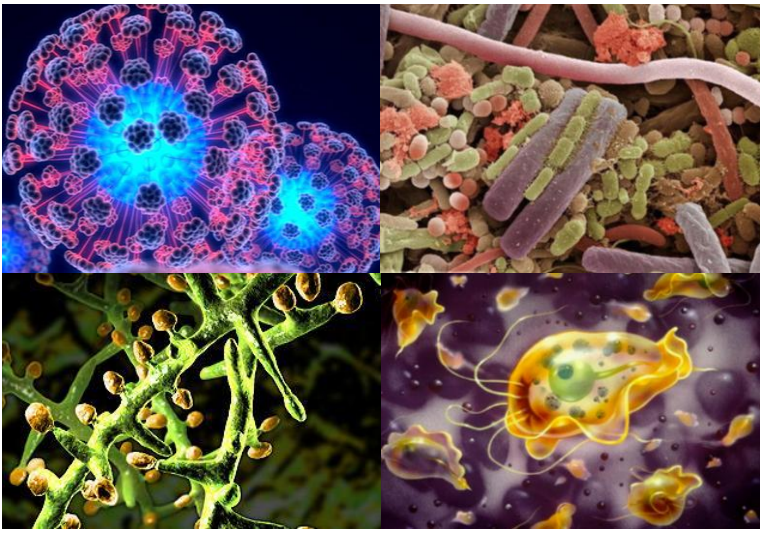

INFECTION. INFECTIOUS PROCESS

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

ІНФЕКЦІЯ. ІНФЕКЦІЙНИЙ ПРОЦЕС

Методичні вказівки з дисципліни "Мікробіологія, вірусологія та імунологія" для студентів II і III курсів медичного та стоматологічного факультетів з англійською мовою викладання

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

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Compilers N. I. Kovalenko,
T. M. Zamaziy

Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III–IV level accreditation.

Learning guide includes sections of characteristics of infectious process and types of infections, epidemiology and specifity of infectious diseases, classification of bacteria due to pathogenicity, virulence factors of bacteria. The most modern information on biological method of diagnosis of infectious diseases is represented.

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Т. М. Замазій.

Theme: Studies about an infection. Experimental method of diagnostics of infectious diseases

Actuality of the theme

The infection (Latin of *infection* – to infect, pollute) is a form of relationship which evolutionarily developed between pathogenic microbes and human in certain environmental conditions.

Manifestations of an infection are various and also depend on properties of a microorganism, state of a macroorganism and environmental conditions.

Infectious process is a collection of physiological protective and pathological reactions that occur in a macroorganism in response to the action of pathogenic microbes. It develops consistently and involves a complex of biochemical, cytochemical, morphological changes that occur in the body.

Infectious disease is the extreme stage of the infectious process. Infectious disease is clinical manifestation of an infectious process, accompanied by characteristic signs or symptoms.

The microbes that cause infectious diseases are commonly called the *causative agents of infectious diseases*. A human or animal organism that is in a state of infection, that is, parasitization of a pathogen in it, is called infected, while environmental objects that have been affected by pathogens should be designated as contaminated with one or another pathogen. The susceptibility of a macroorganism should be understood as the ability of a macroorganism to respond to the introduction of microbes by the development of an infectious process in its various manifestations - from carriage to infectious disease.

Epidemiology of infectious disease

The epidemic process is a continuous course of interconnected infectious diseases caused by the presence of a source, a transmission mechanism of the causative agent of infectious diseases, and a susceptible organism.

Participants of the epidemic process (*Fig. 1*):

– Source (reservoir) of infection

– The mechanism and route of transmission

– Susceptible organism

The source of infection is an abiotic object, a living organism, where the pathogenic microbe lives, from which infection occurs. The source can be a patient, a carrier, abiotic objects (water, food).

Classification by source of infection:

– *sapronous infections* are diseases whose main habitat and reproduction of pathogens are environmental objects, from where they enter the human body (diseases caused by legionella, Pseudomonas aeruginosa, etc.);

– *anthroponous infections* are diseases in which the only source of the pathogen is a person (meningococcal infection, dysentery, cholera, diphtheria, syphilis, hepatitis B, epidemic typhus, epidemic relapsing fever, etc.);

– *zoonotic infections* are diseases in which the only source of the pathogen is animals (tularemia, brucellosis, rabies);

– *zooanthroponic infections* are diseases in which the source is an animal and a sick person, including the corpses of the dead (plague, anthrax, tuberculosis, rickettsioses).

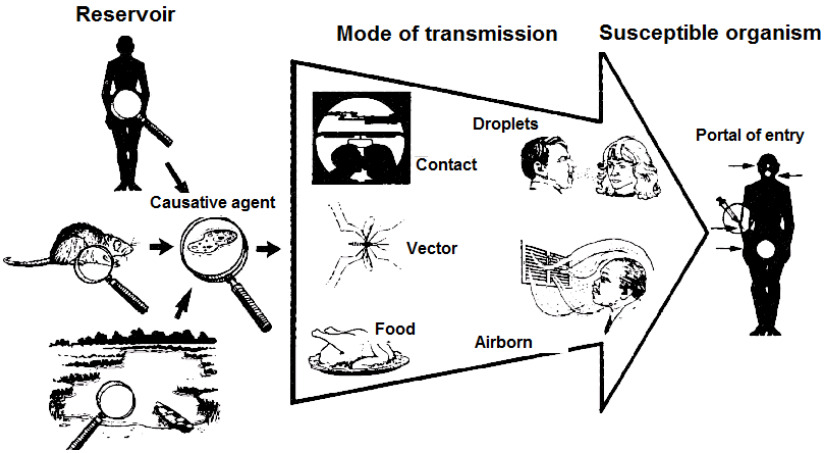

Fig. 1. Participants of the epidemic process

The transmission mechanism is the process of removing the pathogen from the host organism into the environment, its stay on environmental objects, the introduction of the pathogen into a susceptible organism.

Transmission mechanism is a method for moving a pathogen from an infected organism to a susceptible one.

It is carried out in 3 phases:

1. Excretion of the pathogen from the host.
2. Stay in the environment.
3. The introduction of the pathogen into a susceptible organism.

Factors of transmission are environmental objects that transfer microbes from one organism to another.

Routes of transmission are a combination of factors in certain environmental conditions, ensuring the pathogen from one macroorganism to another.

Entrance gates (*portal of entry*) are organs and tissues of the host organism through which pathogenic microbes penetrate.

The entrance gate of infection can determine the clinical form of the disease. The same pathogen microorganism, entering the macroorganism in various ways, causes different clinical forms of the disease, as is the case with anthrax:

• cutaneous form is caused by the penetration of microorganisms into the body through the skin;

• pulmonary – through the mucous membranes of the upper respiratory tract;

• intestinal – gastrointestinal tract.

The susceptibility of society is the presence of an immune layer in the population against the corresponding pathogen.

The allocation of a particular transmission route of infectious diseases is rather conditionaly. According to the mechanism of transmission infectious diseases are divided into:

1. intestinal (fecal-oral);
2. respiratory;
3. infections of the blood (trasmissive);
4. infections of the skin and mucous membranes (contact). In is divided into:

• direct contact – from a source to a host, including sexually transmitted diseases (for example HIV infection);

• indirect contact – through an intermediate object – it can be hands (for wound infections, intestinal infections) or various objects, including medical ones (for purulent-inflammatory diseases and parenteral hepatitis);

5. vertical (from mother to fetus).

Nosological form of the disease depends on the route of transmission of a pathogenic microorganism:

• when ingested, streptococci cause tonsillitis;

• household contact - streptodermia (purulent-inflammatory disease of the skin).

Infectious diseases are characterized by infectiousness and can occur in the form of *epidemics* - diseases registered in large territories (region, country) and associated with the source of infection. Diseases spread to several countries and continents are called *pandemic* (influenza, cholera). Infectious diseases that occur in rare cases are called *sporadic*. Diseases prevalent only in certain areas where there is a reservoir and vector for infection are called *endemic* (tularemia, tick-borne encephalitis). They are usually caused by natural factors that are favorable for the development of pathogens and reservoirs of the pathogen. Natural infections exist naturally in wild animals and from time to time cause outbreaks or *epizootics*. In case of accidental exposure to such area, a person can become infected and get sick (plague, tularemia, tick-borne encephalitis, rickettsiosis).

Depending on the route of penetration of the pathogen into the host, exogenous and endogenous infections are distinguished.

Exogenous infections occur due to the entering of pathogens from the environment. Infection can occur through damaged skin, mucous membranes of the eyes, respiratory, urogenital tract, gastrointestinal tract. Even microtraumas, insect bites, needle punctures can cause infection.

In *endogenous (autoinfection)* infection, pathogens are found in the body in the obligate or transit flora. When the protective properties of the body are weakened, they can be the cause of the disease (candidiasis).

Depending on the manifestations infections are manifest and inapparent.

Manifest – with typical and atypical manifestations of the disease. *Typical* – all clinical signs appear, periods are expressed. *Atypical* – some characteristic

signs are absent. For example: cholera without diarrhea, typhoid fever without rash, etc. It is very difficult to diagnose this disease and only microbiological methods of study are crucial.

This type of infection is characterized by a short stay of the microbe in a macroorganism lasting up to 3 months, which in some cases goes into a protracted form of the infection, lasting from 3 to 6 months. A protracted (or subacute) form is characterized by an increase in the invasive period and the period of convalescence and is often a transition from acute to chronic infection.

Inaparantic (latent, subclinical) – no external manifestations of the disease, but there are immunological, morphological, typical changes for the corresponding disease. The activator is exposed to the pressure of the protective forces. The pathogen will stay in the patient's body for a long time without showing itself. With the weakening the body (cooling, fatigue, fasting) latent infections can turn into a typical disease with a pronounced clinical picture.

Diagnosis of inapparent forms of infection is possible only in the foci of infectious diseases on the basis of specific laboratory methods (change in the rise of antibody titers in dynamics, morphological studies, skin allergic tests, etc.).

This type on infections is characterized by a long stay of the microbe in a macroorganism, or *persistence* (from lat. *Persistentia* - preservation of a previous state, constancy). Persistence confirms the inability of society and the macroorganism to cope with the microbe and the ability of the latter to survive in the macroorganism. The mechanisms for the development of persistence are diverse. An important role is played by: the formation of morphologically modified or defective forms of microbes (L-forms of bacteria, cysts, defective viral particles); the formation of drug resistance, the ability of microbes to intracellular parasitization (pathogens of malaria and Leishmania, viruses, chlamydia, etc.); blocking apoptosis of host cells; the presence of both congenital and acquired immunodeficiencies, including under the influence of microbes; development of immunological tolerance, as well as autoimmune and allergic reactions in a macroorganism, etc. A distinctive feature of persistence is that it develops against the background of acquired immunity, which is ineffective, and also that it is a pathogenetic norm for a number of microbes (viruses, rickettsia and chlamydia, mycobacteria, treponema, brucella, causative agent of four-day malaria). Persistence may occur in several forms.

One form of infection that occurs without signs of the disease is *carriage* - a condition of the body in which pathogens is present in the body and their release into the external environment is not accompanied by clinical symptoms of the disease and disorders by organs and tissues. It is formed more often after the disease, when clinical recovery, but the pathogens continue to remain in the body of the diseased and released into the environment (for example, carrying typhoid, dysentery rods, diphtheria, cholera). In some cases, carriage develops in healthy individuals who had contact with patients or even carriers of pathogens.

Acute carriage occurs when the pathogens persist in the body for 3 months, *chronic* – longer (typhoid throughout life). Usually the carriage occurs in an unacceptable organism against the background of immunity, which was formed as a result of previously transferred disease or vaccinations. Bacterial carriers contribute to the emergence and support the spread of infectious diseases in the inter-epidemic period, when the spread of the pathogen due to natural causes dies down (for example, when cold seasons occur with intestinal infections).

Latent infection (syn. dormant infection) is a peculiar form of carrier, in which the microbe is present in the macroorganism for a long time, but is not released into the environment. As a rule, latent infection is a natural stage of the infectious process in diseases prone to a chronic course (brucellosis, syphilis, herpetic infection, toxoplasmosis).

Chronic infection lasts more than 6 months and can occur in the form of a continuous or relapsing form, characterized by a change in periods of remissions and exacerbations, in which microbes are released into the environment for many months and even years. Primary chronic diseases include brucellosis, tuberculosis, leprosy, malaria and syphilis.

In virology, a *slow virus infection* has been identified as a separate group.

If the infection is caused by one type of pathogen, this infection is called *monoinfection*. When infecting the body 2–3 different pathogens at the same time (for example, diphtheria rods and streptococcus) *mixed* infection occurs.

A secondary infection is distinguished from mixed infections when an underlying disease (such as influenza) is associated with an infection caused by another pathogen (such as staphylococcus or streptococcus), as a result of weakening the body's defenses. In people with immunodeficiency it is called *opportunistic infection*.

Reinfection is called a condition when a new disease has occurred due to a new infection with the same type of pathogen after recovery (gonorrhea). If the disease has returned before recovery as a result of infection with the same pathogen, it is *superinfection* (syphilis).

Relapse is the return of symptoms (typhoid fever, malaria) that occur without re-infection by pathogens remaining in the body.

Depending on the localization of pathogens in the body of the patient there distinguish a *local infection*, in which the microbes persist in one, certain place, not spreading beyond its boundaries (eg, sore throat, furunculosis), and *generalized infection* when the forces of aggression of microorganisms overtake defense mechanism of host and pathogens from the local focus spread throughout the body. A condition where the pathogens circulate for some time in the blood, but do not multiply in it (for example, typhoid fever, brucellosis), is called *bacteremia, virusemia*.

In the case when the pathogen is circulating in the blood for a long time, accumulates and multiplies, *sepsis or septicemia* occurs (from lat. Sepsis - pus). Such accumulation of microorganisms occurs in plague, anthrax. Sepsis is also

caused by purulent cocci. The peculiarity of sepsis is that the clinical picture does not depend on the type of pathogen.

The formation of purulent foci in various organs due to sepsis is called *septicopyemia*. Circulation of the toxin in the blood is called *toxinemia*.

Participants in the infection process

Factors of the onset and outcome of the infectious process:

1. Quantitative and qualitative characteristics of microorganisms determine the specificity of the infectious process.

2. The state of the macroorganism, its susceptibility to the microorganism determines the form of manifestation, duration, severity and outcome of this infectious process.

3. Environmental factors where the meeting of the microorganism with the host has an indirect effect, reducing or increasing the susceptibility of the host or the infectious dose and virulence of the pathogen.

Stages of the infectious process

The infectious process is one of the most dynamic forms of interaction between microbes and a macroorganism that has developed during evolution. The process proceeds with a constant change of cause and effect relationships. Conventionally, it can be divided into several stages.

1. The penetration of the microorganism into the macroorganism, its adaptation at the entrance gate and adhesion. The starting point of the infection process is the introduction and adaptation of microbes (from late Lat. *Adaptatio* - adaptation) at the site of the entrance gate of infection, as well as adhesion of microbes to the cells of the macroorganism. Entrance gates are tissues and organs through which microbes enter the body. In most cases, microbes enter the microorganism through damaged skin and intact mucous membranes permeable to microbes.

2. Colonization and formation of enzymes, toxins and other products during the life and reproduction of microorganisms, which leads to disruption of homeostasis due to local and generalized inflammation.

The second stage is colonization (from Lat. *Colonia* – settlement) – horizontal colonization of the skin and mucous membranes at the site of the entrance gate of infection. In the infectious process, the spread of microbes occurs not only horizontally, on the surface of the cells, but also in the depths of the cells and tissues of the macroorganism. The ability of microbes to penetrate the cells of a macroorganism is called penetration. In this case, the multiplication of microbes and the formation of new generations of the pathogen under favorable conditions, as well as the release of metabolic products of microbes, their enzymes and toxins and, in addition, the formation of toxic decomposition products of macroorganism cells that have local or long-term damaging effects on tissues and organs.

3. The third stage is dissemination (from the Latin. *Disseminare* – scatter, spread) i.e., the spread of microbes beyond the primary focus of the

introduction and colonization of microbes by the hematogenous pathway, bronchogenic or perineural, along the nerve trunks, which leads to the generalization of the infectious process (generalization is a transition from general to particular, spreading throughout the macroorganism).

4. The fourth stage is the mobilization of the protective factors of the macroorganism. In response to the penetration of microbes and their pathogenic effect, the macroorganism mobilizes all initially non-specific and then specific protective factors inherent in it, the action of which is aimed at neutralizing both the microbes themselves and their toxins and at restoring impaired homeostasis in the macroorganism.

5. The fifth stage is the end and outcome of the infectious process. In most cases, sanitation of the macroorganism occurs (from the English. *Sanative* -, healing) that is, the complete release of the macroorganism from the microbe and the acquisition of a new quality by it - the formation of immunity. In some cases, the infectious process ends in death. In those cases when equilibrium is established between the microbe and the macroorganism, carrier state formation takes place.

As a result of the action of many factors, the infectious process does not always go through all its inherent stages and can end already in the early stages, for example, proceeding in an abortive form of an infectious disease in vaccinated or in persons who have previously undergone this disease. Another example is the defeat of the mucous membranes of the urogenital tract in gonorrhea without subsequent generalization of the infection, or colonization of the mucous membranes in intestinal diseases without subsequent generalization of the infection. In diphtheria, the infectious process is also limited by adhesion, colonization and production of exotoxin (histotoxin). The penetration of bacteria into the blood does not occur. If for extracellular parasites the process is limited by adhesion and colonization, then for obligate and facultative intracellular parasites, an important condition is their penetration into the cell and subsequent intracellular multiplication.

The infectious process can occur at all levels of the organization of the biological system of a macroorganism. In addition, each higher level includes lower levels. First of all, the multi-level system of the infectious process includes the body level or the infectious process itself, since infection is a system of reactions that arise in a susceptible macroorganism. The subordinate levels are the tissue-organ, cellular (the interaction of the microbial cell and the host cell) and the subcellular or molecular level, which is based on the competitive interaction of the biological molecules of the microbe and the macroorganism. As a result of the interaction of the microbe with the macroorganism, first of all, the cell suffers in which the microbe makes the gaps through which it subsequently penetrates the macroorganism. The interaction process itself occurs at the level of complementary structures of the microbial macromolecules and the eukaryotic cells of the macroorganism. The

processes taking place at the molecular and cellular levels, namely the interaction of pathogenicity factors of microbes with cellular and humoral factors of defense of a macroorganism, are reflected at the tissue-organ level. Tissue differentiation of host cells determines the specificity of the infectious process and plays a protective role, limiting the breeding zones of microbes. Therefore, the main features of the pathogenesis of the infectious process are formed at the tissue-organ level, reflected subsequently at the organismic level, determining the manifestation of the infectious process. The features of the pathogenesis and clinic of the infectious process at the organismic level are reflected in the course of the epidemic process at the ecosystem level, determining the nature and intensity of the implementation of a particular mechanism of transmission of the pathogen. Thus, the infectious process is characterized by a variety of complex interactions both at each level of the system, and between these levels.

Pathogenicity and virulence of microorganisms

According to this property, microorganisms are divided into:

1. pathogenic;

2. opportunistic microorganisms occur in the environment and in the normal microflora, safe for a healthy person, but can become pathogenic for persons with immunodeficiency, when penetrating into organs where they are not normally found (E. coli when penetrating the abdominal cavity in trauma or during surgery causes peritonitis);

3. non-pathogenic.

Microorganisms that can cause disease in humans and animals are called *pathogenic*, and their ability to cause disease is called *pathogenicity* (from the Latin pathos – suffering, genos – birth). Pathogenicity is a genetically determined species trait. Most pathogens are characterized by specificity that is the ability of this type of microbe to cause a particular disease. For example, cholera is caused by Vibrio cholera, gonorrhea – by gonococcus, dysentery – by Shigella dysentery. However, the specificity of microorganisms is relative. So some microorganisms (staphylococci and streptococci) can cause different diseases and on the contrary the same diseases are caused by different pathogens. So pneumonia can be caused by staphylococci, streptococci, Klebsiella and others microorganisms.

Organotropy is the ability to affect certain tissues selectively (gonococcus affects the epithelium of the genitals, the causative agent of dysentery - enterocytes).

In order to cause an infectious process, pathogenic microbes must penetrate the body in a certain critical infectious dose (pathogenic), i.e. in a minimum dose, which causes persistent adhesion, colonization, penetration of a pathogen into the tissue and the further development of the infectious process. For each type of microbe, there is its own minimum infectious dose, i.e. the number of individuals capable of causing the disease.

Different strains of the same species may have different pathogenic effects. The degree of pathogenicity is called *virulence*.

Virulence (Lat. *Virulentus* – poisonous) is a sign not of a species, like pathogenicity, but of a strain, i.e., it is inherent not to the whole species, but to specific strains. Virulence can also be defined as the phenotypic manifestation of the pathogenic genotype of microorganisms. As a quantitative sign, in contrast to qualitative – pathogenicity, virulence has units of measure: it is measured by the dose of microorganisms that cause a certain biological effect.

Strains are divided into: high, moderate, weak, avirulent.

Laboratory virulence is determined by the magnitude of the lethal (LD) and infectious (ID) doses for experimental animals.

Lethal dose (LD) – the smallest number of pathogen or toxin, causing the death of a specific number (%) of animals in a certain period of time.

• *DCL* (dosis certae letalis) – absolutely lethal dose – the minimum amount of the pathogen that causes the death of 100 % of laboratory animals taken in the experiment;

• *DLM* (dosis letalis minima) – the minimum lethal dose – the minimum amount of the pathogen, causing the death of 95% of laboratory animals taken in the experiment;

• LD_{50} – the minimum amount of the causative agent causing death of 50 % of laboratory animals taken in the experiment (used to measure virulence most often). At the same time, the type of laboratory animal at which this dose was determined is always indicated, since the sensitivity of different types of laboratory animals to various microorganisms is different.

In order to cause an infectious process, pathogenic microbes must penetrate the body in a certain critical infectious dose (pathogenic), i.e. in a minimum dose, which causes persistent adhesion, colonization, penetration of a pathogen into the tissue and the further development of the infectious process. For each type of microbe, there is its own minimum infectious dose, i.e. the number of individuals capable of causing the disease.

Infectious dose (ID) – the minimum number of microorganisms that can cause disease in a certain number (%) of experimental animals.

ID_{100} is the minimum number of living microbes that causes the development of an infectious disease in 100 % of infected experimental animals taken in the experiment.

ID_{50} – 50 % incidence, etc.

As a phenotypic manifestation of the genotype, virulence is subject to changes both in the direction of decrease and in the direction of increase under the influence of physical, chemical and biological factors. A decrease in virulence *(attenuation*) can occur with prolonged cultivation of bacteria on an artificial nutrient medium or as a result of prolonged passage of microbes through the body of unreceptive animals. The complete loss of virulence is associated with a change in genotype. An increase in virulence is observed, on

the contrary, with the passage of microbes through the body of highly susceptible animals, with lysogenesis, as well as due to mutations and recombinations. These features of changes in virulence are taken into account when receiving vaccine strains of microbes.

With a maximum decrease in virulence, pathogenic microorganisms can become avirulent, i.e., non-virulent. But virulent microorganisms are always pathogenic.

Virulence factors

The virulence of microorganisms is determined by three main properties of the pathogen: infectivity, invasiveness, toxicity (*Fig. 2*).

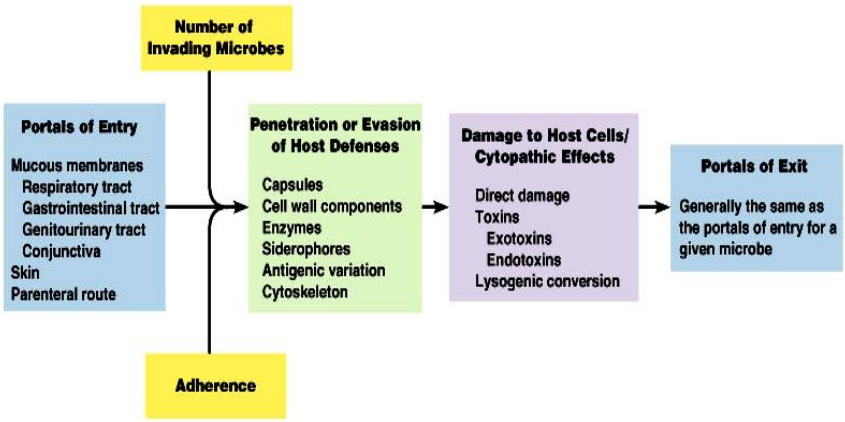

Fig. 2. Bacterial mechanism of pathogenicity

Infectivity, invasiveness and toxicity are not related and are differently detected in various pathogens. The pathogen of diphtheria, tetanus, botulism more pronounced toxigenic properties. The plague agent is invasive. It does not only suppress the body's defenses, it also multiplies so rapidly that the immune system does not have time to react.

Infectiousness is the ability to infect. It is conditioned by its ability to adhere (stick), colonize (multiply). Adhesion and colonization is trigger mechanism of disease development. Microorganisms and toxins realize their pathogenic and toxic properties when they bind to target cell receptors.

Adhesion is the ability to adsorb on cells of host organism that are sensitive to a particular microorganism. It is caused, on the one hand, by the surface structures of the microbial cell (pili, etc.), on the other, by the presence of receptors of the cell of a macroorganism capable of interacting with the microbial cell.

Colonization can be on the surface of cells to which germs adhere (Vibrio cholera adheres to enterocytes), or inside cells into which adherent germs penetrate (eg, S. dysentery multiplys in cells of the colon).

Invasiveness is the ability to penetrate the tissues, overcoming the body's defenses. Invasiveness is related to the ability of germs to produce enzymes that disrupt (increase) the permeability of connective and other tissues. For the implementation of colonization and invasion, many bacteria secrete *enzymes of aggression and defense*:

hyaluronidase (a spread factor), which destroys the hyaluronic acid of the connective tissue and thereby promotes the penetration of germs into the tissue.

Neuraminidase, which cleaves neuraminic acid from glycoproteins, glycolipids, polysaccharides that are part of different tissues, thus increasing their permeability.

Fibrinolysin dissolves a clot of fibrin, which is formed during inflammation, preventing the penetration of the microorganism into the tissues.

Collagenase ferments collagen of muscle fibers, causing their melting.

Lecithinase C ferments the lecithin of the membranes of muscle fibers, red blood cells, and other cells.

Coagulase coagulates blood plasma, promotes the formation of fibrin barriers.

Deoxyribonuclease (DNase) causes depolymerization of DNA.

Proteases destroy proteins (including IgG and others).

Substances with antifagocytic activity. Inhibition of phagocytosis is carried out by bacterial capsules. The substances that make up the capsules of different microorganisms are different, and their functions are also different. Thus, the capsular polypeptide of the *Bacillus anthracis* protects it from capture by phagocytes; polysaccharide of *Pseudomonas aeruginosa* suppresses both capture and intracellular digestion of bacteria. Other capsular polysaccharides and polypeptides of microorganisms: K- and Vi-Ag microcapsules of enterobacteria, M-protein of β-hemolytic streptococci A, A-protein of staphylococci.

In addition to these factors, germs protect against phagocytosis by some enzymes. For example, staphylococcus coagulase promotes plasma coagulation, leading to the formation of a protective "cover" around the microbial cell; fibrinolysin dissolves fibrin, contributing to the spread of germs.

The formation by microorganisms substances capable of withstanding intracellular digestion; preventing the fusion of phagosome and lysosomes; capable of causing lysis of phagocytes (leukocidins).

Antigenic mimicry is the similarity of the Ag-determinants in the microorganism and the host macroorganism, as a result, the microorganism is not recognized by the immune system, which contributes to its persistence in the macroorganism.

Bacteria produce two types of toxins – exotoxins and endotoxins (*table*).

Table

Properties of bacterial exotoxins and endotoxins

Property	Endotoxin	Exotoxin
Source	Gram-negative bacteria	Gram-positive bacteria
Chemical nature	Lipopolysaccharide	Protein
Relationship to cell	Part of outer membrane	Extracellular, diffusible
Denatured by boiling	No	Usually
Antigenicity	Weakly	Highly
Form toxoid	No	Yes
Potency	Relatively low (> 100 ug)	Relatively high (1 ug)
Specificity	Low degree	High degree
Enzymatic activity	No	Usually
Pyrogenicity	Yes	Occasionally

Exotoxins are the products of the metabolism of germs released into the environment. They have a protein origin, which causes their low resistance to external actions. The exceptions are the neurotoxin of the botulinum rods, the enterotoxins of staphylococcus, Vibrio cholera, which withstand short boiling.

Exotoxins are proteins of a bifunctional structure – the transport group interacts with cell receptors, and the toxic group (activator) penetrates the cell and blocks metabolic processes.

Exotoxins are mainly synthesized by Gram-positive bacteria (diphtheria, tetanus, botulism, gas gangrene, *S. aureus*, *S. pyogenes*) and some Gram-negative bacteria (*V. cholera*, *P. aeruginosa*, *E. coli*, *S. dysentery*).

According to the mechanism of action on the cells of a macroorganism, bacterial toxins are divided into several types, although this division is quite arbitrary and some toxins can be assigned to several types at once:

• Type 1 – membranotoxins (hemolysins, leukocidins);

• Type 2 – functional blockers, or neurotoxins (thetanospasmin, botulinum toxin) - block the transmission of nerve impulses in synapses (in the cells of the spinal cord and brain);

• Type 3 – thermostable and thermolabile enterotoxins – activate cell adenylate cyclase, which leads to disruption of enterosorption and the development of diarrhea syndrome. Such toxins produce *V. cholera* (cholerogen), enterotoxigenic *E. coli*;

• Type 4 – cytotoxins – toxins that block protein synthesis at the subcellular level (enterotoxin of *S. aureus*, anthrax bacilli, and *B. pertussis*); here also include anti-elongators – preventing elongation (growth) or translocation, that

is, the movement of i-RNA along the ribosome, and thereby blocking protein synthesis (diphtheria histotoxin, *P. aeruginosa* toxin);

• Type 5 – exfoliatins formed by certain strains of *S. aureus* and erythrogenins produced by group A pyrogenic streptococcus (*S. pyogenes*). They affect the interaction of cells with each other and with extracellular substances and completely determine the clinical picture of infection (in the first case pemphigus of newborns, in the second – scarlet fever).

Many bacteria form not one, but several protein toxins, which have different effects - neurotoxic, cytotoxic, hemolytic: staphylococcus, streptococcus.

The ability of microorganisms to form protein toxins must also be considered when conducting microbiological diagnostics. It should be remembered that all pathogenic strains of this species can produce only one type of toxin according to the antigenic structure and mechanism of action (*C. diphtheriae, C. tetani*), different in antigenic structure, but are identical in mechanism of action *(C. botulinum*). At the same time, some bacteria can simultaneously form both protein exotoxins and endotoxins: *E. coli, V. cholera*.

Exotoxins are characterized by high toxicity and expressed specificity – organotropicity. Each type of toxin affects certain organs or tissues. For example, tetanus toxin affects the motor neurons of the spinal cord, and diphtheria toxin affects the muscles of the heart and adrenal glands, botulinum toxin acts on the end of motor nerves.

The synthesis of protein toxins is encoded by genes localized on the chromosome and linked to genes involved in spore formation or part of the prophage, as well as genes localized in plasmids. These are tox+genes responsible for toxigenicity. The activity of tox+genes is controlled by microbial cell repressor proteins. The initial function of these genes in saprophytes was the synthesis of structural phage proteins, spore membrane components, or the synthesis of enzymes necessary for the absorption of amino acids. As the parasitic lifestyle consolidates, these specialized adaptive enzymes have turned into poisons – protein toxins.

Endotoxins are a lipopolysaccharidoprotein complex that is closely related to the cell of the microorganism and is a part of the cell wall mainly of Gram-negative bacteria, released after cell destruction (typhoid, dysentery, cholera, whooping cough). They are nonspecific. The entire LPS molecule is responsible for the manifestations of the biological activity of endotoxins but not its individual components. Unlike protein toxins, they do not have organotropy and specificity of action. Symptoms of intoxication in diseases caused by Gram-negative bacteria are of the same type, and are associated with the action of the resulting inflammatory mediators. The action of LPS is based on its non-specific lipid-lipid and specific, due to the CD14 receptors, interaction with the membrane components of different types of cells: platelets, granulocytes, red blood cells, lymphocytes, monocytes and macrophages, which release biologically active substances under the influence of LPS. LPS starts the synthesis of more than

20 different biologically active substances in a macroorganism, which determine the pathogenesis of endotoxemia and have a pyrogenic effect.

The main point of its application are macrophages. The formation of large doses of endotoxin is accompanied by inhibition of phagocytosis, symptoms of severe toxicosis, weakness, shortness of breath, diarrhea, impaired cardiovascular-system, decreased pressure, hypoglycemia, leukopenia, followed by leukocytosis, platelet aggregation, hypothermia. With the formation of large amounts of endotoxin in the blood due to increased destruction of a large number of Gram-negative bacteria, the development of endotoxin shock is possible. With the formation of small doses of endotoxin, mild toxicosis and an increase in body temperature, stimulation of phagocytosis are noted. LPS refers to tymine-independent antigens and causes polyclonal stimulation of B-lymphocytes, activates the complement system in an alternative way, and is an adjuvant. Small doses of endotoxin, which are constantly formed by representatives of the normal microflora of the human body in the intestine, have a favorable stimulating effect on the cells of the macroorganism's immune system, which leads to an increase in the nonspecific resistance of the macroorganism, its resistance to infectious diseases, an increase in radioresistance and an increase in the antitumor activity of cells. As a result of polyclonal stimulation and activation of the complement system along an alternative pathway, the macroorganism is in constant readiness to meet with a wide variety of microbes and can resist them until specific defense factors are formed. On the other hand, the prolonged presence of a polyclonal stimulant in a macroorganism can lead to the inclusion of forbidden cell clones and the development of autoimmune reactions. During the immune response, initially, 0-antibodies that do not have antitoxic activity are formed on the administration of LPS. Symptoms of intoxication decrease after the formation of antibodies to the R-core of the polysaccharide and lipid A. Since they have the same structure in Gram-negative bacteria, they try to use antibodies to treat septic processes caused by these microbes. Unlike protein toxins, toxoids cannot be obtained from endotoxins.

Endotoxin causes a general intoxication of the body, showing inflammatory, pyrogenic and allergenic action. The clinical picture that is caused by endotoxins of different microorganisms is of the same type: the reaction of the body is usually accompanied by common phenomena of intoxication such as fever, headache, etc.

The close binding of endotoxin to the cells of the microorganism determines its resistance to temperature and other external factors. To obtain endotoxin, it is necessary to destroy the cell of the microorganism.

It should be remembered that a significant amount of toxin can lead to the development of endotoxic shock and death of the patient, which occurs in the mass death of microorganisms. Therefore, bactericidal antibiotics (penicillin for typhoid) should not be used for diseases caused by Gram-negative microorganisms.

The action of the toxin is determined on animals that are sensitive to this toxin. For example, diphtheria toxin is tested on guinea pigs, botulinum toxin on white mice, etc.

Features of infectious diseases are as follows:

• their etiological factor is a microbial agent;

Depending on the cause (on the etiological basis), infectious diseases are divided into:

a) bacterioses, rickettsioses, mycoplasmoses;

b) viral infections;

c) mycoses.

Infestations (parasitic diseases) are divided into protozoa, helminth infections and infections caused by arthropods.

• they are transmitted from the patient to healthy;

• induce immunity;

• have a number of common syndromes;

• characterized by cyclical course.

Infectious diseases are characterized by a cyclical course, which consists in the presence of successively alternating periods based on the pathogenesis of the disease. The duration of the periods depends both on the properties of the microbe and on the resistance of the macroorganism, the characteristics of immunogenesis. Even with the same disease in different individuals, the duration of these periods can be different.

Clinical stages (periods) of infectious disease:

• *incubation period* (from lat. *incubo* - I rest or incubatio - without external manifestations, hidden) is the period from the moment the infectious agent penetrates the human body until the first precursors of the disease appear.

During the incubation period, the pathogen adapts to the internal environment of the infected macroorganism and overcomes the protective mechanisms of the latter. In addition to the adaptation of microbes, they multiply and accumulate in the macroorganism, move and selectively accumulate in certain organs and tissues (tissue and organ tropism), which are most susceptible to damage. On the part of the macroorganism, already in the incubation period, its protective forces are mobilized. There are still no signs of the disease in this period, however, with special studies you can find the initial manifestations of the pathological process in the form of characteristic morphological changes, metabolic and immunological changes, the circulation of microbes and their antigens in the blood. From the epidemiological point of view, it is important that a macroorganism at the end of the incubation period can be an epidemiological danger due to the release of microbes from it into the environment.

The duration of the incubation period has a certain duration, subject to fluctuations both in the direction of decrease, and in the direction of increase. In some infectious diseases, the duration of the incubation period is calculated in hours, as, for example, with flu; with others – for weeks and even months, such

as for viral hepatitis B, rabies, slow viral infections. For most infectious diseases, the incubation period is 1–3 weeks.

• *The prodromal period* (from Greek *prodromes* – harbinger) – the manifestation of the first nonspecific symptoms of the disease, characteristic of general intoxication of the macroorganism by the products of the vital activity of microorganisms and the possible action of bacterial endotoxins released during the death of the pathogen; they also stand out in the environment, the patient in this period is already epidemiologically dangerous to others.

The prodromal period is not observed in all infectious diseases. It usually lasts 1–2 days, but can be shortened to several hours or lengthened up to 5–10 days or more.

• *The height of the disease* – the manifestation of specific symptoms of the disease. If there is a characteristic symptom complex in this period of the development of the disease, the clinicians call this manifestation of the disease a manifest infection, and in cases where the disease during this period proceeds without pronounced symptoms, it is an asymptomatic infection. This period of development of an infectious disease, as a rule, is accompanied by the release of the pathogen from the body, as a result of which the patient poses an epidemiological danger to others.

It was in this period that the specific pathogenic properties of microbes and the response of the macroorganism find their fullest expression. This period is often divided into three stages: 1) the stage of increase in clinical manifestations (stadium incrementi); 2) the stage of maximum severity of clinical manifestations (stadium fastigii); 3) the stage of attenuation of clinical manifestations (stadium decrementi). The duration of this period varies significantly with different infectious diseases, as well as with the same disease in different individuals (from several hours to several days and even months). This period can end fatally, or the disease goes to the next period, which is called the period of extinction of the symptoms of the disease (early period of convalescence).

• The period of *complete recovery (convalescence*) – the cessation of the pathogen reproduction in the patient's body, the death of the pathogen and the complete restoration of homeostasis. It is characterized by the absence of clinical symptoms, the restoration of the structure and function of organs, the cessation of the multiplication of the pathogen in the macroorganism and the death of the microbe, or the process can go into carrier. The duration of the convalescence period also varies widely even with the same disease and depends on its form, severity, immunological features of the macroorganism, and the effectiveness of the treatment.

There are: a) clinical recovery, in which only the visible clinical symptoms of the disease disappear; b) microbiological recovery, accompanied by the death the microbe; c) morphological recovery, accompanied by the restoration of the morphological and physiological properties of the affected tissues and

organs. Usually, clinical and microbiological recovery do not coincide with the complete restoration of morphological lesions that last a long time.

Sometimes, against the background of clinical recovery, carriage begins to form – acute (up to 3 months), prolonged (up to 6 months), chronic (more than 6 months).

• *Fatal outcome*. It should be remembered that the corpses of infectious patients are subject to mandatory disinfection, as they represent a certain epidemiological danger due to the high content of microbial agent in them.

In the study of infection, there is also the concept of persistence (infection): microorganisms enter the human organism and can exist in it without manifesting themselves for a sufficiently long time.

For clinical purposes, infectious disease is usually divided by type. It is the severity of the characteristics inherent in this nosological form. Typical forms include cases of the disease in which there are all the leading clinical symptoms and syndromes characteristic of the disease. Atypical forms include erased, inapparent, as well as fulminant and abortive forms.

With erased forms, one or more characteristic symptoms are absent, and the remaining symptoms are usually mild.

Inapparent (syn: subclinical, latent, asymptomatic) forms occur without clinical symptoms. They are diagnosed using laboratory research methods, usually in foci of infection.

Fulminant (from Lat. *Fulminare* - kill with lightning, or hypertoxic) forms are characterized by a very severe course with the rapid development of all clinical symptoms. In most cases, these forms end fatally.

In *abortive* forms, an infectious disease develops from the beginning typically, but suddenly breaks off, which is typical, for example, of typhoid fever in vaccinated people.

Biological method

Biological, or experimental, method of the diagnosis of infectious diseases is the study of pathological material in laboratory animals.

The biological method of research is used when it is necessary:

1) to isolate pathogens that do not grow in nutrient media (viruses, rickettsiae);

2) to study some properties of isolated microorganisms (necrotic, toxigenic);

3) to isolate the pure culture of the pathogen from pathological material contaminated with extraneous microflora, which inhibits the pathogen in nutrient media. So, to isolate the pure culture of pneumococcus from sputum, in which there are staphylococci, streptococci, it is emulsified in sterile broth and administered intraperitoneally to white mice, whose body is very sensitive to pneumococcus. Pneumococcus in the body of white mice multiplies very quickly. In 6–8 hours it can be isolated from exudate (from Lat. *Exsudo* – sweat, secrete; fluid leaking from small blood vessels into tissues and body

cavities in case of inflammation) of the abdominal cavity of a white mouse, other microorganisms during this time do not have time to multiply.

Experimental contamination of laboratory animals is carried out for the modeling of infectious disease, the study of the immune response of the macroorganism, to study the effectiveness and safety of immunobiological and medical products, to obtain immune diagnostic and therapeutic sera, immunoglobulins.

Laboratory animals are also used as donors (from Lat. Dono – donate) blood from which serum, plasma, erythrocytes, leukocytes are obtained. Blood and its ingredients (from the Latin. *Ingrediens* - an integral part of the mixture) are used for the production of nutrient media, the performing serological reactions.

In microbiological practice white mice, rats, guinea pigs, rabbits are used most often, chicken, pigeons, sheep, cats, dogs, hamsters, cattle, horses, pigs, donkeys, monkeys are used rarely.

Recently, clean line of animals (inbred animals) have been used. They are obtained by genetically close crossing. These are siblings for 20–40 generations, selected on a specific basis. Genetic homogeneity of animals provides uniformity of reactions of the body to the introduced pathogen.

In specialized laboratories, studies are conducted on non-microbial (sterile) animals kept in aseptic conditions. The main purpose of the research is to study the role of normal microflora in the physiological and pathological processes of animals and humans. Science that studies the life of non-microbial macro-organisms is called *gnotobiology* (from Greek. *Gnotos* - known, obvious and biotos - life), and non-microbial macroorganisms - *gnotobiots*, or *gnotobiotic*.

The purpose, structure and equipment of the vivarium

Vivarium (from Lat. *Vivus* – alive), or experimental-biological clinic, is a unit of the scientific institution in which the laboratory animals are kept and sometimes bred for carrying out biomedical researches.

The vivarium is placed in a separate building or on the upper floors of the laboratory building. In order to ensure anti-epidemic and anti-epizootic regimes, the placement of vivarium must comply with the principle of division into a clean and contaminated area.

The premises where they work with active drugs are potentially contaminated, they are classified as contamination zone III. They must be completely isolated from other rooms and the environment. The premises in which the infected material is handled in sealed equipment, devices and protective boxes, are qualified as conditionally contaminated and they belong to the zone II.

The premises of II and III zones are made especially tight, without windows or with windows that are sealed and are not opened. The sealing these rooms must be such that they can be treated with gaseous (vapor) chemicals. These rooms should be provided by self-contained ventilation systems that dilute the

air, which provides the direction of air movement to these rooms, not the other way around. The air that is vented by the ventilation systems from the premises of zones II and III is filtered through a system of successively installed filters.

An autonomous wastewater disinfection system from these premises ensures their high-temperature sterilization.

The buffer space is allocated to a separate group. These are the preparatory ones serving the premises of zones II and III. They work with materials, laboratory glassware and reagents, and also it is allowed the temporary residence of the active material in a sealed package, which is intended for transfer to the premises of zones II and III. These premises are considered clean, they are referred to zone I.

In addition, there are general-purpose premises in the laboratory: lobbies, locker room, office rooms, dining room, warehouses, maintenance room. This group of premises is assigned to the 0 (zero) zone of the laboratory. At the boundaries of the working areas of different zones barrier systems are created: sanitary passes for people (rooms for the removal of clothing, air showers, washing plant) and transfer facilities for the movement of materials, apparatus and instruments for research (pass sterilizers for heat treatment, gas transmission chambers for chemical treatment with a gas mixture) and transfer baths ("diving chambers") for chemical treatment with disinfectant solutions.

They operate by technical systems from clean area premises.

The following departments are obligatory included in the vivarium: quarantine, section for experimental animals, isolators, operating room, manipulation, fodder kitchen, disinfection and washing department, warehouse for clean spare equipment, sanitary unit (toilet, showers), domestic premises, diagnostic room, service room, refrigerator.

The quarantine unit is intended for keeping animals purchased for the vivarium. Animals are purchased from specialized nurseries. They are housed in clean disinfected (pro-autoclaved) cages. The purchased animals must have a passport indicating the date of departure and receipt of the animals, the cage number, quantity, age and body weight of the animal, the date and results of the clinical examination. If the animals are purchased from a specialized nursery located in the same town, they are kept in that compartment for 3 days to adapt to new conditions. If the animals are purchased from nurseries located in other cities, or non-specialized nurseries, the quarantine period is set according to the incubation period for the most common diseases for these animals. Quarantine term: for mice and rats – 17 days, cats and dogs - 30 days, guinea pigs, rabbits, birds and other animals – 21 days.

During the quarantine, animals are monitored daily for clinical supervision. The quarantine premises are cleaned and disinfected after each batch of animals transferred for work and after each case of detection of infectious disease. At the end of the quarantine period, animals are transferred to the experimental sections.

Isolators are designed to contain animals with suspected infectious diseases or ill animals, the destruction of which is undesirable under the conditions of experience.

Constructive features of operating and manipulation are determined in each case depending on the task and purpose of scientific research.

The fodder kitchen consists of two adjacent premises for processing and production of animal feed.

The disinfection and washing compartment are two adjacent rooms connected by a passing autoclave or a passing dry chamber. It is intended for sterilization of inventory, litter, cages after mechanical cleaning.

Diagnostic and service rooms are laboratory facilities for research on the quality control of animals, feed, and for maintaining documentation.

The refrigeration chamber is intended for storage of carcasses of animals for pathological anatomical examination.

In rooms where animals are kept or experiments are carried out, as well as in the fodder kitchen and in the disinfection and cleaning department, the floor is made of waterproof material, without plinths with a slope to the openings connected to the sewer. The walls from floor to ceiling are covered with glossy tile, the ceiling is painted with oil paint. The doors have a smooth surface, painted with oil paint. The upper part of the door is glazed. Lighting and microclimate (temperature, ventilation) in the premises must meet the established standards.

Rules for the keeping and care of laboratory animals

The correct keeping of laboratory animals and the proper nutrition of animals is of great importance for obtaining reliable results of the experiment. Disruption of the mode of retention and diet causes the weakening of the body of animals, increasing their sensitivity to pathogenic microorganisms, which leads to distortion of the results of the study and incorrect findings. Cages with laboratory animals, boxes, enclosures are placed in the area provided by the established standards.

All work on the care and maintenance of laboratory animals is carried out in accordance with the approved regulations, which determine the time for sanitary treatment of premises and equipment, feeding, carrying out experimental works and manipulations. Animal care and hygiene personnel should wear work clothes (trousers or overalls with elastic seals at wrists and ankles, headgear should cover hair completely, gloves, special footwear), goggles and respirators.

Personnel serving animals infected with pathogens of particularly dangerous infections should wear a bathrobe, rubber apron, sleeves, rubber gloves, rubber boots or galoshes on top of their clothing, and goggles and a cotton gauze mask if necessary.

Cages and equipment are cleaned and washed daily after pre-decontamination. Contaminated litter and other waste from cages are collected in special metal

tanks with lids. The tanks are tightly closed and transferred to the washroom and disinfection room. The waste is disinfected or incinerated. The methods of disinfection, disinsection and autoclaving are set on a case-by-case basis depending on the animal's condition (healthy or infected) and the type of infection.

Laboratory animals are fed 2–3 times a day according to the standards developed by the research institutions. The daily diet is made so that different types of feed are given alternately. The main food for laboratory animals is cereal grains (oats, wheat, corn, millet), oilseeds (sunflower, flax, hemp) and legumes (peas). They are given natural or crushed. It is sterilized in an autoclave or in a dry oven before being fed to animals.

Green, juicy feed, milk, fat, vitamins, and mineral salts are also required for good nutrition. There should always be drinking water in the cages of the animals.

All actions related to the care, keeping animals, carrying out medical and biological experiments (surgery, blood collection, necessary killing of animals) are performed in accordance with the rules of humane treatment for laboratory animals.

Preparation of laboratory animals for experiment

For the experiment, a group of animals of a certain species, age, body weight is selected, sometimes, under the conditions of the experiment, one sex. All animals must be healthy. They are placed in clean disinfected cages and weighed for several days before the experiment, thermometry and marking of animals are carried out.

Weighing. For weighing mice, rats, guinea pigs dial scales are used (without weights) with a loading capacity up to 1 kg; for weighing rabbits cup scales with tare wooden boxes or cardboard boxes (with known weight) with a capacity up to 5 kg are used.

Thermometry. Thermometry is performed with a medical mercury thermometer (there is a special small triangular thermometer for mice) in the rectum. Normal temperature in different animals is not the same: in white mice – 37–39 °C, white rats – 38,5–39,5 °C, guinea pigs – 37,3–39,5 C, rabbits – 37,7–38,0 °C.

Fixation of laboratory animals. During the biomedical experiment, the animals are fixed (limiting their mobility) in a posture that makes it convenient to perform the intended manipulation. There are several ways of fixing animals.

Fixation of animal (rabbit, guinea pig) *with a towel*. The animal is tightly wrapped in a towel. In this case, the front paws are pressed to the chest, the back - to the abdomen, the head remains free.

Fixation of the head of a rabbit. To do this, use a box (wooden box with a movable lid and the top of the front wall). In the front wall cut a hole with a diameter of 5 cm so that it was located in the lower stationary part of the wall and in the upper movable. The rabbit is put in a box, and his head is brought out through this opening. Moving parts of the box fix the trunk and head of the rabbit.

Fixation of animals (rabbit, guinea pig, rat) *in a supine or abdominal position*. To do this, use a special machine. This is a normal board on low legs,

the size of which corresponds to the size of the animal. The side edges of the board have two holes or two hooks to secure the loops (fixation of the legs). At the narrow edge of the board there is a device for fixing the head.

Fixation of guinea pig with hands. The manipulation is performed together. The assistant fixes the animal, and the experimenter performs the appropriate manipulation. The guinea pig is taken with the left hand so that the second finger of the left hand is under the neck, and the first and third fingers – under the forelimbs of the animal, and rotate it with the belly up or out. With the right hand, the pig is stroked to the abdomen until it calms down. The hind legs of the animal are held by the right hand.

Fixation of rats. The fold of the skin on the nape of the neck is taken with a cornice and pressed tightly the animal's head against the table surface. They take the rat's tail with the other hand and, barely lifting it above the table surface, hold it in such a position that the head is slightly drawn away by the cornice.

Fixation of mice. Mouse is let on the surface of the table, holding it by the tail with the first and second fingers of the right hand. After straightening the tail with a quick movement of the left hand, it is grabbed by the fold of skin out of her ears so that it cannot turn her head. Lifting the mouse over the table, the assistant holds it with one hand by the tail and the other by folding the skin on the nape in a comfortable position for the experimenter.

It is possible to work with mice without the assistant, fixing them with the left hand: the first and second fingers of the left hand hold the animal by the fold of skin on the nape of the neck, and the other three fingers press the tail and the skin of the back with the wrist. With this method of fixation, the right arm remains free, allowing various manipulations to be performed.

Methods of infection of laboratory animals

Depending on the purpose of the study, different methods of infection are used: intradermal, cutaneous, subcutaneous, intramuscular, intraperitoneal, intravenous, oral, intranasal, intracerebral, infestation in the eye, intracardiac. In all ways of infection a syringe is used. The area of the body on which the injection or incision is made is called the operative field. When the material is introduced through the skin, the surgical field is pre-released from wool (cut, shaved, plucked or treated with an epilator – a mixture that causes hair to fall out). Before injection, the operating field is treated with a 70 % solution of ethyl alcohol or alcohol solution of iodine. To prevent the formation of contaminated aerosol when removing air from the syringe, a cotton swab moistened with 70 % ethyl alcohol solution is put on the needle. The contaminated cotton swab is lowered into a jar of disinfectant solution. (When working with infectious agents, it is forbidden to remove air from the syringe, so fill the needle with water from the sterilizer).

Intradermal method of infection. For this thin sharp needles with a small bevel are used. Before inserting the material, the skin is stretched with the first and second fingers of the left hand, and the needle of the syringe is inserted by the right hand at a very acute angle. In this case, the end of the needle shines through the epidermis, and a hump is formed at the site of the introduced material; the skin on its surface is transparent and porous, so it is compared to a lemon peel. The material is injected into the skin of the back or abdomen in a volume of 0.1 ml.

In this way they put dermonecrotic and allergy tests.

Cutaneous infection method. After removal of wool, more often in the abdomen, the skin is scarified (from Lat. *Sagifis* – scratch) with a scalpel or Jenner pen. The material is applied to the skin and rubbed into scratches. The method is used to identify pathologic agents of tularemia, plague.

Subcutaneous method of infection. The skin at the site of material insertion is lifted with the first and second fingers of the left hand (*Fig. 3*). The needle of the syringe is inserted at the bottom of the formed folds of the skin. Puncture the skin, insert the needle a few millimeters, then slightly turn it right or left and slowly enter the material. The direction of the needle is changed so that the material does not leak through the puncture. The skin fold is lowered, a cotton wool moistened with alcohol is placed at the injection site, the needle is quickly removed.

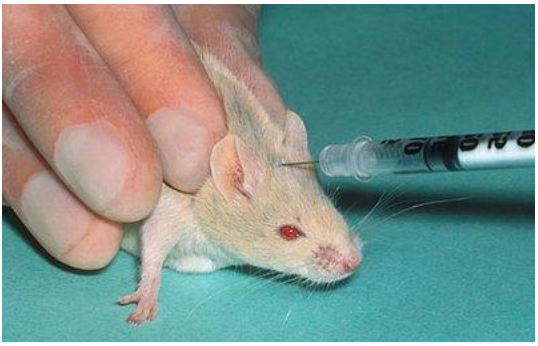

Fig. 3. Subcutaneous method of infection of laboratory animals

The material is introduced to the rabbits and guinea pigs under the skin of the back or lateral surface; rats, mice - under the skin of the back, waist, nape. Rabbits are injected with up to 30 ml of fluid, guinea pigs up to 15 ml, rats up to 10 ml, mice up to 1 ml.

Intramuscular method of infection (*Fig. 4*). The site of insertion of the material is the outer upper third of the hind paw (the area of the body with the most developed muscles). The muscle fold is taken with the first and second

fingers of the left hand, and the needle of the syringe is injected almost at right angle deep into the muscles. Rabbits are administered up to 8 ml of fluid, guinea pigs – 5 ml, rats – 3 ml, mice – 0.5 ml.

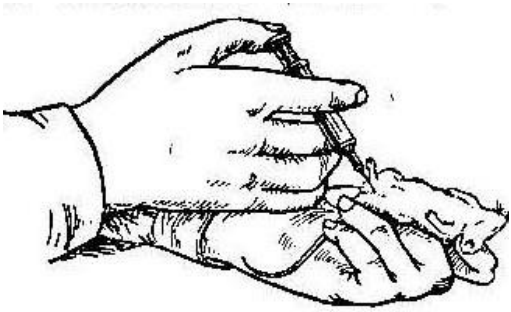

Fig. 4. Intramuscular method of infecting laboratory animals

Intraperitoneal method of infection (*Fig. 5*). The helper holds the animal head down, with the intestines moving to the side of the diaphragm, reducing the possibility of their damage. The material is introduced into the lower third of the abdomen to the left of the midline. In this case, the needle of the syringe is kept perpendicular to the abdominal wall, injected until a feeling of "failure", then remouve the needle upright and inject the material into the abdominal cavity: rabbits – up to 30 ml, guinea pigs – up to 10 ml, rats – up to 5 ml, mice – up to 2 ml.

Fig. 5. Intraperitoneal method infection of laboratory animals

This method is used to detect the exotoxin of the botulinum pathogen.

Intravenous method of infection. Rabbits are infected in the marginal vein of the ear. After wool is removed, the ear is clamped at its base to cause vascular

flushing. The syringe needle is inserted at an acute angle. If the needle enters the vein, the material is easily inserted. Rabbits are injected with up to 20 ml of fluid.

Rats and mice are infected in the lateral vein of the tail. Before introducing the material, the tail of the animals is immersed in water heated to 50 °C; the veins are swollen and shine through the skin. The syringe needle is inserted at a very acute angle. Rats were injected with up to 6 ml of fluid, mice were injected with up to 0.5 ml.

The force of action of microbial exotoxins is determined in this way (DLM, DCL).

Oral infection is infection through the digestive tract. There are two ways to infect animals through the mouth. The material intended for infection is added to the food or to the drinking liquid (milk). This method is simple and convenient, but does not allow to take into account the amount of material that gets into the animal's body. Therefore, the material is more often injected with a syringe. To do this, take a needle with a thickening on the end in the form of olive. It is more convenient and safer to use an elastic probe. Its outer end is attached to the syringe, and the lower end is inserted into the lower esophagus or directly into the stomach. In this case, rats and mice are kept upright, and rabbits and guinea pigs are wrapped in a towel and sit on the assistant's lap. In this way, rabbits are injected with 3.5–5 ml of fluid, guinea pigs 2.5–3.5 ml, rats up to 3.5 ml, mice 1 ml.

This is how the effects of enterotoxins of pathogens of food poisning are studied.

Intranasal method of infection is infection through the respiratory tract. The animal is fixed on a preparation board. A cotton swab moistened with ether or thiopental is broucht to the nose. After signs of light anesthesia appear in the animal, the needle of a syringe or pipette is introduced into the nostrils of the mice to a depth of 1–1.5 mm, rats – 2–3 mm, rabbits and guinea pigs – 4 mm. The material is injected with small drops. To avoid damaging the mucous membranes, blunt needles schould be used.

In this way, the influenza virus is detected in the material being examined.

Intracerebral (subdural) method of infection. The material is injected through the crown of suckling mice under the dura. Intracerebral infection is also carried out to rabbits, guinea pigs, mice. At the same time, after opening the skin in the middle of the forehead, pierce the bone with Frank's needle, then inject the needle of the syringe into this hole. Rabbits are injected with 0.4 ml, guinea pigs 0.2 ml of infected fluid. Mice are pierced with a needle from a syringe and injected with 0.05 ml of infected fluid.

In this way, viruses of encephalitis, and polio, Coxsackie, ECHO viruses are detected in the pathological material.

It is possible *to incorporate infected material through the eye* in various ways. The lower outer corner of the eyelid of the animal is drawn with tweezers (in the left hand), with the right hand (from a pasteur pipette 1–2 drops or a bacteriological

loop) the material is applied to the conjunctiva and distributed on the conjunctiva, moving the eyelids with tweezers. In the *subconjunctival method*, the material is injected with a fine needle under the conjunctiva (0.1–0.2 ml). You can also insert the material into the scarified cornea of the eye by rubbing it with a glass spatula.

The method is used for performing a keratoconjunctival test in the diagnosis of shigellosis, colienteritis caused by enteroinvasive Escherichia.

Intracardiac infection. The material is injected into the ventricle of the heart. This method is used to reproduce anaphylactic shock.

All methods of animal contamination should strictly adhere to the methodological guidelines governing the preparation, conduct of the experiment, the decontamination of pathological material and tools, and the rules of conduct of the staff in order to prevent intra-laboratory contamination.

Methods of blood collection in laboratory animals. Treatment and selection of blood components

Manipulations (from Latin. *Manus* - hand, action of hands at performance of work) at blood sampling from animals are different, they depend on a kind of animals and methods of blood sampling. A small amount of blood is taken from the veins of the ear, tail, inner surface of the wing. More blood is taken from the heart of a rat, a rabbit, a guinea pig, an elbow vein (in a monkey), a thigh vein, a carotid vein of a horse, cattle. In special cases, conduct a total (from Lat. *Totalis* – whole) bleeding. The disadvantage of this method is that the animal dies.

Before blood is collected from laboratory animals, on the area of the body where the puncture is to be punctured the wool is first removed, degreased and disinfected with alcohol, ether or alcohol iodine tincture. After taking the blood, a cotton swab impregnated with an alcoholic iodine solution is applied to the injection site, and the animal is injected subcutaneously with a sterile isotonic sodium chloride solution or glucose solution heated to 37 °C in a volume that is 2 times greater than the volume of taken blood.

Blood collection from the heart. The rabbit, rat, guinea pig are fixed on a special table with the belly up. With the second finger of the left hand feel the heart impulse and in this place enter the needle of the syringe. If the needle hits the heart, blood immediately begins to fill the syringe. The amount of blood that can be taken depends on the species of animal. The rabbit can take 25–30 ml, the guinea pig – 10–12 ml, the rat – 6–8 ml. To prevent blood clotting, the syringe and needle are rinsed with sterile sodium citrate before blood collection, and the manipulation is performed as soon as possible.

Blood collection from a vein. In a rabbit blood is taken from the marginal vein of the ear. To do this, it is fixed with a towel and kneel down. They remove wool on the outer surface of the ear, disinfect the skin, clamp a vein at the root of the ear, causing blood flow to be delayed and a vein swollen. The needle is inserted

into the vein without a syringe, and the vein is released. Blood dripping from the needle is collected into a sterile test tube.

In white mice and white rats, blood is taken from the tail vein. Animals are fixed in metal mesh of appropriate size. The tail is first heated in water at a temperature of 45–50 °C, then amputated (from the Lat. *Amputatio* – cut off) the tip of the tail. Blood flowing from the stump is collected into a sterile test tube. To stop the bleeding the stump is treated with hydrogen peroxide.

Large animals (horses, rams, bulls) are first anesthetized (from Greek. *Narcosis* - numbness), and then blood is taken from the carotid artery.

Total revelation. The animal is fixed with the belly up, give the animal anesthesia. After that, they make a section along the middle line of the neck, dilute the edges of the wound, then separate the muscles, expose the carotid artery and impose on it 2 ligatures (from the Latin *Ligatura* - ligament). Below the second ligature impose clamp. Between the ligature and the clamp insert a cannula (glass tube with the drawn capillary). A rubber tube is placed on the extended part of the cannula and the end of the cannula is lowered into the vial. The clip is removed, the blood flows freely into the vial. With total bleeding, the rabbit you can take 120–150 ml of blood, the guinea pig – 30–40 ml, the rat – 20–25 ml.

In laboratory practice, both whole blood and its individual constituents are widely used: erythrocytes, serum, and plasma.

Production of defibrinated blood. Animal blood is collected into a sterile glass bead vial. The vial is shaken within 10–15 minutes, causing the fibrin to settle on the surface of the necklace and the blood lose its ability to coagulate. Defibrinated blood is poured into another sterile vial. It is used for the manufacture of complex nutrient media, as well as for obtaining a suspension of red blood cells.

Production of suspension of erythrocytes. The defibrinated blood is poured into a centrifuge tube, closed with a stopper and centrifuged at 2 000 –3 000 rpm for 10–15 minutes. The erythrocytes settle to the bottom of the tube, and the transparent part of the blood forms a supernatant. After centrifugation, the liquid level in the tube is indicated by a pencil, the liquid portion is aspirated with a Pasteur pipette with a rubber pear, and a sterile isotonic sodium chloride solution is poured into the test tube and centrifuged again. After centrifugation, the supernatant is aspirated and poured into a disinfectant solution, and a sterile isotonic solution is poured again. Washing the erythrocytes with sterile isotonic solution is repeated 3–4 times. The last dose of wash fluid should be colorless. 3–5 % suspension is prepared from the washed erythrocytes, which can be stored in the refrigerator at 3–4 °C for 5–6 days.

The erythrocyte suspension is used to performe reactions to detect antibodies in the patient's serum, to detect viruses in pathological material.

Production of blood serum. In order to obtain a clear serum, the animals should be fasted. Lipimia (accumulation of excess fat) occurs after ingestion of the animal feed and sometimes with repeated bloodletting at short intervals. Serum obtained from such blood will be cloudy (chylous), so the results of serological reactions may be incorrect. The blood is collected into a test tube, preventing the formation of foam. For this purpose, a stream of blood flowing from the blood vessel is directed to the test tube wall. The tube with the blood can be left at room temperature for 3–4 hours. For more efficient and accelerated coagulation, the blood is placed in a thermostat at 37 °C for 20–30 minutes. (It is not possible to leave blood in the thermostat longer because hemolysis will occur). The formed blood clot is separated from the test tube walls with a sterile bacteriological loop and placed in the refrigerator for 1 hour or centrifuged. Well-settled serum is obtained by settling blood for 18–20 hours. Serum above the precipitate can be stored for no longer than 48 hours (with longer storage hemolysis occurs). If the serum is used later than 48 hours, it is transferred to a sterile tube with a sterile pipette.

Production of citrate blood. Pour 1 ml of 5 % sodium citrate solution into a blood collection tube and select 10 ml of blood. The tube is closed with a stopper and the tube contents is mixed. Citrate blood does not clot.

Production of blood plasma (from the Greek. *Plasma* – molding, design). Plasma is made from citrate blood. For this purpose, citrate blood is placed in the refrigerator for 18–20 hours or centrifuged. A layer of slightly cloudy fluid of light yellow color is formed above the precipitate of the formed elements of blood (erythrocytes, platelets, leukocytes). Plasma differs from serum in that it contains fibrinogen protein. Plasma is used in laboratory practice to detect the plasma-coagulating properties of staphylococcus.

Autopsy of carcasses of laboratory animals, maintenance of the protocol of autopsy

The dissection of corpses of infected laboratory animals is done to determine the cause of death of the animals, isolation of the introduced pathogen, identify the mechanism of spread of germs in the body and the location of their localization, study of pathoanatomical changes resulting from the infection. In the case of an experimental infection, the opening the animal is performed after its death. But there are cases when an animal is killed. There are several ways to do this. The simplest is that the animal is placed in a container, balls of cotton wool soaked in ether or chloroform are lowered into it, and tightly closed.

The animal dies in 3–5 minutes. To kill rabbits an air embolism is used (from the Greek *embole* - insertion, occlusion of blood or lymphatic vessels by foreign particles – emboli brought by blood or lymph, which leads to impaired blood supply to organs and tissues); sometimes mice are decapitated (from Lat.

Deremoval - destruction and *caput* - head). The dissection of the corpse is carried out immediately after the death of the animal, to prevent the penetration of germs from the gut into the blood and other organs. In the absence of such possibility, the corpses are stored in the refrigerator for no more than 1 day.

Experimental study of infected animals with autopsy is performed in the following sequence:

– preparation of the animal for opening;
– fixation of the corpse;
– inspection of external coverings;
– opening and examination of the chest cavity;
– conducting a study of the peritoneal cavity;
– preparing smears-imprints;
– selection of pathological material for seeding on nutrient media.

According to the stages of autopsy, the state of the outer coverings of the body, subcutaneous fat, lymph nodes; presence of exudate in the cavities of the pericardium and pleural (pericarp), appearance of the chest (color, size, consistency of the heart and lungs), the presence of inflammation, hemorrhage; in the abdomen – the presence of exudate, the appearance of the liver, spleen, adrenal glands, mesenteric lymph nodes are noted in the protocol.

Algorithm *"Preparation of an animal corpse for opening":*

Warning! Always observe the safety precautions when working with laboratory animals!

put on rubber gloves;

place the animal in a glass or metal jar;

moisten a cotton swab with ether or chloroform and place it in a jar with the animal;

close the jar with a lid without holes or a towel, hold for 3–5 minutes;

remove the animal's corpse from the jar with long tweezers, place it on a preparation board or tray filled with paraffin.

Algorithm *"Carrying out the opening of an animal's corpse"* (*Fig. 6*):

cover the surface of the preparation board with a gauze soaked in 3 % chloramine solution;

put the body of the mouse on the gauze belly up;

fix the mouse pads with preparatory needles;

take a cotton ball with tweezers, moisten it with 3% chloramine solution, wipe the surface of the animal body;

tighten the skin with surgical forceps;

cut the skin with scissors in a straight line from the pubis to the mandible and to each paw;

Separate (from the Lat. *separatio* - separation) pieces of skin, pulling them with surgical forceps and clipping with a scalpel;

Warning! When separating the skin, it is necessary to expose the anterior and lateral walls of the chest and the walls of the abdominal cavity; inguinal lymph nodes are separated along with the skin, the submandibular and cervical remains on the muscles of the neck.

wipe the tools with a cotton ball soaked in water, place the ball in a jar with disinfectant solution;

lower the instruments in a jar with alcohol, remove them and burn them in the flame of the alcohol;

make two mutually perpendicular cuts of the peritoneum with scissors and open the abdominal cavity;

cut the pectoral muscles, ribs on both sides between the bone and cartilage with scissors, cut the sternum and drop the muscle and bone piece on the animal's neck;

wipe the instruments with water-soaked cotton wool, put them in a jar with alcohol.

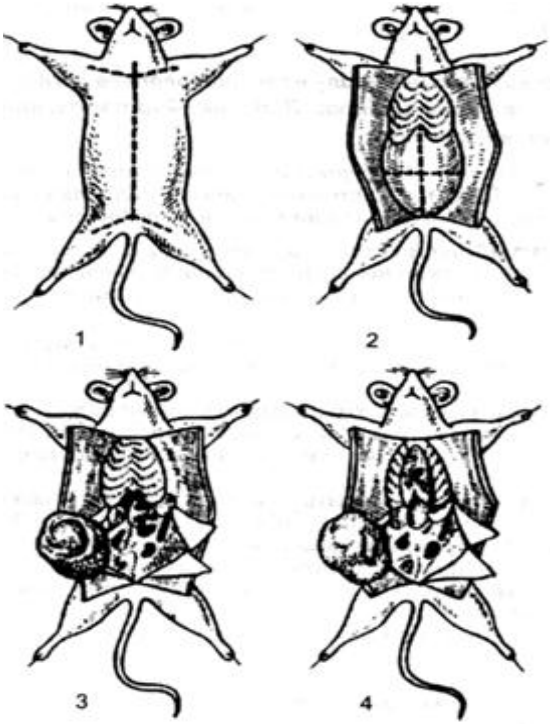

Fig. 6. Stages of dissection of an animal:
1 – skin incision; 2, 3 – section of abdominal cavity, 4 – section of the chest cavity

Warning! In parallel with the opening the corpse of the animals keep a protocol of the opening. The protocol indicates the age of the animal, the number, time and place of infection, the material that was infected, the time of death of the animal (or time of killing).

Algorithm *"Selection of section material, preparation of smears-imprints, seeding section material on a nutrient medium":*

burn anatomic forceps and scalpel in an alcohol flame;

Warning! Do not use surgical forceps, it destroys tissues!

take the organ with tweezers, cut a piece with a scalpel;

make smears-imprints of organ pieces on one slide in the following order: lymph nodes (4 prints), lungs (3 prints), spleen (2 prints), liver (1 print);

lower a piece of lungs into a glass of water (a piece of healthy lungs floats on the surface of the water; a piece of patient lungs sinks);

light the top of the heart with a hot scalpel;

pierce it with a sterile Pasteur pipette, collect blood into the pipette;

apply 1 drop of blood to a slide;

seed 1-2 drops of blood into a test tube with sugar broth;

lower the pipette into a disinfectant solution (5% carbolic acid solution);

make a blood smear from the drop applied to the slide;

lower the slide with smear-imprints and a smear of blood into the Nikiforov solution for fixation;

remove the slides after 20 minutes, air dry;

stain smear-imprints with methylene blue;

stain smear of blood after Romanovsky;

lower the tools into a disinfectant solution;

remove the preparation needles, lower them into the disinfectant solution;

remove the animal's body with a gauze with tweezers, lower it into a disinfectant solution;

pour over the surface of the paraffin with alcohol and set it on fire;

clean up the workplace: hand tools and a tank with an animal corpse to an autoclave technician (the corpse can be burned)

wipe with a disinfectant solution the table surface;

remove the rubber gloves by twisting them, lowering them into a disinfectant solution;

put the Petri dishes wit culture in the thermostat;

Examine the smears and blood smears under the microscope.

Specific goals:

To apply biological research method for isolation from pathological material of infectious disease agent and to identify pathogenicity factors with registration of the research result.

Student should be able:

1. Prepare a laboratory animal for the experiment: mark, weigh, fix.
2. To conduct thermometry.
3. To infect, to open the carcasses of laboratory animals, to keep a protocol of the opening.
4. To make smears-imprints.
5. Selection and seeding section material on nutrient media.
6. To defuse the carcasses of animals after opening.

Theoretical questions:

1. What is a biological method for the diagnosis of infectious diseases.
2. Methods of infection of laboratory animals.
3. Methods of taking blood from a laboratory animal and obtaining from it defibrinated blood, citrate blood, serum, plasma.

Practical tasks performed in the class:

1. Preparation of the workplace.
2. Carrying out fixing, marking, weighing of laboratory animals.
3. Preparation of the animal (white mouse) for opening, carrying out the opening of the corpse, drawing up the protocol of the opening.
4. Preparation of smear-imprints.
5. Selection of section material for microbiological research, seeding it on nutrient media.
6. Carrying out disinfection of corpses after opening.
7. Registration of the protocol.

Short guidelines for working on a practical lesson:

At the beginning of the class, the level of preparation of students for the class is checked. Students study the structure and equipment of the vivarium, the sanitary and hygienic requirements for the keeping laboratory animals; rules for the care of laboratory animals, preparation of laboratory animals for experiment, methods of fixation of animals and methods of their infection, methods of blood collection in laboratory animals, treatment and separation of blood components.

At the end of the class, test control and analysis of the results of each student's independent work are conducted.

Targeted learning objectives:

1. The causes of the pathogenic properties of conditionally pathogenic bacteria are:

A. Biochemical properties of the strain.

B. Toxins of microorganisms.

C. The complex of properties of microorganisms and features of the human body.

D. Decrease in immunity of the macroorganism.

E. Adhesive properties of microbial cells.

2. Patient S. has acute gonorrhea. it is known from the anamnesis that the patient had previously suffered from gonorrhea and was completely cured. To which group of infections can a new disease be attributed?

A. Autoinfection. *C. Reinfection.* *E. Mix-infection.*
B. Relapse. *D. Superinfection.*

3. The factors of spread of pathogenic microorganisms in the macroorganism are:

A. Neuraminidase, hyaluronidase. *D. Exo- and endotoxin.*
B. Capsule substance and coagulase. *E. Spore.*
C. Flagella.

4. Patient B. is registered at the skin and venereological dispensary where he undergoes treatment for syphilis, re-infected with T. pallidum. Which group of infections can be attributed to this new disease?

A. Secondary *C. Reinfection* *E. Mix-infection*
B. Superinfection *D. Relapse*

5. To produce serum test tubes, you can:

A. Leave at room t° for 3–4 hours.
B. Leave in thermostat at t° = 37 °C for 20–30 minutes.
C. Leave in thermostat at t° = 37 °C for 2–3 hours.
D. Leave in the refrigerator for 12 hours.
E. Correctly 1.2.

6. Bacterial endotoxins are generally:

A. Plasma-soluble proteins *D. Cell membrane components*
B. Cell wall components *E. Nucleoid components*
C. Coded for by a temperate phage

7. The role of bacterial capsules as virulence factors is usually related to their ability to interfere with:

A. Antibody binding.
B. B lymphocyte activation.
C. Antibacterial penetration of bacterial cells.
D. Phagocytosis.
E. The release of interferon-γ and other macrophage-activating cytokines.

8. Which of the following bacterial enzymes attacks the intercellular cementing materials of human cells?

A. Myeloperoxidase *C. Hemolysins* *E. Catalase*
B. Streptokinase *D. Hyaluronidase*

9. Septicaemia is:

A. Pus in blood. *D. Presence of viruses in blood.*
B. Toxin in blood. *E. Multiplication of bacteria in blood.*
C. Presence of bacteria in blood.

10. Lowering virulence of bacteria is known as:

A. Pathogenicity. *C. Attenuation.*
B. Exaltation. *D. Toxigenicity.* *E. Communicability.*

11. Vertical transmission of bacteria means:

A. Transplacental transmission. *D. Transfusion transmission.*
B. Man to man transmission. *E. Inhalation.*
C. Animal to man transmission.

12. Endotoxin of Gram negative bacteria has all, EXCEPT:

A. Carbohydrate. *C. Lipid.* *E. All of the above.*
B. Protein. *D. Inorganic substance.*

13. All of the following are characteristics of endotoxins, EXCEPT:

A. Heat stable. *D. Produced by Gram negative bacteria.*
B. Action often enzymatic. *E. Protein-polysaccharide complex.*
C. Can not be toxoided.

14. The patient was taken to hospital with purulent wound infection. *S.aureus* and *P. aeruginosa* were isolated from wound. What type of infection can this disease be attributed to?

A. Reinfection. *C. Relapse.* *E. Mixed infection.*
B. Secondary infection. *D. Superinfection.*

15. Relapsing fever caused by *Borrelia causasica* is found only in certain territories where the carrier is a kind of mite Alectorobius. What is such infection called?

A. Mixed infection. *C. Secondary infection.* *E. Reinfection.*
B. Endemic. *D. Superinfection.*

16. The patient as a result of the activation of own microflora in the oral mucosa, there developed purulent inflammation of periodontal tissues. What type of infection can this disease be attributed to?

A. Autoinfection. *C. Endemic.*
B. Superinfection. *D. Mixed infection.* *E. Secondary infection.*

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