Lecture 4.

Physiology of microorganisms. Metabolism, nutrition, respiration and multiplication. Principles of cultivation of microorganisms

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Physiology of microorganisms

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

Chemical composition of microorganisms

- Microorganisms, like other living cells, are chemically composed of inorganic and organic substances.
- Organic substances include proteins, carbohydrates, lipids and nucleic acids, while inorganic substances 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%;

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 absorption, microorganisms are divided into two types - autotrophs and heterotrophs.

Autotrophs

- Autotrophs (Greek, autos - itself, trophe - nutrition) can use simple inorganic compounds - mainly carbon dioxide and other inorganic carbon compounds - to synthesize all the complex organic substances containing carbon.
- Many bacteria that live in the soil (nitriding, serobacteria, 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 compound 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

Types of nutrition of microorganisms

Catergory	Energy source	Carbon source	Representative
Photoautotroph	light	CO_2	Cyanobacteria, Shibs
Photoheterotroph	light	Organic components	Photosynthetic bacteria
Chemoautotroph	Organic components	CO_2	Sulfur-, iron-oxidizing bacteria
Chemoheterotroph	Organic components	Organic components	Protozoa, Fungus, Most bacteria,

Nutritional mechanisms of microorganisms

Nutrients can enter a microbial cell in several ways:

Passive diffusion

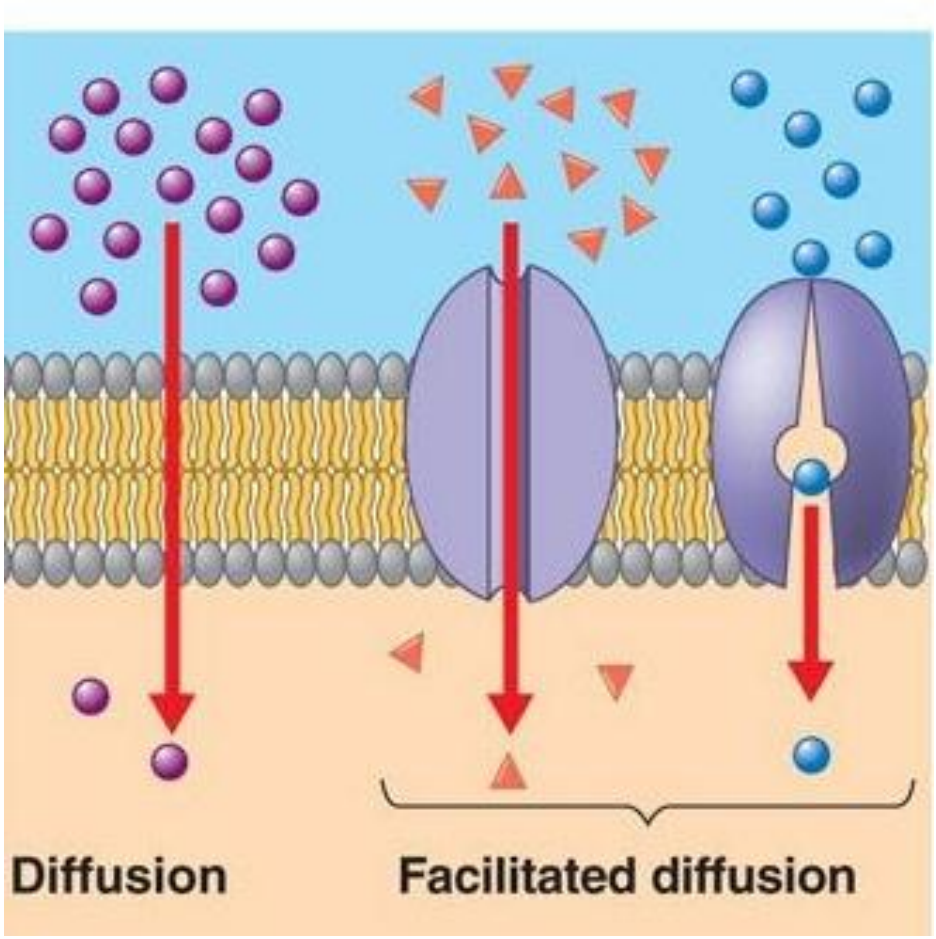
- Simple diffusion (due to the difference in osmotic pressures)
- Facilitated diffusion (carrier proteins - permases)

Active transport

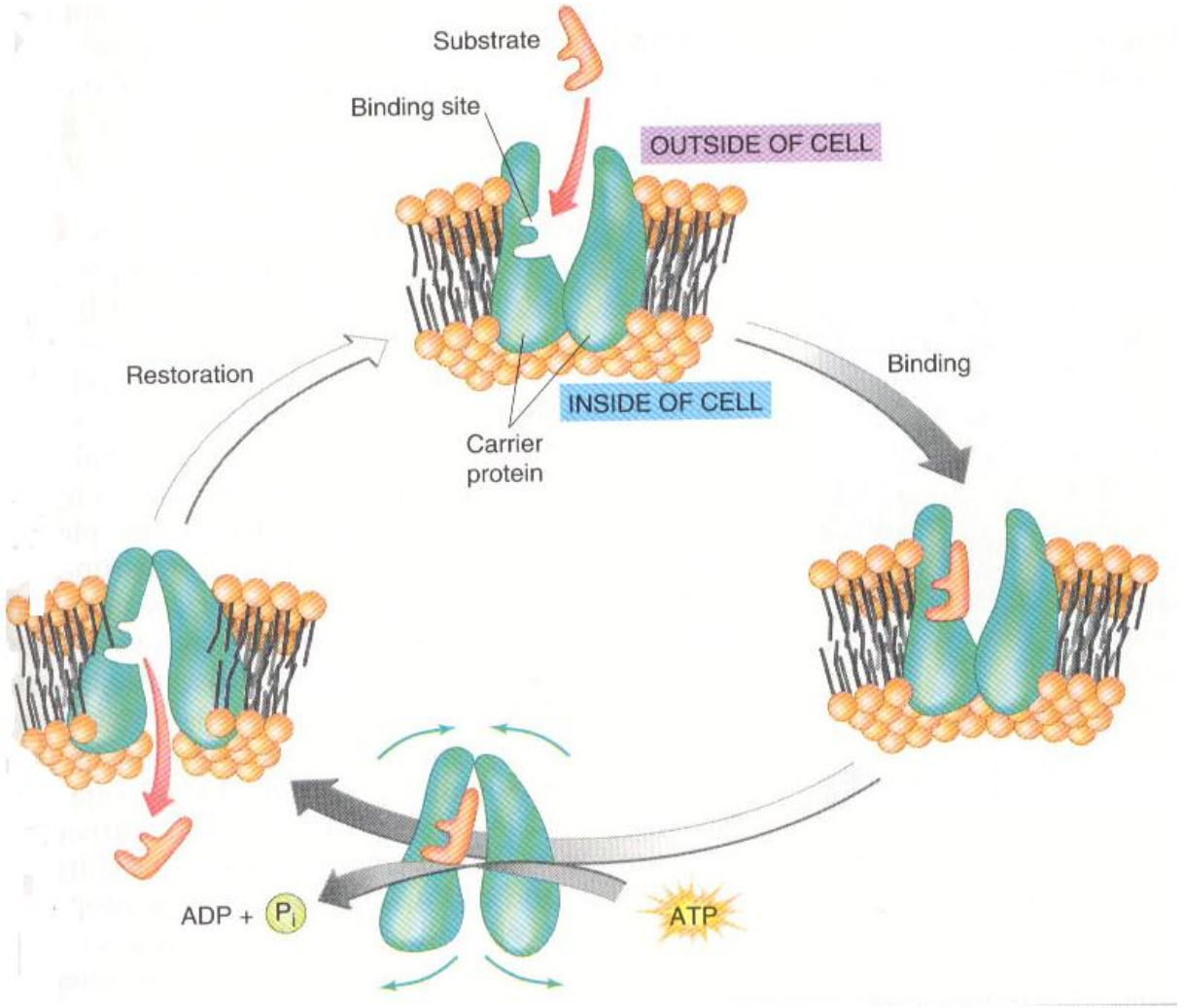
- Ion-transport (uniport, simport, antiport)
- ATF-transport

Transport by translocation mechanism

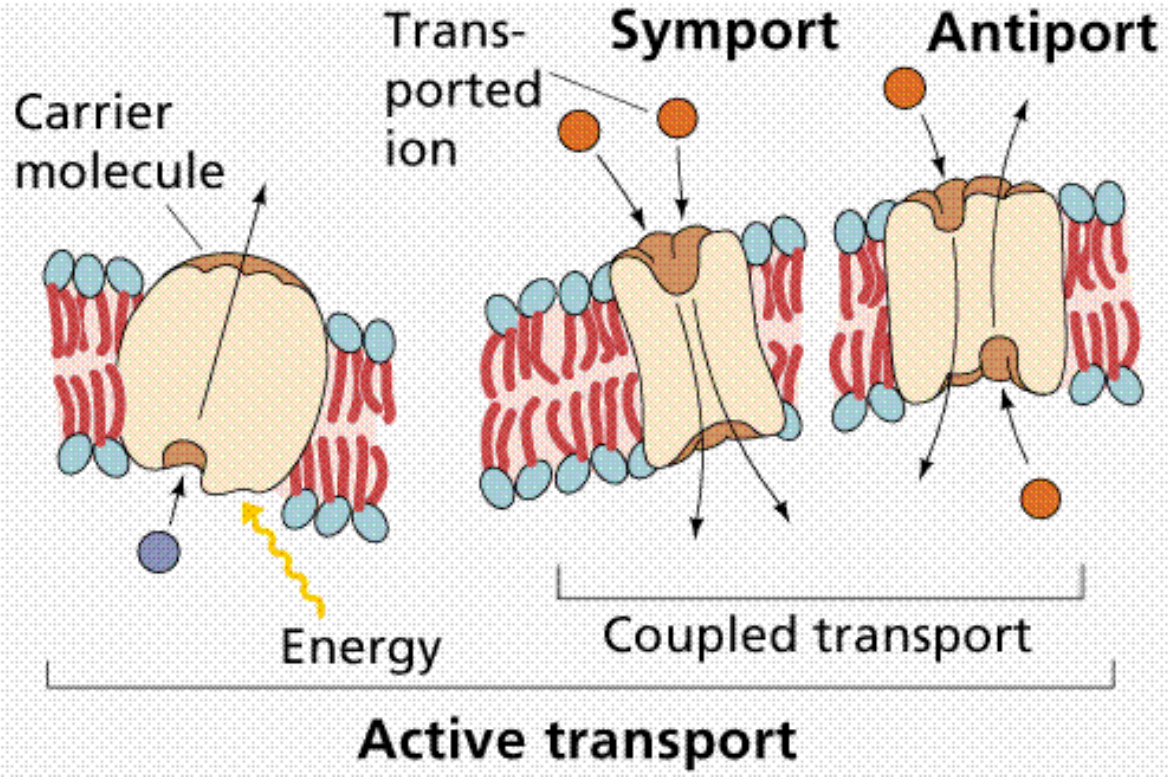
Simple and easy diffusion



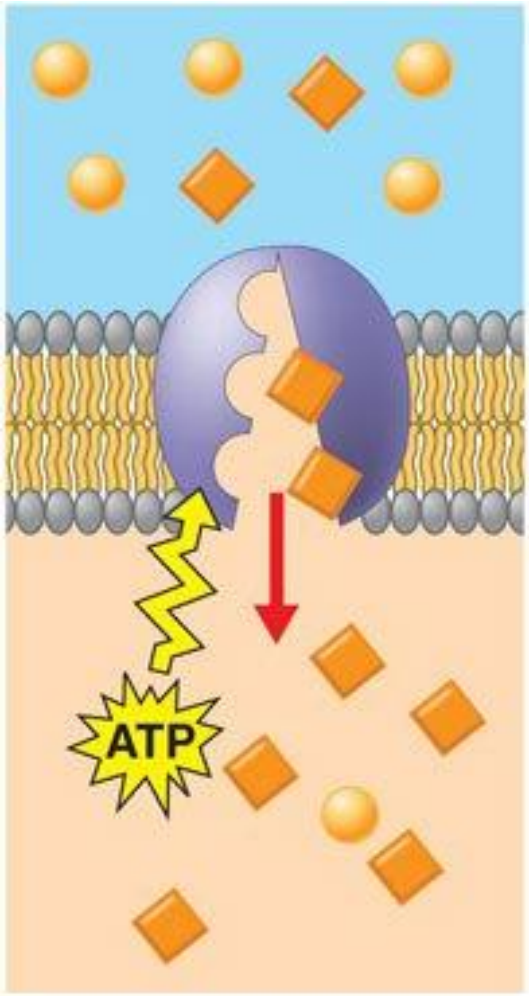
Active transport



Ion-transport (uniport, simport, antiport) is carried out according to the gradient of ion (proton) charges



ATF-transport is carried out using ATF energy



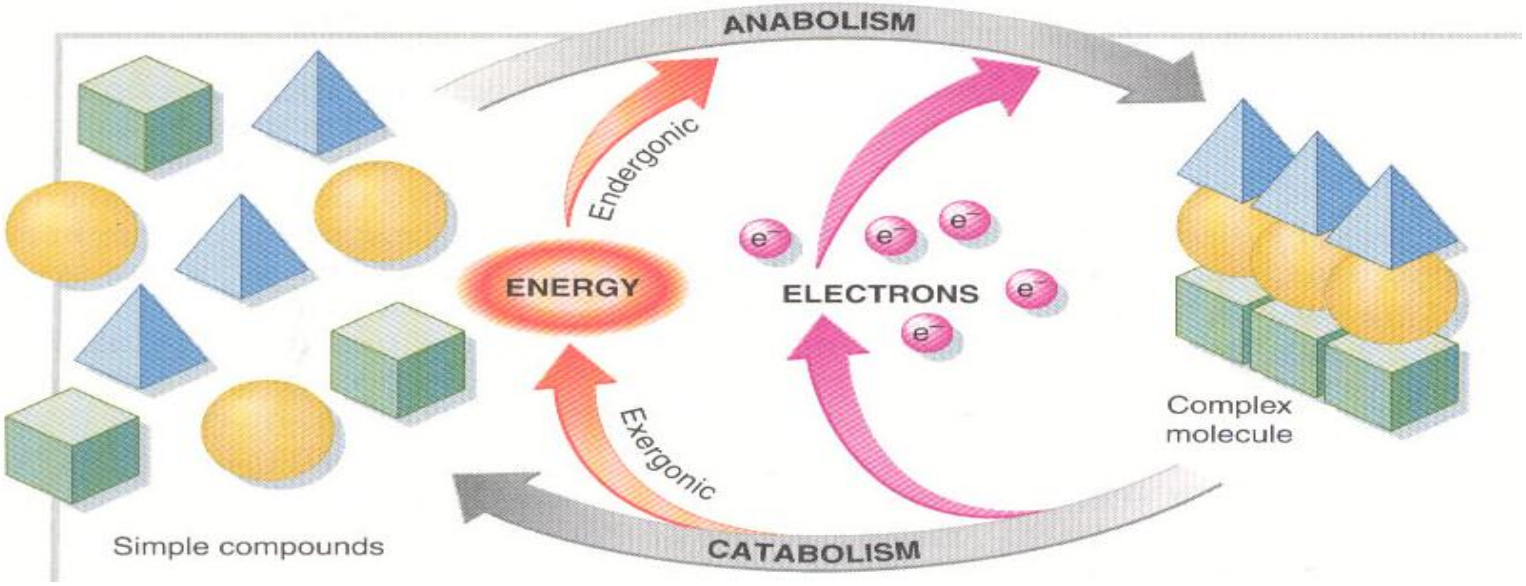
Microbial enzymes and their role in metabolism

- Endoenzymes act within the cell, and exoenzymes are secreted from the microbial cell, breaking down the macromolecules there and making it easier for them to enter the cell.
- Constitutional and inductive enzymes
- Metabolic enzymes - oxyreductases, transferases, lyases, ligases, hydrolases and isomerases
- Aggression or pathogenic enzymes - hyaluronidase, neuraminidase, lecithinase, etc.

Metabolism consists of two opposite processes - catabolism and anabolism

- Catabolism is the process of breaking down large molecules into smaller molecules by releasing energy. As a result, the released energy is stored in the form of macroenergetic bonds in the molecules of adenosine triphosphate (ATF) and is used for vital processes. Therefore, catabolism is sometimes called energy metabolism.
- In the process of anabolism, molecular compounds used to build a cell 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.

Catabolism and anabolism



Energy metabolism (biological oxidation)

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

Brodil metabolism

- During brodil metabolism, ATF is synthesized as a result of phosphorylation of substrates.
- In this case, the decomposing substrate acts as a donor of electrons, and the acceptors of the electrons are reduced, as a result of which the released energy is used for the synthesis of ATF.

Brodil metabolism

- The process of breaking down nitrogen-free organic compounds under anaerobic conditions is called fermentation. The fermentation process consists of two stages.
- In the first stage, glucose is oxidized to pyruvic acid.
- The process of formation of pyruvic acid from glucose consists of a series of biochemical reactions.
- In both brodil and oxidative metabolism, this process can proceed in the same way - in three ways.

Glycolysis pathway (Embden-Meyerhof pathway) predominates in bacteria

- In this case, glucose is first converted to fructose-6-phosphate, and then to pyruvic acid.
- During glycolysis, 2 molecules of ATF are used in the process of glucose breakdown, and 4 molecules of ATF are synthesized. Thus, 2 molecules of ATF are synthesized from 1 molecule of glucose.
- As a result of the reactions, phosphate is transferred from intermediate substrates to the molecule adenosine diphosphate (ADF), and thus ATF is synthesized. Therefore, it is called substrate phosphorylation.

Types of fermentation

- The resulting pyruvic acid undergoes various transformations in anaerobic microorganisms, resulting in different types of fermentation, depending on the final organic matter formed.
- Lactic acid fermentation
- Alcohol fermentation
- Propionic acid fermentation
- Formic acid fermentation
- Butyric acid fermentation

Formic acid fermentation

- This fermentation is mainly characteristic of bacteria of the family Enterobacteriaceae.
- Many bacteria break down formic acid, which is formed during fermentation, into gas (H2 and CO2).
- Thus, some bacteria break down carbohydrates only to form acids, while others break them down to form both acids and gases.
- It is used in the biochemical identification of bacteria (use of the Hiss medium).

Acetymethylcarbinol (acetoin)

- Some bacteria, such as Enterobacter and Serratia, break down carbohydrates to form acetymethylcarbinol (acetoin) in addition to pyruvic acid.
- Determination of acetymethylcarbinol is used in the identification of bacteria. The Voges-Praskauer reaction is used for this purpose

Butyric acid fermentation

- The main products of butyric acid fermentation are butyric acids, as well as other organic acids - acetic, capron, valerian, palmitic acids, as well as butanol, acetone, isopropanol, CO2 and H2.
- Determination of formed acids by gas-liquid chromatography is used as an express method in the identification of obligate anaerobes.
- This type of fermentation is characteristic of bacteria of the genus Clostridium.

A small amount of energy is obtained during the fermentation process

- The breakdown of glucose and other carbohydrates in the fermentation process results in less energy.
- The products of fermentation cannot be used by the cell and are removed.
- However, these products, such as lactic acid, still retain enough energy. When glucose is broken down into CO2 and water, enough energy is generated. As a result of the complete breakdown of 1 molecule of glucose, 38 molecules of ATF are synthesized, which is many times more than the energy obtained in fermentation.
- This is possible due to the breakdown of pyruvic acid under aerobic conditions (by oxidative metabolism).

Oxidative metabolism

- During oxidative metabolism, ATF is synthesized as a result of oxidative phosphorylation.
- In this case, pyruvic acid is completely oxidized to CO2 in the circulation of tricarboxylic acids:
- pyruvic acid NAD, FAD, etc. With the help of coenzymes, acetyl is converted to coenzyme A (activated acetic acid) and joined to the triacetic acid cycle (Crebs cycle).

Tricarboxylic acid cycle (Crebs cycle)

- In the tricarboxylic acid cycle, acetyl groups decompose to form CO2 and 4 pairs of hydrogen atoms.
- Hydrogen atoms combine with NAD, NADF and FAD to reduce them to NADH2, NADFH2 and FADH2.
- In this way, hydrogen atoms are transferred to molecular oxygen along the respiratory chain located in the cytoplasmic membrane of microorganisms.
- The transfer of hydrogen atoms along the respiratory chain to molecular oxygen is provided by dehydrogenase, quinones (ubiquinone, etc.) and cytochromes.

Respiratory chain

- Respiratory chain during oxidative metabolism or respiration (oxidative phosphorylation), electron donors are organic and inorganic substances, and acceptors are only oxygen.
- In this case, the respiratory chain:

NAD ⟶ FAD ⟶ cytochromes ⟶ O2

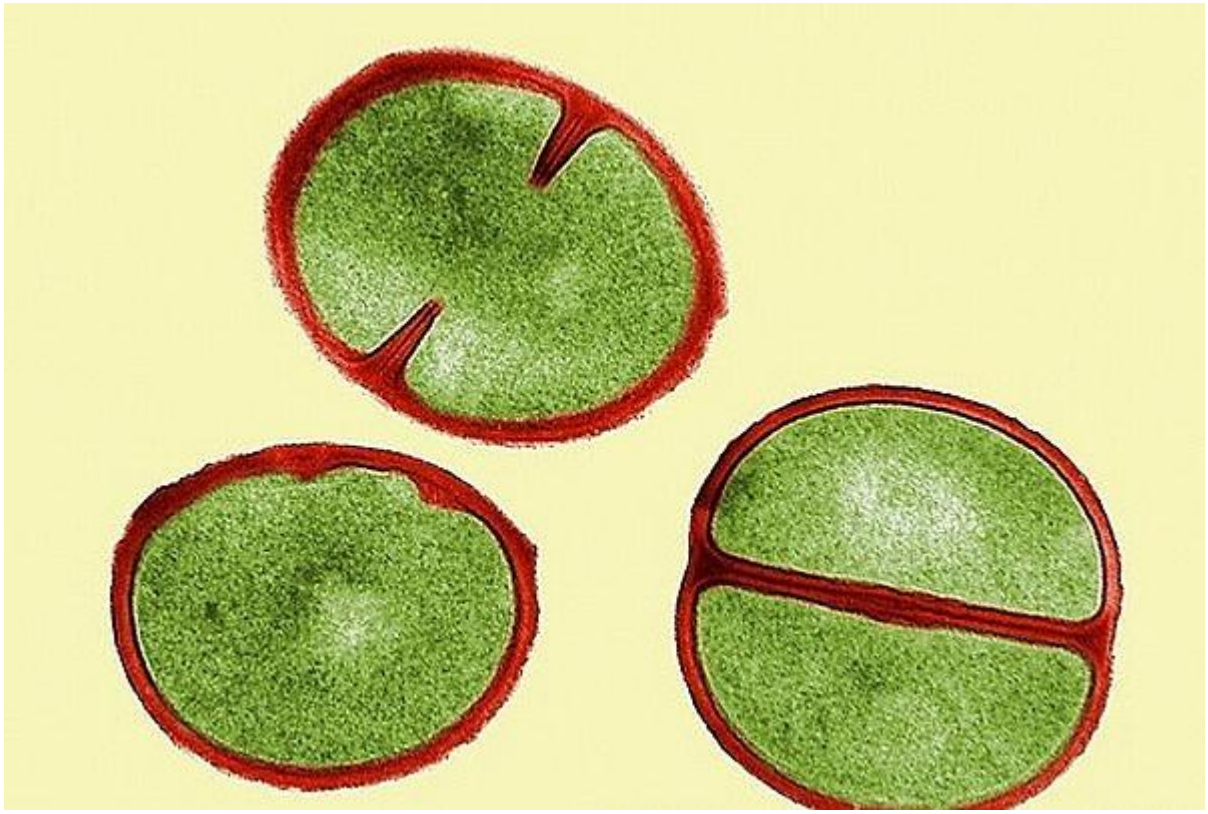
Respiration of microorganisms

- Microorganisms are divided into 3 main groups according to the type of respiration:
- obligate aerobes
  - *Microaerophiles*
  - *Capnophiles*
- obligate anaerobe
  - *obligate anaerobes*
  - *aerotolerant anaerobes*
- facultative anaerobes

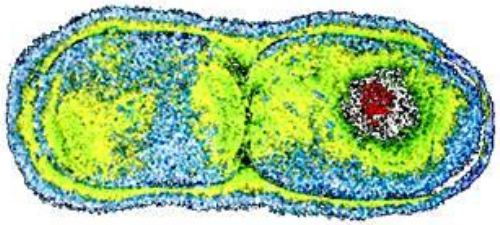
Growth and multiplication of microorganisms

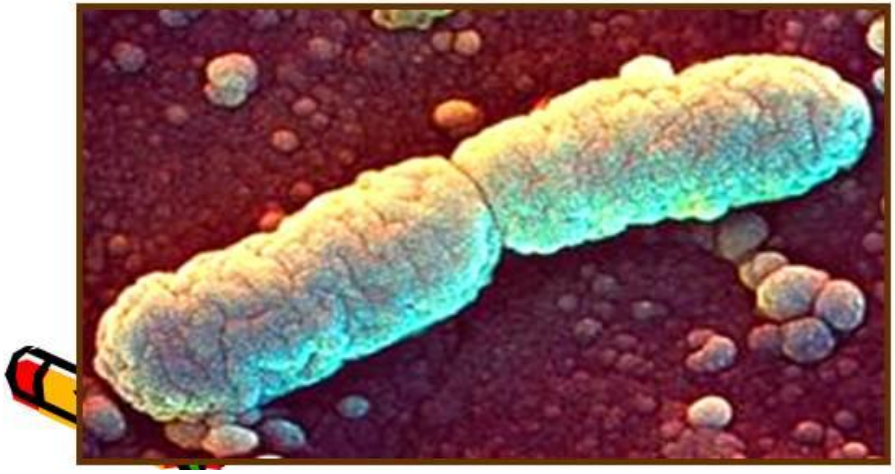
- As microorganisms mature, they begin to multiply
- Multiplication in different microorganisms occurs in different ways
- Bacteria multiply by simple, binary division. The division of a bacterial cell begins with the formation of a transverse partition.
- The transverse partition is provided by mesosomes.

Multiplication of bacteria



Multiplication of bacteria





Generation period

- Bacteria multiply very rapidly. The concept of generation time is used to estimate the rate of multiplication. This period represents the time required for the bacterial cell to double. Generation time is different for each type of bacteria.
- Bacteria, and in general all microorganisms, multiply more rapidly under optimal conditions.
- Most bacteria divide every 15-30 minutes. Some bacteria, such as mycobacterium tuberculosis, divide relatively late (every 20-24 hours).

Multiplication of bacteria

Multiplication of bacteria

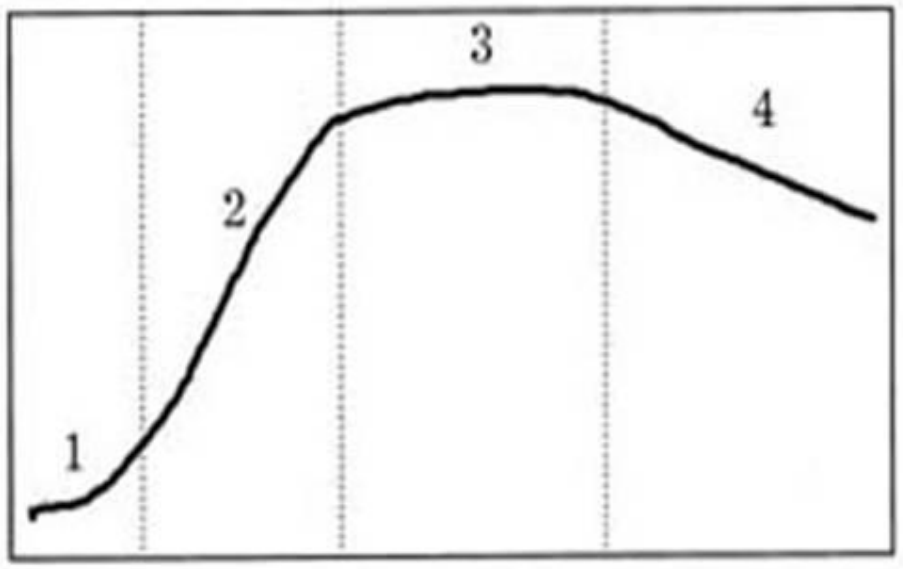
- As the bacterial cell multiplies by dividing in two, their number increases in the culture in a geometric sequence: $2^0 – 2^1 – 2^2 - 2^3 …. 2^n$, so after dividing by n, the number of bacteria in a bacterial family will be 2^n.
- When growing under such conditions, bacteria multiply until the components needed for their development reach a minimum, after which their proliferation stops.
- If no nutrients are added during this period and the metabolic products are not removed, a periodic or static culture is obtained.

Phases of bacterial multiplication in periodic culture

- Periodic culture behaves as if it were a multicellular organism.
- The multiplication of bacteria here is subject to a certain pattern and consists of several phases.
- The graphical description of these phases is called the development curve

Bacterial multiplication phases (development curve)

1. Lag phase
2. Exponential, or logarithmic phase
3. Stationary phase
4. Decline phase or death phase



Continuous culture

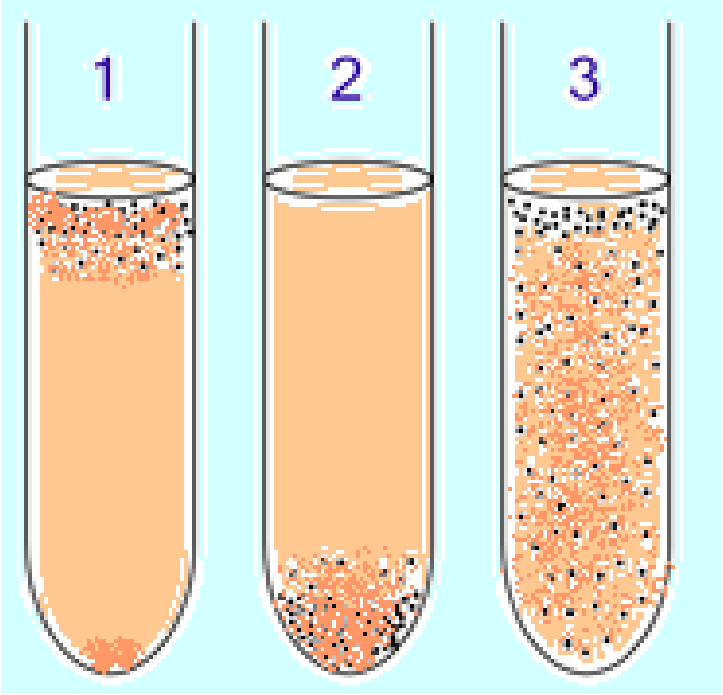
- In biotechnology, conditions are created that require bacterial cells to remain in the exponential (logarithmic) phase for a long time.
- For this purpose, a new nutrient medium is constantly added to the culture in which the bacterial population is developing, and at the same time an appropriate amount of bacterial suspension is removed. Thus, a continuous culture is obtained.
- Continuous cultivation is carried out on special cultivators - **chemostats and turbidostats.** When cultivating in hemostats, as fresh nutrient medium is added to the cultivator, an appropriate amount of bacterial suspension is removed. Cultivation in **turbidostats** is based on maintaining a constant optical density of bacterial suspension in the cultivator.

A population formed by bacteria in nutrient media is called a *culture*

- Under optimal conditions, bacteria form a unique population, which is called culture.
- As they grow in nutrient media, the nature of the cultures produced by each bacterial species is different.
- It is used in the identification of bacteria because their cultural characteristics are relatively stable.

Cultural characteristics of bacteria

Bacterial cultures in liquid nutrient media are accompanied by turbidity of the medium, the formation of sediment at the bottom or on the surface.



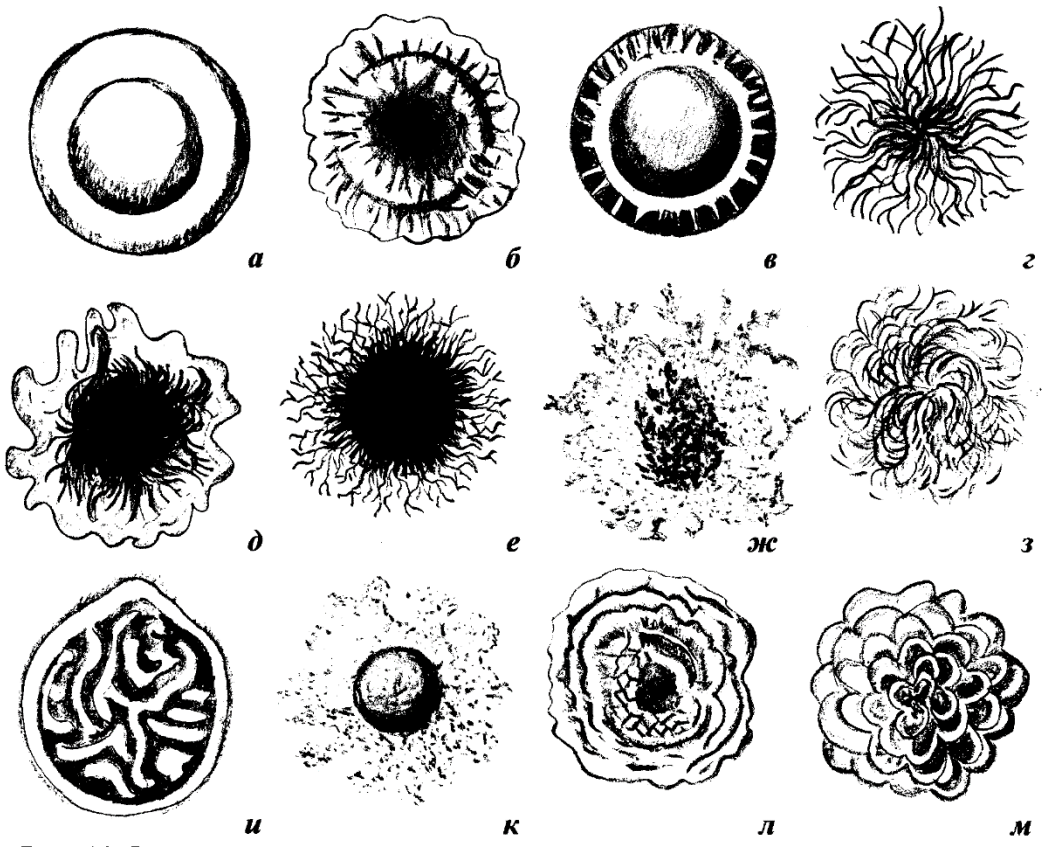
Cultural characteristics of bacteria

•Bacteria form colonies as they grow in solid nutrient media.

•When a bacterial cell multiplies on the surface of a nutrient medium, the cells in the culture form certain clusters, which are called colonies.

•Thus, a colony is a population formed by bacteria on the surface of a solid nutrient medium.

Colonies of different types of bacteria differ from each other in shape and size



Size of colonies

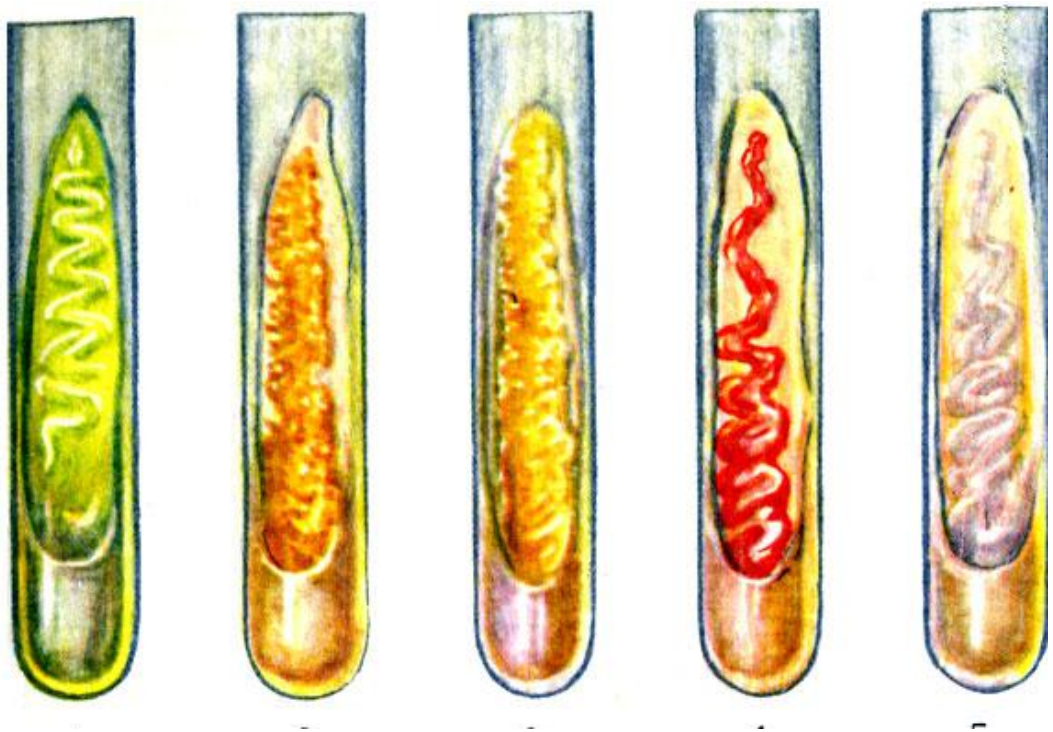
- The size of the colonies can vary. The size of the colonies can vary from one tenth of a millimeter to several centimeters.
- In some cases, the colonies are so small that they cannot be seen with the naked eye. By size:

- very small (tiny colonies, up to 1 mm in diameter),
- small (1-2 mm in diameter)
- medium (diameter 2-4 mm)
- large (more than 4-5 mm in diameter) colonies are distinguished.

Shapes of colonies

- Colonies may be round or irregularly shaped. The shape of the colonies is also assessed by the structure and height of their edges and surfaces.
- The edges of the colonies are smooth, wavy, rough, fringed, etc. can be.
- Colonies also differ in height (flat, convex, erect, etc.).
- The surface of the colonies is smooth (S-colonies, smooth), wrinkled (R-colonies, rough - wrinkled), convex (dome-shaped), on the contrary, the central part is concave, etc. can be.

Colonies can be of different colors, depending on the pigments they form



Pigments of microorganisms

- Carotenoid pigments are yellow, red, orange, insoluble in water (mycobacteria, actinomycetes, sarcins, etc.)
- Quinone pigments are yellow in color and are mainly formed by mycobacteria.
- Melanin pigments are black and brown. Insoluble in water (Bacteroids, fungi, etc.).
- Pyrrole pigments are red, insoluble in water (prodigiozan pigment of the bacterium Serratia marcescens)
- Phenazine pigments are blue-green piocian pigment formed by blue-green pus. Due to its solubility in water, this pigment changes not only the color of the bacterial culture, but also the environment in which they grow, and the color of the pus during purulent processes.

Cultural characteristics of bacteria

- Sometimes the consistency of the colonies and the smell of the culture are also important when identifying bacteria by cultural characteristics.
- Some microorganisms form various aromatic compounds as a result of their vital activity.
- For example, the bacteria of the genus Proteus have a strong odor, so their culture has an unpleasant odor. The culture of blue-green pus has a characteristic lilac odor, and so on.

Assessment of development (multiplication)

- Sometimes in microbiological practice it is necessary to determine the number of bacterial cells in a particular bacterial culture or in suspensions made from them.
- Numerical chambers (Goryayev et al.), As well as special meters (Coulter meter) and filters are used to determine the total number of cells.
- Indirect methods are sometimes used to determine the total number of cells. The most convenient method is the blur standards method. To do this, the turbidity of the sample to be counted is compared with the standards. Currently, the McFarland turbidity standard is more widely used
- The most convenient way to count live cells is by cultivation (colony-forming units - CFU)

Multiplication of other prokaryotes

- The proliferation of spirochetes and rickettsiae, like other bacteria, goes through a simple division. Rickettsiae multiply only within the host cell (nucleus or cytoplasm).
- The proliferation of chlamydia occurs with a complex developmental cycle within the host cells.
- Reproduction of mycoplasmas. The main reproductive forms of mycoplasmas are spherical or ovoid-shaped elementary objects. In the process of development, spherical bodies are formed from the filamentous derivatives formed from them. Thus, chains of spherical bodies are formed. Then, as a result of fragmentation of filamentous derivatives, elementary bodies are formed.
- The proliferation of actinomycetes occurs through the fragmentation of mycelium, or spores formed in aerial mycelium.

Principles of cultivation of microorganisms

- With the exception of obligate parasites (rickettsiae, chlamydia and viruses), it is possible to artificially cultivate all microorganisms, ie to obtain their culture in the laboratory.
- By cultivating, it is possible to obtain a culture of microorganisms and thus study their chemical composition, morphological and biological properties, as well as prepare a number of biological preparations and vaccines of microbial origin.

Nutrient media

- **Special substrates - nutrient media - are used for in vitro cultivation of microorganisms. Nutrient environments should provide optimal (favorable) conditions for the growth of microorganisms to be cultivated. To do this, nutrient media must respond certain requirements:**
- **must have all the components necessary for the growth of microorganisms**
- **should be isotonic**
- **should have an optimal pH**
- **should 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

- In microbiological practice, extremely different nutrient media are used. The modern classification of nutrient media takes into account their physical and chemical properties, composition and purpose.
- Depending on the primary components of the nutrient medium, they are divided into natural and synthetic media.
- Liquid, semi-liquid and solid media are distinguished by their consistency.
- Meat-peptone broth (MPB), peptone water, etc. belong to liquid nutrient media.
- Agar or gelatin is added to liquid media to prepare semi-solid and solid media.

Classification of nutrient media

- Depending on their composition, nutrient media can be simple or complex.
- Meat-peptone broth (MPB), meat-peptone agar (MPA), peptone water, etc. belong to simple nutrient media.
- Complex nutrient media are prepared by adding blood, serum, carbohydrates and other substances to simple media, such as blood agar, whey agar, etc.

Classification of nutrient media

- According to their purpose, nutrient media are basic, special, selective, differential-diagnostic, conservation, etc. divided into media.
- Basic (ordinary) nutrient media are used to cultivate many non-demanding microorganisms. APB, APA, peptone water can be classified as ordinary 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. Such components include all the components necessary for the cultivation of appropriate microorganisms, including growth factors.

Classification of nutrient media

- Elective nutrient media are used only to cultivate a specific microorganism. In such environments, other microorganisms either do not grow at all or grow very poorly. For example, bile added to the environment stops the growth of intestinal bacilli and accelerates the growth of salmonella.
- Liquid environments are sometimes referred to as enrichment or accumulation environments. These environments facilitate the extraction of cultures by providing more intensive growth of the relevant pathogenic microbe in the pathological material. For example, in order to obtain dysentery bacteria - shigella - from the patient's feces, it is advisable to first cultivate the pathological material in a selective broth.

Classification of nutrient media

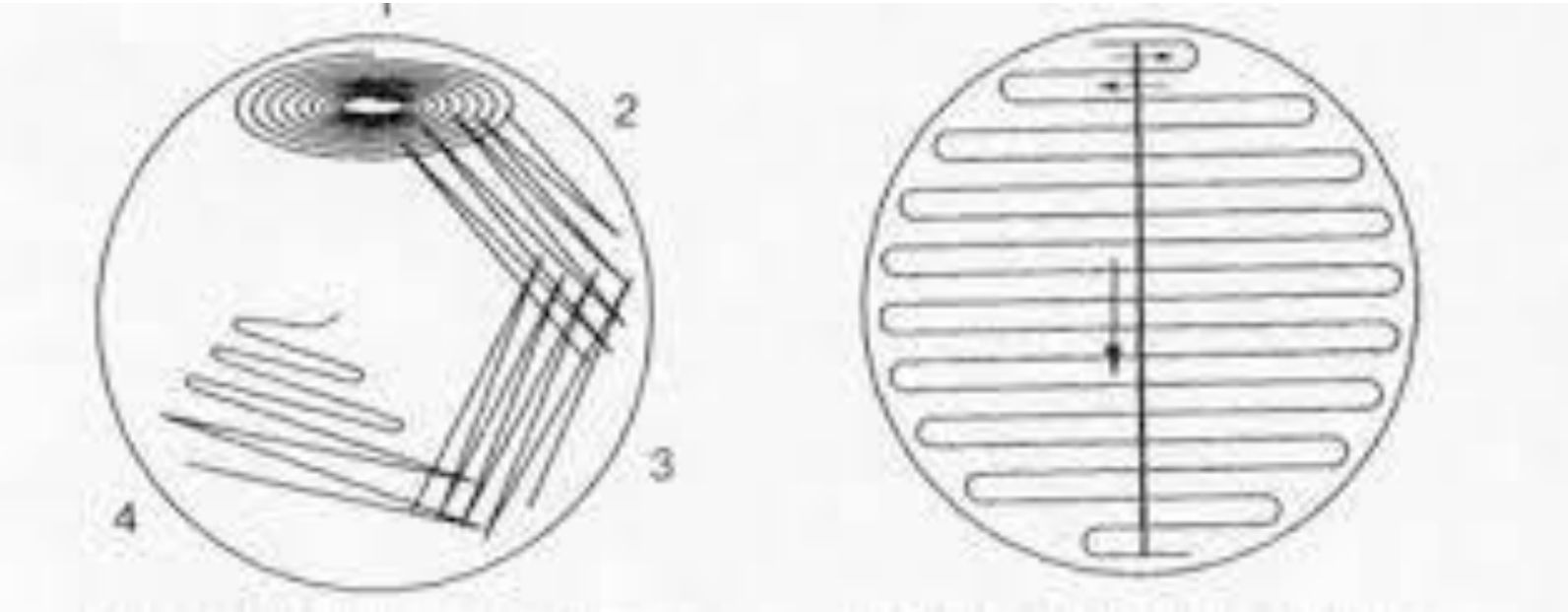
- Differential-diagnostic mediums allow microorganisms to differentiate, and sometimes even identify.
- Differentiation of microorganisms in such mediums is mainly based on their enzymatic properties. Endo medium, Hiss medium, etc. is one of such mediums.

Endo medium

Classification of nutrient media

- Preservation or transport media are used for the initial inoculation and transportation of pathological materials. These medium prevent the destruction of pathogenic microorganisms in pathological materials and inhibit the development of saprophytic microbes

Inoculation technique on the surface of a solid nutrient medium



Cultivation conditions

- Optimal conditions must be created for the cultivation of microorganisms in nutrient media.
- These conditions are primarily ensured by optimal temperature and cultivation period.

Cultivation temperature

- *Depending on the cultivation temperature, all microorganisms are divided into three groups: psychophiles, mesophiles and thermophiles.*
- *The optimum temperature for psychrophilic bacteria is 6-20ºC,is 34-37ºC for mesophylls. Most bacteria that are pathogenic to humans are mesophilic microorganisms for thermophiles, a higher temperature is required. Some members of this group can even grow at 70-75ºC.*

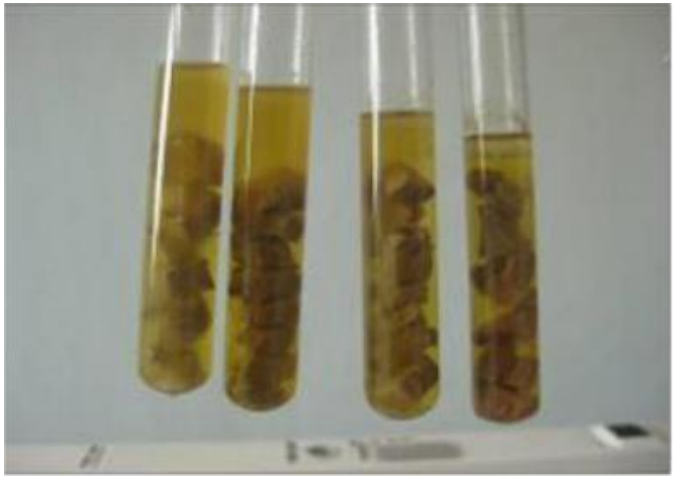
Cultivation period

- The duration of cultivation depends on the type of microorganisms. During this time, the microorganisms usually form visible cultures.
- While 18-24 hours of cultivation is sufficient under optimal conditions for most bacteria, this time varies in some microorganisms. For example, the causative agents of whooping cough are cultivated for 2-5 days, and the causative agents of tuberculosis for 3-4 weeks.
- In the absence of optimal conditions, the cultivation period may be extended.

Cultivation atmosphere

- Oxygen is required for the growth of aerobes. Therefore, aerobes grow well on the surface of solid nutrient media or in the top layer of liquid media.
- Both aerobic and anaerobic conditions can be used to cultivate facultative anaerobes
- Bond anaerobes are cultivated without oxygen.- Special nutrient media are used for this purpose. For anaerobes, the oxidation-reduction potential in the environment is reduced at the expense of various substances - reducing agents. For example, glucose is added as a reducing agent to the Kitt-Tarozzi medium used to cultivate anaerobes.- At present, anaerostats are more commonly used to cultivate anaerobes-
- The Gaspak system is one of the new ways to create anaerobic conditions.

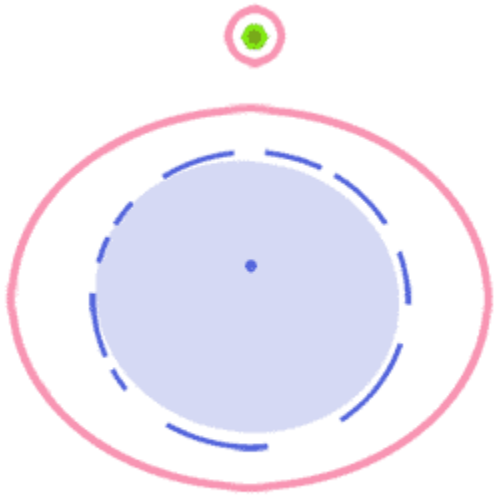
Cultivation of anaerobic bacteria



Multiplication of viruses - reproduction

- As mentioned, viruses are intracellular parasites. They can only multiply inside sensitive cells.
- Once a virus enters the body, it cannot reproduce in all cells, meaning that there are cells that are sensitive to each type of virus.
- The interaction of viruses with sensitive cells takes place in several stages

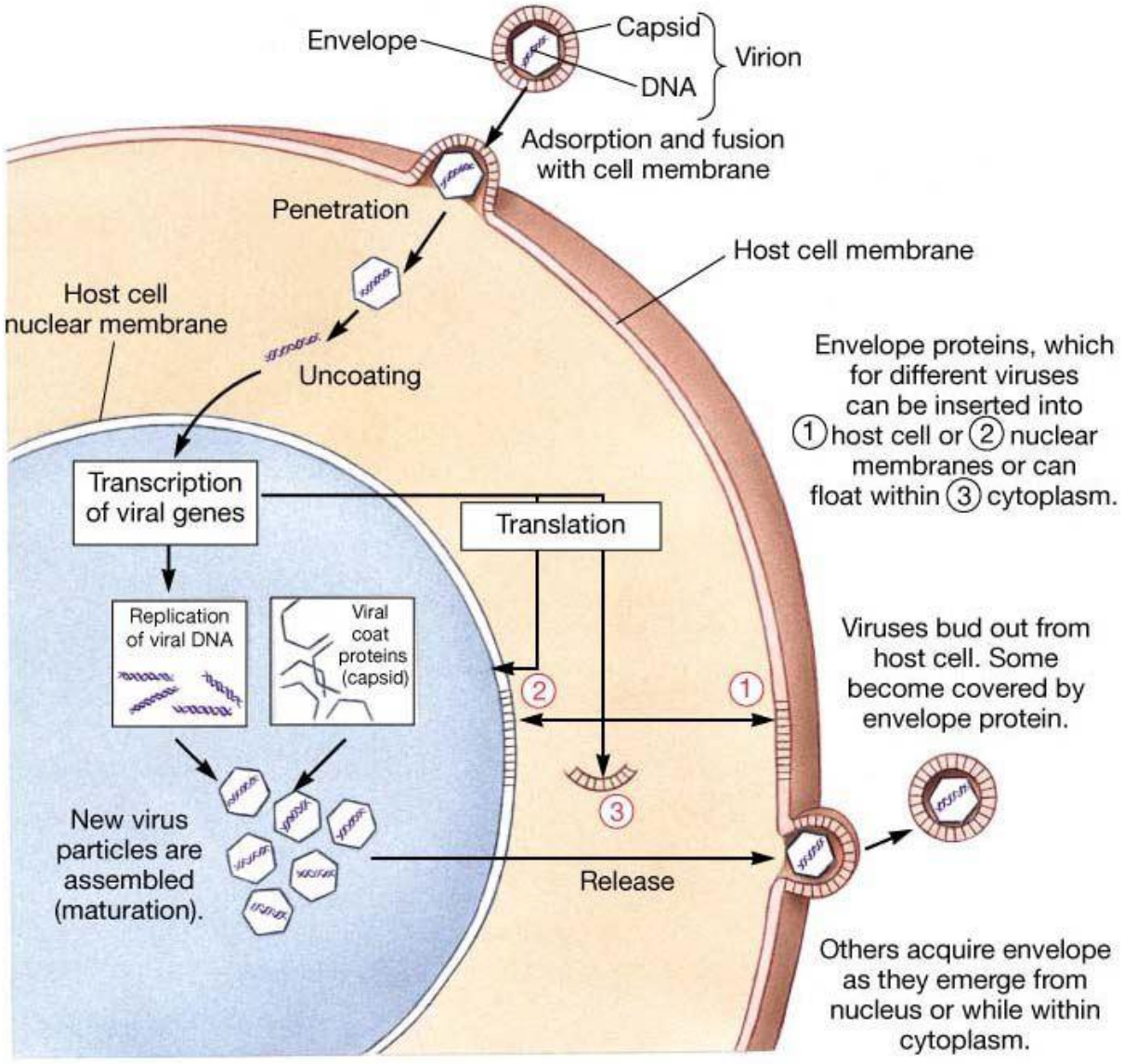
Multiplication of viruses - reproduction



Stages of reproduction

- Adsorption of virion
- Entry of a virus into a host cell (endocytosis - viropexy, combination of a cell membrane with a viral membrane)
- Virion's "uncoatingg", disintegration, or deprecation
- Replication of viral nucleic acids and synthesis of viral proteins
- Formation of virion
- Removal of viruses from the cell (splitting of the host cell, “budding")

Reproduction of viruses



Multiplication of viruses - reproduction

- **In DNA viruses:**
- virus DNA ⟶ iRNA ⟶ synthesis of viral proteins
- **In viruses with a positive strand of RNA:**
- virus RNA ⟶ synthesis of viral proteins
- **In viruses with a negative strand of RNA** :

virus RNA⟶ iRNA ⟶ synthesis of viral proteins

In retroviruses:

virus RNA⟶ complementary DNA ⟶ iRNA ⟶ synthesis of viral proteins

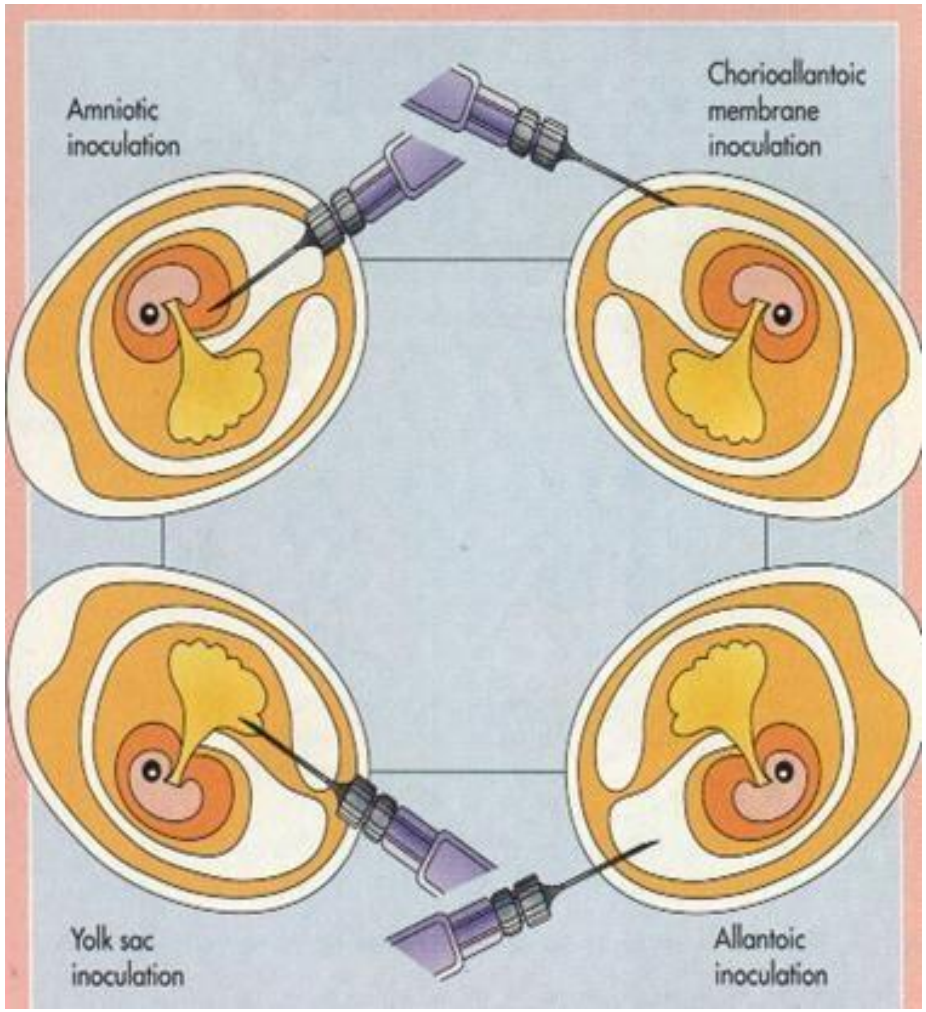
Types of interaction of viruses with host cells

- **Productive infection – reproduction**
- **Abortive infection - incomplete reproduction**
- **Integrative infection - integration (virogenia)**

Basic principles of cultivation of viruses

- In chicken embryosIn
- cell (tissue) cultures
- In the body of laboratory animals

Cultivation of viruses in chicken embryos



Cultivation of viruses in cell (tissue) cultures

- **Cell (tissue) cultures:**

Single layer

Suspended

Organ cultures

Single-celled cell culture is widely used.

- **Single-celled cell cultures:**

Early cell cultures

Transplanted or continuous cell cultures

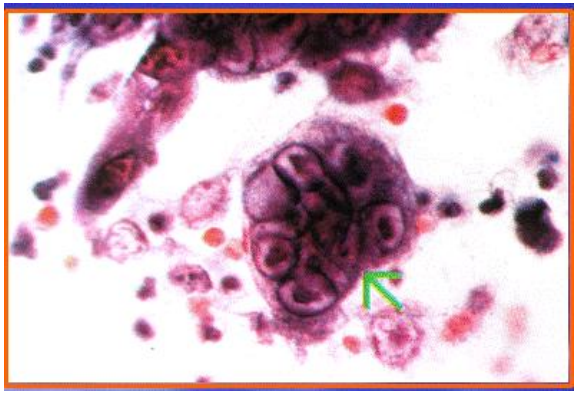
Semi-transplanted cell cultures

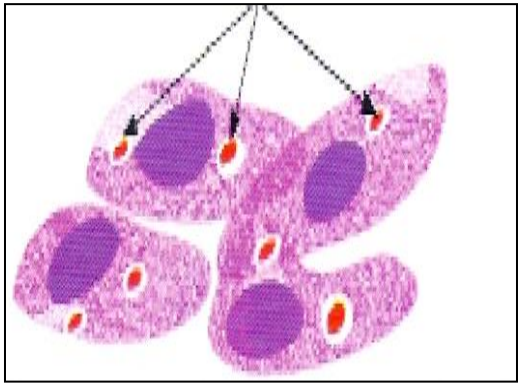
Methods of indication of viruses

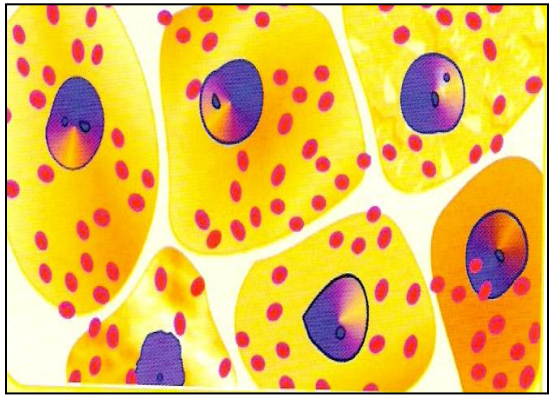
- The multiplication of viruses is not always observed after infecting chicken embryos, cell cultures, as well as laboratory animals with viral material.
- The changes that take place in these objects are taken into account in order to detect (indicate) the multiplication of viruses.

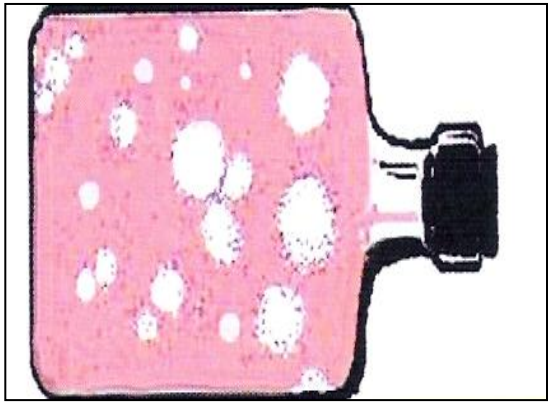
Methods of indication of viruses in cell cultures

Cytopathic effect (SPE), intracellular additives (bodies), hemadosorption phenomenon, "negative colonies", "color test"



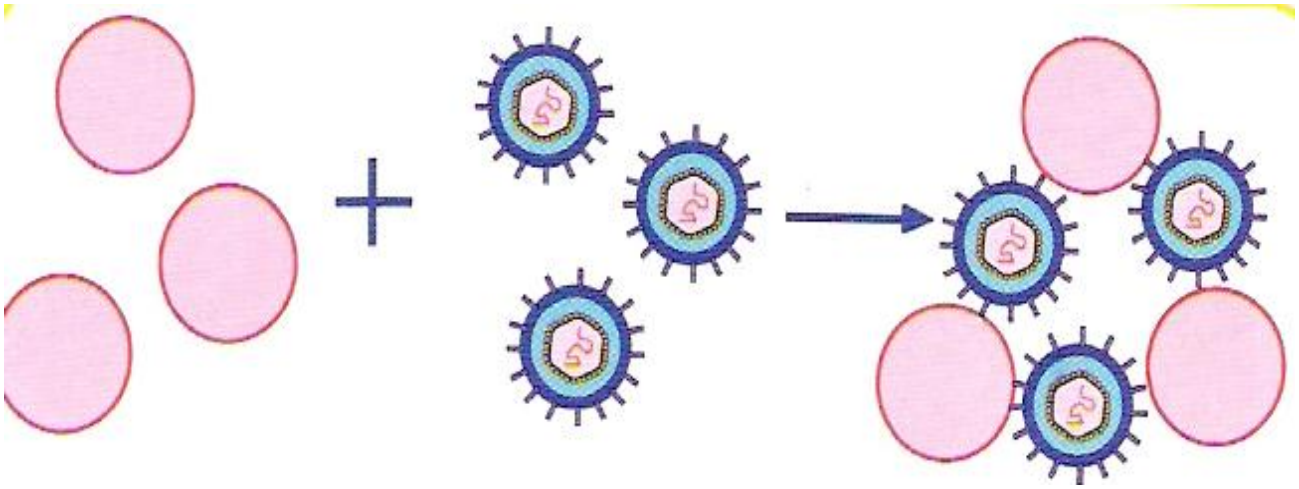






Methods of indication of viruses in chicken embryos

Embryonic death, necrotic areas (ospins) caused by some viruses in the chorionic villus, hemagglutination reaction with amniotic and allantoic fluids, interference



Methods of indication of viruses in the body of laboratory animals

Morbidity and mortality of animals