LESSON 5

Infection. The pathogenesis and diagnosis (microscopic, cultural, biological, serological and molecular genetic) of infections caused by microorganisms (bacteria, fungi, protozoa, and viruses). Modern identification methods of microorganisms

Infection, or infectious process

- An infection, or an infectious process, is a combination of all pathological processes that occur in a macroorganism as a result of the entry and reproduction of a pathogenic microorganism.
- A similar process caused by protozoa, helminths and insects is called invasion (from Latin Invazio - invasion, invasion)
- From a clinical and pathogenetic point of view, the interaction of a macro- and microorganism during an infectious process manifests itself as an infectious disease.

Conditions for the occurrence of an infectious process

- The presence of a pathogenic microorganism
- Presence of a susceptible microorganism
- Environmental conditions

The role of the microorganism in the infectious process

- Saprophytic microorganisms (from Greek, sapros - rotten, phyton - plant) - commensals living in the human body, animals and in the environment, do not cause disease.
- Pathogenic microorganisms (from Latin, pathos - suffering, genos - birth) entering a sensitive macroorganism cause an infectious process.
- Conditionally pathogenic (opportunists) only under certain conditions (the state of reactivity of the macroorganism), have a pathogenic effect.

The concept of pathogenicity and virulence

- The ability of a microorganism to cause a pathological process or disease is called pathogenicity.
- Pathogenicity is a genetic property of each type of microorganism and is specific, i.e. each pathogen causes a specific disease
- Pathogenic properties can differ even among microorganisms of the same species. The degree of pathogenicity is called virulence (from Latin virulentus - poisonous)
- In virology, instead of the term "virulence" they use "infectivity"

Change in virulence

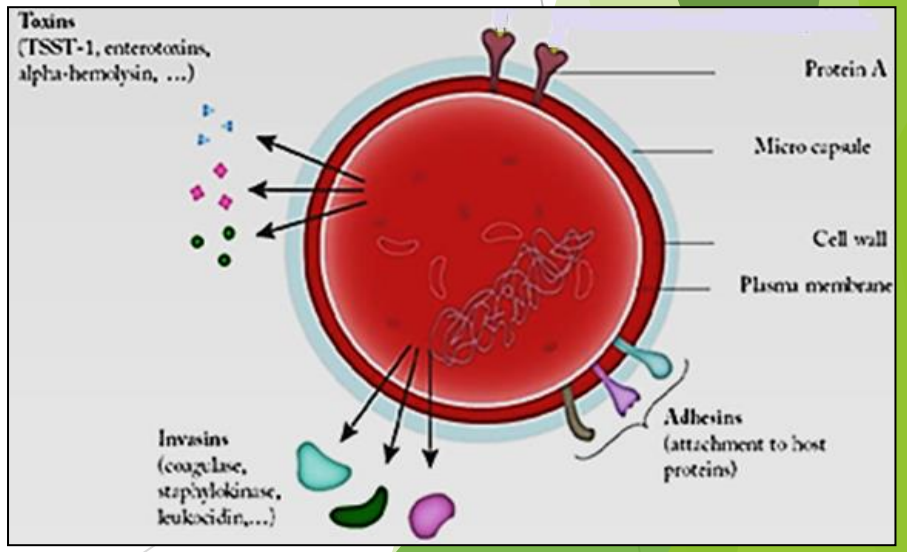
- All strains of a certain type of microorganism by virulence can be divided into high, low and avirulent.
- Changes in virulence - weakening or strengthening, may be phenotypic or genotypic in nature. By eliminating the acting factor leading to phenotypic changes, virulence can be restored.
- If the change in virulence is genotypic in nature, then it will be transmitted from generation to generation.

Factors affecting virulence

- Unfavorable conditions, long-term cultivation in artificial nutrient media, passage to animals with low sensitivity, exposure to various physical and chemical factors can reduce the virulence of microorganisms. Prolonged exposure to these factors can lead to a stable decrease in virulence - attenuation. This principle underlies the production of vaccines.
- It is possible to enhance the virulence of microorganisms by passage into the body of sensitive animals.
- Presumably, in this case, selection of virulent individuals occurs in the population of microorganisms.

Factors of pathogenicity of microorganisms

- The pathogenicity of microorganisms is provided by pathogenicity factors. The presence of these factors distinguish pathogenic microorganisms from saprophytes.
- Pathogenicity factors are morphological structures, enzymes and toxins of microorganisms.
- These factors ensure the introduction of the microorganism into the body, its adhesion to cells and tissues, as well as protection from the protective factors of the body.



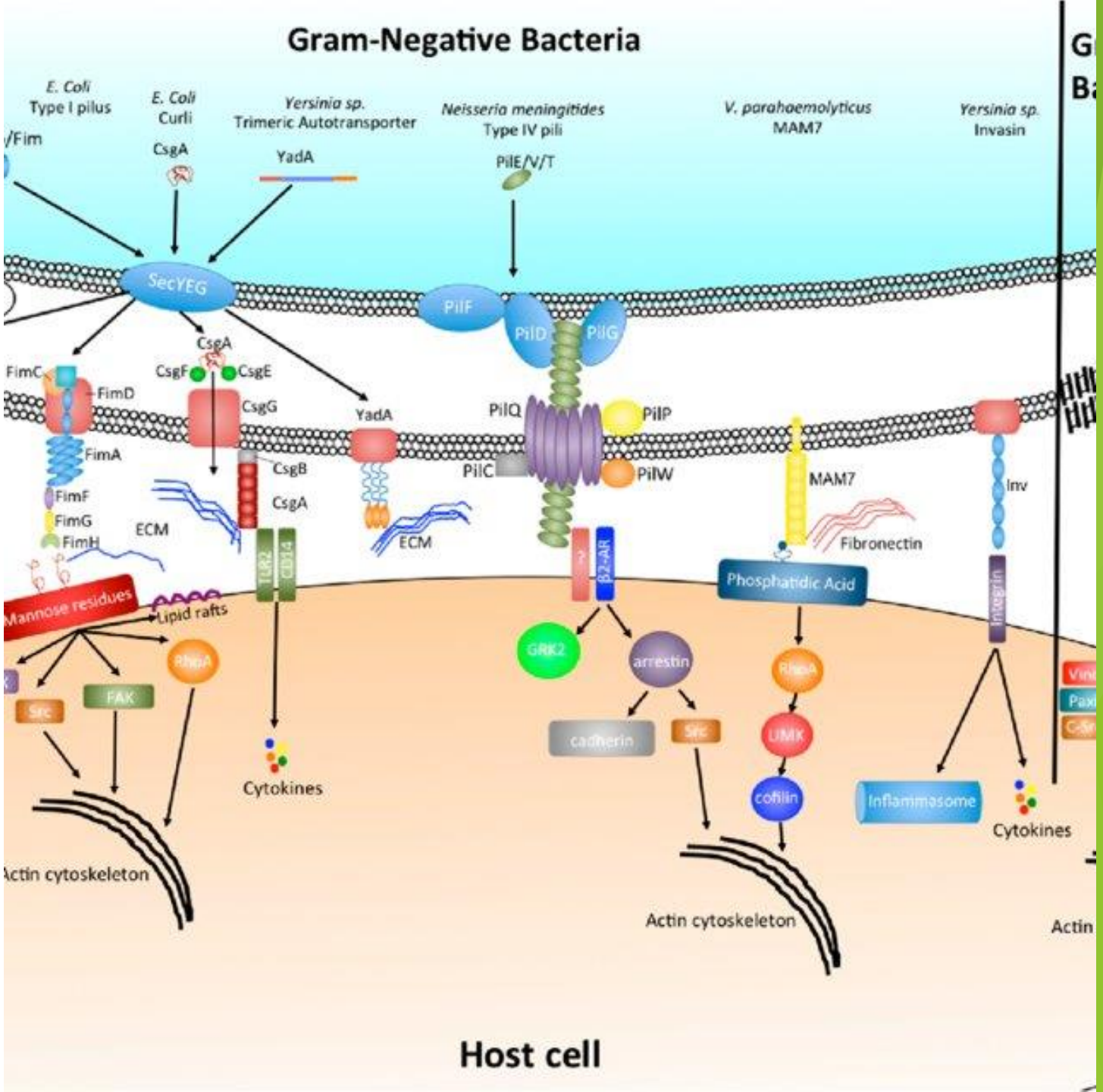
Factors of pathogenicity of microorganisms

- **Adhesion** is a specific connection of a microbe with sensitive cells of a macroorganism.
- **Colonization** is the multiplication of a microbe on the surface of a sensitive cell of a macroorganism.
- **Penetration** - the introduction of certain pathogens into cells (epithelial, leukocyte, lymphocytic, etc.).
- **Invasion** - spread through the mucous and connective tissue barriers in the tissue (neuraminidase and hyaluronidase).

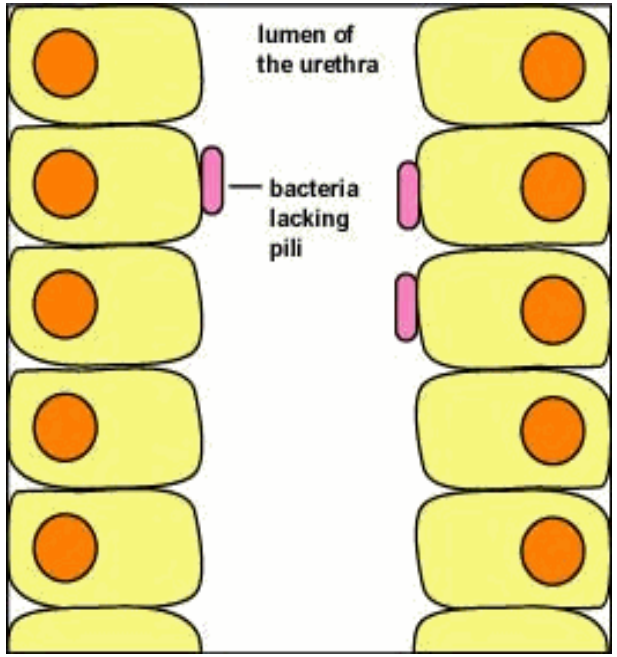
Adhesion

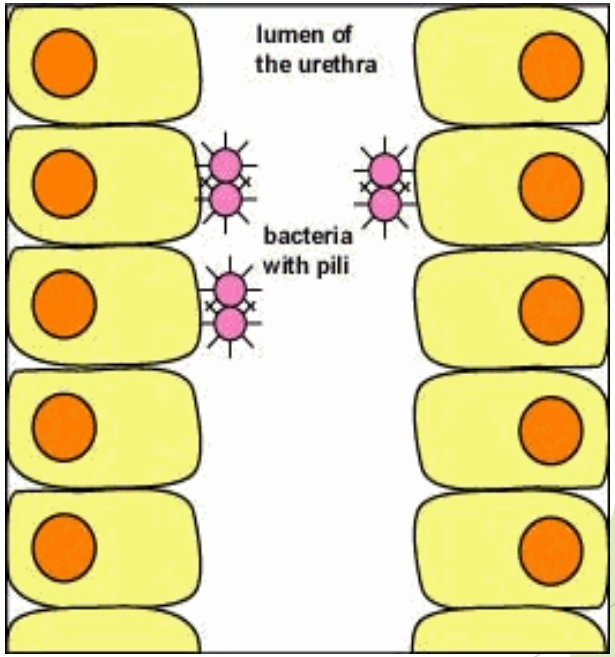
- **Adhesion** (from Latin adhaesio - attraction, sticking) - the ability of microorganisms to attach to the corresponding cells and tissues of the host.
- On the one hand, this process is provided by pili and other surface structures of microorganisms (adhesins or ligands).
- On the other hand - the presence on the surface of cells of the macroorganism of special structures - receptors.
- Thus, the adhesion of microorganisms on cells and tissues is mediated by the ligand-receptor mechanism of interaction.

The role of adhesion in pathogenicity: ligand-receptor interaction mechanism. A-adhesion by means of pili; B - adhesion via adhesins



Adhesion as a factor of pathogenicity

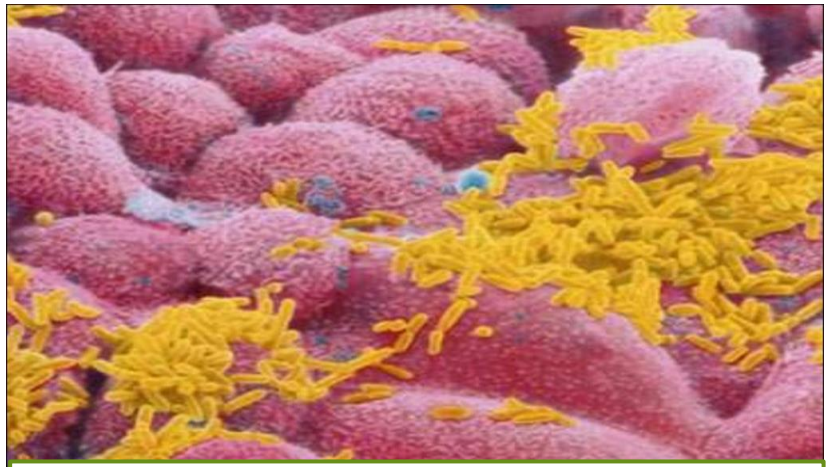




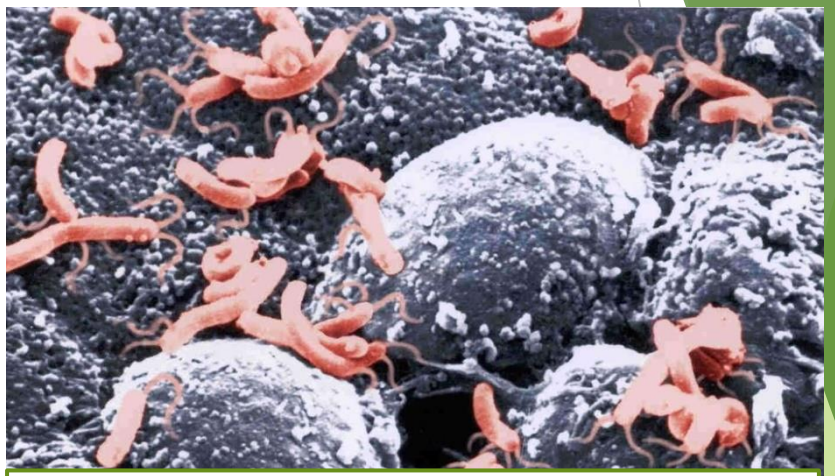
Colonization

- After adhesion, the process of colonization of microorganisms begins - settlement and reproduction.
- Initially, microorganisms colonize the surface of the skin and mucous membranes. They can be both on the surface and inside the cells.
- For example, the causative agent of cholera multiplies on the surface of the epithelium of the small intestine, and the causative agent of dysentery - inside the epithelial cells of the large intestine.

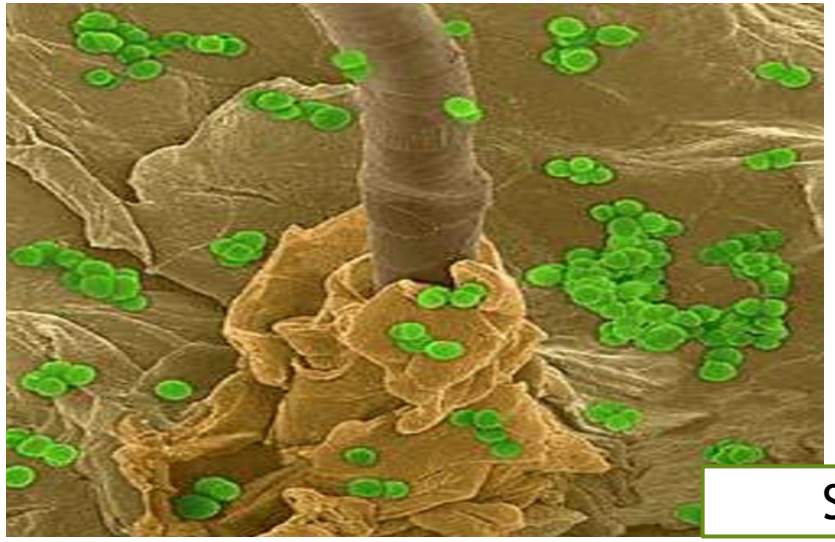
Colonization



Oral mucosa



Gastric mucosa

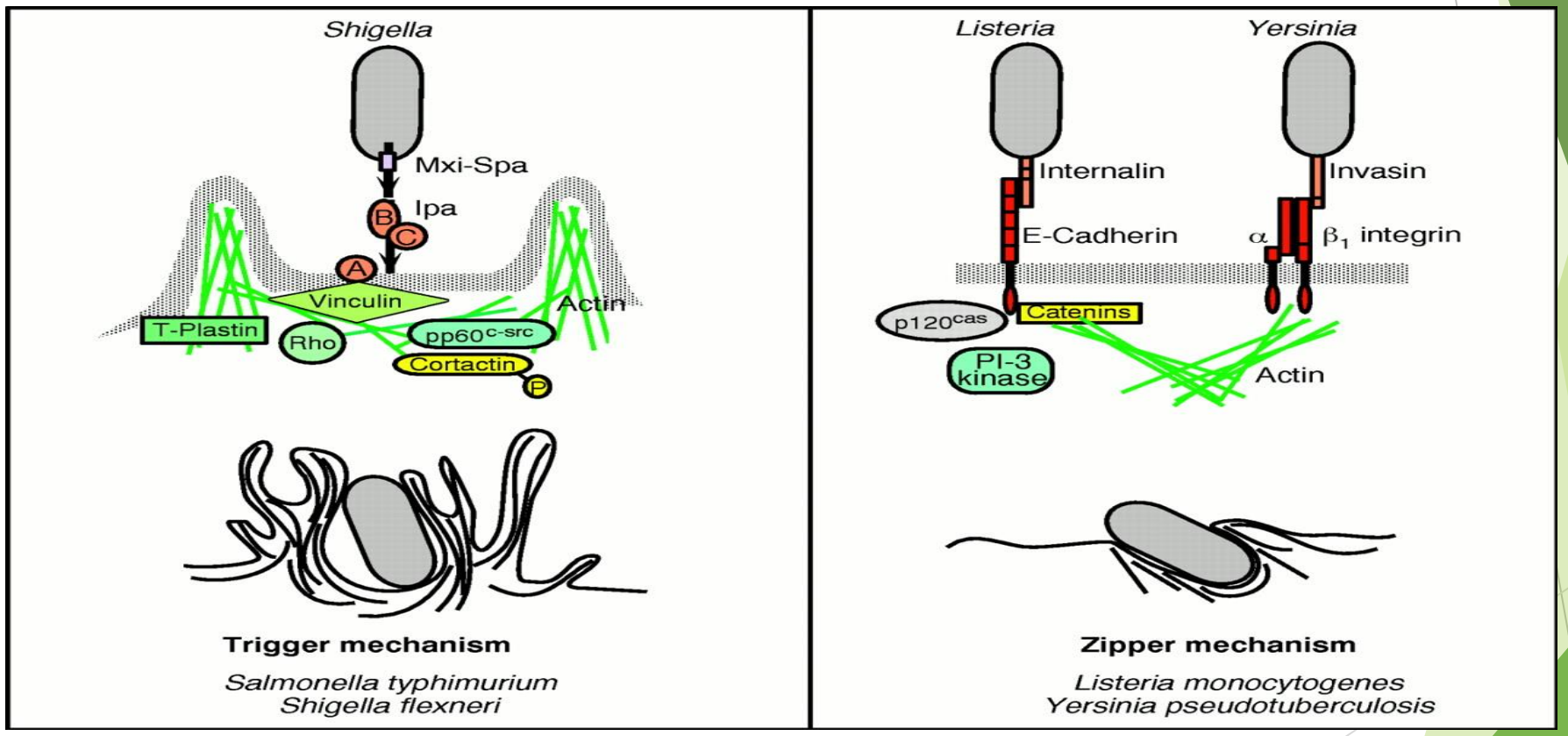


Skin surface

Penetration and Invasion

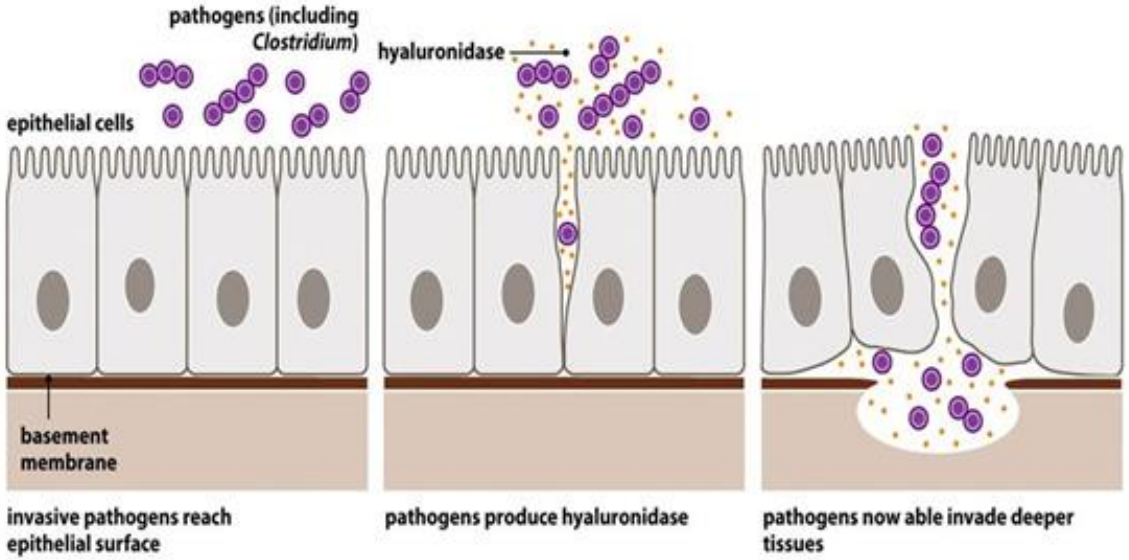
- Introduction - penetration of microorganisms into the inside of the host cell is due to invasiveness.
- Invasiveness is the ability of microorganisms to penetrate tissue cells.
- Colonization of microorganisms is not always limited to the surface of the skin and mucous membranes. The pathogenicity of some microorganisms (Shigella, Yersinia, etc.) is due to their penetration into epithelial cells.
- Penetration is ensured by the presence of specific factors: among them, invasins, proteins of the outer membrane, are the most well studied. The interaction of invasins with integrins - specific receptors on the surface of the host cell, provides endocytosis - the "swallowing" of bacteria.

Features of invasion in various microorganisms



Aggression Enzymes

- The invasiveness of microorganisms is closely related to the ability to synthesize certain enzymes - enzymes of aggression. Their mechanism of action is to destroy membranes and intercellular substance, increase the permeability of the cell wall, which contributes to the spread of microorganisms in tissues.
- Hyaluronidase
- Lecithinase (phospholipase)
- Neuraminidase
- collagenase
- Plasmocoagulase
- fibrinolysin
- Cytolysins (hemolysins), leukocidin, IgA1 protease



Factors preventing phagocytosis

Many microorganisms, in particular bacteria, have such factors as a microcapsule, capsule, mucous membrane that prevent phagocytosis.

Some microbes synthesize substances that inhibit chemotaxis or degrade chemoattractants.

Microorganisms also have factors that protect them from intracellular killing during phagocytosis:

substances that prevent the fusion of the phagosome with the lysosome

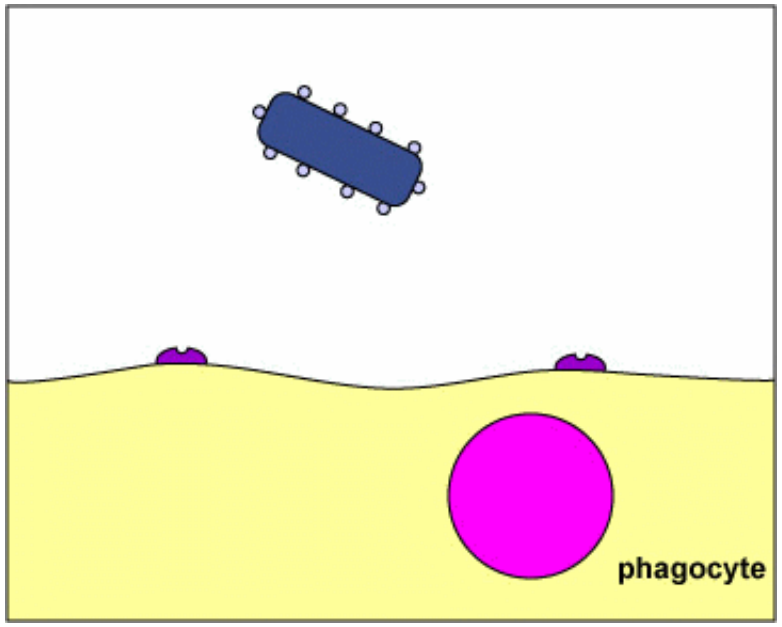
protection against oxidative factors generated inside phagocytes

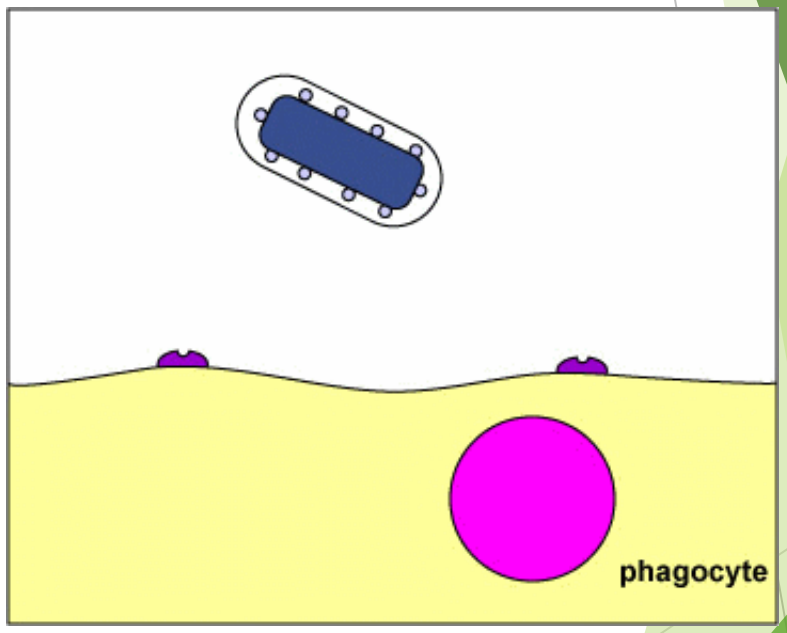
resistance against lysosomal phagocyte enzymes

substances that promote phagosome lysis (for example, listeriolysin)

some microorganisms, such as trypanosomes, leaving the phagolysis, pass into the cytoplasm of the cell, protecting themselves from phagocytosis.

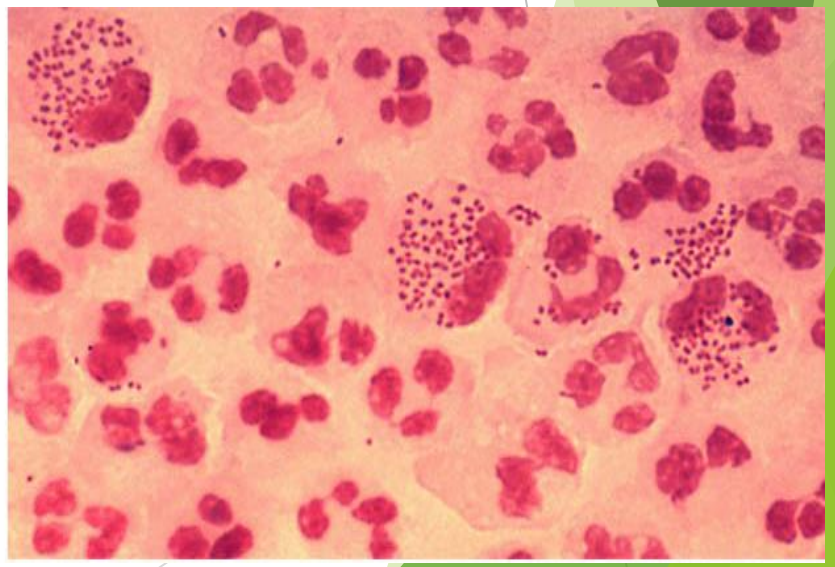
The capsule protects from phagocytosis





incomplete phagocytosis

- These factors provide microorganisms with the ability to survive inside the phagocyte.
- This ability allows not only to survive inside the phagocyte, but also promotes their spread through the blood and lymph (dissemination).



Bacterial toxins

- Toxins are one of the important factors in the pathogenicity of many microorganisms.
- Bacterial toxins are divided into two main groups, exo- and **endotoxins**.

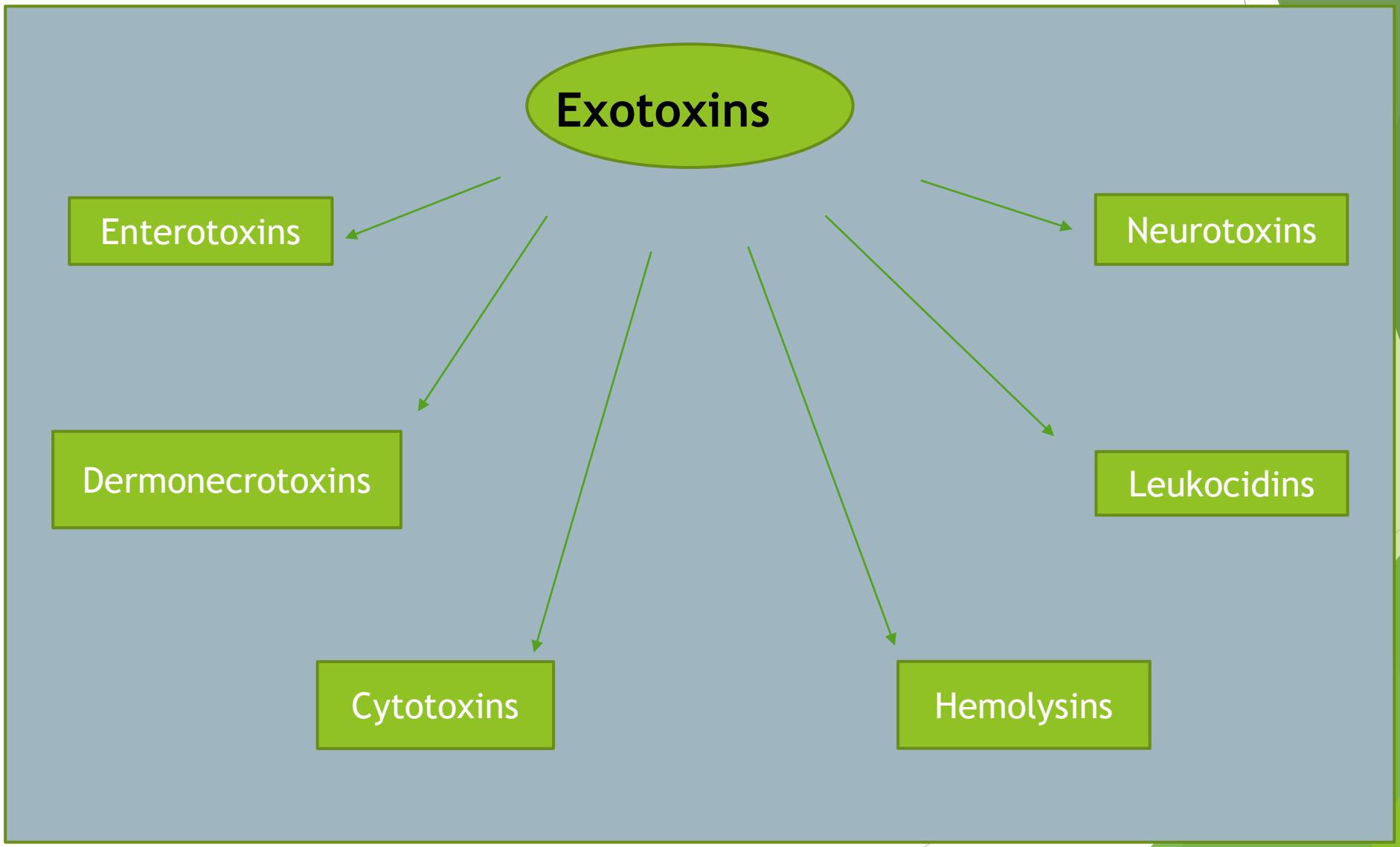
Exotoxins

- Exotoxins are substances of a protein nature (enzymes) that cause the death of macroorganism cells in small doses.
- Exotoxins are secreted by the cell into the environment or are in a bound state with the cell, being released after its autolysis.
- Thus, the release of exotoxins from the cell is not a prerequisite. For this reason, the term "protein toxins" has recently been used instead of the term "exotoxin".

Characterization of exotoxins

- Substances of protein nature (enzymes)
- Not associated with microbial cell
- Have high toxicity
- Relatively thermolabile
- Selective effect on organs and tissues
- Under the influence of formalin, acids, heating can turn into anatoxin (toxoid)
- Synthesized by both Gram-positive and Gram-negative bacteria.

Exotoxins are divided into several groups, depending on the specific interaction with target cell receptors:



Endotoxins

- Endotoxins differ from exotoxins in many ways.
- Endotoxins are lipopolysaccharides (LPS) of the outer membrane of Gram-negative bacteria.

Characterization of endotoxins

- Presented by lipopolysaccharide complex
- Associated with microbial cell
- Relatively low toxicity
- Thermostable
- Cause symptoms of general intoxication
- Do not turn into anatoxin (toxoid)
- Mainly produced by Gram-negative bacteria

Lipopolysaccharide (polysaccharide complex)

LPS chemical composition consists of a complex of polysaccharide and lipid.

The polysaccharide complex consists of the O-antigen and the basic part and provides the antigenicity of LPS. The O antigen has considerable variability and can differ even among members of the same species.

Therefore, within the same species of bacteria, O-serovars are isolated according to the difference in antigenic structure.

The basic part is quite stable and remains constant in microorganisms of the same genus and even family. This explains the presence of cross - reacting antigens in many microorganisms .

Lipopolysaccharide (lipid complex)

- The lipid complex consists of lipid A, which determines the toxigenicity of LPS.
- The structure of lipid A is the same for all types of gram-negative bacteria (the exceptions are Bacteroides fragilis, Bordetella pertussis, Brucella abortus, Pseudomonas aeruginosa, etc.)

Property	Exotoxin	Endotoxin
Source	Some Gram + and some Gram -	Outer membrane of most Gram – and Listeria
Secreted from Cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High	Low
Clinical Effects	Various effects	Fever, shock, hypotension, edema, DIC
Mode of Action	Various	Induces TNF and IL-1
Antigenicity	Induces high titer Abs called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No vaccine
Heat Stability	Destroyed at 60°C	Stable at 100°C for 1 hr

Bacterial pathogenicity factors (FPFs)

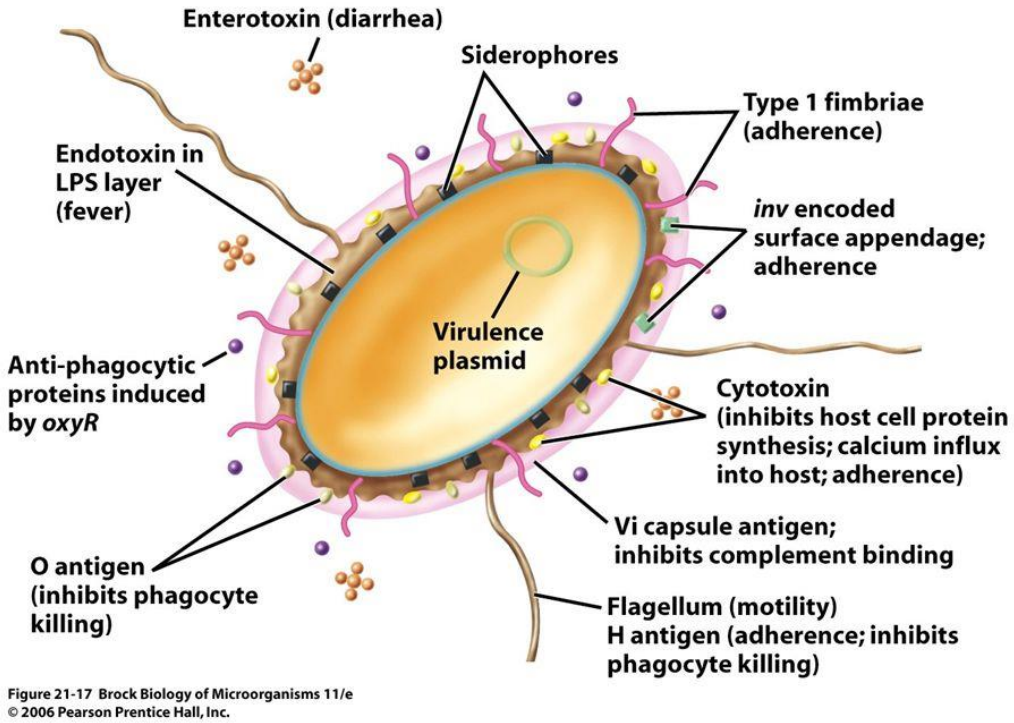


Figure 21-17 Brock Biology of Microorganisms 11/e

The role of the macroorganism in the development of the infectious process

- Age ("childhood infections")
- State of the nervous system
- State of the endocrine system
- The role of nutrition
- Floor
- hereditary factors
- The state of the immune system
- The role of normal microflora (colonization resistance)

The role of the environment in the development of the infectious process

- Exposure to temperature ("colds" diseases)
- The effect of irradiation
- The action of social factors ("social diseases")
- The impact of anthropogenic and environmental factors (natural disasters)
- Action of iatrogenic factors

Features of infectious diseases

- Each infectious disease is caused by a specific pathogen (etiological factor), in other words, each pathogenic microorganism causes only a specific disease (or diseases).
- - Bacterial and viral infections, mycoses
- - Protozooses, helminthiases, infestations
- Infectious diseases are characterized by contagiousness
- - Contagiousness index - shows the ratio of the number of cases after contact with the source of infection to the total number of those in contact with this source.
- Infectious diseases are characterized by a cyclical course
- Acquired immunity develops after infection

Sources of infection

- **Anthroponoses** - the source of infection is only a person
- **Zoonoses** - diseased animals are the source of infection
- **Sapronoses** - the source of infection - environmental objects

Mechanisms of infection

- Airborne mechanism - the pathogen is mainly localized in the upper respiratory tract, when talking, coughing and sneezing, it enters the environment by airborne or airborne dust. This mechanism transmits pathogens of respiratory tract infections.
- The fecal-oral mechanism - the pathogen is mainly localized in the intestine, is excreted into the environment with feces and is transmitted by the alimentary route (food and water ways). This transmission mechanism is inherent in intestinal infections.
- Contact mechanism - pathogens can be localized in different places, and enter the environment in different ways.
- - infection is possible by direct or indirect contact
- Transmissible mechanism - the pathogen is in the blood of a sick person or animal and is transmitted by blood-sucking insects
- (malaria, typhus, etc.)
- - the parenteral route of infection also refers to the transmission mechanism

Periods of infectious diseases

- Incubation, or latent period covers the period from the entry of a pathogenic microbe into the body until the first symptoms appear. For most diseases, this period lasts 1-2 weeks.
- Prodromal (from the Greek prodromos - a harbinger), or the period of precursors occurs after the incubation period and is characterized by non-specific symptoms (fever, headaches, weakness, lethargy)
- The period of clinical manifestations begins after the prodromal period and is characterized by symptoms specific to each infection.
- - common signs, characteristic symptoms, pathognomonic symptoms.
- Recovery (convalescence) is a period of fading of symptoms and restoration of body functions.
- - Recovery, microbe carrying, transition to a chronic form, death.

Forms of an infectious disease

- Based on origin:
- - exogenous, endogenous infection or autoinfection
- Depending on the location of the pathogen in the body
- - focal, generalized infection
- Depending on the spread of the pathogen and its toxin in the body
- - bacteremia (sepsis), viremia, toxinemia
- Depending on the amount of pathogen
- - monoinfection, mixed infection
- Superinfection - repeated infection with the same pathogen until recovery
- Reinfection - repeated infection with the same pathogen
- after full recovery.
- Relapse is the return of the symptoms of the disease without re-infection.

Features of the spread of infectious diseases

- An epidemic is a mass spread of an infectious disease among the population that progresses in time and space.
- Spreading an infectious disease can cover several countries, even continents - a pandemic.
- Sometimes the infection occurs in single - sporadic cases.
- If an infectious disease is distributed only in a certain area, then it is called endemic. Endemias are most often natural focal diseases with a specific source of infection and carriers.

biological method

Infection of laboratory animals is carried out with the aim of:

study of pathogenicity and virulence of microbes,

isolation of pure culture from pathological material,

creating experimental infections

biological method
Animal Modeling of Infection

Material under study
(enrichment or inactivation of the accompanying microflora)

Animal selection

Choice of infection method

animal watching

Animal autopsy, collection and examination of material

Result

Methods of infection of laboratory animals

Infection of laboratory animals (guinea pigs, white mice, rats, rabbits) is carried out in different ways - on the surface of the skin, intradermally, subcutaneously, intramuscularly, intravenously, into the abdominal cavity, intranasally, orally, intratracheally, intracerebral.

Methods of infection of laboratory animals

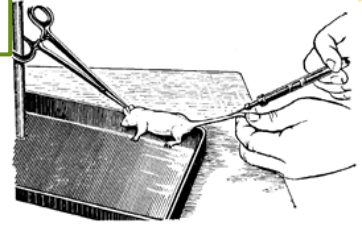
Intradermal

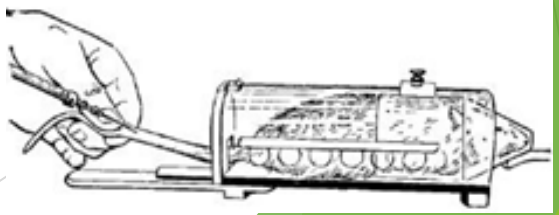
Intracerebral

Intramuscular

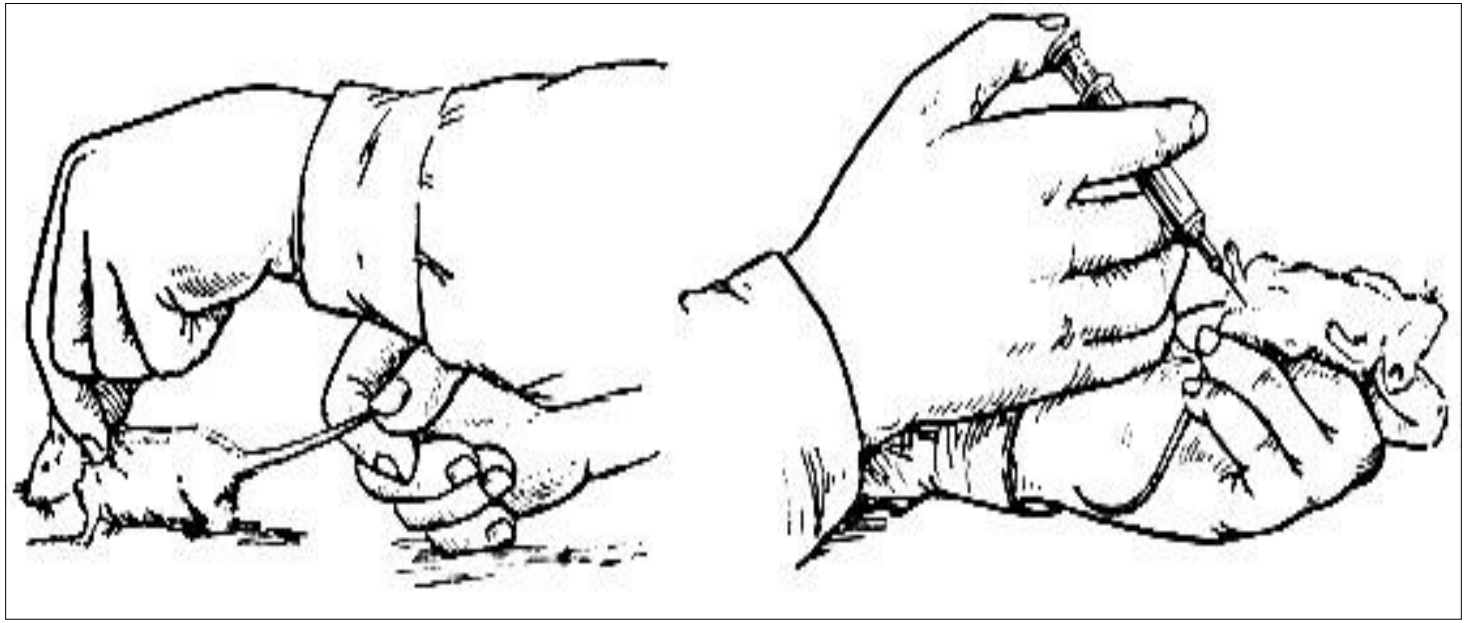
Infection of the digestive tract

abdominal cavity





Peritoneal infection of white mice



Autopsy and bacteriological examination of the corpse of a laboratory animal (white mice)

- The purpose of bacteriological examination of the animal corpse is to isolate the pathogen that caused the death of the animal, to establish the location and obtain a pure culture of the pathogen.
- To prevent contamination, the autopsy of the corpse and the taking of material for inoculation is carried out immediately after the death of the animal under aseptic conditions.
- If necessary, the animal is killed according to the principles of bioethics. According to these principles, the manipulation is carried out under conditions of complete anesthesia of laboratory animals.

Bacteriological study of laboratory animals.

Live animal:

Blood

Exudate from the abdominal cavity, etc.

Dead animal:

Blood

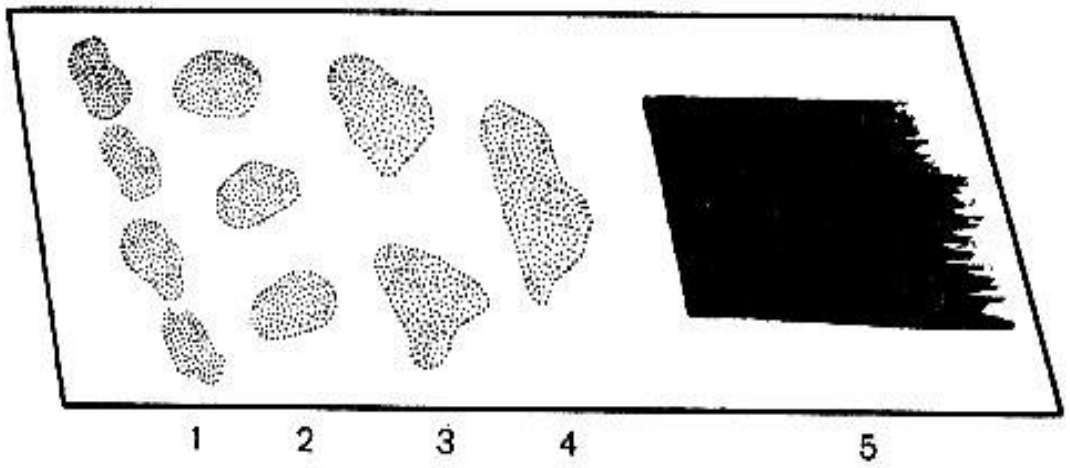
Pieces of various organs

cerebrospinal fluid

Liquids from various cavities, etc.

Bacteriological examination of corpses of laboratory animals

- After the autopsy, the internal organs are examined, a smear-print from the organs is prepared and inoculated into blood agar (the cut surface of the organ touches the nutrient medium)
- At the same time, smears-imprints from the liver, spleen, and kidneys are being prepared. Imprint smears are fixed with Nikiforov's solution (equal concentrations of alcohol and ether) and stained with methylene blue or by the Romanovsky-Giemsa method, microscoped.
- The inoculated culture media are incubated for 24-48 hours at 37°C.
- Microorganisms obtained as a result of cultivation of pathological material are identified by morphological, cultural, biochemical and other properties.



Preparation of impression smears (1-4) and a thin blood smear (5) on one glass object.

Neutralization of animal carcasses

- After autopsy, the animals are cremated, sterilized in an autoclave, or boiled in a phenol solution for 1-2 hours.
- All instruments, cuvette and fixation board are treated with a disinfectant solution or sterilized in an autoclave.

Determination of pathogenicity and virulence (determination of lethal dose)

For this purpose, the median lethal dose (LD50) is determined.

When determining the LD50 of a microbial strain, it is mandatory to standardize the type, sex, weight, and conditions of keeping laboratory animals (mainly white mice).

A microbe culture diluted several dozen times (10-1, 10-2, 10-3, etc.) is introduced into several groups, including at least 4-6 individuals.

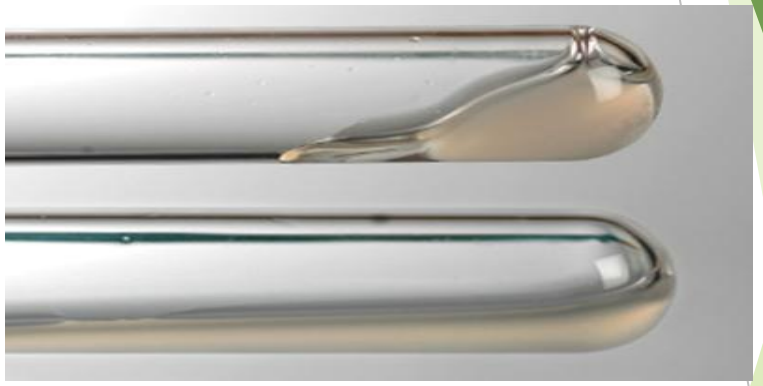
After a certain time, the dead and living individuals in each group are counted to determine the LD50.

Determination of pathogenicity and virulence (pathogenicity enzymes)

- A direct indicator of the pathogenicity of microorganisms is the determination of pathogenicity enzymes.
- In practice, they are determined to identify microorganisms and to differentiate saprophytic species from pathogenic ones..

Determination of the plasmacoagulase enzyme

- The studied microbial culture is inoculated into sterile citrated blood plasma. Incubate for 2-5 hours at 370C.
- The microbes synthesizing plasmacoagulase coagulate the plasma, and in the control tube the plasma remains in a liquid state.



Plasmacoagulase test: positive (top) and negative (bottom)

Determination of the enzyme lecithinase

The detection of the lecithinase enzyme is based on the breakdown of a substrate containing lecithin.

The studied microbial culture is inoculated into Petri dishes with yolk agar and incubated at a temperature of 370C during the day.

Lecithinase activity is manifested by the appearance of turbidity around the colonies.

Determination of the enzyme hyaluronidase

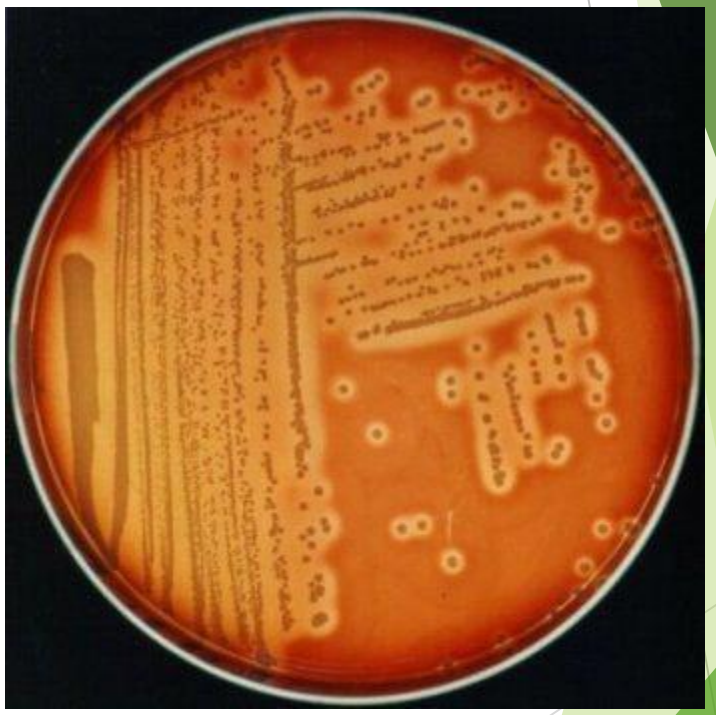
The determination of hyaluronidase is based on the reaction of hydrolysis of hyaluronic acid by this enzyme.

The studied microbial culture is inoculated into a substrate with hyaluronic acid. Incubate at 370C for 15 minutes, then add 2-3 drops of concentrated acetic acid.

In the presence of hyaluronic acid, mucus clots form in the test tubes.

Determination of hemolytic activity

- To determine the hemolytic activity, the studied microbial culture is inoculated into a Petri dish with blood agar.
- Incubated at 370C for a day.
- In the presence of hemolytic activity, zones of hemolysis are observed around the colonies.



Definition of exotoxins

- The main indicator of the pathogenicity of microbes is the synthesis of exotoxins. In classical studies, this property was studied in experiments on laboratory animals.

- Currently, the study of the ability to synthesize exotoxins is carried out on cell cultures, chicken embryos, and protozoan cultures.
- The genetic determinants of microbial toxins, such as toxigenicity genes, are also determined by PCR.

- To determine the exotoxin of the causative agent of diphtheria, a serological method is used - a precipitation reaction (Elek's test)

Toxin neutralization reaction with antitoxin in vivo