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MEDICAL MICROBIOLOGY

GENERAL COURSE

Edited by O.P. Bochkareva and M.R. Karpova

Study guide

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The study guide provides modern information on general microbiology, including the morphology, physiology, and genetics of microorganisms significant in infectious pathology, as well as infection, immunity, immunoprophylaxis, and immunotherapy of infectious diseases.

This study guide is developed in accordance with the Federal State Educational Standards of Higher Education (FSES HE) for students pursuing programs in: 31.05.01 – General Medicine.

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INTRODUCTION

This study guide “Medical Microbiology. General Course” is designed for educating students in medical faculties of higher education institutions. The study guide was translated into English for students studying General Medicine.

Microbiology (from the Greek *micros* – small, *bios* – life, *logos* – study) is a science that studies the structure, vital activity, and ecology of microorganisms – the smallest forms of life of plant or animal origin, invisible to the naked eye. The term “microorganism” includes all representatives of the microbial world (bacteria, fungi, protozoa, viruses). Microbiology is a fundamental biological science that employs methods from other disciplines, primarily physics, biology, bioorganic chemistry, molecular biology, genetics, cytology, and immunology.

Medical microbiology studies microorganisms capable of causing human diseases and is divided into general and specific microbiology.

The general course of medical microbiology examines the principles of the structure and vital activity of microorganisms at the molecular, cellular, and population levels, their genetics, and their interactions with the environment.

The study guide covers in detail the morphology and anatomy of microorganisms that are of medical significance, features of their physiology and genetics, and interactions with environmental factors. Special attention is given to immunity issues. The educational material is illustrated with clear author-developed diagrams, drawings, and tables.

The study guide includes test questions that students can use to assess their progress.

The material is presented in accordance with the approved curriculum for the discipline “Microbiology, Virology” and corresponds to the topics of lectures and practical classes.

BASICS OF MICROORGANISM CLASSIFICATION

Terms and Definitions

Systematics (from the Greek *systema* – whole, composed of parts; systematicos – ordered) is a biological science that comprehensively describes microorganisms, determines their degree of relatedness, and arranges them into hierarchical groups. The goal of systematics is to create a classification.

Classification (from the Latin *classis* – rank, group) is the process of dividing a set of organisms into specific taxonomic groups based on common characteristics.

Taxonomy (from the Greek *taxis* – arrangement in order, law) is a branch of systematics that studies the principles and methods of organizing (classifying) organisms hierarchically.

Taxon is a group of microorganisms united by specific properties within a particular taxonomic category.

Identification (from the Latin *identifico* – to identify) is the process of determining the affiliation of a studied organism to a particular taxon.

A special section of taxonomy – nomenclature – defines the rules for assigning names to described organisms.

In bacterial systematics, the binomial nomenclature of Carl Linnaeus is used, where a biological species is given a name consisting of two words: the first indicates the genus, and the second specifies the species. The genus name often corresponds to the name of the scientist who discovered or studied the pathogen (e.g., the genus *Escherichia* was named after the German pediatrician and bacteriologist Theodor Escherich, who first described it) or the morphology of the pathogen (*Staphylococcus* – a genus of round microorganisms arranged like a bunch of grapes; *Streptococcus* – a genus of round microorganisms arranged in chains). Bacterial species names may be derived from disease symptoms (e.g., *Salmonella enterica* causes enteritis), the source of isolation (e.g., *Brucella suis* from pigs), or colony morphology (e.g., *Staphylococcus aureus* forms golden colonies on nutrient agar).

Bacterial names are assigned in accordance with the rules of the International Code of Nomenclature of Bacteria (ICNB): *Bacillus cereus, Staphylococcus aureus, Salmonella enterica.* When repeatedly mentioning a species, the genus name can be abbreviated to its first letter followed by a period: B. cereus, S. aureus, S. enterica. *B. cereus, S. aureus, S. enterica.*

Classification of Microorganisms

In 1923, the American Society of Bacteriologists published the first international Bergey's Manual of Determinative Bacteriology edited by David Henricks Bergey. Subsequently, an international team of leading specialists in various microorganism groups was formed to compile knowledge on their structure, properties, and systematics.

Currently, information on bacterial systematics and identification is published separately: as a *determinative manual* and a *classification manual*. The determinative manual serves as a reference for practical laboratory workers and is used to identify bacteria based on characteristic phenotypic traits (Gram staining, cell shape and size, chemical composition of the cell wall, motility, presence of capsules, spores, type of respiration, biochemical activity, etc.). The determinative manual reflects the first direction in microorganism systematization – their cataloging based on a limited number of traits.

Bergey's determinative manual divides prokaryotes into four divisions and 35 groups:

1. Gracilicutes – thin-walled, Gram-negative (groups 1–16).
2. Firmicutes – thick-walled, Gram-positive (bacteria of groups 17–21 and actinomycetes of groups 22–29).
3. Tenericutes – lacking a cell wall (group 30).
4. Mendosicutes – archaebacteria, with cell walls lacking peptidoglycan and unique ribosome, membrane, and RNA structures (groups 31–35).

The Bergey's classification manual is based on the phylogenetic relatedness of microorganisms. Microorganisms are classified based on standard traits.

Phenotypic Traits:

1) morphological traits (size, shape, presence of flagella, capsules, spores, etc.);
2) tinctorial traits (staining according to methods: Gram, Ziehl-Neelsen, Neisser, Burri-Gins, etc.);
3) cultural properties (growth characteristics on nutrient medium);
4) biochemical properties (ability to utilize various substrates);
5) physiological properties (type of respiration, nutrition);
6) antigenic structure;
7) susceptibility to bacteriophages.

Genotypic Traits:

1) G+C content ratio;
2) DNA base sequence;
3) Degree of genetic relatedness with other microorganisms;
4) Degree of homology.

Phylogenetic Traits:

1) sequencing of 16S and 23S ribosomal RNA (rRNA);
2) analysis of rRNA nucleotide sequences;
3) RNA-RNA hybridization;
4) Restriction Fragment Length Polymorphism (RFLP) of DNA.

Based on a combination of phenotypic, genotypic, and phylogenetic traits, microorganisms are divided into acellular forms (viruses, kingdom Regnum Vira) and cellular forms, which include three domains:

1) Domain "*Archaea*" – ancestral prokaryotes or archaebacteria (no species pathogenic to humans);
2) Domain "*Bacteria*" – true bacteria (true prokaryotes) or eubacteria;
3) Domain "*Eukarya*" – eukaryotic cells. The Eukarya domain includes: The "Eukarya" domain includes: the kingdom of fungi (*Regnum Fungi*), the kingdom of animals (*Regnum Animalia*) with the subkingdom of *Protozoa*, and the kingdom of plants (*Regnum Plantae*).

Among the microorganisms in domains 2 and 3, there are species pathogenic to humans.

Bergey's classification pertains only to domains 1 and 2. Other microorganisms (viruses, fungi, protozoa) are organized into separate classifications. Currently, most bacteriologists have abandoned the term regnum ("kingdom") for designating prokaryotic taxa. It is used in the systematics of eukaryotes (mycology, protozoology) and acaryotes (virology). The terms "prokaryotes," "eukaryotes," and "acaryotes" are used to refer to corresponding groups of microorganisms based on genome organization and are not used for taxonomic categories. Prokaryotes (from Greek – "pre-nuclear") and eukaryotes (from Greek "possessing a nucleus") differ in their nuclear apparatus.

In Bergey's classification, the following groups or levels (taxa) are used:

Domain – *Domen* (Latin);

Phylum – *Phylum* (Latin); in prokaryotic classification, the term "phylum" is used, while for eukaryotes, the term "type" is used;

Class – *Class* (Latin);

Order – *Ordo* (Latin);

Family – *Familia* (Latin);

Genus – *Genus* (Latin);

Species – *Species* (Latin).

A ***species*** is an evolutionarily formed group of individuals with a common origin and a unified genotype, which, under standard conditions, manifests in similar phenotypic traits: morphological, physiological, biochemical, etc.

In addition to these taxa, other terms are widely used:

Strain – a population of bacteria of one species isolated from a specific source;

Clone – a population of bacteria derived from a single bacterial cell;

Subspecies, infraspecies – a population of bacteria differing from the main species by certain traits, which can be detailed as ***variants*** (*-vars*, but not types); the suffix "-var" is used to avoid confusion with "type" as a eukaryotic taxon;

Morphovars – populations differing from the main species by morphological properties;

Chemovars – by biochemical properties;

Serovars – by antigenic structure;

Phagovars – by susceptibility to bacteriophages;

Colicinovars – by bacteriocin production;

Resistovars – by antibiotic resistance;

Genovars – by the structure of part of the genome;

Patovars – by virulence;

Biovars – by multiple biological properties.

Classification of Prokaryotes

Domain "Archaea": Archaebacteria lack peptidoglycan in their cell walls. They have unique ribosomes and ribosomal RNA. The term "archaebacteria" appeared in 1977, reflecting their status as one of the ancient forms of life, as indicated by the prefix "arche." They include no infectious disease pathogens.

Archaebacteria are a distinct group of microorganisms, differing from eubacteria and eukaryotes. Archaea inhabit many extreme biotopes but are also found in ordinary ones. There is significant morphological diversity among archaebacteria, with Gram-positive and Gram-negative staining, single and filamentous forms, and sizes ranging from 0.1 μm to 15 μm in diameter and up to 100 μm in linear dimensions. Some metabolic pathways are unique, with no analogs among eubacteria, such as the unique enzyme systems found in methanogenic organisms. Archaebacteria inhabit salty and high-temperature biotopes. They are also abundant in ocean depths, constituting over 34% of prokaryotic biomass near the Antarctic coast.

The modern edition of Bergey's Manual divides Archaea into two kingdoms: *Crenarchaeota* and *Euryarchaeota*. Crenarchaeota include well-studied groups of extremophiles and comprise 1 class, *Thermoprotei* (4 orders and 6 families). Euryarchaeota occupy various ecological niches and exhibit diverse metabolic pathways. This phylum is divided into eight classes: *Methanobacteria, Methanococci, Halobacteria, Thermoplasmata, Thermococci, Archaeglobi, Methanopyri, and Methanomicrobia* (9 orders, 16 families).

Domain Bacteria (Eubacteria) is represented by:

- Bacteria with thin cell walls, Gram-negative – *Gracilicutes*;

Features: most Gram-negative bacteria are grouped into the phylum Proteobacteria, based on ribosomal RNA similarity ("Proteobacteria" – named after the Greek god

Proteus, who took various forms). They evolved from a common photosynthetic ancestor;

– Bacteria with thick cell walls, Gram-positive – *Firmicutes*;

Features: according to studied ribosomal RNA sequences, Gram-positive bacteria form a distinct phylogenetic group with two large subdivisions – high and low G+C content (genetic similarity). Like Proteobacteria, this group is metabolically diverse;

– Bacteria without cell walls – Tenericutes (class *Mollicutes* – mycoplasmas);

Features: lack of a cell wall, cells surrounded by a cytoplasmic membrane, Gram-negative staining, pleomorphic round cells, reproduction by binary fission, budding, or fragmentation. They form small colonies that grow into the agar.

The Bacteria domain includes 26 phyla, with those of medical significance listed in Table 1.

Table 1

Taxonomic Scheme of Bacteria
(Representatives of Medical Significance) Domain Bacteria

Phylum	Class	Ordo	Familia	Genus
Phylum BXII. Proteo-bacteria	**Class I.** Alpha-proteobac-teria	**Ordo II.** Rickettsiales	**Familia I**. Rickettsiaceae	Genus I. Rickettsia
				Genus II. Orientia
			Familia II. Ehrlichiaceae	Genus I. Ehrlichia
				Genus III. Anaplasma
		Ordo VI. Rhizobiales	**Familia II.** Bartonellaceae	Genus I. Bartonella
			Familia III. Brucellaceae	Genus I. Brucella
	Class II. Betaproteoba cteria	**Ordo I.** Burkholderiales	**Familia I**. Burkholderiaceae	Genus I. Burkholderia
			Familia IV. Alcaligenaceae	Genus III. Bordetella
		Ordo IV. Neisseriales	**Familia I.** Neisseriaceae	Genus I. Neisseria
		Ordo V. Nitrosomonadales	**Familia II**. Spirillaceae	Genus I. Spirillum
	Class III. Gamma-proteobac-teria	**Ordo II**. Xan-thomonadales	**Familia I**. Xanthomonadaceae	Genus I. Xanthomonas
				Genus VII. Stenotropho-monas
		Ordo IV. Thiotrichales	**Familia III.** Francisellaceae	Genus I. Francisella
		Ordo V.	**Familia I.** Legionellaceae	Genus I. Legionella

Phylum	Class	Ordo	Familia	Genus
		Legionellales	**Familia II**. Coxiellaceae	Genus I. Coxiella
		Ordo VIII. Pseudomonadales	**Familia I.** Pseudomonadaceae	Genus I. Pseudomonas
			Familia II. Moraxellaceae	Genus I. Moraxetta
				Genus II. Acinetobacter
		Ordo X. Vibrionales	**Familia I.** Vibrionaceae	Genus I. Vibrio
		Ordo XII. Enterobacteriales	**Familia I.** Entero-bacteriaceae	Genus I. Escherichia
				Genus X. Citrobacter
				Genus XI. Edwardsiella
				Genus XII. Enterobacter
				Genus XIII. Erwinia
				Genus XV Hafnia
				Genus XVI. Klebsiella
				Genus XXI. Morganella
				Genus XXVIII. Proteus
				Genus XXIX. Providencia
				Genus XXXII. Salmonella
				Genus XXXIII. Serratia
				Genus XXXIV. Shigella
				Genus XL. Yersinia
		Ordo XIII. Pasteurellales	**Familia I.** Pasteurellaceae	Genus I. Pasteurella
				Genus III. Haemophilus
	Class V. Epsilon-proteobacte-ria	**Ordo I.** Campylobacte-rales	**Familia I.** Campylo-bacteraceae	Genus I. Campylobacter
			Familia II. Helicobacteraceae	**Genus I. Helicobacter**
Phylum BXIII. Firmi-cutes	**Class I.** Clostridia	**Ordo I.** Clostridiales	**Familia I**. Clostridiaceae	Genus I. Clostridium
				Genus VIII. Sarcina
			Familia III. Peptostreptococcaceae	Genus I. Peptostreptococcus

Phylum	Class	Ordo	Familia	Genus
			Familia V. Peptococcaceae	Genus I. Peptococcus
			Familia VII. Acidaminococcaceae	Genus XIII. Veillonella
	Class II. Mollicutes	**Ordo I.** Mycoplasmatales	**Familia I.** Mycoplasmataceae	Genus I. Mycoplasma
				Genus IV. Ureaplasma
	Class III. Bacilli	**Ordo I.** Bacillales	**Familia I.** Bacillaceae	Genus I. Bacillus
			Familia IV. Listeriaceae	Genus I. Listeria
			Familia V. Staphylococcaceae	Genus I. Staphylococcus
		Ordo II. Lactobacillales	**Familia IV.** Enterococcaceae	Genus I. Enterococcus
			Familia VI. Streptococcaceae	Genus I. Strepococcus
Phylum BXIV. Actino-bacteria	**Class I.**Actinobacteria **Subclass V**. Actinobacteridae	**Ordo I.** Actinomycetales **SubOrdo I.** Actinomycineae	**Familia I.** Actinomycetaceae	Genus I. Actinomyces
				Genus IV. Mobiluncus
		SubOrdo VI. Micrococcineae	**Familia I.** Micrococcacea	Genus I. Micrococcus
		SubOrdo VII. Corynebacterinea e	**Familia I.** Corynebacteriaceae	Genus I. Coryne-bacterium
			Familia IV. Mycobacteriaceae	Genus I. Mycobacterium
			Familia V. Nocardiaceae	Genus I. Nocardia
		SubOrdo XI. Streptomycineae	**Familia I.** Streptomycetaceae	Genus I. Streptomyces
		Ordo II. Bifidobacteriales	**Familia I.** Bifidobacteriaceae	Genus I. Bifi-dobacterium
				Genus III. Gardnerella
Phylum BXVI Chlamy-diae	**Class I.** Chlamydiae	**Ordo I.** Chlamydiales	**Familia I.** Chlamydiaceae	Genus I. Clamydia
				Genus II. Chlamydophila
Phylum BXVII. Spiro-chaetes	**Class I.** Spirochaetes	**Ordo I.** Spirochaetales	**Familia I.** Spirochaetaceae	Genus I. Spirochaeta
				Genus II. Borrelia
				Genus IX. Treponema

Phylum	Class	Ordo	Familia	Genus
			Familia III. Leptospiraceae	Genus II. Leptospira
Phylum BXX. Bactero-idetes	**Class I**. Bacteroides	**Ordo I.** Bacteroidales	**Familia I**. Bacteroidaceae	Genus I. Bacteroides
			Familia IV. Prevotellaceae	Genus I. Prevotella

MORPHOLOGY OF MICROORGANISMS

All bacteria possess a certain shape and size, which vary widely—from 0.1–0.15 μm (mycoplasmas) to 10–15 μm (clostridia).

The morphological types of bacteria, compared to higher organisms, are few in number. The cells of most microorganisms have a spherical, rod-shaped, or curved form, and there is also a small group of filamentous microorganisms.

Based on morphology, bacteria are distinguished as *cocci* (spherical), *rod-shaped* (cylindrical), *curved* (spiral), and *filamentous* forms.

Cocci (depending on their mutual arrangement in the field of view or relative to each other) are subdivided into the following groups:

Micrococci divide in one plane, have a regular rounded shape, and are arranged singly and randomly (Fig. 1a).

Diplococci divide in one plane to form pairs of cells with a bean-shaped (*gonococci, meningococci*) or lanceolate shape (*pneumococci*) (Fig. 1b).

Streptococci (from Greek *streptos* – chain) divide in one plane and in smears are arranged in chains (*pyogenic streptococci*) (Fig. 1c).

Tetracocci have a regular rounded shape, divide in two mutually perpendicular planes to form tetrads (Fig. 1d).

Sarcinae divide in three mutually perpendicular planes to form packets (cubes or bales), with varying numbers of cells (Fig. 1e).

Staphylococci (from Greek *staphyle* – grape bunch) divide in several planes, have a regular rounded shape, and are arranged in irregular clusters resembling a bunch of grapes (Fig. 1f).

Rod-shaped (cylindrical) microorganisms are subdivided into:

- non-spore-forming rods,
- spore-forming rods: *bacilli* – the spore is small and does not alter the bacterial shape (anthrax pathogen) and *clostridia* – the spore is large, exceeding the diameter of the bacterial cell, resembling a “spindle” or “drumstick” (*pathogens of tetanus, gas gangrene, botulism*).

Rod-shaped microorganisms have various sizes; the ends of the rods may be rounded (*Escherichia coli*, Fig. 2a), pointed (*fusobacteria*), truncated or chopped (anthrax pathogen, Fig. 2b), with club-shaped thickenings at the ends (*corynebacteria – diphtheria pathogen*, Fig. 2c); some rods have an ovoid (egg-shaped) form – coccobacilli (*pertussis pathogen*, Fig. 2d).

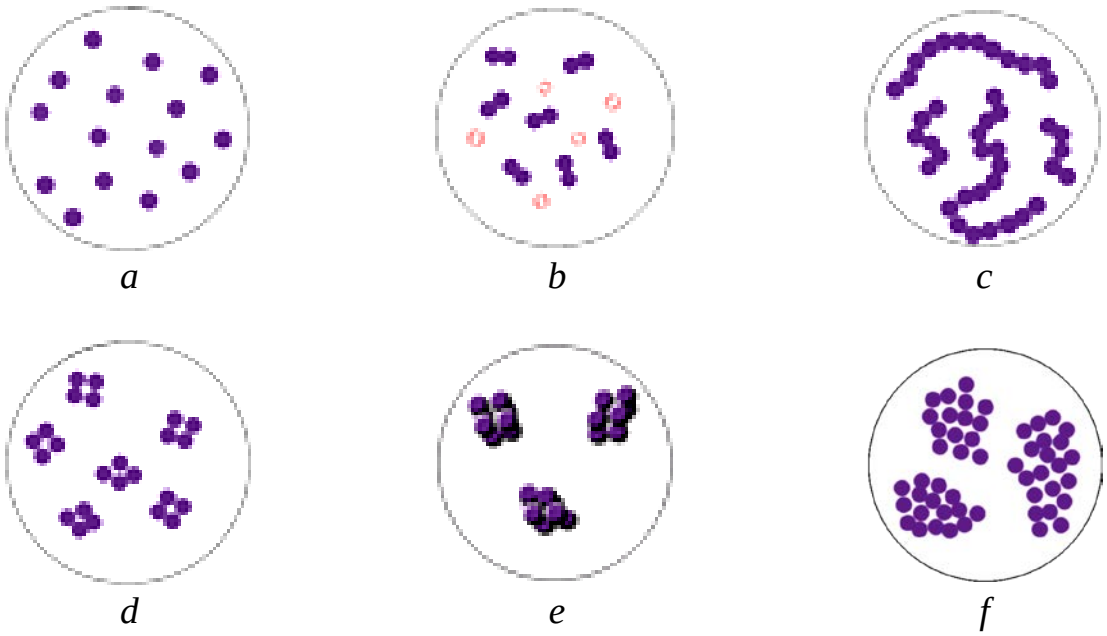


Fig.1. Cocci: a – micrococci; b – diplococci; c – streptococci; d – tetracocci; e – sarcinae; f – staphylococci

Based on their mutual arrangement, they are divided into the following groups:

Monobacteria – arranged randomly, singly; this group includes most rod-shaped forms (*Escherichia coli*, Fig. 2a).

Diplobacteria – arranged in pairs, poles facing each other (*Klebsiella*) or at an angle to each other (diphtheria pathogen, Fig. 2c).

Streptobacteria – arranged in chains (*anthrax pathogen*, Fig. 2b).

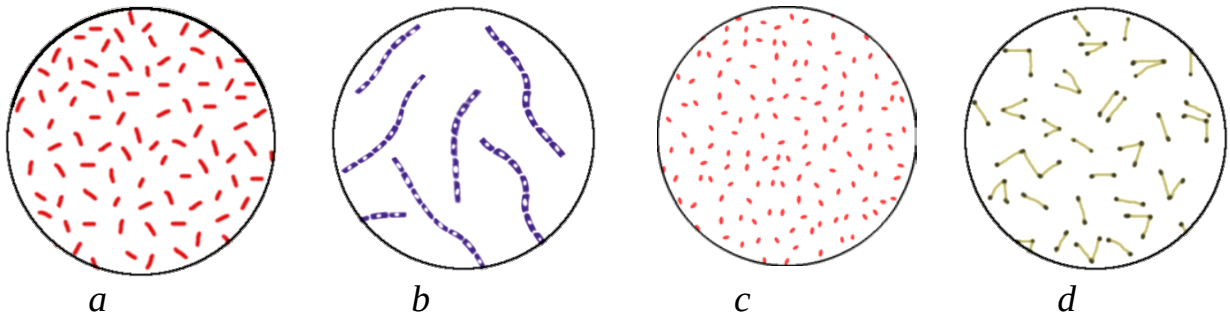


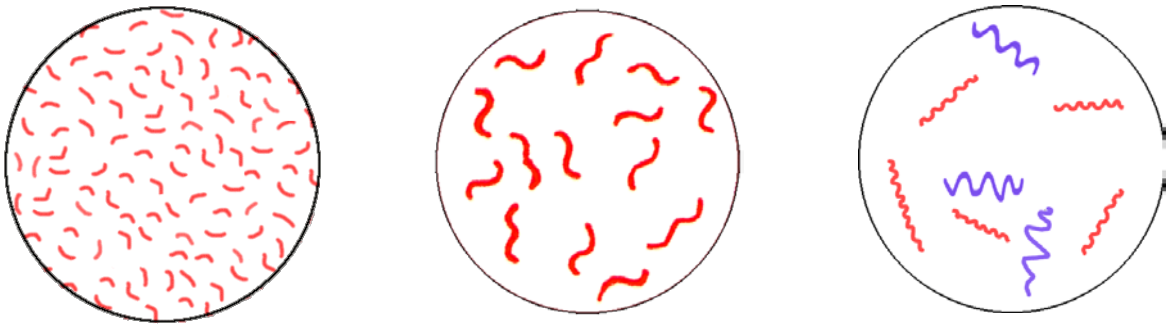
Fig. 2. Rod-shaped microorganisms: a – Escherichia coli; b – anthrax pathogen; c – pertussis pathogen; d – diphtheria pathogen

Curved (spiral) microorganisms differ in the number and nature of curls and are divided into:

Vibrios – have one bend, resembling a comma (Vibrio cholerae, Fig. 3a).

Spirilla – have a spiral shape with two to three curls (campylobacteria, Fig. 3b).

Spirochetes – have more than three curls (treponemes, borreliae, leptospirae, Fig. 3c).



a *b* *c*

Fig. 3. Curved microorganisms: a – vibrios, b – spirilla, c – spirochetes

Filamentous microorganisms have thread-like forms (*actinomycetes*, Fig. 4).

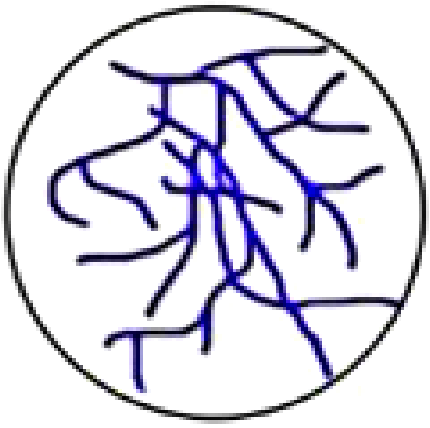


Fig. 4. Filamentous microorganisms

METHODS OF MICROSCOPIC EXAMINATION OF MICROORGANISMS

Due to the small size of microorganisms (no more than 1–15 μm), studying their morphology is possible only with microscopes (from Latin *micros* – small and *scopien* – to examine, observe) that provide sufficient magnification and high resolving power, i.e., the minimum distance at which two points are displayed separately by the microscope.

A modern microscope is a complex optical instrument that allows to study objects in transmitted light, dark field, and in reflected light. Currently, in microbiological research practice, light microscopy, dark-field microscopy, phase-contrast microscopy, and fluorescence microscopy are most commonly used. Electron microscopy is used for specialized microbiological studies.

Light Microscopy

Light microscopy is performed using a conventional light microscope with mechanical and optical systems. The mechanical system includes: a stand, a tube, a specimen stage, a macrometric screw, and a micrometric screw. The main part of the optical system is the objective lens. The objective frames are marked with magnification: 8, 10, 20, 40, 90 (Fig. 5).

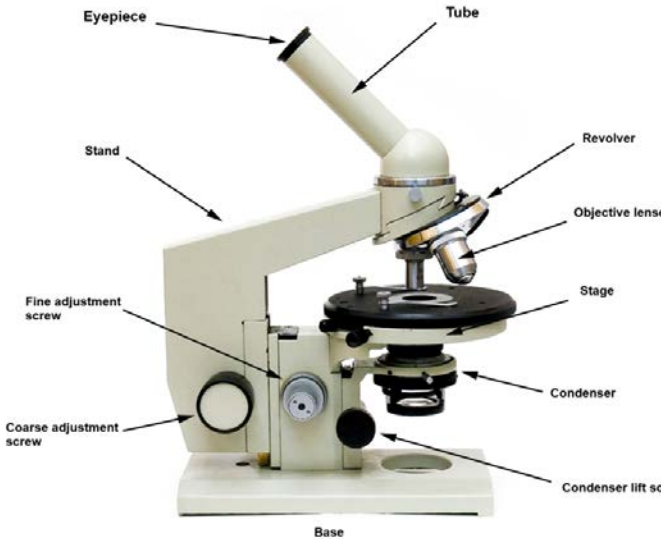


Fig. 5. Structure of a light microscope

When examining microorganisms, a special immersion lens is used. The immersion lens is put in a drop of immersion oil applied to the specimen. Immersion oil has the same refractive index as glass, achieving minimal scattering of light rays (Fig. 6).

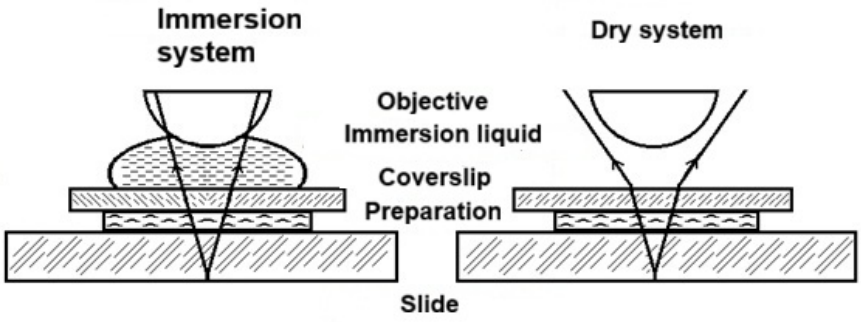


Fig. 6. Diagram of the microscope immersion system

The image obtained by the lens is magnified by the eyepiece, consisting of two lenses. Eyepieces with magnifications of 7, 10, 15 are used in Russian microscopes. The total magnification of the microscope is determined by multiplying the lens magnification by the eyepiece magnification. In microbiology, magnifications of 600–1000 times are typically used. The quality of the microscope depends not on the degree of magnification but on its resolving power. The resolving power of conventional bright-field microscopes with an immersion system is 0.2 μm.

Dark-Field Microscopy

Dark-field microscopy is based on the principle that rays illuminate the object from the side, not from the bottom, and they do not enter the observer's eyes, leaving the field of view dark, while rays are reflected from objects in the field, making the object luminous (Fig. 7). This is achieved using a special condenser (paraboloid) or a regular condenser covered by a circle of black paper in the center.

Native (live) preparations are used for dark-field microscopy, and they are prepared as crushed drops. The examined material (bacterial culture in physiological saline) is applied to a slide covered with a coverslip. The drop of material fills the entire space between the coverslip and the slide, forming an even layer. Dark-field microscopy is used to study live unstained microorganisms.

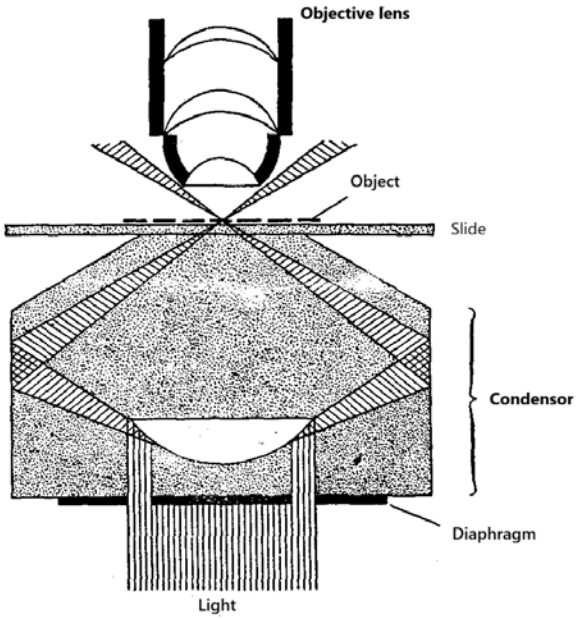


Fig. 7. Diagram of a microscope for observation in dark field (Steiner R. et al., 1979)

Phase-Contrast Microscopy

When a beam of light passes through an unstained object, only the phase of the light wave oscillation changes, which is not perceived by the human eye. Image contrast is achieved by converting the phase variations of a light wave into visible amplitude variations. This is achieved using a phase-contrast condenser and phase objective (Fig. 8).

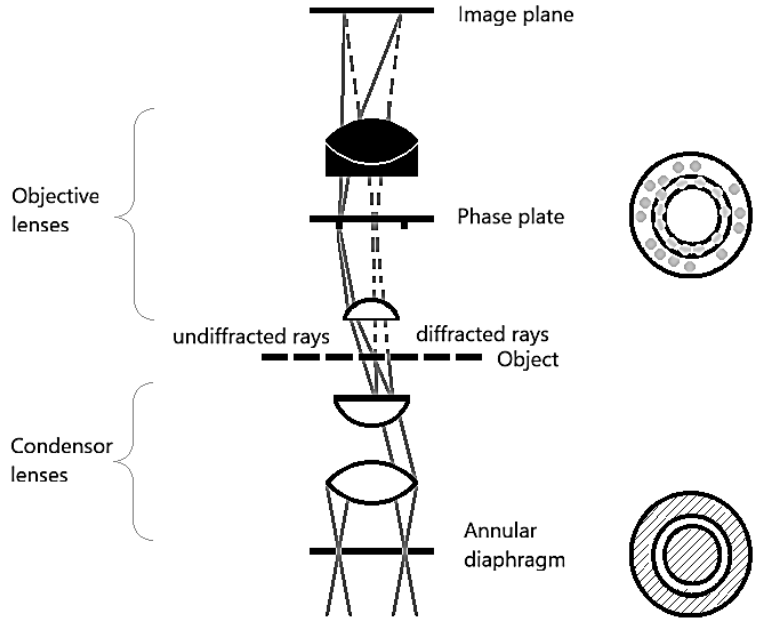


Fig. 8. Diagram of a phase-contrast microscope

The phase-contrast condenser is a regular lens with a turret and a set of annular diaphragms for each lens. The phase objective is equipped with a phase plate obtained by depositing rare-earth metal salts on the lens. The image of the annular diaphragm coincides with the ring of the phase plate of the corresponding lens.

Phase-contrast microscopy significantly increases object contrast and is used to study native (unstained) preparations.

Fluorescence Microscopy

Fluorescence microscopy is based on the ability of certain substances to emit rays with a different (usually longer) wavelength (to fluoresce) under the influence of incident light. Such substances are called fluorochromes (acridine yellow, FITC, rhodamine, etc.). An object treated with a fluorochrome acquires a bright color in a dark field when illuminated with ultraviolet rays.

The main part of a fluorescence microscope is the illuminator, which has an ultraviolet lamp and a filter system (Fig. 9). The use of non-fluorescent immersion oil is very important.

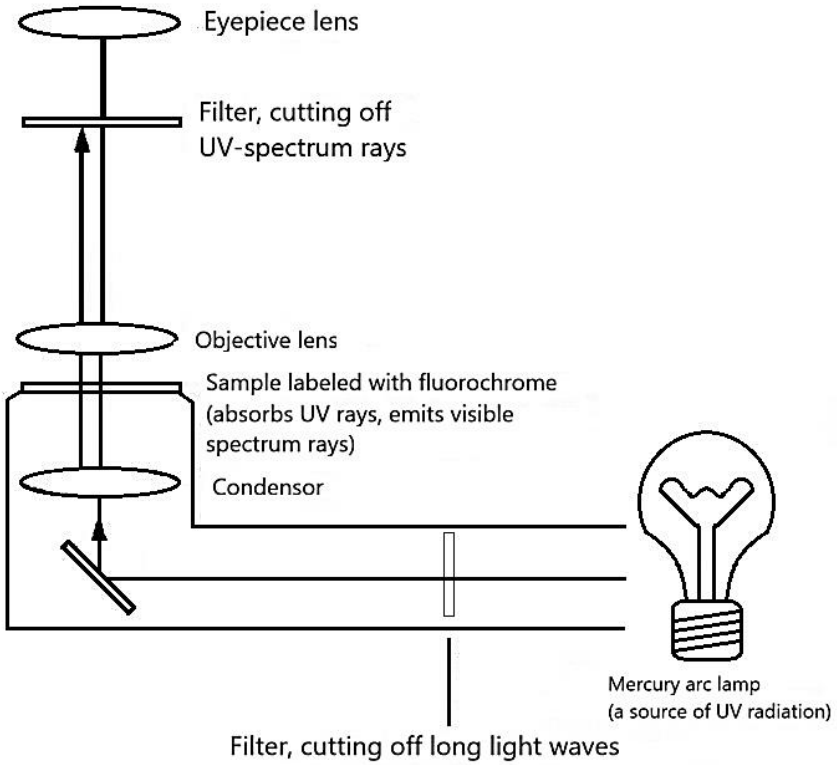


Fig. 9. Diagram of fluorescence microscopy

In practical microbiology, fluorescence microscopy is used for the indication and identification of infectious disease pathogens using the immunofluorescence reaction.

Electron Microscopy

The capabilities of optical microscopes are limited by the excessively long wavelength of visible light. Objects smaller than 0.2 μm are beyond the resolving power of a light microscope. In an electron microscope, electron beams with an extremely short wavelength and high resolving power are used instead of light waves.

Electron microscopy can detect the smallest structures, obtain magnification up to 200,000, and visualize objects as small as 0.002 μm. Currently, there are several types of electron microscopes.

A *conventional transmission electron microscope* (TEM) is similar in many ways to a light microscope, but it uses a beam of electrons instead of light to illuminate samples (Fig. 10a). The electron source is usually a heated cathode made of tungsten or lanthanum hexaboride (1). The cathode is electrically isolated from the rest of the device, and electrons are accelerated by a strong electric field using a special accelerating system (2). For this purpose, the cathode is maintained at a potential of about 100,000 V relative to other electrodes. To reduce electron scattering in the microscope column, a vacuum is created. The electron beam is focused on the sample (5) using condenser magnetic lenses (4). The aperture (3) determines the beam width in the object plane. The sample is placed in the magnetic field of the objective lens with high optical power (6), which creates a magnified image of the object (magnification around 100). Aberrations of the lens are limited by its aperture (7). The projection lens (8) projects the image onto a screen or film (9) and can provide additional magnification.

In a *scanning electron microscope*, electron lenses are used to focus the electron beam into a very small spot (Fig. 10b). This spot continuously scans a section of the sample, similar to the beam scanning a television tube screen. A scanning microscope requires a high-intensity electron source (1). For this, a strong electric field is created near the surface of a pointed thin tungsten wire, extracting electrons without heating. The brightness of such a source is nearly 10,000 times greater than that of a heated tungsten wire source. The electrons are further accelerated using an accelerating system (2) and focused into a small-diameter spot by a magnetic lens (3). Using deflecting magnetic coils (4), the electron beam scans the entire sample section (5). A reflected electron detector (6) located above the sample records reflected electrons. The contrast is mainly related to the angle of electron incidence on the sample, and the image clearly reveals surface structure (scanning microscopy). Detectors located below the sample are used for scanning transmission microscopy to study thin samples. An annular detector (7) records

electrons scattered at angles greater than a few degrees. Electrons that have not undergone scattering in the sample, as well as electrons slowed down due to interaction with the sample, pass through the hole in the annular detector. An energy analyzer (8) located below the annular detector measures the energy lost by electrons during scattering, providing important information about the sample.

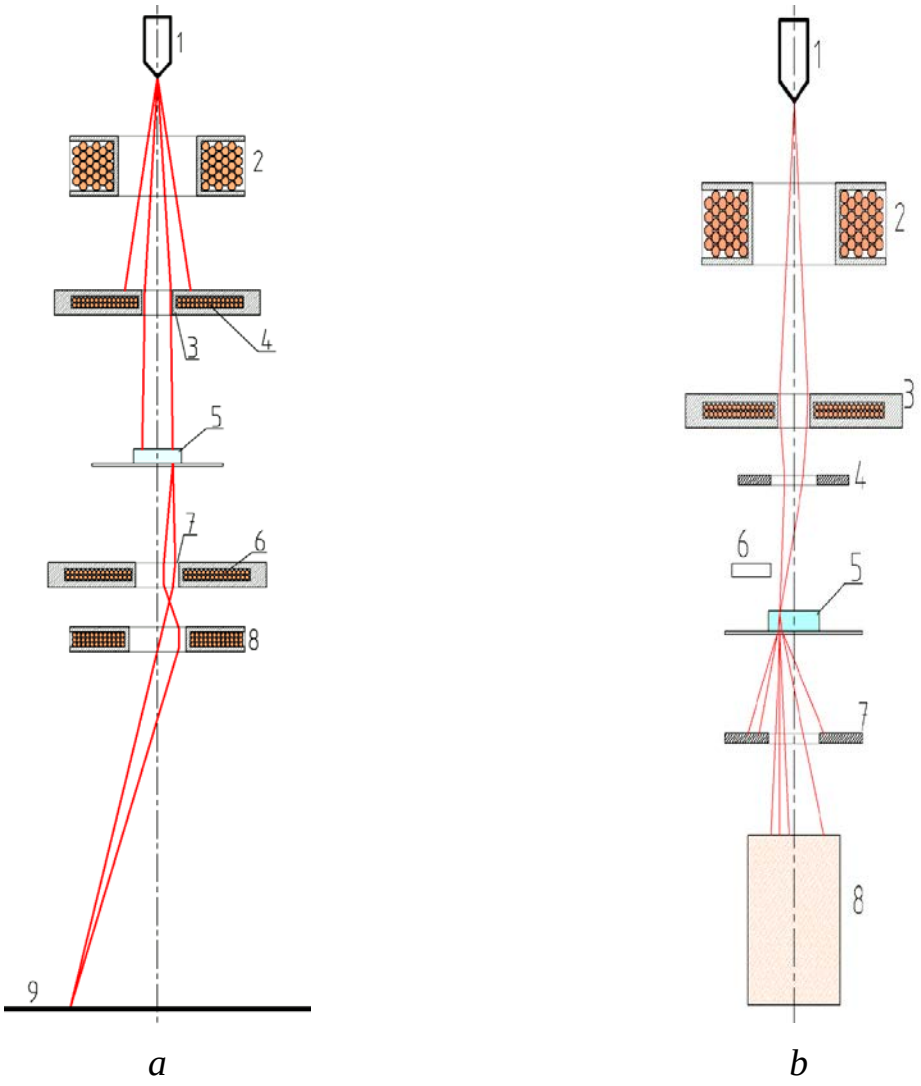


Fig. 10. Diagram of a transmission (a) and scanning (b) electron microscope

STRUCTURE OF THE BACTERIAL CELL

Despite its small size, the bacterial cell has all the main structural components necessary for metabolism. Bacteria are prokaryotes and significantly differ from plant and animal cells (eukaryotes). The main differences between prokaryotic and eukaryotic cells are presented in Table 2.

Table 2

Differences between Prokaryotic and Eukaryotic Cells

Feature	Prokaryotes	Eukaryotes
Defined nucleus	—	+
Cell sizes	0.2–2.0 μm	>2.0 μm
Membrane-bound cytoplasmic organelles: mitochondria, ER, lysosomes, chloroplasts, Golgi apparatus	—	+
Cell envelopes	Cell wall consists of murein	Main component of cell wall is cellulose (in plants) or chitin (in fungi). Animal cells lack a cell wall
Ribosome localization	Scattered in cytoplasm	Attached to ER
Ribosome sedimentation constant	70 S	80 S
Flagellum structure	Consists of one fibril built from flagellin protein	Consists of microtubules assembled in groups: 9+2
Cell division	Binary fission	Mitosis or meiosis
Number of chromosomes	1	Usually >1
Chromosome	Circular	Linear

Bacteria are unicellular organisms composed of a cell wall, a cytoplasmic membrane, cytoplasm, a nucleoid (genophore), ribosomes, and mesosomes — these are the *essential components* of a bacterial cell.

Some bacteria may possess flagella, a capsule, a spore, pili, inclusions, and plasmids — these are the *non-essential components* of a bacterial cell (Fig. 11).

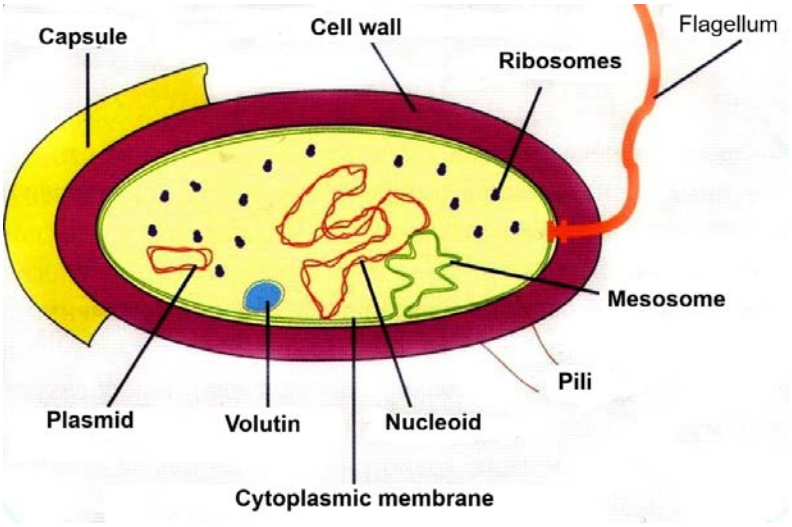


Fig. 11. Schematic structure of a bacterial cell

Cell Wall

The cell wall is an external structure of bacteria with a thickness of 30–35 nm. The cell wall accounts for 5 to 50% of the cell's dry matter. The main component of the cell wall is peptidoglycan (murein). *Peptidoglycan* is a structural polymer consisting of alternating subunits of N-acetylglucosamine and N-acetylmuramic acid, connected by glycosidic bonds. Parallel polysaccharide (glycan) chains are cross-linked by peptide bridges (Fig. 12).

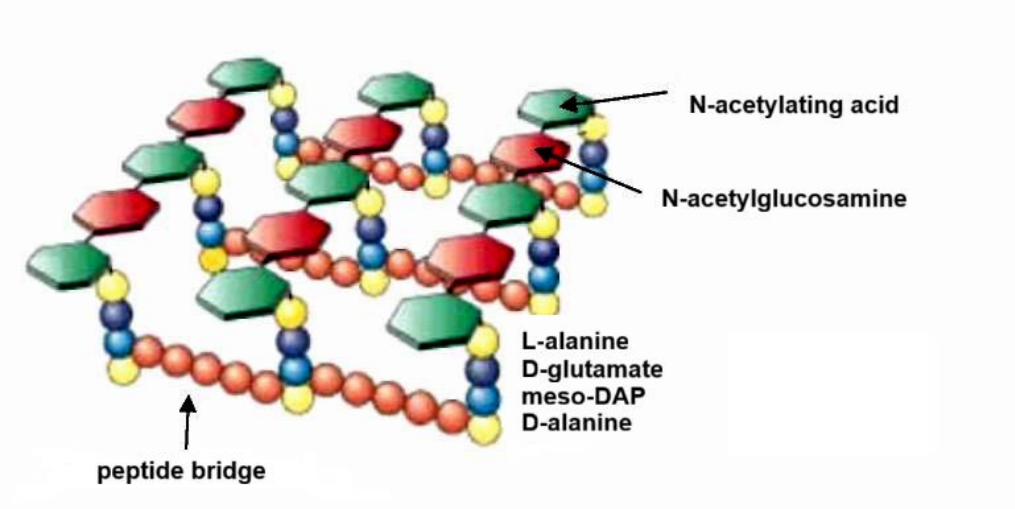


Fig. 12. Schematic representation of the single-layer structure of peptidoglycan (https://scask.ru)

The quantitative content of peptidoglycan affects the ability of bacteria to stain when Gram method is used. Bacteria with a significant thickness of the murein layer (90–95%) are stably stained with gentian violet in purple and are called gram-positive bacteria. *Gram-negative bacteria* with a thin layer of

peptidoglycan (5–10%) in the cell wall lose gentian violet after alcohol treatment and are additionally stained with fuchsin in red. The cell walls of gram-positive and gram-negative prokaryotes differ sharply both in chemical composition (Table 3) and in ultrastructure.

Table 3

Chemical Composition of Cell Walls of Gram-Positive and Gram-Negative Prokaryotes (according to E. Rose, 1971; J. Freer, M. Salton, 1971)

Components of the cell wall	Gram-positive prokaryotes	Gram-negative prokaryotes	
		Inner layer (peptidoglycan)	Outer layer (outer cell membrane)
Peptidoglycan	+	+	-
Teichoic Acids	+	-	-
Polysaccharides	+	-	+
Proteins	±	-	+
Lipids	±	-	+
Lipopolysaccharides	-	-	+
Lipoproteins	-	±	+

The cell wall of gram-positive bacteria under an electron microscope appears as a homogeneous dense layer, with a thickness ranging from 20 to 80 nm. Peptidoglycan in the cell wall of gram-positive bacteria constitutes 50–90% of its dry mass. In addition to peptidoglycan, the cell wall of gram-positive bacteria contains teichoic acids (TA), and in smaller amounts, lipids, polysaccharides, and proteins.

Teichoic acids (polyphosphate compounds) are divided into 2 classes:

1) Wall-associated, linked to the peptidoglycan of the cell wall;
2) Membrane-associated (lipoteichoic), connected to the glycolipid of the cytoplasmic membrane.

Teichoic acids can bind to cell membranes and facilitate the adhesion process necessary for bacterial colonization, which is the first stage of most infections. The phenomenon of teichoic acids inducing inflammatory processes, cytotoxicity, and immunosuppression is particularly interesting to study.

On the surface of the cell wall of gram-positive bacteria, protein molecules may be present – surface, minor proteins that do not form structures of a specific shape (protein A of staphylococci, M-protein of streptococci, etc.). They possess

high biological activity: they inhibit phagocytosis, have toxic properties, and promote bacterial adhesion to cells (Fig. 13). The cell wall also contains major proteins-porins that form diffuse pores through which small hydrophilic molecules can penetrate into the cell.

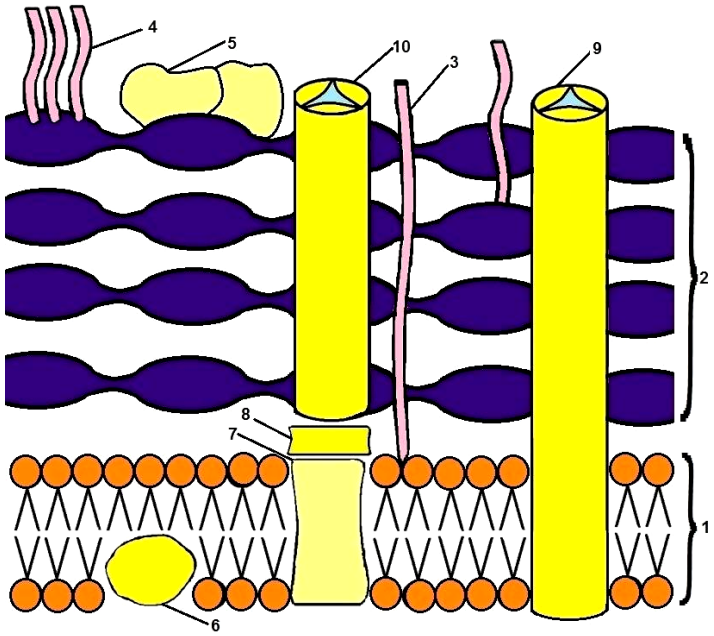


Fig. 13. Structure of the cell wall of gram-positive bacteria.
1 – cytoplasmic membrane; 2 – layers of peptidoglycan; 3 – lipoteichoic (membrane) acids; 4 – teichoic (wall) acids; 5 – surface protein; 6 – minor protein; 7 – major (integral) protein; 8 – inserted protein; 9, 10 – porins

The cell wall of gram-negative bacteria is multilayered, with a thickness of 14–17 nm (Fig. 14). The inner layer of the cell wall is represented by peptidoglycan, which accounts for 1–10% of its dry mass. Gram-negative prokaryotes have an outer membrane (located above the peptidoglycan layer), which includes lipids (averaging 22% of the dry mass of the cell wall), proteins, polysaccharides, and lipoproteins. The outer membrane performs not only mechanical but also physiological functions. It contains major (transmembrane) proteins that completely penetrate the membrane. They represent water-filled channels or hydrophilic pores, also called porins. There are several different types of porins that facilitate the transport of hydrophilic low-molecular-weight substances across the membrane. One of the distinctive features of gram-negative bacteria is the absence of teichoic acids in their cell wall.

In the upper layer of the outer membrane, *lipopolysaccharides (LPS)* are located, they are heteropolymers with a complex structure, possessing diverse biological activity. The LPS of the outer membrane consists of three fragments:

- lipid A – a conserved structure that is almost identical among gram-negative bacteria;
- the core, or core region (Latin core – nucleus), a relatively conserved oligosaccharide structure;
- highly variable *O-specific polysaccharide chain*, formed by repeating identical oligosaccharide sequences.

LPS is “anchored” in the outer membrane by lipid A, which determines toxicity. The destruction of bacteria by antibiotics leads to the release of lipid A (endotoxin), which can cause infectious-toxic (endotoxic) shock in the patient.

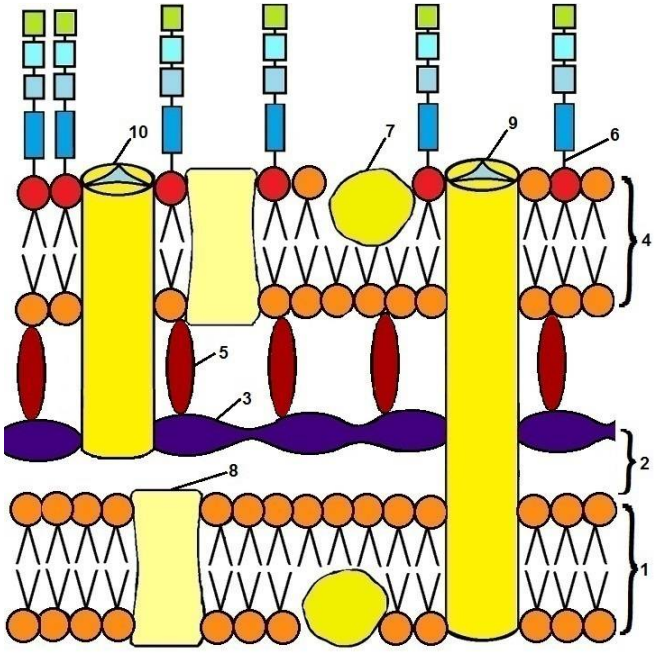


Fig. 14. Structure of the cell wall of gram-negative bacteria.
1 – cytoplasmic membrane; 2 – periplasmic space; 3 – layer of peptidoglycan; 4 – outer membrane; 5 – lipoprotein; 6 – lipopolysaccharide; 7 – minor protein; 8 – major (integral) protein; 9, 10 – porins

The core or core part of LPS extends from lipid A. The O-specific chain, extending from the core part of the LPS molecule, determines the serogroup and serovar of a specific bacterial strain. Thus, the concept of LPS is associated with representations of the O-antigen, by which bacteria can be differentiated. Genetic changes can lead to defects, “shortening” of bacterial LPS and, as a result, to the appearance of “rough” colonies of R-forms. LPS induces the synthesis of Ig M antibodies; in immunology, it is used as an adjuvant and polyclonal activator of B-cells.

The Gram staining method is an important differential method. All bacteria in relation to Gram staining are divided into gram-positive – dark purple color and gram-negative – red ones. The ability to be stained in a particular color depends on

the structure of their cell wall. Gram-positive bacteria, having a significant thickness of the murein layer (90–95%), form a strong connection with the gentian violet-iodine complex, which is not destroyed by short-term alcohol exposure. Gram-negative bacteria with a thin layer of peptidoglycan (5–10%) in the cell wall form with the same violet complex (gentian violet-iodine) a connection that is easily destroyed by alcohol. They are easily decolorized by alcohol. Fuchsin then stains gram-negative microorganisms red.

Gram Method

1. Filter paper is placed on the smear and carbol gentian violet is added for 1–2 minutes.
2. The paper is removed, the dye is drained, the smear is not rinsed with water, and Lugol's solution is added for 1 minute.
3. Lugol's solution is drained and the preparation is decolorized in 96° alcohol for 8–10 seconds.
4. Rinsing with water.
5. Staining for 1–2 minutes with aqueous fuchsin solution.
6. The preparation is rinsed with water and dried.
7. Microscope study.

As a result of staining, gram-positive bacteria stain purple, and gram-negative – red.

The cell wall in bacteria mainly performs shaping and protective functions, ensures rigidity, forms the capsule, determines the ability of cells to adsorb phages. Nutrients enter the cell through the cell wall, and metabolic products are excreted.

Mycobacteria and nocardia are characterized by a complicated cell wall structure. Their basis, as in gram-positive bacteria, is the murein framework, but the latter is linked to lipids, fatty acids (mycolic, phthioid, etc.), waxes, and polysaccharides. Lipid components impart hydrophobicity to the cell surface. Hydrophobicity, on the one hand, makes the cell resistant to the action of various chemical substances (such bacteria are called *acid-fast*), on the other hand, it hinders the cell's exchange with the environment and slows its growth. Acid-fastness of mycobacteria is an important differential feature; to determine it, staining by the Ziehl–Neelsen method is used.

Ziehl–Neelsen Method

1. A filter paper is placed on the fixed smear and carbol fuchsin Ziehl is added, it is carefully heated over a burner until vapors appear. The operation is repeated 2–3 times.
2. When the preparation cools, the filter paper is removed, the dye is drained, and the preparation is rinsed with water.
3. Immerse the preparation 2–3 times in a glass with 5% sulfuric acid for 1–2 seconds.
4. The preparation is thoroughly rinsed with water and it is then counterstained with alkaline methylene blue for 3–5 minutes.

5. The preparation is rinsed with water and dried.

Acid-fast bacteria are not decolorized by sulfuric acid and retain the red color, non-acid-fast lose the dye and are counterstained with methylene blue in blue.

When the synthesis of the bacterial cell wall is disrupted under the influence of lysozyme, penicillin, host defense factors, and other compounds, cells with altered (often spherical) shapes are formed: *protoplasts* – bacteria completely devoid of a cell wall; *spheroplasts* – bacteria with partially preserved cell wall. After removal of the cell wall inhibitor, such altered bacteria can revert, i.e., acquire a full cell wall and restore the original shape.

Bacteria that have completely or partially lost their cell wall but retained the ability to reproduce are called *L-forms* in honor of the Lister Institute (England), where they were first isolated. Regardless of the shape of the original cell (coccus and bacilli), the L-forms of these bacteria are morphologically indistinguishable. They are spherical formations of different sizes. L-forms can arise in natural conditions in the human body as a result of long-term treatment with certain antibiotics, most often penicillin. They are osmotically sensitive, spherical, flask-shaped cells of various sizes, including those passing through bacterial filters.

Unstable and stable L-forms of bacteria are distinguished. The former are capable of reverting to the original form upon elimination of the cause that led to their formation. They restore the ability to synthesize peptidoglycan of the cell wall. The latter, as a rule, are not capable of reversion. L-forms of different bacteria play a significant role in the pathogenesis of many infectious diseases.

Cytoplasmic Membrane

The bacterial cell cytoplasm is separated from the cell wall by a thin semi-permeable structure 5–10 nm thick, it is called the cytoplasmic membrane (CM). The CM consists of a double layer of phospholipids penetrated by protein molecules (Fig. 15).

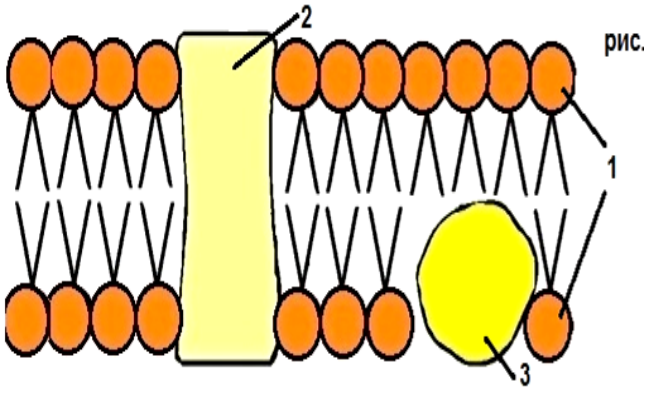


Fig. 15. Schematic structure of the cytoplasmic membrane:
1 – layer of phospholipids; 2 – major protein; 3 – minor protein

Many enzymes and proteins involved in the transport of nutrients, as well as enzymes and electron carriers of the final stages of biological oxidation (dehydrogenases, cytochrome system, ATPase), are associated with the CM. Enzymes that catalyze the synthesis of peptidoglycan, cell wall proteins, and its own structures are localized on the CM. The membrane is also the site of energy transformation in photosynthesis and oxidative phosphorylation.

The cytoplasmic membrane performs vital functions, the disruption of which leads to the death of the bacterial cell. These include, first of all, regulation of the entry of metabolites and ions into the cell, participation in metabolism, DNA replication, and in some bacteria, in sporulation. The CM is associated with the synthesis of the cell wall and capsule due to the presence of specific carriers for the molecules that form them. Flagella are anchored in the cytoplasmic membrane. The energy supply for the operation of flagella is associated with the cytoplasmic membrane.

Between the cell wall and the CM is the periplasmic space (periplasm). The thickness of the periplasm is about 10 nm, the volume depends on environmental conditions and, primarily, on the osmotic properties of the solution. The periplasm can include up to 20% of all the water in the cell; it localizes some enzymes (phosphatases, permeases, nucleases, etc.) and transport proteins – carriers of corresponding substrates.

Cytoplasm

The contents of the cell surrounded by the cytoplasmic membrane constitute the cytoplasm of bacteria, which is a complex colloidal system composed of water (about 75%), mineral compounds, proteins, RNA, and DNA.

The part of the cytoplasm that has a homogeneous colloidal consistency and contains soluble RNA, enzymes, substrates, and metabolic products is called the cytosol. The other part of the cytoplasm consists of various structural elements: mesosomes, ribosomes, inclusions, nucleoid, plasmids.

Ribosomes

Ribosomes are submicroscopic ribonucleoprotein granules with a diameter of 15–20 nm. Ribosomes contain approximately 80–85% of all bacterial RNA. Prokaryotic ribosomes have a sedimentation constant of 70S. They are built from two particles: 30S (small subunit) and 50S (large subunit) (Fig. 16). Ribosomes serve as the site of protein synthesis. Before protein synthesis begins, the large and small subunits combine into one – 70S. Bacterial ribosomes can be a target for

many antibiotics. Depending on growth intensity, a bacterial cell may contain from 5,000 to 50,000 ribosomes; the number is greater with faster cell growth.

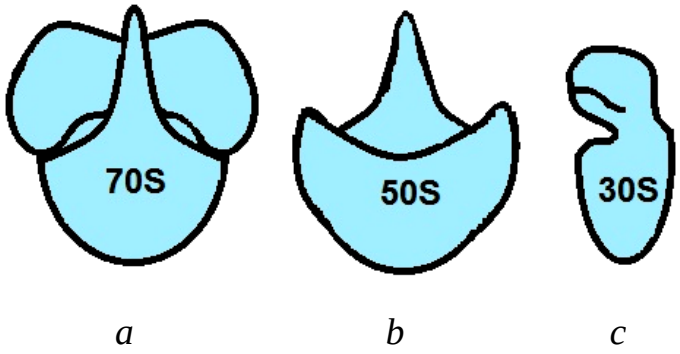


Fig. 16. Ribosomes of a prokaryotic cell:
a – complete 70S ribosome; b – large 50S subunit; c – small 30S subunit

Structurally and functionally, the ribosome is primarily rRNA. The ribosomal RNA of the small ribosomal subunit is referred to as 16S rRNA. The rRNA that forms the structural basis of the large ribosomal subunit is referred to as 23S rRNA. Studying 16S rRNA is the basis of genosystematics, allowing for the assessment of organism relatedness. The ribosome also consists of 30–50% protein. The ribosome contains about 50 different proteins.

Mesosomes

Mesosomes are membrane structures formed by invagination and twisting of the CPM into the bacterial cell. Bacterial mesosomes vary in shape, size, and location within the cell. Three main types of mesosomes are distinguished: lamellar (plate-like), vesicular (bubble-like), and tubular (tube-like) (Fig. 17). In cells of some bacteria, mixed-type mesosomes are also found: consisting of lamellae, tubes, and bubbles. Based on location in the cell, mesosomes are distinguished as those formed in the zone of cell division and cell septum formation (septal mesosomes) and those formed by invagination of peripheral sections of the CPM (lateral mesosomes).

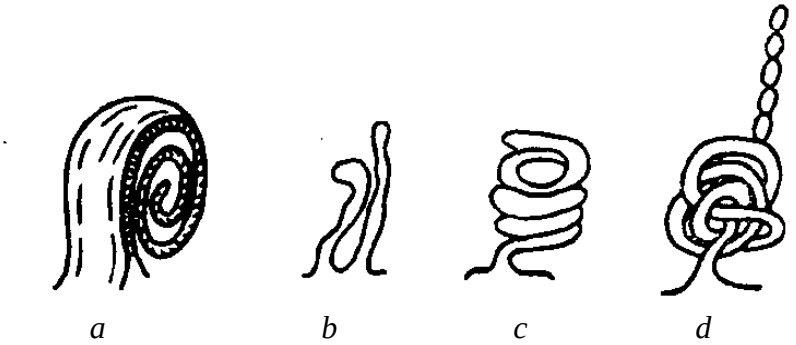


Fig. 17. Types of true mesosome structures:
a – lamellar; b–d – tubular types (Biryuzova, Poglazova, 1977)

Mesosomes are presumed to be polyfunctional, containing various enzyme systems and playing a role in energy metabolism. They are sites for bacterial cell wall formation and nucleoid attachment during DNA replication, they participate in cell division by providing energy for cell wall synthesis, and are involved in substance secretion and sporulation, i.e., processes requiring high energy expenditure.

Nucleoid (Genophore)

The nucleoid (genophore) is the nuclear apparatus of bacteria. The nucleoid is equivalent to the eukaryotic nucleus but differs in structure and chemical composition. It lacks a nuclear membrane, nucleolus, and does not divide by mitosis. The nucleoid lacks basic proteins – histones. Analogous to eukaryotic chromosomes, bacterial DNA is often referred to as a chromosome. It should be remembered that it is present in the cell in single copy, as bacteria are haploid. Before cell division, the nucleoid duplicates (Fig. 18).

Associated with the DNA is a small amount of RNA and RNA polymerase. The DNA is coiled around a central core consisting of RNA and represents a highly ordered compact structure. Chromosomes of most prokaryotes have a molecular weight in the range of $1–3\times10^9$, sedimentation constant 1300–2000 S. The DNA molecule includes $1,6\times10^7$ nucleotide pairs.

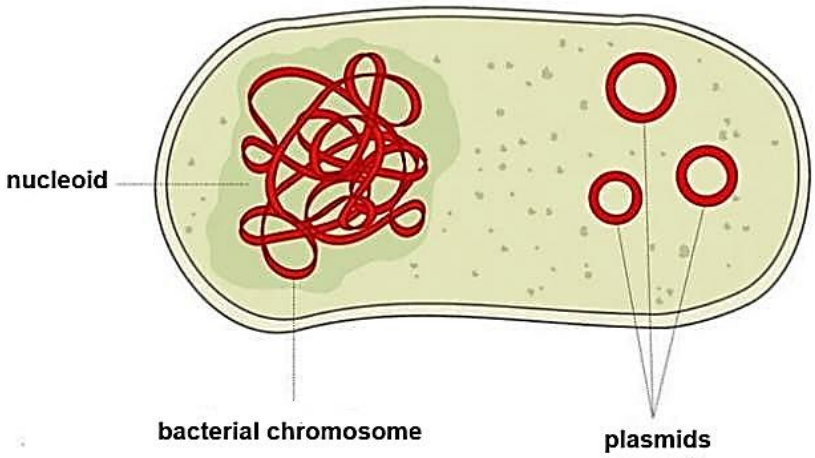


Fig. 18. Nucleoid and plasmids of bacteria (https://news4auto.ru)

The bacterial genophore contains the main hereditary information, realized in the synthesis of specific protein molecules. Each protein corresponds to its gene. The bacterial chromosome contains up to 4000 individual genes. Associated with

the bacterial cell DNA are systems of replication, repair, transcription, and translation.

The nucleoid in a prokaryotic cell can be detected in stained preparations using light or phase-contrast microscopy. The nucleoid is revealed after staining with DNA-specific methods: Feulgen or Romanowsky-Giemsa.

Plasmids

The genetic system of bacteria consists of nuclear and extranuclear structures. In addition to the nucleoid, the bacterial cell has extrachromosomal heredity factors – plasmids. They are closed-loop double-stranded DNA consisting of 1500–40,000 nucleotide pairs and containing up to 100 genes. They also encode hereditary information. However, it is not vital for the bacterial cell.

Plasmids can exist in the cell in an integrated state with the bacterial chromosome, while retaining the ability to revert to autonomy.

Plasmids perform regulatory and coding functions. The former are aimed at compensating for metabolic defects, while the latter provide the bacterium with information about new traits. As part of the bacterial genetic material, plasmids play an important role in its vital activity, determining characteristics such as the ability to produce exotoxins, enzymes, or bacteriocins, resistance to drugs, etc.

Duplication of DNA in some plasmids induces bacterial division, i.e., increases their “fertility”. Such plasmids are referred to as F-plasmids or F-factors (from the English – *fertility*). Plasmids determining drug resistance are called R-plasmids or R-factors (from the English – *resistance*). Pathogenicity plasmids control the virulent properties of microorganisms, determining the synthesis of pathogenicity factors. For example, the Ent-plasmid determines enterotoxin synthesis.

Conjugative (transmissible) plasmids are transferred from bacterium to bacterium within a species or between representatives of closely related species during conjugation. Most often, conjugative plasmids are F- or R-plasmids. Such plasmids are relatively large (25–150 MDa) and are often found in gram-negative rods.

Non-conjugative plasmids usually have small sizes and are characteristic of gram-positive cocci but are also found in some gram-negative microorganisms (e.g., *Haemophilus influenzae, Neisseria gonorrhoeae*). Small plasmids can be present in large numbers (more than 30 per cell), as only such quantities ensure their distribution in progeny during cell division.

Inclusions

The cytoplasm contains various inclusions in the form of glycogen granules, polysaccharides, sulfur, beta-hydroxybutyric acid. They accumulate with excess nutrients in the environment and serve as reserve substances for nutrition and energy needs of the cell. Some bacteria can accumulate phosphoric acid in the form of polyphosphate granules (volutin grains, metachromatic grains, Babes-Ernst grains). They serve as phosphate depots and are found in corynebacteria, spirilla, and yeasts as dense, well-contoured formations in the shape of a sphere or ellipse, mainly located at the cell poles. Usually, there is one granule per pole. In some bacteria, such as the diphtheria bacillus, volutin inclusions in the form of intensely stained cell poles have differential diagnostic value (Fig. 19).

The presence of volutin grains in bacteria is detected by Neisser staining.

Fig. 19. Smear from pure culture of Corynebacterium diphtheriae. Neisser staining

Neisser Method

1. The fixed smear is stained with acetic acid blue for 4 min, then the dye is drained.
2. Rinsing with water and applying Lugol's solution for 20–30 s.
3. Without rinsing with water, staining with vesuvin is made for 1–3 min.
4. The preparation is rinsed with water and dried.

The bacterial bodies are stained a delicate light brown, volutin grains – dark blue, almost black.

Spores

Bacterial spores are a unique form of resting cells, mainly in gram-positive rod-shaped bacteria. Bacterial spores can be considered a form of preserving the hereditary information of the bacterial cell under unfavorable environmental conditions. The transition of bacteria to sporulation is usually observed with nutrient substrate depletion, lack of carbon, nitrogen, phosphorus sources, pH

changes, accumulation of toxic metabolic products, etc. One bacterial cell forms one spore, the location of which can vary (central, terminal, subterminal) (Fig. 20).

If the spore size does not exceed the transverse size of the rod-shaped bacterium, the latter is called a *bacillus* (*anthrax pathogen*). When the spore diameter is larger, the bacteria have a spindle shape and are called *clostridia* (*anaerobic infection pathogens*). Tetanus clostridia have a round spore and resemble drumsticks. Botulism clostridia have large oval spores, giving them the appearance of a tennis racket.

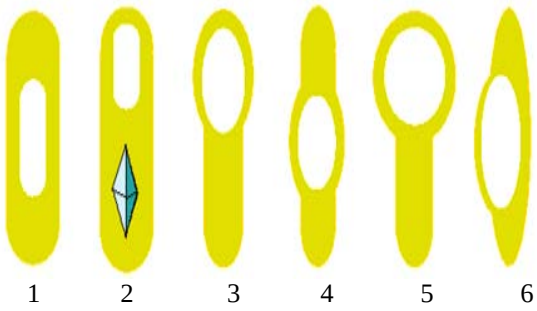


Fig. 20 Typical forms of sporulating cells: 1 – spore located centrally; maternal cell not enlarged (Bacillus megaterium); 2 – spore located terminally, maternal cell not enlarged; protein inclusions visible (Bacillus thuringiensis); 3 – spore located terminally, maternal cell swollen in club shape (Bacillus polymyxa); 4 – spore located centrally; maternal cell deformed into spindle shape – clostridial form (Bacillus polymyxa); 5 – spore located terminally; round maternal cell in drumstick shape – plectridial form (Bacillus sphaericus); 6 – spore located laterally; maternal cell spindle-shaped (Bacillus laterosporus) (Schlegel G., 1987)

Chemically, the difference between spores and vegetative cells is only in the quantitative content of chemical compounds. Spores contain less water and more lipids.

The sporulation process can be divided into three stages or phases (Fig. 21).

The first stage is preparatory. In the vegetative bacterial cell transitioning to sporulation, growth processes cease, the nucleoid (DNA) duplicates, and metabolism changes, namely, a significant portion of the maternal cell proteins breaks down, forming a spore-specific substance – dipicolinic acid, not found in vegetative cells.

The second stage – spore formation begins with cell division. Part of the cytoplasm and bacterial chromosome of the vegetative cell separates, surrounded by an invaginating cytoplasmic membrane – forming a prespore. The prespore is surrounded by two cytoplasmic membranes, between which a thick modified peptidoglycan layer of the cortex is formed. Inside, it contacts the spore cell wall, and outside – the inner spore coat. The outer spore coat is formed by the vegetative cell. As multilayer covers form, the prespore turns into a spore.

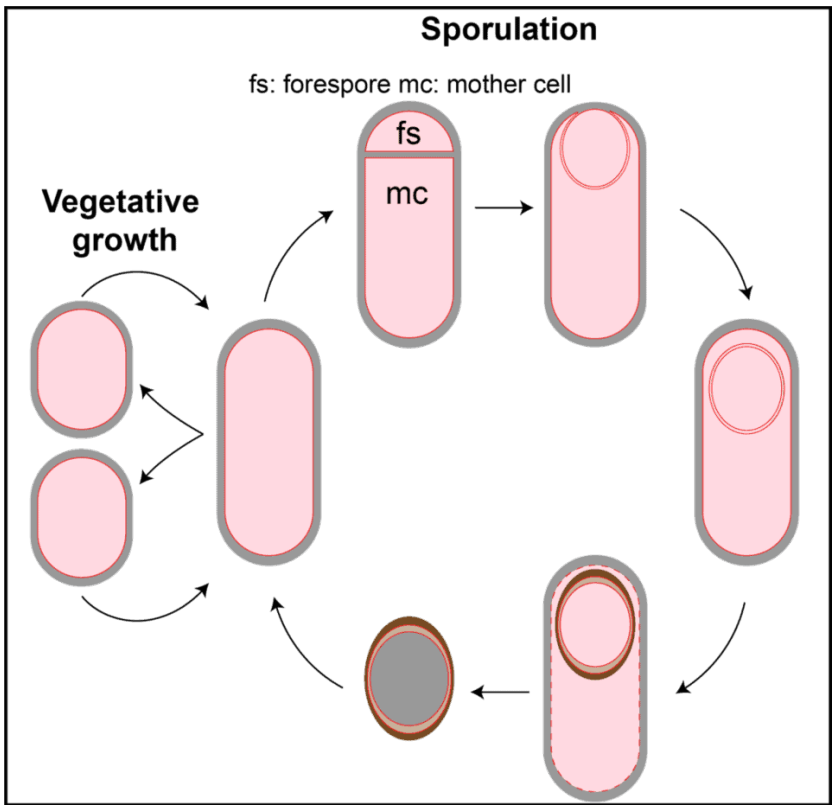


Fig.21. Diagram of the sporulation process (Khanna et al., 2020)

Spores of some bacteria have an additional cover – exosporium, composed of proteins, lipids, carbohydrates. Thus, a multilayer resistant shell is formed. Sporulation is accompanied by intense consumption of dipicolinic acid and calcium ions by the prespore. The spore acquires thermostability, associated with the presence of calcium dipicolinate.

The third stage – spore maturation. The spore acquires a characteristic shape and occupies a specific position in the cell.

Thus, the spore consists of the following structural elements: nucleoid; condensed cytoplasm (due to dehydration, protein binding, reduced activity of some enzymes, and synthesis of calcium dipicolinate); covering layers represented by cytoplasmic membrane, germ cell wall, cortex, inner coat, outer coat, exosporium (Fig. 22).

After full spore maturation, the vegetative part of the cell may lyse. In the spore state, microorganisms are metabolically inactive, they withstand high temperatures (140–150 °C), chemical disinfectants, and persist long in the environment. In soil, for example, anthrax and tetanus pathogens can persist for decades.

Upon entering human and animal organisms, spores germinate into vegetative cells. The spore germination process includes three stages: activation, initiation, and growth. Activating agents disrupting dormancy include higher temperature, acidic pH, mechanical damage, etc. The spore begins absorbing water,

releasing calcium dipicolinate, using hydrolytic enzymes to destroy many own structural components. After outer layer destruction, the vegetative cell formation period begins with biosynthesis activation, ending in cell division. Spore germination occurs within 4–5 hours, while spore formation lasts up to 18–20 hours.

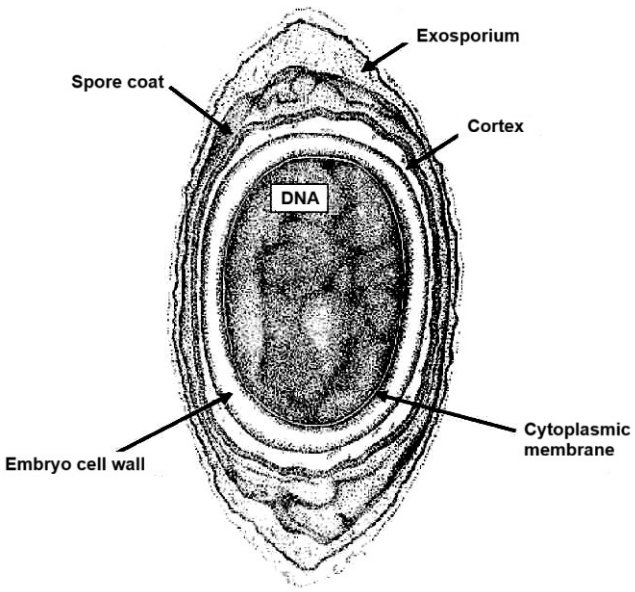


Fig. 22. Diagram of mature spore structure (S. Haley, 2001)

Spore staining is done by the special Ozhieszko method, involving preheating the spore and using concentrated dye solutions at high temperature.

Ozhieszko Method

1. The 0.5% hydrochloric acid solution is applied to the dried smear and heated for 1–2 min.
2. The preparation is rinsed with water and fixed over a burner flame.
3. Filter paper is placed on the fixed smear and Ziehl's carbol fuchsin is applied, gently heated over the burner until steam appears. This step is repeated 2–3 times.
4. When the preparation cools, the filter paper is removed, the dye is drained, and the preparation is rinsed with water.
5. The preparation is immersed 2–3 times in a glass with 5% sulfuric acid for 1–2 s.
6. The preparation is thoroughly rinsed with water and it is then counterstained with alkaline methylene blue for 3–5 minutes.
7. The preparation is rinsed with water and dried.

Spores firmly retain carbol fuchsin and stain red; bacterial cytoplasm is decolorized by 5% sulfuric acid and after counterstaining with methylene blue acquires a blue color.

Capsule

The capsule is a mucous layer of the bacterial cell wall, consisting of polysaccharides (pneumococcus) or polypeptides (anthrax bacillus). A microcapsule (with thickness less than 0.2 μm) can be formed by most bacteria. A clearly expressed macrocapsule (with thickness more than 0.2 μm) is formed by pneumococcus, klebsiella, anthrax pathogen, and some others. In pathogenic bacteria, the capsule forms in the macroorganism. The capsule is visible in impression smears from pathological material. On artificial nutrient medium, it is usually lost (except for klebsiella).

The capsule is hydrophilic, containing a large amount of water. The capsule should be distinguished from mucus – mucoid exopolysaccharides without clear external boundaries. Mucus is water-soluble.

The capsule performs various functions: protective, shielding the cell from unfavorable habitat conditions; adhesive, facilitating attachment (adhesion) to the host cell surface. In human and animal organisms, the capsule protects pathogenic bacteria from bacteriophages, phagocytosis, and humoral immunity factors, determines antigenic specificity of microorganisms. Capsular Ag (K-Ag) of many pathogenic bacteria exhibit pronounced immunogenic properties and are used to prepare immunobiological preparations (e.g., vaccines against pneumococcal and meningococcal infections).

Having a gel consistency, capsules retain dye poorly, and the Burri-Gins negative contrast method is most often used for their detection (Fig. 23).

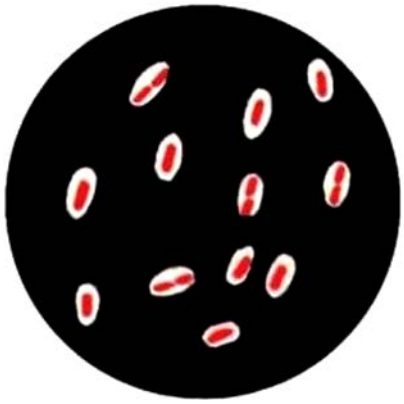


Fig. 23. Capsule detection by negative contrast Burri–Gins method

Burri–Gins Method

1. A drop of black ink is applied to the middle of the slide and mixed with a loop containing a drop of capsular bacterial culture.
2. A smear is made, similar to a blood smear, using the edge of another slide. The smear is dried in air and fixed in a burner flame.

3. The smear is stained for 5 minutes with carbol fuchsin diluted with water in a 1:3 ratio.

4. The smear is gently rinsed with water and dried.

Bacteria stain red, unstained capsules contrast sharply against the dark background of the preparation.

Flagella

Flagella serve as organs of locomotion, allowing bacteria to move at speeds of 20–60 μm/s. Flagella are thin threads originating from the cytoplasmic membrane, longer than the cell itself. Flagella thickness is 12–20 nm, length is 3–15 μm. Flagella consist of the protein flagellin, which is an antigen – the so-called H-antigen. Flagellin subunits are twisted into a helix.

Based on the arrangement and number of flagella on the cell surface, bacteria are subdivided into:

• monotrichous – have one flagellum (e.g., bacteria of genera *Caulobacter* and *Vibrio*);

• lophotrichous – have a bundle of flagella at one or both cell poles (e.g., bacteria of genera *Pseudomonas, Chromatium*);

• amphitrichous – have one flagellum at each pole (e.g., bacteria of genus *Spirillum)*;

• peritrichous – have a large number of flagella distributed over the entire cell surface (e.g., *E. coli* and genus *Erwinia*) (Fig. 24).

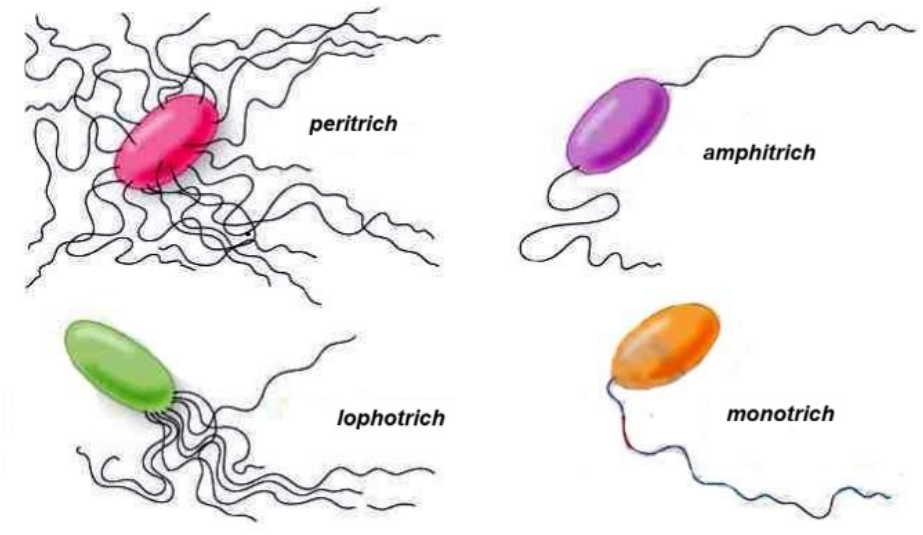


Fig. 24. Types of flagellation in bacteria. (https://ik-ptz.ru)

The flagellum consists of three components – a helical flagellar filament of constant thickness, a hook (knee), and a basal body (Fig. 24). The hook, to which

the flagellar filament is attached, has a length of 30–45 nm and consists of a protein that is different from flagellin. It is connected to the basal body located in the envelope (in the cell wall and CPM). The basal body consists of a central rod enclosed in a system of special rings. The rings serve as a “drive disk” and “bearing” on the inner surface of the peptidoglycan layer. The entire structure functions as a chemomechanical converter (flagellar motor). Removal of the peptidoglycan layer of the cell wall leads to loss of bacterial motility, although the flagella remain intact.

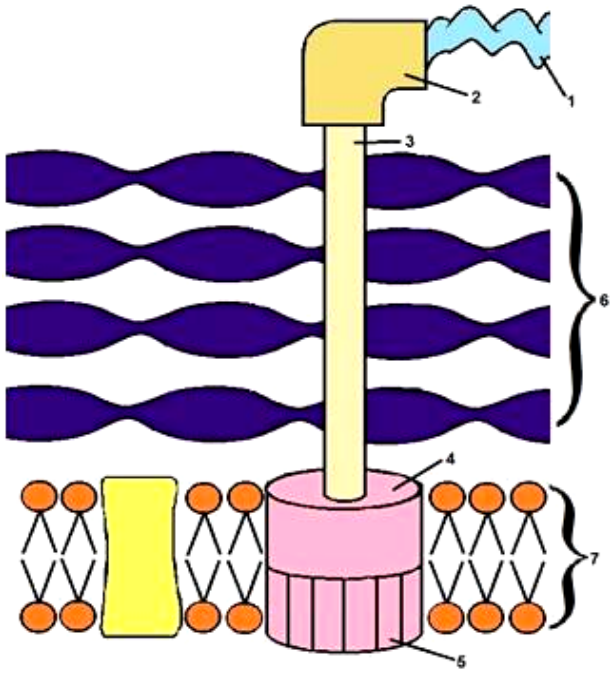


Fig. 24. Structure of the flagellum of a Gram-positive bacterium:
1 – filament; 2 – hook; 3 – rod; 4 – S-disk; 5 – M-disk; 6 – peptidoglycan; 7 – cytoplasmic membrane

In Gram-negative bacteria, the basal body has two pairs of rings (Fig. 25): outer (L and P rings) and inner (S and M rings). The L and P rings are located inside the cell wall (L ring in LPS, P ring in peptidoglycan layer). They apparently serve as a bushing for the rod. The inner pair (S and M rings) is fixed on the CPM, with the S ring in the periplasmic space and the M ring on or in the CPM.

Flagella of Gram-positive bacteria contain only one pair of rings (outer pair absent), so it is believed that only the inner pair (S and M rings) is necessary for flagellar rotation.

Flagellar rotation in the cell wall occurs due to rotational movement of the S and M rings relative to each other and is powered by the energy of the transmembrane gradient of hydrogen or sodium ions. This rotation allows bacteria to move in the most favorable direction. The flagellar apparatus has a special binary switch that allows changing the direction of flagellar rotation from

counterclockwise to clockwise. Thus, bacteria, upon receiving a chemical signal from the environment, change direction and choose optimal habitat conditions.

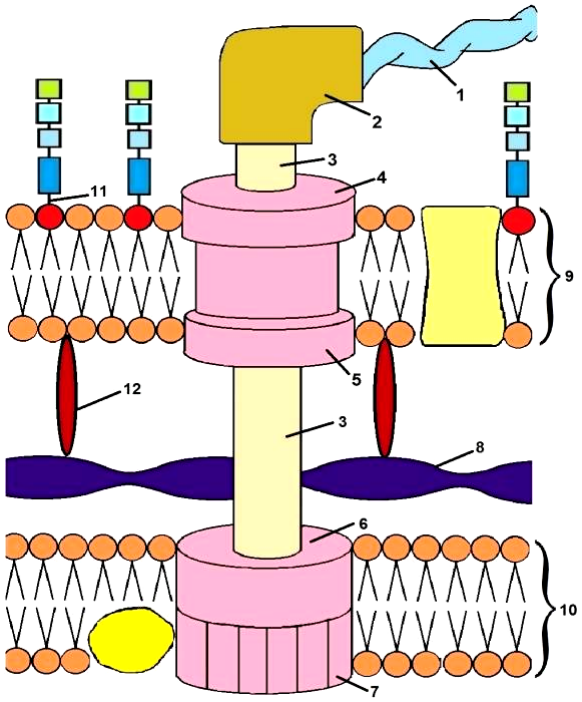


Fig. 25. Structure of the flagellum of a Gram-negative bacterium. 1 – filament; 2 – knee; 3 – rod; 4 – L-disk; 5 – P-disk; 6 – S-disk: 7 – M-disk; 8 – peptidoglycan; 9 – outer membrane; 10 – cytoplasmic membrane; 11 – lipopolysaccharide; 12 – lipoprotein

The proton potential difference on the cytoplasmic membrane is used as an energy source. The rotation mechanism is provided by proton ATP synthase. The flagellar rotation speed can reach 100 rev/s. If the cell has many flagella, they begin to rotate synchronously, intertwining into a single bundle that forms a kind of propeller.

Under the microscope, flagella can be seen only after special mordanting and impregnation methods with silver and mercury salts followed by staining with aniline dyes (Leffler's method). The presence of flagella can be indirectly judged by the directed nature of movement in "hanging" and "crushed" drops in dark-field and phase-contrast microscopes, or in bright-field microscopy with the lowered condenser and a partially closed diaphragm.

Pili

Pili (fimbriae, villi) are thin hollow protein threads covering the surface of bacterial cells. Unlike flagella, they do not perform a locomotor function. As for their size, they are shorter and thinner than flagella, with length of 0.3–10 μm, thickness of 10 nm (Fig. 26).

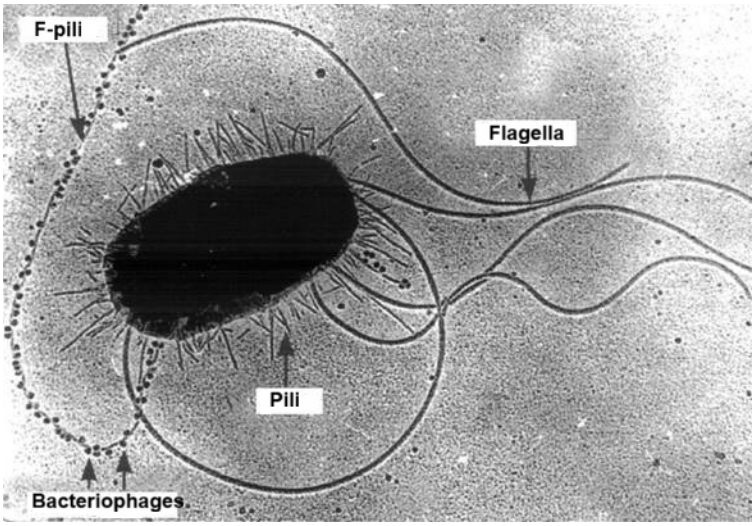


Fig. 26. Flagella and pili of bacteria. Electron microscopy. (Atlas of Medical Microbiology, Virology, and Immunology / Ed. A.A. Vorobyov, A.S. Bykov – M.: Medical Information Agency, 2003. – 236 p.)

Pili are built from one type of protein – *pilin*, whose subunits are organized into a hollow thread and originate from the CPM. Pili have antigenic activity.

Based on functional purpose, they are subdivided into several types. Common type pili and sex pili are distinguished.

Common type pili mediate attachment or adhesion of bacteria to specific host organism cells. Their number is large – from several hundred to several thousand per bacterial cell. Adhesion is the initial stage of any infectious process.

Sex pili (conjugative pili) participate in genetic material exchange (conjugation) between bacteria, ensuring transfer of part of the genetic material from donor cell to recipient. They are present in limited numbers (1–4 per cell) only in donor bacteria, i.e., in the cells containing the sex factor (F-plasmid) or other donor-specific plasmids.

SEPARATE GROUPS OF PROKARYOTES

Actinomycetes

Actinomycetes (from the Greek *aktis* – ray, *mykes* – fungus) represent a group of non-motile bacteria that occupy an intermediate organizational level between bacteria and fungi. They appear as small or long non-septate branching filaments called hyphae. An aggregation of hyphae is called mycelium. The similarity to fungi is purely external, as actinomycetes have a prokaryotic cell type with a cell wall that does not contain chitin or cellulose. The peptidoglycan may include galactose, arabinose, xylose, and other sugars not typical for other bacteria, allowing differentiation of actinomycetes. Actinomycetes are Gram-positive, non-motile, many forms are acid-fast, and some actinomycetes have a capsule around the filaments.

When cultured on nutrient medium (cultural form), actinomycetes form substrate mycelium, which grows into the nutrient medium, and aerial mycelium, which grows on the surface of the medium. In affected tissues (tissue form), actinomycetes can form druses-granules (Fig. 27), accumulations of densely intertwined hyphae in the form of rays extending from the center and ending in club-shaped thickenings.

Actinomycetes reproduce asexually by forming spores – conidia or sporangiophores with sporangia at the ends of aerial mycelium, or by budding, or by fragmentation of mycelium. Sporangiophores can be straight, wavy, or spiral. Spores are oval, round, cylindrical, with a smooth surface or spines, sometimes motile due to flagella (zoospores). Spores serve for the reproduction of actinomycetes; they are not heat-resistant but can withstand desiccation. In budding, a hypha develops from a small bud, which gradually elongates into a rod, and then into a short filament with lateral branches. In reproduction by fragmentation, the mycelium breaks into individual rods or cocci, each of which gives rise to a new cell – hypha.

Actinomycetes are widely distributed in nature, inhabiting water and humus-rich soil. They participate in the cycling of substances in nature. Individual species of actinomycetes are used as producers of antibiotics, vitamins, lipids, proteases, amino acids, and steroids.

As symbionts of humans and animals, actinomycetes are present in the oral cavity, in the matrix of dental calculus, tonsillar crypts, and in stones of the urinary and biliary tracts.

Actinomycetes belong to the order *Actinomycetales*, which includes the families:

- *Actinomycetaceae*, genus *Actinomyces*;
- *Nocardiaceae*, genus *Nocardia*;

- *Streptomycetaceae*, genus *Streptomyces*;
- *Mycobacteriaceae*, genus *Mycobacterium*.

Representatives of the genus *Actinomyces* appear as long or short branched rods, not forming aerial mycelium. *Actinomyces israelii*, *A. bovis*, *A. albus*, *A. violaceus*, and others cause actinomycosis in humans and form druses in affected tissues.

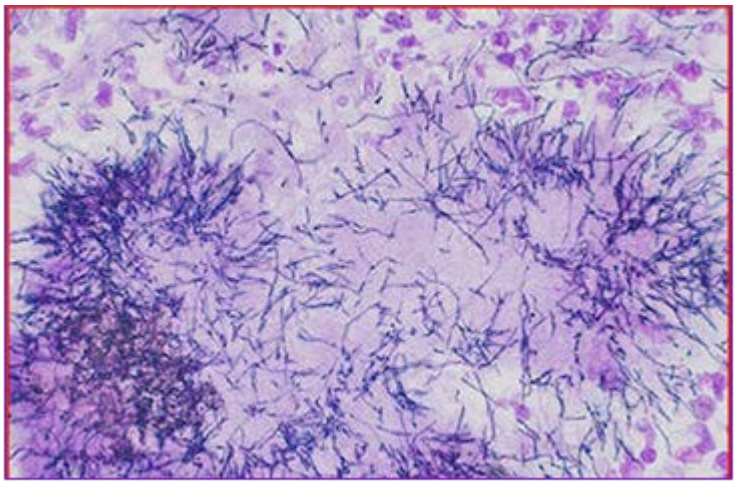


Fig. 27. Actinomycete druses in the affected tissue

Nocardia have filamentous cell shapes and form aerial and substrate mycelium on nutrient media. They reproduce by fragmentation and binary fission. Pathogenic nocardia, for example, *N. asteroides* and *N. farcinica*, cause nocardiosis.

Actinomycetes of the genus *Streptomyces* form branched mycelium, divided into substrate and aerial. Mycelial filaments do not break into fragments; reproduction occurs via spores. The main habitats of streptomycetes are soil and layers of seawater. Representatives of this genus produce a large number of antibiotics active against fungi, bacteria, and tumor cells (streptomycin, nystatin, tetracyclines, etc.). Some representatives of the family *Streptomycetaceae* cause cutaneous mycetomas in humans.

The family Mycobacteriaceae differs in several features from true actinomycetes. They are usually thin, curved rods that do not form mycelium and branch only during prolonged cultivation. Mycobacteria are acid-fast due to the presence in the cell wall of a large amount of lipids (up to 40% dry weight), mycolic acids, etc. They cause tuberculosis in humans – *M. tuberculosis* and leprosy – M. leprae.

Detection methods. Actinomycetes are stained by Gram and Ziehl-Neelsen methods. A druse is extracted from pathological material with a loop, placed in a drop of water on a slide, slightly pressed with a cover slip, then a drop of alkaline methylene blue solution is introduced under the glass, and it is examined under a microscope with a dry lens; phase contrast can be used.

Spirochetes

Spirochetes (from the Latin *spira* – coil and the Greek *chaite* – hair) are spirally coiled bacteria with active motility. The sizes of spirochetes vary in thickness from 0.1–0.3 μm, in length from 7–500 μm. Movements are diverse – from screw-like to bending. Electron microscopic examination has revealed in spirochetes a *protoplasmic cylinder* (the cell body), an axial filament (axostyle), and envelopes: a cell wall built like that of Gram-negative bacteria and a cytoplasmic membrane. The *axial filament* is located in the periplasmic space between the cell wall and the cytoplasmic membrane and consists of individual fibrils (endoflagella), the number of which varies among species: in treponemes and leptospires – 3–4; in borreliae – up to 30. Each fibril (endoflagellum) is attached to attachment disks (*blepharoplasts*) located at the ends of the protoplasmic cylinder and extends to the opposite end, wrapping around it and ending freely. The chemical composition of the fibrils is similar to that of flagella (protein – flagellin) (Fig. 28).

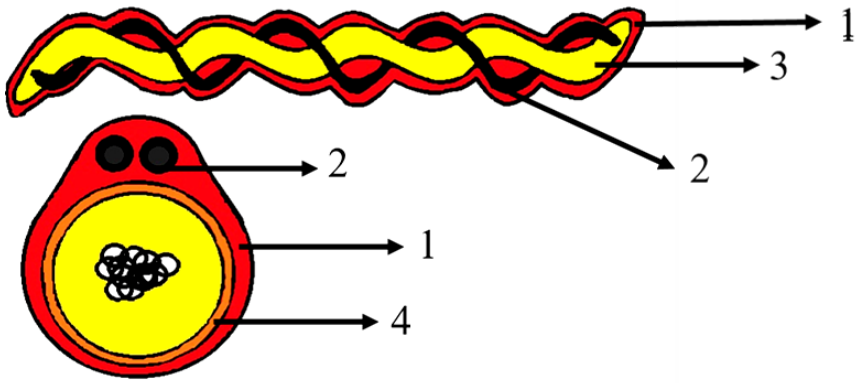


Fig. 28. Structure of a spirochete: 1 – cell wall; 2 – axostyle consisting of axial fibrils; 3 – protoplasmic cylinder; 4 – cytoplasmic membrane

The protoplasmic cylinder contains: nucleoid, ribosomes, mesosomes, inclusions. The cell wall contains a thin layer of peptidoglycan, is elastic, and lacks rigidity. These bacteria do not form endospores, capsules, or exoflagella; they are Gram-negative, and in smears, they are arranged randomly.

Spirochetes belong to the *Spirochaetales order*, consisting of two families: *Spirochaetaceae* and *Leptospiraceae*. The family *Spirochaetaceae* includes two genera: *Borrelia* and *Treponema*; the family *Leptospiraceae – Leptospira* genus.

1. Genus *Borrelia* – representatives have 3–10 uneven, shallow coils, pointed ends, length 10–30 μm. The movement is jerky. By Romanowsky-Giemsa, they stain blue-violet. Pathogenic species – *Borrelia burgdorferi*, *B. afzelii*, *B. garinii* – cause Ixodes tick-borne borreliosis – Lyme disease.

2. Genus *Treponema* – spirochetes have 8–14 tightly coiled, uniform amplitude coils, length 5–15 μm. The movement is smooth, slow with rotation around the longitudinal axis. By Romanowsky-Giemsa, they stain pale pink. *Treponema pallidum species* is the causative agent of syphilis.

3. Genus *Leptospira* – representatives with length 5–15 μm, have up to two dozen small frequent coils, ending in a hook with a button-like thickening. Movement is very active, with forward progression, bending, and rotation around the axis. By Romanowsky-Giemsa, they stain weakly in pinkish-lilac. *Leptospira interrogans* representative is the causative agent of leptospirosis (Fig. 29).

Detection methods. In the living state, spirochetes are studied in a phase-contrast microscope and dark-field microscope, the active characteristic movement of spirochetes is observed, and features of their shape.

For studying the morphological features of spirochetes, preparations are stained by Burri (the light coiled filaments of spirochetes become visible on a dark background of the preparation), by Romanowsky-Giemsa, and by Morozov's method.

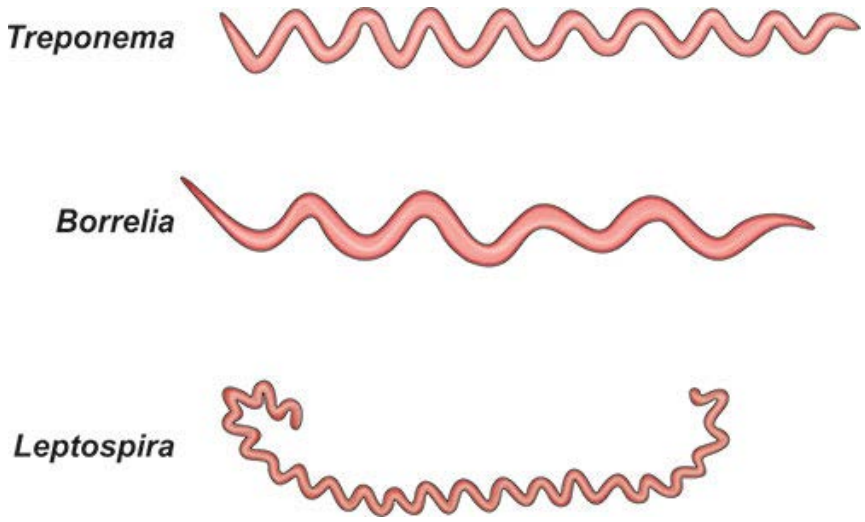


Fig. 29. Morphology of spirochetes

Mycoplasmas

Mycoplasmas are the smallest prokaryotes (125–150 nm) capable of independent reproduction. It is believed that mycoplasmas are the closest descendants of the original prokaryotic cells. The mycoplasma genome is minimal for a cell, five times smaller than that of Escherichia coli, and is 0.45 MDa. The main feature of mycoplasmas is the absence of a cell wall. They are surrounded by a capsule-like layer, under which there is only a thin three-layered membrane 7.5–10 nm thick, containing a significant amount of cholesterol. Due to structural features, mycoplasmas belong to the class *Mollicutes* ("tender skin"), order

Mycoplasmatales. The family *Mycoplasmataceae* includes two genera – *Mycoplasma* and *Ureaplasma*.

Due to the absence of a cell wall, mycoplasmas (Fig. 30) are osmotically sensitive and have diverse shapes:

a) small spherical or ovoid cells sized 0.2 μm (elementary bodies) that pass through bacterial filters;

b) larger spherical, up to 1.5 μm;

c) filamentous, branching cells up to 150 μm.

Mycoplasmas are Gram-negative, do not form spores or flagella; some species have gliding motility. They reproduce by binary fission of spherical and filamentous cells, budding.

Mycoplasmas are characterized by a unique need for sterols (cholesterol) among prokaryotes. Cholesterol stabilizes the mycoplasma membrane. In infected tissues, mycoplasmas are membrane parasites of eukaryotic cells and can persist on them for a long time.

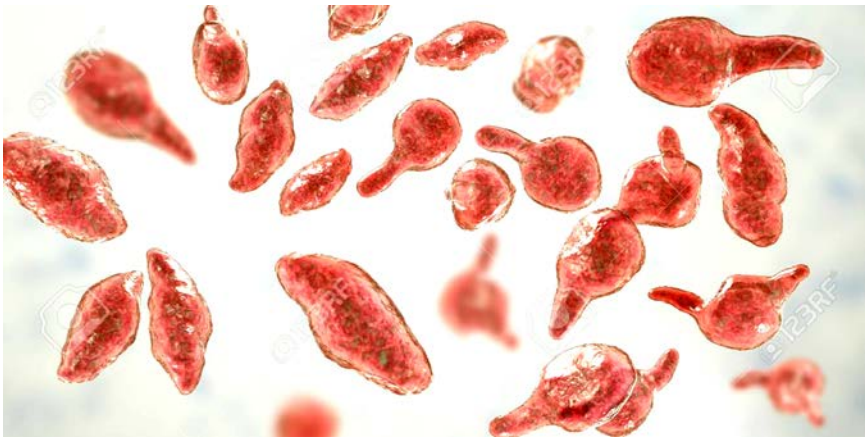


Fig. 30. Morphology of mycoplasmas. Cited from "Illustration – Bacteria Mycoplasma genitalium, 3D illustration. The causative agent of sexually transmitted infections and infertility», https://www.123rf.com/photo_124508103_stock-illustration-bacteria-mycoplasma-genitalium-3d-illustration-the-causative-agent-of-sexually-transmitted-infection.html

Mycoplasmas are facultative anaerobes. Due to their small genome, mycoplasmas have limited biosynthetic capabilities, and they must be cultivated on nutrient medium enriched with lipids, proteins, and nucleic acid precursors. They grow slowly on nutrient media, forming small colonies with a dense center ingrowing into the medium, resembling "fried eggs" (Fig. 31) (dark center and lighter lacy periphery).

Most mycoplasmas are harmless commensals of the mucous membranes of the eyes, respiratory, digestive, and urogenital tracts of humans.

In human pathology, several representatives of the genus *Mycoplasma* play the greatest role: *M. pneumoniae, M. fermentans, M. hominis, M. genitalium, M. arthritidis,* and of the genus *Ureaplasma – U. parvum and U. urealyticum* (named for urease activity). Pathogenic mycoplasmas cause diseases (mycoplasmoses) of the respiratory, urogenital tracts, and joints with diverse clinical manifestations. In treating these diseases, it should be remembered that mycoplasmas are insensitive to beta-lactam antibiotics and other drugs that inhibit cell wall synthesis (due to the absence of a cell wall in the pathogen).

Detection methods. In a light microscope, only the largest forms of mycoplasmas are detected. In the living state, they are studied in dark-field and phase-contrast microscopes; ultrastructural components are revealed using electron microscopy. They stain poorly by Gram. Mycoplasmas stain better by Romanowsky-Giemsa.

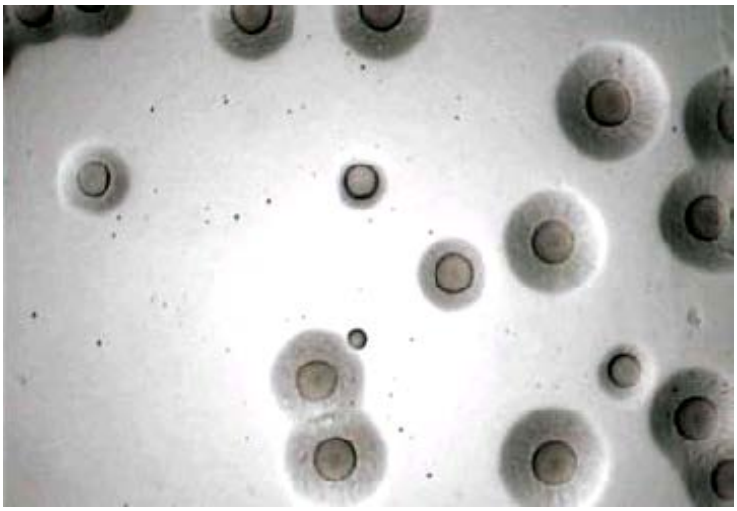


Fig. 31. Colonies of Mycoplasma bovis. Cited from «Development of a Second-Generation Vaccine against Mycoplasmosis: Preparation of a Fraction Candidate from Mycoplasma bovis and its Evaluation as a Vaccine; Global Veterinaria 16 (2): 137–144, 2016»

Rickettsiae

Rickettsiae are small (0.35–1.0 μm) Gram-negative, polymorphic bacteria that are obligate intracellular parasites.

Rickettsiae are diverse in shape; the following types are distinguished:

1) coccoid single-grained (up to 0.5 μm);
2) rod-shaped double-grained (1–1.5 μm);
3) bacillary three- to four-grained (3–4 μm);
4) filamentous multi-grained (10–40 μm).

Grains (nucleoproteins) are detected by Romanowsky-Giemsa staining. All forms are interconvertible. Structurally, they have all components of a bacterial cell: cell wall, CPM (cytoplasmic membrane), lipid capsule, cytoplasm, nucleoid,

ribosomes, pili. Rickettsiae contain both DNA and RNA, have high phospholipid content, low carbohydrate content.

The life cycle of rickettsiae depends on the host cell's activity and consists of two stages: vegetative and resting (elementary bodies). Rickettsiae in the vegetative stage (Fig. 32) actively reproduce by binary fission and have active motility, apparently due to flagellar structures. Rickettsiae in the resting stage (elementary bodies) have a spherical shape and are inactive.

Rickettsiae are capable of protein biosynthesis but cannot independently obtain high-energy compounds, so they can be called "energy parasites" of eukaryotic cells. Accordingly, for cultivating rickettsiae, chicken embryos are usually infected in the yolk sac (Cox method), cell cultures. Less commonly, sensitive laboratory animals are infected: guinea pigs, white mice.

Rickettsiae belong to the order *Rickettsiales*, *Rickettsiaceae* family, genus *Rickettsia*. The genus *Rickettsia* includes species pathogenic for warm-blooded animals and humans, transmitted by lice, fleas, ticks. The diseases are called rickettsioses. Most human-pathogenic species of rickettsiae are in the family *Rickettsiaceae*, genus *Rickettsia*, causing epidemic typhus (*Rickettsia prowazekii*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), tick-borne rickettsiosis (*Rickettsia sibirica*), and others. Parasitic species are associated with reticuloendothelial cells and vascular endothelial cells or erythrocytes.

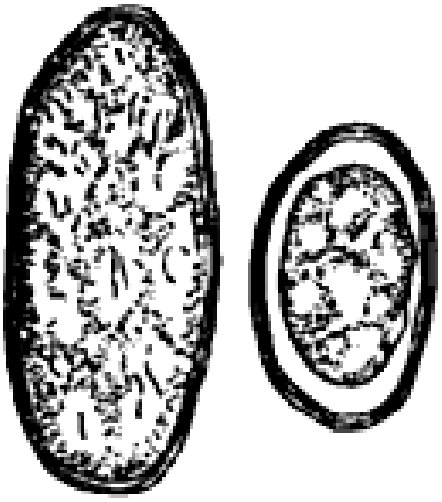


Fig. 32. Longitudinal section of vegetative (left) and resting (right) forms of Rickettsia prowazekii (schematic) [from Zdrodovsky P.F. and Golinevich E.M., 1972]

Detection methods. Rickettsiae stain well by Romanowsky-Giemsa in lilac color, by Morozov (silvering method) in black. A specific method for detecting rickettsiae is Zdrodovsky staining.

Rickettsiae stain ruby-red and are easily detected against the blue cytoplasm and blue nucleus of cells.

Chlamydiae

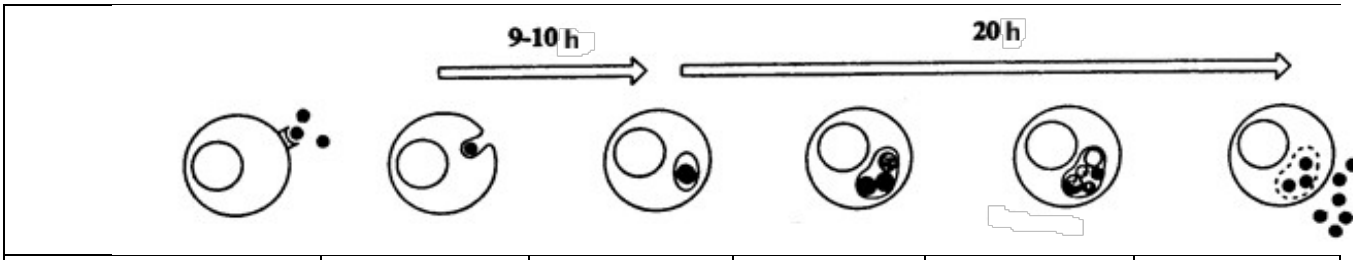
Chlamydiae are non-motile, obligate parasitic, coccoid bacteria. They reproduce only inside membrane-bound vacuoles in the cytoplasm of human, mammalian, and bird cells. Arthropods do not serve as hosts or vectors. Reproduction occurs through a unique developmental cycle. The main stages of the chlamydial life cycle are:

1. Elementary bodies – small (0.2–0.5 μm) electron-dense spherical structures, lacking metabolic activity, with a compact nucleoid and rigid cell wall, which pass through bacterial filters. They are the infectious principle of chlamydiae and ensure survival in the extracellular environment and infection of new cells.
2. Reticular bodies are larger (0.8–1.5 μm), spherical formations with a reticular structure, thin cell wall, and fibrillar nucleoid. They form from elementary bodies inside cells, lack infectivity, and by dividing ensure chlamydial reproduction. Hence the other, historically first name for reticular bodies – “initial bodies.” Reticular bodies are the vegetative form of chlamydiae.
3. Intermediate bodies – intermediate stage between elementary and reticular bodies.

The chlamydial life cycle begins with elementary bodies being phagocytosed by the host cell, then over several hours reorganizing, increasing in size, and transforming into reticular bodies, which reproduce by transverse division. The life cycle ends when emerging intermediate forms condense, decrease in size, and transform into elementary bodies. Reproducing inside cytoplasmic vacuoles, chlamydiae form microcolonies (chlamydial inclusions) surrounded by a membrane. The microcolonies contain all three developmental stages of chlamydiae. After rupture of the vacuole wall and host cell membrane, newly formed chlamydiae are released, and elementary bodies, infecting other cells, repeat the developmental cycle. Under optimal growth conditions in eukaryotic cells, the chlamydial life cycle lasts 17–40 hours (Fig. 33).

Chlamydiae grow well in the yolk sac of chicken embryos at temperatures from 33–44 °C, as well as in cell cultures of various vertebrates. The dependence of chlamydiae on eukaryotic cells is explained by their inability to accumulate and utilize energy, as they cannot synthesize ATP. In this regard, chlamydiae are similar to rickettsiae, which is why these microorganisms are also called “energy parasites.”

The uniqueness of chlamydiae is also evident in the structure of their cell wall. It lacks peptidoglycan and consists of a bilayer membrane, whose rigidity is determined by peptides cross-linked by disulfide bridges. Otherwise, chlamydiae resemble Gram-negative bacteria, as they contain glycolipids similar to lipopolysaccharides.



Adhesion Elementary bodies (EB) attach to specific receptors on the cell	Penetration EB - induced endocytosis	Differentiation into RB	Multiplication of RB	Differentiation into EB	Release EB spread to neighboring cells

Fig. 33. Schematic diagram of the chlamydial replicative cycle. EB – elementary bodies, RB – reticular bodies (C.A. Mims et al. Medical Microbiology. Mosby, 1993)

The order *Chlamydiales* includes one family *Chlamydiaceae* with two genera: *Chlamydia* and *Chlamydophila*. Pathogenic for humans are species *Chlamydia trachomatis, Chlamydophila psittaci, Chlamydophila pneumoniae*. Chlamydiae cause diseases of the eyes, respiratory, and urogenital systems in humans, collectively called "chlamydioses."

Detection methods. For microscopic detection of chlamydial inclusions (microcolonies) in infected cells (tissues), various staining methods are used: Romanowsky-Giemsa, etc. Chlamydiae acquire a blue or violet color when stained by Romanowsky-Giemsa method (Fig. 34). In addition, chlamydiae are well visible in unstained state when microscopying wet preparations under glass using phase-contrast microscopy. Currently, immunofluorescence can be used to detect chlamydiae.

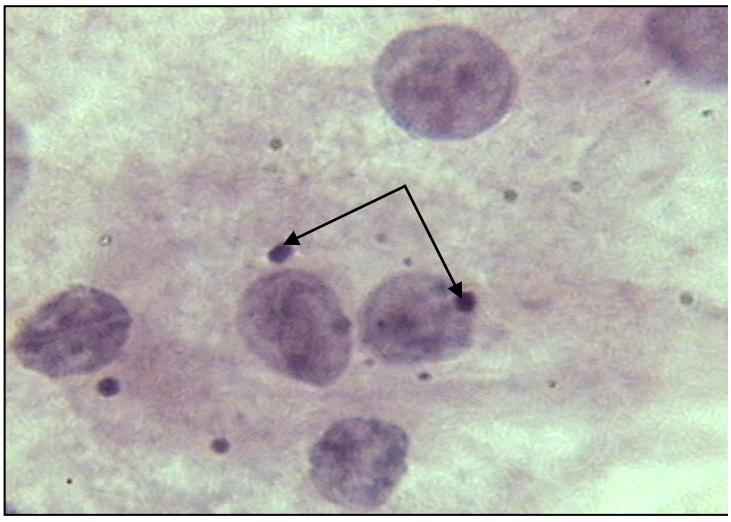


Fig. 34. Chlamydial inclusions in vaginal cells of a woman with acute chlamydiosis

Eukaryotic Microorganisms

Fungi

Fungi are unicellular or multicellular eukaryotic organisms that occupy an intermediate position between plants and animals. In terms of nutrition type and vitamin requirements, presence of chitin in the envelope, sterols in the cytoplasmic membrane, and glycogen in the cytoplasm, they resemble animal-origin cells, but in the presence of a cell wall consisting of polysaccharides close to cellulose and vacuoles filled with cell sap; in the ability for unlimited growth, reproduction by spores, immobility in the vegetative state – plants. Approximately 98% of all organisms attributed to fungi belong to the kingdom Fungi (true fungi). Humans use fungi in many areas of activity. Since ancient times, edible fungi have been used as food products, fermenting yeasts are used in baking, cheese-making, distilling, and brewing. The biosynthetic features of fungi are used to obtain antibiotics (penicillin, griseofulvin, cephalosporin, etc.), carotenoids, citric acid, etc. The use of fungi for waste disposal, such as household garbage, is promising. Growing edible fungi and fodder yeasts on garbage is an environmentally safe and effective method, alternative to widely used incineration or burial. The negative effects of fungi are also diverse. Colonization of all available substrates by fungi inevitably leads to damage of natural products. Fungi cause rotting of fruits, spoil milk, meat, fish, destroy wood, wool, flax, cotton, etc. They cause diseases in plants, animals, and humans.

Classification and Structure of Fungi

Of the more than 250 thousand known species of fungi, no more than 500 are pathogenic to humans. There is no single universally accepted classification of fungi, and existing classifications pursue mainly practical goals, based on morphostructural organization and mode of reproduction.

Fungi are assigned to the kingdom *Fungi (Mycota)*, subdivided into divisions *Myxomycota* (slime molds) and *Eumycota* (true fungi). True fungi, whose cells lack septa, are known as *lower* fungi. These include classes *Chytridiomycetes, Hyphochytridiomycetes, Oomycetes, Zygomycetes*. Representatives of classes *Ascomycetes, Basidiomycetes,* and *Deuteromycetes* are *higher* fungi, as their filamentous cells have septa. Some representatives of these classes cause diseases in humans.

The structure of a fungal cell is typical for eukaryotes. Fungal cells can have the most diverse appearance, but all are similar in the set of basic cellular organelles. These include: cell nucleus, mitochondria, ribosomes, elementary membranes, cell wall, microsomes, and similar organelles, etc.

The fungal cell is mostly covered by a hard envelope – the cell wall, which consists 80–90% of nitrogen-containing and non-nitrogen polysaccharides (microfibrillar matrix). In most fungi, the main polysaccharide is chitin, a fungal marker, a polymer of N-acetylglucosamine. In addition, the wall contains small amounts of proteins, lipids, and polyphosphates. Under the cell wall is the cytoplasmic membrane surrounding the inner part of the cell – the protoplast.

The fungal cytoplasm contains structural proteins and enzymes not associated with cell organelles, amino acids, carbohydrates, lipids. Fungal cells have organelles: mitochondria (mostly similar to those in higher plants), lysosomes with proteolytic enzymes that cleave proteins, segresomes and chitosomes. They are unique to fungi. Segresomes are vacuole-like structures that limit the entry of hydrophobic substances, such as hydrocarbons, into the cell. Chitosomes are organelles containing the enzyme chitin synthase, necessary for chitin synthesis. The cell cytoplasm also contains vacuoles filled with reserve substances such as volutin, lipids, glycogen, as well as fatty acids.

A fungal cell has from one (in yeast-saccharomycetes) to several nuclei (up to ten in lower fungi). The nucleus is surrounded by a double membrane, contains a nucleolus and several chromosomes of deoxyribonucleic acid (DNA).

Fungi have two types of growth: hyphal growth (hyphomycetes) and yeast growth *(blastomycetes*). The vegetative body of *hyphomycetes* (molds) consists of filamentous cells that are highly branched and called hyphae. The diameter of hyphae varies depending on the fungal species and external conditions from 2 μm to 100 μm and more. Lower fungi are unicellular, as they lack transverse septa in hyphae. Higher fungi are multicellular; their hyphae are divided by septa into separate cells with a common envelope; the septa grow inward and have a pore in the center through which cytoplasm can flow from one cell to another (Fig. 35).

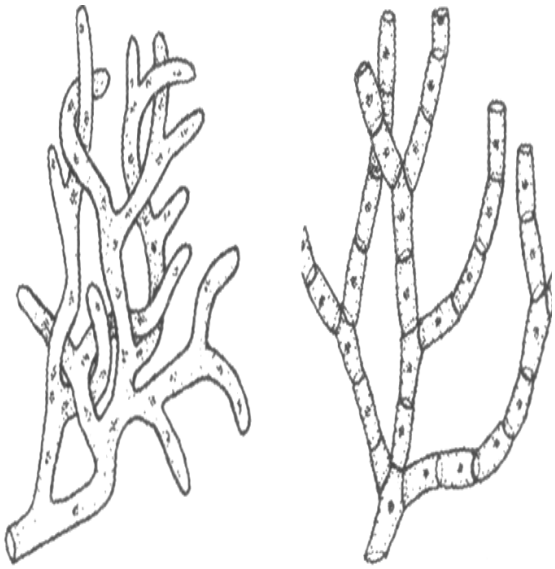


Fig. 35. Structure of mycelium in lower and higher fungi (https://studopedia.org)

The aggregate of hyphae forms mycelium (mycelial network). It represents intertwined branching hyphae; branching occurs through lateral outgrowths. Mycelium can be substrate (vegetative), growing into the nutrient medium. Such mycelium is usually very abundant, with a large total surface area, through which osmotic absorption of water and nutrients occurs. Mycelium growing on the surface of the medium is called aerial or reproductive, as it contains special structures – spores that ensure fungal reproduction. Mycelium is called true if all septate hyphae have a common cell wall; if mycelium consists of isolated elongated cells formed by budding of unicellular fungi without detachment of daughter cells, then it is pseudomycelium. Pseudomycelium, unlike true, does not have a common envelope. When grown on nutrient media, molds form fluffy, often pigmented colonies, which usually appear after 4–7 days (Fig. 36).

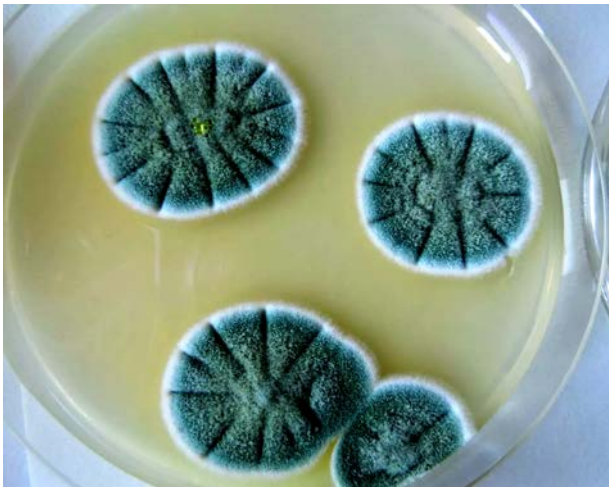


Fig. 36. Colonies of mold fungi

Blastomycetes (yeasts) are unicellular non-motile organisms. They can have various shapes: elliptical, oval, spherical, and rod-shaped. Cell length varies from 5 to 12 μm, width from 3 to 8 μm. The shape and size of yeast cells are not constant and depend on the genus and species, as well as cultivation conditions, nutrient medium composition, and other factors. When cultivating blastomycetes in laboratory conditions on solid nutrient media, a yeast colony represents an accumulation of a huge number of cells of different sizes (result of budding); they resemble bacterial ones – smooth, pasty, growing in 24–48 hours (Fig. 37).

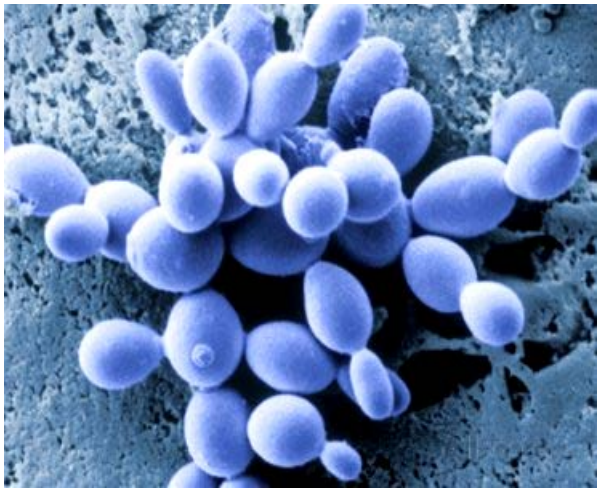
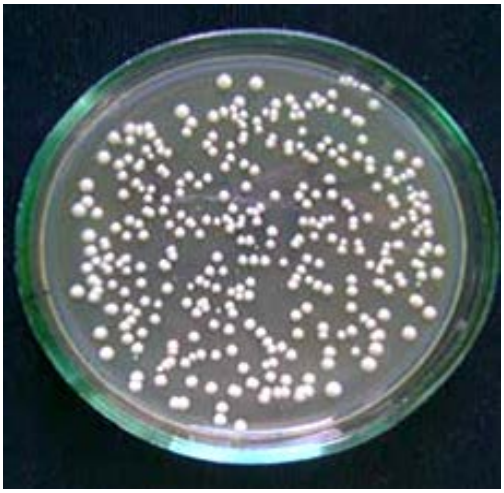



Fig. 37. Appearance of blastomycetes and their colonies (https://bio-x.ru)

Blastomycetes and hyphomycetes are completely dissimilar, but there are fungi that, depending on conditions, grow as yeasts or as molds. This phenomenon is called dimorphism, and such fungi – dimorphic fungi. Dimorphism is characteristic of causative agents of systemic (deep) mycoses in humans – blastomycosis (*Blastomyces dermatitidis*), histoplasmosis (*Histoplasma capsulatum*), and coccidioidomycosis (*Coccidioides immitis*). In the host organism, they form yeast-like cells, and on nutrient media, they grow as mycelial forms. This is related to the response to temperature regime where fungal reproduction occurs (temperature-dependent dimorphism). Growth in the yeast phase occurs at 37 °C (i.e., at human body temperature); room temperature initiates mycelium formation.

Dimorphism can manifest somewhat differently: both morphological variants coexist in the infected organism. This is characteristic of fungi of the genus *Candida*, which is the causative agent of one of the most common fungal diseases (mycoses) in humans. Usually, *C. albicans* are represented by yeast-like cells that can form chains of elongated cells – pseudohyphae.

Fungal Reproduction

A feature of fungi is the great diversity of reproduction methods and organs. Fungal reproduction can be *vegetative* and *reproductive*. *Vegetative reproduction* occurs without formation of any specialized organs. In *reproductive,* specialized cells – spores – are formed, through which reproduction is carried out.

In *vegetative reproduction*, hyphomycetes reproduce by individual fragments or individual hyphal cells capable of forming new cells, i.e., giving rise to new mycelium. Vegetative reproduction can also occur by budding of growing hyphae into separate cells; buds can form along the entire hypha or only on apical hyphae. Cells formed this way are called oidia (Fig. 38-1). Another method of vegetative reproduction is the formation of chlamydospores, hyphal cells with thickened

envelopes. They contain many reserve inclusions and can endure prolonged periods of starvation, desiccation, and other adverse conditions (Fig. 38-2). Oidia and chlamydospores are formed by higher fungi.

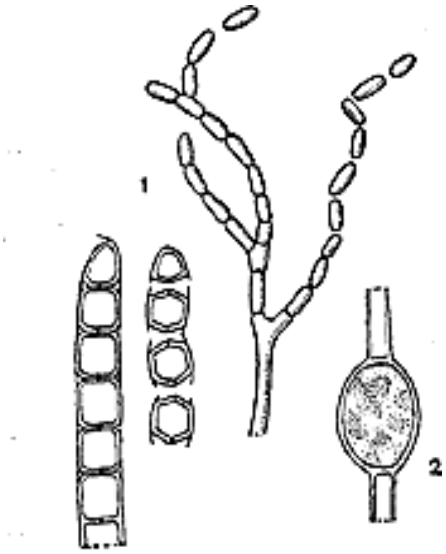


Fig. 38. Vegetative reproduction of fungi: 1 – oidia; 2 – chlamydospore (https://sites.google.com)

Reproductive reproduction of hyphomycetes can occur *sexually* and *asexually*. Fungi reproducing sexually are called *perfect* (*fungi perfecti*); those lacking a sexual process are *imperfect* (*fungi imperfecti)*, or deuteromycetes. Asexual reproduction is carried out with spores, which are spread by water, animals, insects, air currents. Formation of asexual spores is not preceded by cell fusion. Asexual spores are diverse in structure, formation methods, biological significance. In asexual reproduction of fungi, special fruiting hyphae of aerial mycelium are formed. In higher fungi, specialized elongated cells called sterigmata form at the tip of the fruiting hypha, where chains of exospores (conidia) are formed, located on the outside of the hypha. Such a hypha on which conidia are formed is called a *conidiophore*. Conidiophores have different shapes. The apex can be branched like brushes (*Penicillium glaucum*, Fig. 39-1) or club-shaped thickened (*Aspergillus niger*, Fig. 39-2). The shape and arrangement of conidia are characteristic of various fungal species and are used as identification signs. In lower fungi (*Mucor mucedo,* Fig. 39-3), spores are formed inside spherical sacs – sporangia, inside which internal spores – endospores – are formed. The fruiting hypha on which sporangia are formed is called a sporangiophore.

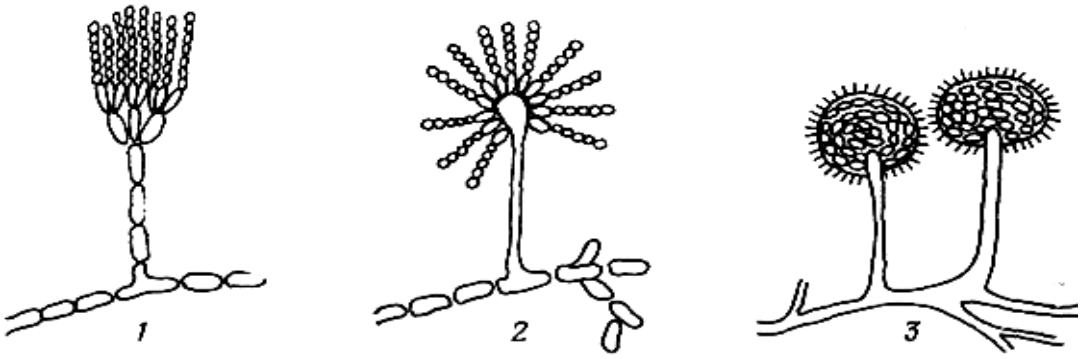
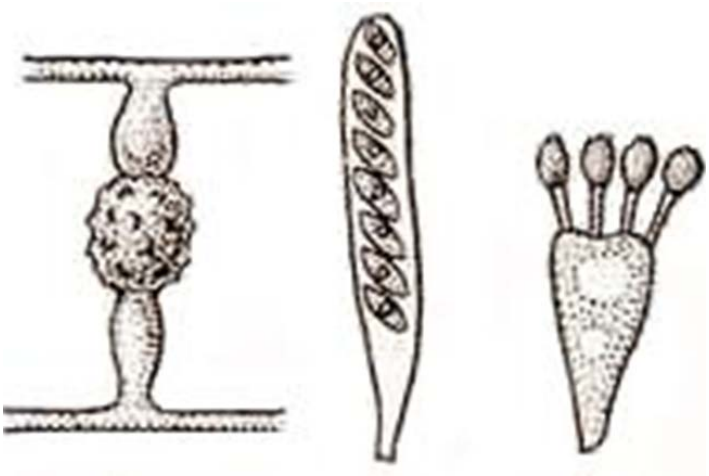


Fig. 39. Mold fungi. 1 – Penicillium; 2 – Aspergillus; 3 – Mucor (https://spravochnick.ru)

In many lower fungi, asexual reproduction occurs via motile zoospores equipped with flagella and capable of independent movement in water. Zoospore development occurs in zoosporangia.

Sexual reproductive reproduction in hyphomycetes is intended for gene recombination, for the survival of the fungus in unfavorable conditions, and it also contributes to the genetic stabilization of species. *Sexual spores* are formed as a result of fusion of two cells. Fusion can be isogamous (fusion of identical cells) and oogamous (fusion of female and male sex cells). Sexual reproduction represents the emergence and development of a new generation from a fertilized cell – zygote, arising from fusion of the contents of two cells. In fusion of two heterosexual cells called gametes, the sexual process proceeds in two phases. The first phase of the sexual process is fusion of the protoplasmic contents of two cells, i.e., plasmogamy. After plasmogamy, either immediately or after some interval (different in different organisms), fusion of nuclei occurs, i.e., karyogamy. As a result of fusion of two cells, a special cell is obtained, inside which the contents of both the first and second (male and female) cells are concentrated. After fusion of two nuclei, a diploid nucleus is obtained, in which a double set of chromosomes is concentrated. After formation of the diploid nucleus, in some fungi immediately, in others somewhat later, the diploid nucleus undergoes division accompanied by reduction in chromosome number, i.e., reductive division (meiosis). As a result of division, haploid cells are subsequently obtained, differing from diploid ones by half the number of chromosomes. At the same time, each of these cells contains the contents of two previous haploid cells that participated in the sexual process, i.e., recombination of hereditary properties occurs, and the two cells are no longer identical to the first cells. The sexual process thus contributes to the emergence of great diversity of forms.

Examples of sexual spores are ascospores, basidiospores, and zygospores (Fig. 40).



a *b* *c*

Fig. 40. Organs of sexual sporulation:
a – zygospores;
b – sac (ascus) with ascospores;
c – basidia with basidiospore
(https://spravochnick.ru)

Ascospores are internal spores. They are formed in sacs or asci, and the number of ascospores in a sac can vary from 4 to 16 or more. Such spores are characteristic of sac fungi – ascomycetes. *Basidiospores* are external spores and arise four each on a stalk (sterigma), characteristic of basidiomycetes (cap fungi). *Zygospores* – in some lower fungi (zygomycetes), the tips of hyphae located close to each other fuse, meiosis occurs, and large zygospores with thick walls are formed.

For blastomycetes, the typical reproduction method is *budding*; binary fission and ascospore formation are rare. *Budding* occurs due to formation on the cell surface of a small protrusion, into which part of the protoplasm and nucleus of the mother cell enters – a bud is formed, which then separates and becomes an independent cell. As a result of budding, accumulations of round, yeast-like cells are formed, which at low microscope magnification resemble balls or granules (Fig. 41).

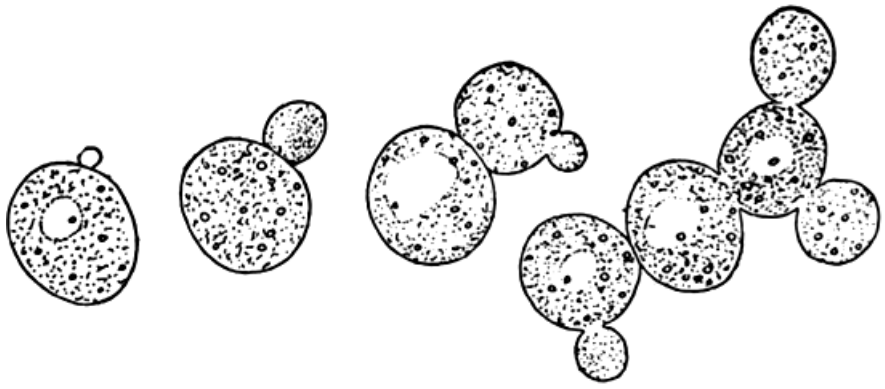


Fig. 41. Yeast budding (https://studme.org)

Role of Fungi in Human Pathology

Many fungi harm the health of humans and animals. They are commonly called parasitic or pathogenic fungi.

Three main types of direct human damage by fungi are distinguished:

- infectious (mycoses of skin and other organs);
- toxic (mycetism, mycotoxicosis);
- sensitizing (mycogenic allergy).

Currently, about 500 fungal species are counted that can always cause infectious diseases in humans: some species cause systemic infections, others affect skin and subcutaneous tissue, some – only skin. A larger number of fungi are pathogenic and opportunistic pathogens, causing diseases only with reduced colonization resistance of skin, mucous membranes, and overall body resistance (most often in immunodeficiency states).

Based on the nature of primary localization of pathogenic fungi, pathogenesis, and clinical manifestations, four groups of mycoses are distinguished:

1) keratomycoses, superficial mycoses (lichen, trichosporia) affecting hair and the stratum corneum of the epidermis;

2) dermatophytoses, epidermomycoses (athlete's foot, trichophytosis, microsporosis) affecting epidermis, hair, nails;

3) subcutaneous mycoses affecting skin, subcutaneous tissue, fasciae, bones;

4) deep, systemic mycoses (blastomycoses, histoplasmosis, coccidioidomycosis, sporotrichosis) characterized by damage to internal organs, frequent dissemination.

Fungi most commonly causing mycoses belong to classes Zygomycetes, Ascomycetes and Deuteromycetes.

Class Zygomycetes. Representatives of this class are characterized by well-developed non-septate multinucleate mycelium. Zygomycetes develop in soil, on food products, in manure, in plants, and can parasitize in animal and human organisms. Asexual reproduction is by non-motile sporangiospores, less often conidia. Sexual process – zygogamy (zygospores). Medical significance have fungi of the genus *Mucor (Mucor mucedo)*. Mucor fungi form branched mycelium resembling a web, usually creeping along the substrate. It consists of colorless hyphae, highly branching and usually without septa. On fairly thick hyphae, asexual reproduction organs are formed – sporangiophores with sporangia. Mucor sporangia appear as dark, well-visible heads, which is why these fungi are called pin molds. When the sporangium matures, a huge number of sporangiospores are formed inside, which after release from the sporangia germinate and give rise to new mycelium. Pin mold can cause mucormycoses in humans – lung damage

(pseudotuberculosis), keratitis, otitis, vulvovaginitis, damage to liver, kidneys, and other organs.

Class Ascomycetes. Ascomycetes or sac fungi – one of the largest classes, includes about 30% of all known fungal species. Ascomycetes live as saprophytes in soil, on various plant substrates (wood, dead plants), in seas or freshwater bodies on submerged wood. Most species of Ascomycota are parasitic on various organisms—on plants (algae, lichens, and higher plants), on animals, and humans, often causing serious diseases. Sac fungi have multicellular septate mycelium. In sexual process, they reproduce by ascospores (spores develop in special sacs – asci). Asexual reproduction is by conidia. Fungi in this class are extremely diverse in structure. Pathogenic fungi belong to genera *Aspergillus, Penicillium, Saccharomyces*. Representatives of genus *Aspergillus (Aspergillus niger, Aspergillus flavus)* have upright conidiophores, with spherical swellings at the ends bearing sterigmata arranged radially on the entire swelling surface. At low microscope magnification, the apex of the Aspergillus conidiophore bearing chains of conidia externally resembles the tip of a watering can from whose holes water streams flow. Therefore, the Russian name for Aspergillus is "watering can fungus." However, the exact translation of Aspergillus is "shaggy head." "Watering can" mold causes aspergillosis in humans – damage to lungs, bronchi, cornea of eyes, ears, urogenital and other organs. Genus *Penicillium* ("brush fungus") has multicellular conidiophores that branch in the upper part and end in sterigmata arranged in brushes. From sterigmata, conidia are pinched off, unicellular, round or oval, often greenish in mass. The brush structure varies among Penicillium species and forms the basis of the genus systematics. More than 30 species of genus *Penicillium* cause diseases in humans, for example, *Penicillium glaucum, P. minutum, P. album,* etc. Diseases are called penicillioses, affecting skin, nails, ears, upper respiratory tract and lungs, as well as generalized infection with formation of internal foci in internal organs. Genus *Saccharomyces* includes yeasts. Yeast cells have round, oval, or elongated shape, size 8–10 μm, double-contoured envelope. In cytoplasm, inclusions in the form of glycogen granules, volutin, lipids. Reproduction by budding and ascospores. Yeasts cause numerous yeast mycoses.

Class Deuteromycetes (Fungi imperfecti). The vegetative body of deuteromycetes is a well-developed, branched, haploid mycelium, usually consisting of multinucleate cells, similar in structure to the mycelium of sac fungi. Mycelium always has septa (partitions), usually with simple pores, as in ascomycetes. They reproduce only asexually by conidia, and sexual (perfect) stages are absent. Deuteromycete conidia are diverse in structure. They can be unicellular or with varying number of septa. In shape, they are spherical or ellipsoidal, but some species have filamentous, star-shaped, or spirally coiled conidia. Conidial color is light or dark, brownish. A number of imperfect fungi representatives are causative agents of dermatomycoses: favus (*Achorion schoenleinii*), trichophytosis

(*Trichophyton violaceum, T. mentagrophytes*), microsporosis (*Microsporum lanosum, M. canis*), epidermophytosis (*Epidermophyton inguinale*).

Diseases are also caused by representatives of genus *Candida*. They are very widespread in nature. The most pathogenic and commonly encountered are *Candida albicans* and *C. tropicalis*. In humans, these fungi are found on skin, mucous membranes of oral cavity, gastrointestinal tract, urogenital organs. They are often found on fruits, vegetables, and other food products, in bath wastewater, in wash waters from canteens, buffets, restaurants, etc. Human infection can occur exogenously from the environment and endogenously – by fungi from oral cavity, gastrointestinal tract. Candidas affect interdigital areas of hands and feet, inguinal folds, nails, nail folds, mucous membranes of lips, tongue, mucous of pharynx, esophagus, internal organs. The development of candidiasis is facilitated by various factors: lesions of the mucous membrane, chronic gastrointestinal diseases, vitamin imbalance, dysbiosis, immunosuppression, prolonged treatment with antibiotics and corticosteroids, radiation exposure, and others. Recently, pathogenic deuteromycetes often cause generalized lesions. Severe generalized mycoses with fatal outcome in humans are caused by *Coccidioides immitis* (coccidioidomycosis), *Histoplasma capsulatum* (histoplasmosis), and *Cryptococcus neoformans* (cryptococcosis).

Since fungi can synthesize toxins, when they enter the human body, they exert toxic effects, causing mycetism or mycotoxicosis. Mycetism is poisoning either by primarily poisonous fungi when accidentally consumed as food, or by edible fungi when preparation or storage technologies are violated. Mycetism occurs due to the action of fungal toxic peptides on the digestive, nervous systems or less specific damage to body cells and tissues. Mycotoxicoses are poisonings by toxic substances of toxin-producing fungi parasitizing on plants (e.g., cereals) used for food production. Toxins of these fungi retain their poisonous properties during harvest storage, as well as during plant processing. Consumption of such products causes human poisonings.

Many fungi cause specific sensitization of the human body, manifesting as peculiar allergic diseases. Mycogenic sensitization can manifest as general symptoms (e.g., hay fever type) or skin rashes (mycids).

Methods of Fungal Research

For microscopic examination, both native (unstained) and stained preparations are prepared. Native preparations are made by the “crushed drop” method and microscopied in phase-contrast or light microscope at magnification ×200, ×400. For studying fungi in stained form, simple stains (lactofuchsin or lactophenol) or complex methods (Romanowsky-Giemsa, Gram, Ziehl-Neelsen) are used.

Protozoa

Protozoa are unicellular eukaryotes, similar in structure to cells of complexly organized animals. They are widespread in nature (about 2500 species) and lead a free-living or parasitic lifestyle. Most protozoa have sizes from 30 to 150 μm. Shape can be pear-shaped (*trichomonads, giardias*), ovoid (*balantidium*), spindle-shaped (*trypanosomes, leishmanias*); they can take the most bizarre configuration (*amoebae*). Protozoan cells, like all eukaryotes, contain a nucleus (sometimes several), cytoplasm; most protozoa have an elastic dense membrane – pellicle, which maintains a certain shape of these microorganisms. In addition to general-purpose organelles (ribosomes, mitochondria, lysosomes, endoplasmic reticulum, Golgi apparatus), there are specific organelles. For giardias, these are two elastic threads – axostyle and a sucking disk; for trichomonads and trypanosomes – undulating membrane; for toxoplasmas – conoid and microtubule system; for balantidium – mouth-like (cytostome) and anal pore (cytoproct), contractile vacuoles, micronucleus.

Most protozoa are motile and move using pseudopodia (amoebae), flagella (giardias, leishmanias), cilia (balantidium).

Pseudopodia are temporary cytoplasmic protrusions, by releasing which protozoa constantly change body shape.

Flagella are long thin outgrowths consisting of fibrils, there are two central and nine peripheral ones.

Cilia are similar in structure to flagella but, unlike them, short and work like oars.

Protozoa are characterized by certain life cycles, during which under adverse conditions, vegetative forms turn into cysts. The protozoan rounds up, loses motility, and is covered by a double-layered dense envelope. Features of cyst shape and structure have diagnostic value. Thus, a mature cyst of Entamoeba histolytica has size 8–14 μm, round shape, and four nuclei; giardia cysts are oval and tetranucleate, with visible axostyle with flagella; balantidium cysts are large 30–60 μm, oval with bean-shaped nucleus. Protozoa can reproduce asexually and sexually. Reproduction of some species is quite complex, with alternation of asexual and sexual cycles.

Protozoa belong to the subkingdom *Protozoa* (from Greek *protos* – first, *zoon* – animal), to the kingdom *Animalia*. Protozoa play a significant role in human infectious pathology. Some are harmless inhabitants of the intestine (e.g., *Entamoeba coli*), others realize their pathogenicity usually with massive infection against immunodeficiency states (*giardias, pneumocysts*), and finally, some species are tissue and blood parasites causing acute or chronic diseases (*leishmanias, trypanosomes, malarial plasmodia*). Of medical significance are:

1. Phylum Sarcomastigophora, subphylum *Sarcodina*. The body of sarcodines lacks pellicle; they move using pseudopodia, with which capture and

immersion of nutrients into cytoplasm also occurs. Sexual reproduction is absent in sarcodines. Under adverse conditions, they form cysts. This subphylum includes various amoeba species. In humans, *Entamoeba histolytica* parasitizes (Fig. 42), causing amoebic dysentery or amoebiasis.

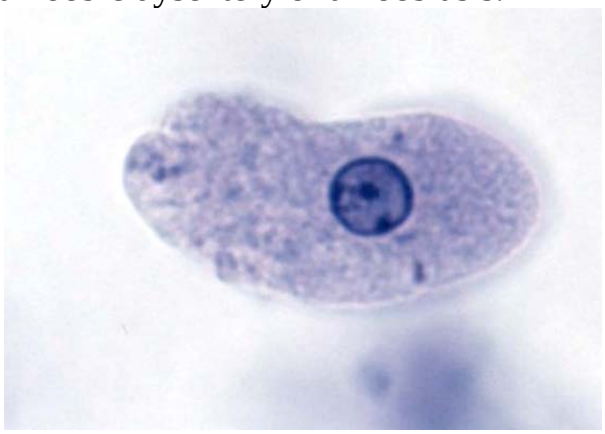
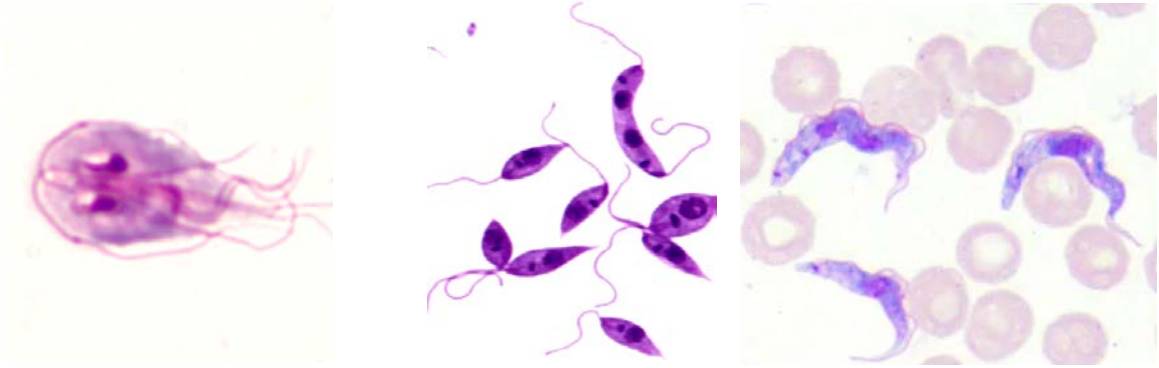


Fig. 42. Entamoeba histolytica (https://autogear.ru)

2. Phylum Sarcomastigophora, subphylum Mastigophora (flagellates).

Characteristic feature – presence of one or several flagella. A fundamental difference of protozoan flagella from bacterial flagella is the presence of kinetoplast – a special organelle located at the flagellum base and producing energy for its movement. In some flagellate species, movement is provided by undulating membrane – a thin comb-like fold longitudinally connecting the flagellum to the protozoan body. Among parasitic protozoa, this subphylum includes trypanosomes (*Trypanosoma gambiense*), giardias (*Giardia lamblia*), leishmanias (*Leishmania donovani, Leishmania tropica*), causing respectively African sleeping sickness (trypanosomiasis), giardiasis, and leishmaniasis (Fig. 43).



a *b* *c*

Fig. 43. Morphology of flagellates:
a – giardias, b – leishmanias, c – trypanosomes (https://medicine-live.ru)

3. Phylum Ciliophora (ciliates, infusoria). The body of these protozoa is covered by pellicle with numerous short cilia, by means of which they move. A pathogenic representative of ciliates is *Balantidium coli* – causative agent of balantidiasis, affecting human large intestine (Fig. 44). Balantidia are motile, have numerous cilia, thinner and shorter than flagella. Their life cycle consists of two

phases – asexual (reproduction by transverse division) and sexual (conjugation). After conjugation, balantidium encysts.

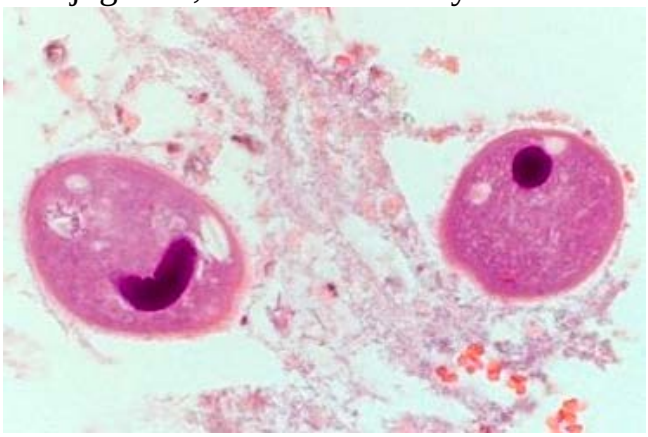
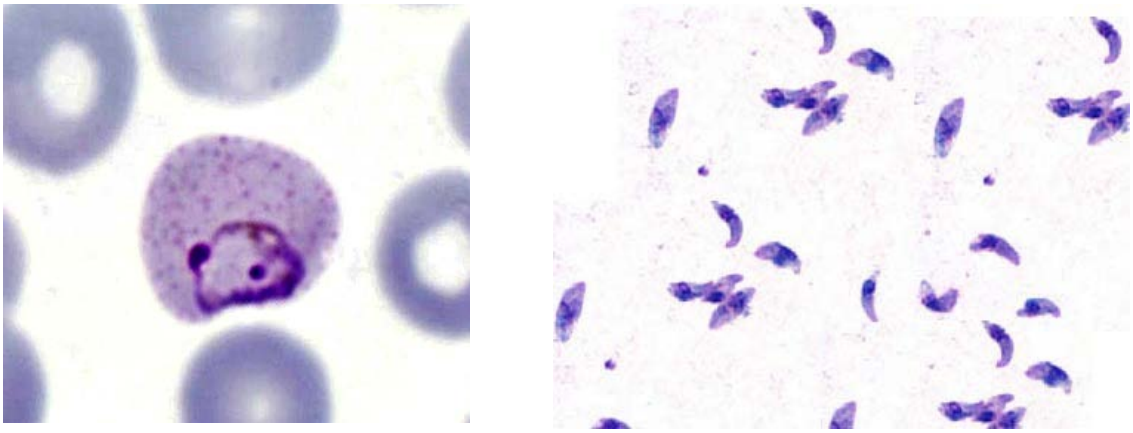


Fig. 44. Balantidium coli (https://medicine-live.ru)

4. Phylum Apicomplexa, class Sporozoa (sporozoans). The class consists exclusively of parasitic protozoa; many sporozoans are intracellular parasites. Most lack movement organelles. They are characterized by both exclusively sexual development and alternation of sexual and asexual generations, usually associated with host change. The class includes malarial plasmodia (*Plasmodium vivax, malariae, ovale, falciparum*), causing malaria, and toxoplasmas (*Toxoplasma gondii*), causing toxoplasmosis (Fig. 45).

Research methods. For studying protozoa, temporary (native) and permanent (stained) preparations are prepared. Native preparations are made by “crushed drop” or “hanging drop” method with addition of warm physiological saline or vital supravital dyes: trypan blue or neutral red. In microscopy of native preparations, low (×80) or high magnification (×400) of light-field or phase-contrast microscopes is used. When studying protozoa in stained form, Romanowsky-Giemsa staining is most often used, resulting in protozoan cytoplasm staining blue, nucleus and flagella – reddish-violet.



a *b*

Fig. 45. Morphology of sporozoans: a – malarial plasmodium (schizont stage), b – toxoplasmas (https://medicine-live.ru)

PHYSIOLOGY OF MICROORGANISMS

The ***physiology of microorganisms*** is a section of general microbiology that studies the features of development, nutrition, energy metabolism, and other vital processes of microorganisms. The study of the physiology of pathogenic and opportunistic microorganisms is important for establishing a microbiological diagnosis, understanding pathogenesis, prescribing treatment, and conducting prevention of infectious diseases, as well as for regulating human interactions with the environment, etc.

Microorganisms, like all other organisms, require a constant exchange of substances with the environment for existence and reproduction of their kind. The incorporation of certain compounds from the external environment into cellular reactions of any nature determines bacterial nutrition.

The transformations of substances in the cell (*metabolism*) are represented by opposing but interconnected processes of *catabolism*, or energy metabolism, and *anabolism*, or plastic (constructive) metabolism. During enzymatic catabolic reactions, energy is released, which is accumulated in ATP molecules. In anabolic reactions, this energy is expended on the synthesis of organic compounds.

Bacterial Nutrition

Transport of substances into the bacterial cell. To carry out metabolic processes, nutrients enter the bacterial cell from outside through the cytoplasmic membrane, while the cell wall does not serve as a barrier to the passage of ions and small molecules. High-molecular-weight compounds are first broken down by microbial cell enzymes and then absorbed by the cell.

The following transport mechanisms are distinguished:

1) simple or passive diffusion;
2) facilitated diffusion;
3) active transport;
4) translocation of radicals.

Simple (passive) diffusion occurs along a concentration gradient (Fig. 46a), with no energy expenditure. Water, foreign substances such as poisons, inhibitors, and drugs penetrate the cell via simple diffusion. Simple diffusion is non-specific; only the size of the molecules matters.

Facilitated diffusion occurs along a concentration gradient, without energy expenditure, involving membrane carrier proteins called *permeases*. Permeases on the outer side of the cell bind to the substrate, transport it into the cell, where the complex dissociates, releasing the substrate (Fig. 46b). Via facilitated diffusion, molecules enter the cell where their concentration in the cytoplasm is lower than in the surrounding environment.

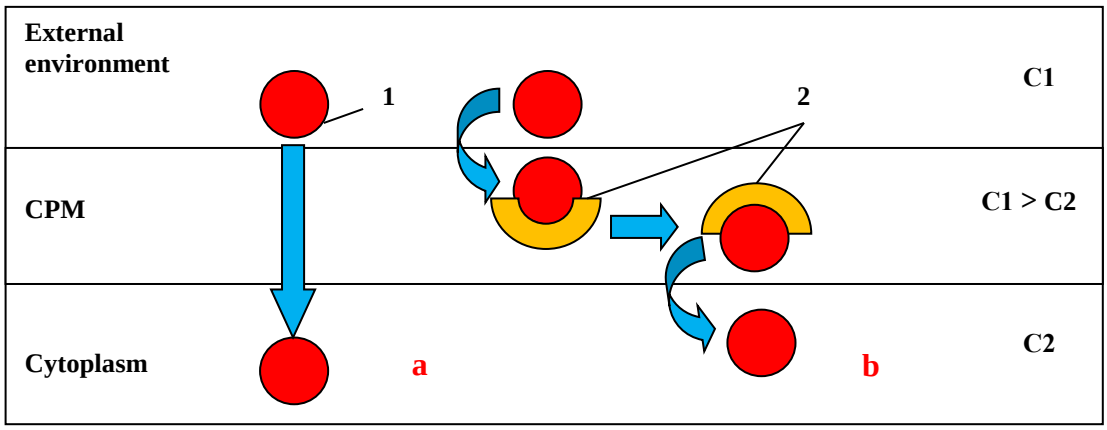


Fig. 46. Simple (a) and facilitated (b) diffusion: CPM – cytoplasmic membrane, C – concentration of substances/ions, transported substances (1), permease proteins (2)

Active transport occurs against a concentration gradient. It also involves substrate-specific enzyme proteins, but requires energy expenditure, and the substances that enter the cell accumulate in it (Fig. 47a). Translocation of radicals. In translocation of radicals, chemical modification of the transported molecules occurs, whereas in passive diffusion, facilitated diffusion, and active transport, they enter the cell in a chemically unchanged form (Fig. 47b).

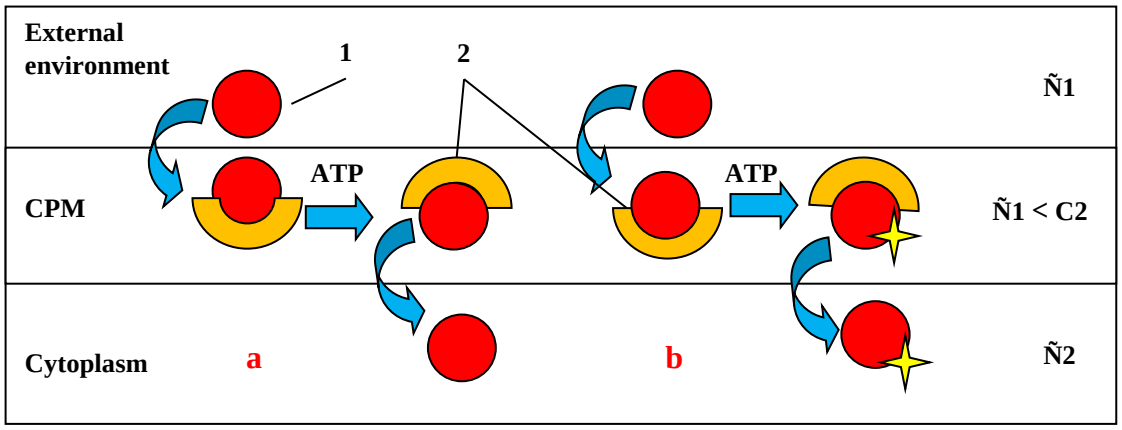


Fig. 47. Active transport (a) and translocation of radicals (b): CPM – cytoplasmic membrane, C – concentration of substances/ions, transported substances (1), permease proteins (2)

The bacterial cell excretes metabolic products, enzymes, and toxins. Compounds synthesized in bacterial cells exit them in three ways:

1. Phosphotransferase reaction. Occurs during phosphorylation of the transported molecule.

2. Cotranslational secretion (signal transport). In this case, the synthesized molecules must have a special leader sequence of amino acids to attach to the membrane and form a channel through which protein molecules can exit into the environment. In this way, toxins of tetanus, diphtheria, etc., exit from the cells of corresponding bacteria.

3. Membrane budding. Molecules formed in the cell are surrounded by a membrane vesicle, which "buds off" into the environment.

Chemical Composition of the Bacterial Cell

Microorganisms contain the same chemical substances as the cells of all living organisms.

The basis of the microbial cell is water – 80–90% of the total mass. Water is present in free and bound states. Free water in the cell is necessary for bacteria as a solvent for organic and mineral compounds, a dispersion medium for colloids, a source of hydrogen and hydroxyl ions, and a factor in osmotic pressure (cell turgor). Water, as the main chemical component, is associated with the basic vital processes of the bacterial cell – nutrition, respiration, growth, and reproduction.

The dry residue (10–20% of the bacterial cell mass) is a mixture of organic and mineral compounds (Fig. 48). The organic components of the bacterial chemical composition include proteins, nucleic acids, carbohydrates, lipids, vitamins, etc.

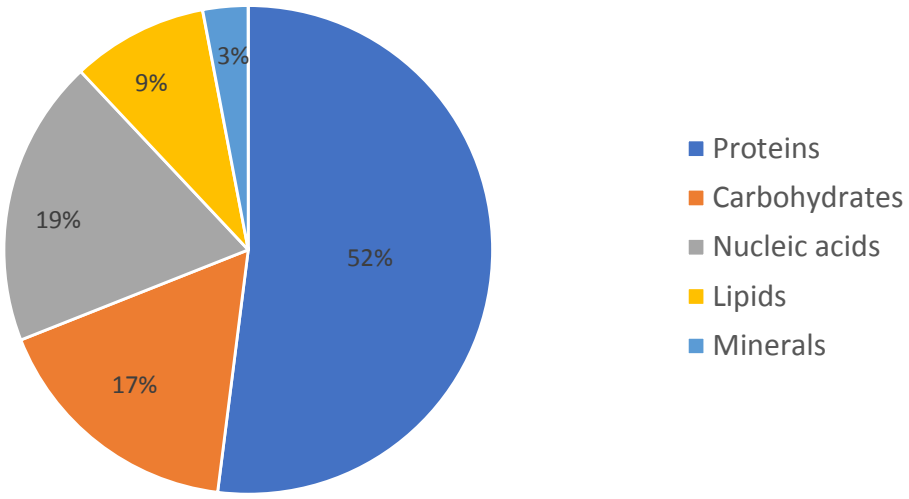


Fig. 48. Chemical composition of the bacterial cell dry residue

Proteins make up more than 50% of the microbial cell dry residue. Proteins are distributed in the cytoplasm, nucleoid, and are part of the cell wall structure. They perform plastic and structural functions, participate in growth and reproduction processes, determine bacterial species characteristics; characterize antigenic and immunogenic properties, possess toxicity and virulence; as enzymes,

they characterize the biochemical activity of bacteria. Simple and complex bacterial proteins are distinguished. Simple proteins (proteins) hydrolyze to amino acids, which the bacterial cell uses as a carbon source. Complex proteins, depending on the nature of non-protein compounds included in them, are divided into nucleoproteins, chromoproteins, lipoproteins, etc. Complex proteins are most important for the vital activity of microorganisms.

Nucleic acids are represented by two types – DNA and RNA. Nucleic acids in the microbial cell perform the same functions as in animal-origin cells. DNA is contained in the nucleoid and determines the genetic properties of the microorganisms. RNA participates in the biosynthesis of cellular proteins and is contained in ribosomes and cytoplasm. The total amount of nucleic acids ranges from 10 to 30% of the microbial cell dry matter and depends on its species and age.

Carbohydrates (12–18% of dry matter) are used as a source of energy and carbon. They make up many structural components of the cell (cell envelope, capsule, and others). Carbohydrates are also part of teichoic acids in Gram-positive bacteria. Microbial cells contain simple (mono- and disaccharides) and high-molecular-weight (polysaccharides) carbohydrates. Some bacteria may have inclusions chemically similar to glycogen and starch, which serve as reserve nutrients in the cell. The carbohydrate composition varies among different microbial species and depends on their age and developmental conditions.

Lipids in most bacteria constitute 5–10%, reaching up to 40% of the dry residue in yeast-like fungi and mycobacteria. A significant portion of lipids is in complex association with proteins and carbohydrates. They are essential components of the cytoplasmic membrane and cell wall and serve as reserve nutrients; energy material; a factor in microbial resistance to environmental effects (spores, mycobacterial cell wall).

The basis of organic substances consists of four elements: nitrogen, carbon, hydrogen, and oxygen. These elements are classified as macroelements along with sulfur, potassium, calcium, phosphorus, magnesium, and iron. Microelements are needed by bacteria in very small, trace amounts. They include manganese, molybdenum, zinc, copper, cobalt, nickel, chlorine, bromine, and some other metals and non-metals. Most of them are present as impurities in macroelements or can enter nutrient media from glassware, water, or air. Some bacteria can do without microelements.

One of the most essential chemical elements for bacteria is carbon, as it forms the basis of proteins, carbohydrates, fatty acids, etc. Based on the carbon source, bacteria are divided into two major groups: *autotrophs* and *heterotrophs* (Fig. 49).

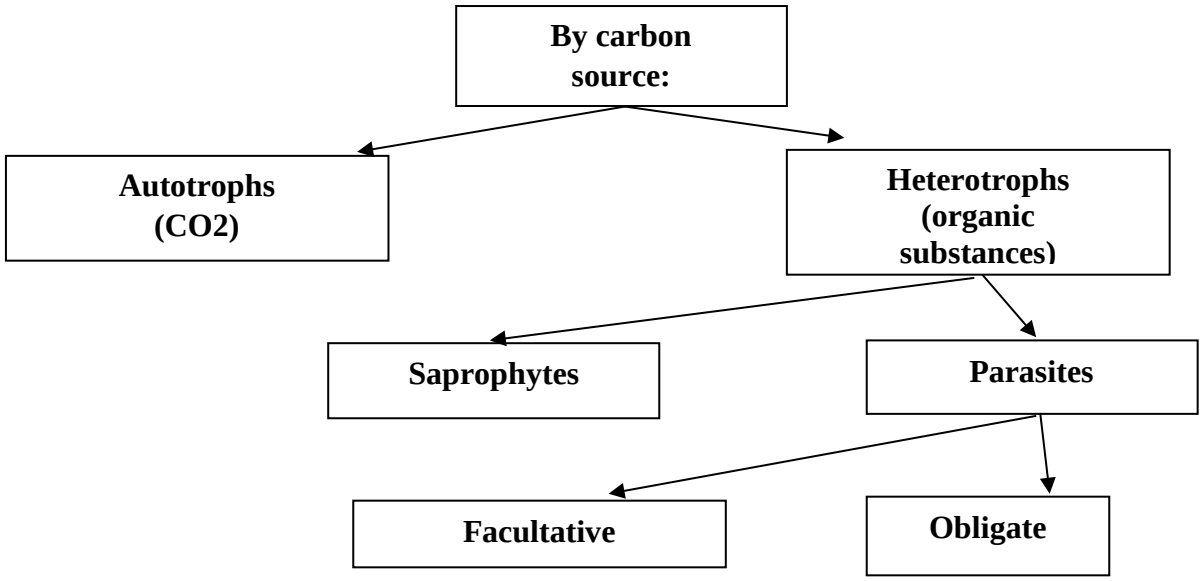


Fig. 49. Classification of bacteria by carbon source

Autotrophs (from Greek *autos* – self and *trophe* – food, nutrition) are bacteria that synthesize all necessary organic substances for life from inorganic substances (mainly carbon dioxide, carbonates). When cultivating autotrophs, it is necessary to provide cells with carbon dioxide, as the CO2 concentration in air does not exceed 0.03%, and its diffusion into the medium is insufficient for microbial growth. Nutrient media for autotroph cultivation include calcium carbonate (CaCO3) or sodium bicarbonate (NaHCO3). Sometimes air enriched with 1–5% CO2 is bubbled through the nutrient medium. *Heterotrophs* (àùêü åðó Greek *heteros* – other, different and *trophe* – nutrition) are organisms that use ready-made organic compounds for nutrition. Depending on the individual characteristics of the microorganisms, the carbon source can be alcohols, carbohydrates, aromatic compounds, organic acids. Heterotrophs, in turn, are divided into *saprophytes*, which live on organic compounds entering the bacterial cell from the external environment (decay products), and *parasites* (paratrophs), which can utilize only metabolic products inside a living cell. Parasitism can be facultative or absolute (obligate).

For microbial growth, nitrogen is also necessary, which is part of organic compounds or salts in varying degrees of reduction.

These can be ammonium salts, nitrates, or individual amino acids. To meet bacterial nitrogen needs, products of incomplete breakdown of animal proteins are also used – hydrolysates, peptones, and complex protein mixtures – native animal serum, ascitic fluid, etc.

In addition to carbon, nitrogen, and other chemical elements, many bacteria require growth factors, which include vitamins, nucleic acid bases, and other biologically active substances. Based on this feature, microorganisms can be divided into two groups: *auxotrophs*, for which the medium must contain one or

more growth factors, and *prototrophs*, which can synthesize growth factors themselves and do not require their addition to nutrient media.

To carry out biochemical processes, bacteria need energy. Based on the method of obtaining energy, bacteria are divided into two groups: *chemotrophs* and *phototrophs*. Phototrophs use light energy to meet energy needs. Chemotrophs use energy from the oxidation of various compounds. Depending on the oxidized substrate, chemotrophic organisms are divided into *lithotrophs* and *organotrophs*. Chemolithotrophs use inorganic substances as electron donors, while chemoorganotrophs use organic compounds as electron donors (Fig. 50).

Energy in bacteria is accumulated in the form of adenosine triphosphoric acid (ATP). ATP synthesis in bacteria can occur as a result of:

- respiration (oxidative metabolism);
- fermentation (enzymatic or fermentative metabolism);
- mixed metabolism.

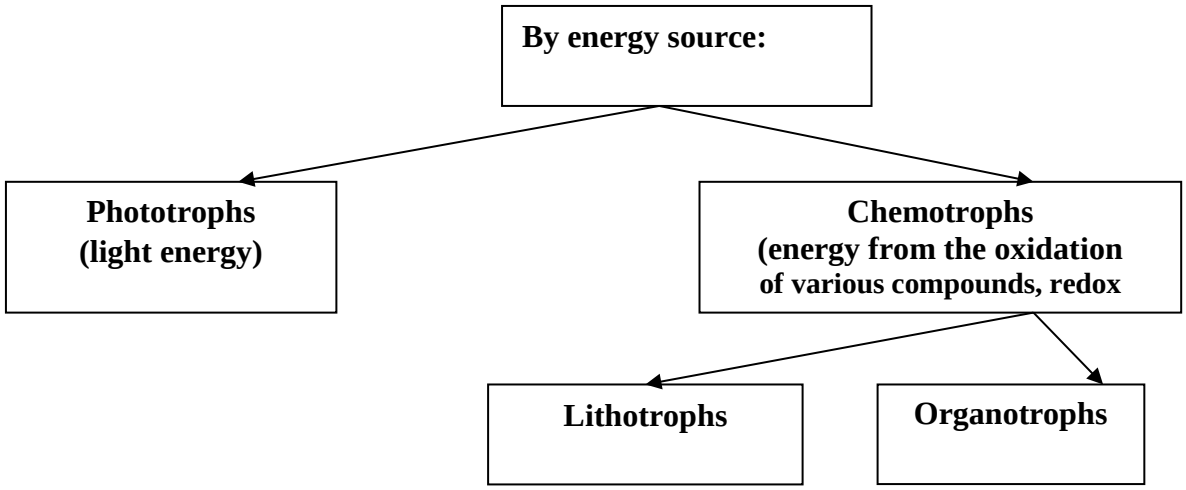


Fig. 50. Classification of bacteria by energy source

Bacterial Respiration

Bacterial respiration (energy metabolism) is a chain of sequential oxidation-reduction reactions accompanied by electron transfer from the oxidized substance to the reduced one with enzyme participation. During respiration, energy (ATP) is released. Respiration involves the functioning of the *respiratory chain*. Depending on the final electron acceptor, *anaerobic* and *aerobic* respiration are distinguished. If the final electron acceptor is molecular oxygen (O2), aerobic respiration occurs. If inorganic compounds (NO2, SO4, SO3) serve as electron acceptors, anaerobic respiration arises. When organic substances serve simultaneously as hydrogen donors and acceptors, the metabolic process is called *fermentation*. As a result of

fermentation, organic acids, alcohols, and gases are formed. Depending on the end products, alcoholic, lactic acid, acetic acid, and butyric acid fermentation are distinguished.

Based on oxygen needs, microorganisms can be divided into 5 groups (Fig. 51):

1. Strict (obligate) aerobes – growth and reproduction of these microorganisms cease in the absence of O2. These include, for example, *meningococci*.

2. Strict (obligate) anaerobes do not tolerate the presence of oxygen, as the toxic oxygen derivatives formed (hydrogen peroxide, superoxide and hydroxyl radicals, singlet oxygen) are lethal to the bacteria themselves due to the absence of enzymes that could destroy these toxic products (catalase, peroxidase, superoxide dismutase). Strict anaerobes include *clostridia of tetanus, botulism, gas gangrene*, and some *bacteroides*. Among the many pathogenic bacteria, their number is small.

3. Facultative anaerobes are the most extensive group of pathogenic microorganisms, capable of using both molecular oxygen and organic compounds as electron acceptors, as well as switching to fermentation in the absence of molecular oxygen.

4. Microaerophilic bacteria grow well at reduced partial pressure of oxygen but at increased $\tilde{N}\hat{I}_2$ content (representatives of the genus *Brucella*).

5. Aerophiles require increased oxygen content (*Vibrio cholerae, causative agents of diphtheria, tuberculosis*).

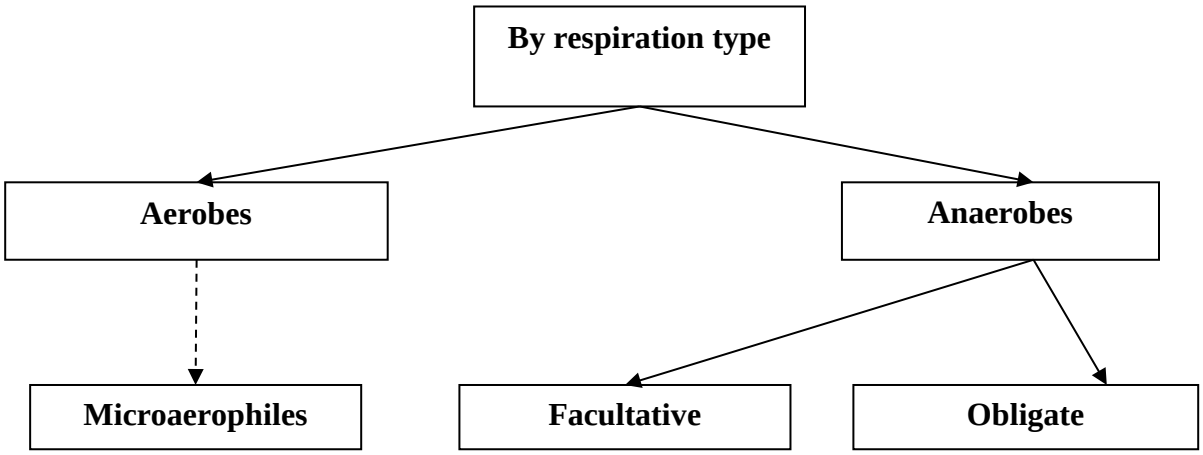


Fig. 51. Division of bacteria by respiration type

During respiration, a huge amount of energy (ATP) is produced, which is used for the synthesis of organic substances, movement, maintenance of osmotic pressure, etc. ATP formation in aerobes and anaerobes occurs via different pathways. However, the biological significance of respiration is not limited to this. As a result of chemical reactions accompanying respiration, a large number of

intermediate compounds are formed, from which amino acids, fats, proteins, and vitamins can be synthesized.

Bacterial Growth and Reproduction

The nutrients and energy obtained by the microbial cell are used for bacterial growth and reproduction. *Growth* is an increase in the mass of an individual cell due to the synthesis of the cellular material. During growth, the cell size increases. After reaching certain sizes, the cell stops growing and begins to divide (reproduce). *Bacterial reproduction* is the ability of bacteria to self-reproduce (increase the number of individuals). Most bacteria typically undergo binary transverse division. Before division, bacterial cells undergo duplication of the DNA molecule. Each daughter cell receives a copy of the maternal DNA. The process of microbial cell division is considered complete when the cytoplasm of the daughter cells is separated by a septum or a constriction (Fig. 52).

Division of bacteria can occur in various planes, forming diverse cell combinations: chains of streptococci, paired associations (diplococci), tetrads of cocci, packets (sarcina), clusters (staphylococci). Rod-shaped and spiral forms divide transversely, only in one plane. In some microorganisms, reproduction occurs by budding, where the bud is smaller than the original cell.

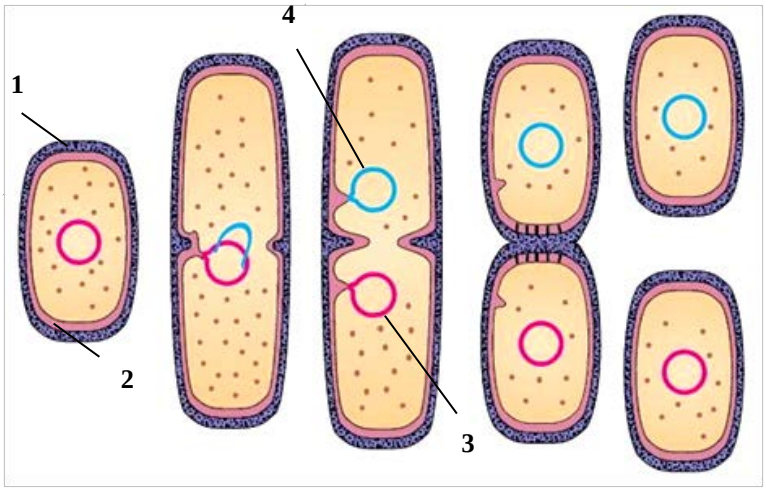


Fig. 52. Schematic of binary division of a bacterial cell: 1 – cell wall; 2 – cytoplasmic membrane; 3, 4 – chromosomes (https://studfile.net)

Bacterial reproduction rate is high, due to the intensity of their metabolism. In most bacteria, each cell divides every 15–30 minutes. There are bacterial species that divide slowly, once every 24 hours, for example, Mycobacterium tuberculosis. For each bacterial species, the reproduction rate can vary depending on the culture age, nutrient medium, temperature, pH value, and many other factors.

Nutrient Media

Media used for cultivating bacteria in laboratory or industrial conditions must meet their nutritional needs, have an appropriate pH value, be isotonic, sterile, and, if possible, transparent. The specificity of most nutrient media is determined by carbon and nitrogen compounds, but since microbial constructive and energy processes are diverse, their nutrient requirements are also different.

Nutrient media are typically classified into several groups based on their composition and origin, physical state or consistency, and functional or intended purpose.

By origin, media are natural and artificial (synthetic). Natural media include those composed of plant or animal products. They contain all components necessary for bacterial growth and development but have an inconsistent chemical composition, i.e., they are unstable. Therefore, such nutrient media are not suitable for studying bacterial metabolism and are mainly used for biomass accumulation, maintaining bacterial cultures in a viable state, and for diagnostic purposes, such as isolating pure bacterial cultures. Natural media include milk, blood and blood serum, decoctions and extracts from natural substrates, peptone and meat water, meat-peptone broth and agar, yeast extracts, potato, egg, and bile-containing media. *Synthetic (artificial) media* have a defined chemical composition and precise quantitative content of nutrients. They are used to study microbial physiology. Examples of synthetic media are Koser's and Simmons' media, used to study bacterial ability to utilize citrates. In addition to other salts, these media contain sodium citrate and an indicator.

In practical microbiology, combined nutrient media typically integrate natural components with inorganic salts. Examples include Zeissler's medium, which includes meat-peptone agar (MPA), blood, and sugar; Hiss's media containing peptone, agar, sugar, and indicator; Rappoport's medium consisting of bile broth, glucose, and indicator, etc.

By composition, media can be divided into simple and complex. *Simple* ones include meat and peptone water, meat-peptone broth and agar. Adding one or more ingredients such as carbohydrates, blood, serum, and other components makes them *complex*.

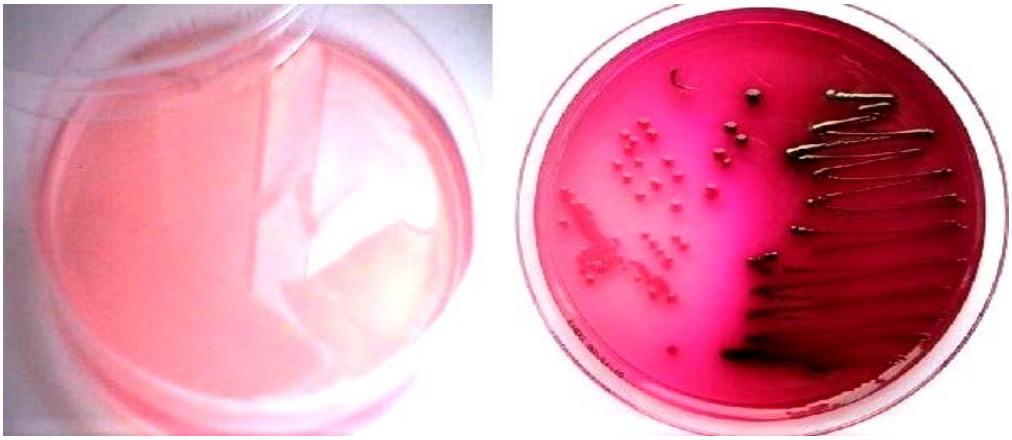
By physical state (consistency), nutrient media can be liquid, semi-liquid, solid or firm, powdery or dry. *Liquid* media are usually aqueous solutions of substances necessary for life. They are used for biomass accumulation, culture enrichment, and metabolism studies. *Semi-liquid* and *solid* nutrient media are obtained from liquid ones by adding agar-agar or gelatin. Agar concentration for semi-liquid media is 0.5–0.7%, and for solid 1.5–2%. The polysaccharide agar is obtained from certain types of seaweeds, dried and stored as plates or powder. Bacteria do not use agar as a substrate, so the composition of the solid nutrient medium depends on the composition of the liquid medium to which agar is added.

Agar melts at about 100°C and solidifies at 40°C. Agarized media are poured into test tubes or Petri dishes in molten state and then cooled. The use of gelatin is limited because it is liquefied by bacterial proteolytic enzymes and is mainly used in nutrient media for diagnostic purposes. Silica gel and carrageenan, obtained from red seaweeds, are also used to solidify media. Gel plates impregnated with nutrient medium are used for cultivating autotrophic bacteria. *Dry* nutrient *media* are mixtures of nutrient medium components of a specific composition. Before use, they are dissolved in water according to the label instructions, the required pH is set, and they are sterilized. The use of dry nutrient media facilitates the preparation of complex media in laboratories.

By intended purpose, nutrient media are divided into several groups. *Basic* or *universal* media, such as meat-peptone agar (MPA), meat-peptone broth (MPB); many species of undemanding microorganisms can grow on them. *Special media* are used for cultivating bacteria that cannot grow on universal media; for example, carbohydrates are added for streptococci, bile for salmonellae, defibrinated blood for diphtheria bacillus. Among special media, *selective* (*elective, selctive*) media can be distinguished. They are intended for isolating and cultivating a specific bacterial species from material containing a large number of different microbial species. For example, to isolate the tuberculosis causative agent from patient sputum, Löwenstein–Jensen medium is used; for salmonellae from feces – Ploskirev's medium. The complex composition of such media includes substances that inhibit the growth of extraneous microflora but do not affect the viability of the target bacterial species. Such substances can be aniline dyes, bile, sodium chloride in concentrations above 1%. Some media contain not only substances suppressing the growth of certain microbial groups but also growth stimulants for specific bacterial species.

Differential-diagnostic media (DDM) are intended for identifying bacteria by biochemical properties. The use of these media is based on differences in bacterial enzymatic composition and the ability of enzymes to break down a particular substrate (Fig. 53). There are media for determining the glycolytic activity of bacteria, which include one (Hiss media) or two (Ressel media) sugars. Proteolytic activity of bacteria is studied on MPB, media with gelatin, and coagulated serum. The ability to ferment simpler nitrogen-containing compounds is studied on nutrient media with amino acids and urea broth.

A variety of differential-diagnostic media are *chromogenic nutrient media*. These are nutrient media with special chromogenic substrates that, in the presence of a certain type of microorganisms, cause the appearance of specific colony coloring (Fig. 54). Chromogenic nutrient media are suitable for cultivating, differentiating, and selecting microorganisms.



a *b*

Fig. 53. Differential-diagnostic Endo medium:
a – medium without inoculation; b – medium with inoculation

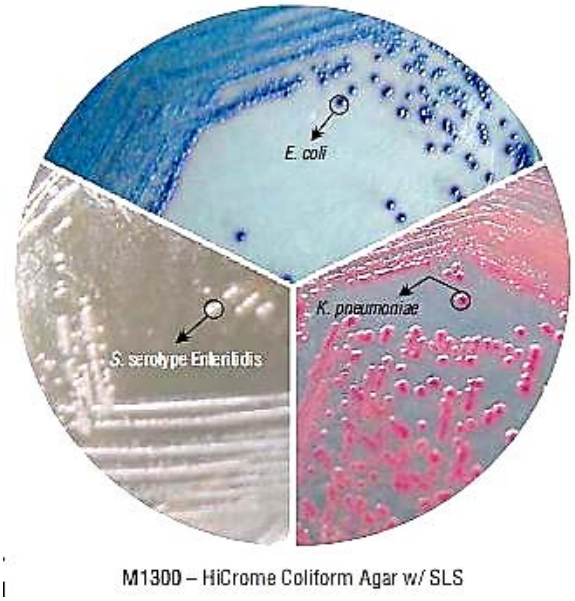


Fig. 54. Chromogenic media.
M 1300 medium – HiCrom Coliform Agar w/SLS
(https://bio-media.ru)

The ability of bacteria to produce toxins and aggression enzymes is investigated on blood agars, yolk-salt agar, serum-free phosphate agar, and other similar media.

Preservative media are used for transporting and storing material containing bacteria for extended periods. Their composition includes glycerol, sodium chloride, and phosphate-buffered solutions.

Conditions for Bacterial Cultivation

For bacterial growth, in addition to the composition of the nutrient medium, the acidity of the medium, aeration, temperature, light, and humidity are important. Most bacteria grow at pH 6.8–8.0; that is, in a neutral environment. Maintaining a neutral pH value is especially important for acid-producing bacteria. During industrial cultivation of bacteria in large volumes, the pH of the medium is automatically regulated by adding solutions of sodium bicarbonate or alkalis.

The gas composition of the medium is also important for bacteria. A significant portion of them requires a constant supply of molecular oxygen. Such microorganisms are grouped as obligate aerobes. A smaller portion of bacteria—obligate anaerobes—can develop only in the absence of oxygen. However, most bacteria are facultative anaerobes; they grow both in the presence of oxygen and without it. For bacteria cultivated on solid nutrient media or in small volumes of liquid media, the oxygen present in the atmosphere is sufficient. For cultivating aerobic bacteria on an industrial scale, forced aeration is required by bubbling oxygen into the reactor or fermenter with the culture, while for cultivating anaerobes, oxygen-free conditions must be created. To do this, bacteria are inoculated by stabbing into a column of solid nutrient medium, the inoculations are placed in special devices—anaerostats, where the gas phase consists of an inert gas or a vacuum is created, and oxygen is removed from the medium by boiling or chemical methods (Fig. 55). For isolating pure cultures of anaerobes, the special Kitt-Tarozzi medium is used, as well as cultivation in glass tubes using the Vignal-Veillon method.

a *b*

Fig. 55. Equipment for cultivating anaerobic bacteria: 1-CUBE anaerostat (a) and GasPak 100 anaerostat (b)

Growth and reproduction of microorganisms occur at favorable temperatures. Therefore, their cultivation is carried out in special cabinets—thermostats or temperature-controlled rooms, where the optimal specified temperature is maintained.

When cultivating microorganisms in laboratory or industrial conditions to obtain large quantities of biomass, two different technological systems are used: *batch* (or periodic) and *continuous* (or flow-through) *cultivation*. In the first case, bacterial multiplication occurs in a closed vessel until the cell population density reaches a critical concentration, the nutrient reserves are depleted, and metabolic products begin to exhibit toxic properties. Bacterial cultures, when grown in liquid media in closed vessels of a certain volume where microorganisms are in a closed system, are called *batch cultures*. In this case, bacterial growth follows a regular pattern, characterized by a growth curve (Fig. 56). On this curve, the following phases can be distinguished:

1. Initial – lag phase. The period from bacterial inoculation to the start of multiplication. During this time, bacterial cells adapt to the new cultivation conditions. The duration averages 2–5 hours.

2. Exponential (logarithmic) phase. During this period, constant cell division occurs at maximum rate. This speed depends on the bacterial species and nutrient medium. The cell doubling time is called the *generation time.*

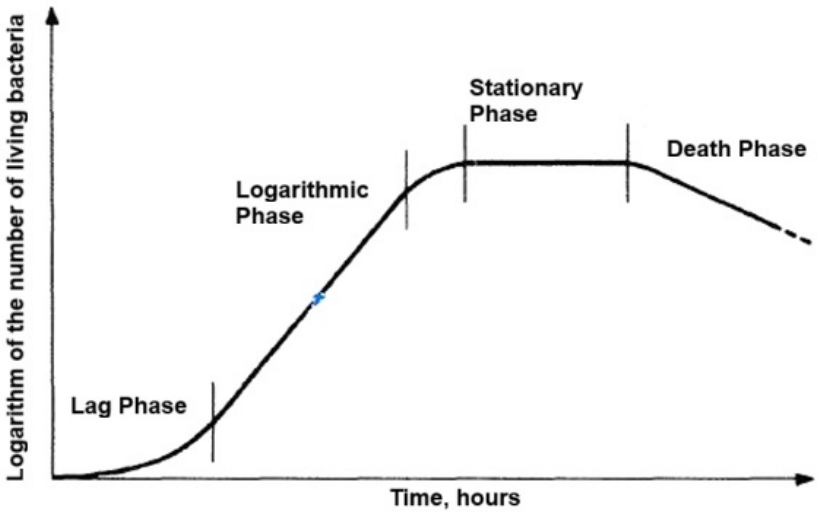


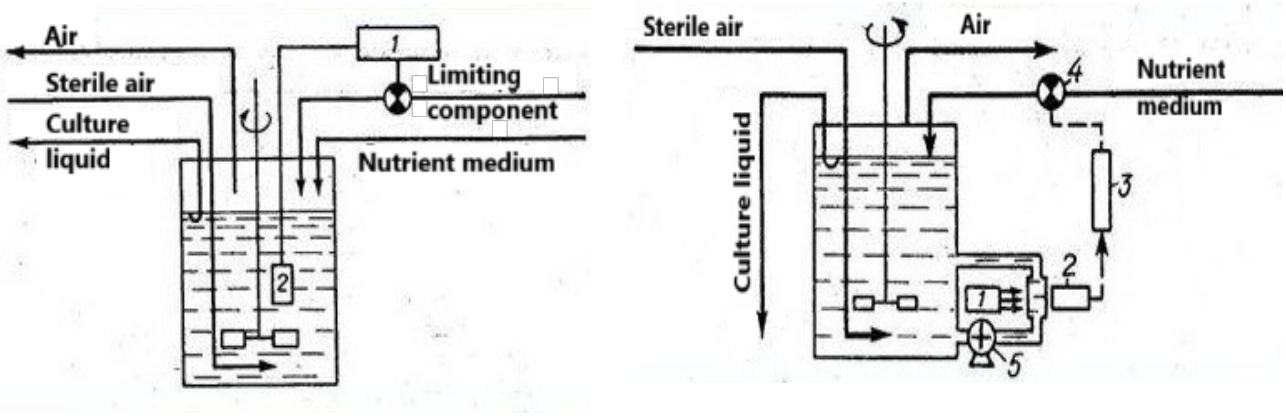
Fig. 56. Bacterial growth curve in liquid nutrient medium. Phases of bacterial reproduction

3. Stationary phase. Characterized by a constant number of bacterial cells, decreased nutrient concentration in the medium, accumulation of toxic metabolic products.

4. Death phase. Occurs as a result of accumulation of toxic metabolic products in the medium or due to bacterial autolysis.

h

In industrial conditions, flow-through or continuous cultivation is often used. In this case, fresh nutrient medium is continuously supplied to the reactor or fermenter with stirring, and metabolic products and accumulated bacterial mass are automatically removed. Such cultivation can be carried out in special apparatuses – *chemostats* and *turbidostats*, where the required volume of nutrient medium is supplied automatically, depending on the bacterial cell concentration (Fig. 57).



a *b*

Fig. 57. Schematic diagram of a chemostat (a) and turbidostat (b)

The isotonicity of the nutrient medium depends on the content of inorganic salts. For most bacteria, a medium with a sodium chloride concentration of 0.5–0.6% is considered isotonic.

The cultivation time for bacteria depends on the time of the next cell division in the given population. The generation time for most pathogenic bacteria is 20–30 minutes. Such cultures form within 18–24 hours. Cells of some bacterial species divide at longer intervals, so the population increase of such cultures occurs over a long period, for example, leptospires grow in 8–10 days, mycobacterium tuberculosis – in 3–4 weeks.

Cultural Properties of Bacteria

Cultural or macromorphological properties refer to the characteristic features of microbial growth on solid and liquid nutrient media. Depending on the inoculation, microorganisms can grow as colonies, streaks, or continuous lawns on *solid nutrient media*. A colony is an isolated accumulation of cells of one species grown from a single cell (cell clone). Depending on where the microorganism grows (on the surface of the solid nutrient medium, in its depth), *surface, deep*, and *bottom colonies* are distinguished. Surface colonies (grown on the medium surface) are diverse, species-specific, and their study aids in determining the species affiliation (identification) of the culture being analyzed (Fig. 58).

When describing colonies, the following features are considered:

- colony shape – round, amoeboid, rhizoid, irregular, etc.;
- colony size (diameter) – very small (pinpoint) (0.1–0.5 mm), small (0.5–3 mm), medium-sized (3–5 mm), and large (more than 5 mm in diameter);
- colony surface – smooth, rough, folded, wrinkled, with concentric circles or radially striated;
- colony profile – flat, convex, conical, crater-like, etc.;
- transparency – dull, matte, shiny, transparent, floury;

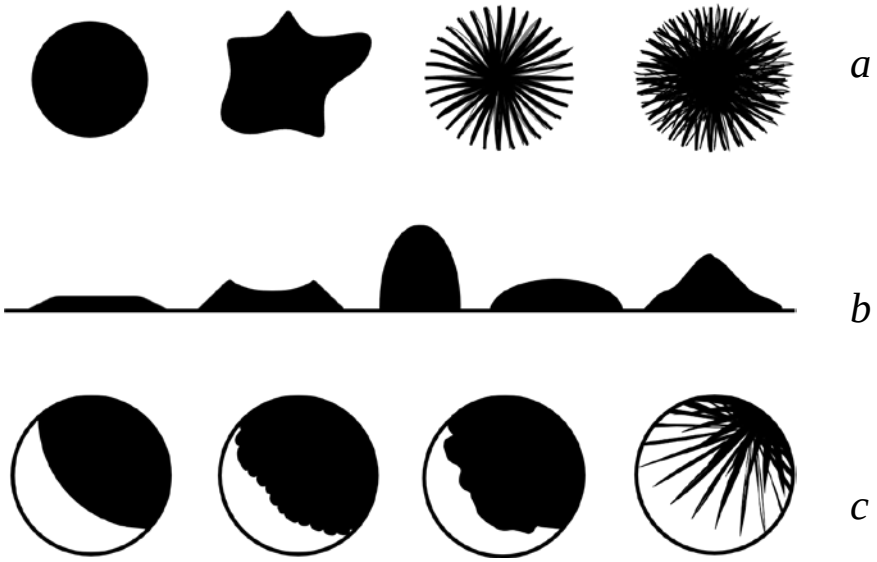


Fig. 58. Bacterial growth pattern on solid nutrient medium: colony shape (a); surface (b); colony edge characteristics (c) (https://lifelib.info)

- colony color (pigment) – colorless or pigmented (white, yellow, golden, red, black), with special note of pigment release into the medium with its staining;
- colony edge – even, wavy, toothed, fringed, etc.;
- colony structure – homogeneous, fine- or coarse-grained, streaky; the edge and structure of the colony are determined using a magnifying glass or at low microscope magnification, placing the Petri dish with the culture on the microscope stage lid down;
- colony consistency – determined by touching the surface with a loop; the colony can be dense, soft, ingrowing into agar, mucoid (stretches behind the loop), brittle (easily breaks upon contact with the loop).

Two main colony types are distinguished – S and R types. *S-type colonies* are round, smooth, shiny, convex, with even edges, moist. *R-type colonies* are flat, rough, matte, irregular in shape, with striated edges. However, colony shape is subject to variability. R-forms of colonies can transition to S-forms and vice versa. *Deep colonies* most often resemble more or less flattened lentils (oval shapes with pointed ends), sometimes cotton-like clumps with filamentous outgrowths into the

nutrient medium. Deep colony formation is often accompanied by rupture of the solid medium if microorganisms release gas. *Bottom colonies* usually appear as thin transparent films spreading along the bottom.

Colonies can change with age; their properties depend on the medium composition and cultivation temperature.

Description of microbial growth by streak inoculation includes its features: scanty, moderate, abundant; continuous with even or wavy edge; diffuse; feathery; rhizoid; tree-like. Color, surface, and consistency are also characterized.

Microbial growth on liquid nutrient media is assessed using 24-hour cultures grown under stationary conditions.

In *liquid nutrient media*, microbial growth results in turbidity of the medium, film formation, or sediment (Fig. 59).

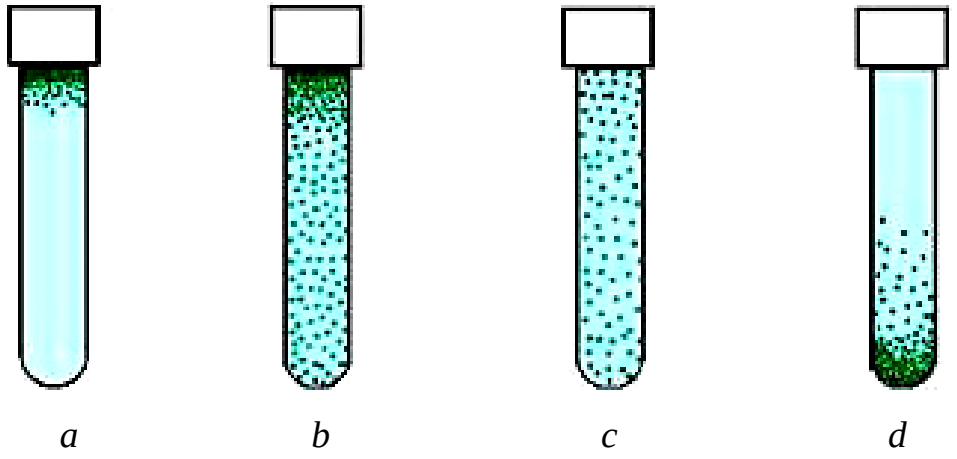


Fig. 59. Bacterial growth pattern in MPB: surface growth (a); diffuse turbidity (b and c); bottom growth (d) (http://old.gsu.by)

1. Uniform turbidity of the medium (diffuse growth) is characteristic of facultative anaerobes. The degree of turbidity can be weak, moderate, strong.

2. Bottom bacterial growth is typical for strict anaerobes, resulting in sediment: scanty, abundant, loose, mucoid, flocculent, granular. The nutrient medium can be transparent or turbid.

3. Wall-adherent growth – formation of grains, loose flakes on the inner surface of vessel walls. The nutrient medium remains transparent.

4. Surface bacterial growth is characterized by the appearance of a film on the medium surface: thin, dense, loose, smooth, folded, moist, dry, ring-like, or continuous. Such growth is observed in aerobic bacterial cultivation.

In semi-liquid nutrient media, motile microorganisms cause pronounced turbidity, while non-motile forms grow only along the inoculation stab in the medium.

Microbial growth is often accompanied by odor appearance, medium pigmentation, gas release. The characteristic odor of cultures of some bacterial species is associated with the formation of various esters (ethyl acetate, amyl acetate, etc.), indole, mercaptan, hydrogen sulfide, skatole, ammonia, butyric acid.

The ability to form pigments is inherent in many microbial species. The chemical nature of the pigments is diverse: carotenoids, anthocyanins, melanins. If the pigment is insoluble in water, only the culture overlay is colored; if soluble, the nutrient medium is also colored. It is believed that pigments protect bacteria from the destructive effects of sunlight, which is why there are so many pigmented bacteria in the air; additionally, pigments participate in the metabolism of these microorganisms.

In nature, there are so-called phosphorescent bacteria, whose cultures glow in the dark with greenish-blue or yellowish light. Such bacteria are found mainly in river or sea water. Luminescent bacteria (photobacteria) include aerobic bacteria (vibrios, cocci, rods).

Bacterial Enzymes

Microbial biochemical processes are carried out due to the presence of various enzymes in the cell that catalyze all vital reactions. Since the enzyme composition is determined by the genome, it is a species characteristic. The set of enzymatic properties of bacteria is called the *biochemical properties of bacteria.*

Enzymes are biological catalysts of protein nature. The microbial cell, like cells of higher organisms, is equipped with a sufficiently active enzymatic apparatus. Microbial enzymes possess the same properties and functions as enzymes of higher organisms. According to the catalyzed reactions, all enzymes are divided into six classes:

1. Oxidoreductases – catalyze oxidation-reduction reactions.
2. Transferases – catalyze the transfer of various groups from donor to acceptor.
3. Hydrolases – catalyze bond cleavage in substrates with water addition.
4. Lyases – catalyze bond cleavage in substrates without water addition or oxidation.
5. Isomerases – catalyze transformations within one molecule (intramolecular rearrangements).
6. Ligases (synthetases) – catalyze the joining of two molecules using energy from phosphate bonds.

Despite the small size of the microbial cell, the distribution of enzymes in it is strictly ordered. Enzymes of energy metabolism and nutrient transport are localized in the cytoplasmic membrane and its derivatives. Enzymes of protein

synthesis are associated with ribosomes. Many enzymes are not bound to specific cell structures but are present in dissolved form in the cytoplasm.

Bacterial enzymes are subdivided into exo- and endoenzymes. *Endoenzymes* function only inside the cell. They catalyze biosynthesis and energy metabolism reactions. *Exoenzymes* are secreted by the cell into the environment and catalyze hydrolysis reactions of complex organic compounds into simpler ones accessible for microbial cell assimilation. These include hydrolytic enzymes, which play an exceptionally important role in microbial nutrition.

Depending on formation conditions, enzymes are divided into constitutive and inducible. *Constitutive enzymes* are synthesized by the cell regardless of the substrate on which bacteria develop. For example, glycolysis enzymes. *Inducible enzymes* are synthesized only in response to the presence in the medium of a substrate-inducer necessary for the cell. Induced enzyme synthesis continues as long as the inducer is present in the medium. At the same time, enzymes are synthesized anew in all cells simultaneously. Many nutrients act as inducers of biosynthesis. Most hydrolytic enzymes are inducible.

Allosteric enzymes are also known. In addition to the active site, they have a regulatory or allosteric center, which is spatially separated from the active site in the enzyme molecule. It is called allosteric (from Greek *allos* – other, foreign) because molecules binding to this center are structurally (sterically) unlike the substrate but influence the binding and transformation of the substrate in the active site by changing its configuration. The enzyme molecule can have several allosteric centers. Substances binding to the allosteric center are called allosteric effectors. They affect the active site function through the allosteric center: either facilitating or hindering it. Accordingly, allosteric effectors are called positive (activators) or negative (inhibitors). Allosteric enzymes play an important role in the fine regulation of bacterial metabolism. Since virtually all reactions in the cell are catalyzed by enzymes, metabolic regulation reduces to regulating the intensity of enzymatic reactions.

Infectious disease causative agents have *pathogenicity enzymes* that destroy host tissues and cells, thereby enabling the spread of pathogenic microorganisms and their toxins in infected tissues. Such enzymes include *plasmocoagulase, neuraminidase, collagenase, lecithinase, hyaluronidase*, and some other enzymes. For example, streptococcal hyaluronidase breaks down hyaluronic acid in connective tissue cell membranes of the host, facilitating the spread of causative agents and their toxins in the body, determining the high invasiveness of these bacteria. Plasma coagulase is the main pathogenicity factor of staphylococci, as it participates in converting prothrombin to thrombin, which causes fibrinogen formation, resulting in each bacterium being covered by a film that protects it from phagocytosis.

Microbial enzymes have found wide application in biotechnology, including genetic engineering, for obtaining immunobiological preparations (e.g., vaccines),

various biologically active substances, as well as a number of products in light and food industries.

Microbial enzymes characterize their species properties and are therefore studied for bacterial identification. Depending on the substrate, hydrolytic enzymes are conventionally divided into two major groups:

1. *Glycolytic* or saccharolytic enzymes, whose substrates are various sugars, with breakdown products being acids, alcohols, aldehydes, $Í_2Î$, and $ÑÎ_2$;

2. *Proteolytic enzymes*, which break down proteins to form polypeptides, amino acids, ammonia, indole, hydrogen sulfide.

To study enzyme activity in microbial identification, differential-diagnostic media containing specific substrates – sugars or proteins – are widely used.

When studying bacterial saccharolytic activity, monosubstrate differential-diagnostic Hiss media (Hiss's colored series), lactose-containing Endo, Levin, Ploskirev media, disubstrate Ressel media (Fig. 60), polysubstrate Kligler and Olkenitsky media are common. The latter can also be used to study bacterial proteolytic properties, as they contain components capable of detecting ammonia and hydrogen sulfide released during amino acid breakdown by some microorganisms (Fig. 61).

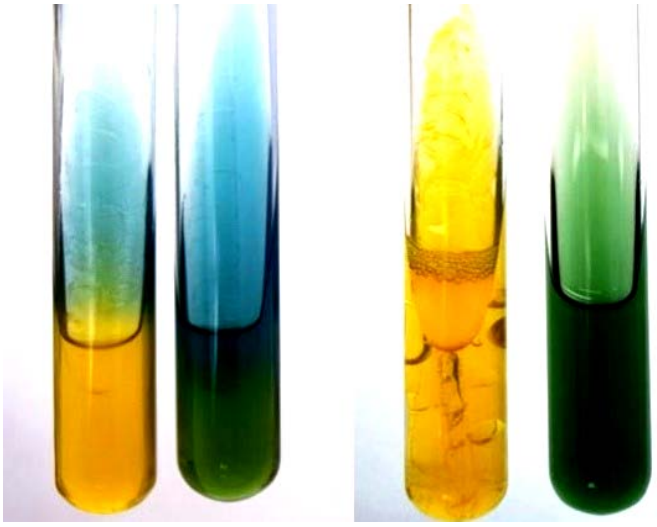


Fig. 60. *Bacterial identification by glycolytic properties.*
Ressel medium I: S. flexneri (a); A. faecalis (b); E. coli (c); control (d)

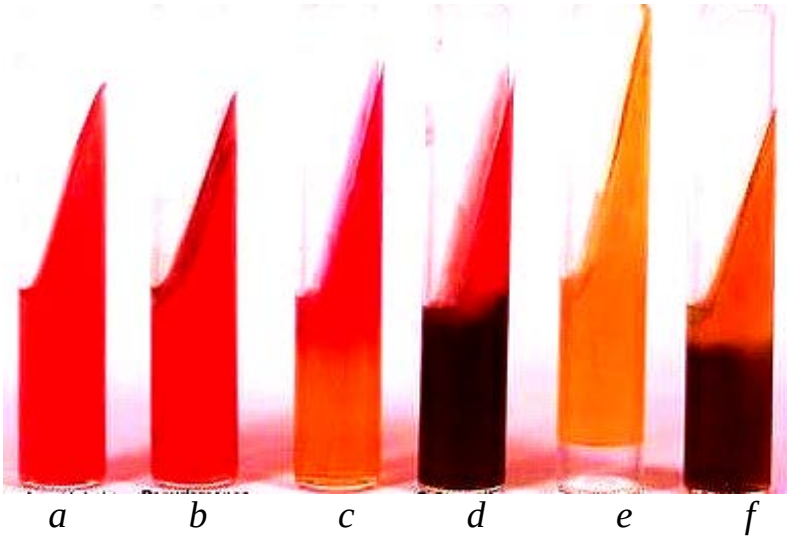


Fig. 61. Bacterial identification by glycolytic and proteolytic properties. Kligler medium: control (a); P. aeruginosa (b); S. sonnei (c); S. typhi (d); E. coli (e); P. mirabilis (f)

Bacterial proteolytic enzymes are determined by indole release, hydrogen sulfide, breakdown of certain amino acids, e.g., phenylalanine, lysine, cystine. Proteolytic enzymes can alter (liquefy) gelatin, with different bacterial species altering the gelatin "column" in a test tube with microbial inoculation differently (Fig. 62). Thus, with Vibrio cholerae growth, the gelatin column takes the shape of a nail; with staphylococcus – a stocking; with Pseudomonas aeruginosa, layered liquefaction of the medium is observed.

Fig. 62. Action of proteolytic enzymes. Gelatin liquefaction (http://biologylib.ru)

In practical bacteriological laboratories, test systems and rapid methods are widely used for preliminary study of microbial biochemical properties. Indicator paper systems (IPS) are most commonly used. IPS are filter paper disks impregnated with solutions of sugars or other substrates combined with indicators. Such disks are placed in a test tube with a culture grown in liquid nutrient medium.

The enzyme activity is judged by the change in disk color with the substrate. Microtest systems for enterobacterial identification are disposable plastic containers with media containing various substrates and indicators. Inoculating pure microbial cultures into such test systems allows quick detection of bacterial ability to utilize citrates, glucose, sucrose, release ammonia, indole, degrade urea, lysine, phenylalanine, etc. (Fig. 63).

Fig. 63. API test system for enterobacteria. Upper plate – all tests positive, lower – all negative

Isolation of Pure Microbial Cultures

A ***pure culture*** is a culture containing microorganisms of one species. Isolation of pure bacterial cultures is a mandatory stage of bacteriological investigation in microbiological diagnosis of infectious diseases, in studying microbial contamination of various environmental objects, and in general, in any work with microorganisms. The studied material (pus, sputum, feces, and other material from patients; water, soil, air, food products) usually contains microbial associations. Isolation of a pure culture allows study of morphological, cultural, biochemical, antigenic, and other characteristics, by which the species and type affiliation of the causative agent is determined, i.e., its identification is performed.

Methods for isolating pure microbial cultures include:

1. Sector method involves inoculating the studied material with a loop onto the surface of agarized medium in Petri dishes. In the first stage, a loop with culture applies a series of parallel streaks on the agarized medium (Fig. 64A). The loop is sterilized and a series of streaks is applied in a direction perpendicular to the first one (Fig. 64B). Then the loop is sterilized again, and streaks are applied in direction C (Fig. 64), and after another sterilization – in direction D (Fig. 64). The dishes are placed in a thermostat, and after a certain time, the results are evaluated. Usually, a large number of colonies grow on streaks A and B (sometimes it is a continuous growth), while on streaks C and D, isolated colonies form.

2. Koch's method ("plate dilutions") is sequential dilution of the studied material in molten agar (temperature 48–50 °C), followed by pouring into Petri dishes where the agar solidifies. Inoculations are usually made from three to four last dilutions, where bacteria become few, and subsequently, during growth on

dishes, isolated colonies appear, formed from one original maternal cell. Pure bacterial cultures are obtained from isolated colonies in the agar depth by reinoculating onto fresh media.

3. Shukevich's method is used to obtain pure cultures of Proteus and other microorganisms with "crawling" growth. The studied material is inoculated into condensation water at the base of the slanted agar. Motile microbes (proteus) can climb up the slanted agar, while non-motile forms remain growing at the inoculation site below. By reinoculating the upper edges of the culture, a pure culture can be obtained.

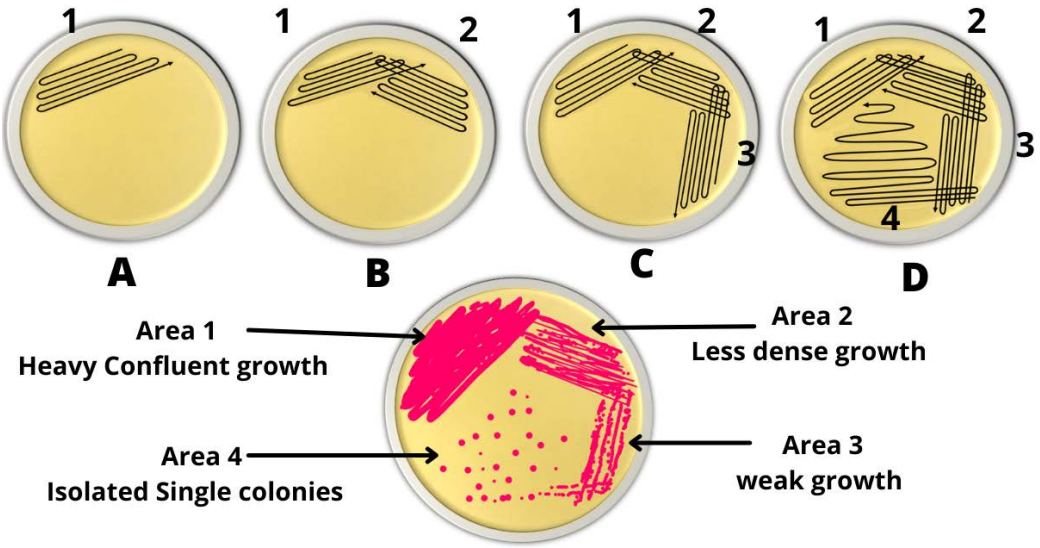


Fig. 64. The scheme of streaking bacteria to obtain isolated colonies

4. Drigalski's method is widely used in bacteriological practice, where the studied material is diluted in a test tube with sterile physiological saline or broth. One drop of material is introduced into the first dish and distributed over the medium surface with a sterile glass spatula. Then, with the same spatula (without flaming), inoculation is done in the second and third dishes. With each inoculation, fewer bacteria remain on the spatula, and when inoculating the third dish, bacteria will be distributed on the nutrient medium surface separately from each other. After 1–7 days of incubation in the thermostat (depending on microbial growth rate), each bacterium on the third dish gives a cell clone, forming an isolated colony, which is reinoculated onto the slanted agar to accumulate a pure culture.

In the process of isolating a pure bacterial culture, the following stages can be distinguished:

I. Studied material. Microscopy and inoculation of native material onto nutrient/elective medium to obtain isolated colonies.

II. Isolated colonies. Study of "suspicious" colonies (by cultural properties). Microscopy of the stained smear (determination of bacterial morphological properties). Reinoculation onto accumulation medium.

III. Pure culture. Microscopy of the stained smear. Identification of the isolated pure culture by biochemical properties, antigenic structure, sensitivity to bacteriophages.

Methods for cultivating and isolating pure cultures of obligate anaerobes have their peculiarities. An essential condition for isolating pure cultures is the absence of oxygen at all stages of anaerobe isolation. The following methods for creating anaerobic conditions are distinguished:

1. Physical
2. Chemical
3. Biological

Physical methods include: boiling the medium followed by isolating the inoculation in the medium with sterile vaseline oil or paraffin; stab inoculation of anaerobes into a tall agar column or semi-liquid medium; removal of air from special hermetic vessels – anaerostats with inoculations.

Chemical methods involve using chemical substances in sealed vessels – oxygen adsorbents (alkaline pyrogallol solution, sodium hydrosulfite), oxygen reductants (sodium thioglycolate, ascorbic acid, cystine, etc.). Anaerobic conditions can be created using gas-generating systems in anaerostats: sodium borohydride tablets, which interact with water to form hydrogen. Hydrogen, binding with air oxygen in the presence of a catalyst, forms water.

Biological methods: joint cultivation of aerobes and anaerobes on solid nutrient media (Fortner's method), cultivation of some obligate anaerobes in laboratory animal organisms.

Methods for isolating pure anaerobe cultures:

1. Weinberg's method. Its essence is that the studied material is diluted in molten and cooled to 45–50°C agarized nutrient medium. 6–10 sequential dilutions are made, then the medium in test tubes is quickly cooled and the surface is covered with a layer of paraffin and vaseline oil mixture to prevent air penetration into the nutrient medium depth.

2. Veillon-Veillon method. Dilutions of the studied material are prepared in sugar agar. From each dilution, material is drawn into Pasteur pipettes, whose ends are sealed. After cultivation, the pipette is filed, broken, and the colony is transferred to accumulation medium (e.g., Kitt-Tarozzi medium).

3. Zeissler's method. The studied material is streaked on the surface of solid nutrient medium, placed under anaerobic conditions (e.g., anaerostat), and cultivated at optimal temperature in a thermostat. The obtained isolated colonies are reinoculated into accumulation medium.

For cultivating strict anaerobes, media such as Kitt-Tarozzi, Wilson-Blair, Willis-Hobbs are used.

BASICS OF VIROLOGY

Structure and Classification of Viruses

A virus is a non–cellular form of life that possesses a genome (RNA or DNA) but lacks synthetic machinery; therefore, it is capable of reproduction only within the cells of more highly organized organisms.

The morphology and structure of viruses are studied using an electron microscope, as their sizes are small and comparable to the thickness of bacterial cell walls. The shape of virions can vary: rod-shaped (e.g., tobacco mosaic virus), bullet-shaped (e.g., rabies virus), spherical (e.g., poliomyelitis viruses, HIV), or resembling a sperm (many bacteriophages). The sizes of virions range from 20–30 nm (picornaviruses, parvoviruses) to 150–250 nm (herpesviruses, rhabdoviruses), and even 350–400 nm (poxviruses).

Viruses exist in one of two states: extracellular (dormant) and intracellular (reproducing, vegetative). The extracellular form is called a viral particle or virion. Virions consist of nucleic acid surrounded on the outside by a protein shell – called a *capsid* (from the Latin *capsa* – case). The capsid, together with the contained nucleic acid, is referred to as the *nucleocapsid*. The morphological subunits of the capsid visible under an electron microscope are called capsomers – protein subunits composed of one or more protein molecules. There are three types of capsid structure based on the arrangement of morphological subunits (Fig. 65):

1) Virions with spiral symmetry;
2) Virions with cubic (icosahedral) symmetry;
3) Virions with a mixed type of symmetry.

In the first type, the capsomers are arranged in a spiral pattern, and the nucleic acid (primarily RNA) is also coiled in the form of a spring, located between the coils of protein molecules. In viruses with cubic symmetry, the capsomers are arranged to form a regular icosahedron with a twisted strand of DNA or RNA. An icosahedron has 20 faces (each an equilateral triangle) and 12 vertices. The third type exhibits both types of symmetry; this mixed symmetry is typical of bacterial viruses – bacteriophages. The head of a phage (the capsid) has an icosahedral symmetry, while the tail exhibits a spiral symmetry.

The virus symmetry type and the arrangement pattern of capsomers determine its shape, which can be observed under an electron microscope. When capsomers are arranged in a polyhedron form, they give the virus a spherical appearance. Consequently, viruses with icosahedral symmetry appear rounded under electron microscopy. A spiral symmetry is characteristic of larger viruses; in these cases, capsomers form rod-shaped structures, and virions with spiral symmetry appear elongated rod-shaped or filamentous under an electron

microscope. Bacteriophages with mixed symmetry often have a shape resembling a sperm cell.

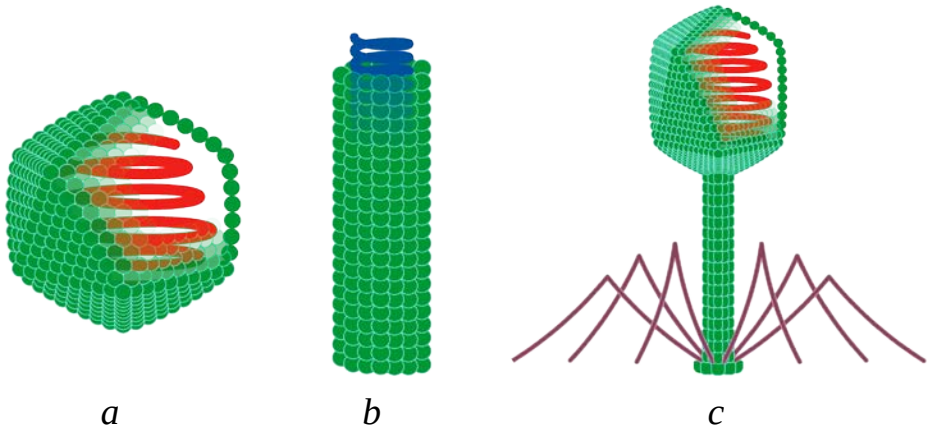


Fig. 65. Types of viral capsids structure: icosahedral (a), spiral (b) and mixed (c). Quoted from "Introduction to Viruses" (https://ru.khanacademy.org/science/biology/biology-of-viruses/virus-biology/a/intro-to-viruses?modal=1)

According to their structure, viruses are typically classified as simple or complex. *Simple* (naked) viruses, such as picornaviruses and parvoviruses, consist of a nucleocapsid, while *complex* (enveloped) viruses have an additional outer shell – referred to as a *supercapsid* or peplos (a derivative of the host cell membrane structures). The shape of such virions is generally close to spherical. Proteins of the supercapsid form morphological subunits called peplomeres, which appear as spikes under an electron microscope (e.g., togaviruses, coronaviruses, orthomyxoviruses, etc.) (Fig. 66).

The capsid and supercapsid protect virions from environmental influences, facilitate selective interaction (adsorption) with specific cell receptors, and contribute to the antigenic and immunogenic properties of the virions.

In terms of their chemical composition and potential pathogenicity, viruses are referred to as infectious nucleoproteins. Virions are composed of *nucleic acids, proteins, lipids, and carbohydrates*.

Nucleic acids. Viruses contain only one type of nucleic acid, either DNA or RNA, resulting in a distinction between DNA-containing and RNA-containing viruses. The majority of viruses are RNA-based. Viruses are typically haploid, meaning they possess a single set of genes. The simplest viral genome encodes 3-4 proteins, while the most complex genomes encode more than 50 polypeptides.

The DNA of viruses can be:

- Linear, single-stranded DNA (parvoviruses);
- Circular, single-stranded DNA (bacteriophages);
- Linear, double-stranded DNA (herpesviruses);
- Circular, double-stranded DNA (hepatitis B viruses).

The RNA of viruses can be:

- Single-stranded non-segmented RNA (paramyxoviruses);
- Single-stranded segmented RNA (influenza viruses);
- Double-stranded segmented RNA (reoviruses);
- Single-stranded circular RNA (hepatitis D virus);
- Two identical single-stranded RNAs (retroviruses).

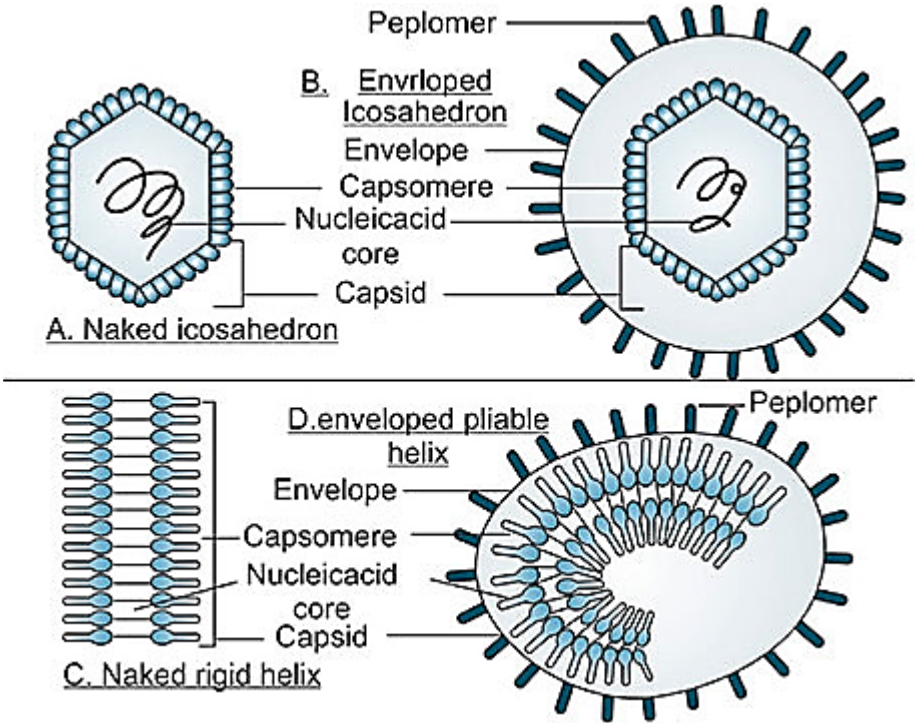


Fig. 66. The structure of simple and complex viruses (Kumar Surinder, 2012, New Delhi, India)

Among RNA-containing viruses, viruses with a positive genome are distinguished (plus-strand RNA, +RNA). The plus-strand RNA of these viruses performs both a hereditary function and the role of messenger RNA (mRNA). It is infectious, meaning that when it enters a cell, it can independently initiate an infectious process. There are also RNA-containing viruses with a negative genome (minus-strand RNA, –RNA). The minus-strand RNA of these viruses serves only a hereditary function and lacks messenger activity; therefore, before translation can occur, a complementary positive RNA must be synthesized on it using the RNA-dependent RNA polymerase enzyme.

The viral genome can function independently of the host cell genome or can be integrated into the host cell's genetic apparatus in the form of a provirus, acting as a genetic parasite. The nucleic acids of some viruses (e.g., herpesviruses) can be found in the cytoplasm of infected cells, where they resemble plasmids.

Viral proteins are typically classified into two groups: structural and non-structural (functional). Structural viral proteins (VP) are all those proteins that

constitute the virion; their number varies from virus to virus, depending on the degree of organization and the size of the virus. They are divided into (Fig. 67):

Capsid proteins:

- Nucleocapsid (N) proteins;
- True capsid (core) proteins;

Supercapsid proteins:

- Outer proteins (spikes, peplomeres, receptor proteins);
- Membrane proteins;
- Matrix proteins.

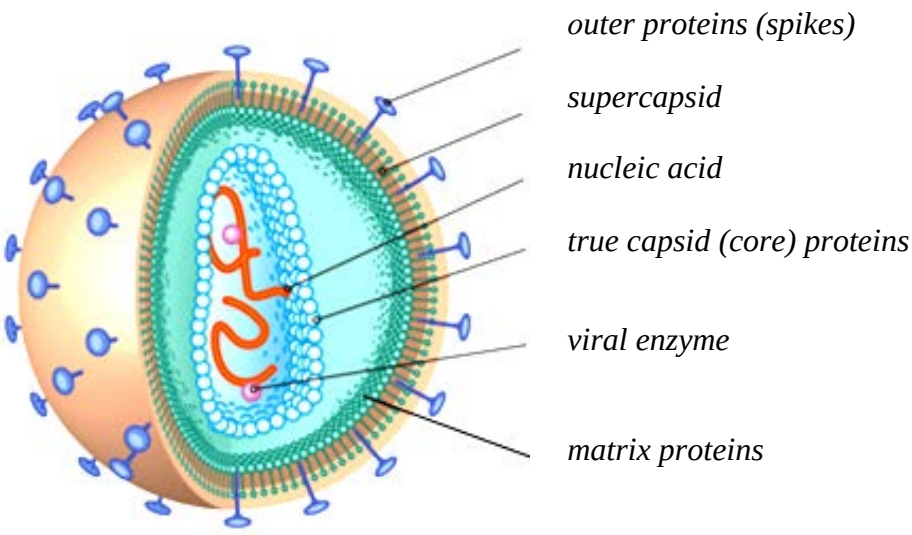


Fig. 67. Viral structural proteins

Non–structural proteins (NS) are enzymes that are either part of the virion (virionic) or encoded by the viral genome (virus-induced). Viral enzymes function at various stages of viral ontogenesis: during attachment to a host cell, infection, replication of the viral genome, virion assembly, or release from the host cell. Such enzymes include neurominidase, polymerases, reverse transcriptase, proteases, endonucleases, ligases, and others.

Viral lipids (phospholipids and glycolipids) are of cellular origin. They can constitute up to 20-30% of the total virion weight. Lipids are incorporated into the viral envelope during virus release from the cell and are also present in the supercapsid. Lipids stabilize the viral particle, influence the conformation of supercapsid proteins, and facilitate virus penetration through the hydrophobic cell membrane. Most lipid-containing viruses are sensitive to ether and detergents.

Carbohydrates. The virion contains ribose and deoxyribose, which are essential components of nucleic acids. It also contains galactose and mannose. All carbohydrates participate in the capsid packaging. Additionally, carbohydrates are present in the glycoproteins of the supercapsid. A typical example of such a

glycoprotein is hemagglutinin receptor, which mediates binding to red blood cells and exhibits antigenic specificity.

The modern taxonomy and classification of viruses are developed by the International Committee on the Taxonomy of Viruses (ICTV). This committee is part of the Virology Department of the International Union of Microbiological Societies. The ICTV establishes principles and rules for the classification of existing viruses and for the recognition of new viral taxa. Information on the current taxonomy of viruses is published in the periodic Reports and releases of the ICTV.

All known viruses belong to the realm *Vira*, which is divided into two sub-realms: *Deoxyviruses* (DNA viruses) and *Riboviruses* (RNA viruses). In modern virus classification, the following taxonomic levels are distinguished: order, family, subfamily, genus and species. The name of a virus order ends with "*-virales*", the family with "*-viridae*", the subfamily with "*-virinae*", and the genus with "*-virus*". For example, the varicella-zoster virus belongs to the order *Herpesvirales*, the family *Herpesviridae*, the subfamily *Alphaherpesvirinae*, and the genus *Varicellovirus*. The name of a virus species typically consists of more than one word and may include information such as the location where it was identified, the diseases it causes, its host organism, or a serial number. Currently, over 300 virus species belonging to more than 50 genera and 30 viral families are known to cause diseases in humans. The number of pathogenic viruses continues to increase.

According to the classification, the basic taxonomic unit in virology is the species, although it is not defined in all known viruses. When identifying viruses, their intraspecific differentiation into variants is essential. The main types of variants are genetic variants (virus *genotypes*) and serological (antigenic) variants or viral *serotypes*. For the purposes of medical virology, such a division is particularly important, as different variants of the virus often differ significantly in their pathogenicity. *The serotype* of a virus is determined through reactions with specific antibodies: neutralization tests (NT), enzyme immunoassay (EIA), direct immunofluorescence (DIF), and hemagglutination inhibition tests (HI tests). *The genotype* (or genogroup) of the virus is identified using methods such as molecular hybridization (MH), polymerase chain reaction (PCR), and nucleic acid sequencing. The International Committee on Taxonomy does not establish or conduct intraspecific differentiation of viruses into variants; this task is performed by working groups of virologists.

In addition to conventional viruses, there are also so-called non-canonical infectious agents, such as *prions* and *viroids*. *Prions* are proteinaceous infectious particles characterized by fibrils measuring approximately 10-20×200 nm. They cause encephalopathies in animals and humans under conditions of slow viral infection, including Creutzfeldt–Jakob disease and kuru. *Viroids* are small molecules composed of single–stranded circular, supercoiled RNA (250-370 nucleotides) that lack protein and cause diseases in plants.

Reproduction of Viruses

Viruses are obligate intracellular parasites, requiring a living cell for replication. There are three types of virus-cell interactions:

1) Productive or cytocidal type — an interaction in which a new generation of virions is produced within infected cells;

2) Abortive type is characterized by the interruption of the infectious process in the cell, resulting in no new virions being formed;

3) Integrative type, or virogeny, involves integration of viral DNA into the host cell's chromosome as a provirus, leading to their coexistence within the cell.

The productive interaction of the virus with the cell involves multiplication, i.e., the reproduction of the virus (from the Latin *reproducere*, where *re-* means “again” and *producer* means “to produce”). Virus reproduction occurs in several stages (see Fig. 68), which vary depending on the virus:

1) Virion adsorption onto a cell;
2) Virus entry into the cell;
3) Deproteinization or “uncoating” of the virus and release of the viral genome;
4) Biosynthesis of virus components;
5) Assembly of a viral particles;
6) Release of virions from the cell.

Viral infection of a cell begins with the adsorption of the virus onto the cell surface. *Adsorption* is mediated by the interaction between viral surface proteins and specific membrane receptors on permissive cells. The number of cellular receptors can reach up to 10^4–10^5 molecules per single cell membrane. This interaction is primarily facilitated by complementarity between viral and cellular receptors, as well as by non-specific intermolecular forces such as charge differences, hydrogen bonds, or hydrophobic interactions. Initially, adsorption is reversible due to single interactions between the virus and the cell; irreversible adsorption is achieved through multiple polyvalent attachments of the virus. The specificity of the interaction between cellular receptors and viral surface proteins is determined by viral tropism (from Greek *trópos* – turn, direction), which refers to the ability of a virus to selectively infect certain cell types. For example, viruses that replicate in the liver cells are known as hepatotropic, while those that infect cells of the nervous system are called neurotropic, and so on.

The virus enters the host cell either by viropexis (receptor-mediated endocytosis), by fusion of the viral envelope with the cell membrane (in the presence of a fusion protein), or through a combination of these two mechanisms. In receptor-mediated endocytosis, invagination of the cell membrane occurs, leading to the formation of an intracellular vacuole (endosome). This vacuole can then migrate to different parts of the cytoplasm or the cell nucleus, followed by the release of the virus from the endosome. The fusion process is initiated when viral

proteins of the capsid or supercapsid shells bind to cellular receptors. The viral envelope then fuses with the host cell's cytoplasmic membrane, driven by hydrophobic interactions. Some causative agents, such as paramyxoviruses, possess a specialized F protein that induces fusion between the viral and cellular membranes. Endocytosis is the mechanism of cell entry for simple viruses, such as adenoviruses or picornaviruses, as well as for some complex viruses, including orthomyxoviruses (influenza virus), rhabdoviruses (rabies virus), and togaviruses. Fusion is primary entry pathway for complex viruses such as herpesviruses, paramyxoviruses, and retroviruses (HIV).

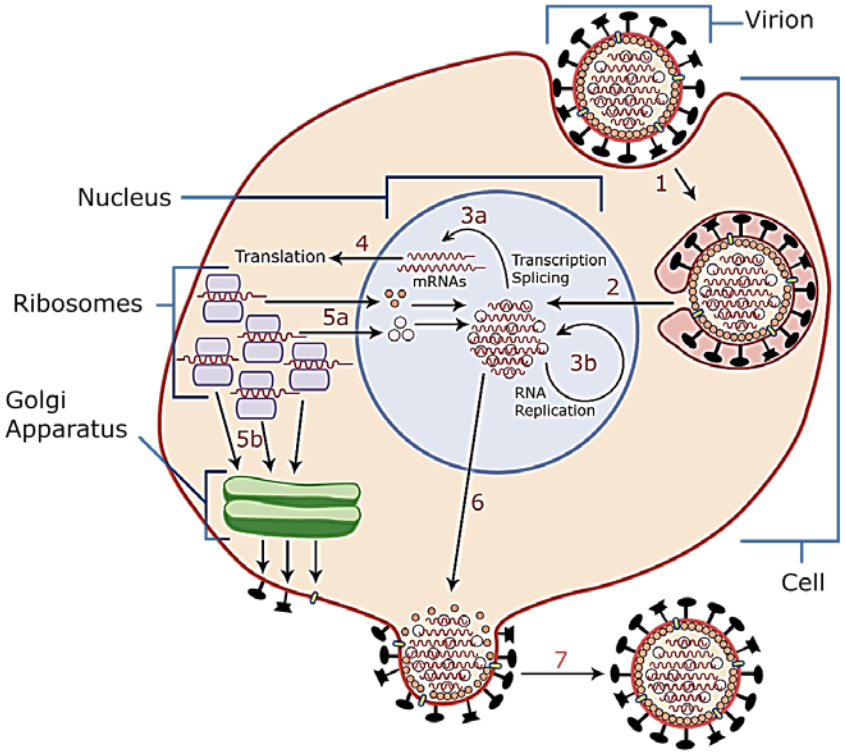


Fig. 68. Reproduction of viruses in a cell: virus adsorption on a cell (1), virus entry into the cell via endocytosis (2), transcription (3a), replication of viral nucleic acid (3b), synthesis of viral proteins on cellular ribosomes (5a), virion assembly (5b), viral budding and release from the cell (7) (https://students-library.com)

Virion entry into the cell is accompanied by its *deproteinization* (mediated by cellular enzymes), which involves removal of surface structures and subsequent release of its internal components (core, nucleocapsid, or nucleic acid).

The biosynthesis of virus components occurs in different parts of the cell, which is why it is referred to as disjunctive (from the Latin *disjunctus* – fragmented). All RNA viruses, except for influenza and retroviruses, are replicated in the cytoplasm of the host cell. In contrast, DNA viruses (except for poxviruses)

replicate in the nucleus, where transcription and replication take place, while viral proteins are synthesized in the cytoplasm. Viral proteins are produced through transcription, which involves “rewriting” information from the viral genome into messenger RNA (mRNA), followed by translation — reading the mRNA on ribosomes to synthesize viral proteins. The synthesis of viral nucleic acids occurs during replication (from the Latin *replicatio* – repetition).

Virions are assembled through self-assembly: viral components are transported to assembly sites within the nucleus or cytoplasm. The assembly process involves hydrophobic interactions, ionic bonds, hydrogen bonds, and steric complementarity. As a result of self-assembly, capsomers — formed from viral polypeptides — interact with viral nucleic acids to form nucleocapsids. In addition to virus-specific proteins, the supercapsid envelope of complex viruses includes other cellular membrane components.

Virions are released from the cell primarily via two mechanisms. The first is explosive release, where a large number of virions exit a dying cell simultaneously. This method is typical for simple viruses lacking a supercapsid. The mechanism is budding, characteristic of viruses with a supercapsid. In budding, the synthesized nucleocapsid is transported to regions of the cell membrane embedded with virus-specific proteins. These areas then protrude outward, forming a bud that separates from the cell as a mature complex virus particle. This process allows the host cell to remain viable for an extended period and continue producing viral progeny.

In addition to the productive interaction between the virus and the cell, an integrative or virogenetic type of interaction is also possible. Virogeny is characterized by the integration (embedding) of viral nucleic acid into the host cell genome, as well as by the replication and functional utilization of the viral genome as a component of the cell's genetic material. The nucleic acid of DNA viruses is directly integrated into the host's DNA (e.g., hepadnaviruses, papovaviruses). In contrast, the nucleic acid of RNA viruses cannot be directly integrated into the host DNA due to differences in their chemical structures. Therefore, RNA viruses (retroviruses) first synthesize a DNA strand from their RNA genome. This reverse synthesis is possible only because of a specialized enzyme present in retrovirus virions — reverse transcriptase (revertase). Viral DNA integrated into the host chromosome is known as a provirus. The provirus replicates within the chromosome and is passed on to the genome of daughter cells, making virogeny heritable. Certain physical or chemical factors can induce the provirus to enter an autonomous state, leading to productive interactions with the host cell. The additional genetic information carried by the provirus can confer new properties to the cell, potentially resulting in tumor development, autoimmune disorders, or chronic diseases. The ability of viruses to integrate into the host cell genome underpins their persistence (from the Latin *persisto* — to constantly reside, to remain) within the body and contributes to persistent viral infections. For example,

hepatitis B virus can cause chronic lesions and is often associated with chronic hepatitis and liver tumors.

Methods of Virus Culturing and Indication

Since viruses are obligate intracellular parasites, they are cultured in living cellular systems: *in laboratory animals bodies*, *developing chicken embryos, and cell cultures*. The main objectives of culturing include:

- Studying the mechanisms of viral pathogenesis;
- Developing methods for laboratory diagnosis of viral diseases;
- Producing drugs for the prevention, treatment, and diagnosis of viral infections.

Virus Culturing in the Bodies of Laboratory Animals. Laboratory animals used for virus culturing are selected based on the purpose of the study and the species' sensitivity to the virus being investigated. Monkeys, rabbits, guinea pigs, hamsters, white rats, and mice are commonly employed for infection studies.

Animals are infected through various routes depending on the tropism of the virus for specific tissues. For example, neurotropic viruses (such as rabies virus and tick-borne encephalitis virus) are typically introduced into the brain; respiratory viruses (such as influenza) are cultured via intranasal inoculation; and dermatotropic viruses (such as smallpox virus) are inoculated through cutaneous and intradermal routes. The most common routes of inoculation include cutaneous, intradermal, intramuscular, intraperitoneal, and intracerebral administration.

During initial infection, animals may not exhibit clinical symptoms, therefore after 5–7 days, seemingly healthy animals are euthanized. Their organs are then used to prepare suspensions for infecting subsequent batches of animals. These successive infections are referred to as *"passages"*.

The indication or confirmation of viral reproduction is based on the development of characteristic disease markers, pathomorphological changes in organs and tissues, or a *positive hemagglutination reaction* (HA). The hemagglutination reaction relies on the ability of certain viruses to cause agglutination (clumping) of erythrocytes from various species — animals, birds, and humans — due to the surface viral protein – *hemagglutinin*.

Currently, the use of animals for virus culturing is limited.

Culturing Viruses in Chicken Embryos. Chicken embryos provide a convenient and simple model for culturing viral cultures. Their cavities are protected by a hard shell and are sterile. Additionally, most known viruses have the ability to replicate in chicken embryos. Virus culturing in chicken embryos is widely used in industrial settings. To grow viruses, embryos aged between 8 and 14 days are used, depending on the virus species, the method of infection, and the objectives of the study. For example, influenza viruses are cultured in 9- to 10-day-old embryos; smallpox vaccines are produced in 12-day-old embryos; and mumps

virus is grown in 7-day-old embryos. The virus multiplies in different parts of the chicken embryo depending on its tropism. Therefore, a developing chicken embryo is infected by inoculating the virus into various sites, such as the chorioallantoic membrane, allantoic and amniotic cavities, yolk sac, and embryo body (Fig. 69).

The presence of the virus in the chicken embryo is confirmed based on specific lesions of the membranes and body, such as pocks or hemorrhages, as well as by hemagglutination reaction (HA).

Culturing Viruses in Cell Culture. This method is currently the most widely used.

A cell culture is a collection of similar cells derived from various organs and tissues of humans, animals, or plants, which are living and capable of reproduction in an artificial culture medium in vitro.

Cell cultures are grown using specialized laboratory glassware and utensils, such as mattrasses, flasks, test tubes, plates, Petri dishes, and other containers. Due to their ability to grow and reproduce more actively compared to normal cells of an adult organism, cell cultures derived from embryonic or malignant tissues have become widely utilized.

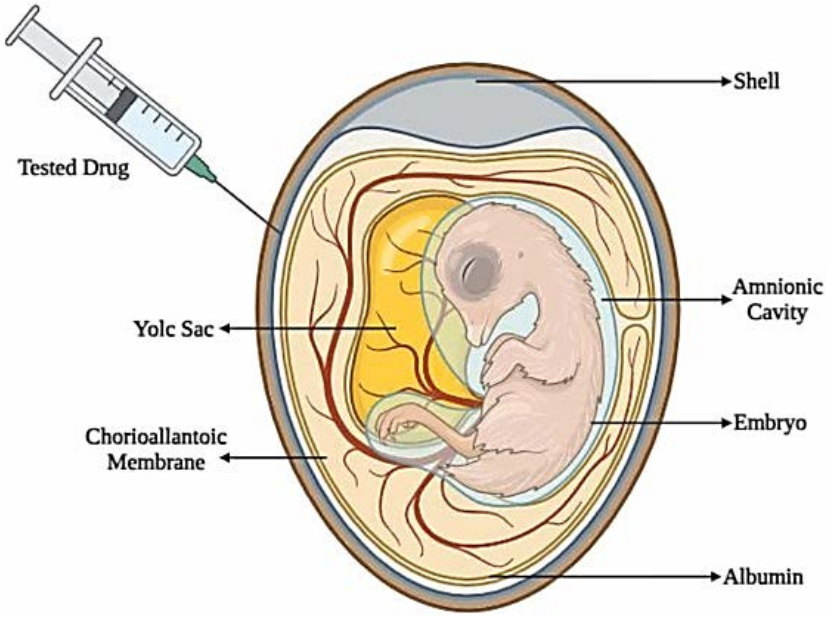


Fig.69. The structure and methods of chicken embryo infection (https://studopedia.ru)

There are three types of cell cultures depending *on their growth technique*:

1) Single-layer culture, which consists of a group of cells capable of attaching and multiplying on the surface of chemically inert laboratory glassware in a single layer;

2) Suspension culture, a type of cell culture in which cells multiply throughout the entire volume of the nutrient medium, which is continuously mixed. This type of culture is actively used in biotechnology to produce large quantities of viral biomass in industrial bioreactors for vaccine production or viral diagnosticums;

3) Organ culture, which involves whole pieces of organs and tissues that retain their original structure outside the body. Such cultures are used to a limited extent.

Single-layer cell cultures are the most widely used. They can be distinguished based on the *number of viable generations*, which depends on the number of passages. *Passage* (or subculturing) refers to the transfer of a small portion of cells from a cultured cell population into a new flask or other culture vessel with fresh medium for further growth. During conventional culturing, cells tend to age rapidly due to high density and accumulation of metabolic products; passages help ensure the long-term maintenance of the cell culture.

1. Primary cultures are obtained from human or animal tissues (e.g., monkey kidney cells, human or chicken fibroblasts). They are capable of reproducing only in the initial generations — i.e., in several passages after isolation — and then they die.

2. Semi-continuous or diploid cultures contain an unaltered diploid genome and have a limited lifespan (approximately 40-50 passages). These cells are derived from fetal tissues of humans or animals (such as human lung and pancreatic fibroblasts, breast epithelial cells). They are actively used both for culturing viruses and for producing viral vaccines for the prevention of diseases such as poliomyelitis, rubella, rabies, and others.

3. Continuous or stable cultures are capable of indefinite reproduction under laboratory conditions through ongoing passaging. Many originate from malignant tumor cell lines (malignant transformation). These cultures typically have an altered chromosomal set. Examples include *HeLa* cervical adenocarcinoma cells (obtained from a patient died in 1951), *Hep-2* laryngeal carcinoma cells, Rhabdomyosarcoma *RD* cells, and others. Continuous cell lines of animal origin are widely utilized, including cells from the golden Syrian hamster kidney (*BHK* cells), African green monkey kidney cells (*Vero* cells), and many others. An additional source of continuous cell lines can be primary culture cells that have been transformed in vitro.

To obtain cell cultures, tissues containing the desired cells are isolated and homogenized. Subsequently, they are treated with proteases (such as trypsin or collagenase) to disrupt intercellular septa, and the resulting cell suspension is transferred to a laboratory vessel containing culture medium. After incubation in a thermostat, the cells begin to divide and form a single layer (monolayer) at the bottom of the vessel. Once a confluent monolayer is established, cell proliferation ceases due to contact inhibition. If necessary, further passages of the cell culture are performed. Culture media are classified into growth and maintenance media. *Growth media* are enriched with nutrients to stimulate active cell division and monolayer formation. *Maintenance media* support cell viability in an already established monolayer and during viral reproduction within the cells. All nutrient media are based on a balanced buffered salt solution (e.g., Hanks' solution with a

pH of 6.8–7.2). To prevent bacterial or fungal contamination, growth factors such as amino acids or vitamins, as well as antibiotics, are added to the media. The most widely used standard synthetic media include Medium 199, Eagle's Minimal Essential Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), etc.

Any type of cell culture can be used for virus culturing. The infectious dose depends on the purpose and objectives of the experiment. Cell cultures are particularly valuable for isolating new or poorly studied viruses when traditional methods — such as infection of animals or chicken embryos — are insufficient to establish the viral nature of the pathogen. The selection of specific cell cultures is determined by their sensitivity to particular groups of viruses.

Viruses are identified in cell culture based on the following phenomena:

1. Cytopathic effect (CPE) refers to morphological changes in cells observable under a microscope, including detachment from the glass surface, resulting from intracellular viral replication. The nature of CPE varies among different viral infections. For example, some viruses such as paramyxoviruses and herpesviruses induce cell fusion and syncytium formation; others, including enteroviruses and reoviruses, cause cell shrinkage and destruction; while some, like adenoviruses, lead to cell aggregation.

2. Viral inclusion bodies are aggregates of viral particles or individual viral components within the cytoplasm or nucleus of infected cells, detectable under a microscope with special staining techniques. Inclusion bodies differ in size, shape, and abundance. Cells infected with herpesviruses, adenoviruses, influenza virus, rabies virus, smallpox virus, and others exhibit characteristic nuclear inclusion bodies.

3. Plaques, or negative colonies are localized areas of cell degeneration formed by viral infection in a monolayer under an agar overlay. They appear as light spots visible to the naked eye against a background of stained cells (e.g., with neutral red) during their development. Each plaque corresponds to progeny derived from a single virion. The size and shape of plaques vary among different viruses. Plaque assays are used for virus differentiation, selection, and quantification of viral concentration in the sample. The viral titer is expressed as plaque-forming units (PFU) per milliliter.

4. "Colored" test (Salk test). If viruses do not replicate in the cell culture, living cells secrete acidic metabolic products that change the pH of the medium and the color of phenol red indicator to yellow. When viruses are produced successfully, normal cellular metabolism is disrupted; cells die, and the medium retains its original red color. Thus, a red medium indicates the presence of virus and cessation of cell activity.

5. Hemadsorption refers to the ability of virus-infected cell cultures to adsorb erythrocytes from certain animal or bird species onto their surface. Hemadsorption appears as clusters of erythrocytes attached to virus-infected cells.

6. *Interference* describes the inhibitory effect one virus exerts on the replication of another virus. In this phenomenon, cells infected with one virus become resistant to subsequent infection by a different virus.

Bacteriophages

Bacteriophages (phages) are viruses that specifically parasitize bacterial cells. They are incapable of reproducing in eukaryotic cells. Bacteriophages are ubiquitous in nature, particularly in environments where bacteria are present. They are found in water, soil, human and animal discharges, food products, and other settings. Due to their stability, phages can persist in environmental objects for extended periods. During infections, bacteriophages are excreted from the body with feces, urine, and saliva alongside bacterial pathogens. Consequently, they serve as important epidemiological markers of infection. Bacteriophages are considered to be the most numerous and phylogenetically ancient group of viruses. The estimated number of phage particles on Earth (10^{30}–10^{31}) exceeds the total number of any other organisms, including bacteria. Phages play a crucial role in maintaining ecological balance by regulating microbial populations on the planet. They facilitate gene transfer between bacteria, which accelerates bacterial evolution and promotes the selection of adapted microorganisms. On one hand, phages are viewed as a potential tool for combating antibiotic-resistant bacteria, on the other hand, they must be controlled where bacteria are used for producing beneficial products, such as lactic acid.

Bacteriophages belong to 19 families. Representatives of nine families infect bacteria, nine infect archaebacteria, and one family includes phages capable of infecting both. Phages can be classified based on the type of nucleic acid they contain and their particle structure. Only two families consist of RNA-containing bacteriophages; the remaining families contain DNA genomes. Among these, two families have single-stranded DNA genomes, while the others possess double-stranded DNA. The genomic DNA can be either circular or linear.

When examined morphologically by electron microscopy, most phage particles resemble a spermatozoon or tadpole in shape. The main structural elements of the phage are the head, which contains the packed nucleic acid, and the “phage tail”, ending in a terminal tail plate or basal plate with short spikes and threads (tail fibers) for attachment to the bacterial cell (Fig. 70).

Some phages have a head covered with a lipid supercapsid, and the tail may be surrounded by a contractile protein shell (sheath). Phages with this structure exhibit a mixed symmetry type: the head is cubic, and the tail is helical. The average size of phages ranges from 20 to 200 nm, while filamentous phages can reach up to 800 nm. The total protein content in a phage particle is approximately 50–60%, nucleic acid can constitute up to 40–50%, and lipids account for about 1.5–3%.

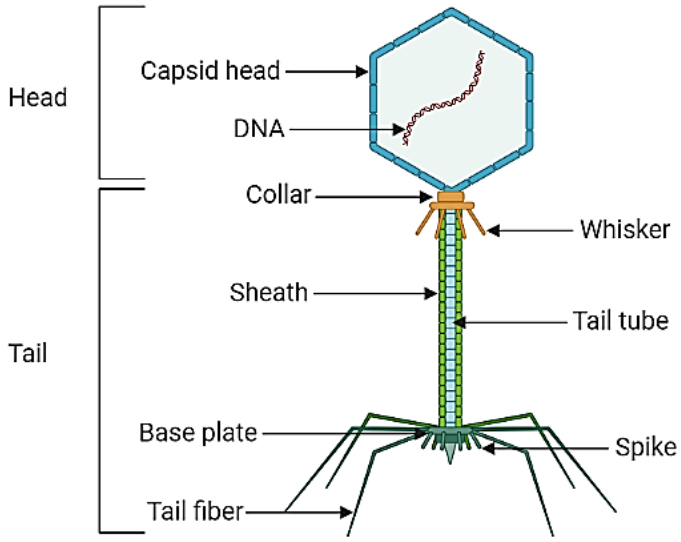


Fig. 70. The structure of bacteriophage (https://nikafarm.ru)

Escherichia coli T-phages (from the English *type* – typical), including seven representatives (T1-T7), have been extensively studied. Based on morphology, five main types of bacteriophages are distinguished:

1) DNA-containing filamentous phages;
2) RNA-containing phages with a tail rudiment (analog);
3) DNA-containing phages with a short tail;
4) DNA-containing process phages with a long tail and a non- contractile sheath;
5) DNA-containing phages with a contractile tail sheath and a basal plate.

To infect a microbial cell, phages contain the enzyme endolysin (also known as murein hydrolase) beneath their sheath, which functions similarly to lysozyme. Phages also contain ATPase and calcium ions, which are necessary for sheath contraction. The internal proteins within the phage head include polyamines such as spermine and putrescine, which bind to nucleic acids. This binding promotes supercoiling of nucleic acid and its tight packaging. Additionally, the head contains the enzyme transcriptase, responsible for transcribing phage DNA.

According to their interaction with bacterial cells, bacteriophages are divided into two groups:

1) *Virulent phages* – capable of productive infection, leading to bacterial lysis and the formation of new phage particles;
2) *Moderate* (*temperate*) phages – capable of integrating their DNA into the bacterial genome, resulting in integrative infection (*lysogeny*) and lysogenic conversion of the bacterial cell.

The interaction of bacteriophages with susceptible bacterial cells involves the following stages:

1) Adsorption;
2) Entry of the phage nucleic acid into the bacterium;

3) Phage replication;

4) Release of phage particles (for virulent phages) or establishment of lysogeny (for moderate phages).

Adsorption occurs through interactions between the phage particle's receptors – particularly spikes and fibers of the basal plate – and receptors on the bacterial cell membrane, such as lipopolysaccharides, teichoic acids, and proteins. Some phages adsorb to bacterial pili. The strength of binding depends on factors such as pH, temperature, and ionic strength of the medium. A single bacterial cell can adsorb up to approximately 300 phages. *The entry* of the phage nucleic acid into the bacterial host cell depends on the contractile proteins of the phage sheath. Endolysin degrades a section of the bacterial cell wall, activating ATPase; this causes the sheath proteins to contract, and the central tube of the tail pierces the cell membrane. Subsequently, the phage nucleic acid is injected into the host cell, while the head and tail proteins remain outside. *Phage reproduction* occurs in several stages. Initially, cellular metabolism ceases with the inhibition of bacterial nucleic acid and protein synthesis. Then, processes such as translation, transcription, and replication proceed, leading to the synthesis of phage proteins and nucleic acids. The maturation of phage particles, or the assembly of bacteriophages, involves filling the newly formed hollow capsids of the head with phage DNA or RNA. Simultaneously, the basal plate and the tail are assembled and connected to the phage head. The duration of reproduction ranges from 10–15 minutes to several hours. *The release* of mature phages most often occurs via lysis, involving destruction of the bacterial host cell by phage endolysins. A single bacterial cell can host several hundred phage particles. Some filamentous phages exit the host cell by seeping or being extruded through the bacterial membrane without killing it.

The interaction between phages and bacterial cells is highly specific. *Monovalent phages* react only with bacteria of a specific species, while *typical phages* interact only with certain variants (types) within a given bacterial species. Type-specific bacteriophages are used for identifying corresponding bacteria, a process known as phagotyping. *Polyvalent phages* can bind to multiple related bacterial species.

During the interaction of *moderate (temperate) phages* with a bacterial cell, the following processes occur: adhesion, injection of the phage nucleic acid, and its integration into or incorporation into the bacterial chromosome (prophage). Sometimes, the DNA of moderate phages can be found in the cytoplasm of a bacterium in the form of a plasmid. As part of the nucleoid, the prophage replicates simultaneously with the bacterial genome during bacterial reproduction. There is no bacterial lysis; however, the phage DNA is inherited from cell to cell by an unlimited number of descendants. The integration of the bacterial genome with a temperate phage is called *lysogeny*, and bacteria containing a prophage are referred to as *lysogenic*. A bacterial cell carrying a prophage becomes resistant to infection by an identical phage. Such cells produce repressors – phage proteins that inhibit

their own reproduction and prevent entry of identical phages into the cell. Under external influences such as ultraviolet or radioactive radiation, as well as chemical compounds, a prophage can be induced to enter the lytic cycle, transforming into a virulent bacteriophage. This process results in the formation of mature phage particles and subsequent lysis of the bacterial cell. A change in the properties of a microorganism due to the presence of an integrated prophage is known as phage or lysogenic conversion. An additional factor that can induce conversion is activation of silent bacterial genes if prophage genes act as promoters. Lysogenic conversion is common among many bacterial species and often leads to significant changes in their cultural, biochemical, antigenic, or other properties. The emergence of antibiotic-resistant or highly virulent bacterial strains through transduction is critically important in clinical settings. Notably, the transfer of toxin (TOX) genes by bacteriophages determines the production of exotoxins in pathogens responsible for diphtheria, botulism, and cholera.

The use of bacteriophages is based on their strict specificity for bacterial cells that are sensitive to them. In medicine, phages are empolyed for diagnosis, treatment, and prevention of bacterial infections. During diagnosis, bacteriophages are utilized to identify isolated bacterial cultures. Identification is performed using phages specific either to individual species, such as Shigella flexneri or Shigella sonnei, or to bacterial variants, for example, bacteriophages targeting Vibrio cholerae biovars. Type-specific phages are used for phagotyping, i.e., for determining the phagotype of an unknown isolated bacterial culture. The phagotype serves as a significant epidemiological marker of the isolated pathogenic strain, since the same bacterial species may contain dozens of different phagotypes. Determining phagotypes and comparing them across different strains of the same species allows for tracing the source and routes of infection.

Additionally, the detection of specific bacteriophages in environmental objects or clinical material may indicate the presence of corresponding bacteria (for example, detection of coliphages in water).

Phages are also used to treat and prevent infectious diseases. Due to the widespread occurrence of antibiotic-resistant bacterial strains, there is an increased interest in bacteriophages as highly specific agents for treating bacterial infections. Phages can also penetrate and multiply within microbial biofilms. Several types of phages – including those targeting typhoid, salmonella, dysentery, Klebsiella, Proteus, Pseudomonas, staphylococcal phages, coliphages, and combined phages – have been successfully produced. They are available in liquid formulations, tablets with acid-resistant coatings, ointments, and aerosols.

Bacteriophages serve as vectors for genetic engineering in biotechnology, enabling the insertion of human genes that encode hormones, cytokines, antibodies, or other substances into bacteria. They are also used for studying the functions of protein molecules and for creating gene and protein libraries, for example, through the use of “phage display” technology.

PHYSICAL AND CHEMICAL FACTORS AFFECTING MICROORGANISMS

Physical factors (such as *temperature, pressure, radiation,* etc.) can exert both adverse and beneficial effects on microorganisms. Adverse effects may slow down microbial growth and reproduction (static effect) or cause their death (bactericidal or fungicidal effect). Beneficial effects, on the other hand, promote the growth and reproduction of microorganisms, which is actively utilized in their culturing.

Temperature. Microorganisms are classified by their temperature preferences into *mesophilic*, *psychrophilic*, and *thermophilic* groups.

Mesophiles thrive at moderate temperatures, typically between 25°C and 40°C. This group includes the majority of both saprophytic and pathogenic bacteria.

Psychrophiles are microorganisms that inhabit cold environments such as ocean depths and tundra soils; they reproduce at temperatures below 20°C. *Thermophiles,* which can be found in environments like hot springs, are capable of multiplying at temperatures above 70°C.

Mesophilic bacteria, in the vegetative state, are sensitive to temperature increases, typically becoming inactivated at temperatures between 50 and 55°C. Such temperature increases can disrupt their cellular components, leading to protein denaturation, ultimately resulting in cell death. Spore-forming bacteria, particularly those from the genera Bacillus and Clostridium, are renowned for their remarkable resistance to heat. Many species can withstand temperatures between 100–110 °C for extended periods. However, the sensitivity of bacteria to elevated temperatures varies significantly due to factors like cultivation conditions, nutrient medium composition, exposure duration, and other variables. At temperatures 5-10°C below a bacteria's optimal growth temperature bacteria do not die; however, they significantly slow down their metabolic processes, thereby reducing their rate of reproduction. To preserve vegetative bacterial forms at low temperatures, substances with high viscosity, known as *cryoprotectants*, are used to protect the cytoplasm from damage by ice crystal formation. These include gelatin, albumin solution, glycerin, and 40% sucrose solutions. Cryoprotectors are used for the long-term storage of bacterial cultures at sub-zero temperatures and for the lyophilization of microorganisms.

Dehydration. When the relative humidity is less than 30%, most bacteria experience a slowdown in all life processes. Pathogenic microorganisms (causative agents of gonorrhea, meningitis, cholera, typhoid fever, dysentery, etc.) are most sensitive to dehydration. Microorganisms protected by sputum mucus are more resistant. Freeze-drying, or *lyophilization*, which is dehydration under vacuum from

a frozen state, is used to prolong viability and preserve microorganisms during the preparation of immunobiological products. Lyophilization involves the transition of a substance from a frozen state to a dry state, bypassing the liquid phase. Lyophilized cultures of microorganisms can be preserved for several years without changing their initial properties.

Osmotic and Hydrostatic pressure. Bacteria, yeasts, and molds are resistant to hydrostatic pressure. They can withstand pressures of 1,000–3,000 atmospheres (atm), while spore-forming bacteria can tolerate up to 20,000 atm. At such high pressures, the activity of bacterial enzymes and toxins decreases. Osmotic pressure negatively affects the biochemical activity of microorganisms. An increase in salt concentration inhibits the growth of many bacteria; however, some species can thrive in concentrated salt solutions – these are known as *osmophilic* (or *halophilic*) bacteria. The cytoplasmic membrane regulates the osmotic pressure within the cell. Under high osmotic pressure in the surrounding environment, *plasmolysis* occurs. Plasmolysis is a reversible phenomenon; if the osmotic pressure of the surrounding solution is reduced, water re-enters the cell, leading to a phenomenon opposite to plasmolysis, known as *plasmoptysis*.

Effects of Radiation. Most pathogenic bacteria poorly tolerate direct sunlight. This principle underlies the use of ultraviolet (UV) light to disinfect (sterilize) the air in healthcare facilities. Both UV light and X-rays, as well as other types of ionizing radiation, exert lethal or mutagenic effects on microorganisms. Short-wavelength UV rays, with a wavelength of approximately 280 nm, is the most effective. Such rays are absorbed by the cell's nucleic acids, damaging pyrimidine bases and leading to cell death due to lethal mutations. Some irradiated cells can recover through DNA *reparation* processes. Repair of irradiated DNA molecules occurs during photoreactivation of cells, which requires repeated exposure to longer-wavelength rays (520–550 nm) or can be achieved through "dark reactivation".

Radioactive radiation also exerts a detrimental effect on microorganisms; however, bacteria resistant to ionizing radiation have been identified. For example, *Deinococcus radiodurans (*formerly *Micrococcus radiodurans)* was isolated from a nuclear reactor.

Resistance to radiation depends on the microorganism's morphological and physiological state, exposure time, and radiation dose. Bacteria are generally more sensitive to ionizing radiation than viruses. The mechanism of action of ionizing radiation involves changes in cellular nucleic acids. In certain cases, ionizing radiation is used in healthcare practice for sterilizing drugs, plastic medical products, and surgical materials.

Ultrasonic Waves with an oscillation frequency of 1–1.3 MHz, applied for 10 minutes, exert a bactericidal effect on microbial cells. Ultrasound causes the rupture of cell walls and membranes and damages flagellin in motile microorganisms. The effect of ultrasound is based on the mechanical destruction of

microorganisms resulting from high intracellular pressure or the generation of hydroxyl radicals and atomic oxygen in the aqueous medium of the cytoplasm. This property allows ultrasound to be used as a sterilizing agent, as well as for the inactivation and disintegration of viruses to facilitate the production of antigens and viral vaccines.

Chemicals and their concentrations in the culture medium significantly influence the vital activity of microbes. They can serve as nutrient sources, have no effect, or either stimulate or inhibit growth. The various effects of chemicals also depend on the contact duration and the individual properties of the microbe. Chemicals capable of exerting a bactericidal effect are used for *disinfection*, which means the destruction of infectious disease agents in the environment.

Disinfection using chemicals is a part of comprehensive measures aimed at eliminating microorganisms not only in the environment but also within the macroorganism, such as in a wound. It forms the basis of *asepsis* and *antisepsis*. Disinfectants and antiseptics provide a non-specific microbicidal effect.

Antimicrobial antiseptic substances can be classified into several groups based on their chemical composition:

- Halogens (e.g., iodine and chlorine preparations) disrupt the enzymatic structures of bacterial cells, inhibit hydrolytic and dehydrogenase activities, and inactivate enzymes such as amylases and proteases.
- Hydrogen peroxide and potassium permanganate, similar to halogens, possess an oxidizing action.
- Acids and their salts, alkalis, alcohols, and aldehydes damage the surface structures of bacterial cells, including cell walls and membranes, thereby disrupting their selective permeability and other functions.
- Heavy metal compounds act through an antienzymatic mechanism by binding to sulfhydryl (SH) groups of protein molecules, which alters the structure of respiratory enzymes and interferes with oxidation and phosphorylation processes in mitochondria.
- Dyes exert a denaturing and lytic effect.

Antiseptic chemicals also include groups of 8-hydroxyquinoline derivatives (such as quinozole, nitroxaline, and quinolone) and nitrofuran (such as furacilin and furazolidone), which also disrupt biosynthetic and enzymatic processes within the bacterial cell.

The most commonly used disinfectants include chlorine-containing, phenolics, peroxides, and ammonium compounds.

ANTIMICROBIAL THERAPY OF INFECTIOUS

Antibiotics are specific products of cellular activity or their modifications that exhibit high physiological activity against certain groups of microorganisms (such as viruses, bacteria, actinomycetes, fungi, and protozoa) or malignant tumors. They selectively inhibit microbial growth or completely suppress their development.

There are *three methods of obtaining* antibiotics:

1. Biological synthesis. This method involves using highly productive strains of microorganisms and specialized nutrient media. The microorganisms produce an antibiotic, which is then isolated in pure form from the nutrient medium. Such antibiotics are referred to as natural antibiotics.

2. Chemical synthesis. This approach is used to produce all synthetic antibiotics or chemotherapeutic agents.

3. Combined method. This combines the first and second methods. The so-called core (for example, 6-aminopenicillic acid from penicillin) is isolated from an antibiotic produced by biological synthesis, and various chemical radicals are then added to it. This process imparts specific properties to the antibiotic. Antibiotics produced by this method are called semisynthetic (e.g., methicillin, oxacillin). Microorganisms resistant to natural antibiotics tend to remain sensitive to semisynthetic variants for a longer period. Additionally, the combined method is the most cost-effective way to produce antibiotics: from one natural antibiotic – which production cost is very high – it is possible to synthesize approximately 100 semisynthetic drugs with different properties.

Classification of Antibiotics

There are several approaches to classifying antibiotics; here are the main ones.

I. Classification of Antibiotics by Biological Origin

1. Antibiotics produced by microorganisms belonging to the order *Eubacteriales*.

A. Produced by representatives of the genus *Pseudomonas* (e.g., pyocyanin).

B. Produced by representatives of the genera *Micrococcus, Streptococcus, Chromobacterium, Escherichia, Proteus* (e.g., diplomycin, coliphin).

C. Produced by bacteria of the genus *Bacillus* (e.g., gramicidin, polymyxins).

2. Antibiotics produced by microorganisms belonging to the order *Actinomycetales*.

A. Produced by representatives of the genus *Streptomyces* (e.g., streptomycin, tetracyclines, and erythromycin).

B. Produced by representatives of the genus *Nocardia* (e.g., rifamycins, ristomycin).

C. Produced by representatives of the genus *Actinomadura* (e.g., carminomycin).

D. Produced by the genus *Micromonospora* (e.g., gentamicin).

3. Antibiotics produced by cyanobacteria (e.g., malingolide).
4. Antibiotics produced by imperfect fungi (e.g., penicillin, griseofulvin).
5. Antibiotics produced by fungi belonging to the classes Basidiomycetes and Ascomycetes (e.g., chaetomin).
6. Antibiotics produced by lichens, algae, and lower plants (e.g., usnic acid, chlorellin).
7. Antibiotics produced by higher plants (e.g., allicin, phaseolin).
8. Antibiotics of animal origin: (e.g., lysozyme, interferon).

II. Classification of Antibiotics by Spectrum of Biological Action

1. Narrow-spectrum antibacterial antibiotics, primarily active against Gram-positive microorganisms:
   - Penicillin and cephalosporin groups: Biosynthetic penicillins, semisynthetic penicillins, and semisynthetic cephalosporins;
   - Vancomycin, Ristomycin;
   - Lincomycin;
   - Novobiocin;
   - Macrolides.
2. Broad-spectrum antibacterial antibiotics:
   - Biosynthetic tetracyclines and semisynthetic tetracyclines (e.g., doxycycline);
   - Chloramphenicol;
   - Aminoglycosides (e.g., streptomycin, kanamycin, gentamicin);
   - Polymyxins.
3. Anti-tuberculosis antibiotics:
   - Streptomycin;
   - Kanamycin;
   - Viomycin;
   - Cycloserine.
4. Antifungal antibiotics:
   - Nystatin, Griseofulvin, Amphotericin B;

- Miconazole, clotrimazole, oxiconazole.

5. Antitumor antibiotics:
   - Adriamycin (Doxorubicin).
   - Daunomycin, Rubomycin.

III. Classification of Antibiotics by Chemical Structure

The classification based on chemical properties is founded on the fundamental similarity of molecular structures.

1. β-Lactams (penicillins, cephalosporins).
2. Aminoglycosides (streptomycin, gentamicin).
3. Tetracyclines (tetracycline, doxycycline).
4. Macrolides (erythromycin, oleandomycin).
5. Lincosamides (lincomycin).
6. Glycopeptides (vancomycin).
7. Oxazolidinols (linezolid).
8. Rifampicins (rifampicin).
9. Polymyxins.
10. Sulfonamides.
11. Quinolones, fluoroquinolones.
12. Nitrofurans (furazolidone).
13. Nitramidazoles (metronidazole).
14. Quinoxaline derivatives (dioxidine).
15. Sulfonamides with trimethoprim (co-trimoxazole).
16. Polyenes (nystatin, levarin, amphotericin B).
17. Chloramphenicol.

Mechanisms of Antibiotic Action

Antibiotics act selectively on bacteria. This means that the bacterial cell components targeted by antibiotics have structures that differ from those of eukaryotic cells, primarily human cells. Consequently, antibiotics exert their effects on bacteria without harming human cells. Antibiotics can either lead to the bacterial death (a *bactericidal effect*) or inhibit their growth and reproduction (a *bacteriostatic effect*).

All antibiotics share the characteristic that they primarily affect bacteria during their active growth and reproduction phases. This is because antibiotics (and other chemotherapeutic agents) interfere with the metabolism of bacterial cells and typically do not damage the preformed structures of dormant bacteria. The exceptions are antibiotics with *a membranotropic* activity – such as tyrothricin and polymyxins.

Consequently, antibiotics predominantly target pathogenic microorganisms, with their effects on normal microflora becoming significant only during prolonged

use. Pathogenetic colonization requires rapid bacterial reproduction and hightened metabolic activity, which increases bacterial susceptibility to antibiotics. This leads to a practically important conclusion: *antibiotics are less effective in chronic infections than in acute ones and are virtually ineffective in asymptomatic or oligosymptomatic carriage of pathogenic bacteria*.

Depending *on the mechanism of action*, antibiotics are classified as follows:

1. Antibiotics that inhibit cell wall synthesis, also known as anti-peptidoglycan antibiotics (e.g., penicillins, cephalosporins, bacitracin, vancomycin, D-cycloserine). Peptidoglycan is unique and essential for bacterial cells, making it an ideal target for antibiotics. Exceptions include *Mycoplasma* and *Chlamydia* (Mycoplasma genetically deficient in a cell wall, and Chlamydia lack a peptidoglycan layer in their cell wall), rendering them inherently insensitive to antibiotics that block cell wall synthesis.

Peptidoglycan synthesis involves three stages: 1) the formation of glycopeptide precursors and their transport to the cytoplasmic membrane; 2) the polymerization of these precursors into glycopeptide chains; and 3) the cross-linking of peptidoglycan chains by interpeptide bonds, catalyzed by enzymes called *transpeptidases*. These are known as penicillin-binding proteins (PBPs), which serve as targets for β-lactam antibiotics. Anti-peptidoglycan antibiotics exert bactericidal effects, but they only initiate the process; its completion is carried out by the bacteria's own enzymes – the *autolysins*.

2. Antibiotics that inhibit protein synthesis, also known as anti-ribosomal antibiotics (e.g., aminoglycosides – streptomycin, neomycin, kanamycin, etc.; macrolides – erythromycin, oleandomycin; tetracyclines; lincosamides; chloramphenicol, etc.). Prokaryotic ribosomes differ from eukaryotic ribosomes in key aspects, which underpins the selective targeting of the ribosomal cycle by these antibiotics. Aminoglycosides and tetracyclines bind to the 30S ribosomal subunit, disrupting the formation of *the ribosomal initiation complex*. Tetracyclines have a bacteriostatic effect by reversibly blocking the attachment of initiator aminoacyl-transfer RNA (tRNA) to the ribosomes. Aminoglycosides, however, kill bacteria by causing the formation of functionally incompetent 70S ribosomes and by distorting messenger RNA (mRNA) information. Macrolides, chloramphenicol, and lincomycin bind to the 50S subunit near aminoacyl-tRNA binding site, thereby terminating peptide chain elongation – an effect that is bacteriostatic. Fusidic acid inactivates the enzyme (translocase, also known as elongation factor G), which is responsible for the ribosome’s movement along the mRNA, facilitating the incorporation of the next amino acid into the peptide chain.

3. Antibiotics that disrupt membrane functions (e.g., polymyxins, gramicidins, nystatin, levorin, amphotericin B, trichomycin, candicidins, ascosin, albomycin, tyrothricin, endomycin, etc.).

4. Antibiotics that selectively inhibit nucleic acid synthesis (metabolism):

a) RNA synthesis inhibitors (e.g., actinomycin, griseofulvin, kanamycin, neomycin, novobiocin, etc.);

b) DNA synthesis inhibitors (e.g., mitomycins, novobiocin, sarcomycin, etc.).

5. Antibiotics that inhibit purine and pyrimidine biosynthesis (e.g., azaserine, sarcomycin, etc.).

6. Antibiotics acting as respiratory inhibitors (e.g., oligomycins, pyocyanin, usnic acid, etc.).

7. Antibiotics that inhibit oxidative phosphorylation (e.g., gramicidins, colicins, tyrocidin, etc.).

8. Antibiotics with antimetabolite properties (e.g., furanomycin – an antimetabolite of leucine, etc.).

Antibiotic Side Effects

Antibiotics can cause side effects, negatively affecting the host organism, other microorganisms, and altering the efficacy of other medications.

Complications of antibiotic therapy affecting the host organism include:

1. *Toxic reactions*. The severity of an antibiotic's toxic effect on the host organism depends on the drug's properties, its dosage, the route of administration, and the patient's condition. For example, tetracyclines and erythromycin exhibit *hepatotoxic effects*, while aminoglycosides are *nephrotoxic*. Additionally, tetracyclines interfere with *skeletal development and tooth enamel* formation, making them contraindicated for pregnant women and children under 12 years old. Chloramphenicol and sulfonamides can affect *hematopoietic organs*. Some cephalosporins may cause *bleeding* due to disruption of vitamin K synthesis.

2. *Dysbiosis*. The use of broad-spectrum antibiotics results not only in the death of pathogenic bacteria but also in the elimination of susceptible normal microflora. This creates an environment conducive to the proliferation of antibiotic-resistant microorganisms, which can lead to secondary endogenous infections. While it is impossible to completely prevent dysbiosis, its consequences can be minimized by several measures: first, using narrow-spectrum antibiotics whenever possible; second, prescribing antifungal agents concurrently with antibacterial therapy; third, employing probiotics and prebiotics to restore the normal microflora

3. *Negative impact on immunity*. *Allergic reactions* may occur following antibiotic administration. Their occurrence depends on the drug's properties (penicillins and cephalosporins are among the most allergenic), the method of administration (allergic reactions are more frequent with repeated antibiotic exposure), and the patient's individual sensitivity to the antibiotic. Many antibiotics also have *immunosuppressive effects*; for instance, chloramphenicol inhibits antibody formation, while tetracyclines impair phagocytosis.

Antibiotic-Induced Changes in Microorganisms. In addition to their adverse side effects on the host organism, antibiotics can induce changes in microorganisms themselves that are undesirable for humans.

1. Emergence of atypical microbial forms. Microbes can undergo changes in their morphological, biochemical, and other properties. For example, antibiotic therapy can lead to the formation of L-forms of bacteria. Microorganisms with altered properties are often difficult to detect.
2. Development of antibiotic resistance.

Another undesirable effect of antibiotics is the inactivation of other drugs. For instance, erythromycin induces the production of liver enzymes that destroy many co-administered medications.

Mechanisms of Bacterial Antibiotic Resistance and Strategies to Overcome Them

In a medical context, bacteria should be considered ***resistant*** *if they are not neutralized by antibiotic concentrations achieved in the body with pharmacological (i.e., clinically relevant) dosages*.

Understanding the nature of bacterial drug resistance is facilitated by a grasp of the fundamental principles of antimicrobial action, which can be summarized as follows:

1) The antibiotic must bind to the bacteria and penetrate their cell walls;
2) The antibiotic must be transported to the site of action;
3) The antibiotic must interact with intracellular targets.

Resistance can develop at each of these stages.

The resistance of a microbial cell to the antibiotic action can be either *intrinsic* (natural, innate or species-specific), meaning present from the moment of bacterial “birth”, or acquired, which develops as a result of antibiotic therapy. *Natural resistance* is encoded by chromosomal genes and is a species-specific characteristic. True innate resistance is characterized either by the absence of an antibiotic’s target within the microorganism or by the inaccessibility of that target due to inherently low permeability or enzymatic inactivation. For example, *Mycoplasmas* are inherently resistant to penicillin, as they lack the target for this antibiotic – peptidoglycan. Similarly, natural resistance of anaerobes to

aminoglycosides is explained by the fact that their transport across the cytoplasmic membrane is linked to electron transfer systems absent in anaerobes. Consequently, facultative anaerobes become significantly more resistant to aminoglycosides under anaerobic conditions than under aerobic conditions. When aminoglycosides and β-lactams act together on a microbial cell, β-lactams disrupt the structure of the bacterial cytoplasmic membrane, facilitating the transport of aminoglycosides. This results in a pronounced synergism between β-lactams and aminoglycosides.

Natural resistance is a constant species-specific property and is easily predictable.

Acquired resistance is associated with the acquisition of resistance genes (*r-genes*), carried by transposons (mobile or "jumping" genes) and plasmids (such plasmids are called R-plasmids). The emergence of acquired antibiotic resistance occurs either through changes resulting from spontaneous mutations in the bacterial chromosome or via the acquisition of R-plasmids by the bacterial cell. Resistance is then transmitted to other cells through reproduction or genetic exchange, leading to the spread of antibiotic resistance. In this scenario, the antibiotic acts as a *selective factor*: 1–3 years after the introduction of a new antibiotic, resistant bacterial strains emerge, and after 10–20 years, complete resistance to the drug may develop. Through mutation, a bacterium may acquire resistance to a single antibiotic, while the presence of R-plasmids can confer *multiple drug resistance*, often to 5–6 antibiotics simultaneously. Furthermore, a bacterial cell can possess multiple R-plasmids, resulting in the emergence of *polyresistant strains*.

One reservoir for r-genes is the normal microflora, which abounds with numerous bacterial species. This environment creates favourable conditions for the exchange of transposons and plasmids, especially since endogenous bacteria are often indirectly affected by antibacterial agents and are forced to survive in their presence, straining their genetic reserves for adaptation.

The following resistance mechanisms are identified:

1. Impaired cell wall permeability to the antibiotic and suppression of its transport to intracellular targets. This occurs due to the complete or partial loss of porin proteins. The *MAR system* (Multiple Antibiotic Resistance) has been extensively studied. Activation of the MAR system leads to a simultaneous decrease in the expression of one of the porin proteins (OmpF) and an increase in the activity of active efflux systems.

2. Modification of targets, meaning structural changes at the site of antibiotic action. For example, the targets of β-lactams are transpeptidases enzymes (PBPs – penicillin-binding proteins) involved in bacterial cell wall synthesis. Modifications can reduce the affinity for β-lactams, resulting in increased Minimum Inhibitory Concentrations (MICs) and decreased clinical efficacy. Resistance among staphylococci and pneumococci has significant clinical relevance. Staphylococcal resistance is due to the emergence of an additional PBP

(PBP2a) in microorganisms. The presence of PBP2a is indicated by resistance to methicillin or oxacillin. Regardless of *in vitro* susceptibility results, all β-lactams should be considered clinically ineffective and not used in therapy for infections caused by MRSA (methicillin-resistant *Staphylococcus aureus*). The primary mechanism of quinolone/fluoroquinolone resistance involves target modification – alterations in DNA gyrase and topoisomerase IV – bacterial enzymes responsible for conformational changes in the bacterial DNA molecule necessary for its normal replication. The main target of macrolides, ketolides, and lincosamides is the 50S ribosomal subunit of bacteria; methylation of this target confers high level resistance to these antibiotics.

3. Inactivation of the antibiotic, i.e., the synthesis of enzymes by the bacterial cell that destroy or modify the antibiotic. Examples include β-lactamases, which, through hydrolysis, cleave a bond in the β-lactam ring in penicillins and cephalosporins. Approximately 95% of Staphylococcus strains produce penicillinase, conferring resistance to penicillin. Penicillinase hydrolyzes penicillin into penicilloic acid, which lacks antibiotic activity. Of greatest significance are extended-spectrum β-lactamases (ESBLs), which destroy first- to fourth-generation cephalosporins but are susceptible to inhibitors. Resistance to aminoglycosides primarily involves *enzymatic inactivation* through modification. Modified aminoglycoside molecules lose their ability to bind to ribosomes and inhibit protein biosynthesis.

4. Active efflux of the antibiotic from the bacterial cell. For example, *Pseudomonas aeruginosa* possesses a system for actively expelling carbapenems from the cell, while several transport systems facilitate the active efflux of macrolides and lincosamides.

5. Formation of a metabolic shunt, which decreases target physiological significance by duplicating pathways for vital metabolite synthesis. Resistance to trimethoprim may result from acquisition of dihydrofolate reductase genes insensitive to inhibition, while resistance to sulfonamides may involve acquisition of dihydropteroate synthetase genes.

6. Competitive binding or interception of antimicrobial agent.

Principles of Rational Antibiotic Therapy

I. Microbiological principle. Antibiotics must be used strictly according to indications. To select the appropriate drugs, it is necessary to collect patient's samples for examination, isolate a pure culture of the pathogen, and determine its sensitivity to antibiotics – an antibiogram – before initiating treatment.

II. Pharmacological principle. This principle is based on correct drug dosage, adherence to necessary intervals between drug administrations, appropriate duration of antibiotic therapy, methods of administration, knowledge of drug's

pharmacokinetics, and consideration of potential drug combinations. Typically, infectious diseases are treated with a single antibiotic (monoantibiotic therapy). For chronic conditions (e.g., subacute septic endocarditis, tuberculosis), combination therapy with multiple chemotherapeutic agents is employed to prevent the development of antibiotic resistance.

III. Clinical principle. When prescribing antibiotics, factors such as the patient's overall condition, age, sex, pregnancy status, immune status, and presence of concomitant diseases should be considered.

IV. Epidemiological principle. The choice of antibiotic should be informed by knowledge of local resistance patterns – specifically, which antibiotics microorganisms in the patient's environment (in the ward, hospital, or geographical region) are resistant to, and how prevalent antibiotic-resistant strains are. The prevalence of resistance to a particular antibiotic is not constant but varies depending on its usage frequency.

V. Pharmaceutical principle. It is essential to consider the expiration date and storage conditions of the medication since prolonged or improper storage can lead to loss of antibiotic activity and may result in the formation of toxic degradation products.

Determination of Microbial Susceptibility to Antibiotics

Serial Dilution method

This method is based on the direct determination of the *Minimum Inhibitory Concentration* (MIC), defined as the lowest concentration of an antibiotic that inhibits visible growth of the test microorganism. Depending on the type of culture medium used, there are serial dilution methods performed on agar or in broth. Based on the volume of liquid culture medium employed, methods are categorized into macro- and micro-dilution techniques.

A variation of the serial dilution method involves using only two antibiotic concentrations, which correspond to the threshold levels that distinguish susceptible microorganisms from intermediate ones, and intermediate from resistant ones.

To perform the method, serial dilutions of the tested antibiotic are prepared in sterile, transparent nutrient broth. A specified number of test microbial cells is then added to each test tube (see Fig. 71). The tubes are incubated in a thermostat for 20–24 hours at the optimal temperature for the test microorganism. Afterward, the presence or absence of test culture growth is determined in the tubes. Microbial growth in the broth (indicated by turbidity) signifies that the antibiotic concentration is insufficient to inhibit its viability. The lowest antibiotic concentration at which no visible bacterial growth occurs is considered the Minimum Inhibitory Concentration. The MIC is expressed in mg/L or μg/mL.

Disk Diffusion Method (Disk method)

To determine microbial susceptibility to antibiotics using the disk diffusion method, a suspension of the test microorganism is evenly spread over the surface of agar in a Petri dish. Disks impregnated with a specific amount of antibiotic are then placed onto the agar surface. The diffusion of the antibiotic into the agar creates a zone of inhibited microbial growth around the disks. After incubating the plates in a thermostat at 35–37°C for 20–24 hours, the results are interpreted by measuring the diameter of the inhibition zone around each disk in millimeters (see Fig. 72).

Based on the diameter of the inhibition zone, the microorganism's susceptibility to the antibiotic is determined as follows:

- Up to 10 mm: Resistant
- 11–15 mm: Low Susceptibility
- 16–25 mm: Susceptible
- More than 25 mm: Highly Susceptible

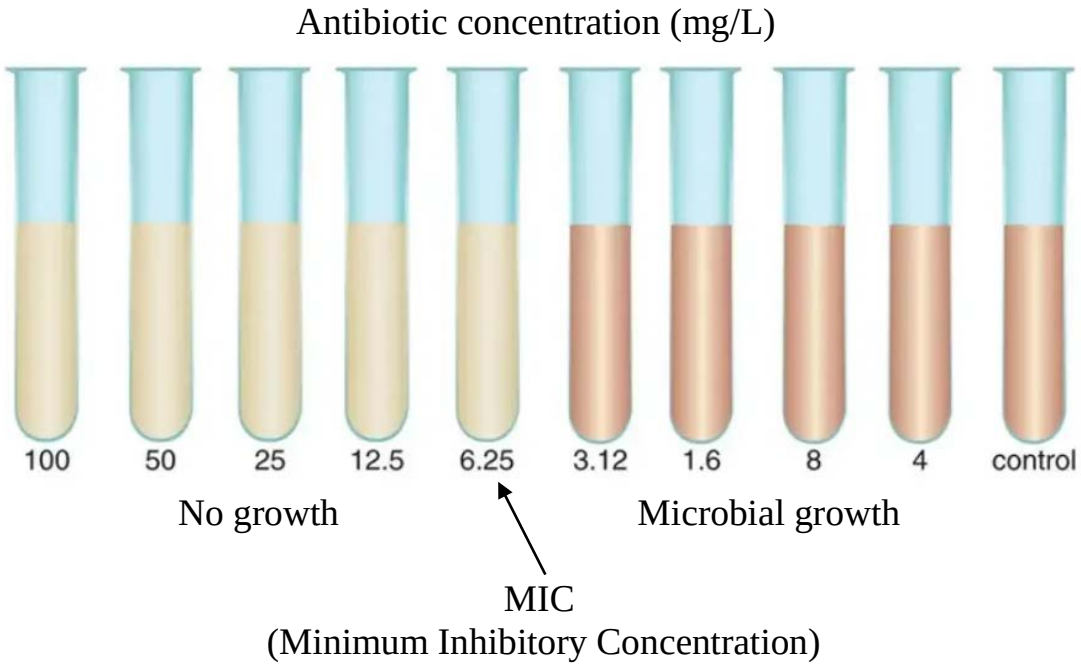


Fig. 71. Scheme of the Serial Dilution Method for Determining the MIC of an Antibiotic

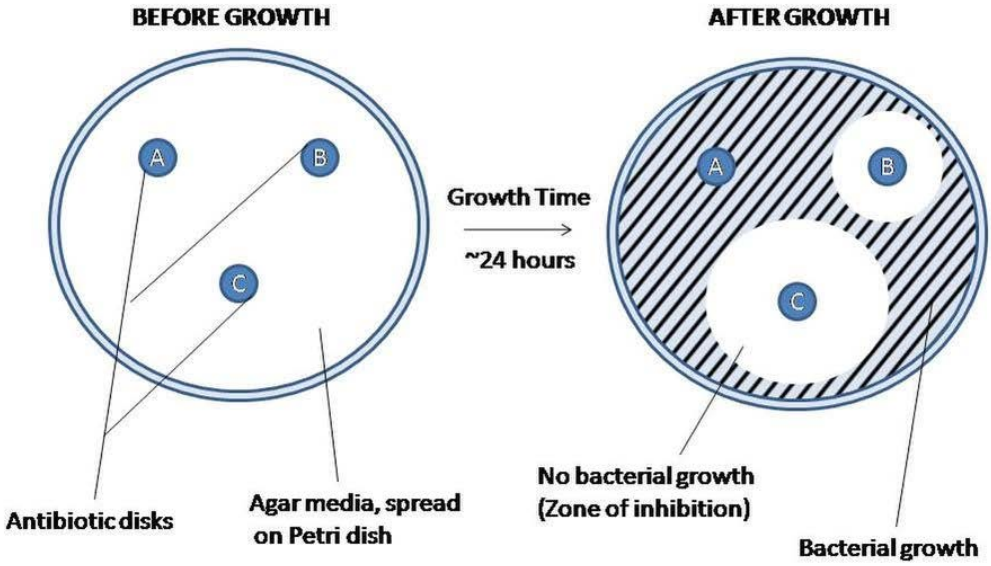
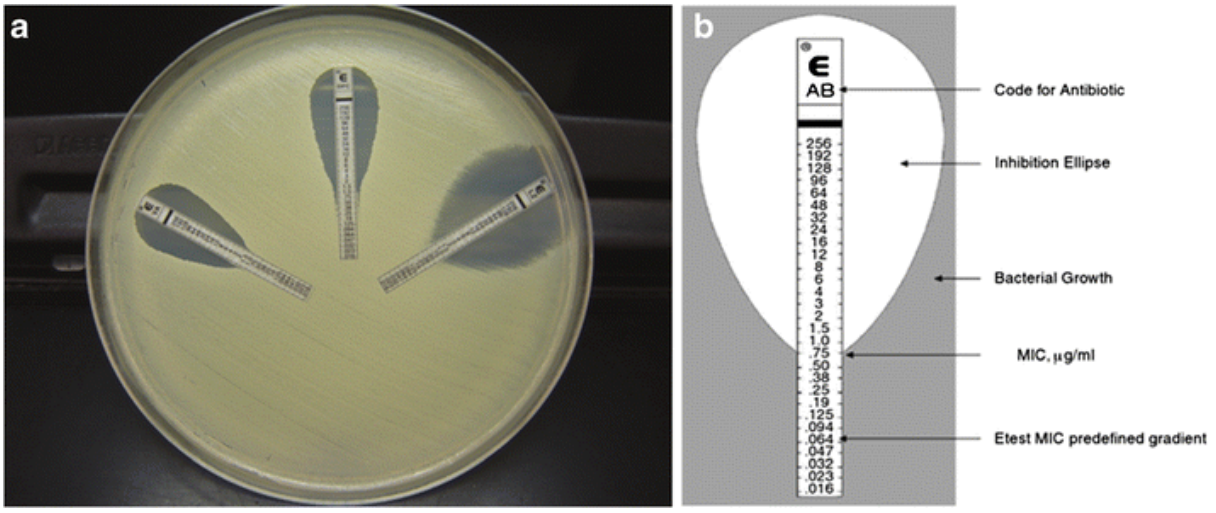


Fig. 72. Scheme of the Disk Diffusion Method for Determining Microbial Susceptibility to Antibiotics (https://www.wikilectures.eu/w/Disk_diffusion_test)

E-test

The determination of microbial susceptibility using the E-test is performed similarly to the disk diffusion method. The key difference is that, instead of a disk impregnated with an antibiotic, an E-test strip is used. This strip contains a gradient of antibiotic concentrations ranging from maximum to minimum (see Fig. 73). The point where the elliptical zone of growth inhibition intersects the E-test strip indicates the Minimum Inhibitory Concentration (MIC) value.



a *b*

Fig. 73. E-test Photo (a) and Scheme (b). (https://www.researchgate.net/publication/319477187_In_vitro_antimicrobial_susceptibility_testing_methods_agar_dilution_to_3D_tissue-engineered_models/figures?lo=1)

SANITARY MICROBIOLOGY

Sanitary microbiology is a branch of general microbiology focused on the study of microflora in the environment and its impact on human health and his environment. Studying microflora and microbiological processes in the human environment is essential for the hygienic assessment of his relationship with the surroundings. Sanitary microbiology develops methods for controlling the state of water, soil, air, food products, and various household items. Sanitary-microbiological techniques are employed to determine total microbial content (total microbial count), identify and quantify sanitary indicator microorganisms, detect pathogenic microorganisms and their metabolites in the examined samples, and assess the extent of spoilage in the examined samples or products caused by microbial activity.

Practical sanitary microbiology employs two primary methods to assess the sanitary and epidemiological state of the external environment: *direct detection of pathogens or their toxins* in environmental objects, and *identification of indirect signs of pathogen presence in the external environment*.

Direct detection methods are the most accurate and reliable criteria for assessing the epidemiological risk posed by the external environment. Typically, they involve culturing the tested material on nutrient media. The limitations of these methods include the low concentration of pathogens in the sample and their uneven distribution within the environment. Direct methods involve multifaceted procedures, which are practically challenging to implement. This limitation is further compounded by the increasing role of opportunistic microorganisms capable of causing epidemic outbreaks.

Indirect identification methods rely on assessing two key indicators that allow for an indirect assessment of the possible presence of pathogens in the environment: *the total microbial count (TMC)* and the presence of *sanitary indicator microorganisms (SIM).*

The *TMC* is determined by counting all microorganisms that grow on nutrient media in a given sample — per 1 gram (g), 1 milliliter (ml), or 1 cubic meter (m^3) of substrate. Using this indicator, it is generally assumed that higher levels of organic matter contamination tend to correlate with increased TMC and a greater probability of pathogenic microorganisms being present.

Sanitary indicator microorganisms (SIM) are those that can be used to indirectly assess the potential presence of pathogens in the external environment. This approach is based on the assumption that a higher degree of contamination of an object with human and animal excreta typically indicates a higher number of SIM and, consequently, a greater likelihood of pathogen presence.

Sanitary indicator microorganisms must meet several key requirements:

1) They must constantly inhabit the natural cavities of the human and animal bodies and are excreted into the environment.

2) Their survival time in the environment should be equal to or longer than that of pathogenic microorganisms excreted from the body via similar routes.

3) They should not multiply in the environment.

4) They should not significantly alter their biological properties when released into the environment.

5) They should not have “doubles” or analogues in the external environment that could cause confusion.

6) The detection, identification, and quantitative assessment of SIM should be performed using modern, simple, accessible, and cost-effective microbiological methods.

All SIM are indicators of biological pollution. There are several groups of SIM, and the detection of these in environmental samples indicates different types of contamination.

1. Group A includes microorganisms that inhabit the intestines of humans and animals. They serve as indicators of fecal contamination. This group comprises coliform group bacteria (CGB), such as *Escherichia, Citrobacter, Enterobacter*, and *Klebsiella*. Additionally, this group includes *Enterococci, Proteus, Salmonella, Clostridia, Thermophiles, Bacteroides, Bacteriophages*, and others.

2. Group B consists of microorganisms that inhabit the upper respiratory tract and nasopharynx. They are indicators of aspiration contamination. This group includes *Staphylococci* (e.g., *S. aureus*), as well as *Viridans* and *Hemolytic streptococci*, which constantly inhabit the mucous membranes of the upper respiratory tract and are released into the air during talking, coughing, or sneezing.

The content of SIM is expressed in titers and indices. The *SIM titer* is defined as the minimum volume of material under study (in milliliters (mL) or cubic meters (m^3)) or weighted quantity (in grams (g)) in which at least one individual SIM is detected. The SIM index represents the amount of SIM detected in a specific volume or quantity of the sample. The index is the inverse of the titer. Knowing one value allows for the calculation of the other.

Soil microflora

Soil is composed of mineral and organic compounds. It is a product of the metabolic activity of microorganisms that facilitate its formation, self-purification, and the cycling of nitrogen, carbon, sulfur, and iron in nature. Soil microorganisms fix nitrogen from the air, produce soil humus, and release nutrients for plant uptake, thereby performing a sanitary function for the soil. Soil serves as a primary habitat for a vast array of microorganisms, and these microbes can be transferred from soil into water bodies and even dispersed into the air. The soil microflora encompasses all known microbial groups, including spore-forming and non-spore-forming

bacteria, actinomycetes, fungi, spirochetes, protozoa, blue-green algae, mycoplasmas, and viruses. A single gram of soil can contain up to 6 billion microbial cells. Actinomycetes and aerobes are typically found in the upper layers of soil. Conversely, fungi and anaerobes are more common in the lower layers. The quantitative distribution of microorganisms in soil is uneven, with the topmost layer (1 – 2 mm thick) having relatively fewer microorganisms due to their rapid death under the influence of factors like sunlight and dryness. The subsequent layer, between 10 and 20 centimeters deep, is typically the most densely populated with various microorganisms, where they drive significant biochemical processes. As soil depth increases, the overall number of microbes gradually decreases. However, microbes are still found at significant depths. Regardless of depth, the rhizosphere, the soil zone surrounding plant roots, is always the most densely populated area. The qualitative composition of microorganisms in the rhizosphere is influenced by the specific plant species, but fungi tend to be a dominant group across different plant types. The rhizosphere typically contains several thousand times higher concentration of microorganisms compared to the soil away from plant roots.

The qualitative and quantitative composition of soil microflora is influenced by various environmental factors. These include soil type, fertility, moisture content, aeration, and the soil's physical and chemical properties. Human activities have a substantial impact on soil microbiocenosis. These impacts stem from various practices, including tillage, fertilization, land improvement, and pollution from industrial waste. Pathogenic microorganisms can enter the soil through human and animal excrements. Untreated livestock waste, including manure, urine, and carcasses, exhibit particular sanitary risks.

Soilborne pathogenic microorganisms that pose a threat to human health can be categorized into three groups. The first group consists of microorganisms that are permanent residents of the soil, and this includes actinomycetes and fungi that can cause mycoses in humans. The second group consists of spore-forming microorganisms, which use soil as a secondary reservoir for long-term survival (often decades). This group includes the causative agents of anthrax, tetanus, botulism, and gas gangrene. The third group comprises pathogenic microbes and viruses that, when introduced into soil via human or animal waste, can survive for varying durations, ranging from a few hours to several months (e.g., *Escherichia coli*, *Salmonella*, *Shigella*). The risk of disease transmission through soil contaminated with these pathogens is generally considered low and heavily influenced by the level of contamination.

Sanitary and Microbiological Soil Analysis

Soil analysis can involve both comprehensive and brief analyses. A comprehensive sanitary and bacteriological soil analysis is conducted for the following purposes:

a) for a detailed and in-depth assessment of the soil's sanitary condition;

b) for determining soil suitability for finding of sites for residential and recreation areas, childcare centers, and water supply facilities;

c) for epidemiological investigations.

A brief soil analysis is recommended for routine sanitary surveillance and includes determining total bacterial count (TBC), coliforms (coliform titer and coliform index), clostridia (*Clostridium perfringens* titer), and thermophilic and nitrifying bacteria.

A comprehensive sanitary and bacteriological soil analysis additionally involves the identification and quantification of *Actinomycetota*, fungi, *Salmonella*, *Shigella*, and pathogens responsible for tetanus, botulism, brucellosis, and anthrax.

Preparing soil for testing starts with clearing of inclusions like stones and glass fragments, etc. Large soil aggregates are crushed. The number of soil samples taken for testing is related to the testing purpose and objectives. The soil sample is thoroughly mixed with distilled (or purified) water at a ratio of 1:10. The resulting suspension is shaken for 10 minutes and allowed to settle for 2–5 minutes. From the initial 1:10 dilution, a series of subsequent tenfold dilutions are prepared, ranging from 1:10 to 1:1,000 for testing clean soils, and up to 1:10,000 for analyzing heavily contaminated soils.

Determining Total Bacterial Count (Soil TBC). The soil microbial count refers to the total number of microorganisms present in one gram of soil. A sample of the prepared soil suspension is introduced onto the surface of the nutrient medium (Meat-Peptone Agar, also known as MPA) in Petri dishes, using 1 ml of each dilution. Subsequently, 7–10 mL of melted agar, cooled to 45°C, is poured over the inoculated dishes. The cultures are incubated at 28–30 °C for 72 hours, and the number of colonies formed is counted. If more than 150 colonies develop on a Petri dish, counting is performed on one-quarter of the surface area, with subsequent recalculation for the entire area. The arithmetic mean of colonies counted across all Petri dishes is calculated, and then the number of microorganisms per gram of soil is determined by accounting for the dilutions.

Determining Coliform Bacteria. ***The coliform index*** indicates the number of viable *Escherichia coli* bacteria in one gram of soil. If a low degree of fecal contamination is suspected, coliform bacteria in the soil are quantified using either the titrimetric (fermentation) method or the membrane filter technique. In cases of high contamination levels, direct inoculating Endo medium with a 1:10 dilution of a soil suspension is performed.

Membrane filter method involves centrifuging 1:10 soil suspension with subsequent filtering of 5–10 mL of the suspension through membrane filters. The filter, with bacteria trapped on its surface, is then placed onto a Petri dish containing Endo medium. The filter is carefully positioned using sterile forceps, ensuring the bacterial side faces upwards. The Petri dishes are incubated at 37°C for 24 hours. If coliform bacteria are present in the soil, colonies typical of *Escherichia coli* will

appear on the filters — these are dark red with a metallic sheen or pink with a red center. After observing these colonies, smears are prepared and then stained using Gram's method. To differentiate between the families of *Enterobacteriaceae* and *Pseudomonadaceae*, an oxidase test is employed. Colonies are considered coliforms if they are Gram-negative rod-shaped bacteria that ferment glucose and produce acid and gas, and lack proteolytic activity. Colonies are counted on those filters where the colony count falls between 30 and 50 from the corresponding dilution of soil suspension. The number of colonies grown on the filters is recalculated per gram of soil, and the coliform index is subsequently determined.

Soil Coliform Titer refers to the smallest quantity of soil in which viable *E. coli* can be detected. *Titrimetric method*. From prepared soil suspension dilutions, inoculations are made into Kessler's nutrient medium containing peptone, bile, lactose, and gentian violet. For the 1:10 dilutions, 10 mL are inoculated into a flask containing 50 mL of medium, which corresponds to 1 gram of soil. For smaller quantities (0.1 mL and 0.01 mL), 1 mL from the corresponding soil suspension dilutions is inoculated into test tubes containing 9 mL of medium. If growth or growth accompanied by gas formation is observed in these cultures, further inoculation is performed onto Petri dishes with the medium, incubated for 24 hours at 37 °C. Subsequent identification of the isolated bacteria is then carried out. The result is expressed as the coliform titer. The absence of growth and gas formation in all tubes indicates the absence of coliform bacteria.

Determining Clostridium Perfringens Titer. ***Clostridium Perfringens Titer of soil*** refers to the smallest weight quantity of soil, expressed in grams, in which a viable cell of *C. perfringens* is detected. Determining the perfringens titer is a crucial criterion for soil sanitary assessment and its self-purification capacity, as *E. coli* typically disappear from fecally contaminated soil within 4–5 months, whereas *C. perfringens* can still be found at a titer of 0.01. The perfringens titer allows for an estimation of the duration of fecal contamination. From the prepared dilutions of the soil suspension, 1 mL is transferred into two parallel rows of test tubes. One row is heated at 80°C for 15 minutes. Then, 9–10 mL of melted and cooled-to-45°C Wilson-Blair medium are added into all test tubes. Cultures are incubated at 43°C for 24 hours; however, after 2–3 hours, a positive result can be indicated by the formation of round black colonies within the agar. Smears prepared from these colonies typically reveal characteristic gram-positive rods.

Determination of thermophilic bacteria. Thermophilic bacteria are counted on Meat-Peptone Agar (MPA), which is poured into Petri dishes in a thicker layer than usual. The inoculation is performed using dilutions of 1:10, 1:100, and 1:1000, with 2–3 parallel dishes recommended for each dilution. Thermophilic bacteria are cultured at approximately 60°C. The results are calculated 24 hours after inoculation. The number of bacteria is calculated per gram of soil.

The sanitary and microbiological analysis of soil is performed using a set of indicators. For sanitary analysis of soil, the indicators outlined in Table 4 shall be used.

Table 4

Scheme of Soil Sanitary Status Based on Microbiological Indicators

Soil category	Titers		Number of thermophilic bacteria (per gram)
	coli titer	perfringens -titer	
Clean	1 and higher	0.01 and higher	100 – 1,000
Contaminated	0.9 – 0.01	0.009 – 0.0001	1,000 – 100,000
Heavily contaminated	0.09 and lower	0.00009 and lower	100,000 – 4,000,000

Water Microflora

Water is a natural habitat for microorganisms. Within aquatic environments, specific biocenoses develop, dominated by microorganisms adapted to the local conditions. The qualitative and quantitative composition of water microflora depends on the composition and concentration of mineral and organic substances, their temperature, pH, water flow velocity, and the volume of stormwater, domestic sewage, and industrial wastewater entering the water body. The microbial count is directly proportional to the degree of water body pollution. Ponds, streams, and lakes in densely populated areas are particularly rich in microorganisms. In enclosed water bodies such as lakes and ponds, a distinct pattern in bacterial distribution is observed. The microbial composition varies between the surface and the bottom of these water bodies. Water at depths of 10–100 cm is most abundantly populated with microorganisms, while their numbers significantly decrease at greater depths. Spring waters and artesian well waters are among the purest. Water microflora actively participates in the self-purification process from organic waste. Microorganisms that are part of the autochthonous (permanent) microflora present in water facilitate organic waste recycling. There are various bacteria in freshwater bodies: rod-shaped (e.g., *Pseudomonas*, *Aeromonas*), coccoid (e.g., *Micrococci*), as well as twisted and filamentous bacteria (e.g., *Actinomycetota*). The number of anaerobic bacteria increases at the bottom of water bodies within the silt layer. When water is contaminated with organic substances, a large number of non-permanent (allochthonous) water microflora appear, which then disappear during the natural self-purification process. Water can serve as a vector for transmitting pathogens responsible for many infectious diseases. Along with polluted rainwater, meltwater, and wastewater, lakes and rivers receive normal human and animal microflora — such as *Escherichia coli*, *Citrobacter*, *Enterobacter*, *Enterococci*, and *Clostridia* — as well as pathogens causing intestinal infections (e.g., typhoid

fever, paratyphoid fever, dysentery, cholera, leptospirosis, enterovirus infections, cryptosporidiosis, etc.). While many of these bacteria are not adapted to survive in water and die after some time, they can persist in water for a certain period, and some pathogens can even multiply within aquatic environments (e.g., *Vibrio cholerae*, or *Legionella*). Therefore, water can act as a vector for transmitting infectious diseases, necessitating sanitary and microbiological monitoring of water quality.

Sanitary and Microbiological Water Analysis

Water from centralized supply systems, wells, open water bodies, swimming pools, and wastewater are all subjects to analysis. The following indicators are used to assess the sanitary and bacteriological status of water:

1) Determining total microbial count (TMC);
2) Identification of bacteria of Enterobacteriaceae family and thermotolerant coliform bacteria;
3) Detection of sulfite-reducing spores of *Clostridia*;
4) Identification of coliphages;
5) Detection of pathogenic intestinal bacteria.

The analysis of drinking water for coliphages and pathogenic intestinal bacteria is performed in accordance with epidemiological criteria. The detection of sulfite-reducing *Clostridia* spores is carried out to evaluate the effectiveness of water treatment technologies.

Determining Total Microbial Count (*TMC)*. To determine the TMC, water samples are inoculated onto nutrient media, and the resulting colonies are subsequently counted. The volume of water used for inoculation is selected to yield between 30 and 300 colonies per plate. For tap water, 1 mL is inoculated; for water from open reservoirs, volumes of 1 mL, 0.1 mL, and 0.01 mL are employed (with tenfold dilutions prepared). Each dilution is inoculated using the pour plate method. This involves aseptically pipetting 1 mL of water into two sterile Petri dishes, each containing 6–8 mL of melted and cooled-to-45°C nutrient medium (Meat-Peptone Agar, MPA), cooled to 45°C. The contents are mixed thoroughly, allowed to solidify, and then incubated for 24 hours. After incubation, the number of colonies on each plate is counted, and the arithmetic mean is calculated. The results are expressed as the number of colony-forming units (CFUs) per 1 mL of water.

Identification of Bacteria of Enterobacteriaceae Family and Thermotolerant Coliform Bacteria. Thermotolerant coliform bacteria possess all the characteristics of the family Enterobacteriaceae but are distinguished by their ability to ferment lactose to produce acid and gas at 44°C within 24 hours.

The membrane filter method is most commonly used to detect bacteria of the family Enterobacteriaceae and thermotolerant coliform bacteria. This technique involves filtering a specified volume of water through membrane filters, culturing colonies on a differential diagnostic medium, and subsequently identifying colonies

based on cultural and biochemical characteristics. Filtration is performed using specialized equipment. The volume of water sample depends on the aim of the study. For tap water analysis, 300 mL is passed through three membrane filters, 100 mL per filter. The filters are then placed onto the Endo medium in Petri dishes and incubated at 37°C for 24 hours. Colonies are counted based on their color and appearance: red colonies or red colonies with a metallic sheen. Bacterial identification involves performing an oxidase test and assessing acid and gas production during lactose fermentation, with at least 10 colonies examined for this purpose.

All lactose-positive colonies are inoculated into two test tubes containing lactose-enriched media. One tube is incubated at 37°C to cultivate microorganisms of the Enterobacteriaceae family, while the other at 44°C for cultivating thermotolerant coliform bacteria.

Bacteria from the Enterobacteriaceae family serve as indicators of long-standing fecal contamination of water, whereas thermotolerant coliform bacteria indicate recent fecal contamination.

The analysis results are expressed as the number of colony-forming units (CFUs) of Enterobacteriaceae and thermotolerant coliform bacteria per 100 mL of water.

Detection of Sulfite-Reducing Bacteria Spores. Sulfite-reducing *Clostridia* species, primarily *C. perfringens*, are spore-forming anaerobic rods that reduce sodium sulfite on iron sulfite agar at 44°C within 24 hours. To detect sulfite-reducing *Clostridia*, a sample of water is added to melted and cooled Wilson–Blair medium. This medium contains thiosulfate (hyposulfite) and a colorless iron salt. Upon spore germination and *Clostridia* growth, reduction of sulfite occurs, resulting in the formation of iron sulfide, which imparts a black color to the medium.

Identification of coliphages. *Coliphages* are viruses that infect *Escherichia coli* and produce lysis zones (plaques) on a bacterial lawn. The presence of coliphages is identified using the agar layer method according to Grazia. The water sample is added to five sterile Petri dishes, each containing 20 mL of medium. A sixth dish serves as a control and does not contain water. Then, melted agar cooled to 45°C, is mixed with a 24-hour *E. coli* culture. The mixture is poured onto the dishes, which are then left to solidify and incubated at 37°C for 24 hours. The results are evaluated by counting plaques in the Petri dishes, expressed as plaque-forming units (PFU) per 100 mL of water. No plaques should be present in the control dish.

Detection of pathogenic intestinal bacteria (*Salmonella* and *Shigella* Genera). To detect pathogenic enterobacteria, the investigated volume of water (at least 2–3 mL) is inoculated into enrichment media, such as Müller-Kauffmann medium or magnesium medium, followed by subculturing onto solid, selective, and differential diagnostic media, including Ploskirev's and Endo's medium, Levine's E.M.B. and

bismuth-sulfite agar. The isolated cultures are identified based on their morphological, tinctorial, biochemical, and serological properties.

Table 5

Tap Water Quality Standards

Indicators	Units of measurement	Standards
1. Total Microbial Count (TMC)	CFU* per 1 mL of water	No more than 50
2. Enterobacteriaceae Bacteria	Number of enteric bacteria in 100 mL of water	Absence
3. Thermotolerant Coliform Bacteria	Number of enteric bacteria in 100 mL of water	Absence
4. Sulfite-Reducing Bacteria Spores	Number of spores in 20 mL of water	Absence
5. Coliphages	PFU** per 100 mL of water	Absence

Note: *CFU – colony-forming unit; **PFU – plaque-forming unit

Air microflora

Air is an environment that does not support the reproduction of microorganisms, primarily due to the lack of nutrients and moisture. Additionally, the microbicidal effect of ultraviolet (UV) rays is more evident in the air. Microbial contamination of the air occurs from soil, water, animals, humans, and plants. The composition of air microflora is diverse and varies significantly with conditions. The air in the upper atmosphere, as well as mountain and sea air, contains very few microorganisms. In populated areas, particularly during summer, microbial presence is markedly higher. The atmospheric air above large cities is especially saturated with microorganisms. This is because microorganisms in the air exist predominantly in an aerosol state. There are three main phases of bacterial aerosols, including:

The Droplet or Large-Nuclear Phase consists of bacterial cells surrounded by a water-salt membrane. The particle diameter is approximately 0.1 mm or larger. These particles settle relatively quickly, remaining in the air for only several seconds, with an average movement speed of about 30 cm/s.

The Small-Nuclear Phase forms when the particles from the first phase dry out and comprises bacterial cells that have retained only chemically bound water on their surface, with free water remaining inside the cells. In this phase, the particles are the smallest, easily transported by air currents, and can remain suspended for extended periods. This is the most stable phase, as most particles do not exceed 0.05 mm in diameter, and their sedimentation rate averages around 0.013 cm/s. Despite their slow sedimentation velocity, these particles can move at speeds

exceeding 30 cm/s, allowing them to disperse over long distances. This phase presents the greatest epidemiological risk because it contains most pathogens of airborne infections, particularly those less resistant to external factors (e.g., whooping cough pathogen).

The "Bacterial Dust" Phase occurs when bacteria from the first two phases aggregate into larger particles that settle as dust on various surfaces, forming what is known as "bacterial dust". A key property of this dust is its ability to disperse easily under even minor air currents. Particle sizes range from 0.01 to 1 mm. Depending on particle size and air current velocity, their movement speed varies between 0.5 and 30 cm/s. Due to their prolonged suspension in the air and the ability of particles to penetrate into the distal regions of the lungs, fine bacterial dust also poses an epidemiological hazard. This phase of bacterial aerosol predominates in indoor environments and disperses pathogenic microorganisms that are resistant to drying – such as mycobacteria, *Clostridia*, staphylococci, streptococci, and fungi.

The microflora of atmospheric air and the microflora of indoor air in residential premises differ significantly.

Microflora of Atmospheric Air. Among the microorganisms present in atmospheric air, the dominant species are those originating from soil. Staphylococci and streptococci are found in only 3.7% of samples collected in crowded areas. The main groups of microorganisms in atmospheric air include:

- On sunny days, pigment-producing cocci constitute 70–80% of the total flora, as the pigment protects bacteria from insolation.
- Soil spore-forming and putrefactive microorganisms, which numbers increase sharply in dry and windy weather.
- Mold fungi and yeast, with their content increasing as air humidity rises.

Self-purification processes continuously occur in atmospheric air due to factors such as precipitation, insolation, temperature effects, and others. Conversely, atmospheric air itself acts as a factor in purifying the air within residential premises.

The microflora of indoor air is more uniform and relatively stable. It is predominantly composed of microorganisms originating from the human nasopharynx, including pathogenic species that enter the air through coughing, sneezing, or talking. These include staphylococci, streptococci, diphtheroids, pneumococci, meningococci, various viruses, and others. The primary source of pathogenic bacteria in indoor air is bacterial carriers. The level of microbial contamination depends mainly on factors such as population density, human activity levels, sanitary conditions of the premises (including dust contamination), ventilation practices, frequency of ventilation, cleaning methods, illumination degree, and other environmental factors. Therefore, regular ventilation and wet cleaning can reduce airborne microbial contamination by up to 30 times compared

to control premises. Self-purification of indoor air does not occur naturally in enclosed premises.

Sanitary and Microbiological Air Analysis

Air can act as a vector for the transmission of respiratory viral diseases, including acute respiratory viral infections (such as ARVIs), influenza, diphtheria, meningococcal infection, tuberculosis, chickenpox, and others.

The objectives of sanitary and microbiological air analysis are to conduct hygienic and epidemiological assessments of the air environment, and, consequently, to develop measures aimed at preventing the airborne transmission of infectious agents. The objects of sanitary and microbiological air analysis in enclosed premises include: air in hospitals (operating rooms, intensive care units, labour wards in maternity hospitals, etc.), kindergartens, schools, outpatient hospitals, pharmacies, as well as production facilities and auxiliary premises at enterprises of various profiles (e.g., food industry, microbial synthesis, etc.), and places of mass gatherings such as cinemas and gyms.

During sanitary and bacteriological air analysis, the following assessments are conducted:

1) Determination of the total bacterial contamination of the air (the total number of microorganisms per 1 m^3);
2) Identification of sanitary indicator microorganisms;
3) Assessment of the release of viruses and pathogenic bacteria from the air of enclosed premises, performed according to epidemiological indications.
4) When studying atmospheric air, additional determination of the qualitative composition of microflora is performed, taking into account the presence of spore-forming aerobes and anaerobes, which serve as indicators of soil microorganism contamination.

When evaluating the sanitary condition of enclosed premises (see Table 6), depending on the study's objectives, the total microbial count, the presence of sanitary indicator microorganisms (such as staphylococci, α- and β-hemolytic streptococci), as well as directly pathogenic microorganisms (depending on the type of premises – e.g., *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, yeast, and mold fungi, etc.) are determined. For example, when assessing the air quality in medical facilities, the presence of opportunistic flora such as *Pseudomonas aeruginosa*, bacteria from the genus *Proteus*, and other gram-negative rods that can cause nosocomial infections is evaluated. The presence of spore-forming rods is considered an indicator of dustiness and inadequate wet cleaning, while mold fungi indicate increased humidity.

Table 6

Standard Indicators of Air in Residential Premises

Degree of Pollution	Winter	Summer
Clean air	TMC no more than 4500, hemolytic streptococci - up to 35	TMC no more than 1500, hemolytic streptococci - up to 16
Dirty air	TMC no more than 7000, hemolytic streptococci - up to 124	TMC no more than 2500, hemolytic streptococci - up to 36

Air sampling methods can be classified into *sedimentation* and *aspiration* techniques.

The sedimentation method is the oldest and most widely used due to its simplicity and accessibility; however, it is less accurate. It is exclusively employed for studying the air in enclosed spaces. This method was proposed by R. Koch and relies on the principle that microorganisms under the influence of gravity and air currents (along with dust particles and aerosol droplets), settle onto the surface of a nutrient medium in open Petri dishes.

The dishes are placed on a horizontal surface at the chosen sampling points. When assessing total microbial contamination, the dishes containing Meat-Peptone Agar (MPA) are left open for 5–10 minutes or longer, depending on the suspected level of bacterial contamination. To identify sanitary indicator microorganisms, different media are used: blood agar (for detecting streptococci), milk-salt or yolk-salt agar (for identifying staphylococci), and wort agar or Sabouraud medium (for yeasts and fungi). When identifying sanitary indicator microorganisms, the dishes are left open for 40–60 minutes. After exposure, all dishes are closed, incubated in a thermostat for 24 hours at an optimal temperature for microbial growth, and then, if necessary, kept at room temperature for additional 48 hours to allow pigment formation by pigment-producing microorganisms.

Aspiration methods are based on the forced deposition of microorganisms from the air onto the surface of a dense nutrient medium or into a collecting liquid (such as meat-peptone broth, buffer solution, isotonic sodium chloride solution, etc.). Aspiration methods are used for analyzing both indoor and outdoor air. Currently, specialized air aspirators or sampling devices are widely used for assessing indoor air quality. Examples include the Rechmensky sampler, the Air Sampling Device (POV-1), the Portable Aerosol Bacteriological Sampler (PAB-1), the Bacterial-Viral Electroprecipitator (BVEP-1), the PU-1B aspirator, SAS (Surface Air System) microbial air samplers (Fig. 74), and others. The operating principle of these devices is based on drawing air through a wedge-shaped slit in the device’s lid, which directs the airflow onto the surface of a nutrient medium. Dust and aerosol particles, along with airborne microorganisms, stick to the

medium. A Petri dish with a thin layer of nutrient medium is mounted on the device's rotating table, ensuring an even distribution of bacteria across its surface.

After sampling for a specified exposure time, the Petri dish is removed, covered with a lid, and incubated in a thermostat for 48 hours. Typically, sampling is performed at a flow rate of 20–25 L/min for 5 minutes, allowing for the analysis of flora present in 100–125 L of air. If sanitary indicator microorganisms are detected, the volume of air examined is increased to 250 L.

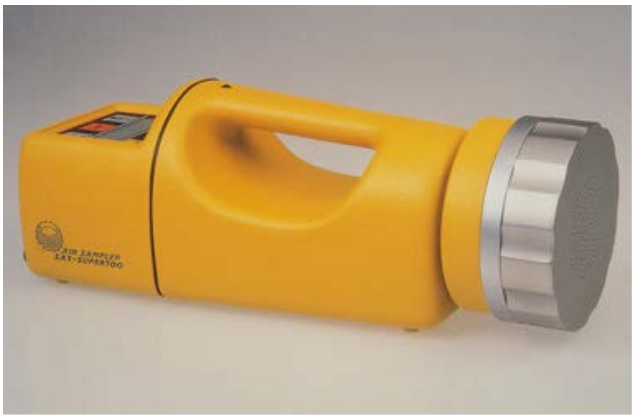


Fig. 74. SAS (Surface Air System) microbial air samplers

(*https://biosci-intl.com/products.htm)*

Determination of Total Microbial Count

To determine the total bacterial count in indoor air, two samples (100 L each) are collected onto Petri dishes containing Meat-Peptone Agar (MPA), using any suitable device – most commonly the Krotov apparatus – or by the sedimentation method. The inoculated dishes are incubated in a thermostat for 24 hours, then left at room temperature for additional 48 hours. The number of colonies on both dishes is counted, the arithmetic mean is calculated, and the result is converted to the number of microorganisms per 1 m^3 of air.

Determination of Staphylococci. Air samples for staphylococci are collected using the Krotov apparatus, with a volume of 250 L per 2–3 dishes containing yolk-salt agar and one dish with blood agar. The dishes are incubated at 37°C for 48 hours. The cultural characteristics of all colony types are examined, and smears are prepared from suspicious colonies and stained using Gram's method. The number of staphylococcal colonies is counted, and the concentration of microbes per 1 m^3 of air is determined. In cases of nosocomial staphylococcal infections, further investigations are conducted to identify sources and routes of infection. This involves phage typing to determine the identity of staphylococci isolated from environmental objects, patients, and healthcare personnel.

Determination of Streptococci. Air samples for studying α- and β-hemolytic streptococci are collected using the Krotov apparatus onto blood agar dishes. A volume of 200–250 L of air is sampled, and the inoculated dishes are incubated in a thermostat for 18–24 hours, and then kept at room temperature for an additional 48 hours (after preliminary examination and counting). The number of colonies grown is counted per 1 m^3 of air, followed by confirmatory microscopic examination.

NORMAL HUMAN MICROBIOTA

The human ***microbiota*** is a collection of microbial biocenoses typically found in healthy people, formed through the evolution process. These biocenoses are characterized by relative stability; however, the qualitative and quantitative composition of the human body's microflora varies throughout life and depends on factors such as gender, age, nutrition, climate, and other influences. Additionally, changes in microbial biocenoses can be triggered by disease onset or the use of chemotherapeutic and immunological agents.

Under physiologically normal conditions, the human body hosts hundreds of microbial species, with bacteria being predominant, while viruses and protozoa are represented by a significantly smaller number of species. Similar to the external environment, microorganisms within the human body exist as microbiocenoses – specific microbial communities that develop over the lifetime.

Currently, a distinct form of microflora organization is identified within the human body: the formation of a microbial community covering surfaces such as the intestinal wall, mucous membranes, skin, and teeth. This balanced collective microbial community is known as a biofilm. Microbial biofilms can take various forms – either as a layer attached to epithelial cells or as separate conglomerates of cells.

Organs and tissues that do not contact the external environment are typically free of microorganisms. Under normal conditions, the heart, blood, lymph, brain, cerebrospinal fluid, bladder, uterus, and deep tissues remain sterile.

The primary body sites colonized by bacteria include the skin, upper respiratory tract, gastrointestinal tract, and genitourinary system, as well as the conjunctiva of the eye and the outer ear.

The human body's microflora can be divided into two groups: obligate (also known as resident or autochthonous microbiota) and facultative (or transient microbiota). *Obligate microflora* consists of microorganisms that are well-adapted to survive within the human body and are consistently found in its organs and cavities. Members of the obligate microflora are further classified into dominant species and filler species. *Facultative microflora* is temporary and non-essential, determined by the microbial contamination of the environment and the host organism's resistance status. Both resident and transient microflora include saprophytic and opportunistic microorganisms.

Recently, hospital-acquired or nosocomial infections have gained increasing importance in human pathology. Their causative agents are opportunistic microorganisms belonging to the human resident microflora. The pathogenic power is realized when the host organism's resistance is weakened.

The microflora of different biotopes within the human body varies significantly and shall be considered separately.

Skin Microbiota

The surface of human skin, particularly its exposed areas, is a habitat for a wide variety of microorganisms; the microbial number here ranges from 25 million to 1 billion microbial cells.

The resident microflora of human skin includes *Sarcina*, *staphylococci*, *diphtheroids*, *certain species of streptococci*, *bacilli*, *fungi*, and other microorganisms. In addition to this typical skin microflora, there may be also transient microorganisms, rapidly disappearing due to the skin bactericidal properties. Cleanly washed skin exhibits a strong capacity for self-cleanse. The bactericidal properties of the skin reflect the overall resistance of the organism.

The microbiota on hair-covered areas is similar in composition to that of the skin. Typically, 10^3–10^4 microorganisms are detected per one square centimeter, but in areas with increased humidity, this number can reach up to 10^6. In some individuals, streptococci and Gram-positive spore-forming rods are also present on their skin.

The highest number of microorganisms is observed in skin folds, where fungi of the genus *Candida* are commonly found. In areas with a high concentration of sebaceous glands – such as outer ear and genitals – non-pathogenic acid-fast mycobacteria, corynebacteria, and yeasts are frequently present.

Intact skin acts as an impenetrable barrier against most microorganisms, including pathogenic species. However, if the skin integrity is compromised or the host's resistance is diminished, skin diseases may develop.

Sanitary and bacteriological analysis of the skin is performed using two methods:

1. Inoculating handprints onto Meat-Peptone Agar (MPA) in Petri dishes, followed by macroscopic and microscopic examination of the grown colonies.
2. Inoculating of skin swabs to determine the total microbial count and specifically identify *Escherichia coli*.

Oral Microbiota

The oral cavity provides favorable conditions for microbial growth, including the presence of nutrients, an optimal temperature, a moist environment, and a slightly alkaline pH maintained by saliva. Saliva plays a vital role in maintaining the healthy balance of the oral microflora by influencing both qualitative and quantitative stability in this biotope, exhibiting antibacterial activity

due to the enzymes it contains (lysozyme, lactoferrin, peroxidase, and nuclease) as well as secretory immunoglobulins.

By the end of the first week of life, the oral cavity of newborns contains *streptococci, Neisseria, lactobacilli, yeast-like fungi,* and *Actinomycetota*. The quantitative and species composition of oral microbes depends on factors such as the child's diet and age. Obligate Gram-negative anaerobes appear during tooth eruption.

More than 350 microbial species have been identified in the oral cavity, most of which are anaerobes. The majority of oral microorganisms are localized in dental plaque: just 1 mg of dry plaque mass contains approximately 250 million microbial cells. A high concentration of microorganisms is found at the neck of the tooth, in interdental spaces, and in other areas of the oral cavity that are difficult to cleanse with saliva, as well as on the mucous membranes of the pharyngeal tonsils. The qualitative and quantitative composition of oral microflora is not static and can fluctuate due to several factors. These include a person's age, their dietary habits, how well they maintain oral hygiene, the strength of their mucosal resistance, and the presence of any pathological processes affecting their teeth and gums.

The *resident group* of oral bacteria includes ïram-positive cocci (such as streptococci and peptococci), *Veillonella*, non-pathogenic staphylococci, saprophytic *Neisseria* species, corynebacteria, lactobacilli, *Bacteroides* bacteria, fusiform bacteria, yeast-like fungi, *Actinomycetota*, *Mycoplasma* (e.g., *M. orale*), and protozoa (such as *Entamoeba buccalis*).

Among the *facultative microorganisms* are enterobacteria (genera *Escherichia, Klebsiella, Enterobacter,* and *Proteus*), *Pseudomonas aeruginosa,* spore-forming bacteria (genera *Bacillus* and *Clostridium*), and microorganisms of the genus *Campylobacter* (e.g., *C. consicus*, *C. sputorum*).

Qualitative and quantitative analysis of the oral microflora is conducted using bacterioscopic and bacteriological methods.

Microbiota of the Gastrointestinal Tract

The composition of the gastrointestinal (GI) tract microbiota varies significantly depending on its specific section. In a healthy stomach, the acidic environment of gastric juice and the high activity of hydrolytic enzymes create a hostile environment that effectively eliminates most microorganisms. Consequently, only acid-resistant species are present in the stomach in small quantities – such as lactobacilli, yeasts, *Sarcina ventriculi,* and others – typically amounting to 10^6–10^7 cells per 1 mL of contents.

In the duodenum and upper small intestine, microorganisms are relatively scarce, despite the transition from an acidic to an alkaline environment. This is attributed to the inhibitory effects of digestive enzymes present in these regions. There are enterococci, lactic acid bacteria, fungi, and diphtheroids here (10^6 cells

per 1 mL of contents). In the lower small intestine, where microbial populations gradually increase, the microflora approaches the composition of the large intestine.

The large intestine hosts the most diverse and abundant microbiota, comprising over 200 species and containing between 10^9–10^{11} cells per 1 mL of contents. Microbes constitute about one-third of the dry mass of feces.

The *obligate microflora* primarily consists of obligate anaerobic bacteria, making up 96–99% of the microflora (including *Bacteroides* bacteria, *Bifidobacteria*, and *Veillonella* species), as well as facultative anaerobes, accounting for 1–4% of the microbiota and including lactobacilli, fecal enterococci, and coliform bacteria. The latter group encompasses four genera within the family *Enterobacteriaceae*: *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella*. These microorganisms are classified together based on shared features: they are Gram-negative, non-spore-forming rods that are oxidase-negative and capable of fermenting glucose and lactose to produce acid and gas at 37°C within 24 hours.

The *transient microflora* includes the following genera and species: *Proteus*, *Clostridium, Pseudomonas aeruginosa*, *Campylobacter*, yeast-like fungi of the genus *Candida*, and other species. *Campylobacter* species (e.g., *C. fennelliae, C. cinaedi, C. hyointestinalis*) are found in the human large intestine during immunodeficiency states of various origins.

The composition of the intestinal microflora changes throughout a person's life. During the first hours after birth – in the aseptic phase – the newborn's meconium is sterile. The second phase involves increasing bacterization, occurring during the first three days of life. During this period, *Escherichia coli*, staphylococci, enterococci, and yeast-like fungi predominate in the intestine. The third phase begins around the fourth day of life and is characterized by the transformation of the intestinal flora, with establishment of lactic acid bacteria, lactobacilli, and acidophilic bacteria. After stopping breastfeeding, a permanent biocenosis gradually begins to develop in the digestive tract.

The GI tract microflora is comprised of distinct *mucosal microbiota (MAM)* and *luminal microbiota (LM)*, which differ in their composition. *MAM* is closely associated with the mucous membrane, is more stable, and is primarily composed of bifidobacteria and lactobacilli. It prevents the penetration of pathogenic and opportunistic microorganisms into the mucous membrane. *LM*, in addition to bifidobacteria and lactobacilli, includes other permanent inhabitants of the intestine.

To study the microflora of the large intestine, fecal samples are examined; their species composition is determined using microscopic and bacteriological methods.

Microbiota of the Respiratory Tract

The upper respiratory tract exhibits a particularly high microbial load, as it is anatomically adapted to deposit bacteria from inhaled air. Dust particles and microorganisms enter the respiratory tract with the air, most of which are retained in the nasal cavity. These microorganisms eventually die over time due to the bactericidal action of lysozyme and mucin, the protective functions of the epithelium, and the phagocytic activity. The *obligate microflora* of the nasal passages includes staphylococci and corynebacteria. The *facultative microflora* comprises *Staphylococcus aureus*, streptococci, and non-pathogenic *Neisseria bacteria*. The microflora of the nasopharynx consists of streptococci, *Bacteroides* species, *Neisseria* bacteria, *Veillonella* species, and mycobacteria. Some individuals consistently carry *Staphylococcus aureus*, indicating resident carriage.

Tracheal and bronchial mucosa, alveoli and parenchyma are practically free of microorganisms.

For microbiological examination, samples from the nose are collected using a sterile swab, while material from the nasopharynx is obtained with a sterile posterior pharyngeal swab. These samples are then inoculated onto blood agar and yolk-salt agar. The isolated colonies are subsequently identified.

Microbiota of the Conjunctiva

In a significant percentage of cases, the conjunctiva is free of microflora, owing to the bactericidal properties of tear fluid. When present, the conjunctiva microflora may include staphylococci, corynebacteria (*Corynebacterium xerosis*), and mycoplasma. A weakened immune system, visual impairment, or hypovitaminosis can allow the normal microflora on the ocular mucous membranes to cause various diseases, such as conjunctivitis, blepharitis, and other suppurative processes.

Microbiota of the Ear

The external auditory canal is normally populated by non-pathogenic staphylococci, corynebacteria, yeast-like fungi, and mold fungi (such as *Aspergillus*), where they can become pathogenic under certain conditions. Under normal circumstances, the middle ear and inner ear are generally considered sterile environments.

Microbiota of the Urogenital System

The microbial composition of urogenital system biotopes varies depending on gender, age, and the certain organ.

The kidneys, ureters, and urine in the bladder are typically sterile. In men, the urethra harbor microorganisms such as staphylococci, diphtheroids, *Bacteroides* species, mycobacteria, and non-pathogenic Gram-negative bacteria. The female urethra, however, is sterile.

On the external genitalia of both men and women, smegma bacteria (*M. smegmatis*) staphylococci, corynebacteria, mycoplasmas (*M. hominis*), and saprophytic treponemes can be found.

The composition of vaginal microflora is diverse and variable, depending on the level of glycogen in epithelial cells and the pH of vaginal secretions, which are associated with ovarian function. Colonization of the vagina by lactobacilli occurs immediately after birth. Subsequently, enterococci, streptococci, staphylococci, and corynebacteria become part of the microbiocenosis. Coccal flora becomes predominant and specific to childhood until puberty. With the onset of adolescence, aerobic and anaerobic lactic acid bacteria predominate, with *Lactobacillus* species (the Döderlein group) being dominant.

In healthy women, several categories of vaginal cleanliness are distinguished:

- Category 1: Döderlein’s bacilli are identified in smear preparations; other microorganisms are almost absent.
- Category 2: In addition to lactic acid bacteria, a small number of Gram-positive diplococci are found.
- Category 3: The number of lactic acid bacteria decreases; leukocytes and other microflora increase.
- Category 4: Abundant leukocytes and diverse microflora are present; Döderlein’s bacilli are nearly absent.

Categories 1 and 2 are typical in healthy women, while categories 3 and 4 are associated with inflammatory processes in the vagina. The uterine cavity in healthy women remains sterile.

The Significance of Normal Human Microbiota

The normal microbiota of a healthy individual plays a vital role in maintaining overall health and ensuring the proper functioning of the entire body.

Obligate microflora (such as *Escherichia coli*, lactobacilli, bifidobacteria, and certain fungal species) exhibits pronounced antagonistic properties against some infectious disease agents. These antagonistic properties are associated with the production of antibiotic substances, bacteriocins, alcohols, lactic acid, and other products that inhibit the multiplication of pathogenic microorganisms.

Some enterobacteria, including *E. coli*, synthesize essential nutrients such as B vitamins, vitamin K, pantothenic acid, and folic acid, which are essential for the host organism. Lactic acid bacteria are also active producers of vitamins.

The microflora plays a significant role in the development of the body's immune resistance. Disruption of the normal microflora composition in gnotobiotic (germ-free) animals leads to hypoplasia of lymphoid tissue and a decrease in both cellular and humoral immune factors.

The gastrointestinal tract microflora influences the morphological structure of the intestinal mucosa and its absorptive capacity; by breaking down complex organic substances, these microorganisms facilitate digestion. It has been established that *C. perfringens*, a permanent inhabitant of the intestine, possesses the ability to produce digestive enzymes.

During the normal colonization of mucous membranes, bacteria, acting as antigens, stimulate the production of antibodies (such as IgA), forming the basis of local immunity. They prevent pathogens from penetrating tissues and help maintain the homeostasis of the mucous membranes themselves.

For the normal functioning of the human body, the relationship between the host organism and its resident microflora is essential. When this established relationship is disrupted by factors such as hypothermia, hyperthermia, ionizing radiation, psychological stress, or other influences, microbes can spread from their usual habitats, invade the internal environment, and induce pathological processes.

However, normal microbiota can also cause infectious diseases, most of which are opportunistic (coexisting) in nature. For example, intestinal anaerobes (*Bacteroides*) can cause abscess formation when they penetrate the intestinal wall following injury. Similarly, microorganisms residing in the nasopharynx of any individual are considered the primary causative agents of post-influenza pneumonias. In such cases, the development of disease is less dependent on the virulence of the pathogen itself and more on the weakening of the host's defense systems (immunodeficiency).

Dysbiosis

Dysbiosis refers to a qualitative and quantitative disruption in the ecological balance among microbial populations within the human microbiota. It arises from the influence of destabilizing factors such as the irrational use of broad-spectrum antibiotics, antiseptics, or a sharp decline in the body's resistance due to chronic infections, radiation, and other influences. In dysbiosis, antagonistic microbes that regulate the composition of the microbial biocenosis and inhibit the reproduction of opportunistic microorganisms are suppressed. This allows for the proliferation and spread of opportunistic microorganisms. Consequently, there is an overgrowth and dissemination of microorganisms from genera such as *Pseudomonas*, *Klebsiella*, and *Proteus*, which are common causes of nosocomial infections, as well as yeast-like fungi like *Candida albicans*, responsible for candidiasis, and *E. coli*, which can cause colienteritis, and others.

The most prevalent form is intestinal dysbiosis, manifesting as digestive disturbances accompanied by general malaise, abdominal pain, and increased gas production. Prolonged imbalance in the intestinal microbial composition can also trigger the development of allergic diseases, including bronchial asthma, chronic bronchitis, rheumatoid arthritis, and others.

To correct dysbiosis, probiotics, prebiotics, and synbiotics are used. ***Probiotics*** are preparations derived from live microorganisms that are representatives of the normal human microflora. Examples include *Colibacterin* (live *Escherichia coli M-17*), *Bifidumbacterin* (a suspension of live *Bifidobacterium bifidum N1*), *Lactobacterin* (a suspension of live *Lactobacillus bacteria* strains), and *Bifikol* (a complex preparation consisting of a suspension of live *Bifidobacterium bifidum N1*, and *Escherichia coli Ì-17*). ***Prebiotics*** are preparations of non-microbial origin that are indigestible in the intestine but capable of stimulating the growth and/or metabolic activity of the normal microflora. Prebiotics must meet three key criteria: they must not be hydrolyzed by human digestive enzymes, must not be absorbed in the upper gastrointestinal tract, and they must selectively promote the growth of a certain group of microorganisms. An example of a prebiotic is Hylak® Forte. ***Synbiotics*** are formulations that combine prebiotics and probiotics (for example, Maxilac®).

Intestinal dysbiosis is diagnosed using bacteriological methods. Based on fecal culture results, the following parameters are assessed:

1) The content of total number of intestinal bacteria with typical enzymatic activity;
2) The presence of hemolytic strains of *E. coli*;
3) The presence of other opportunistic microorganisms;
4) The presence of bacteria from the genus *Proteus*;
5) The presence of fungi from the genus *Candida*;
6) The quantitative content of bifidobacteria, lactobacilli, and *Bacteroides*.

Table 7 illustrates the composition of the intestinal microbiota.

Table 7

Bacterial Content in the Feces of Healthy Individual

Bacteria	Quantity (per 1 g of feces)
Bifidobacteria	$10^8 – 10^9$
Bacteroides	$10^9 – 10^{10}$
Lactobacilli	10^6 -10^8
Spore-forming anaerobic clostridia	10^5
Escherichia coli: • Lactose-positive • Lactose-deficient • Non-lactose fermenting	 $10^7 – 10^8$ $10^5 – 10^7$ $10^5 – 10^7$

Bacteria	Quantity (per 1 g of feces)
• Hemolytic	10^6
Proteus	10^4
Klebsiella	10^3
Other Gram-negative bacteria	10^3
Staphylococci (*S. epidermidis*, hemolytic, non-hemolytic saprotrophic)	10^4
Enterococci	$10^5 – 10^6$
Yeast-like fungi	10^4
Molds	10^4

GENETICS OF MICROORGANISMS

The Genetic System of Bacteria

The genetic system of bacteria comprises nuclear and extranuclear structures. The prokaryotic equivalent of the nucleus differs significantly from the nucleus of eukaryotic cells. It is represented by *a nucleoid*, which lacks a surrounding membrane and contains nearly all bacterial DNA. The bacterial chromosome consists of a single, *double-stranded DNA molecule shaped like a circle* (or ring). This DNA molecule is composed of two polynucleotide chains. Each *nucleotide* consists of a nitrogenous base, deoxyribose sugar, and a phosphate group. The nitrogenous bases include purines (adenine and guanine) and pyrimidines (thymine and cytosine). Each nucleotide has polarity, with a deoxyribose 3′-end (3 prime end) and a phosphate 5′-end (5 prime end). Individual nucleotides are linked together into a polynucleotide chain by phosphodiester bonds. These bonds form between the 5′-end of one nucleotide and the 3′-end of the adjacent nucleotide. The two chains are held together by hydrogen bonds between complementary nitrogenous bases: adenine pairs with thymine, and guanine pairs with cytosine. The nucleotide chains run in opposite directions, or antiparallel. This means that at each end of the molecule, one strand will have a 5′-end and the other strand will have a 3′-end. Hereditary information in bacteria is stored as a sequence of DNA nucleotides, which determines the sequence of amino acid residues in a protein molecule. Each protein is encoded by its own *gene* – a discrete segment of DNA characterized by the number and specificity of its nucleotide sequence. A bacterial chromosome can contain up to approximately 4,000 individual genes. The complete set of genes constitutes *the genome*. The observable expression of the genome is called *the phenotype*. Bacterial chromosome sizes vary among different prokaryotic species, ranging from approximately 3×10^8 to $2{,}5\times10^9$ Da. The bacterial cell is haploid; thus, chromosome duplication is always accompanied by cell division.

In bacteria, genetic information can also be found within *extranuclear* (extrachromosomal) DNA molecules, such as plasmids, transposons, and insertion sequences. These elements are not essential for survival, as they do not encode enzymes involved in the core metabolism of the bacterial cell.

Bacterial plasmids are double-stranded DNA molecules ranging in size from approximately 10^6 to 10^8 Da, typically carrying between 40 and 50 genes. The number of plasmids present in a single bacterial cell can vary from 1 to 200. Plasmids can exist as independent, circular DNA molecules called *episomes*, or as integrated sequences within the bacterial chromosome (*integrated plasmids*).

Plasmids perform both regulatory and coding functions. Regulatory functions compensate for metabolic deficiencies, while coding functions introduce new traits

into the bacterium. As components of the bacterial genetic material, plasmids play a vital role in its life processes by determining characteristics like exotoxin production, enzyme synthesis, bacteriocin production, and resistance to various drugs.

The replication of certain plasmids can induce bacterial cell division, effectively increasing their “fertility”. Such plasmids are termed as *F-plasmids* or F-factors (from the English *fertility*). Integrated F-plasmids are called *Hfr-plasmids* or Hfr-factors (from the English *high frequency of recombinations*). Hfr-factors facilitate the transfer of a part of the genetic information from their chromosome to a recipient cell.

Plasmids that determine resistance to antimicrobial drugs are called *R-plasmids* or R-factors (from the English *resistance*). R-plasmids contain genes responsible for synthesizing enzymes that either inactivate antibacterial agents or are responsible for other resistance mechanisms. As a result, the bacterial cell becomes resistant to an entire group of drugs. Many R-plasmids are transmissible, meaning they can spread throughout bacterial populations and transfer resistance traits across different strains or species.

Pathogenicity plasmids regulate the virulent properties of microorganisms by determining the synthesis of pathogenicity factors. For example, the Ent plasmid determines the enterotoxin biosynthesis. The development of infectious processes caused by the causative agents of plague, anthrax, intestinal yersiniosis, and Ixodes tick-borne borrelioses is associated with the activity of pathogenicity plasmids.

Conjugative plasmids are transferred from one bacterium to another within the same species or between closely related species through a process called conjugation. The most common conjugative plasmids are F- or R-plasmids. These plasmids are relatively large, ranging from approximately 25 to 150 million Da, and are often found in Gram-negative rods. Large plasmids are typically present in one or two copies per cell, with their replication closely linked to the replication of the bacterial chromosome.

Non-conjugative plasmids are typically small and are characteristic of Gram-positive cocci; however, they are also found in some Gram-negative microorganisms (e.g., *Haemophilus influenzae*, *Neisseria gonorrhoeae*). Small plasmids may be present in large quantities within a cell (more than 30 copies per cell), since their abundance ensures their distribution to daughter cells during cell division. If a bacterium contains both conjugative and non-conjugative plasmids, the donor can transfer non-conjugative plasmids by binding of their genetic material with factors facilitating their transfer during conjugation.

Mobile genetic elements are components of the bacterial genome, which includes the bacterial chromosome and plasmids. They encompass *insertion sequences* and *transposons*. Insertion sequences (IS elememts) are DNA segments capable of moving from one location to another, containing only the genes necessary for their transposition. IS elements coordinate interactions among

plasmids, temperate phages, transposons, and the nucleoid to ensure reproduction; they also regulate the activity of bacterial cell genes. They can inactivate genes in which they are inserted (“gene silencing”) or, by integrating into the chromosome, exert a promoter effect, activating or repressing transcription of adjacent genes.

Transposons (Tn) are DNA segments composed of insertion sequences and structural genes that encode molecules, confering specific biological functions, such as toxicity or antibiotic resistance. Transposons are incapable of independent replication and can only multiply as part of the bacterial chromosome.

Replication of Bacterial DNA

The reproduction of bacterial genetic material occurs through a process called *replication*, which in bacteria follows a semi-conservative mechanism. This means that each of the two DNA strands of a chromosome or plasmid serves as a template for the synthesis of a complementary daughter DNA strand. The replication process relies on a complex of enzymes. It begins with the unwinding of the double-stranded DNA structure, carried out by the enzyme *DNA helicase*. This unwinding results in the formation of two replication forks, which move in opposite directions until they meet. The synthesis of new daughter strands is carried out by the enzyme *DNA polymerase*. A key feature of DNA polymerase is its ability to add nucleotides complementary to the template strand only to the free 3′-end of the growing chain. Therefore, to initiate nucleotide polymerization on the parental strand template, DNA polymerase requires a pre-existing starting point called a primer (from the English *primer*). A primer is a short nucleic acid sequence complementary to the template strand and has a free 3′-end. This property of DNA polymerase is the foundation of the polymerase chain reaction (PCR), widely used in infectious disease diagnosis. The two strands of the DNA double helix are complementary. New strands are built on each parental strand using structural DNA elements – deoxyribonucleoside triphosphates. During this process, each base pairs specifically with its complementary base, ensuring that each new strand is complementary to its parent strand. As a result, both resulting double-stranded molecules consist of one original (parental) strand and one newly synthesized strand. This highly accurate process of DNA replication ensures the preservation of genetic information.

Bacterial DNA, while carrying hereditary information, does not itself serve as a direct template for polypeptide synthesis. Protein biosynthesis occurs on ribosomes, which do not directly interact with DNA. The transfer of information encoded in DNA to the sites of protein synthesis is mediated by *messenger RNA* (mRNA). mRNA consists of a single strand and differs from a single DNA strand by replacing the base thymine (T) with uracil (U). mRNA is synthesized on one of the DNA strands through a process similar to DNA replication. The formation of mRNA begins at the 5′-end, and its base sequence is complementary

to the DNA strand. This process is called *transcription*, and the subsequent process of translating the nucleotide sequence into an amino acid sequence is called *translation*.

Each gene corresponds to a specific segment of the DNA molecule. The specific information encoded within a gene is determined by the sequence of bases in the DNA strand. The specificity of enzyme proteins, which synthesis is regulated by genes, is determined by the sequence of amino acids in their polypeptide chains. This sequence also dictates the three-dimensional structure of the protein – the *conformation*.

The polypeptide chain elongates as the ribosome moves along the mRNA. During this process, the chain begins to twist and fold into a globular shape, a process determined by the sequence of amino acids and the properties of their side chains (hydrophobic and hydrophilic groups). This results in a structure that gives the protein its specific properties and function. Several ribosomes typically attach to a single mRNA molecule, allowing several polypeptide chains to be synthesized simultaneously on the same template. At the end of the mRNA, a stop codon trigger the release of the newly formed polypeptide chain from the ribosome.

Thus, the DNA nucleotide sequence functions as an encoded “instruction” that determines the structure of a specific protein. This universal process of information transfer, encompassing DNA replication, transcription, and translation, is fundamental to both eukaryotic and prokaryotic organisms.

Regulation of Gene Expression in Bacteria

A bacterial cell can initiate or cease the synthesis of a particular enzyme depending on the presence of the corresponding substrate. To facilitate this, bacterial genes are organized into groups (*clusters*), so that all enzymes necessary for a specific biosynthetic pathway are determined by genes linked to each other. The entire group of genes can be transcribed into a single polycistronic mRNA, which is then sequentially translated by ribosomes to produce multiple, different proteins. This organizational structure allows for coordinated regulation of all genes expression within a single transcriptional unit.

Gene expression in prokaryotes is primarily regulated at the transcriptional level. *Effector molecules*, which are low-molecular-weight compounds, can act as signalling substances to initiate transcription. These effectors are either substrates that the enzyme acts upon, or the products of the enzymatic reaction. Induction and repression are different aspects of the same regulatory mechanism. Small molecules that induce the production of enzymes capable of metabolizing them are called *inducers*. Conversely, molecules that inhibit the synthesis of enzymes responsible for their own production are known as *corepressors*.

Effector molecules cannot directly interact with DNA; instead, they bind to specific *regulatory proteins*, which then act as intermediaries to influence the

transcription of genes. A regulatory protein that binds to DNA in the absence of an inducer is called *a repressor*.

Regulatory genes are responsible for synthesizing regulatory proteins. In the presence of a repressor protein, transcription is inhibited. Its removal allows RNA polymerase to access the genes and initiate transcription. The process of halting enzyme synthesis through the action of a repressor protein is called *repression*. Repression enables a bacterial cell to conserve resources by preventing the synthesis of enzymes that are not immediately needed. If an inducer is present in the cell at high concentrations, it specifically binds to the regulatory protein, inducing a conformational change that reduces its ability to bind to DNA. This type of regulation is illustrated in Figure 75.

Transcriptional control is achieved through the interaction between regulatory proteins and regulatory sites, often referred to as *operators*. The operators are located between the structural genes and the *promoter* (the region recognized by DNA-dependent RNA polymerase). The promoter acts as the binding site for RNA polymerase, initiating the process of transcription. The promoter, operator, and structural genes together form an *operon*. An operon is a functional genetic unit that regulates the expression of one or more genes.

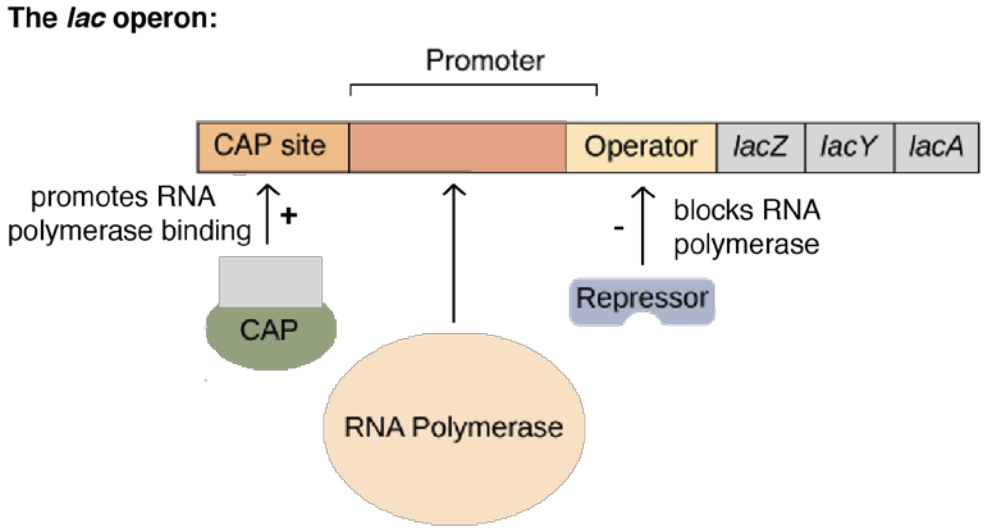


Fig. 75. Structure of the Lac operon of E. coli. The lac operon regulates the synthesis of enzymes involved in lactose catabolism, such as β-galactosidase. In the absence of lactose in the medium, the lac operon is in a repressed state: the active repressor protein binds to the operator gene, preventing transcription of the structural genes lacZ, lacy, lacA; as a result, β-galactosidase is not produced. When lactose is present in the medium, it inactivates the repressor protein, which loses affinity for the operon and thereby giving the way for RNA polymerase. This enables transcription to proceed

Transfer of Bacterial Genetic Material

The exchange of genetic material in bacteria occurs through genetic recombination. *Genetic recombination* refers to the interaction between two genomes that leads to the formation of recombinant DNA and a daughter genome combining genes from both parent cells. The unique features of recombination in bacteria are influenced by the absence of a true sexual process and meiosis in prokaryotes, as well as their haploid set of genes. During recombination, bacteria are conventionally classified as donor cells, which transfer genetic material, and recipient cells, which receive this material. Not all of the donor cell's chromosome is transferred; usually, only a portion – one or more genes – is incorporated into the recipient cell. The resulting recombinant genotype is primarily derived from the recipient's original genotype, with additional fragments of donor DNA integrated into its genome.

Recombination can be *homologous*, involving exchange between DNA regions that share a high degree of similarity (homology) during DNA breakage and repairing. There is also *site-specific* recombination, which occurs only at specific regions (sites) of the genome and does not require a high degree of DNA homology. An example is the integration of a plasmid into the bacterial chromosome. The transfer of genetic material between bacteria occurs via three main mechanisms: conjugation, transduction, and transformation.

Conjugation is the transfer of genetic material through direct contact between two cells. A prerequisite for conjugation is the presence of a transmissible plasmid in the donor cell. Transmissible plasmids encode sex pili, which act as a bridge between donor and recipient cells, forming a conjugation tube that facilitates the transfer of plasmid DNA from the donor to the recipient. As a result of this transfer, the recipient cell acquires donor properties. The integrative transmissible plasmid is known as the F factor. Donor cells containing the F factor are called F^+ cells, while recipient cells lacking the F factor are referred to as F^- cells. If the F factor is integrated into the donor cell's chromosome and begins to function as a single transmissible replicon with the chromosome, the donor chromosome can be transferred to a recipient cell. Donor cells with the F factor integrated into their chromosomes are called *Hfr cells* (high-frequency recombination cells). The chromosomal DNA is replicated, and one strand of the chromosome copy is transferred to the recipient F^- cell, while the other remains in the Hfr^+ cell, preserving its genetic integrity.

The transfer of genetic material during conjugation begins with the cleavage of the DNA a specific site, where the F factor is located. One strand of donor DNA is transferred through the conjugative bridge into the recipient cell. This process is accompanied by the synthesis of a complementary strand, resulting in a double-stranded structure. The DNA strand is transferred to the recipient cell, becoming

double-stranded and then aligning with a homologous region of the recipient's DNA to form a stable genetic structure. The biological significance of conjugation is clearly demonstrated by the spread of bacterial antibiotic resistance. Bacteria can acquire antibiotic resistance through mutation, which occurs approximately once in every 10^6 cell divisions. However, once altered, genetic information can rapidly spread among similar bacteria via conjugation, since approximately one-third of closely related bacteria are capable of this type of genetic transfer (Fig. 76).

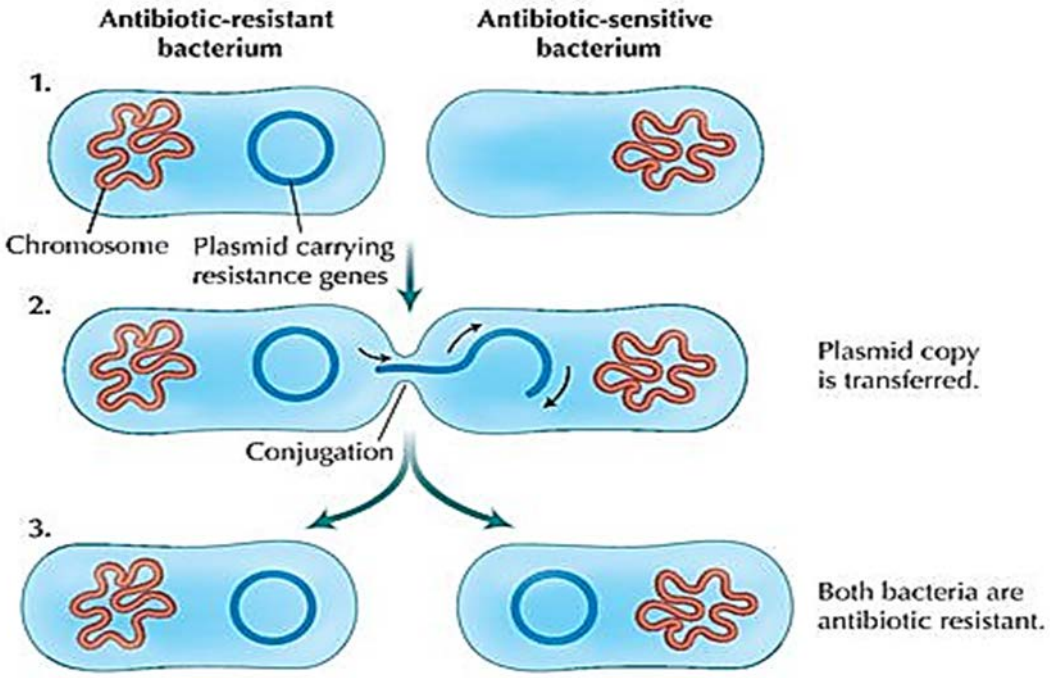


Fig. 76. Diagram of Conjugation Illustrating the Transfer of Resistance Plasmids to Antibiotics (https://www.huvepharma.com/news/article/new-research-further-confirms-inhibition-of-flavomycin-on-resistance-gene-transfer/)

Transformation is the transfer of genetic information via DNA isolated from a donor cell. This process can occur spontaneously in nature in certain bacterial species, predominantly Gram-positive ones, when DNA released from lysed cells is taken up by recipient cells. Typically, any foreign DNA entering a bacterial cell is cleaved by restriction endonucleases; however, under specific conditions, such DNA can be integrated into the bacterial genome. The DNA can be either plasmid or chromosomal in origin and carry genes that transform the recipient cell. Similarly, transformation processes can facilitate the spread of genes encoding virulence factors within bacterial populations; however, its overall role horizontal gene transfer is relatively minor. The transformation process is illustrated in Figure 77.

Transformation serves as a valuable tool for chromosome mapping because transformed cells incorporate various DNA fragments. By analyzing the frequency of simultaneous acquisition of two specific traits (the closer the genes are located

on the chromosome, the higher the likelihood they will be transferred together to the same DNA segment) researchers can infer the relative positions of those genes within the genome. The transfer of extracted DNA is a fundamental method in genetic engineering, used for constructing recombinant strains with a desired genome.

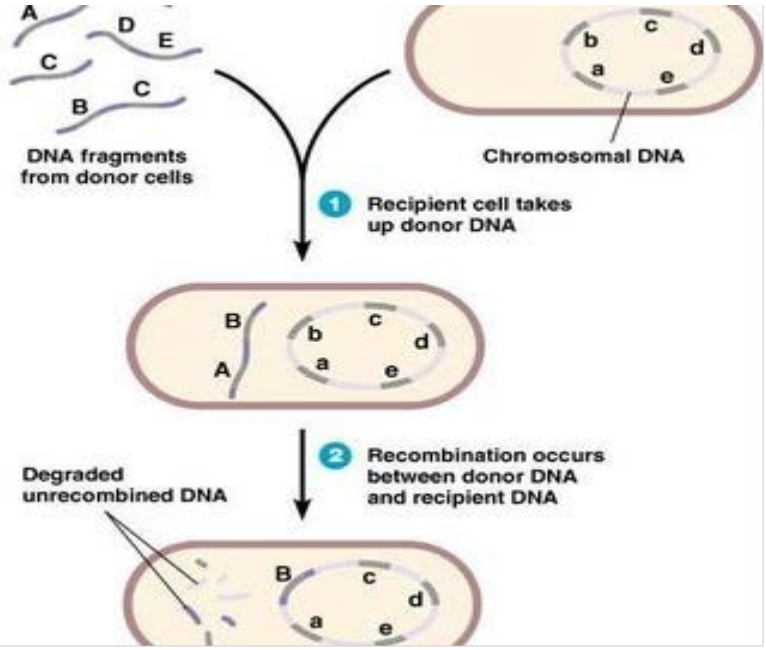


Fig. 77. Diagram of Bacterial Transformation

Transduction is the transfer of bacterial DNA mediated by a bacteriophage. During phage replication within bacteria, a fragment of bacterial DNA enters the phage particle and subsequently is transferred to a recipient bacterium. In this process, phage particles are typically defective and lose their ability to reproduce. Since only small DNA fragments are transduced, the probability that recombination will affect a specific trait is very low, ranging from 10^{-6} to 10^{-8}. There are three types of transduction: generalized (nonspecific), specialized, and lateral (abortive).

Generalized (nonspecific) transduction involves the transfer of a fragment from any part of the bacterial chromosome by a bacteriophage. During the assembly of progeny phage particles in a bacterium infected with a bacteriophage, fragments of bacterial DNA or plasmids may be packaged into the phage head, either alongside or instead of the viral DNA. This occurs because bacterial DNA is fragmented following phage infection and a piece of bacterial DNA approximately the same size as the phage DNA enters the viral particle with a frequency of about 1 in 1,000 phage particles. This form of transduction allows for the transfer of virtually any gene to recipient cells. The phenomenon of nonspecific transduction can be used for mapping the bacterial chromosome. The mechanisms of nonspecific and specialized transduction are illustrated in Figure 78.

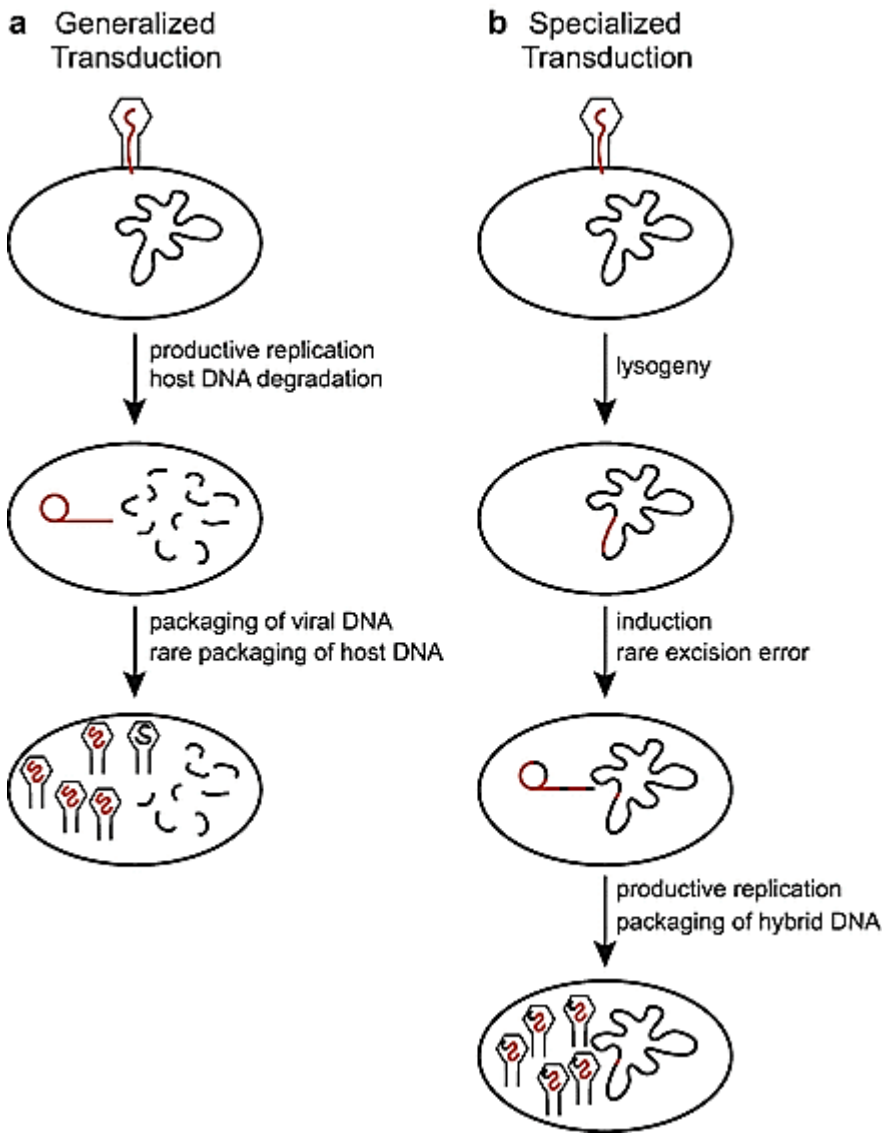


Fig. 78. Diagram of Generalized (Nonspecific) (a) and Specialized (b) Transduction

Specialized transduction occurs when phage DNA integrates into a bacterium to form a prophage. During the excision of phage DNA from the bacterial chromosome, a random process may capture a fragment of bacterial DNA adjacent to the site of phage DNA insertion. Since most temperate phages integrate into bacterial DNA at specific sites, such bacteriophages are characterized by the transfer of a particular region of the donor bacterial DNA into the recipient cell. Specialized transduction can serve as a mechanism for transferring virulence genes among bacteria, provided these genes are located near the sites of prophage integration.

A typical example is transduction mediated by phage λ. This phage usually transduces specific genes such as *gal* (which encodes galactose synthesis) and *bio* (which encodes biotin synthesis). When transitioning to a prophage state, phage λ

integrates into a specific region of the host bacterium's chromosome – located between the *gal* and *bio* genes. The excision of phage DNA from the bacterial chromosome may not be perfectly precise, leaving some bacterial DNA fragments within the prophage. Consequently, closely linked genes near the integration site are captured by the phage DNA. In cases where cells infected with a transducing defective phage – lacking a functional copy of a particular gene such as *gal* – recombination can occur, replacing the defective gene in the bacterial genome with an intact transduced gene. This results in a recombinant bacterium, or transductant, with a functional *gal+* gene.

Lateral (abortive) transduction occurs when the introduced fragment of donor DNA does not integrate into the recipient's chromosome but remains in the cytoplasm, where it functions independently. This DNA is subsequently transmitted to one of the daughter cells (i.e., inherited unilaterally) and is eventually lost in subsequent generations.

Genetic Variability of Bacteria

Changes in the bacterial genome can occur as a result of mutations. ***Mutations*** are alterations in the DNA nucleotide sequence, manifested by a heritable loss or modification of one or more traits. They arise from errors in copying hereditary information during replication. The phenotypic manifestations of a mutation may include changes in bacterial cell morphology; the emergence of auxotrophy (the requirement for growth factors such as amino acids or vitamins); the development of antibiotic resistance; alterations in temperature sensitivity, or reduced virulence (*attenuation*). Mutations in bacteria are non-directional.

Mutations can be spontaneous, occurring naturally without external influence, or induced. *Spontaneous* mutations result from DNA replication errors, incorrect base pairing, structural distortions of DNA, or the movement of mobile genetic elements during bacterial growth and reproduction. Spontaneous mutations can lead to both beneficial and detrimental genetic alterations. The probability of specific mutations occurring per cell per generation is known as the mutation frequency. At high growth rates, this frequency remains relatively constant and is typically determined for cells in exponential growth phase under optimal environmental conditions. Not all mutations are phenotypically expressed; unexpressed mutations are called *silent* mutations. A mutant may undergo a *reverse* mutation or *reversion*, restoring the properties of the wild-type strain. A true reverse mutation occurs only when the second mutation precisely restores the original genotype. If only the phenotype is restored, it is referred to as a *secondary reversion* or a *suppressor mutation*. Suppressor mutations can occur either within the same gene as the original mutation (*intragenic*) or in other chromosome regions (*extragenic*).

Induced mutations occur under the influence of external factors known as *mutagens*. Mutagens can be physical (such as UV rays and gamma radiation), chemical (including purine and pyrimidine base analogs, for example, 2-aminopurine, nitrous acid and its analogs, alkylating agents, etc.), or biological (such as transposons).

Depending on the extent of damage, mutations are classified into *point* mutations – where damage is limited to a single pair of nucleotides – and *large-scale* mutations (*aberrations*). Mutations are further divided into *chromosomal* mutations, leading to a new trait, when two or more chromosome regions are altered, and *gene mutations*, resulting in new traits due to changes within a gene. In this case, there may be base *modifications* (changes in individual nucleotides), the *deletion* of several nucleotide pairs, the movement of a group of nucleotides within a chromosome (*transposition*), a breakage with foreign DNA insertion (*insertion*), or the addition of nucleotide pairs (*duplication*), and DNA helix deformations. Point mutations tend to have a relatively high frequency of reversions, whereas reversions are less common in chromosomal aberrations.

The primary effect of a mutagenic factor does not necessarily produce a true mutation. A new phenotype appears only when the altered gene begins to function. Various methods allow for the accumulation and isolation of mutants with different types of defects, such as impaired transport processes or substrate utilization, defects in intermediate metabolism, increased temperature sensitivity, etc.

Although mutations caused by radiation, chemicals, or other factors could theoretically lead to the extinction of a bacterial population, any living cell possesses biochemical mechanisms capable of fully or partially restoring the original DNA structure. The set of enzymes catalyzing DNA damage correction reactions constitutes *repair systems*, which differ fundamentally in their biochemical mechanisms for restoring the genome. There are three main mechanisms for correcting DNA defects:

1) Direct reversal from damaged DNA to the original structure.
2) Excision (“dropout”) of the damage followed by restoration of the original structure.
3) Activation of mechanisms ensuring resistance to damage.

Reversal of DNA Damage. Mechanisms of direct reversal of DNA damage include *light repair*, also known as *photoreactivation*, which specifically addresses DNA damage caused by UV radiation. Light repair is mediated by several enzymes, including photolyase (which splits thymine dimers and restores the integrity of adjacent thymine bases); O6-methylguanine-DNA methyltransferase (*MGMT*) (which removes the O6 methyl group from guanine residues that have been damaged by methylating agents); DNA-purine insertase (which inserts a missing purine base into an apurinic site), and DNA-glycosylase (which removes

damaged bases from DNA). All these processes occur in a single step under the action of a specific enzyme, accurately restoring the original DNA structure.

Excision repair systems remove mispaired or damaged bases from DNA and then replace them with new, correct sequences (Fig. 79).

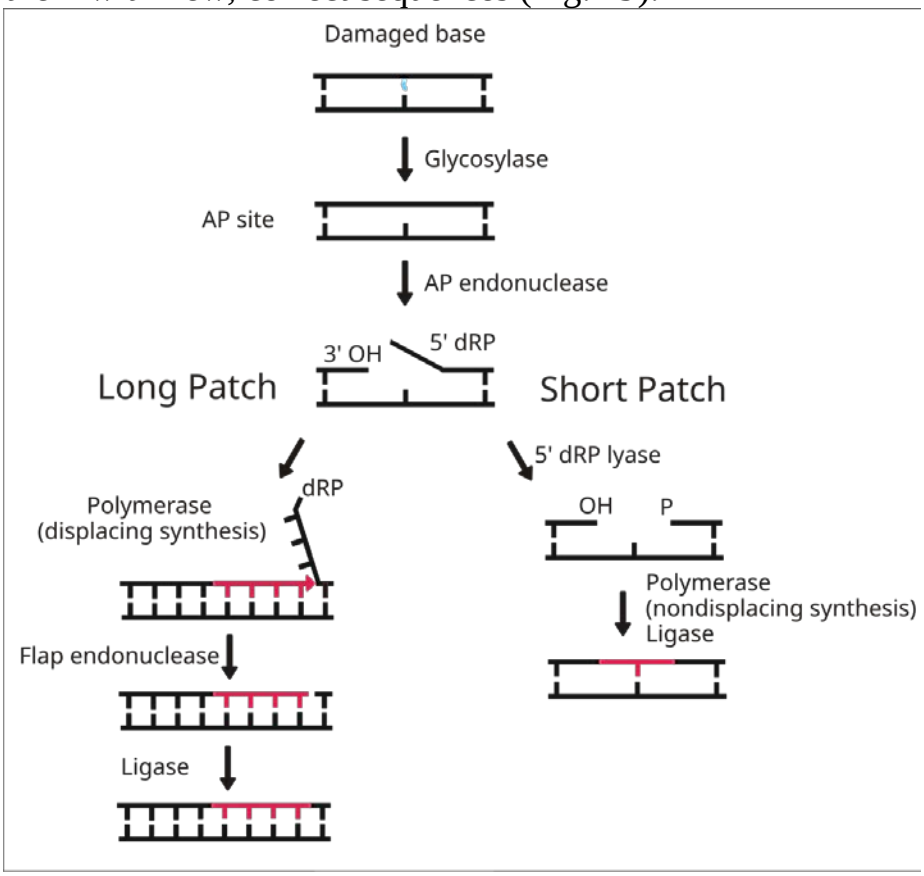


Fig. 79. Diagram of Excision Repair

The site of damage is recognized by an endonuclease, which cleaves the DNA strand near the defect. The damaged fragment is then removed, and the gap is filled by DNA polymerase, which enters the gap and synthesizes the missing nucleotides, using the undamaged DNA strand as a template. DNA ligase covalently joins the 3′-end of the newly synthesized DNA segment to the existing strand. Since this repair system relies on the resynthesis of a nucleotide chain based on an undamaged template, it is also virtually error-free.

Repair Mechanisms for Resistance to DNA Damage. In addition to damage correction mechanisms, cells have the ability to “bypass” DNA replication blocks caused by damage, for example, through recombination repair.

Phenotypic Variability of Bacteria

Temporary, non-hereditarily fixed changes are called ***modifications***. Modifications are also regulated by the bacterial genome but are not accompanied by alterations in the coding structure and are quickly lost, unlike mutations. Most commonly, bacteria exhibit *morphological* modifications (leading to reversible changes in shape) and *biochemical* modifications (manifested by inducible synthesis of certain products, most often enzymes). Modifications arise as adaptive responses of bacterial cells to environmental changes, allowing them to rapidly adapt and maintain the population at a viable level. Once the inducing factor is removed, bacteria revert to their original phenotype.

A standard manifestation of modification is the division of a homogeneous population into several types. This phenomenon is known as *microbial dissociation*. Dissociations usually occur under conditions unfavorable for the original population. An example of dissociation is a change in the appearance and structure of bacterial colonies on solid nutrient media. The first letters of the corresponding English names are used to designate dissociating colonies: *S-colonies* (from the English *smooth*), R-colonies (from the English *rough*), M-colonies (from the English *mucoid*), and D-colonies (from the English *dwarf*). Dissociations are often accompanied by changes in biochemical, morphological, antigenic, and pathogenic properties of the causative agents.

A phenotypic change should be considered a modification if three basic conditions are met: 1) certainty (a clear relationship between the phenotypic change and a specific factor); 2) universality of changes within the population; 3) reversibility (restoration of the original trait after cessation of the factor’s action).

Genetics of Viruses

Like all living organisms, viruses possess heredity and variability. A key feature of the viral genome is that hereditary information in viruses can be encoded in either DNA or RNA. The genomes of DNA-containing viruses are double-stranded (with a few exceptions like parvoviruses which have single-stranded DNA genomes), nonsegmented, and exhibit infectious properties. In viruses belonging to the genera *Poxvirus* and *Hepadnavirus*, the genome consists of two DNA strands of different lengths. Most RNA-containing viruses have single-stranded genomes. However, there are exceptions, most notably reoviruses with double-stranded RNA genomes and retroviruses with two identical single-stranded RNA strands. The genomes of RNA-containing viruses can be either segmented (e.g., *Retrovirus*, *Orthomyxovirus*, *Arenavirus*, and *Reovirus*) or nonsegmented.

Viral RNAs are classified into two groups based on their functions. The first group includes RNAs capable of directly translating genetic information onto the

ribosomes of a susceptible cell, i.e., performing the functions of mRNA. They are called *positive-sense (+) RNAs* and are designated as +RNA (or positive genome). These RNAs typically possess characteristic ends (“caps”) that are crucial for ribosome recognition.

In another group of viruses, RNA is not capable of directly translating genetic information onto ribosomes and functioning as mRNA (*minus-sense RNA*). Instead, such RNA serves as a template for the synthesis of mRNA; during replication, a complementary (+) RNA strand is initially synthesized to produce the −RNA. This type of RNA is referred to as the minus strand and is designated as −RNA (negative-sense genome). In viruses of this group, RNA replication differs from transcription in the length of the molecules produced: during replication, the RNA length corresponds to that of the parental (template) strand, whereas during transcription, shortened mRNA molecules are synthesized. +RNA molecules are infectious on their own, while −RNA molecules lack infectious properties and must be transcribed into +RNA for viral reproduction.

An exception to this are retroviruses, which contain single-stranded +RNA that serves as a template for viral RNA-dependent DNA polymerase (reverse transcriptase). Using this enzyme, information is transcribed from RNA into DNA, resulting in the formation of a DNA provirus that integrates into the host cell genome.

Viral nucleic acids are subject to *mutations*. Phenotypically, mutations in the viral genome manifest as changes in antigenic structure, inability to cause productive infection in a susceptible cell, altered thermal stability, and variations in size and shape of plaques formed under agar overlay. Most mutations tend to revert to the wild type, and each mutation has a characteristic reversion frequency that can be precisely measured. Viruses are classified based on whether their mutations occur spontaneously or are induced by external factors.

The rate of *spontaneous mutagenesis* in DNA genomes is significantly lower (approximately 10^{-8}–10^{-11} per incorporated nucleotide) than in RNA genomes (10^{-3}–10^{-4} per incorporated nucleotide). The higher frequency of spontaneous mutations is associated with the low fidelity of RNA genome replication, which is likely due to the absence of proofreading activity in RNA-dependent RNA polymerases (RNA replicases), an activity characteristic of DNA-replicating enzymes. Spontaneous mutations are most frequently observed in retroviruses, which is related to a higher rate of errors during reverse transcription that cannot be corrected by self-correction mechanisms.

Induced mutations in viruses occur under the influence of various chemical and physical mutagens and are classified into those acting in vivo and those acting in vitro.

Viral mutations are categorized based on changes in phenotype and genotype. *Phenotypically*, viral mutations are divided into four groups:

1. Mutations with no phenotypic expression.

2. Lethal mutations, which completely disrupt the synthesis or function of vital proteins, leading to loss of reproductive capacity.

3. Conditionally lethal mutations, which result in the loss of ability to synthesize a specific protein or impair its function only under certain conditions.

4. Mutations with phenotypic expression, such as changes in plaque size under agar overlay or alterations in thermal stability.

By changes in genotype, mutations are subdivided into *point mutations* (localized within individual genes) and *gene mutations* (affecting larger regions of the genome).

Infection of susceptible cells by viruses is often multiplicative, meaning that multiple virions can enter a single cell simultaneously. In such cases, viral genomes can interact during replication, either cooperating or interfering with each other. *Cooperative interactions* among viruses include genetic recombination, genetic reactivation, complementation, and phenotypic mixing.

Genetic recombination is more common in both DNA-containing and RNA-containing viruses with fragmented genomes (e.g., influenza virus). In genetic recombination, there is an exchange between homologous regions of viral genomes.

Genetic reactivation occurs between the genomes of related viruses carrying mutations in different genes. When genetic material is redistributed, a complete functional genome is created.

Complementation takes place when one of the co-infecting viruses synthesizes a non-functional protein due to a mutation. A non-mutant virus, by synthesizing a complete functional protein, can compensate for the absence of that protein in a mutant virus.

Phenotypic mixing occurs during mixed infection, when a susceptible cell is infected by two different viruses, resulting in some progeny acquiring phenotypic traits from both parental viruses while maintaining an unchanged genotype.

In cases of multiple infection of a susceptible cell, interfering interactions can also occur between viruses. ***Viral interference*** describes the phenomenon of immunity to a secondary infection in a cell already infected with a virus. *Heterologous interference*, in particular, occurs when infection with one virus completely blocks the replication of a second, different virus within the same cell. The mechanisms underlying heterologous interference include the suppression of adsorption of the second virus through blocking or destroying specific receptors, as well as the inhibition of mRNA translation of any heterologous mRNA in the infected cell. Additionally, the primary infection can induce the production of interferon, which inhibits the replication of the subsequent virus.

Homologous interference, i.e., interference between identical viruses, is characteristic of many viruses, especially during repeated in vitro passages and at

high multiplicities of infection. Under these conditions, numerous *defective viral particles* are produced, which are usually incapable of independent reproduction. However, defective viruses can replicate when co-infecting a cell with a complete, functional virus (also known as a helper virus). In such cases, the defective virus may interfere with the helper virus's replicative cycle and generate daughter *defective interfering (DI) viral particles*. DI particles possess three main properties: defectiveness, which means they have damage in essential genes; interference, where DI particles inhibit the replication of the complete virus or other homologous viruses; and self-enrichment through replication at the expense of a standard virus. The circulation of DI particles and co-infection with a complete functional virus can lead to persistent, long-term infections.

In addition to interactions among viruses, mixed infections also involve interactions between the virus and the host cell. The interaction of cells with DNA-containing viruses can lead to *viral transformation of the cell*. Because of transformation, the morphological, biochemical, and growth characteristics of the cells are altered, potentially resulting in tumorigenic growth. The transformation of cells induced by RNA-genomic retroviruses is of particular interest to scientists . In retroviruses, transformation and replication are not mutually exclusive, as transformed cells are capable of replicating the virus. The genomes of transforming viruses typically integrate into the genome of the transformed cell.

Application of Genetic Methods in Diagnosing Infectious Diseases

In the diagnosis of infectious diseases using genetic methods, the marker of the pathogen is its gene. Nucleic acid detection methods are employed for diagnosing viral infections, identifying bacteria – particularly those that are difficult to isolate –and determining the precise taxonomic position of microorganisms. These methods allow for the detection of a microorganism in various samples (including water, food products, patient samples) by identifying its DNA without the need for isolation into a pure culture.

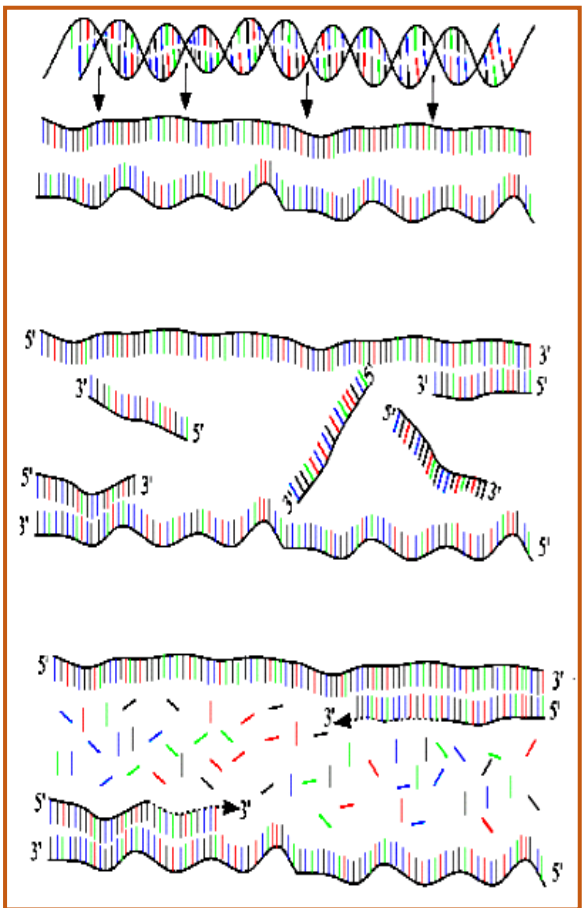
The *molecular hybridization method* relies on the ability of DNA and RNA to specifically bind (*hybridize*) with complementary oligonucleotide fragments that are artificially synthesized and labeled with an enzyme, fluorochrome, or isotope. These fragments are known as *probes*.

To perform molecular hybridization, the DNA molecule under study is denatured, and one strand is immobilized on a special filter. This filter is then placed in a solution containing the labeled probe. Conditions are optimized to facilitate the formation of double helices. If there is complementarity between the probe and the target DNA, they will hybridize to form a double helix. After hybridization is complete and unbound products are washed away, the resulting complex is detected using the appropriate label.

Polymerase Chain Reaction (PCR) is based on the repeated increase in the number of copies (*amplification*) of a specific DNA segment, catalyzed by the enzyme DNA polymerase. PCR is a highly sensitive method; in theory, the presence of a single DNA molecule in the sample is sufficient to produce a detectable result.

PCR typically involves three main stages: sample preparation (DNA or RNA isolation), the PCR reaction itself, and detection of the amplified PCR product. When using RNA as a template in a PCR reaction, complementary DNA (cDNA) is first synthesized from this RNA template using the enzyme RNA-dependent DNA polymerase (reverse transcriptase). The cDNA then serves as the template for the PCR amplification. With advances such as thermostable DNA polymerase, originally derived from the bacteria *Thermus aquaticus*, which possesses both DNA polymerase and reverse transcriptase activities, it has become possible to combine these reactions into a single process. This variant of PCR is widely used for detecting RNA-containing viruses and for analyzing gene expression of viral, bacterial, and cellular genes through their RNA.

Five main components are required for PCR to work: 1) DNA polymerase enzyme; 2) a pair of oligonucleotide primers; 3) a set of nucleotides; 4) template DNA; and 5) Mg^{2+} ions, which are essential for DNA polymerase activity. A diagram of PCR is illustrated in Figure 80.



1. Denaturation

2. Primer Annealing

3. DNA Strand Synthesis

Fig. 80. Diagram of Polymerase Chain Reaction

For amplification (i.e., synthesis of the DNA template), the most conserved region, a unique gene is selected. To initiate synthesis on the DNA template, two primers are used: short, single-stranded DNA fragments approximately 20–30 bases in length that are complementary to the 3′-ends of the target gene's DNA. The DNA isolated from the sample is first heated, causing it to denature into two separate strands. Primers are then added, and the mixture of DNA and primers is cooled. If the mixture contains the DNA of the target gene, the primers anneal to its complementary regions (*annealing*). Next, DNA polymerase and nucleotides are added. At a temperature optimal for DNA polymerase activity, nucleotides are incorporated into the 3′-ends of the primers, resulting in the formation of a specific DNA fragment known as an amplicon. This cycle is repeated again, with each cycle doubling the amount of target DNA. It is estimated that after 30–40 cycles, from a single template molecule, approximately 10^8 amplicons can be produced. The reaction is performed in specialized devices called thermal cyclers (also known as thermocyclers, PCR machines or DNA amplifiers). After 30–80 cycles, the amplified DNA products are typically analyzed by gel electrophoresis and visualized under UV light after staining with ethidium bromide. To confirm that the amplified DNA belongs to a specific pathogen, hybridization techniques can be employed.

INFECTION. INFECTIOUS PROCESS

Main Forms of Infection

Infection is a process of interaction between a microorganism and a macroorganism, occurring under specific conditions of the external and social environment.

The infectious process comprises a series of physiological and pathological reactions that develop within the macroorganism during infection.

An infectious disease represents one of the clinical manifestations of the infectious process.

The development of infection is influenced by several factors, including the state of the host organism's defense mechanisms, the properties of the pathogen and its infectious dose, environmental conditions, transmission routes, and the portals of entry for infection.

Forms of Infection. Depending on the properties and nature of the pathogen, its localization within the macroorganism, its transmission routes, and the state of the host organism, the following main forms of infection are distinguished:

1) Exogenous infection occurs as a result of the entry of pathogenic microorganisms from external sources, such as infected patients or bacterial carriers, or via water, food, air, or soil.

2) Endogenous infection is caused by opportunistic microorganisms – members of the body's normal microflora – that become pathogenic due to a decrease in the macroorganism's resistance (e.g., hypothermia, trauma, surgical interventions, immunodeficiency states).

Infections are also classified as acute and chronic. An acute infection is characterized by a sudden onset and a short course. A chronic infection persists over an extended period, with the pathogen potentially remaining in the host organism for several months or years.

Depending on where pathogens are located in a host's body, infections are classified as either focal or generalized. In focal infections, the microorganism is confined to a specific site, whereas in generalized infections, the pathogen spreads throughout the entire host organism via lymphogenous and hematogenous routes. In such cases, bacteremia or viremia may develop. During sepsis, the pathogen proliferates in the patient's bloodstream. In cases where purulent foci form within internal organs, septicopyemia may develop. The presence of microorganism toxins in the blood is referred to as toxinemia.

Depending on the number of microorganism species involved in causing the disease, infections are further divided into monoinfection and mixed (or polymicrobial) infection.

A monoinfection is caused by a single type of microorganism, while a *mixed* infection involves two or more microbial species

Reinfection occurs when the same pathogen causes disease again after recovery.

Superinfection refers to an ongoing infection with the same pathogen during the course of treatment until complete recovery.

Relapse is characterized by the recurrence of clinical symptoms without a new infection, stemming from the persistence of the original pathogen within the host.

Secondary infection involves a new infection caused by a different pathogen that joins an existing primary infection.

Autoinfection describes an infectious process initiated by the body's own microflora, most often by opportunistic microorganisms.

Infections can be also categorized based on the presence or absence of symptoms. Manifest infections exhibit noticeable clinical signs, while asymptomatic infections show minimal or no symptoms.

Typical infections are characterized by the development of clinical symptoms that are specific and distinctive to the particular disease.

Atypical infections often present with subtle and indistinct clinical symptoms, often due to low virulence of the pathogen, strong immune response, or effective treatment.

Slow infections are characterized by a prolonged incubation period, a progressive course of the disease, a weak immune response, and an unfavorable prognosis. The pathogen remains in the human body for an extended period (months or years) in a latent state. Under favorable conditions, it begins to actively multiply and can lead to severe disease.

Persistent infection occurs when the pathogen, upon entering the host, causes a disease but undergoes L-transformation under the influence of active chemotherapy and acquired specific immunity. Such bacterial forms are resistant to many chemotherapeutic agents and antibodies, allowing them to persist in the patient's body for a long time. Under certain conditions (such as a decreased host resistance or cessation of treatment), the pathogen can restore its pathogenic properties and trigger a relapse of the disease.

Latent (inapparent) infection refers to a condition where the disease progresses covertly without any noticeable clinical symptoms.

Bacterial carriage describes a state following latent infection or a resolved initial illness, when the host is unable to completely eliminate the pathogen. This form of infection is referred to as a bacterial or viral carriage. This condition develops when post-infectious immunity is weak. In this condition, after clinical recovery, an individual remains a carrier of the pathogen for many months or years, serving as a source of infection for others.

Abortive infection occurs when the pathogen penetrates the host organism but does not multiply within it. Due to the high resistance of the host body, the infectious process does not develop.

Main Sources of Infection. Routes and Modes of Transmission. Portals of Entry for Infection

The primary ***sources*** of infection include:

1) Infected individuals, bacterial or viral carrier, and convalescents;
2) Animals;
3) Environmental objects.

Infection of a person from a sick individual can occur throughout the entire course of the disease. In cases of bacterial carriage, the release of the pathogen can continue even after a patient's clinical recovery. Diseases that exclusively affect humans, such as cholera and typhoid fever, are referred to as *anthroponoses*.

Animals also serve as sources of infection. Humans become infected directly through contact with sick animals or by consuming contaminated food, or via bites from blood-sucking vectors. Diseases that affect both humans and animals are called *zoonoses* (e.g., brucellosis, plague, leptospirosis).

Environmental objects can serve as a natural habitats for certain pathogenic bacteria and also become contaminated with human and animal waste.

A transmission mechanism is a set of evolutionarily developed methods by which an infectious (parasitic) pathogen moves from the source to a susceptible organism. Each transmission mechanism can operate through one or multiple transmission routes.

There are several mechanisms for the transmission of infectious agents, each involving specific routes:

1. *Aspiration* (Aerosol) Mechanism. Transmission routes: airborne particles, dust. Transmission factors: liquid and dry aerosols.
2. *Fecal-Oral* (for anthroponoses), or *Alimentary* Mechanism. Transmission routes: water, food, household contact. Transmission factors: contaminated water and food, hands, flies, soil.
3. *Contact* Mechanism. Transmission routes: direct contact (including sexual contact), household contact. Transmission factors: mucus, pus, serous discharges, household items, soil, water.
4. *Transmissive* Mechanism. Transmission routes: inoculation, contamination. Transmission factors: blood-sucking arthropods, saliva of blood-sucking animals, tick coxal fluid.
5. *Vertical* Mechanism. Transmission route: transplacental. Transmission factor: mother's blood.

6. *Artificial* Mechanism. Transmission route: parenteral. Transmission factors: medical and non-medical instruments.

Portals of Entry for Infection. The site through which the pathogen enters the internal environment of the host organism is known as the portal of entry for infection. Human become infected via damaged skin, mucous membranes of the digestive and respiratory tracts, and the genitourinary system. Infection through intact skin is rare (e.g., leptospirosis).

Depending on the type of pathogen and its properties, further dissemination throughout the body occurs via lymphogenous, hematogenous, or neurogenic routes. Some microorganisms begin to multiply at the site of entry, leading to a focal infection. The spread of the pathogen throughout the entire body results in the generalization of the infectious process.

Periods of the Infectious process

A distinctive feature of infectious diseases is their cyclical course, consisting of alternating periods: *incubation, prodrome, disease development* (*full onset of illness*), and *recovery* (*convalescence*).

The incubation period is the interval from the moment the pathogen enters the host organism until the first clinical symptoms of the disease appear. The duration of the incubation period varies widely depending on the specific infectious disease – from several hours (e.g., influenza) to several months (e.g., hepatitis B). It depends on factors such as the type of microorganism, the infectious dose, its virulence, route of entry into the body, and the host organism's state. The incubation period is associated with the pathogen's adhesion to and colonization of the host cells at the portal of entry. During this period, there are no overt signs of disease; however, initial manifestations of the pathological process are already occurring within the body, including morphological alterations, metabolic and immunological shifts, etc. If the macroorganism proves unable to neutralize the pathogen, the disease progresses to subsequent phases.

The prodromal period is characterized by the appearance of initial general signs of the disease without clear, specific symptoms characteristic of the particular infectious process. Nonspecific symptoms common to many diseases develop, such as fever, malaise, loss of appetite, general weakness, headache, and a low-grade fever. The duration of the prodromal period typically ranges from 1 to 3 days but can extend up to 10 days, depending on the etiology of the infectious disease. For certain diseases (e.g., leptospirosis or influenza), the prodromal period may be absent. The absence of a prodromal period may indicate a more severe form of the infectious process. During this period, the pathogen actively multiplies at its site of localization, produces corresponding toxins, and invades tissues.

The period of disease development (full onset of illness). During the development phase, alongside general nonspecific signs, characteristic symptoms

specific to the disease emerge. The most typical signs of an infectious disease include fever, inflammation, damage to the central and autonomic nervous systems, and dysfunction of the cardiovascular and digestive systems. In some diseases, skin rashes, jaundice, and other symptoms may appear. In this period, the pathogen actively multiplies within the body; toxins and enzymes accumulate and enter the bloodstream, leading to intoxication syndrome or toxic shock syndrome. During the full onset of the illness, there is an active restructuring of the body's immune response, marked by the production of specific IgM antibodies followed by subsequent synthesis of IgG antibodies.

During this period, the patient is most infectious to others due to the shedding period, where the pathogen is actively released from the body into the environment.

The duration of the peak and the development phases of the disease is influenced by several factors including the type of pathogen, the host organism's immunological reactivity, how quickly the disease is diagnosed, the effectiveness of treatment, and other conditions.

Recovery Period (Convalescence). If the disease progresses favorably, the recovery period begins. Recovery is characterized by the gradual disappearance of clinical symptoms, restoration of impaired body functions, and the neutralization and elimination of pathogens and toxins from the body. Recovery can be complete, with full restoration of all impaired functions, or incomplete, if residual effects remain (e.g., muscle atrophy in poliomyelitis, sequelae of tick-borne encephalitis, or skin defects in smallpox). Clinical recovery often precedes the pathomorphological restoration of damaged organs and complete clearance of the pathogen from the body. In most infectious diseases, during convalescence, the body is fully cleared of the pathogen and immunity is established.

In some cases, recovery may lead to microbial carriage, or a relapse may occur after apparent recovery.

The Concept of Pathogenicity and Virulence of Bacteria. Toxins

Pathogenicity refers to the inherent potential of a microorganism to cause an infectious process. Pathogenicity is a species-specific trait has developed during the evolution of the microorganism and its adaptation to parasitize the human body. Pathogenicity is characterized by specificity, meaning the ability to induce pathomorphological and pathophysiological changes in particular tissues and organs.

To quantitatively evaluate the degree of pathogenicity of a microorganism, the concept of ***virulence*** is used. Virulence is measured in conventionally accepted units such as DLM, DcL, and DL_{50}. DLM (Dosis letalis minima) represents the minimum dose of microorganisms that causes death in 95% of susceptible laboratory animals. DL_{50} causes death in 50% of infected animals, while DcL results in the death of all tested animals.

The degree of a microorganism's pathogenicity depends on numerous factors and is determined both by the presence of enzymatic systems that enable the pathogen's survival within the host organism and by its ability to resist the host body's defense factors aimed at eliminating the pathogen. Based on the degree of pathogenicity, microorganisms are classified as either pathogenic or opportunistic. *Pathogenic* microorganisms are capable, in most cases, of causing an infectious disease, whereas *opportunistic* microorganisms are often naturally found in the human body that cause disease only when immunity is compromised or when exposed to a sufficiently large infectious dose. The pathogenic potential of a microorganism is associated with its ability to adhere, colonize, invade, suppress phagocytosis, and other factors.

Pathogenicity factors include the ability of microorganisms to attach to host cells (*adhesion*), multiply on their surface (*colonization*), penetrate cells and tissues (*invasion*), and resist the body's defense factors (*aggression*).

Adhesion is the initiating mechanism of the infectious process. It refers to the ability of a microorganism to adsorb to susceptible cells, leading to colonization. The structures responsible for binding a microorganism to a host cell are called adhesins and are located on the microbial surface. Adhesins are highly diverse in structure and determine the pathogen tropism – the ability of certain microorganisms to attach specifically to the epithelial cells of the respiratory tract, while others attach to the intestinal tract, the genitourinary system, or other tissues. The adhesion process can be influenced by physicochemical mechanisms related to the hydrophobicity of microbial cells and the balance of attractive and repulsive forces. In Gram-negative bacteria, adhesion is mediated by common pili. In Gram-positive bacteria, adhesins are surface proteins and teichoic acids of the cell wall. In other microorganisms, this function is performed by various cellular structures such as surface proteins, lipopolysaccharides, and others.

Invasion. Invasiveness refers to the ability of microorganisms to penetrate through mucous membranes, skin, or connective tissue barriers into the internal environment of the host and subsequently spread through its tissues and organs. Microbial invasion involves the production of enzymes, as well as factors that suppress cellular defenses. For example, the enzyme hyaluronidase breaks down hyaluronic acid – a component of intercellular substance – thereby increasing the permeability of mucous membranes and connective tissue. Neuraminidase breaks down neuraminic acid, which is part of surface receptors on mucous membrane cells, facilitating pathogen entry into tissues.

Aggression. Aggressiveness refers to the pathogen's ability to resist the host's protective factors. Factors contributing to microbial aggression include proteases – enzymes that degrade immunoglobulins; coagulase – an enzyme that that causes blood plasma to clot; fibrinolysin – an enzyme that dissolves fibrin clots; and lecithinase – an enzyme that acts on phospholipids in the membranes of muscle

fibers, erythrocytes, and other cells. Pathogenicity may also be associated with other microbial enzymes, which can act both locally and systemically.

An important role in the development of infectious process play toxins. Based on their biological properties, bacterial toxins are classified into *exotoxins* and *endotoxins*.

Exotoxins are produced by both Gram-positive and Gram-negative bacteria. Chemically, exotoxins are protein-based substances. Depending on their mechanism of action on host cells, exotoxins are categorized into several types: cytotoxins, membrane toxins, functional blockers, exfoliants, and erythrogenins. The pathogenic mechanism of protein toxins involves damaging vital cellular processes: increasing membrane permeability, inhibiting protein synthesis, disrupting other biochemical pathways within the cell, or interfering with intercellular interactions and coordination. Exotoxins are potent antigens that stimulate the production of antibodies (specifically, antitoxins) in the host organism.

According to their molecular organization, exotoxins are divided into two groups:

1) Exotoxins composed of two fragments;
2) Exotoxins comprising a single polypeptide chain.

Based on the degree of association with the bacterial cell, exotoxins are conventionally divided into three classes:

- Class A: Toxins secreted into the external environment.
- Class B: Toxins that are partially secreted and partially bound to the microbial cell;
- Class C: Toxins that are bound to the microbial cell and released into the environment upon cell destruction.

Exotoxins are highly toxic. When treated with formalin or exposed to heat, exotoxins lose their toxicity but retain their immunogenic properties. Such toxins are called toxoids and are used to prevent diseases such as tetanus, gas gangrene, botulism, and diphtheria. They are also used as antigens to immunize animals to produce antitoxic sera.

Chemically, endotoxins are lipopolysaccharides contained in the cell wall of Gram-negative bacteria and released into the environment during bacterial lysis. Endotoxins are nonspecific in their effects, thermostable, less toxic than exotoxins, and exhibit weak immunogenicity. When present in large doses, endotoxins can inhibit phagocytosis, increase capillary permeability, and exert cytotoxic effects. Microbial lipopolysaccharides can destroy blood leukocytes, induce mast cell degranulation with the release of vasodilators, and activate the Hageman factor, leading to leukopenia, hyperthermia, hypotension, acidosis, and disseminated intravascular coagulation (DIC).

Endotoxins stimulate the synthesis of interferons, activate the complement system via the classical pathway, and possess allergic properties. Administration of small doses of endotoxin can enhance host resistance by increasing phagocytosis and stimulating B-lymphocytes.

The pathogenicity of bacteria is regulated by three types of genes: those located on their own chromosome, as well as genes introduced by plasmids or temperate phages.

IMMUNOLOGY OF THE INFECTIOUS PROCESS

Immunity. Types of Immunity

Immunity (from the Latin *immunitas* – relief, deliverance from something) is a system of biological mechanisms aimed at maintaining the constancy of the body's internal environment. It enables the organism to recognize and eliminate all entities that are genetically foreign, whether exogenous (pathogenic microorganisms, helminths, their toxins, proteins, foreign tissues, and organs) or endogenous (the body's own mutated or damaged cells).

The recognition and a specific response to foreign agents are functions of the *immune system.*

There are two primary mechanisms of the body's defense against foreign agents:

1. An evolutionarily developed system of cellular and humoral factors of resistance (from the Latin *resistentia* – resistance), controlled by genetic mechanisms, which is known as the *innate immunity*. This constitutes the body's first line of defense against microorganisms and comprises a set of pre-immune biological responses.
2. In cases of defects or failure of the body's resistance factors, an infectious process can develop under natural conditions. During this process, a second line of defense is activated through the immune system that is referred to as the *acquired immunity* and characterized by the development of specific immune responses directed against particular foreign agents.

Depending on their mechanisms of formation, several types of acquired immunity are distinguished (Fig. 81).

Acquired active immunity occurs following contact the host organism with pathogen antigens, during which the immune system actively participates in the development of an immune response. The duration of actively acquired types of immunity is typically significant.

Acquired natural active immunity develops after an infectious disease, a silent infection, or repeated community exposures without the manifestation of clinical illness. It is often referred to as post-infectious immunity and, depending on the extent of the body's clearance of the pathogen, is subdivided into sterile and non-sterile. The duration of this immunity can vary widely, lasting for years, decades, or even a lifetime (e.g., typhoid fever, diphtheria, measles).

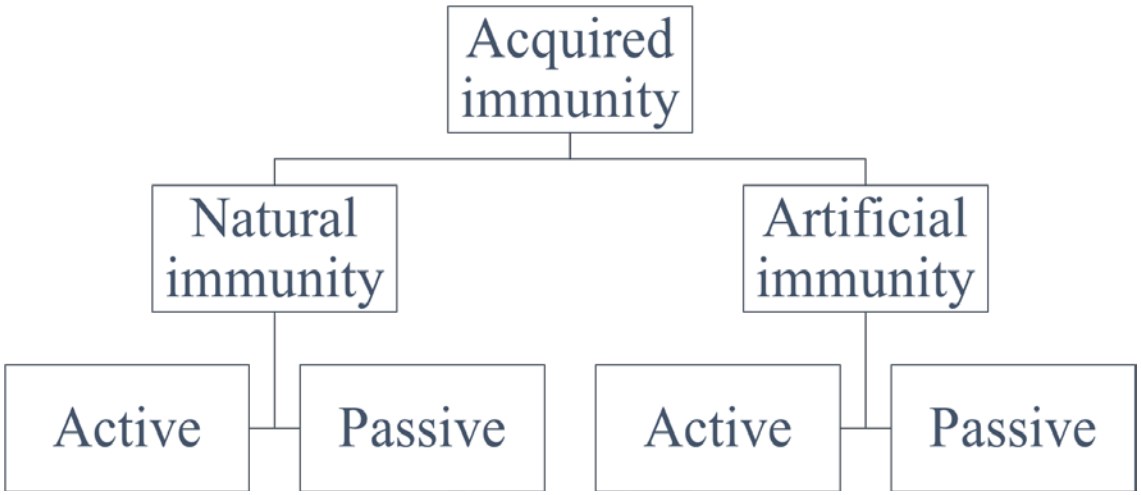


Fig. 81. Types of Acquired Immunity

Acquired passive immunity is formed by the transfer of antibodies into a host organism, either naturally or artificially, without the participation of the immune system in its production.

Acquired natural passive immunity occurs when maternal antibodies are transferred to the fetus via the bloodstream (IgG) and through breast milk during breastfeeding (secretory IgA). This type of immunity (placental, maternal) provides the newborn with resistance to the pathogens of certain infectious diseases (e.g., measles, diphtheria, or scarlet fever) for approximately 6–7 months.

Acquired artificial passive immunity is established through the administration of specific antibodies produced by another organism (heterologous sources such as animals, or homologous sources such as humans). The duration of this immunity ranges from 2–3 weeks to about 1.5 months.

None of these forms of acquired immunity are transmitted to offspring genetically. Their effectiveness is relative and typically diminishes over varying periods.

Acquired anti-infectious immunity is comprised of two types of immune responses: humoral and cellular. The strength of humoral immunity depends on the class and level of circulating specific antibodies, while the cellular immunity depends on the functional activity of macrophages and various subpopulations of T-lymphocyte. Usually, both responses work together to develop protection against infectious agents, with one or the other predominating during different phases of an infectious disease.

Depending on the nature and biological properties of microorganisms, acquired anti-infectious immunity is divided into different categories, including antitoxic, antibacterial, antiviral, anti-fungal and anti-protozoan immunity. However, this division is largely conventional and primarily serves didactic purposes.

Along with acquired immunity, there is *species immunity*, which is a genetically mediated insusceptibility of certain species of animals and humans to pathogens that infect other species. For example, humans are not susceptible to the canine distemper virus or cattle plague, as human cells lack the receptors for these viruses to infect. Similarly, some animals are resistant to certain viruses (such as varicella (chickenpox), hepatitis A and B, and measles) and bacteria (such as gonococci and syphilis pathogens). Rats and mice are resistant to diphtheria toxin, while cats and dogs are resistant to tetanus toxin.

Species immunity is typically caused by the absence of specific receptors on host cells for the pathogen (bacteria, viruses, toxins) or by the lack of membrane substrates required by enzymes that facilitate microbial invasion (e.g., gonococcus).

Additionally, there are intraspecific (racial or ethnic) differences in susceptibility to infectious diseases. For instance, in some African populations, a gene has been identified that leads to the production of abnormal hemoglobin, known as sickle cell hemoglobin or hemoglobin S (Hb-S). Erythrocytes containing this hemoglobin take on a sickle shape. Individuals who are heterozygous for this gene exhibit resistance to malaria caused by *Plasmodium falciparum*.

The Concept of Organismal Resistance. Factors of Resistance

The body's resistance (from the Latin *resistentia* – resistance) refers to its ability to withstand the effects of various factors, including foreign agents (antigens).

The body's resistance is determined by the action of nonspecific defense factors (resistance). Non-specific defense mechanisms are established under genetic control during the development of the organism (innate and species immunity) and determine the non-selective nature of the response to an antigen.

The ability to interact with a microorganism and respond to it as a factor disrupting normal physiological functions reflects the general physiological reactivity of the organism.

The susceptibility of a macroorganism depends on the site of infection (portals of entry) through which pathogens penetrate. The entry of a parasitic microorganism into the host triggers a complex cascade of protective and adaptive responses, aimed ultimately at eliminating the pathogen and restoring the structure of the body's organs and systems. The protective reactions of the macroorganism mobilized during an infectious process ensure the chemical stability of the body's internal environment, as well as its genetic and antigenic composition (homeostasis). These responses represent a specific form of the body's adaptive reaction to any damaging factor.

The body's resistance depends on the impermeability of the normal skin and mucous membranes to most microorganisms, the presence of bactericidal substances in skin secretions, the acidity of stomach contents, and the presence in

blood and other body fluids (such as saliva, tears, etc.) of enzyme systems like lysozyme, properdin, and others. It also depends on the number and activity of phagocytes in the blood and tissues.

All these factors are nonspecific, formed during prenatal development (ontogenesis), and are genetically determined. From birth until natural death of the macroorganism (the postnatal period of ontogenesis), their functional activity is constantly changing, either due to age-related features or as a result of exposure to various external factors (physical, chemical, biological, psychotropic, etc.). At any given moment in an individual's life, the collective influence of these factors determines the degree of resistance of their organism. Their primary role is to prevent the development of disease during an incipient infectious process.

Thus, resistance factors include:

1. The insusceptibility of host cells to pathogenic microorganisms and their toxins, caused by the absence of adhesion receptors on the cell surface.
2. A temperature response, which inhibits the reproduction of most pathogenic microbes.
3. External barriers (skin, mucous membranes, and microbiota).
4. Internal barriers (lymph nodes, tissue and cellular barriers).
5. Cellular factors (phagocytes, Natural Killer (NK) cells, mast cells).
6. Humoral factors (complement system proteins, lysozyme, interferons, acute phase proteins, cytokines, properdin, and lysins).

Skin

The skin is an important resistance factor, serving as a mechanical barrier against the entry of parasites. The shedding of the upper layers of the epidermis, along with secretions from sebaceous and sweat glands, facilitates the removal of microorganisms from the skin surface. Various microbicidal substances (such as lactic acid, fatty acids, etc.) that contribute to the skin's "self-cleaning" function play a significant role in eliminating microorganisms. As a result, various microorganisms that are not permanent residents of the skin cannot remain its surface for extended periods. The bactericidal properties of skin secretions are largely dependent on their acidity.

Mucous Membranes

For most pathogenic microorganisms, the mucous membranes of various organs serve as a barrier, preventing their penetration into the body. The permeability of mucous membranes depends on the physiological state of the host and on the biological activity of a given pathogen type or strain.

Pathogenic microorganisms do not find optimal conditions for reproduction on mucous membranes due to the bactericidal action of tissues and secretions, as well as being washed away by saliva and other fluids. Consequently, their reproduction is significantly slowed in natural environment.

Normal Microbiota

On the surface of the skin and on all mucous membranes of an adult, there are approximately 10^1 to 10^4 representing normal and opportunistic flora at any given time. Members of the normal microbiota perform essential functions that contribute to the colonization resistance of the host, as well as the maturation and maintenance of immune system activity.

Lymph Nodes, Tissue, and Cellular Barriers

Lymph nodes, along with clusters of lymphoid cells in various internal organs, serve as primary barriers against most microorganisms. Within lymphoid tissue, cellular factors such as cytotoxic effect and phagocytosis facilitate the fixation and destruction of a significant portion or all of the pathogens.

The skin and mucous membranes of the gastrointestinal and respiratory systems are particularly rich in lymphatic vessels. When these barriers are damaged, microbes on their surface can penetrate the lymphatic vessels and be transported by the lymph flow to the lymph nodes, which function as unique biological filters for pathogens carried with the lymph.

Histohematic barriers prevent pathogens from crossing from the bloodstream into the brain, reproductive system, and eyes. Additionally, the membrane of each cell acts as a barrier, preventing the penetration of microorganisms.

Inflammation. Cellular factors

When the body is exposed to various damaging factors of physical, chemical, or biological origin, a complex nonspecific protective and adaptive response occurs. This process is known as inflammation. *Inflammation* is a localized reaction of blood vessels, connective tissue, and the nervous system to injury. The process of inflammation involves lymphatic structures, the vascular network, and the locally damaged tissues. Initially, fluid exchange is disrupted, leading to impaired circulation within the capillaries. One of the most significant changes during inflammation is an increase in capillary permeability.

The outcome of the inflammatory response is the localization of microorganisms or the delimitation of the lesion caused by them, followed by their elimination and partial or complete restoration of the affected structures.

The first step in the inflammatory process involves vascular changes, primarily manifested by increased permeability of the vessel walls. The permeability increase is mediated by biologically active substances (such as histamine, serotonin, and kinins) released upon cell damage – particularly from mast cells – and results in perivascular exudation of plasma (“serous edema”) and migration of phagocytic cells (neutrophils and macrophages) to the affected tissue areas.

Inflammatory edema, characterized by the accumulation of exudate in inflamed tissue, plays a crucial role as a factor capable of binding and immobilizing bacterial toxins at the site of inflammation, thereby preventing their absorption and spread throughout the body. Of particular protective significance are the phagocytic and proliferative functions of cells such as histiocytes and macrophages. The granulation tissue formed by these cells constitutes a powerful protective barrier against infection.

Phagocytes can be considered the primary factors of inflammation. In particular, tissue macrophages play a vital role due to their ability to engulf and digest various foreign agents, including microorganisms. The protective properties of phagocytes are associated with the presence of numerous lysosomes within their cytoplasm – organelles rich in various hydrolytic enzymes that facilitate the breakdown of many chemical bonds. The process of phagocytosis often results in the death of the phagocytic cell; however, this process also leads to the destruction of a large number of microorganisms.

Phagocytosis (from the Greek *phagos* – “to devour”, and *cytos* – *cell*) is a general biological, nonspecific phenomenon that, phylogenetically, reflects a high level of recognition of foreign substances. Phagocytic cells of the mesenchyme have acquired a certain specialization over the course of evolution, enabling them to engulf a wide range of foreign particles such as microorganisms, macromolecular antigen-antibody complexes, cells and their organelles, viruses, colloidal dye solutions, and others. The most important property of phagocytes is their ability to recognize “non-self” issues, which places this reaction at a transitional stage between resistance and specific immunity.

Phagocytes include polymorphonuclear cells (neutrophils, eosinophils, and basophils), mononuclear cells (blood monocytes), as well as fixed macrophages such as alveolar macrophages, peritoneal macrophages, dendritic cells, and others.

The process of phagocytosis occurs in several stages.

In the first stage, the phagocyte approaches the foreign object through positive *chemotaxis*. Next, the foreign agent is *recognized* and *adsorbed*; this process primarily involves the cellular membrane of the phagocyte, which uses specific receptors (such as Fc receptors) to establish contact with the target. The engulfment of the foreign particle involves complex changes in the physicochemical properties of the phagocyte’s cytoplasm. Initially, actin-like protein polymerizes; subsequently, these polymers contract under the action of myosin with a cofactor. This results in the formation of pseudopodia that surround and capture the particle. The ingested particle is enclosed within a *phagosome* – a membrane-bound vesicle formed by invagination of the plasma membrane – inside the cytoplasm. When a phagosome fuses with lysosomal granules, a *phagolysosomal* (*digestive*) *vacuole* is formed.

The final stage of phagocytosis involves the *digestion* of the foreign agent (e.g., a bacterium) into biologically inert, low-molecular-weight compounds. The

outcome of phagocytosis is the death of the foreign agent (complete phagocytosis). However, in some cases, death does not occur, and certain pathogens can even multiply within the phagocyte and kill it, leading to incomplete phagocytosis. The defense mechanisms of pathogenic microorganisms that lead to incomplete phagocytosis include obstruction of lysosomal fusion with phagosomes (as seen with *Toxoplasma* and *Mycobacteria*), resistance to the action of lysosomal enzymes (such as gonococci, staphylococci, and streptococci), or escape from the phagosome, or avoidance of microbicidal factors (as with *Rickettsiae*). Incomplete phagocytosis results in the persistence of pathogens within the body and can contribute to chronic infection.

Microbicidal activity of phagocytes can be classified into *oxygen-dependent* and *oxygen-independent* mechanisms.

Oxygen-dependent mechanisms include the respiratory burst.

The respiratory burst is characterized by an increase in the production of reactive oxygen species (ROS) within phagocytic cells. These reactive oxygen forms are categorized into primary and secondary species. Primary reactive oxygen species include superoxide radicals (O_2^-) and nitric oxide (NO). These molecules exert regulatory and moderate bactericidal effects and are produced enzymatically within cells. Superoxide radicals are generated by the enzyme NADPH oxidase, while nitric oxide is produced by nitric oxide synthase.

A complex of enzymes is activated that synthesizes secondary ROS, including hydrogen peroxide (H_2O_2), produced by superoxide dismutase from superoxide radicals, and hypochlorous acid (HOCl), generated by myeloperoxidase from H_2O_2. All secondary ROS possess potent bactericidal activity. They are secreted into the phagosome, where the ingested object – such as anaerobic microorganisms – is broken down.

Oxygen-independent mechanisms operate within the phagolysosome through the action of lysosomal enzymes such as lysozyme, lactoferrin, and hydrolases. Microbicidal activity is also mediated by lactic acid, which is formed during glycolysis and lowers the intravacuolar pH to approximately 4.0. Lysosomes contain over thirty different enzymes capable of hydrolyzing most of the captured objects. Additional microbicidal substances have been identified in leukocyte granules, including cationic proteins, lactoferrin, and various peroxidases, which are also active against viruses.

Mast cells (tissue basophils) are cells containing cytoplasmic granules that store heparin and biologically active substances such as histamine and serotonin. Upon degranulation, mast cells release inflammatory mediators (including leukotrienes and cytokines). This process increases vascular permeability and facilitates the infiltration of complement components into the tissues at the site of injury, thereby preventing pathogen penetration into the internal environment of the body.

Natural killer (NK) cells are large granular lymphocytes capable of spontaneously destroying tumor cells and virus-infected cells. They also exhibit anti-parasitic activity. NK cells are primarily located in the liver, red pulp of the spleen, and mucous membranes.

Humoral Factors

Alongside cellular resistance mechanisms, a number of substances have evolved that exist in a colloidal-soluble state or are released into the body's liquid environments. These substances perform the function of primary defense against foreign antigenic and non-antigenic particles. The number of these humoral factors is extensive. Among the most active and well-studied are normal antibodies, lysozyme, complement system, properdin, leukins, β-lysins, interferon, cytokines, and others.

Normal (natural) antibodies against various microbial antigens are present in the serum of humans and animals. They exhibit agglutinating, complement-binding, lytic, and neutralizing activities against microbial antigens. Notably, blood serum may contain immunoglobulins directed even against antigens that have never entered the organism. Such antibodies are termed "natural" or "normal". They are typically found in low titers but play a significant immunological role, especially concerning infectious agents.

It is generally believed that natural antibodies arise as a result of so-called imperceptible immunization with pathogens or antigens ingested with food; however, one cannot exclude the spontaneous (genetically determined) mechanism of their formation.

Reactions of antibodies to antigens that have not penetrated the organism can be considered cross-reactive, which explains their lower titers. Normal antibodies may also cross the placental barrier or be transferred through breast milk. Their titer is determined through serological reactions and typically ranges from 1:5 to 1:20.

β-Lysins. Many sera exhibit bactericidal activity primarily against gram-positive bacteria, especially spore-forming bacteria and micrococci. This activity is independent of complement and persists after heating the serum at 60–65°C for 30 minutes. Notably, β-lysins are found in serum following the formation of a coagulated clot of whole blood and are absent in plasma. It is believed that β-lysins are released by platelets during blood coagulation.

The bactericidal effect of β-lysins appears to result from their influence on the cytoplasmic membrane, leading to the autolysis of the cell wall mediated by enzymes of the cytoplasmic membrane. β-lysins are active only in the presence of calcium ions (Ca^{++}).

Blood Serum Proteins. The "acute-phase reaction" is an early response that increases the concentration of certain blood plasma proteins. Acute phase proteins

include C-reactive protein, serum amyloid A and P, blood clotting factors, metal-binding proteins, protease inhibitors, mannan-binding lectin, and others.

The complement system (from the Latin *complementum* – "supplement", "means of replenishment") is a system of serum proteins that participate in nonspecific defense reactions, including phagocytosis, chemotaxis, cell lysis, participation in anaphylaxis, and activation of mast cells, etc. Complement proteins are globulins or glycoproteins according to their structure. They are produced by macrophages, leukocytes, and liver cells and constitute approximately 5–10% of blood proteins. According to the nomenclature adopted by the WHO, the complement system is designated by the symbol C, with individual components labeled as C1, C2, etc. The system comprises 20–26 serum proteins, nine of which are primary components (C1-C9), along with one complex: four proteins of the properdin system and one inhibitor of the activating enzyme.

Each protein fraction possesses specific properties. Under normal conditions, complement fractions are in an inactive state. Activation occurs through a cascade of enzymatic reactions, where products of preceding reactions serve as catalysts for subsequent steps, involving the inclusion of additional components and subcomponents.

There are *three mechanisms (pathways) of complement activation* (Fig. 82):

1) The classical pathway.
2) The lectin pathway.
3) The alternative pathway.

In the classical pathway, the initial activator of the complement system is the antigen-antibody immune complex. The system is primarily activated by IgM and most subclasses of IgG. The immune complex binds to the C1 complement component, which contains an active protease. This enzyme cleaves components C2 and C4 to form C3 convertase. The convertase then activates the C5 component, leading to the formation of the Membrane Attack Complex (MAC). The MAC inserts into cell wall of the microorganism, creating a "pore" that disrupts osmotic pressure inside the cell, ultimately resulting in cell lysis.

In the alternative pathway, activators include microbial cells, polysaccharides, bacterial lipopolysaccharides (endotoxins), viruses, and other antigens, without the involvement of antibodies. The reaction is initiated by the C3b component, which is present in small quantities in blood serum. This component binds to the antigen and, with the participation of factors B (a proteinase), D (a glycoprotein), and P (*properdin* – a γ-globulin), forms C3 convertase. The convertase then activates component C5, leading to the formation of the membrane attack complex (MAC) and subsequent cell lysis.

The lectin pathway of complement activation is triggered by a calcium-dependent protein in the blood called mannan-binding lectin (MBL). MBL binds to mannose residues on the surface of microbial cells, activating a protease that

cleaves components C2 and C4, subsequently forming C3 convertase. Further reactions proceed along the classical pathway of complement activation.

The complement system plays a crucial role in maintaining homeostasis and is regulated by specific homeostatic mechanisms.

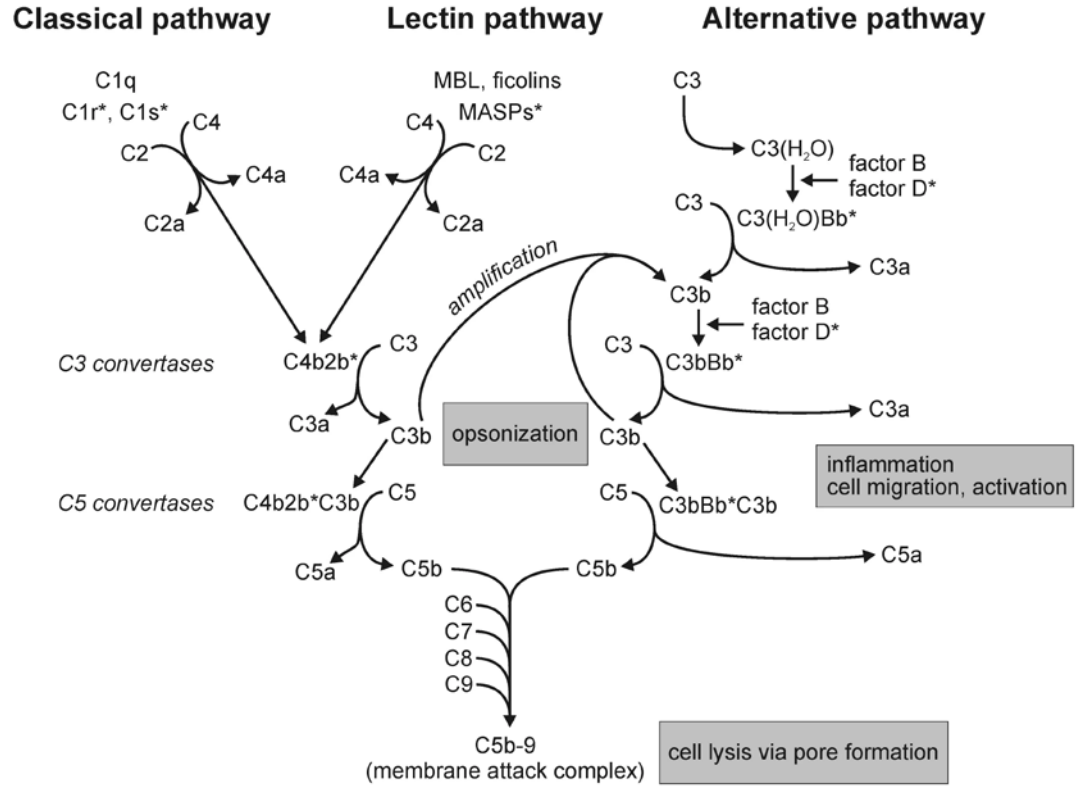


Fig. 82. Pathways of Activation and Physiological Functions of Complement Components

Unlike immunoglobulins, complement proteins do not increase in concentration in blood serum following immunization.

The concentration and level of complement in the blood can serve as a diagnostic marker reflecting the state of the macroorganism’s natural resistance. A high complement level in the blood is considered a favourable sign, whereas a decrease in complement levels is a negative prognostic indicator.

Lysozyme is a proteolytic enzyme known as muramidase (from the Latin *murus* – wall). Chemically, lysozyme is a polypeptide composed of approximately 130–160 amino acid residues. It is soluble in a mildly acidic media, resistant to short-term boiling, and stable in the presence of trypsin.

Lysozyme (muramidase) has the ability to cleave the main component of the bacterial cell wall, murein, by breaking the bond between the first carbon atom of N-acetylmuramic acid and the fourth carbon atom of N-acetylglucosamine. This enzymatic activity alters the permeability of the bacterial cell wall.

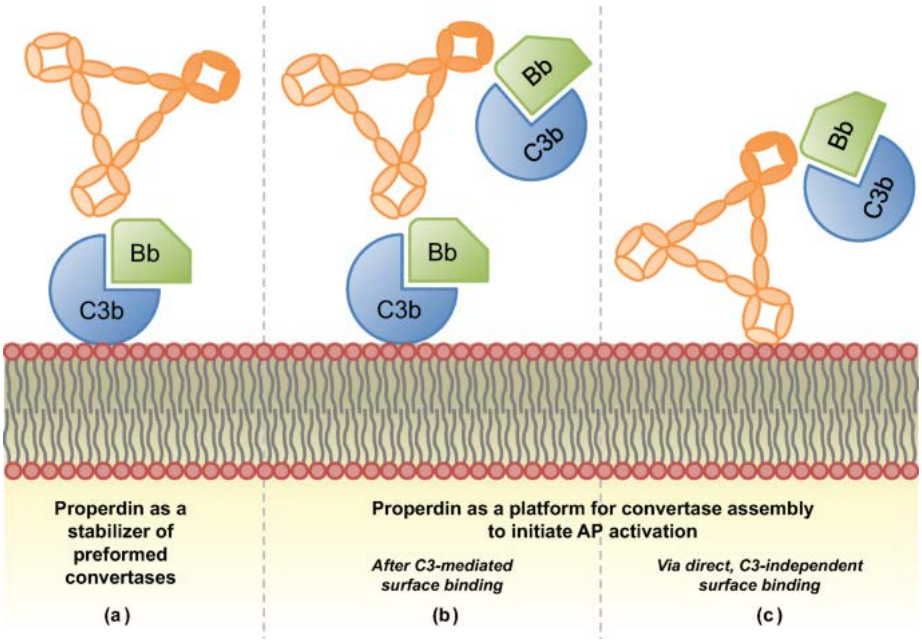


Fig. 83. Role of properdin in the alternative complement activation pathway (https://link.springer.com/article/10.1007/s00467-018-4042-z)

Lysozyme is synthesized by phagocytes and serves as a potent protective factor of the mucous membranes of the oral cavity and eyes. It is also present in tears, saliva, blood, breast milk, and the tissues of various internal organs. A high concentrations of lysozyme are found in the amniotic membranes and fetal fluids.

Lysozyme performs several important biological functions in the body, including bactericidal activity, stimulation of phagocytosis and antibody production, the ability to neutralize certain microbial toxins, and anti-inflammatory effects.

Cytokines are mediators of intercellular interactions produced by various cells of the immune system. They are proteins that exert their effects by binding to specific receptors on the surface of target cells. The activity of cytokines is interconnected; for example, one cytokine can activate the production of another.

Cytokines can be classified into several groups:

1) Interferons
2) Hemopoietins
3) Tumor necrosis factors
4) Chemokines

Interferons are a group of proteins produced by eukaryotic cells in response to the introduction of certain biological agents known as interferonogens. They possess antiviral, immunomodulatory, antitumor, and radioprotective activities. Interferons are classified into α-, β-, and γ-interferons (IFN-α, IFN-β, IFN-γ). Chemically, they are glycoproteins.

IFN-α (leukocyte interferon) is synthesized by peripheral blood leukocytes. It interacts with target cells that express the CD118 receptor on their surface. Its

primary function is to increase the expression of HLA class I molecules, which promotes recognition and destruction of virus-infected cells by cytotoxic lymphocytes and induces the production of antiviral proteins that inhibit viral replication.

IFN-β (fibroblast interferon) is produced by fibroblasts. Its functions are the similar to those of IFN-α.

IFN-γ (immune interferon) is secreted by immune T lymphocytes – specifically CD4+ T helper (Th1) cells, CD8+ cytotoxic T lymphocytes, and natural killer (NK) cells. It interacts with the CD119 receptor on the cell surface. IFN-γ activates macrophages, enhances the activity of cytotoxic lymphocytes and NK cells, and upregulates the expression of HLA class I and HLA class II molecules.

Interferons inhibit the reproduction of many viruses, not only those that induce their production but also a variety of unrelated viruses. The possible mechanisms underlying the antiviral effects of interferons are illustrated in Fig. 84.

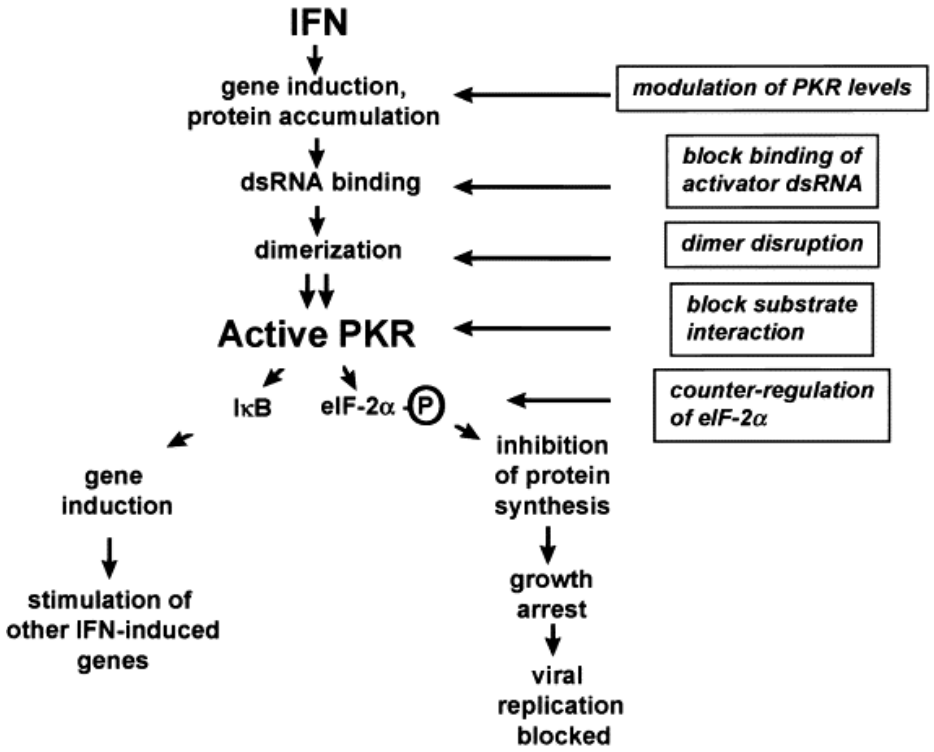


Fig. 84. Mechanism of antiviral action of interferon

Interferon does not affect the attachment or penetration of the virus into the cell, nor does it influence the release of the virus from the cell; primary action is to limit or inhibit the synthesis of viral proteins. In humans, interferon circulates for a relatively short duration – approximately two weeks – which should be taken into account when considering its use as a preventive or therapeutic agent.

Hemopoietins are cell growth factors that include interleukins (IL-2–IL-7, IL-9, IL-11, IL-13, and IL-15), which are produced by activated T and B

lymphocytes, macrophages, and thymus stromal cells. These interleukins stimulate the activation and differentiation of lymphocytes, leukocytes, macrophages, and other immune cells.

Hemopoietins also encompass colony-stimulating factors (CSFs), which regulate the activation, proliferation, and maturation of cells within the immune system.

Tumor necrosis factors (TNF-α, TNF-β) are cytokines that promote adhesion, antibody production, and mononuclear cell activity; they are also capable of lysing certain tumors. These factors are primarily produced by macrophages.

Chemokines are signaling proteins that attract leukocytes, monocytes, and lymphocytes to sites of inflammation. Examples include IL-8, macrophage migration inhibitory factor (MIF), and others.

Antigens

Antigens (from the Latin *anti* – against, *genos* – origin) are genetically foreign substances of colloidal structure that, upon entering the internal environment of the body, are capable of eliciting a specific immunological response. This response is manifested, in particular, by the production of specific antibodies, the appearance of sensitized lymphocytes, or the development of immune tolerance to the substance.

The main properties of antigens are immunogenicity and specificity (antigenicity).

The term *specificity* refers to the ability of a foreign antigen to induce the formation of antibodies that are specific to it and to form a precise binding with them.

Immunogenicity is the capacity of an antigen to provoke an immune response; such an antigen is called an *immunogen*. The immunogenicity is determined by factors such as its foreignness, molecular weight, and chemical nature. In other words, substances that qualify as antigens must be foreign to the body, macromolecular in size, in a colloidal state, and introduced parenterally (bypassing the gastrointestinal tract, where substance is typically degraded and lose its foreignness).

The *foreignness* of antigens refers to a certain degree of chemical difference between the antigen and the macromolecules of the organism it enters. Simple elements (iron, copper, sulfur, etc.), simple and complex inorganic compounds (acids, salts, etc.), as well as simple organic molecules such as monosaccharides, disaccharides, and amino acids, are not considered antigens. The biosynthesis of these molecules results in chemically identical molecules, regardless of whether it occurs in an animal, plant, or microbial cell; thus, these substances do not possess specificity. Specificity appears at a higher level of biological macromolecular

organization. For example, amino acids linked in a polymer chain acquire antigenic properties if the chain contains more than eight amino acids.

The antigenic properties are related to the molecular weight of the macromolecule – it should be at least 10,000 Da. Generally, the higher the molecular weight of a substance, the greater its immunogenicity. However, it is incorrect to assume that high molecular weight is an obligatory property of an antigen. For example, glucagon (a pancreatic hormone with a molecular mass of approximately 3,800 Da) and vasopressin and angiotensin (molecular mass around 1,000 Da) also exhibit antigenic properties.

It is customary to distinguish between *complete* antigens, *incomplete* antigens (*haptens*), and *semi-haptens*.

Complete antigens are those that induce the formation of antibodies or sensitize lymphocytes and are capable of reacting with them both within the body and in laboratory tests. Proteins, polysaccharides, high-molecular-weight nucleic acids, and complex compounds of these substances possess the properties of complete antigens.

Incomplete antigens, or haptens, are not capable of inducing antibody production or lymphocyte sensitization on their own. This property manifests only when they are combined with complete antigens ("carriers"). Among the antibodies or sensitized lymphocytes formed, some are specific to the "carrier", while others are specific to the hapten. These can react with both in vivo and in vitro.

Semi-haptens are relatively simple substances that, upon entering the internal environment of an organism, can chemically bind with the body's proteins and confer antigenic properties. Some medications (such as iodine, bromine, antipyrine, etc.) may also belong to this category.

The antigen molecule consists of two unequal portions. The active (small) portion, with a molecular weight of approximately 350–1,000 Da, is called the *antigenic determinant (epitope)* and determines the antigenic specificity. Antigenic determinants are located in regions of the antigen molecule that are most accessible to the microenvironment. In a protein molecule, for example, they can be situated not only at the ends of the polypeptide chain but also within other regions. Antigenic determinants contain at least three amino acids with a rigid structure (such as tyrosine, tryptophan, and phenylalanine). The specificity of an antigen is also associated with the sequence order of amino acids in the polypeptide chain and their relative positions. Approximately one antigenic determinant (epitope) is present per every 5,000 Da of the molecular weight of the antigen molecule. The number of antigenic determinants in an antigen molecule determines its valence; higher valence indicates a greater number of determinants and correlates with a larger molecular mass. For example, diphtheria toxin has eight valences, while hemocyanin has 231, and so on.

The remaining (inactive) portion of the antigen molecule is believed to serve as a carrier for the determinant, facilitating the penetration of the antigen into the

internal environment of the body, its pinocytosis or phagocytosis, and cellular responses to antigen entry. This process involves the formation of mediators of intercellular interaction in the immune response, with T lymphocytes possessing receptors for the carrier and B lymphocytes for the antigenic determinant. In some cases, antigenic determinants are artificially synthesized. When introduced into animals without a carrier, these determinants elicit a weak immune response.

The route of administration and dosage of the antigen are crucial factors influencing its antigenicity. For most bacterial and viral antigens, intradermal and subcutaneous injections are the most effective routes. Both are significantly more effective than intramuscular or intravenous administration. The enteral route is often ineffective for many antigens. Overdosing with slowly excreted antigens can lead to immunological paralysis. Introducing an antigen into an embryo can induce tolerance, which persists after birth. Depending on the route of administration, antigens tend to accumulate preferentially in specific organs: intravenous injection favors accumulation in the spleen, bone marrow, and liver; subcutaneous injection favors regional lymph nodes. Antigens enter the body cells via phagocytosis or pinocytosis. The duration of antigen persistence in the body depends on the size and chemical structure of its molecule, all other factors being equal. The longest retention (several hundred days) occurs when the antigen binds to a substance with a long half-life. Excretion of antigens primarily occurs through urine and, to a lesser extent, through feces.

Antigens of Bacteria and Viruses

Due to the complexity of their structure and chemical composition, various microorganisms contain a range of antigens: proteins (complete antigens), carbohydrates, lipoid compounds (haptens), and their complexes.

Based on the anatomical structures of the bacterial cell, the following are distinguished:

- *H-antigens* (flagellar antigens, present if the bacteria possess flagella).
- *K-antigens* (surface or capsule antigens – polysaccharides, lipopolysaccharides, proteins).
- *O-antigens* (somatic antigens – proteins, nucleoproteins, bacterial enzymes).
- Antigens excreted by bacteria into their environment (exotoxins – proteins, capsule polysaccharides).

Among the numerous antigens of a microbial cell, some are specific only to a particular type of microbe (*type-specific antigens*), some are specific to a species (*species-specific antigens*), and others are common to a group of microorganisms (genus or family) – *group-specific antigens*. These antigens are extracted from destroyed microorganisms; animals are immunized with them to produce type-specific, species-specific, and group-specific sera. Such sera are used for

identifying bacteria isolated from a patient's body or the environment, allowing determination not only of the species but also of the serotype within that species.

Antigens of a viral nature include *glycoproteins and proteins of the viral supercapsid, capsid proteins,* and *viral enzymes*.

Thus, a bacterial cell (like microorganisms from other kingdoms – viruses, protozoa, and fungi) represents a complex of numerous antigens. When it enters the internal environment of a host organism, many of these antigens induce the formation of specific antibodies. Some antigens stimulate only minimal antibody production, while others provoke rapid and significant antibody formation. Accordingly, antigens are classified as "weak" and "strong".

Additionally, there are antigens capable of binding directly to T lymphocytes without the assistance of antigen-presenting cells (APCs), bypassing their active centers. This results in polyclonal activation of T lymphocytes (constituting approximately 2 to 20% of all peripheral lymphocytes), which release a huge amount of cytokines into the bloodstream. This cytokine release can cause systemic intoxication of the body and lead to apoptosis-mediated death of T-lymphocytes, ultimately resulting in immunodeficiency. Such antigens are known as superantigens. Examples of superantigens for T lymphocytes include staphylococcal enterotoxins, associated with toxic shock syndrome, HIV antigens, and rabies virus antigens.

Not all bacterial cell antigens contribute equally to the induction of non-susceptibility (immunity) when pathogenic microbes of the same species re-enter the host organism. It has also been established that certain antigens from some microorganisms can trigger various types of hypersensitivity reactions (allergies). These antigens are referred to allergens.

Antigens of the Human Body

Proteins and carbohydrates present in the blood and internal organs are generally non-antigenic for the organism in which they are synthesized. However, these molecules can be antigenic for other members of the same species. Such antigens are called *alloantigens* or *isoantigens*. Individuals differ in their isoantigens; examples include the ABO blood group system, the Rh (Rhesus) blood group system, and antigens of the major histocompatibility complex (MHC). Additionally, there are so-called sequestered organs, which are organs protected from the general bloodstream by specialized physiological barriers, such as the blood-brain barrier (BBB) and the blood-testis barrier (BTB). The proteins of these organs do not normally enter the bloodstream and are therefore considered antigens for the body itself. These organs include the brain, lens of the eye, parathyroid glands, and testes.

Antigens of the *major histocompatibility complex* (*MHC*) are unique to each individual and determine biological individuality. In humans, MHC antigens are historically been referred to as "*human leukocyte antigens*" (*HLA*).

The set of loci (genes) of the major histocompatibility complex is located on the short arm of human chromosome 6 and is divided into three distinct classes.

Class I includes the three most studied loci: HLA-A, -B, and -C (Fig. 85), each of which can have multiple variants of the same gene (alleles).

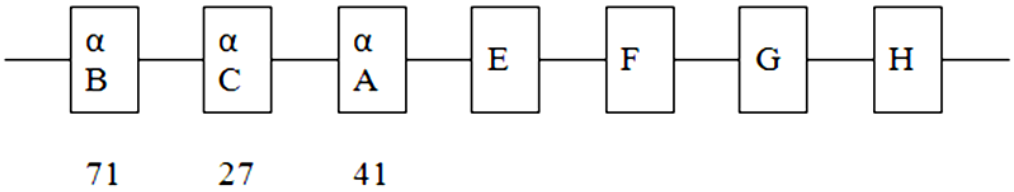


Fig. 85. Loci (genes) of Class I of the major histocompatibility complex

The A locus includes 41 variants, B has 71, and C has 27. The loci (genes) of class I regulate the synthesis of glycoproteins – human leukocyte antigens (HLA I) – that function as cellular receptors. The structure of HLA class I molecule is illustrated in Fig. 86.

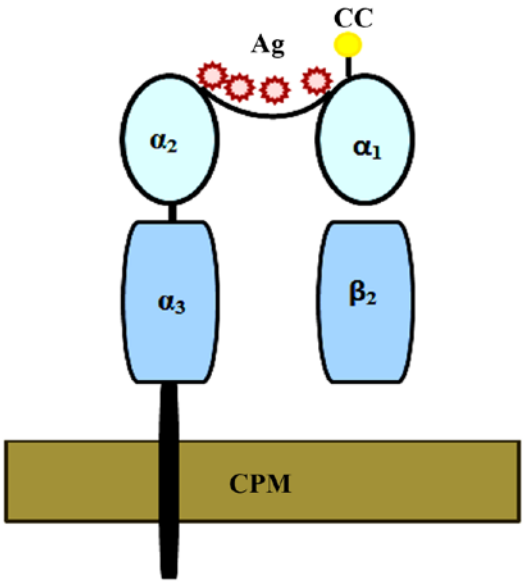


Fig. 86. Structure of an HLA class I molecule. CPM – cytoplasmic membrane; α1, α2, α3 – three extracellular domains of the heavy chain; β2 – microglobulin; CC – carbohydrate chain; Ag – antigen

The heavy chain of the glycoprotein dimer (45 kDa) consists of a hydrophilic cytoplasmic domain, a hydrophobic transmembrane domain, a constant extracellular domain (α3), and two variable extracellular domains (α2, α1). The α1 domain contains a short carbohydrate chain (CC). The light chain of the dimer is represented by β2-microglobulin (12 kDa), which is part of its constant region. It has no variants and is encoded by one of the genes on chromosome 15, which is not related to the MHC. The cleft between α1–α2 domains and β2-microglobulin constitutes the active site for binding and presenting antigens (antigenic determinants) to cytotoxic T lymphocytes (Tc).

HLA class I molecules are expressed on the surface of all nucleated cells, forming the basis of individual antigenic specificity. They facilitate self-recognition, intercellular cooperation, and antigen presentation.

The class II loci include several genes (Fig. 87). The loci (genes) DP, DQ, and DR regulate the synthesis of α and β chains of cellular receptors and can have multiple alleles (alternative gene variants), as indicated in the diagram. The structure of HLA class II antigens is depicted in Fig. 88.

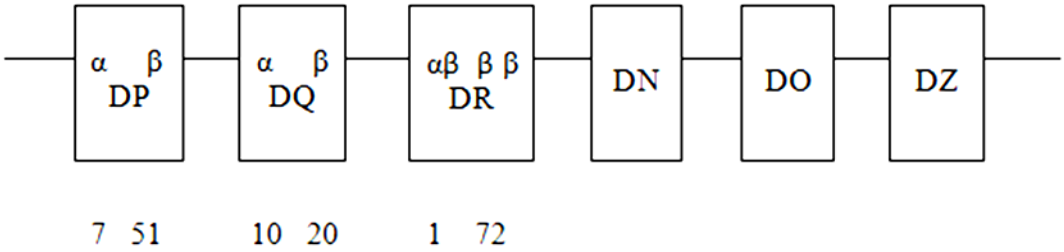


Fig. 87. Loci (genes) of class II of the major histocompatibility complex

The HLA class II receptor is a glycoprotein composed of two chains. Chain A consists of a variable extracellular α1 domain (34 kDa), a constant extracellular α2 domain (34 kDa), as well as hydrophobic transmembrane and hydrophilic cytoplasmic domains. The α1 and α2 domains each contain one short carbohydrate chain. Chain B includes a variable extracellular β1 domain (28 kDa) with one carbohydrate chain, a constant extracellular β2 domain (28 kDa), along with hydrophobic transmembrane and hydrophilic cytoplasmic regions. The cleft between the α1 and β1 domains constitutes the active site of the receptor. HLA class II antigens are expressed only on macrophages, B lymphocytes, and some activated T lymphocytes.

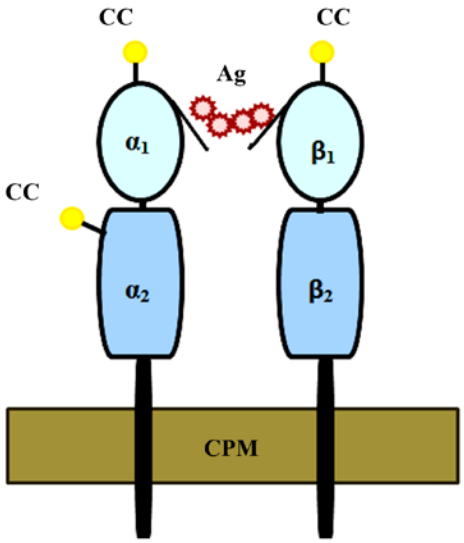


Fig. 88. Structure of the class II MHC molecule. CPM – cytoplasmic membrane; α1, α2, β1, β2 – extracellular domains; CC – carbohydrate chain; Ag – antigen

The class III MHC loci contain genes that regulate the synthesis of certain components involved in the activation of the C3 component of complement. The complement system components (C4, factor B, C2) encoded by these genes are secreted into the bloodstream, circulate with it, but are not anchored to the membranes of the body's cells.

Antibodies

Antibodies are globulin proteins (immunoglobulins) produced by the body in response to an antigen and capable of specifically binding to it. There are five classes of immunoglobulin molecules, with molecular weights ranging from 150,000 to 900,000 Daltons: *IgM, IgG, IgA, IgE,* and *IgD*. Immunoglobulin molecules consist of two light (L) chains and two heavy (H) polypeptide chains linked together by disulfide bonds (Fig. 89).

Both types of chains, when joined, possess antigenicity. The antigenic specificity of the heavy chains is unique to each immunoglobulin class; accordingly, the H-chains are designated with the Greek letters μ (mu), γ (gamma), α (alpha), ε (epsilon), and δ (delta). Light chains are classified into two types based on their antigenic properties: κ (kappa) and λ (lambda), which are consistent across different classes. The antigenic differences in heavy chains are utilized to produce antisera that enable the detection of specific immunoglobulin classes in various samples.

The light chains of IgG consist of two regions (domains): a variable region (VL) and a constant region (CL). Heavy chains contain one variable region (VH) and three constant regions (CH1, CH2, and CH3). The variable regions of both light and heavy chains form the active sites of antibodies (VL–VH). The CL–CH1 regions account for minor amino acid sequence variations among individuals of the same species (alloantigenic differences in IgM molecules). The CH2–CH2 regions are involved in the complement fixation and activation, while the CH3–CH3 region is involved in antibody binding to cells such as lymphocytes, macrophages, and mast cells. This structural organization is characteristic of all immunoglobulin classes; however, additional structural features distinguish each class. For example, the H-chain of IgM comprises five domains instead of four, and the entire IgM molecule is a pentamer of the IgG molecule, connected by additional polypeptide J-chains. IgA can exist as monomers (serum IgA) or dimers (secretory IgA), with the latter containing additional J and S chains. Other properties of antibodies are summarized in Table 8.

The antibody molecule binds to the antigenic determinant not entirely, but only with a specific region known as *the active site*. The active site is a cavity or cleft that corresponds to the spatial configuration of the antigenic determinant group. One of the active sites may be functionally inactive for various reasons; such antibodies are called *incomplete*. Their formation is typically preceded by the

production of complete antibodies, which possess two active sites (as in IgG). Incomplete antibodies are present across different classes of immunoglobulins.

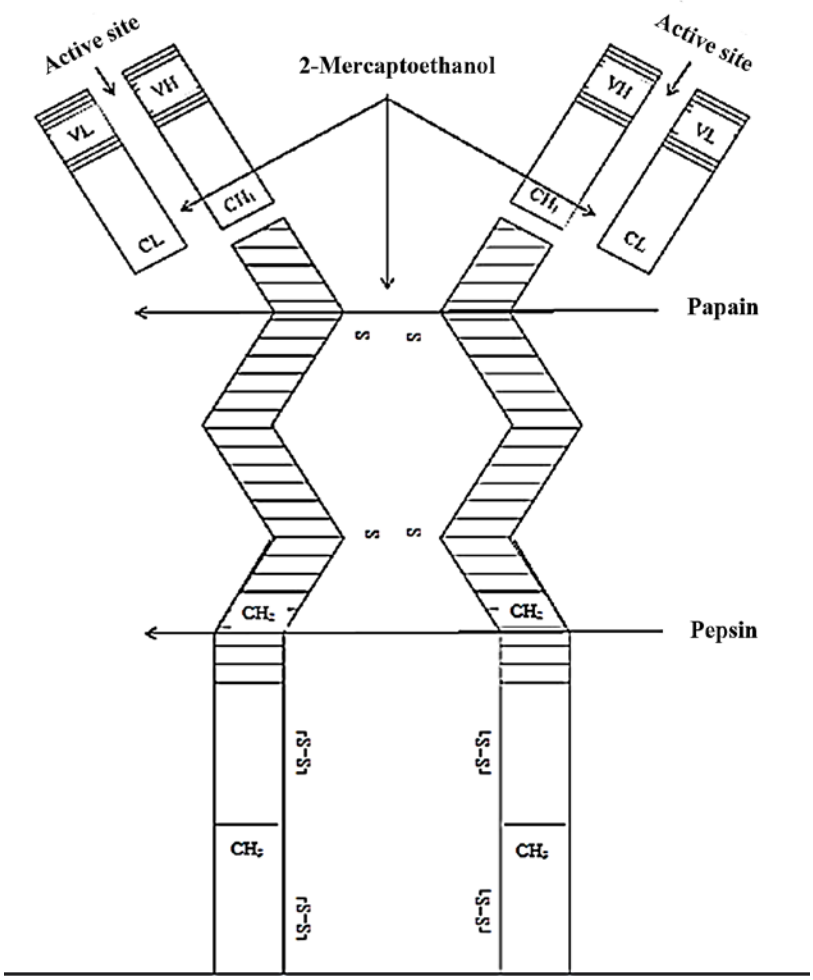


Fig. 89. Structure of the IgG molecule with cleavage sites indicated for fragmentation by 2-mercaptoethanol, papain, and pepsin

Table 8

Major Characteristics of Human Immunoglobulins

No.	Features	IgM	IgG	IgA	IgE	IgD
1	Molecular weight	900,000	150,000	170,000 and 300,000	190,000	180,000
2	Level in blood (g/L)	0.5 – 1.8	6 – 16	1 – 5	0,00002	0.03 – 0.04
3	Heavy chain type	μ 1 – μ2	γ 1 – γ4	α 1 – α2	ε	δ
4	Formula	5H5L	2H2L	4H4L	2H2L	2H2L
5	C Fixation	++++	++	+S	–	–
6	Toxins neutralization	+	+	+	–	–
7	Agglutination	+	+	+	–	–
8	Bacteriolysis	+	+	?	–	–
9	Placental transfer	–	+	–	–	–

The vast majority of antibodies are produced by cells of the plasmacytic series, which include plasmablasts, proplasmacytes, and plasmacytes. Each of these cells produces antibodies with only one specificity, i.e., targeting a single antigenic determinant. These cells are primarily located in the spleen, lymph nodes, bone marrow, and lymphoid tissues of the mucous membranes.

During the initial contact between the organism and an antigen and subsequent antibody production, two phases are distinguished: the inductive phase and the productive phase. The duration of the inductive phase is approximately two days. During this period, proliferation and differentiation of lymphoid cells occur, along with the development of the plasmablastic reaction. The productive phase follows the inductive phase. Antibodies can be detected in the blood serum starting from the third day after contact with the antigen; these early antibodies belong to the IgM class. Between days five and seven, there is a gradual shift from IgM synthesis to IgG synthesis of the same specificity. Typically, by days 12 to 15, the antibody production curve reaches its peak; afterward, antibody concentrations begin to decline. However, a certain amount of antibodies can be detected for many months or even years later. Upon re-exposure to the same antigen, the inductive phase is shortened to only a few hours. The productive phase proceeds more apidly and intensely, predominantly involving IgG synthesis (Fig. 90).

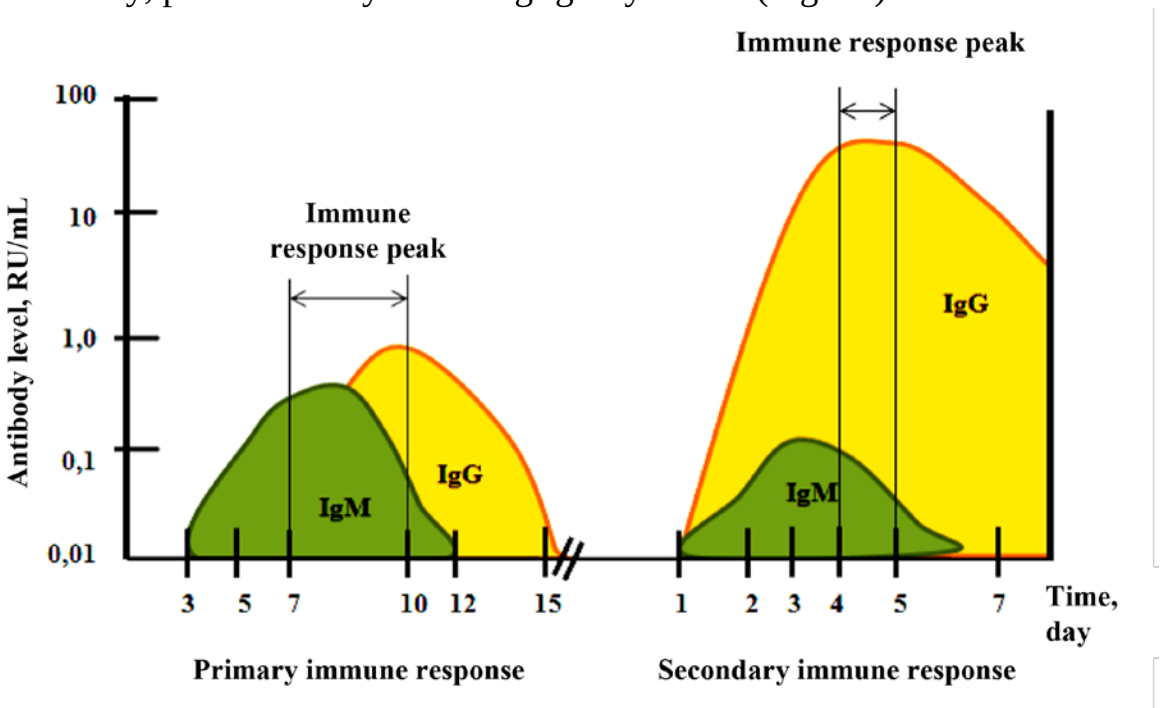


Fig. 90. Primary and secondary immune response

Genetic Control of Antibody Biosynthesis

A typical immunoglobulin molecule consists of two heavy (H) chains and two light (L) chains, with both κ (kappa) chains accounting for approximately 70%

and λ (lambda) chains for about 30%. The polypeptide chains are synthesized on the ribosomes of B lymphocytes, assembled into a globular molecule (globule), and then transported either to the cell surface, where they function as B cell receptors, or into the bloodstream, where they serve as antibodies.

The synthesis of polypeptide chains is regulated by three gene loci located on different chromosomes. The loci have various combinations depending on the specific chain being controlled and may contain the following regions:

1) L – encodes a leader peptide necessary for the secretion of antibodies onto the cell surface;
2) V – genes encoding the variable region of the antibody;
3) C – genes encoding the constant region;
4) J – genes encoding the joining (connecting) region of the polypeptide;
5) D – genes contributing to additional variability.

The functional organization of the genes controlling the synthesis of different chains is illustrated in Fig. 91 (the numbers indicate the number of variants).

Thus, in each B cell that synthesizes only one class (or subclass) of antibodies, one locus responsible for light chain synthesis (one of the numerous V and J variants or J-C combinations) and the locus controlling heavy chain synthesis (one of the V, D, J, and C variants) are active.

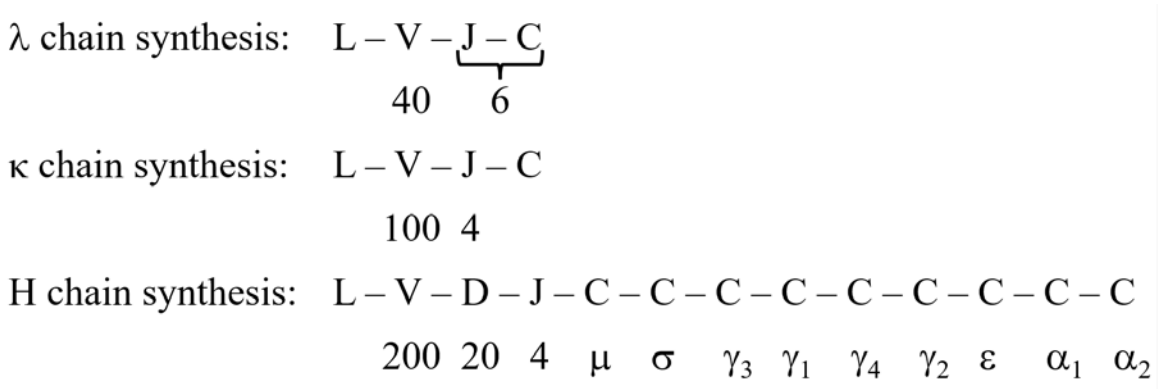


Fig. 91. Functional organization of genes regulating immunoglobulin synthesis

The diversity of gene variants within a single B cell provides a potential for up to 10^7 different antibody variants across all B cells in the body. Furthermore, when accounting for splicing (which introduces inaccuracies in reading the DNA information from these loci in B lymphocytes), the potential variability in the formation of specific antibodies increases to approximately 10^8.

Cellular Cooperation in the Immune Response

Antigen Processing and Presentation. Every microbial cell is a complex antigenic entity that includes dozens of antigens, each of which elicits its "own" specific immune response. Therefore, the overall immune response to a microorganism is the combined effect of responses to most of its structural and functional antigens. The breakdown of a microbial cell into individual antigens and their antigenic determinants (epitopes) is carried out by accessory cells, primarily macrophages, and is known as processing. The role of HLA antigens is to transport epitopes to the cell surface and present them to other immune cells involved in the cellular cooperation in the immune response.

When somatic cells of the host organism are infected by microorganisms characterized by intracellular parasitism (such as viruses, rickettsia, chlamydia, mycobacteria, etc.), antigen processing occurs within proteasomes. Subsequently, epitopes are transported by HLA class I antigens to the surface of these cells. The HLA I–epitope complex serves as a signal for the CD8+ cytolytic T cell, initiating the lysis of the infected cell along with the pathogen (Fig. 92).

In contrast, in diseases where intracellular parasitism is not obligatory (such as salmonellosis, shigellosis, etc.), antigen processing occurs after phagocytosis of the pathogen or endocytosis of its components by macrophages within endosomes. Epitopes are transported by HLA class II molecules to the macrophage surface and presented to other immunocompetent cells, leading to subsequent antibody production (Fig. 93).

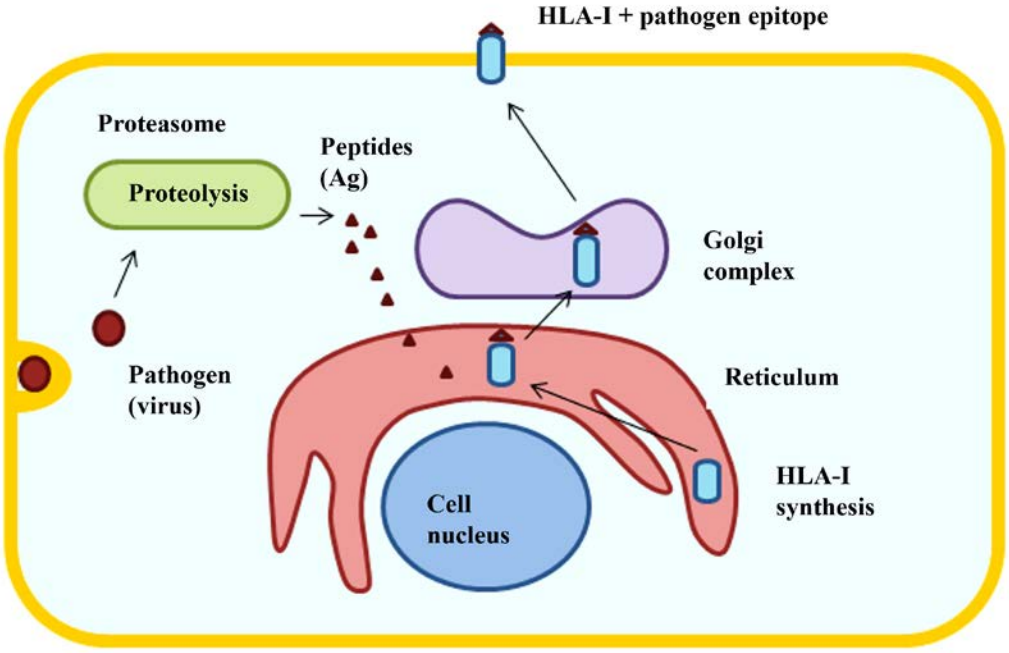


Fig. 92. Antigen presentation by somatic cells

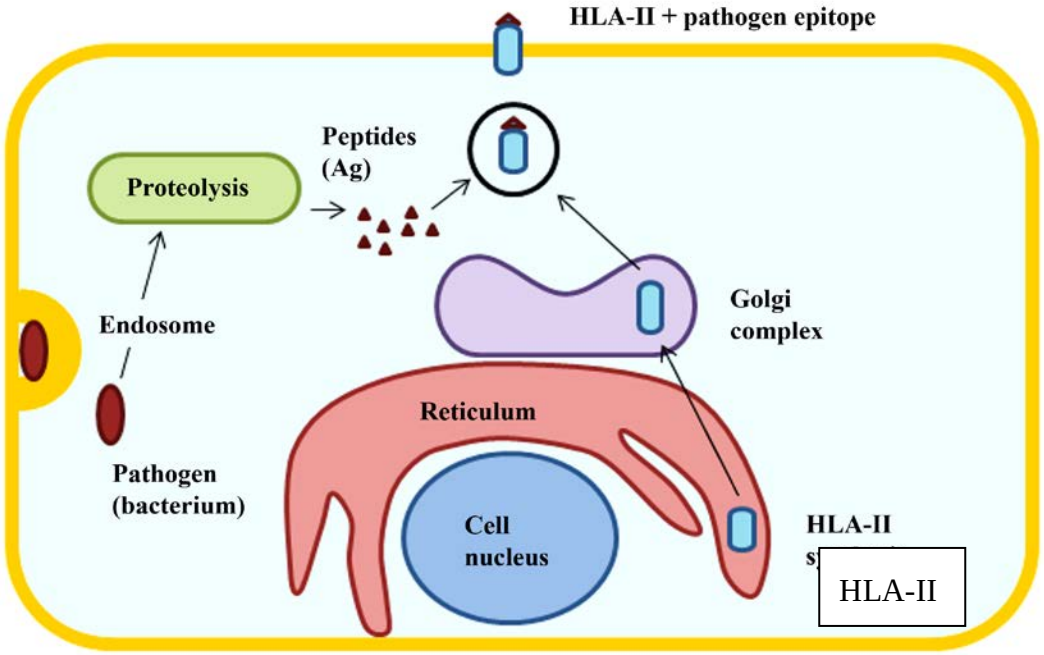


Fig. 93. Antigen presentation by macrophages

T-Cell Receptor. The T-cell receptor (TCR) consists of two chains (Fig. 94). The α-chain comprises four domains: a variable extracellular domain, a constant extracellular domain, a hydrophobic transmembrane domain, and a hydrophilic cytoplasmic domain (MW – 50 kDa). The β-chain has a similar structure, with a molecular weight of approximately 45 kDa.

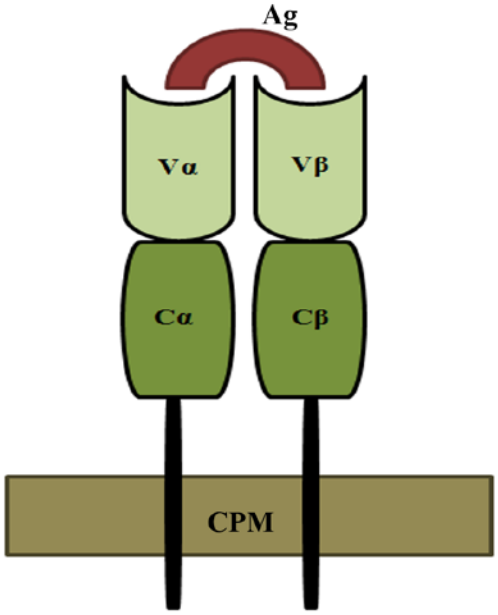


Fig. 94. Structure of the T-cell receptor (TCR): CPM – cytoplasmic membrane; Vα – variable region of the α-chain; Vβ – variable region of the β-chain; Cα – constant region of the α-chain; Cβ – constant region of the β-chain; Ag – antigen

Coreceptors for Intercellular Interactions (CD Receptors)

On the surface of cells involved in the immune response, there are protein molecules (molecular complexes) that function as coreceptors for intercellular interactions. More than 300 coreceptors are known, and they are designated by numbers according to the accepted classification of such complexes (From the English *cluster of differentiation*, *cluster of designation*, or CD). These include CD1, CD2, CD3, and up to CD100, etc. CD receptors can be expressed on T lymphocytes, B lymphocytes, monocytes, macrophages, granulocytes, and other immune cells. The function of some coreceptors is to recognize their "own" antigen-presenting cell. For example, a T lymphocyte expressing CD4 (T-helper cell) or CD8 (cytolytic T lymphocyte) recognizes its respective antigen-presenting cell through "recognizing" HLA class II or HLA class I antigens. Additionally, the cytolytic T lymphocyte (CTL) mediate "recognition" via the CD28 coreceptor and the CD80/86 of the macrophage (Figs. 95, 96).

Other CD receptors (such as CD25, CD121, CD122, CD124, CD126, CD127, CD128, CD130, etc.) exhibit properties similar to certain interleukins. The interaction between specific receptors – for example, CD154 on Th2 cells and CD40 on B lymphocytes – leads to the formation of a complex that acts as a potent inducer of B lymphocyte proliferation. The roles of many other coreceptors remain insufficiently studied.

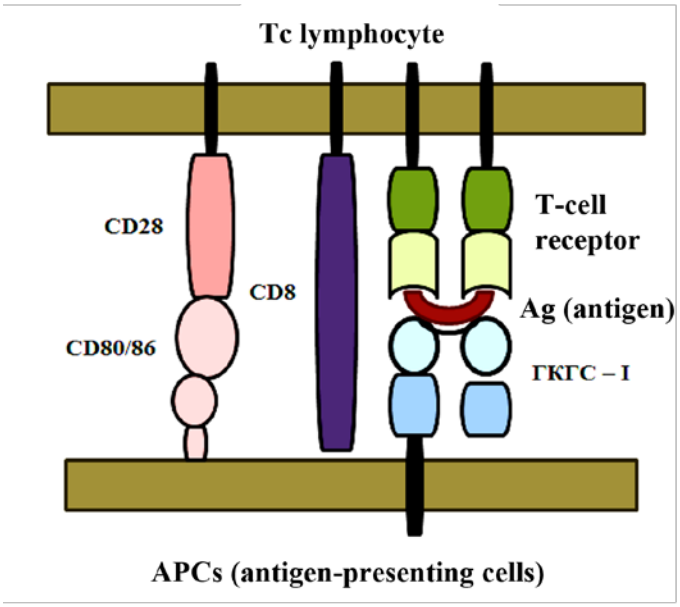


Fig. 95. Coreceptors involved in intercellular interactions of a cytotoxic T lymphocyte (Tc lymphocyte)

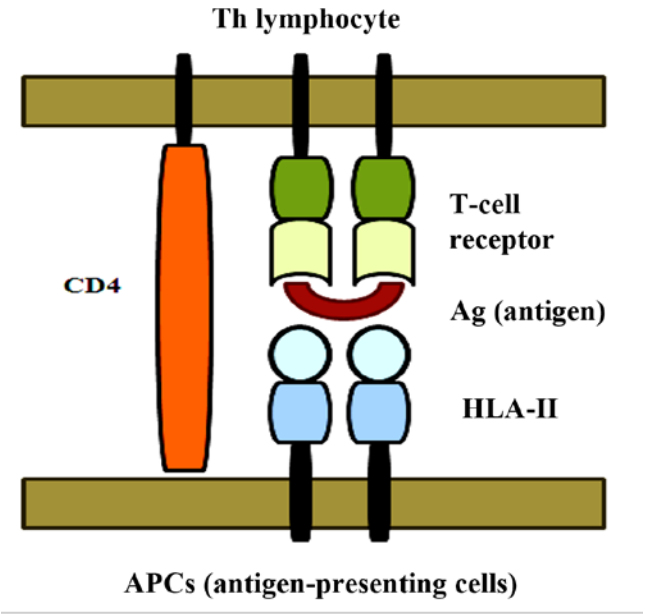


Fig. 96. Coreceptors for intercellular interactions of a T-helper cell

Cellular (Th1) Type of Immune Response

The cellular type of immune response is mediated by the T-cell system of immunity, involving cytotoxic T lymphocytes (CTLs). Antigens that trigger a Th1-type immune response include intracellular parasites, tumor cells, virions, and others. The macrophage (antigen-presenting cell – APC) adsorbs the antigen on its surface, engulfs it, processes it, and presents it on its surface (antigen processing) in complex with HLA-II molecules to a naive T-helper cell (Th0). During antigen recognition, the macrophage becomes activated and secretes cytokines such as IL-12 and IFN-γ).

Under the influence of IL-12, the naive T-helper (Th0) transforms into Th1 cell and produces IFN-γ, TNF-α, and IL-12. These cytokines stimulate the recruitment of cytotoxic T lymphocytes (CTLs or T-killers) from the bone marrow and promote their activation. Activated T-killers (CD8+) interact with target cells or macrophages presenting the same antigenic determinant in conjunction with HLA class I molecules. Upon direct contact with such a cell, the CTL releases granules containing the proteins perforin and granzyme. Perforin inserts into the somatic cell membrane, forming channels, also known as “pores” and can act as a membrane attack protein. Granzyme (serine proteases) induces a form of apoptosis in the somatic cell and facilitate the destruction of intracellular microbes. Secretion of IL-2 by Th1 cells stimulates proliferation of antigen-specific CTLs. Additionally, a clone of memory T cells is generated, which circulates in the body for extended periods and can rapidly differentiate into activated CTLs. Thus, the primary function of CTLs in anti-infectious defense is to destroy somatic cells of

the body harboring intracellular pathogens while displaying specific HLA I molecules complexed with pathogen epitopes on their surface.

Humoral (Th2) Type of Immune Response

The development of a humoral immune response involves the production of *antibodies (immunoglobulins)* against a foreign antigen.

The macrophage digests and presents the antigenic determinant in a complex with HLA class II molecules to a naive T-helper cell (Th0). Under the influence of IL-4, which is produced by the macrophage, Th0 transforms into Th2 cells. The most important interleukins produced by Th2 include IL-4, IL-5, IL-6, and IL-10, which activate B lymphocytes. On the surface of B lymphocytes, there is an immunoglobulin receptor (BCR) that recognizes, captures, and internalizes the antigen.

A B-lymphocyte can acquire a microbial peptide (antigen) through different mechanisms:

1. Obtaining a soluble antigen from the surrounding microsphere. Since this antigen has already been processed by another cell, it does not require further processing. The antigen selectively chooses the B lymphocyte (or B-lymphocytes) that has pre-existing γ-globulin receptors on its surface that are most specific to this antigen.
2. Binding a soluble antigen through its γ-globulin receptor, followed by processing within the B lymphocyte and presentation on its membrane in complex with the B lymphocyte's HLA class II molecules.
3. Acquiring an antigen from the surface of a macrophage. This involves recognition by γ-receptors on B lymphocytes, processing of the antigen within the B cell, and presentation to T lymphocytes.

The signal received from the antigen-binding receptor, along with stimulation by interleukins, triggers proliferation and differentiation of B lymphocytes. As a result, a clone of highly active lymphocytes is generated, synthesizing antibodies specific to this antigen. A clone of memory B cells is also formed; these cells persist in the body for a long time (immunological memory). The humoral type of response is particularly important in combating extracellular microorganisms. Antibodies facilitate their uptake and digestion by phagocytes.

Features of Immunity in Bacterial, Fungal, and Protozoal Infections

Antibacterial Immunity

The formation of sanogenesis mechanisms (elimination of antigens) during various bacterial infections underlies some specific features of the immunity during these diseases. In infections caused by bacteria that produce exotoxins (such as

diphtheria, tetanus, botulism, gas gangrene, etc.), antibodies (antitoxins) produced by the host play a leading role in the immunity. The interaction between antitoxin molecules and toxin molecules can result in various outcomes:

a) blockage of the toxin molecule's receptor-binding site, thereby preventing the toxin from attaching to target cell receptors;
b) direct neutralization of the catalytic (enzymatic, or toxic) domain of the toxin molecule;
c) formation of immune complexes that neutralize the toxin's toxic, receptor-binding, and/or translocation sites (subunits). These complexes are phagocytized and eliminated by the host cells.

However, antitoxic antibodies do not block bacterial adhesion to target cell surfaces or their colonization. Consequently, artificial antitoxic immunity does not provide complete protection to the macroorganism; it does not inhibit bacterial attachment to the surface of target cells, colonization of cells and tissues, or bacterial proliferation.

In another group of bacterial infections – such as meningococcal disease, whooping cough (pertussis), legionellosis, etc. – the primary protective mechanism involves immune-mediated lysis and phagocytosis of bacteria. IgG antibodies generated during these infections initiate a series of antibody- dependent biological reactions:

a) when antibodies bind to bacterial surfaces, complement activation occurs via the classical pathway, leading to the formation of a membrane attack complex (MAC) and subsequent lysis of exposed bacterial membrane areas;

b) opsonization of bacteria by antibodies, followed by the interaction of the Fc-fragments of the antibodies with the Fc-receptors of macrophages, which enhances the engulfing and digestive activity of the phagocyte;

c) formation of immune complex "bacterial Ag–Ab–$C_{1,4,2,3b}$" that binds to the C_{3b} receptors on macrophages, which also promotes the engulfing activity of these complexes by phagocytes;

d) neutralization by antibodies of bacterial antiphagins – substances secreted by bacteria that inhibit pseudopodia formation or macrophage migration—or structural components such as M-protein in streptococci and capsular polysaccharides in pneumococci.

Thus, the immunity developed in these diseases depends on the level of circulating antibodies, the content and activity of complement components, as well as the functional state of phagocytes.

The next group of bacterial infections, characterized by their own specific features of immunity response development, includes those caused by intracellular parasites capable of surviving for extended periods within phagocytes and even reproducing inside them (e.g., tuberculosis, tularemia, brucellosis, listeriosis, etc.).

The main mechanisms that allow bacteria to carry out intracellular parasitism are:

1) blockade of phagolysosomal fusion (e.g., Mycobacterium tuberculosis);
2) resistance of bacteria to lysosomal enzymes (e.g., gonococci, staphylococci);
3) ability of bacteria to rapidly exit phagosomes after engulfment and persist in the cytoplasm for prolonged time (e.g., Listeria).

In diseases characterized by prolonged intracellular persistence and reproduction of the pathogen (persistence), granuloma formation in affected tissues is typical. Such bacteria become inaccessible to the action of antibodies and humoral antibacterial factors. The mechanisms of sanogenesis and immunity formation in these diseases are primarily associated with the formation of cytotoxic T lymphocytes (CTLs), which exert a killing effect on infected target cells containing parasitic bacteria marked with HLA class I receptors presenting bacterial antigens.

Features of Immunity in Fungal Diseases

The characteristics of antifungal immunity depend on the morphological properties of fungi (such as cell size and shape), the complexity of their antigenic composition, and their variability influenced by environmental conditions, as well as the form and stage of mycosis.

Most fungi are free-living organisms, and only some are capable of causing disease. Moreover, for an individual to develop a fungal disease, the presence of immunodeficiency is a necessary condition – particularly involving polymorphonuclear leukocytes, T lymphocytes, and the C3 component of complement. Functional defects of leukocytes include their inability to form pseudopodia (as in “lazy leukocyte syndrome”), inability to form phagolysosomes (as in Chediak-Higashi syndrome), and impaired ability to produce reactive oxygen species essential for microbial digestion. C3 deficiency also results in decreased phagocytic activity. Finally, mycoses in humans most often occur with reduced production of T lymphocytes (cytotoxic T cells and helper T cells).

The development of immunity is associated with the restoration of polymorphonuclear leukocyte function and increased production of T lymphocytes. Specific antibody formation occurs only in certain forms of deep mycoses. It is generally believed that these antibodies do not participate directly in defense mechanisms but rather serve as markers indicating immune restructuring within the body.

Features of Immunity in Protozoal Diseases

The characteristics of immune responses in protozoan diseases are determined by the intracellular localization of the pathogens, the variability of their surface antigens, the presence of antigens shared with human cells, and the immunosuppressive properties of the parasites.

In protozoan infections, both IgM and IgG antibodies can be produced; however, their specificity is often extremely low due to their formation resulting from polyclonal activation of B lymphocytes and the antigenic variability of the parasites.

Recovery typically occurs with the activation of T lymphocytes (Tc and Th). Complete post-infection immunity is rarely established.

Antiviral Immunity

The specific features of antiviral immunity are influenced by the unique anatomical structure of viruses, relatively limited set of antigens present in their membranes, viral antigenic drift of surface proteins, obligate parasitism of viruses, as well as the specific nature of their interactions with susceptible cells.

Within the host organism, a virus can exist in various states:

a) extracellular (virion);
b) intracellular, at different stages of rapid or slow productive interaction with a susceptible cell (virus);
c) integrated into the genome of the target cell (non-productive interaction, provirus).

According to these primary states, antiviral immune responses are aimed at neutralizing and eliminating the virus and its antigens from the body. This is achieved through the action of antibodies, as well as by destroying the body's own virus-infected target cells via cytotoxic T lymphocytes (CTLs).

IgG antibodies produced during viral infections can participate in various biological reactions.

1. Neutralization of the invasive properties of virions. The resulting immune complex binds to the surface of a macrophage via its Fc receptors. Engulfment of the complex typically leads to the death of the pathogen; unabsorbed immune complexes can dissociate, releasing free virions that may infect susceptible cells. Prolonged circulation of unabsorbed and undissociated immune complexes throughout the body can result in their deposition in various tissues, inducing local inflammatory reactions through activation of the complement system or interleukins after the complexes are fixed by cells bearing a receptor for the Fc fragment of antibodies (e.g., in hepatitis B, infectious mononucleosis, subacute sclerosing panencephalitis, etc.).

2. Antibody-mediated complement-dependent cytolysis. The membrane of the infected cell is lysed due to the formation of the membrane attack complex (MAC) of the complement system. The released virions are then exposed to the action of antibodies.

3. Antibody-mediated cytolysis of target cells by macrophages and granulocytes occurs when these effector cells release granzymes and cytolysins upon contact with the infected cell. Such macrophages and granulocytes must express Fc receptors but do not have specificity for the viral antigen. Cytotoxic T

lymphocytes do not participate in this process; their activity is independent of antibody presence.

Destruction of virus-infected cells is also mediated by cytotoxic T lymphocytes (CTLs). CTLs are capable of lysing virus-infected cells by reacting to the viral antigen presented on the cell surface via HLA class I molecules. For the cytotoxic effect to occur, CTLs require direct contact with the target cell. Upon contact, the CTL releases granzymes or cytolysins, which induce changes in the membrane permeability of the target cell. This results in osmotic swelling, membrane rupture, and the release of cytoplasmic contents into the surrounding microenvironment.

DNA-containing viruses and retroviruses have the ability to integrate their viral nucleic acid into the genome of the target cell. The progeny of an infected cell can inherit a provirus. In such cases, viral antigens (proteins) are not synthesized within the cell and are not presented on HLA class I molecules. Consequently, these infected cells evade immune surveillance.

SEROLOGICAL DIAGNOSTIC METHODS

Serological diagnostic methods are used for:

a) Detection of antibodies in the blood serum of the subject for diagnostic purposes;

b) Identification of the genus, species, and type of a microorganism.

Serological diagnostic methods are based on the reaction of a specific interaction between an antigen and an antibody. Typically, these reactions are performed *in vitro* in a laboratory and proceed through two phases:

1) The specific phase

2) The non-specific phase.

In the specific phase, a specific interaction occurs between the antigen and the antibody. In the non-specific phase, an “antigen–antibody” complex appears as a precipitate, a zone of turbidity, or similar formations. This phase requires certain conditions, such as the presence of electrolytes and an optimal pH of the medium.

Laboratory serological reactions include those based on direct antigen-antibody interactions (e.g., agglutination and precipitation reactions) and indirect reactions (e.g., indirect hemagglutination and complement fixation reactions). Additionally, reactions using labeled antibodies or antigens – such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent antibody techniques – are employed.

Antibodies are detected using known antigens. Diagnostic solutions containing suspensions of inactivated microorganisms or their antigens serve as antigens. Typically, serological diagnostic results are obtained by analysing paired blood serum samples collected from patients during the early days of illness and at certain intervals thereafter.

The identification of the pathogen or its antigens is performed using known diagnostic sera, which contain highly specific antibodies.

Agglutination Test (AT)

The agglutination test (AT), also known as *agglutination assay*, (from the Latin *agglutinare* – to glue) is used in laboratory practice to identify isolated microorganisms or to detect specific antibodies in blood serum.

The assay mechanism is based on the interaction between the determinant groups of the particulate antigen and the active sites of an immunoglobulin in an electrolyte medium. The reaction proceeds in two phases: first, the binding of antigen to antibody; second, the precipitation of the formed antigen-antibody (Ag+Ab) complex. The nature of the resulting precipitate depends on the type of

antigen: flagellated bacteria produce a large-flocculated precipitate; capsular bacteria produce a stringy or ropy precipitate; non-flagellated and non-capsular bacteria produce a fine-grained precipitate (Fig. 97).

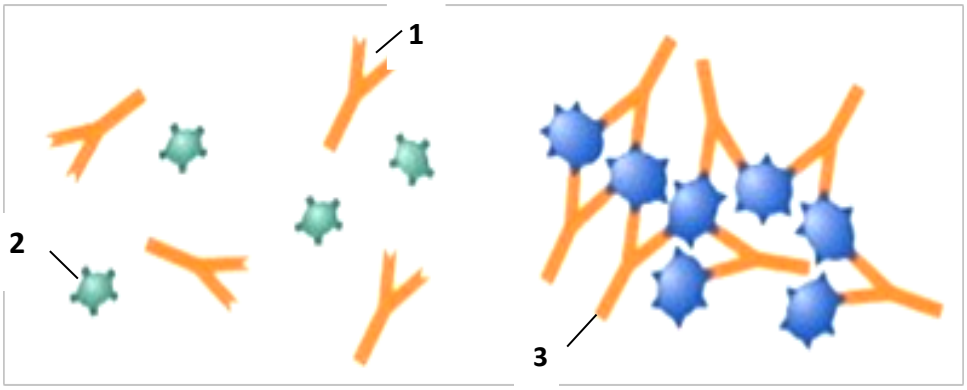


Fig. 97. The Agglutination Reaction: 1 - microbial cell; 2 - antigen; 3 – antibodies

There are two options for performing the agglutination test:

- Rapid or direct slide agglutination test (preliminary)
- Slow agglutination test performed in tubes (expanded)

Slide agglutination test is a qualitative reaction used for preliminary identification of microorganisms (Fig. 98). The slide agglutination assays are typically read after 5–10 minutes.

Fig. 98. Slide agglutination assay: negative result (a); positive result (b)

Tube agglutination assay is used to determine the quantitative content of antibodies, involving a comprehensive (expanded) agglutination test conducted in test tubes.

A positive reaction is indicated by the formation of a precipitate (agglutinate) at the bottom of the test tube. The antibody titer is considered as the highest dilution at which clear agglutination is observed (Fig. 99). The procedure should be

accompanied by serum and antigen controls. The results of the tube agglutination assay are typically read after 18–20 hours.

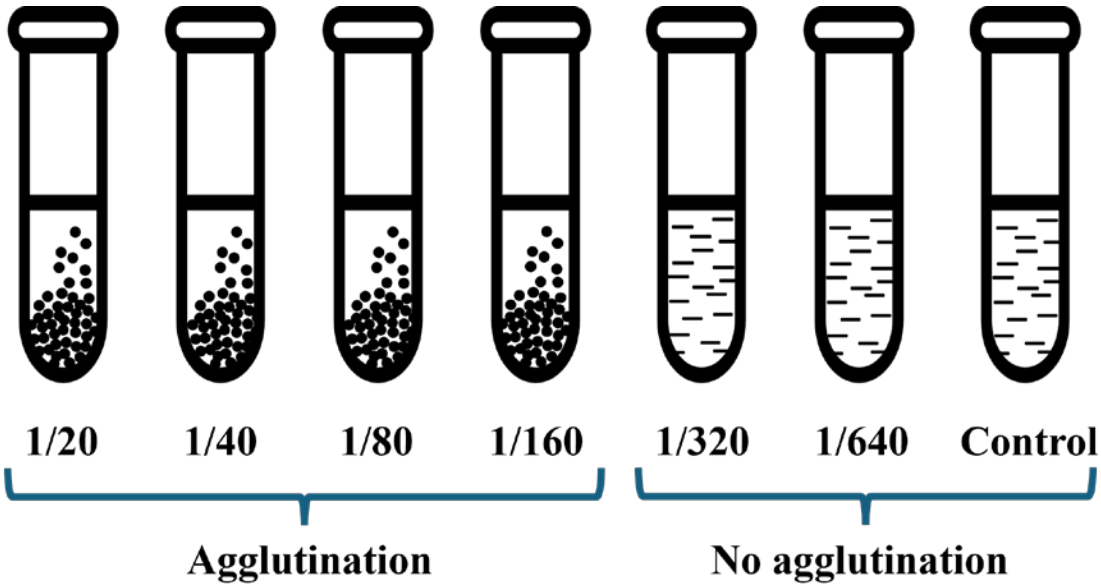


Fig. 99. Expanded agglutination test performed in tubes

Indirect Hemagglutination Assay (IHA)

Indirect (or mediated) agglutination assays (IHA), also known as indirect hemagglutination tests (IHAT), are based on the interaction of specific antibodies with finely dispersed antigens that are adsorbed onto corpuscular carriers (such as erythrocytes, latex beads, or staphylococcal cells). Depending on the type of corpuscular carrier, the following reactions are distinguished:

- Indirect hemagglutination assay (IHA)
- Latex agglutination test
- Co-agglutination (Co-A) test

The indirect hemagglutination test (IHAT) is used in two variants: with known antigens to detect antibodies, or with known antibodies to detect antigens. This reaction is specific and is employed in the diagnosis of diseases caused by bacteria and rickettsiae. To perform IHAT, erythrocyte diagnostic suspensions are prepared by adsorbing either antigens or antibodies onto erythrocytes, depending on the purpose of the study (Fig. 100). The reaction is conducted in specialized polystyrene plates with wells.

In positive cases, the precipitate appears as a thin film with scalloped, lace-like edges of adherent erythrocytes covering the bottom of the well (“umbrella” pattern). The titer is defined as the highest dilution of the test material that still causes erythrocyte agglutination (Fig. 101).

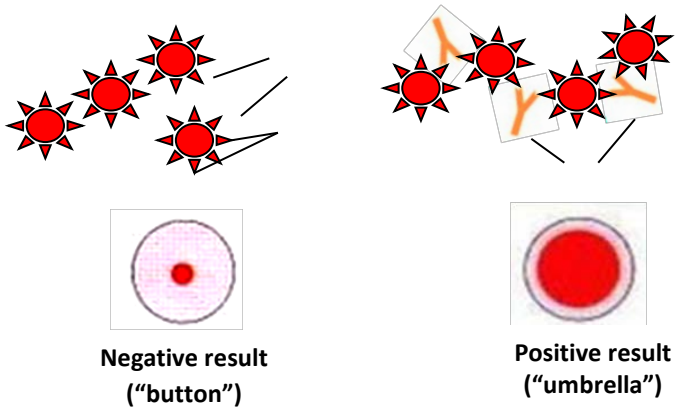


Fig.100. The indirect (mediated) agglutination assay (IHA):
1 – erythrocytes; 2 – conjugated antigen; 3 – antibodies to the antigen

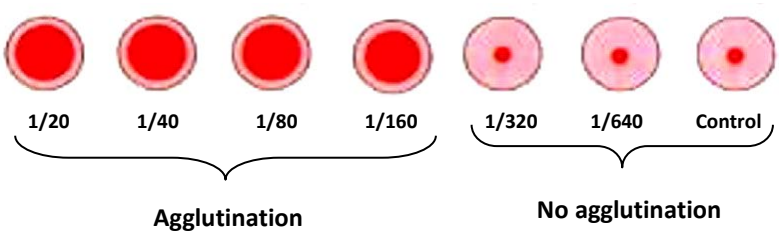


Fig. 101. The indirect (mediated) agglutination assay: serum dilutions (1:20; 1:40; 1:80; 1:160; 1:320; 1:640)

The presence of "normal" (cross-reacting) antibodies in a patient's serum can lead to false-positive results in agglutination assays (IHA, Co-A, latex agglutination). To prevent this, the concept of a diagnostic titer was introduced. The diagnostic antibody titer is defined as the threshold serum dilution at which agglutination is primarily caused by specific antibodies. The value of the diagnostic titer varies depending on the pathogen and the type of serological assay. For example, for typhus, the diagnostic titer of the agglutination assay is 1:100; for typhoid fever, it is 1:200; and for indirect hemagglutination in typhoid fever, it is 1:40. An assay is considered positive when the titer exceeds the diagnostic threshold by at least 2–4 times.

A false-positive result at or above the diagnostic titer may occur if an individual has previously experienced the infectious disease being diagnosed. To exclude such results, serological testing with paired sera is performed. This involves testing the patient's serum twice, with an interval of 7 to 14 days. During an active infection, the antibody titer will increase; if there has been a prior infection, the titer will remain unchanged. When analyzing paired sera, a fourfold or greater increase in titer is considered diagnostically significant.

Indirect Coombs Test

An indirect Coombs test (or indirect antiglobulin test) is used to detect the so-called incomplete (monovalent) antibodies. These antibodies specifically interact with antigens but do not induce agglutination. Such antibodies are found, for example, in certain infectious diseases (e.g., brucellosis) and in Rh incompatibility situations. The reaction is performed in two stages. In the first stage, antigens (such as brucellosis diagnostic reagents or Rh-positive erythrocytes) are added to the patient's serum. In the second stage, a test system – antiglobulin serum – is added, which causes the agglutination of the antigens (Fig. 102).

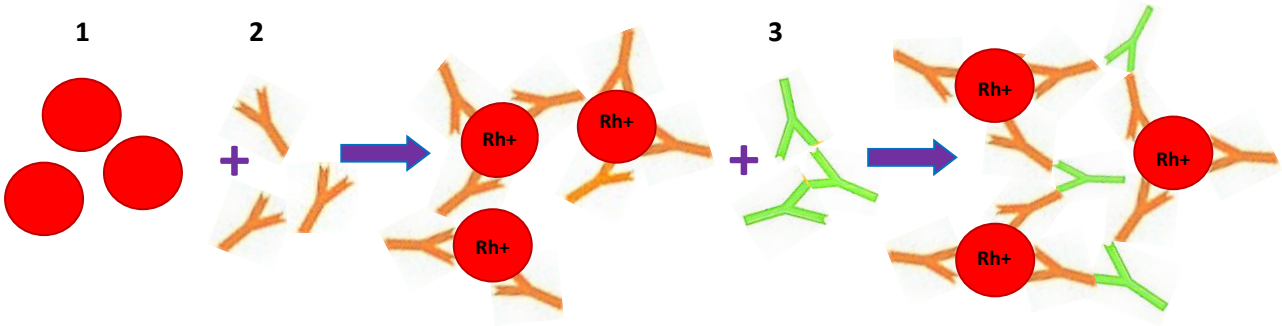


Fig. 102. Coombs test: erythrocytes (1);
anti-Rh antibodies (2); rabbit antibodies against human Ig (3)

Precipitation Reaction (PR)

The phenomenon of precipitation (from Latin *praecipito* – "to cast down" or "throw headlong") involves the interaction of finely dispersed antigens (precipitinogens) with their corresponding antibodies (precipitins), resulting in the formation of a precipitate (Fig. 103). Unlike the agglutination assay, in this method the antigen has a molecular structure and creates a transparent solution.

The precipitation reaction (PR) is performed using two methods: in a liquid medium, such as a flocculation, ring precipitation reaction, or in a solid medium such as agar (gel). The PR is used for two main purposes: to detect antigens using a known precipitating serum, or to detect antibodies using known antigens. Several methods are available for conducting this reaction, but the most commonly used include: Ouchterlony double immunodiffusion, Radial immunodiffusion (RID) or Mancini immunodiffusion, immunoelectrophoresis, flocculation, and ring precipitation.

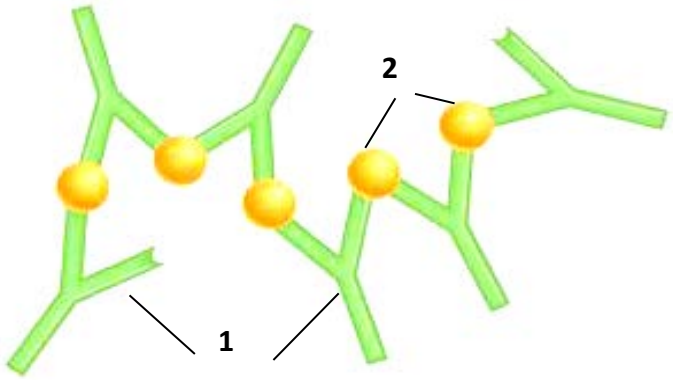


Fig. 103. Precipitation reaction: 1 – antibody; 2 – antigen

Ouchterlony Double Immunodiffusion. To perform this reaction, a 1% Difco agar is poured in a melted state onto slides or Petri dishes, forming a layer approximately 0.5 cm thick. Once solidified, a series of holes ("wells") with a diameter of about 5 mm are cut out using a special device. A suspension containing the test antigen is placed in one well, and a precipitating serum is placed in another. The antigen and antibodies diffuse into the nutrient medium, interact immunologically, and form visible precipitation bands. The reaction is initially read after 4 hours and final results are recorded after 24–48 hours. In multicomponent systems, multiple precipitation bands may appear (Fig. 104).

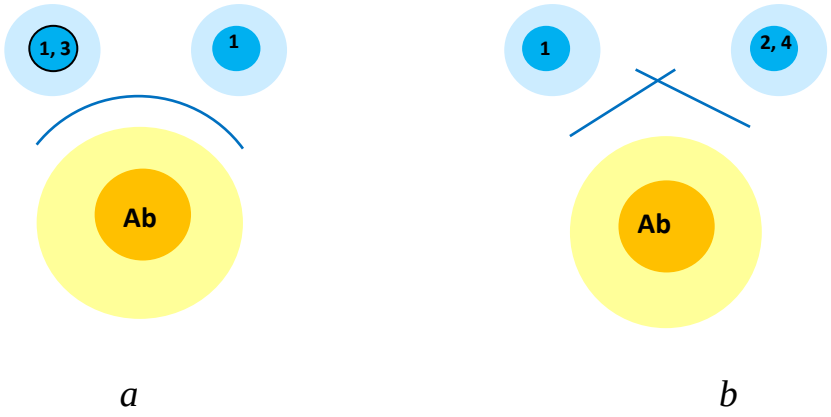


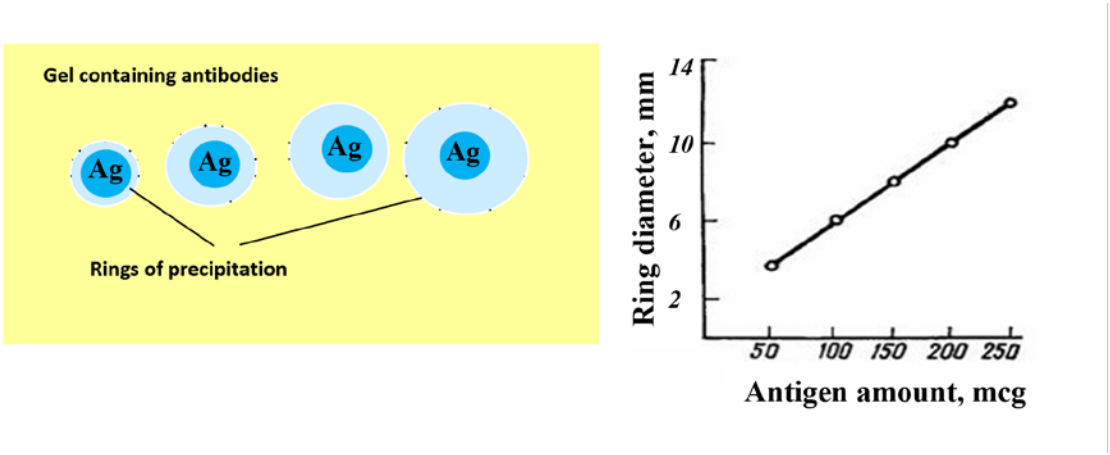
Fig. 104. Ouchterlony Double Immunodiffusion. Precipitation bands of identical antigens merge (a); precipitation bands of non-identical antigens cross (b)

The Ouchterlony reaction can be used to determine the toxicity of bacteria, antibody titers, and the activity of standard diagnostic reagents or specific immune serums.

Radial immunodiffusion (RID) or Mancini immunodiffusion. Radial immunodiffusion, as described by Mancini, allows the use of monospecific antisera and a standard with a known antigen content. The test antigen and dilutions of the solutions being tested for the presence of a specific antigen are placed in wells

arranged in rows within a gel plate that has been pre- coated with the corresponding monospecific antiserum. The antigen diffuses into the gel and, upon binding with specific antibodies, forms visible rings of precipitation. The diameters of these rings depend on the concentration of the antigen in the wells. The results are used to construct a calibration curve that expresses the relationship between the diameter of the precipitates and the antigen concentration in the tested solutions (Fig. 105).

The principle of radial diffusion underpins a method used to study bacterial culture toxicity and to select clones with high toxicity from a bacterial population. In this method, cultures are inoculated onto agar plates containing antitoxic serum. Precipitation rings form around individual colonies, with diameters directly proportional to the toxicity level of each strain.



a b

Fig. 105. Simple radial immunodiffusion: rings of precipitation (a); calibration curve (b)

The *immunoelectrophoresis (IEP)* reaction is based on the principle of precipitation. It is commonly used to study the antigenic structure of microorganisms. The procedure is performed in two stages. First, the antigen is separated by electrophoresis in a buffered agar gel. The antigenic complex is placed in a well located at the center of the gel, which is poured onto a glass plate. An electric current is then applied through the gel, causing the antigens to migrate over varying different distances according to their electrophoretic mobility (Fig. 106-I). Next, a specific immune serum is placed to a groove along the edge of the plate, which is then placed in a humid chamber (Fig. 106-II). The antigens and antibodies diffuse toward each other within the gel. At their point of contact, arcual lines of precipitation are formed (Fig. 106-III).

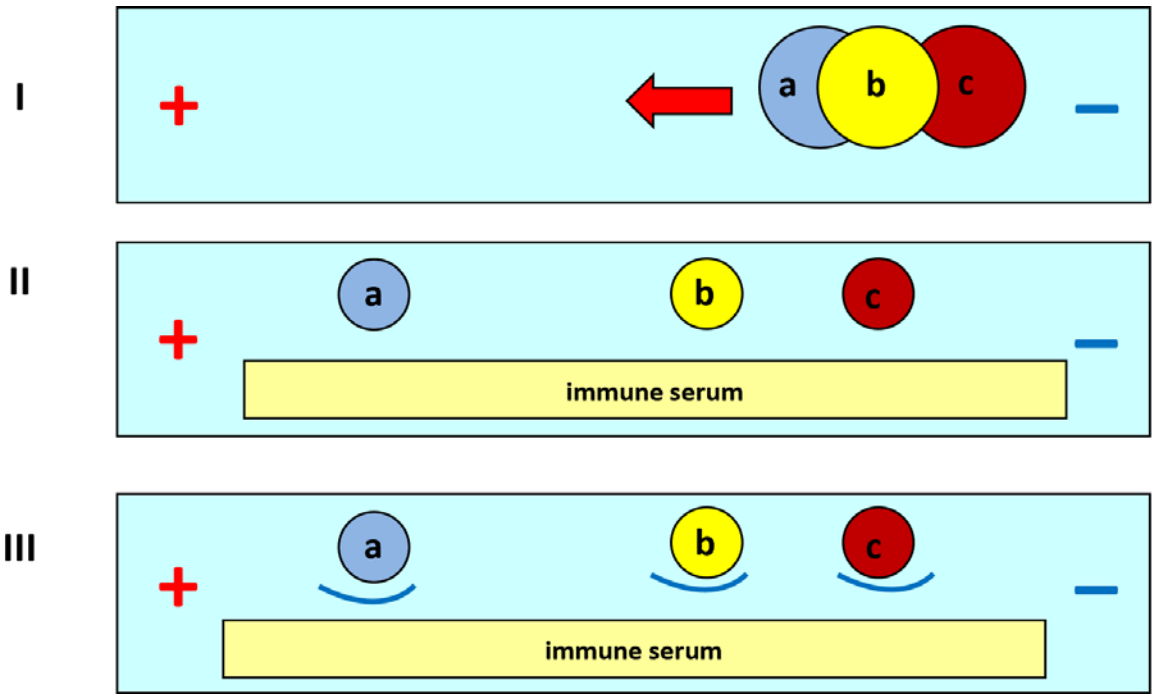


Fig. 106. Immunoelectrophoresis (IEF) reaction with different antigens (a, b, c)

IEF is used to analyse the composition and quantity of proteins in blood serum, cerebrospinal fluid, and microbial proteins.

Ring Precipitation Test. The ring precipitation test is used to detect antigens with precipitating serum containing specific antibodies. It is a qualitative method. The test is performed by layering a medium containing a specific antigen onto an immune serum. The procedure is conducted in narrow test tubes with a volume of 0.1–0.5 mL. If the antigen and antibody are compatible, a cloudy, opalescent precipitation ring forms at the interface between them within 3–5 minutes (Fig. 107).

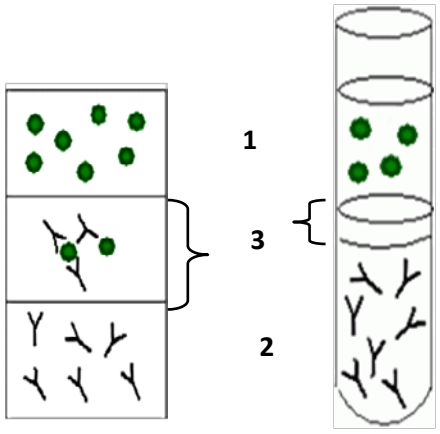


Fig. 107. Ring precipitation test: antigens (1);
immune serum antibodies (2); precipitate (3)

A necessary condition for the formation of an insoluble immune complex is an equivalent ratio of antigens and antibodies.

If boiled and filtered extracts of infected tissues are used as antigens in the assay, the process is called *a thermoprecipitation test* (*Ascoli's Precipitation Test* for the detection of anthrax antigen).

Immunofluorescence (IF)

The immunofluorescence (IF) is based on the binding of bacterial, rickettsial, and viral antigens with specific antibodies labeled with fluorescent dyes (such as fluorescein isothiocyanate (FITC), Rhodamine, B-isothiocyanate, Lissamine rhodamine B-200 (RB 200), sulfochloride, and others) that contain reactive groups (such as sulfochloride, isothiocyanate, etc.). These groups bind to free amino groups of antibody molecules without compromising their specific affinity for the corresponding antigen. The resulting antigen-antibody (Ag–Ab) complexes become clearly visible as brightly luminescent structures under a fluorescent microscope (Fig. 108). IF be used to detect small amounts of bacterial and viral antigens. There are three main types of IF: direct, indirect, and those involving complement.

The direct method involves the direct binding of the antigen to a labeled antibody. *The indirect method* relies on a stepwise detection process of the Ag–Ab complex using fluorescent dyes. The first step is the formation of immune complexes between a specific antigen and its corresponding antibodies. The second step involves detecting this complex by treating it with labeled anti-gamma-globulin.

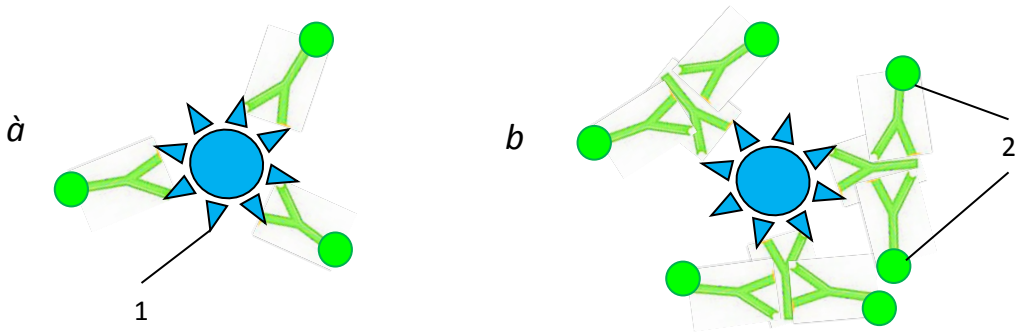


Fig. 108. Immunofluorescence – direct IF (a);
indirect IF (b): 1 – bacterium, 2 – fluorochrome

The advantage of the indirect IF is that it enhances the detection of weakly expressed antigens. Additionally, the indirect IF method is used to detect antibodies in a patient's serum.

The simplicity, high sensitivity, and rapidity of obtaining results make IF suitable for early express-diagnosis of diseases such as influenza, dysentery,

malaria, plague, tularemia, syphilis, and others. Such studies are conducted using a fluorescent microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) is used to detect antigens or antibodies by employing their corresponding antibodies conjugated with a labeling enzyme, such as horseradish peroxidase, β-galactosidase, or alkaline phosphatase. After the antigen binds to the enzyme-labeled immune serum, a substrate and chromogen are added to the mixture.

For example, the substrate for peroxidase is hydrogen peroxide, while the chromogen can include tetramethylbenzidine, orthophenylenediamine, or 5-aminosalicylic acid. The enzyme cleaves the substrate, producing degradation products that chemically modify the chromogen. This results in a color change—its intensity being directly proportional to the number of bound antigen-antibody molecules.

The most common form is solid-phase ELISA, in which one component of the immune reaction (either antigen or antibody) is adsorbed onto a solid carrier. Polystyrene microarrays are typically used as solid carriers. When detecting antibodies, the following components are added sequentially into the wells containing the adsorbed antigen: the patient’s blood serum along with enzyme-labeled antiglobulin serum, and a mixture of substrate solutions for the enzyme and chromogen. After adding each component, any unbound reagents are thoroughly washed away. A positive result is indicated by a color change in the chromogen solution (Fig. 109 A).

The solid carrier can also be bound not only to an antigen but also to an antibody. In this case, the desired antigen is introduced into wells with adsorbed antibodies, followed by addition of enzyme-labeled immune serum against the antigen, and then a mixture of substrate and chromogen solutions (Fig. 109 B).

ELISA is widely used for diagnosing diseases caused by viral and bacterial pathogens.

Immunoblotting (IB)

Immunoblotting (IB), also widely known as Western Blotting (from the English *blot* – spot), is a highly sensitive method for detecting protein antigens or antibodies against them. It combines electrophoresis with either ELISA or radioimmunoassay (RIA).

Pathogen antigens are first separated by electrophoresis in a polyacrylamide gel, and then transferred onto an activated paper or nitrocellulose membrane (film).

Subsequently, the presence of these antigens or specific antibodies is detected using ELISA (Fig. 110).

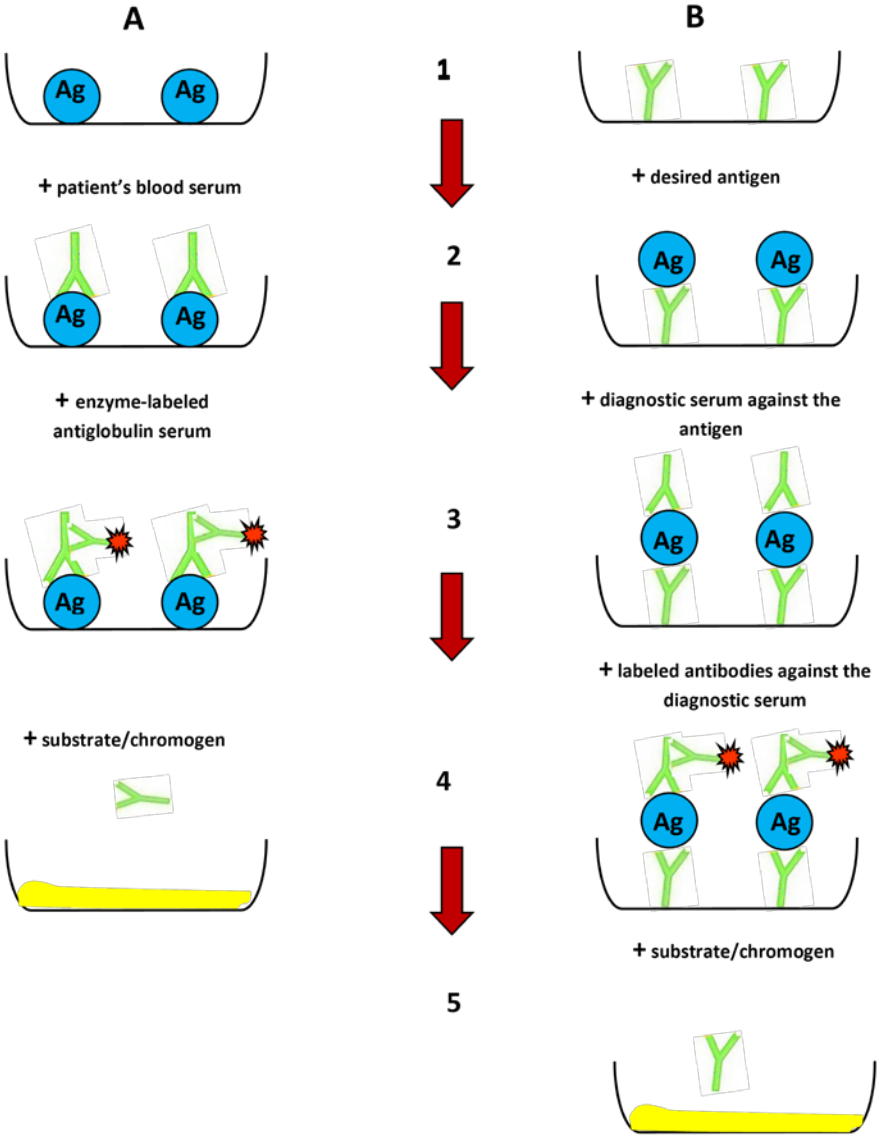


Fig. 109. ELISA: stages (1-5) of antibody detection in the patient's blood serum (A); antigen detection in the test material (B)

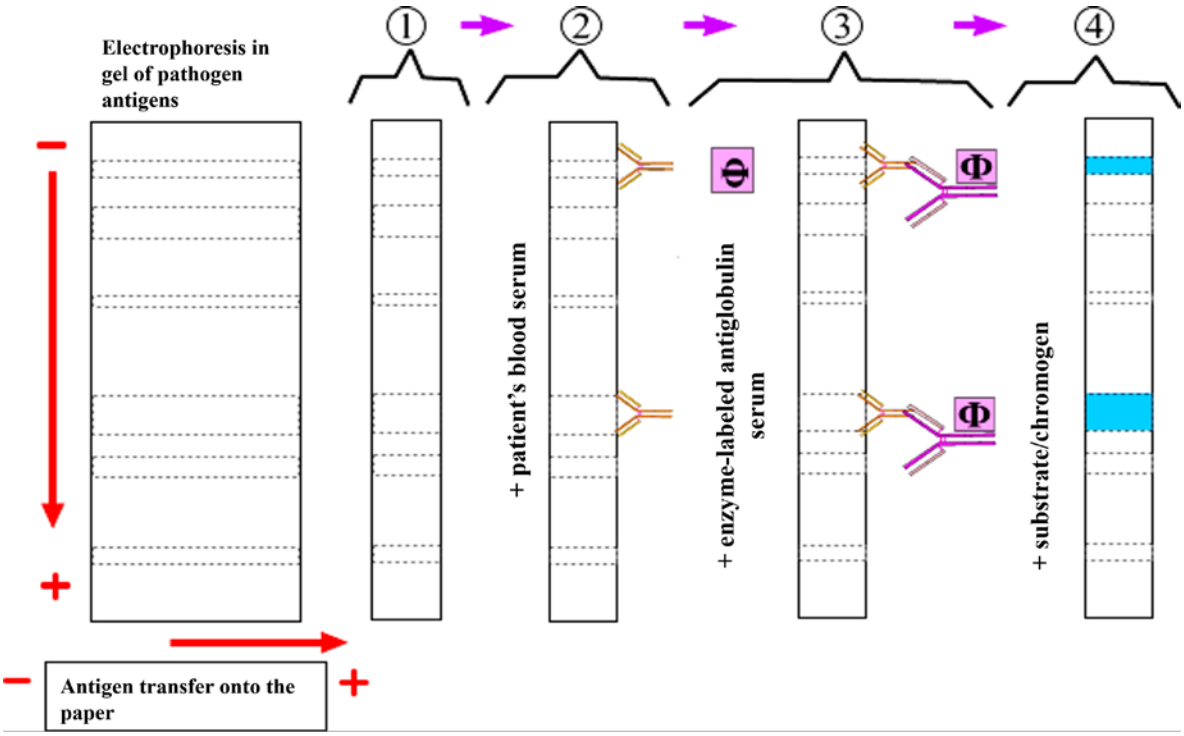


Fig. 110. Immunoblotting : stages (1-4) of antibody detection pathogen antigens in a patient's serum

ALLERGY

Allergy (from the Greek *allos* – other) is a specific hypersensitivity to antigens that arises and develops because of an inadequate immune response. Antigens that trigger allergic reactions are called ***allergens***. Allergens can be classified as follows:

- Inhalant (e.g., plant pollen, epidermal antigens, dust, etc.);
- Food (e.g., eggs, chocolate, nuts, etc.);
- Medicinal (e.g., antibiotics, hormones, vitamins, etc.);
- Infectious (antigens of bacteria, fungi, protozoa);
- Industrial (e.g., polymers, pesticides, metals, etc.).

For an allergy to develop, prior sensitization of the host by an allergen is necessary. ***Sensitization*** is the process by which the host acquires increased sensitivity to an allergen. Clinical manifestations of allergy occur upon subsequent exposures to the allergen. The dose of antigen (allergen) that induces sensitization is referred to as the *sensitizing* or *priming* dose. Repeated exposure to the same antigen after a certain period can trigger an allergic reaction. The dose of antigen that causes an allergic reaction is called the ***challenging*** or ***anaphylaxis-provoking*** dose.

Allergies can manifest as Immediate-Type Hypersensitivity (ITH) or as Delayed-Type Hypersensitivity (DTH). Immediate-Type hypersensitivity is mediated by antibodies including IgE, IgG, and IgM. It develops within a few minutes or hours and can be desensitized. Delayed-Type hypersensitivity is mediated by macrophages and Th1 lymphocytes, which are responsible for stimulating the cellular response. It develops within 6–8 hours and is not amenable to desensitization.

Based on the mechanism, the following types of hypersensitivity (i.e., allergic reactions) are distinguished (The 1963 Gell and Coombs classification):

I. Anaphylactic
II. Cytotoxic
III. Immune complex
IV. Cell-mediated

Hypersensitivity I, II, and III correspond to Immediate-Type Hypersensitivity (ITH), while type IV corresponds to Delayed-Type Hypersensitivity (DTH).

Allergic reactions develop in a predictable manner and proceed through the following stages:

1. Immunological stage
2. Pathochemical stage
3. Pathophysiological stage

The immunological stage begins with contact between an allergen and an organism already sensitized to it, leading to the formation of an “allergen-antibody” complex.

The pathochemical (biochemical) stage is characterized by the release of biologically active substances (BAS), which are allergy mediators such as histamine, serotonin, bradykinin, acetylcholine, and heparin.

The pathophysiological stage involves functional and structural disturbances resulting from the action of biologically active substances on effector tissues. This stage is characterized by blood formation disorders, bronchial and intestinal smooth muscle spasms, changes in blood serum composition, coagulability disturbances, cytolysis of cells, and other effects. The pathophysiological stage underlies the clinical manifestations of an allergic reaction.

Type I Hypersensitivity (Anaphylactic, Immediate)

The mechanism development of Type I hypersensitivity, also known as anaphylactic, immediate or reaginic allergic reactions, is mediated by reaginic antibodies belonging to the IgE and IgG4 classes, which are formed during the initial contact with an allergen. These antibodies bind to mast cells and tissue basophils via their Fc fragments. Upon subsequent exposure to the same allergen, cross-linking occurs between the allergen and IgE or IgG4 antibodies. This cross-linking destabilizes the cytoplasmic membrane of the cells, leading to the release of biologically active substances such as histamine, heparin, prostaglandins, cytokines, enzymes, and others (Fig. 111). The allergic response develops rapidly, typically within 5–10 minutes.

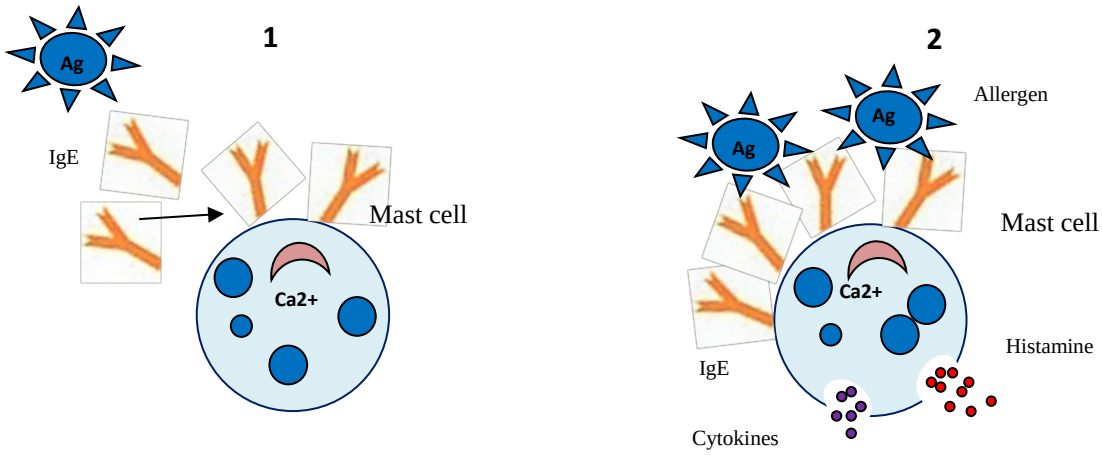


Fig. 111. Type I hypersensitivity: primary (1) and repeated penetration of antigen (2)

Biologically active mediators induce contraction of smooth muscles, weaken cardiac activity, cause collapse, increase vascular permeability, and lead to swelling, itching, and other symptoms.

Clinical Manifestations. A key feature of the clinical manifestations of Type I hypersensitivity is that they typically develop against a background of atopy.

Atopy (from the Greek *atopia* – strangeness or unusualness) is a hereditary predisposition to the development of Immediate-Type Hypersensitivity (ITH). Atopy is induced by:

- Increased production of IgE in response to allergens;
- An increased number of Fc receptors for these antibodies on mast cells;
- Increased permeability of tissue barriers.

Type I hypersensitivity manifests as urticaria, rhinitis, rhinoconjunctivitis, pollinosis, and food allergy. It can also lead to bronchial asthma. The most severe complication of a Type I allergic reaction is anaphylactic shock, which is an acute condition characterized by collapse, edema, and spasm of smooth muscles. It often results in death.

Anaphylaxis can be passively transferred from a sick individual to a healthy person via IgE antibodies specific to the allergen. This phenomenon is known as the *Prausnitz–Küstner reaction.*

Laboratory Diagnostics. Tests include the determination of total IgE in serum; measuring specific IgE and IgG levels to suspected allergens; and levels of histamine and interleukins (IL-4, IL-5). Provocative nasal and inhalation tests are also performed. Skin tests (scarification tests) are conducted using atopic allergens (e.g., household, food, pollen, etc.).

Scarification skin tests are read 15–20 minutes after contact with the allergen. A positive outcome is indicated by the appearance of a skin blister (2–10 mm) with surrounding hyperemia at the scarification site. A negative reaction shows no skin blister or significant hyperemia. Outcomes are compared to controls – reactions to the allergen solvent and histamine.

Type II Hypersensitivity (Cytotoxic, Cytolytic or Antibody-dependent)

This type of reaction is based on the interaction between antigens (endogenous or exogenous chemicals, or drugs) fixed on the cell membrane and antibodies (IgG or IgM). Subsequently, cytolysis of the cells occurs as a result of:

- Activation of the complement system (complement-dependent cytolysis);
- Immune phagocytosis;
- The development of antibody-dependent cellular cytotoxicity (ADCC), mediated by natural killer (NK) cells, leading to cell damage and lysis.

Complement-Dependent Cytolysis involves antibodies interacting with antigens on the cell surface. The complement components then attach to the Fc fragment of these antibodies, which activate via the classical pathway, resulting in the formation of anaphylatoxins (e.g., C3a) and the membrane attack complex (MAC). This process leads to the complement-dependent cell lysis (Fig. 112).

Fig. 112. Complement-dependent cytolysis: target cell (1); antigens (2); antibodies (3); complement (4); MAC (5)

Immune phagocytosis. As a result of the interaction between antibodies and the antigens of the target cell, as well as participation of the complement component (the opsonin C3b), the cell undergoes opsonization, which enhances phagocyte recognition and ingestion of the target cell (Fig. 113).

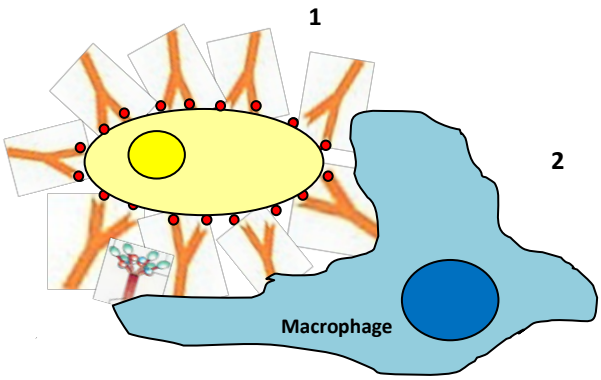


Fig. 113. Immune phagocytosis: opsonization (1); phagocyte/macrophage (2)

Antibody-dependent cellular cytotoxicity (ADCC) occurs when target cells opsonized with antibodies interact with NK cells via the Fc fragment. NK cells then release perforins and granzymes, which induce lysis of the target cell (Fig. 114).

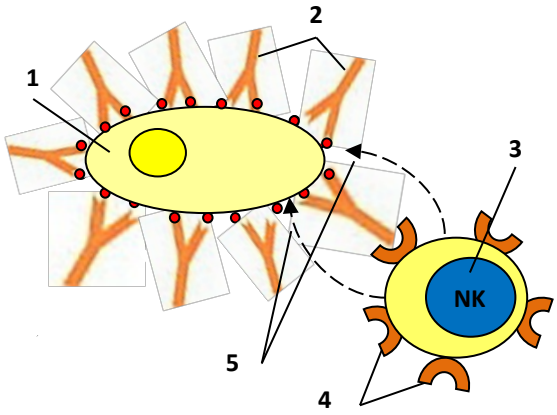


Fig. 114. Antibody-dependent cellular cytotoxicity: target cell (1); antibodies (2); NK cell (3); Fc receptor (4); perforins and granzymes (5)

Clinical Manifestations. Type II hypersensitivity can lead to conditions such as drug-induced hemolytic anemia and thrombocytopenia, where antibodies lyse erythrocytes or platelets containing specific antigens. This type of allergic reaction underlies certain autoimmune diseases, including myasthenia gravis, pemphigus vulgaris, Goodpasture syndrome, and autoimmune hyperthyroidism.

Laboratory diagnostics include the detection of circulating anti-tissue antibodies and the assessment of antibody and complement presence in affected tissues using immunofluorescence (IF) on biopsy specimens.

Type III Hypersensitivity (Immune Complex)

The mechanism of development is based on the formation of soluble immune complexes (antigen-antibody-complement), primarily involving IgG and, less frequently, IgM.

Type III hypersensitivity is initiated by an excess of antigens or a deficiency of complement. These antigens can be exogenous (such as those from chronic bacterial, viral, fungal, or protozoal infections) or endogenous. The resulting immune complexes are deposited on structures bearing Fc receptors, such as vascular walls and basement membranes (Fig. 115). Under conditions of a significant antigen excess, these immune aggregates exert a toxic effect. Activation of complement components (C3a, C3b, and C5a) at the sites of complex deposition leads to the generation of anaphylatoxins, which change vascular permeability, attract polymorphonuclear leukocytes, and promote inflammation. Damage to granulocytes results in the release of proteolytic enzymes that destroy body tissues, as well as vasoactive amines (e.g., histamine). Anti-inflammatory cytokines,

including TNF-α and chemokines, are also produced. In the later stages, macrophages participate in the process.

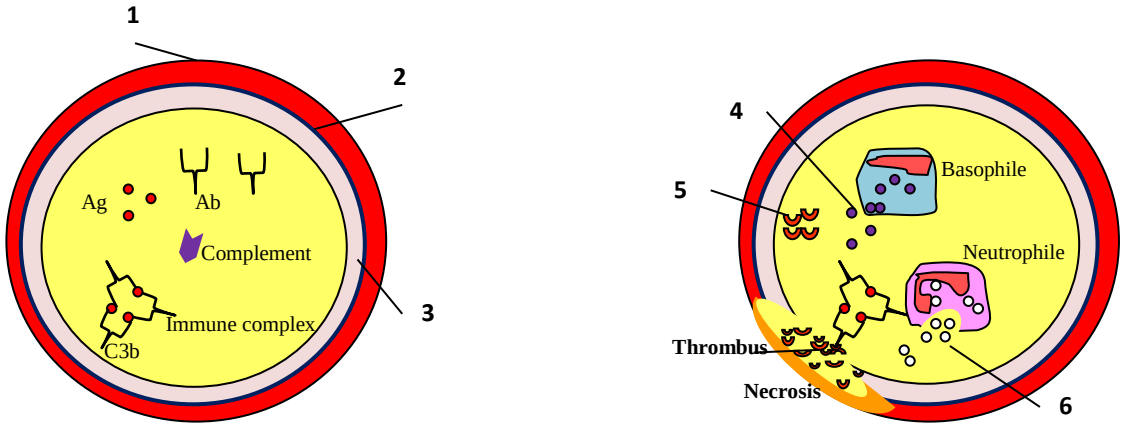


Fig. 115. Hypersensitivity Type III (deposition of immune complexes in the vascular walls): vessel wall (1); basement membrane (2); endothelium (3); vasoactive amines (4); aggregated platelets (5); enzymes (6)

Clinical manifestations. The reaction can be systemic (e.g., serum sickness) or localized to specific organs (e.g., lupus nephritis, vasculitis, aspergillosis) or tissues (e.g., systemic lupus erythematosus, Arthus reaction).

Laboratory diagnostics include determining immunoglobulin and complement deposits in tissue biopsies using immunofluorescence (IF). IgG levels in immune complexes precipitated from blood by polyethylene glycol can also be measured.

Type IV Hypersensitivity (Cell-Mediated or Delayed)

Unlike types I, II, III, and IV hypersensitivity is not mediated by antibodies but is caused by Th1 lymphocytes, macrophages, and cytokines secreted by these cells. The reaction develops 1–3 days after repeated exposure to the allergen: tissue consolidation and inflammation occur due to infiltration by T lymphocytes (Th1) and macrophages. The allergens involved in delayed-type hypersensitivity (DTH) include antigens from intracellular parasites (bacteria, fungi, viruses, and protozoa), transplant antigens, and natural or synthetic haptens (drugs, food dyes, metal ions, etc.). DTH is most strongly manifested by low-immunogenic antigens; optimal responses are elicited by small doses of antigens administered intradermally.

During initial exposure to the allergen, it is processed by macrophages; subsequent presentation leads to the activation of T lymphocytes and the formation of a pool of sensitized T lymphocytes through proliferation and differentiation. These cells are predominantly Th1 cells derived from Th0 precursors (CD4+ cells). In some cases, the pool of sensitized T cells may include cytotoxic T lymphocytes (CD8+ cells) (Fig. 116).

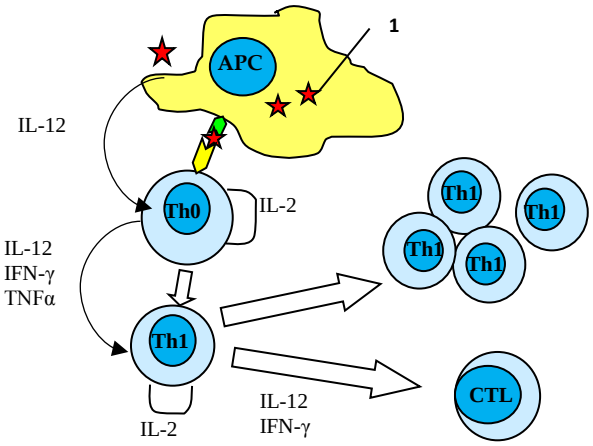


Fig. 116. Type IV hypersensitivity – DTH.
First stage of sensitization: allergen penetration (1), cellular immune response, formation of DTH effector T lymphocytes

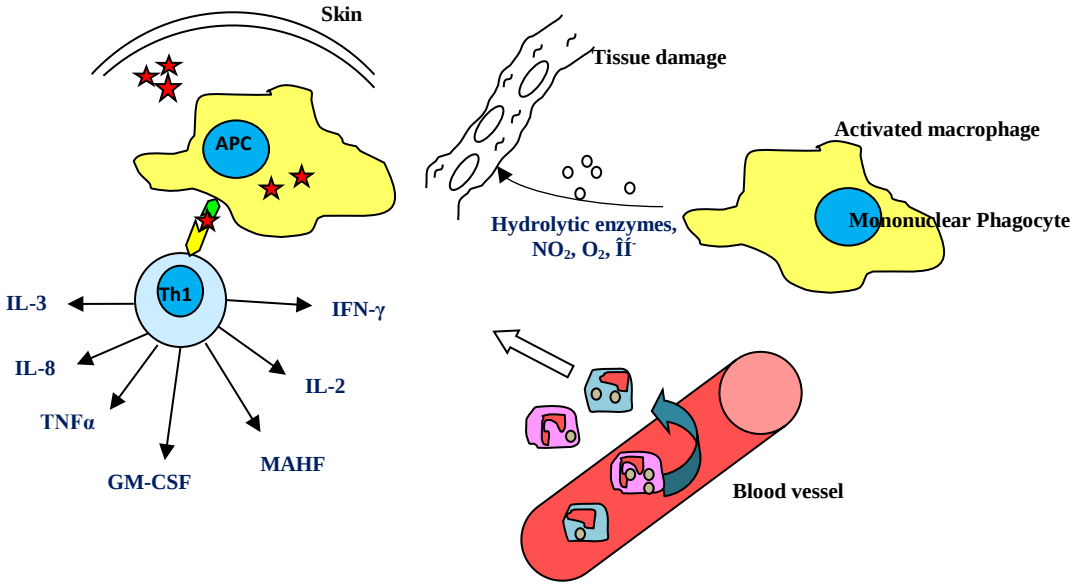


Fig. 117. The second stage of the DTH reaction: allergen penetration, enhanced production of cytokines by T effectors, cell accumulation and proliferation, increased vascular permeability, inflammation

Repeated contact with the same allergen activates sensitized T-lymphocytes and results in excessive cytokine production: IFN-γ, IL-2, TNF-β, IL-3; chemokines such as IL-8 and MIF (macrophage migration inhibitory factor); and macrophage activation factors like MAHF. These mediators attract macrophages to the site of allergen contact and stimulate their phagocytic and antigen-presenting functions as well as metabolic activity. Macrophages then produce hydrolytic enzymes, reactive oxygen species (ROS), nitric oxide, active oxygen forms, and other biologically active substances into surrounding tissues. The effects of these

substances lead to inflammation and localized degenerative-destructive processes (Fig. 117).

Clinical Manifestations. Three main types of Type IV hypersensitivity are recognized: tuberculin reaction, dermatitis, and granulomatous inflammation (Table 9).

Table 9

Cell-Mediated Hypersensitivity

Form of DTH	Reaction time	Histology	Clinical Manifestations
Contact	48–72 hours	Lymphocytes, later macrophages	Eczema, edema
Tuberculin	48–72 hours	Lymphocytes, monocytes, macrophages	Local induration (hardening)
Granulomatous	21–28 days	Macrophages, epithelioid cells, giant cells; fibrosis	Hardening (granuloma formation) in skin, lungs, etc.

It should be noted that a single antigen can induce different types of reactions that may overlap. Diseases characterized by granulomatous reactions include tuberculosis, brucellosis, tularemia, and Crohn's disease, among others. Activation of macrophages by lymphocytes can help contain infections but may also cause tissue damage with persistent stimulation. Contact DTH can be triggered by drugs, cosmetics, low-molecular-weight substances (haptens), low-molecular-weight substances (haptens), or autoimmune processes such as insulin-dependent diabetes mellitus.

Laboratory Diagnostics. For infectious diseases involving cell-mediated hypersensitivity responses, such as tuberculosis or brucellosis—skin tests with specific pathogen allergens are used: tuberculin for tuberculosis; brucellin for brucellosis; tularin for tularemia. Histological examination of the skin may also be performed.

IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF INFECTIOUS DISEASES

Immunotherapy involves the therapeutic administration of immunobiological preparations containing antibodies (such as specific sera and immunoglobulins), which create artificial passive immunity by introducing pre-formed antibodies, interferons, cytokines, and some other immunomodulators.

Immunoprophylaxis refers to the administration of immunobiological preparations containing either antigens or antibodies, aimed at inducing artificial immunity in the infected organism.

In addition to sera, immunoglobulins, vaccines, and interferons, other immunobiological preparations (IBPs) are used for the diagnosis and treatment of infectious diseases. These include, firstly, diagnostic reagents and diagnostic sera employed in serological reactions for the serological identification of microorganisms and/or for determining the presence and quantity of specific antibodies in human blood serum. Secondly, bacteriophages are utilized for diagnostic, therapeutic, and prophylactic purposes due to their high specificity. Thirdly, probiotics – preparations containing microorganisms from the normal human microbiota – are used to restore its composition during dysbiosis and also exert antimicrobial (antibiotic) activity against pathogenic microorganisms. Fourthly, allergens are preparations used to detect sensitization to infectious and non-infectious antigens.

A distinctive feature of all the aforementioned preparations, except for probiotics and immunomodulators, is their specificity, which sets immunobiological drugs apart from other pharmacological agents.

Based on their composition, all immunobiological drugs can be classified into two groups:

Preparations containing antigens:

- Vaccines
- Anatoxins
- Diagnostic reagents, including erythrocyte diagnostic reagents
- Probiotics, symbiotics, synbiotics
- Allergens
- Bacteriophages

Preparations containing antibodies:

- Therapeutic and prophylactic sera
- Therapeutic and prophylactic immunoglobulins
- Diagnostic sera

Vaccines

Vaccines are among the most widely used immunobiological preparations. The branch of immunoprophylaxis dedicated to the development and application of vaccines is called vaccinology. The use of vaccines remains the most effective and cost-efficient for combating many infectious diseases. Vaccination relies on the body's ability to develop acquired immunity and immunological memory against specific pathogens. Through vaccination efforts, smallpox has been eradicated globally, mass vaccination campaigns have nearly eliminated poliomyelitis and diphtheria; and the epidemic risks associated with measles, pertussis, tetanus, brucellosis, tularemia, anthrax, tick-borne encephalitis, rabies, and other infections has been significantly reduced.

The first vaccine was named after a cattle disease – *vaccinia* (cowpox). Over two centuries ago, English physician Edward Jenner used it to protect people against smallpox. This marked the first scientifically grounded attempt at preventing an infectious disease in humans. Only a century later, Louis Pasteur formulated the fundamental principle of vaccination: to induce strong immunity against highly virulent microorganisms using preparations derived from the same pathogens but attenuated in virulence.

Vaccines are complex immunobiological preparations. Besides the active component – the antigen – they contain stabilizers, adjuvants that enhance immune response activation, and preservatives. The active component used in vaccines include:

– Live attenuated pathogens;
– Whole pathogens inactivated by various methods;
– Individual antigenic components of pathogens (protective antigens);
– Secondary metabolites produced by the microbial cell that contribute to pathogenicity or immunity (e.g., toxins and their neutralized derivatives—anatoxins);
– Molecular antigens produced via genetic engineering or chemical synthesis, which are analogues of natural bacterial or viral antigens

Live attenuated vaccines contain modified forms of pathogenic organisms (vaccine strains) that have lost their virulence but retain their ability to induce immunity. Attenuation is achieved through methods such as:

a) Cultivating bacteria on media with altered composition or temperature (e.g., vaccines against tuberculosis, anthrax, plague, and tularemia).

b) Passage through non-susceptible or minimally susceptible animals (e.g., rabies vaccine).

c) Creation of recombinant strains in a laboratory setting.

Additionally, natural attenuation involves isolating pathogen strains that have lost virulence for humans in nature. Live vaccines typically cause subclinical infections that confer effective protection but may also pose risks – they can cause

persistent infections or damage cellular genetic material. Therefore, maximum caution is essential when immunizing individuals with immunodeficiency.

Live divergent vaccines are developed based on divergent strains. Such strains of microorganisms are closely antigenically related to infectious pathogens. The antigens of these microorganisms elicit an immune response that is cross-reactive with the antigens of the pathogen. The most well-known and historically significant examples are the smallpox vaccine, first developed by Jenner from the cowpox virus, and the Bacillus Calmette-Guérin (BCG) vaccine for the prevention of human tuberculosis, derived from an attenuated strain of bovine Mycobacterium tuberculosis.

Live vaccines that lack pathogenicity but retain specific antigenic properties when introduced into the body induce the so-called vaccination process. This process involves the multiplication of the vaccine strain within the host and its effect on immunocompetent cells. Consequently, it leads to the development of specific immunity against the pathogen of a particular infectious disease. Immunity acquired after inoculation with most live vaccines tends to last significantly longer than after inactivated ones. For example, a single dose of vaccines against measles, rubella, and mumps can confer immunity lasting up to 20 years, while the yellow fever vaccine provides approximately 10 years of protection; and tularemia vaccine offers about 5 years of immunity.

Live vaccines are produced by cultivating the vaccine strain under controlled production conditions on nutrient media and substrates that allow for sufficient growth. Bacterial vaccine strains are cultivated on liquid or solid artificial nutrient media, while viral vaccine strains are typically propagated in chicken embryos or cell cultures. The pure culture of the vaccine strain obtained through these methods is quantified according to bacterial or viral count and then subjected to lyophilization (freeze-drying) along with stabilizers that prevent inactivation during drying and storage. An exception is the oral polio vaccine, as it is produced in liquid form.

Stabilizers such as human albumin, sucrose combined with gelatin, or other non-antigenic and harmless substances are used to maintain vaccine stability. The quality control of vaccines involves assessing key parameters such as the content (concentration) of live bacteria or viruses in the vaccine strain, residual moisture content, safety, allergenicity, immunogenicity, and other relevant indicators.

Killed corpuscular (inactivated) vaccines are derived from freshly isolated virulent and immunogenic strains of pathogens that have been inactivated by heat or with chemical substances. Depending on the chemical used, include formalin (formaldehyde), alcohol, acetone, merthiolate, or chloroform vaccines. Killed vaccines are considered safer than live ones but tend to be less effective and often require repeated administration (revaccinationû). Currently, they are used against diseases such as pertussis, cholera, leptospirosis, tick-borne encephalitis, and other diseases. Killed vaccines can be prophylactic (the majority) or therapeutic (e.g.,

gonococcal or brucellosis vaccines). They are employed to stimulate specific immunity in chronic, indolent infectious processes. Production of killed vaccines involves cultivating the vaccine strain under controlled conditions on nutrient media or substrates that promote sufficient accumulation of the vaccine strain. The pure culture of the vaccine strain is then inactivated – most commonly with 0.4% formaldehyde at 37–40°C for approximately four weeks – followed by purification from residual components and formulation into doses, often with freeze-drying.

Killed subunit (also called ***subcellular*** or ***subvirion) vaccines*** contain antigens extracted from microorganisms using chemical agents or ultrasound techniques. They include various antigenic components of bacteria such as cell wall antigens, Vi antigens, H antigens, ribosomal antigens; and viral surface antigens. The use of purified antigens enhances immunogenicity of the preparations while reducing their disadvantages: sensitization of the macroorganism, high immune, reactogenicity, and toxicity due to lipids and other chemical compounds. To produce subunit vaccines from bacteria or viruses, protective antigens (typically protein complexes like lipopolysaccharide-protein conjugates) are isolated from bacteria or viruses after cultivation under industrial conditions using physicochemical methods such as alcohol precipitation, salting out with neutral salts, chromatography techniques, or ultracentrifugation. Subcellular vaccines were historically referred to as chemical vaccines due to their extraction methods.

Vaccines based on protective antigens often include preservatives like merthiolate (at a concentration of 1:10,000) and adjuvants. Such vaccines have been developed against numerous bacterial and viral infections, including typhoid fever, dysentery, influenza, brucellosis, etc.

The quality control parameters for killed vaccines include residual virulence testing, the content analysis for bacteria or viruses of the vaccine strain, residual moisture levels, sterility testing, safety assessments, allergenicity evaluation, and immunogenicity testing.

Anatoxins are detoxified bacterial exotoxins. The toxin is detoxified through treatment with formaldehyde (0.4%) at 37–40°C over four weeks until toxicity is abolished while preserving antigenic and immunogenic properties. Anatoxins are obtained by cultivating a toxigenic strain of the corresponding microorganism under industrial conditions on liquid nutrient media that facilitate sufficient accumulation of the toxin. The microorganism releases the exotoxin into the medium. The resulting pure culture is filtered through bacterial filters to separate the toxin, which is then detoxified with formalin. The detoxified toxin, called an anatoxin or toxoid, is then purified from residual medium components, microbial cell components, and concentration. An adjuvant is added to the purified anatoxin to enhance its immunogenic properties.

Anatoxins are evaluated for key parameters: residual toxicity, concentration, sterility, safety, allergenicity, and immunogenicity. They are produced both as single-component and associated preparations. Anatoxins are used against tetanus,

diphtheria, botulism, and staphylococcal infections. A key feature of anatoxins is their ability to induce long-lasting immunological memory in the vaccinated person. Therefore, re-administration after many years can rapidly stimulate high titers of protective antibodies—making them valuable for post-exposure prophylaxis during outbreaks, for example: tetanus booster shots following injury or diphtheria vaccination campaigns.

The immunogenicity of an inactivated vaccine is determined by the size of the molecule, its chemical composition, and physical state of the administered antigen. The larger and more complex the molecule, the higher its immunogenicity. Soluble proteins are weaker immunogens than insoluble antigens. To enhance the immune response, special substances called *adjuvants* are used. ***Adjuvants*** (from Latin *adjuvare* – to help) are non-specific immunostimulants of inorganic and organic origin that increase immunogenicity when added to antigens and vaccines. The use of adjuvants is also associated with the high degree of purification of the vaccine antigen, which reduces its inherent immunogenicity. Adjuvants can include mineral compounds; microbial structures such as proteins, nucleic acids, and lipopolysaccharides; synthetic substances like polynucleotides, glycopeptides, and polyoxidonium; as well as cytokines and peptides. Currently, only aluminum salts and oil-based *adjuvants* are approved for widespread use in vaccines globally. Aluminum compounds adsorb the antigen, retaining it near the injection site for an extended period, thereby facilitating better interaction with antigen-presenting cells. Mineral adjuvants primarily stimulate a Th2 immune response.

In Russian vaccines like Grippol (against influenza) and HEP-A-in-VAC-POL (against hepatitis A) utilize polyoxidonium, which enables induction of a T-independent immune response. This ensures a high level of immunogenicity even in individuals with genetically predetermined low immune responsiveness.

The mechanisms by which adjuvants exert their effects include:

a) Formation of an antigen “depot” at the injection site, prolonging antigen action and providing sustained action on the immune system;

b) Induction of an inflammatory reaction that activates immunocompetent cells;

c) Enhancement of antigen capture and processing by phagocytic cells.

Adjuvants can increase vaccine immunogenicity by tenfold or more, especially for protein-based molecular antigens such as anatoxins, which are rapidly resorbed and degraded by body enzymes without adjuvants, resulting in a short-lived immune response.

Recombinant (genetically engineered) vaccines are developed using recombinant strains of bacteria and viruses. Their potential advantage lies in utilizing only those antigens necessary for eliciting protective immunity. These vaccines are also relatively inexpensive to produce and involve safe manufacturing processes. In cases where cultivating sufficient quantities of the pathogen is challenging or impossible, a genetic engineering approach is the only viable option.

The general principle behind genetically engineered vaccine development involves “inserting” gene(s) encoding protective antigens into the genome of viruses, bacteria, or eukaryotic cells without disrupting their biological activity. This process causes the host organism to produce necessary protective antigen along with its own antigens.

Recombinant strains, such as *Escherichia coli*, adenoviruses, yeast cells, and other microbes, have been created with genes for various pathogens integrated into their genomes.

Currently, two main types of genetically engineered vaccines under development: subunit vaccines and live recombinant vaccines. The efficacy of subunit vaccines stems from the fact that the immune response to several viral infections is often driven by one or a few surface protective antigens of the virus. For example, the hepatitis B vaccine consists solely of purified HBsAg produced via genetic engineering – where the gene encoding HBsAg was inserted into yeast cells that then produce this antigen.

Live genetically engineered vaccines often use a well-studied attenuated DNA-containing virus containing inserted genes encoding protective antigens from other pathogens. The key is that the infectivity of the original vector virus remains intact while enabling simultaneous expression of both native and foreign antigens within the host organism. This vector-based virus stays in the vaccinated person’s body, and the vaccinal process induce strong immunity against both the vector virus and the inserted pathogen antigen; an example is the *Sputnik V COVID-19* vaccine.

Associated vaccines. The body can form a complete immune response even when multiple antigens are administered simultaneously. This resulted in the development of single and complex, also known as associated vaccines for simultaneous immunization against multiple infections. The principles of designing these vaccines involve ensuring compatibility among different antigens within a single formulation, appropriate dosing ratios, and their interference with immune responses and acceptable reactogenicity.

Associated vaccines are widely used in practice for immunization against pertussis, diphtheria, and tetanus (DTaP vaccine – combining killed *Bordetella pertussis* bacteria adsorbed on aluminum hydroxide with diphtheria and tetanus anatoxins), Poliomyelitis vaccines containing strains types I and III. In some countries there are also associated vaccines against pertussis, diphtheria, tetanus, and polio (Tetracoq); vaccines against measles, mumps, and rubella (MMR); DTaP combined with *Haemophilus influenzae type b* (Hib) polysaccharide-protein conjugate vaccine; vaccines against hepatitis A and B, as well as vaccines against hepatitis B and *Haemophilus influenzae type b*, etc.

Synthetic peptide vaccines. are produced when the structure of a natural protective antigen is known; they involve chemical synthesis of short peptides, including antigenic determinants, which can serve as the basis for designing

vaccine and diagnostic preparations, as well as semi-synthetic vaccines. Semi-synthetic vaccines refer to a complex consisting of an antigen or its determinant, carriers such as high-molecular-weight polymers and adjuvants; an example is *EpiVacCorona*, developed for coronavirus prevention.

DNA vaccines consist of plasmid DNA encoding protective antigens from infectious agents. Once introduced into an animal, this DNA enters cell nuclei where it persists outside the chromosomes without replicating for a long time but is transcribed to produce antigens that stimulate immunity. DNA vaccines can be produced in large quantities, are stable, and free from infectious agents. Developing multicomponent DNA vaccines, containing multiple plasmids encoding different antigens, is a promising area.

Animal studies have explored DNA vaccines against HIV, influenza, rabies, lymphocytic choriomeningitis virus, hepatitis B and C, herpes simplex virus, papillomavirus, malaria parasites, leishmaniasis pathogens, and *Mycobacterium tuberculosis*.

Plant-based vaccines are vaccines developed using transgenic plants. A structural gene encoding a viral or bacterial antigen is integrated into the nuclear chromosome of a plant cell via a plant vector. The resulting transgenic plant expressing infectious antigens can be used orally to induce natural mucosal immunity when consumed.

Vaccine Production and Quality Control. Vaccines are produced at specialized facilities. The technology, production conditions, and quality standards for vaccines are determined by manufacturing instructions, technical specifications, and instructions approved by official state authorities.

The ability of vaccines to induce immunity is assessed biologically – by infecting previously vaccinated laboratory animals with pathogenic microbes – and epidemiologically – by monitoring disease incidence among vaccinated populations.

All countries adhere to WHO recommendations when developing national vaccine standards. The WHO Expert Committee on Biological Standardization reviews, approves, and publishes guidelines for almost all types of vaccines. These guidelines cover all stages of vaccine production and quality control, from the certification of the vaccine strain and cell cultures used to develop vaccines to final product testing.

General requirements focus primarily on the vaccine safety and its specific activity. To ensure vaccine safety, the following parameters should be considered: properties of vaccine strains, cell substrates, and properties of the intermediate and final products (sterility, toxicity, pyrogenicity, chemical and biological impurities, additives, contamination risks, etc.).

Specific safety criteria include complete inactivation of toxins, bacteria, and viruses; absence of residual virulence or reversion to virulence; absence of contamination; genetic stability and genetic homogeneity in the vaccine strain.

The specific activity of vaccines encompass indicators such as the Antigen content per unit volume, number of live or killed microbial cells forming the basis of the vaccine, levels of specific antibodies in the blood serum of immunized animals, and the protection degree of these animals demonstrated through challenge studies.

Vaccines may be formulated as liquids, adsorbed preparations, dry powders, tabs, pills, or capsules. Most are administered parenterally, meaning by injection: subcutaneously, intramuscularly, intradermally, or orally.

Vaccine Administration Schedule. Vaccination involves the administration of the vaccine either once or multiple times. Live vaccines are typically administered once, while inactivated vaccines are given multiple times. There are two types of vaccination: primary immunization and revaccination (repeated vaccine administration). Primary vaccination establishes ground immunity and an enhanced ability to respond to the antigen (immunoreactivity and immunological memory). Consequently, upon subsequent vaccination, the body responds to the antigen more rapidly and vigorously. Revaccinations serve to maintain immunity at a protective level over the long term.

Vaccine-based prophylaxis of infectious diseases is conducted within the framework of routine immunizations and epidemic-based vaccination campaigns. Routine vaccinations can be divided into two groups.

The first group includes vaccinations performed implemented nationwide according to the national immunization schedule. The immunization schedule in Russia encompasses immunization against hepatitis B, tuberculosis, pneumococcal infection, poliomyelitis, pertussis, diphtheria, tetanus, measles, mumps, and rubella. The schedule specifies the administration schemes and timing from birth and through various stages of life.

The second group comprises vaccinations given to populations residing in areas endemic for natural focal and zoonotic infections, high-risk groups (professional, social, etc.), and individuals who pose a risk to others if infected. Routine vaccinations in this group include immunization against influenza, tick-borne encephalitis, Japanese encephalitis, hepatitis B (for adults), hepatitis A, rabies, yellow fever, brucellosis, tularemia, anthrax, plague, cholera, leptospirosis, typhoid fever, meningococcal infection, and rickettsioses.

Epidemic (emergency) vaccinations are performed in response to unfavorable epidemiological situations or after exposure of a susceptible (unvaccinated) individual to an infectious source. The first group may include vaccinations against influenza, meningococcal infection, and particularly dangerous infections. The second group involves vaccinations at outbreak foci, as well as emergency prophylaxis for tetanus and anti-rabies vaccinations.

Contraindications to Vaccination. Contraindications to vaccination are specified in the instructions accompanying each vaccine and must be carefully considered before administration. These primarily include acute diseases affecting

the cardiovascular system, kidneys, liver, respiratory or nervous systems; certain chronic diseases; immunodeficiencies; severe allergic reactions, etc. The complete list of contraindications is provided in the instructions for each vaccine.

Preparations for Passive Immunization

For the treatment and emergency prophylaxis of infectious diseases, immune sera and immunoglobulins (specific active fractions extracted from sera) are used. These preparations contain antibodies and are employed to induce passive immunity as quickly as possible. Following intramuscular or subcutaneous injection, non-susceptibility develops as the drug is absorbed from the injection site; antibody concentrations in blood peak within 12–24 hours post-injection. Intravenous administration results in almost immediate immune protection.

The production of therapeutic and prophylactic serum preparations involves complex processes with advanced technology. There are two main methods for obtaining specific sera:

- Hyperimmunization of animals (heterologous serum preparations);
- Vaccination of human donors (homologous serum preparations).

Serum preparations are obtained by specially processing blood from immunized animals or from humans vaccinated with the relevant antigen or recovered from a specific infectious disease.

Heterologous Serum Preparations. To produce heterologous serum preparations for therapeutic or prophylactic use under industrial conditions, large animals such as horses, oxen, bulls, cows, or mules are primarily used due to their capacity for large blood volume collection. Horses are most frequently employed because they exhibit high immunological reactivity; they can produce serum containing high titers of specific antibodies within a relatively short period. Additionally, they are convenient for handling and care. Hyperimmunization schedules are typically individualized for each animal. Blood is collected during periods when antibody titers peak. The collected blood is defibrinated using calcium chloride solutions to remove formed blood elements. The resulting serum is then bottled and preserved.

To reduce adverse reactions and enhance efficacy of serum preparations, they are purified and concentrated by isolating immunologically active serum protein fractions. Depending on whether antibodies are antitoxic, antibacterial, or antiviral in nature, different purification methods are employed.

Homologous Serum Preparations. Immunoglobulins derived from human blood – from volunteer donors vaccinated against specific pathogens or their toxins – offer advantages over animal-derived serum preparations because they are not heterologous to the human body, they are practically non-reactogenic. When these preparations are administered to a patient, the antibodies circulate in the body for a

longer time than immunoglobulins from heterologous preparations, providing passive immunity (therapeutic effect) for 4–5 weeks.

Such products are prepared from collected donor blood (at least 1,000 individuals). Currently available preparations include purified concentrated donor serums obtained via alcohol precipitation (e.g., anti-tetanus or anti-botulinum), as well as donor-derived immunoglobulins targeting staphylococci, influenza viruses, tick-borne encephalitis virus, and normal human immunoglobulin used for prophylaxis and treatment of mumps, measles, poliomyelitis, and other infections.

Use of Serum Preparations. Heterologous serum preparations have widespread applications in treating and prophylaxis of numerous infectious diseases, particularly those caused by bacterial toxins and viruses. Their primary benefit lies in inducing passive immunity (therapeutic effect) immediately after administration. Timely administration of the preparation can prevent disease development by extending incubation periods or resulting in milder disease courses with reduced mortality. Homologous immunoglobulins and purified serums are generally non-reactogenic. However, they contain a mixture of IgG molecules belonging to different allotypic variants, and therefore, potentially leading to isoimmunization or formation of anti-gammaglobulins upon repeated administration. Preparations enriched with IgM and IgA may provoke formation of isoantibodies against these subclasses. Consequently, although very rarely, anaphylactic-type reactions can occur with repeated administrations of homologous serum preparations.

Reactions to Heterogeneous Serum Administration. *Anaphylactic reactions:* When foreign serum is introduced into the body, sensitization occurs due to exposure to heterologous proteins. Subsequent injections of the same serum may trigger anaphylactic reactions up to shock severity. The introduction of a sensitizing dose of the antigen (foreign protein) induces the formation of specific IgE and IgG antibodies that fix to the membranes of tissue basophils (mast cells). Upon re-entry into the body, the specific antigen engages in an immune response with the antibodies fixed on the target cells. This leads to the activation of cellular proteases, degranulation of the target cells, and releasing biologically active mediators such as histamine, SRS-A, serotonin, etc. that cause smooth muscle contraction, increased vascular permeability, hypersecretion, etc. The patient’s condition is complicated by bronchospasm, skin rashes, swelling of mucous membranes including larynx and nasal passages, and vascular collapse. These phenomena can escalate into anaphylactic shock. Anaphylactic reactions can develop within a few minutes after the allergen introduction, or more rarely, after several hours.

To prevent anaphylactic complications, a desensitization state is developed by administering small eliciting doses of the specific antigen (e.g., horse protein). A.M. Bezredka proposed a method of administering a small amount of serum (0.5–1.0 mL) subcutaneously or several smaller but gradually increasing doses

intravenously with an interval of 15–30 minutes (fractional administration), followed by a larger remaining dose of serum.

Serum sickness may develop 7–12 days after administration of foreign sera – especially following large doses – even during initial exposure. It results from immune complexes formed by antigens combined with precipitating antibodies settling around small blood vessels, leading to endothelial damage and local thrombosis that impair tissue perfusion.

The prodromal period is characterized by hyperemia and enlarged lymph nodes. Full-blown disease features skin rashes, fever, acute pulmonary emphysema, arthralgia, mucosal edema, albuminuria, leukopenia, and an elevated erythrocyte sedimentation rate (ESR) – all persisting for approximately for 6–7 days. The condition does not induce desensitization of the organism.

TEST TASKS

Select one or more correct answers.

1. THE FIRST WORD IN THE BINOMIAL NAME OF MICROORGANISMS IS THE NAME OF THE:
    1) species
    2) genus
    3) families
    4) class

2. ACCORDING TO THEIR MORPHOLOGICAL PROPERTIES, THE FOLLOWING SPECIES ARE KNOWN AS COCCI:
    1) clostridia
    2) bacilli
    3) sarcina
    4) borrelia

3. THE FOLLOWING BELONG TO SPIROCHETES
    1) vibrios
    2) campylobacter
    3) sarcina
    4) borrelia

4. A CONSTANT COMPONENT OF A BACTERIAL CELL IS THE:
    1) cytoplasmic membrane
    2) capsule
    3) spore
    4) flagella

5. THE CYTOPLASMIC MEMBRANE IS COMPOSED OF:
    1) proteins and lipopolysaccharides
    2) lipopolysaccharides and phospholipids
    3) proteins and phospholipids
    4) proteins and teichoic acids

6. IN A PROKARYOTIC CELL, RIBOSOMES ARE LOCATED IN THE:
    1) endoplasmic reticulum

2) Golgi apparatus
3) cytoplasm
4) cytoplasmic membrane

7. THE MAIN COMPONENTS OF PEPTIDOGLYCAN ARE:
   1) N-acetylglucosamine and N-acetylmuramic acid
   2) N-acetylglucosamine and teichoic acids
   3) N-acetylmuramic acid and teichoic acids
   4) N-acetylglucosamine and lipopolysaccharide

8. ACCORDING TO THE GRAM METHOD, GRAM-NEGATIVE BACTERIA STAIN:
   1) pink
   2) crimson
   3) ruby red
   4) purple

9. STAINING BY THE NEISSER METHOD IS USED TO DETECT:
   1) inclusions
   2) spores
   3) acid-fast bacteria
   4) capsules

10. ACCORDING TO THE ZIEHL-NEELSEN METHOD, ACID-FAST BACTERIA STAIN:
   1) purple
   2) light blue
   3) red
   4) blue

11. THE CELL WALL OF ACID-FAST BACTERIA CONTAINS A LARGE AMOUNT OF:
   1) mycolic acids
   2) teichoic acids
   3) diaminopimelic acid
   4) proteins

12. THE MAIN FUNCTION OF BACTERIAL SPORES IS:
   1) reproduction
   2) movement
   3) preservation under unfavorable conditions
   4) protection from the body’s immune mechanisms

13. THE MAIN METHOD FOR STAINING SPORES IS THE:
    1) Gram method
    2) Ozheshko method
    3) Burri - Gins method
    4) Neisser method

14. THE MAIN METHOD FOR DETECTING BACTERIAL CAPSULES IS THE:
    1) Gram method
    2) Ozheshko method
    3) Burri - Gins method
    4) Neisser method

15. ACTINOMYCETE CELLS ARE CALLED:
    1) sporangia
    2) hyphae
    3) cysts
    4) conidia

16. IN DAMAGED TISSUES, ACTINOMYCETES FORM:
    1) grains
    2) granules
    3) drusen
    4) cysts

17. THE MAIN CONTRACTILE PROTEIN OF BACTERIA IS:
    1) actin
    2) myosin
    3) flagellin
    4) albumin

18. A BACTERIUM WITH A LARGE NUMBER OF FLAGELLA ARRANGED OVER ITS ENTIRE CELL SURFACE IS CALLED A:
    1) monotrichous
    2) lophotrichous
    3) amphitrichous
    4) peritrichous

19. THE GENERAL NAME FOR DISEASES CAUSED BY RICKETTSIA IS:
    1) mycoses
    2) chlamydiosis
    3) rickettsiosis
    4) actinomycosis

20. CHLAMYDIAL MICROCOLONIES IN INFECTED CELLS ARE CALLED:
    1) reticulate bodies
    2) chlamydial inclusions
    3) elementary bodies
    4) Guarnieri bodies

21. FUNGI THAT FORM HYPHAE ARE:
    1) blastomycetes
    2) yeast-like
    3) spore-forming
    4) hyphomycetes

22. FUNGI CAN REPRODUCE:
    1) sexually
    2) asexually
    3) both sexually and asexually
    4) by binary fission

23. THE PHYLUM SARCOMASTIGOPHORA HAS TWO TYPES OF MOTILITY ORGANELLES:
    1) pseudopods and flagella
    2) pseudopods and cilia
    3) fins and cilia
    4) flagella and cilia

24. WHEN STAINING PROTOZOA WITH THE ROMANOWSKY-GIEMSA METHOD, THE NUCLEUS AND FLAGELLA STAIN:
    1) blue
    2) red
    3) purple
    4) brown

25.THE MAIN REQUIREMENT FOR NUTRIENT MEDIA IS:

1) high salt content
2) sterility
3) presence of enzymes
4) presence of lipids

26.PHOTOTROPHS ARE MICROORGANISMS THAT:

1) use light as a source of energy
2) obtain energy through oxidation-reduction reactions
3) feed on inert organic material
4) depend on the nutrients of a macroorganism

27.THE FOLLOWING IS NOT A DIFFERENTIAL DIAGNOSTIC MEDIUM:

1) Meat-Peptone Agar MPA
2) Endo's medium
3) Ploskirev's medium
4) Hiss's medium

28.FOR ISOLATING MICROORGANISMS, IT IS PREFERABLE TO USE:

1) simple media
2) complex media
3) elective media
4) glycerol-containing media

29.AUTOTROPHS ARE BACTERIA THAT:

1) use organic carbon
2) use CO_2 to build cells
3) use light for nutrition
4) use organic compounds as electron donors

30.THE FOLLOWING ARE NOT GROWTH FACTORS

1) amino acids
2) purine and pyrimidine bases
3) vitamins
4) lipids

31.THE TRANSPORT OF SUBSTANCES INTO A BACTERIAL CELL DOES NOT OCCUR VIA:

1) simple diffusion
2) facilitated diffusion

3) active transport
4) endocytosis

32. CULTIVATION OF ANAEROBES TAKES PLACE UNDER CONDITIONS OF:
1) increased blood pressure
2) increased O_2 content
3) increased CO_2 content
4) reduced temperature

33. THE FOLLOWING MEDIUM IS USED FOR CULTIVATING ANAEROBES:
1) Löwenstein-Jensen medium
2) Kitt-Tarozzi medium
3) Endo agar
4) Kligler Iron Agar

34. HE DETERMINATION OF SACCHAROLYTIC ENZYMES IS CARRIED OUT ON:
1) Ru medium
2) Hiss medium
3) Zeissler agar
4) Meat-Peptone Broth (MPB)

35. THE DETERMINATION OF BACTERIAL ENZYMES IS USED FOR:
1) vitamin synthesis
2) hereditary information transfer
3) motility of the microbial cell
4) bacterial identification

36. AMONG PATHOGENIC BACTERIA, THE MOST COMMON ARE:
1) obligate aerobes
2) obligate anaerobes
3) facultative anaerobes
4) microaerophiles

37. ACCORDING TO THEIR CHEMICAL NATURE, BACTERIAL ENZYMES ARE:
1) carbohydrates
2) proteins
3) lipids
4) nucleic acids

38. TRANSFERASES ARE:
    1) carrier enzymes
    2) stitching enzymes
    3) enzymes that perform intramolecular rearrangements in the substrate
    4) pathogenicity enzymes

39. THE COMPLETE DESTRUCTION OF ALL MICROORGANISMS IN A MATERIAL IS CALLED:
    1) disinfection
    2) sterilization
    3) antiseptics
    4) asepsis

40. COLD STERILIZATION IS:
    1) tyndallization
    2) autoclaving
    3) filtration
    4) pasteurization

41. THE SET OF MEASURES AIMED AT REDUCING THE NUMBER OF PATHOGENIC AND OPPORTUNISTIC MICROORGANISMS ON ENVIRONMENTAL SURFACES IS CALLED:
    1) sterilization
    2) asepsis
    3) disinfection
    4) chemotherapy

42. A BACTERIUM EXPOSED TO A BACTERICIDAL ANTIBIOTIC:
    1) stops growing
    2) stops dividing
    3) dies

43. ANTIBIOTICS OBTAINED THROUGH BIOLOGICAL SYNTHESIS ARE CALLED:
    1) natural
    2) synthetic
    3) semi-synthetic

44. THE PRIMARY TARGET FOR ANTIBIOTICS THAT INHIBIT PROTEIN SYNTHESIS IS THE:
    1) cell wall
    2) nucleoid
    3) ribosomes
    4) cytoplasmic membrane

45. MYCOPLASMAS ARE RESISTANT TO PENICILLIN DUE TO THEIR LACK OF A:
    1) cytoplasmic membrane
    2) cell wall
    3) flagella
    4) inclusions

46. A PLASMID THAT CONTROLS ANTIBIOTIC RESISTANCE IS CALLED A:
    1) R-factor
    2) S-factor
    3) J-factor
    4) A-factor

47. THE EXTRACELLULAR FORM OF A VIRUS IS CALLED A:
    1) capsid
    2) prophage
    3) virion
    4) elementary body

48. THE SIZE OF A VIRUS PARTICLE IS MEASURED IN:
    1) µm
    2) nm
    3) kDa
    4) cm

49. VIRUSES BELONG TO THE KINGDOM:
    1) Fungi
    2) Animalia
    3) Protista
    4) Vira

50. BACTERIOPHAGES ARE:
    1) animal viruses
    2) human viruses
    3) plant viruses
    4) bacterial viruses

51. A VIRAL NUCLEIC ACID TOGETHER WITH A CAPSID IS CALLED A:
    1) supercapsid
    2) peplos
    3) nucleocapsid
    4) capsomere

52. THE BEST BIOLOGICAL MODEL FOR CULTIVATING VIRUSES IS CULTIVATION IN:
    1) laboratory animals
    2) chicken embryos
    3) organs
    4) cell culture

53. AGGREGATES OF VIRUS PARTICLES OR INDIVIDUAL VIRUS COMPONENTS IN THE CYTOPLASM OR NUCLEUS OF CELLS, VISIBLE UNDER A MICROSCOPE WITH SPECIAL STAINING, ARE CALLED:
    1) viral granules
    2) viral particles
    3) viral inclusions
    4) viral plaques

54. THE INTERACTION OF A TEMPERATE PHAGE WITH A BACTERIAL CELL IS CALLED:
    1) phage conversion
    2) lysogeny
    3) adsorption
    4) bacteriophagy

55. THE TOTAL BACTERIAL COUNT OF SOIL IS THE:
    1) total number of microorganisms contained in 1 g of soil
    2) minimum number of microorganisms contained in 1 g of soil
    3) total number of microorganisms contained in 1 kg of soil
    4) number of viable E. coli in 1 g of soil

56. THE COLI-INDEX OF SOIL IS THE:
    1) total number of microorganisms contained in 1 g of soil
    2) minimum number of microorganisms contained in 1 g of soil
    3) total number of microorganisms contained in 1 kg of soil
    4) number of viable E. coli in 1 g of soil

57. TO ASSESS THE SANITARY AND BACTERIOLOGICAL CONDITION OF WATER, THE FOLLOWING IS USED:
    1) total microbial count
    2) coli-titer
    3) perfringens-titer
    4) all of the above indicators

58. THE METHOD OF AIR SAMPLING BASED ON THE SETTLING OF BACTERIAL PARTICLES AND DROPLETS UNDER THE INFLUENCE OF GRAVITY IS CALLED THE:
    1) aspiration method
    2) sedimentation method
    3) titration method
    4) membrane filter method

59. MICROFLORA THAT IS MAXIMALLY ADAPTED TO EXIST IN THE HUMAN BODY IS CALLED:
    1) obligate
    2) transient
    3) facultative
    4) there is no correct answer

60. A DISRUPTION IN THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE MICROFLORA OF THE HUMAN BODY IS CALLED:

1) biocenosis
2) dysbiosis
3) anabiosis
4) enterobiasis

61. THE POTENTIAL ABILITY TO CAUSE AN INFECTIOUS PROCESS IS CALLED:
1) an infectious disease
2) pathogenicity
3) virulence
4) exogenous infection

62. A FORM OF INFECTION IN WHICH THE PATHOGEN IS PRESENT IN THE MACROORGANISM AND SHED IN THE ABSENCE OF CLINICAL SYMPTOMS IN THE PATIENT IS CALLED:
1) carrier state
2) mixed infection
3) sepsis
4) localized infection

63. THE PATHOGENICITY ENZYME THAT PROVIDES BACTERIA WITH THE ABILITY TO INVADE UNDERLYING TISSUES IS:
1) hyaluronidase
2) hemolysin
3) interferon
4) DNase

64. EXOTOXINS ARE, BY THEIR CHEMICAL NATURE:
1) proteins
2) carbohydrates
3) lipids
4) polysaccharides

65. A CELLULAR FACTOR OF INNATE IMMUNITY IS:
1) lysozyme
2) complement
3) phagocytosis
4) interferon

66. A HUMORAL FACTOR OF INNATE IMMUNITY IS:
    1) complement
    2) phagocytosis
    3) inflammatory response
    4) NK cells

67. THE ACTIVATION OF COMPLEMENT VIA THE CLASSICAL PATHWAY IS INDUCED BY A:
    1) antigen
    2) antibody
    3) microorganism
    4) antigen-antibody complex

68. THE SEROLOGICAL REACTIONS THAT USE A LABEL ARE:
    1) ELISA, IFT
    2) RPHA, RLA
    3) IFT, RA
    4) RP

69. THE MICROSCOPE USED FOR THE IMMUNOFLUORESCENCE ASSAY IS A:
    1) light microscope
    2) dark-field microscope
    3) phase-contrast microscope
    4) fluorescence microscope

70. WHEN PERFORMING AN INDIRECT IMMUNOFLUORESCENCE ASSAY, THE DYE LABELS:
    1) the antigen
    2) the specific antibodies
    3) anti-globulin antibodies
    4) both antigens and antibodies

71. THE STRIPS USED FOR THE IMMUNOBLOT ASSAY TO DIAGNOSE HIV CONTAIN:
    1) pathogen antigens
    2) specific antibodies
    3) anti-globulins
    4) immunoglobulins of class M

72. IN THE PAIRED SERUM METHOD, A DIAGNOSTICALLY SIGNIFICANT INCREASE IN ANTIBODY TITER IS:
    1) a 2-fold increase
    2) a 3-fold increase
    3) a 4-fold increase
    4) a 5-fold increase

73. IN AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TO DETECT ANTIBODIES IN BLOOD SERUM, THE BOTTOM OF THE PLASTIC MICROPLATE WELL IS COATED WITH:
    1) antigen
    2) antibodies
    3) immunoglobulins of class A
    4) anti-globulins

74. A CENTRAL ORGAN OF THE IMMUNE SYSTEM IS THE:
    1) lymph nodes
    2) spleen
    3) thymus
    4) tonsils

75. CD MARKERS ARE:
    1) cytotoxic enzymes
    2) marker molecules of immune cells
    3) markers of tumor cells
    4) markers of antibodies

76. HLA CLASS I ANTIGENS ARE FOUND ON THE SURFACE OF:
    1) only erythrocytes
    2) all body cells
    3) only antigen-presenting cells
    4) all body cells except erythrocytes

77. HLA CLASS II ANTIGENS ARE FOUND ON THE SURFACE OF:
    1) only erythrocytes
    2) all body cells
    3) only antigen-presenting cells
    4) all body cells except erythrocytes

78. THE SYNTHESIS OF IMMUNOGLOBULINS IS THE FUNCTION OF:
    1) neutrophils
    2) B-lymphocytes

3) T-lymphocytes
4) Macrophages

79. THE MAIN CELL THAT REGULATES THE IMMUNE RESPONSE IS THE:
1) B-lymphocyte
2) T helper cell
3) T killer cell
4) Macrophage

80. THE MAIN CYTOKINES OF THE HUMORAL IMMUNE RESPONSE ARE:
1) C3a, C3b
2) histamine, serotonin
3) IL-4, IL-6
4) IL-12, IFNγ

81. IMMUNOGLOBULIN E PLAYS A KEY ROLE IN:
1) anaphylactic allergic reactions
2) cytotoxic allergic reactions
3) immune complex allergic reactions
4) T-cell allergic reactions

82. ANATOXINS ARE USED FOR THE PROPHYLAXIS OF:
1) viral infections
2) toxinemic (toxic, toxigenic) infections
3) bacterial infections
4) mixed infections

83. A MICROORGANISM STRAIN WITH REDUCED VIRULENCE THAT IS USED TO PREPARE A LIVE VACCINE IS CALLED:
1) attenuated
2) divergent
3) recombinant
4) inactivated

84. TO CREATE ACQUIRED ARTIFICIAL ACTIVE IMMUNITY IN HUMANS, THE FOLLOWING ARE USED:
1) immunoglobulins
2) vaccines
3) allergens
4) bacteriophages

85. ADJUVANTS ARE INCLUDED IN VACCINES TO:
1) increase shelf life
2) increase immunogenicity
3) reduce reactogenicity
4) preserve

86. INFECTIOUS ALLERGENS ARE USED FOR:
1) treatment of infectious diseases
2) prevention of infectious diseases
3) diagnosis of infectious diseases
4) detection of atopic allergies

87. A DIAGNOSTIC REAGENT IS USED FOR:
1) detecting antibodies in blood serum
2) detecting the pathogen
3) identifying the pathogen
4) detecting the pathogen's nucleic acids

88. PROBIOTICS ARE USED FOR:
1) treating viral infections
2) correcting dysbiosis
3) diagnosing infectious diseases
4) preventing infectious diseases

ANSWERS TO TEST TASKS

Question No.	Answer	Question No.	Answer	Question No.	Answer
1.	2	34.	2	67.	4
2.	3	35.	4	68.	1
3.	4	36.	3	69.	4
4.	1	37.	2	70.	3
5.	3	38.	1	71.	1
6.	3	39.	2	72.	3
7.	1	40.	3	73.	1
8.	1	41.	3	74.	3
9.	1	42.	3	75.	2
10.	3	43.	1	76.	4
11.	1	44.	3	77.	3
12.	3	45.	2	78.	2
13.	2	46.	1	79.	2
14.	3	47.	3	80.	3
15.	2	48.	2	81.	1
16.	3	49.	4	82.	2
17.	3	50.	4	83.	1
18.	4	51.	3	84.	2
19.	3	52.	4	85.	2
20.	2	53.	3	86.	3
21.	4	54.	2	87.	1
22.	3	55.	1	88.	2
23.	1	56.	4		
24.	2	57.	4		
25.	2	58.	2		
26.	1	59.	1		
27.	1	60.	2		
28.	3	61.	2		
29.	2	62.	1		
30.	4	63.	1		
31.	4	64.	1		
32.	3	65.	3		
33.	2	66.	1		

RECOMMENDED READING

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