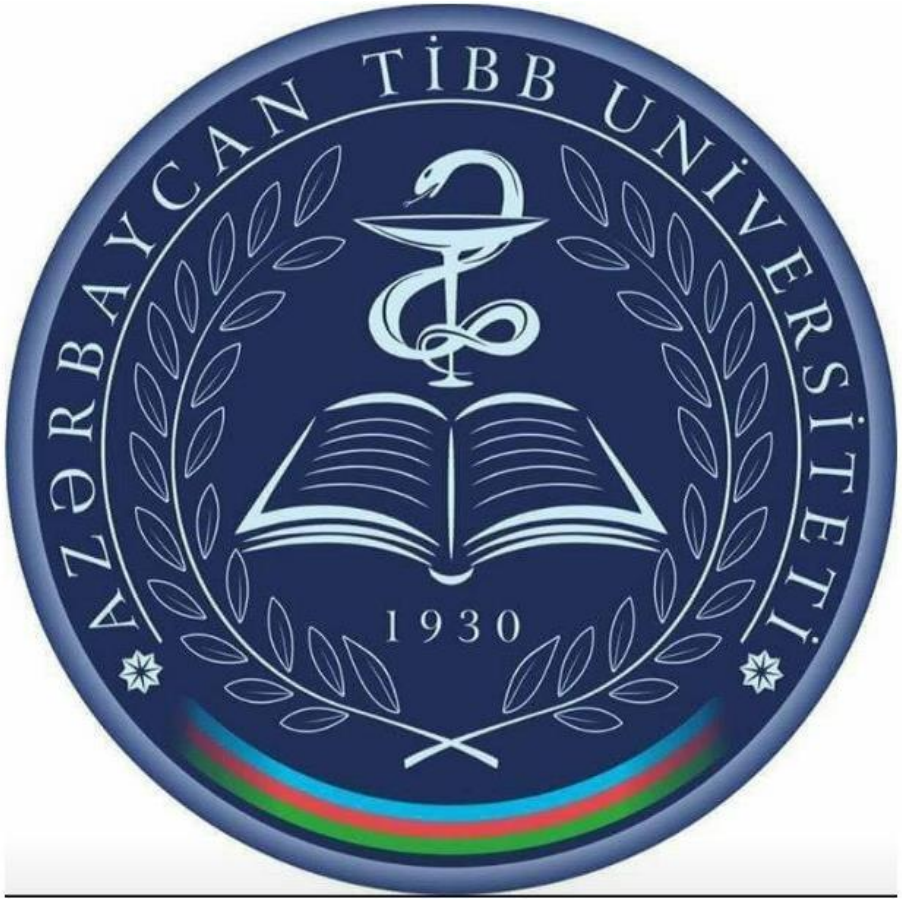
AZERBAICAN MEDICAL UNIVERCYTI



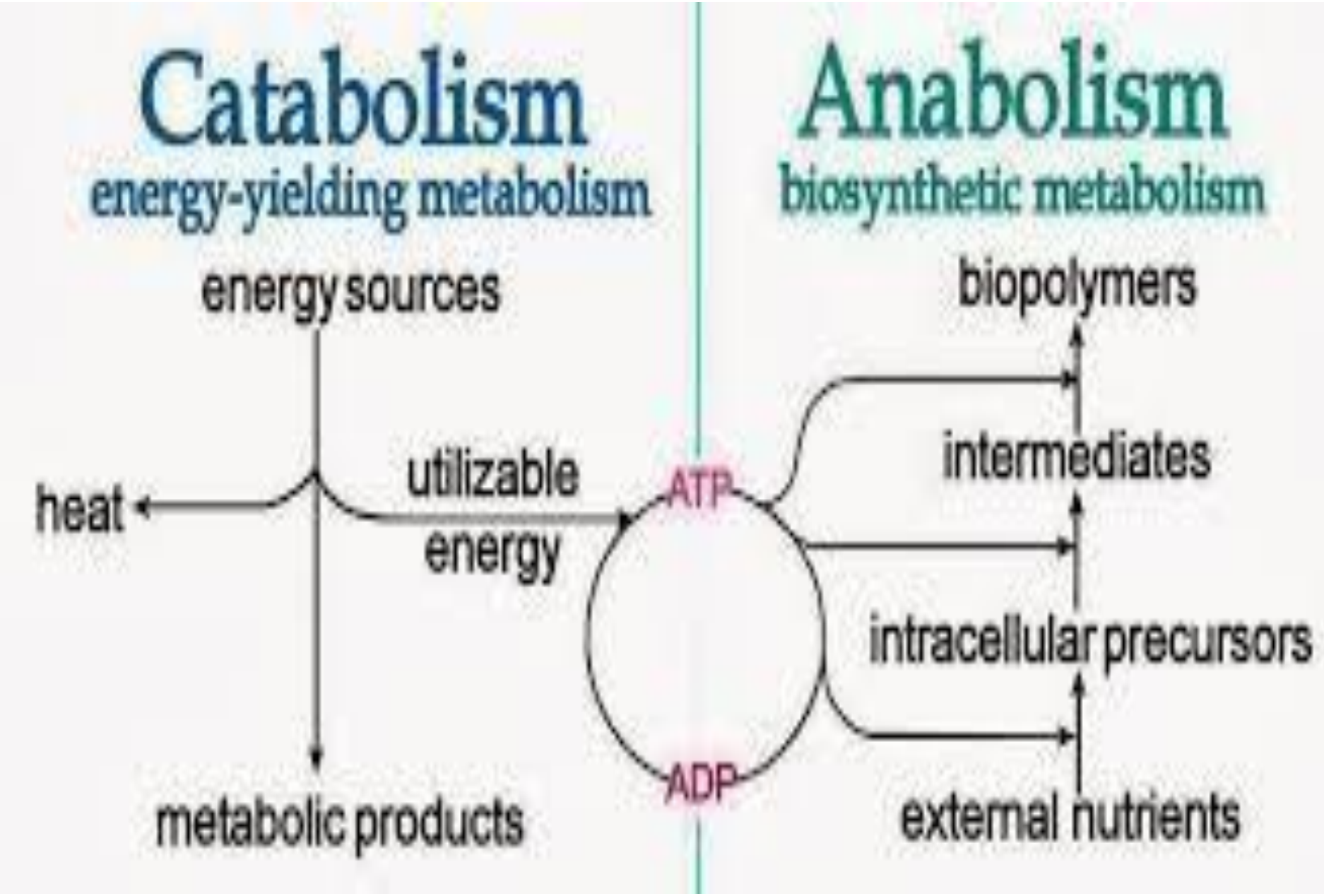
DEPARTAMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY

Lecture III

Physiology of microrganizms. Metobolism and growth.Microflora of pharmaceututecal raw materials and medicinal preparations. Effect of physical, chemical and biological factors on microorganisms. Bacteriophages. Genetics of microorganisms.

Physiology of microorganisms

- Physiology studies the vital functions of microorganisms: nutrition, breathing, growth and division. At the core of physiological functions lies continuous metabolism.
- The essence of the metabolism consists of two opposite but interconnected processes: assimilation (anabolism) and dissimilation (catabolism)


Catabolism
energy-yielding metabolism
Anabolism
biosynthetic metabolism
energy sources
biopolymers
intermediates
heat
utilizable
energy
ATP
intracellular precursors
ADP
metabolic products
external nutrients

Nutrition of microorganisms.

- It is a process during which bacterial cell gains components from the environment around them, which are the basic components for building their organoids.

Chemical Composition of Bacteria

- Water - 70%
- Dry weight - 30% composed of:
  - DNA - 5% MW 2,000,000,000
  - RNA - 12%
  - protein- 70% found in:
    - Ribosomes(10,000) – RNA
    - Protein particles - MW 3,000,000
    - Enzymes
    - Surface structures
  - polysaccharides - 5%
  - lipids - 6%
  - phospholipids - 4%

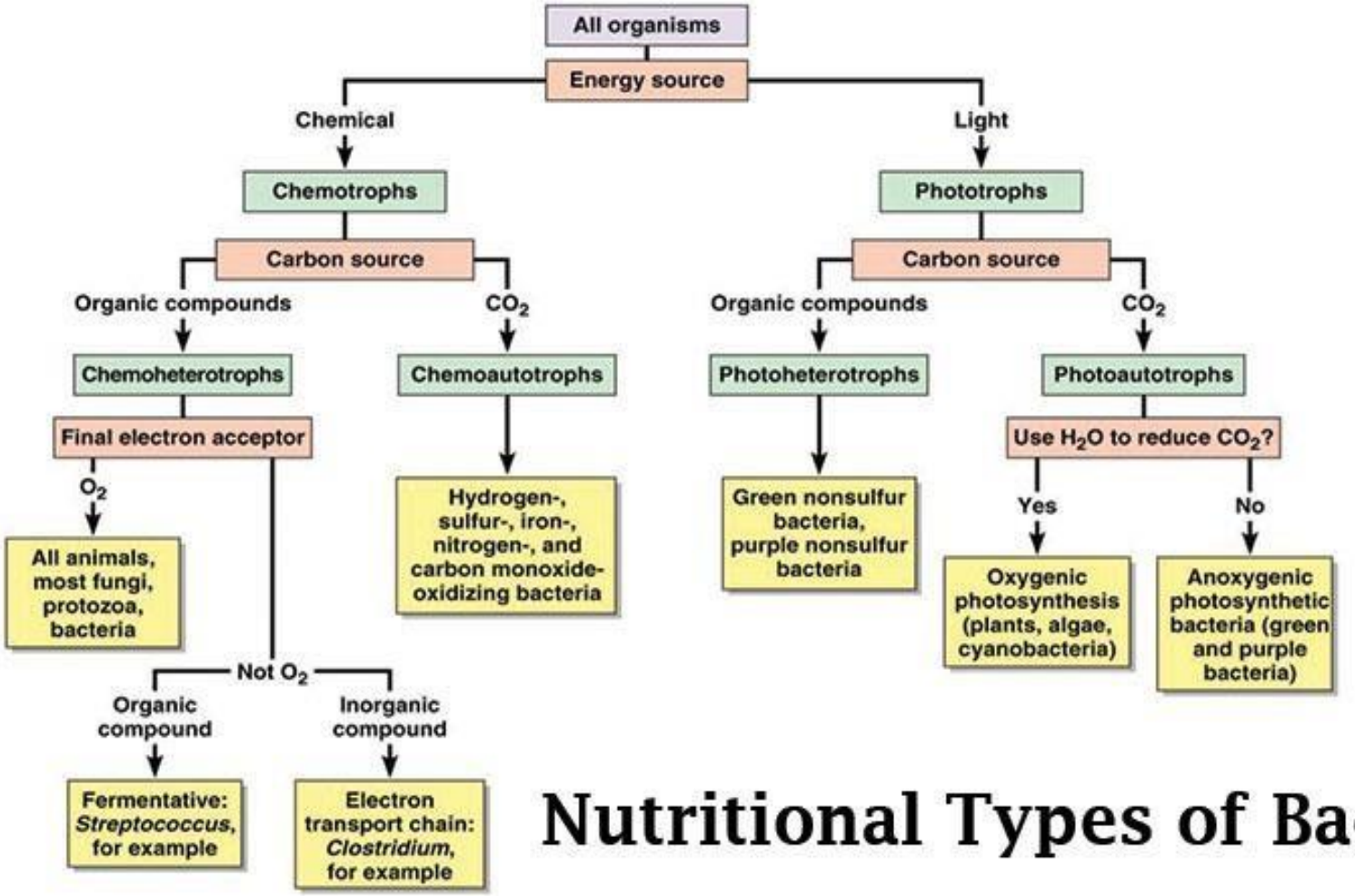
Chemical components of bacterial cell.

- By chemical composition (proteins, polysaccharides, lipids, phospholipids, enzymes), prokaryotes do not differ from eukaryotes.
- Organoids- oxygen, hydrogen, carbon, nitrogen and phosphorus.

Types of bacteria nutrition.

- Source of nutrition
  - Carbon
  - Energy
  - Electron donors
- Groups of microorganisms
  - Autotrophy, Heterotrophy
  - Phototrophy, Hem trophy
  - Littrophy, Organ trophy

Classification of bacteria by the types of nutrition



Nutritional Types of Bacteria

Bacteria enzymes

- Nutrition of microorganisms is carried out due to the presence in the cell of various enzymes that catalyze all vital reactions.
- Enzymes are biological protein-based catalysts.
- In accordance with the catalytic reactions, all enzymes are divided into six classes:

Ferments are divided into 6 classes.

- Oxidorectases- ferments, catalyses redox reactions. Plays a crucial role in gaining energy by biological processes.
- Transferase- ferments, catalyses the transfer of individual radicals, parts of molecules or the whole atomic groups (non hydrogen), from one compounds to another.

- Hydrolases- ferments (enzymes), catalyses reactions of splitting and synthesizing of complex compounds.
- Lyases- ferments, catalyses the cleverage from substrates of particular chemical groups with creation of double bond or by connection of individual groups or radicals with double bonds
- Isomerase- ferments, makes transformation of organic compounds into isomers . Carbohydrates and their derivatives, organic acids, amino acids etcare exposed to isomerization.
- Ligases- ferments, catalyses synthesis of complex organic bonds from simple ones.

Classification of bacteria's enzymes

- **Endo and exoenzymes**
- **Constitutive and inductive enzymes**
- **Enzymes of metabolism**
  oxidoreductases, transferases, hydrolases, ligases, lyases, isomerases
- **Aggression enzymes.**

Endo and exoenzymes

- **Endo enzymes** Endo enzymes functioning only inside of the cell. They catalyze reaction of biosynthesis and energy exchange.
- **Exoenzymes** they are secreted by the cell into the environment and catalyze the hydrolysis of complex organic compounds into simpler ones available for assimilation by the microbial cell. These include hydrolytic enzymes, which play an extremely important role in the nutrition of microorganisms.

Constitutive and inducible enzymes.

- Depending on the conditions for the formation of enzymes, they are divided into constitutive and inducible.
- The constitutive name is the enzymes synthesized by the cell, regardless of the substrate on which the bacteria develop. For example, glycolysis enzymes.
- Inducible enzymes are synthesized only in response to the presence of the inducer substrate necessary for the cell in the medium. It interacts with the repressor, inactivates it, as a result of which the genetic apparatus of the cell is turned on and the synthesis of the corresponding enzyme begins. Induced synthesis of enzymes occurs while an inductor is present in the medium.

Enzymes of aggression.

- Some enzymes, the so-called aggression enzymes, destroy the tissues and cells of a macroorganism, thereby causing the spread of pathogenic microorganisms and their toxins in infected tissues.
- Such enzymes include plasmocoagulase, neuraminidase, collagenase, lecithinase, hyaluronidase and some other enzymes.

Transport of nutrients to the bacterial cell.

- Passive nutrient transfer (only by concentration gradient without energy consumption):
- simple diffusion facilitated diffusion (involving carrier protein)
- Active transfer (against concentration gradient with energy expenditure):
- active transport
- chemical radical translocation

Passive transportation of substances .

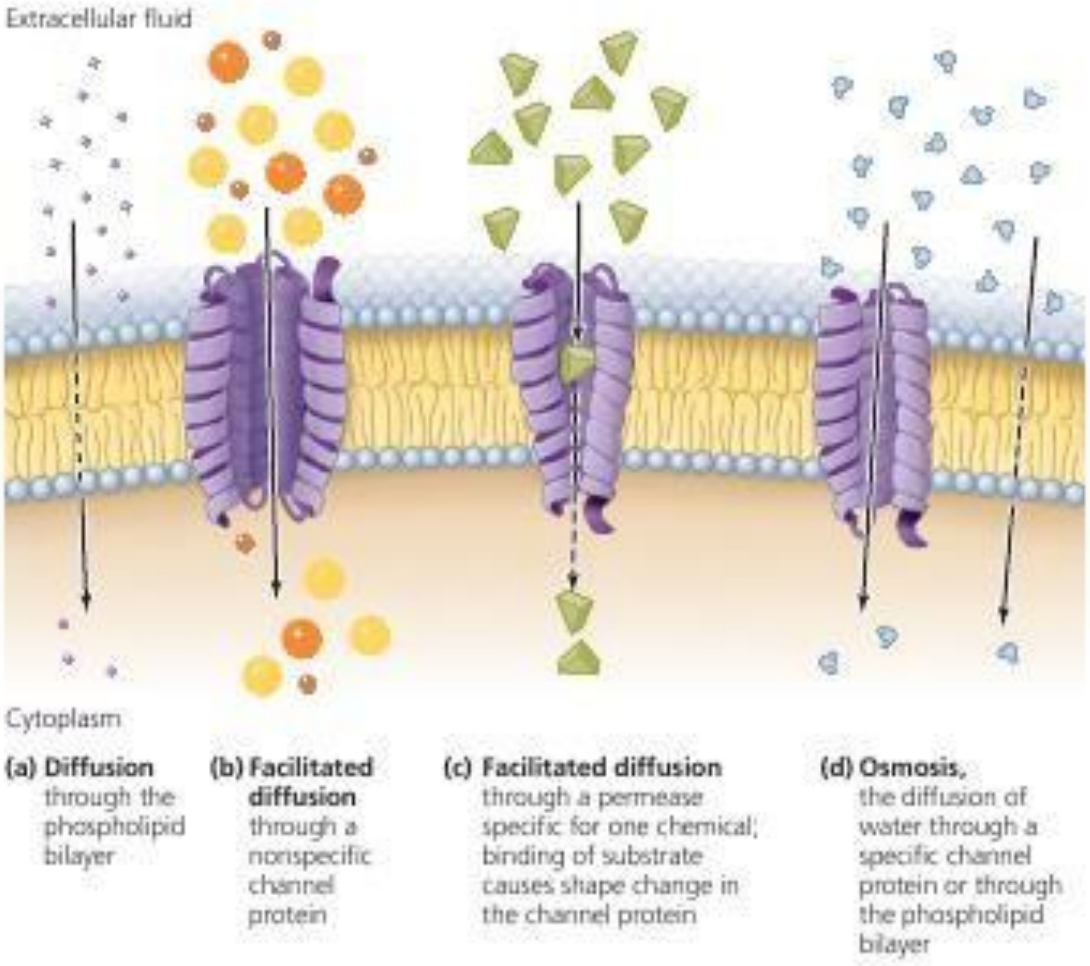
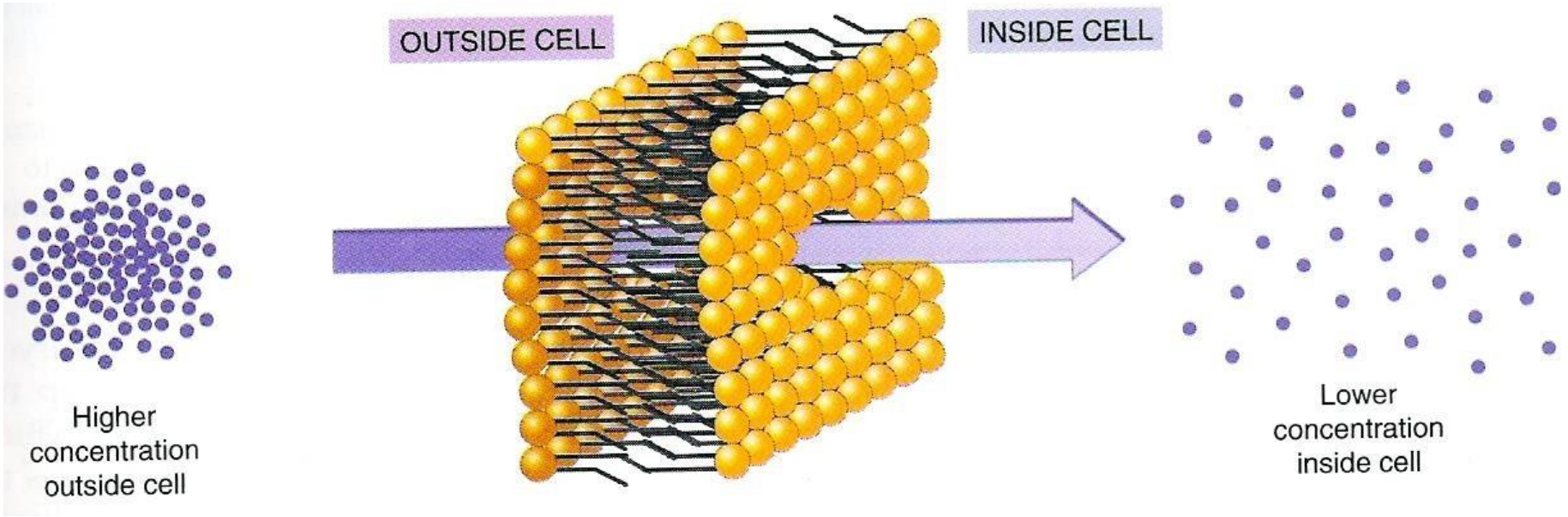
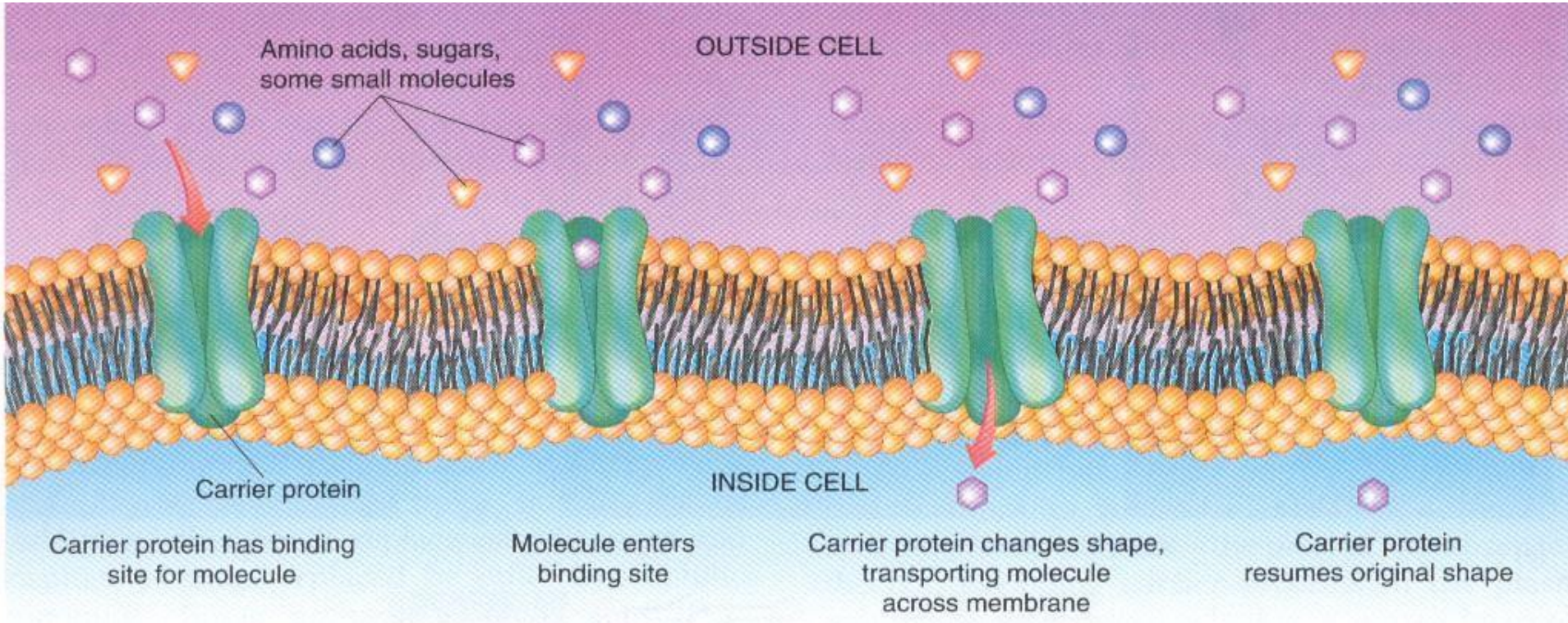


Figure 3.18 Passive processes of movement across a cytoplasmic membrane. Passive processes always involve movement down an electrochemical gradient. **(a)** Diffusion. **(b)** Facilitated diffusion through a nonspecific channel protein. **(c)** Facilitated diffusion through a specific channel protein. **(d)** Osmosis through a nonspecific channel protein or through a phospholipid bilayer.

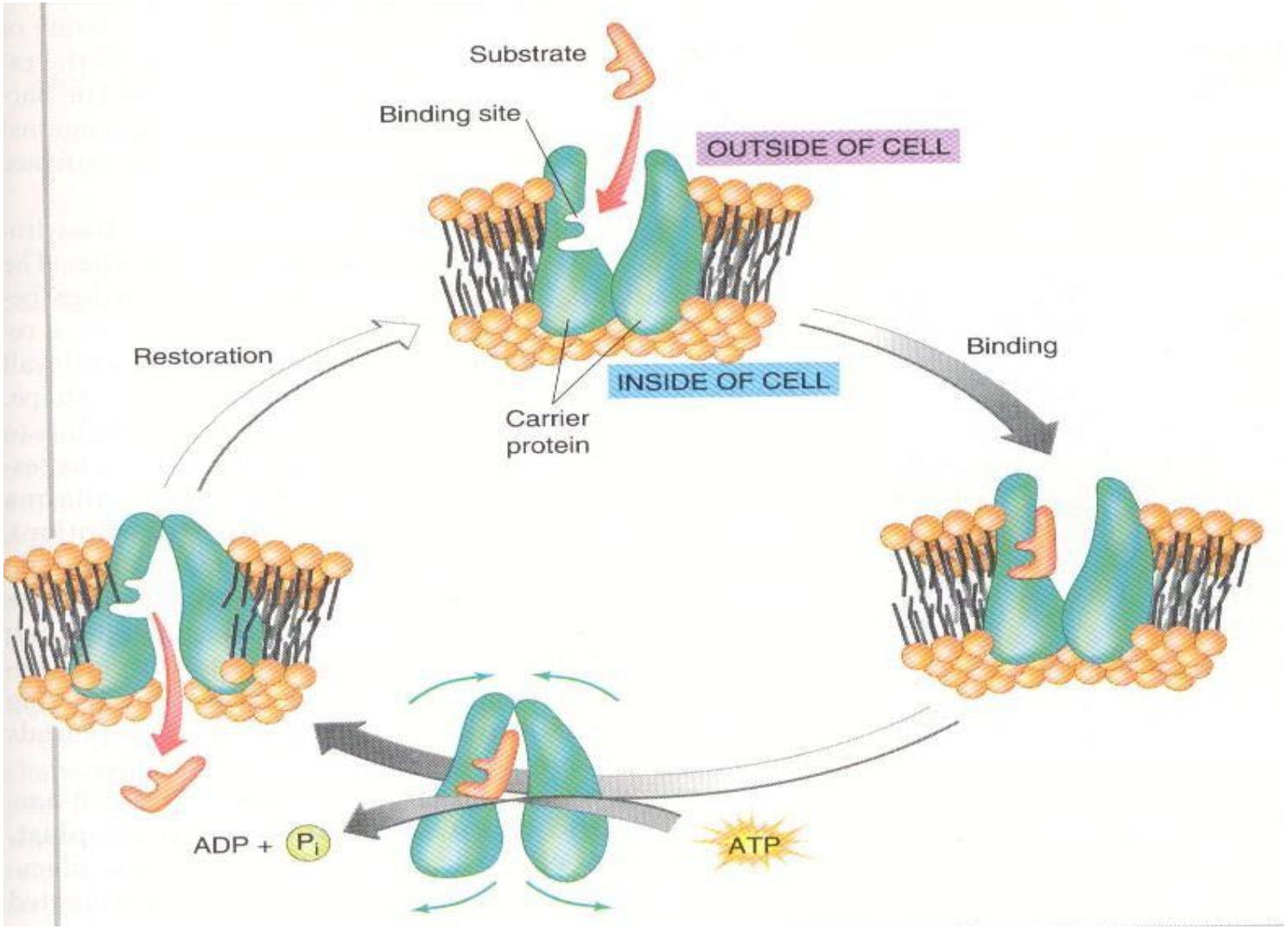
Mechanism of simple diffusion.



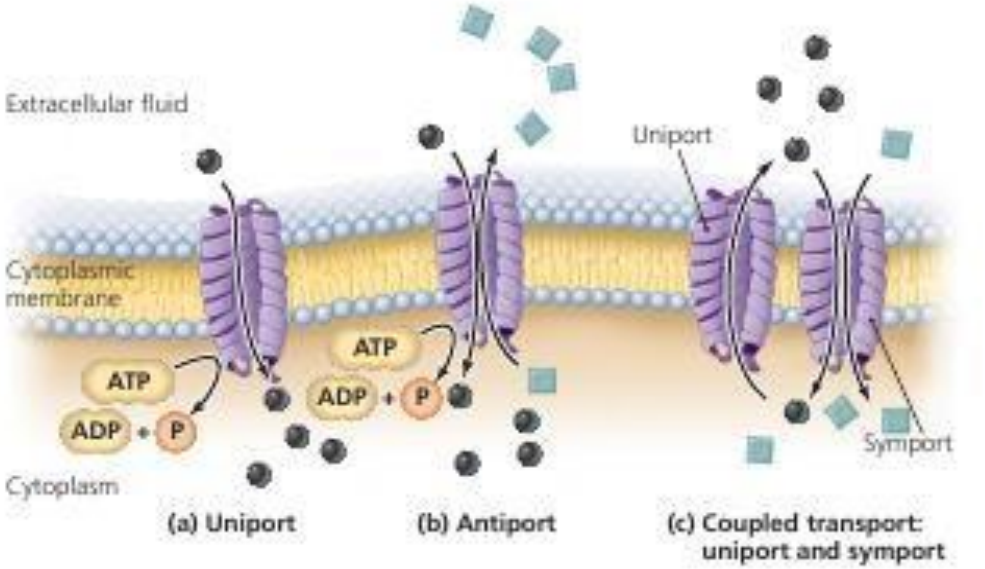
Mechanism of lightweight diffusion.



Mechanism of active diffusion.



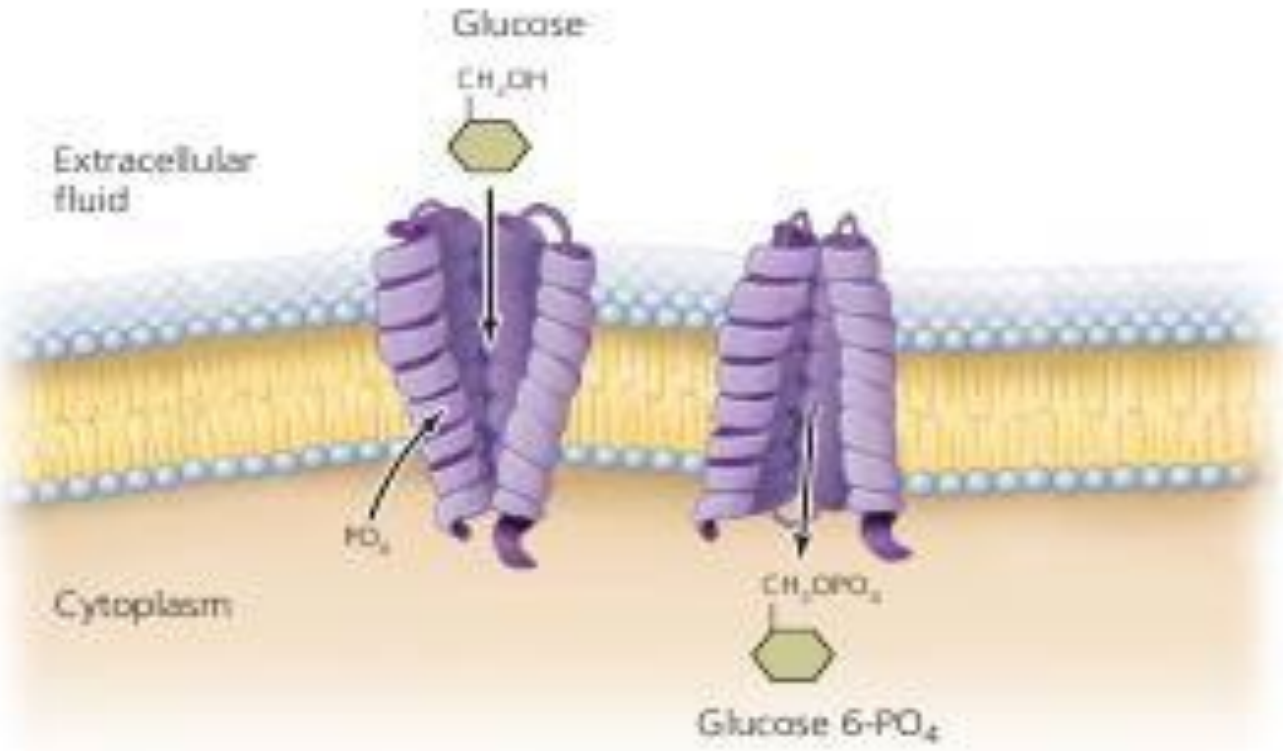
Types of active transport.



◀ **Figure 3.21 Mechanisms of active transport.** (a) Via a uniport. (b) Via an antiport. (c) Via a uniport coupled with a symport. In this example, the membrane uses ATP energy to pump one substance out through a uniport. As this substance flows back into the cell, it brings another substance with it through the symport. *What is the usual source of energy for active transport?*

Uniport - transport of substances independently of other substances
Antiport - transport of a substance is associated with oppositely directed transport of another
Simport - substance transfer is associated with unidirectional transport of another

Translocation of groups



▲ **Figure 3.22 Group translocation.** This process involves a chemical change in a substance as it is being transported. This figure depicts glucose being transported into a bacterial cell via group translocation.

Constructive metabolism (anabolism)

- Amino acid biosynthesis;
- Nucleotide biosynthesis;
- Fat biosynthesis;
- Carbohydrate biosynthesis.

Prototrophs are bacteria that synthesize all components of a cell from a single source of carbon and energy.

Auxotrophs are bacteria whose growth and reproduction require growth factors (amino acids, purine and pyrimidine bases, vitamins)

Energy metabolism (catabolism) in bacteria .

- The following types of metabolism are available depending on the method of generating energy:
- oxidative (respiration)
- fermentative (enzymatic)
- mixed

Oxidative metabolism, or respiration .

Breathing is the process of generating energy in oxidation-reduction reactions coupled with oxidative phosphorylation reactions, in which organic and inorganic compounds can be electron donors, and only inorganic compounds can be acceptors.

In bacteria with oxidative metabolism, molecular oxygen is the electron (hydrogen) acceptor.

Stages of aerobic breathing

- Oxidative decarboxylation
- Krebs cycle
- Electron transport chain

Fermentative (enzymatic) metabolism in bacteria.

Fermentation metabolism (substrate phosphorylation) is a process of energy production in which hydrogen cleaved from the substrate is transferred to organic compounds. Depending on the final product, the following types of fermentation metabolism are distinguished:

- Alcohol fermentation
- Lactic acid fermentation
- Butyric acid fermentation
- Formic acid (mixed) fermentation

Comparison of aerobic and anaerobic respiration.

TABLE 5.4

Comparison of Aerobic Respiration, Anaerobic Respiration, and Fermentation

	Aerobic Respiration	Anaerobic Respiration	Fermentation
Oxygen required	Yes	No	No
Type of phosphorylation	Substrate-level and oxidative	Substrate-level and oxidative	Substrate-level
Final electron (hydrogen) acceptor	Oxygen	NO_3^-, SO_4^{2-}, CO_3^{2-}, or externally acquired organic molecules	Cellular organic molecules
Potential molecules of ATP produced per molecule of glucose	38 in prokaryotes, 36 in eukaryotes	2–36	2

Separation of bacteria by type of respiration.

- Obligatory aerobes (causative agents of tuberculosis, plague, cholera)

-Microaerophiles (lactic acid bacteria)

- Obligate anaerobes (clostridia of tetanus, botulism, gas anaerobic infection, bacteroids, fusobacteria)
- -Aerotolerant microorganisms (some clostridia)
- Optional anaerobes (most pathogenic bacteria)
- Capnephilic bacteria (bovine brucellosis pathogen)

- **Obligate aerobes-** are able to gain energy by respiration only and needs molecular oxygen. For them the type of redox processes oxidation is typical, in which the end electron acceptor is oxygen.

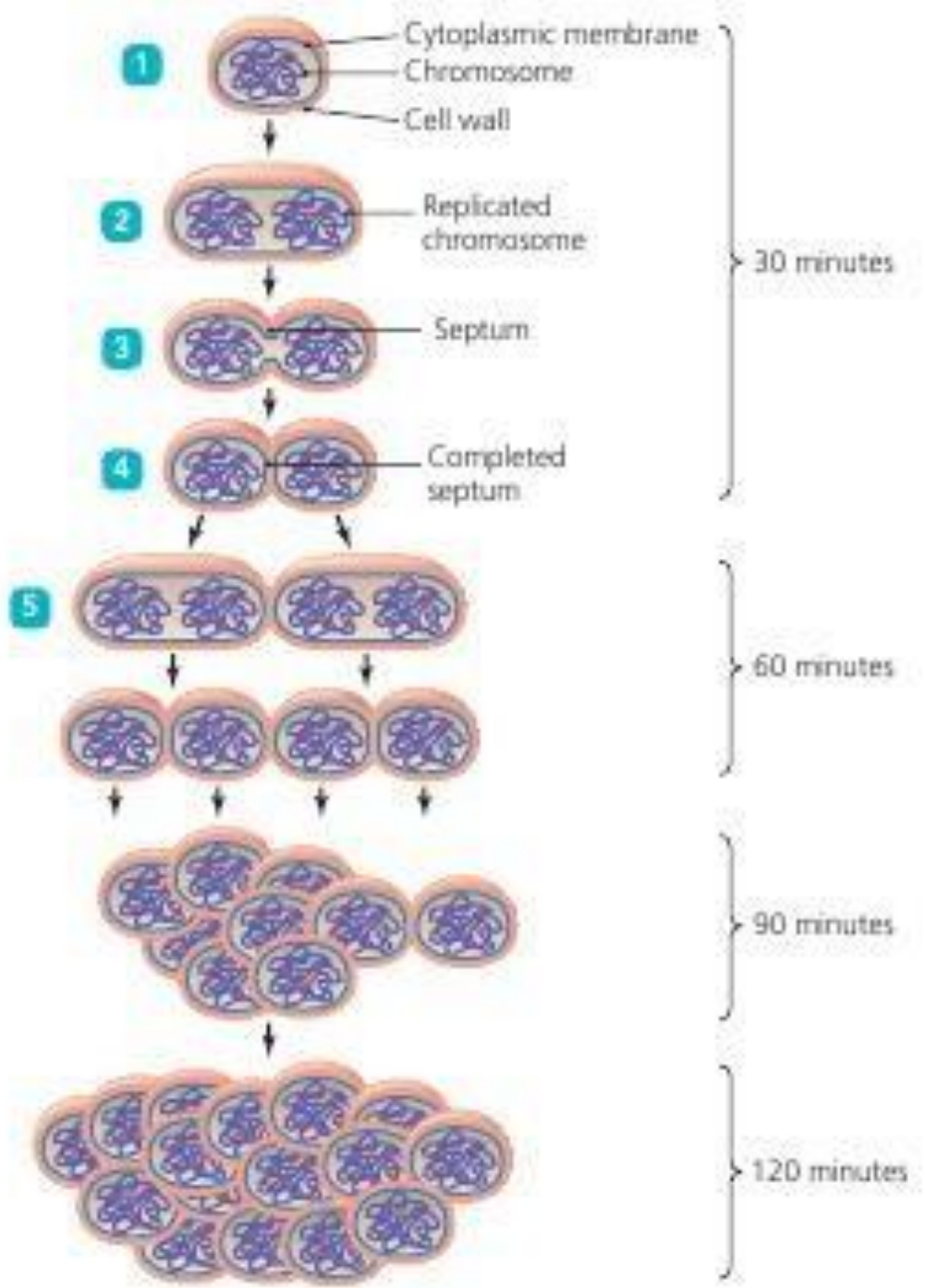
- **Obligate anaerobes-** bacteria's which are able to grow only in the environment without oxygen. Fermentation is typical for them in the redox process. In which the transformation of electrons from substrate-donor to substrate- acceptor happens.

- **Facultative anaerobes-** bacteria's, are able to grow in environment with or without oxygen and use terminal acceptors of electrons as a molecular oxygen and also organic compounds.

Growth and reproduction of bacteria

Growth - increase in the mass of a bacterial cell

Reproduction - increase in the number of bacteria



Division of bacterial cell

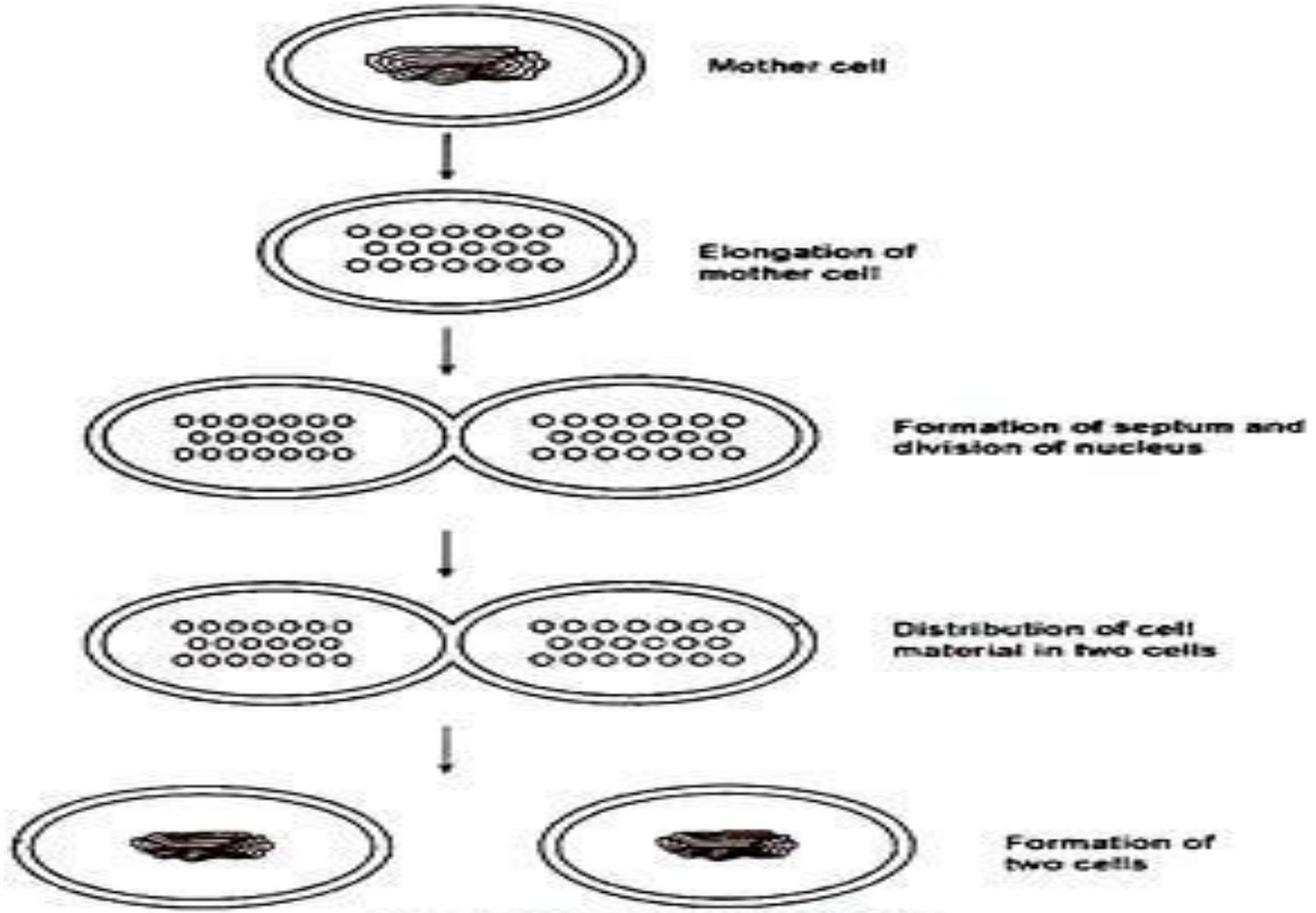
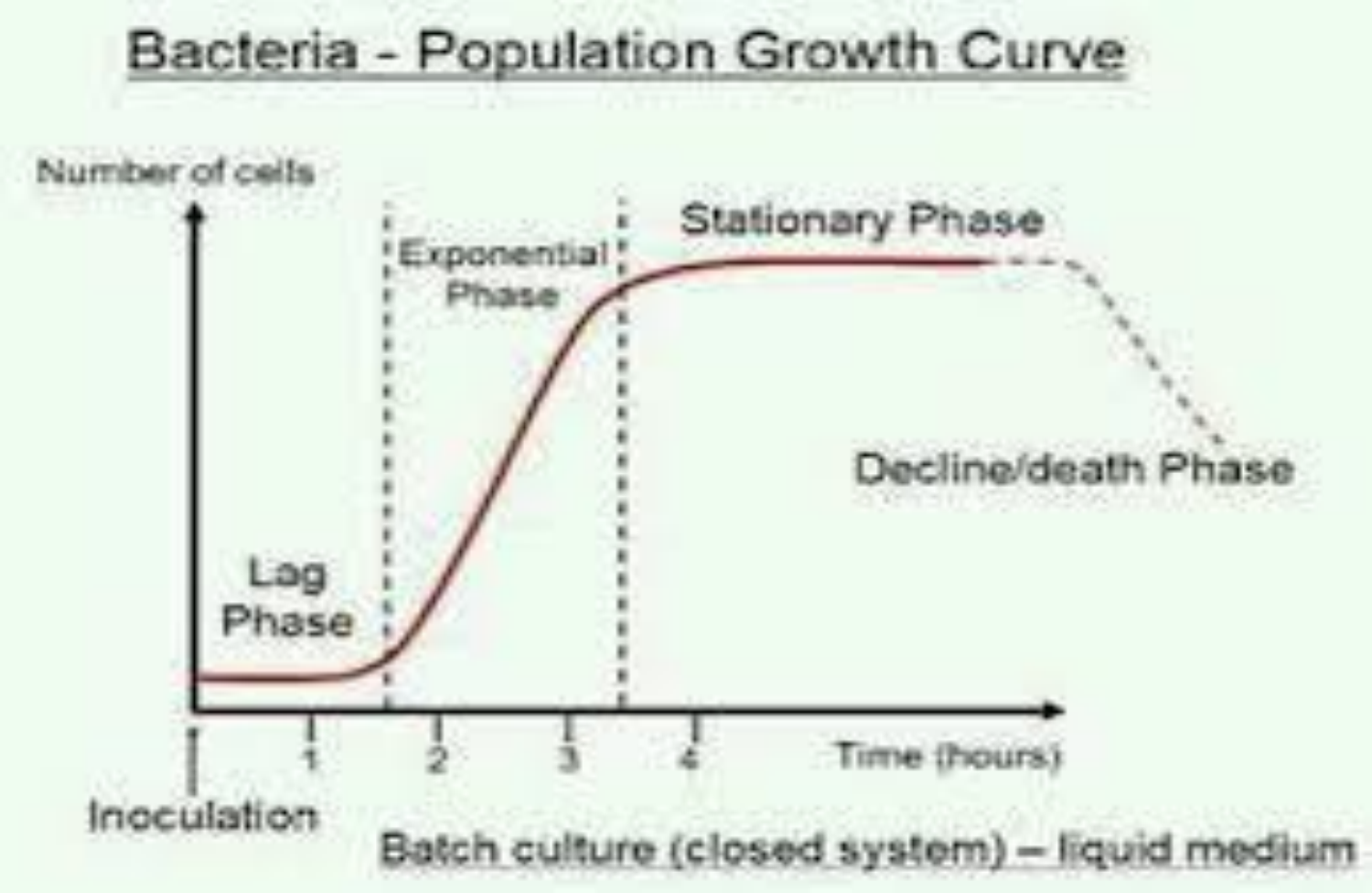


Fig. 1. Bacterial cell division.

The growth curve of bacteria in a liquid medium



Types of Culture Media.

- By composition: - simple environments:
- MPB and MPA
- - complex media: blood, sugar, whey broth or agar. By appointment:

- elective: alkaline broth
- enriched: with sodium selenite
- differential diagnostic: Giss environment, Endo environment

- By origin:

- natural
- artificial

Media Requirements.

- the presence of water;
- the presence of a source of carbon and energy;
- the presence of sources of nitrogen, sulfur, phosphates, trace elements;
- pH of the medium;
- specific osmotic pressure (usually isotonic); sterility.

Cultivation of bacteria on artificial nutrient media

- On solid nutrient medium grow in the form of colonies
- On a liquid nutrient medium can grow in the form of diffuse turbidity, surface film and sediment.

Growth of bacteria in a nutrient medium depending on the type of respiration

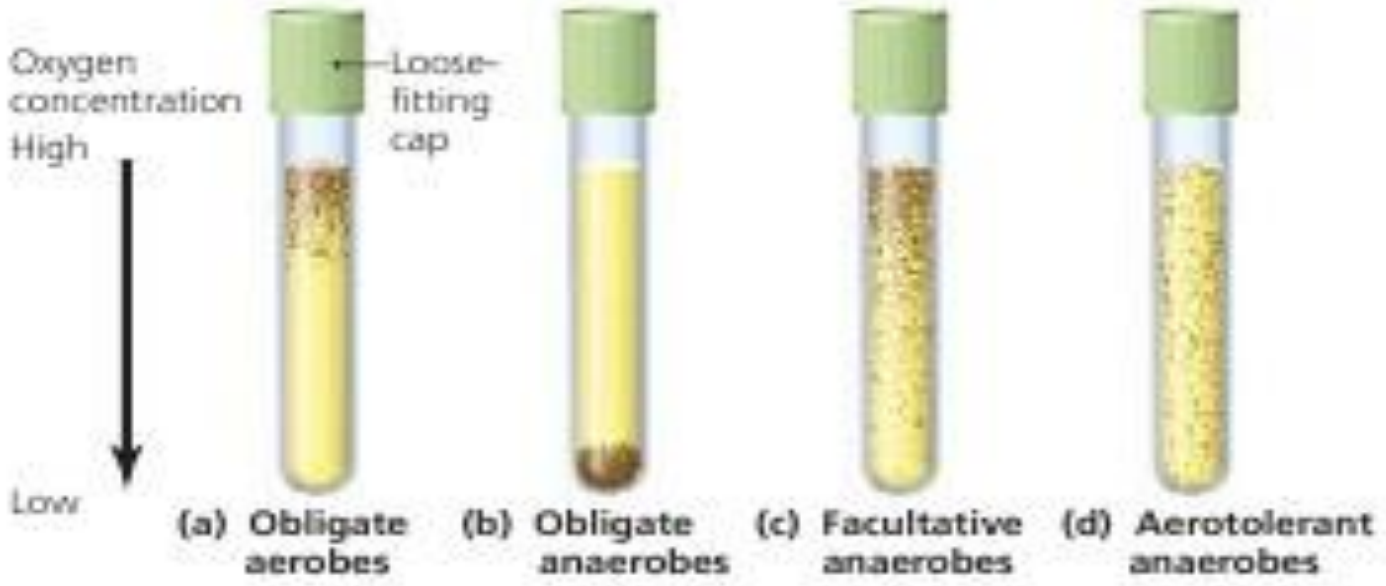
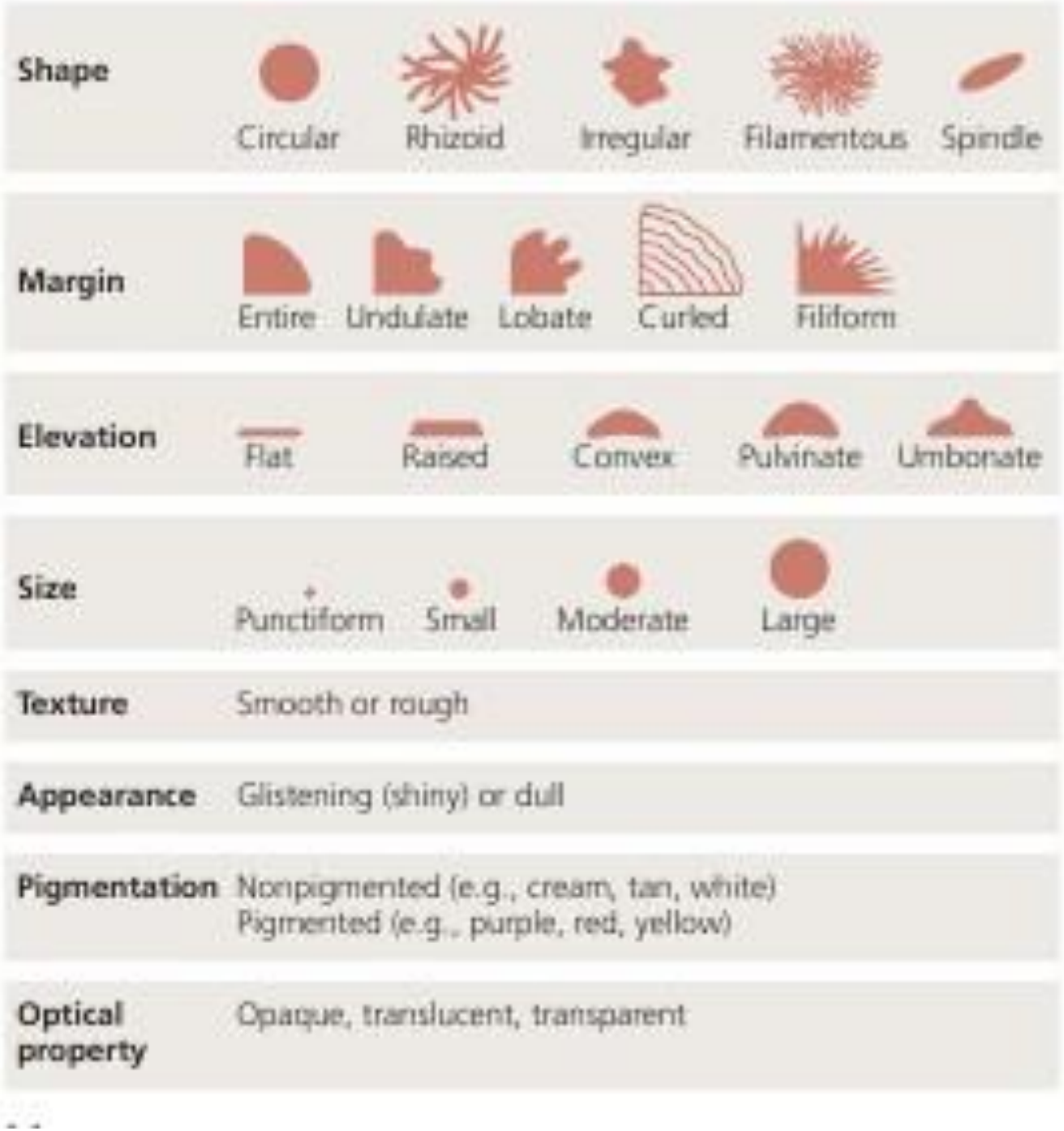
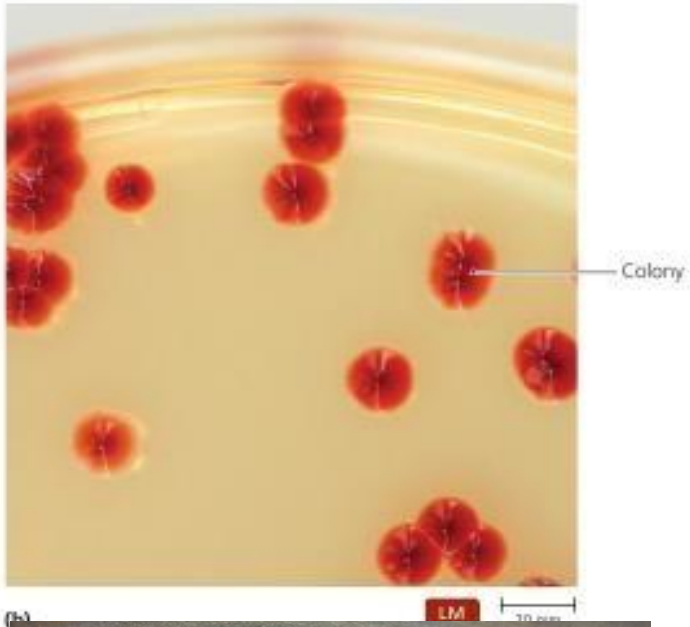


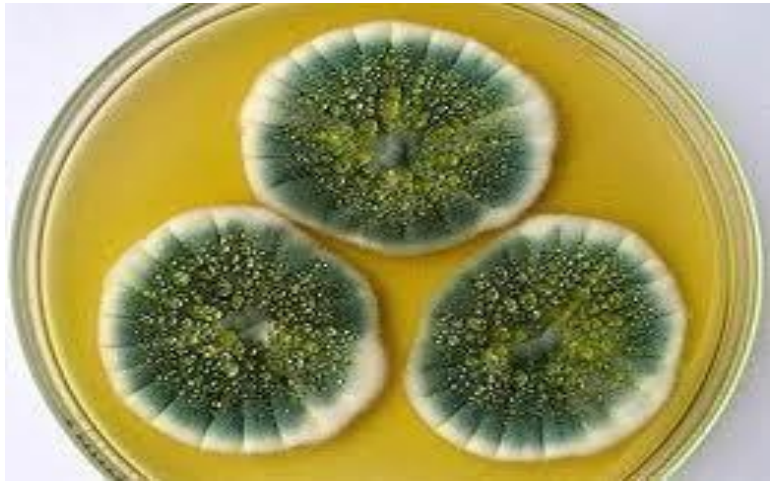
Figure 6.3 Using a liquid thioglycollate growth medium to identify the oxygen requirements of organisms. The surface is exposed to atmospheric oxygen and is aerobic. Oxygen concentration decreases with depth; the bottom of the tube is anaerobic. **(a)** Obligate aerobes cannot survive below the depth to which oxygen penetrates the medium. **(b)** Obligate anaerobes cannot tolerate any oxygen. **(c)** Facultative anaerobes can grow with or without oxygen, but their ability to use aerobic respiration pathways enhances their growth near the surface. **(d)** Aerotolerant aerobes can grow equally well with or without oxygen; their growth is relatively evenly distributed throughout the medium. *Where in such a test tube would the growth zone be for a microaerophilic aerobe?*

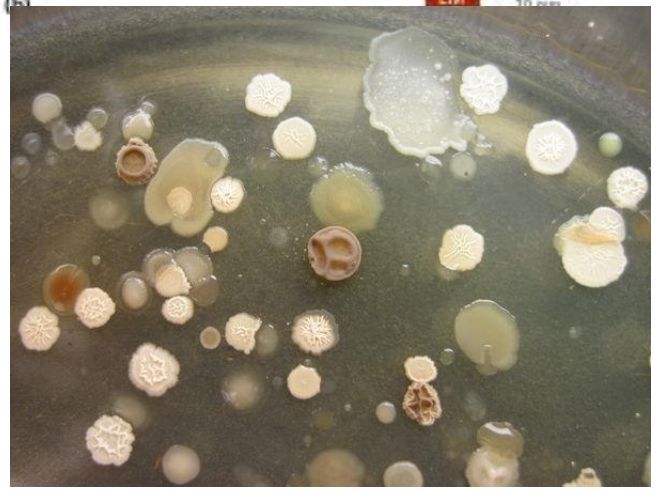
The main features of the colonies



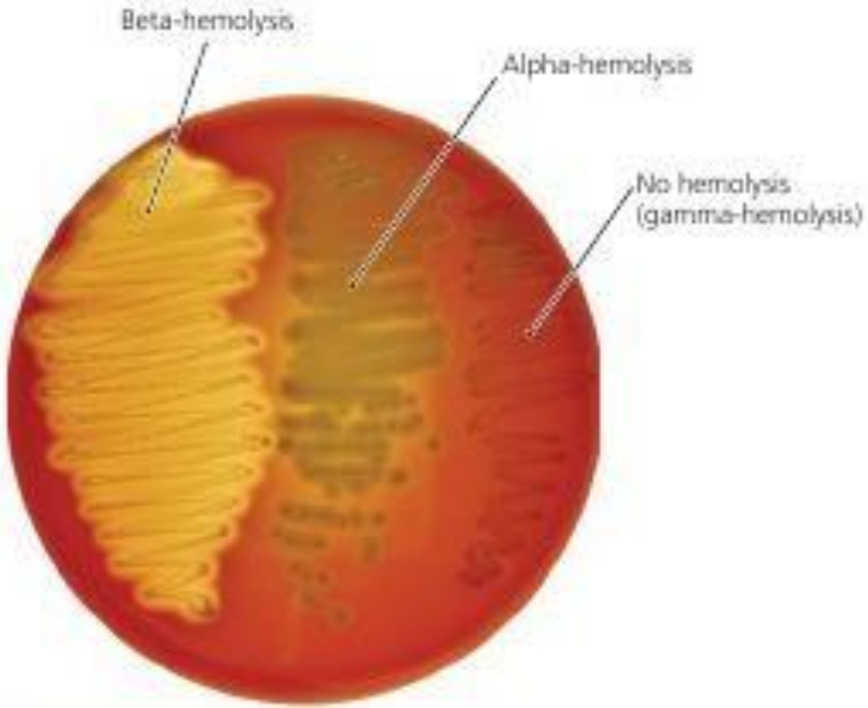
Types of bacterial colonies.





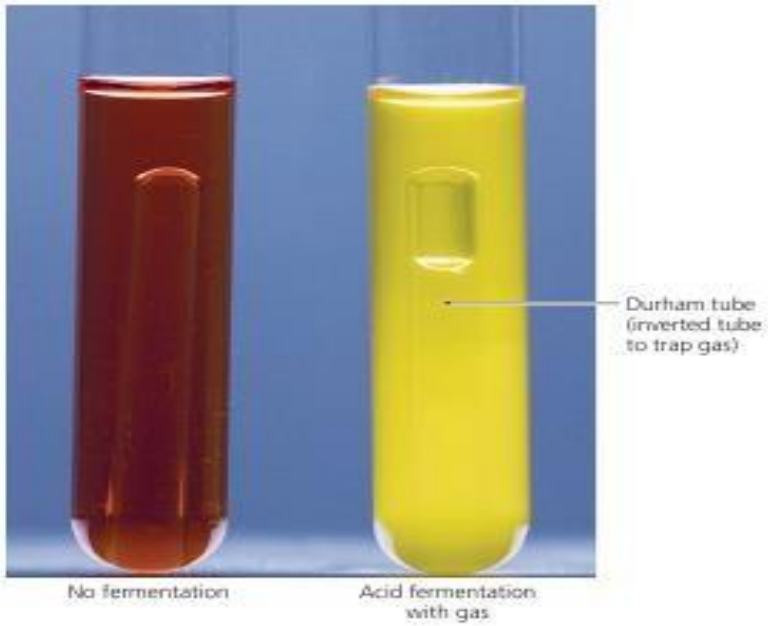


The nature of hemolysis on blood agar.



▲ **Figure 6.13 The use of blood agar as a differential medium** *Streptococcus pyogenes* (left) completely uses red blood cells, produci a clear zone termed *beta-hemolysis*. *Streptococcus pneumoniae* (middl partially uses red blood cells, producing a discoloration termed *alpha-hemolysis*. *Enterococcus faecalis* (right) does not use red blood cells; th lack of any change in the medium around colonies is termed *gamma-hemolysis*, even though no red cells are hemolyzed.

Carbohydrate Breakdown (Giessa Medium).



▲ **Figure 6.14 The use of carbohydrate utilization tubes as differential media.** Each tube contains a single kind of simple carbohydrate (a sugar) as a carbon source, and the dye phenol red as a pH indicator. *Alcaligenes faecalis* in the tube on the left did not ferment this carbohydrate; because no acid was produced, the medium did not turn yellow. *Escherichia coli* in the tube on the right fermented the sugar, producing acid and lowering the pH enough to cause the phenol red to turn yellow. This bacterium also produced gas, which is visible as a small bubble in the Durham tube.

Microflora of pharmaceutical raw materials and medicinal preparations.

While there are no publications about the use of medicines for the treatment of microorganisms and the possibility of infection.Orally taken orally administered contaminant products after 1963 caused some infections. It was understood that the drugs could also be a source of infection.Oral medications include food type infections-Salmonella, eye ointments containing P. aeruginosa, eye drops are common eye infections. In the past - when the pharmacist prepared the medicine according to the patient's prescription and consumed it in a short time. Today - the drug is being prepared in factories and used by a large patient population after a long time in the factory.

Microflora of pharmaceutical raw materials and medicinal preparations.

Standard, set of rules for quality production = GMP (Good Manufacturing Practice)

- Reduce the risk of error in production to a minimum
- A concept that provides quality production suitable for its intended use
- First introduced in 1963 by the Food and Drug Administration (FDA) in the United States,
- It was accepted and published by the World Health Organization (WHO) in 1968,
- In 1984, practiced in our country as a compulsory drug producer.

Microflora of pharmaceutical raw materials and medicinal preparations.

The rules governing the minimum requirements of the methods, installations and controls applied to the production, packaging and presentation of a product (medicine)

• The aim is; It is safe to use the drug, and it ensures that it carries the desired purity and quality

• GMP; A quality system that directly influences human health is a quality system that guides the conditions under which products such as medicines, cosmetics, food, medical devices should be produced.

• The quality of each serial product in the production depends on its suitability to all required standards. So;

• Adequate training of staff, provision of suitable buildings and equipment • Use of the right materials

• Implemented trial actions •

Availability of suitable storage and transport equipment

• Correct record keeping means - GMP

Microflora of pharmaceutical raw materials and medicinal preparations.

Microbiological quality controls should be performed at each stage of production to minimize microbial contamination and microbial quality in pharmaceutical products and to minimize the risk of secondary infection.

- The microbial contamination in the pharmaceutical product causes the product and the patient's health to deteriorate, causing material and moral loss for the manufacturer.
- A statistically insignificant error in the medication may pose a serious hazard to the patient using the product.

Causes of Microbiological Contamination of Pharmaceutical Preparations

Raw material properties and characteristics:

- Many drug substances and adjuvants are suitable for the proliferation of microorganisms.
- The most important factors that play a role in the microbiological contamination of medicines are natural raw materials with a broad microflora of vegetable and animal origin.
- Pharmaceutical form:
- It is directly related to the microbiological contamination of a drug.
- For example; Liquid and semi-solid preparations are extremely dangerous.

Antimicrobial substances such as ethanol and sugar are added to some preparations to inhibit the growth of bacterin.

- Sterile products and non-sterile products can not be produced in the same environment.

Manufacture of medicines

• Manufacturing stage- Fabricated Hygiene: During the manufacture of medicines
1-unsuitable environmental conditions
2-used tools and equipment
3-staff
4-Raw
5-Water
6-packaging
7-storage and waiting time to raft; the causes of
All factors that cause contamination during manufacture should be removed. The water used must comply with microbiological standards. Deionized water used for the preparation of noninjectable drugs and freshly drawn (4 hours prior) distilled water for injectable and eye preparations which must be sterile should be used after microbiological controls. Filtered air should be delivered to the area where the production is made. Trained personnel should be employed.

All factors that cause contamination during manufacture should be removed.The water used must comply with microbiological standards.Deionized water used for the preparation of non-injectable drugs and freshly drawn (4 hours prior) distilled water for injectable and eye preparations which must be sterile should be used after microbiological controls.Filtered air should be delivered to the area where the production is made.Trained personnel should be employed. Sterile production should be done in units built separately and purposefully from other production areas.Attention should be paid to particulate contamination during sterile production.This is why walls, ceilings and floors. Dust and other particulate matter.Provides continuous cleaning and disinfection. The surfaces must be smooth and air, non-water permeable. Staphylococcus, Micrococcus and Diphtheroid bacilli, which are present in the normal hand flow of contaminated hands by hand, cause contamination of the drug and reach the organism through contaminating drugs. Cross-contamination: Pathogenic bacteria or viruses are said to pass from a contaminant surface to another surface.Therefore, the contamination spread can be reduced by methods such as not using the spoon, needles, injectors for the second time, and disposing of the applicators after the use of the topical products - disposing of the applicators. Drugs that are kept open may be contaminating with airborne microorganisms.In terms of homes and hospitals, the drugs used in hospitals are more likely to be infected with pathogenic microorganisms.In the investigations conducted, it has been determined that the drugs are mostly in high-level contaminants during use. Bacillus subtilis, yeast in the majority of daily used tablets and the land was found

A pharmaceutical preparation

Contains pathogenic or potentially pathogenic microorganisms.Possession of toxic metabolic residues of microorganism. In the case of obvious and obvious physical and chemical changes, the preparation is regarded as completely degraded in terms of microbiology.Contamination is the activation of the active substances in the drug and may lead to some. Types of microorganisms contained in a drug that is contamine; Air, water, human, animal and vegetal fluoride.Aeropers are the dominant microorganisms. The majority, except Bacillus anthracis, are saprophytic bacteria. Sports forms are particularly resistant to heat and antimicrobial agents. Gram (-) basil is another group of bacteria that can be found in contaminating prep. E. coli, Klebsiella, Enterobacter, Hafnia, Serratia, Citrobacter, Salmonella, Proteus and Pseudomonas group microorganisms. Most of these microorganisms are opaque (opportunistic, potential pathogen). These bacteria, which are found in human and animal normal microflora, gain pathogenicity . Yeast and Mold (Aspergillus, Penicillium, Saccharomyces) Are among the microorganisms encountered in medicines and most of them are heat resistant.

Environmental factors

- Physical, Chemical and biological environmental factors have bactericide, bacteriostatic and mutagen influence on microbes.

Physical factors

- Temperature
- Atmosphere pressure
- Drying
- Light (UV-rays)
- Ionizing radiation
- Ultrasound

High pressure. Bacteria's and mostly their spores

are resistant to mechanical pressure. Bacteria's are found in nature that are living in deep ocean at the depth 1000- 10 000 m under the pressure of 100-900 atmospheres. Some types of bacteria withstand 3000-5000 atm, but bacterial spores are resistant even 20 000 atm

Drying.

- Water is needed for normal life activity of microorganisms. Drying can cause dehydration of cytoplasma, violates integrity of cytoplasmic membrane, which brings to death of the cell.
- Some microorganisms under the influence of drying die after a couple of minutes: those are mostly cocci’s.

Liofilization drying.

- The essence of this method is that microorganisms are freeze first from -25 to -73 Celsius, and then dry with the + temperature in the vacuum. Wherein the cytoplasma of bacteria is frozen and becomes ice and then this ice evaporates under positive temperature and the cell stays alive (transfer of water from frozen condition into gaseous, bypassing the liquid phase- sublimation).

Ray energy

- In nature, microorganisms are constantly exposed to solar radiation. Direct sunlight causes the death of many microorganisms within a few hours, with the exception of photosynthetic bacteria (green and purple sulfur bacteria). The destructive effect of sunlight is due to the activity of ultraviolet rays (UV rays). They inactivate cell enzymes and damage DNA. Pathogenic bacteria are more sensitive to UV rays than saprophytes. Therefore, it is better to store microbial cultures in the laboratory in the dark. In this regard, Buchner's experience is demonstrative.

Buchner's experiment.

In a Petri cup with a thin layer of agar, an abundant inoculation of any bacterial culture is performed. Then the letters cut out of black paper and is glued onto the outer surface of the seeded cup, forming, for example, the word "typhus".
The cup, turned upside down, is exposed to direct sunlight for 1 hour. Then the pieces of paper are removed, and the cup is put in a thermostat for a day at 37 ° C. Bacterial growth is observed only in those places of the agar that were protected from the action of UV rays by glued letters. The rest of the agar remains transparent, i.e. there is no growth of microorganisms.

- X-ray radiation, Alfa, beta and ultraviolet rays have a devastating effect on microorganisms only in big quantities. Those rays destroy cells. In recent years with the use radiation method Petri's cups, syringes, suture materials are sterilized for one time usage.
- UVH-energy. Causes heating of environment, haves devastating effect on microorganisms and damages cells.

-Influences on genetic features of microorganisms

-Influences on intensity of cell division

-activity of some ferments

-hemolytic features

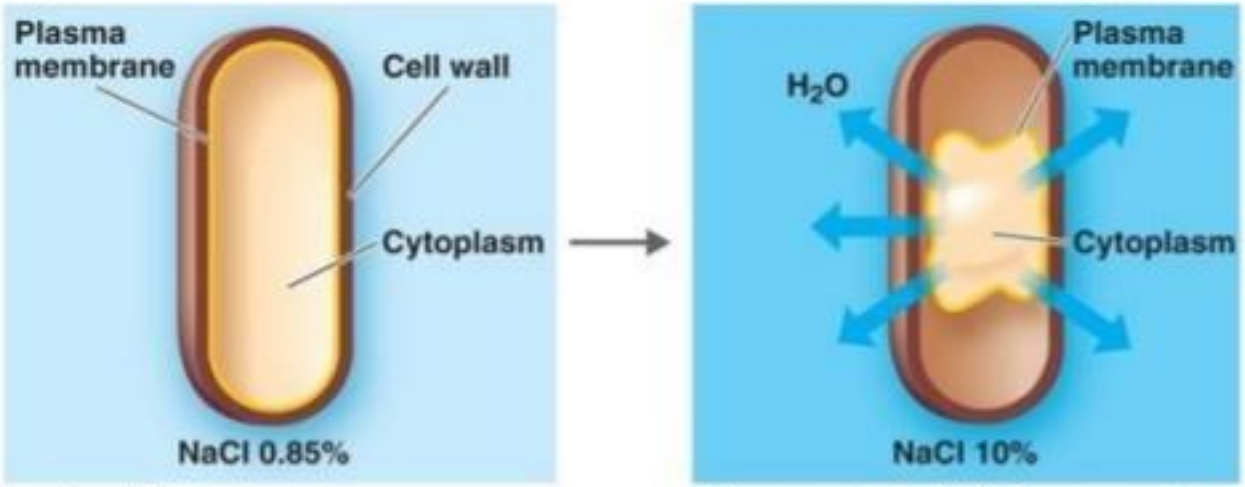
- Ionizing radiation. The main feature of this radiation is causing the ionization process.

Ultrasound.

- Haves a big amount of energy and with the help of this energy they can cause physical, chemical and biological phenomenon. With the help of US waves we can cause inactivation of ferments, vitamins, toxins, destroy different materials and substances, multicellular and unicellular organisms.

Effect of Osmotic Pressure

- Osmotic pressure is the pressure exerted on bacterial cells by their environment



- **Isotonic**
- **Hypertonic (plasmolysis)**

- **Hypotonic:** the bacterial cell gains water and swells to the limit of its cell wall
- Some opportunistic pathogens are **facultative halophiles**
  - ***Staphylococcus aureus* - colonizes the surface of the skin (salt)**

Sterilization

- **Sterilization- it is a process of total destruction of microorganisms.**
- **Methods of sterilization**
  - **Heating**
  - **Chemical**
  - **Ray or beam affection**
  - **Mechanical (filtration)**

Dezincification, aseptic and antiseptics.

- **Dezincification – process of destruction of pathogen microorganisms**
- **Aseptic – set of measures directed to prevent microbes to get into the wounds or organs during surgery and other medical procedures.**
- **Antiseptics – меры, направленные на уничтожение микробов в ране или патологическом очаге. Set of measures needed to destroy microbes in the wounds and pathological focus.**

Influence of chemical factors on microorganisms.

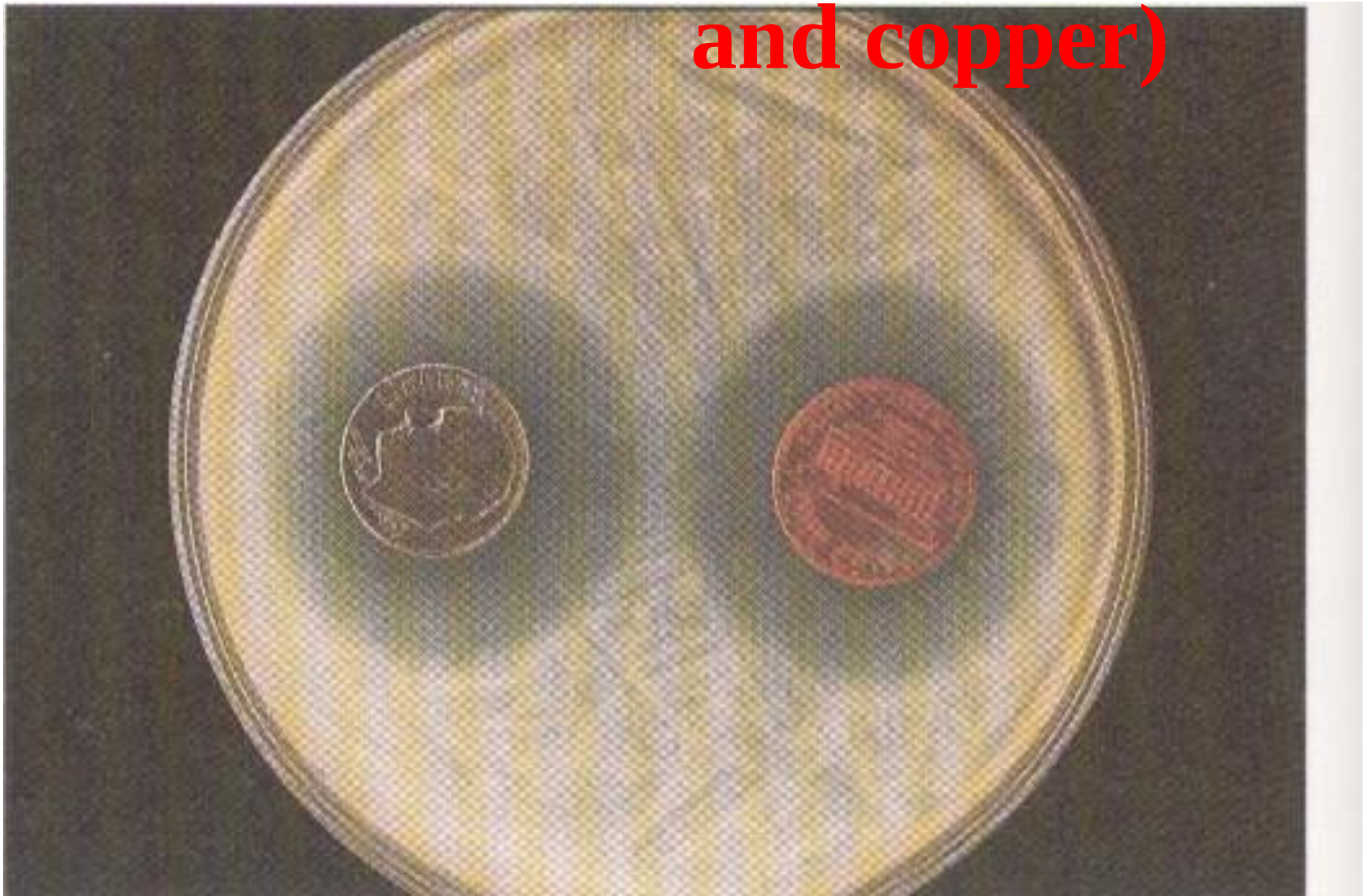
- **Main groups of antiseptics:**
- surfactants (detergents)
- - acids and their salts (boric, salicylic) and alkalis (ammonia)
- - alcohols (70-80 about ethanol)
- - phenol and its derivatives
- - aldehydes (formaldehyde)
- - oxidizing agents (hydrogen peroxide, potassium permanganate)
- - halogens (iodine, chlorine preparations)
- - salts of heavy metals
- - derivatives of 8-hydroxyquinolone
- - derivatives of nitrofuran (furatsilin)
- - dyes (diamond green)

Influence of chemical factors on microorganisms

The main groups of disinfectants:

- Chlorine-containing compounds (bleach, sodium and calcium hypochlorite, chloramine B, dichloro-1, sulfochlantin, etc.)
- Phenolic compounds (lysol, chloro-beta-naphthol, hexachlorophen, etc.)
- Quaternary ammonium compounds (nirtan, ampholan, etc.)
- Peroxide compounds (perhydrol, deoxone-1)

Antimicrobial salt activity of heavy metals (silver and copper)



Influence of biological factors on microorganisms.

Symbiosis is the coexistence of various organisms.

Forms of symbiosis:

- - metabiosis
- -mutualism
- - commensalism
- - satelliteism
- - synergism
- - antagonism

Metabiosis.

- One of the microbes uses product of other life activity, creates conditions for progress.

Ammonifiers —(ammonia)→ nitrification

Cellulose-breaking bacteria —(carbohydrates)→ nitrogen fixing

Mutualism

Cohabitation of microorganisms is –Mutualism.

atmospheric nitrogen, proteins

Rhizobia(nodule bacteria) thermophilicbacteria

Commensalism is

One microbe lives off the other, without harming him.

Normal micro flora of animals

Epiphytic microbes

Satelliteism

- Stimulation of growth and multiplication of one microbe with the products of other living activity.

Factors of growth, vitamins gr.B

Hay stick → Hemophilic bacteria

Synergism.

- Amplification of physiological features and functions during co-cultivation.

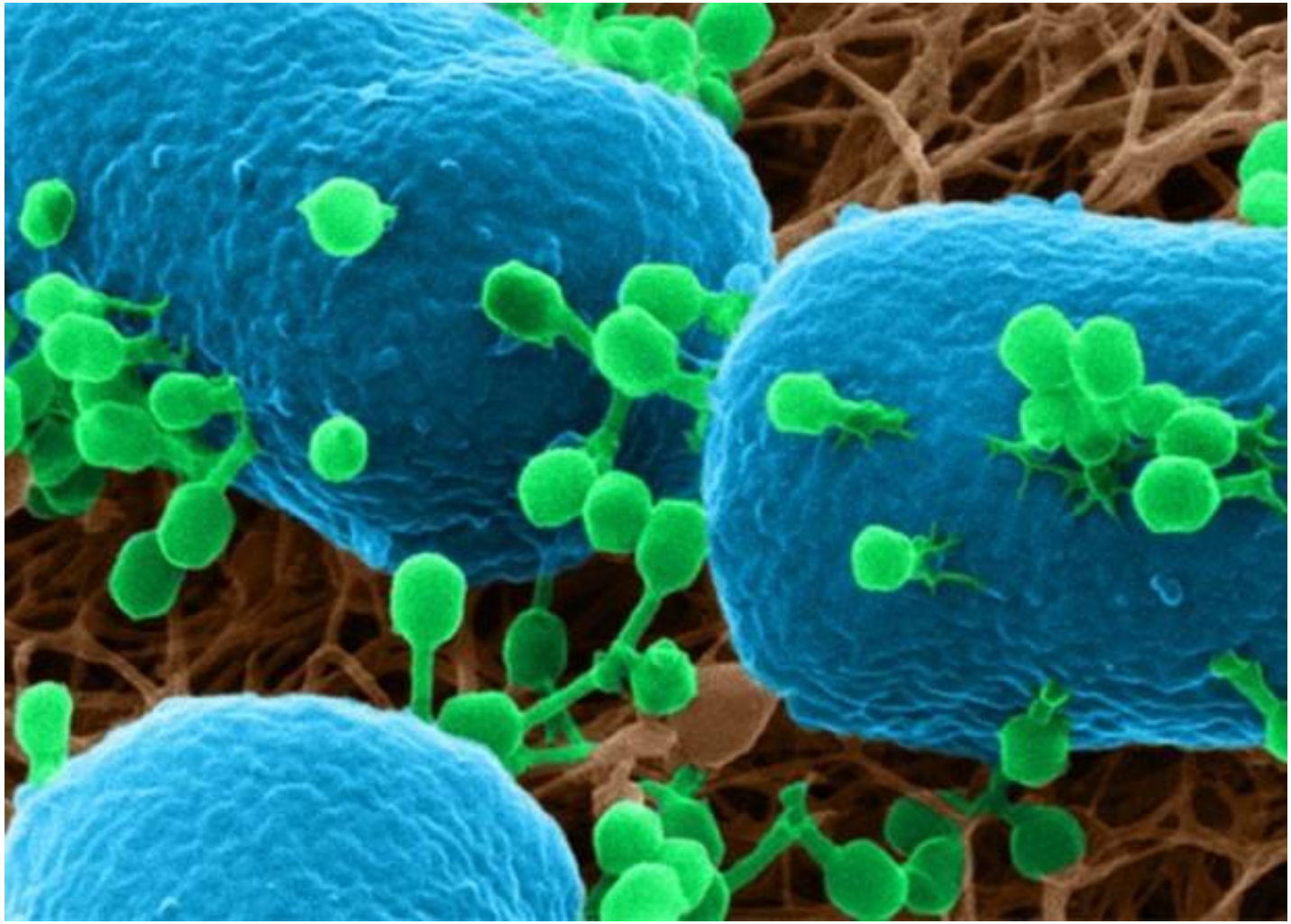
revitalization of lactic acid bacteria

Hay stick ⟷ Lactic acid bacteria

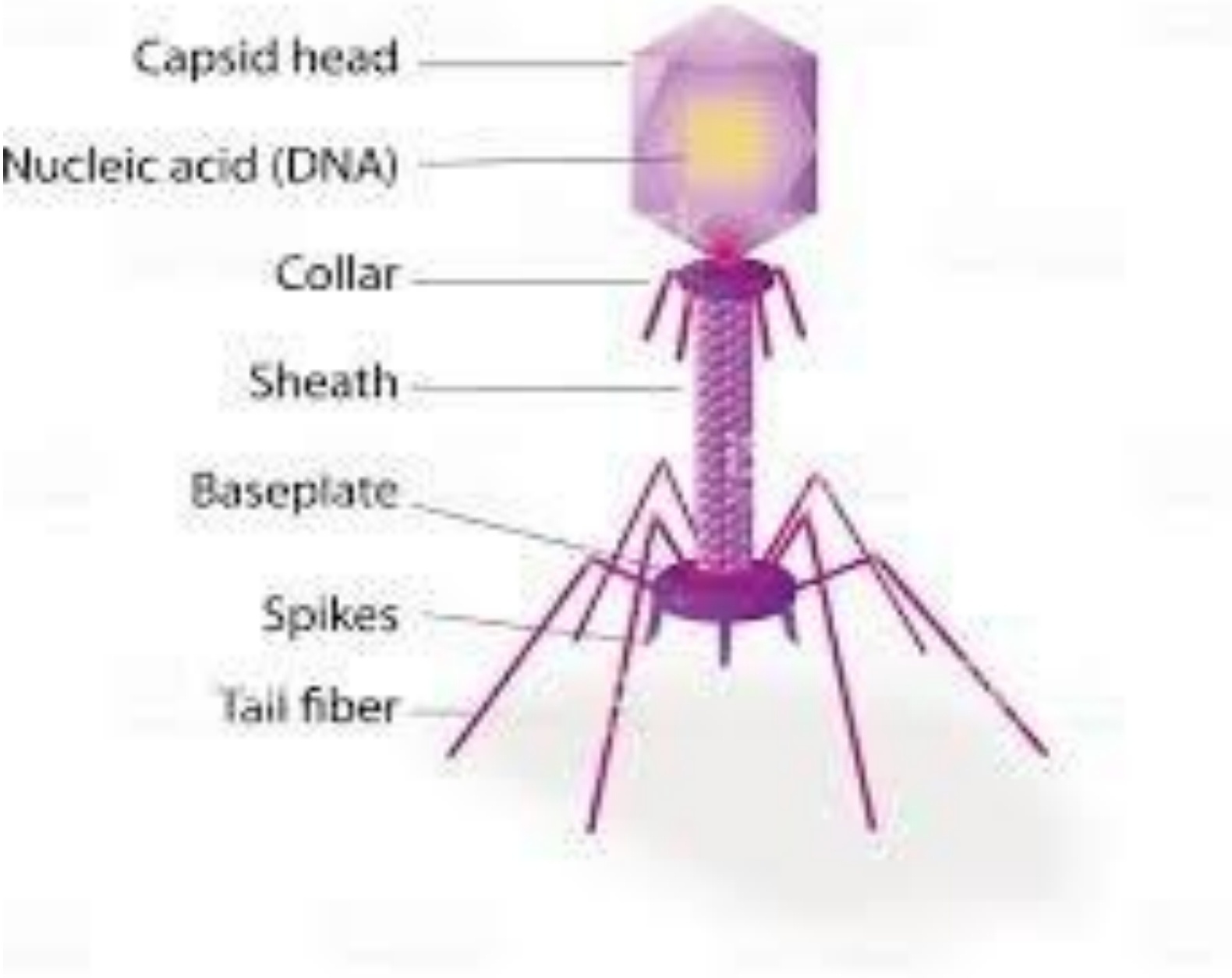
Bacteriophages.

- Bacteriophages (Greek "faqos" -fatting) are viruses that specifically penetrate bacteria that multiply inside them, as a result of which a bacterial cell dies or becomes a prophage carrier.
- Opened in 1917 scientist D ‘Errell.

Attack of bacteriophages on bacteria.



Structure of bacteriophage



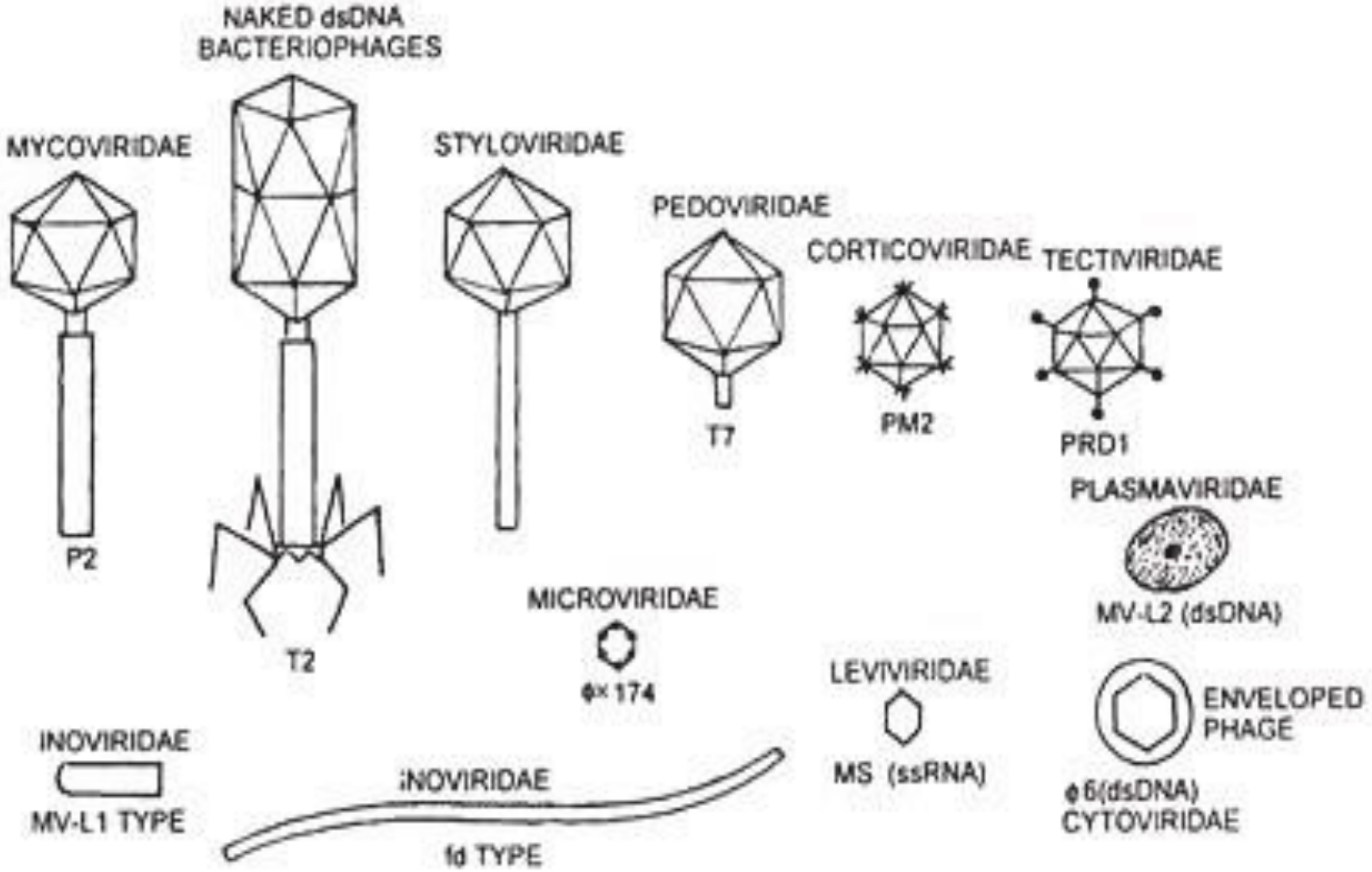


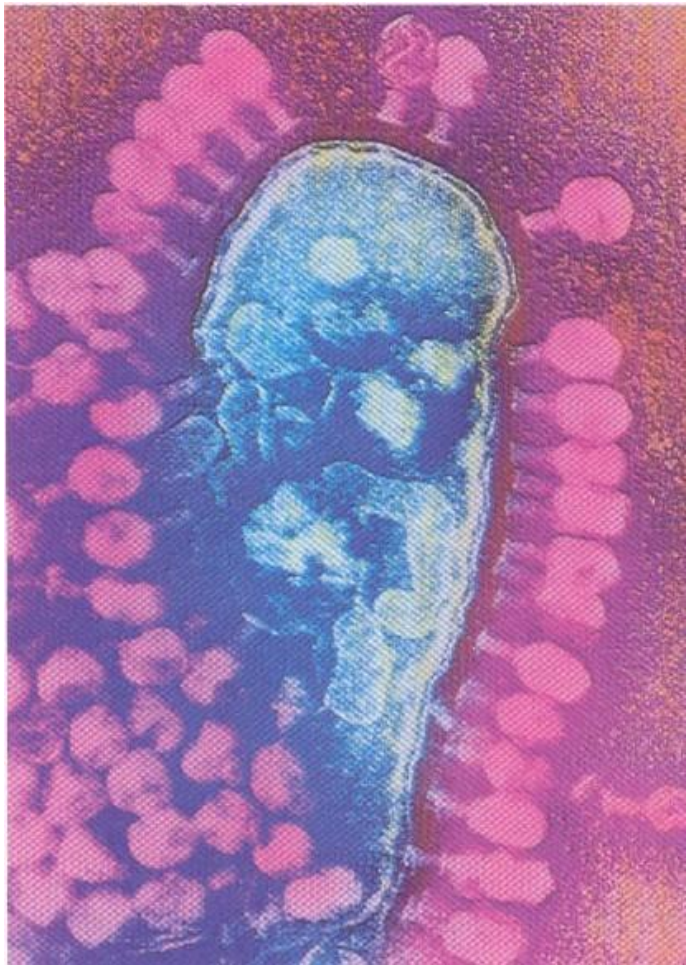
FIG. 13.1. Families of bactenophages (diagrammatic).

Types of bacteriophages

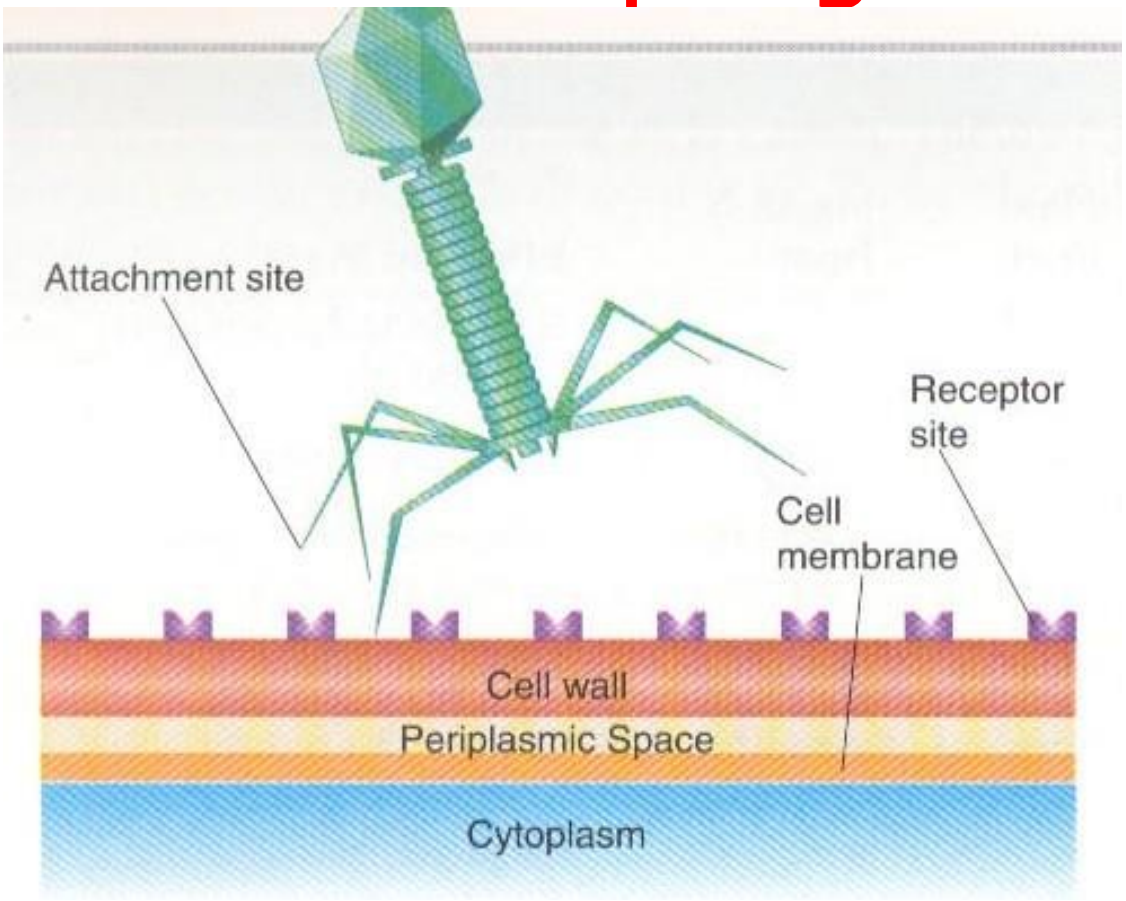
- Virulent bacteriophages (a productive type of interaction with a bacterial cell)
- Moderate bacteriophages (integrative type of interaction)

Phases of interaction of a virulent phage with a sensitive cell.

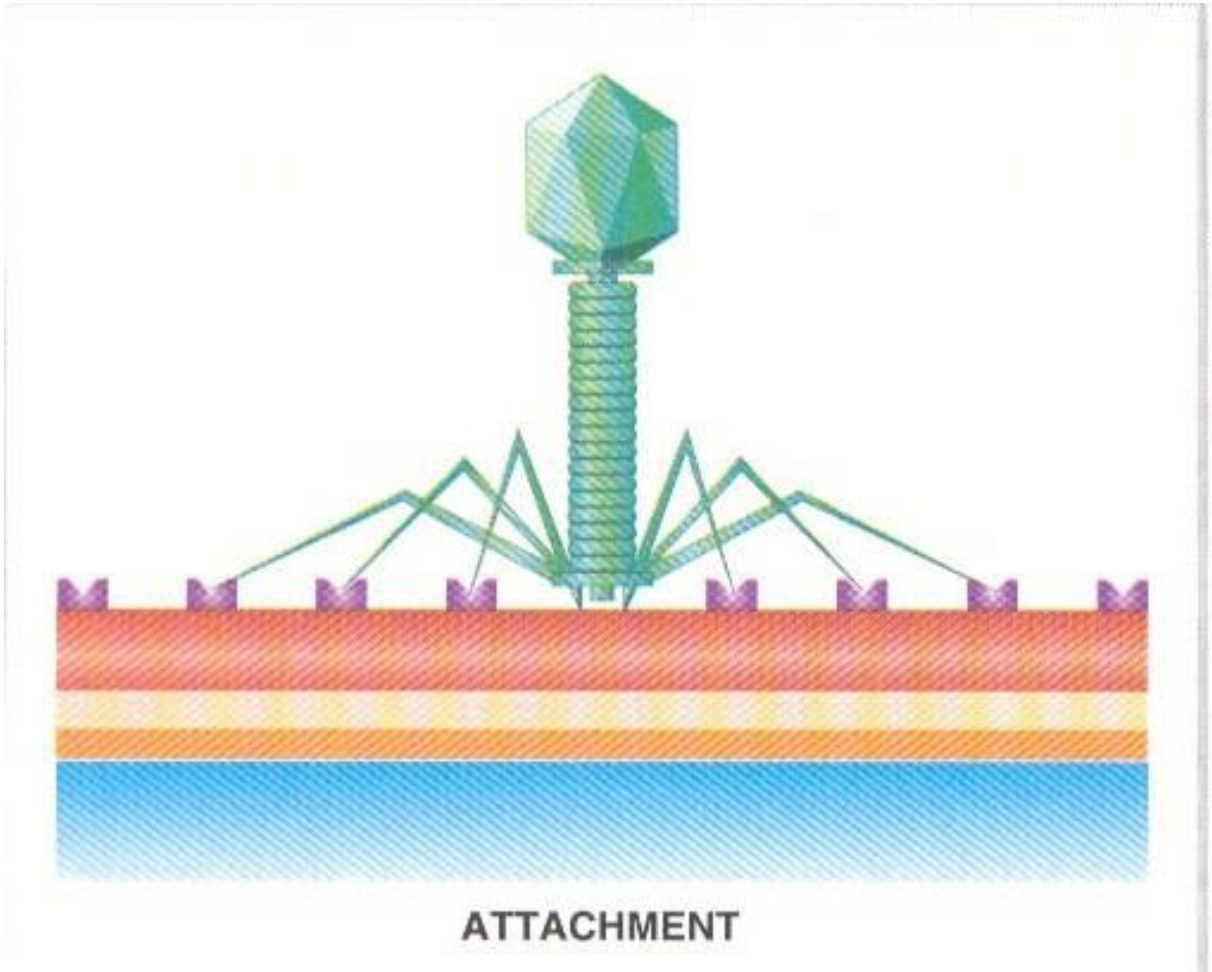
- Adsorption of phage on the surface of a bacterial cell
- Penetration of the phage into the bacterial cell
- Reproduction of phages in a bacterial cell
- Lysis of the bacterial cell and the release of phage offspring



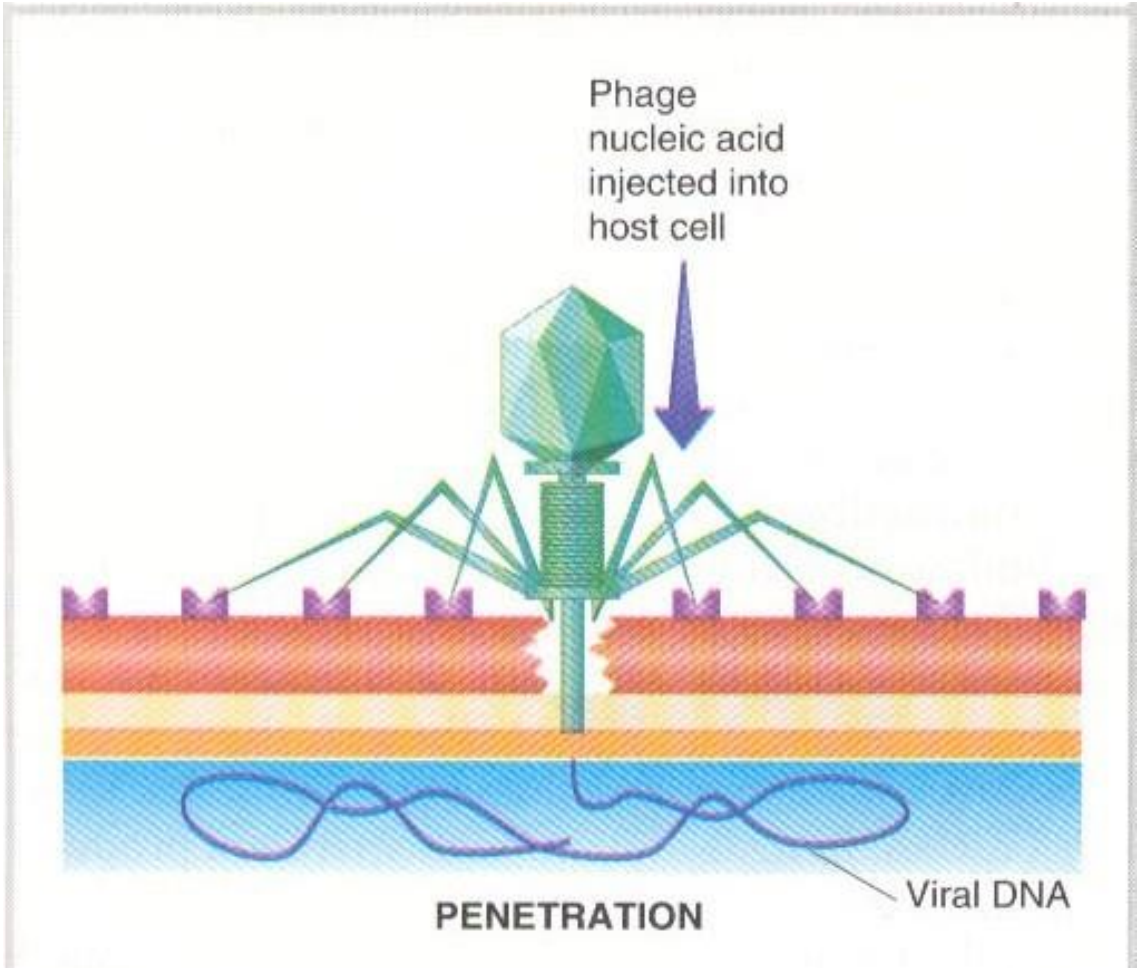
The mechanism of specific adsorption of a bacteriophage on a



Irreversible adsorption of bacteriophage on the cell wall.



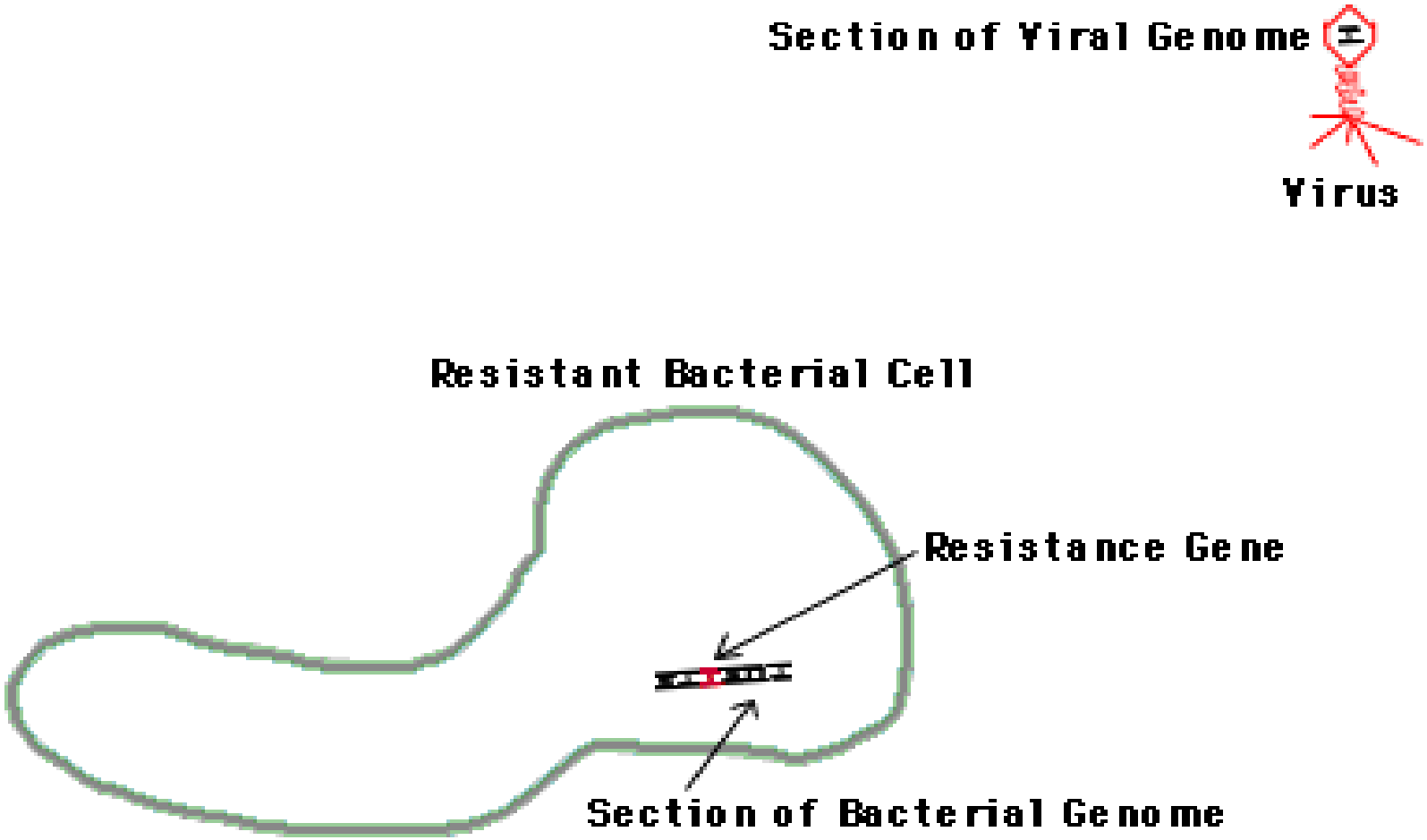
Penetration of nucleotide acid phages into bacterial cell.



Phases of interaction of a moderate phage with a sensitive cell.

- Adsorption of phage on the surface of a bacterial cell
- Penetration of the phage into the bacterial cell
- Integration of phage DNA into the bacterial chromosome and the formation of prophage
- Formation of Lysogenesis

Transduction of resistance.



Bacteriophages infect only particular bacteria's, interacting with specific cell receptors.

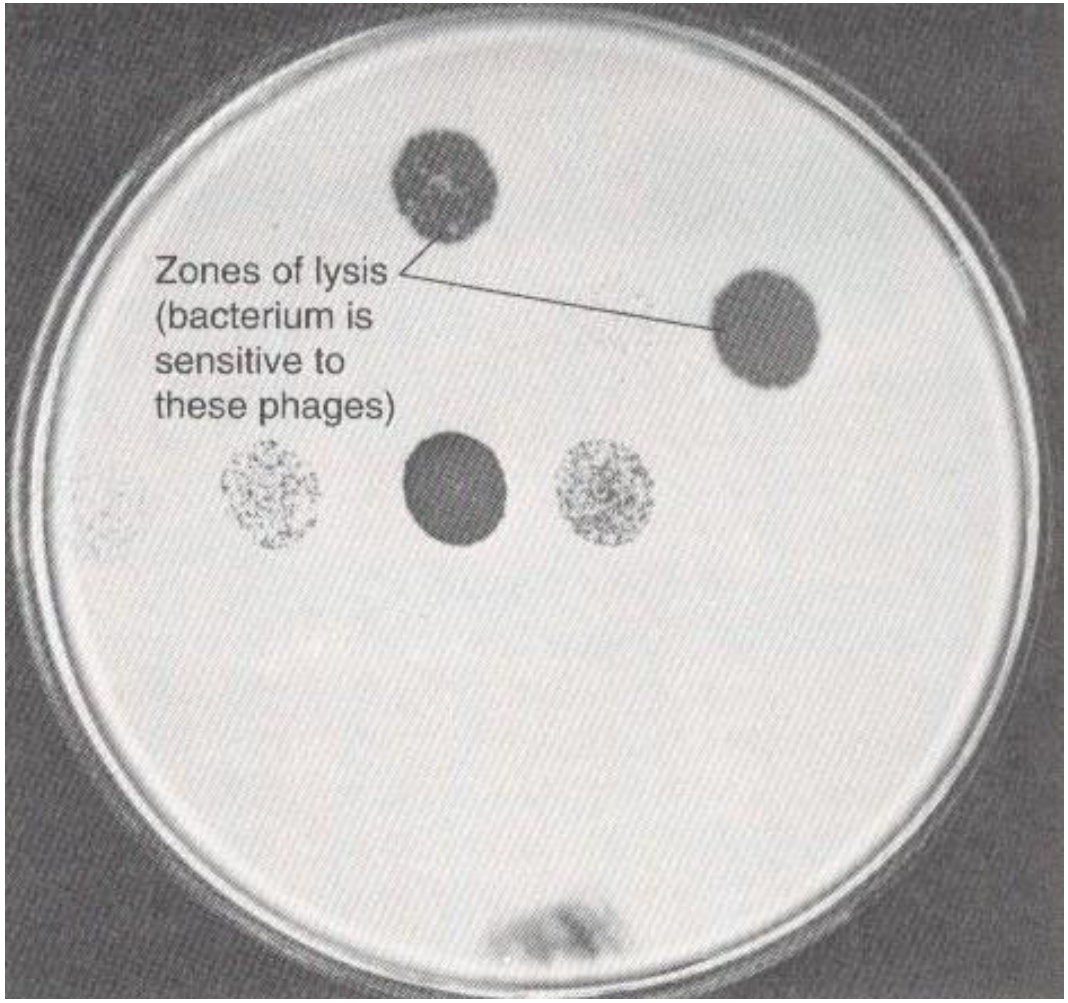
- By the spectrum of activity on bacteria, phages divide into:

  -polyvalent- lysed with familiar bacteria's.

  -monovalent- lysed with same gender bacteria's.

  -Type specific –lysing particular bacteria, bacteria's inside.

Phagodiagnosis.



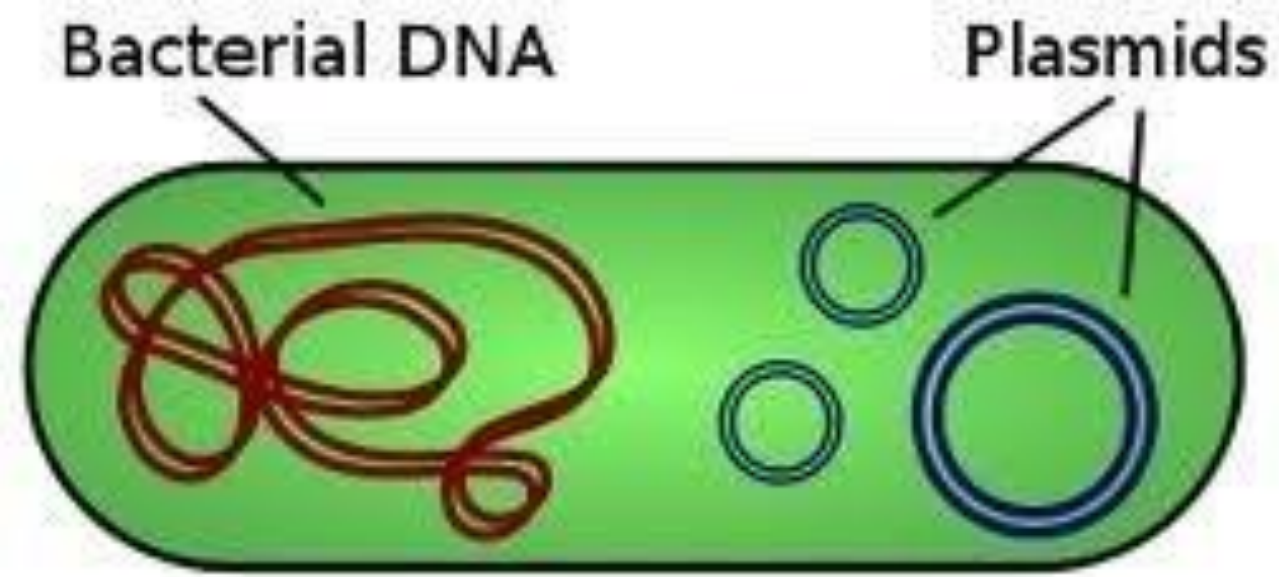
Защита и уход за кожей

Genetics of microorganisms. Application of genetics in microbiological diagnostics.

Genetic appararus of bacteria

- Hereditary information in bacteria can exist in **nucleoid(chromosome), plasmids** – extrachromosomal structures, and in **migrating genetic elements**.
- The material basis of heredity is DNA. All features of organism are coded in DNA in form of nucleotide sequences.
- Only in some viruses (RNA viruses) the genetic information is coded by RNA.
- DNA molecule is formed by two spiral strands(chains). Each strand of the DNA is formed by nucleotides.

Bacterial genetic apparatus



Genes

- A part of DNA molecule responsible for synthesis of one protein is called gene. All organism features are coded by chromosomal genes.
- Structure and regulatory genes exist. ***Structural genes*** code information about protein, while ***regulatory genes*** regulate the activity of structure genes.

Genotype

- The whole set of cell genes comprises its genotype
- The genes responsible for synthesis of substance is named by initial letters of corresponding substance. For example, aminoacide arginine gene *arg*$^{+}$, lactase gene - *lac*$^{+}$
- Susceptibility to antibiotics and phages is denoted by **s** (*sensitive*), resistance – by **r** (*resistanse*). For exp., gene responsible for susceptibility to streptomycin is named as *str*^{s}, for resistance – as *str*^{r}.

Phenotype

- *Phenotype* refers to observable properties of an organism.
- In contrast to genotype phenotype can change. Manifestation of genitype in form of phenotype is called **expression**. However, genotype is not always expressed.
- Phenotype of bacteria is named as genotype (the first letter of phenotype name is written in capital).For example *arg*$^+$ *genotype corresponds to Arg*$^+$ phenotype, *lac*$^+$ - to *Lac*$^+$ phenotype.

Extrachromosomal genetic elements

- Some bacteria have extrachromosomal genetic elements – **plasmids and migrating genetic elements**.
- They are not of vital importance for bacteria, but support their variability and adaptation to environmental conditions.

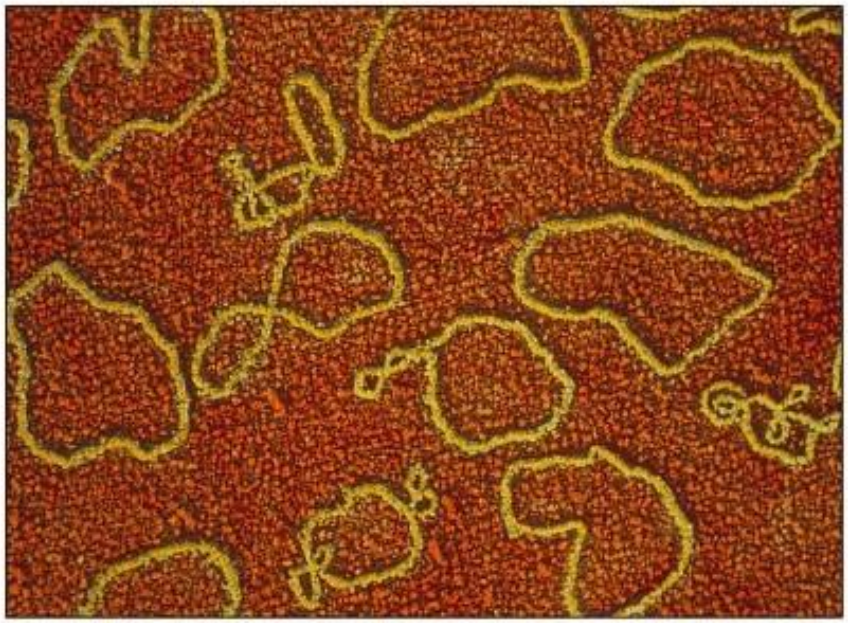
Plasmids

- **Plasmids are extrachromosomal DNA fragments consisiting of 40-50 genes.**
- Some circular plasmids are located in cytoplasma(**episomes**), some – integrated to chromosome(**integrated plasmids**).
Plasmids features:
- extrachromosomal DNA molecules;
- Multiply independently of chromosome;
- Can be transferred between bacteria;
- Exist in circular and linear forms;

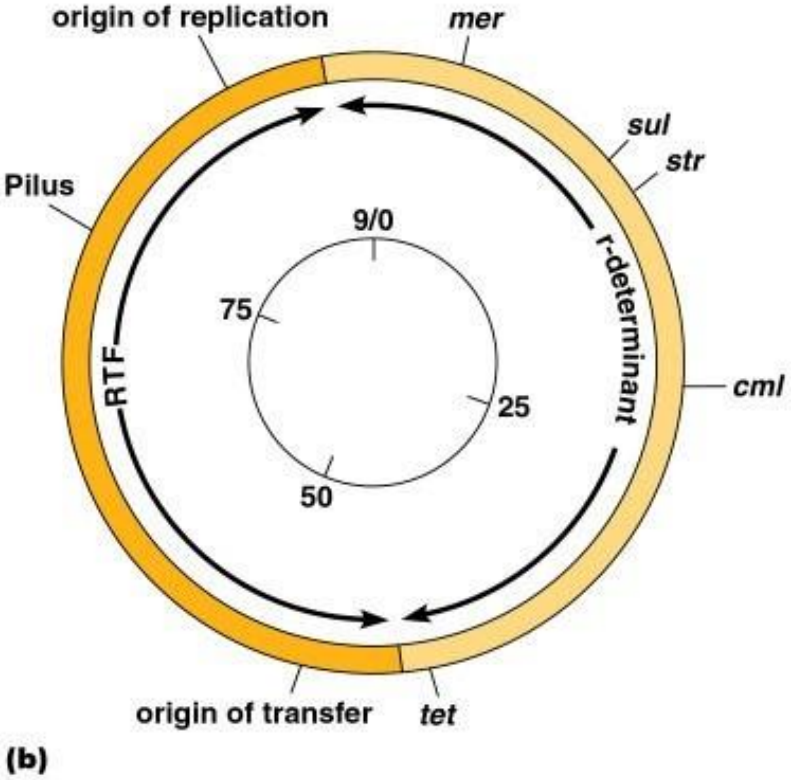
Plasmids

- Plasmids are a part of genetic apparatus of bacteria and responsible for antimicrobial resistance, toxin production, bacteriocin synthesis etc. Genes responsible for synthesis of these molecules are located in plasmids.
- ***F-plasmids*** (eng, *fertility*) – participate in conjugation
- ***R-plasmids*** (eng, *resistanse*) – antimicrobial resistance
- ***tox^{+}-plasmids***- synthesis of exotoxins (exp., diphtheria and botulism, prototoxins)
- ***Col^{+}-plasmidsr -*** synthesis of colicin and other bacteriocins by E.coli

Plasmids



(a)



(b)

Figure 8.29

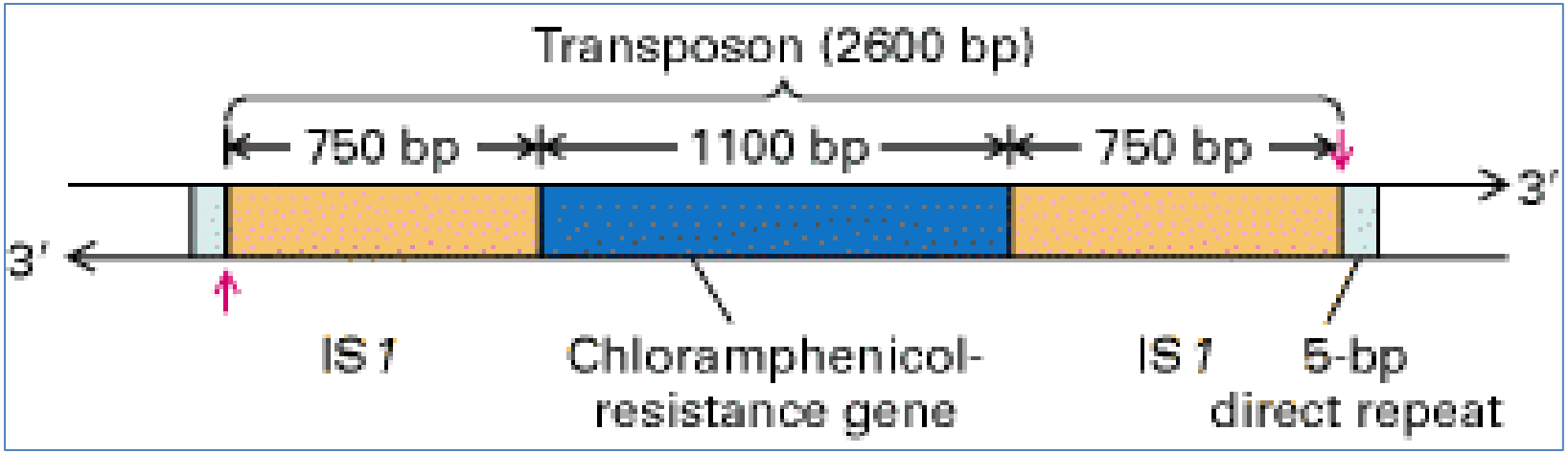
Migrating genetic elements

- Small DNA fragments are able to **migrate (transposition)** from one chromosome to another, from chromosome to plasmid, from plasmids to chromosome. This feature is due existence in migrating elements of enzyme – **transposase.**
- Migrating genetic elements

  - insertion sequences (IS-elements),
  - transposons(Tn-elements),
  - defective phages.

Transposons

- ***Transposons (Tn-elements).*** DNA fragments with 2000-25000 nucleotide pairs.
- Have specific structure gen and 2 IS-elements.
- Structure gene of transposon can transmit to bacteria special feature, for exp. Antimicrobial resistance, ability to produce toxin, bacteriocin etc.
- After entering bacterial cell they can cause duplication, deletion and inversion.



Types of genetic transfer

- **Nonhereditary variability(modification). It is also called phenotypic variability** as it is accompanied only by phenotypic changes.
- **Genetic variability**. Also called genotypic variability. In microorganisms genotypic variability occurs through **mutation** and **genetic recombination.**

Modification

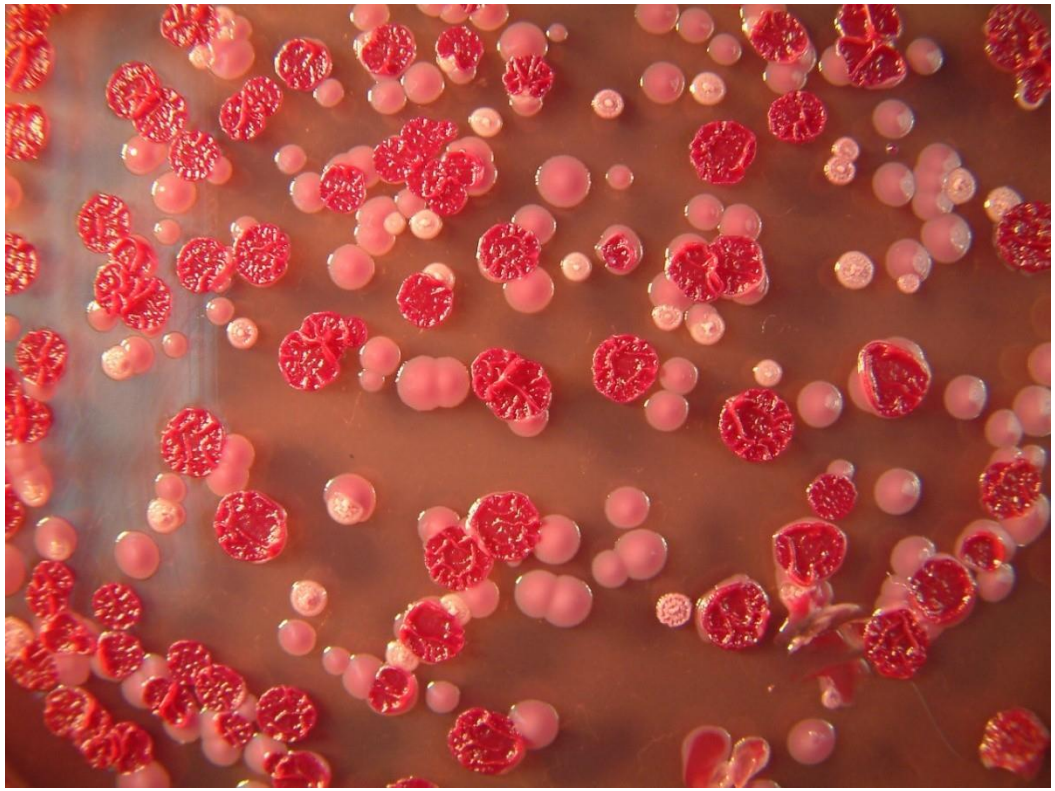
- Through modification microorganisms attain morphological, cultural, biochemical changes.
- Modification in ***morphological features*** is accompanied by changes in form and size of microorganisms***.***
- Modification can be represented by changes in***:***
- ***cultural features,***
- ***Biochemical features*** of microorganism
- Modification is manifested in microorganism population as **dissociattion** phenomenon.

Dissociation

- During dissociation some bacteria when cultivated in solid media form different types of colonies (2 or more types).
- Smooth ***S-colonies***, rough ***R-colonies***.
- Sometimes mucoid ***M-colonies, very small D-colonies*** (*dwarf) are formed*

R - S dissociation

- Under some circumstances S-colonies can change to R-colonies and vice versa. R-S dissociation is not frequently observed phenomenon
- Majority of human pathogens form S-colonies. Exceptions are *Mycobacterium tuberculosis, Yersinia pestis, Bacillus anthracis* etc.



Comparison of R- and S-colony forming microorganisms

S-colonies	R-colonies
Smooth, bright, convex	Irregular, turbid, wrinkled
Cause turbidity in broth	Sediment in broth
Motile species have flagella	Flagellalar olmaya bilər
Some species have capsule	Do not have capsule
High biochemical activity	Weak biochemical activity
High virulence	Weak virulence
Commonly isolated during active diseases	Commonly isolated during chronic diseases

Genetic variability

- As it is related to genotype it is called also genotypic variability.
- In microorganisms genotypic variability occurs through ***mutation*** and ***genetic recombinations***.

Mutation

- **Mutation** (lat, *mutatio* - change) – occurs in chromosomes and genes. As a result of mutation microorganism can obtain or loose some features. This variability is passed on future generations.
- In order to distinguish strains passed through mutation from ***wild strains*** they are called ***mutant strains.***

Mutations

- ***Spontaneous mutations***

  - ***reversible***

- ***inducible mutations***

  - ***mutagens*** (chemical substances, radiation– UV, ionizing, X-rays.)

- ***Point mutations***

  - ***frameshift mutations***

  - ***missens mutations*** –change in aminoacide

  - ***nonsens mutations***

- ***Chromosome mutations(deletion, inversion, duplication)***

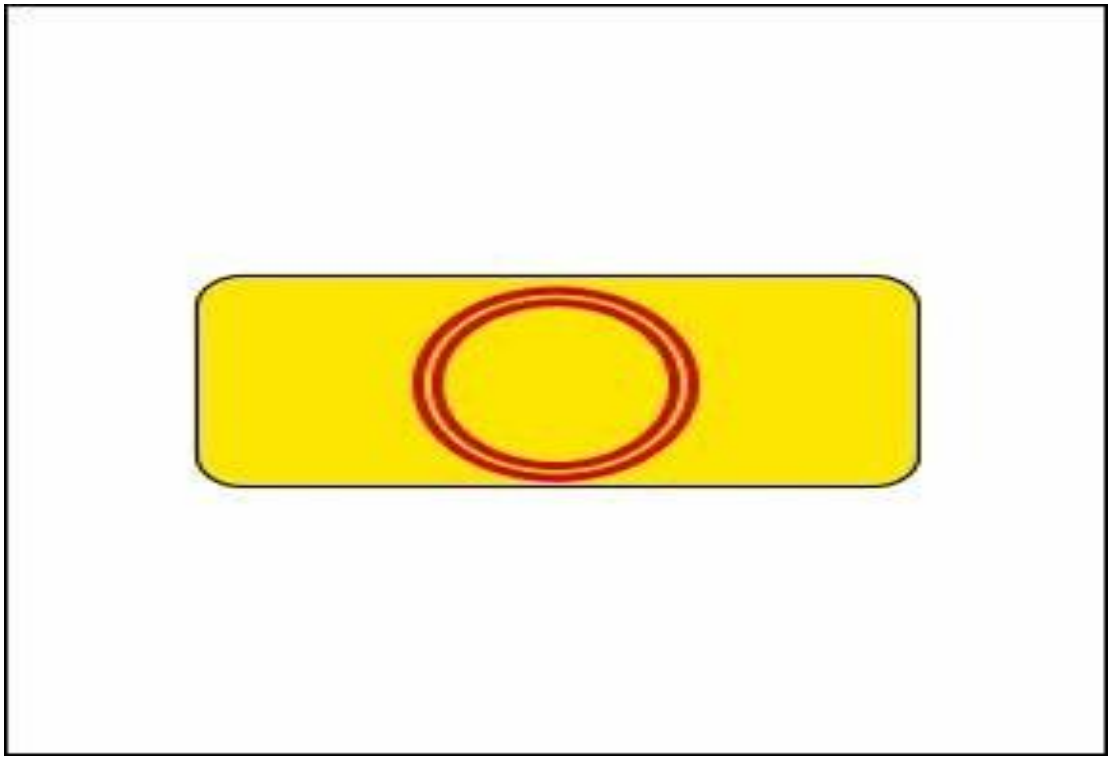
- ***According to phenotypic results- neutral mutations, conditional lethal, lethal mutations***

Genetic recombinations

- Exchange of genes occurs between two microorganisms. An isolate passing genetic material is called **donor**, while isolate receiving it – **recipient**.
- During recombination recipient cell receive a part of chromosome which leads to formation of noncomplete zygote – **merozygote**.
- After recombination from recipient cell **recombinant** cell is formed. Thus, recombinant cell posses recipient cell genotype and some genes of of donor.
- Transfer of genetic material in microorganisms occur through **transformation**, **transduction** and **conjugation**.

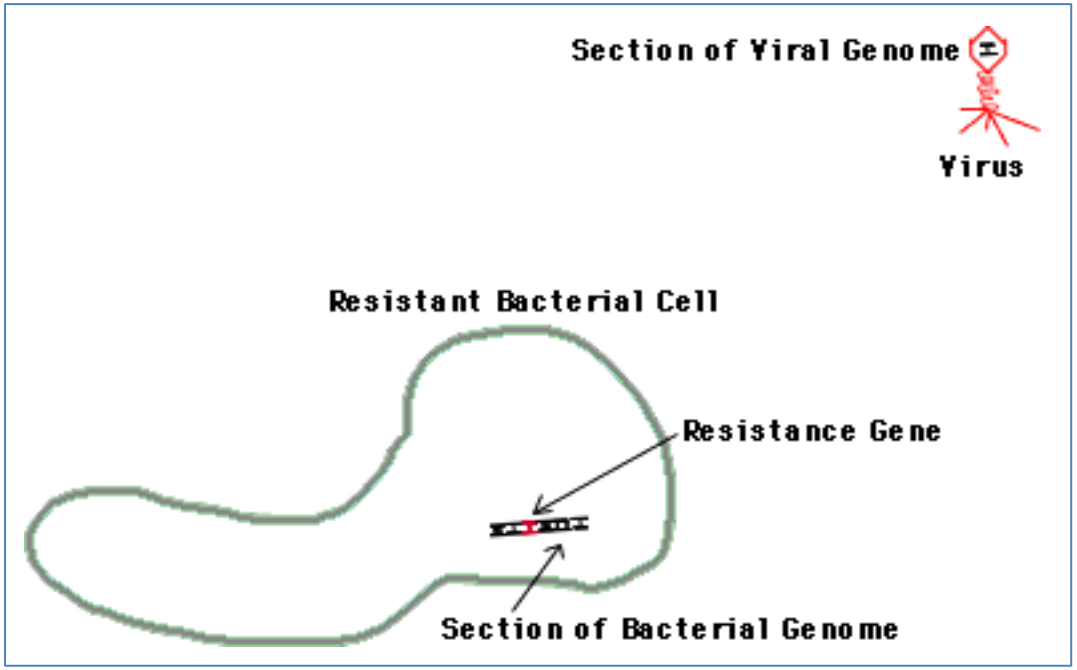
Transformation

Transformastion – direct transfer of genetic material (DNA)from donor to recipient



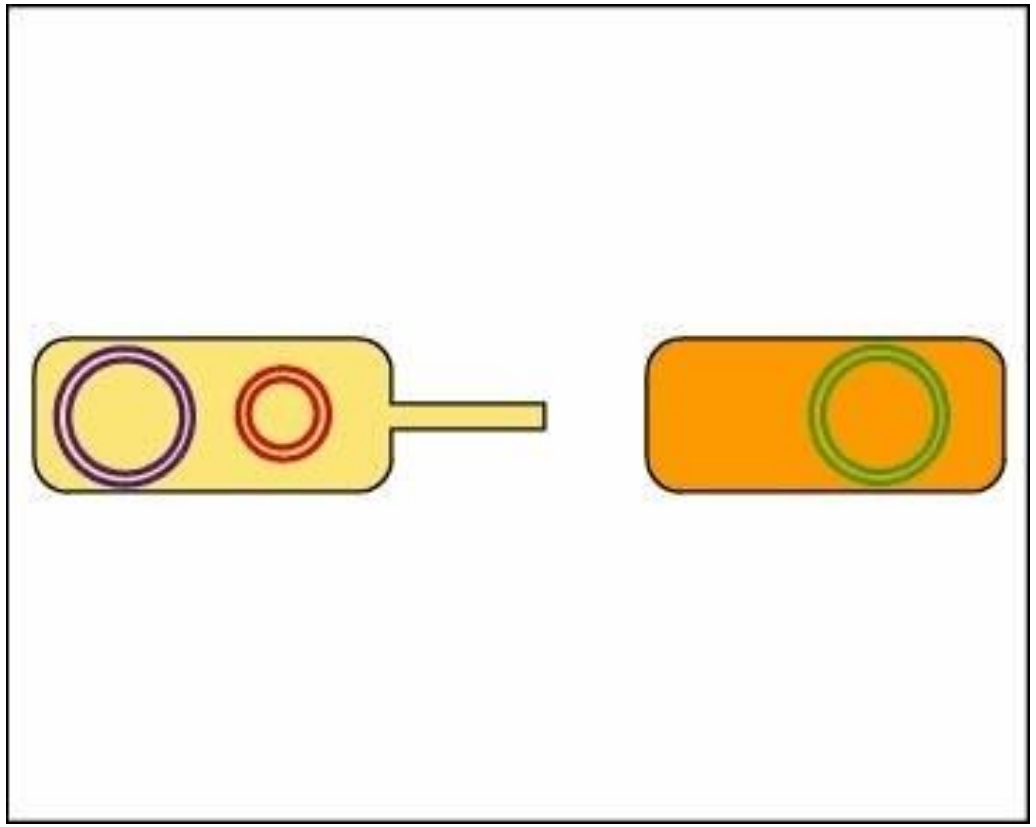
Transduction

Transduction – transfer of genetic material (part of a DNA molecule) from a donor to a recipient by bacteriophages

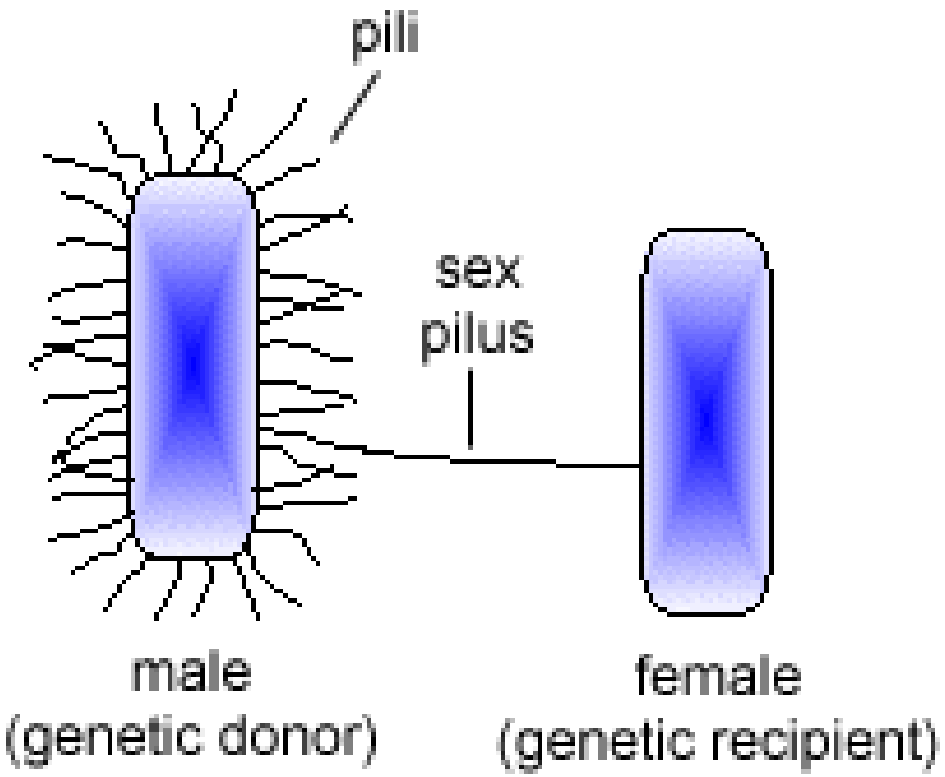


Conjugation

Conjugation- the most frequent mechanism of transfer of genetic material.
In this case, the genetic material is transferred from the donor to the recipient by direct contact.



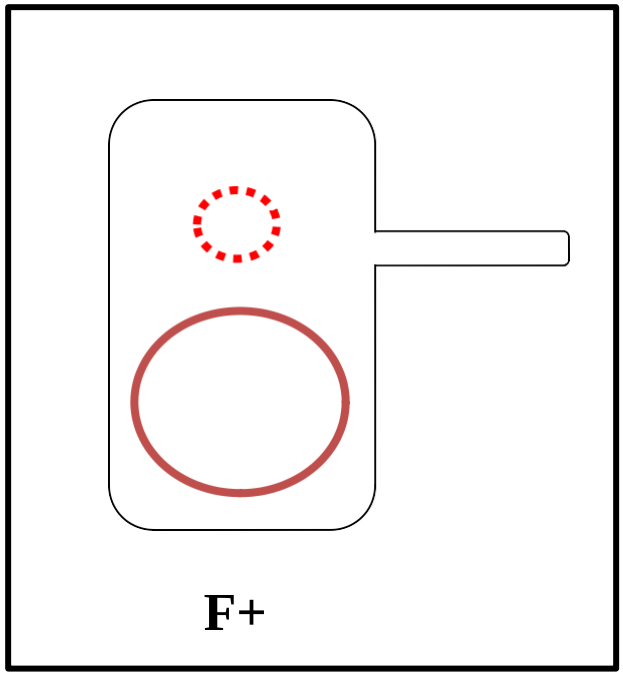
Conjugation



Conjugation

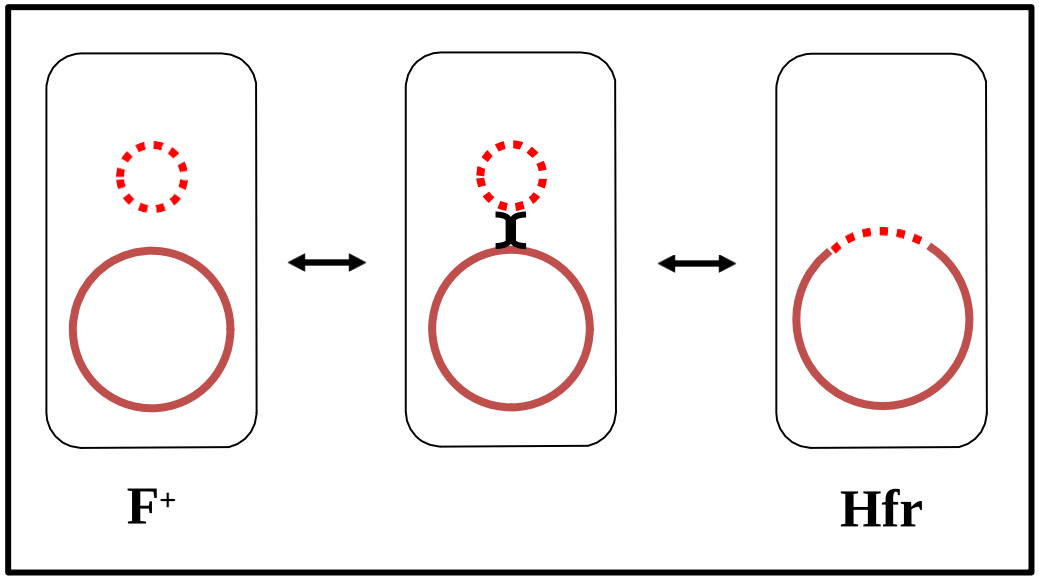
- As other recombination mechanism 2 cells participate in conjugation. The donor must have F-plasmid or F-factor (fertility), and called F + cell. Since this factor is not present in the recipient cell, it is referred to as F- cell.
- During conjugation the F-factor is transferred to the recipient cell in almost all cases, regardless of the donor chromosome.
- F-factor encodes conjugative pili (F-pili).
- After conjugation recipient cell becomes F^+-cell,which can transfer F-factor to other cells.

F^+ cell



Hfr-strains

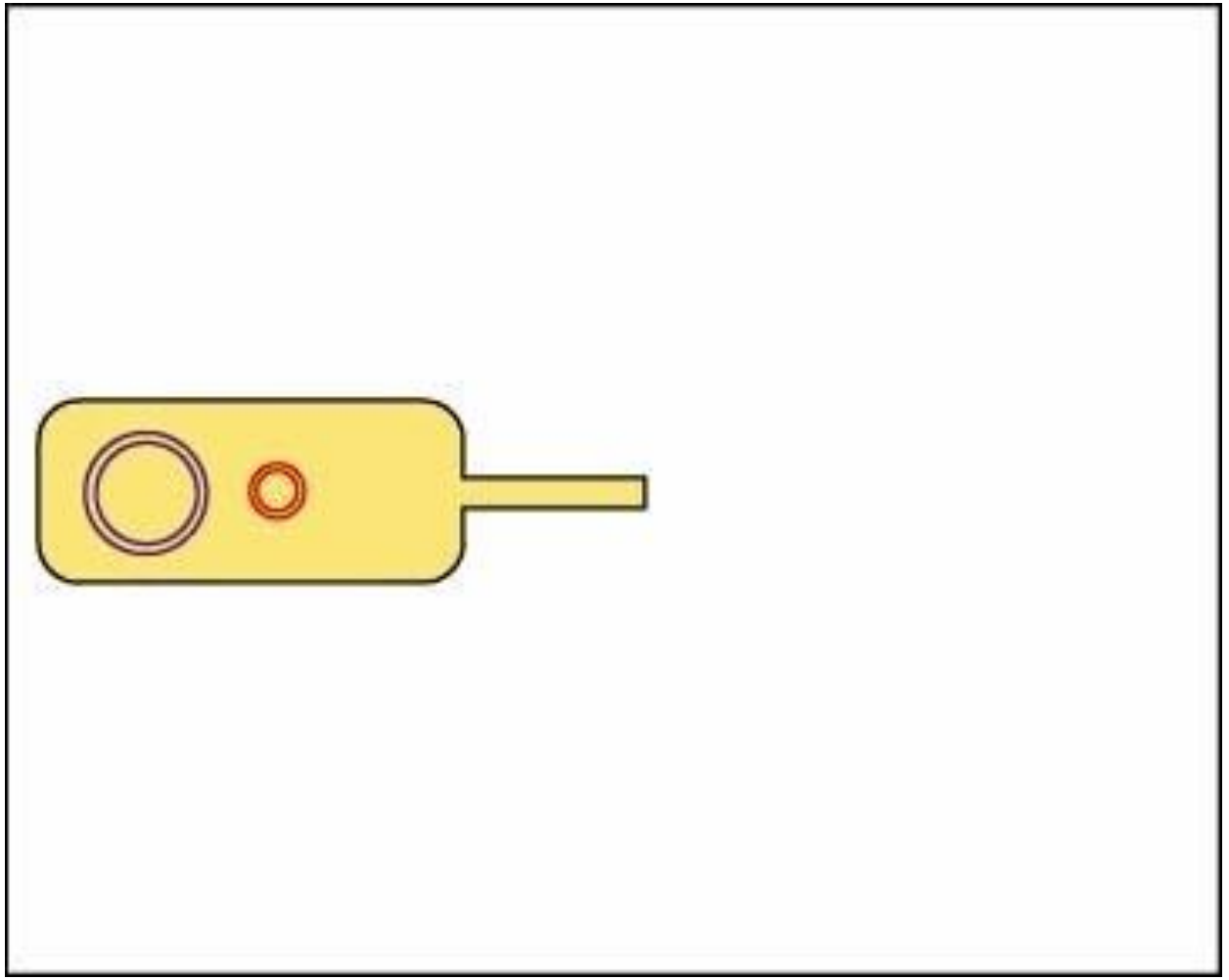
If F-plasmid integrates to cell chromosome it forms Hfr-cell (*high frequency of*). They are able to transfer chromosomal genes to recipient cells with high frequency



Conjugation between Hfr strain and *F*$^{-}$ cell

- During conjugation between Hfr-strain and F^{-} **cell** F-factor is not transferred, in contrast chromosome DNA is transferred with high frequency.
- After such conjugation, **the recipient still remains an F-cell**.
- During ***Hfr-conjugation*** chromosome DNA is replicated, as a result one strand of synthesized DNA copy is transferred to F^{-} cell. Thus, donor strain remains genetically stabile.

Conjugation between Hfr strain and *F*$^{-}$ cell



Genetics of viruses

Characteristics of viral genome

- Viral genome consists of only one type nucleic acid - DNA or RNA;
- While the genome of other organisms consists of DNA, in viruses RNA also can play a genome role(RNA viruses);
- DNA viruses have 2-strand, nonsegmented genome with infectious properties (except *Poxvirus* and *Hepadnovirus* as their DNA strands have different lengths);
- Except Reoviruses and retroviruses majority of RNA viruses have single strand RNA;
- Genome of RNA viruses may be segmented(fragmented) or nonsegmented;
- Genome of positive (+RNA) viruses possess infectious properties;
- Genome negative (-RNA) viruses does not possess infectious properties

Types of variability in viruses

- **Modification**
- **Mutation**

  - *Without phenotypic manifestation(neutral),*
  - *with phenotypic manifestation*
    - *lethal,*
    - *conditional-lethal- temperature sensitive mutants* (*ts-mutantlar*)
  - *Increase of viral infectious spectrum*
  - *resistance to antiviral drugs*

Genetic interactions between viruses

- When at the same time different viruses infect a cell they interact with each other during reproduction.
- ***Genetic recombination*** is exchange of genes between two or more viruses. It is common in DNA-containing viruses, resulting in the formation of recombinant viruses with two or more parental genes.
- ***Genetic reactivation*** occurs between to relative viruses with nonactive genes. After recombination these genes become activated (reactivation).

Nonspecific interaction between viruses

- ***Complementation*** – a protein encoded by genome of one virus supports reproduction of other virus. Complementation is observed between two defective viruses that cannot be reproduced separately, resulting in the reproduction of one or both of these viruses.
- **Phenotypic mixing** - when a susceptible cell is infected with two different viruses, sometimes one generation of the virus has the phenotypic characteristics of the both parental viruses.
- **Phenotypic masking** - the genome of one virus is surrounded by the capsid membrane of another virus, resulting in ***pseudotypes***.

Genetic engineering(obtaining genes)

- First a gene (DNA molecule) encoding the product or feature is obtained or synthesized. The DNA molecule is then broken down into fragments using enzymes called restriction enzymes. This enzyme belongs to the endonucleases and has the ability to break down the DNA molecule only in certain places.
- Fragments of DNA molecules obtained by the action of restriction enzymes are called restricts. If necessary, it is possible to combine the ends of the restricts with **DNA ligases**.

Genetic engineering(transfer of gene)

- DNA fragments are bound to vectors. **Vector** is agent transferring foreign DNA fragments to recipients.
- **Plasmids** and **phages** or their combinations – **cosmids** and **phasmids** are used as vectors.
- Recombinant DNA is transferred by vector to recipient via **transformation, transfection** or **microinjection.**

Genetic engineering (gene transfer)

- *By **native transformation** r-DNA* can be transferred to *Bacillus subtilis*, *Streptococcus pneumoniae* and *E.coli strains*.
- Transfer of r-DNA to prokaryotic or eukorayotic cells by phage is called **transfection**. In some cases eukaryotic cell is infected by vector virus (polioma and SV-40 viruses).
- ***During microinjection DNA or r-DNA is transferred to animal and plant cells via glass microneedles.***
- R-DNA can be transferred to recipients via liposomes as well. Lipososmes are prepared from equal mixture of phosphatidylserine and cholesterol. A mixture of rDNA and liposome is treated with ultrasound, and then incubated with the recipient cell.

Genetic engineering

(obtaining the final product)

- rDNA is transferred to recipient cells called **permissive cells**. In these cells transferred r-DNA is not broken down and can be expressed.
- Prokaryotic *E.coli, B.subtilis*, eukaryotic *Saccharomyces cerevisiae* are often used in genetic engineering
- Nowadays, insuline, somatotrope hormone, interferons etc. synthesizing bacterial and fungal strains are obtained and used in biotechnology.

Modern opportunities of genetic engineering

- Microinjection of r-DNA in animal embryo cells made possible obtaining of **transgenic animals.**
- Via same the method phytopathogen, cold resistant **transgenic plants are obtained.** By transferring of immune dominant antigen genes of microorganisms to plants “vaccine” containing fruits and carrots were obtained.
- One of the recent successes of genetics is the creation of a genetic clone. The genetic clone was first created at the end of the last century by Scottish scientists Jan Welhmut and Ken Campbell.

Application of genetic methods in diagnostics

- Polymerase chain reaction
- Molecular hybridization
- Restriction analysis
- Sequenation

Thank you for your attention.