AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 9.

Physiology of microorganisms. Metabolisms of microorganisms, nutrition, nutrient media. Action of physical and chemical factors on microorganisms. Sterilization and disinfection

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

- Physiology of microorganisms,
- Chemical composition of microorganisms.
- Bacterial metabolism: anabolism and catabolism
- Nutrition of bacteria, types of nutrition: carbon (autotrophic and heterotrophic), energy phototrophic, chemotrophic), electronic source (lithotrophs and organotrophs), nitrogen (aminoautotrophic and aminoheterotrophic) sources, growth factors, saprophytes, parasites.
- Mechanism of nutrition: passive (simple and facilitated diffusion), active transport, translocation
- Nutrient media: classification by composition (natural, synthetic), consistency (liquid, semi-liquid, solid) and purpose (universal, special, elective, differential-diagnostic)
- The physical factors effects on microorganisms: temperature, drying, radiation energy (light, ultraviolet, radioactive rays), ultrasound, pressure.
- The chemical factors effect on microorganisms, disinfection.
- The main groups of disinfectants used in microbiological practice (surfactants, phenols, oxidizers, halogens, heavy metal salts, acids, alkalis, alcohols, dyes, etc.).
- The sterilization methods: physical, chemical, mechanical.
- The concept of asepsis and antiseptics (mechanical, physical, chemical, biology).

Purpose of the lesson:

- To acquaint students with the physiology, metabolism, nutrition of microorganisms, to inform them about the types of nutrition, mechanisms and nutrient media. To teach them the effect of various factors on microorganisms, to explain sterilization, sterilization methods, disinfection, disinfection methods, the main groups of disinfectants.

Physiology of microorganisms

The physiology of microorganisms studies their metabolism, nutrition, respiration, growth and reproduction, and all vital processes in general.

Chemical composition of microorganisms

- Microorganisms are chemically composed of inorganic and organic substances.
- **Organic compounds** include proteins, carbohydrates, lipids and nucleic acids, while **inorganic compounds** include water and minerals.
- In general, 80-85% of a microbial cell consists of water and 15-20% of dry matter.

The composition of the dry matter:
protein - 50-75%;
carbohydrate - 10-25%;
lipid - 0.2 - 40%;
RNA– 16%;
DNA– 3%;
mineral– 3%;

Energy metabolism (biological oxidation)

- There are two types of biological oxidation (energy metabolism), depending on whether they are oxygen-free and oxygen-free:
- fermentation metabolism
- oxidative metabolism

Metabolism consists of two opposite processes - catabolism and anabolism

- ***Catabolism*** *is the process of breaking down large molecules into smaller molecular compounds with the release of energy. The resulting energy is stored in the molecules of adenosine triphosphate (ATF) in the form of macroenergetic bonds and is used for vital processes. Therefore, catabolism is sometimes called* ***energy metabolism****.*
- *In the process of* ***anabolism****, large-molecule compounds used in cell construction are synthesized, so it is sometimes called* ***constructive metabolism.*** *This process involves the consumption of energy, which uses the energy released as a result of energy metabolism.*

Types of nutrition of microorganisms

- Different types of nutrition are distinguished in microorganisms due to their absorption of **carbon** and **nitrogen**
- According to the properties of carbon uptake, microorganisms are divided into two types - **autotrophs** and **heterotrophs**

Autotrophs

- Autotrophs (Greek, ***autos*** - self, **trophe** - nutrition) can use simple inorganic compounds - mainly carbon dioxide and other inorganic carbon compounds - to synthesize all complex organic substances containing carbon
- Many bacteria that live in the soil (nitriding, serobacteria (Thiobacteria), etc.) belong to autotrophs
- Depending on the use of the energy source - photoautotrophs that use light and chemoautotrophs that use organic compounds are distinguished

Heterotrophs

- Heterotrophs (Greek, *heteros* - other, *trophe* - nutrition) use organic matter as a source of carbon
- They assimilate carbon from carbohydrates (mainly glucose), amino acids and other organic compounds
- Depending on the use of the energy source - photoheterotrophs using light and chemoheterotrophs using organic compounds are distinguished
- Currently, the terms autotroph and heterotroph are replaced by the new terms organotroph and lithotroph, respectively. Lithotrophs are so named because they can grow in a pure mineral environment.

- Aminoautotrophs - use either atmospheric nitrogen or ammonium salts as nitrogen sources for protein synthesis.
- Aminoheterotrophs - use organic matter - amino acids and proteins as nitrogen sources. All pathogenic and most saprophytic microorganisms belong to this group.
- Prototrophs are microorganisms capable of synthesizing all the substances they need using only glucose as a carbon source and ammonium salts as a nitrogen source.
- Auxotrophic microorganisms cannot synthesize any substance from glucose and ammonium salts, respectively, as a single source of carbon and nitrogen. Growth factors are required for their development.

- Saprophytes (Greek sapros - decay, phyton - plant) receive ready-made organic substances from dead organisms.
- Parasites (Greek: parasitos - omnivores, live at someone else's expense) take organic matter from living plants, animals and human organisms. Obligate and facultative parasites are distinguished. Obligate parasites are adapted to live inside the cell. For example, rickettsia and chlamydia, etc.

Types of nutrition of microorganisms

Category	Energy source	Carbon source	Type
Photoautotroph	light	CO_2	Cyanobacteria, Lichens
Photoheterotroph	light	Organic compounds	Photosynthetic bacteria
Chemoautotroph	Organic compounds	CO_2	Sulfur-, iron-oxidizing bacteria
Chemoheterotroph	Organic compounds	Organic compounds	Protozoa, Fungi, Most of bacteria

Nutritional mechanisms of microorganisms

Nutrients can enter a microbial cell in several ways:

Passive diffusion

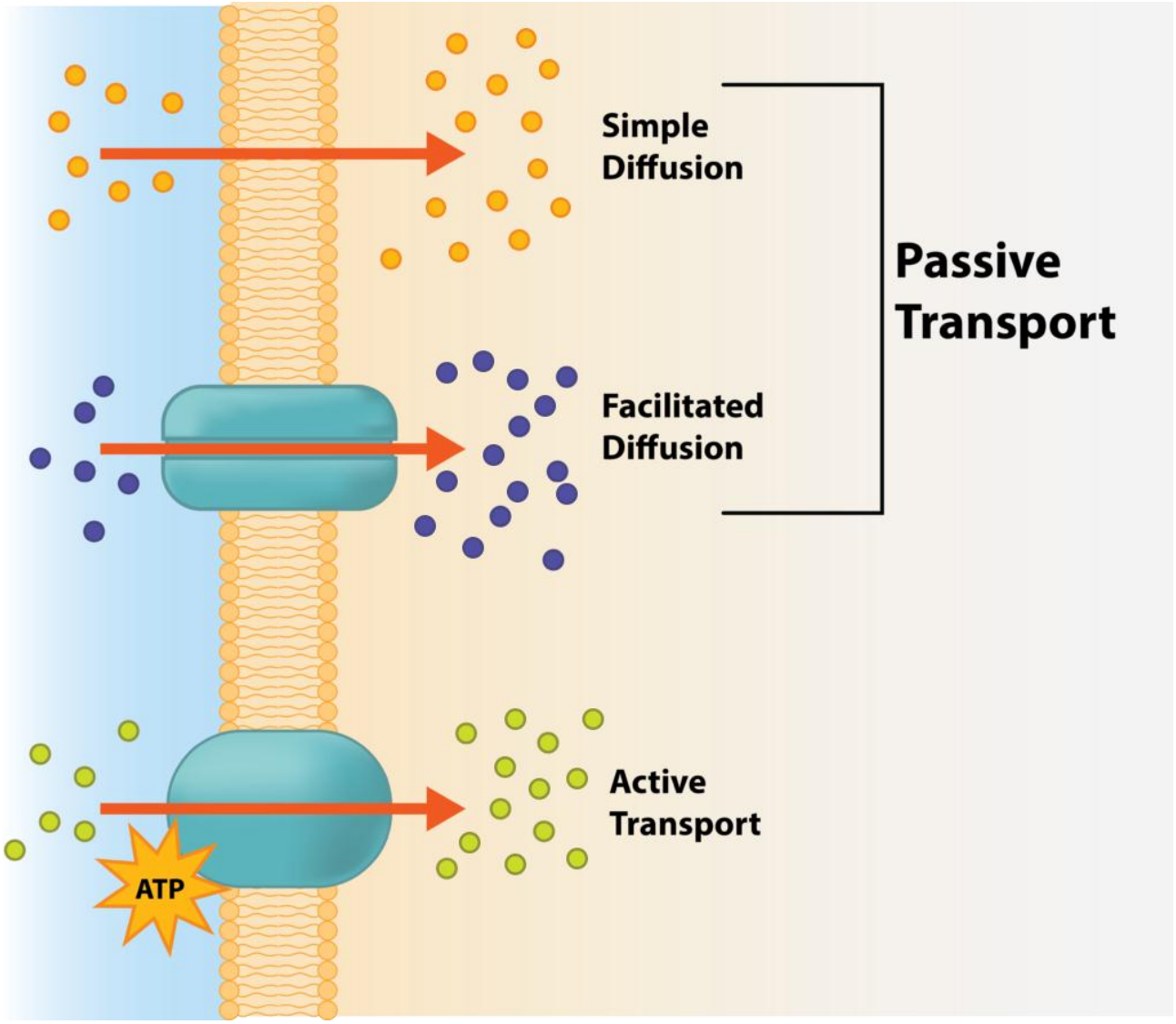
- ***Simple diffusion (due to the difference in osmotic pressures)***
- ***Facilitated diffusion (carrier proteins - permeases)***

Active transport

- ***Ion transport (uniport, simport, antiport)***
- ***ATF-transport***

Transport by translocation mechanism

Nutrition mechanisms of microorganisms:



Nutrient media

- Special substrates - nutrient media - are used for in vitro cultivation of microorganisms. Nutrient media must provide optimal conditions for the growth of microorganisms to be cultivated. To do this, nutrient media must meet certain requirements:
- must have all the components necessary for the growth of microorganisms
- must be isotonic
- should have an optimal pH
- must be sterile
- must have a certain oxidation-reduction potential
- the composition should be sufficiently standardized
- they must have a certain viscosity and be sufficiently transparent.
- It should be easy to prepare and economically viable, ie cheap.

Classification of nutrient media

- Different nutrient media are used in microbiological practice. Modern classification of nutrient media takes into account their **physico-chemical properties**, **composition** and **purpose**.
- Depending on the primary components of the nutrient medium, they are divided into **natural** and **synthetic** media.

Classification of nutrient media

- **Liquid**, **semi-liquid** and **solid** media are distinguished by their consistency.
- Liquid nutrient media include meat-peptone broth (PPB) and peptone water, etc.
- Agar or gelatin is added to liquid media to prepare semi-solid and solid nutrient media.

Classification of nutrient media

- Due to its composition, nutrient media can be **simple** or **complex**.
- *Simple* nutrient media include meat-peptone broth (MPB), meat-peptone agar (MPA), and peptone water, etc.
- **Complex** nutrient media are prepared by adding blood, serum, carbohydrates, and other substances to simple media, such as blood agar, etc.

Classification of nutrient media

- According to their purpose, nutrient media are divided into basic, special, elective, differential-diagnostic media.
- **Basic (ordinary) nutrient media** are used to cultivate many microorganisms. MPB, MPA, peptone water can be classified as basic nutrient media.
- **Special nutrient media** allow the cultivation of some microorganisms that do not grow in normal nutrient media. For example, bloody and serum media are used to cultivate pneumococci and meningococci. Thus, these microbes do not grow in normal nutrient media.
- Specific nutrient media also include enriched nutrient media. All components necessary for the cultivation of appropriate microorganisms, including growth factors, are added to media.

Nutrient media

Meat-pepton agar (MPA)

Blood agar

Classification of nutrient media

- ***Elective nutrient media*** are used only for the cultivation of certain microorganisms. In such media, other microorganisms either do not grow at all or grow very poorly. For example, the action of bile added to the media stops the growth of *Escherichia coli*, and induce the growth of *Salmonella*
- Elective media with a liquid consistency are sometimes referred to as enrichment or accumulation media. These media facilitate the extraction of cultures of pathological materials by ensuring more intensive growth of the relevant pathogenic microbe. For example, in order to obtain dysentery bacteria - Shigella from the feces of a patient, pathological material must first be cultured in ***Selenium broth***

Classification of nutrient media

- ***Differential-diagnostic media*** allow microorganisms to differentiate (differentiate) from each other, and sometimes even identify them.
- Differentiation of microorganisms in such media is mainly based on their enzymatic properties. Endo medium, EMB etc. is one of the such media.

Classification of nutrient media

- ***Preservation or transport media*** are used for the initial inoculation and transportation of pathological materials. These media prevent the destruction of pathogenic microorganisms in pathological materials and slow down the growth of saprophytic microbes.

The effect of external environmental factors on microorganisms

- The vital activity of microorganisms, their development, reproduction and destruction depend on environmental factors.
- Factors that can affect microorganisms can be divided into three groups: ***physical***, ***chemical*** and ***biological***.
- The effect of these factors varies depending on both their nature and the nature of the microorganism. Thus, each of these factors can have a lethal effect on microorganisms, as well as beneficial for their development.

The effect of physical factors on microorganisms

- **Temperature.** All microorganisms are divided into three groups according to temperature:
- ***Psychrophilic*** (Greek, psychros-cold, phileo-love) microorganisms
- - minimum temperature– 0^0C, optimal – 6-20^0C, maximum– 30^0C
- ***Mesophilic (Greek, mesos-middle) microorganisms***
- - minimum temperature – 10^0C, optimal – 34-37^0C, maximum– 45^0C
- ***Thermofilic*** (***Greek***, *termos*-hot), , or heat-loving microorganisms grow at relatively high temperatures, usually above 55^0C

  - minimum temperature – 30^0C, optimal – 50-60^0C, maximum– 70-75^0C
- ***The effect of low and high temperatures***

The effect of physical factors on microorganisms

- ***Dryness*** - results in dehydration of the cytoplasm of microbial cells and disruption of the permeability of the cytoplasmic membrane, which leads to disruption and destruction of their nutrition. Some microorganisms, such as meningococci, gonococci, leptospira, the causative agent of syphilis, etc. die within a minute or two of drying. then they are destroyed. The causative agent of cholera can last for 2 days, the causative agent of typhoid fever for 2 months, and the causative agent of tuberculosis can last up to 3 months. ***Lyophilic drying*** or ***lyophilization*** is widely used in the storage of microbial cultures and preparations made from them, as well as many biological preparations. To do this, the drugs are first frozen and then dried under vacuum. In this case, the microbial cells become anabiotic and retain their biological properties for a long time.

The effect of physical factors on microorganisms

- **Radiation energy.** Under natural conditions, microorganisms are mainly exposed to light rays.
- Direct exposure light rays to bacteria, especially pathogenic bacteria, has a destructive effect.
- The destructive effect of light on microorganisms is due to its ***ultraviolet rays (UVR)*** with a wavelength of 254-300 nm. UVS inactivates enzymes in microbial cells and causes changes in the DNA molecule.
- Other rays, such as ***X-rays,*** as well as ***alpha-, beta- and gamma-rays***, have a destructive effect on microorganisms only in large doses. As a rule, doses of 44,000 r and more of these rays have a lethal effect on microbes.
- The bactericidal effect of ionizing radiation is sometimes used for canning food, ***sterilization*** of biological products (serum, vaccines, etc.).

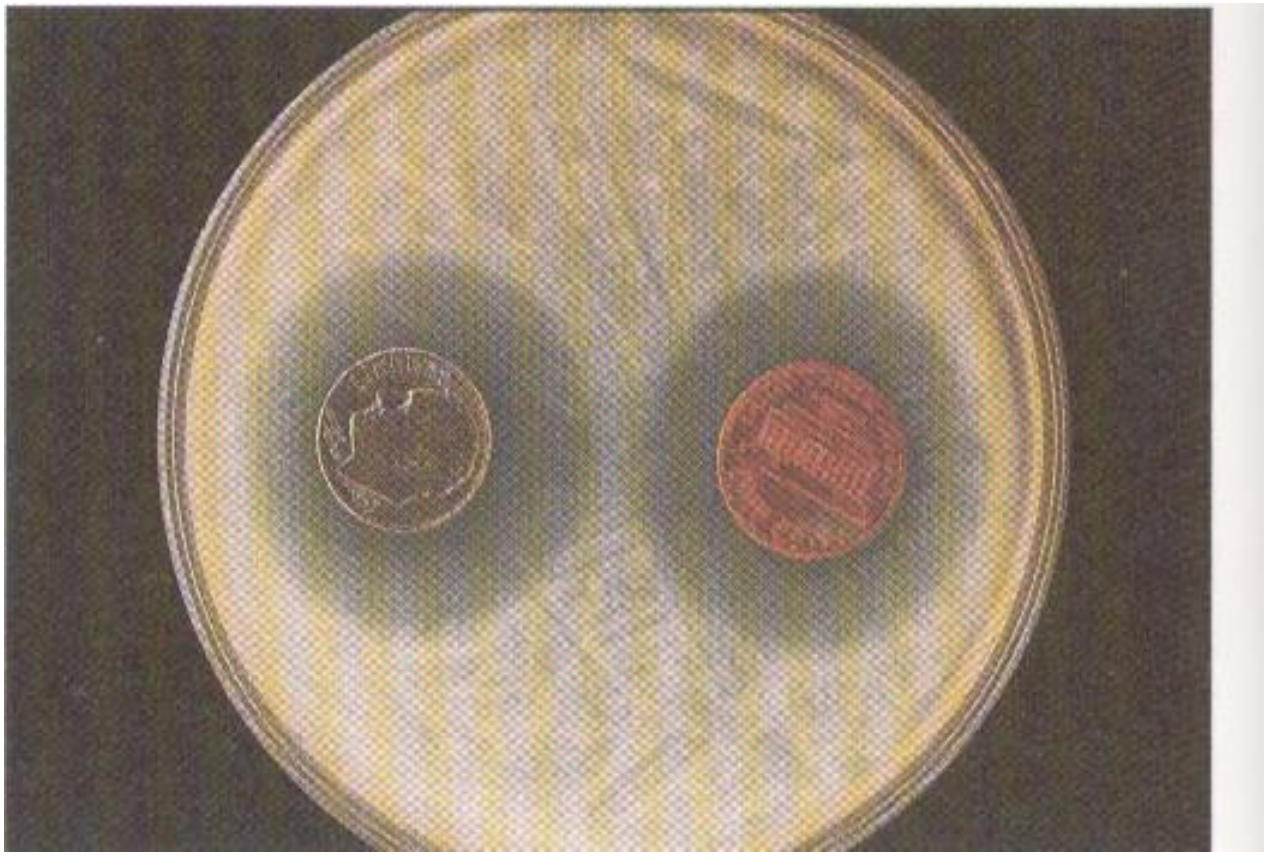
The effect of physical factors on microorganisms

- ***Ultrasound***. Sound waves with a frequency greater than 20,000 Hz are called ultrasound. Ultrasound waves cause some effects when passing through the environment. The most important of these is the **cavitation effect** (Latin, cavitum - space). Under the influence of ultrasound waves with a high pressure of 10,000 atm. are formed cavitation space in the cytoplasm of microorganisms, which results in the breakdown of the microbial cell. Ultrasound waves are also used to sterilize some foods (milk, fruit juices, etc.) and drinking water.
- **High pressure.** High atmospheric pressure is harmless to most microorganisms. Some microorganisms withstand a pressure of 3000-5000 atm, and bacterial spores withstand a pressure of even 20,000 atm. Interestingly, saturated water vapor under high pressure has a destructive effect on all microorganisms and their spores. Sterilization of materials in autoclaves is based on this principle.

Disinfectants and antiseptic preparations

- ***Surfactants -*** soaps and detergents (decamine, chlorhexidine, etc.)
- ***Phenol*** and its derivatives (tricresol, phenylresorcinol, phenylsalicylate)
- ***Oxidizers*** (hydrogen peroxide, potassium permanganate, etc.)
- ***Halogens*** (alcoholic solution of iodine, lugol's solution, iodoform, iodinol), chlorine (chlorinated lime, chloramines, pantoside)
- ***Alcohols*** (ethyl alcohol, etc.)
- ***Acids, their salts*** (boric, salicylic, benzoic, acetic acids) and alkalis (ammonia and its salts, zinc);
- ***Aldehydes*** (formaldehyde and its 40% solution - formalin, hexamethylenetetramine - urotropin, glutar aldehyde, etc.)
- ***Heavy metal salts*** (mercury dichloride, silver nitrate, copper sulfate, etc.).
- ***Dyes*** (diamond green, methylene blue, ethacridine lactate - rivanol, etc.)

Experiments showing the antimicrobial effect of heavy metals (silver and copper)



Effect of chemical factors on microorganisms

- **Disinfection** is the destruction of pathogenic microorganisms in the environment.
- Chemicals used for this purpose are called disinfectants.
- Substances that do not have a harmful effect on the human body are used to remove microorganisms from the skin and mucous membranes, wounds. In such cases, these substances are called antiseptics and are used for antiseptic purposes.
- A set of measures applied to remove microorganisms from different parts of the human body, as well as from wounds, is called antiseptic.
- Asepsis is a set of measures taken to prevent contamination of various objects (various areas of the body, skin and mucous membranes, including wounds) with microorganisms.

Aseptic and antiseptic

Sterilization

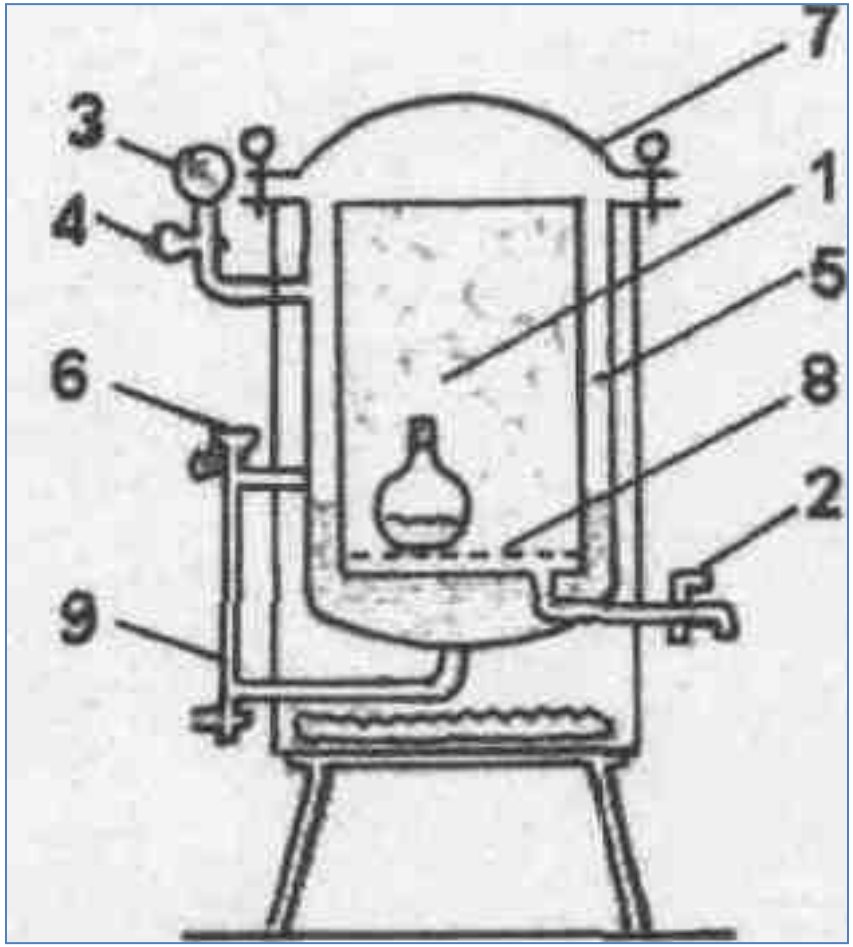
- It is the complete destruction of microorganisms, as well as their spores, in various objects.
- Sterilization is carried out in different ways:
- ***Physical methods*** (under the influence of high temperatures and various rays);
- ***Chemical methods*** (under the influence of various disinfectants and antiseptics, as well as antibiotics);
- ***Mechanical methods*** (application of bacterial filters)

Physical sterilization (thermal sterilization)

- **Burning and boiling sterilization** is one of the simplest and most convenient methods of heat sterilization
- Dry heat and high pressure saturated water vapor are mainly used for thermal sterilization.
- **Dry hot sterilization** is carried out in pasteurization furnaces (air sterilizers). The most common mode is sterilization at 165-170^{0}C for 1 hour, in which case all microorganisms, as well as their spores are completely destroyed.
- High pressure saturated water vapor is used to sterilize materials that change their properties and quality at high temperatures. Autoclaves (steam sterilizers) are used for this purpose. The most common mode of operation is sterilization at 2 atm at 121^{0}C for 30 minutes, in which case all microorganisms, as well as their spores, are completely destroyed.
- ***Pasteurization*** can be considered conventional sterilization. 1-hour exposure at 65^0-70^{0}C allows to destroy vegetative forms of microorganisms in food (milk, wine, beer, fruit juices, etc.).

Air sterilizer

Autoclave and his working principle



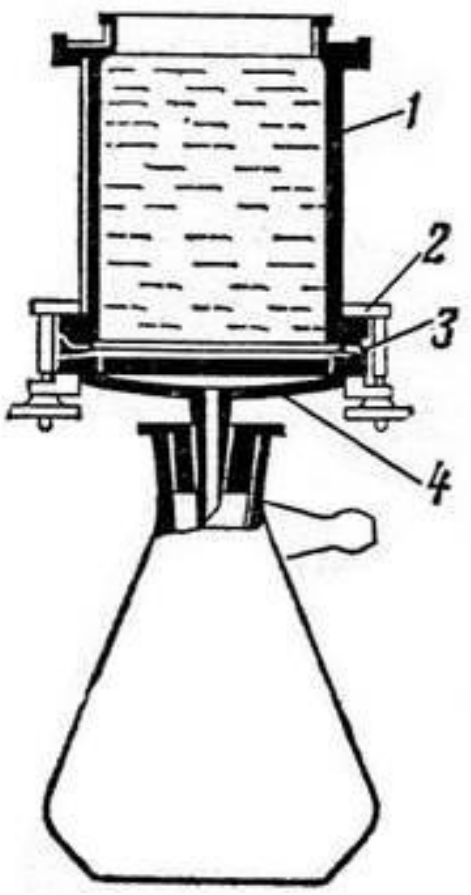
Physical sterilization (radiation sterilization)

- It is used for sterilization of thermolabile materials.
- The sterilizing effect of ultraviolet rays is limited by its low permeability and high absorption when passing through water and glass.
- Although gamma and X-rays have effective sterilizing properties, their application requires strict adherence to safety regulations. Biological preparations (serum, vaccines, etc.), disposable syringes, petri dishes, surgical sutures are sterilized by these rays.
- In some cases, microwave radiation and ultrasound are also used for sterilization.

Sterilizing effect of ultraviolet rays (bactericidal lamps)

Mechanical sterilization

- ***Sterilization by filtration*** through bacterial filters is used for sterilization of thermolabile liquid solutions.
- In microbiological practice are widely used ***Zeitz*** filters made from a mixture of asbestos and cellulose, ***membrane filters*** made from nitrocellulose**,** ***Chamberlain and Berkfeld*** filters made from a mixture of kaolin sand and quartz.
- Filters allow to get release from most microorganisms and sometimes viruses protein-rich media, blood serum and various drugs



Chemical sterilization

- Antimicrobials, disinfectants and antiseptics, as well as selective antibiotics and synthetic antimicrobials are used for **chemical sterilization** for the destruction of all microorganisms (see below).
- In some cases, toxic gases, such as ethylene oxide, are used for this purpose.

Quality control of sterilization

- Chemical control - substances with known melting point are used (sulfur - 119^0 C, benzoic acid - 120-122^0 C, benzonaftol - 110^0 C, mannose and urea - 132-133^0 C), as well as temperature indicator papers. Judgments are made based on changes in these ingredients, which are placed in an autoclave along with the materials to be sterilized.
- Biological control - biotests (paper boards or strips with heat-resistant spore bacteria on the surface). These paper strips, which are placed in the autoclave along with the materials to be sterilized, are judged on the basis of whether the spore bacteria are destroyed.