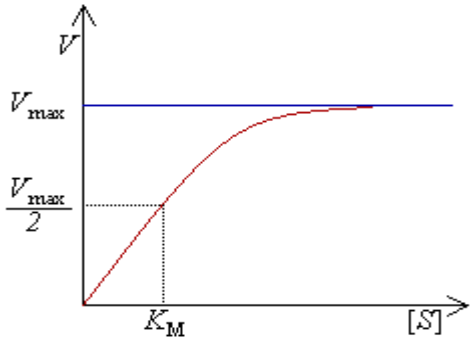


Figure 9. **Dependence activity of enzyme (V) on concentration of substrate (S)**

The rate of an enzymic reaction is seen as a measure for catalytic activity of the enzyme involved and is referred to simply as the activity of enzyme. The enzymic activity can be estimated only in an indirect fashion, by a decrease in the amount of substrate converted, or by an increase in the concen-

tration of products formed per unit time. The dependence of reaction rate on the enzyme amount is linear in character.

Dependence of reaction rate on pH of medium

Usually, the curve relating the enzymic reaction rate to the medium pH has a bell-like shape, since each enzyme is characterized by a pH range within which the rate of an enzyme-catalyzed reaction attains a maximum. Any change in the pH value to either side leads to a decrease in the enzymic reaction rate.

Optimal pH values for certain enzymes:

- pepsin = 1,5 – 2,5
- urease = 6,4 – 7,2
- trypsin = 7,5 – 7,8
- arginase = 9,5 – 9,9.

Dependence of enzymic reaction rate on temperature

When the temperature of medium is raised, the rate of an enzymic reaction increases, attains a maximum at an optimal temperature, and then drops down to zero. The optimal temperature for most enzymes falls within the range of 20-40^{o}C. Thermal lability of enzymes is related to the proteinic structure. Certain enzymes are denatured at temperatures above 40-50^{o}C. A number of enzymes are inactivated on cooling, i.e. their denaturation occurs at temperatures close to 0^{o}C.

Estimation of enzymic activity: methods and units

The enzymes contained in cells, tissues, and organs must be preparative extracted by making use of special techniques. The enzyme solution (extract from biological materials) is then used for enzyme testing. Serum or blood plasma, as well as other biological fluids are ready enzyme solutions and can be used for testing immediately. Enzyme tests, both qualitative and quantitative, are carried out in an indirect manner by measuring a decrease in the substrate amount, or by estimating reaction products accumulated in the medium.

The rate at which the substrate is consumed, or the reaction products are accumulated per unit time, is taken as a measure of *enzymic activity.*

The determination is carried out using any suitable method (colorimetry, spectrophotometry, fluorometry, polarography, etc.) either by recording the signal after the reaction is interrupted in a certain period of time, or by taking measurements continually during the reaction. The latter technique is more convenient, especially if the substrate or reaction products exhibit characteristic absorption in a definite spectral region (the light absorption change in the course of the reaction is recorded by a spectrophotometer), or fluorescence (the fluorescence change is continually recorded within a certain time interval using a spectrofluorimeter), etc. In other words, the choice of a method for estimating the enzymic activity is primarily determined by the possibility of reliable assessment of the substrate and reaction product concentrations.

Units of enzyme activity

According to the Nomenclature Committee of the International Union of Biochemistry, to specify the activity of an enzyme, the use of the *Unit (**U**)* is recommended which is the amount of enzyme required to turn over one micromole of substrate per minute under standard conditions. *Specific activity* of enzyme is expressed as the amount of enzyme (in milligrams) needed to consume one micromole substrate per minute under standard conditions; its dimension is *Mmol/min·mg* protein. A new unit of catalytic activity, the *katal (*denoted *kat*), has also been recommended (1972), which expresses the amount of enzyme required to turn over one mole substrate per second (*mol/sec)* under standard conditions.

Regulation of enzyme activity

The enzymes are catalysts with controlled activity. Therefore, the rates of chemical reactions in the organism can be monitored through the intermediacy of enzymes. The regulation of enzyme activity can be effected through the interaction of enzymes with various biological components or foreign compounds (for example, pharmaceuticals or toxins) which are commonly called *enzyme modifiers,* or *enzyme regulators.* The modifiers that are capable, by their action on enzymes, of accelerating reactions are called ***activators***; if the modifiers retard the reactions, they are referred to as ***inhibitors.***

Activation of enzymes. The activation of an enzyme is defined by the acceleration of enzyme-catalyzed reactions observable after the onset of modifier action. One group of activators is made up of compounds affecting the *active center region* of an enzyme. This group includes substrates and enzyme cofactors. The cofactors (metal ions and coenzymes), while being essential structural elements of conjugated enzymes act actually as cofactors for the latter. Metal ions, coenzymes, and substrates

(as precursors and active analogues of enzymes) can be used in practice as agents for activating the enzymes.

The activation of certain enzymes can be accomplished via structural modifications thereof, leaving the active center of enzyme unaffected. A number of approaches to such a modification may be envisaged:

1. The activation of an inactive precursor referred to as proenzyme, or zymogen.
2. The activation via addition of a specific modifying group to the enzyme molecule.
3. The activation via dissociation of an inactive complex "*protein-active enzyme*".

Inhibition of enzymes. The investigation of enzymic inhibitory reactions is important from the standpoint of practical applications for the synthesis of medicinal drugs, pesticides, etc., and in the elucidation of the mechanisms of their action.

Still, a certain degree of caution should be exercised in employing the term *inhibitor*. In principle, the inhibitor is understood as an agent capable of exerting a specific deterrent action on the activity of an enzyme. The inhibitors are divided into two groups: *reversible* and *irreversible inhibitors.*

According to the mechanism of their action, the enzyme inhibitors are subdivided into the following major types:

1. competitive
2. noncompetitive
3. uncompetitive
4. allosteric
5. substrate-linked.

Competitive inhibition is the enzymic reaction retardation produced by binding the enzyme active center with an inhibitor structurally related to the substrate and capable of preventing the formation of an enzyme-substrate complex. Under competitive inhibition conditions, the inhibitor and the substrate, being structurally related species, complete for the active center of enzyme. The following scheme holds true for this type of inhibition:

$$\mathbf{E + I \rightarrow EI,}$$ where, **I** is the inhibitor, and **EI**, the enzyme-inhibitor complex.

The removal of inhibitory blocking can be accomplished by an excess of the substrate whose molecules eliminate the inhibitor from the active center of the enzyme molecules and reactivate the latter to catalytic activity. For example, malonic acid is a competitive inhibitor for succinate dehydrogenase.

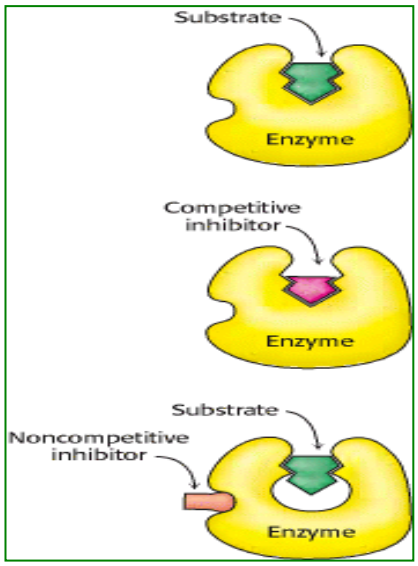


Figure 10. **Scheme of competitive and noncompetitive inhibition**

Antimetabolites are promising for use as specific pharmaceuticals. Competitive relations are possible not only between the substrate and the inhibitor, but also between the inhibitor and the coenzyme.

Anticoenzymes (analogues of coenzymes, incapable of enzymic activity) likewise act as competitive inhibitors by "putting out of action" the enzyme molecules to which they are bound. Anticoenzymes (or their precursors, antivitamins) are widely used in biochemical studies and medicinal practice as effective drugs.

Noncompetitive inhibition of enzymes is the retardation associated with the effect of an inhibitor on the catalytic conversion rather than on the substrate-enzyme binding. A noncompetitive inhibitor either directly binds the catalytic groups of the enzyme active center or, on binding with the enzyme, leaves the active center free and induces conformational changes in it. The conformational changes affect the structure of the catalytic site and hinder its interaction with the substrate. Since the noncompetitive inhibitor exhibits no effect on the substrate binding, in this case, as distinct from competitive inhibition, formation of a ternary complex **E·S·I** according to the scheme below is observed:

$$E + S + I = E \cdot S \cdot I$$

However, no conversion of this complex to any reaction products occurs. Noncompetitive inhibitors are exemplified by cyanides which are capable of strongly binding with the trivalent iron forming part of the catalytic moiety of hemin enzyme, cytochrome oxidase. In intoxication, the toxin can be bound or eliminated from the enzyme-inhibitor complex using reactivators, or antidots. These include all SH-containing complexones (cysteine and dimercaptopropanol), citric acid, ethylenediaminetetraacetic acid (EDTA), etc.

Allosteric regulation of enzymic activity. The allosteric regulation is characteristic only of a special group of enzymes with quaternary structure possessing regulatory centers for binding allosteric effectors. The negative effectors, which retard the conversion of a substrate in the enzyme active center, function as *allosteric inhibitors.*The positive effectors, on the contrary, accelerate enzymic reactions and are therefore, assigned to *allosteric activators.* Most commonly, various metabolites, as well as hormones, metal ions, and coenzymes act as allosteric effectors for enzymes. The greater the number of allosteric centers and effectors in an enzyme, the more is the enzyme responsive to metabolic alterations.

The mechanism of action of an *allosteric inhibitor* on enzyme is effected via a change of the enzyme's active center conformation. Allosteric enzymes play an important role in the cell metabolism. They take a "key position" in metabolism, since, being extremely responsive to metabolic change they control the rate at which the materials are supplied through the whole enzymic system.

Multiple molecular forms of enzymes

A family of molecules might be envisaged for the same enzyme, and the term "multiple enzyme forms" was coined to this effect. Commonly, a reference to multiple forms of enzyme implies the occurrence of enzyme proteins that differ among themselves in physico-chemical properties but can catalyze the same reaction in the organism of a definite species. Depending on their origin, multiple enzyme forms are divided into two groups:

- isoenzymes (synonym – isozymes),
- simply multiple forms of enzymes.

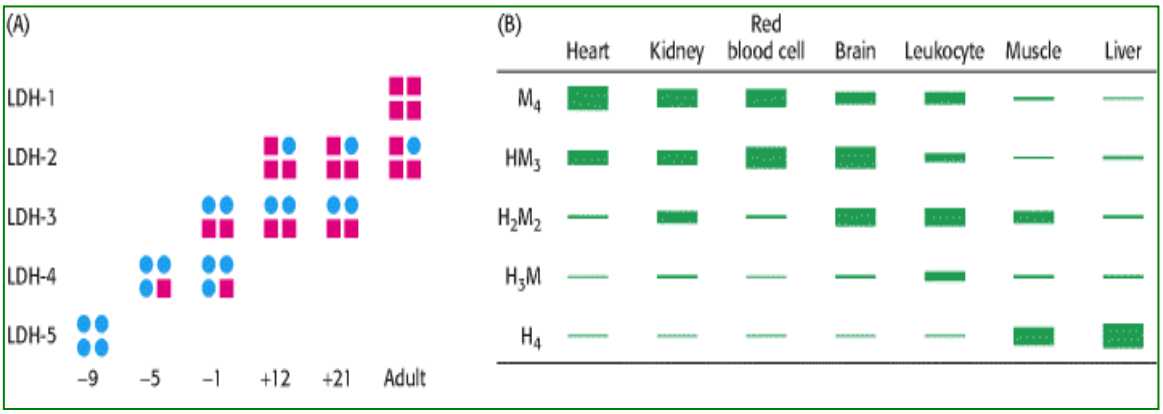


Figure 11. **LDH-isoenzymes**

Isoenzymes are molecular forms of an enzyme that arise due to *genetic* differences in the primary structure of enzyme protein, i.e. distinctions in physico-chemical properties of isoenzymes are of genetic origin. All *nongenetically originated* forms of the same enzyme are referred to as *multiple enzyme forms.*

Isoenzymes of lactate dehydrogenase are hybrids of two subunits of independent *genetic origin* (LDH_1 (4H), LDH_2 (3HM), LDH_3 (2H2M), LDH_4 (H3M) and LDH_5 (4M)). Isoenzymes of glutamate

dehydrogenase are polymers produced from subunits of the same type (different homopolymers) – *nongenetic origination*.

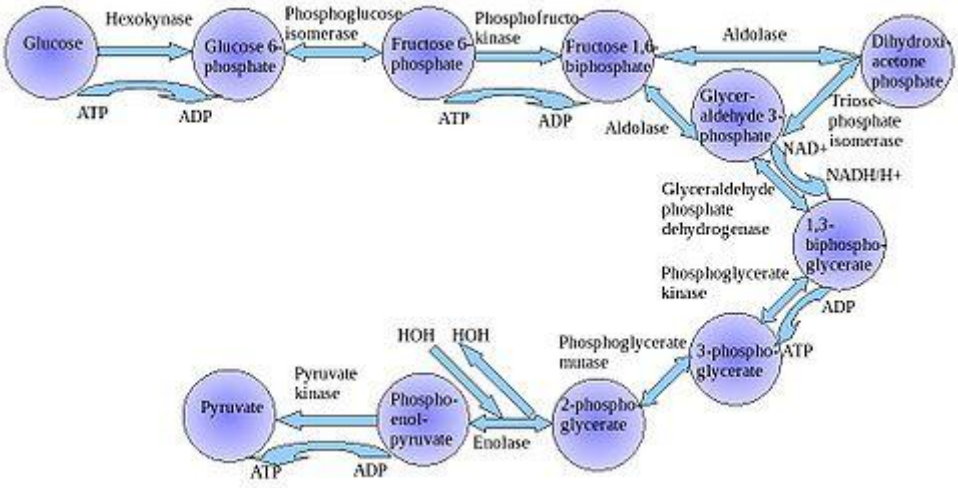
Multienzyme systems

The functioning of a multienzyme system is defined by the specificity of its cellular organization. Types of multienzyme system organization may be envisaged: *functional, structure-functional*, and *combined* types.

The *functional* organization is remarkable in that the individual enzymes are united in a function-oriented multienzyme system through the agency of metabolites that are capable of diffusing from one enzyme to another. In a functionally organized multienzyme system, the reaction product of the first enzyme in the conversion chain serves as a substrate for the second enzyme, etc.

The *structure-functional* organization consists in that the enzymes form structural function-oriented system via enzyme-enzyme (protein-protein) interactions. Such multienzyme complexes are tightly bound and resist decomposition into constituent enzymes. This is their major distinction from functionally organized multienzyme systems. For example, the enzymes involved may become fixed on the biomembrane to form a chain. This is a pattern for the mitochondrial respiratory chain involved in energy generation and transport of electrons and protons.

Polyenzymic complex – Glycolytic enzymes



The *combined* type of multienzyme system organization is a combination of the two above types, i.e. one part of the multienzyme system has a structural, and the other one, a functional organization. This type of organization may be exemplified by the multienzyme system of the Krebs cycle in which some of the enzymes are united into a structural complex (2-oxoglutarate dehydrogenase complex), while other enzymes are functionally interrelated through metabolite mediators.

Immobilized enzymes

Immobilized, or *insoluble*, enzymes are an artificially derived complex of soluble enzymes bound to a water-insoluble organic or inorganic carrier. The immobilization (from the Latin *immobilis*, fixed, stable) is effected by:

- physical adsorption of an enzyme onto an insoluble material,
- entrapment of an enzyme in gel cells,
- covalent binding of an enzyme to an insoluble carrier,
- cross-linkage of enzyme molecules to form insoluble multienzyme complexes.

Glass, silica gel, hydroxylapatite, cellulose and its derivatives are commonly used as adsorbents. However, immobilized enzymes are, as a rule, less active as compared with the free ones, since binding with the carrier weakens the enzyme-substrate contact. Insoluble enzymes can readily be removed from a reaction medium; they can be washed off the reaction products and repeatedly used in further experiments.

Practical utility of enzymes

Enzymes are widely used in practical activities of man. They are employed in various branches of agriculture and technology, let alone their exceptional importance in medical practice.

In medicine the diagnostic importance of enzymes should be emphasized: the detection of individual enzymes in clinical analyses is an aid in the identification of the nature of a disease. The enzymes are used as substrates for a deficient enzyme in the organism, or as agents for the decomposition of a

substrate whose excess may be thought to be the cause of a presumed disease. Digestive enzymes (pepsin, trypsin, etc.) are most commonly used in the clinic. Immobilized enzymes are used in the technological syntheses of a number of hormonal preparations, in high-sensitive analyses of drugs, in proximate analysis of biological components, and in many other applications. Proteolytic enzymes (trypsin, chymotrypsin), immobilized on gauze bandages or tampons, are used in surgery for cleansing purulent wounds and necrotic tissues; their action consists in enzymic degradation of dead cell proteins discharged in purulent wounds. Currently, immobilized and soluble enzymes are most commonly employed drugs of biological origin.

LECTURE 6

Subject: **NUCLEIC ACIDS: STRUCTURE AND LEVELS OF NUCLEIC ACIDS ORGANIZATION**

Nucleic acids (*nuclein* from the Latin "*nucleus"*), or polynucleotides, are high- molecular compounds composed of mononucleotides linked to chains by 3',5'-phosphodiester bonds. The concentrations of DNA and RNA in the cells are dependent on the functional state of the cells.

99% of DNA is present in nucleus, and 1% - in mitochondria. RNA, as distinct from DNA, is distributed more uniformly over the cell. In the cells of higher organisms, the nucleus accounts for about 11%, the mitochondria for about 15%, the ribosomes for 50 %, and the hyaloplasm for 24 % of the total of RNA. In the cell chromosomes of human and other higher organisms, the DNA is bound to histones and nonhistone proteins. Such DNA-protein complexes are referred to as deoxyribonucleoproteins (DNP).

The molecular mass of RNA is significantly inferior to that of DNA. Depending on the function assigned, molecular mass, and nucleotide composition, the following major types of RNA are distinguished: messenger RNA (m-RNA), transfer RNA (t-RNA), and ribosomal RNA (r-RNA). In the cell, most RNA is bound to various proteins. Such complexes are called ribonucleoproteins (RNP).

Concise characterization of nucleic acids occurring in cells of higher organisms

DNA is storage of genetic information and mediation in transfer of its parent DNA in the course of the cell division, or in transfer of RNA in the course of biological activity.

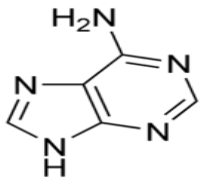
m-RNA is carrier of the DNA region containing structural information on protein polypeptide chain. It participates in the information transfer from DNA (replication) to the site of protein synthesis (on ribosome).

t-RNA participates in the activation of amino acids, their transport to ribosome, and assemblage of polypeptides from amino acids on ribosome.

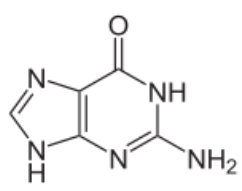
r-RNA forms of the skeleton of cytoplasmic ribosome (or mitochondria), to which ribosomal proteins are linked in protein assemblage on ribosome.

Components of nucleic acids

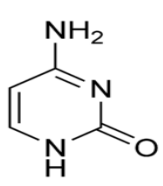
Nitrogenous bases. By their chemical structure, the nitrogenous bases of nucleic acids are divided into two groups: *purine bases* and *pyrimidine bases.* Purine bases are *adenine* and *guanine*; pyrimidine bases are *cytosine*, *uracil,* and *thymine*. DNA contains adenine, guanine, cytosine, and thymine. RNA contains the same bases excluding *thymine*, which is replaced by *uracil.*



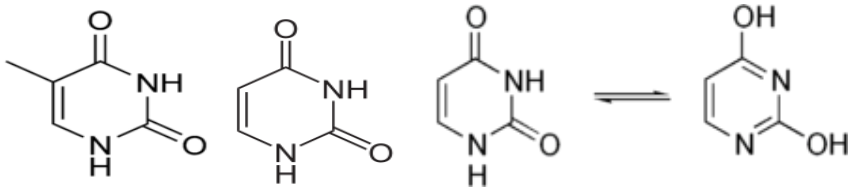
adenine



guanine



cytosine

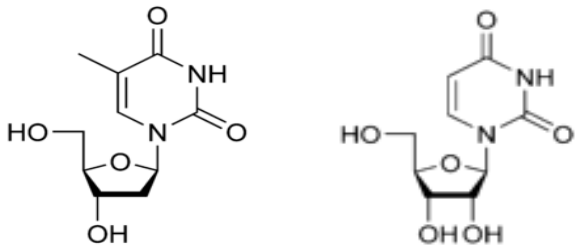


thymine uracil uracil tautomers

Figure 12. **Structures of nitrogenous bases. Uracil tautomers: amide or lactam structure (left) and imide or lactim structure (right)**

A very important feature of these bases is their ability to undergo a lactam-lactim tautomerism. For example, uracil.

Nucleosides. Compounds that contain nitrogenous bases linked to pentose are called nucleosides. With reference to the pentose type involved, two different species of nucleosides are distinguished: *deoxyribonucleosides* containing 2'-deoxyribose, and *ribonucleosides* containing ribose.



thymidine uridine

Figure 13. **Chemical structure of nucleosides**

Nucleotides. Nucleotides are phosphoric acid esters of nucleosides. They are also divided into *ribonucleotides*, which contain ribose, and *deoxyribonucleotides*, which contain 2'-deoxyribose. The phosphate group can add to the pentose ring at various positions: at positions 2', 3', and 5' in ribonucleotides, and at positions 3' and 5' in deoxyribonucleotides. Free nucleotides, present in the cell, carry a phosphate group in the 5' position. Nucleoside 5'-phosphates are involved in the biological synthesis of nucleic acids and are formed by decomposition of the latter. Since nucleoside 5'-phosphates, or mononucleotides, are derived from the corresponding nucleosides, major and rare ribomononucleotides and deoxyribomononucleotides are distinguished.

The lengthening of the mononucleotide phosphate end by linking on additional phosphate group to it leads to the formation of nucleoside polyphosphates:

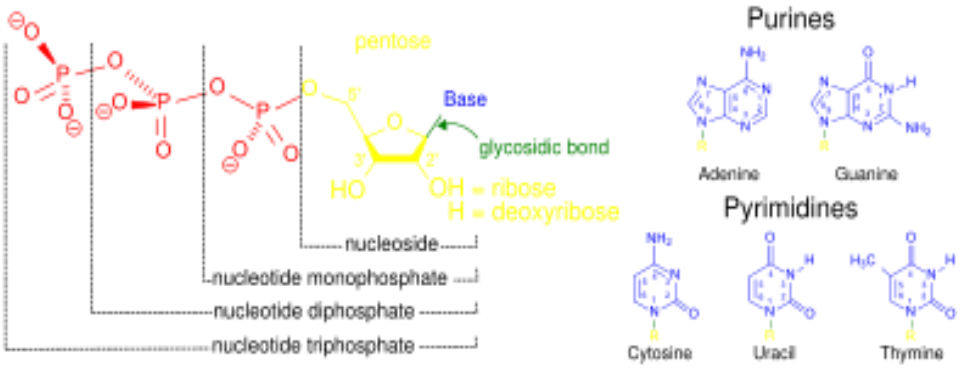
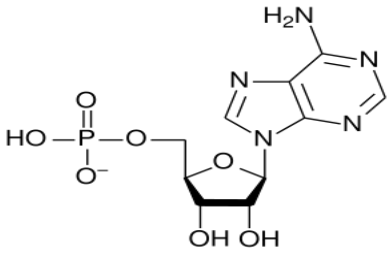


Figure 14. **Structural elements of the most common nucleotides**

Nucleoside diphosphates and nucleoside triphosphates are those most frequently occurring in the cells. The names and abbreviated notations for nucleoside phosphates are given below:

Name	***Notation***
	Ribonucleoside phosphates
Adenosine mono-, di-, triphosphate	AMP, ADP, ATP
Guanosine mono-, di-, triphosphate	GMP, GDP, GTP
Cytidine mono-, di-, triphosphate	CMP, CDP, CTP
Uridine mono-, di-, triphosphate	UMP, UDP, UTP

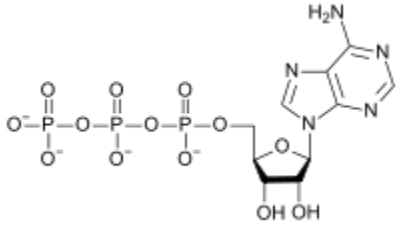


AMP

Deoxyribonucleoside phosphates

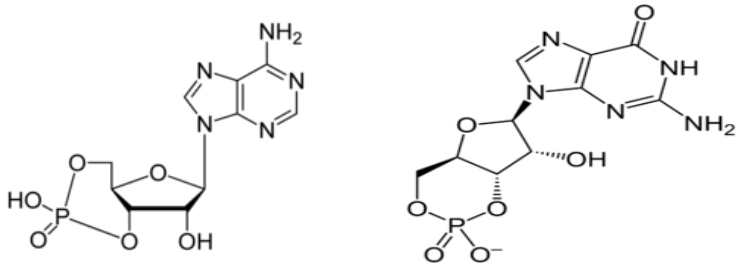
Deoxyadenosine mono-, di-, triphosphate	dAMP, dADP, dATP
Deoxyguanosine mono-, di-, triphosphate	dGMP, dGDP, dGTP
Deoxycytidine mono-, di-, triphosphate	dCMP, dCDP, dCTP
Thymidine mono-, di-, triphosphate	TMP, TDP, TTP

The entities ADP and ATP are macroenergetic, i.e. rich in energy; their chemical energy is used by the organism for performing various functions. The other nucleoside diphosphates and triphosphates are also implicated in the reactions of biological synthesis.



ATP

Nucleotide derivatives. Cyclic nucleotides (for example, 3',5'-cAMP and 3',5'-cGMP), which are universal regulators of intracellular metabolism, merit special mention. A large number of nucleotide derivatives serve as *coenzymes* for diverse enzymes in metabolic reactions. Coenzymes, derivatives of mononucleotides and dinucleotides, are also known (FAD and FMN, NAD and NADP, etc., see Lecture 4 "Enzymes").



Cyclic adenosine monophosphate (3',5'cAMP) and guanosine monophosphate (3',5'cGMP)

Structure and levels of organization of nucleic acids

Nucleic acids possess *primary, secondary, tertiary and quarternary* levels of organization.

Primary structure of nucleic acids. The primary structure of DNA and RNA is linear polynucleotide chain made up of mononucleotides which are linked by 3',5'-phosphodiester bonds. The build-up of both DNA and RNA primary structures follows the same basic principle: each pentose 3'-hydroxyl group of one mononucleotide is linked covalently to pentose 5'-hydroxyl group of the neighboring mononucleotide. Thence the name is derived: *3',5'-phosphodiester bond.* Linear chains of DNA and RNA, whose lengths are determined by the number of constituent nucleotides, have two ends called the *3'-end* and the *5'-end*.

Since nucleoside 5'triphosphate is the starting material for assemblage of nucleic acids in the cell, the 5'-end of the chain contains a triphosphate, and the 3'-end, a hydroxyl group. Consequently, the chains of nucleic acids are polar and are directed either 5'→3' or 3'→5'. Exceptions to the rule are circular DNA and RNA of certain viruses and bacteria.

The secondary structure of DNA. In 1953, Watson and Crick proposed the *double-helix model* for the DNA secondary structure. The DNA double helix is formed via specific pairing of a base of

one polynucleotide chain with a base of the other chain: the interaction of adenine with thymine is effected through involvement of two hydrogen bonds, and that of guanine with cytosine, through three hydrogen bonds.

Such a mutual correspondence of the base pairs is called the complementarity. The DNA chains are directed antiparallelly (one of the two chains runs in 5'→3' direction, and the other one, in 3'→5' direction). The sugar phosphate moieties of both chains are directed outwards, while the bases protrude into the interior of the helix, pair-wise against each other. The DNA helix is regular in structure: one full turn of the helix contains 10 nucleotides and is 3.4 nm long. The length of each nucleotide in axial direction is therefore 0.34 *nm*. Such a form of the *Watson-Crick* double helix is called the *B-form.*

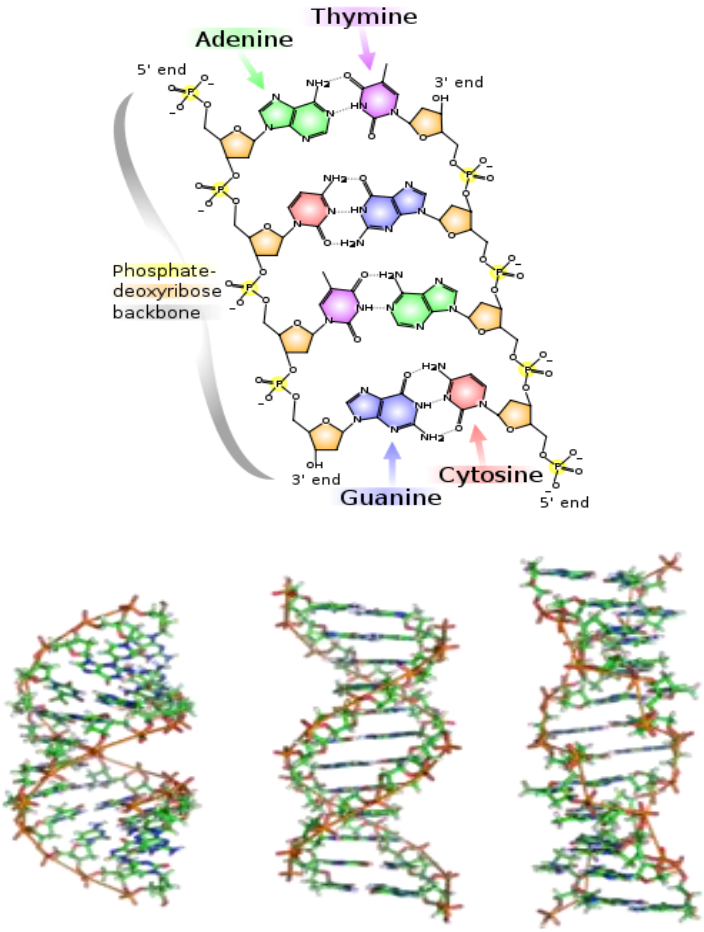


Figure 15. **DNA secondary structure (**from left to right: the structures of **A**, **B** and **Z** DNA)

The tertiary DNA structure emerges as the double-helical DNA molecule is twisted in space. Outwardly, it looks like a super-coil or like a bent (broken) double helix. In higher organisms, the DNA is located in chromosomes. Each chromosome contains a single giant DNA molecule of molecular mass about 10^{11} and a few centimeters long; this molecule constitutes a basis for chromatin.

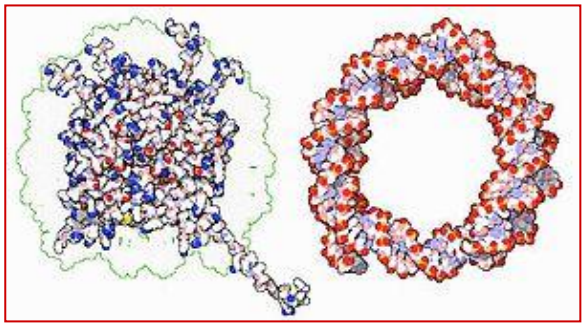
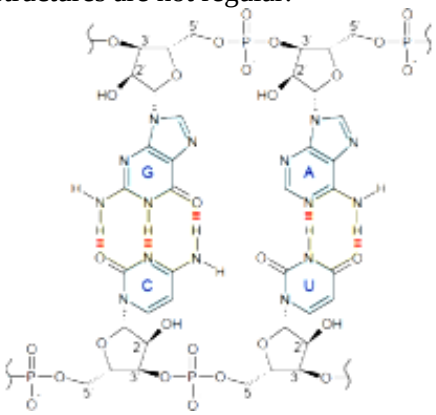


Figure 16. **Nucleosome structure**

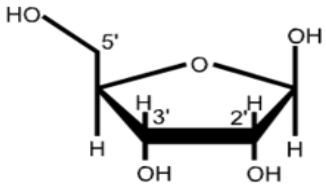
Chromatin is a super-molecular structure made up of a double-stranded DNA which is completed primary with proteins and to a lesser extent with RNA and inorganic compounds. The major part of chromatin is inactive. It contains a closely packed DNA. The active part accounts for 2-11% of the total chromatin, depending on the nature of the cell. Chromatin resembles a string of beads, i.e. globular bulges of about 10 nm in diameter spaced apart by thread-like linkers. The globular bulges are called *nucleosomes.* Each nucleosome contains a length of double-stranded DNA equal in extension to 140 base pairs, and eight molecules (an octet) of histones. The histone octet is composed of four pairs of histones of $H_{2\alpha}$, $H_{2\beta}$, H_3 and H_4 type. The thread-like linkers of double-stranded DNA that is composed of 30-60 base pairs are bound with histone H_1, each, the linker lengths being different in various cells.

The quaternary structure of DNA is superspiralization of the tertiary structure of it (chromosome).

The secondary structure of RNA. RNA are single-stranded molecules, therefore their secondary and tertiary structures are not regular.



Base pairing in RNA



Ribose structure indicating numbering of carbon atoms

mRNA is formed in the cell from a precursor, pre-mRNA. The pre-mRNA secondary structure contains hairpins and linear regions. The secondary structure of mRNA is a bent chain; while the tertiary structure looks like a thread wound round a spool. Functionally, the part of spool is played by a special transport protein particle called an *informofer*.

tRNA. The secondary structure of tRNA has the shape of *"clover leaf"*. This structure is produced owing to intra-chain pairing of complementary nucleotides in certain regions of the tRNA chain. *Acceptor region (end or terminus)* made up of four linearly linked nucleotides, three of which show the same sequence, *CCA*, in all types of tRNA. The 3'-OH hydroxyl of adenosine is free. The amino acid bound to the adenosine 3'-OH hydroxyl group is transported by tRNA to ribosomes, where the protein synthesis takes place.

rRNA exhibits a secondary structure composed of helical regions which alternate with nonhelical bent regions. The tertiary structure of rRNA constitutes a framework for the ribosome and is shaped as a rod or a coil. Ribosomal proteins adhere to the tertiary structure on the outside.

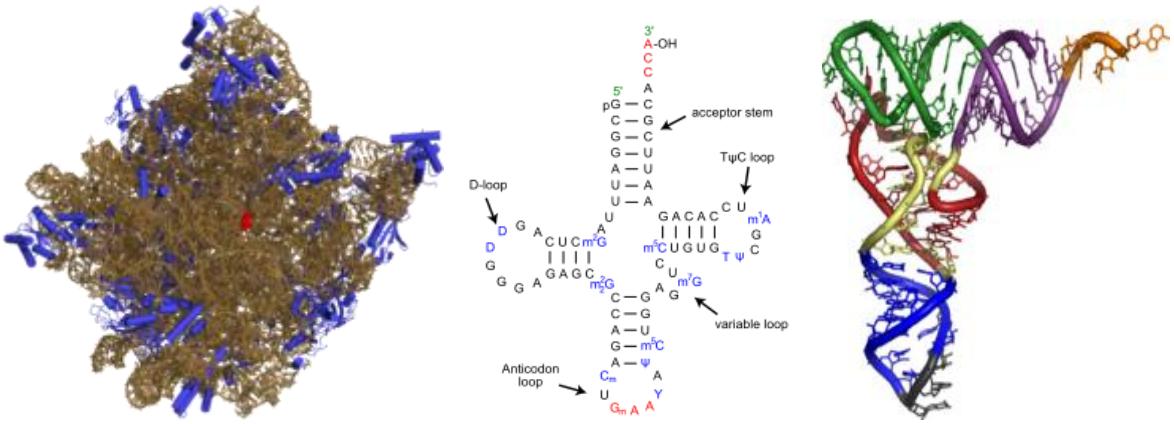


Figure 17. **Structures of RNAs: rRNA (left) and tRNA (right from yeast)**

Physico-chemical properties of nucleic acids

Physico-chemical properties of nucleic acids are primarily determined by their high molecular mass and by the level of structural organization. Specific features of nucleic acids show up in their:

- colloidal and osmotic properties,
- high viscosity and density of solutions,
- optical properties,
- aptitude to denaturation.

In solution, the molecules of nucleic acids exist as polyanions with distinctly pronounced acidic properties.

Denaturation is a property inherent in the macromolecules possessing a spatial organization. Denaturation is produced by heating and by the action of chemical agents which break hydrogen and vander-Waals bonds stabilizing the secondary and tertiary structures of nucleic acids. DNA regains its native double helix. This phenomenon is called *renaturation*.

Molecular hybridization of nucleic acids. The aptitude of nucleic acids to renaturate after denaturation has provided for a very valuable method of estimating the *homology*, or *congenerity*, of nucleic acids. This method is referred to as the molecular hybridization. It is based on the complementary pairing of single-stranded regions of nucleic acids.

LECTURE 7

Subject: TYPES OF GENETIC TRANSFORMATION TRANSFER. MOLECULAR FUNDAMENTALS OF REPLICATION AND TRANSCRIPTION

Three variants for genetic information transfer occurring in different organisms may be distinguished.

1. **Replication** (or, less commonly, *duplication)* is the transfer of genetic information within a single class of nucleic acids, i.e. from DNA to DNA or, as in certain viruses, from RNA to RNA.

2. **Transcription** is the transfer of information between different classes of nucleic acids, via DNA-to-RNA scheme. As distinct from replication, transcription is effected as a transfer of a certain portion of information stored in the DNA molecule. In the course of transcription, all the RNA types (mRNA, tRNA, and rRNA) are formed. Distinguished are forward transcription (from DNA to RNA) and reverse transcription (from RNA to DNA).

3. **Translation** is the transfer of genetic information from mRNA to a protein; it confined within the macromolecules of different classes. The specified direction of genetic information transfer from DNA via RNA to protein is called the central dogma of molecular genetics; it has been formulated by Crick. According to the central dogma, no information transfer is allowed from protein to RNA. However, the RNA-to-DNA information transfer is not, in principle, forbidden. If, by any chance, information transfer from protein to RNA is ascertained, this will require an immediate revision of the currently accepted basis of molecular genetics.

All the types of genetic information transfer are based on the template mechanism. This signifies that each type requires an appropriate template. During replication, one of the two DNA chains (or RNA in viruses) serves as a template. In transcription, a DNA section (forward transcription), or a RNA section (reverse transcription), and in translation, mRNA, that is, only a nucleic acid is capable of acting as a template. The precision of reproduction from the corresponding nucleic template is warranted by the complimentary rule for the nitrogenous bases of nucleotides according to which the base pairing occurs as A-to-T (or A-to-U in RNA) and G-to-C. Owing to this rule, the alternation order of nucleotides in each new polynucleotide chain is complimentary to that of the template.

Molecular fundamentals of replication

In 1957, *Meselson* and *Stahl* established that DNA replication in living organisms proceeds by the semiconservative mechanism. Untwisting proteins (helicase) break hydrogen bonds between the complementary bases of the double-stranded DNA. As a result, the double helix becomes untwisted and separates into single strands. Outwardly, this resembles the unzipping of a "*zipper*". The untwisted portion of DNA is called the *replicative fork*.

The initial step of replication (initiation) is the production of the RNA primer in the 5'→3' direction, assisted by RNA-polymerase (*primase*). After the synthesis of the short chain of RNA on the DNA template is completed, the enzyme is detached from DNA. Subsequently, deoxyribonucleotides

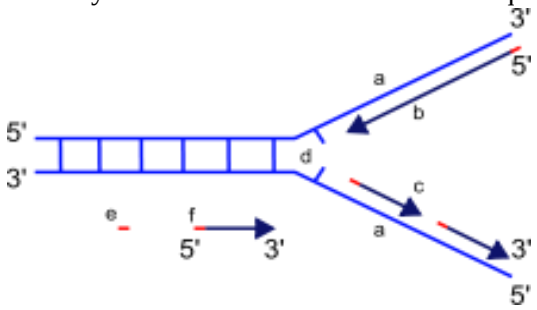
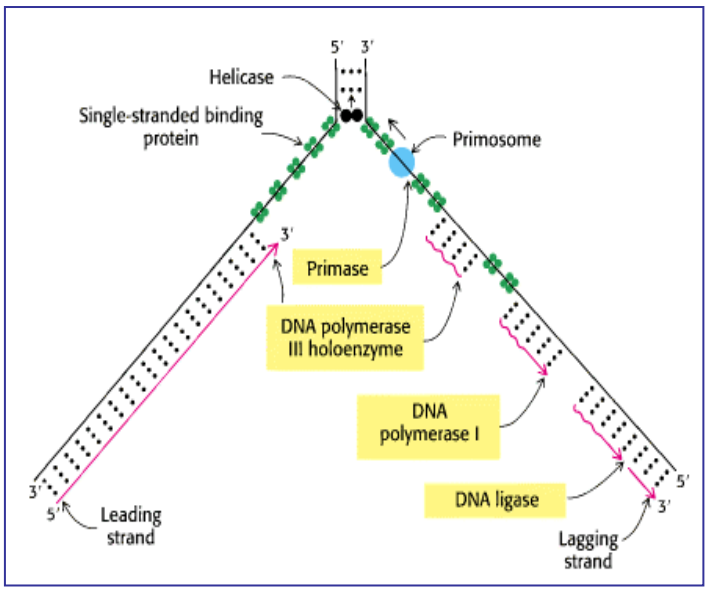


Figure 18. **Replication of DNA: Scheme of the replication fork:** *a:* **template,** *b:* **leading strand,** *c:* **lagging strand,** *d:* **replication fork,** *e:* **primer,** *f:* **Okazaki fragment**

are added to the RNA primer through the assistance of DNA-polymerase III in 5'→3' direction. A hybrid chain RNA-DNA is thus formed.



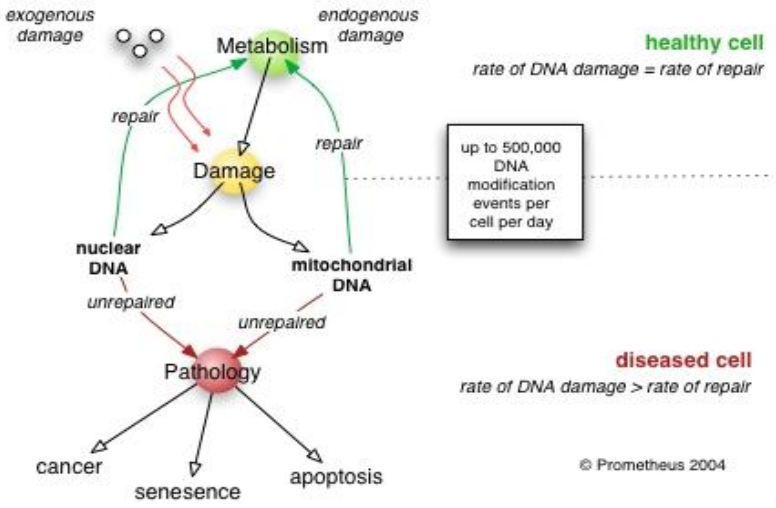
In this process, DNA-polymerase III synthesizes short DNA fragments (the so-called *Okazaki fragments*) on the other parental strand of the replicative fork. An RNA primer is also needed for the synthesis of Okazaki fragments. Interestingly, DNA-polymerase III is able, in the course of the synthesis, to correct the errors in a mismatched pairing of nucleotides. If an error in base pairing occurs, the mismatched nucleotide is immediately split off by the enzyme operative within the controlled nuclease activity mechanism. At the same time, a correctly paired new nucleotide is routinely added, through the agency of the enzyme, to the preformed DNA fragment.

The RNA primer, after the termination of DNA-polymerase III activity, is removed from the synthetic chain either by specific *ribonuclease H*, or by DNA-polymerase I. At the site of the removed RNA primer, the missing fragment of DNA strand is completed by means of DNA-polymerase I. The colligation of the presynthesized DNA fragments (Okazaki fragments) in the 3'→5' direction is effected through the aid of DNA-ligase.

Repair of DNA. The DNA repair, or correction of damaged sites in either of the DNA strands, may be regarded as a limited replication. The repair process of DNA strands damaged by UV radiation has been studied in greater detail (for example, radiation damage of epithelial cells of the skin).

UV radiation causes "cross-links" between the neighboring thymine residues in DNA. These dimerized thymine residues are thus rendered incapable to perform the template function during replication. To provide against this contingency, the cell has a complement of enzymes whose function is to repair damage inflicted on DNA. At first, the damaged spot is recognized and excised by DNA-ases. Then the correct sequence in the 5'→3'direction is synthesized by a DNA-polymerase I enzyme type.

Finally, the ends of the new section are joined to the former strand by DNA-ligase. Inherited mutations that affect DNA repair genes are strongly associated with high cancer risks in humans.



DNA repair rate is an important determinant of cell pathology

Molecular fundamentals of transcription

During the biological activity of the mature cell, only a portion of the genetic information that has been written down in the chromatin DNA is used in the transcription as RNA copies. In procaryotes and eucaryotes, the elementary transcription unit, i.e. a DNA segment liable to transcription, is called *transcription.* The section is the structural gene, that carry information are called *exons*, while those that carry none, *introns.* Possibly, the introns perform a supplementary regulatory function for the exons. In the chromosomal DNA, mobile genes, or *transposons*, have been identified.

Mechanism of transcription. The starting point of transcription is called a *promotor*. Proteins that facilitate the transcription initiation and the transcription enzyme RNA polymerase are added to the promotor from the medium. The operator is the section of DNA capable of binding proteins that act as transcription regulators. In procaryotes, the transcription regulator protein is the repressor. In eucaryotic DNA, the transcripton section adjacent to the promotor is called the *acceptor,* or *regulatory site.* At the transcript on end, there is a nucleotide sequence whose function is to signal the termination of transcription, the so-called *terminator.* The RNA produced by transcription is called the *transcript.* The transcript is a complementary copy of transcript on extending from the promotor to the terminator.

The transcription of DNA may be considered as proceeding via three stages: *initiation, elongation,* and *termination* of RNA synthesis.

The initiation of transcription occurs as DNA-dependent RNA-polymerase becomes bound to the DNA promotor exhibiting a high affinity for this enzyme. The promotor is the starting site of transcription. The procaryotic RNA-polymerase is composed of five different subunits. Four of these constitute an aggregate called the *core enzyme* whose function is to catalyze the formation of phosphodiester bonds between RNA nucleotides. The fifth subunit called *sigma* (σ)*-factor,* is readily detachable from the core enzyme. This σ-factor acts to select the starting site for the transcription by binding with the promotor. The transcription site thus having been specified, the core enzyme is added to the σ-factor to initiate the transcription. It remains unclear as yet what makes the strands of double-helix DNA separate at the transcription site. Probably this function is performed by RNA-polymerase itself, or, alternatively, there is a protein specially intended for this purpose (like untwisting proteins in reparation).

Eucaryotes have RNA-polymerases of three types: I, II, and III. These are proteins composed of several subunits and differing in transcription specificity. RNA-polymerase I is responsible for the transcription of rRNA genes, RNA-polymerase III, for the transcription of tRNA, and RNA-polymerase II participates in the synthesis of a precursor to mRNA. RNA-polymerases make the chain grow in the 5'→3' direction only; for this reason, the 5'-end always bears a triphosphate (P~P~P), and the 3'-end, a free hydroxyl, OH.

The elongation (or polymerization) of transcription proceeds as RNA-polymerase move along the DNA template. Every next nucleotide becomes paired to a complementary base in the codogenic

DNA template, and RNA-polymerase "fastens" the nucleotide through phosphodiester bonds to the growing RNA chain.

The elongation rate is about 40 to 50 nucleotides per second. Within 1 second after the transcription onset the nascent transcript is about 30 nucleotides long, a protective chemical group, the so-called "*cap"*, is formed at its 5'-end. In other words, simultaneously two different acts of transcript growth are operative: the transcription proper and the post-transcriptional modification of transcript ("*capping*"). The cap is present in all eucaryotic mRNA. It is composed of 7-methylguanosine linked through a chain of three phosphate groups and 5'→5', rather than 5'→3', phosphodiester bond to the first mRNA nucleotide, CH_3-G-P-P-P-mRNA. This methylated cap protects the mRNA from an attack by 5'-exonucleases. Length of primary transcript is 5000-8000 nucleotides, but it may be long as 20,000 and even more.

The termination of transcription takes place as RNA-polymerase has reached the DNA nucleotide sequences, called termination codons, which signal to stop the polymerization. It is believed that such termination codons in a transcription may be *poly (A)* sequences, since in transcripts at the 3'-end, the corresponding complementary *poly(U)* sequences occur. A special termination factor, *rho (ρ)*-factor, which is a protein, has been identified. The *ρ*-factor interrupts the transcription by recognizing in a specific, as yet not quite clear, manner the transcription termination codons. Owing to the terminators, the RNA chains of a definite length only are formed.

As the transcription proceeds to its termination, the synthesized RNA separates itself from the codogenic DNA strand. The primary products of transcription, i.e. RNAs, are complete copies (in complementary representation) of the DNA transcriptons. This implies that the newly synthesized RNA contains both informative and noninformative portions. The primary transcript is also called the RNA precursor. The mRNA precursor, or pre-mRNA, is represented by linear chain that does not close to a ring. It is longer as compared with functionally active RNA molecules. Pre-mRNA is especially liable to variation in the molecular mass: from 6-7 *S* to giant sizes of 50-70 *S*. In eucaryotic nuclei, all the precursors are bound to proteins to form ribonucleoproteins.

Post-transcriptional alterations in RNA

In the nucleus, all the RNA precursors are involved in the stage of *post-transcriptional maturation*, or *processing.* During processing, all noninformative, "redundant" fragments are removed from the pre-RNA to form "mature", functionally adequate RNA molecules. Processing includes three steps:

1. Excision of noninformative portions from pre-RNA.
2. Splicing of the informative portions of the "split" genes.
3. Modification of the 5'- and 3'-ends in RNA.

Pre-mRNA processing. In the course of processing, the modified primary transcript is excised at the *intron* sites, and the *exons* are spliced by their ends to form mRNA. This function is carried out by the so-called small nuclear ribonucleoproteins, rich in uracil. One of these, denoted U_1-RNA, is believed to play a major role in excising the introns and in splicing the exons, i.e. this molecule possesses a function of both ribonuclease and ligase. The introns are bent to form a loop, which facilitates their excision, while the exon ends become linked through phosphodiester bonds.

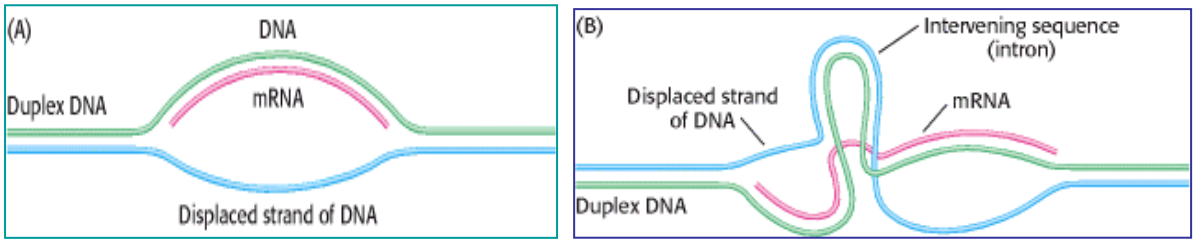


Figure 19. **Splising of mRNA**

In the nucleus, the 5'- and 3'-ends of the mRNA formed are modified. A polyadenyl fragment, poly (A), composed of about 200 nucleotides, is added to the 3'-end of eucaryotic mRNA. This addition is accomplished by means of poly (*A*)-polymerase. The poly (A) fragment is apparently needed for the transport of mRNA from nucleus to cytoplasm.

Transport of mature RNAs from the nucleus to the cytoplasm. Eucaryotes, in distinction from procaryotes, possess a nucleus membrane, and the ready RNA must pass across the membrane to be

supplied to the cytoplasm where protein synthesis occurs. All the mature RNAs are transported from the nucleus into the cytoplasm as complexes with proteins; the proteins facilitate the transport and provide an additional protection from RNA environmental factors. The mRNA becomes bound to a special protein, *informofer* (which means "information carrier"). RNA, complexed with the informofer, is supplied to the cytoplasmic ribosomes, within which the assembly of proteins from amino acids, or transcription, takes place.

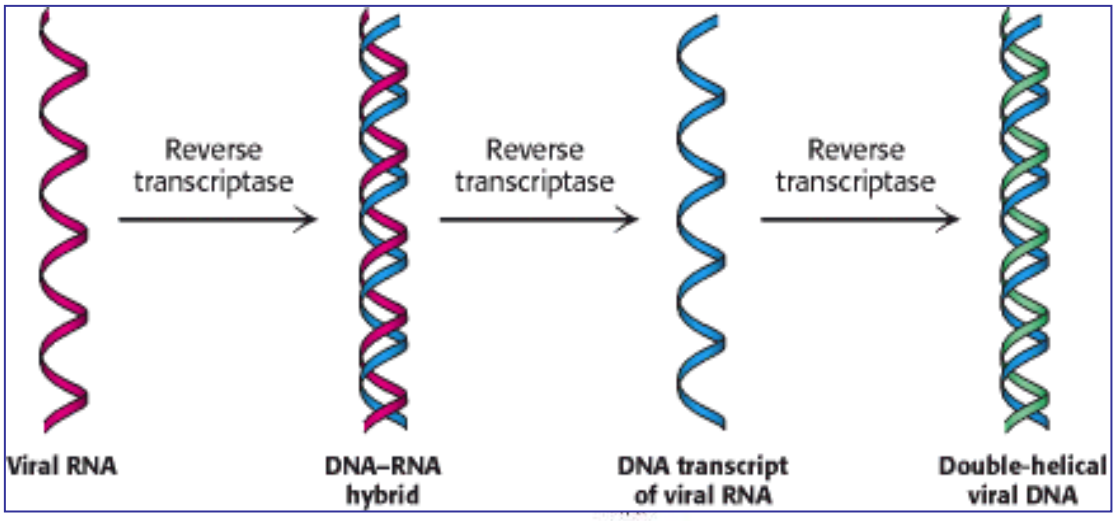
Reverse transcription. In the fields of molecular biology and biochemistry, a ***reverse transcriptase,*** also known as ***RNA-dependent DNA polymerase***, is a DNA polymerase enzyme that transcribes single-stranded RNA into double-stranded DNA. It also helps in the formation of a double helix DNA once the RNA has been reverse transcribed into a single strand cDNA. Normal transcription involves the synthesis of RNA from DNA; hence, reverse transcription is the *reverse* of this. Well studied reverse transcriptases include:

- HIV-1 reverse transcriptase from human immunodeficiency virus type 1 (PDB 1HMV).
- M-MLV reverse transcriptase from the Moloney murine leukemia virus.
- AMV reverse transcriptase from the avian myeloblastosis virus.
- Telomerase reverse transcriptase that maintains the telomeres of eukaryotic chromosomes.

The enzyme is encoded and used by reverse-transcribing viruses, which use the enzyme during the process of replication. Reverse-transcribing RNA viruses, such as retroviruses, use the enzyme to reverse-transcribe their RNA genomes into DNA, which is then integrated into the host genome and replicated along with it. Reverse-transcribing DNA viruses, such as the hepadnaviruses, can allow RNA to serve as a template in assembling, and making DNA strands. HIV infects humans with the use of this enzyme. Without reverse transcriptase, the viral genome would not be able to incorporate into the host cell, resulting in the failure of the ability to replicate.

Process of reverse transcription. Reverse transcriptase creates single stranded DNA from an RNA template. In virus species with reverse transcriptase lacking DNA-dependent DNA polymerase activity, creation of double-stranded DNA can possibly be done by host-encoded DNA polymerase δ, mistaking the viral DNA-RNA for a primer and synthesizing a double-stranded DNA by similar mechanism as in primer removal, where the newly synthesized DNA displaces the original RNA template.The process of reverse transcription is extremely error-prone and it is during this step that mutations may occur. Such mutations may cause drug resistance.

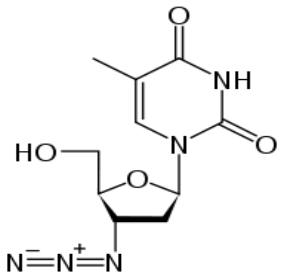
In eukaryotes. Self-replicating stretches of eukaryotic genomes known as *retrotransposons* utilize *reverse transcriptase* to move from one position in the genome to another via a RNA intermediate. They are found abundantly in the genomes of plants and animals. *Telomerase* is another reverse transcriptase found in many eukaryotes, including humans, which carries its own RNA template; this RNA is used as a template for DNA replication.



Scheme of reverse transcription

Antiviral drugs. As HIV uses reverse transcriptase to copy its genetic material and generate new viruses (part of a retrovirus proliferation circle), specific drugs have been designed to disrupt the process and thereby suppress its growth. Collectively, these drugs are known as reverse transcriptase inhibitors

and include the nucleoside and nucleotide analogues zidovudine (trade name *Retrovir*), lamivudine (*Epivir*) and tenofovir (*Viread*), as well as non-nucleoside inhibitors, such as *nevirapine* (*Viramune*).



The molecular structure of zidovudine (AZT) used to inhibit HIV reverse transcriptase

Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. This region of repeated nucleotide repeats called telomeres contains non-coding DNA material and prevents constant loss of important DNA from chromosome ends. As a result, every time the chromosome is copied only 100-200 nucleotides are lost, which causes no damage to the organism's DNA. Telomerase is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates Carol W. Greider and Elizabeth Blackburn in 1984 in the ciliate *Tetrahymena*. Together with Jack W. Szostak, Greider and Blackburn were awarded the 2009 Nobel Prize in Physiology or Medicine for their discovery.

The protein composition of human telomerase was identified in 2007 by Scott Cohen and his team at the Children's Medical Research Institute in Australia. It consists of two molecules each of human telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC), and dyskerin (DKC1). The genes of telomerase subunits, which are TERT, TERC, DKC1, and TEP1 *etc*, are located on the different chromosomes in human telomeres, which are shortened after each replication cycle. The existence of a compensatory shortening of telomere (*telomerase*) mechanism was first predicted by Soviet biologist Alexey Olovnikov in 1973, who also suggested the telomere hypothesis of aging and the telomere's connections to cancer. Telomerase was discovered by genome. Human TERT gene (*hTERT*) is translated into a protein of 1132 amino acids. TERT proteins from many eukaryotes have been sequenced. TERT polypeptide folds with TERC, a non-coding RNA (451 nucleotides long in human). TERT has a '*mitten*' structure that allows it to wrap around the chromosome to add single-stranded telomere repeats.

TERT is a reverse transcriptase, which is a class of enzyme that creates single-stranded DNA using single-stranded RNA as a template. Enzymes of this class (not TERT specifically, but the ones isolated from viruses) are utilized by scientists in the molecular biological process of reverse transcriptase PCR (RT-PCR), which allows the creation of several DNA copies of a target sequence using RNA as a template. As stated above, TERT carries its own template around, TERC.

The high-resolution protein structure of the *Tribolium castaneum* catalytic subunit of telomerase TERT was decoded in 2008 by *Emmanuel Skordalakes* and his team at The Wistar Institute in Philadelphia. The structure revealed that the protein consists of four conserved domains (RNA-Binding Domain (TRBD), fingers, palm and thumb), organized into a ring configuration that shares common features with retroviral reverse transcriptases, viral RNA polymerases and bacteriophage B-family DNA polymerases.

By using TERC, TERT can add a six-nucleotide repeating sequence, 5'-TTAGGG (in all vertebrates, the sequence differs in other organisms) to the 3' strand of chromosomes. These TTAGGG repeats (with their various protein binding partners) are called telomeres. The template region of TERC is 3'-CAAUCCCAAUC-5'. This way, telomerase can bind the first few nucleotides of the template to the last telomere sequence on the chromosome, add a new telomere repeat (5'-GGTTAG-3') sequence, let go, realign the new 3'-end of telomere to the template, and repeat the process.

Aging. The enzyme telomerase allows for replacement of short bits of DNA known as telomeres, which are otherwise shortened when a cell divides via mitosis.

In normal circumstances, without the presence of telomerase, if a cell divides recursively, at some point all the progeny will reach their Hayflick limit. With the presence of telomerase, each dividing cell can replace the lost bit of DNA, and any single cell can then divide unbounded. While this unbou-

nded growth property has excited many researchers, caution is warranted in exploiting this property, as exactly this same unbounded growth is a crucial step in enabling cancerous growth.

Embryonic *stem cells* express telomerase, which allows them to divide repeatedly and form the individual. In adults, telomerase is highly expressed in cells that need to divide regularly (e.g., in the immune system), whereas most somatic cells express it only at very low levels in a cell-cycle-dependent manner.

A variety of premature aging syndromes are associated with short telomeres. These include *Werner syndrome*, *Ataxia telangiectasia*, *Ataxia-telangiectasia* like disorder, *Bloom syndrome*, *Fanconi anemia* and *Nijmegen breakage syndrome*. The genes that have been mutated in these diseases all have roles in the repair of DNA damage, and their precise roles in maintaining telomere length are an active area of investigation. While it is currently unknown to what extent telomere erosion contributes to the normal aging process, maintenance of DNA in general and telomeric DNA, to be specific, have emerged as major players. Dr. Michael Fossel has suggested in an interview that telomerase therapies may be used not only to combat cancer but also to actually get around human aging and extend lifespan significantly. He believes human trials of telomerase-based therapies for extending lifespan will occur within the next 10 years. This timeline is significant because it coincides with the retirement of Baby Boomers in the United States and Europe.

Some experiments have raised questions on whether telomerase can be used as an anti-aging therapy, namely, the fact that mice with elevated levels of telomerase have higher cancer incidence and hence do not live longer. In addition, although certain premature aging syndromes have been associated with telomere shortening, mice without active telomerase do not appear to suffer from premature aging. Telomerase also favors tumorogenesis, leading to questions about its potential as an anti-aging therapy. On the other hand, one study showed that activating telomerase in cancer-resistant mice by overexpressing its catalytic subunit extended lifespan. The potential remains for telomerase activators to contribute to the development of cancer.http://en.wikipedia.org/wiki/Telomerase_-cite_note-Nobel_Prize_2003-2#cite_note-Nobel_Prize_2003-2

LECTURE 8

Subject: **MOLECULAR PRINCIPLES OF TRANSLATION**

During translation, the mRNA genetic text is translated into a linear sequence of amino acids that constitute the polypeptide chain of a protein. Since the product of translation is a specific protein, the translation process may with equal right be called protein biosynthesis.

Genetic code and its characterization

The genetic, or amino acid, code is understood as the relationship between the sequence of bases in a nucleic acid and the sequence of amino acids in the polypeptide synthesized from it; otherwise stated, this is the correspondence of codons (code words) to definite amino acids. The genetic code may be regarded as a specific dictionary for translating a text, recorded by means of four nucleotides, into a protein text, recorded by means of 20 amino acids. The other amino acids found in a protein are modified forms of one of the 20 amino acids. The genetic code exhibits the following features:

1. ***Triplicity:*** a triplet of nucleotides corresponds to each amino acid. There are four nucleotides available; it can easily be seen that, taken as triplets, they can form 4^3=64 codons. Of these, 61 are sense codons, and 3, nonsense codons (or termination codons).
2. ***Nonoverlap:*** The genetic text codons are independent of each other.

|CCA| |CGG| |AAC|
Nonoverlapping

3. ***Degeneracy,*** or ***redundancy:*** certain amino acids may have more than one codons. A simple comparison testifies to this: 61 sense codons account for 20 amino acids, i.e. on the average, are slightly more than 3 codons per amino acid.
4. ***Specificity:*** definite codons correspond to each amino acid. They cannot be used for another amino acid.
5. ***Colinearity:*** correspondence between the linear sequence of codons in mRNA and that of amino acids in protein.

6. ***Universality:*** all the above mentioned features of the genetic code are characteristic of any living organism.

Mechanism of translation process

The translation process may be divided into two stages:

- the recognition of amino acids
- protein biosynthesis in its proper sense.

The spatial localization of these stages in the cell is different: recognition occurs in the hyaloplasm, and protein biosynthesis proceeds on ribosome.

Recognition of amino acids. In essence, the amino acid recognition process consists in binding an amino acid to its tRNA. The tRNA structure exhibits the properties of a potential "translator", since the tRNA is capable of both "reading" a nucleotide text (the tRNA anticodon is specifically paired with the mRNA codon) and carrying its amino acid (at the acceptor end). However, the tRNA is incapable of linking with its amino acid. For this purpose, there are available special enzymes in the cell sap (cytosol) that actually act as "translators", i.e. provide for the conditions enabling the synthetases (for short, ARSases). At least 20 types of ARSases are known (by the number of proteinogenic amino acids involved). They recognize specifically tRNA and its amino acid, and catalyze their addition according to the reaction:

$$\mathbf{R\text{-}CH(NH_2)\text{-}COOH + HO\text{-}tRNA + Mg^{2+} + ATP \rightarrow ARSase \rightarrow}$$

$$\mathbf{R\text{-}CH(NH_2)\text{-}C(=O)\text{-}O\text{-}tRNA + AMP + H_4P_2O_7}$$

ATP is required for this process, with Mg^{2+} acting as a cofactor. The ATP energy is supplied to the production of a macroergic bond in the aminoacyl-tRNA, i.e. the reaction simultaneously involves the activation of amino acid at the carboxyl end and the addition of amino acid to the adenosine hydroxyl (3'-OH) at the acceptor end (CCA) of tRNA. The cell contains about 40 to 60, rather than 20, tRNAs, since some of the amino acids stand in need of specifically using more than one tRNA.

Further, the tRNA, with the amino acid linked to it, is transferred by simple diffusion to the ribosome. The ribosomes perform the assembly of proteins from amino acids supplied as a variety of aminoacyl-tRNAs.

Ribosomal protein biosynthesis. Protein biosynthesis (second stage of translation) requires:

- mRNA as a genetic template whose program defines the amino acid sequence order in a given protein;
- aminoacyl-tRNA (for reading the genetic mRNA text, as a source of amino acids for the protein assembly);
- ribosomes as molecular assembly units for the successive linking of amino acids into a polypeptide chain in accordance with the mRNA program;
- GTP as an energy source in the ribosomal protein synthesis;
- protein "factors" assisting at various steps of the ribosomal protein synthesis;
- certain metal ions for using as cofactors (Mg^{2+}, K^{+}, and others).

Mechanism of ribosomal protein synthesis. Protein biosynthesis, or translation, proper is conventionally divided into three stages:

1. *initiation* (start of synthesis),
2. *elongation* (polypeptide chain lengthening),
3. *termination* (end of synthesis).

Initiation. The starting stage is the slowest one in the overall translation process. In an Inoperative stage, the ribosomal subunits are separated. The mRNA, transported from the nucleus into the cytoplasm becomes bound to a small subunit at the site in its surface shared with the large subunit.

To be noted, the mRNA binding site is always located close to the 5'-end of mRNA, since the RNA program "reading" always proceeds, in the 5'→3' direction. Within the subunit limits only two mRNA codons can be accommodated. The first mRNA codon at the 5'-end is *AUG* or *GUG*. These codons are called *initiation codons* (or start codons), since they always serve as the start signal for the ribosomal translation. In the tRNA, an *anticodon* of methionyl-tRNA is correspondent to these codons. Eucaryotic cells have two different methionyl-tRNAs. One of the two is always involved in the initia-

tion, while the other one is engaged in the elongation process. In procaryotes, protein biosynthesis starts from formylmethionyl-tRNA in which the NH_2 group is blocked by formyl moiety.

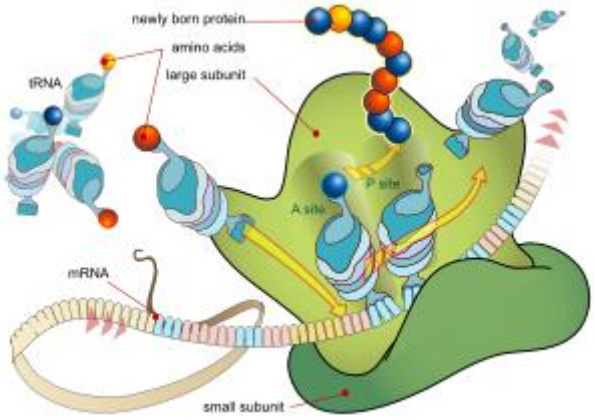


Figure 20. **Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome**

In addition, at least three proteinic *initiation factors* (IF_1, IF_2, and IF_3), which are not ribosomal constituents, and GTP take part in the initiation. The initiation factors facilitate the binding of mRNA with the small subunit (S) and GTP. A large subunit is added to the primary complex thus formed (IF-S-mRNA-GTP) to complete the closure of all the ribosomal subunits; this having been performed, the initiation factors are removed from the ribosome. The energy needed for the ribosomal subunit closure is supplied by GTP hydrolysis. The initiator complex thus formed (mRNA-ribosome-methionyl-tRNA) is ready to start elongation. To be noted, methionyl-tRNA, through its anticodon, becomes specifically paired to the AUG codon of mRNA, i.e. it becomes as if "suspended" from the mRNA by hydrogen bonds, while its acceptor site (for binding an amino acid) is attached to the large ribosomal subunit.

Elongation. The polypeptide synthesis always starts from the N-end and terminates in the C-end. The addition of one amino acid to the polypeptide chain is accomplished in three steps:

- binding of aminoacyl-tRNA
- transpeptidation (or peptide transfer)
- translocation (or displacement of mRNA by one triplet).

The ***first step.*** In the ribosome tRNA is located on the right; tRNA is bound to the mRNA codon through its anticodon, while its acceptor end is "linked" to the growing peptide. This peptide, which is a constituent of peptidyl-tRNA, is bound to the ***P-site*** (peptide, or donor site), which is a kind of protein pocket in the large subunit. As the first step is being performed, the second mRNA codon is free. This codon becomes coupled to the anticodon of the incoming aminoacyl-tRNA. The aminoacyl end of tRNA becomes bound to the ***A-site*** (acceptor site) of the large ribosomal subunit. This brings to termination the first step, i.e. binding. Energy for the binding is provided by breaking the GTP phosphate bond.

The ***second step,*** transpeptidation, proceeds in such a manner that peptidyl is transferred from the left-side tRNA onto the amino group of aminoacyl-tRNA. A peptide bond buildup is catalyzed by ribosomal proteins exhibiting a peptidyl transferase activity.

The ***third step*** involves separation of the ribosomal subunits using the energy supplied by one GTP molecule. Peptidyl-tRNA that carries the tripeptide moves, together with the mRNA to which it is coupled, a distance of one codon from the A-site to be positioned at the P-site. This movement is called translocation; it is accompanied by the release of free tRNA from the ribosome. For the synthesis of one peptide bond (or lengthening of the polypeptide by one amino acid), the energy of two GTP molecules is consumed.

Elongation is assisted by the so-called *elongation factors* (or transfer factors), proteins catalyzing the elongation of peptide chains. Elongation continues until all the mRNA text has been completely read out.

Termination, or the completion of polypeptide synthesis, is dependent on the occurrence of termination codons, or "stop-signals" (UAA, UGA, UAG), and elongation factors in mRNA. The tRNAs

are not capable of binding with termination codons, since they are not in possession of the corresponding anticodons. It is possible that the termination factors release the synthesized polypeptide chain. In the cell mRNA uses more than one ribosome for protein biosynthesis purposes. Such along the length of a strand of mRNA is called *polyribosome*. The polypeptide chain is lengthened at a rate of one amino acid per second; and during an intensive phase of cell growth, the biosynthesis rate rises to 20 amino acids per second.

Post-translational modifications of proteins

During translation, the protein starts to be folded into a three-dimensional structure which is completed to the final form after the protein has been released from the ribosome. Some proteins are synthesized as precursors. These are subject to a *limited hydrolysis* in the cell. Apparently, most proteins are amenable to the treatment by proteases, i.e. figuratively speaking they pass a stage of maturation.

A significant part of the synthesized proteins remain inside the cell, while some proteins are particular active in protein secretion. As a rule, the proteins that have been synthesized on ribosome bound with the endoplasmic reticulum (EPR) membrane are exported from the cell. In the cisterns of endoplasmic reticulum, the proteins become concentrated. The storage and secretion of proteins take place in the *Golgi apparatus*, where a carbohydrate component is added to the proteins. The transport of proteins from the cell is accomplished by exocytosis. The ATP energy is consumed in this process; when ATP is in short supply, the proteins are retained within the cell.

Conservation of mRNA. The mRNA intended for storage and subsequent re-usage of its genetic program for assembling the required proteins must be protected against an attack by nucleases. In the cell, the conservation of mRNA is done by binding it to special cytoplasmic proteins. Such protein-mRNA arises for protein biosynthesis, mRNA binding to the small ribosomal subunit to be engaged in the translation. The mRNA conservation procedure is used during cell development.

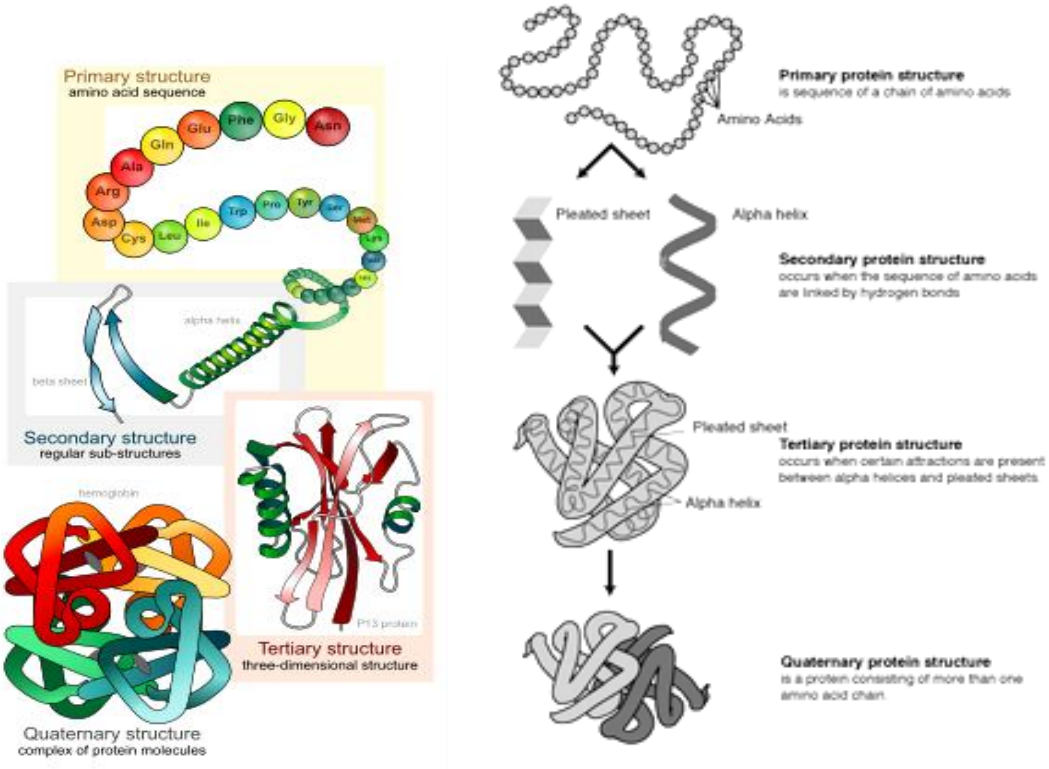
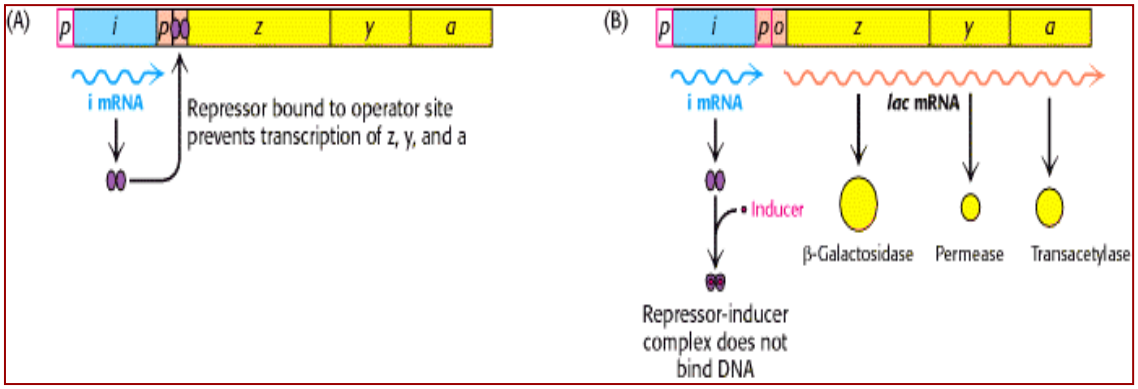


Figure 21. **Post translation modification of protein (folding of protein: from primary to quaternary structure)**

Regulation of protein biosynthesis

Proteins determine the vital activity of the cell. Therefore, within the framework of the overall scheme of cellular protein synthesis, the cell must exercise a precise control over the synthesis of the proteins needed at the given moment.

Proteins that are synthesized at a constant rate are called *constitutive proteins*, while those for which the synthesis rate may be varied within a wide range depending on the extant conditions are called *adaptive*, or *inducible*, *proteins.* The concentrations of inducible (adaptive) proteins are subject to wide variation. The synthesis of constitutive proteins appears to be independent control, and in contrast, the synthesis of inducible proteins is liable to a very strict regulation.



The stimulation of protein biosynthesis, which leads to an increased amount of proteins, is called *induction*, while the inhibition of protein biosynthesis is referred to as *repression.* Apparently, there are substances in the cell that are capable of signaling the state of metabolism in the cell or in the organism. This allows switching protein synthesis *on* or *off*. In procaryotes, such signal compounds may be nutrients supplied to the cell, metabolites, and certain intracellular regulators (of cyclic nucleotide type). In multicellular systems, especially highly organized ones, in addition to autonomous intracellular regulators, an important role is given to extracellular protein biosynthesis regulators which govern the activity of the genetic apparatus of protein biosynthesis for a given tissue or organ cell in accordance with the global strategy of the whole organism.

Genetic engineering

Genetic engineering is a branch of molecular genetics dealing with the development of methods for constructing desired genes and incorporating them into the host cell with the purpose of changing the genetic properties of the cell. In future methods of genetic engineering have proved to be effective in the transplantation of numerous genes, including those of insulin, somatotropin, ovalbumin, and others. This opens the way for the potential commercial production of proteinic drugs by the genetic engineering method.

Preparations affecting protein biosynthesis

Preparations that affect protein biosynthesis are widely used in practice. Inducers are applied to stimulate protein synthesis in impaired cells, or in the organs affected by long-lasting inactivity (atrophied organs). This inducer effect facilitates the functional restoration of the cells in a damaged organ. Protein synthesis inhibitors are used for the opposite effect, i.e. to suppress the division and growth of cells.

Preparations for stimulating protein synthesis belong to the so-called anabolic agents. Hormonal and nonhormonal anabolic agents are distinguished. The group of hormonal agents is the larger one. Among these agents, the most pronounced ability to protein synthesis induction (at the transcription level) is manifest in the *anabolic steroids* (methandrostenolone, phenobolin, and the most active retabolil) which are derivatives of male sex hormones (androgens) and are used with the exclusive purpose to stimulate protein synthesis in the organism. *Insulin* exhibits a distinct anabolic activity; this protein hormone appears to activate protein biosynthesis at the translation level.

Nonhormonal anabolic agents that have gained acceptance in practice include precursors of nucleotides and nucleic acids. For example, *sodium orotate* (orotic acid is a key compound in the biosynthesis of pyrimidine nucleotides) and *inosine* (or hypoxantine riboside).

Protein synthesis inhibitors. These constitute a larger group of preparations used in biochemical studies and practical medicine. All the protein synthesis inhibitors may be classified into:

- transcription inhibitors
- processing and RNA transport inhibitors
- translation inhibitors.

The transcription inhibitors are: α-*amanitin* (poison of the death cup fungus, *Amanita phalloides*), antibiotics *rifamycins, actinomycin D* (used in biochemical experiments), antibiotics *olivomycin* and *dactinomycin,* plant alkaloids *vinblastine* and *vincristine* (used in medicine as antitumoral agents), *5-fluorouracil.*

Processing and m-RNA transport inhibitors

Cordycepin (3'-deoxyadenosine) may be referred to as an mRNA transport inhibitor since it impedes the addition of polyadenyl moiety to mRNA, the polyadenyl moiety facilitating the transport of mRNA from the nucleus to cytoplasm.

Translation inhibitors may be exemplified by antibiotics used as antibacterial agents in medicine: *chloramphenicol, lincomycin, erythromycin, tetracyclines, streptomycin,* etc.

LECTURE 9

Subject: GENERAL CHARACTERIZATION OF METABOLISM AND ENERGY METABOLISM

Characterization of metabolism

The vital activity of any living organism is determined by the specific organization of biological structures, metabolic processes, energy metabolic processes, energy metabolism, genetic information transfer, and regulatory mechanism. Damage of any of these links develops a pathological process and a disease in the organism. An understanding of the molecular mechanisms involved in the vital activity or malfunction of the organism constitutes the basis for the search and clinical applications of biological medicinal preparations.

In the metabolism of the living organism distinguished are:

- *exogenous metabolism,* which comprises extra-cellular transformations of materials on the way to their uptake and excretion by the cells
- *intermediary metabolism,* which occurs in the cells.

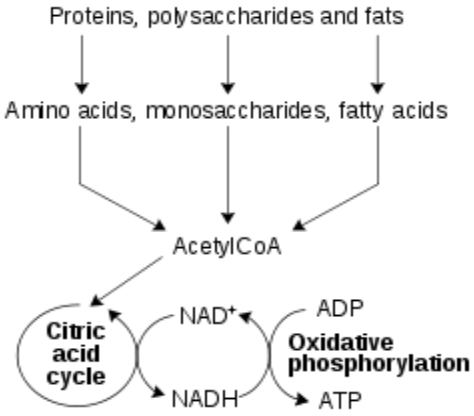
The intermediary metabolism is conceived as the total sum of chemical reactions that occur in the living cell.

Functionally, metabolism encompasses the following major processes:

1. Accumulation of energy from decomposition of compounds or supplied by light.
2. Utilization of energy for synthesis of essential molecular components (monomers, macromolecules) and the performance of work (osmotic, electric, mechanical).
3. Decomposition of renewable structural components of the cell.
4. Synthesis and decomposition of specialized biological molecules (hormones, mediators, hormonoids, cofactors, etc.).

The sequences of chemical reactions involved form *metabolic pathways,* or cycles, each of these performing a definite function. Conventionally, *central* and *special* metabolic pathways are distinguished. Central pathways are common to the decomposition and synthesis of major macromolecules. Actually, they are much alike in all representatives of the living world. Special cycles are characteristic of the synthesis and decomposition of individual monomers, macromolecules, cofactors, etc. Special cycles are extremely diversified.

In the metabolism, two oppositely directed processes, or phases are commonly distinguished: *catabolism* and *anabolism.*



Catabolism of substances (3 stages)

Catabolism is the sum of degradation processes leading to the cleavage of large molecules into smaller ones. Catabolism is accompanied by a release of energy that can be stored as energy-rich ATP.

Anabolism is the sum of metabolic processes leading to the synthesis of complex molecules from simpler ones. Anabolic processes proceed through consumption of ATP and decomposition of the latter into ADP and H_3PO_4.

However, ATP is not the only linking component shared by catabolism and anabolism. Other simple metabolites are also formed by the catabolic pathway from macromolecules and monomers to be used as starting materials for the subsequent synthesis of monomers and macromolecules, i.e. in the process of anabolism. This linking pathway, or cycle, unifying degradation and synthetic routes, is called the ***amphibolic*** pathway. This signifies that the catabolic and anabolic pathways are coupled not only via the energetic ATP-ADP system, but also through their common metabolites, which renders the metabolism more versatile and economical (for example, pyruvate, oxaloacetate, α-ketoglutarate, acetyl-ScoA, etc.).

Energy transfer in biochemical processes

The biological activity of the cell is closely associated with the continuous redistribution of the energy delivered by the compounds that enter the cell. The storage of energy in the specific phosphate bonds of ATP constitutes the basis for the energy transfer mechanism in the living cell. The living cell is a nonequilibrated chemical system the circumstance that permits storing the energy, produced by catabolic reactions of nutrients, in the ATP phosphate bonds.

The ATP energy in the cell can be converted, via three major routes, to energy of chemical bonds, to thermal energy, and to energy for performing work. We know consider in general terms the transfer of *chemical bond energy*.

The chemical reaction $\mathbf{ADP + H_3PO_4 \rightarrow ATP}$ is associated with the generation of ATP energy. The chemical energy of ATP phosphate bonds can be spent on osmotic, electric, mechanical, and other types of work. In doing so, not all of the ATP energy is used for performing work; a portion of it is dissipated as heat. All chemical processes in the living organism can proceed only with the involvement of enzymes.

Bioenergetics

The energy resources stored in the cells are used to provide for the cells' energetic requirements. They include monosaccharides, amino acids, glycerol, and fatty acids. These materials, when crossing the cytoplasmic membrane, may either be used immediately as energy sources or, by forming part of biopolymers (polysaccharides, lipids, and proteins), be reserved as consumed for generating energy. In the organism, the role of various tissues and organs involved in storing the energy sources, especially such valuable ones as fats and carbohydrates, is different.

Stages of energy release from nutrients

In the process of energy release from various substrates, three conventional stages may be defined. The ***first stage*** is a preliminary one. This stage is necessary for converting biopolymers (supplied in food or found in the cell) to a monomeric form suitable for energy extraction. This is accomplished by hydrolyses in the intestine or within the cell. The intracellular hydrolysis is effected with the invol-

vement of cytoplasmic enzymes and lysosomes. The energetic value of this stage is rather inferior, since only about 1% of the substrate energy is released, and it is dissipated as heat.

The ***second stage*** is partial degradation of monomers to key intermediates, chiefly to acetyl-ScoA and to a number of the Krebs cycle acids (oxaloacetate and 2-oxoglutarate, or α-ketoglutarate). At the second stage, a large number of initial substrates become reduced to three only. This stage is characterized by partial (to 20%) release, under anaerobic (oxygen-free) conditions, of the energy contained in the initial substrates. Part of this energy accumulates in ATP phosphate bonds; the other part is dissipated as heat. The conversion of monomers proceeds in the hyaloplasm, and the final reactions reach completion in the mitochondria.

The ***third stage*** represents an ultimate, oxygen-assisted degradation of the materials to CO_2 and H_2O. This phase comprises the aerobic biological oxidation of materials and proceeds with a complete release of energy. The specific feature of chemical transformation at this stage is that out of the three metabolites of the foregoing stage, after the so-called the Krebs cycle, only the hydrogen bound to carries (NAD or FAD) remains. Hydrogen is the universal energy fuel which is employed in the respiratory chain for producing ATP and water. About 80% of the total chemical bond energy of compounds involved is released at this stage. This energy, generated by the oxidation of substrates, is localized in the ATP phosphate bonds, and a part of it is dissipated as heat. All the reactions of this stage are confined within the mitochondrial space.

Biological oxidation

Biological oxidation reactions are catalyzed by enzymes. The oxidation may be associated with:

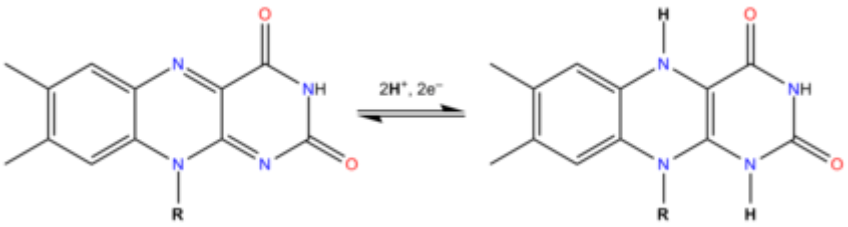
- cleavage of hydrogen from the substrate to be oxidized (dehydrogenation);
- loss of an electron;
- addition of oxygen.

All of the three reaction types are equivalent and occur in the living cell.

Oxidation is not an isolated process, it is coupled to a reduction reaction, i.e. addition of hydrogen or electron. Both compounds involved, i.e. the oxidant and the reductant, form a reduction-oxidation pair, or *redox-pair*. The oxidative or reductive ability of a compound is characterized by its electron affinity. The easier the substrate donates electrons, the higher is its reductive ability. On the contrary, a high electron affinity of the substrate attests to its oxidative ability. The aptitude of a redox-pair for reduction reactions is characterized by the standard ***oxidation-reduction potential,*** or ***redox potential.*** This potential is expressed by the electromotive force (in volt, **v**) that develops in a half-element in which the oxidant and reductant both have a concentration of 1,0 mol/liter at 25°C, pH=7,0 and are in equilibrium with an electrode which can reversibly accept electrons from the reductant. The standard redox potential of oxidation-reduction pair according to the reaction $\mathbf{H_2 \leftrightarrow 2H^+ + 2e}$ has been set at zero. The redox potential for the system $\mathbf{H_2/2H^+ + 2e}$ is equal to –0,42v. The potential for the pair $\mathbf{NADH^+ + H^+/NAD^+}$ is 0,32v, which attests to a high ability of the pair to donate electrons, while the redox potential for the pair $\mathbf{½\ O_2/H_2O}$ shows a large positive value +0,81v; therefore, oxygen exhibits the highest ability for accepting electrons. The redox-potential value enables one to predict a route for electron transfer under biological oxidation conditions and to estimate the energy change on electron transfer from a redox pair to another one.

As has been pointed out above, oxidation substrates are formed during the catabolic conversion of proteins, carbohydrates, and lipids. These substrates are liable to dehydrogenation, the most common type of biological oxidation with the involvement of intracellular dehydrogenases. The dehydrogenation reactions that proceed with the participation of a substrate other than oxygen as a hydrogen acceptor, is referred to as the *anaerobic oxidation.* The biological oxidation reactions involving oxygen as a hydrogen acceptor with the resultant production of water are known as *tissue respiration.*

The anaerobic oxidation is nothing other than hydrogen generation. Such reactions proceed with the involvement of *nicotinamide-dependent dehydrogenases* (with NAD^+ and $NADP^+$ as acceptors for the hydrogen split from an organic substrate), and flavin-dependent gehydrogenases (with FMN and FAD as hydrogen acceptors). FAD can be reduced to $FADH_2$, whereby it accepts two hydrogen atoms (a net gain of two electrons):



Dehydrogenation substrates are formed in the extra-mitochondrial environment, but then are transported into the mitochondria within which oxidative metabolic processes take place.

Aerobic generation of energy in mitochondria

The major part of cellular energy is generated in the mitochondria, and for this reason these are figuratively referred to as power plants of the cell. Mitochondria are organelles present in all plant and animal cells. Mitochondria vary in shape and size, and outwardly look like an oval, rod, or thread. The average size of mitochondria is about 0,5·3 μm.

Any mitochondrion consists of two concentric membranes (membrane sacs). The inter-membrane space filled with an aqueous medium lies between the outer and inner membranes.

The outer membrane is made up in half of proteins and lipids. In the inner membrane, proteins account for about ¾, and lipids, mostly *cardiolipin*, for about ¼. The inner membrane is folded in a characteristic manner to form *cristae* which extend into the mitochondrion inferior. The space between the cristae is filled with an aqueous phase called *matrix*. On the matrix side of the inner membrane surface there are attached small knob-like particles, earlier referred to as oxysomes.

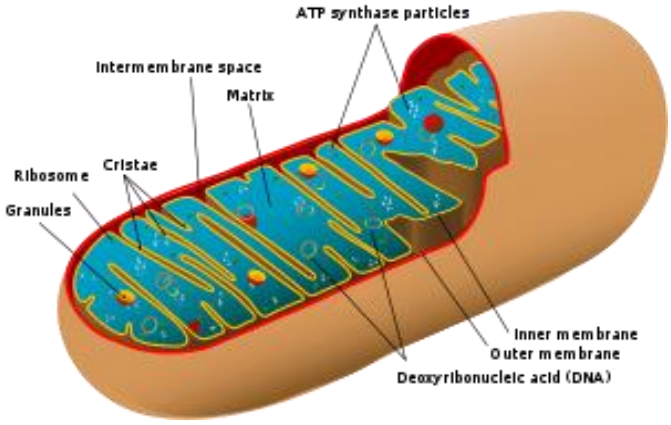


Figure 22. **Fifure of mitochondrium**

Structure and function of respiratory chain

The hydrogen produced by dehydrogenation of substrates in the mitochondria becomes bound to dehydrogenase-specific coenzyme carriers. The total of intra-mitochondrial hydrogen is supplied to the respiratory chain via three channels. For the most part, hydrogen is bound with NAD as $NADH_2$ complexes; since the majority of intra-mitochondrial dehydrogenases are NAD-dependent. The next part of intra-mitochondrial hydrogen is bound with NADP (as $NADPH^+ + H^+$). The hydrogen supplied through the third channel is produced by the action of flavin dehydrogenases (flavoproteins) on the substrates, with FAD acting as a coenzyme.

The functional involvement of hydrogen bound to various coenzymes is different: $NADH_2$ and $FADH_2$ are employed in oxidative processes, while $NADPH_2$ is chiefly used for reductive syntheses. The inner mitochondrial membrane contains an enzyme, transhydrogenase, which controls the distribution of hydrogen between $NADP^+$ and NAD^+. Transhydrogenase catalyzes the hydrogen transfer between the two nicotinamide-adenine-nucleotides:

$$\mathbf{NADPH_2 + NAD^+ \rightarrow NADP^+ + NADH_2}$$

Tissue respiration and oxidative phosphorylation

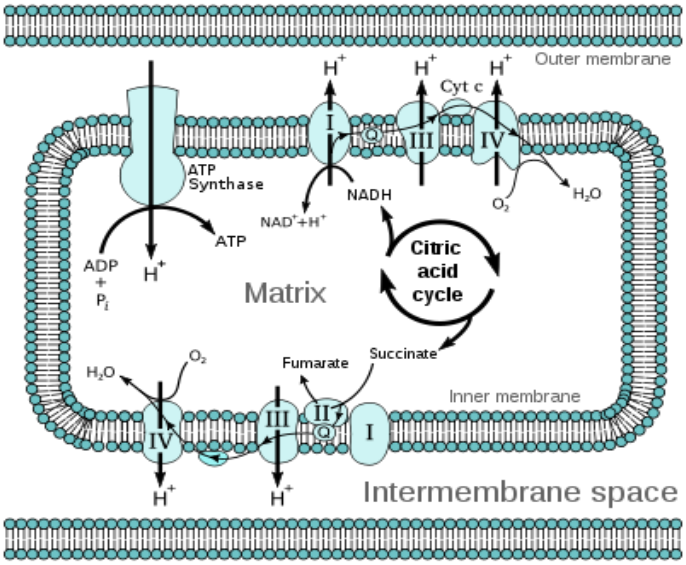


Figure 23. **Scheme of Respiratory chain**

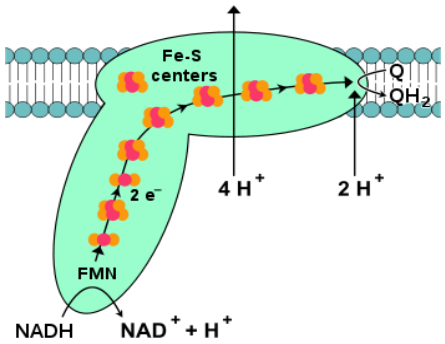
Hydrogen is utilized as the major fuel for energy generation. In the mitochondria, the flow of electrons from hydrogen is channeled towards their terminal acceptor, oxygen. This results in the formation of water which is the least efficient on the energy scale of biological materials and is the end product of tissue respiration. It follows therefore that the tissue respiration is a redox process associated with formation of water due to the transfer of electrons from hydrogen onto oxygen.

The *respiratory chain* may be likened to a specific conveyer for the transport of protons and electrons from the reduced NAD ($NADH^+ + H^+$), which is formed by the action of NAD-dependent dehydrogenase on a substrate, or from the reduced FAD ($FADH_2$), formed by action of flavin-dependent dehydrogenases on a substrate, to oxygen.

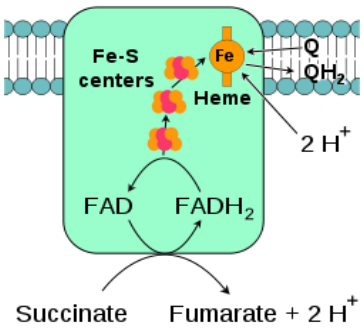
The respiratory chain is made up of the following proton and electron carriers:

- flavoprotein-1 (FP) containing FMN for a coenzyme;
- coenzyme *Q* (ubiquinone);
- two iron-sulphur proteins containing nonheme iron (Fe);
- cytochromes *b*, c_1, *c*, *a*, and a_3.

In the liver, there occur about 5,000 respiratory chains per mitochondrion, and in the heart, about 20,000.



Complex I or NADH-Q oxidoreductase



Complex II: Succinate-Q oxidoreductase

Flavoprotein (FP) is a *NADH-dehydrogenase*. This conjugated protein contains FMN acting as an acceptor for protons and electrons supplied by NADH. Bound to flavoprotein is an *iron-sulphur protein* likewise participating in the transport of protons and electrons onto CoQ. The active center of $NADH^+$-dehydrogenase is located on the inner side of the inner membrane; for this reason, dehydrogenation of $NADH^+$ proceeds at this particular side.

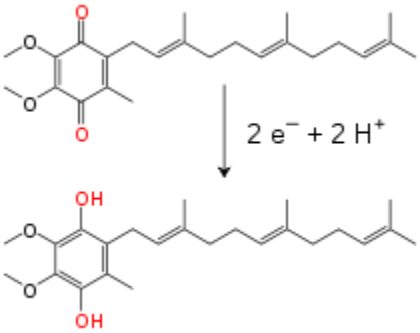


Figure 24. **Reduction of coenzyme Q from its ubiquinone form (Q) to the reduced ubiquinol form (QH_2)**

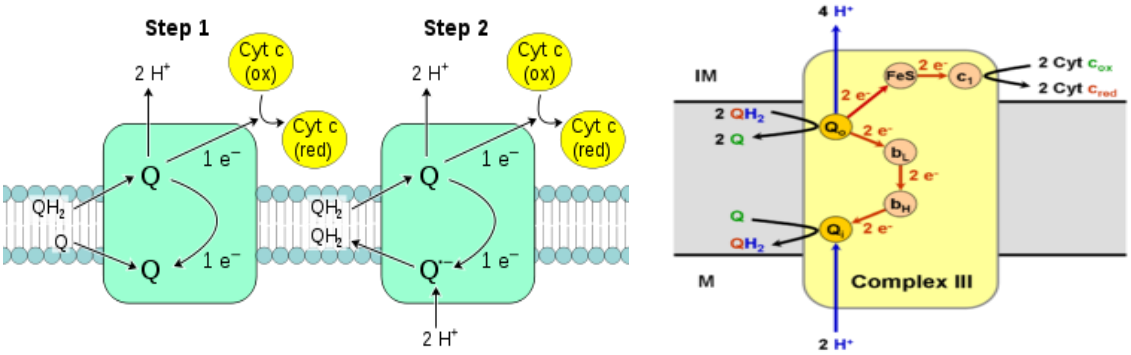
Coenzyme Q , or *ubiquinone,* is dissolved in the lipid layer of the membrane and can diffuse both across and along the membrane.

Cytochromes b,c, c_1, a, and a_3,, are heme proteins. Cytochromes *a* and a_3 form a complex called cytochrome oxidase. As distinct from other cytochromes, the cytochrome oxidase contains $Cu^{+.}$

As a matter of fact, tissue respiration in a simplified form resembles an explosive combustion of hydrogen in oxygen according to the reaction:

$$2H^+ + 2e + ½\ O_2 \rightarrow H_2O$$

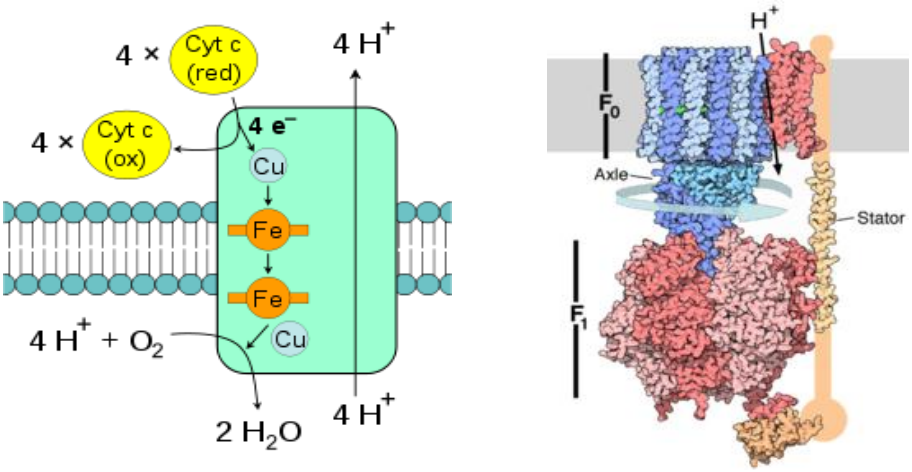
A coupling of respiration with phosphorylation is called ***oxidative phosphorylation.*** The P/O ratio ia a measure for respiration-phosphorylation coupling. This ratio was called *phosphorylation ratio.* The uptake of one oxygen atom (or transfer of an electron pair from a substrate to oxygen) proceeded with the involvement of about three, rather than one, inorganic phosphate moieties, i.e. the P/O or P/2e, ratio was equal to about 3.



Complex III: Q-cytochrome *c* oxidoreductase

Other vise stated, the respiratory chain possessed a minimum of three coupling, or phosphorylation, sites involved in the formation of ATP with the participation of inorganic phosphate according to the scheme:

$$ADP + H_3PO_4 \rightarrow ATP + H_2O$$



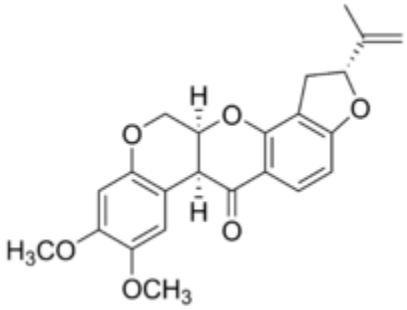
Complex IV: cytochrome c oxidase **H^+- ATP synthase**

For the formation of one macroergic ATP bond with energy expenditure of at least 40 Kj/mol, a redox-potential drop of about 0,22 v per electron pair transferred between the respiratory chain steps is required: $\triangle E = \pm 0{,}22$ v.

Agents affecting energy metabolism in cells

Numerous materials, including toxins and therapeutic drugs, are capable of affecting the cell energetic through intervening in the energy-generating glycolysis and oxidative phosphorylation processes.

Isonicotinic acid hydrazides (phthivazid) are competitive inhibitors for NAD-dependent dehydrogenases. *Malonate* is a competitive specific inhibitor for succinate dehydrogenase.



Rotenone

Amytal, rotenone (fish poison), and *progesterone* (female sex hormone) inhibit the first coupling link (proton potential generation) and render inoperative the portion of respiratory chain ahead of the block.

Cyanides (NaCN, KCN), *azides* (NaN_3), and *carbon monoxide* (CO) inhibit cytochrome oxidase and render thereby the very process of respiration impossible.

Respiration-phosphorylation coupling mechanism in mitochondria

A basically novel mechanism for respiration-phosphorylation coupling has been developed by the British biochemist Mitchell (1961). The hypothesis that has been put forward by him is currently known as *chemiosmotic,* or *proton-driving,* hypothesis. According to Mitchell, the energy of electron/proton transfer along the respiratory chain is initially stored as a *proton potential,* or *H^+ion electrochemical gradient* which is generated as the protons move across the membrane. The reverse proton diffusion across the membrane is coupled to phosphorylation, which is accomplished by H^+-ATP-synthetase. Respiration performs an osmotic work (i.e. makes the protons concentrate in the extramitochondrial medium) and an electric work (by generating an electric potential difference), both of which are converted by ATP-synthetase into a chemical stored energy, i.e. the synthesis of ATP takes place. The junction of these two functions, respiration and phosphorylation, has provided a good reason to name the hypothesis *chemiosmotic,* or *proton-driving,* since here the proton potential is a driving force for phosphorylation.

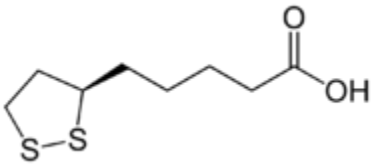
LECTURE 10

Subject: ENZYMIC MITOCHONDRIAL SYSTEMS AS HYDROGEN GENERATORS

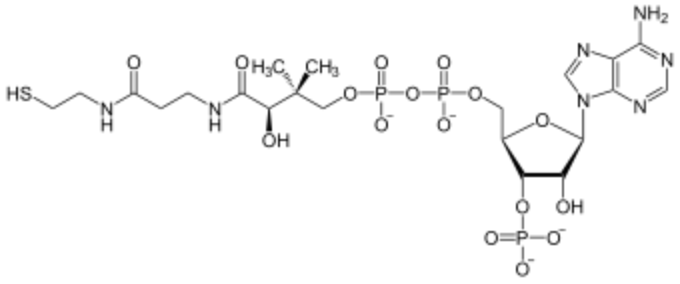
Pyruvate, the anion of pyruvic acid, is an important oxidation substrate formed as a metabolic intermediate from carbohydrates, proteins, amino acids, and glycerol. Pyruvate is subjected to oxidation in the mitochondria where it penetrates from the cytoplasm. In addition to pyruvate, a number of substrates undergo mitochondrial oxidation. Some of them are involved in accepting cytoplasmic hydrogen and transferring it within the mitochondria to the respiratory chain. The significance of pyruvate as an oxidation substrate resides also in that it can be oxidatively decarboxylated to acetyl-ScoA, a major producer of hydrogen in the mitochondria. For that matter let us consider the enzymic system of pyruvate oxidation.

Oxidation of pyruvate to acetyl-ScoA

The oxidative decarboxylation of pyruvic acid is effected by a multienzyme *pyruvate dehydrogenase complex.* This complex is found in the matrix not in a dissolved state, but rather attached to surface proteins of the inner mitochondrial membrane exposed to the matrix. The pyruvate dehydrogenase complex exemplifies the structural organization of a number of different enzymes, and features all the advantages of such an organization. The complex is composed of three enzymes: *pyruvate dehydrogenase (E_1), dihydroxylipoylacetyl transferase (E_2),* and *dihydrolipoyl dehydrogenase (E_3).* Pyruvate dehydrogenase is made up of 24 enzyme molecules, each containing a *thiamine diphosphate (TDP* or *TPP) residue* acting as coenzyme for pyruvate dehydrogenase. The quaternary structure of dihydrolipoylacetyl transferase includes 24 subunits. Each subunit of dihydrolipoylacetyl transferase contains a *lipoic acid (LA) residue*:



The complex includes 12 molecules of dixydroxylipoyl dehydrogenase, each containing a *FAD residue.* In addition, during the oxidation of pyruvate, two outer (complex-nonbound) coenzymes, coA-SH and NAD, are involved, which act as acceptors for the pyruvate oxidation products.



Coenzyme A

The pyruvate is successively acted on by the enzymes of the pyruvate dehydrogenase complex. The following stages for pyruvate oxidation may be considered:

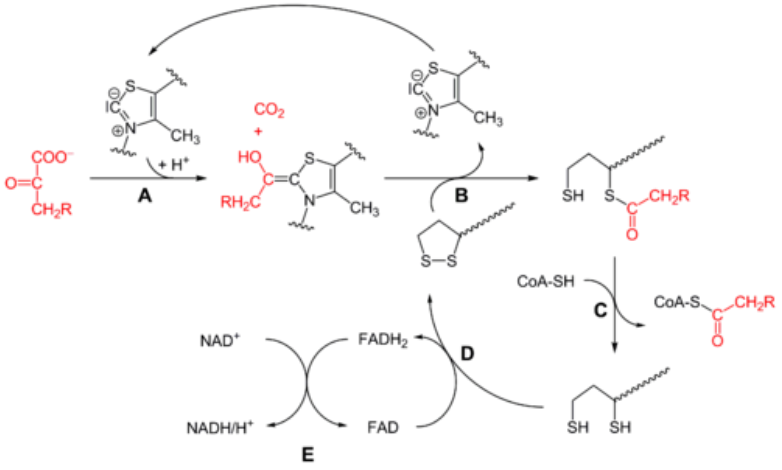


Figure 25. **PDHcomplex reactions**

The overall reaction for oxidation of pyruvate with the pyruvate dehydrogenase complex enzymes is expressed as:

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

Practically, the total of the pyruvate supplied to the mitochondria is rapidly oxidized to acetyl-ScoA. Of the pyruvate oxidation products, the end metabolite CO_2 is of little energetic value. In contrast, the reduced NAD is an energy-rich material which supplies hydrogen to the respiratory chain ($NADH_2$=3ATP). Acetyl-ScoA is engaged in the Krebs cycle operative in the mitochondria.

Oxidative enzyme system of the Krebs cycle

Krebs cycle is a major enzymic system acting as a hydrogen generator for the respiratory chain. In 1937, Krebs, a German biochemist in England suggested, on the basis of his experiments and the data obtained by *Szent-Guorgyi*, that an oxidative cyclic system of reactions might be operable in the cells. He proposed the name ***citric acid cycle*** because at that time there was no reliable evidence that citric acid could be the first substrate of the cycle. As has been shown later, this cycle is the major enzymic system for oxidation of citric acid residue (acetyl-coA) and that the first reaction of the cycle is the synthesis of citric acid. However, most commonly this cycle is referred to as the *Krebs* as a tribute to Sir *Hans Adolf Krebs* who was the first to establish the reaction sequence in this cycle.

Separate reactions involved in the Krebs cycle

Acetyl-ScoA, produced by oxidation of pyruvate, fatty acids, and amino acids, is included in the Krebs cycle.

Biochemical functions of the Krebs cycle

Integrative function: the Krebs cycle acts as a specific metabolic "collector" that unifies the catabolic pathways of carbohydrates, lipids, and proteins.

Amphibolic function: the Krebs cycle performs a dual function: catabolic one, since the cycle substrates are used in the synthesis of other materials. For example, oxaloacetate is utilized in the synthesis of glutamic acid; and succinate, in the synthesis of heme.

Energetic function: in the course of the Krebs cycle reactions, one substrate level ATP molecule is formed per 1 mole of acetyl-ScoA supplied.

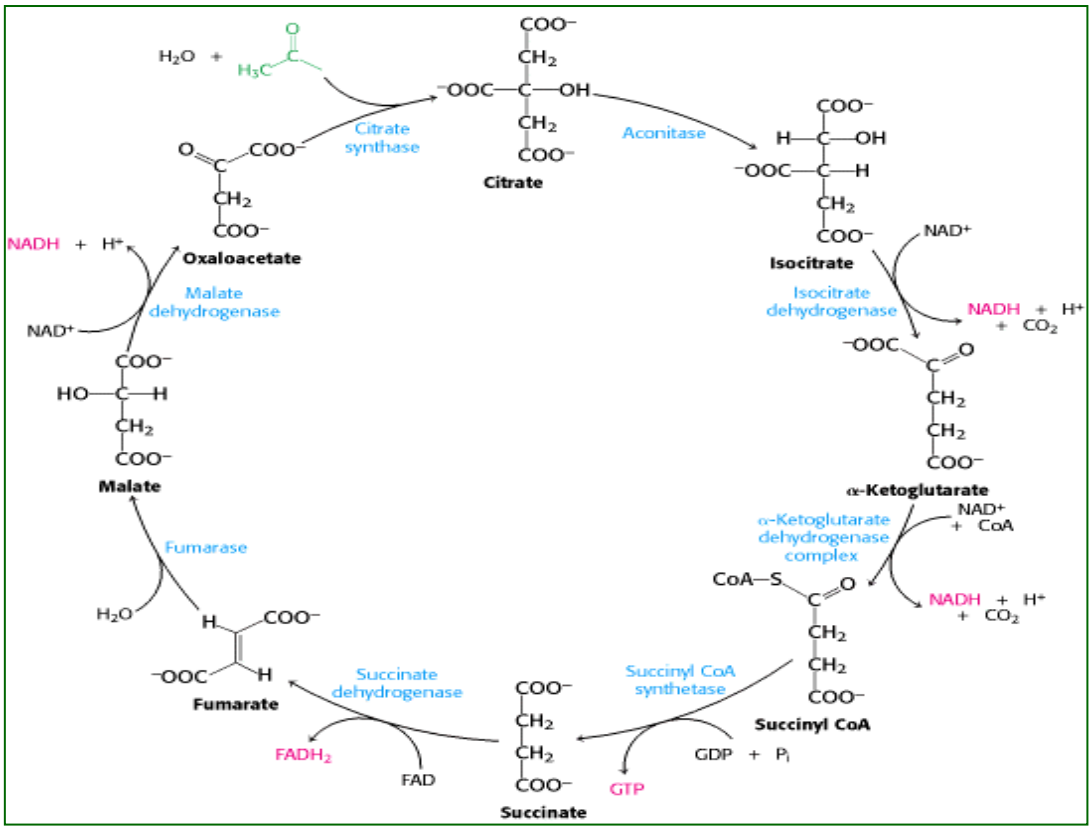


Figure 26. **Reactions of the Krebs cycle**

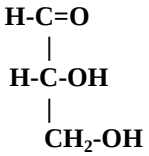
Hydrogen-donating (or *hydrogen-generating) function:* the Krebs cycle is a major hydrogen generator for the respiratory chain. In the Krebs cycle, four pairs of hydrogen atoms are formed, of which three pairs are bound with NAD ($3NADH^+$=9ATP), and one, with FAD ($FADH_2$ =2ATP).

The terminal stage of the Krebs cycle merits a closer look. Firstly, it should be kept in mind that all the processes that "supply" the Krebs cycle with acetic acid residues (acetyl-ScoA) and other intermediates (di- and tricarboxylic acids), ensure the operation of the Krebs cycle and its function of generating hydrogen for the respiratory chain. These processes include the cycle for oxidation of fatty acids and pyruvate (as sources of acetyl-ScoA) and the reactions for degradation of the amino acid carbon skeleton (as sources of acetyl-ScoA and dicarboxylic acids).

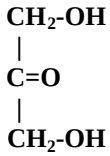
LECTURE 11

Subject: CARBOHYDRATES: CHEMICAL STRUCTURE, DIGESTIVE MECHANISM OF SUGARS

Carbohydrates are the most widespread compounds involved in the buildup and biological functions of the cell. Carbohydrates (sugars) are polyhydroxycarbonyl compounds and their derivatives. The term "*carbohydrate*" is rather obsolete and reflects properly neither the chemical nature nor the composition of the species in question; still, the suggested alternative term "*glycide*" has not gained acceptance. A characteristic feature of carbohydrates is the occurrence in them of a carbonyl (*aldo* or *keto*) group and at least two hydroxyl groups. Therefore, glyceraldehyde and dihydroxyacetone may be referred to as the simplest carbohydrates:



glyceraldehyde



dihydroxyacetone

The classification of carbohydrates is based on their structure and physico-chemical properties.

Carbohydrates bearing an *aldo* group are called *aldoses* and those bearing a *keto* group, *ketoses*. Based on their physico-chemical properties, carbohydrates are divided into:

- *neutral* species (those containing hydroxyl and carbonyl groups only: glucose),
- *basic* (those containing, alongside the aforementioned groups, an amino group: aminosaccharides),
- *acidic* (those containing, except hydroxyl and carbonyl groups, carboxyl moieties also: glucuronic acid).

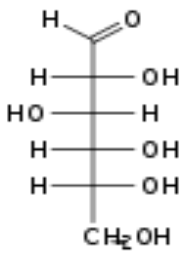
Based on their chemical structure, carbohydrates are divided into:

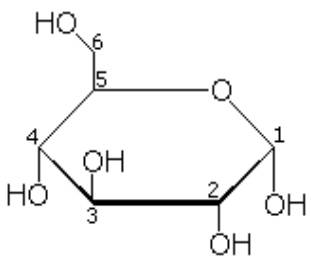
- *monosaccharides*, or *monoses*, which are simple carbohydrates,
- *olygosaccharides*, which are carbohydrates possessing two to ten monosacchride units linked by glycoside bonds,
- *polysaccharides*, or *glycans*, which are high-molecular carbohydrates containing more than ten monosaccharide units linked by glycoside bonds.

Monosaccharides, or *monoses*, are simple carbohydrates. The names of all monosaccharides end in – *ose.* The group name of monosaccharides indicates the number of carbon atoms and the presence of an appropriate carbonyl group (aldo or keto). For example, monosaccharides possessing five carbon atoms are called *pentoses* and taking into account the occurrence of an aldehyde group, *aldopentoses.* If the monosaccharides in question contain a keto group, they are called *ketopentoses.* Carbohydrates with six carbon atoms are called, respectively, *aldohexoses* and *ketohexoses*.

Monosaccharides belong to either *L*-(levorotatory) or *D*-(dexterorotatory) series depending on the *L*- or *D*-configuration of substituents at the asymmetric carbon atom farthest from the carbonyl group in a given monosaccharide. For example, in hexoses, this carbon is C-5'.

In aqueous solution, monosaccharides exist either in the form of an unfolded chain, or as a cyclic structure. Cyclic monosaccharides do not occur in trioses and tetroses but, starting with pentoses, a spontaneous reaction takes place between one of the hydroxyl groups and the carbonyl group leading





D-Glucose (aldohexose) **D,α-Glucose** depicted in Haworth projection

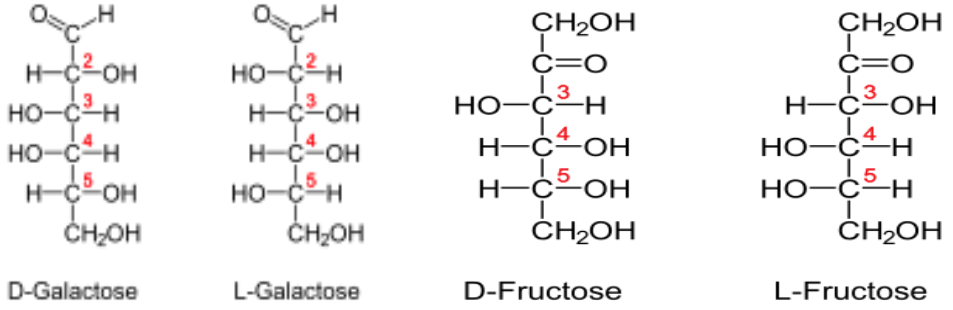


Figure 27. **Chemical structures of carbohydrates-monosaccharides**

to the closure of a ring. In such a manner, for example, five-membered (*furanose*) and six-membered (*pyranose*) rings are formed. The hydroxyl at the first carbon atom of a cyclic monosaccharide is called the *half-acetal* hydroxyl. It is essential for reducing properties of carbohydrates.

Monosaccharide derivatives. The modification of available functional groups, or the introduction of substituents into the molecule of a monosaccharide produces various monosacchride derivatives. These are used in generating diverse polymeric carbohydrates. Some of the derivable species may act as intermediary metabolites.

*2-deoxy-D-ribose (*or simply: *deoxyribose*) forms part of deoxyribonucleosides and deoxyribonucleotides, which are structural monomers of DNA.

Many monosaccharides are involved in the synthesis of a very important group of compounds called glycosides. In particular, ribose and deoxyribose make part of the aforementioned nucleosides and nucleotides which are *N-glycosides.* Of importance is the role of *aminosugars*, or *aminodeoxysugars*. D-glucosamine is used in the buildup of essential structural polysaccharides, i.e. hyaluronic acid. D-galactosamine takes part in the synthesis of polysaccharides of cartilage tissue, chondroitin sulphates and certain glycolipids.

Biological importance of monosaccharides

In the cell, the monosaccharides are used as a source of energy. Monosaccharides (as well as polysaccharides) as distinct from other compounds, are an energy substrate for the cells of human and animal organisms both in the presence and in the absence of oxygen. Moreover, monosaccharides and their derivatives are involved in the buildup of diverse biological molecules, i.e. in the performance of a *plastic function.*

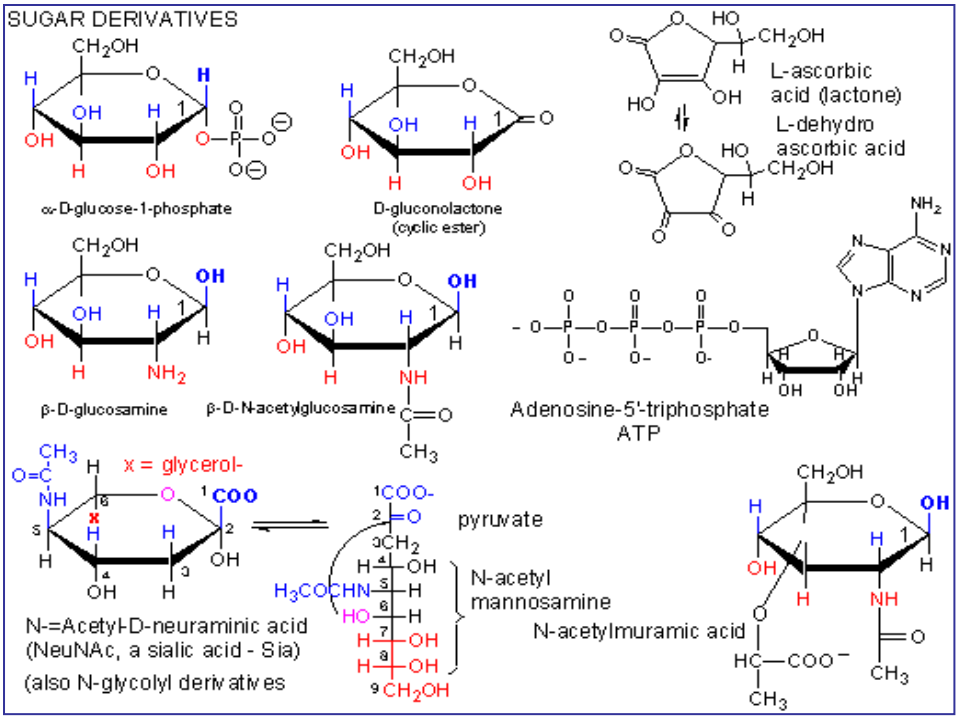


Figure 28. **Chemical structure of monosaccharide derivatives**

Olygosaccharides, are carbohydrates possessing two to ten monosaccharide units linked by glycoside bonds. They differ from one another in the monosaccharide composition and in the type of glycoside bond. Among the most widespread olygosaccharides worth mentioning are:

- *sucrose* (cane sugar, beet sugar) widely distributed in plants,
- *maltose* (malt sugar), a product of partial hydrolysis of starch in plants and glycogen in animals,
- *lactose* (milk sugar) found in the milk of all mammals,
- *trehalose*, present in numerous lower and higher fungi.

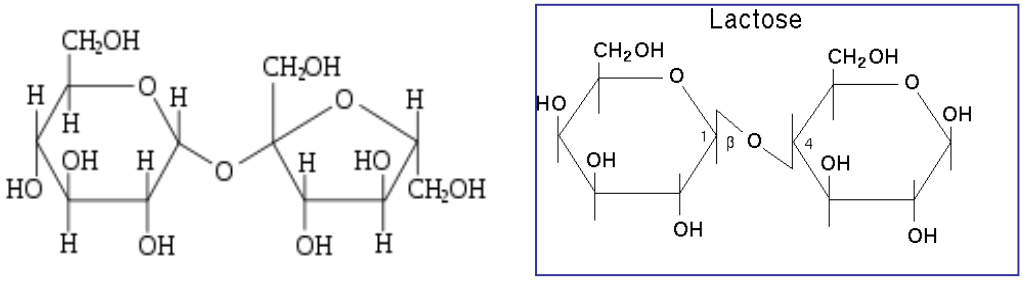


Figure 29. **Chemical structures of disaccharides: sucrose** (left) **and lactose**

Olygosaccharides are found in the cells and biological fluids in a free state as well as constituents of covalently bonded carbohydrate-protein complexes (glycoproteins and proteoglycans).

Polysaccharides, or *glycans,* are high-molecular carbohydrates containing more than ten monosaccharide units linked by glycoside bonds. Polysaccharides differ from one another in the nature of monosaccharides involved, in molecular mass, and in the type of chain-linking glycoside bonds. The most common monomeric unit in polysaccharides is D-glucose. Other monosaccharides that occur are galactose, mannose, fructose, and monosaccharide derivatives.

Distinguished are *homopolysaccharides* (homoglycans) built of only one type of monosaccharides and *heteropolysaccharides* (heteroglycans) composed of different types of monosaccharides. For example, starch is a homopolysaccharide, since it contains D-glucose only, while hyaluronic acid is a heteropolysaccharide, since it is made up of alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. Polysaccharides are also subdivided into linear and branched species, according to the structural type of their polymeric chains.

General properties of polysaccharides

Polysaccharides exhibit typical properties common to high-molecular compounds bearing polar groups. Thence, polysaccharides are hydrophilic; when placed in water, they swell (similar to fibrous proteins) and then dissolve partially to form colloidal solutions.

Polysaccharides are contained both inside the cells and in the extracellular matter. This signifies that the characteristic properties of polysaccharides show up under a variety of conditions. As a rule, neutral polysaccharides inside the cells are intended as reserve material (starch or glycogen). Acidic polysaccharides (hyaluronic acid and chondroitin sulphate) are commonly found in the extracellular space.

Individual polysaccharides

Neutral polysaccharides. The structures for the most widespread homopolysaccharides are presented below:

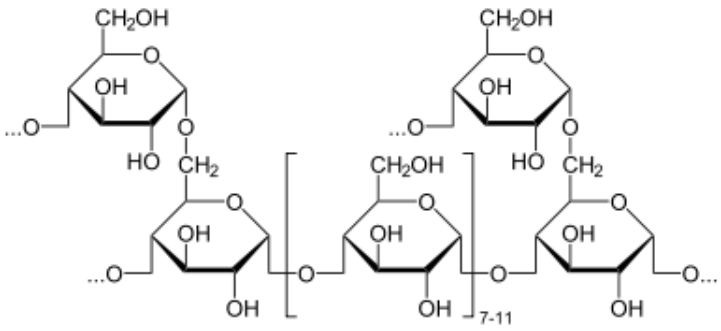


Figure 30.**Glycogen structure**

Starch is a homopolysaccharide found in plants; it consists of α-amilose and amylopectin. *Glycogen* is the major short-term storage polysaccharide found in all human and animal tissues. In small amounts, glycogen is also present in bacteria and plants. *Cellulose* is a structural homopolysaccharide of plants.

Acidic heteropolysaccharides, or ***mucopolysaccharides*** (from the Latin *mucus*, slime). They owe this name to their slime-like consistency. These polysaccharides are highly hydrated, gel-like sticky materials bearing a distinct negative charge. They all are found in the extracellular material, not in a free state but rather bound with proteins. Such heteromacromolecules are referred to as *proteoglycans* or *glucosamine proteoglycans*, since the basic properties of these macromolecules are mainly due to the carbohydrate, rather than the protein, moiety (to be noted, the functional behavior of glycogen-protein and starch-protein complexes is also determined by the carbohydrate component).

The acidic heteropolysaccharides, depending on their chain structure, are divided into seven major types. Six of them, represented by hyaluronic acid, chondroitin 4-sulphate, chondroitin 6-sulphate, dermatan sulphate, heparin, and heparan sulphate, possess similar repeating disaccharide units (for example: *D*-glucuronic acid, *N*-acetyl-*D*-glucosamine, *D*-galactose, etc.).

Hyaluronic acid is a nonsulphated linear heteropolysaccharide. It has the greatest molecular mass among the heteropolysaccharides. It serves as the biological cement filling the intercellular space. The molecules of hyaluronic acid a gel-like network which acts as a biological filter retaining microbiotic and other large molecules that invade the organism.

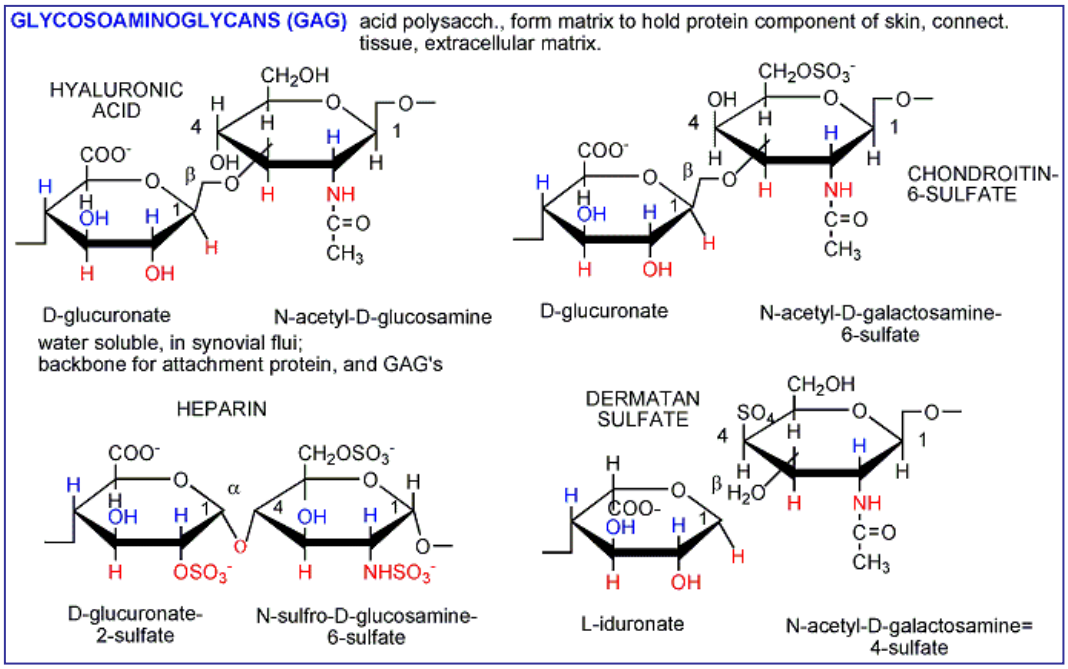


Figure 31. **Glucosoamineglycans**

Chondroitin sulphates are the most abundant acidic heteropolysaccharides in human and animal tissues. They occur in skin, bone tissue, cartilage, in tissues of trachea, aorta, arteries, etc. In tissues,

they are bound to the protein basis, or protein core, which makes up about 17-22% of the heteromolecule mass.

Dermatan sulphate is contained in the aorta and in distinction from other chondroitin sulphates, possesses anticoagulative properties.

Keratan sulphate is found in the cornea, where it is bound covalently to protein. *Heparin* and *heparan sulphate* are structurally related species. As distinct from other acidic heteropolysaccharides, they are not structural components for the extracellular material. In blood heparin is noncovalently bound to specific proteins. The complex of heparin with glycoprotein of plasma exhibits an anticoagulatory activity, while the complex of heparin with the enzyme lipoprotein lipase is capable of cleaving lipids found in the blood as chylomicrons.

Biological functions of polysaccharides

The essential functions of polysaccharides are:

- energetic
- supportive
- protective
- colligative (structural)
- hydroosmotic and ion-regulating
- cofactor.

Digestive mechanism for carbohydrates

The digestion of carbohydrates starts in the oral cavity, mainly through the agency of salivary *α-amylase*. Certain dietitians believe that another enzyme, *maltase*, is also present in the saliva. α-Amylase is composed of a single polypeptide chain; it is stabilized by calcium and activated by chloride ions, its optimum pH is 7,1. The enzyme belongs to endoamylases and acts on the endo α-1,4-glycoside bonds of dietary starch and glycogen. It is incapable of hydrolyzing the α-1,6-glycoside bonds of these polysaccharides. By the action of salivary α-amylase, the polysaccharides are split into a limit-dextrin, maltose, and a small amount of glucose. Dietary disaccharides, the major ones of which are sucrose, lactose, suffer no cleavage in the oral cavity.

In the stomach, α-amylase is inactivated by gastric acid components, and digestion of carbohydrates ceases. Hydrolysis of polysaccharides, including α-limitdextrin, formed in the oral cavity, and of disaccharides to monosaccharides proceed in intestine. The enzymic activity is favoured by neutralization of acidic food by hydrocarbonates dissolved in the alkaline contents of pancreatic juice and bile.

The intestinal hydrolysis of carbohydrates is carried out by the enzymes of pancreas and intestine. The former enzymes are pancreatic α-amylase and oligo-1,6-glucosidase. The hydrolytic action of pancreatic α-amylase is similar to that of salivary α-amylase. Within a short span of time (4-5 minutes), pancreatic α-amylase hydrolyzes the delivered starch and glycogen to α-limit-dextrin and maltose. Hydrolysis of α-limit-dextrin is effected through the aid of oligo-1,6-glucosidase, which is capable of specifically disrupting α-1,6-glycoside bonds at "branch" points of polysaccharide. Maltose is formed as the end product. Disaccharides undergo hydrolysis in the intestinal wall, rather than in the intestinal cavity, and the monosaccharides formed are immediatelly absorbed.

α-Oligosaccharidases include maltase, sucrase, lactase:

Maltase

Maltose + $H_2O \rightarrow$ 2 glucose

Sucrase

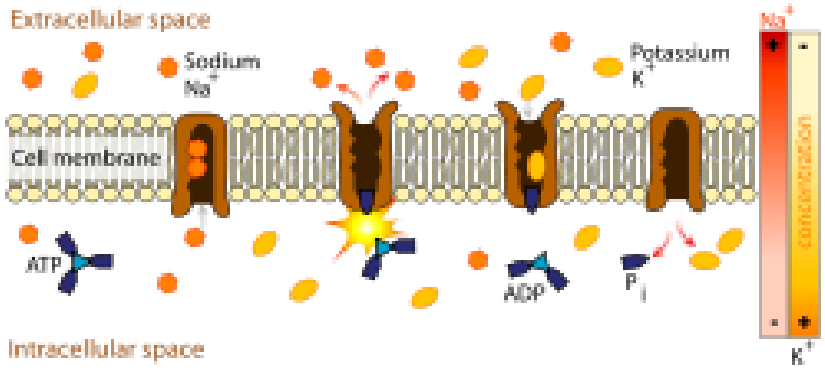
Sucrose + $H_2O \rightarrow$ glucose + fructose

Lactase

Lactose + $H_2O \rightarrow$ glucose + galactose

The end products of carbohydrate digestion are monosaccharides, mostly glucose, fructose, and galactose. Further absorption of monosaccharides occurs in the small intestine.

The *uptake of monosaccharides* produced by digestion of carbohydrates is accomplished via the secondary active transport (K, Na-ATPase).



Na^+/K^+ATPase

The transport of monosaccharides is dependent on Na^+ ions and is mediated by a special carrier, the Na^+ gradient being the driving force for the monosaccharide transport, like in the amino acid absorption. The absorption rates for separate monosaccharides (hexoses and pentoses) differ. Galactose is absorbed at the highest rate, followed by glucose. The absorbed monosaccharides are delivered from the intestinal wall into the portal vein, to the liver, and then are supplied in the blood to other tissues. In the liver, the rest of hexoses (galactose, fructose and mannose) are converted to glucose or its metabolites. In addition to the liver, the major consumers of glucose are the brain and skeletal muscles wherein glucose is utilized as an easily oxidizable source of energy. In the adipose tissue, glucose is employed for the synthesis of neutral fat. Usually, about 65% of glucose supplied from the intestine is consumed for oxidation in the cells (for generation of energy). Further 30% and 5% are used, respectively, in the syntheses of fat and glycogen. These percentages may vary, depending on the physiological state of the organism, age, and a number of other factors.

LECTURE 12

Subject: GLYCOLYSIS–ANAEROBIC OXIDATION OF CARBOHYDRATES

Energy in the cells is produced not only by oxidative phosphorylation (via the aerobic route), but also by degradation of nutrients, omitting the intervention of molecular oxygen. Such an oxygen-free, or anaerobic, route to energy generation is referred to as fermentation. Fermentation is a pristine and simplest mode of energy extraction as compared with the aerobic route. In the cells hexoses are the major source of energy generation by the anaerobic route. Here, of prime importance is D-glucose.

Degradation of glucose: glycolysis

In the cells of humans and other higher organisms (animals and plants) lactic acid fermentation, commonly referred to as *glycolysis* (from the Greek "*glykis*", sweet, and "*lysis*", degradation, decomposition) takes place. Thus, glycolysis is the anaerobic degradation of glucose to two molecules of lactic acid.

Separate steps of glycolysis are catalyzed by 11 enzymes. These form a chain of functionally interrelated enzymes (multienzyme functional system), in which the product of a reaction catalyzed by an enzyme serves as a substrate for the enzyme at the next step. Glycolytic enzymes are found outside the mitochondria, in the cell sap, where they either persist in a dissolved state, or are loosely bound to the endoplasmic reticulum membrane. Degradation of glucose is an exergonic process. The energy liberated in this process accumulates in the ATP phosphate bonds.

Separate reactions of glycolysis

1. *Phosphorylation of D-glucose.* This is very starting reaction that triggers the glycolysis.
2. *Isomerization of glucose-6-phosphate to fructose-6-phosphate.*
3. *Phosphorylation of fructose-6-phosphate to fructose-1,6-biphosphate.*

Phosphofructokinase is a "key" enzyme in the glycolysis, since, being the ratelimiting factor for the overrall process (due to reaction irreversibility), is controlled by various iso- and allosteric regulators.

4. Cleavage of fructose-1,6-biphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Fructose-1,6-biphosphate splits into two phosphotrioses; because of this, glycolysis was earlier referred to as the dichotomic route of glucose conversion.

5. *Interconversion of triose phosphates.*

6. *Oxidation of glyceraldehyde-3-phosphate to 1,3-biphodphoglycerate.*

7. *Transfer of phosphate group from 1,3-biphosphoglycerate onto ADP (first glycolytic phosphorylation).*

8. *Isomerization of 3-phosphoglycerate to 2-phosphoglycerate.*

9. *Dehydration of 2-phosphoglycerate to phosphoenolpyruvate.*

10. *Transfer of phosphate group from phosphoenolpyruvate onto ADP (second glycolytic phosphorylation).*

11. *Reduction of pyruvate to lactate.*

This reaction is catalyzed by concluding enzyme of glycolysis, *lactate dehydrogenase* (for short LDH).

In human and animal tissues, five lactate dehydrogenase isoenzymes occur whose composition is defined by the specific features of oxidative tissue metabolism. Isoenzymes LDH_1 and LDH_2 (H type, heart-muscle) are effective under aerobic conditions, when pyruvate undergoes a fast oxidation, rather than a reduction to lactate. In contrast, isoenzymes LDH_4 and LDH_5 (muscle, of M type) are effective under anaerobic conditions.

Glucose is transformed to lactate via the glycolytic process. Lactate is an "impasse" in the metabolic pathway, since it is incapable of participating in any biochemical process, except the reverse conversion to pyruvate. When lactate is accumulated in the cells, the intracellular medium pH may be disturbed and the glycolysis suppressed. For this reason, lactate is scavenged from the cells as the metabolic "ballast". However, in certain organs (for example, heart) it is amenable to oxidation and is used as an energetic material.

Energy balance and biological function of glycolysis

Glycolysis may be regarded as an internal redox process involving the formation of two molecules of $NADH_2$ at the glyceraldehyde-3-phosphate dehydrogenation step with the subsequent acceptance of hydrogen by two pyruvate molecules to produce lactate. The overall scheme for glycolysis is shown below:

$$\textbf{Glucose} + 2H_3PO_4 + 2ADP \rightarrow \textbf{2 lactate} + 2ATP + H_2O$$

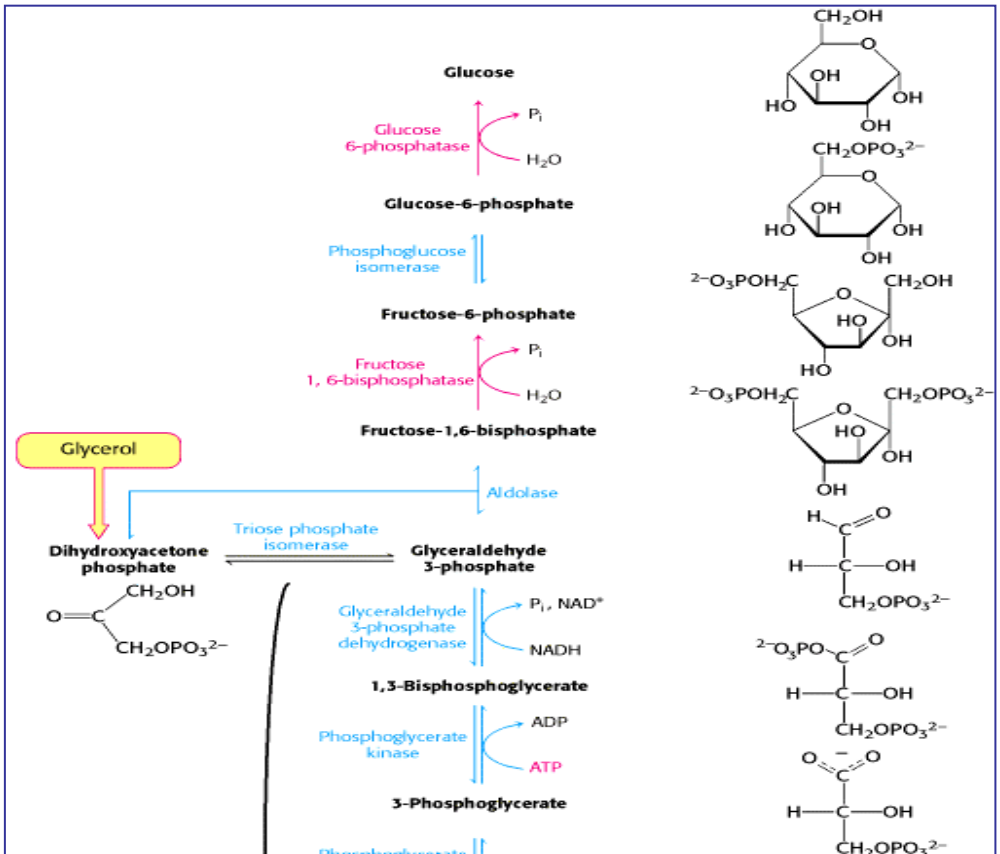


Figure 32. **Glycolysis**

At the steps of glucose and fructose phosphorylation (steps 1 and 3), two ATP molecules are consumed. Taking into account that the total of dihydroxyacetone phosphate (at step 5) is converted to glyceraldehyde-3-phosphate, i.e. the reaction yields a molecule of glyceraldehyde-3-phosphate more to the available one, the further route is actually a successive conversion of the two molecules of this phosphotriose. Therefore, at each of the two steps of glycolytic phosphorylation catalyzed by phosphoglycerate kinase and pyruvate kinase, two ATP molecules are formed. In total, the net yield in ATP is two molecules per molecule of split glucose. The energetic value of glucose is thus readily perceived.

In critical situations in the organism, when for some reasons the delivery or consumption of oxygen is hindered, glycolysis is the only way to urgent energy supply for sustaining the vital activity of the cells. Therefore, in hypoxia (reduction of oxygen supply to tissues), glycolysis plays an important energetic role. Glycolysis is operable in all cells and tissues; commonly, its potentiality is superior to the need in carbohydrate cleavage, i.e. glycolysis enzymes are present in the cells in excess. For the erythrocytes that lack mitochondria, glycolysis remains the only possibility to produce ATP and maintain the functional and structural integrity.

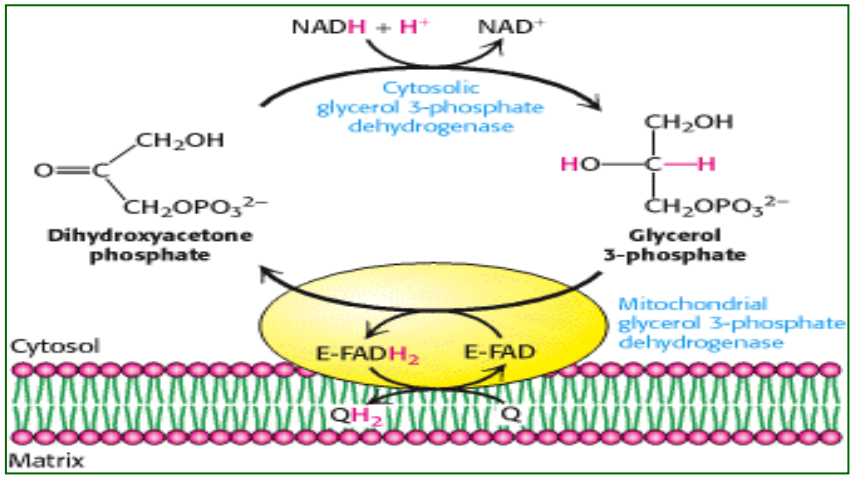


Figure 33. **Glycerophosphate shuttle mechanism**

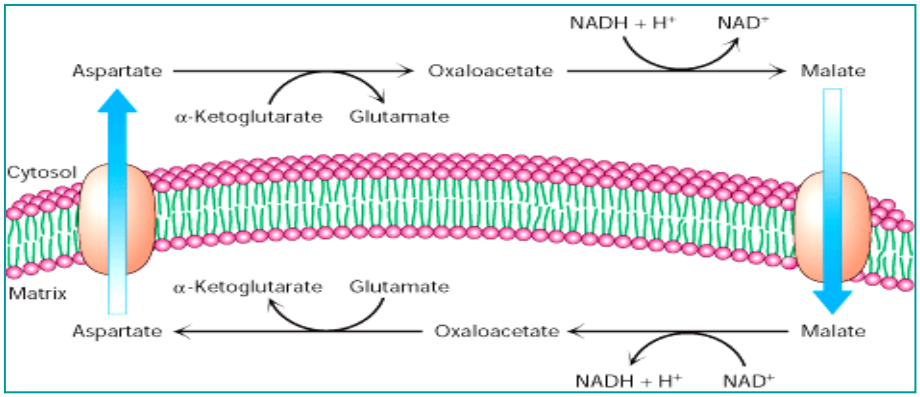
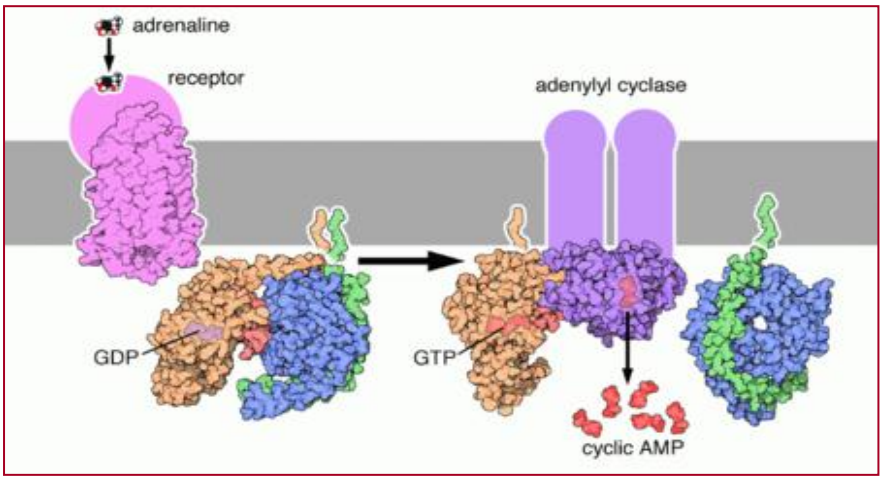


Figure 34. **Malate-aspartate shuttle mechanism**

The yield in energy under aerobic metabolism conditions amounts to 38 ATP molecules per molecule of split glucose provided the cytoplasmic $NADH_2$ hydrogen is supplied to the respiratory chain via malate-aspartate cycle (via glycerophosphate shuttle mechanism – 36 ATP).

Degradation of glycogen (glycogenolysis)

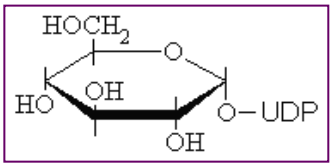
In the mammalian cells, glycogen is a reserve carbohydrate whose breakdown produces energy. The process by which glycogen is degraded is called *glycogenolysis*. The breakdown of glycogen is accomplished via *phosphorolysis* and *hydrolysis*. Phosphorolysis of glycogen is carried out through the aid of glycogen phosphorylases and oligo-1,6-glycosidase, an accessory enzyme in this process. Mechanism for phosphorylation of *phosphorylase B* (according to Sutherland):



Biosynthesis of glycogen (glycogenogenesis)

Synthesis of glycogen is carried out in all cells of organism, but this process is especially active in skeletal muscles and in the liver. Two routes for glycogen synthesis are possible. One route involves a successive addition of glucose units to the extant glycogen moiety (glycogen primer); the other one originates in glucose molecules. The source of glucose, uridine diphosphate glucose (UDP-glucose), which is produced from glucose-1-phosphate and uridine triphosphate (UTP) through the agency of the enzyme *glucose-1-phosphate uridyltransferase* according to the scheme:

Glucose-1-phosphate + UTP → UDP-glucose + $H_4P_2O_7$



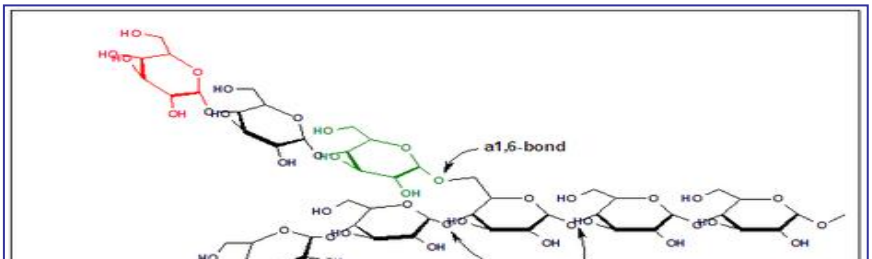
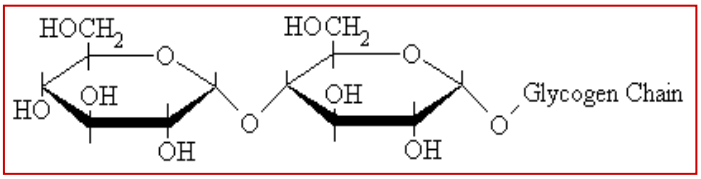


Figure 35. **Structure of glycogen**

The next step involves a transfer of the glucose residue from UDP-glucose onto the glycogen primer through the aid of the enzyme *glycogen synthetase*:

UDP-glucose + $(Glucose)_n \rightarrow$ UDP + $(Glucose)_{n+1}$



To be noted, the glycogen synthetase catalyzes the formation of α-1→4 glycoside bonds only.

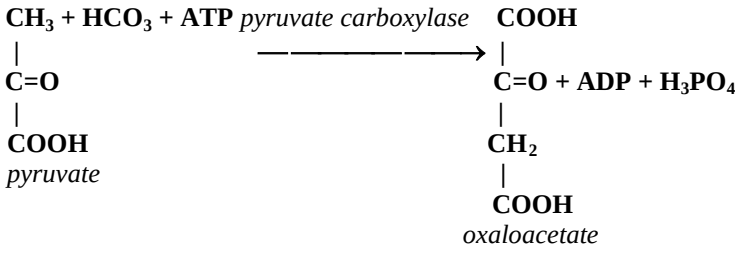
The "branching" enzyme, amylo-(α-1,4→α-1,6)-trans-glycosylase, transfers short fragments (two or three glucose residues) from one portion of the glycogen molecule onto another and forms α-1→6-glycosidic bonds (branch points). The alternating action of these two enzymes results in the lengthening of the glycogen molecule.

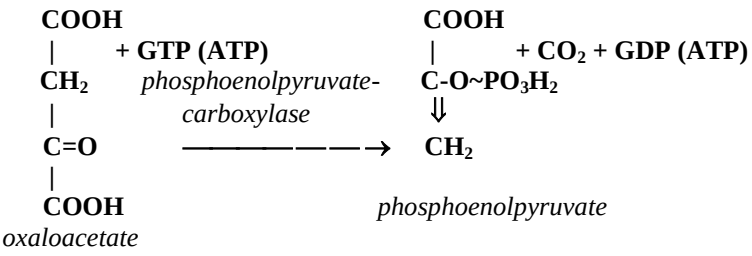
Gluconeogenesis

The synthesis of glucose from noncarbohydrate sources is referred to as the *gluconeogenesis*. It is feasible only in certain organism tissues. The major site for gluconeogenesis is the liver.

Mechanism for gluconeogenesis. Since the glycolysis involved three energetically irreversible steps at the pyruvate kinase, phosphofructokinase, and hexokinase levels, the production of glucose from simple noncarbohydrate materials , for example, *pyruvate* or l*actate*, by a reversal of glycolysis ("from bottom upwards") is impossible. Therefore, indirect reaction routes are to be sought for:

1. ***The first indirect route*** in glucose synthesis involves the formation of phosphoenolpyruvate from *pyruvate* without the intervention of pyruvate kinase. This route is catalyzed by two enzymes:





2. ***The second indirect route*** involved the formation of fructose-6-phosphate from fructose-1,6-biphosphate without the intervention phosphofructokinase reaction. This route is catalyzed by *fructose biphosphatase:*

fructose-1,6-biphosphate + H_2O → fructose-6-phosphate + H_3PO_4

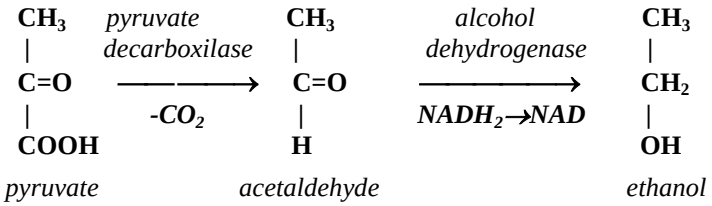
3. ***The third indirect route*** involves the formation of free glucose from glucose-6-phosphate by circumventing the hexokinase reaction. This route is catalyzed by glucose-6-phosphatase:

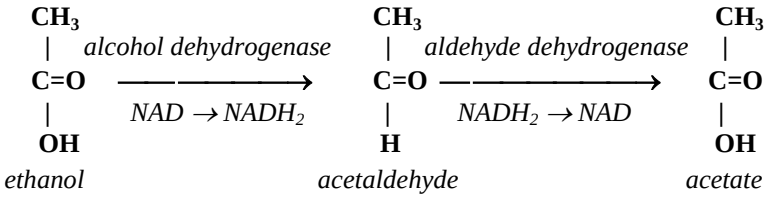
glucose-6-phosphate + H_2O → glucose + H_2O

The free glucose produced by this reaction is supplied to the blood from the tissues. Amino acids involved in the gluconeogenesis are referred to as *glycogenic amino acids* (for example, alanine, serine, cysteine, etc.).

Alcoholic fermentation

This route of carbohydrate fermentation diverges from the glycolysis route at the step of pyruvate conversion:





Alcoholic fermentation is typical of yeast and certain microorganisms. Alcohol dehydrogenase, present in human tissues (especially in liver) oxidizes ethanol to acetalaldehyde; the latter becomes involved in the metabolism through *aldehyde dehydrogenas*e.

Acetate enters the Krebs cycle in an activated form:

Acetate + CoA-SH + ATP → *acetyl-coA synthetase* **→ acetyl ScoA +AMP +PP**

LECTURE 13

Subject: PENTOSE PHOSPHATE CYCLE. CARBOHYDRATE METABOLISM CONTROL IN THE HUMAN ORGANISM

There is known one more catabolic route for carbohydrates commonly referred to as the ***pentose phosphate cycle*** (also called *hexose monophosphate shunt,* or *phosphogluconate pathway).* The pentose phosphate cycle represents a multienzyme system in which the important intermediates are, the name implies, pentose phosphates. This cycle may be regarded as a branching, or shunt, at the glucose 6-phosphate step in the overall glycolysis. To provide for all steps of the pentose phosphate cycle, at least three glucose-6-phosphate molecules are required.

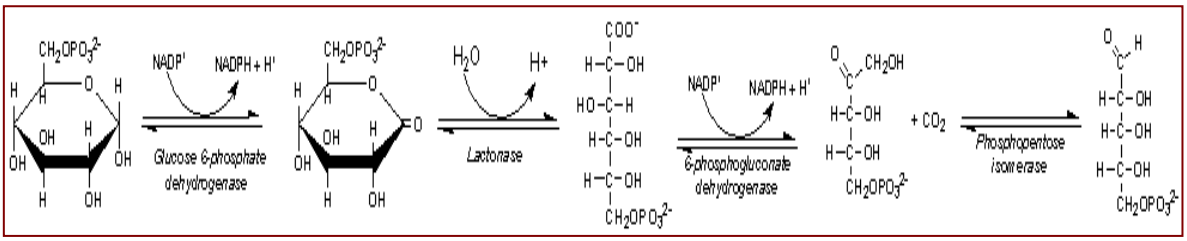


Figure 37. **Reactions of pentose phosphate cycle (PPC)**

Then fructose-6-phosphate and glyceraldehyde-3phosphate produce and enter the glycolysis. Thus, in the course of reactions catalyzed by the intrinsic enzymes of the pentose phosphate cycle, two fructose-6-phosphate molecules, one glyceraldehyde-3-phosphate molecule, and three carbon dioxide molecules are produced from three glucose-6-phosphate molecules. In addition, six $NADPH_2$ molecules are formed. The overall scheme for the pentose phosphate cycle is:

3 Glucose-6-phosphate + $6NADP^+$ →
2 Fructose-6-phosphate + glyceraldehyde-3-phosphate + $6NADPH_2$ + CO_2

Interrelation of the pentose phosphate cycle and glycolysis

These two pathways for carbohydrate conversion are closely related. The products of the pentose phosphate route – fructose-6-phosphate and glyceraldehyde-3-phosphate – are likewise glycolysis metabolites; for this reason, they are involved in glycolysis and undergo conversion by glycolytic enzymes. Two molecules of fructose-6-phosphate can regenerate to two glucose-6-phosphate molecules through the agency of the glycolytic enzyme glucose-phosphate isomerase. Here, the pentose phosphate pathway functions as a cycle. The other product, glyceraldehyde-3 phosphate, enters the glycolysis to be either converted to lactate (under anaerobic conditions) or oxidized to CO_2 and H_2O (under aerobic conditions). As can easily be estimated, the conversion of glyceraldehyde-3-phosphate to lactate leads to the formation of two ATP molecules, while the combustion to CO_2 and H_2O produces 20 ATP molecules. It follows there for that under physiological conditions, when the pentose phosphate pathway for carbohydrate conversion is included in the glycolysis, the overall process of glucose-6 phosphate conversion may be expressed via the pentose phosphate cycle.

Under anaerobic conditions:

3 glucose-6-phosphate + $6NADP^+$ + $2H_3PO_4$ →
→ 2 glucose-6-phosphate + lactate + 2 ATP + $6NADPH_2$ + $3CO_2$

Under aerobic conditions:

3 glucose-6-phosphate + $6NADP^+$ + 20ADP + $20H_3PO_4$
→ 2 glucose-6-phosphate + $6NADPH_2$ + $6CO_2$ + $6H_2O$ + 20ATP

At the first glance, the energetic value of this conversion of glucose-6-phosphate via the pentose phosphate cycle appears to be inferior to that of the aerobic glycolysis pathway, the latter providing a maximum of 38 ATP molecules. However, it should be borne in mind that a major portion of energy is stored in $NADPH_2$, and 6 $NADPH_2$ molecules are energetically equivalent to 18 ATP molecules. Consequently, the energetic effect remains the same.

The biological function of the pentose phosphate cycle

The biological function of the pentose phosphate cycle involves the production of two compounds: $NADPH_2$, which is a "*reductive force*" in the synthesis of various materials, and the metabolite ribose-5-phosphate, which is used as a building material in the synthesis of various species. The ***major functions*** of the pentose phosphate cycle are:

- *amphibolic function:* the cycle is a route to degradation of carbohydrates and, simultaneously, to the supply of materials used in synthetic reactions ($NADPH_2$ and ribose-5-phosphate);
- *energetic function,* since the involvement of pentose phosphate cycle products (glyceraldehyde-3-phosphate) in the glycolysis produces energy;
- *synthetic function,* as a major function associated with the use of $NADPH_2$ and ribose-5-phosphate.

$NADPH_2$ is used*:*

- in the detoxification of drugs and poisons in the monooxygenase oxidation chain of the endoplasmic reticulum of the liver;
- in the synthesis of fatty acids and other structural and reserve lipids;
- in the synthesis of cholesterol and its derivatives – bile acids, sterol hormones (corticosteroids, female and male sex hormones), and vitamins D;
- in the neutralization of ammonia under reductive amination.

Ribose-5-phosphate is used in the synthesis of histidine, nucleosides and nucleotides (nucleotide mono-, di-, and triphosphates), nucleotide coenzymes (NAD, NADP, FAD, and HS-coA), and polymeric nucleotide derivatives (DNA, RNA, and short-chain oligonucleotides). A high activity of this pathway is observed in fat tissue, liver mammary gland (especially during lactation), adrenal glands, gonad glands, marrow, and lymphoid tissue. Relatively high is the activity of pentose phosphate shunt dehydrogenases in the erythrocytes. A low activity of the pentose phosphate pathway is observed in muscular tissue (heart and skeletal muscle).

Carbohydrate metabolism control in the organism

From the standpoint of activity of the whole organism, certain specializations of the carbohydrate metabolic routes in individual tissues are profitably complementary. For example, strenuous muscular exertion requires energy which is initially supplied by the breakdown of glycogen to lactic acid. The latter compound is excreted into the blood to be supplied to the hepatic tissue, where it is used for the synthesis of glucose during gluconeogenesis. From the liver, glucose is delivered in the blood to the skeletal muscles to be consumed for energy generation and to be deposited as glycogen. This intertissue (or interorgan) cycle in the carbohydrate metabolism is referred to as the *Cori cycle* (called also glucose-lactate cycle).

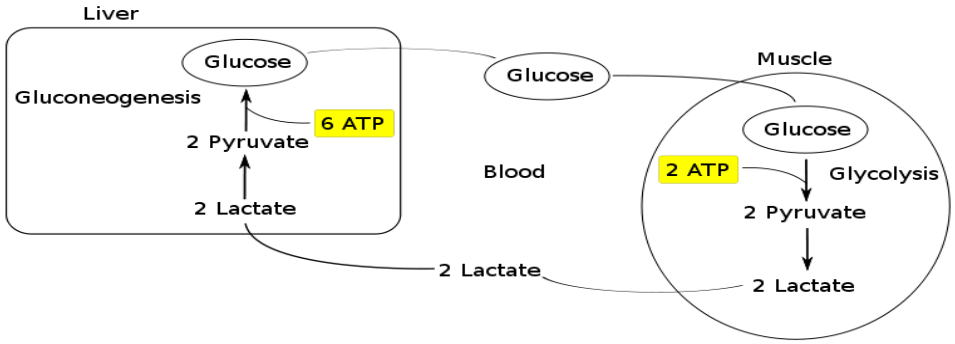


Figure 38. **Cori cycle**

The maintenance of a constant glucose level in the blood is of primary importance for the organism, since glucose is the major energy substrate for the nervous tissue. The normal glucose content in the blood is 3,3 to 5,5 mmol/liter.

An increased concentration of glucose in blood is known as *hyperglycemia*. If hyperglycemia reaches as high as 9 to 10 mmol/liter, the glucose excess is released into the urine, i.e. *glucozuria* sets in. On the contrary, a decreased glucose percentage in the blood is known as *hypoglycemia*. Hypoglycemia as low as about 1,5 mmol/liter leads to the syncopal state, while a still lower glucose concentration results in high excitability of the nervous system and ultimately leads to convulsing and coma.

Processes leading to hyperglycemia:

- absorption of glucose from the intestine (alimentary hyperglycemia);
- breakdown of glycogen to glucose (commonly, in liver)
- gluconeogenesis (in liver and kidney).

Processes leading to hypoglycemia:

- transport of glucose from the blood to tissue followed by glucose oxidation to end products;
- synthesis of glycogen from glucose in liver and skeletal muscles;
- production of triacylglycerol from glucose in fat tissue.

The dietary intake of carbohydrates leads to a short-term (within 1 or 2 hours) hyperglycemia and, occasionally, glucosuria. The glucose level in the blood is monitored by neuron-hormonal nervo-

us system increases the glucose level in the blood, while excitation of the parasympathetic portion produces a reverse effect. The only hormone capable of reducing the glucose content is *insulin.* It stimulates all of the three processes of glucose assimilation (intracellular transport and degradation of glucose, synthesis of glycogen, and synthesis of triglyceride from glucose in fat tissue). All other hormones make the glucose level increase; for this reason, they are occasionally referred to as *contrainsular* hormones. These include adrenalin, glucagon, thyroxin and triidothyronin, somatotropin (which stimulate glycogen degradation), and glucocorticoids (which stimulate gluconeogenesis).

Pathology of carbohydrate metabolism. Patients with *diabetes mellitus* type I have the deficiency of active insulin, and patients with *diabetes mellitus* type II have the deficiency of number receptors for insulin on the cell membranes. Patients with "steroid" diabetes mellitus have hyperproduction of glucocorticoids (cortisol).

In diabetes mellitus take place the changes in carbohydrate, lipid, protein, and water-mineral metabolisms. All patients with different types of these diseases have the character disturbances of metabolism: hyperglycemia, glucosuria, ketonemia, ketonuria, hyperaminoacidemia and hyperaminoaciduria, increased concentrations of fatty acids, glycerol, and cholesterol in blood. Insulin preparations are used in the therapy of diabetes mellitus.

Enzymopathies of carbohydrate metabolism. Enzymopathies associated with an impaired glycogen metabolism occur rather frequently. They manifest themselves either in glycogen accumulation in the organs (accumulation diseases), or in lack of glycogen. The enzymopathies that lead to glycogen deposition are called *glycogenoses,* and those, preventing glycogen deposition, *aglycogenoses.* Depending on the site of preferential glycogen deposition, three disease forms are distinguished: *hepatic, muscular,* and *generalized* (Gierke's disease, Pompe's disease, Hardy's disease, etc.*).*

Galactosemia is a molecular disease evoked by a defective *galactose-1-phosphate-uridyl transferase.*This disease leads to the accumulation of galactose-1-phosphate, which, when in norm, rapidly converts to uridine diphosphate galactose with subsequent involvement in the glucose conversion pathway. Galactose-1-phosphate is toxic for the human organism. Since galactose (which makes part of lactose) is consumed in large quantities by the infant from the breast milk, the organism of an infant suffering from galactosemia rapidly accumulates this toxic galactose derivative. The infant loses in weight, its mental and physical development becomes retarded, its liver increases in size, and the lenticular opacity develops. If not withdrawn from the breast milk feeding, the infant usually dies from alimentary toxicosis. In patients with galactosemia, galactose must be excluded from the diet.

LECTURE 14

Subject: LIPIDS: CLASSIFICATION, CHEMICAL STRUCTURE AND DIGESTION OF LIPIDS

Lipids are organic compounds of biological nature, insoluble in water but soluble in nonpolar solvents such as chloroform, ether, or benzene. The main features enabling assignment of a compound to the lipid class are: biological origin, hydrophobicity, solubility in nonpolar liquids and insolubility in water, occurrence of higher alkyl radicals or carbohydrates.

Classification of lipids. A number of classifications have been proposed for lipids on the basis of their *structural, physico-chemical, biological,* or physiological properties. The most sophisticated classification appears to be the *structural* one, which takes into account structural features of lipid compounds.

Simple molecules:

- *glycerides* (acylglycerides),
- *waxes,*
- *steroids.*

Complex lipids (heterolipids):

- *phospholipids,*
- *glycolipids,*
- *sphingolipids.*

The differentiation of lipids according to their *physico-chemical* properties takes into account the extent of polarity exhibited by the lipid molecules. By this property, the lipids are classified into *neutral* (or nonpolar) and *polar* species. Lipids possessing no charge belong to the former type. The latter

type includes charged lipids exhibiting distinct polar properties (for example, phospholipids and fatty acids).

According to their *physiological* importance, the lipids are divided into *reserve lipids* and *structural lipids.* The reserve lipids are stored in large amounts and then consumed to supply the energetic requirements of the organism. Acylglycerides rank among reserve lipids. The former are primarily used in the buildup of biological membranes, covering (protective) layers in plants, insects, and skin in vertebrates.

Lipids account for about 10-20% of the total mass of the human organism. The body of an adult human contains on the average 10 to 12 kg of lipids, of which the structural lipids (major constituents of biomembranes) account for 2 or 3 kg; the rest are reserve lipids. About 98% of the latter are deposited in adipose tissues. Among the tissues of the organism, the structural lipids are distributed nonuniformly. The nerve tissue is the most rich one is structural lipids (to 20-25%). In biomembranes of the cell, the lipids make up about 40% (by dry weight).

Simple lipids

Glycerides, or ***acylglycerides,*** constitute the most widespread group of simple lipids. By their chemical structure, they are esters of fatty acids with a tribasic alcohol, *glycerol.* Glycerides (acylglycerides) are also called *neutral lipids* because of their rather inert character. Glycerides are divided into mono-, di-, and triacylglycerides containing one, two, and three acyl groups (R-CO-), respectively:

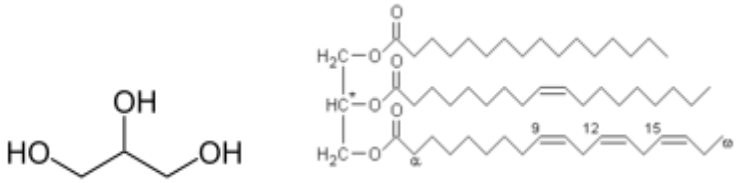


Figure 39. **Chemical structures of glycerol and triglycerides**

The name for a neutral lipid is derived from the name of the constituent fatty acid and the ending "glyceride". For example, palmitoylglyceride is a monoglyceride containing a palmitic acid moiety; tripalmitoylglyceride is a triglyceride containing three identical acyl residues.

Differentiated are simple acylglycerides containing residues of the same fatty acid (as typified by the above case), and complex acylglycerides composed of residues of two or three different fatty acids. Natural neutral lipids are mixtures of simple and complex glycerides with a prevalence of unsaturated triglycerides.

$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**$(CH_2)_7COOH$

Linoleic acid

$CH_3(CH_2)_4$**CH=CH**CH_2**CH=CH**CH_2**CH=CH**CH_2**CH=CH**$(CH_2)_3COOH$

Arachidonic acid

$CH_3(CH_2)_7$**CH=CH**$(CH_2)_7COOH$

Oleic acid

Physico-chemical properties of neutral lipids are largely determined by their composition. The melting points are higher in acylglycerides containing higher saturated acyl groups. Saturated fatty acids (for example, tristearin) are found, as a rule, in solid fats, and in liquid fats, unsaturated acids (for example, triolein). All acylglycerides are less dense than water. They are soluble in nonpolar solvents (chloroform, benzene, ether, and hot ethanol). Only mono- and diacylglycerides containing free polar hydroxyl groups are water-soluble. They form micelles in water. Triacylglycerides are insoluble in water and are incapable of micellation. When subjected to basic hydrolysis or saponification, acylglycerides regenerate glycerol and free fatty acids. In the organism, acylglycerides are hydrolyzed by special enzymes, *lipases.*

Waxes are mixtures of ethers and esters derived from higher monobasic alcohols and higher fatty acids. High hydrophobicity of waxes form a waterrepellent protective layer (grease) on the leaves and fruit of plants, skin and hair of animals, feathers of birds, and the external skeleton of insects. They constitute part of honey wax.

Sterids are also known as of frequent occurrence. They are contained in products of animal origin (butter and egg yolk). In human and animal organisms, most of the tissue cholesterol (about 60-70%) occurs as cholesterol esters. In blood, cholesterol esters as constituents of transport lipoproteins make up the major content of the overall cholesterol. It is quite probable that cholesterol esters provide for a specific form of storing cholesterol in tissues.

Lanolin (wool fat) is also of sterid origin (a mixture of fatty acid esters of lanosterol and agnosterol) and is widely used in the pharmaceutical industry as an ointment base. Sterids such as fatty acid esters of stigmasterol, ergosterol, and β-sitosterol constitute a significant part of overall sterols inplants.

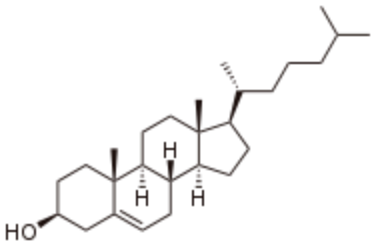


Figure 40. **Chemical structure of cholesterol**

Cholesterol has been classified as a structural lipid. It forms part of biological membranes of the cells with a predominant content in the plasmatic (cellular) membrane rather than in other membranes (those of mitochondria, microsomes, nuclei, etc.).

Among the steroid compounds of animal and plant origin, the following biologically active cholesterol derivatives should be mentioned bile alcohols, bile acids, steroid hormones, D vitamins, steroid glycosides, steroid alkaloids. The most widespread bile acids in the human bile are *cholic* and *chenodeoxycholic acids.*

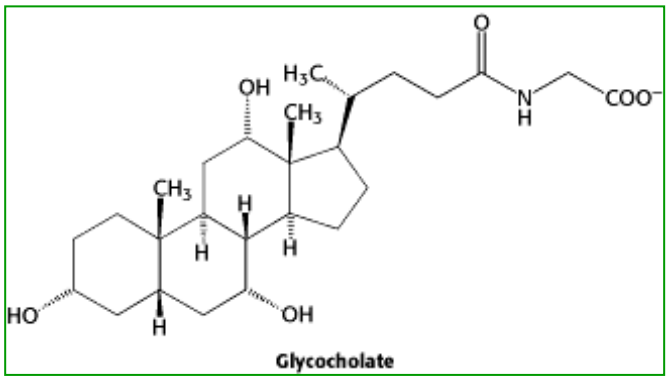


Figure 41. **Chemical structure of bile acid: glycocholic acid**

In liver these acids react easily with glycine and taurine to form coupled compounds, or conjugates, for example, *glycocholic* and *taurocholic acids.*

In the intestine and bile, the bile acids occur as anions (bile salts). Viewed structurally, the bile acids are amphipathic molecules, since they possess a hydrophobic ring and hydrophilic groups, viz. hydroxyl properties and favour thereby the emulgation of lipids in the intestine. The bile acids are essential for normal digestion and absorption of lipids in the intestine. They also assist in the absorption of fat-soluble vitamins in the digestive tract. Sparing solubility of bile acids is the reason for the formation of gallstones (in humans, the bile acids account for about 1% of the gallstone composition).

Complex lipids or heterolipids

Complex lipids (heterolipids), as distinct from simple lipids, contain a nonlipid component, which may be a phosphate, carbohydrate, etc. According to their chemical structure, the representatives of complex lipids, i.e. phospholipids, glycolipids and sphingolipids, belong to molecular species with a higher level of structural organization.

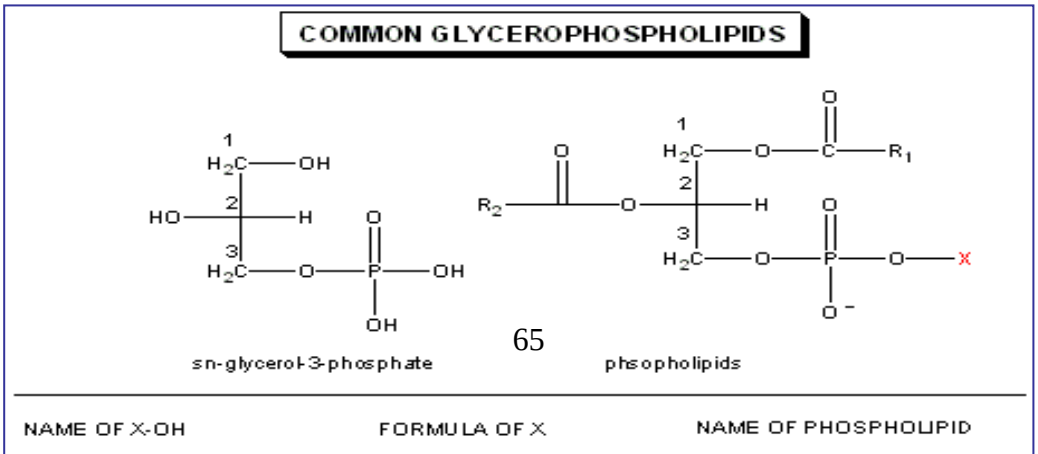


Figure 42. **Phospholipid structure**

Phospholipids are complex representative of phosphate-substituted esters of diverse organic alcohols (glycerol, sphingosines, and diols). All of the phospholipids are polar lipids and are predominantly contained in the cell membranes. In the fat depots, their occurrence is insignificant.

Phosphoglycerides. In phosphoglycerides, one of the hydroxyl groups forms an ester bond with a phosphate, rather than with a fatty acid.

Phosphatidic acid is the simplest representative of naturally occurring phosphoglycerides. Phosphatidylethanolamines and phosphatidylcholines are very widespread phosphoglycerides present in the membranes of all types. They account for 20% and 50% of the cell membrane lipids. Plasmalogens are especially abundant in the brain and spinal marrow, constituting 50-90% of the whole of lipids. A specific feature of plasmalogen structure is the formation of an ether bond with a higher aliphatic aldehyde.

Glycolipids are complex lipids containing a carbohydrate component. They include the simplest glycolipids, *glycosyldiacylglycerols,* in which one hydroxyl group of the glycerol moiety is replaced by a monosaccharide. Glycoshpingolipids occur in the nerve cells where they appear to be essential for the normal electric activity and transmittance of nervous impulses. Cerebrosides, gangliosides, and sulpholipids belong to these lipids.

Cerebrosides were first isolated from the brain (cerebrum), to which they owe their name. Their carbohydrate moiety is most commonly represented by galactose or, very rarely, by glucose. Fatty acids forming part of cerebrosides are quite numerous and include lignoceric (n-tetracosanoic), cerebronic (2-hydroxytetracosanoic), nervonic, and hydroxynervonic fatty acids.

Sulpholipids are sulphated derivatives of cerebrosides. The sulphate moiety is added to the third hydroxyl group of galactose, sulpholipids exhibit pronounced acidic properties and are capable of easily binding cations. Presumably, they participate in the cation transport across the membranes of nervous cells and fibers. Therefore, sulpholipids are necessary for the normal electrical activity of the nervous system.

Gangliosides, in contrast to other glycosphingolipids, contain an olygosaccharide composed of different monosaccharides. Ganglioside components are diversified and their masses vary over a wide range. The cerebral cortex cells are rich in gangliosides.

***Sphingolipids* (*sphingophosphatides*)**. In the group of sphingolipids, sphingosine is the alcohol entity. Its N-acylated derivative, *ceramide,* is the ancestor for all of the sphingolipids. Sphingolipids are divided into *sphingomyelins,* or *sphingophosphatides,* and *glycosphingolipids.*They differ in that the sphingomielins, like all phospholipids, have the hydroxyl group substituted by a phosphate or a residual alcohol (mostly choline), while glycolipids bear, in place of the phosphate group, a monosaccharide (in cerebrosides) or an oligosaccharide (in gangliosides) containing galactose and a number of aminosaccharides.

Sphingomyelins are contained in the nerve tissue in large amounts and form part of myelin which serves as a material for the nerve fibre sheath. They have been found in the lung, liver, kidney, spleen, blood, and other organs. A special period, the so-called *myelinization period,* is singled out during the

human and animal brain development. In man, the myelinization period, i.e. the formation of sphingomyelin in the nerve sheath and nervous system, starts immediately after the birth and continues for about four months.

In large amounts sphingomyelins are found in the blood plasma and the erythrocyte envelope: 8-15% and 30-40% of the total lipid mass, respectively. This appears to be associated with the function of erythrocytic membranes.

Biological functions of lipids are determined by their structural and physicochemical properties.

Digestion mechanism for lipids

Digestion of lipids takes place in the sections of digestive tract where the following necessary conditions have been provided for:

1. Availability of lipolytic enzymes capable of hydrolyzing lipids.
2. Conditions providing for emulsifying lipids.
3. Optimal pH of medium for the action of lipolytic enzymes (the medium should be neutral or weakly alkaline).

All of these conditions are provided for in the intestine of adult human. In infants, especially newborns, similar conditions are created for digesting milk triacylglycerides by gastric lipase. The medium pH for the infant's stomach contents is about 5,0 (weakly acidic medium), and milk fat occurs in an emulsified state; therefore fat is amenable to cleavage by lipase to a certain extent. In the adult man, a strongly acidic medium inactivates the gastric lipase.

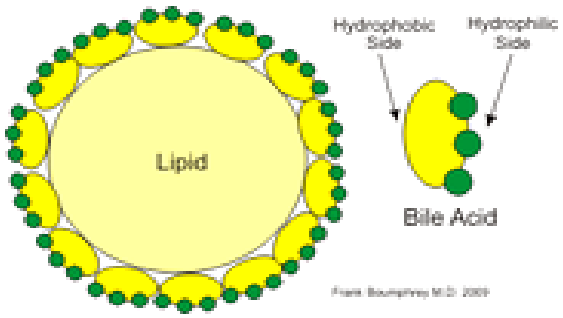
In the intestine, the food delivered from the stomach becomes neutralized, and the fat undergoes emulsification. Emulsification of lipids is effected by bile acids supplied to the intestine as bile components. The major bile acids contained in the bile are: *cholic* and *chenodeoxycholic acids* and their *glycine* and *taurine* derivatives: *glycocholic* and *taurochenodeoxycholic acids.*

Bile acids perform biological functions:

- as emulsifying agents;
- as activators of lipolytic enzymes;
- as transport means, since, by forming a transport complex with fatty acids, bile acids facilitate the intestinal absorption of the former. All bile acids are amphiphilic compounds and therefore exhibit emulsifying properties.

Bile acid molecules adsorbed at the fat/water interface prevent the phase separation. The intestinal peristalsis facilitates dispersion of large fat droplets, while the bile acid molecules retain finely dispersed fat particles on a suspended state and prevent their coalescence. Free fatty acids and monoacylglycerides produced by digestion of lipids, dietary phospholipids, and their partly digested products (phosphatidylcholine) are auxiliary emulsifying agents.

Hydrolysis of triacylglycerides which constitute the major mass of dietary lipids is effected by the action of *pancreatic lipase.* The lipase is supplied in an inactive form. In the intestine, it is activated by a special cofactor, *colipase,* and by bile acids. The activated lipase acts on the triacylglycerides of a



Action of bile salts in digestion

suspended fat droplet. The enzyme itself is dissolved in the aqueous phase; nonetheless, it splits a substrate found in the lipid phase. Lipase possesses a special hydrophobic site (head) which comes into contact with triacylglyceride. The hydrolyzates are mostly 2-monoacylglyceride and free fatty acids.

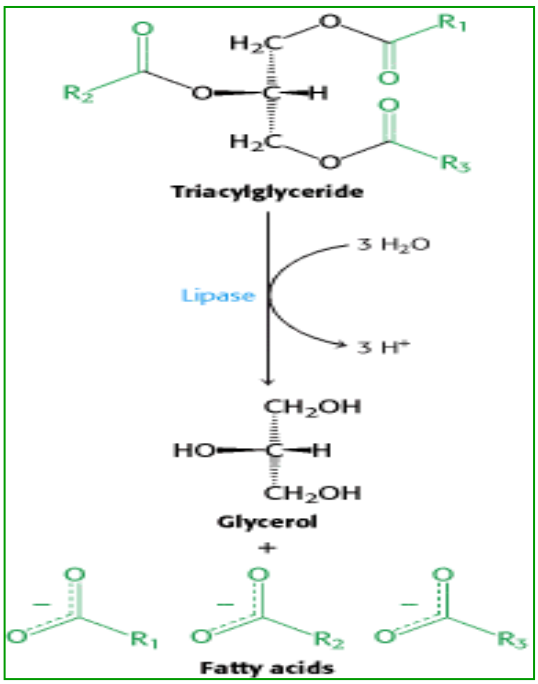


Figure 43. **Reaction of TG hydrolysis by lipase**

The intestinal and pancreatic juice carboxyesterases split 2-monoacylglyceride into a free acid and glycerol. The hydrolysis of triacylglycerides is assisted by calcium ions that form complexes with free fatty acids.

Hydrolysis of phospholipids is carried out by a group of lipolytic enzymes called *phospholipases.* A variety of phospholipases are known, denoted A_1, A_2, C and D. They hydrolyze various bonds in the phospholipid molecule, as exemplified by phosphatidylcholine.

Activation of phospholipase A_2 occurs in the intestinal juice, via splitting a hexapeptide from the proenzyme by trypsin. Besides, the intervention of bile acids and calcium ions is needed for the activity of phospholipase A_2 and other phospholipases. Bile acids assist in the approach of a substrate to the active centre of enzyme, while calcium ions remove free fatty acids from the site of enzymic activity (as in the case of lipase) and prevent inactivation of phospholipase.

The action of phospholipase A_2, which is the chief digestive phospholipase, results in the formation of extremely toxic lysophosphatides that are immediately hydrolyzed by *lysophospholipase*.

The end products of hydrolysis are glycerol, fatty acids, an inorganic phosphate and a residual alcohol (choline, ethanolamine, inositol, or serine).

Hydrolysis of steroid esters. Dietary cholesterol esters which are abundant in certain food products (egg yolk, butterfat, caviar, etc.) are hydrolyzed in the emulsion droplets of intestinal contents by *pancreatic cholesterolesterase*. This enzyme is also activated by bile acids. The enzymic hydrolysis leads to free cholesterol and fatty acids. The hydrolyzates of all dietary lipids are absorbed in the intestine.

The uptake of lipid hydrolyzates and their transport. The absorption of digested lipid products exhibits a number of specific features. For example, the uptake of fatty acids depends on the carbon chain length. Short-chain fatty acids (to 10-12 carbon atoms) are transported into the intestinal epithelium by simple diffusion. Long-chain fatty acids (over 14 carbon atoms) form transport complexes with bile acids. The complexes thus formed are called *choleinic acids.* Therefore, the fatty acids pass across the intestinal epithelium membrane in a complexed state. This kind of transport may be regarded as a facilitated transport in which bile acids act as carriers. Within the intestinal wall, the choleinic complexes dissociate, and the bile acids are excreted into the blood of portal vein and transferred to the liver. From the liver, the bile acids return with bile to the intestine. This turnover is referred to as the *hepatoenteric circulation of bile acids.*

Besides, easily absorbed are glycerol, phosphates (as sodium and potassium salts), choline and other alcohols, sphingosine and cholesterol.

Glycerol and fatty acids are the starting materials for the resynthesis of triacylglycerides. The rest of phosholipids, all the triacylglycerides, free and esterified cholesterol are transported in the lymph. The intestinally resynthetized lipids are transported as constituents of *chylomicrons.* From the thoracic

lymphatic duct, chylomicrons are supplied into the blood which becomes milky turbid and distinctly opalescent (such a blood plasma is called lipemic). In blood, chylomicrons or more exactly, their constituent triacylglycerides are split by *lipoprotein lipase.* This enzyme hydrolyzes the chylomicron triacylglycerides into glycerol and fatty acids.

LECTURE 15

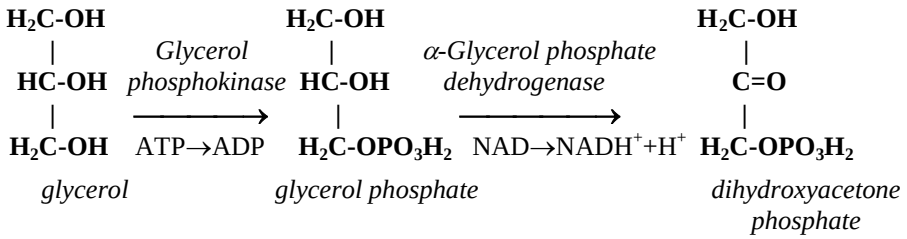
Subject: **CATABOLISM OF LIPIDS IN TISSUES**

Hydrolysis of triacylglycerides in tissues is effected by a tissue enzyme, *triacylglyceride lipase,* which hydrolyzes triacylglycerides to glycerol and free fatty acids. There are a variety of tissue lipases that differ primarily in their optimum pH and their location in the cell. The acidic lipase is contained in lysosomes; the basic lipase, in microsomes; and the neutral lipase, in cytoplasm. A specific feature of the tissue lipase is its sensitivity to hormones which, by activating adenylate cyclase, elicit the transition of the inactive tissue lipase to its active form via phosphorylation with protein kinase. Lipases mobilize triacylglycerides. This process is also known as the tissue *lipolysis.*

The cell membrane phosphoglycerides are hydrolyzed with phospholipases A_1, A_2, C, and D which are located chiefly in lysosomes. In the adipose tissue, glycerol and fatty acids as produced by triglyceride hydrolysis are not subject to oxidation and are released into the blood to be supplied to other organs.

Oxidation of glycerol

The glycerol metabolism is closely related to the glycolysis involving glycerol metabolites according to the following scheme:



At first, glycerol converted to glycerol phosphate through the agency of *glycerol phosphokinase.* Glycerol phosphate, by action of NAD-dependent *glycerol phosphate dehydrogenase,* is converted to dihydroxyacetone phosphate, which, as a common glycolysis metabolite, enters glycolysis to be reduced by enzymes to lactate under anaerobic conditions, or to CO_2 and H_2O under aerobic conditions. Conversion of one glycerol molecule yields one ATP molecule under anaerobic and 22 ATP molecules, under aerobic conditions. Glycerol is a profitable energy substrate and it used as an energy source practically by all organs and tissues.

Oxidation of fatty acids

Oxidation of higher fatty acids was first studied in 1904 by *Knoop*. Knoop coined the fatty acid oxidation mechanism as β-oxidation.

The fatty acids, as produced by intracellular hydrolysis of triacylglycerides or supplied to the cell from the blood, must be brought into a state of activation. Their activation is effected in the cytoplasm with the participation of *acyl-coA synthetase:*

$$\text{R-CH}_2\text{-CH}_2\text{-COOH+ATP + coA-SH} \xrightarrow{\textit{acyl-coA synthetase}} \text{R-CH}_2\text{-CH}_2\text{-CO\sim ScoA+ AMP +P}$$

Since the activation process is effected extramitochondrially, transport of acyls across the membrane into the mitochondria is necessary. The transport is accomplished with the participation of *carnitine,* which takes up the acyl from acyl-coA on the outer membrane side. Acylcarnitine assisted by carnitine translocase diffuses to the inner side of the membrane to give its acyl to the HScoA located in the matrix. The process of reversible acyl transfer between HScoA and carnitine on the outer and inner sides of the membrane is effected by the enzyme *acyl-coA carnitine transferase.*

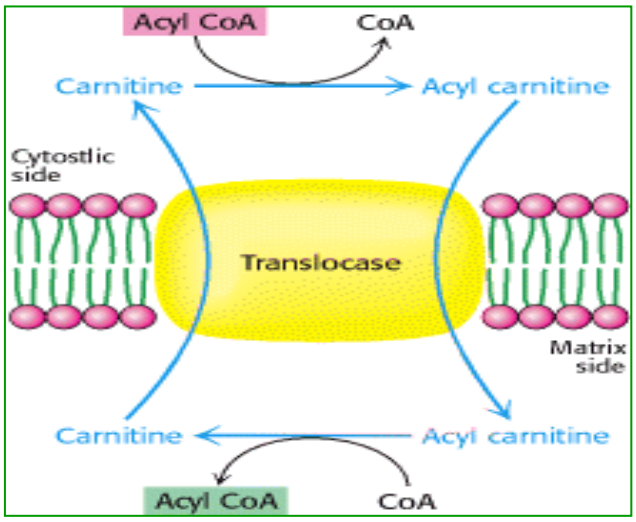
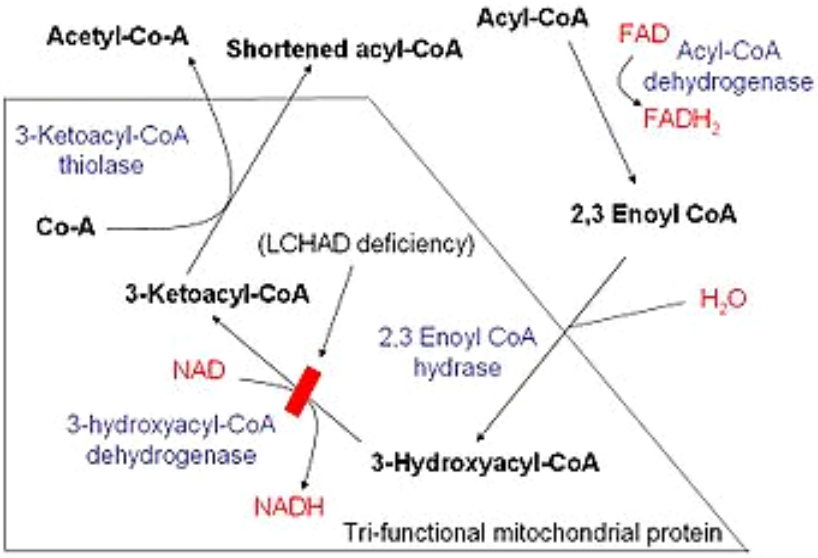
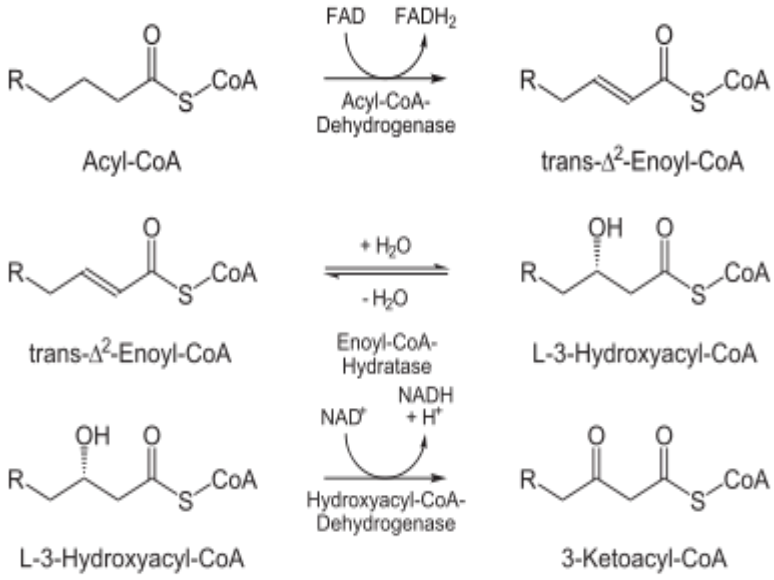


Figure 44. **Fatty acid transport into mitochondrium**

The β-oxidation of fatty acids occurs in the matrix of mitochondrium.





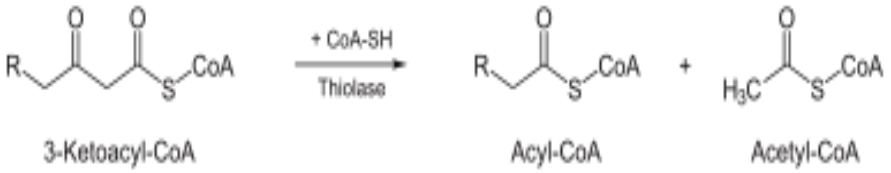


Figure 45. **Scheme of fatty acid oxidation**

Each turn, or revolution, of the "fatty acid spiral" produces an acetic acid residue split from the fatty acid as acetyl-coA to yield one $FADH_2$ molecule and one $NADH_2$ molecule. The cycle turns are then repeated until the fatty acid chain becomes shortened to a four-carbon fragment, i.e. butyryl-coA. In the last turn, butyryl-coA splits apart, and two, rather than one, acetyl-ScoA molecules are formed:

$$\mathbf{CH_3\text{-}CH_2\text{-}CH_2\text{-}CO\sim ScoA}$$
$$\mathbf{CH_3\text{-}CO\sim ScoA + CH_3\text{-}CO\sim ScoA}$$

The oxidation products of an even-numbered fatty acid are acetyl-coA, $FADH_2$ and $NADH_2$. Subsequently, acetyl-coA enters the Krebs cycle, and $FADH_2$ and $NADH_2$ are directly supplied to the respiratory chain.

Energy balance of fatty acid oxidation

The ***energetic value of an even-numbered fatty acid*** is estimated in the following manner. Complete oxidation of a fatty acid composed of **n** carbon atoms yields (**n÷2**) acetyl-ScoA molecules (each acetyl containing two carbon atoms) and [**(n÷2) – 1**] $FADH_2$ and $NADH_2$ molecules (since the last turn of the "fatty acid spiral" yields two acetyl-coSA molecules, one $FADH_2$ molecule and one $NADH_2$ molecule). Oxidation of $FADH_2$ gives two ATP molecules, and oxidation of $NADH_2$, three ATP molecules, i.e. a total of 5 ATP molecules, or, in the general case, 5[(n÷2 –1] ATP molecules. As has been noted above, the complete oxidation of one acetyl-ScoA molecule results in the formation of (12 n÷2) ATP molecules. One ATP molecule being used for the fatty acid activation, [(12n÷2) –1] ATP molecules remain. For example, the palmitic acid molecule, which contains 16 carbon atoms, produces 130 ATP molecules.

The energetic value of fatty acids is superior, for example, to that of glucose. For example, the complete oxidation of capronic acid (whose molecule contains the same number of carbon atoms as glucose) yields 45 ATP molecules as compared with 38 molecules which can be derived from glucose. However, the acetyl-ScoA molecules as produced by β-oxidation require a sufficient amount of oxaloacetate to be degraded by the Krebs cycle. In this respect, carbohydrates have an advantage over fatty acids, since the breakdown of the former species leads to pyruvate serving as a source for both acetyl-coA and oxaloacetate (pyruvate-carboxylase reaction), i.e. the acetyl-coA conversion within the Krebs cycle is thus facilitated. It is not without reason that in the older biochemical literature the notion that "*fats burn down in the carbohydrate flame*" was popular, since the ATP from glycolysis can be used for the cytoplasmic activation of fatty acids, while the pyruvate-derived oxaloacetate facilitates the insertion of fatty acid acetyl residues into the Krebs cycle.

The specific behaviour of odd-numbered fatty acids under oxidation is that one propionyl-ScoA molecule ($CH_3\text{-}CH_2\text{-}CO\sim ScoA$) per oxidized fatty acid molecule, alongside the products acetyl-coA, $FADH_2$, and $NADH_2$ (common to even-numbered fatty acids), is formed. Propionyl-ScoA converts to succinyl-ScoA:

$$\mathbf{CH_3\text{-}CH_2\text{-}CO\sim ScoA \xrightarrow{CO_2,\ ATP\rightarrow ADP} CH_3\text{-}CH(COOH)\text{-}CO\sim ScoA \rightarrow HOOC\text{-}CH_2\text{-}CH_2CO\sim ScoA}$$

propionyl-ScoA ***methylmalonyl-ScoA*** ***succinyl-ScoA***

Succinyl-ScoA enters the Krebs cycle.

The specific behaviour of unsaturated fatty acids under oxidation is determined by the position and the number of double bonds in the fatty acid molecule. The stepwise oxidation of an unsaturated acid to the position of a double bond in it proceeds in a manner similar to that of saturated acid oxidation. The oxidation reaction proceeds with the involvement of an accessory enzyme, *3,4-cis-2,3-trans-*

enoyl-ScoA isomerase; this facilitates the translocation of the double bond to an appropriate position and alters the double-bond configuration from *cis* to *trans.*

LECTURE 16

Subject: BIOSYNTHESIS OF LIPIDS IN TISSUES. REGULATION AND PATHOLOGY OF LIPID METABOLISM

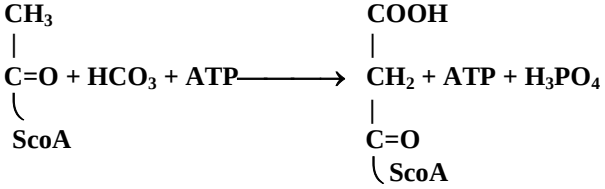
Biosynthesis of fatty acids. In the organism tissues, fatty acids are continually renewed in order to provide not only for the energy requirements, but also for the synthesis of multicomponent lipids (triacylglycerides, phospholipids, etc.). In the organism cells, fatty acids are resynthetized from simpler compounds through the aid of a supramolecular multienzyme complex referred to as *fatty acid synthetase.* Since in mammals palmitic acid in this process is a major product, this multienzyme complex is also called palmitate synthetase. Biosynthesis of fatty acids exhibits a number of specific features:

1. Fatty acid biosynthesis, being distinct from oxidation, is localized in the endoplasmic reticulum.
2. The source for the synthesis is malonyl-ScoA, which is produced from acetyl-ScoA.
3. Acetyl-ScoA is involved in the synthetic reaction as a primer only.
4. $NADPH_2$ is used to reduce fatty acid biosynthesis intermediates.
5. All the steps of malonyl-ScoA fatty acid biosynthesis are cyclic processes that occur on the surface of palmitate synthetase.

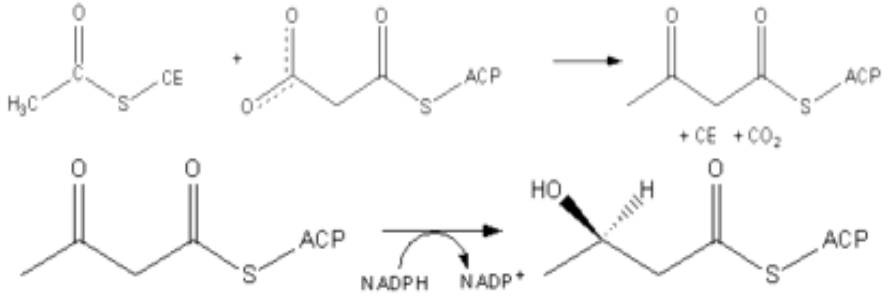
Production of malonyl-ScoA for fatty acid biosynthesis. Acetyl-ScoA serves as a substrate in the production of malonyl-ScoA. There are several routes by which acetyl-coA is supplied to the cytoplasm. One route is the transfer of acetyl residues from the mitochondrial matrix across the mitochondrial membrane into the cytoplasm. This process resembles a fatty acid transport and is likewise effected with the participation of carnitine and the enzyme *acetyl-ScoA-carnitine transferase.* Another route is the production of acetyl-ScoA from citrate. Citrate is delivered from the mitochondria and undergoes cleavage in the cytoplasm by the action of the enzyme *ATP-citrate lyase:*

Citrate + ATP + HScoA → acetyl-ScoA + oxaloacetate + ADP + H_3PO_4

The acetyl-ScoA supplied to the cytoplasm via the above routes is used for the synthesis of malonyl-ScoA:



Steps of fatty acid biosynthesis assisted by palmitate synthetase. The *acyl carrier protein* (ACP) is located at the centre of the multienzyme complex. ACP acts both as an acceptor and a distributor of acyl groups. The cyclic process of fatty acid synthesis may be represented by a series of consecutive reactions:



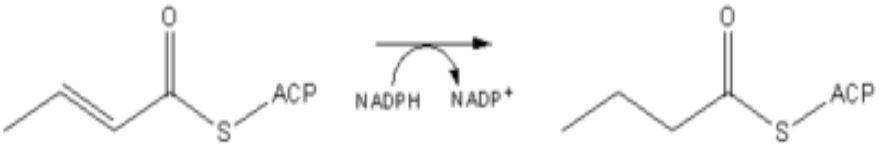


Figure 46. **Scheme of fatty acid biosynthesis**

The synthetic cycle is thus repeated. For example, seven cycles are implicated in palmitc acid biosynthesis and, accordingly, seven malonyl residues and one acetyl are required.

Fatty acid chain elongation. In the mitochondria, the chain elongation is achieved through the aid of an enzyme complex by adding acetyl residues from acetyl-ScoA. In the endoplasmic reticulum, the chain elongation is accomplished by an enzyme complex through making use of malonyl-ScoA.

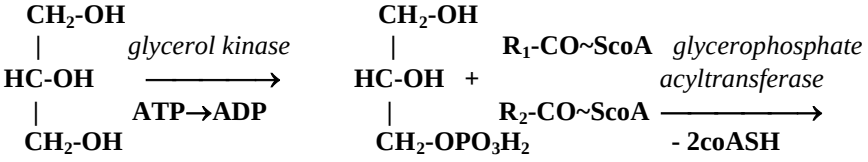
Biosynthesis of unsaturated fatty acids

In the mammalian tissues, the formation of monoene fatty acids is only possible. Oleic acid is derived from stearic acid, and palmitooleic acid, from palmitic acid. This synthesis is carried out in the endoplasmic reticulum of the liver cells via the monooxigenase oxidation chain. Any other unsaturated fatty acids are not produced in the human organism and must be supplied in vegetable food. Polyene fatty acids are essential food factors for mammals (vitamin like substances).

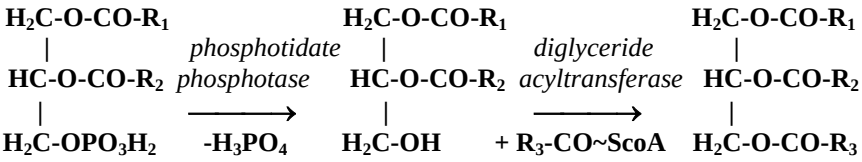
Biosynthesis of triglycerides

Triglyceride biosynthesis proceeds with the involvement of the lipids deposited in fat tissue or in other tissues of the organism. This process is localized in the hyaloplasm of cells.

α-Glycerol phosphate and acyl-ScoA, rather than corresponding free glycerol and free fatty acid, are utilized in the direct synthesis of triglycerides. α-Glycerol phosphate is produced either by phosphorylating the glycerol supplied to the tissue, or by reducing dihydroxyacetone phosphate as an intermediary product of glycolysis.



α-glycerol *α-glycerol phosphate*



phosphatidic acid *diglyceride* *triglyceride*

Phospholipid biosynthesis

Biosynthesis of phospholipids is associated with the renewal of membranes. This process is accomplished in the tissue hyaloplasm. The first steps of phospholipid and triglyceride biosyntheses coincide; subsequently, these routes diverge at the level of phosphatidic acid and diglyceride. Two routes of phospholipid biosynthesis are known; in either, the participation of CTP is necessary.

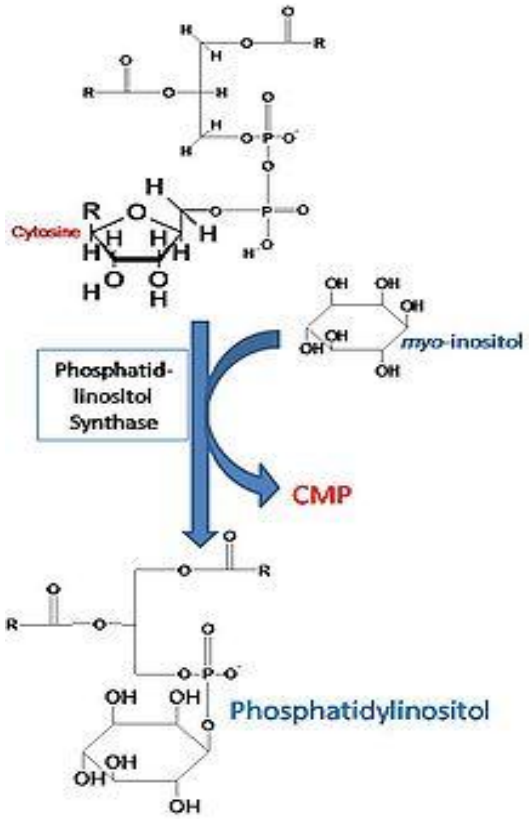


Figure 47. **Scheme of phospholipid synthesis**

The first route involves phosphatidic acid in phosphoglyceride biosynthesis. Phosphatidic acid reacts with CTP to yield CDP-diglyceride which, as a coenzyme, can participate in the transfer of diglyceride onto serine (or inositol) to produce phosphatidylserine (or phosphatidylinositol). Serine phosphatides are liable to decarboxylation (pyridoxal phosphate acting as a coenzyme) to yield ethanolamine phosphatides. The latter species are subject to methylation by S-adenosylmethionine (which donates three methyl groups), tetrahydrofolic acid and methylcobalamine acting as methyl group carriers.

The second synthetic route involves activation of an alcohol (for example, choline) to produce CDP-choline. The latter participates in the transfer of choline onto diglyceride to form phosphatidylcholine.

The phospholipids thus obtained are transported by lipid-carrier cytoplasmic proteins to the membranes (cellular or intracellular) to replace the used or impaired phospholipid molecules.

Because of the competition between the phospholipid and triglyceride synthetic routes for common substrates, all substances that favour the former route impede the tissue deposition of triglycerides. Such substances are referred to as *lipotropic factors*. They include: choline, inositol, and serine, as structural components of phospholipids; pyridoxal phosphate, as an agent facilitating the decarboxylation of serine phosphatides; methionine, as a donor of methyl group transfer coenzymes (tetrahydrofolic acid and methylcobalamine). They may be used as drugs preventing excessive deposition of triglycerides in tissues (the so-called fatty infiltration).

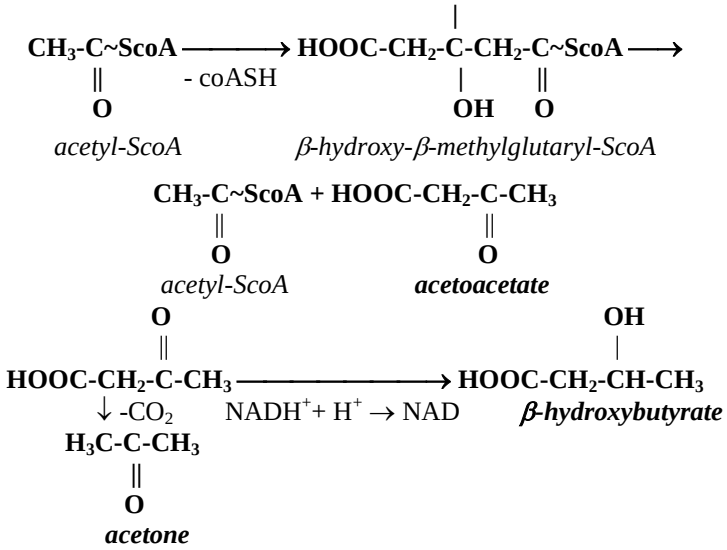
Biosynthesis of ketone bodies

Three compounds: acetoacetate, β-hydroxybutirate, and acetone, are known as ketone bodies. They are suboxidized metabolic intermediates, chiefly those of fatty acids and of the carbon skeletons of the so-called ketogenic amino acids (leucine, isoleucine, lysine, phenylalanine, tyrosine, and tryptophan). The ketone body production, or ketogenesis, is effected in the hepatic mitochondria (in other tissues, ketogenesis is inoperative). Acetyl-ScoA is the starting compound for the biosynthesis of ketone bodies.

$$CH_3\text{-}C(=O)\sim ScoA + CH_3\text{-}C(=O)\sim ScoA \rightarrow CH_3\text{-}C(=O)\text{-}CH_2\text{-}C(=O)\sim ScoA + coASH +$$

acetyl-ScoA *acetyl-ScoA* *acetoacetyl-ScoA*

CH_3



Biosynthesis of cholesterol

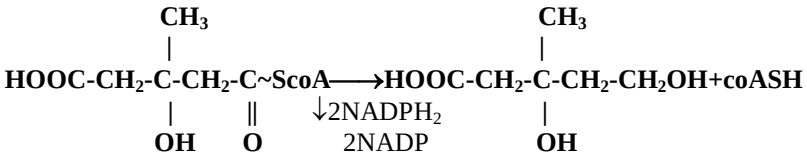
Biosynthesis of cholesterol from acetyl-ScoA proceeds as assisted by the enzymes of endoplasmic reticulum and hyaloplasm, in many tissues and organs. This process is especially active in the liver of adult humans. Cholesterol biosynthesis is a multistage process; in general, it may be divided into three steps;

1. Production of mevalonic acid from acetyl-ScoA.
2. Synthesis of an "active isoprene" from mevalonic acid followed by the condensation of the former to squalene.
3. Conversion of squalene to cholesterol.

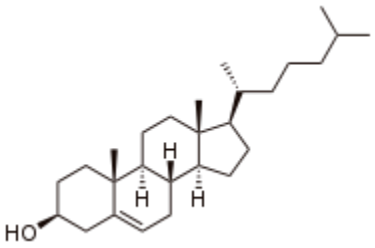
The initial reactions in the first step, prior to the formation of β-hydroxy-β-methylglutaryl-ScoA, resemble those involved in ketogenesis with the only distinction that ketogenesis occurs in the mitochondria, while cholesterol biosynthesis is carried out extramitochondrially:

2 Acetyl-ScoA → acetoacetyl-ScoA + acetyl-ScoA
→ β-hydroxy-β-methylglutaryl-ScoA

Further, β-hydroxy-β-methylglutaryl-ScoA is converted with hydroxymethylglutaryl-ScoA into mevalonic acid:



This reaction is irreversible and is a rate-limiting stage of the overall cholesterol biosynthesis.

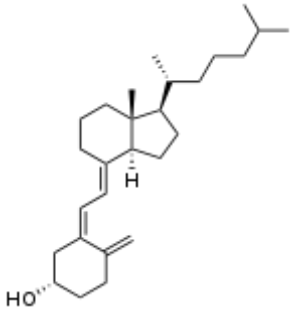


Cholesterol

During the second step, the two compounds constitute the "active isoprene", which is consumed in the production of squalene: **squalene → lanosterin → cholesterol**

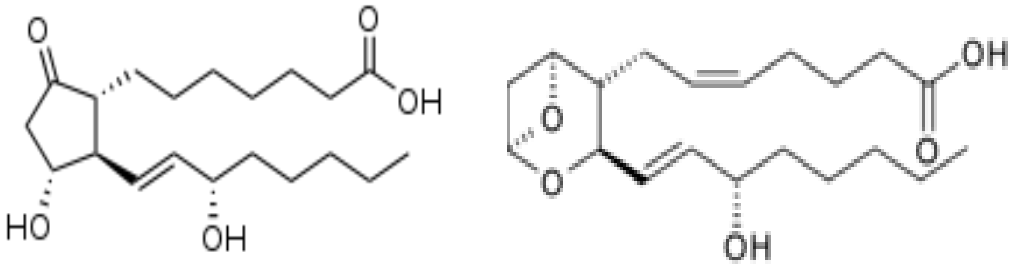
Cholesterol, mostly esterified, is utilized in the buildup of cell biomembranes. Besides, cholesterol is a precursor of biologically important steroid compounds: *bile acids* (in liver), *steroid hormones*

(in adrenal cortex, male and female sexual glands, and placenta), and *vitamin D_3*, or *cholecalciferol* (in skin).



Cholecalciferol

Prostaglandins are hormone-like compounds (hormonoids) derived from C_{20}-polyene fatty acids containing a cyclopentane ring. They are short-lived species that are synthetized at need in small amounts to exert a local biological effect at the site of their formation.



Prostaglandin E_1 (left) **and thromboxane A_2**

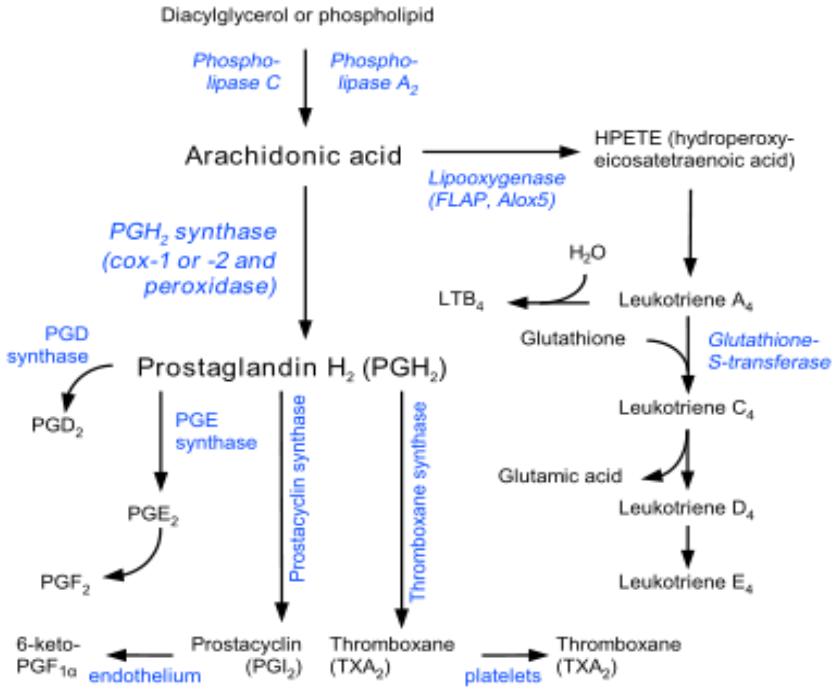


Figure 48. **Prostaglandines: pathways in biosynthesis of eicosanoids from arachidonic acid**

Regulation of lipid metabolism in the organism

The rate of lipid metabolism in the organism tissues is dependent on the dietary supply of lipids and on the neurohormonal regulation. An excessive intake of high-calory food (carbohydrates and triglycerides) impedes the consumption of endogenic triglyceride reserves stored in the fat tissues. Moreover, carbohydrates provide a very favourable basis for the neogenesis of various lipids; for this rea-

son, a large dietary intake of carbohydrate-rich food exerts a significant influence on the production of triglycerides and cholesterol in the organism.

Synthesis of endogenic cholesterol is also controlled by exogenous cholesterol supplied in food: the more dietary cholesterol is digested, the less endogenic cholesterol is produced in the liver. Exogenous cholesterol inhibits the activity of hydroxymethylglutaryl-ScoA reductase and the cyclization of squalene to lanosterol.

The dietary ratio of various lipids plays an important role in the lipid metabolism in the organism. The available amounts of polyene fatty acids and phospholipids acting as solvents for fat-soluble vitamins affect not only the absorption of the latter species, but also the solubility and stability of cholesterol in the organism fluids (blood plasma and lymph) and biliary ducts. Vegetable oils with a high percentage of phospholipids and polyene fatty acids impede an excessive accumulation of cholesterol and its deposition in blood vessels and other tissues, and facilitate the removal of cholesterol excess from the organism. Polyene fatty acids are needed in the production of prostaglandins.

The lipotropic factors exercise a marked effect on the biosynthesis of phospholipids and triglycerides. The dietary deficiency of lipotropic factors favours the triglyceride production in the organism.

The neuro-hormonal control of lipid metabolism chiefly affects the mobolization and synthesis of triglycerides in the fat tissue. The lypolysis in tissues is dependent upon the activity of triglyceride lipase. All the regulators that favour the conversion of the inactive (nonphosphorylated) lipase to the active (phosphorylated) one stimulate the lipolysis and the release of fatty acids into the blood. Adrenalin and noradrenalin (secreted in the sympathetic nerve endings) hormones, (glucagon, adrenalin, thyroxin, triiodothyronine, somatotropin, β-lipotropin, corticotropin, etc.), tissue hormones, including biogenic amines (histamine, serotonin, etc.), act as stimulators for this process. Insulin, on the contrary, inhibits the adenylate cyclase activity; preventing thereby the formation of active lipase in the fat tissue, i.e. retards the lipolysis. In addition, insulin favours the neogenesis of triglycerides from carbohydrates, which, on the whole, provides for lipid deposition in the fat tissues as well as for the cholesterol production in other tissues. The thyroid hormones thyroxin and triiodthyronine assist in the oxidation of the cholesterol side chain and in the biliary excretion of cholesterol in the intestine.

Pathology of lipid metabolism

Most commonly, the lipid metabolism pathology is manifest as *hyperlipemia* (elevated concentration of lipids in blood) and *tissue lipidoses* (excessive lipid deposition in tissues). Normally, the lipid contents in the blood plasma are:

- total lipids: 4-8 g/L
- triglycerides: 0,5-2,1 mmol/L
- total phospholipids: 2,0-3,5 mmol/L
- total cholesterol: 4,0-9,8 mmol/L (esterified cholesterol accounts for 2/3 of total cholesterol).

Hyperlipemia may manifest itself by an increased concentration of lipids, or certain groups thereof. For example, hypercholesterolemia and hypertriglyceridemia may be mentioned in this connection. Since practically all the blood plasma lipids make part of lipoproteins, hyperlipemias may be reduced to one of the hyperlipoproteinemia forms which differ in the varied rations of plasma lipoproteins of different groups.

Hyperlipoproteinemia is characterized by the enhanced content of chylomicrons in the blood plasma; simultaneously, the percentage of α- and β-lipoproteins may be lowered. The triglyceride content is 8-10 times above the norm, while cholesterol does not exceed the normal level.

Secondary hyperlipoproteinemias, which arise from a disordered lipid tissue metabolism or its impaired control, are observed in diabetes mellitus, thyroid gland hypofunction, alcoholism, etc.

Tissue lipidoses. Hyperlipoproteinemias may lead to tissue lipidoses and also arise from hereditary defects of the enzymes involved in the synthesis and breakdown of lipids in the tissues.

Atherosclerosis is a wide-spread pathology, manifested chiefly by the deposition of cholesterol in arterial walls, which results in the formation of lipids plaques (atheromas). Lipid plaques are specific foreign bodies around which the connective tissue develops abnormally (this process is called sclerosis). β-Lipoproteins and, partly, pre-β-lipoproteins containing much cholesterol exhibit atherogenic properties.

Fatty infiltration of the liver. In this pathology, the triglyceride concentration in the liver is 10-fold superior to the norm. The accumulation of fat in the cytoplasm is numerous; one of these may be a deficiency in lipotropic factors and the associated therewith synthesis of excess of triglycerides.

Ketosis is a pathologic state produced by an excess of ketone bodies in the organism. Ketosis may be regarded as lipid metabolism pathology with a certain reserve, since excessive biosynthesis of ketone bodies in the liver is sequent upon an intensive hepatic oxidation not only of fatty acids, but also of ketogenic amino acids. The breakdown of carbon frameworks of these amino acids leads to the formation of acetyl-ScoA and acetoacetyl-ScoA, which are used in ketogenesis. The ketosis is accompanied by *ketonemia* and *ketonuria,* which is manifested by the increased concentration of ketone bodies in blood and their excretion in the urine. In an aggravated form of ketosis, the ketone body concentration in blood may be as high as 10-20 mmol/L. The ketone bodies are normally present in the daily urine in trace amounts, while in pathology, 1 to 10 g (or even more) of ketone bodies per day is excreted in the urine. Most commonly, ketonemia and ketonuria are observed in diabetes mellitus (the manifest ketosis symptoms are dependent on the extent of diabetes mellitus), as well as in prolonged starvation or in "*steroid" diabetes.*

LECTURE 17

Subject: CATABOLISM OF SIMPLE PROTEINS

The biological value of proteins of animal and vegetable origin is determined by the constituent amino acids, especially the essential ones. If food proteins contain all of the essential amino acids such proteins are referred to as *complete* proteins. Any other food proteins are referred to as *incomplete* (or *partial*) proteins. Plant proteins, as distinct from animal proteins, are, as a rule, less complete. The hen's egg protein has been recognized as an appropriate reference protein optimally corresponding to physiological requirements of the organism. All food proteins should be compared for the amino acid composition to the reference protein. The daily dietary protein intake for the adult human should amount to 80-100 g, of which a half must be of animal origin.

Digestive mechanism for proteins

Proteolytic enzymes involved in the digestion of proteins and peptides are synthetized and secreted in the digestive tract cavity as proenzymes, or *zymogens.* Zymogens are inactive and incapable of digesting the intrinsic cell proteins. Proteolytic enzymes are activated in the intestinal lumen where they act on dietary proteins.

The human gastric juice contains two structurally related proteolytic enzymes, *pepsin* and *gastricsin*, which are derived from a common precursor.

Pepsin is produced as a proenzyme, *pepsinogen,* in the major cells in the gastric mucosa. The activity of pepsinogen is elicited either by hydrochloric acid (HCl) excreted from the cover cells of stomach or autocatalytically, i.e. by the pepsin molecules themselves. The molecular mass of pepsinogen is 40,000. The pepsinogenic peptide chain contains pepsin (molecular mass 34,000), and a residual polypeptide-inhibitor. Pepsin belongs to carboxyproteinases containing dicarboxy amino acid residues in the active center, at pH optimum 1,5-2,5.

Proteins, both native and denatured, serve as substrates for pepsin. Denatured proteins are more easily hydrolyzed. Food proteins are denatured by cooking or by treating with hydrochloric acid. The following biological functions of hydrochloric acid are to be noted:

- activation of pepsinogen,
- provision for optimal pH in hydrolysis by pepsin and gastricsin juice,
- denaturation of food proteins,
- antimicrobial action.

The intrinsic proteins of stomach wall are protected against the denaturing action of hydrochloric acid and digestive activity of pepsin by glycoprotein-containing mucous secretum. Pepsin, which is an endopeptidase, exerts a fast cleavage of protein endo-peptide bonds formed by the carboxyl groups of aromatic amino acids (phenylalanine, tyrosine, and tryptophan).

Gastricsin is close to pepsin by molecular mass (31,500). The optimal pH for gastricsin is about 3,5. Gastricsin hydrolyzes the peptide bonds formed by dicarboxylic acids. The pepsin/gastricsin ratio in the gastric juice is 4:1. In patients with peptic ulcer, this ratio alters in favour of gastricsin.

Pepsin and gastricsin hydrolyze proteins to a mixture of polypeptides (also called peptones or albumoses). The extent of protein digestion in the stomach is dependent on the food residence time therein. Usually, this time is rather short (from 3 to 6 hours, depending on the digestive regime), therefore, the main portion of proteins is degraded in the intestine.

***Proteolytic enzymes in intestine*.** The proteolytic enzymes are excreted to the intestine from the pancreas as proenzymes: trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, and proelastase. The activation of these enzymes is effected via partial proteolysis of their polypeptide chains, i.e. by cleaving off the fragment that acts as a shield for the active center of enzyme. The key process for activating all of the proenzymes is the formation of trypsin. Trypsinogen secreted by the pancreas is activated by the intestinal enterokinase, or enteropeptidase. In addition, the generated trypsin favours the autocatalytic conversion of trypsinogen to trypsin. The mechanism for activation of trypsinogen consists in the hydrolysis of one peptide bond resulting in the release of N-terminal hexapeptide as an inhibitor for trypsin. Further, trypsin splits peptide bonds in other proenzymes to form active enzymes. In such a way, three varieties of chymotrypsin, carboxypeptidases A, B, and elastase, are formed.

The intestinal proteinases hydrolyze the peptide bonds of dietary proteins and polypeptides produced by the gastric enzymes, to yield free amino acids. The *endopeptidases* (*trypsin* and *chymotrypsin*) and *elastase* facilitate the cleavage of the endopeptide bonds and reduce proteins and polypeptides to smaller fragments. Trypsin mainly attacks the peptide bonds formed by the carboxyl groups of lysine and arginine; vis-a-vis the isoleucine peptide bonds, it is less active.

Chymotripsins are the most active towards the peptide bonds involving tyrosine, phenylalanine, and tryptophan. The specificity of hydrolytic action of chymotrypsin is similar to that of pepsin.

Elastase hydrolyzed the bonds formed by aliphatic amino acids and imino acids, proline and hydroxyproline.

Carboxypeptidase A is a zinc-containing enzyme. It cleaves C-terminal aromatic and aliphatic amino acids from polypeptides. ***Carboxypeptidase B*** splits C-terminal lysine and arginine residues only.

In the intestinal mucosa, ***dipeptidases*** occur that are capable of hydrolyzing dipeptides to their two constituent amino acids. The dipeptidases are activated by cobalt and manganese ions and by cysteine.

The available variety of proteolytic enzymes provide for complete degradation of proteins to free amino acids even in the contingency when the proteins remain unaffected by the action of gastric pepsin. Due to this happy circumstance, the patients that have been operated for a partial or complete excision of stomach retain the capability to assimilate dietary proteins.

Absorption of protein hydrolyzates

Amino acids are the major products of protein hydrolysis. Their intestinal uptake, as well as the transport across other cell membranes, occurs via special amino acid transport systems. The amino acid transport is an active transport and requires an appropriate Na^+ ion gradient, which is produced by Na^+, K^+-ATPase of the intestinal epithelium membrane. Amino acids are absorbed in the intestine by the secondary active transport. This is evidenced by the fact that the glycoside *ouabain*, an inhibitor for Na^+,K^+-ATPase, also exerts a retarding effect on the amino acid transport.

There are known at least five amino acid carrier systems that specialize in the transport of:

1. *neutral aliphatic amino acids,*
2. *cyclic amino acids,*
3. *basic amino acids,*
4. *acidic amino acids,*
5. *proline and hydroxyproline.*

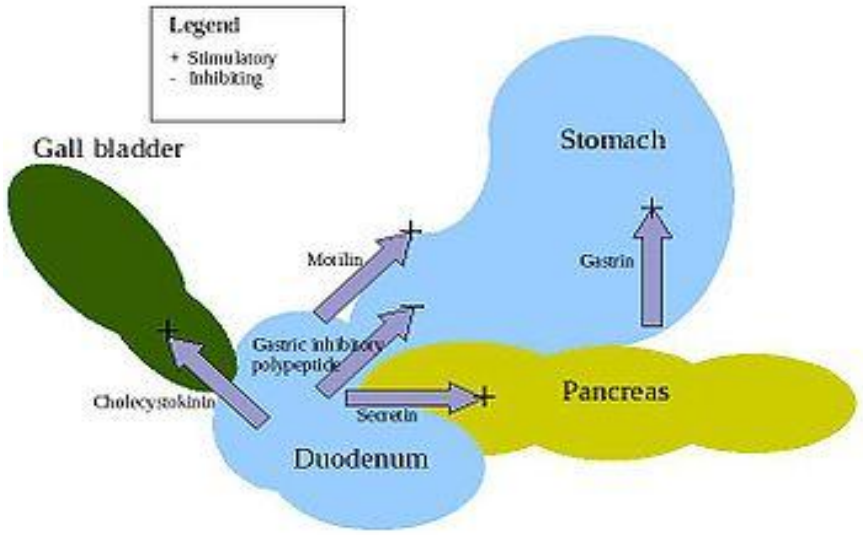
The amino acids of these groups complete for binding with a carrier of the corresponding transport system. In the transport of amino acids across the intestinal epithelium membrane Na^+ ions occurs via a special carrier system. The sodium ions are pumped out of the cell by *Na^+,K^+-ATPase*, while the amino acids remain inside the cell.

There is also an alternative mechanism for the transport of amino acids across the cell membrane of intestinal epithelium and of other cells; it is the so-called *γ-glutamyl cycle*. The amino acid transfer is accomplished through the agency of a special enzyme, *γ-glutamyl transferase*, which is found within the membrane of intestinal epithelium and other cells. The cofactor for this enzyme is a tripeptide, *glutathione,* which is sufficiently abundant in the cell. The energy of glutathione peptide bonds is used for the transfer of amino acid from the environment into the cell. The wide occurrence of the major enzymes of this transport cycle in the tissues is indicative of the enzymes importance for the transport of amino acids in a large number of cells. In the intestine, the uptake of small amounts of dipeptides and nonhydrolyzed proteins is possible. These are absorbed by endocytosis, and inside the cell they are hydrolyzed by lysosomal proteinases.

The absorbed amino acids are delivered to the portal vein, then to the liver and, in a dissolved state, are supplied in the blood to the tissues and organs. Amino acids are most actively consumed by liver and kidney and, to a lesser extent, by other organs, especially brain.

Regulation of digestion

Digestion of food components is controlled by a system of hormone-like compounds formed in the cells of the digestive tract: *histamine, gastrin, secretin, cholecystokinin*, etc. The secretion of digestion regulators is elicited by the ingested food and is determined by its composition. On delivery of food into the stomach histamine and gastrin are secreted which, in turn, provide for the secretion of hydrochloric acid and pepsin involved in the digestion of proteins. The transit of stomach contents into the duodenum stimulates the secretion of *enterogastrone* which, when excreted in the blood, inhibits the gastric juice secretion.



Action of the major digestive hormones

The delivery of food into the intestine stimulates the secretion of a number of regulators (secretin, cholecystokinin, pancreosimine, chymodenin, and enterocrinin) which, in turn, provide for a fast secretion of pancreatic and intestinal juices for food digestion. Disturbances in secretory regulation cause a disharmony of the digestive processes.

Pathology of digestion and absorption

Digestive disturbances are produced by a deficiency in enzymes and cofactors as well as by biochemical malfunction of absorption in the intestine. A reduced secretion of hydrochloric acid and pepsin (the so-called *subacid gastritis* or *hypoaciditas*), and, occasionally, a complete cessation (such a state is referred to as *achylia*) do not affect significantly the overall digestibility of dietary protein. This functional deficiency is a compensated for by an essential set of proteolytic enzymes under the intestinal digestion conditions. However, a failure of hydrochloric acid production leads to the development of microbial flora and putrefactive processes in the stomach.

A shortage of proteolytic pancreatic enzymes (congenital or produced by mechanical obstruction) leads to the discharge of undigested proteins with feces and, as a consequence, to a relative protein deprivation. The indigested proteins are subject to the digestion by microorganisms of large intestine. This process is called *intestinal putrefaction of proteins*. The protein putrefaction is concomitant with the production of toxic materials: ammonia (NH_3), hydrogen sulphide (H_2S), amines (putrescine, cadaverine), and alcohols (phenol, cresol, scatole, and indole). These products may exert a deleterious effect on the organism. Some of the noxious products are neutralized in the intestine, and others are rendered harmless during absorption, mostly in the liver. Amines are inactivated by *diaminooxidase* (DAO) type. The major route to detoxication of ammonia is *urea* synthesis in the liver. Indole, scatole, phenol, and cresol are inactivated by conjugation with UDP-glucuronic acid and PAPS (3'-phosphoadenosine-5'-phosphosulphate).

LECTURE 18

Subject: **AMINO ACID CATABOLIC PATHWAYS IN TISSUES**

The first step of protein renewal is proteolysis which effected by *tissue proteinases*, or *cathepsins*. Cathepsins are located chiefly in the lysosomes, as many other hydrolytic enzymes. However, cathepsins also occur in other cell organelles: mitochondria, endoplasmic reticulum, and hyaloplasm. The lysosomal cathepsins are most active in an acidic medium therefore they are referred to as acidic cathepsins. In the cell cytoplasm and other organelles, cathepsins are optimally active in neutral and weakly basic media.

Proteolysis, as it is effected in tissues, is important for the renewal of proteins, for the disposal of defective protein molecules, and for the energetic mobilization of endogenic proteins (especially in starvation). Therefore, cathepsins perform not only a destructive function, but are also involved in reconstructive processes. Cathepsins are capable of a limited proteolysis, i.e. they are apt to split off a particular fragment from a polypeptide chain (this phenomenon is reminescent of the production of enzymes from digestive proenzymes). The limited proteolysis liberates neuropeptides that perform mediatory and hormonal functions. Prohormones produced by the endocrine glands are converted to active protein hormones by the same mechanism.

***Amino acid catabolic pathways*.** The catabolic pathways by which amino acids are degraded to the end products may be divided into three groups:

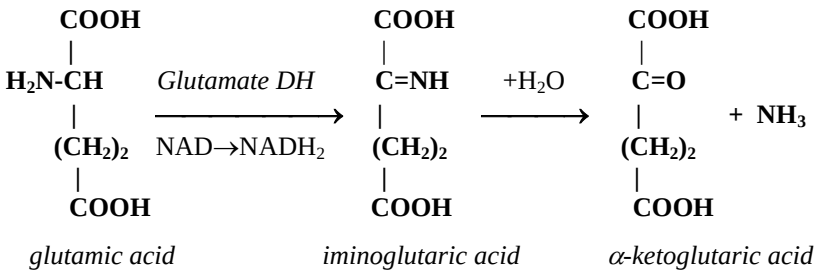
1. catabolic pathways involving the conversion of NH_2 groups (any amino acid has at least one α-NH_2 group),
2. pathways for the breakdown of amino acid carbon frameworks,
3. decarboxylation of amino acid α-COOH groups.

The third pathway is a version for the amino acid carbon framework conversion. It is employed in the production of biogenic amines and will be dealt with later in discussing the processes involving formation and degradation of mediators.

Conversion of α-amino groups of amino acids

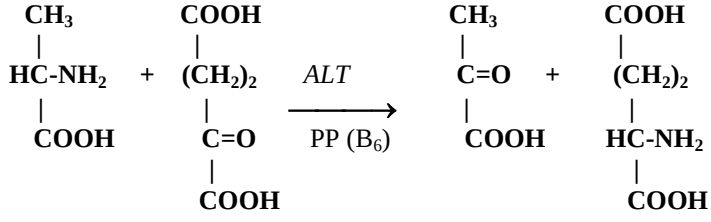
In the organism tissue, the cleavage of an amino group from an amino acid leads to the production of ammonia. This process is called deamination. Four types of deamination are known in nature: *reductive, hydrolytical, intramolecular, and oxidative.* ***Oxidative deamination*** can proceed via a *direct route* or an *indirect route* (transdeamination) in the human organism.

Direct oxidative deamination is effected for the glutamic acid:

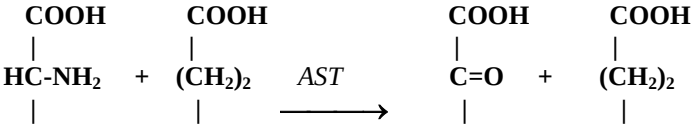


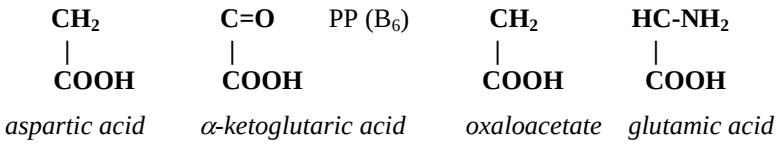
glutamic acid *iminoglutaric acid* *α-ketoglutaric acid*

Transdeamination is the major route to amino acid deamination. It is accomplished in two steps. The transamination reaction is a reversible one; it is catalyzed by enzymes *aminotransferases*, or *transaminases*.



alanine *α-ketoglutaric acid* *pyruvic acid* *glutamic acid*





aspartic acid *α-ketoglutaric acid* *oxaloacetate* *glutamic acid*

An aminotransferase is composed of an apoenzyme and coenzyme. Derivatives of pyridoxine (vitamin B_6), pyridoxal 5-phosphate (PP) and pyridoxamine 5-phosphate are coenzymes for aminotransferases. Amino transferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), have clinico-diagnostical value. They are organospecific enzymes (ALT-liver, AST-heart).

Indirect oxidative deamination is effected for the other amino acids. This deamination is accomplished in two steps: transamination and direct deamination of glutamic acid.

Biosynthesis of nonessential amino acids

In the mammalian tissues, biosynthesis of only nonessential amino acids is possible; the essential amino acids must be supplied in food. The starting materials for the biosynthesis of nonessential amino acids are intermediates of carbohydrate catabolism, the Krebs cycle metabolites, and essential amino acids. For example, alanine is produced from pyruvate via two routes:

1. transamination with the participation of *alanine aminotransferase* (ALT):

Pyruvate + glutamate → *ALT*→ alanine + α-ketoglutarate

2. reductive amination involving *alanine dehydrogenase* (ADH):

Pyruvate + NH_3 + $NADH^+$ + H^+ + H_2O → *ADH*→ alanine + NAD^+.

Decarboxylation of amino acid α-COOH groups

Production of histamine. Histamine is synthetized from histidine by the action of *histidine decarboxylase:*

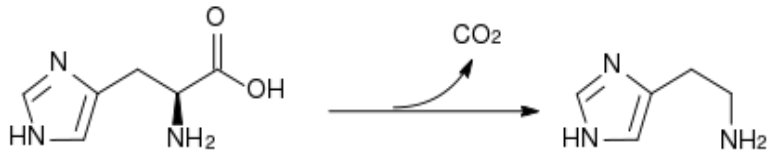


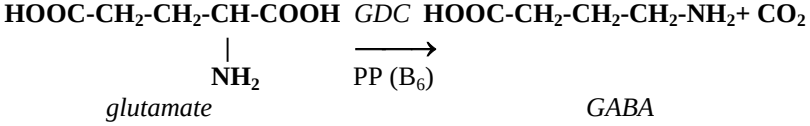
Figure 49. **Conversion of histidine to histamine by histidine decarboxylase**

Nearly all the tissues and organs contain histamine. Histamine is released from the mast cells by the agents called histamine liberators. In large amounts histamine is produced in the gastric mucosa, where it affects the secretion of pepsin and hydrochloric acid. Histamine in small amounts is always present in the blood plasma and other biological fluids. Histamine serves as a mediator of allergic reactions and is released in large amounts in pathologic processes.

Production of serotonin. Serotonin is derived from tryptophan.

Serotonin acts as a mediator in the nervous system and as a local functional regulator for the peripheral organs and tissues.

Production of γ-aminobutiric acid (GABA). GABA is produced from glutamic acid by the action of *glutamate decarboxylase:*



glutamate *GABA*

The synthesis proceeds in the inhibitory synapses of the nervous system; for these, GABA acts as a mediator.

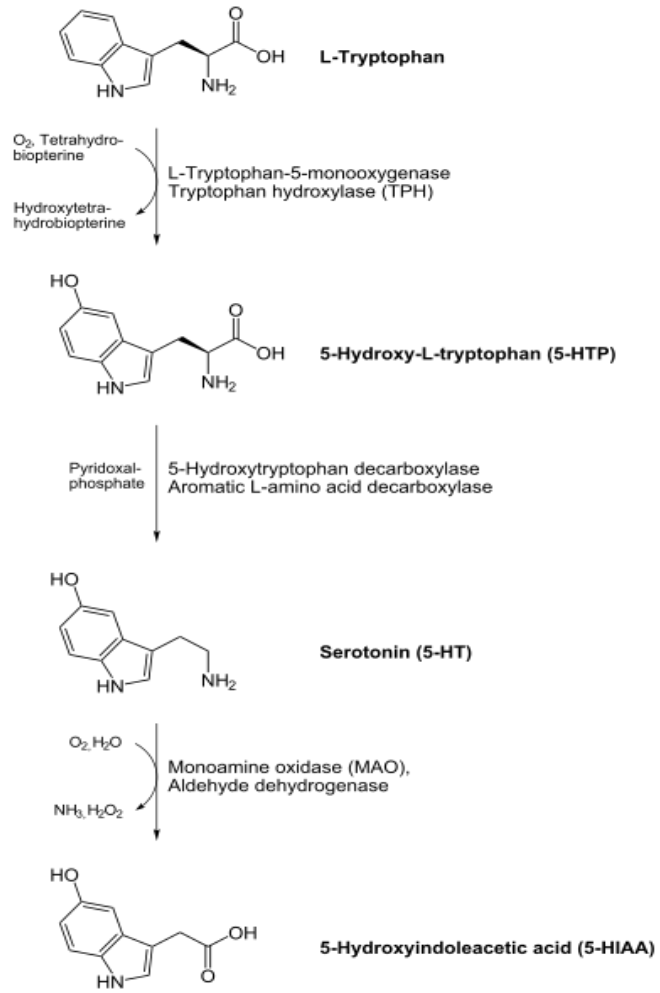


Figure 50. **Serotonin production**

Inactivation of biogenic amines. A route to detoxication of biogenic amines is the oxidative deamination with the involvement of aminooxidases: *monoaminoaxidase (MAO)* type and *diaminooxidase (DAO)* type.

$$\mathbf{R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \rightarrow} \textit{Aminooxidase} \mathbf{\rightarrow R\text{-}HC{=}O + NH_3 + H_2O_2}$$

biogenic amine *aldehyde*

Aldehydes produced by deamination of biogenic amines are oxidized to organic acids by *aldehyde dehydrogenases*:

$$\mathbf{R\text{-}HC{=}O + NADH^+ + H^+ + H_2O \rightarrow R\text{-}COOH + NAD^+}$$

LECTURE 19

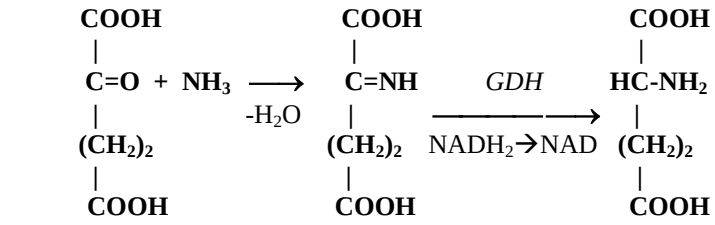
Subject: ROUTES TO METABOLIC DETOXIFICATION OF AMMONIA

In the organism, ammonia (NH_3) is produced by the following processes *deamination* of:

1. amino acids,
2. biogenic amines (histamine, serotonin, etc.),
3. purine bases (adenine and guanine),
4. pyrimidine bases (uracil, thymine, cytosine),
5. amino acid amides (asparagine and glutamine).

Ammonia is a very toxic compound, especially for nervous cells. Its accumulation in the organism elicits excitation of the nervous system.

Mechanism of toxication of ammonia



NH_3 is inhibitor of Krebs cycle. For this reason, to counteract the adverse effects, mechanisms for neutralization of ammonia are operative in the organism tissues. These mechanisms include:

1. production of urea;
2. reductive amination, or transreamination;
3. production of amino acid amides (asparagine and glutamine);
4. production of ammonium salts.

The major route to detoxification of ammonia is urea synthesis. In the liver urea was formed from ammonia and carbonic acid. The urea cycle also refer to as the *arginine-urea cycle*, or *ornithine cycle.*

Urea production cycle (ornithine cycle)

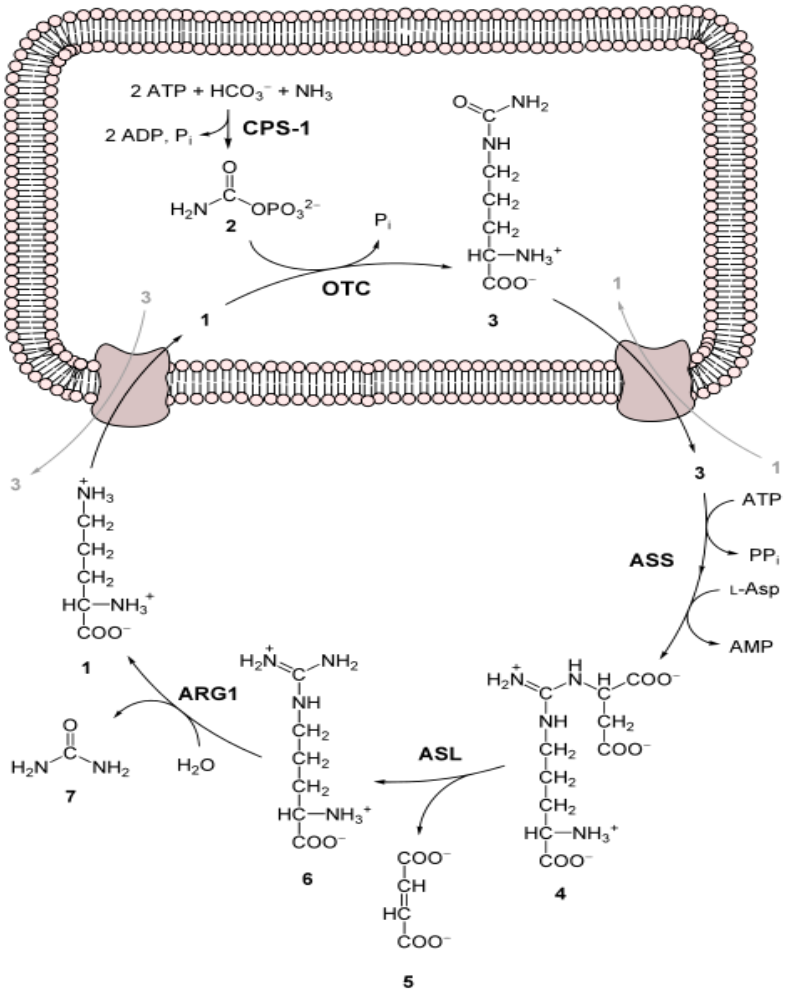
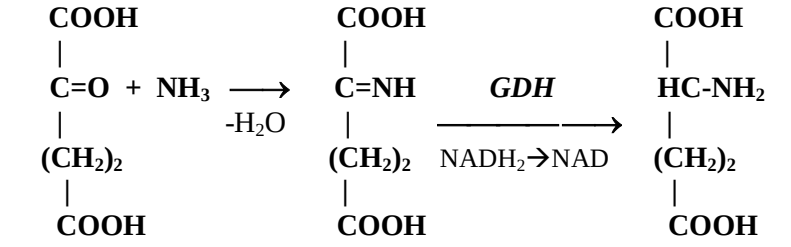


Figure 51. **Scheme of urea production:** *1* – L-ornithine; *2* – carbamoyl phosphate; *3* – L-citrulline; *4* – argininosuccinate; *5* – fumarate; *6* – L-arginine; *7* – urea; L-Asp: L-aspartate; CPS-1: carbamoyl phosphate synthetase I; OTC: Ornithine transcarbamylase; ASS: argininosuccinate synthetase; ASL: argininosuccinate lyase; ARG1: arginase 1.

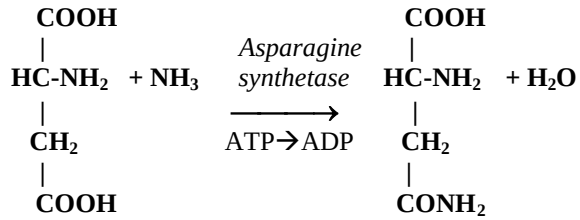
To produce one urea molecule, three ATP molecules are needed to be consumed. Urea is a material which is inert towards the living organism. The major site for its formation in the organism is the liver which contains all the enzymes involved in urea production.

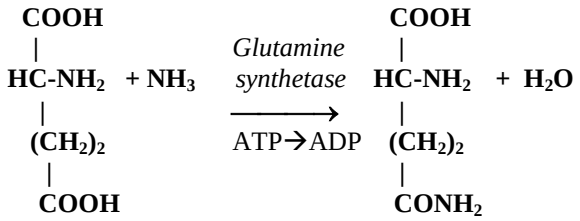
Disturbances in the hepatic function lead to a decreased urea production, with the resultant diminution of urea blood concentration and urea excretion in the urine.

Reductive amination. Viewed from the standpoint of an ammonia-binding process, it is little effective, since it requires a significant amount of α-ketoglutarate for its effectuation.



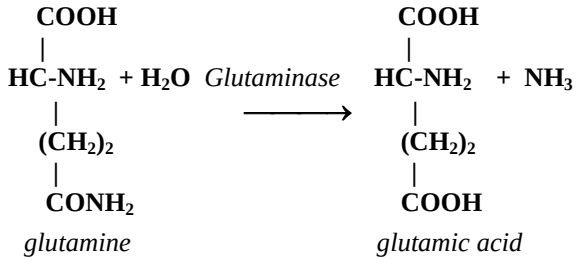
Production of asparagine and glutamine. It is important a subsidiary route to binding ammonia. It proceeds with the involvement of *asparagine synthetase* and *glutamine synthetase*:





This process is active in nervous and muscle tissues and in kidney.

Ammonium salt production. Glutamine and, to a lesser extent, asparagine are believed to be a kind of vehicles for ammonia, since two species, after their formation in the tissues, are delivered in the bloodstream to the kidneys to be hydrolyzed by the action of specific enzymes, *glutaminase* and *aspartaginase*:



Ammonia, liberated in the tubules of the kidney, is neutralized to ammonium salts:

$NH_3 + H^+ + Cl^- \rightarrow NH_4Cl$, which are excreted in the urine.

Amino acids as medicinal preparations. In the practical medicine, preparations on the basis of protein hydrolyzates and individual amino acids are used. Methionine and other methionine-rich hydrolyzates are used as lipotropic factors, and in treating protein deficiency in chronic diseases. For cli-

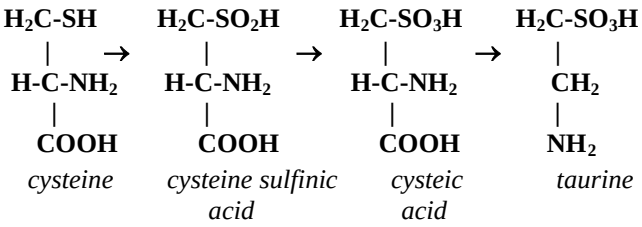
nical purposes, glutamic and aspartic acids are widely used, which play a decisive role in the detoxification of ammonia and in other synthetic reactions of amino acid metabolism.

LECTURE 20

***Subject*: CONVERTION OF AMINO ACIDS TO SPECIALIZED PRODUCTS**

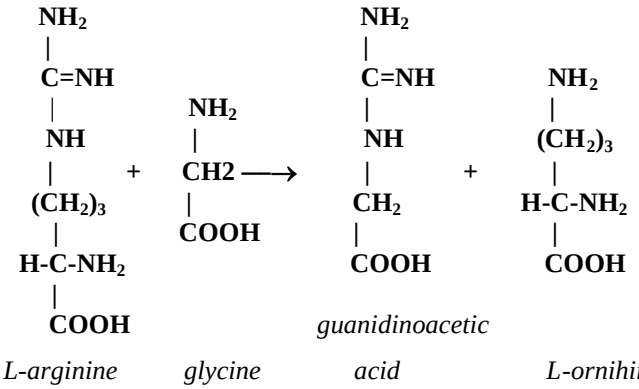
Amino acids are widely used for the biosynthesis of a large variety of nonproteinic nitrogenous materials: choline, phosphatides, creatine, mediators (including biogenic amines), pigments, vitamins, coenzymes, porphyrins, purine and pyrimidine bases, etc.

Production of taurine. Taurin is a biogenic amine; it is produced from cysteine:

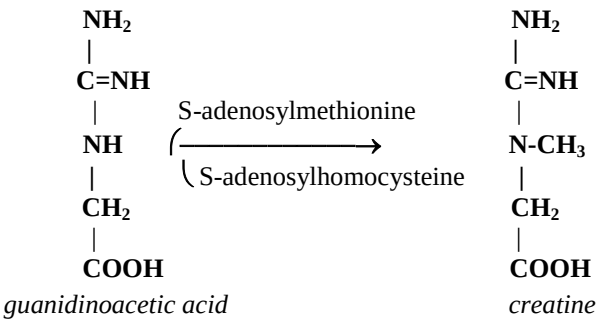


This compound is synthetized in various organs. In the liver, taurine is involved in conjugation reactions with bile acids. In the nervous system, taurine is presumably carable of acting as a synaptic mediator.

Biosynthesis and breakdown of creatine. In the human and animal tissues, creatine synthesis proceeds with the involvement of three amino acids: *arginine*, *glycine*, and *methionine.* Creatine synthesis proceeds in two steps. The first step involves the formation of guanidinoacetic acid from arginine and glycine with the participation of the enzyme *glycine-amidine transferase:*

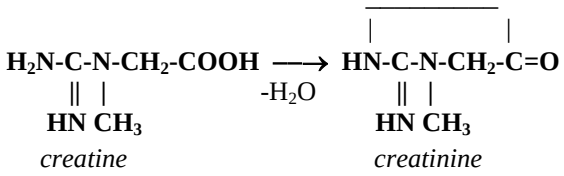


The first step proceeds actively in the kidney and pancreas. At the second step, methylation of guanidinoacetic acid through the assistance of *guanidinoacetate methyltransferase* takes place. The active form of methionine, S-adenosylmethionine, acts as a methyl group donor:



This step takes place in the liver and the pancreas which provide for the conditions and for the appropriate enzyme required for the synthesis of creatine from guanidinoacetic acid. It is commonly believed that the creatine that has been synthesized in the liver and in the pancreas is supplied via blood circulation to other organs and tissues: brain, skeletal muscles, heart, etc. In the cells, creatine is

involved in the energy transfer via reversible transphosphorylation with ATP. A product of creatine breakdown is creatinine produced via a nonenzymic pathway:



creatine *creatinine*

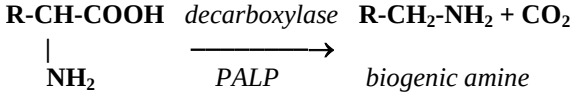
About 2% of creatine contained in the organism is liable to conversion to creatinine. In small amounts, creatine and creatinine are present in the blood plasma. In the creatine-synthetising organs (kidney, liver, pancreas), creatine concentrations are small (0.1-0.4 g/kg). In highest amounts, creatine is found in skeletal muscles (25-55 g/kg), heart (15-30 g/kg), and brain tissue (10-15 g/kg).

Urinary creatine is found only in children. In adult humans, creatinine is excreted in the urine (4.4 to 17.6 mmol per day); creatinine excretion has been found to be directly related to the muscular development of the human body. Creatine found in the urine of an adult human is indicative of pathology.

Production of certain vitamins and coenzymes from amino acids. In human tissues, *nicotinamide* (vitamin PP) is produced from tryptophan. Therefore, tryptophan supplied in food can in part compensate for a deficit of dietary nicotinamide. Cysteine is used for the biosynthesis of coenzymes of pantothenic acid (vitamin B5), *4-phosphapantetheine*, and *HS-coA*. *Glutathione*, which acts as a coenzyme for certain oxidoreductases and transferases (glutathione reductase, glutathione peroxidase, glutathione-S-transferase, γ-glutamyltranspeptidase, etc.), is produced from cysteine, glutamic acid, and glycine by a two-step reaction. At the first step of this synthesis, glutaminylcysteine synthetase is operative, and at the second step, glutathione synthetase.

Production and breakdown of mediators. Mediators are produced in the nervous tissue and certain other cells. Neuromediators, which are generated in the nerve endings, are transmitter substances that participate in the transmission of the nervous impulse to other nervous cells or peripheral organs and tissues. Tissue mediators take part in the metabolic tissue regulation.

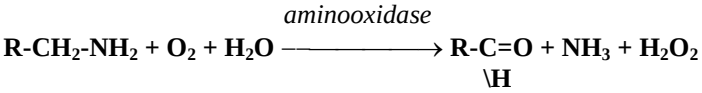
In the production of a number of mediators, of special importance is the reaction of amino acid decarboxylation specifically catalyzed by decarboxylases (enzymes that contain pyridoxal phosphate as a coenzyme):



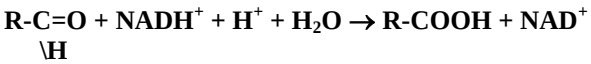
amino acid

The decarboxylation products are amines exhibiting a high biological activity. For this reason, they are referred to as *biogenic amines.* Most mediators belong to this group of compounds.

involvement of aminooxidases:



Aminooxidases are differentiated into a monoaminooxidase (*MAO*) type and a diaminooxidase (*DAO*) type. FAD serves as a coenzyme for MAO, and pyridoxal phosphate, for DAO (the reaction requires the presence of Cu^{2+} ions). MAO is bound with the cell mitochondria, and DAO is found in the cytoplasm. In small amounts these enzymes are present in the blood. Primary, secondary, and tertiary amines are inactivated by MAO, while histamine, putrescine, cadaverine, and to a lesser extent, aliphatic amines are mostly inactivated by DAO. Aldehydes produced by deamination of biogenic amines are oxidized to organic acids by *aldehyde dehydrogenases:*



Production of catecholamines. Catecholamines constitute a group of biogenic amines with mediatory and hormonal functions. They are produced from phenylalanine and tyrosine. The following biogenic amines are derived from phenylalanine and tyrosine: phenylethylamine, phenylethanolamine,

3,4-dihydroxyphenylalanine (DOPA), noradrenalin, adrenalin, dopamine (3,4-dihydroxyphenethylamine).

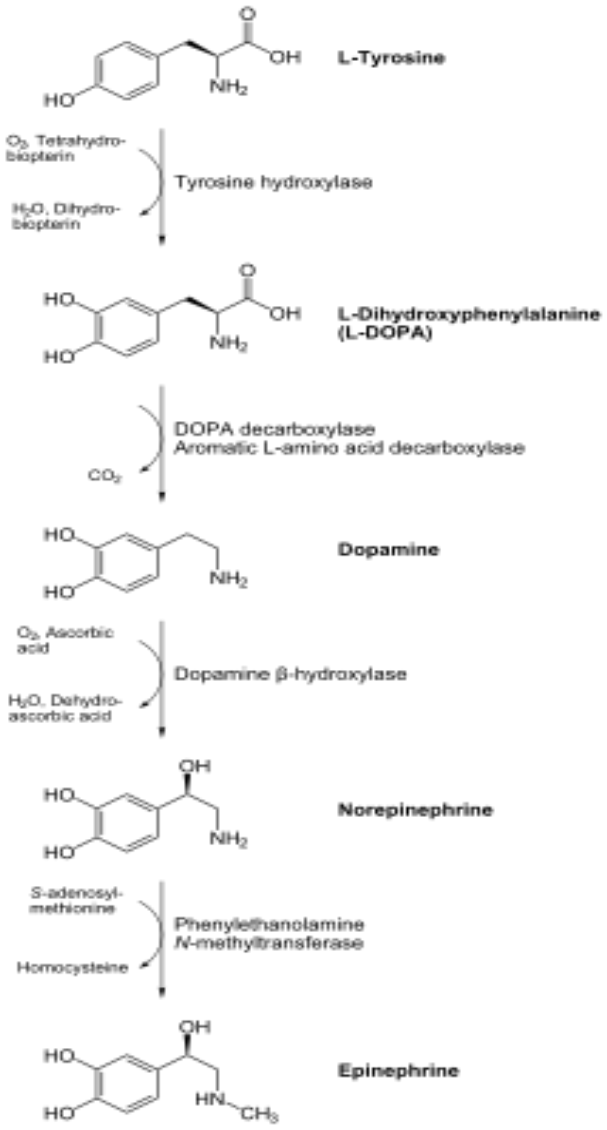


Figure 52. **Scheme of adrenalin and noradrenalin production**

The catecholamines that exhibit mediatory and hormonal functions to a great extent are adrenalin and noradrenalin. Noradrenalin and adrenalin are synthesized in the chromaffin cells of *substantia medullaris* of the adrenal glands (for performing a hormonal function), in the adrenergic synapses of the brain, and in the sympathetic nerve endings of the vegetative nervous system.

Adrenalin and noradrenalin are inactivated via two pathways:

- involvement of *monoaminooxidase,* via deamination,
- involvement of *catechol O-methyltransferase,* via methylation.

Glycine. Metabolites and pharmaceuticals excreted as water-soluble glycine conjugates include glycocholic acid and hippuric acid formed from the food additive benzoate. Many drugs, drug metabolites, and other compounds with carboxyl groups are excreted in the urine as glycine conjugates. Glycine is incorporated into creatine, the nitrogen and α-carbon of glycine are incorporated into the pyrrole rings and the methylene bridge carbons of heme, and the entire glycine molecule becomes atoms 4, 5, and 7 of purines.

Cysteine. L-Cysteine is a precursor of the thioethanolamine portion of coenzyme A and of the taurine that conjugates with bile acids such as taurocholic acid.

Methionine. S-Adenosylmethionine, the principal source of methyl groups in the body, also contributes its carbon skeleton for the biosynthesis of the 3-diaminopropane portions of the polyamines

spermine and spermidine. It is donor of methyl groups for synthesis of adrenalin, thymine, choline, acetylcholine, phosphatidylcholine, etc.

Histidine. Decarboxylation of histidine to histamine is catalyzed by a broad-specifity aromatic L-amino acid decarboxylase. Histidine compounds present in the human body include ergothioneine, carnosine, and dietary anserine. Urinary levels of 3-methylhistidine are unusually low in patients with Wilson's disease.

Ornithine, and ***arginine.*** Arginine is the formamidine donor for creatine synthesis and via ornithine to putrescine, spermine, and spermidine. Arginine is also the precursor of the intercellular signaling molecule nitric oxide (*NO*) that serves as a neurotransmitter, smooth muscle relaxant, and vasodilator. Synthesis of NO, catalyzed by *NO synthase*, involves the NADPH-dependent reaction of L-arginine with O_2 to yield L-citrulline and NO.

Tryptophan. Following hydroxylation of tryptophan to 5-hydroxytryptophan by liver *tyrosine hydroxylase*, subsequent decarboxylation forms serotonin (5-hydroxytryptamine), a potent vasoconstrictor and stimulator of smooth muscle contraction. Catabolism of serotonin is initiated by *monoamine oxidase* catalyzed oxidative deamination to 5-hydroxyindoleacetate. The psychic stimulation that follows administration of iproniazid results from its ability to prolong the action of serotonin by inhibiting *monoamine oxidase*. In carcinoid (argentaffinoma), tumor cells overproduce serotonin. Urinary metabolites of serotonin in patients with carcinoid include *N*-acetylserotonin glucuronide and the glycine conjugate of 5-hydroxyindoleacetate.

Kidney tissue, liver tissue, and fecal bacteria all convert tryptophan to tryptamine, then to indole 3-acetate. The principal normal urinary catabolites of tryptophan are 5-hydroxyindoleacetate and indole 3-acetate.

Tyrosine. Neutral cells convert tyrosine to epinephrine and norepinephrine. While DOPA is also an intermediate in the formation of melanin, different enzymes hydroxylate tyrosine in melanocytes. *DOPA decarboxylase*, a pyridoxal phosphate-dependent enzyme, forms dopamine. Tyrosine is also a precursor of triiodothyronine (T_3) and thyroxine (T_4).

Aspartic and glutamic amino acids. Ammonia may be toxic to the brain in part because it reacts with α-ketoglutarate to form glutamate. The resulting depleted level of α-ketoglutarate then impairs function of the tricarboxylic acid (TCA) cycle, or the Krebs cycle in neurons. Formation of glutamate is catalyzed by mitochondrial *glutamine synthase.* One function of glutamine is to sequester ammonia in a nontoxic form. Also production of asparagine is catalyzed by *asparagine synthase*. In kidney tissue glutamine is donor of ammonia for production of ammonium salts, which excreted with urine. Glutamine and asparagine are donors of ammonia for urea synthesis in liver. Aspartate is needed for urea production. Glutamate and aspartate participate in synthesis of purines, pyrimidins, and malate-aspartate shuttle mechanism. GABA is a product of glutamate decarboxylation. *L-glutamate dehydrogenase* occupies a central position in nitrogen metabolism.

Amino acid metabolism control in the organism. Amino acid balance in the human organism is dependent on the nutritive value of dietary proteins. Lack of any of the essential amino acids in the alimentary diet or a prolonged deficit of half-essential amino-acids entails a disorder in the utilization of other amino acids (both essential and nonessential) in protein biosynthesis. Amino acids transport to the cells of different organs is apparently determined by the efficiency of the carrier systems involved with respect to different groups of amino acids. In the kidneys the reabsorption of amino acids from the urine into the blood occurs; owing to this recovery mechanism, only a small amount of amino acids is lost into the urine.

The dietary supply of amino acids is one of the factors that regulates amino acid metabolism. An excessive consumption of food high in proteins results in an increased delivery of amino acids to the liver. The amino acids supplied favour an increased activity of the liver enzymes in the catabolic conversion of amino acids to the end products. This mechanism provides for the removal of an amino acid excess supplied to the organism. During starvation, on the contrary, an active breakdown of tissue proteins to free amino acids takes place.

The amino acid balance control at various stages of amino acid transport can be effected by means of the intestinal epithelium (in absorption), peripheral tissues (in intracellular penetration), and the tubules of the kidney (in reabsorption). Transmembrane amino acid transport is stimulated chiefly by insulin. Under physiological conditions, insulin, somatotropin, thyroid hormones, and male and female sex hormones facilitate the utilization of amino acids in protein biosynthesis and other synthetic processes.

Since amino acids constitute the major source of nitrogen for all the nitrogenous materials, they are responsible for the nitrogen balance in the organism. *Nitrogen balance* is the difference between the amount of nitrogen supplied to the organism in nitrogen-containing nutrients (chiefly proteins and amino acids) and that of nitrogen-containing materials discharged from the organism in the urine, feces, and sweat. If the amount of nitrogen supplied is equal to that lost from the organism, a *nitrogen equilibrium* sets in. If the amount of nitrogen ingested is larger than that excreted from the organism, the *nitrogen balance is said to be positive.* With the nitrogen eliminated superior to that supplied, the *nitrogen balance is negative.* The positive nitrogen balance is observed during the active synthesis of proteins from amino acids (during prenatal development of the fetus, during juvenile growth, in the diet rich in meat, in the administration of anabolic hormones, etc.). The negative nitrogen balance develops under complete protein deprivation, under prolonged reduction of motor activity, in grave chronic diseases, burns, and other states adverse to the normal protein digestion and amino acid absorption, or conducive to the degradation of tissue protein.

LECTURE 21

Subject: INTERRELATION OF MAJOR METABOLIC ROUTES IN THE ORGANISM. METABOLISM OF NUCLEOPROTEINS

Interrelation of metabolic routes in the human organism. The metabolisms of the monomers basically important for the living systems: amino acids, monosaccharides (glucose), fatty acids, and mononucleotides, are intimately interrelated. This all-pervading interdependence effected through the so-called key metabolites that intervene as common links in the catabolic and anabolic pathways of the monomers. Pyruvate, acetyl~ScoA, α-glycerol phosphate, and the Krebs cycle intermediates (oxaloacetate, malate, fumarate, succinyl~ScoA, α-ketoglutarate, isocitrate, and citrate) can be cited among these key metabolites. Pyruvate is a crossing point for the routes of synthesis and breakdown of glucose, other monosaccharides, and some of the amino acids. Acetyl~ScoA is a more branched site for metabolic pathways. Acetyl~ScoA bridges the gaps between monosaccharides, amino acids, and lipids, i.e. opens up possibilities for conversions of amino acids (finally, proteins) and glucose (in a wider sense, carbohydrates) to lipids. α-Glycerol phosphate serves as an accessory link between carbohydrates and lipids. It participates in the conversion of carbohydrates to certain lipids (triglycerides and phosphoglycerides) and, vice versa, of glycerol-containing lipids to carbohydrates.

Ample opportunities for the interconversion of monomers are provided by the intermediary products of the Krebs cycle. The Krebs cycle is a switching centre for all major catabolic and anabolic routes. For example, fatty acids, serving as a source for acetyl-ScoA, are convertible to carbohydrates (gluconeogenesis proceeds via the Krebs cycle oxaloacetate), to amino acids (via oxaloacetate and α-oxoglutarate to aspartic and glutamic acids) and to porphyrins (via succinyl-ScoA).

Since monomers are structural units for biopolymers, relative interchangeability of proteins, carbohydrates, and lipids as major nutritional components of the food, is possible. The acceptable limits for this interchangeability provide the clue to an understanding both of an eventual metabolic disorder as produced by monotonous dietary regimen and of the mechanism of metabolic compensation in starvation, i.e. when the essential materials are produced at the expense of the endogenic reserves of other materials in the organism.

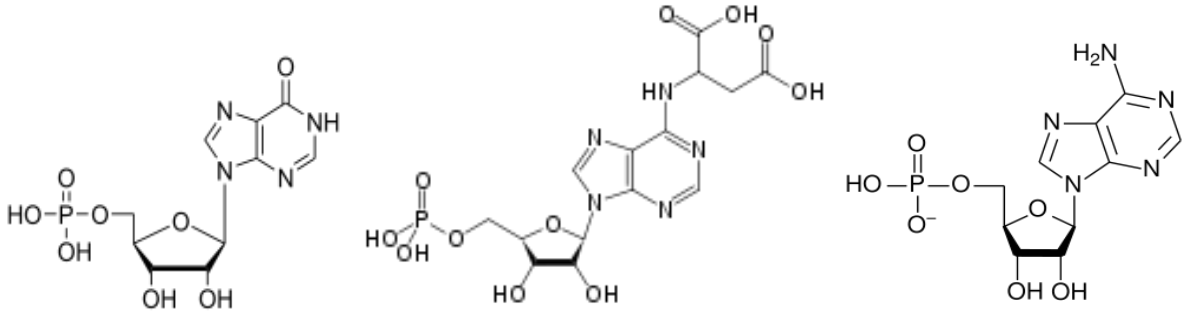
Metabolism of purine and pyrimidine mononucleotides

Mammals are not in need of dietary supply of nitrogenous bases or nucleotides despite the ability of the mammalien organism to assimilate directly these nutrients with food rich in nucleuc acids. In mammalian tissues the consumed purine and pyrimidine nucleotides are continually renewed via their synthesis from simple compounds.

Nucleotide phosphates, which are present in normal cells in small and relatively constant amounts, act as a link in the pathways of supply and consumption of mononucleotides:

Nucleic acids			Synthesis of nucleic acids
	→	Nucleotide →	Synthesis of nucleotide
Synthesis from		phosphate	coenzymes
simple compounds			Breakdown to end products

Biosynthesis of purine mononucleotides. The starting compound in the synthesis of purine nucleotides is phosphoribosyl pyrophosphate (PRPP), which can be derived from ribose-5-phosphate and ATP. The prime purine mononucleotide that crowns a long chain of synthetic reactions is inosine monophosphate (IMP), which is a parent species for generating other purine nucleotide phosphates via AMP and xantosine monophosphate (XMP). The genesis of the purine mononucleotide (AMP):

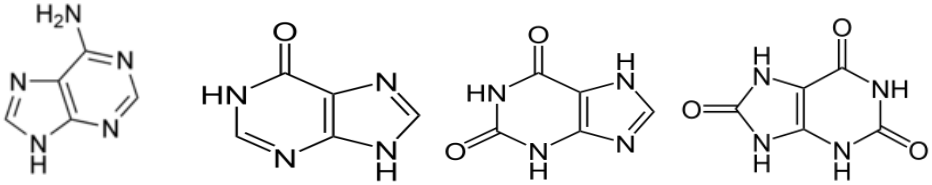


IMP Adenylosuccinate AMP

IMP + aspartate → Adenylosuccinate → fumarate + AMP

Figure 53. **Scheme of purine synthesis**

Breakdown of purine mononucleotides. Hypoxanthine is a naturally occurring purine derivative. It is also a deamination product of adenine. Xanthine is a product on the pathway of purine degradation, oxidation of hypoxanthine by xanthine oxidase. Also, by action of xanthine oxidase on xanthine forms the end product of purines degradation – uric acid, which excretes with urine.



AMP Hypoxanthine Xanthine Uric acid

Biosynthesis of pyrimidine mononucleotides (AMP, GMP)**.** The starting compounds in the synthesis of pyrimidine mononucleotides are carbomoyl phosphate and aspartic acid. They lead, through a long chain of reactions, to uridine 5'-monophosphate (UMP) and other pyrimidine mononucleotides. The constituent atoms of the pyrimidine ring are supplied by carbomoyl phosphate and aspartic acid.

Breakdown of pirimidine mononucleotides (TMP, UMP, CMP). The end products of pirimidine nucleotides degradation in the tissues are NH_3, CO_2, H_2O and β-alanine. Ammonia and CO_2 are used for urea production in the liver. In the muscles β-alanine is used for production of anserin and carnosin. So, the end product of pirimidine nucleotides breakdown is urea. Urea and β-alanine are excreted with urine.

Biosynthesis of deoxyribonucleotides, precursors to DNA, is effected by reducing ribose to 2'-deoxyribose within the preformed nucleotide. A special protein, thioredoxin, bearing two SH groups in its molecule, acts as a reductant in this process.

LECTURE 22

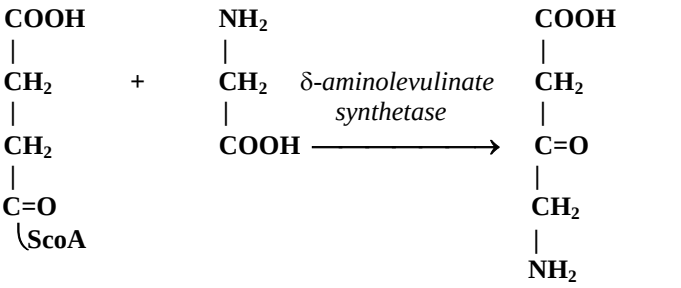
Subject: HEMOPROTEIN METABOLISM

A specific feature of hemoproteins is the metabolic involvement of the nonprotein moiety of these conjugated proteins. The hemoglobin of blood erythrocytes and of marrow cells accounts for a major portion (about 83%) of hemoproteins in the human organism. The remainder is myoglobin of skeletal muscles and heart (about 17%) and cellular hemoproteins – cytochromes, catalase, and peroxidase (1%).

Synthesis of hemoproteins

Glycine and succinyl~ScoA are the starting compounds in heme synthesis. The reaction involving the pyridoxal-assisted enzyme δ-*aminolevulinate synthetase* yields δ-aminolevulinic acid. Two mole-

cules of δ-aminolevulenic acid are combined with the participation of *porphobilinogen synthetase,* to form *porphobilinogen,* a direct precursor of porphyrins. One of these is *coproporphyrin III* which is directly converted to *protoporphyrin IX*. The insertion of iron ions (Fe^{2+}) into the protoporphyrin IX ring is effected with the assistance of *ferrochelatase*. At the ultimate step, heme becomes complexed with globin to form *hemoglobin* or *myoglobin.* In the synthesis of other hemoproteins, heme adds to the specific protein moiety of cytochromes or other hemo-containing enzymes. It these is not a sufficient quantity of protein to bind the reaction step of porphyrin synthesis.



The enzymes involved in heme biosynthesis are found in the marrow, nucleated erythrocytes, liver, kidneys, and intestinal mucosa. The reactions leading to δ-aminolevulinic acid proceed in the mitochondria; the production of porphobilinogen and the subsequent synthesis of coproporphyrinogen III occur in the cytoplasm, and the synthesis of heme from coproporphyrinogen III, in mitochondria.

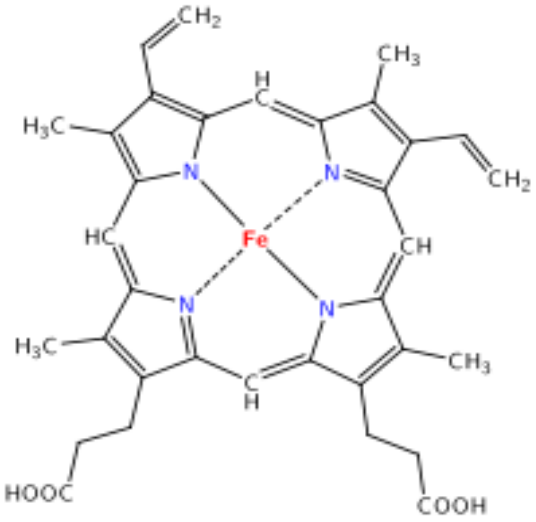


Figure 54. **Scheme of heme**

Breakdown of hemoproteins

The ***first stage of hemoprotein catabolism.*** In the human organism, about 9g of hemoproteins per day is degraded, mainly owing to the breakdown of erythrocytic hemoglobin. Erythrocytes undergo degradation (hemolysis) in the bloodstream or in the spleen in the range of about 120 days.

Hemoglobin, released from the erythrocytes in the blood, becomes bound with haptoglobin (a group of glycoproteins in the α_2-globulin fraction of blood plasma) and is supplied, as a hemoglobin-haptoglobin complex, to the cells of the reticulo-endothelial system (*RES*), chiefly to those of the spleen. Hemoglobin is oxidized to methemoglobin (Fe^{3+}) to be subsequently degraded. Haptoglobin is split off to pass into the blood. In RES cells, the first stage of hemoglobin degradation takes place, leading to bilirubin production. In the first step, heme is converted to biliverdin by the enzyme *heme oxygenase* (HOXG).

$$\text{heme} \xrightarrow[H^+ + NADPH^+ \; / \; O_2]{HMOX} \text{biliverdin} + Fe^{3+} \; / \; NADP^+ \; / \; CO$$

$NADPH^+$ is used as the reducing agent, molecular oxygen enters the reaction, carbon monoxide *CO* is produced and the iron is released from the molecule as the ferric ion (Fe^{3+}).

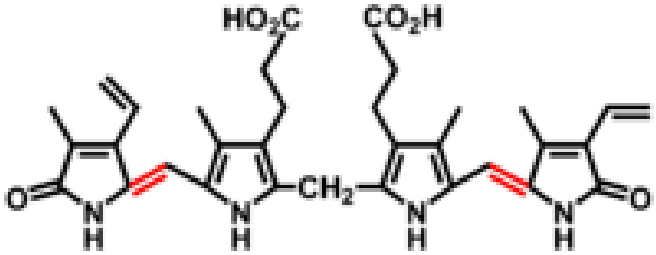
In the second reaction, biliverdin (*biliverdin* is a bile pigment of green colour) is converted to bilirubin by *biliverdin reductase* (BVR). Biliverdin can be reduced to bilirubin, a yellow-red pigment:

BVR

biliverdin → bilirubin

/ \

$H^+ + NADPH^+$ $NADP^+$



Bilirubin

Globin is hydrolyzed by the spleen cathepsins to amino acids. ***Bilirubin*** is sparingly soluble in water and, when released into the blood, it becomes bound with the plasma protein albumin. The albumin-bilirubin complex is the most important normal transport form for bile pigments. In the bloodstream, bilirubin is carried over to the liver cells. Since bilirubin is a lipophil, it passes easily across the liver cell membrane and, in doing so, liberates itself from albumin.

The ***second stage of hemoprotein conversion*** takes place inside the liver cells. Conjugated forms of bilirubin, *bilirubin glucuronides*, are produced in the liver. UDP-glucuronic acid acts as a donor of glucuronic acid.

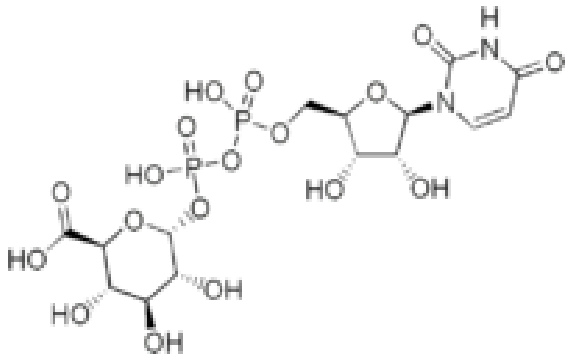


Figure 55. **Chemical structure of UDP-GA**

Bilirubin is transported into the liver, where it is conjugated with glucuronic acid to become more water soluble. The reaction is catalyzed by the enzyme *UDP-glucuronide transferase* (UDP-GUTF) *glucuronosyltransferase* to yield bilirubin monoglucuronide (20%) and bilirubin diglucuronide (80%). Bilirubin glucuronides are compounds readily soluble in water.

UDP-GUTF

bilirubin + 2 UDP-glucuronate → bilirubin diglucuronide

\

2 UMP + 2 Pi

Bilirubin glucuronides are capable, to a small extent through, of diffusing into the blood capillary. For this reason, two bilirubin forms are present in the blood plasma: *unconjugated* (also referred to as indirect, or free) bilirubin, and *conjugated* (direct or bound) bilirubin. The former one accounts for about 75% of the total blood plasma bilirubin, and the latter, for about 25%.

The ***third stage of hemoprotein degradation.*** Bilirubin glucuronides are excreted in the intestinal bile where the third, final stage of hemoprotein degradation occurs. In the bile tracts, glucuronic acid

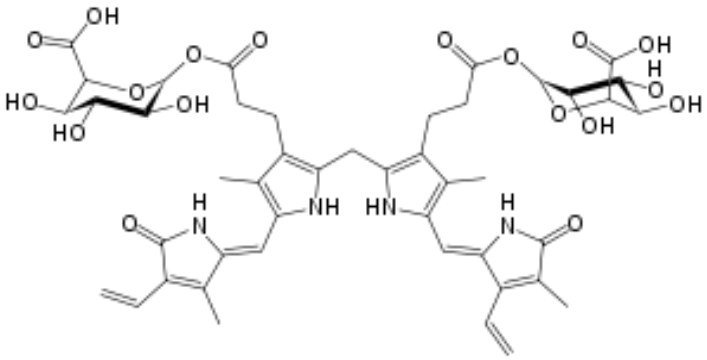


Figure 56. **Bilirubin diglucuronide**

is split off bilirubin glucuronides, with the recovery of unconjugated bilirubin. In the small intestine, a small portion of bilirubin can be absorbed and, through the portal vein, be supplied to the liver to be again excreted in the bile from the intestine. This hepatoenteric circulation, bearing resemblance to bile acid circulation, is presumably by the intestinal bacteria or by the reductases of intestinal mucosa. In the small intestine, bilirubin is converted to *mesobilirubin* and then to *mesobilinogen* (or *urobilinogen*). The latter is absorbed in the small intestine and through the portal vein is delivered to the liver in which urobilinogen is irreversibly degraded to mono- and dipyrroles. The intact urobilinogen is returned in the bile to the intestine. In the large intestine, mesobilirubinogen (urobilinogen) is reduced by anaerobic bacteria to stercobilinogen which like urobilinogen, is a colourless substance. Most stercobilinogen is excreted in the feces and undergoes rapid oxidation with atmospheric oxygen to stercobilin, an orange-yellow pigment, chiefly responsible for the colour of the feces.

A small amount of stercobilinogen is absorbed in the rectum, and through the hemorrhoidal vein system, in bypass to the liver, is delivered in the bloodstream to the kidneys to be excreted in the urine. Urinary stercobilinogen, like fecal stercobilinogen, is oxidized to stercobilin which is in part responsible for the light-yellow colour of the normal urine. It was formerly believed that urobilinogen was excreted in the urine (hence the name coined for this material) to be oxidized to urobilin exhibiting the same colour as stercobilin. Presumably, the resemblance in colour might have led to erroneous conclusions, since urobilin and stercobilin structurally are different species. Normally, urobilinogen is excreted neither in urine, nor in feces.

Thus, the intermediate product of heme breakdown, bilirubin, is normally not accumulated in the blood, but is rapidly entrapped by the liver cells. The concentrations (absolute and relative) of different forms of bilirubin in the blood allow on the other hand, on the bilirubin conversion in the liver. Normally, the total bilirubin concentration in the blood serum amounts to 8-20 mcmol/litre (here unconjugated bilirubin accounts for 75%).

In humans, the end product of bilirubin conversion, stercobilinogen, is chiefly excreted in the feces (about 300 mg per day) and, to a lesser extent, in the urine (about 1-4 mg per day). The composition of the pigments excreted in the feces and urine is influenced by the intestinal microflora. In neonates, whose intestine is devoid of microflora, bilirubin being excreted is oxidized to biliverdin, which is green in colour; for this reason, the meconium (newborns' feces) retains a dark green grass-like colour. In infants, within the first year of life, as the intestine becomes populated with microflora, the composition of excreted pigments and the colour of feces gradually change. The greenish fecal colour, produced by the presence of biliverdin, disappears, since the intestinal bacteria become increasingly active in the reduction of bilirubin to stercobilinogen.

Pathology of bile pigment metabolism

The production, conversion, and excretion of bilirubin in the organism may become disturbed owing to a variety of factors. An increased concentration of bilirubin in the blood leads to its deposition in tissues, including skin and mucosa, and imparts to these a yellow-brownish colour (the color of bilirubin). Such states of the organism are referred to as *jaundices.* A variety of jaundices are known, of which typical are:

- *hemolytic jaundice,*
- *parenchymatous* (or hepatocellular) *jaundice,*
- *obstructive jaundice.*

Hemolytic jaundice may arise from a number of causes conducive to an extensive intravascular (or tissue) breakdown of erythrocytes, or to their tissue breakdown (in RES cells). A large amount of unconjugated bilirubin, excreted from RES cells in the bloodstream, fail to become conjugated in the liver, which results in a high bilirubin level in the blood. Owing to an excess of excreted stercobilin and urobilin, the feces acquire an intense, nearly dark coloration, and the abundantly excreted urobilin colours the urine intensely yellow-orange.

Parenchymatous jaundice arises because of impaired liver cells (as afflicted with viruses or toxic hepatotropic compounds); the liver cells become more permeable to invading agents, including bilirubin glucuronides normally excreted from hepatic cells in the blood in small amounts. The impaired hepatic cells exhibit a reduced activity towards the uptake of bilirubin from the blood and fail to provide for the normal production of bilirubin glucuronides in them. For this reason, despite the normal hemolysis, the concentrations of unconjugated and conjugated bilirubin increase, albeit to a lesser extent as in hemolytic jaundice. Because of a diminished excretion of stercobilin and urobilin, the feces and the urine are but slightly coloured. However, a small quantity of urinary unconjugated bilirubin, normally absent in the urine, is observed.

Obstructive jaundice as its name implies arises due to an impeded outflow of bile in the intestine. As a consequence, conjugated bilirubin is reexcreted from the liver cells in the blood. The blood contains an increased amount of conjuated bilirubin which, being a readily soluble compound, is excreted at a higher rate in the urine. Because of this, the urine acquires a beer-like colour, and is discharged with yellow bright foam. The feces, devoid of bile pigments, are imparted a grey-white, clay-like colour.

Inogenous jaundice (jaundice in newborns) is believed to be of physiological origin. This disease arises from the developmental insufficiency of glucuronosyltransferase, the bilirubin conjugation enzyme. Therefore, an increased degradation of erythrocytes, irrespective of its origin, leads to an enhanced level of unconjugated bilirubin in the blood and, consequently, to an icteric indisposition. Commonly, physiological jaundice in newborns is transient within two weeks as the hepatic secretion of glucuronosyltransferase increases. In premature infants, physiological jaundice may be of longer duration. However, a prolonged increase of unconjugated bilirubin concentration in the blood may constitute a health hazard due to the toxic effect of bilirubin on the developing brain. Occasionally, convulsions and irreversible nervous system disturbances are observed in infants. In adults, the cerebral cells are little permeable to bilirubin and, as a rule, hyperbilirubinemia evokes no complications. In certain instances, in order to stimulate the activity of glucuronosyltransferase, the administration of phenobarbital is recommendable, which increases the enzyme secretion and alleviates the icteric processes.

The impaired pigment metabolism is also observed in intestinal dysbacteriosis produced by suppression of normal microflora in the intestine (for example, as a result of prolonged therapy with tetracycline antibiotics). In dysbacteriosis, intermediary products of bilirubin reduction are excreted in the feces; under deep suppression of the intestinal microflora, bilirubin itself, when oxidized to biliverdin, colours the feces greenish.

LECTURE 23

Subject: **NEUROENDOCRINE CONTROL OF METABOLISM**

The endocrine system includes special glands whose cells function to secrete chemical regulators, commonly referred to as ***hormones***, into the internal media of organism, i.e. blood and lymph. The notion "hormone" (from the Greek *hormaion,* to incite, to set in motion) was coined by Beyliss and Starling in 1905. Currently, hormones are called compounds that are produced in the gland cells and secreted into the blood or lymph to exercise control over metabolism and development of the organism.

The following general biological signs are characteristic of hormones:

- Remote action, i.e. the hormones control metabolism and the functions of effector cells at a distance.
- Strict specificity of biological action, i.e. functionally, no hormone can be entirely replaced by another one.
- High biological activity, i.e. quite small amounts of hormone, in some instances of the order of 10^{-5}g, are sufficient to provide for the vital activity of the organism.

The hormone-secreting glands are occasionally subdivided into the *central glands* (anatomically related to specialized portions of the central nervous system) and the *peripheral glands.*

Endocrine functions are also exercised by other cells capable of secreting biologically active compounds whose properties resemble those of hormones. For this reason, these agents are conventionally referred to as *hormone-like compounds,* or *hormonoids* (local hormones, parahormones). Their action is, a rule, confined to the site where they are produced; they are secreted by cells dispersed about different tissues. Hormonoids are secerted by the cells of gastrointestinal tract (they control digestion), by the intestinal enterochromaffin cells (which produce serotonin – a regulator of intestinal function), by the mast cells of connective tissue (heparin and histamine), and by the cells of kidneys, seminal vesicles, and other organs (prostaglandins). Presumably, these endocrine cells are specific peripheral representatives of the endocrine system. This is borne out by the occurrence of certain true hormones in the peripheral organs, for example, somatostatin in the pancreas and liver.

By their chemical structure, hormones are subdivided into:

1. Protein-peptide hormones (those of hypothalamus, pancreas, pituitary and parathyroid glands, thyroidal calcitonin).
2. Amino acid derivatives (adrenalin, derived from amino acids phenylalanine and tyrosine; iodothyronines, derived from tyrosine; melatonin, derived from tryptophan).
3. Steroids (sex hormones: androgens, estrogens, and gestagens; corticosteroids).

Neuroendocrine relationship scheme

Information on the state of internal and extaernal media of the organism is transmitted to the nervous system to be processed therein; in response, regulatory signals are transmitted to the peripheral tissues and organs. The production and secretion of hormones by the peripheral glands are accomplished incessantly. This is essential for maintaining the hormones at appropriate level in the blood, since they are liable to fast inactivation and elimination from the organism. The required level of a hormone in the blood is maintained owing to the self-regulation mechanism. This mechanism is based on interhormonal relationships which are referred to as "*plus-minus"* interactions, or mutually exclusive relationships.

Hormonal control: general outline

Hormones, which are secreted by the glands into the blood, usually become bound to specific blood plasma transport proteins (or, in certain cases, adsorbed on blood cells) to be carried to the peripheral tissues, where they exert influence on metabolism and tissue function. Various tissues respond to hormonal action differently. Tissues (cells, organs) exhibiting a high sensitivity to hormones, i.e. the tissues in which the hormones elicit pronounced charges in metabolism and function, are called "*targets*" for a given hormone, while other, less responsive, tissues are assigned to "*nontarget*" tissues; it should be admitted that this classification is rather arbitrary. The hormonal influence extends, in a wider sense, to all metabolic manifestations. However, in order to get a better understanding of the mechanism of action of any hormone or, in fact any extracellular regulator, it is worthwhile to consider the general principles of hormonal metabolic control.

The following possible types of action for extracellular regulators (often referred to as *first messengers*), including hormones, are distinguished:

- membrane, or local, action,
- membrane intracellular, or indirect, action,
- cytosolic, or direct, action.

Membrane type of hormonal action. The membrane type of hormonal action consists in that the hormone, at the site of its binding with the cell membrane, renders the membrane permeable to *glucose, amino acids,* and *certain ions* (Ca^{2+}, K^{+}). In this case, the hormone acts as an allosteric effector for membrane transport systems. The glucose and amino acids supplied exert, in turn, influence on biochemical cellular processes, while a change in ion partition on both sides of the membrane affects the electric potential and function of the cell. As a typical example, insulin may be cited which exhibits both a membrane type (by eliciting local changes in the transport of certain ions, glucose, and, possibly, amino acids) and a membrane-intracellular type of action.

Membrane-intracellular mechanism for metabolic control. The membrane-intracellular type of action in characteristic of hormones or functionally related to them extracellular regulators called *first messengers*. These are not capable of entering the cell and cannot therefore influence the intracellular processes directly. Their action is effected indirectly, through the agency of an intracellular mediator

(or *second messenger*) which triggers a chain of successive biochemical reactions leading to a modification of cellular functions.

The sequence of information transfer from diverse external stimuli into the cell may be outlined within the following general scheme: external signal (first messenger) → membrane receptor → transducer→ →chemical amplifier→second messenger→internal effector→cell response.

The first messenger (for example, a hormone) becomes bound to the receptor on the outer side of plasma membrane. The membrane receptors are a kind of "*start buttons"* on a control desk of cellular metabolism: the *hormone – receptor complex* thus formed acts on a protein, the membrane transducer, which, in turn, transmits the signal to an enzyme (chemical amplifier) acting as a catalyst for the production of a second messenger inside the cell. The second messenger binds to a special protein (internal effector) which exerts influence on the activity of definite enzymes or on the properties of nonenzymic proteins conducive to alterations of cellular processes, viz. chemical conversion rates, permeability, secretion, structural contractility, activity of genes, etc.

There are known several groups of compounds that are candidates for the function of a second messenger. These are:

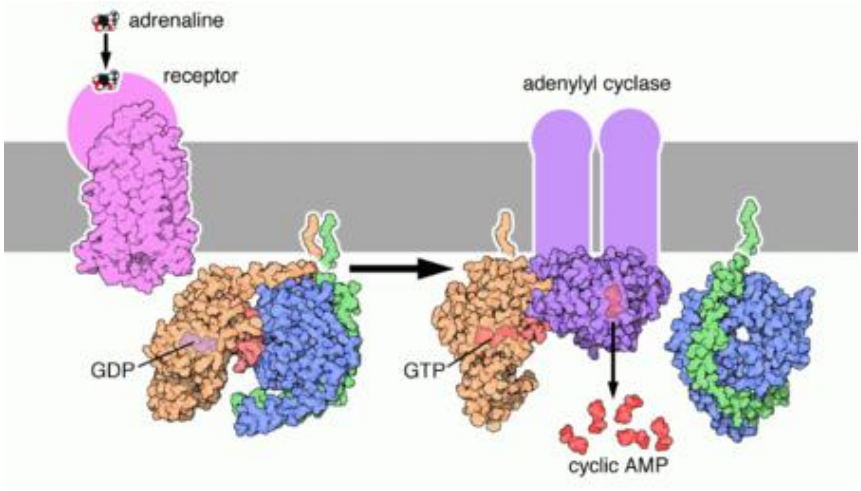
1. Nucleotides: cyclic nucleotides (3',5'-cAMP and 3',5'-cGMP), 2,5-oligo(A)*n* (adenyl oligonucleotide).
2. Lipids: diacylglyceride, inositol triphosphate.
3. Ca^{2+} ions.
4. Peptides.

Metabolic control effected via cyclic nucleotides. The intracellular regulators affect the production of cyclic nucleotides via either of the two signal systems: adenylate cyclase or guanilate cyclase. The former controls the production of cAMP and the latter of cGMP.

Adenylate cyclase is built into the membrane. This enzyme catalyzes the production of cAMP according to the scheme:

$$\mathbf{ATP \rightarrow 3',5'\text{-}cAMP + H_4P_2O_7}$$

The interaction of the external regulator with ***R***-type receptors brings about a conformational change in the latter, and this effect is transmitted to the ***G***-protein which thus acquires an ability to bind GTP. The ***G-GTP*** complex acts as an allosteric activator towards adenylate cyclase. Its activation initiates the production of cAMP from the intracellular ATP. Thus, at the moment as the G-GTP complex is formed, information is transferred from the external signal molecule to an internal molecule, the second messenger. Until the G-GTP complex persists, adenylate cyclase continuously catalyzes the cAMP synthesis, owing to which the external chemical signal becomes multiply amplified: per one molecule of R-bound first messenger, as many as 100-1000 molecules of cAMP, the second messenger, are generated.



The production of cAMP ceases as the G-GTP complex becomes inactivated, which is effected by the action of GTPase acting to hydrolyze GTP to GDP in this case, G loses its ability to activate adenylate cyclase. The *cholera toxin* is known to inhibit GTPase extending thereby the lifespan of G-GTP complex which elicits the continuous production of cAMP. In the intestinal cells, cAMP makes the

secretion of fluid in the organism sharply increase, which explains the acute diarrhea in patients with cholera.

The othere cyclic nucleotide, a potential candidate for the second messenger, is cGMP. It is produced from GTP with the participation of guanylate cyclase. In distinction from adenylate cyclase, this enzyme is loosely bound to the plasma membrane.

Further transmission of the signal through the aid of cAMP and cGMP is effected with the participation of protein kinases as internal effectors. The protein kinase function is phosphorylation of definite proteins according to the scheme: ***protein + ATP → phosphoprotein + ADP.***

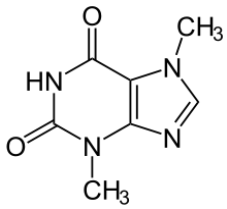
Since the cyclic nucleotides transmit information to protein kinase which is catalyst, an added, 10-to-100-fold increase in chemical signal occurs, otherwise stated, each of the protein kinase molecules, while persisting in an activated state, is able to phosphorylate and, therefore, to functionally modify, to about a hundred diverse proteins. Thus a single hormone molecule is potentially capable of modifying the structure and affecting the function of nearly a million molecules in the cell.

In the cell are systems that offset the influence of cyclic nucleotides on biochemical processes. The hydrolysis of cyclic nucleotides is carried out by phosphodiesterases according to the scheme:

3',5'-cAMP → *phosphodiesterase* **+ H_2O → 5'-AMP**
or **3',5'-cGMP →** *phosphodiesterase* **+ H_2O → 5'-GMP**

The 5'-AMP and 5'-GMP thus formed are incapable of activating the protein kinases. Numerous regulators act to increase cAMP concentration. They encompass all the hypothalamic and hypophyseal hormones, calcitonin, parathyrine, glucagon, catecholamines (acting via β-adrenergic receptors), vasopressin, histamine, and certain prostaglandins. The action exerted by hormones is reversible; it is operable within a few seconds or minutes after the contact of hormone with a membrane receptor. The hormones whose action is mediated by cAMP produce not only short-term metabolic changes (i.e. stimulate lipolysis, glycogenolysis, ion transport, etc.), but long-term ones also. *Caffeine* and *theobromine* are the competitive inhibitors of enzyme – *phosphodiesterase*.

Caffeine



Theobromine

Metabolic control via Ca^{2+} ions. The intracellular concentration of Ca^{2+} ions is negligeably small 10^{-7} mol/L, while outside the cell, their concentration is as high as 10^{-3} mol/L. The Ca^{2+} ions are delivered into the cell from the external medium via several "*calcium channels"* in the cell membrane. The Ca^{2+}ions flow is controlled by the Ca^{2+}-ATPase of cell membrane which pumps Ca^{2+} ions out at the expense of ATP energy. The elimination of Ca^{2+} ions from the intracellular depots is accomplished in a manner similar to their supply from the exterior via calcium channels. The calcium supplied from the external medium or from intracellular depots by the action of various external stimuli interacts with cytoplasmic calcium-binding proteins. As has been mentioned earlier, the most important of them is *cadmodulin* (CaM).

The Ca^{2+}-CaM complex by activating the protein kinase sensitive to it or other enzymes exerts control over biochemical processes and other functional activities of the cell related to them.

Cell-invading drugs inhibit either of the phosphodiesterases cAMP or cGMP, they may simulate a metabolic effect due to natural hormones and mediators operative via cyclic nucleotides. The phosphodiesterase activators inhibit the action of these hormones and mediators. This possibility is made use of in practical medicine by applying drugs that can inhibit phosphodiesterase. These include xantine derivatives, such as *caffeine, theophylline, theobromine,* and *euphylline*. These drugs mainly increase the concentration of cAMP, while such a drug as *trental* increases chiefly the cGMP concentration.

Cytosolic mechanism of hormone action. Cytosolic mechanism is typical of the hormones that can penetrate through the lipid layer of plasmatic membrane, i.e. are related by their physico-chemical properties, to lipophilic compounds, for example, steroid hormones (vitamin D, by its metabolic effect, is also close to steroid hormones, and for this reason it is often assigned to hormonal agents).

Hormones with cytosolic type of action penetrate the cell to be complexed with cytosolic or nuclear receptors. The receptor-complexed hormones control the enzyme concentration in the cell by selectively affecting the gene activity of nuclear chromosomes and exerting thereby influence on metabolism and functions of the cell. Since the cell-invading hormone is involved in the mechanism of enzyme concentration control, this type of action is called *direct action* in contrast to the membrane intracellular mechanism, when a hormone controls *metabolism indirectly*, through the agency of second messengers. In terms of lipophilicity, iodothyronines are found midway between steroids and other water-soluble hormones. Probably, for this reason they exhibit a *combined type* of action on cell metabolism, i.e. both membranes intracellular and cytosolic.

Production and practical application of hormones. For their practical applications, hormones are produced by extraction from biological materials, by chemical synthesis, or by genetic engineering methods. The first of the above techniques is employed in the production of insulin and glucagon from bovine pancreas, of corticotropin and melanotropin from bovine pituitary, of folitropin (serum gonadotropin) from blood serum, and of lutropin (chorionic gonadotropin) from the urine of pregnant mares. Currently, chemical synthesis is widely used for production of all steroid hormones, their analogues and derivatives iodothyronines and other hormones-amino acid derivatives and peptide hormones of oxytocin type. Practically all the protein hormones can be prepared by laboratory synthesis. Insulin, somatostatin and related hormones have been prepared by the genetic engineering method in the laboratory.

In medical practice, hormones are used for *substitution therapy* and *pathogenetic therapy*. In the latter instance, certain specific properties of hormones (antiinflammatory, anabolic, etc.) are made use of, even through the hormonal concentrations in the patient's organism may be at normal level.

LECTURE 24

Subject: HORMONES OF HYPOTHALAMUS-HYPOPHYSEAL SYSTEM. HORMONES OF PERIPHERAL GLANDS

Production and secretion of hormones by hypothalamus and hypophysis are closely related and for this reason it appears expedient to discuss the relevant processes in parallel. In the anterior lobe of the pituitary gland (*adenohypophysis*), tropic hormones are produced, while the posterior pituitary lobe (*neurohypophysis*) releases only neurohormones (vasopressin and oxytocin), which are produced in the hypothalamus nuclei.

By their chemical structure, thyrotropin, follitropin, and lutropin are glycoproteins. They are composed of two subunits, α and β. In all these glycoproteins, the α-subunits are identical, while the β-subunits are structurally different and determinative of the specificity of hormonal action. Other relevant hormones are simple proteins possessing a single polypeptide chain; vasopressin and oxytocin are cyclic octapeptides.

The secretion of tropic hormones is controlled by hypothalamic peptides. To date the following hypothalamic neuropeptides, which are regulators of hypophyseal hormonal secretion, have been isolated:

Tropic (pituitary) hormone	**Hypothalamic hormone**
Somatotropin	Somatoliberin, somatostatin
Corticotropin	Corticoliberin, corticostatin
Thyrotropin	Thyroliberin, thyrostatin
Follitropin	Folliberin
Lutropin	Luliberin
Prolactin	Prolactoliberin, prolactostatin
Melanotropin	Melanoliberin, melanostatin

Mechanism of action and functions of hypophyseal hormones

All the tropic hormones exert their action either on the function of peripheral glands, or directly on the peripheral tissues by binding to the membrane receptors and by activating adenylate cyclase. cAMP effects the hormone production or metabolism in the target cells.

The effects that the hypophyseal hormones can produce may be divided into four groups:

1. Control of biosynthesis and hormonal secretion by peripheral glands (thyrotropin, follitropin, lutropin, corticotropin, and somatotropin).

2. Control of sex cell production (follitropin).

3. Functional and metabolic control of effector tissues and organs (somatotropin, α- and β-lipotropins, corticotropin, lutropin, follitropin, melanotropin, prolactin, oxytocin, and vasopressin).

4. Functional control of the nervous system (corticotropin, β-lipotropin, and others).

Direct effect of hypophyseal hormones on peripheral tissues

Corticotropin exerts a direct action on fat tissue by stimulating the tissue's glucose absorption and release of fatty acids and glycerol. The hormonal fat-mobilizing effect is related to adenylate cyclase activation; the cAMP formed stimulates *triacylglyceride lipase*, which splits triacylglycerides into glycerol and fatty acids. In addition, the hormonal action of corticotropin on melanin production and skin pigmentation is similar to that of melanotropin.

α- and β-lipotropins exert a specific fat-mobilizing action by the mechanism typical of all c-AMP-stimulating hormones.

Gonadotropins produce a fat-mobilizing effect similar to that of lipotropins. Moreover, prolactin stimulates protein biosynthesis and lactose production by the mammary gland epithelium.

Vasopressin, or ***antidiuretic hormone***, in addition to its fat-mobilizing action, exerts a selective control of water reabsorption in the distal tubes and collecting ducts of the kidneys and activates adenylate cyclase. cAMP activates protein kinases, which phosphorylate the cell membrane proteins to increase their permeability for water. The water reabsorption reduces *diuresis*, makes increase of the urine density and urinary concentrations of sodium and chlorides. Vasopressin stimulates the contraction of the muscular tissue of the capillaries and arterioles and produces a moderate rise in blood pressure.

Deficiency in vasopressin develops a disease called *diabetes insipidus.* It is manifested by a large discharge of urine (4 to 10 liters per day) of low density (1.002-1.006); excessive thirst (polydipsia) develops. Administration of vasopressin preparations removes the symptoms of this disease.

Oxytocin stimulates contraction of uterine muscles; this action is associated with an increased intracellular Ca^{2+} concentration and with cGMP production. Oxytocin accelerates protein synthesis in the mammary glands during the lactation period (in part, this effect is also exhibited by vasopressin) and stimulates the release of breast milk owing to an enhanced contractile activity of the milk duct myoepithelium. This hormone produces an insulin-like effect on the fat tissue, i.e. increases glucose consumption and triglyceride synthesis in it.

Melanotropin. α- and ***β-melanotropins*** are distinguished. The two hormones are secreted by the anterior pituitary and affect the production of *melanin* in skin, iris of the eye, and the epithelial pigment of the retina. They produce a fat-mobilizing action on the fat tissue by stimulating cAMP generation in it.

Somatotropin, or ***growth hormone***, is the only hormone that exhibits a biological species-specific effect. The animal somatotropin produces no effect on the humans. It also controls the growth of internal organs and soft tissues of the face and oral cavity. Its direct action is associated with the activation of adenylate cyclase and formation of cAMP, for example, in muscles and insular pancreatic tissue.

Somatotropin deficiency in the young organism is conducive to a premature growth cessation, with on eventual development of *dwarfism*. The stature of an adult dwarf is 100-120 cm. Hypophyseal dwarfism in distinction to those afflicted with hypothyroid dwarfism, has a proportionate constitution, with no signs of mental retardation. Somatotropin hypersecretion in a juvenile period shows up as *gigantism*; in a maturity period, it is conductive to a state called *acromegalia*. Acromegalia is manifested by an enlargement of the prominent parts of the face (nose, chin, superciliary eyebrows).

Hormones of peripheral endocrine glands

Thyroidal hormones. The thyroid gland secretes hormones of two groups, which affect metabolism differently. ***Iodothyronines-thyroxine*** and *iodothyronine-control* energy metabolism and exert influence on cell division and differentiation, determining thereby the development of the organism. ***Calcitonin*** (polypeptide hormone with a molecular mass of about 30,000) controls phosphorus-calcium metabolism: it appears more expedient to discuss calcitonin action in parallel to that of parathyroid glands.

Iodothyronines exert action on numerous tissues of the organism, the highest sensitivity being exhibited by the tissues of liver, heart, kidney, skeletal muscles, and, to a lesser extent, adipose and nervous tissues. In the organism, the thyroid hormones affect to a higher degree cell division, cell dif-

ferentiation, and energy metabolism. Alterations in energy metabolism due to the so-called calorigenic property of thyroid hormones; outwardly show up as an increased oxygen consumption and production of heat. Iodothyronines exert influence on metabolism via cytosolic receptors by effecting the nuclear chromosomes, and via cAMP.

For the synthesis of iodothyronines essential are iodide as supplied by active transport from blood to thyroidal epithelium and thyroglobulin which is produced in the epithelium and fills the follicular cavity to form colloid. Iodothyronine synthesis proceeds by several steps which are:

1. Formation of an "active" iodine from iodide through the aid of *iodide peroxidase* by reaction:

$$\mathbf{I^- - e\ (2e) \rightarrow I^\bullet}$$

H_2O_2 serves as an electron acceptor. The active iodine is capable of iodinating tyrosine.

2. Iodination of tyrosine constitutive of thyroglobulin with the participation of *tyrosine iodinase.* The products formed are monoiodotyrosine or diiodotyrosine.

3. Oxidative condensation of mono- and diiodotyrosines with the formation of triiodothyronine and tetraiodothyronine (thyroxine) within the thyroglobulin molecule. The process is carred out on the surface of *tyrosine iodinase*.

4. Uptake of thyroglobulin from the colloid by epithelium cells and its translocation to the outer membrane surface bathed by the extracellular fluid. This process is reminiscent of endocytosis.

5. Secretion of iodothyronines, which is accomplished by hydrolysis of thyroglobulin by proteases enabling the release of thyroxine (T_4) and triiodothyronine (T_3) into blood.

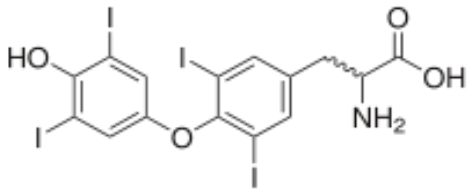


Figure 57. **Chemical structure of thyroxine (T_4)**

The thyroid gland malfunction may entail an excess or deficiency of iodothyronines in the organism. In the *hyperfunction of thyroid gland,* or *hyperthyroidism,* excessive production of iodothyronines is observed. Acute hyperfunctional forms have been named *thyrotoxicosis*, or *Basedow's disease,* since the symptoms of disturbed metabolism and malfunction may be likened to iodothyronine intoxication. In *hypofunction of the thyroid gland*, or *hypothyrosis*, the organism is in short supply of iodothyronines. Hypothyrosis in neonates or infants is called *cretinism,* or *infantile myxedema;* in adults, it is called simply *myxedema.*

Hormones of parathyroid glands. The parathyroid glands secrete two protein hormones *calcitonin* (also secreted by the thyroid gland) and *parathyrin* (*parathormone).* Calcitonin and parathyrin control the balance of Ca^{2+} ions and inorganic phosphate in the organism. In turn, the secretion of calcitonin and parathyrin, which lack appropriate tropic hormones, is feedback-controlled by Ca^{2+} ions. An increased concentration of Ca^{2+} ions in blood plasma elicits the secretion of calcitonin, on the contrary, a decreased percentage of Ca^{2+} in blood plasma is a stimulus for liberation of parathyrin from the glands. Parathyrin to a significant extent exerts influence on phosphorus-calcium metabolism through vitamin D.

Hormones of pancreatic gland

Hystologically, in the pancreas distinguished are *insular* (Sobolev-Langerhans islands) and *acinous* tissues which contain cells responsible for synthesis and secretion of a number of hormones. The cells of *A-type (α-cells), B-type (β-cells),* and *D-type* (all of them contained in insular tissue) secrete, respectively, ***glucagon***, ***insulin*** and ***somatostatin.***

Glucagon is a protein with M.m. of 3,485; it is composed of 29 amino acids. It binds to the membrane receptors of target tissues. The targets for glucagon are liver, fat tissue, and, to a lesser extent, muscles. By activating adenylate cyclase and by making increase the cAMP concentration, glucagon elicits mobilization of glycogen in liver and, partly, in skeletal muscles, and triacylglycerides in fat tissue. Mobilization of these energy reserves leads to increased levels of glucose, fatty acids, and glycerol in blood.

Insulin is formed in the β-cells as preproinsulin which, when subjested to hydrolysis, yields proinsulin. Proinsulin, containing 84 amino acid residues, suffers the cleavage of a polypeptide fragment (called C-peptide) composed of 33 amino acids. This results in the formation of insulin (of a molecular mass of about 6,000) composed of 51 amino acid residues. Insulin has two chains: a short one (*A-*

chain) made up of 21 amino acids and a long one (*B-chain*), of 30 amino acids. The two chains are cross-linked through disulphide bridges.

It supplied to the blood occurs in a free state or as bound to plasma proteins. Free insulin exerts influence on the metabolism of all insulin-sensitive tissues, while the bound insulin, on fat tissue only. The insulin-sensitive tissues include muscular and connective tissues. Membrane insulin receptors of glycoprotein nature have been found in the tissues. The insulin-receptor complex is capable of drastically changing the cell membrane permeability for glucose, amino acids, Ca^{2+}, K^{+}, and Na^{+} ions, or to be more precise, of accelerating the transport of glucose, amino acids, and Ca^{2+} and K^{+} ions into the cell.

The *excessive insulin* may be observed in tumoral islands (insulomes) or in overdosed insulin therapy. With *deficient insulin,* a widely spread disease, *diabetes mellitus,* develops. Diabetes mellitus develops under true and false insulin deficiency. The true insulin deficiency is due to a disorder in production and secretion of insulin in the insular β-cells (*insulin-depending diabetes mellitus,* type I). In patients with deficiency of receptors to insulin disease called *insulin-independent diabetes mellitus*, type II.

In diabetes mellitus take place the changes in carbohydrate, lipid, protein, and water-mineral metabolisms. The metabolic disturbances are: hyperglycemia and glucosuria, hyperaminoacidemia and hyperaminoaciduria, increased concentrations of fatty acids, glycerol, and cholesterol in blood, and ketonemia, and ketonuria.

Insulin preparations are used in the therapy of diabetes mellitus, as well as anabolic stimulators in dystrophy of organs.

Hormones of the adrenal glands

Hormones of adrenal medulla. In the medulla of the human adrenal glands, ***adrenalin*** and, to a lesser extent, ***noradrenalin,*** are formed.

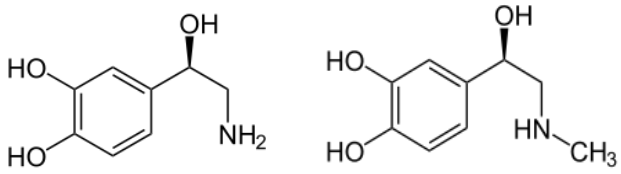


Figure 58. **Chemical structure of noradrenalin** (left) **and adrenalin**

Adrenalin exerts a dual effect on the metabolism of target tissues depending on the predominant occurrence in them of either α-, or β-adrenoreceptors to which the hormone becomes bound. The binding of adrenalin to β-adrenoreceptors stimulates adenylate cyclase and produces metabolic alterations characteristic of cAMP. The adrenalin binding with α-adrenoreceptors stimulates guanylate cyclase and produces metabolic alterations typical of cGMP. The alterations that occur in carbohydrate and lipid metabolisms are very much the same as those produced by glucagon.

Adrenal cortex hormones. In the adrenal cortex, *steroid hormones*, or ***corticosteroids***, are formed from cholesterol. Corticosteroids, by the physiological effect they produce, are subdivided into three groups:

- ***glucocorticoids*** chiefly affecting carbohydrate metabolism,
- ***mineralocorticoids*** chiefly affecting mineral metabolism,
- ***sex hormones*** (male hormones ***androgens,*** and female hormones ***estrogens***).

The human adrenal glands secrete glucocorticoids (*hydrocortisone* and *corticosterone)* and a mineralocorticoid (*aldosterone*).

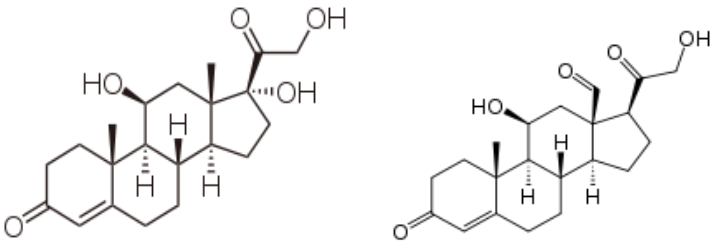


Figure 59. **Chemical structure of corticosteroids: cortisol** (left) **and aldosterone**

The secretion of *glucocorticoids* is controlled by corticotropin. ***Glucocorticoids*** (***cortisol***) become bound to α1-globulin of blood plasma, called *transcortin*, to be transported in the complexed stat to peripheral tissues. The targets for glucocorticoids are liver, kidney, lymphoid tissue (spleen, lymph nodes, lymphoid plaques of the intestine, lymphocytes, and thymus), connective tissues (bones, subcutaneous connective tissue, and adipose tissue), and skeletal muscles. These tissues contain cytosolic receptors for binding glucocorticoids. To be noted, a hormone-cytoreceptor complex may exert an entirely opposite effect on protein synthesis in different tissues. In the liver and kidneys, glucocorticoids favour the utilization of amino acids in gluconeogenesis, since they act as specific inducers of the synthesis of gluconeogenesis enzymes.

Since glucocorticoids enhance the secretion of adrenalin from the adrenal medulla, the action of glucocorticoids becomes "augmented" by the metabolic effect of adrenalin. Thus, glucocorticoids mobilize triacylglycerides from the adipose tissue at the expense of adenylate cyclase activation, although the membrane intracellular activity is not typical of them. Apparently, the mobilization of fat from the fat depots is associated with adrenalin. As a result, glycerol and fatty acids are supplied to the blood; glycerol is used in gluconeogenesis, while fatty acids are consumed in the liver to produce ketone bodies which are excreted in the blood.

Glucosuria, aminoaciduria, and ketonuria set in, as the concentrations of glucose, amino acids, fatty acids, glycerol, and ketone bodies in the blood increase. On the whole, these metabolic changes resemble a picture of *diabetes mellitus*. This diabetic state is of different nature and for this reason is referred to as *"steroid"* diabetes.

Glucocorticoids produce changes in the water-salt metabolism: they increase the Na+ ion reabsorption and renal excretion of K+. They return sodium and water in the extracellular space of the organism tissues (which may lead to *oedemas*). This action is similar to the effect due to mineralocorticoids, only less pronounced. Inhibition of the bone tissue protein synthesis leads to local deossification of the bones. Calcium and phosphorus are eliminated from the affected bone tissue into the blood and then are excreted in the urine.

Glucocorticoids and their analogues are widely applied in clinics. The medicinal effect of glucocorticoids is based on their ability to affect lymphoid and connective tissues. Glucocorticoids inhibit the formation of antibodies in the lymphoid tissue to produce the state of sensitization towards invaders, and thus prevent further development of allergic response and inflammation.

Mineralocorticoids (***aldosterone***) controls the balance of Na^+ and K^+ cations. At low Na^+ and high K^+ concentrations in blood, the synthesis and secretion of aldosterone become increased.

Aldosterone controls the balance of Na^+, K^+, Cl^- ions and water in the organism; for this reason, the normal function of this hormone is of utmost importance for the vital activity of the organism.

Aldosterone is transported in the blood to tissues by using plasma albumins as carrier adsorbents. The targets for aldosterone are epithelial cells of distal tubules of the kidney, which contain a large number of cytoreceptors for binding this hormone. The aldosterone-cytoreceptor complex penetrates the nuclei of the renal tubule cells and activates the transcription of chromosomal genes that carry information on the proteins involved in the transport of Na^+ ions across the membranes of tubular epithelium. Owing to this, the reabsorption of Na^+ and its counterion Cl^- from the urine into intercellular fluid and further into blood becomes increased. Simultaneously, K^+ ions are excreted (in exchange for Na^+) in the urine from the epithelium of renal tubules. On the whole, the aldosterone effect is manifested by the retention of Na^+, Cl^-, and water in the tissues and by urinary loss of K^+ ions.

Disturbances of hormonal function of the adrenal glands. In such forms of *hypercorticoidism,* or *Cushing's disease* (which occurs due to the impaired hypothalamohypophyseal system conducive to corticotrophin hypersecretion) and *corticosteroma* (a tumor active chiefly in the synthesis of hydrocortisone), a hyperproduction of glucocorticoids is observed.

There occurs *hypercorticoidism* attended by excessive secretion of aldosterone (*hyperaldosteronism,* or *Konn's disease*). In this disease, the symptoms of influence of aldosterone excess on the water-salt balance are observed, viz. *oedemas*, high blood pressure, and myocardial hyperexcitability.

Hypocorticoidism, also called *Addison's disease,* or *bronze disease,* is manifested by a deficiency in all the corticoids and attended by manifold alterations in metabolism and functions of the organism.

Sex hormones

The sex glands (gonads) are paired organs represented by the testes in males and by the ovaries in females. The male sex hormones, ***androgens,*** are produced by Leidig's cells, and the female sex hor-

mones (***estrogens)*** are produced in the ovarian follicles. The male and female hormones are synthesized from their common precursor, cholesterol.

Female sex hormones, ***estrogens*** (*estradiol)* and ***progesterone***, have the cytosolic mechanism of action. Most physiological effects due to estrogens are basically determined by the hormonal influence of estrogens on the activity of specific chromosomal genes. These effects ultimately induce the synthesis of specific proteins which are decisive for characteristic alterations in metabolism, cell growth, and cell differentiation. The pronounced *anabolic action* of estrogens, i.e. their ability to stimulate protein synthesis in target organs, provides for a *positive nitrogen balance* in the organism. Estrogens are inducers of glycolysis and pentose phosphate cycle. The estrogen hormones accelerate the renewal of lipids, inhibit accumulation of lipids in liver and fat tissues, favour the elimination of cholesterol from the organism and lower the cholesterol level in blood.

Progesterone exerts physiological and biochemical effects only during the luteal phase of ovarian cycle.

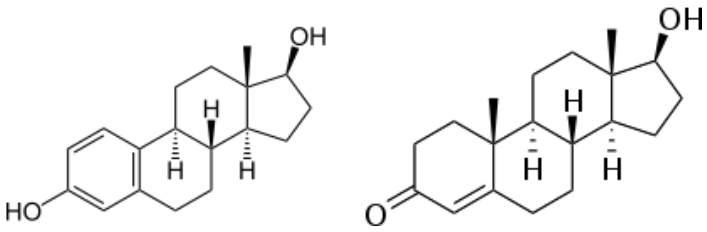


Figure 60. **Chemical structure of sex hormones: estradiol** (left) **and testosterone**

Androgens (*testosterone)*, prior to be supplied to the tissues become bound to glycoprotein of blood plasma called *testosterone-estradiol-binding globulin* (which is a specific binder for testosterone and estradiol). The androgens become bound to androgenic receptors and act on the nuclear chromatin of target cells to facilitate DNA synthesis activation during replication and to accelerate the specific gene transcription. The anabolic action of testosterone is much superior to that of estrogens. In males, this results in the development of powerful skeletal musculature in the development and mineralization of the epiphyseal growth zones of the bones.

Testosterone preparations and their synthetic analogues, ***anabolic steroids***, are used clinically. Anabolic steroids (methylandrostenediol, nerobolil, retabolil) are used to treat various dystrophies, diabetes mellitus. Female sex hormones are also used clinically.

Antihormones are compounds that exhibit antihormonal activity through binding to cytosolic receptors. While being active antagonists towards other hormones, they may either exhibit or not the intrinsic hormonal activity. The molecular mechanism of antihormonal action is based on the competition for binding sites with the corresponding cytosolic receptors. Owing to this, the complex "*antihormone-cytosolic receptor*"is incapable of acting as a protein synthesis inducer in the cells. An antihormone displaces a true hormone only when its concentration in the cell is very high.

Natural antihormones include estrogens and androgens which complete with each other for binding with the receptors of opponent hormone: estrogens block the androgenic receptors, and androgens, the estrogenic ones.

Antihormones are used in treating hormone-dependent tumors when a need arises to prevent the action of hormone on proliferating tumoral cells, in therapy of abnormal sexual behaviour (hypersexuality), etc.

Prostaglandins are hormone-like compounds (hormonoids) derived from C_{20}-polyene fatty acids containing a cyclopentane ring (see Lecture 15 "Biosynthesis of lipids"). Prostaglandins are short-lived species that are synthetized at heed in small amounts to exert a local biological effect at the site of their formation. An excessive production of prostaglandins or their deficiency may lead to pathologic processes such as inflammation, thrombosis, gastric ulcer, and others.

LECTURE 25

Subject: **BIOCHEMISTRY OF BLOOD: CHEMICAL COMPOSITION OF BLOOD PLASMA**

Blood is fluid tissue composed of cells (formed elements of the blood) and an extracellular liquid

medium. The overlying liquid (supernatant) of blood sample obtained on precipitation of the blood cells in the presence of an anticoagulant is called *blood plasma*. The plasma is an opalescent liquid containing all extracellular components of the blood. The blood cells account for about 45%, and the plasma, for about 55% of the blood volume. The clear liquid that separates from the blood when it is allowed to clot completely is called *blood serum*. Actually, the blood serum is the plasma from which fibrinogen has been removed in the process of clotting.

Water accounts for about 83% of blood accounts for the rest. By its physico-chemical properties, blood is a viscous liquid of specific density 1.050-1.060. The blood viscosity and density are dependent on the relative contents of blood cells and plasma proteins. The blood pH value (7.36-7.44) is maintained by buffer systems at a constant level, its variations not exceeding 0.05-0.10 pH unit.

Blood components. Cellular and extracellular blood components are different origin, and for this reason they may be regarded as indicators of biochemical physiological processes occurring in the tissues and organs from which they are supplied to the blood. Alterations in the blood composition are therefore diagnostic of the state of health of the human organism.

Alterations in the quantitative and qualitative composition and in biochemistry of the blood cells are warning signals on disturbances in the bone marrow; any abnormality in lymphocytes is indicative of an impaired function of both bone marrow and lymphoid tissue. Most chemical components of blood plasma are supplied from various organs. Since the cell membranes are impermeable or poorly permeable to macromolecules (proteins and enzymes), macromolecules are secreted from tissues and organs actively (by exocytosis). Major sources of macromolecules are liver, endocrine glands, partially intestinal mucosa, and blood cells themselves. Under physiological conditions, other organs and tissues are but to a small extent involved in the generation of blood plasma proteins. Low-molecular plasmic components are of more diversified origin and specify various aspects of tissue metabolism.

Biochemical and physiological functions of the blood are defined by a cooperative participation of blood cells and chemical components of blood plasma in metabolism.

Biochemical functions of blood and their characterization. The blood performs the following functions:

- transport,
- osmoregulatory,
- buffering,
- detoxifying,
- defensive, or immunologic,
- regulatory, or hormonal,
- hemostatic.

Chemical composition of blood plasma

I. Proteins

1. Total protein	65-85 g/L
2. Albumins	35-50 g/L
3. Globulins	25-35 g/L
4. Fibrinogen	2.0-7.0 g/L
5. Haptoglobin	0.28-1.90 g/L
6. Prothrombin	10-15 mg/dL
7. Plasminogen	1.4-2.8 μmol/L (20-40 mg/dL)
8. Transferrin	19.3-45.4 μmol/L (170-400 mg/dL)
9. Ceruloplasmin	1.52-3.31 μmol/L (23-50 mg/dL)
10. β-Lipoproteins	3.0-6.0 g/L (300-600 mg/dL)
HDL – high density lipoprotein (α-LP)	1.063-1.210 mmol/L (80-400 mg/dL)
LDL – low density lipoprotein (β-LP)	1.006-1.063 mmol/L (360-640 mg/dL)

II. Enzymes

1. Alanyl aminotransferase (ALT) (glutamate pyruvate transferase, GPT)	0.16-0.68 mmol/h ·L or (15-75 IU/L)
2. Aspartate aminotransferase (AST) (glutamate oxaloacetate transferase, GOT)	0.10-0.45 mmol/h · L or (10-50 IU/L)
3. Lactate dehydrogenase	0.8-4.0 mmol/h · L
4. Creatine kinase	< 1.2 mmol/h · L or (< 90 IU/L)
5. Fructose-biphosphate aldolase (F-1,6-PA)	3.6-21.8 mmol/h · L

6. Acetylcholine esterase	160-340 mmol/ h · L
7. α-Amylase	15-30 g/h · L or (< 300 IU/L)
8. Alkaline phosphatase	30-150 IU/L
9. Acidic phosphatase	< 62 nkat/L
10. γ-Glutamyl transferase (γGT or GGT)	< 60 IU/L

III. Nonproteinic nitrogenous compounds

1. Nitrogen residual (nonproteinic)	19.5-30.0 mmol/L
2. Nitrogen of amino acids	3.5-5.5 mmol/L
3. Creatine	15-70 mmol/L
4. Creatinine	60-150 μmol/L
5. Urea	3.3-6.7 mmol/L
6. Uric acid	0.1-0.4 mmol/L
7. Bilirubin total	8-20 μmol/L
8. N-Acetylneiraminic acid	1.8-2.2 mmol/L
9. Histamine	17.99-71.94 nmol/L (0.2-0.8 μg/dL)
10. Adrenalin	1.91-2.46 nmol/L (0.35-0.45 μg/L)
11. Serotonine	0.3-1.7 μmol/L (5.0-30.0 μg/dL)
12. Thyroxine	64.36-141.59 nmol/L (5-11μg/dL)

IV. Carbohydrates and metabolites

1. Glucose	2.8-6.0 mmol/L
2. Lactate	0.5-2.0 mmol/L
3. Pyruvate	< 0.1 mmol/l
4. Citric acid	88.5-156.1 μmol/L (1.7-3.0 mg/dL)

V. Lipids and metabolites

1. Total lipids	4.0-8.0 g/L
2. Triacylglycerides	0.5-2.1 mmol/L
3. Total phospholipids	2.0-3.5 mmol/L
4. Total cholesterol	4.0-8.6 mmol/L
5. Free fatty acids	0.3-0.8 mmol/L
6. Ketone bodies	100-600 μmol/L

VI. Mineral components

1. Sodium (Na^+)	135-155 mmol/L
2. Potassium (K^+)	3.6-5.0 mmol/L
3. Chlorides (Cl^-)	97-108 mmol/L
4. Calcium (Ca^{2+})	2.25-2.75 mmol/L
5. Phosphate inorganic	0.8-1.4 mmol/L
6. Magnezium (Mg^{2+})	0.7-1.0 mmol/l
7. Sulphates	0.4-0.6 mmol/L
8. Iron (Fe)	14-32 μmol/L (65-175 μg/dL)
9. Copper (Cu)	12-19 μmol/L
10. Zinc (Zn)	12-20 μmol/L
11. Ammonia	10-47 μmol/L

Indices of blood

1. Hemoglobin: males	130-180 g/L (13-18 g/dL)
females	120-160 g/L (12-16 g/dL)
2. Hydrogen ion: arterial blood	35-46 nmol/L (pH= 7.36-7.44) (38^o C)
3. Oxygen (Po_2) in arterial blood:	11-15 kPa (85-105 mm Hg)
4. Bicarbonate total (CO_2)	22-30 mmol/L

5. Carbon dioxide (Pco_2) in arterial blood: 4.5-6.0 kPa (35-46 mm Hg)

Osmotic function of blood. Blood maintains osmotic pressure inside the blood vessels. This function is carried out by blood proteins, chiefly albumins, and by Na^+ cations. Inside the erythrocytes, the role of osmotic pressure regulation is assigned to hemoglobin and K^+ ions. The lowered blood plasma concentration of proteins, or *hypoproteinemia*, leads to a decrease in the *oncotic pressure* in blood capillaries and to *oedematous* disturbances. This has been observed in starvation (nutritional oedema), in impaired hepatic albumin formation, and in other disturbed states. An increase in concentration of

proteins and sodium ions in blood plasma is conducive to retention of water in vascular beds; this state is referred to as *hypervolemia*.

Buffer systems of blood. Since the blood is not a simple extracellular fluid, but rather a suspension of cells in a liquid medium, its acid-base equilibrium is maintained by a cooperative participation of the buffer systems of blood plasma and blood cells, primarily erythrocites. The following buffer systems of the blood are distinquished: plasmic systems (bicarbonate, phosphate, organic phosphate, proteinic), and erythrocytic systems (hemoglobinic, bicarbonate, phosphate).

All buffer systems resist changes in the acid-base equilibrium. The equilibrium may be perturbed as acidic materials are accumulated, for example, ketone bodies in diabetes mellitus. This state is called *acidosis.* Acidosis may be compensated, when the pH of blood remains unchanged, or uncompensated, when the pH is altered. Two forms of acidosis, *metabolic* and *gaseous*, are distinquished. Metabolic acidosis occurs due to retention of acidic metabolites, chiefly organic acids, in the organism. It is accompanied by diminution of the alkali reserve of the blood, since acids displace H_2CO_3 from bicarbonates. Gaseous acidosis results from accumulation of carbonic acid in the organism. In gaseous acidosis, the blood alkali reserve is increased, since H_2CO_3 makes part thereof.

Accumulation of alkaline substances in the blood is referred to as *alkalosis*, of which two forms, *metabolic* and *gaseous*, are also distinquished. The former one is concomitant with an increase of the alkali reserve in blood; the latter one develops due to an excessive expiration of carbonic acid through the lungs, with a depletion of the alkali reserve.

Detoxifying function of blood. This function provides for detoxification and neutralization of materials supplied to the blood. Detoxication is effected by diluting the toxicants and their binding mainly to the albumins of blood plasma. This reduces the probability for penetration of extraneous materials into the tissues and facilitates their expulsion from the organism. Alongside this passive neutralization, toxicants can be actively detoxified by enzymes found in blood plasma and blood cells. To exemplify, the detoxification of alcohol by alcohol dehydrogenase, of various amines by amine oxidases, of curare-like compounds (succinyldicholine) by choline esterase of the blood, etc., may be quoted.

LECTURE 26

Subject: RESPIRATORY FUNCTION OF THE BLOOD. HEMOSTATIC FUNCTION OF BLOOD

In the blood, a variety of materials are transported by mechanical transport: nutrients, gases (O_2 and CO_2), hormones, vitamins, etc.

Oxygen transport. The human organism requires an incessant supply of oxygen which is taken up by the lungs from the ambient atmosphere to be carried to the tissues where oxygen is consumed in reactions of aerobic oxidation of materials. In the normal state, the human tissues consume 200 to 250 ml of oxygen per minute. The human daily requirement of oxygen is of the order of 300 liters; under heavy physical exertion, the oxygen consumption is increased by a factor of several tens. The mean lung capacity is taken to be 6 litres, and the amount of oxygen contained in the alveolar air does not exceed 850 ml, which is sufficient to sustein the human organism for a span of merely 4 minutes.

The blood is saturated with oxygen in the lungs. Diffusion of oxygen from the alveoli pulmonis to the blood is effested owing to the alveolar-capillary partial oxygen pressure drop, which is: 13.83 kPa (P_{O2} in alveoli) – 5.98 kPa (P_{O2} in lung capilllaries) = 7.85 kPa. Oxygen that has passed through the capillary wall dissolves in the blood plasma and then, across the erythrocytic membrane, penetrates into the erythrocytes to become bound to hemoglobin. One gram of hemoglobin is capable of binding 1.34 ml of oxygen. Taking into account that the blood concentration of hemoglobin is 140-160 g/liter, one will have easily estimated that one liter of blood binds a maximum of 180 to 210 ml of oxygen (providing that all available hemoglobin is saturated with oxygen). The quantitative measure for hemoglobin-bound oxygen in blood is called the *oxygen capacity of blood.* It chiefly depends on the hemoglobin concentration in blood.

During respiration under normal atmospheric conditions, hemoglobin is never saturated completely with oxygen, but to some 95-97% (to obtain complete saturation, the O_2 percentage in the inspired air should be equal to 35% as compared to the usual 21%). It follows therefore that under normal atmospheric pressure, the oxygen concentration in blood (in the form of oxyhemoglobin) is about 200

ml/liter. A small amount of oxygen (about 3 ml/liter) remains dissolved in blood plasma. Thus, hemoglobin, through binding a 60-fold excess of oxygen, as compared to simple dissolution of O_2 in blood plasma, provides for a 60 times more efficient transport of oxygen to the tissues; otherwise, the bloodstream rate should have been increased by a factor of 50-60. For this reason, the human organism cannot exist without hemoglobin.

Saturation of hemoglobin with oxygen depends upon the partial oxygen pressure. This relationship is represented by a sigmoid curve (*S-curve*) whose shapr, as has been noted previously, is defined by mutual influence of the hemoglobin subunits on their ability to bind oxygen. Owing to this relationship, the oxygen supply of tissues can be ensured at small drops in the partial oxygen pressure: from 13.03 kPa in lung capillaries to 3.99-5.32 kPa in tissue capillaries.

The erythrocytes possess a special regulatory mechanism which varies the affinity of hemoglobin to O_2. These facilities are provided by 2,3-bisphosphoglycerate. Its low concentration in lung capillaries increases the affinity of hemoglobin to O_2 and favours the formation of oxyhemoglobin, i.e. the dissociation of oxyhemoglobin at low 2.3-bisphosphoglycerate level is suppressed. An increased production regulatory sites of hemoglobin molecule, it makes the hemoglobin-to-O_2 affinity decrease and facilitates the uptake of oxygen by tissues. Actually, this process is observed in the tissue capillaries.

At the arteriolar end of the blood capillary, oxygen diffuses through the capillary wall into the intercellular medium and then enters the cells. Initially, the plasma-dissolved oxygen passes across the erythrocyte membrane, becomes dissolved in blood plasma and transported to the cell's interior. The delivery of oxygen to peripheral tissues leads to a drop in the partial oxygen pressure at the venular end of the capillary and to a loss in oxyhemoglobin concentration along the capillary length gives a general idea of the oxygen requirement of tissues: the higher this oxygen concentration difference, the greater the requirement in oxygen for a given tissue. The release of oxygen from oxyhemoglobin at the arteriolar capillary end is also dependent on oxygen consumption in oxidative reactions within the cells. If, because of certain reasons, the oxygen consumption in tissues becomes smaller, then, owing to its sparing solubility in liquid medium, oxygen stops to be delivered from the arterial blood channel. In this case, a drop in oxygen arteriolar-venular difference and a high oxyhemoglobin pressure at the venular end are observed.

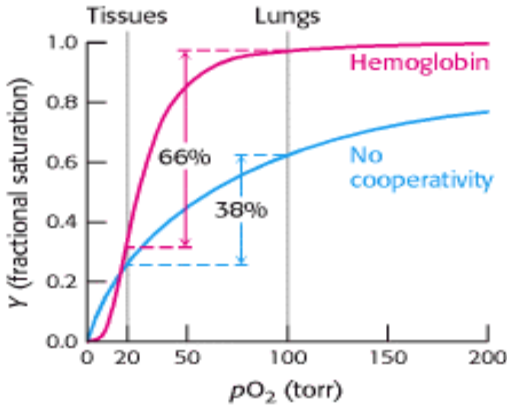


Figure 61. **Scheme of HbO_2 production, curve of cooperative effect**

The acidic properties of hemoglobin during oxygen transfer are liable to variations. Hemoglobin is a weaker acid than oxyhemoglobin, and for this reason, as oxyhemoglobin is formed, the medium in lung capillaries becomes more acidic: $\mathbf{HHb + O_2 \leftrightarrow Hb{\cdot}O_2 + H^+}$

As the blood pH value is lowered (e.g. due to the accumulation of acidic ketone bodies in diabetes mellitus or prolonged starvation), the saturation of hemoglobin with oxygen in the lungs is seen to difinish. In return, the release of oxygen from oxyhemoglobin in tissues is facilitated. An increase in pH leads to the opposite effect.

Transport of carbon dioxide **(CO_2).** The transport of carbon dioxide from tissues to lungs is also associated with hemoglobin. Carbon dioxide, as supplied to the tissue capillary blood, is consumed for production of carbonic acid in the erythrocytes through the aid of *carboanhydrase* acting as a catalyst for the reversible reaction: $\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3}$**.** Carbonic acid dissociates into H^+ and HCO_3^-. However, no acidification of the medium occurs, owing to hemoglobin which binds the H^+ ions to facilitate the oxyhemoglobin dissociation. HCO_3^-anions interact with K^+cations (whose concentration within the erythrocytes is very high) to produce hydrocarbonates: $KHCO_3$ in erythrocytes, and $NaHCO_3$

in blood plasma, the Na^+ concentration in blood plasma being higher than that in erythrocytes. Owing to the hemoglobin buffering action, carbonic acid in the tissues becomes neutralized and the blood pH varies only within a narrow range (by less than 0.1 pH unit). Partially, carbon dioxide is bound by the NH_2 groups of hemoglobin to produce carbohemoglobin.

It is to be inferred, therefore, that the CO_2 transport from tissues to lungs effected through the agency of bicarbonates ($KHCO_3$ and $NaHCO_3$) and carbhemoglobin, hemoglobin being involved, directly or indirectly, in both processes.

In the lungs, the processes reverse to those that take place in the tissues occur. In the blood of the lungs, the formation of oxyhemoglobin leads to a release of H^+ ions within the erythrocyte cells. The H^+ ions released from oxyhemoglobin react with the erythrocytic $KHCO_3$ to yield H_2CO_3, and the K^+ ions become bound to oxyhemoglobin: $\mathbf{Hb{\cdot}O_2 + K^+ \leftrightarrow K^+Hb{\cdot}O_2}$.

H_2CO_3 suffer decomposition by erythrocytic carboanhydrase, and the CO_2 formed is expired through the lungs.

Thus, there exists a close relationship between the supply of tissues with oxygen and the discharge of carbon dioxide from them owing to the participation of hemoglobin in these two processes.

Hemostatic function of blood. Hemostasis, or arrest of bleeding to prevent blood loss, is an important function of the blood. Involved in this process are the blood coagulation system, thrombocytes, and vascular wall.

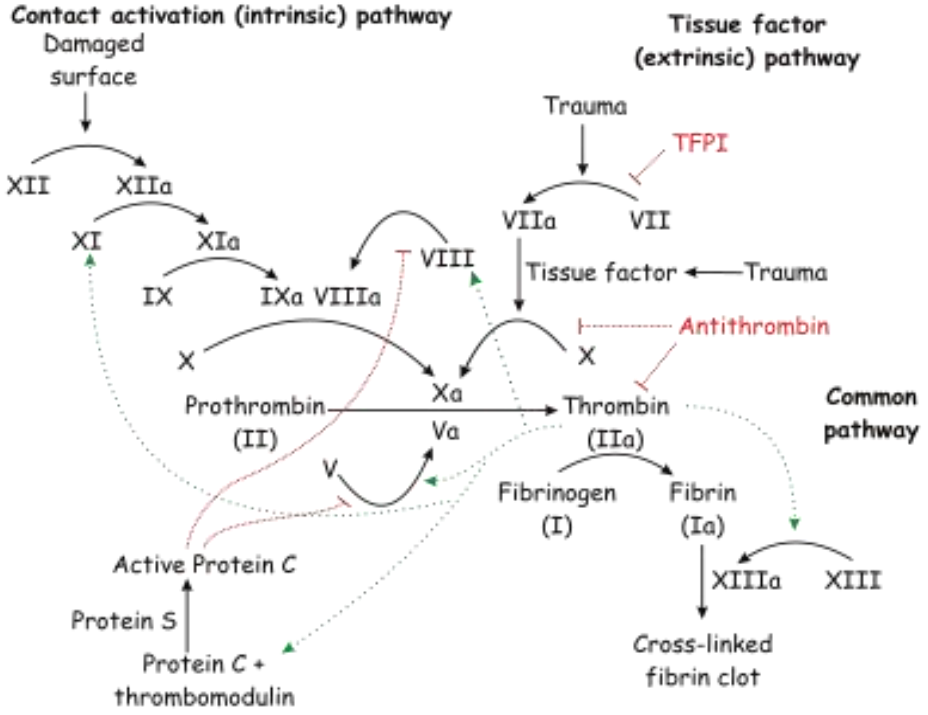


Figure 62. **Scheme of blood coagulation**

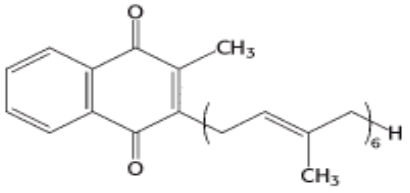
The vascular traumatoses and other interferences causing stripping of the endothelium of vessels expose the subendothelial *collagen* of vascular wall serving as a *matrix* on which a blood clot, or *thrombus*, composed of blood components, is formed. The primary thrombus is formed from thrombocytes adhering to the damaged site of vascular wall. But the primary thrombus does not arrest bleeding it rather elicits the release of thrombocyte components that trigger off the enzymic system of blood coagulation to form a polymeric fibrin mesh from the blood plasma fibrinogen. Blood cells become entangled in the mesh fibres and condense to form a thrombus which stops bleeding at the damaged spot and plugs the lumen of the vessel. The vessel patency may be restored by dissolving the thrombus. This process is accomplished by the anticoagulative, or fibrinolytic, enzymic system. Both systems, coagulative and fibrinolytic, are involved in monitoring the hemostasis. The former system leads to the formation of polymeric fibrin wall, and the latter, to hydrolysis of fibrin.

Blood coagulation has two mechanisms: *intrinsic* and *extrinsic*.Blood clotting is a multistage self accelerating process in which plasmic and thrombocytic factors are involved. To date, 13 plasmic and 11 thrombocytic factors have been described. We wish to merely point out that three major stages are constitutive of the basis of this process. On the first stage takes place activation of prothrombokinase

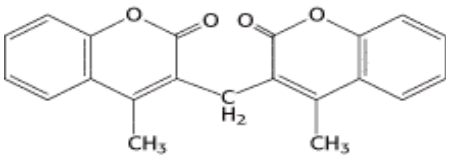
(X factor, or *Hageman factor*), which participates in the formation of thrombin plasma from prothrombin, and then thrombin participates in the thrombin-assisted formation of fibrin from fibrinogen.

Ошибка! Объект не может быть создан из кодов полей редактирования.

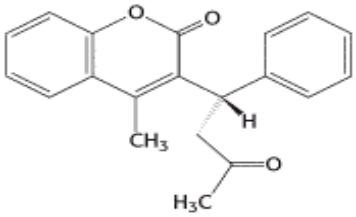
Fibrin is the building material for the thrombus. To dissolve the thrombus, plasminogen should be activated to produce plasmin which is responsible for fibrin hydrolysis in the thrombus.



Vitamin K



Dicoumarol



Warfarin

In the organism, *blood coagulation is controlled* by agents that either accelerate the process (*procoagulants*), or decelerate it (*anticoagulants*). Natural anticoagulants include heparin (heteropolysaccharide), fibrinolysin, and antithrombin. Synthetic anticoagulants-antivitamins K: neodicumarin, pelentan, sincumar, dicumarol, warfarin. Procoagulant-vitamin K is cofactor (coenzyme) of prothrombin synthesis.

Regulatory, or hormonal, function of blood. The blood cells and blood plasma are sources for production of various extracellular regulators involved in the control of metabolism and functions of tissues and organs. These regulators belong to local hormones, or hormonoids. Basophils generate *heparin* and *histamine*; eosinophils – *histamine* and *serotonin*; and thrombocytes – *serotonin*. The secretion of histamine and serotonin produces local changes in the capillary permeability, contractility of vascular smooth muscles, and leads to the development of allergic reactions. Heparin as an activator for lipoprotein lipase and as an anticoagulator takes part in the respective controls of lipids metabolism and blood clotting.

The blood plasma proteins serve as substrates for the formation of biologically active polypeptides grouped under the common name *kinins*. They include *bradykinin, kallidin* and *methionyl-lysyl-bradykinin.* Kinins are produced from their inactive precursors called *kininogens.* Kinins are released from the blood plasma kininogens by the action of proteolytic enzymes called *kininogenases.* These include *kallikreins* of blood plasma and tissue, derived from inactive enzymes *kallikreinogens*. The tissue kallikreinogens are activated by katepsins or trypsin in the pancreas, and the blood plasma kallikreinogens, by blood plasma proteinases – *Hageman factors* (blood coagulation system enzyme), plasmin (fibrinolytic enzyme), and trypsin (supplied to blood plasma, especially in affected pancreas).

The physiological role of kinins consists in the control of the bloodstream rate, blood pressure, and capillary permeability. Kinins produce dilatation of peripheral citculatory and coronary vessels, decrease arterial pressure, increase capillary permeability, and stimulate the cardiac cycle. In addition, kinins are capable of eliciting the contraction of smoth muscles of nonvascular organs (bronchi, uterus, and intestine); they stimulate intracranial baro- and algesic receptors and perturb the intracranial pressure of the cerebrospinal fluid.

Under physiological conditions, the systems for production and inactivation of kinins are equilibrated. Pathologic alterations occur due to an excessive kinin generation, which is concomitant with the development of local inflammations and impaired blood circulation.

Blood as source for medicinal preparations. The blood is used as a raw material for producing a variety of medicinal preparations which, by their therapeutic applications, are divided into four groups:

- systemic effect agents (albumin, protein, native blood plasma)
- immunologically active preparations (gamma-globulin, antistaphylococcic, interferon)
- hemostatic preaparations (antihemophilic plasma, thrombin, fibrin sponge, fibrin film, fibrinogen)
- antianemic and stimulating preparations (polyobolin – dry powdered protein components of blood plasma, eryheme-dehydrated hemolyzate of erythrocytes, etc.).

LECTURE 27

Subject: FUNCTIONAL BIOCHEMISTRY OF THE LIVER

The liver takes a central place in the organism metabolism. Specific organization of the enzymic hepatic apparatus and its anatomic routes to other organs enable the liver to participate practically in all types of metabolism and to maintain at appropriate concentration levels many vitally important blood components in the organism. One may safely assert that the functional specialization of the liver exemplifies a specific "biochemical altruism", i.e. provision of essential conditions for normal functioning of other organs and tissues in the organism. This bridge forward an explanation of the specificity of biochemical hepatic processes which, on the one hand, are oriented to the production of various compounds for other organs and, on the other hand, provide defense for these organs from toxicants formed therein, or from extraneous compounds invading the organism.

The liver is involved in the following biochemical functions:

1. regulatory-homeostatic
2. ureapoietic
3. biligenic
4. excretory
5. detoxifying.

Regulatory-homeostatic function. The liver participates in the metabolic control of nutrients: carbohydrates, lipids, proteins, vitamins, and, in part, water-mineral compounds as well as in the metabolism of pigments and nitrogenous nonproteinic materials.

The ***carbohydrate metabolism control*** effected owing to the fact that the liver is actually the only organ capable of maintaining a constant glucose level of the blood, even under starvation conditions. Glucose produced by the liver in the course of glycogenolysis and gluconeogenesis, is supplied to the blood to be consumed primarily by the nervous tissue or, when its supply from the intestine is excessive, to be stored as glycogen (see "Carbohydrate metabolism").

The ***lipid metabolism control*** effected through a variety of liver-biosynthesized lipids (cholesterol, triacylglycerides, phosphoglycerides, etc.) which are secreted into blood to be delivered to other tissues. The amount of cholesterol produced by the liver is larger than that ingested in food: in humans, the daily dietary intake of cholesterol is about 0.3-0.5 g, while the cholesterol that is daily produced in the liver amounts to 2-4 g,. The distribution of lipids over the organs and tissues is accomplished by the liver. Apoproteins of α- and β-lipoproteins are formed in the liver. In addition, fatty acids are catabolized in the liver to yield ketone bodies which are used as an energy source for extrahepatic tissues.

The ***protein metabolism control*** is effected by the liver owing to the intense synthesis of proteins and oxidation of amino acids therein. The human organism synthesized about 80-100 g of proteins per day of which a half is accounted for by the liver. Unlike other organs and tissues a major portion of proteins synthesized by the liver (mainly albumin) is supplied for consumption by other organs. The liver produces daily about 12 g of blood plasma albumins and most α- and β-globulins of the blood. In addition, the liver is a producer of other blood plasma proteins involved in the coagulative and anticoagulative blood systems), choline esterase, transport proteins (ferritin, ceruloplasmin).

In the liver *amino acid metabolism* is the most active process, viz. synthesis of nonessential amino acids, of nonproteinic nitrogenous compounds from amino acids (creatinine, glutathione, nicotinic acid, purines and pyrimidines, dipeptides), oxidation of amino acids with a release of ammonia. During starvation, the liver is the first to loose its reserve proteins in supplying other tissues with amino acids. The protein losses in the liver amount to about 20%, while in other organs they do not exceed 4%.

The involvement of the liver in ***vitamin metabolism*** is associated with deposition of vitamins in it, mostly fat-soluble ones, synthesis of certain vitamins (nicotinic acid) and coenzymes, and conversion of calciferols to 25-hydroxycalciferols.

The involvement of the liver in ***water-mineral metabolism*** consists in that the liver supplements the function of the kidneys in maintaining the water-salt equilibrium and acts as a kind of an internal buffer for the organism. The available evidence indicates that the liver retains Na^+, K^+, Cl^-. Ca^{2+} ions and water and secretes them into the blood. Besides, the liver stores microelements (iron, copper) and participates in their distribution among other tissues through the agency of transport proteins.

The involvement of the liver in the ***metabolism of nitrogenous bases of nucleic acids*** shows up in the synthesis of these species from simpler compounds and their subsequent oxidation to uric acid. Nitrogenous bases are utilized by other organs for the synthesis of nucleosides, nucleotides, and nucleic acids, uric acid being the end metabolite.

Ureapoietic function. Liver is the only organ in possession of all the enzymes involved in the cycle for production of urea from ammonia. The ammonia generated within other tissues becomes converted in the liver to a neutral product (*urea*), which is excreted into blood. An intense catabolism of proteins and nonproteinic nitrogenous compounds (amino acids, purines, pyrimidines, and biogenic amines) is accompanied by enhanced hepatic ureapoiesis, increased blood concentration of urea and increased urinary discharge of urea.

Biligenic and excretory functions. Liver produces a special liquid excretion, bile, which is released in the small intestine. Liver is the only producer of bile acids and their conjugates, which are used in digestion and absorption of lipids in the intestine. The bile is composed of bile acids, proteins (albumins and globulins), cholesterol and its esters, mineral compounds (Ca^{2+}, Na^+, K^+), water, pigment metabolites (bilirubin glucuronides), inactive products of hormone and vitamin metabolism, extraneous invaders in the organism. A disorder of the excretory hepatic function disfavours the digestion and uptake of lipids and leads to an accumulation of toxic metabolites derived from pigments and foreign substances.

Detoxifying function. Liver is the major organ involved in the detoxification and neutralization of intrinsic metabolites and extraneous substances. The participation of the liver in pigment metabolism is manifested by conversion of chromoproteins to bilirubin in the cells of reticuloendothelial system (*RES*) present in the liver, by conjugation of bilirubin in the liver cells, and by hepatic breakdown of intestinal urobilinogen to nonpigmentary products.

Disturbances of liver functons. Damages inflicted on the liver by infections agents or chemicals disturb its functions, all or some of them. A change in the concentration of substances secreted into the blood from the liver can indicate such disturbances. These substances are glucose, cholesterol, phospholipids, vitamins and 25-hydroxycalciferol, uric acid, urea, etc.

Disturbances in the *regulatory-homeostatic function* are distinctly visible in compositional alterations of the blood plasma proteins; the relevant symptoms are:

- dysproteinemia, i.e. disbalanced ratio of plasmic protein fractions (albumin/globulin quotient is low);
- reduced concentrations of albumin, fibrinogen, prothrombin, and other proteinic clotting factors, transport proteins (α- and β-lipoproteins, transferrin, ceruloplasmin, hormone- and vitamin-transporting proteins);
- reduced activity of choline esterase, and some others.

In hepatic pathology, the disturbances of *ureapoiesis* are manifested in a reduced blood concentration of urea and in a decreased daily urinary content of urea, while the disordered detoxifying function shows up in reduced rates of conversion and conjugation of metabolites (e.g. bilirubin, indole, scatole) and foreign compounds (benzoic acid, arylamines, alcohols) supplied in food or administered to the organism.

The *inhibition of bile acid production* manifests itself in reduced concentrations of bile acids in the bile, and in symptoms of disordered digestion and uptake of lipids. The impaired excretory function of the liver leads to the retention in the organism of substances normally excreted in the bile (bile

acids, bilirubin glucuronides, various drugs and toxins). Their increased concentrations in the blood and urine are diagnostic of alterations in the excretory function of the liver.

LECTURE 28

Subject: **FUNCTIONAL BIOCHEMISTRY OF THE KIDNEY**

Specific functions performed by the kidney are the following:

1. uropoietic and excretory
2. regulatory-homeostatic
3. detoxifying
4. incretory (endocrine).

The major, vitally important function of the kidney is uropoiesis (production of the urine), which is followed by the excretion of substances, including extraneous ones that enter the organism.

The *functional unit* of the kidney is the *nephron.* The uropoiesis in the nephrons is effected via ultrafiltration of blood plasma at the glomerules, via tubular reabsorption of materials (in tubules and collecting ducts), via secretion in the urine of certain excrets (in tubules). About 180 liters of blood plasma ultrafiltrate (*primary urine*) is filtrated by the kidney per day, at a rate of 120 ml/min. Over 99% of the total of ultrafiltrate is returned to the extracellular fluid by reabsorption. Urine discharged from the organism amounts to 1.0-2.0 liters per day.

All compounds contained in the primary urine are differentiated into threshold and no-threshold substances. Normally, the former are reabsorbed completely and are excreted into the urine only when their concentration in the primary urine exceeds a certain threshold value. The latter substances are not reabsorbed and are excreted in the urine in proportion to their concentration in the blood plasma. The reabsorption proceeds either by simple diffusion, or by active transport. Most materials are reabsorbed by active transport, which requires large energy expenditure. For this reason, the active transport system in the renal tubules is highly developed: the activity of Na^+, K^+-ATPase which provides for the Na^+, K^+ gradient in the secondary active transport, and the activity of protein carrier systems subserving various substances are quite efficient. The kidneys are rich in mitochondria, and differ in oxygen consumption. This enables the kidneys to produce much energy in the course of oxidative phosphorylation. In the renal generation of energy, glucose, fatty acids, acetone bodies, and amino acids are utilized as energy sources.

The primary urine is produced by ultrafiltration of blood through the pores of the glomerular basal membrane whose size is about 4 nm. The ultrafiltrate contains all the blood plasma components, except the proteins with a molecular mass above 50,000.

The ***uropoietic function*** of the kidney is closely related to the renal capacity to control osmotic pressure, salt-and-water balance, and acid-base equilibrium in extracellular fluids, blood included, of the organism.

The kidney regulates the ***acid-base equilibrium*** in the blood and favours the urinary excretion of acidic substances and the retention of the alkali reserves (bicarbonates) in the organism. In the course of metabolism, acidic compounds (lactate, ketone bodies, and carbonic acid) are chiefly formed. The removal of volatile acidic substances is effected by pulmonary expiration, and of nonvolatile substances, by renal filtration. Acid anions are mainly neutralized by sodium cations and, because of this, are excreted in the urine as sodium salts (Na_2HPO_4, $NaHCO_3$, NaCl, and sodium salts of organic acids). To preserve hydrocarbonate HCO_3^- (which is the alkali reserve of the blood), Na^+ ions are reabsorbed in the distal tubules and the replaced in the urinary salts by H^+ and NH_4^+ that are produced by the tubular epithelium. The reabsorption of Na^+ is followed by reabsorption of HCO_3^-anions these remain in the organism. Salts of greater acidity (NaH_2PO_4 and NH_4Cl) and acids (carbonic, lactic, acetylacetic, and β-hydroxybutiric) are excreted in the urine. The urine test becomes distinctly acidic, and the pH may be as low as 4.5 while the alkali reserve of the blood is preserved.

In the kidney, the detoxication of foreign compounds is accomplished by binding the toxicants to glycine, acetic and glucuronic acids. Moreover, the renal oxidation of certain alcohols and other materials also occurs. In the cells of renal connective tissue, extracellular regulators of hormonoid type (for example, prostaglandins) are formed. The kidney is also involved in the hormonal control of vascular tension and pressure.

Characterization of urine components in norm and in pathology

The urinary excretion of various materials reflects alterations in the processes that occur in the kidney and other tissues and organs of the organism. The daily volume of final urine amounts to 1.0-2.0 liters and the dry weight of final urine is about 60 g. Since the urine is a filtrate of blood plasma, it appears expedient to consider the urinary concentrations of various groups of biological materials from the standpoint of their occurrence in the blood plasma.

Proteins. *In norm*, the daily urinary excretion of proteins amounts to about 30 mg, which is not detectable by common laboratory techniques and routinely specified as "*traces, or absence of urinary proteins".* Among the urinary proteins, enzymes are also present. The origin of normal urinary proteins is different.

In pathology, the urinary protein concentration may be increased; depending on the location of the damage, prevalent in the urine may be either plasmic proteins, or cellular proteins of the urinary tract. In inflammatory renal diseases (glomerulonephritides), the permeability of the basal membrane of nephron glomerulus increases; proteins are filtered in an amount above normal and fail to be reabsorbed completely. Disturbances in the tubular protein reabsorption (nephroses) are conducive to a similar pathology. For this reason, in patients with glomerulonephritides and nephroses the urinary excretion of proteins may vary from 1 to 15-40 g per day. Nonetheless, even in such a contingency, the urinary proteins concentrations are small and can be detected only using special techniques. For example, in pancreatites, an enhanced activity of α-amylase and trypsin is observed both in blood and urine.

Nonproteinic nitrogenous urinary components

Urea is a major nitrogenous component of the urine. The normal excretion of urea is 333 to 583 mmol per day, which accounts for 60% to 80% of the overall urinary nitrogen. An increased urinary concentration of urea is observed in the states with pronounced catabolism of proteins and other nitrogenous components (starvation, burns, traumatism, atrophy of tissues, etc.). A decreased excretion of urea is observed in affected liver (urea-producing organ) and in impaired in the blood (this state is called azotemia).

Uric acid. Normally, the urinary excretion of uric acid is 2.35 to 5.90 mmol per day. Its increased urinary concentration is observed in a diet rich in nucleic acids or as produced by breakdown of cells and tissues, for example, leucocytes in patients with leucosis.

Creatinine. In norm, the urinary excretion of creatinine is 4.4 to 17.6 mmol per day; variations in creatinine concentration are dependent on muscular development. Physiological excretion of creatinine is normal only in children. In adult humans, creatinuria is a sign of pathology (e.g. muscular dystrophy).

Amino acids. In norm, the urinary excretion of amino acids is 0.29 to 5.35 mmol per day (as based on nitrogen). The urinary concentrations of glycine, histidine, and alanine are higher than those of other amino acids. In pathology (e.g. burns, diabetes mellitus, affected liver, and muscular dystrophy) *hyperaminoaciduria* may occur. Heriditary hyperaminoaciduria is associated with defective proteins-carriers for amino acids in the proximal renal tubules. In a disordered amino acid tissue metabolism, the urinary excretion of normally nonexcretable amino acid metabolites occurs (e.g. homogentisine acid, in alcaptonuria; phenylpyruvic acid in phenylketonuria).

Ammonium salts. In norm, the urinary excretion of ammonia as a component of ammonium salts (ammonium chloride) is 30-60 mmol per day. In pathology, an increased urinary elimination of ammonium salts may be observed (in diseases accompanied by acidosis). A diminished excretion of ammonium salts occurs in diseases associated with alkalosis, in renal diseases due to affected distal tubules in which ammoniogenesis takes place.

Hippuric acid. The urinary excretion of hippuric acid is dependent solely on the amount of ingested vegetable food, since in the organism this acid endogenically is not produced. Commonly, the daily urine contains to 5.5 mmol of hippuric acid.

Indican (indoxyl sulphuric acid). Normal urine contains indican (in the form of potassium indoxyl sulphate) in trace amounts. In detectable quantities, indican appears in the urine on excessive alimentary intake of meat products; it also occurs as a byproduct of putrefactive processes in the intestine.

Nitrogenous pigments. Representative of these is stercobilinogen, a product of hemoprotein breakdown. Stercobilinogen is convertible to stercobilin and normally is excreted in the urine. In pathology, urinary excretions contain bile acids and a variety of bile pigments, for example, in affected liver and in toxicoses conducive to hemolysis.

Nitrogen-free components of urine

Glucose and other monosaccharides. *In norm,* the daily urine contains a more 0.3-1.1 mmol of glucose. Such amounts escape detection by conventional analytical techniques; for this reason, glucose is not reckoned as a component of normal urine. However, in excessive dietary intake of carbohydrates, when the glucose concentration in blood attains a threshold value, i.e. of the order of 8.3-8.8 mmol/liter, alimentary glucosuria may develop in the organism. *In pathology,* glucosuria occurs due either to an increased blood glucose concentration, or to a defective carrier protein involved in glucose reabsorption in the renal proximal tubules. The former case is the most commonly encountered in the clinic, for example, in diabetes mellitus or in steroid diabetes. The latter case is the so-called renal diabetes. For example, the occurrence of fructose or pentose in the urine (renal *fructosuria* or renal *pentosuria*) is indicative of affected transport systems of the renal tubules.

Lactic and ***pyruvic acids.*** *In norm,* the daily urinary excretions of lactic and pyruvic acids amount to 1.1 and 0.11 mmol, respectively. An increased concentration of lactic acid in the urine is observed under intensive muscular work and in hypoxia. An increased urinary excretion of pyruvic acid occurs in diabetes mellitus and in B_1 hypovitaminosis.

Ketone bodies. *In norm,* the daily urine contains 20 to 50 mg of ketone bodies. At this level, they are not detectable by the analytical methods currently employed in the clinic. *In pathology,* increased concentrations of ketone bodies, i.e. a state called ketonuria, occur in diabetes mellitus, steroid diabetes, and starvation.

Mineral salts. *In norm,* the daily urine contains (in mmol): sodium, 174-222; potassium, 61-79; calcium, 4.02-4.99; inorganic phosphorus, 33. *In pathology,* an increase in urinary excretion of sodium and a decrease in excretion of potassium are observed in the *adrenal hypofunction*; the reverse situation occurs in *hyperaldosteronemia* and when mineralocorticoids and glucocorticoids are prescribed as drugs. A decreased urinary concentration of calcium and a distinct *phosphaturia* are observed when large doses of vitamin D and parathyrin are administered; a high urinary loss of calcium is characteristic of *rickets* and *hypoparathyroidism*.

LECTURE 29

Subject: BIOTRANSFORMATION OF DRUGS AND POISONS

All the substances supplied to the organism in a variety of ways pass through several basically similar stages such as absorption, distribution (mechanical transport), and excretion. The transit rate of substance at these stages may either be increased, or lowered, depending on the structural features and physico-chemical properties of a substance as well as on its affinity to biological molecules. The discipline, dealing with rate characteristics at the stages in which any substance entering the organism is involved, is referred to as chemobiokinetics which treats, in a broader sense, movements of substances in the living organism. Conceptually, chemobiokinetics is divided into three subdisciplines: *pharmacokinetics, toxicokinetics,* and *biokinetics.* Pharmacokinetics confines itself to the study of drugs; toxicokinetics, to the study of toxic substances; and biokinetics, to the study of substances not alien to the organism. In many respects, this classification is rather arbitrary, since the distinction between a drug and a poison in many instances may be evasive. Moreover, even autobiogenous compounds taken in improper doses may exhibit toxic properties. The subsequent history of a substance after its uptake by the organism is dependent to a significant degree on the rates at which it is converted by various enzymes, i.e. on its metabolic transformations. In point of fact, the metabolism of biogenous substances and xenobiotics used as drugs is governed by the laws of enzymic kinetics. Biogenous substances, being natural substrates for enzymes, are converted at the rates characteristic of catalytic properties of the enzymes involved. The metabolic evolution of xenobiotics is dependent on the occurrence of enzymes capable of catalyzing the conversion of these xenobiotics. If no enzymes that are potentially capable of catalytic intervention of the xenobiotics are available, the xenobiotics behave as metabolically inert. Apparently, in the course of evolution, highly substrate-specific enzymes have laid a basis for the intrinsic metabolism in living organisms, while the enzymes with low specificity towards substrates have taken up defense functions aimed at the inactivation of extraneous invaders.

Biochemistry studies enzyme-assisted conversions of drugs in the organism by making use of appropriate methods and techniques. The drug metabolism in the organism may be represented within the framework of a general scheme:

Drug → *Enzymes* → **Metabolites** → **End Metabolites**
absorption *intrinsic metabolism* *excretion* or *storage*

The drug metabolism is studied by determining the drugs and their metabolites in biological fluids, tissues, and excretions as well as by estimating the activity and kinetics of enzymes involved in the drug metabolism.

Experimentally, the two approaches are used in the studies on metabolism of xenobiotics. In the clinic, the drug metabolism is assessed, as a rule, by measuring the concentration of administered drug and its metabolites in blood, urine, and other excretions.

Stages in the metabolism of xenobiotics

Biogenous substances, as distinct from xenobiotics, are involved in the conventional metabolic process. Xenobiotics, in the course of their conversion, are subject to two major stages: *modification* (nonsynthetic stage) and *conjugation* (synthetic stage).

The ***modification stage*** is an enzyme-assisted modification of the initial structure of a xenobiotic resulting either in a cleavage of bonds within the xenobiotic molecule, or in the insertion of additional functional groups (e.g. hydroxyl or amino groups) into its molecule, or in a release of its functional groups blocked in the initial structure (for example, by hydrolysis of ester or peptide bonds). The modification leads to an *increased solubility* of the xenobiotic (xenobiotic becomes more *hydrophilic*). Additional functional groups are needed to enable the xenobiotic to enter the conjugation stage.

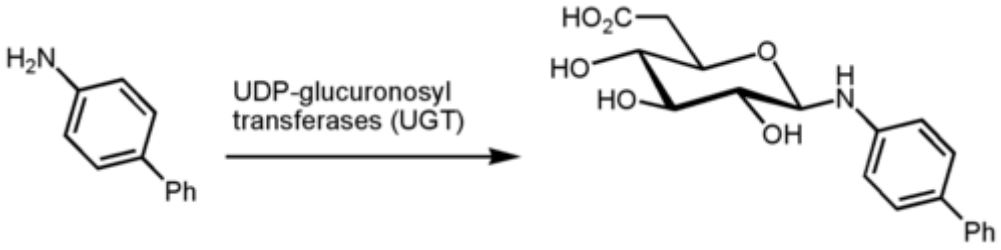
Conjugation stage is viewed as an enzyme-assisted process for building covalent bonds between the xenobiotic and biomolecules occurring in the organism's media (e.g. glucuronic acid, sulphates, and others). The conjugation stage terminates in the *synthesis of a novel compound* whose constituents are, on the one hand, the xenobiotic moiety and, on the other hand, a conjugate (biomolecule).

Relationship between metabolism of xenobiotics and their structure Xenobiotics invaded into the organism are liable to a chain of modifications, or nonsynthetic conversions (oxidation-reduction, isomerization, cyclization, ring opening, and hydrolysis) carried out by the respective enzymes (oxidoreductases, isomerases, lyases, and hydrolases):

$$\mathbf{RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O}$$

Depending on the number of functional groups in the molecule of modified xenobiotic, its conjugation can proceed by a variety of routes in which each of the xenobiotic functional groups becomes bound with a conjugating agent. If the xenobiotic is not functionalized (e.g. benzene), it cannot enter the conjugation stage. In contrast, if the introduced xenobiotic is in possession of an appropriate functional group (e.g., 4-aminobiphenyl); it may become immediately engaged in the conjugation stage with UDP-glucuronic acid.

The knowledge of principles that govern the enzyme-assisted conversions of xenobiotics provides an opportunity to prognosticate metabolic behaviour of any xenobiotic taking into account its structural specificities.



Xenobiotic routes in the organism

Xenobiotics are either eliminated from the organism, or become accumulated in tissues. Xenobiotics are excreted as:

a) supplied (unmodified by enzymes);
b) metabolites (modified by enzymes);
c) conjugates (by action of conjugating enzymes);

d) complexed with biomolecules (for example, metal-containing xenobiotics become bound to cysteine by glutathione and excreted as complexes).

The xenobiotics that accumulate in the organism are those capable of interacting with macromolecules (proteins, nucleic acids, and lipid entities). For example, organochloric compounds, which are readily soluble in lipids, are quite resistant to catabolic conversion and are difficult to eliminate from the organism. They tend to accumulate in lipid-rich tissues. Heavy metals (mercury, cadmium, silver, arsenic, and lead) and preparations containing organometallic compounds become bound with proteins and likewise accumulate in the organism.

Metabolism and physiological action of drugs

Substances introduced into organism may exhibit either medicinal or toxic properties. Commonly, any drug can exert both medicating and side (toxic) effects. Therefore, generally speaking, the more active the drug, the faster its toxic properties become manifest. During metabolism, the specific activity and toxicity of xenobiotics are susceptible to alterations.

Biological activity alterations show up in:

a) deactivation, i.e. a loss of medicinal or biological activity of drugs;

b) activation, i.e. induced activity of an inactive preparation;

c) modification of the major effect, i.e. when the administered drug, on having metabolized, exhibits properties different from those of the initial preparation.

The alterations of toxicity are manifested in:

a) deintoxication, i.e. a loss or reduced toxicity of drug;

b) toxification, i.e. enhanced toxicity of drug.

The above instances may be exemplified as follows:

Deactivation is observed as the functional groups responsible for the biological activity of a drug are either eliminated from, or blocked in the drug molecule. For example, the active *sulphanilamide*, after its conjugation with *acetyl-ScoA*, is converted to an inactive *acetylsulphanilamide*.

Activation is observed when the biologically active groups that have been blocked in the initial preparation become deblocked during metabolism:

Phthalsulphathiazole →*hydrolysis*→**Sulphathiazole + Phthalic acid**
inactive drug *in organism* *active drug*

or acquire functional groups that are necessary for eliciting the drug activity:

Benzopyrene → *hydrolysis in organism* → **Hydroxybenzopyrene**
inactive procarcinogen *carcinogen,i.e.tumor-producing agent*

Modification of the major drug effect manifests itself as a variant of activation. For example, codeine (morphine 3-methyl ether) exhibits mainly antitussive and mildly analgesic action. When codeine undergoes demethylation in the organism, it converts to morphine, which is a strong analgetic.

Deintoxication resembles deactivation and is a defense reaction to the toxic effect of a drug. For example, phenol is a toxic compound, while phenol sulphate, which is a product of phenol conjugation in the organism, is nontoxic.

Toxication shows up as an enhanced side effect due to a drug administered into the organism. By mechanism, toxification resembles activation. Occasionally, toxification is produced by "lethal" molecules synthesized from the introduced compounds during their metabolism in the organism. The lethal synthesis with the involvement of a xenobiotic leads to a metabolic block and to the death of organism. For example, the administered fluoroacetate enters the Krebs cycle in tissues to produce a toxic product, fluorocitrate, which blocks aconitate hydratase and interrupts conversion steps in the Krebs cycle. Toxification effects are taken into account in the development of chemicals against rodents and other vermin.

Localization of drug metabolism in the organism

Depending on the site of conversion of biogenous preparations and xenobiotics in the organism, the drug metabolism is classified into cavitary (enteral), extracellular (humoral), and cellular, or tissue, types of metabolism.

The ***cavity,*** or ***enteral, drug metabolism*** is effected by hydrolytic enzymes supplied to the cavity of gastrointestinal tract. Hydrolysis of biogenous preparations occurs with the involvement of pancrea-

tic and intestinal digestive enzymes. Xenobiotics whose molecules contain peptide, carboxyester, glycoside, amide and phosphamide bonds are also liable to hydrolysis. This process involves proteolytic and lipolytic enzymes as well as enzymes capable of hydrolyzing glycoside bonds. In addition, a large group of esterases (e.g. carboxyesterases and phosphatases) and phosphamidases (involved in hydrolysis of phosphamide bonds in drugs) are found in the intestine. Trypsin, while being a proteolytic, exhibits also an esterase activity and is capable of hydrolyzing the ester bonds in xenobiotics.

Extracellular, or ***humoral, drug metabolism*** takes place in the extracellular fluids (after uptake and subsequent circulation of a drug in the organism), i.e. in the blood, lymph, cerebrospinal, and extracellular proper, fluids. Possibly, metabolic conversions therein are chiefly confined to hydrolysis of the preparations delivered (both biogenous and xenogenous). In the blood and other fluids, this function is performed by proteinases and esterases (e.g. pseudocholine esterase, phosphatases). In the extracellular fluids, other enzymes, for example, alcohol dehydrogenase, aminooxidases, etc., are available in small amounts, but the activity of these enzymes is rather low. The contribution of the humoral metabolic link to the overall drug metabolism is insignificant. At the humoral level, drug hydrolysis plays a role in drug inactivation; this metabolic link should be taken into account.

Cellular (tissue) drug metabolism. In the cells, the whole varieties of metabolic transformations, including those of xenobiotics, are being accomplished. However, the substances, before being subjected to the action of enzymic systems, should be transported from the site of their introduction to the cells and allowed to penetrate the intracellular space through the cell membrane. Xenobiotics are transported by the same mechanisms as biogenous substances. In the blood plasma, they either become dissolved in the liquid medium, or adsorbed, mostly on albumin. In a dissolved or in a protein-bound state, xenobiotics are delivered to the cells (tissues). They gain access to the cells mostly by simple and facilitated diffusion; large molecules enter the cells by endocytosis. Xenobiotics synthetically derived from biogenous substances can be actively transported across the cell membranes using natural substance transport systems.

Not all the tissues and organs are equally active when they convert xenobiotics. The most actively engaged organ is liver which is in possession of enzymes that perform modification and conjugation of drugs. The other organs and tissues are less active in the metabolism of xenobiotics.

The metabolic conversion of xenobiotics occurs in various organelles of the liver cells. The most powerful metabolic system is found in endoplasmic reticulum (in microsomes). The microsomes are fragments of endoplasmic reticulum that are formed, for example, on trituration of a tissue sample and spontaneously close into small bladder-like structures (vesicles). Thus, with reference on its localization, the metabolism of xenobiotics is differentiated into *microsomal* and *extramicrosomal.* The extramicrosomal metabolism occurs in hyaloplasm, lysosomes, peroxisomes, and mitochondria.

The enzymic reactions conducive to conversion of xenobiotics may be divided into the following major groups:

1. oxidation-reduction reactions;
2. hydrolytic reactions
3. synthetic reactions, or conjugation reactions;
4. other reactions (isomerization, ring opening, etc., which are effected by isomerases and lyases).

Microsomal oxidation of substances

In the microsomes, there are found enzymic chains for oxidation of substances. These chains are represented by two short electron-proton transfer chains built into the membranes of endoplasmic reticulum or into microsomal membranes. Microsomal oxidation is connected with these chains. One of these chains is a *monooxigenase oxidation chain* (in which the source of electrons and protons is reduced NADP), and the other is a *reductase oxidation chain,* with reduced NAD as a suplier of electrons and protons. The source of $NADPH^+$ in the monooxigenase chain is the pentose phosphate cycle, and the source of $NADH^+$ is glycolysis.

The ***microsomal $NADPH^+$-dependent monooxigenase chain*** is composed of flavoprotein (FP_2), with FAD for a coenzyme, and cytochrome P_{450}. Flavoprotein exhibits a $NADPH^+$-dehydrogenase activity, FAD acting as an acceptor for two protons and two electrons. From flavoproteins, electrons are transported onto cytochrome P_{450}, and protons are lost into the environment (cytosol). Cytochrome P_{450} is the terminal self-oxidizable link of this chain. Like all the cytochromes, it belongs to hemoproteins. Its protein moiety is represented by a single polypeptide chain. The molecular mass of cytochrome P_{450} is about 50,000. The P_{450} is capable of complexing with carbon monoxide, *CO*. The light absorption maximum for these complexes is at 450 nm; hence the name for the given cytochrome.

Cytochrome P_{450} performs a *dual function*: it activates molecular oxygen by transferring electrons onto it, and uses the activated oxygen to oxidize substances **R**, with the concomitant formation of water. Consequently, one oxygen atom adds to the oxidizable substance (**RO**), and the other, by accepting two H^+ ions from the medium, makes up water.

The ***NADH$^+$-dependent reductase oxidation chain*** occurs not only in the microsomal membranes; it is also available in the outer mitochondrial membrane, in the nuclear membrane, and in the erythrocytic cell membranes. The reductase chain is thus included among the most rapid reactions of biological oxidation, but its function in the cell remains still unclear. The self-oxidizable component of this chain capable of activating the oxygen has never been identified either; quite probable that this function is exercised by cytochrome P_{450} itself. The $NADPH^+$- and $NADH^+$-dependent chains can exchange electrons. For example, the electrons from FP_2 and cytochrome b_5 may be transferred onto cytochrome P_{450} to be used in the oxidation of substrates.

Cojugation of xenobiotics, its mechanism and role

The conjugation stage, or synthetic stage, is essential for the formation of nontoxic and easily excretable drug metabolites. By their mechanism, the conjugation reactions are divided into two groups:

- ***Reactions of I type.*** Initially, conjugating agents, i.e. biomolecules, are activated and then transferred onto xenobiotics to form conjugates. This type of conjugation reactions occurs in all tissues of the organism.
- ***Reactions of II type.*** Initially, a xenobiotic is activated to be transferred onto a conjugating biomolecule to form a conjugate. This conjugation type is of rare occurrence and is only observed in liver and kidney.

Various groups for conjugation reactions of I and II types are distinguished, depending on the nature of a conjugating species involved. In the I type reactions, glucuronide, sulphate, acetyl, methyl, thiosulphate conjugations are to be noted, and in the II type, glycine and glutamine conjugations.

Glucuronide conjugation. UDP-glucuronic acid is the source for glucuronic acid residues in this process. Endogenous substances and xenobiotics are subject to glucuronide conjugation (known are glucuronides of bilirubin, steroid hormones, vitamin D, etc.). Xenobiotics can enter glucuronide conjugation if they possess or have acquired, during modification, a hydroxyl, carboxyl, and amino group (commonly, in the aromatic ring), or, at least, a SH-group. The conjugation reaction proceeds with the participation of UDP-glucuronosyltransferase by the scheme:

$$\underset{\text{xenobiotic}}{\mathbf{RXH}} + \underset{\text{UDP-glucuronic acid}}{\mathbf{UDP\sim C_6O_9O_6}} \rightarrow \mathbf{UDP} + \underset{\text{glucuronide of xenobiotic}}{\mathbf{RX\text{-}C_6H_9O_6}}$$

Among xenobiotics (drugs and poisons), susceptible to glucuronide conjugation are phenols, polyphenols, phenolic steroids, aromatic amino acids, and others.

Sulphate conjugation. Active form of a conjugating agent is *3′-phosphoadenosine-5′-phosphosulphate* (*PAPS* for short). PAPS, which may also be designated as $PAP\sim SO_3H$, is a source of labile sulphate groups used in the conjugation of natural compounds and xenobiotics. The natural substances as subject to sulphate conjuation include endogenous toxic products, e.g. indole, scatol, phenol as well as steroids, iodothyronines, tocopherols, and others.

The sulphate conjugation reaction proceeds with the involvement of a special enzyme, *sulphotransferase*, according to the scheme:

$$\underset{\text{xenobiotic}}{\mathbf{RXH}} + \underset{\text{PAPS}}{\mathbf{PAP\sim SO_3H}} \rightarrow \underset{\text{sulphate of xenobiotic}}{\mathbf{RX\text{-}SO_3H}} + \underset{\text{3'-phosphoadenosine-5'-sulphate}}{\mathbf{PAP}}$$

Acetyl conjugation. The source of labile acetyl groups in this variety of conjugation reactions is *acetyl~ScoA*, which is produced by degradation of carbohydrates, triacylglycerides, and amino acids. Endogenous substances and xenobiotics containing a free NH_2 group may be acetylated.

N-acetylation is an essential biochemical reaction in the synthesis of monosaccharide derivatives (N-acetylglucosamine, N-acetylgalactosamine, and neuramic acid) that are further used in the synthesis of heteropolysaccharides. N-acetylation is also a route to neutralization of biogenous amines: serotonine, histamine, GABA, and others. N-acetylation of histones and nonhistonic chromatin proteins is an important regulatory mechanism of DNA transcription. For endogenous substances, the only case of *O*-acetylation has been reported, which is a reaction of acetylcholine formation.

Xenobiotics possessing a free NH_2 group (commonly, on the aromatic ring) are subject to acetylation. This reaction is effected by means of a special acetyltransferase called *arylamine-N-acetyltransferase.* This enzyme exhibits a low specificity to xenobiotics to be acetylated. The reaction proceeds by the scheme:

$$\mathbf{RNH_2 + CH_3\text{-}CO\sim SCoA \rightarrow R\text{-}NH\text{-}CO\text{-}CH_3 + CoASH}$$

Among the xenobiotics susceptible to acetylation, sulphanylamides, isonicotinic acid hydrazides, and aniline derivatives can be mentioned; these preparations are widely used in medical practice.

Methyl conjugation. In this reaction methyl groups derived from the active form of methionine, *S-adenosylmethionine,* serve as a conjugating agent. S-adenosylmethionine is a participant in numerous reactions of methylation of endogenous compounds. It is also a methyl group donor for conjugation reactions of xenobiotics (RXH), which proceed with the involvement of methyltransferases, according to the scheme:

$$\mathbf{RXH + S\sim Adenosylmethyonine \rightarrow RX\text{-}CH_3 + S\sim Adenosylhomocysteine}$$

Xenobiotics containing an NH_2 group or heterocyclic nitrogen, as well as OH and SH groups, are subject to methylation by addition of methyl groups to N, O, and S atoms. Among the preparations used in therapy, liable to methylation are mono- and polyphenols, and heterocyclic compounds of pyridine, quinoline, isoquinoline, and thiouracil type.

Thiosulphate conjugation. This kind of conjugation is used in the enzymic detoxication of cyanides. The transfer of sulphur from thiosulphate onto a cyanide ion is catalyzes by a specific enzyme, *thiosulphatesulphide transferase:*

$$\underset{\text{cyanide}}{CN^-} + \underset{\text{thiosulphate}}{S_2O_3^{2-}} \longrightarrow \underset{\text{thiocyanate}}{SCN^-} + \underset{\text{sulphite}}{SO_3^{2-}}$$

Glycine conjugation. This reaction belongs to type II conjugations, which require a prior activation of the substrate rather than of the conjugating agent. In principle, any carboxylic acid can serve as a conjugation substrate. The mechanism of glycine conjugation may be exemplified by the formation of hippuric acid. According to the mechanistic concept of type II conjugation reactions, the initial step is activation of benzoic acid with the involvement of arylacyl~ScoA synthetase. Then benzoyl (or, in a wider sense, any activated substrate in the reactions of this type) is transferred onto the glycine amino group. This process is catalyzed by *acyl-N-glycine transferase,* which is specific to acylation of only glycine, barring other amino acids.

Similarly, glycine conjugates of other compounds are formed: aromatic acids (nicotinic), phenyl-substituted acetic acids (phenylacetic and hydratropic), steroid acids (cholic and deoxycholic).

Glutamine conjugation is a rare variety of conjugation, distinctly observable in patients with phenylketonuria. In normal humans, the glutamine conjugation of xenobiotics has never been reported.

Factors affecting drug metabolism

Drug metabolism is affected by a variety of factors. These include genetic, age- and organ-specific, neuroendocrine, environmental factors, and the manner a drug has been administered. The rate at which a drug supplied to the organism is metabolized is dependent on the number of enzymes involved in modification and conjugation of the drug. In *enzymophathies* associated with defective enzymes that are involved in drug metabolism, a decrease in the drug metabolism rate is observed. Molecular diseases due to a defective *UDP-glucuronosyltransferase* are known. These molecular diseases are characterized by the disturbed glucuronide conjugation not only of bilirubin, but of other endogenous substrates and drugs too. For this reason, the prescription of sulphanilamides, salicylates, and phenol-derived preparations, which are metabolized by glucuronide conjugation, leads to aggravated symptoms of the disease; even normal doses of these drugs produce a negative effect.

The *age is an important factor* in drug metabolism. In neonates and infants, the enzymic apparatus of xenobiotic metabolism is poorly developed. As the young organism develops, the physiological enzymic deficiency disappears, while hereditary enzymopathies in adult humans persist.

Liver is the major organ responsible for drug metabolism. Environmental factors such as light, ambient temperature, radiation have been noted to influence the drug metabolism. The action of these factors is accomplished indirectly, via the neuroendocrine system. Over 200 preparations are known to be drug metabolism enzymes, primarily microsomal ones. They include butadion (antiinflammatory), amidopyrine (analgesic), novocain (local anesthetic), ethanol, and others. Phenobarbital (soporific)

acts as the most powerful inducer. It drastically enhances the synthesis of microsomal oxidation enzymes in the liver by affecting the genetic apparatus of the liver cells. Phenobarbital elicits the synthesis of UDP-glucuronosyltransferase and facilitates the conjugation stage in the metabolism of various materials.

Inducers for drug metabolism enzymes are biogenic preparations such as thiamine, riboflavin and their coenzymes, carnitine, pantothenic acid, androgens, and anabolic steroids; preparations of progesterone and estrogens inhibit these enzymes.

LECTURE 30

Subject: BIOCHEMISTRY OF CONNECTIVE TISSUE

Connective tissue is a system of insoluble protein fibers embedded in a continuous matrix called the ground substance. It is widely distributed in the body, composing, in addition to the dermis, the tendons, ligaments, cartilage, and matrix of bone. Its chief function is supportive and is performed by the fibrils of insoluble proteins – *collagen* and *elastin*.

The characteristics of connective tissues depend on the proportions of collagen and elastin as well as the amount of ground substance. The Achilles tendon, for instance, a tissue of great strength, is 32% collagen and 2.6% elastin. The ligamentum nuchae, an elastic tissue, contains 32% elastin and only 7% collagen. Although cartilage is rich in ground substance, the areolar connective tissue contains little of the liquid matrix.

Collagen is the most abundant protein in the animal world. It serves as an extracellular framework for all multicellular animals. It is most abundant in fibrous connective tissue, but also is present in some form in practically every tissue. Examples are the "*ropes"* and "*straps"* in tendons and ligaments, the woven sheets in skin and fascia, porous membranes (glomeruli of the kidney), the supporting structure of bones and dentin (with embedded calcium salts, etc.), lubricating fluid with proteoglycans in cartilage, bone joints, and intervertebral disks, and special tissues requiring strength yet with unusual properties, such as fatigue-resistant heart valves, and translucency, as in the cornea and lens of the eye. At least five different types of collagen molecules have been found in the tissues of higher animals, each apparently adapted for special extracellular functions. There are variations in hydroxylation, glycosylation, and cross-linking. These are related to factors such as tissue types, hormonal factors, and even age. Abnormalities may occur in the structure and biosynthesis of collagen, leading to a variety of human diseases.

The protein collagen, which represents almost 30% of the total body proteins, is formed by fibroblasts embedded in the connective tissues. The prior formation of small soluble subunits can be produced by the cell in accord with the usual processes of protein synthesis. These subunits presumably leave the cell and aggregate in a regular manner in the extracellular fluid or ground substance. The solubilized collagen is then called tropocollagen and is considered to be the subunit of the mature fibers.

Tropocollagen is a highly asymmetrical molecule, a relatively rigid rod, with a molecular weight of approximately 300,000 and dimensions of 150 to 28,000 nm. It is composed of three polypeptide chains, each in a left-handed helical conformation having only three residues per turn. Helices of this type have their amide and carbonyl groups extending perpendicular to the polypeptide chains so that two of every three such groups may participate in inter-chain hydrogen bonding. The three helices together then wind is a right-handed coil (super-helix). The inter-chain hydrogen bonds appear to be the primary linkage holding the structure together.

Collagen is unique in its high concentration of proline and glycine. These prevent the formation of the usual α-helical conformation, which requires the presence of 3.6 amino acid residues per turn. Instead, collagen has 3 amino acid residues per turn. Each of the constituent polypeptides has 1000 residues and a molecular weight of approximately 95,000.

In connective tissues such as bone, mature dermis, tendon, and dentin, collagen has two identical polypeptide chains called the α1-chains and a third chain of different composition called the α_2-chain. This type of collagen is referred to as α_1 $(I)_2$ α_2. However, the composition of α-chains varies in other connective tissues. For example, in cartilage the collagen is α_1 $(II)_3$, meaning that the three polypeptides are identical but that the α_1 is different from α_1 (I) and also the α_2 just mentioned.

The primary structure of these polypeptides consists of alternate regions of highly polar amino acids separated by regions rich in glycine, proline, and hydroxyproline. The results of amino acid se-

quencing suggest the separation of polar region by nonpolar segments that average about 15 residues in length. Summarization of information now available shows that the nonpolar regions have the following sequence: gly-hydroxypro-ala-gly-pro-ala-gly-hydroxypro. Significant in this sequence are the triads gly-hydroxypro-ala, gly-pro-hydroxypro, and gly-hydroxypro-pro. For the left-handed helix to exist, every third residue must be glycine. It is now clear that the α_1- and α_2-chains are not synthesized in their final form as found in fibroblasts. For example, neither hydroxyproline nor hydroxylysine is incorporated directly; there are no codons for these two growing polypeptide. Hydroxylation requires a *proline* or *lysine hydroxylase*, ascorbic acid, ferrous iron, and oxygen. The need for ascorbic acid (vitamin C) in these reactions is evidence for the profound effect of scurvy on connective tissue formation and wound healing.

Proline + α-ketoglutarate + $\mathbf{O_2}$ + $\mathbf{Fe^{2+}}$ → **4-hydroxyproline** + $\mathbf{Fe^{3+}}$ + $\mathbf{CO_2}$ + **succinate**

Hydroxyproline is a major component of the protein collagen. Hydroxyproline and proline play key roles for collagen stability. They permit the sharp twisting of the collagen helix.http://en.wikipedia.org/wiki/Hydroxyproline - cite_note-3#cite_note-3

Nascent collagen polypetyde chains are also modified by the insertion of carboxyhydrate. Thus in the α_1-chains lysine-10^3 is hydroxylated and then glycozylated by the sequential addition of galactose (UDP-galactose) and glucose (UDP-glucose). The side chains are peptide-hydroxylysine-galactose-glucose. Peptide-hydroxylysine-galactose also is present.

The extracellular aggregation of tropocollagen molecules involves an orderly parallel alignment of individual molecules to create a quarter-staggered effect. In tissue the newly formed tropocollagen is readily extractable with neutral salt solutions because little or no cross-linking has occurred. On aging the collagen becomes increasingly cross-linked and no longer readily extractable. Collagen is unique in its transformation into gelatin on heating.

Elastin, is a protein having distinctly elastic properties as well as high mechanical strength. Compared with that of collagen, knowledge of elastin remains meager. Elastin fibers consist of two components: one is microfibrillar, about 1100 nm in diameter, and the other is amorphous. Elastin has a low content of polar side chains, especially of the basic and acidic amino acids. Like collagen it has a high content of glycine (27%) and proline (13.5%). In contrast, however, there is little hydroxyproline, no hydroxylysine a preponderance of nonpolar amino acids, i.e. valine, alanine, leucine, and isoleucine. Elastin is also insoluble in all solvents that do not change its chemical nature. It is not, however, converted to gelatin, as is collagen.

Elastin is probably composed of long-chain precursor molecules (*tropoelastin*) that are polymerized by a system of cross-links. Distinctive amino acid derivatives have been obtained from elastin and shown to have a cross-linking function. Two of these are called *desmosine* and *isodesmosine*. In the structure of desmosine four units of lysine are required to produce the compound having a pyridine nucleus. Three lysines are first converted to the α-semialdehydes (*allysine*). Then the three allysines condense with an intact peptide-bound lysine to produce the pyridine nucleus. Desmosine and an isomer called isodesmosine are capable of linking together two, three, or four polypeptide chains.

Reticulin. Third fibrous protein found in connective tissues is known as reticulin, which is the component protein of the reticular fibers. This protein resembles collagen in terms of its amino acid composition. It appears to be distinctive, however, because of its association with lipids and carbohydrates.

Proteoglycans or ground substance (mucopolysaccharides)

Ground substance, a proteoglycan, may be viewed as a modified dialysate of plasma. It contains some proteins derived from plasma by a certain degree of capillary permeability. The fluid matrix is unique because of its high content of glycosaminoglycans or mucopolysaccharides. In fact, up to as much as 95% carbohydrate may be present. The mucopolysaccharides present are hyaluronic acid and chondroitin sulfates A, B, and C. These polysaccharides are synthesized by the fibroblasts.

A good example of ground substance is the lubricating fluid of all joints, called the synovial fluid.

Hyaluronic acid is an unbranched polymer of high molecular weight (I to 1.5·10) of a repeating disaccharide consisting of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine.

The ***chondroitin sulfates*** are sulfated polysaccharides. Chondroitin sulfate A, now termed *chondroitin-4-sulfate,* is a polysaccharide of glucuronic acid and *N*-acetylgalactosamine bearing a sulfate ester group on carbon-4 of amino sugar, whereas *chondroitin-6-sulfate* (chondroitin sulfate *C*) is sulfa-

ted on carbon-6 of the amino sugar. Chondroitin sulfate *B*, now called *dermatan sulfate*, is similar to chondroitin sulfate *A* except that the uronic acid is of the sugar *L*-idose rather than *D*-glucose.

Mucopolysaccharides are believed to be complexed with protein found in the ground substance. The chondroitin sulfates combined covalently with a single protein core (M.m.=89,000) to produce a total unit of large molecular weight (3 to $6 \cdot 10^6$). Therefore the chondroitin sulfates are a special type of glycoproteins and are usually called proteoglycans. The oligosaccharide chains are attached to the single polypeptide chain at its seryl residues. A polysaccharide made up of a repeating disaccharide in which *L*-iduronic acid replaces *D*-glucuronic acid. These polysaccharides are found in connective tissues in their sulfated forms.

Two other proteoglycans (mucopolysaccharides) are found in some tissues: *keratosulfate* (keratin) I and II in the cornea, cartilage, spinal disks, and bone; and *heparin* in liver, lung, arterial walls, mast cells. The repeating disaccharide unit in the keratosulfates is mainly *D*-galactose-*N*-acetyl-*D*-glucosamine-6-sulfate. In heparin and heparin sulfate the repeating disaccharide unit is *D*-dlucuronic acid-2-sulfate, *N*-acetyl-*D*-dlucosamine-6-sulfate, with variable amounts of *N*-sulfo derivatives of *D*-glucosamine, rather than *N*-acetyl-*D*-glucosamine and of iduronic and glucuronic acids.

The biosynthesis of proteoglycans probably occurs by a sequential addition of monosaccharides to the core polypeptide chain. Each monosaccharide is added to the growing polysaccharide chain by a specific *glycosyl transferase* using the appropriate nucleotide-bound carbohydrate. This process may occur in the smooth endoplasmic reticulum and the *Golgi* apparatus. The production of proteoglycan probably terminates in an unsulfated glycoprotein such as the chondroitin that is found in corneal tissue. The final step in the synthesis of the sulfated proteoglycans is the sulfation process itself. Sulfation requires a mixed phosphosulfate anhydride: 3'-phosphoadenosine-5'-phosphosulfate, or *PAPS*.

The proteoglycans (mucopolysaccharide-protein complexes) play numerous roles. They have the particular ability to bind water and cations (even Na^+ and K^+), forming a gel. Hyaluronic acid is an important constituent of vitreous humor and the umbilical cord. It imparts the lubricative quality to the synovial fluid of the joints. Mucopolysaccharides help prevent the invasion of the body through the skin by disease-producing microorganisms. Many bacteria produce an enzyme, *hyaluronidase*,that catalyzes the depolymerization of mucopolysaccharides, thereby increasing the invasive capability of the bacteria. Hyaluronidase is a *β-glucosaminidase* and hydrolyzes the β-1,4-glycosidic bond between *N*-acetylhexosamine and the neighboring uronic acid. This enzyme is also found in spermatozoa and may be functional in facilitating the penetration and thus the fertilization of the ovum. Highly purified hyaluronidase is used clinically for the intradermal administration of large volumes of fluid when intravenous injections are contraindicated.

The composition of ground substance changes with aging. In children chondroitin sulfate A predominates, whereas in the adult chondroitin sulfate C is major component.

Cartilage is also a connective tissue and contains collagen, some noncollagenous protein, and ground substance. Fibers of elastin are present in elastin cartilage, tending to give added flexibility to this tissue. Vitamin D is quite possibly related to the development and health of cartilage, in addition to being involved in the normal conversion of cartilage to bone.

Chronic *rheumatouid arthritis* in humans is characterized by disruption of collagenous structures. Recent investigations have indicated that this may result from an excessive production of the enzyme collagenase by cells of the proliferating synovium. Several investigators reported high collagenase activity in synovial specimens from patients with rheumatoid arthritis, whereas none was demonstrable in specimens from control subjects. The amount of collagenase activity was directly proportional to the degree of local and systemic disease activity. This could account for the destruction of collagen in and about the joints, tendons, capsules, ligaments, cartilage, and bone in patients with this type of arthritis. A more recent hypothesis regarding the effects of arthritis on joint function suggests a depolymerization of hyaluronic acid by the superoxide radical. It is proposed that superoxide is released by phagocytic cells that have entered the synovial fluid. Superoxide dismutase derived from bovine red blood cells is being investigated as a potential therapeutic agent.

LECTURE 31

Subject: **BIOCHEMISTRY OF BONE AND TEETH**

Bone. The organic matrix of bone or cartilage is the supporting lattice in which the bone salts are deposited to from a rigid structure. The cartilaginous matrix is flexible and extremely strong. Collagen is the principal protein present in bone. Collagenous fibers, formed by the osteoblasts, mature in the mucopolysaccharide-containing ground substance. The ground substance varies in consistency from interstitial fluid to a thick gel, and forms the communicative medium between the tissue fluid and blood, thereby permitting an exchange of ions and other substances.

Analysis of bone ash reveals a preponderance of calcium, a small amount of sodium, and less magnesium and other cations. As a matter of fact 99% of the body's calcium is located in bone. The anions are chiefly phosphate, with some carbonate, citrate, and small amounts of chloride and fluoride. In general, the mineral of bone is in form of a *hydroxyapatite* crystal structure having the following formula: $\mathbf{3Ca_3(PO_4)_2 \cdot Ca(OH)_2}$

This formula accounts for the calcium and phosphate but not for the carbonate, citrate, and small amounts of chloride, fluoride, and other ions that are occasionally found. The belief was formerly held that bone salt was an apatite of changing composition and that these other elements or ions were introduced and withdrawn as they fluctuated in concentration in the blood plasma. Now there is evidence that these other salts are present in an intercrystalline semiliquid medium, which allows the transport of materials from the blood to the bone and vice versa. These two phases of the mineral structure of bone have quite different physical, chemical, and probably physiologic properties. The hydroxyapatite crystals are subject to rapid ion-exchange reactions at their surfaces. They are extremely-small and thus have an enormous surface area. The intercrystalline fraction is far smaller in amount than the crystalline fraction, about 4% as great. However, it is much more soluble, and its calcium and other elements readily exchange with those of the blood.

The hardness and rigidity of bone are attributable chiefly to the hydroxyapatite, whereas elasticity and toughness are attributable to the proteins.

Composition of bone:

Ash	71.0%
Water	8.2%
Collagen	18.6%
Fat	0.1%
Sugars	0.2%
Proteins	1.2%
Total:	99.3%

Citrate has been discovered as a constituent of human bone, to the extent of about 1% of the dry weight of bone, representing as much as 70% of the body's citrate content. The function of bone citrate is as yet unknown; it may play a special role in the metabolism of calcium by virtue of its power to bind calcium. The calcium-citrate complex is soluble and diffusible but is un-ionized.

Ossification, the formation of bone salt, requires that the concentration of calcium and phosphate must exceed the saturation point at the site of deposition.

Two enzymes are involved: *glycogen phosphorylase* and *alkaline phosphatase*. The latter hydrolyzes the glucose 1-phosphate formed by glycogenolysis and yields high local concentrations of inorganic phosphate. It may also hydrolyze other phosphoric acid esters that may be available, e.g., glycerophosphates and nucleotides. The phosphate that is found in high concentration wherever bone is formed is probably produced by the osteoblasts; the concentration of phosphate ions is raised locally near these cells as a result of enzyme action. The un-ionized calcium is partly diffusible and partly not. The nondiffusible calcium is largely that fraction combined with protein, whereas the calcium-citrate complex forms most of the un-ionized diffusible part. Probably the chondroitin sulfates of growing bone unite with calcium to provide a local surplus of available calcium. Normally the concentrations are such that the product of ionic calcium and phosphate is about 36 to 40 mg/100 ml of plasma. Products above 40 mg are found when bone growth or healing is taking place, whereas products below 40 mg generally are seen in active rickets and in other conditions in which bone formation is not occurring properly. When the local concentration of calcium and phosphate ions is increased beyond the saturation point, calcium phosphate is produced. This is changed to hydroxyapatite in a series of steps.

Another enzyme, *carbonic anhydrase*, may be involved in the deposition of calcium salts in bones and teeth. This enzyme apparently makes available the carbonate ion for the formation of the bone salt, carbonate hydroxyapatite. The long axes of the bone salt crystals are invariably oriented with the long axes of the collagen fibrils and are arranged around the cross-banding of the fibrils, forming a type of sheath.

Note that the bone calcium is in equilibrium with the calcium of the blood. Consequently the blood calcium can be kept at a fairly steady concentration by a slight shift of calcium from the bones to the blood or vice versa. The concentration of total calcium in the blood is approximately 10 mg/100 ml (2.5 mmol/L). If the level of blood calcium ions diminishes, calcium is derived from the inter-crystalline material. More calcium is dissolved into the surrounding fluid and enters the blood. This process usually keeps the level at about 7 mg/100 ml. The remaining 3 mg, more or less are supplied in just the amount needed by means of a feedback mechanism under the control of the parathyroid glands.

Osteoporosis. In patients with osteoporosis in bone calcium salts decrease. In ***rickets*** the amount of calcium phosphate in the bones is much below normal. This deficiency is usually the result of inadequate *vitamin D*, which decreases the absorption and use of calcium and phosphorus. Since the concentration of minerals in the bones is low, the bones become less rigid and consequently bend, resulting in bowlegs or other deformities. *Vitamin A deficiency* retards the growth of bone, particularly endochondral bone formation in rats. If the deficiency is established very early in life, skeletal growth is inhibited considerably before the effect on total increase in weight can be observed. *Vitamin C* also is essential to bone development specifically the formation of tropocollagen. In *skurvy* there are lesions of the epiphyseal junctions of growing bones. Subperiosteal hemorrhages are likely to occur in both growing and adult bone. Rarefaction of the alveolar bone leads to loosening of the teeth; dentine is resorbed, and the gums become spongy.

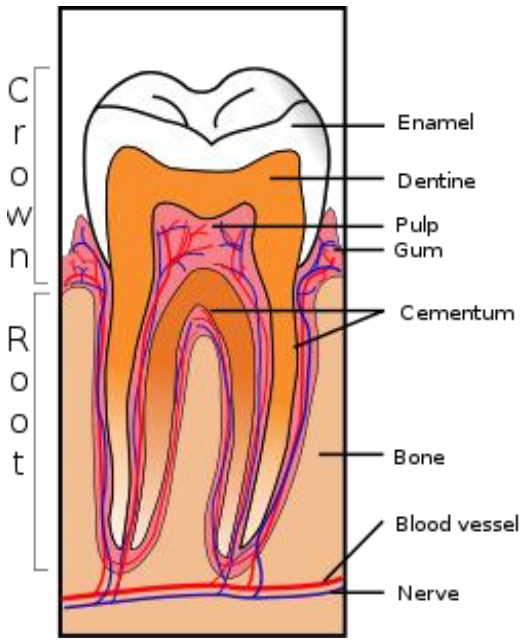
Teeth. The teeth resemble bone chemically to a certain extent. Over the upper surface of the tooth is the *enamel*. This is the hardest substance in the body, a property of great value for the masticating and grinding action of the teeth. Only about 5% of enamel is water. The remaining 95% consists of inorganic material chiefly embedded in an organic matrix.

This organic matrix of enamel is composed of a protein, resembling keratin but containing no cystine, and a mucopolysaccharide. The inorganic material is hydroxyapatite, a calcium phosphate with the formula $\mathbf{Ca_{10}(PO_4)_6{\cdot}Ca(OH)_2}$, and possibly also containing fluoroapatite $\mathbf{Ca_5(PO_4)_3F}$. It is an important constituent of tooth enamel. The greater part of the tooth is *dentin*, which is identical to bone from a chemical standpoint although different histologically. Dentin protein is largely collagen, and there is chondroitin sulfate present. The inorganic basis is again an apatite, similar to the bone salt.

Administration of labeled phosphorus is followed by rapid uptake of the tracer by developing teeth. Once the teeth are completely formed and calcified, this continuing metabolism is reduced to a minimum. Thus the teeth are not drawn upon for calcium in time of need, as are the bones.

Vitamins A, C, and D are all necessary for proper tooth development and calcification. Lack of vitamins A and C affects the functional activities of the formative cells. Deficiency of vitamin A results in hypoplastic enamel imperfectly calcified. Lack of ascorbic affects the formation of the organic matrix of dentin, as it does in bone. Vitamin D not only aids in the absorption of calcium but also apparently promotes the deposition of calcium and phosphorus in teeth.

Dental caries. When the enamel breaks and the underlying dentin exposed, dental caries develops. The cause of this formation of tooth cavities has been a matter of dispute for years and is still unsettled. Dental caries is one of the most widespread of human diseases, and a tremendous amount of investigation has been instituted to determine the cause and the effect a cure. In caries the enamel and dentin are dissolved by chemical action and washed away, thus producing a cavity. Demineralization occurs for several reasons, but the most important cause of tooth decay is the ingestion of sugars.



Tooth cavities are caused when acids dissolve tooth enamel:

$$Ca_{10}(PO_4)_6(OH)_2(s) + 8H^+(aq) \rightarrow 10Ca^{2+}(aq) + 6HPO_4^{2-}(aq) + 2H_2O(l)$$

The formation of cavities in the teeth is not only a source of pain and discomfort, necessitating dental attention, but also is likely to lead to interference with mastication and thus with proper nutrition. Furthermore, infections processes occurring in cavities may result in absorption of toxins or lead to secondary infections in other parts of the body. Local bacterial factors are entirely or at least chiefly responsible. Food particles lodged between the teeth or in recesses in the surface of teeth become breeding spots for bacteria, forming bacterial *plaques*. If they are not removed promptly, enough organic (e.g. lactic) acids are produced to dissolve mineral constituents from the enamel and dentin to form a cavity and thus dental caries. Local pH values as low as 4.0 to 5.0 on the tooth surface lend support to this view. Foods particularly rich in easily fermentable sugars (sucrose, glucose, and fructose), especially *sticky sugars* of candies and certain pastries, are most likely to lead to the formation of caries. *Streptococcus mutants* found to be highly *cariogenic*, as were several species of *lactobacilli* and *actinomyces*.

The influence of *fluoride* must be emphasized. If the amount of fluoride in drinking water is adequate (1.0 to 1.2 ppm), the enamel seems to be *more resistant* to the development of dental caries. Note that fluoride is probably most effective if it is present during the period of tooth development. The fluoride content of the surface layers of enamel is normally approximately 10 times higher than that of the layers near the dentin enamel function. It is hypothesis that fluoride acts as an enzyme inhibitor, thus interrupting the chain of fermentative reactions and preventing the formation of organic acids in proximity to the enamel.

Another view is that some component or components of saliva play a role in the prevention of dental caries. Various proteins present in saliva have been alleged to exert *anticariogenic* effects. Some propose that they act as buffers of the organic acids produced in the mouth by the fermentation of sugars. Other, believe that the effect is immunological, certain proteins acting as antibodies. Indeed, there are claims that a group of arginine peptides, termed *sialins,* are the principal agents in saliva conferring resistance to caries. Another cariostatic peptide is alleged to control the incorporation of calcium and phosphorus into teeth. It has been given the name *statherin.*

Thus vigorous biochemical and immunological research continues today in an effort to determine the basic mechanisms involved in the prevention and control of dental caries, one of humankind's and most prevalent chronic diseases.

Gingivitis ("inflammation of the gum tissue") is a term used to describe non-destructive periodontal disease. The most common form of gingivitis is in response to bacterial biofilms (also called *plaque*) adherent to tooth surfaces, termed *plaque-induced gingivitis*, and is the most common form of periodontal disease. In the absence of treatment, gingivitis may progress to periodontitis, which is a destructive form of periodontal disease. While in some sites or individuals, gingivitis never progresses to periodontitis, data indicates that periodontitis is always preceded by gingivitis.

The *etiology*, or cause, of plaque-induced gingivitis is bacterial plaque, which acts to initiate the body's host response. This, in turn, can lead to destruction of the gingival tissues, which may progress to destruction of the periodontal attachment apparatus. The plaque accumulates in the small gaps between teeth, in the gingival grooves and in areas known as *plaque traps*: locations that serve to accumulate and maintain plaque. Examples of plaque traps include bulky and overhanging restorative margins, claps of removable partial dentures and calculus (tartar) that forms on teeth. Although these accumulations may be tiny, the bacteria in them produce chemicals, such as degrative enzymes, and toxins, such as *lipopolysaccharide* (LPS, otherwise known as endotoxin) or *lipoteichoic acid* (LTA), that promote an inflammatory response in the gum tissue. This inflammation can cause an enlargement of the gingiva and subsequent *pseudopocket* formation. The *primary etiology* (cause) of gingivitis is poor oral hygiene which leads to the accumulation of a mycotic and bacterial matrix at the gum line, called *dental plaque*. Other contributors are poor nutrition and underlying medical issues such as diabetes.

Microbic plaque calcifies to form *calculus*, which is commonly called *tartar*. Calculus above and below the gum line must be removed completely by the dental hygienist or dentist to treat gingivitis and periodontitis. Although the primary cause of both gingivitis and periodontitis is the microbic plaque that adheres to the tooth surface, there are many other modifying factors. A very strong risk factor is one's genetic susceptibility. Several conditions and diseases, including Down syndrome, diabetes, and other diseases that affect one's resistance to infection also increase susceptibility to periodontitis.

In some people, gingivitis progresses to periodontitis - with the destruction of the gingival fibers, the gum tissues separate from the tooth and deepened sulcus, called a periodontal pocket. Subgingival microorganisms (those that exist under the gum line) colonize the periodontal pockets and cause further inflammation in the gum tissues and progressive bone loss.

Dental plaque is a biofilm, usually colorless, that develops naturally on the teeth. It is formed, as in any biofilm, by colonizing bacteria trying to attach itself to a smooth surface (of a tooth) It has been also speculated that plaque forms part of the defense systems of the host by helping to prevent colonization by microorganisms which may be pathogenic.

The film is soft enough to come off if scraped with a fingernail. If not removed it starts to harden within 48 hours; in about 10 days the plaque becomes *dental calculus* (tartar), rock-hard and difficult to remove.

Dental plaque can give rise to *dental caries* (tooth decay)—the localised destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars—and periodontal problems such as *gingivitis* and *chronic periodontitis*.

The mechanisms of plaque formation include:

- Absorption of proteins and bacteria to form a film on the tooth surface.
- The effect of van der Waals and electrostatic forces between microbial surfaces and the film to create reversible adhesion to the teeth.
- Irreversible adhesion due to intermolecular interactions between cell surfaces and the pellicle.
- Secondary colonisers attach to primary colonisers by intermolecular interaction.
- The cells divide and generate a biofilm.

Components of plaque. Plaque consists of *microorganisms* and *extracellular matrix*. The microorganisms that form the biofilm are mainly *Streptococcus mutans* and anaerobes, with the composition varying by location in the mouth. Examples of such anaerobes include fusobacterium and actinobacteria. The extracellular matrix contains proteins, long chain polysaccharides and lipids. The microorganisms present in dental plaque are all naturally present in the oral cavity, and are normally harmless. However, failure to remove plaque by regular tooth brushing means that they are allowed to build up in a thick layer. Those microorganisms nearest the tooth surface convert to anaerobic respiration; it is in this state that they start to produce acids.

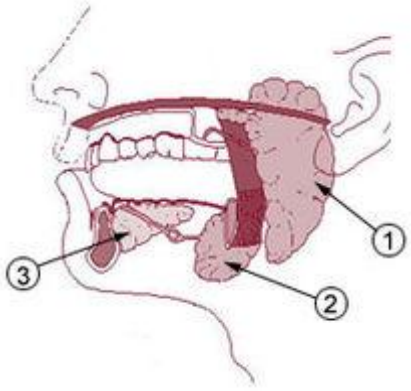
- Acids released from dental plaque lead to demineralization of the adjacent tooth surface, and consequently to dental caries. Saliva is also unable to penetrate the build-up of plaque and thus cannot act to neutralize the acid produced by the bacteria and remineralize the tooth surface.
- They also cause irritation of the gums around the teeth that could lead to gingivitis, periodontal disease and tooth loss.
- Plaque build up can also become mineralized and form calculus (tartar).

Dental fluorosis is a health condition caused by a child receiving *too much fluoride* during tooth development. The critical period of exposure is between 1 and 4 years old; children over age 8 are not at risk. In its mild form, which is the most common, fluorosis appears as tiny white streaks or specks that are often unnoticeable. In its severest form, which is also called mottling of dental enamel it is characterized by *black* and *brown stains*, as well as cracking and pitting of the teeth. The severity of dental fluorosis depends on the amount of fluoride exposure, the age of the child, individual response, as well as other factors including nutrition. Although water fluoridation can cause fluorosis, most of this is mild and not usually of aesthetic concern. Severe cases can be caused by exposure to water that is naturally fluoridated to levels well above the recommended levels, or by exposure to other fluoride sources such as *brick tea* or pollution from high fluoride coal. Dental fluorosis occurs because of the excessive intake of fluoride, either through fluoride in the water supply, naturally occurring or added to it; or through other sources. The damage in tooth development occurs between the ages of 3 months to 8 years, from the overexposure to fluoride. Teeth are generally composed of *hydroxyapatite* and *carbonated hydroxyapatite*; when fluoride is present, some fluorapatite is generated. Excessive fluoride can cause white spots, and in severe cases, brown stains or pitting or mottling of enamel. Fluorosis cannot occur once the tooth has erupted into the oral cavity. At this point, fluorapatite is beneficial because it is more resistant to *dissolution by acids* (demineralization). Although it is usually the permanent teeth which are affected, occasionally the primary teeth may be involved. The differential diagnosis for this condition may include *Turner's hypoplasia* (although this is usually more localized), some mild forms of *amelogenesis imperfecta*, and other environmental enamel defects of diffuse and demarcated opacities.

LECTURE 32

Subject: **BIOCHEMISTRY OF SALIVA**

Saliva is the mixed secretion of the parotid, submaxillary, sublingual, and buccal glands. It contains 99.3% to 99.7% water and has a specific gravity of 1.002 to 1.008. Approximately 1500 ml is believed to be the daily secretion in man. The secretion of saliva is entirely under the control of the nervous system. A variety of stimuli cause an increased flow by reflex stimulation. This is true whether the stimulus is *psychic* (sight, smell, or thought of food), *mechanical* (chewing), or *chemical* (action of acids, salts, etc. on the taste buds). There seems to be no hormonal control of salivary secretion.



1 **– Parotid gland;** ***2*** **– Submandibular gland;** ***3*** **– Sublingual gland**

Saliva is almost colorless and rather viscid, and if a quantity of saliva is a vessel is exposed to air, the surface becomes covered with an incrustation consisting of calcium carbonate with a small proportion of organic matter. The reaction of the saliva of a given individual is not constant. Resting saliva is slightly acidic, pH 6.4 to 6.9, whereas saliva obtained during active stimulation of the glands is neutral to slightly alkaline, pH 7.0 to 7.3.

The solid constituents of saliva comprise albumins, globulins, mucins, enzymes, urea, uric acid, glucose, and inorganic salts. The salivary mucins are glycoproteins and yield 30% to 45% carbohydrate. Ovine submaxillary mucin, for example, contains approximately 45% carbohydrate and has about 800 disaccharide units per protein molecule. The disaccharide units are known to be *N*-acetylneuraminyl (2→6) *N*-acetylgalactosamine. The disaccharide units are attached to every sixth amino

acid residue of the protein component – a single polypeptide chain. The protein portion is rich in threonine, with a low isoelectric point, about 3.5, probably because of the carbohydrate moiety. This material has a relatively high viscosity and a high degree of hydration, which in part account for the protective and lubricating functions described below. The inorganic components differ greatly in concentration from those of blood serum, but the nonprotein nitrogen-containing constituents (urea, uric acid, NH_4^+ salts) appear to bear some relation to these same constituents in the blood. Amino acids and glucose occur in extremely small amounts in the saliva of healthy individuals (11 to 30 mg glucose per 100 ml). Both salivary cholesterol and lipid phosphorus values are very low as compared with blood. The salivary glands therefore appear to be quite selective in secretary action.

The chief inorganic ions present are K^+, PO_4^-, and Cl^-, with smaller amounts of Na^+, Ca^{2+}, and SO_4^-. Some of these may combine to form insoluble precipitates. This may be added by changes in the pH brought about by decomposing food material left between the teeth or by the loss of carbon dioxide, held in solution in the saliva, as soon as it meets atmospheric conditions. Thus tartar may be formed. This consists chiefly of calcium carbonate and phosphate. Salivary calculi sometimes are formed in the ducts and the similar in composition to tartar (namely, $Ca_3[PO_4]_2$ or $CaCO_3$). It is usually stated that a clump of bacteria or a foreign body establishes a nucleus around which the precipitation of these salts occurs. However, calcium oxalate may be the precipitated salt, which, together with mucin and globulin, may form the calculus. Increased acidity is necessary for oxalate calculus formation.

Functions of saliva. Saliva has a digestive function because of the enzymes present, but it also has other functions. It moistens and lubricates the food, permitting it to be swallowed easily. Saliva holds the taste-producing substances in solution and so brings them into contact with the taste buds. It dilutes salts, acids, etc., thereby protecting the mucosa and, to some extent the teeth. It also has a cleansing action on the teeth, gums, and buccal mucosa. It owes its viscous and lubricating property to its content of mucin. This protein is present as an alkaline salt, which is soluble at the pH of saliva but is precipitated on acidification. It is one of the chief buffers present in saliva. A major function of epithelial mucins in general is the protection of the mucosal lining of the mouth, the gastrointestinal tract, and the inner surfaces of other body cavities. They form water-soluble films. In the stomach the acidity probably results in the formation of insoluble gels and although the mucins are not completely resistant to proteolytic enzymes, the action is slow and thus there is considerable protection. Some authorities maintain that saliva has an excretory function, since certain elements and drugs are found in it after administration. Among these are mercury, lead, and potassium iodide. Any part of them lost in expectoration could be considered excreted, but some of the part swallowed may be reabsorbed. Hence it is difficult to see how these elements can be called a true excretion. The same is true of the traces of urea, uric acid, and ammonium salts ordinarily found in saliva.

The parotid and submaxillary salivary glands have been implicated in the deiodination of the hormone thyroxine and hence in the regulation of the thyroxine level of the blood. The iodine thus released is excreted in the saliva and is reabsorbed in the small intestine for reuse, completing an iodine cycle.

Enzymes. The principal enzyme of human saliva is an *α-amylase* (ptyalin). There are also traces of possibly a maltase, catalase, urease, protease, and others.

Some investigators maintain an important function of salivary α-amylase is a cleansing agent for the oral cavity; i.e. digests starch particles or pastes left in or near the teeth. Salivary amylase is 1,4-glycosidase and serves to hydrolyze only 1,4-glycosidic bonds found in starch and glycogen. The specificity of this enzyme is such that it can only hydrolyze alternate glucosidic bonds. Consequently the result of the amylolytic process beginning in the saliva involves the conversion of starch and glycogen into maltose and dextrin. The latter is a mixture of large-chain, branched oligosaccharides or partial digestion products.

Indices of saliva

Daily volume (V)	500-1500 ml (500-1500 ml/24 h)
pH	6.07-7.9
Density (ρ)	1.08-1.32
Nitrogen-containing organic substances	
Proteins	1.4-6.4 g/L
Mucine	0.8-6.0 g/L
Ammonia	0.01-0.12 g/L
Urea	0.14-0.75 g/L

Uric acid	0.005-0.029 g/L
Creatinine	0.005-0.750 g/L
Choline	0.005-0.036 g/L
Nitrogen-noncontaning organic substances	
Glucose	0.10-0.30 g/L
Citric acid	< 0.020 g/L
Lactic acid	0.01-0.05 g/L
Cholesterol	0.025-0.500 g/L
Inorganic substances	
Sodium (Na)	5.2-24.4 mmol/L
Potassium (K)	14-41 mmol/L
Calcium (Ca)	2.3-5.5 mmol/L
Chlorides (Cl^-)	15.1-31.6 mmol/L
Phosphate inorganic	0.080-0.217 g/L
Bicarbonate (HCO_3^-)	2.13-13.00 mmol/L
Fluor (F^-)	0.8-2.5 g/L
Brom (Br^-)	0.2-7.1 mg/L
Tiocianate (SCN^-)	0.12-0.33 g/L
Magnezium (Mg^{2+})	0.16-1.06 mmol/l
Sulphates	0.04-0.2 g/L
Copper (Cu)	0.5-7.6 mg/L
Zinc (Zn)	0.06-0.80 g/L
Vitamins	
Thiamine (B_1)	0.7 μg/dL
Riboflavin (B_2)	5.0 μg/dL
Pyridoxine (B_6)	60 μg/dL
Nicotinic acid (PP)	3.0 μg/dL
Pantotenic acid	8.0 μg/L
Ascorbic acid (C)	0.58-3.78 mg/L
Biotin (H)	0.08 μg/dL
Phyllochinon (K)	1.5 μg/dL

LECTURE 33

Subject: **BIOCHEMISTRY OF MUSCLE TISSUE**

Muscles form a large proportion of the active tissue of the body. In normal adults it is fully two fifths of the body weight, but about half the metabolic, or chemical and physical, activity of the body takes place in muscles even during rest. When the muscles are contracting, while doing work, fully three fourths of the total metabolism can be assigned to them.

The three types of muscle-striated (voluntary) skeletal, cardiac, and nonstriated (involuntary) smooth-differ somewhat in their chemistry, but they have the same general characteristics. In skeletal muscle are found the following:

- water,
- proteins (albumins, globulins, nucleoproteins, myoglobin),
- lipids (cholesterol, phospholipids, triglycerides),
- extractives (soluble in hot water),
- nonnitrogenous (creatine, creatine phosphate, creatinine, inosinic acid, adenylic acid, adenosine triphosphate, glutathione, purines, pyrimidines, carnosine, anserine, choline, acetylcholine),
- enzymes,
- hormones,
- vitamins,
- inorganic salts.

Adult muscle has 72% to 78% water. As in the case of nerve tissue, the water content of the muscle of the young and of the fetus is even higher. The solids of muscular tissue are largely protein in

nature, whereas those of nerve tissue are largely lipid. The total lipid of muscle amounts to only about 3% and the glycogen less than 1%, but the protein content is about 20%.

Structure of skeletal muscle. A typical skeletal muscle consists of many muscle fibers, or *fascicules*, the structural unit of muscle. Numerous fibers are bound together by areolar connective tissue and are arranged parallel to the line of its attachments via tendons to the bones or other structure whose movements they control. Typically, muscle fibers are approximately 0.01 to 0.1 mm in diameter and from 1 to 40 mm in length. Groups of fibers are encased in a thin but tough elastic sheath, known as the *sarcolemma*, beneath which are embedded numerous nuclei and mitochondria. The sarcolemma is an electrically excitable membrane, important in the transport of motor nerve impulses. The sarcolemma also contains numerous longitudinal and transverse *sarcotubules* (transport ducts) and the *sarcoplasmic reticulum*. Bundles of muscle fibers are surrounded by a semi-fluid substance, the muscle plasma or *sarcoplasm.* The sarcoplasm contains the red protein *myoglobin* (muscle hemoglobin), which transports oxygen from adjacent blood capillaries to the sites of oxidation in mitochondria. Myoglobin, like erythrocyte hemoglobin can be converted to *metmyoglobin* by various oxidants (e.g., certain drugs) and reconverted to myoglobin by mechanisms similar to those present in red blood cells (antioxidant defense systems).

Muscular fibers are made up of numerous smaller structures called *myofibrils*. These contain transverse alternating *light* (*isotopic*) *I* bands bisected by a *dark* Z *line* (membrane) and dark (*anisotropic*) *A bands*, having a lighter central *H zone.* These bands are aligned in adjacent myofibrils to appear as continuous bands, or striations, across an entire muscle fiber.

Myofibrils also contain numerous fine longitudinal lines, sometimes termed myofilaments, consisting of *actin* (thin) filaments and *myosin* (thick) filaments containing a number of cross-bridges. A single *sacromere*, the functional unit of the myofibril is bounded by two Z lines.

Proteins of muscle. As started before, about 20% of muscle tissue is protein. Over half the muscle proteins are the structural proteins: actin, myosin, tropomyosin, and troponin. Other proteins include myoglobin, a number of enzymes involved in the metabolism of muscle tissue, and collagen and other proteins of connective tissue.

Myosin, a globulin, is the most abundant muscle protein. The myosin molecule consists of two globular heads, each joined to a tail-like appendage and twisted into a double-stranded α-helix. The molecular weight is approximately 500,000. The two identical major chains have molecular weights of about 20,000 each. Several hundred myosin molecules bind together tail-to-tail to form the *thick filament* of the sarcomere *A* band. Their double heads project at regular intervals forming the cross-bridges of the sarcomere. The tails meet at the central *H* band of the sarcomere. The head portion of *myosin* has a marked ability to bind with *actin* to form *actomyosin*, which has a pronounced ATPase activity.

Actin also is a globulin, with a molecular weight of about 60,000. It is a major constituent of the *thin filaments* of the sarcomere. A lower molecular weight form of actin, called *G-actin* (M.m.= 42,000), with a globular configuration, is obtained by extracting muscle tissue with a low-ionic strength medium. If the ionic strength is increased and Mg^{2+} ions and ATP are present, *G*-actin polymerazes into *F-actin,* a fibrous form. It has a double-helical structure resembling a double chain of beads.

F-actin combines with myosin to form actomyosin, which has the remarkable property of contraction in the presence of ATP, K^+, and Mg^{2+}, as shown in the classic experiments of *Szent-Gyorgyi*. He correctly concluded that the force of muscular contraction comes from an interaction of actin, myosin, and ATP.

Two other proteins, *tropomyosin* and *troponin* are now known to be involved in the contraction of muscle. Tropomyosin is a double-stranded α-helical rod with a molecular weight of 70,000. In the thin actin filaments it is intertwined between the two helical strands of *F*-actin.

Troponin, which is positioned at regular intervals along actin filaments, is a spherical molecule containing three different types of subunits, with a molecular weight of approximately 70,000. It is the third component of the thin filament. The three subunits are named according to their functions: *Tn-T* binds to tropomyosin; *Tn-I* is the inhibiting unit; and *Tn-C* is the calcium-binding unit. The *Tn-T* subunit has a molecular weight of 37,000 and contains 259 amino acid residues. Its action appears to be to prevention of the interaction of the head of myosin molecules with actin, thus preventing the binding to actin and ATPase activity. Contraction of the myofibril is thus prevented. Troponin-C, which binds calcium ions, has a molecular weight of about 18,000 and contains 159 residues, a relatively large number being aspartic and glutamic acids. Tn-C also competitively binds Mg^{2+} ions.

Sequence of events during muscle contraction. Contraction of the myofibrils, indeed the sarcomeres of the myofilaments, is initiated by nerve impulses transmitted via motor nerves across the neu-

romuscular junction to the sarcolemma of muscle fibers. A wave of depolarization spreads from this point along the entire fiber and then inward through openings in the transverse tubules into sarcoplasmic reticulum of the myofibrils. This stimulates a release of Ca^{2+} ions by some as yet uncertain mechanism. The Ca^{2+} ions bind to troponin-*C* (of the thin actin filaments), producing a change in its conformation that is followed by a shift of tropomyosin, permitting the attachment of the cross-bridges of the myosin (thick) myofilaments to the actin (thin) myofilaments. Simultaneously, energy is released by the hydrolysis of the ATP by the ATPase action of actomyosin. This energy is used to cause a ratchet-like movement of the myosin cross-bridges attached to the actin filaments, causing the latter to slide along the stationary myosin (thick) filaments towards the center of the sarcomere. These results in the shortening of the sarcomere and in turn the myofibril, the muscle fiber, and the muscle as a whole. The foregoing events occur quite rapidly, the contraction process requiring only a 10 to 20 msec.

When the neurostimulatory phase ends, Ca^{2+} ions are drawn back into the sarcoplasmicc reticulum network, the cross-bridges of myosin retract, and the sarcomeres and the muscle fibers relax, returning to the resting state. The sequence may be repeated for as long as the supply of ATP and other components essential for muscle contraction permit.

Source of energy for muscle contraction. ATP is the immediate source of energy for muscle contraction. However, the amount of ATP in muscle is relatively small, so a back-up reserve supply of readily available energy is necessary. This is supplied in the form of phosphocreatine ("phosphagen"), the high-energy phosphate of which is transferred to ADP by the enzyme *creatine phosphotransferase (creatine kinase* or *CPK)* – the *Lohmann* reaction. Mammalian skeletal muscle contains about five times as much phosphocreatine as ATP. This still is a fairly limited supply of ATP, so replenishment from other sources is soon necessary. Muscle glycogen is available to a limited extent, forming ATP by means of glycolysis and the citric acid cycle. Any lactic acid formed in muscle contraction under anaerobic conditions can be converted to glucose-glycogen in the liver and then into ATP. Free fatty acids and ketone bodies also can be used as a source of ATP in most skeletal muscles. Gluconeogenesis is another source. ATP also may be derived from ADP by the muscle *myokinase* reaction:

$$\mathbf{2ADP \leftrightarrow ATP + AMP}$$

The reserve supply of phosphocreatine in muscle is regenerated by the transfer of the high-energy phosphate of ATP to creatine via the reversible action of *creatine phosphokinase.* The resynthesis of ATP and phosphocreatine can be blocked by agents, such as iodoacetate, that inhibit glycolysis. Glycolysis thus is the ultimate major source of energy for the contraction of skeletal muscle. Other types of muscle (aerobic), however, can use additional types of fuel efficiently, such as free fatty acids, lactate, and ketone bodies.

Metabolism in other types of muscle

Cardiac muscle, which is characterized by its capability for long, sustained contractions, can readily use fuels requiring aerobic conditions, such as free fatty acids, lactate, and ketone bodies. Indeed, there is some evidence that free fatty acids are a preferred source of energy for the myocardium. The mechanisms of contraction of cardiac muscle are essentially the same as those described for skeletal muscle.

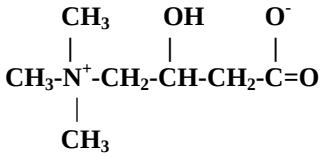
Smooth muscle (involuntary) differs sharply from skeletal muscle in its structure, location in the body, innervations, mode of action, and general functions. However, the chemical processes involved in the contraction of smooth muscle are similar to those found in skeletal and cardiac muscle, e.g., the requirement for calcium ions for initiation and ATP for the force of contraction.

Muscle lipids. Besides variable amounts of fat, muscle is found to contain small amounts of cholesterol and larger quantities of phospholipids. Here there are definite differences among the three types of muscle. Smooth muscle has the greatest amount of cholesterol, cardiac muscle next, and striated muscle the least. The ratio of phospholipids to cholesterol is high for skeletal and cardiac muscle and low for smooth muscle. These findings indicate that cholesterol has some relation to the spontaneous muscular activity of cardiac and smooth muscle, and phospholipids are involved in some way with the greater energy production of cardiac and striated muscle.

Extractives. If muscle tissue is ground and repeatedly extracted with hot water, a light tan fluid with droplets of fat floating on its surface and particles of coagulated protein suspended is obtained. When this is filtered and concentrated, a dark brown sticky material, commonly known as beef extract, is left. This is composed of all the soluble inorganic salts and all the extractives mentioned earlier. Aside from the small amounts of carbohydrate, amino acids, and peptides present, there is little food value in beef extract.

Some of the individual extractives deserve mention at this point. One of them is inositol, $C_6H_{12}O_6$, or better, $C_6H_6(OH)_6$. There are a number of natural isomers of inositol. The most important one was renamed *myoinositol*, instead of *mesoinositol.*

At least four peptides have been isolated from muscle extracts. *Carnosine* (β-alanylhistidine), *anserine* (β-alanyl-1-methylhistidine), and β-alanyl-3-methylhistidine are compounds found only in skeletal muscle of vertebrates. The fourth peptide present in muscle extractives is *glutathione,* a tripeptide, with the composition *glutamylcysteinylglycine.* This compound is a hydrogen acceptor and as such plays a role in tissue reactions, such as the reduction of methemoglobin. It is found in many tissues other than muscle, especially liver, red blood cells, brain, and kidney. It is also present in the lens of the eye and is reduced in amount when cataract occurs. *Carnitine,* a betaine, is also a muscle extractive and is also found in most tissues. Its formula is as follows:



Carnitine plays a role in the oxidation of fatty acids in muscle tissue, as well as in other tissues, by facilitating their transfer to fatty acid oxidation sites.

Other constituents. Other constituents include g*lycogen* (0.5% to 1.0%), important as a source of energy for the formation of ATP, traces of free amino acids, a number of enzymes, and inorganic ions, including *potassium*, *magnesium*, and *sodium* as the principal cations in descending amount, and *phosphate*, *sulfate*, and *bicarbonate* as the main anions similarly.

LECTURE 34

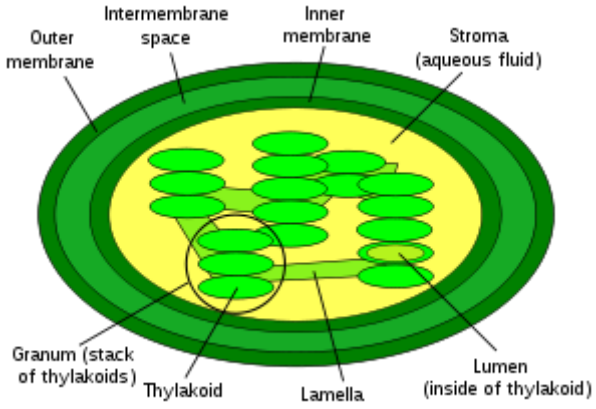
Subject: **PRODUCTION OF ENERGY IN PHOTOSYNTHETIC ORGANISMS**

Photosynthetic organisms (*photoautotrophs*) convert luminous energy to chemical energy to be used in the synthesis of carbohydrates and other materials. Photosynthetic cells possess an apparatus for acquisition and conversion of energy and enzymic systems for CO_2 assimilation. Photosynthetic organisms are diversified. They include *multicellular organisms* (higher green plants and their lower forms: green, brown, and red algae) and *unicellular organisms* (euglenaceae, diatoms, and dinoflagellata). A large group of photosynthetic organisms is represented by procaryotes (green-blue algae, green and purple bacteria). About half the overall work in the photosynthesis on the Earth accomplished by higher green plants, and the other half, chiefly by unicellular algae.

Photosynthetic structures. In bacteria, the photosynthetic structures are represented by invaginated portions of the cell membrane, which form lamellar organelles called *mesosomes.* Isolated mesosomes produced from disintegrated bacteria are called *chromatophores;* they accommodate the light-sensitive apparatus of bacteria. In eukaryotes, the photosynthesis apparatus is located in special intracellular organelles, *chloroplasts.* Chloroplasts, similar to mitochondria, contain also DNA, RNA, and the apparatus for protein synthesis, i.e. potentially they are capable of self-reproduction. In size, chloroplasts are several times as large as mitochondria. The number of chloroplasts per cell varies over a range from 1 (in algae) to 40 (in higher plants). The green plant cells contain, alongside of chloroplasts, mitochondria which are employed for energy generation at night time by the respiration mechanism, like in heterotrophic cells.

Chloroplasts are of spherical or oblate (lense-like) shape. A chloroplast possesses two-outer and inner-membranes. Stacks of flattened vesicle-like disks extend from the inner membrane. These stacks are called the *grana.* The number of grana per chloroplast in algae does not exceed 1, while in higher plants, 50 grana link with each other through membraneous bridges. The aqueous medium of the chloroplast in which the grana are immersed is called the *stroma.* The vesicle-like structures, which the grana are composed of, are referred to *thylakoids*. One granum contains 10 to 20 thylakoids.

The elementary structural and functional photosynthetic unit of the thylakoid membrane, which contains necessary light-trapping pigments and components of the energy conversion apparatus, is called the *quantasome.* This particle has a molecular mass of about $2 \cdot 10^6$ daltons and measures about 17.5 nm.



The simplified internal structure of a chloroplast

Stages of photosynthesis. Photosynthesis is the conversion of luminous energy to chemical energy, with the sequent use of the latter in the synthesis of carbohydrates from carbon dioxide. The overall reaction for the photosynthesis is:

$$6CO_2 + 12H_2O \rightarrow nh\nu \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2 + 2861 \text{ kJ/ml}$$

This process is endergonic and requires much energy. Therefore, the overall process of photosynthesis is made up of two stages, commonly referred to as the *light* (or energetic) *reaction* and the *dark* (or metabolic) *reaction.* In chloroplasts, these two stages are spatially separated: the light reaction is carried out in the quantasomes of thylakoid membranes, while the dark *reaction* is accomplished extrathylakoidally, in the stroma. The relationship between the light and dark reactions may be presented schematically as:

$$12\ H_2O \quad 6O_2 \quad 12\ NADPH^+ + H^+ \longrightarrow \downarrow \quad 6H_2O$$
$$light \rightarrow \qquad \rightarrow \qquad 6CO_2 \longrightarrow C_6H_{12}O_6$$
$$Light\ reaction \quad 18\ ATP \longrightarrow \uparrow \quad Dark\ reaction$$

The light reaction proceeds in the light. At this stage, the sunlight energy is converted to the ATP chemical energy, and the energy-deficient water electrons are transferred to become the energy-rich $NADPH_2$ electrons. The oxygen liberated during the light reaction is a byproduct of this process. The energy-rich light-reaction products, ATP and $NADPH_2$, are used at the sequent stage which can proceed in the dark. The reductive synthesis of glucose from CO_2 occurs during the dark reaction. The dark reaction cannot be accomplished independently of the light reaction.

Mechanism of the light (photochemical) reaction in photosynthesis. In the thylakoidal membrane, there are two photochemical centers, or photosystems, commonly denoted as *photosystems* I and II. Either of the two photosystems cannot replace the other one, since functionally these photosystems are different. The photosystems contain various pigments: green pigments, *chlorophylls a* and *b*; yellow pigments, *carotinoids;* and red or blue pigments, *phycobilins.* In this variety of pigments, only ***chlorophyll a*** is photochemically active. The other pigments play a minor role and act merely as photon collectors (a kind of focusing lenses) or light guides to the photochemical center. The function of the photochemical center is assigned to special forms of *chlorophylls a* which are: in the photosystem I, the *pigment 700* (P_{700}) absorbing light at a wave-lenght of about 700 nm, and in the photosystem II, the *pigment 680* (P_{680}) absorbing light at a wavelenght of 680 nm. In the photosystems I and II, there occurs only one molecule of the photochemically active pigment, *chlorophyll a*, per 300 to 400 molecules of light-collecting pigments. The absorption of protons by photosystem I induces a transition of the P_{700} pigment from the ground state into an excited state, P^*_{700}, in which the pigment molecule easily loses an electron. The electron loss leads to the formation of an electron hole P^*_{700} according to the scheme:

$$P_{700} \rightarrow h\nu \rightarrow P^*_{700} \rightarrow P^+_{700} + e$$

The electron hole is apt to easily recapture an electron. Thus, absorption of photons by the photosystem I leads to a separation of charges: the positive charge formed as an electron hole (P^+_{700}), and

the negatively charged electron, which is initially accepted by a special iron-sulphur protein (FeS centre), further to be either transported via a carrier chain back to P_{700} (to fill the electron hole), or transferred via another carrier chain through ferredoxin and flavoprotein to the permanent acceptor $NADPH_2$. In the former instance, a closed *cyclic electron transport*, and in the latter instance, a *noncyclic electron transport* occurs. The return of excited electrons onto P_{700} is associated with a release of energy (transition from a higher onto a lower energy level), which is accumulated in the ATP phosphate bonds. This process is called *photophosphorylation.* Under cyclic electron transport conditions, the *cyclic photophosphorylation,* and under noncyclic electron transport, respectively, the *noncyclic photophosphorylation* occurs. In the thylacoids, both processes are operative, the latter one being somewhat more complicated, since it is coupled to the operation of photosystem II.

Absorption of photons (H^+) by the photosystem II leads to decomposition (photooxidation) of water in the photochemical centre P_{680} according to the scheme:

$$\mathbf{H_2O \rightarrow h\nu \rightarrow 2H^+ + 2e + {}^1/_2O_2} \text{ (}\textit{Hill reaction)}$$

Photolysis of water is referred to as the *Hill reaction.* The electrons released by water decomposition are initially accepted by the substance denoted *Q* (occasionally, it is also called *cytochrome* C_{550} by its light absorption maximum, although this compound is not a cytochrome). Then from the substance *Q*, the electrons are transferred towards P^+_{700} to fill the electron hole a carrier chain compositionally similar to the mitochondrial chain.

Consequently, the lost P_{700} electrons are replenished with the electrons derived from water photodecomposed in the photosystem II. The observed noncyclic electron flow from H_2O to $NADPH_2$, which is produced by the interaction of the two photosystems and their coupling electron-transport chains, formally is reverse to the respective redox potentials: E^o=+0.81v for ${}^1/_2O_2$ /H_2O and E^o=-0.32v for $NADP^+/NADPH^+$. In other words, the luminous energy turns the electron flow "*upstream".* To be noted, on the II-to-I photosystem electron transfer, a portion of electron energy is stored on the thylacoid membrane as a proton potential which is further converted to the ATP energy.

The mechanism by which the proton potential is produced in the electron transfer chain to be used for the ATP formation in chloroplasts is similar to that in mitochondria. However, the photophosphorylation mechanism exhibits certain specific features. In a sense, thylacoids may be likened to mitochondria turned "*inside out"*; therefore, the direction of electron and proton transport across the thylakoid membrane is reversed to that observed for the mitochondrial membrane. The electrons are transferred towards the outer side, while the protons are stored within the thylakoid matrix. The matrix acquires a positive charge, and the outer thylakoid membrane, a negative charge, i.e. the direction of the proton potential is opposite to that of the mitochondrial proton potential. Another specific feature is a substantially larger contribution of pH to the proton potential as compared with the mitochondria. The thylakoid matrix is more acidic.

The H^+-ATP-synthetase, denoted in chloroplasts as the complex *"CF_1 + F_o",* is also oriented in the opposite direction. Its knob (coupling factor F_1) faces outwards, towards the chloroplastic stroma. The protons are forced outwards from the matrix through the CF_1+F_o complex, and ATP in the active centre is produced through the use of proton potential energy.

As distinct from the mitochondrial chain, there appear to be only two coupling sites in the thylakoidal chain; therefore three instead of two protons are required for the synthesis of one ATP molecule, i.e. the molar H^+/ATP ratio is 3:1.

Mechanism for the dark reaction in photosynthesis. The products of light reaction, ATP and $NADPH_2$, which are formed in the chloroplast stroma, are immediately used in the CO_2 synthesis of glucose. Assimilation of carbon dioxide (photochemical carboxylation) is a cyclic process commonly referred to as the photosynthetic pentose-phospate cycle, or *Calvin cycle*. Three major phases are distinguished in the cycle:

1. fixation of CO_2 with ribulose biphosphate,
2. production of triose phosphates by reduction of 3-phosphoglycerate,
3. regeneration of ribulose biphosphate.

The fixation of CO_2 with ribulose biphosphate is catalyzed by the enzyme *ribulose-biphosphate carboxylase*:

Ribulose biphosphate + CO_2 → 3-phosphoglycerate

3-phosphoglycerate is further reduced by $NADPH_2$ and ATP to glyceraldehyde 3-phosphate. This reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphate dehydrogenase*. Glyceraldehyde 3-

phosphate is readily isomerizable to dihydroxyacetone phosphate. Both triose phosphates are used in the synthesis of fructose biphosphate (reverse reaction, catalyzed by fructose-biphosphate aldolase). The fructose biphosphate produced is partly involved, alongside triose phosphates, in the regeneration of ribulose biphosphate, and partly is stored as carbohydrate reserve in the photosynthetic cells.

As has been estimated, the synthesis of one glucose molecule from CO_2 in the *Calvin cycle* requires 12 $NADPH^+ + H^+$ and 18 ATP (12 ATP molecules are spent on the reduction of 3-phosphoglycerate, and 6 molecules are used in the ribulose bisphosphate regeneration reactions). The minimal ATP-to-$NADPH_2$ ratio is 3:2.

One may perceive a generality of the principles that constitute the basis for photosynthetic and oxidative phosphorylations, the photophosphorylation being a kind of reversed oxidative phosphorylation:

$$\mathbf{SH_2 \rightarrow AH_2 \rightarrow} \boldsymbol{ATP} \mathbf{\rightarrow {}^1/_2\, O_2 \rightarrow H_2O}\ \textit{oxidative phosphorylation}$$
$$\mathbf{SH_2 \leftarrow AH_2 \longleftarrow H_2O \rightarrow {}^1/_2\, O_2 \longleftarrow}\ \textit{Sunlight}$$
$$\boldsymbol{ATP} \qquad \textit{photosynthetic phosphorylation}$$

The sunlight energy is a driving force for phosphorylation and synthesis of organic materials (SH_2) under photosynthesis and, vice versa, the energy of organic material oxidation is a driving force under oxidative phosphorylation. Precisely for this reason, plants ensure existence for animals and other heterotrophic organisms:

$$\textbf{Sunlight} \longrightarrow \underset{{}^1/_2\,\mathbf{O_2}}{\overset{\mathbf{H_2O}}{\downarrow}} \longrightarrow AH_2 \rightarrow SH_2 \rightarrow \Big| \rightarrow SH_2 \rightarrow AH_2 \longrightarrow \underset{\boldsymbol{ATP}}{\overset{\mathbf{H_2O}}{\uparrow}}\ {}^1/_2\,\mathbf{O_2}$$
$$\textit{Plants} \qquad\qquad \textit{Animals}$$

Carbohydrates produced by photosynthesis serve for building the carbon frameworks of numerous organic materials in plants. Ultimately, photosynthesis provides for the buildup of not only essential proteins, nucleic acids, carbohydrates, lipids, and cofactors, but also numerous products of secondary synthesis, which exhibit valuable medicinal properties (alkaloids, flavanoids, polyphenols, terpenes, steroids, organic acids, etc.).

Photosynthesis and environment. Photosynthesis is feasible only if light, water, and carbon dioxide are available. In cultivated plants, the photosynthesis efficiency does not exceed 20%; usually, it is not higher than 6-7%. The atmospheric occurrence of CO_2 is about 0.03% (by volume); an increased CO_2 content (to 0.1%) is favourable for photosynthesis and plant productivity. It is therefore expedient to use hydrogen carbonates as fertilizer additives in plant cultivation. However, an atmospheric CO_2 content higher than 1.0% exerts a deleterious effect on photosynthesis. Land plants assimilate 3% of the Earth's atmospheric CO_2, i.e. about 20 thousand million tons per year. The byproduct of photosynthesis, oxygen, is of vital importance for higher organisms and aerobic microorganisms. In preserving the plant kingdom we are saving life on Earth.

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