

Lesson 3

Ecology of microorganisms. Normal microbiota of the human organism. Microbiota of the oral cavity and saliva. Influence of physical, chemical and biological factors to microorganisms. Sterilization and disinfection. Bacteriophages

Ecology of microorganisms

- ▶ Microorganisms are found in soil, water, air, plants, humans and animals.
- ▶ Ecology of microorganisms (from the Greek oikos-home, habitat) studies the relationship of microorganisms with each other and with the environment

Ecosystem and its components

- ▶ An **ecosystem** is a biological system consisting of a community of living organisms, their habitat, a system of connections that exchange substances and energy between them.
- ▶ The **biotic components** of the ecosystem form biocenoses - microbial populations that differ in number and species composition.
- ▶ Abiotic components are the physical and chemical factors of the ecosystem in which organisms live.

Microorganisms living in an ecosystem

- ▶ All microorganisms living in the ecosystem are divided into two categories - autochthonous and allochthonous.
- ▶ **Autochthonous** microflora is a set of microorganisms that constantly live and reproduce in a particular ecosystem.
- ▶ (eg, soil, intestines). Such an ecosystem has all the conditions for
- ▶ the activity of these microorganisms.
- ▶ **Allochthonous** (zymogenic) microorganisms are microorganisms that are not capable of long-term existence in a particular ecosystem, since it does not have the necessary conditions for their existence.
- ▶ An example is bifidobacteria - permanent
- ▶ (autochthonous) intestinal microorganisms and fungi of the genus *Candida* -
- ▶ allochthonous intestinal microflora.

Types of relationships between microorganisms

- ▶ In the environment, as well as in the host organism, microorganisms form biocenoses in which they are in various relationships with each other. The coexistence of two different organisms (symbionts) is called **symbiosis**.
- ▶ There are several variants of symbiosis:
 - ▶ mutualism
 - ▶ antagonism
 - ▶ neutralism

Types of relationships between microorganisms

- ▶ Mutualism is a mutually beneficial relationship of different symbionts, which is beneficial for each of them. An example of a mutualistic symbiosis is lichens - a symbiosis of a fungus and blue-green algae (cyanobacteria).
- ▶ - **Metabiosis** - the relationship of microorganisms, in which one of them uses the waste products of another for its life.
- ▶ - **Commensalism** is the cohabitation of individuals of different species, in which one species benefits without harming the other.
- ▶ - **Satellism** - increased growth of one type of microorganism under the influence of another type
- ▶ **Antagonism** is expressed in the form of an adverse effect of one type of microorganism on another, leading to damage and even death.

Microorganisms in the environment Fundamentals of sanitary microbiology

- ▶ **Sanitary microbiology** - a branch of medical microbiology that studies microorganisms contained in the environment (soil, water, air, food, etc.) and the processes they cause
- ▶ **The main goal of sanitary microbiology** is to identify pathogens of infectious diseases.
- ▶ in the environment, implementation of measures to prevent
- ▶ environmental pollution by microorganisms, and
- ▶ as well as the prevention of infectious diseases.

Sanitary-indicative microorganisms

- ▶ Direct detection of pathogenic microorganisms in the environment is somewhat difficult, since their content in the external environment is relatively small, and the pathogenic microflora is unevenly distributed in the external environment.
- ▶ And therefore, sanitary-indicative microorganisms are used to indirectly determine the possible presence of pathogenic microorganisms in the environment. Each of the environmental objects (water, air, soil, food products, etc.) has sanitary-indicative microorganisms characteristic of it, by the number of which one can judge the sanitary condition of this object. SPMO:
- ▶ They live permanently in the human and animal body and are constantly released into the environment.
- ▶ Survive in the environment longer or similar to pathogens, and are unable to multiply in the environment

Normal microflora of the human body

- ▶ Most representatives of the normal microflora are harmless saprophyte commensals.
- ▶ Representatives of normal microflora can be found on the skin and mucous membranes of the upper respiratory tract, gastrointestinal tract and urinary tract, etc.
- ▶ The distribution of normal microflora in the mucous membranes is subject to a special "geographical specialization". For example, the distal sections of the mucous membranes that communicate with the external environment are richer in microorganisms.
- ▶ Many tissues and organs of the human body that do not communicate with the external environment do not contain microorganisms. They are sterile. These include blood, lymph, internal organs, brain, cerebrospinal fluid, etc.

Normal microflora of the human body

- ▶ Distinguish between permanent and transient microflora
- ▶ Permanent or resident (indigenous, autochthonous) is represented by microbes that are constantly present in the body.
- ▶ It is represented by obligate (bifidobacteria, lactobacilli, bacteroids, E. coli) and facultative (staphylococcus, streptococcus, klebsiella, clostridia) microflora
- ▶ Transient microflora (allochthonous) is not capable of long-term existence in the body

Microbiota of the oral cavity and saliva

Anatomical area	Microorganism	Morphological features
Oral cavity saliva and teeth	p.Streptococcus p. Lactobacillus p.Veilonella p.Bacteroides Fusobacteria p.Actinomyces	Gram(+) cocci in the form of chains Gram(+) sticks Gram(-) diplococci Gram(-) pleomorphic rods Gram(-) sticks Gram (+) sticks, or filamentous, forming mycelium

NORMAL MICROFLORA OF THE MOUTH

AUTOCHTHONIC

ALLOCHTHONOUS

**RESIDENT
(PERMANENTLY
RESIDENT)**

**TRANSITOR
(TEMPORARY PRESENT)**

**GET INTO THE ORAL
CAVITY FROM OTHER
BIOTOPES**

Factors affecting the formation of the microflora of the oral cavity

- ▶ The species composition of the microbial flora of the oral cavity is normally quite constant. However, the number of microbes can vary significantly. The following factors can influence the formation of the microflora of the oral cavity:
- ▶ 1) the state of the oral mucosa, structural features (mucosal folds, gingival pockets, desquamated epithelium);
- ▶ 2) temperature, pH, redox potential of the oral cavity;
- ▶ 3) secretion of saliva and its composition;
- ▶ 4) the condition of the teeth;
- ▶ 5) food composition;
- ▶ 6) hygienic condition of the oral cavity;
- ▶ 7) normal functions of salivation, chewing and swallowing;
- ▶ 8) natural resistance of the organism

The dynamics of the formation of microbiocenosis of the oral cavity

- ▶ The formation of microbiocenosis of the oral cavity is a multi-stage process of interaction of its various components. Colonization of the oral cavity by microbes depends on:
- ▶ from the ability of microorganisms to adhere to various surfaces, primarily to the epithelium and enamel;
- ▶ from the interrelationship of the metabolism of various groups of microorganisms.

The mechanism of formation of microbial associations

- ▶ To settle in the oral cavity, microorganisms must first attach to the surface of the mucous membrane or to the teeth. Adhesion (sticking) is necessary to ensure resistance to saliva flow and subsequent colonization (reproduction).
- ▶ Adhesion is mediated by bacterial surface adhesins and oral cavity epitheliocyte receptors, tooth enamel structures.
- ▶ Gram-negative bacteria may involve pili or fimbriae in the process of adhesion, while lipoteichoic acids may act as adhesins in Gram-positive bacteria.

The mechanism of formation of microbial associations

- ▶ Specific receptors of epithelial cells of the oral cavity are involved in the adhesion process (specific interactions are also present during adhesion to the surface of the teeth).
- ▶ Some bacteria do not have their own adhesins, then they are fixed on the surface of mucous membranes using adhesins of other microorganisms, i.e. there is a process of coaggregation between bacterial species of the oral cavity.
- ▶ Streptococci of different species coaggregate with actinomycetes, *F.nucleatum*, *Veillonella*, *Haemophilus parainfluenzae*. *F.nucleatum* binds to *Porphyromonas gingivalis*, *Haemophilus parainfluenzae* and *Treponema* spp.

Relationships in the microbial community of the cavity

mouth can be mutually beneficial and antagonistic and aimed at preserving homeostasis of the oral flora. On the microflora of the cavity mouth significantly influenced by the presence of food substrates, vitamins, ORP, medium pH, isolation reproductive inhibitors.

The normal composition of microorganisms in a given ecological niche is maintained largely due to antagonistic relationships between microbes.

P Defend airways against pathogens
(as competitors and as production
of bacteriocins)

Lungs

Brain

Indirect roles through:

- Activation of the vagus nerve
- Activation of the immune system
- Secretion of neurotransmitters

P Production of defensive anti-
microbial peptides

Skin

Gut

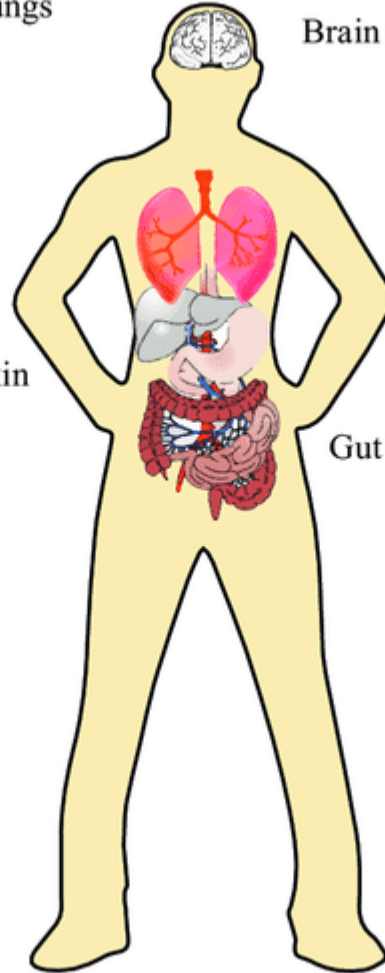
- S** Wound healing
- S** Inflammation control
- S** Immune system development

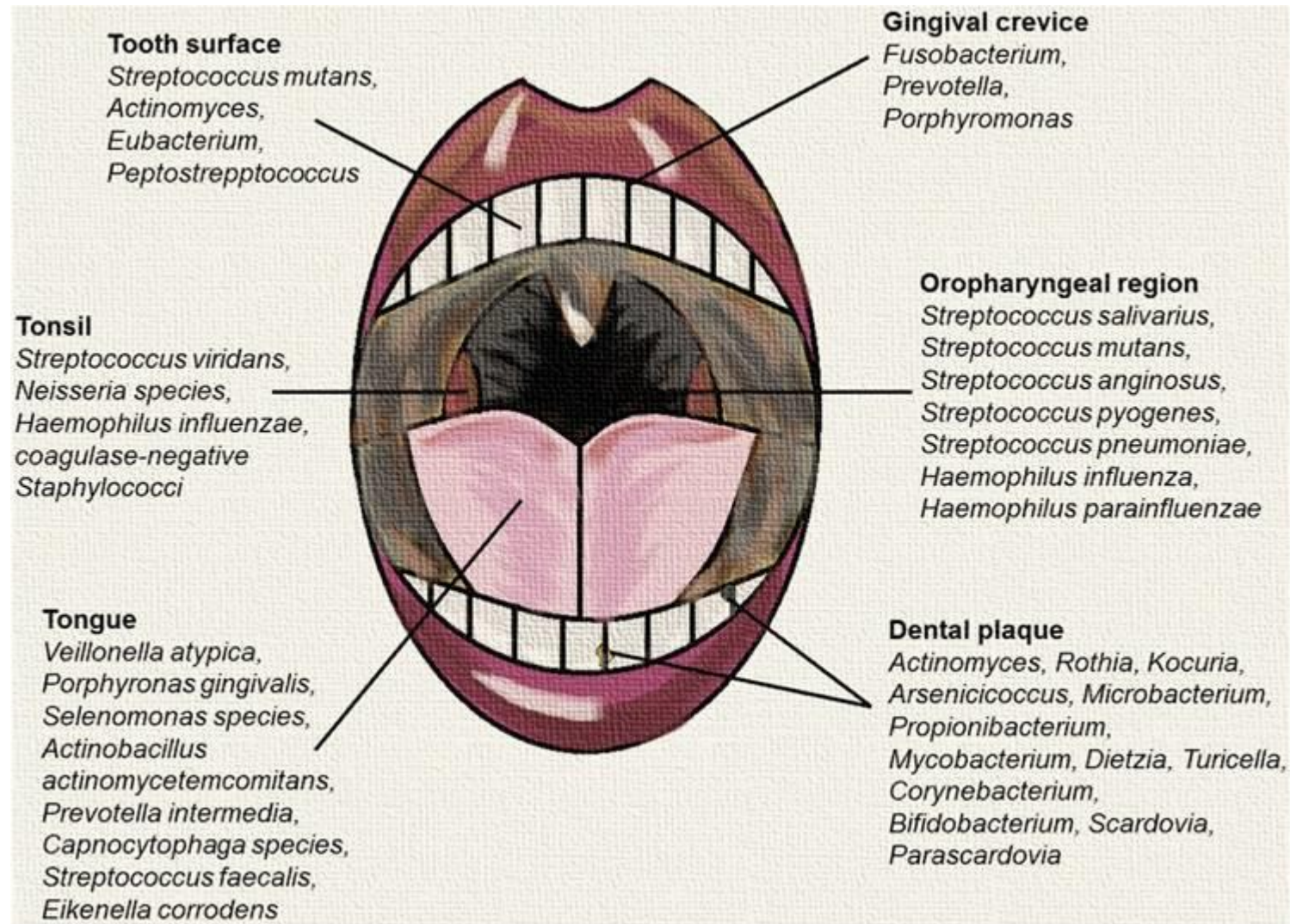
- P** Pathogen displacement
- P** Antimicrobial factors

- S** Barrier fortification
- S** Tightening of junctions
- S** Immune system development

- P** Protective functions
- S** Structural functions
- M** Metabolic functions

- M** Vitamins synthesis
- M** Metabolize carcinogens
- M** Ferment non-digestible residues
- M** IEC differentiation & proliferation
- M** Metabolite transformation and
absorption





Oral fluid (Saliva)

- ▶ Oral fluid is the most important biotope of the oral cavity, because. through it, the interaction between all biotopes of the oral cavity and the regulation of microflora by the macroorganism are carried out.
- ▶ Microbes constantly enter the oral fluid, multiplying on the oral mucosa, in the gingival groove, pockets, mucosal folds and in dental plaque (plaque). In the oral fluid, they remain viable for a long time, and many species, especially those that do not have adhesion factors to the mucous membrane and enamel, actively multiply.
- ▶ The oral fluid contains a significant amount of streptococci (*S. salivarius*), neisseria, veillonella. In addition, mobile species are found - vibrios, spirilla and spirochetes.

The composition and properties of oral fluid

- ▶ Organic components - proteins, carbohydrates, free amino acids, enzymes, vitamins, etc. The main organic substance is protein
- ▶ By origin, they are divided into 2 groups: 1) entering the saliva from the blood serum (amino acids, urea)
- ▶ 2) synthesized by glands (amylase, glycoproteins, mucin, immunoglobulins)

Composition and properties of the oral fluid. Enzymes

- ▶ Enzymes - carbohydrases, esterases, proteases, transferases
- ▶ According to their origin, they are divided into 3 groups:
 - ▶ 1. Secreted by salivary glands
 - ▶ 2. Formed as a result of the enzymatic activity of bacteria
 - ▶ 3. Formed as a result of the breakdown of leukocytes in the oral cavity

The composition and properties of oral fluid

- ▶ Amylase - hydrolyzes carbohydrates, turning them into dextrans and maltose.
- ▶ Phosphatase (acid and alkaline). Alkaline is involved in phosphorus-calcium metabolism, detaching phosphate from phosphoric acid compounds and transferring it to bones and teeth.
- ▶ Hyaluronidase and kallikrein - enzymes that change tissue permeability
- ▶ Proteases (elastase) - are involved in the development of the inflammatory process

The role of the microflora of the oral cavity in the nonspecific resistance of the body

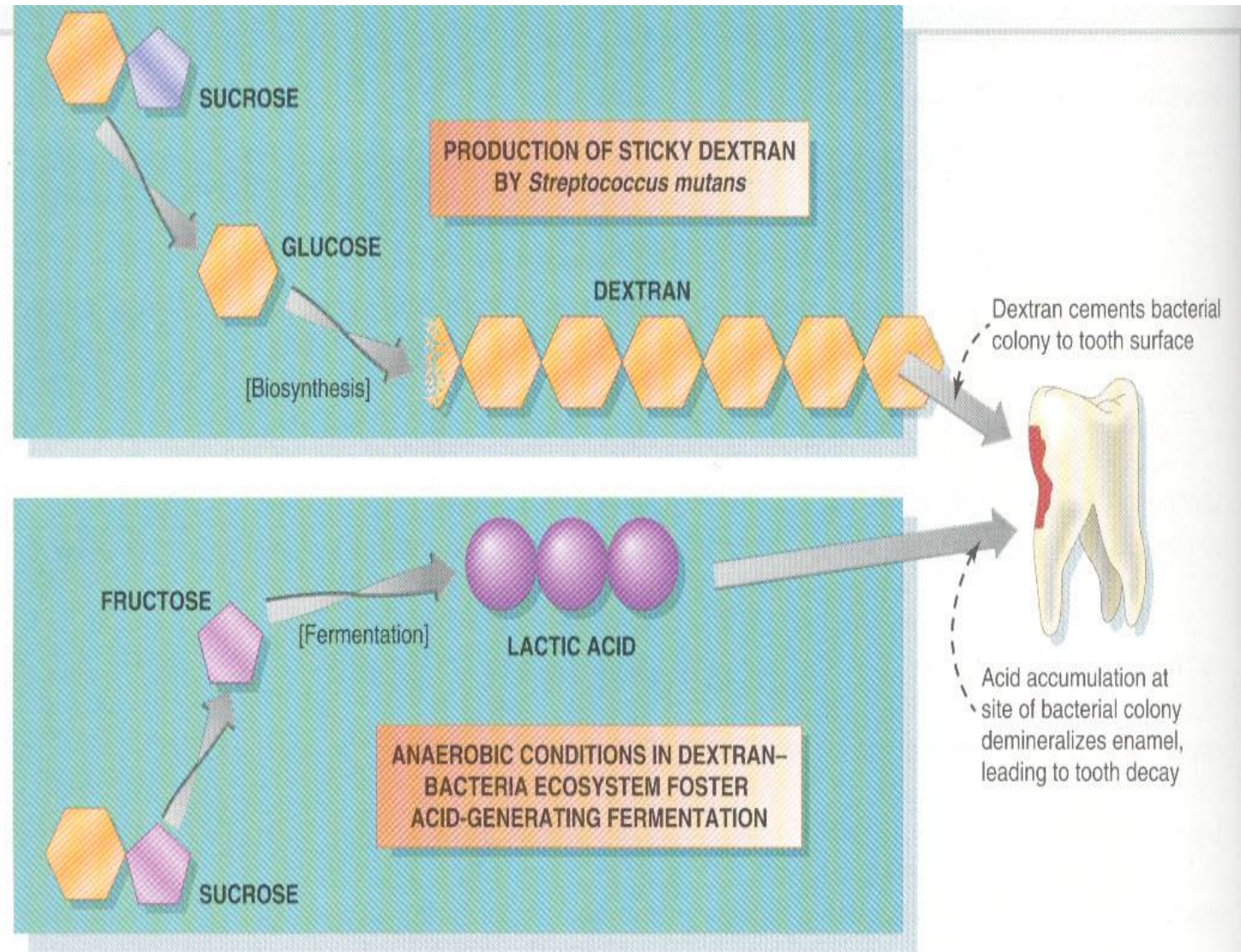
- ▶ The oral cavity, its mucous membrane and lymphoid tissues of the maxillofacial region play a crucial role in the interaction of the human body with the environment. Microorganisms living in the oral cavity contribute to the digestion of food and the synthesis of vitamins, on the one hand, and on the other hand, form products that damage tooth tissues.
- ▶ The oral cavity contains more than 700 types of microorganisms
- ▶ 1 ml of saliva contains up to 10^9 microorganisms
- ▶ Microbes of the oral cavity are antagonists of pathogenic flora, and at the same time can cause the development of serious diseases.
- ▶ The spectrum of microflora may vary due to hygiene habits, dental condition, age.

The role of the normal microflora of the oral cavity

1. has an antagonistic effect in against various pathogenic bacterial species, entering the oral cavity.
2. stimulates the development of lymphoid tissue
3. supports physiological inflammation in mucous membrane and increase readiness for immune reactions
4. provides self-cleaning of the oral cavity
5. Promotes the supply of the body amino acids and vitamins that secreted m / o during metabolism
6. waste products of microorganisms can stimulate the secretion of salivary and mucous membranes glands
7. are pathogens and main culprits major dental diseases.



The etiological role of microorganisms in the development of caries



The value of normal microflora

- ▶ Most representatives of normal microflora, especially obligate ones, have antagonistic activity against pathogenic and opportunistic microorganisms.
- ▶ This activity is associated with the ability to produce organic acids (lactic, acetic, etc.), antibiotics, bacteriocins, and other compounds that prevent the colonization of pathogenic microorganisms.
- ▶ Thus, the microflora is involved in colonization resistance, which is a combination of the protective properties of the body and the competitive properties of the normal intestinal microflora, which give stability to the microflora and prevent the colonization of the body by foreign microbes.

The value of normal microflora

- ▶ Normal microflora is an important factor in innate immunity. Antigens of microflora non-specifically stimulate the immune system
- ▶ Normal microflora induces the synthesis of antibodies that can be found in the serum of healthy people.
- ▶ Intestinal normoflora is involved in water-salt metabolism, metabolism of proteins, carbohydrates, fatty acids, in the production of biologically active compounds (antibiotics, vitamins (K and group B))

The value of normal microflora

- ▶ The significance of the microflora was established after microbial-free gnotobiont animals were obtained.
- ▶ Animals-gnotobionts are kept in special microbial-free conditions (sterile air, food, water are supplied).
- ▶ Underdevelopment of the main immunocompetent organs in gnotobionts (for example, thymus, intestinal lymphoid tissue) makes these animals susceptible to infections and unable to survive under normal conditions.

The influence of environmental factors on microorganisms

- ▶ Environmental factors affect the vital activity, growth, reproduction and death of microorganisms.
- ▶ Factors affecting microorganisms are divided into physical, chemical and biological
- ▶ The effect of these factors may be different depending on their nature and characteristics of microorganisms. For example, the influence can be detrimental or favorable to the growth of microbes.

The influence of physical factors on microorganisms

- ▶ Temperature. In relation to temperature, all microorganisms are divided into three groups:
- ▶ Psychrophilic (from Greek psychros - cold, phileo - love) microorganisms
 - ▶ - minimum t - 00C, optimal - 6-200C, maximum - 300C
- ▶ Mesophilic (from Greek mesos - medium) microorganisms
 - ▶ - minimum t - 100C, optimal - 34-370C, maximum - 450C
- ▶ Thermophilic (from the Greek termos - heat, heat) or heat-loving microorganisms develop at temperatures above 55 ° C
 - ▶ - minimum t - 300C, optimal - 50-600C, maximum - 70-750C

The influence of physical factors on microorganisms

- ▶ Drying leads to dehydration of the cytoplasm, disruption of the integrity of the cytoplasmic membrane, as a result of which the nutrition of microbial cells is disturbed and their death occurs.
- ▶ For example, pathogenic *Neisseria* (meningococcus, gonococcus), *leptospira*, *treponema pallidum*, etc. die when dried after a few minutes. *Vibrio cholerae* can withstand drying for 2 days, *salmonella typhoid* - 2 months, and *Mycobacterium tuberculosis* - up to 3 months.
- ▶ For the storage of cultures of microorganisms, vaccines and other biological preparations, the freeze-drying method is widely used. The essence of the method lies in the fact that microorganisms or preparations are first subjected to freezing, and then they are dried under vacuum. At the same time, microbial cells enter a state of suspended animation and retain their biological properties for several months or years.

The influence of physical factors on microorganisms

- ▶ Beam energy. In nature, microorganisms are constantly exposed to solar radiation. Direct sunlight causes the death of many microorganisms within a few hours.
- ▶ The destructive effect of sunlight is due to the activity of ultraviolet rays (UV rays) with a wavelength of 254-300 nm. They inactivate cell enzymes and damage DNA. Pathogenic bacteria are more sensitive to UV rays than saprophytes.
- ▶ Other types of radiant energy - X-rays, α -, β -, γ -rays have a detrimental effect on microorganisms only in large doses, about 440-280 J/kg. The death of microbes is due to the destruction of nuclear structures and cellular DNA. Small doses of radiation stimulate the growth of microbial cells.
- ▶ The bactericidal effect of ionizing radiation is used for the preservation of certain foodstuffs, sterilization of biological preparations (sera, vaccines, etc.)

The influence of physical factors on microorganisms

- ▶ Ultrasound is sound waves with frequencies above 20,000 Hertz. One of the main effects of the influence of ultrasound on microorganisms is the effect of cavitation (from Latin cavitum - cavity).
- ▶ Ultrasound causes significant damage to the microbial cell. Under the action of ultrasound, the gases in the liquid medium of the cytoplasm are activated, high pressure arises inside the cell (up to 10,000 atm.) And cavitation cavities are formed. This leads to rupture of the cell membrane and cell death.
- ▶ Ultrasound is used to sterilize food products (milk, fruit juices), drinking water.
- ▶ High pressure . High atmospheric pressure is not detrimental to most microorganisms. In nature, there are even bacteria living in the seas and oceans at a depth of 1000-10000 m under pressure from 100 to 900 atm. Some types of bacteria can withstand pressure up to 3000-5000 atm, and bacterial spores - even 20,000 atm.
- ▶ It is noteworthy that exposure to saturated water vapor at a pressure above atmospheric leads to the death of both vegetative and spore forms of microorganisms. This method of sterilization with steam under pressure is carried out in an autoclave.

Disinfectants and antiseptics

- ▶ Surfactants-fatty acids, soaps and other detergents (decamine, chlorhexidine, etc.)
- ▶ Phenol, cresol and their derivatives (tricresol, phenol-resorcinol, phenyl salicylate)
- ▶ Oxidizing agents (hydrogen peroxide, potassium permanganate, etc.)
- ▶ Halogens-iodine preparations (iodine alcohol solution, Lugol's solution, iodoform, iodinol), chlorine preparations (bleach, chloramine, pantocid)
- ▶ Alcohols (ethyl, etc.)
- ▶ Acids and their salts (boric, salicylic, benzoic, acetic acids) and alkalis (ammonium salts, alum);
- ▶ Aldehydes (formaldehyde-used in the form of a 40% solution (formalin), hexamethylenetetramine-urotropine, glutaraldehyde, etc.)
- ▶ Heavy metal salts (mercury, lead, zinc, gold, etc.)
- ▶ Dyes (brilliant green, rivanol, ethacridine lactate, methylene blue, etc.)

Influence of chemical factors on microorganisms

- ▶ Disinfection (disinfection) is the destruction of pathogenic microorganisms in various environmental objects. Chemicals used to kill microorganisms are called disinfectants.
- ▶ Substances that have a pronounced antimicrobial effect, but do not have toxicity to the macroorganism, are called antiseptics and are used to kill or suppress the growth of microbes that come into contact with the surface of the skin, mucous membranes and wounds.
- ▶ Antiseptics - a set of measures aimed at the destruction of microorganisms in the wound, the whole organism or on environmental objects, using various disinfecting chemicals.
- ▶ Antiseptics include a set of measures aimed at the destruction of microbes in a pathological focus, wound or other object.
- ▶ Asepsis is a set of preventive measures that prevent microbial contamination of various objects (wounds, surgical fields, skin and mucous membranes, etc.).

STERILIZATION

- ▶ Sterilization is the complete release of environmental objects from microorganisms and their spores.
- ▶ Sterilization is carried out in various ways:
- ▶ Physical (exposure to high temperature, UV rays);
- ▶ Chemical (use of various disinfectants, antiseptics and antibiotics);
- ▶ Mechanical (use of bacterial filters)

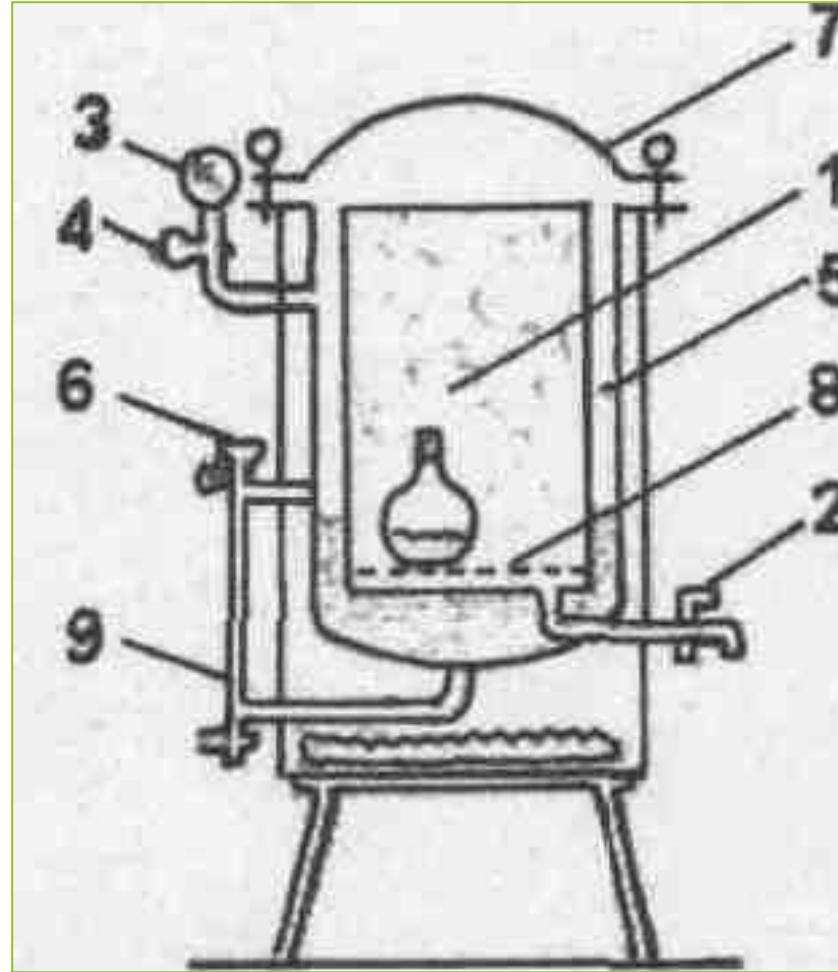
Sterilization by physical methods (thermal sterilization)

- ▶ Boiling sterilization and calcination can be considered the simplest and most affordable methods of heat sterilization.
- ▶ For heat sterilization, mainly dry heat and steam under pressure are used.
- ▶ Sterilization by dry heat or hot air is carried out in Pasteur ovens (drying ovens) at 165-170°C for 1 hour. The method allows you to destroy not only vegetative cells, but also spores of microorganisms.
- ▶ Steam sterilization under pressure is carried out in steam sterilizers (autoclaves), the method is based on exposing the materials to be sterilized to saturated water vapor at a pressure above atmospheric. In the operating mode at 2 atm. at 121°C for 30 min. both vegetative and spore forms of microorganisms die. Pasteurization can be conditionally considered sterilization. As a result of an hour exposure at 65-70°C, vegetative forms of microorganisms in food products (milk, juices, wine, beer, etc.) are destroyed.

AIR STERILIZER



Working principle of the autoclave

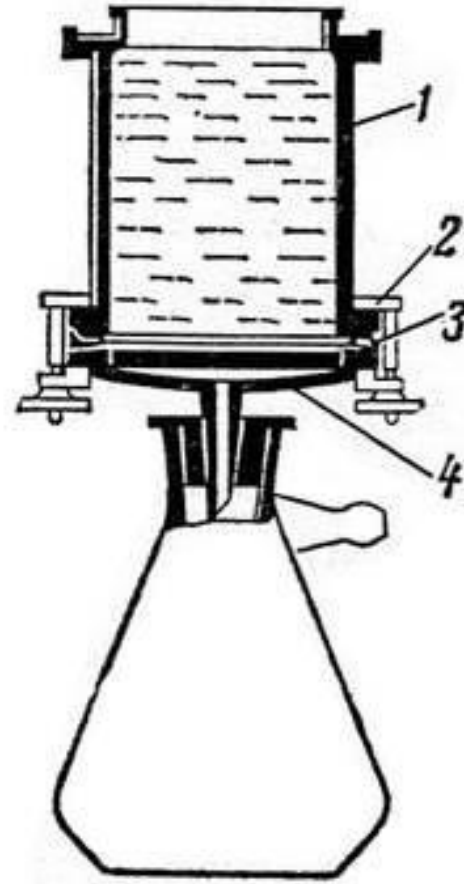


Physical sterilization (radiation sterilization)

- ▶ It is used for sterilization of thermolabile materials.
- ▶ The use of UV radiation for sterilization is limited by its low permeability and high absorption activity of water and glass.
- ▶ X-ray and gamma radiation. Working with them requires strict adherence to safety rules. They are used for sterilization of bacteriological preparations (sera, vaccines, etc.) of disposable syringes, Petri dishes, suture materials, etc.
- ▶ microwave radiation. Based on the effect of rapid temperature rise, used to re-sterilize long-term stored media.

Mechanical sterilization

- ▶ Sterilization through bacterial filters is used in cases where the objects to be sterilized change when heated.
- ▶ In microbiological practice, Seitz asbestos filters, nitrocellulose membrane filters, chamberland filters (candles) made from kaolin with an admixture of sand and quartz, and filters from Berkefeld diatomaceous earth are used.
- ▶ The filtration method sterilizes nutrient media containing protein, serum, some antibiotics, and also separates bacteria from viruses, phages and exotoxins.



Chemical sterilization

- ▶ Chemical sterilization is carried out using antimicrobial preparations that are detrimental to microorganisms - disinfectants and antiseptics, as well as selective antibiotics and synthetic antimicrobials.
- ▶ For this purpose, toxic gases are also used, for example, ethylene oxide

Quality control of sterilization

- ▶ Chemical control - substances with a known melting point are used (sulfur - 1190 C, benzoic acid - 120-1220 C, benzonaphthol - 1100 C, mannose and urea - 132-1330 C) and temperature indicator papers. The assessment of control is carried out on the basis of changes occurring with the indicated substances, which are placed in the autoclave along with the material to be sterilized.
- ▶ Biological control - carried out using biotests (paper strips with temperature-resistant spore bacteria applied to the surface). The assessment of control is carried out on the basis of the death of spore bacteria on the surface of the pieces of paper, which are placed in the autoclave along with the material to be sterilized.

Asepsis and antisepsis



Asepsis

- ▶ Sterilization of linen and dressings is carried out in autoclaves, to control sterilization, benzoic acid and pyramidon or resorcinol are collected in a test tube, and if at the end of sterilization these substances melt and turn into a mass, this means that sterilization has been carried out properly.
- ▶ Sterilization of surgical instruments is carried out in special sterilizers, but first they are washed mechanically, then placed in a container with boiling water, 1-2% soda solution is added, boiled for 20-30 minutes
- ▶ In order to prevent airborne infections, persons entering the operating room must wear masks and shoe covers. Conversations are strictly prohibited. It is advisable to use germicidal lamps

Antiseptic

- ▶ Antiseptic is a system of measures that helps to reduce and destroy microbes in the wound. There are mechanical, physical, chemical, biological antiseptics.
- ▶ Mechanical antiseptics - the primary surgical treatment of an infected wound, i.e., the removal of dead and non-viable tissues from the edges and from the bottom of the wound.
- ▶ Physical antiseptic - carried out using hygroscopic cotton swabs, drying swabs, powders, drainage, etc. in order to prevent the multiplication of microbes and the accumulation of toxic substances in the wound

Antiseptic

- ▶ Chemical antiseptics - the use of chemicals that create unfavorable conditions for microbes, stop the growth, development or cause their death. Most commonly used:
- ▶ Silver nitrate 1:500-1:3000 for washing, 1% brilliant green solution - for treating wounds, 1% iodine, iodinol - for washing wounds, surgical field, for washing hands, 2-5% solution of carbolic acid - for washing gloves and tools, formalin (40%), furacillin (1:5000), chloramine (0.5-2%), etc.
- ▶ Biological antiseptics - the use of injections (in / in, in / m, into various cavities), inhalations, as well as by introducing antibiotics on the surface or inside the wound

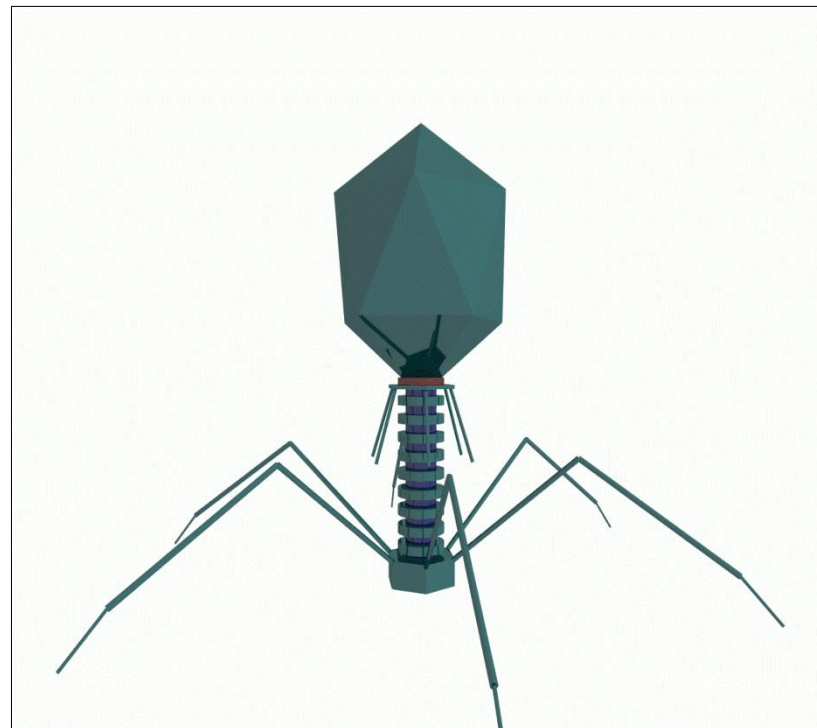
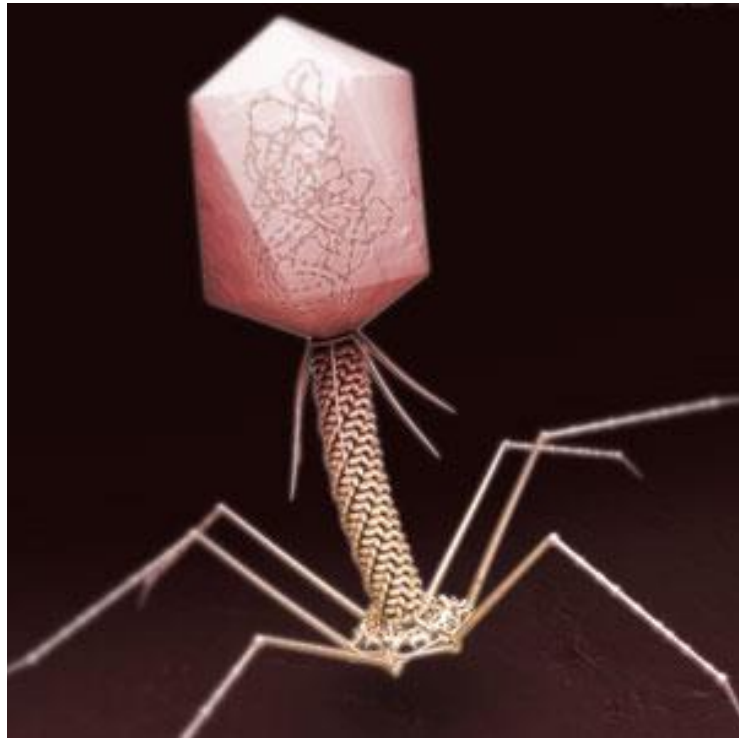
Bacteriophages

- ▶ Phages are widely distributed in nature, they are able to parasitize in the cells of bacteria and other microorganisms, contributing to their death (lysis).
- ▶ In 1917, Fr. the scientist F. D'Herelle observed that when the feces filtrate of a patient with dysentery is added to the broth culture of dysentery bacteria, their complete lysis occurs.
- ▶ F. D'Herelle concluded that the lytic agent he observed, passing through bacterial filters, is a virus of bacteria, and called them "bacteriophage" (eater of bacteria).

The structure of bacteriophages

- ▶ Phage sizes range from 20 to 800 nm. They are divided into several morphological types: filamentous, cubic, spermatozoon, etc.
- ▶ The most studied coliphages are T (from the English type - typical). There are 7 representatives of T group phages, among which there are single (T1, T3, T5, T7) and paired phages (T2, T4, T6).
- ▶ T2 phages have the most complex structure

Scheme of the structure of bacteriophages



The nature of the interaction with the bacterial cell

- ▶ Depending on the type of interaction with a bacterial cell, virulent and temperate bacteriophages are distinguished.
- ▶ As a result of the interaction of virulent phages with a bacterial cell, bacterial lysis occurs.
- ▶ This process is characterized by the clarification of the broth culture, i.e. the formation of a phagolysate. In cultures growing on a dense nutrient medium, bacterial lysis sites appear as negative phage colonies.

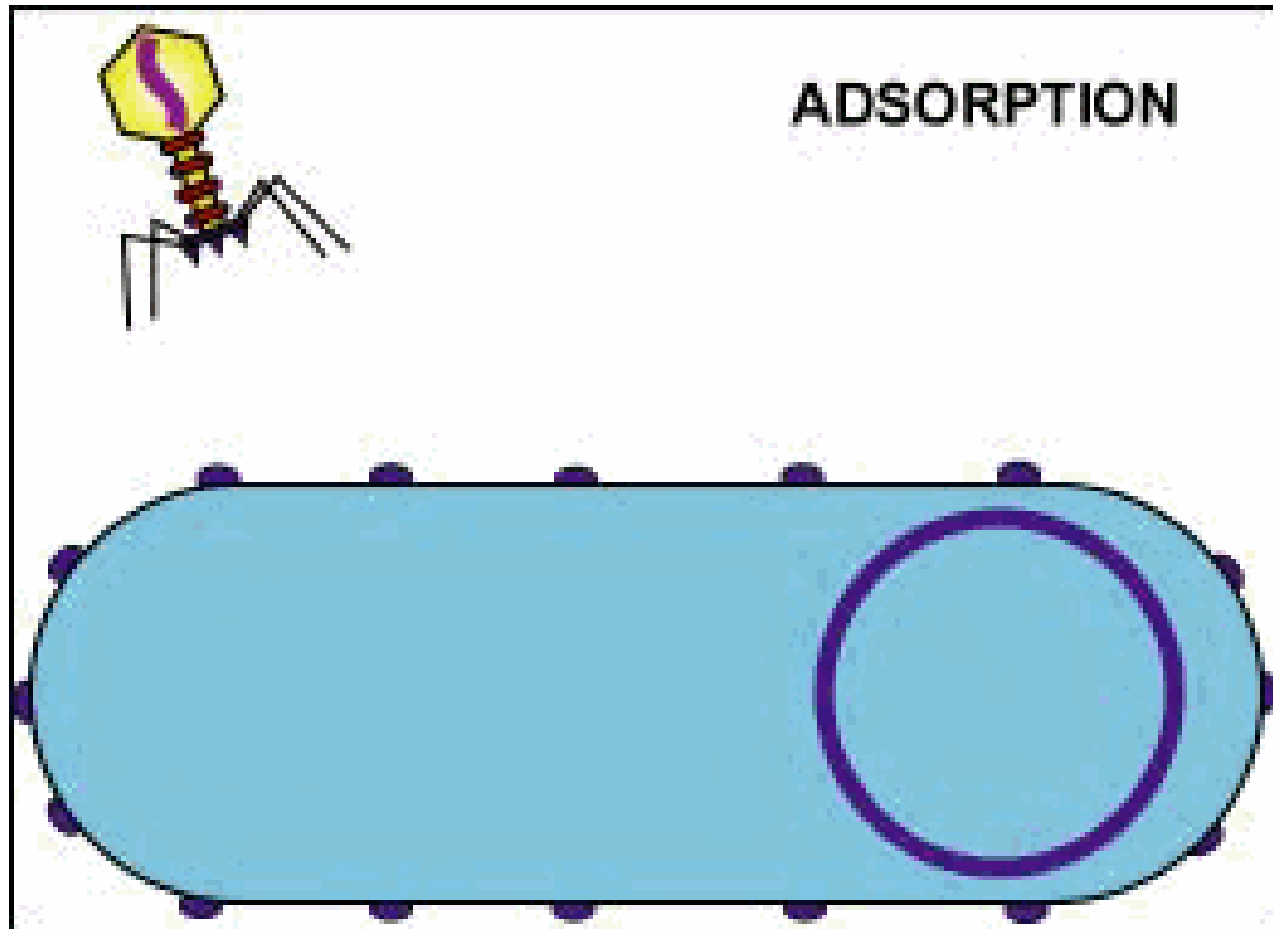
Interaction of virulent phages with a bacterial cell

1. Adsorption of phages on a bacterial cell
2. Penetration of the phage nucleic acid into the bacterial cell
3. Nucleic acid replication and phage protein synthesis
4. Phage particle formation
5. The exit of the phage from the bacterial cell

Interaction of temperate phages with a bacterial cell

- ▶ After the penetration of a temperate phage into a bacterial cell, the phage DNA is integrated into the chromosome of the bacterium and exists with it, that is, an integrative infection develops. Cell death does not occur.
- ▶ The DNA of a bacteriophage embedded in the chromosome of a bacterium is called a prophage.
- ▶ Such coexistence of a bacterium and a temperate bacteriophage is called lysogeny, and a bacterial culture infected with such a phage is called lysogenic.
- ▶ Prophages of some part of lysogenic bacteria can be excluded from the chromosomes and go into a vegetative state. This process ends with the production of phages and the lysis of bacteria.
- ▶ The transformation of a temperate phage into a virulent one is possible under the influence of various factors, for example, ionizing radiation, UV rays, etc.

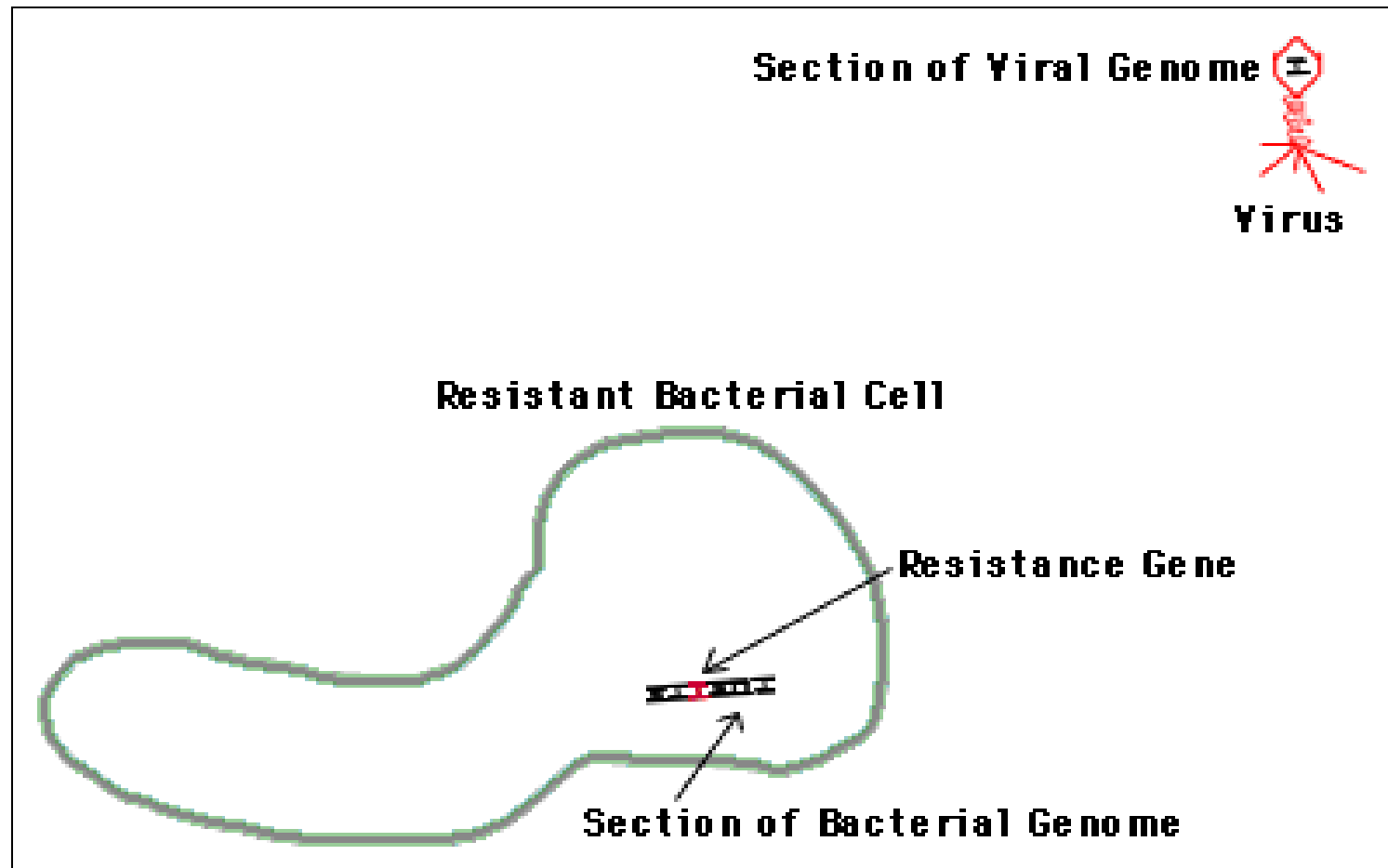
Interaction of phages with a bacterial cell



Defective phages

- ▶ Defective phages are formed as a result of fragmentation of bacterial DNA after phage infection and insertion of a piece of bacterial DNA into the DNA of the phage.
- ▶ Defective phages carrying a piece of bacterial DNA in the genome, when integrated into the genome, can impart new (morphological, cultural, biochemical, toxigenic, etc.) properties to bacteria. This phenomenon of changing properties under the influence of a prophage is called phage or lysogenic conversion.
- ▶ For example, the toxigenicity of the causative agent of diphtheria is due to the presence of the tox gene, the source of which is a lysogenic bacteriophage integrated into the same state.
- ▶ Defective bacteriophages are used as a vector in genetic engineering

Defective phages



Obtaining bacteriophages

- ▶ The test material (water, feces, wound discharge) is suspended and filtered. The filtrate and the homologous test culture are inoculated into nutrient broth and incubated at 37°C for 18-24 hours.
- ▶ The inoculum is then centrifuged and filtered to remove bacteria.
- ▶ The filtrate and test culture are plated on agar plates and incubated. As the bacterial culture grows, spots (negative colonies) appear on the agar.
- ▶ The material taken from the negative colonies is transferred into a test tube with broth, a test culture is added to it and incubated. Phages reproducing inside bacteria cause their lysis, in a test tube a phagolysate is obtained, consisting of numerous phages and completely freed from bacteria.

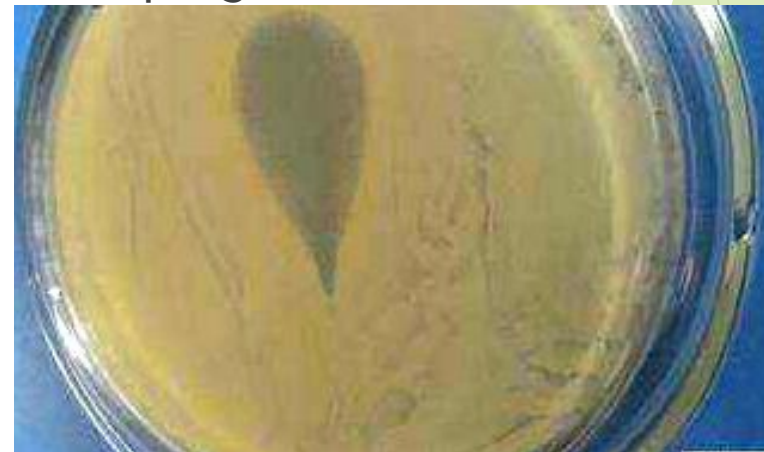
Determination of the sensitivity of bacteria to phages by the method of "flowing drop"

The determination of sensitivity to phages is based on the strict specificity of their action.

The use of diagnostic (known) phages makes it possible to identify an unknown culture of microbes

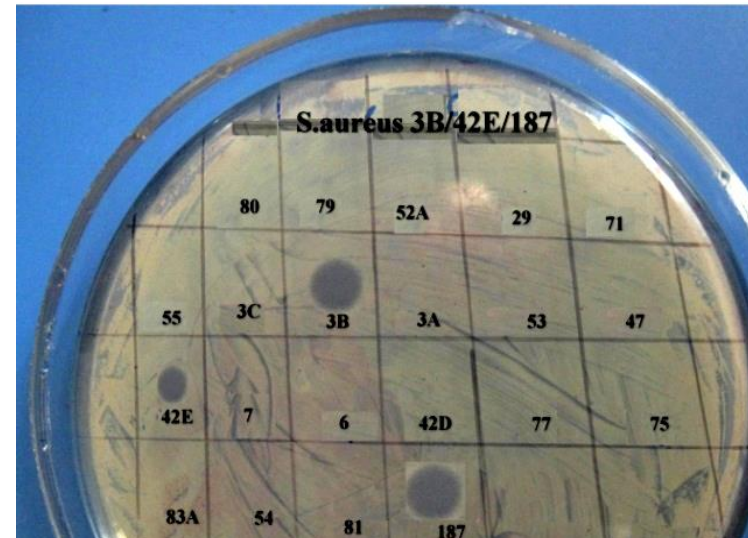
The studied bacterial culture is seeded with a lawn on the surface of a dense nutrient medium in a Petri dish. Then, a suspension of a known phage is applied to the surface of the agar, and by tilting the dish, the liquid spreads. Cups are incubated in a thermostat.

The sensitivity of the studied culture to the phage is judged by the presence or absence of a zone of lysis in the area of contact with the phage.



Definition of phage type (phage type)

- ▶ Bacterial phage type is determined to identify the source of infection
- ▶ The test daily broth culture is seeded on a dense nutrient medium in a Petri dish, the back surface of which is divided into squares.
- ▶ One drop of various type-specific phages is applied to each square with a Pasteur pipette.
- ▶ After daily incubation, the cup is examined, marking those squares in which bacterial lysis is observed. The phage type of a bacterial culture is determined by the type of phage that caused the lysis.



Practical applications of phages

- ▶ The specificity of phages forms the basis of phage diagnostics
- ▶ - The use of diagnostic phages allows identification of an unknown microbial culture
- ▶ - Phage typing (phage typing) is used to identify the source of the disease
- ▶ Phage prophylaxis and phage therapy is based on the ability of phages to destroy bacteria that are sensitive to them in the patient's body. For this purpose, phages are produced in the form of drugs.

Phagodiagnosics

