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Medical Microbiology, Virology and Immunology

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AIMS AND PROBLEMS, SHORT HISTORICAL OUTLINE ON THE DEVELOPMENT OF MICROBIOLOGY

Microbiology (GK-micros-small, bios-life, logos-science) - is the science studying minute organisms, invisible to the naked eye, named microbes. Microbiology studies the laws of the life and development of microorganisms and also the changes which they bring about in animal and plant organisms and in non-living matter.

The microbes are found everywhere; they are on all the subjects around us. And these microbes are subdivided into:

- Pathogenic – causative agents of infectious diseases
- Conditionally pathogenic – this can become pathogenic according to the condition.
- Non-pathogenic – saprophytes, which are in nature and participate in the circulation of the substances (matters).

According to the requirements of society MB divided into:

- general
- agricultural
- veterinary
- sanitary
- medical

We should study medical microbiology. Modern medical microbiology has become an extensive science. It studied the microorganisms - bacteria, viruses, fungi, which are pathogenic for the human organism.

Medical microbiology is subdivided into – **bacteriology** – the science of bacteria, the causative agents of a number of infectious diseases; **virology –** the science of viruses – no cellular living systems, capable of causing infectious diseases in man; **immunology** – the science which is concerned with the mechanisms of body protection against pathogenic microorganisms and foreign cells and substances; **mycology –** the study of fungi, pathogenic for man; **protozoology** – which deals with pathogenic, unicellular animal organisms.

Each of these disciplines studies the following problems (items):

1. Morphology and physiology which includes microscopic and other kinds of research. It also studies metabolism, nutrition, respiration, growth and reproduction, genetics of pathogenic microorganisms.

2. The role of microorganisms in the origin and development of the infectious processes.

3. The main clinical manifestation and widespreadness of infectious diseases.

4. Specific diagnoses, prophylactic and treatment of infectious diseases.

5. Ecology.

Besides, medical microbiology involves the study of the mechanisms of infection and the methods of specific therapy and prophylaxis of infectious diseases. Microbiology as a separate science has special methods of investigation which help to solve a number of important problems regarding public health. These methods are extensively used in the theoretical and clinical medical sciences.

Second half of the 20^{th} century was marked by great discoveries in the field of natural science. The using of isotopes, chromatography, spectroscopy, phase contrast, luminescent and electron microscopy and modern methods in genetics greatly speeded up the development of medical microbiology and virology and they permitted more detailed investigation into the life of microbes in order to reveal the unknown mechanisms of their relation to the environment.

Long before the discovery of microorganisms certain processes caused by their life activities, such as fermentation of wine, juice, milk, yeast etc.; were known to mankind. In ancient times at the beginning of civilization, by using these processes man

learned to prepare koumiss, sour milk and other products. But mankind couldn't give the scientific explanation before the discovery of microorganisms and development of MB.

H I S T O R Y

In the early stages of the science development physicians and naturalists tried to learn the causes of infectious diseases. In the works of Hippocrates (460-375B.C.), Veron (second century B.C.), Galen (130-200 A.D.) and other great scientists of that time the hypothesis of the living nature of contagious diseases was stated.

The people of Asia had certain ideas on the contagiousness of some diseases and they isolated those diseased people.

Avicenna (980-1037) thought that all infectious diseases were caused by minute living creatures, invisible to the naked eye and transmitted through air and water. There were ideas about these invisible creatures but they couldn`t see and describe or show them. The first person who saw and described microbes was Dutch microscopist **Antony Van Leeuwenhoek** (1632-1723). He himself made simple lenses which magnified 160-300-fold and observed water, various infusions, feces and teeth scribes. He discovered microbes, made drawing of them. In 1695 he published his scientific work “The secrets of Nature” discovered by A. V. Leeuwenhoek. He not only discovered the microbes, but also made accurate drawings of them and he described the main shapes of microbes: cocci, rods, spiral shape. This terminology is used nowadays. The discovery by Leeuwenhoek stimulated vivid interest among scientists, and these became the starting point for studying of micro world. Scientists discovered many microbes and described their morphological features. This is the **morphological stage** of development of microbiology.

Edward Jenner, (17 May 1749 – 26 January 1823) was an English physician and scientist who was the pioneer of smallpox vaccine, the world's **first vaccine**. The terms "vaccine" and "vaccination" are derived from *Variolae vaccinae* (smallpox of the cow), the term devised by Jenner to denote cowpox. He used it in 1796 in the long title of his *Inquiry into the Variolae vaccinae known as the Cow Pox*, in which he described the protective effect of cowpox against smallpox.

In the second half of the 19th century more advanced microscopes appear which improved the methods of microscopy. During the study of microorganisms attention was paid to the biochemical processes - the ability of microbes to ferment organic substances. This was the **physiological stage** (Pasteur’s stage) of microbiology, which associated with the great French scientist chemist and **microbiologist Louis Pasteur** (1822-1894) whose name was linked with the most important discoveries in the field of microbiology. His works lie in the basis of medical microbiology development. He found out the microbes which cause diseases of wine and beer. He proved the microbial nature of alcoholic, lactic acid and butyric acid fermentations. He demonstrated a new anaerobic type of respiration in some microbes. He discovered the putrefaction caused by the activity of certain species of microorganisms. Pasteur’s great investigations are vaccines of chicken cholera, anthrax, and rabies. The next great investigation is the rejection of spontaneous germination theory.

Thus the great investigations of Pasture are:

1. protective inoculations /vaccines/ against **chicken cholera, anthrax, rabies.**
2. anaerobic type of respiration /anaerobiosis/.
3. pasteurization.
4. putrefaction.
5. fermentation(established that fermentation was the result of microbial activity
6. rejection of spontaneous germination theory

L. Pasture is the **founder of scientific microbiology.**

The most eminent scientists were pupils and followers of Pasture.

An immediate application of Pasteur's work was the introduction of **antiseptic techniques** in surgery by **Joseph Lister**(1867). He revolutionized surgery by developing effective methods that prevent surgical wounds from becoming infected.

The next great scientist is German scientist **R. Koch (1843-1910)**. He tried to establish the role of microorganisms in etiology of infectious diseases by setting specific criteria. It is known in the history as the **Henle-Koch triad** according to which:

1.The bacterium should be constantly associated with the lesions of the disease. It should be possible to isolate the bacterium in pure culture from the lesion
2.Inoculation of such pure culture into suitable laboratory animals should reproduce lesions of the disease
3.It should be possible to re-isolate the bacterium in pure culture from the lesions produced in experimental animals

He and his pupils introduced **solid nutrient media** (meat-peptone agar, potatoes, gelatin and etc.); **aniline dyes,** the **immersion system of microscopy, microphotography.** Besides, Koch established the **etiology of anthrax** (1876), discovered the **causative agents of tuberculosis (1882), cholera (1883),** and **obtained tuberculin** from tubercle bacilli. Koch made a detailed investigation of wound infections and worked out a **method for pathogenic bacteria pure culture isolation in solid nutrient media**. He developed a large school of microbiology and E. Klebs, F. Loeffler, S. Kitasato and many others were among his pupils. R. Koch is the **founder of disinfection.** Lister is the **founder of antisepsis.**

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Next outstanding /remarkable/ scientist was **E. Metchnikoff (1845-1916), the founder of cellular theory of immunity.** It was a new stage in the development of medicine. The study of **phagocytosis** became the basis for the understanding of the processes of inflammation. Metchnikoff showed that inflammation is an active reaction against pathogenic microbes. He organized the first Russian bacteriological station in Odessa.

Metchnikoff's studies of the phagocytosis problem was the starting point for the appearance of a number of works, which demonstrated that in the defense reactions of the body an important role is played by certain substances in the blood, serum, especially secreted under the influence of microbes and their toxins.

At the same time the German scientist **P. Erlich (1854-1915) created the theory of humoral immunity,** which promotes the difference of opinions dividing the scientists into two schools; supporters of Erlich and his opponents headed by I. Metchnikoff. Due to widespread discussions, it was established that insusceptibility to infectious diseases depends on cellular as well as humoral immunity. In 1908 I. Metchnikoff and P. Erlich were awarded the Nobel Prize for elaborating the science of immunity. Erlich was the founder **of chemotherapy.**

In the second half of 19th century (the golden era of Microbiology) and in the 20th century very important investigations were made in the field of prophylaxis of infectious diseases. And the scientists who discovered the causative agents of a number of infectious diseases have made a great contribution to the advancement of medical

microbiology. The Russian scientists took part in these advancement and they laid the foundations to study a new field in biology, especially in the studies of viruses carried out by **D. Ivanovsky (1864-1920).** In 1892 while working on the problem of tobacco mosaic disease causing great damage to tobacco plantations, D. Ivanovsky revealed that this disease is produced by a virus which has a high virulence and a strictly selective activity. D. Ivanovsky showed that these organisms passed through filters of small pore size and lacks a cellular structure. Due to this discovery the causative agents of many **viral infections** of man, animals, plants were discovered and studied. **This period is known immunological and development of virology**.

Many eminent scientists contributed to the development of medical microbiology, virology and immunology. Due to development of science and technology, development of genetics, gene engineering new era of microbiology is developed. This period is **modern stage** of microbiology.

The stages of development of microbiology are:

- **morphological stage** (XVII-XIX century)
- **physiological stage** -Pasteur's stage (XIX-XX century)
- **immunological and development of virology** (XX century)
- **modern –molecular-genetic stage** (XX-XXI century)

M O R P H O L O G Y A N D B A C T E R A L T A X O N O M Y

During the development of microbiology scientists discovered great majority of microbes and it becomes necessary to classify microorganisms. Bacterial taxonomy or systematic comprise three components:

- Classification or the orderly arrangement of units. A group of units is called a **taxon**, irrespective of its hierarchic level.
- Identification of an unknown with a defined and named unit.
- Nomenclature, or the naming of units

The classification of Bergey was accepted in 1974. Contemporary classification of microorganisms is based on phenotypic properties: morphological, physiological, biochemical. Morphology is characterized by shape and structure of microbial cell; physiological properties are characterized by peculiarities of growth on artificial nutrient media, morphology of colonies on solid nutrient media and character of growth on liquid media; biochemical properties are based on type of metabolism, fermentation of carbohydrates, proteolytic activity and others. According to this systemization the highest taxonomic categories are **Empire** and **Kingdom.**

The microbial world, in fact all living organisms, can be classified into one of three major groups called **domains.** Organisms in each domain share properties of their cells that distinguish them from members of the other domains and the three domains may have a common ancestor. The three domains are the **Bacteria** (formerly called Eubacteria), **the Archaea** (meaning ancient) and the **Eukarya.** Microscopically, members of the ***Bacteria*** and ***Archaea*** look identical. Both are single- celled organisms that do not contain a membrane- bound nucleus nor any other intracellular lipid –bound organelles. Their genetic information is stored in deoxyribonucleic acid (DNA) in a region called the **nucleoid.** This simple cell types have their cytoplasm surrounded by a rigid cell wall and are termed **prokaryotes,** which means **«prenucleus». All bacteria and archaea are prokaryotes**. Although members of these two domains are prokaryotes because of their structural similarities, they differ in their chemical composition and are unrelated. Members of the Eukarya, termed eukaryotes, which means **«true nucleus»,** are distinctly different from members of the *Bacteria* and *Archaea.*

A kingdom is divided successively into following taxonomic categories: **division, class**, **order, family**, **genus and species.** An important difference between the classification of bacteria and that of other organisms is that in the former, the properties of a population are studied, and not of an individual. **Species** is the lowest taxonomic category. Species is the standard taxonomical unit in biology. A bacterial species is defined simply as a population of cells with similar characteristics. The members of bacterial species are essentially indistinguishable from each other, but are distinguishable from the members of other species, usually on the basis of several features.

Binominal System of Nomenclature is accepted in the classification: The first word in the name is the **genus; the** second is the **species.**

BINOMINAL NOMENCLATURE

Staphylococcus aureus **Escherichia coli**
Salmonella typhi **Vibrio cholera**

Genus may indicate the form (morphological feature) of bacteria or indicate the name of the discoverer of the microorganism. For example: ***Staphylococcus aureus-*** *Staphylococcus* showed that this bacterium has a form of grapes cluster. Genus may show the discoverer of bacteria – e.g., **Escherichia coli** – Escherichia- the discoverer of this bacterium is Escherich. Genus is written in capital letter.

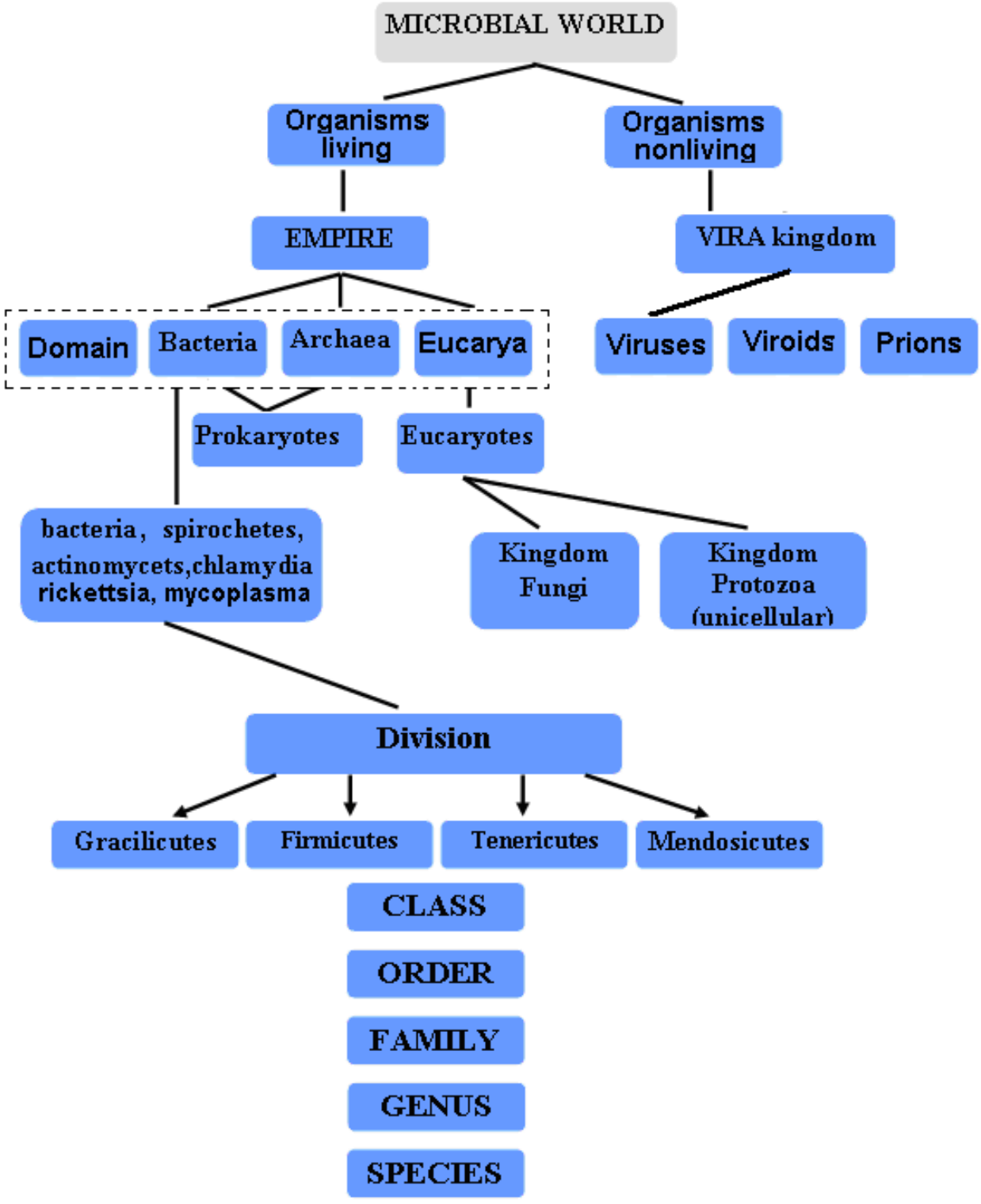
Species indicates the source origin - e.g. *E. coli – coli* shows that this bacterium is obtained from the intestine, or it indicates the name of diseases. For example: V. cholera - cholera is the name of disease. The species is written in small letter.

S**pecies -** is a distinct group of organisms that have certain distinguishing features and generally bear a close resemblance to one another in the more essential features of organization**.**

Clone – a population derived by binary fission from single cell **(**is the progeny of a single cell - unicellular culture, genetically identical cells).

Strain - is the culture of microorganisms discharged from the certain sources (or from the same sources but during different time).

CLASSIFICATION OF MICROORGANISMS



T**able: Comparative characteristics of Prokaryotic and Eukaryotic cells**

Characteristic	Prokaryotic cells	Eukaryotic cell
1. DNA within a nuclear membrane	No	Yes
2. Mitotic division	No	Yes
3. Multiplication by binary fission	Yes	No
4. DNA associated with histones	No	Yes
5. Chromosome number	One (haploid)	More than one
6. Membrane – bound organelles such as mitochondria and lysosomes	No	Yes
7. Size of ribosome	70s	80s
8. Cell wall containing peptidoglycan	Yes	No

THE MAIN FORMS OF BACTERIA

The main forms of bacteria: Morphologically bacteria possess three main forms:

1.Spherical (cocci).

2.Rod-shaped (bacteria bacilli and clostridia).

3.Spiral shaped (vibrios and spirilla/.

Cocci – (GK-coccus-berry) - cocci are subdivided into six groups according to plan of cell division, cell arrangement; and biological properties.

1. Micrococci (micrococcus) - divide in one plane. The cells are arranged singly or irregularly. They are saprophytes and live in water and in air.

2. Diplococci (GK-diplos-double) - divide in one plane and remain attached in pairs. These include: meningococcus, causative agent of epidemic cerebrospinal meningitis and gonococcus causative agents of gonorrhoea and blennorrhoea. These cocci resemble coffee bean. Another diplococcus is pneumococcus – the causative agent of pneumonia. This diplococcus looks like a lancet.

3. Streptococci (GK–Streptos-chain) divide in one plane and are arranged in chains of different length. They are probably responsible for a number of illnesses and cause a greater variety of diseases than any other group of bacteria. Scarlet fever, pharyngitis (sore throat) and others are among the diseases caused by streptococci.

4. Tetracocci (GK–tetra–four) divide in two planes and form groups of fours. They are saprophytes.

5. Sarcinae (GK-Cartio-to tie) - divide in three planes and resemble packets of 8, 16 or more cells. They are frequently found in the air. These are conditionally pathogenic organisms.

6. Staphylococci (GK–Staphyle–cluster of grapes) - divide in several planes resulting in irregular bunch of cells, sometimes resembling clusters of grapes. The most important staphylococcal species is S. aureus, named for its yellow pigmented colonies (aureus means golden), causative agents of diseases in man and animals. S. aureus produced many toxins that contribute to the bacterium's pathogenicity by increasing its ability to invade the body or damage tissue.

Rods - rod-shaped or cylindrical forms are subdivided into **bacteria** (include those micro-organisms which as a rule do not produce spores – e.g. organisms responsible for enteric fever-paratyphoids, dysentery, diphtheria, etc.) **bacilli and clostridia (spindle form)** include organisms the majority of which produce spores (e.g. bacilli responsible for anthrax, clostridia - tetanus, and etc.). Bacillus (B. anthracis) is rod shape spore forming aerobic microorganism. Clostridium (C. tetani, C. botulinum) are rod shape, spore forming, anaerobic microorganisms. Rods exhibit **differences** in the form of ends, in shape and size as well as in arrangement. By the form of end they may be rounded, pointed, thickened, truncated, etc.. By size they may be small (0.5-1.5μm) middle (2-5μm) and large (6-10μm). By their arrangement they may be irregular arranged, in pairs, in chains.

Spiral shaped bacteria – *Vibrions, Spirilla and Spirochetes* belong to this group of bacteria. Spirills are coiled forms of bacteria exhibiting twists with one or more turns. Only one pathogenic species

is known (Spirillum minus) which is responsible for a disease in humans, transmitted through the bite of rats and other rodents (Sodoku).

MORPHOLOGY, ULTRASTRUCTURE AND FUNCTIONS OF BACTERIA

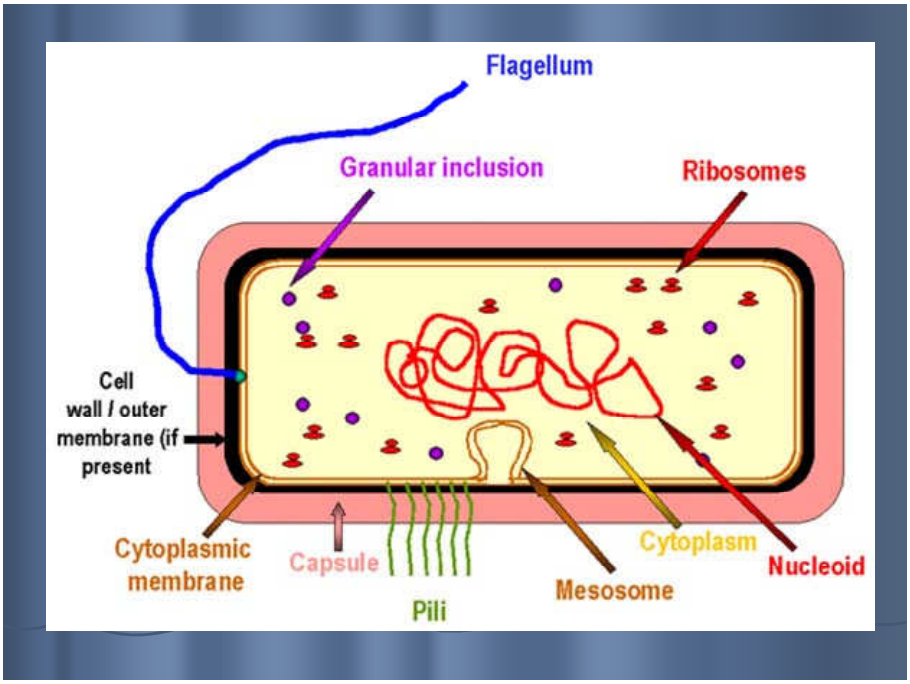
Bacteria differ essentially from plant and animal cells in structure.
They consist of the main and supplementary (additional) structures:
The main structures are:

- cell membrane
- cytoplasm
- nucleoid

The supplementary (additional) structures are:

- flagella
- pili (fimbriae)
- Spores

MORPHOLOGY AND ULTRASTRUCTURE OF BACTERIA



The cell membrane consists of three components:

1. the external layer – glycocalyx which may form a capsule
2. the middle layer – cell wall
3. the internal layer – cytoplasmic membrane

CAPSULE(the external layer) - glycocalyx is a viscous (sticky), gelatinous polymer that is external to the cell wall and is composed of polysaccharide, polypeptide, or both. Its chemical composition varies widely with the species. This external layer can take the form of **capsule** in some bacteria. Capsule usually forms in human or animal organisms (B. anthracis, S. pneumonia, etc.), but some bacteria can form capsule in nutrient media too (eg. K. pneumonia, K. ozenae, K. rhinoscleromatis), which called **constantly** capsule forming bacteria (capsular). The capsule is composed of: **polysaccharides** (Streptococcus pneumonia, Clostridium perfringens); **polypeptides** (Bacillus anthracis, Yersinia pestis).

Capsule forming bacteria are:

1.Streptococcus pneumonia
2.Clostridium perfringens
3.Klebsiella pneumonia
4.Klebsiella ozenae
5.Klebsiella rhinoscleromatis
6.Neisseria. meningitis

7.Bacillus anthracis
8.Yersinia pestis

The functions of capsule are:

1.Protective: protection of microorganism from biological, physical, chemical agents (phagocytosis, antibodies, antibacterial drugs, drying.)

2.Virulence: it is determinant of virulence of many bacteria, since it limits the ability of phagocytes to engulf the bacteria. Loss of the capsule may render the bacterium avirulent.

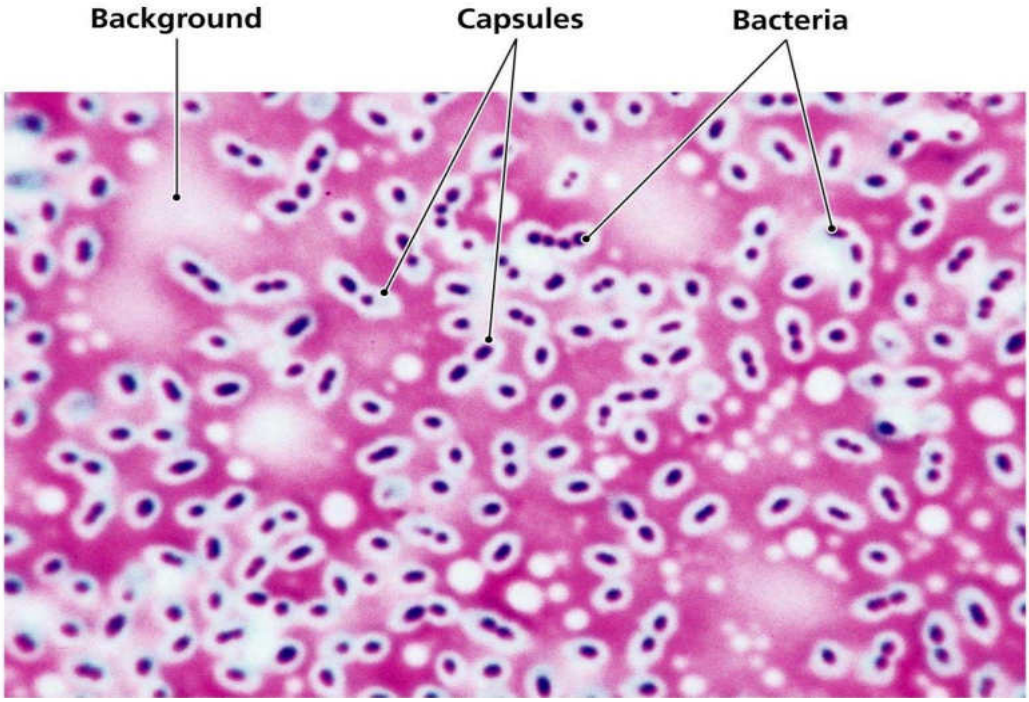
3.Antigenicity: capsular polysaccharides and polypeptides ensure the antigenicity.

4.Adhesive: The capsule may play a role in the adherence of bacteria to human tissues, which is an important initial step in causing infection.

Capsule cannot be stained with ordinary stains like gram staining.

It can be visualized by **Ionne, Burry-Hins** staining methods and it may also be visualized by reaction with specific antibody(capsular material is antigenic and may be demonstrated by serological methods) which causes a characteristic swelling of the capsule. It is known as **Quellung reaction.** This phenomenon is seen in and allows rapid identification of capsular serotypes of Streptococcus pneumoniae, Neisseria meningitidis and etc.

Capsule:



CELL WALL (the middle layer) - the bacterial cell wall is composed of a macromolecular network called **peptidoglycan** (murein) which consists of N-acetyl glucosamine (NAG) and N-acetylmuramic acid (NAM) which are combined with glycosyle bonds. NAM includes tetrapeptide side chains, which consist of four amino acids: D-alanine, L-alanin, D-glutamine and diaminopimelic acid which is found only in bacteria cell. These tetrapeptides are combined with peptide bonds.

Since peptidoglycan is present in bacteria but not in human cells, it is a good target for antibacterial drugs. Several of these drugs, such as penicillins and cephalosporins, inhibited synthesis of cell wall.

The enzyme lysozyme, which is present in human tears, mucus and saliva, can cleave the peptidoglycan backbone by breaking its **β-glycosyle bonds**, thereby contributing to the natural resistance of the host to microbial infection.

The structure, chemical composition and thickness of the cell wall differ in gram-positive and gram-negative bacteria. In most gram-positive bacteria, the cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure (by contrast, gram-negative cell walls contain only a thin layer of peptidoglycan). It constitutes 50-90% of the dry weight of the cell wall. The cell wall of gram-positive bacteria contains ***teichoic acid*,** which is ribitol or glycerol polymers. Two types of teichoic acid are present: **cell**

wall teichoic acid, which is linked to peptidoglycan; and **membrane** teichoic acid (lipoteichoic acid)-linked to membrane glycolipid. Teichoic acid provides, much of the wall's, antigenic specificity and thus make it possible to identify bacteria by serological means.

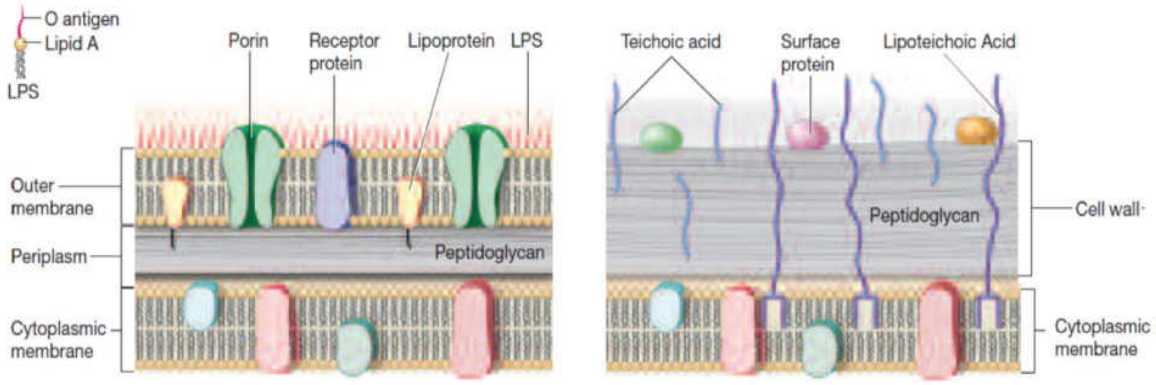
The cell wall of gram-negative bacteria is thinner than that of gram- positive bacteria but is structurally more complex. It consists of one or a very few layers of peptidoglycan and an outer membrane. The peptidoglycan is bonded to lipoproteins (lipids covalently linked to proteins) in the outer membrane and is *periplasmic space*, a space between the outer membrane and the plasma membrane. P*eriplasmic space* contains the murein layer and a gel – like solution of proteins. The periplasmic space contains a high concentration of hydrolytic enzymes and transport proteins. Gram-negative cell wall does not contain teichoic acid. Because the cell wall of gram-negative bacteria contains only a small amount of peptidoglycan, they are more susceptible to mechanical breakage.

The outer membrane of gram-negative cell consists of lipoproteins, lipopolysaccharides (LPS), and phospholipids. The outer membrane has several specialized functions. Its strong negative charge is an important factor in evading phagocytosis and the action of complement, two components of the defences of the host. The outer membrane also provides a barrier to digestive enzymes such as lysozyme, detergents, heavy metals, bile salts, dyes and certain antibiotics (e.g. penicillin), because this bacteria consist enzymes called beta-lactamases that degrade penicillins and other beta-lactam drugs. The lipopolysaccharide component of gram-negative bacteria provides two important characteristics: first, the **polysaccharide** is an antigen and is useful for distinguishing species of gram-negative bacteria; second, the **lipid portion (lipid A)** of the lipopolysaccharide is ***endotoxin*** and is toxic when in the host's bloodstream or gastrointestinal tract. It causes fever and shock.

Functions of the cell wall:

1.determines the *shape* of bacteria
2.has *protective* meaning
3.ensures the *tinctorial* properties (staining by Gram method)
4.ensures *virulence*
5.contains *receptors* for bacteriophages, bacteriocines and different chemical structures (ensures attachment of bacteriophage to the host cells)
6.provides *antigenicity* (somatic O antigen)
7.has *taxonomic* meaning
8.participates in *metabolism*

Gram-positive bacteria stained **blue-violet** and gram-negative bacteria stained **red-pink** by gram staining method.



Cell Wall of Gram Negative Organism Cell Wall of Gram Positive Organism

Removal of the bacterial cell wall may be accomplished by hydrolysis with lysozyme or by blocking peptidoglycan biosynthesis with an antibiotic such as penicillin. In osmotically protective media, such treatments liberate **protoplasts** from gram positive cells and **spheroplasts** (which retain outer membrane and entrapped peptidoglycan) from gram-negative cells. If such cells are able to grow and divide, they are called **L forms.** Some L forms can revert to the normal bacillary form upon removal of the inducing stimulus. Thus, they are able to resume normal cell wall synthesis. Others, however, are stable and never

revert (Mycoplasma). Some bacterial species produce L forms spontaneously. The spontaneous or antibiotic-induced formation of L forms in the host may produce chronic infections, the organisms persisting by becoming sequestered in protective regions of the body. Since L-form infections are relatively resistant to antibiotic treatment, they present special problems in chemotherapy. Their reversion to the bacillary form can produce relapses of the overt infection.

Plasma (Cytoplasmic) membrane – or inner membrane is a thin structure lying inside the cell wall and enclosing the cytoplasm of the cell. Electron microscopy shows the presence of three layers: bilayer phospholipids and the protein layer. It acts as a semipermeable membrane controlling the inflow and out flow of metabolites to and from the protoplasm. It permits the passive diffusion inward and outward of water and other small molecular substances, but it actively affects the selective transport of specific nutrients into the cell and that of waste products out of it.

The plasma membrane has following **functions**:

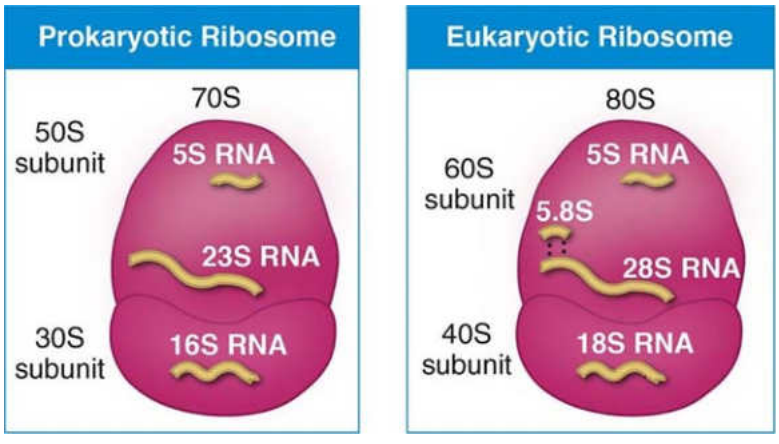
1. active transport of molecules into the cell
2. energy generation by oxidative phosphorylation
3. synthesis of precursors of the cell wall
4. secretion of enzymes and toxins
5. protects against osmotic pressure
6. participates in spore-forming
7. participates in cell division

Mesosome is the invagination of cytoplasmic membrane into the cytoplasm. Mesosome is important during cell division. They are the principal sites of respiratory enzymes in bacteria and are analogous to mitochondria of eukaryotes and are more prominent in gram – positive bacteria. They participate in sporulation.

Cytoplasm is about 80% water and contains primarily proteins (enzymes), carbohydrates, lipids; inorganic ions are present in cytoplasm. Cytoplasm is thick, aqueous, semitransparent and elastic. The major structures in the cytoplasm are DNA, particles called ribosomes, and reserve deposits called inclusions, plasmids.

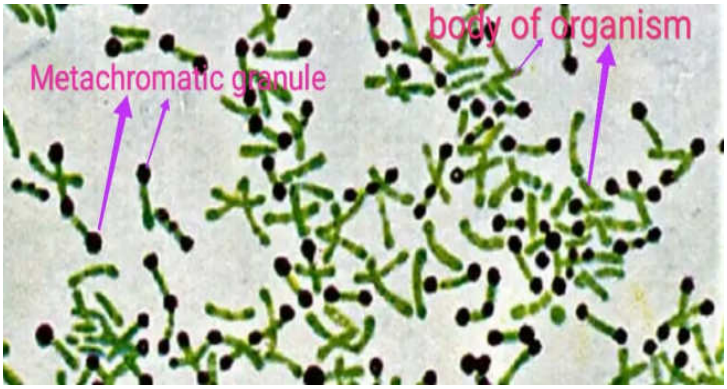
Ribosomes - all eukaryotic and prokaryotic cells contain ribosomes (60% RNA and 40% proteins), which function as the sites of protein synthesis. But prokaryotic cells differ from eukaryotic ribosomes in size and chemical composition. Bacterial ribosomes are 70S in size (with 50S and 30S subunits), whereas eukaryotic ribosomes are 80S in size (with 60S and 40S subunits). The letter S refers to Svedberg units, which indicate the relative rate of sedimentation during ultra-high-speed centrifugation. Sedimentation rate is a function of the size, weight, and shape of a particle. Several antibiotics work by inhibiting protein synthesis on the ribosomes. Each cell contains thousands of ribosomes strung together on strands of messenger RNA (mRNA) to form polysomes and it is at this site that code of mRNA is translated into peptide sequences. Several antibiotics, such as neomycin, streptomycin, work by inhibiting protein synthesis on the ribosomes. Because of differences in prokaryotic and eukaryotic ribosomes, the microbial cell can be killed by the antibiotic while the eukaryotic host cell remains unaffected.

Ribosome:



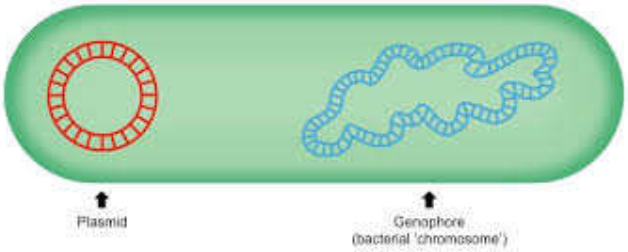
Inclusions – the cytoplasm contains several different types of granules (inclusions) that serve as storage areas for nutrients. Some inclusions are common to a wide variety of bacteria (e.g. polysaccharide granules-glycogen, lipid inclusions and etc.), whereas others are limited to a small number of species and therefore serve as a basis for identification. E.g. **volutin granules** (metachromatic granules, Babesh – Ernst granules) – these large inclusions, which take their name from the fact that they sometimes stain red with certain blue dyes such as methylene blue, are collectively known as volutin granules. Volutin granules represent a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. It is generally formed by cells that grow in phosphate – rich environments. Metachromatic granules are found in algae, fungi, and protozoa, as well as in bacteria. These granules are characteristic of *Corynebacterium diphtheriae*, the causative agent of diphtheria; thus, they have diagnostic significance. Characteristically, they possess irregular swellings at one or both poles of pathogenic Corynebacteria that give them a club-shaped appearance. In conditionally pathogenic Corynebacteria volutin granules spread on all the surface of the rod or they are absent.

Volutin granules in Corynebacterium diphtheria:



Bacterial **nuclear area or nucleoid (**bacterial chromosome) contains a single long circular molecule of double stranded DNA and fewer quantity of RNA. This is the cell's genetic information, which carries all the information required for the cell's structures and functions. Unlike the chromosomes of eukaryotic cells, bacterial chromosomes do not include histones and are not surrounded by a nuclear envelope (membrane), do not include nucleolus, no mitotic apparatus. The DNA of prokaryotes has a molecular weight of approximately 2.109 and contains about 2000 genes. (By contrast, human DNA has approximately 100000 genes). In actively growing bacteria, as much as 20% of the cell volume is occupied by DNA because such cells pre-synthesize nuclear material for future cells. The chromosome is attached to the plasma membrane are believed to be responsible, for replication of the DNA and segregation of the new chromosomes to daughter cell's in cell division.

Plasmids – are extrachromosomal, double-stranded circular DNA molecules that are capable of replicating independently of the bacterial chromosome. Although plasmids are usually extra-chromosomal, they can be integrated into the bacterial chromosome. Plasmids may be gained or lost without harming the cell. Under certain conditions, however, plasmids are an advantage to cells. Plasmids may carry genes for such activities as antibiotic resistance (R-plasmids), tolerance to toxic metals, production of toxins (tox-plasmids) and synthesis of enzymes. Plasmids can be transferred from one bacterium to another. In fact, plasmid DNA is used for gene manipulation in biotechnology.



Flagella – are long, whip like appendages, that, move the bacteria toward nutrients and other attractants, a process called chemotaxis. The long filament which acts as a propeller is composed of many subunits of a single protein-flagellin, which belongs to the same chemical group as myosin- the contractile protein of muscle, arranged in several intertwined chains. The energy for movement, the proton, motive force is provided by adenosine triphosphate (ATP) derived from the passage of ions across the membrane.

The functions of flagella:

1.gives motility to bacteria

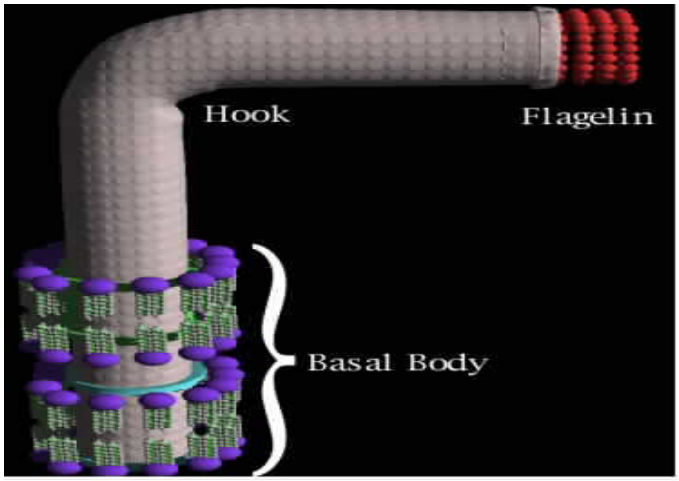
2.gives antigenicity to bacteria (flagellar "H" antigen)

Flagellated bacteria have a characteristic number and location of flagella: some bacteria have one and others have many. And to these characteristic motile microbes can be divided into **4 groups**:

1. **monotrichous** - bacteria having a single flagellum at one pole of the cell (e.g. cholera vibrio);
2. **amphitrichous** - bacteria with two polar flagella or with a tuft of flagella at both poles (e.g. Spirillum volutans);
3. **lophotrichous** - bacteria with a tuft of flagella at one pole (blue green milk bacillus);
4. **peritrichous** - bacteria having flagella distributed over the whole surface of their bodies (e.g. E. coli, Salmonella).

The type of motility in bacteria depends on the number of flagella, age and properties of the culture, temperature, amount of chemical substances and other factors. Monotrichous bacteria move with the greatest speed (60 μm per second). Peritrichous bacteria move at rates ranging from 25-30 μm per second. Motility in bacteria can be observed by the hanging drop in wet conditions, by phase contrast microscopy. The determination of motility in microbes is employed in laboratory, practice as a means to identify the microbes.

The flagellum consists of three parts: the **filament,** the **hook** and the **basal body.**



Pili or fimbriae) - hair like microfibrils, 1 to 1.5μm in length and 4 to 8 nm in diameter, that extend from the cell surface. They are shorter and straighter than flagella and are composed of subunits of a protein called pilin, arranged in helical strands. They are found mainly on gram negative organisms.

Pili have **two important roles**:

1.Common which ensure enlargement of the bacterial surface and participate in metabolism and gives antigenicity to bacteria organisms.

2.Specialized (fimbria, pili): which mediate the

- **attachment**(adhesion) of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection (adhesive pili).
- **F -pili** (fertility-sex pili, conjugative pili) form the attachment between the male (donor) and the female (recipient) bacteria during conjugation.

Spores – are highly resistant structures which are formed in response to adverse conditions, by two genera of medically important gram-positive rods: the genus Bacillus, which includes the agent of anthrax, and the genus Clostridium, which includes the agents of tetanus, botulism, gas gangrene.

Sporulation occurs in the environment (in soil and on nutrient media), and is not observed in human or animal tissues. This is anabiotic form of bacteria. The sporulation process occurs in following stages:

1.**formation of sporogene zone** inside of bacterial cell. The process is characterized by a thickening of the cytoplasm in a certain region and the formation of a **fore-spore.**

2.formation of membranes: invagination of cytoplasmic membrane which surrounds the sporogene zone by two layers. Between these two layers syntheses of **cortex-peptidoglycane like structure;** instead of diaminopimelinic acid presence of **dipicolinic acid**. After it the next step is covering of external part by dense membrane, which includes proteins, lipids, which is absent in vegetative cell.

3.maturation of spores: the rest of the cell gradually disappears. Instead of a vegetative cell, a mature spore, one-tenth the size of the parental cell, is produced. Sporulation is completed within 18 to 20 hours. When conditions become favorable, the spores germinate and transform again into vegetative cells (within 4 to 5 hours).

Spore is stable to physical and chemical agents and it depends on:

- presence of multilayer membrane
- presence of dipicolonic acid
- presence of calcium compounds
- inactivation of enzymes
- absence of free water (bonded water)

Sporulation is completed within 18 to 20 hours. When conditions become favorable, the spores germinate and transform again into vegetative cells. Usually germination takes place more quickly than sporulation (within 4 to 5 hours). The sporulation process in bacilli is not one of multiplication since most rod-shaped forms produce only one spore each.

In bacilli and clostridia, spores are located: **1. Centrally** (in the centre of the cell – B. anthracis);

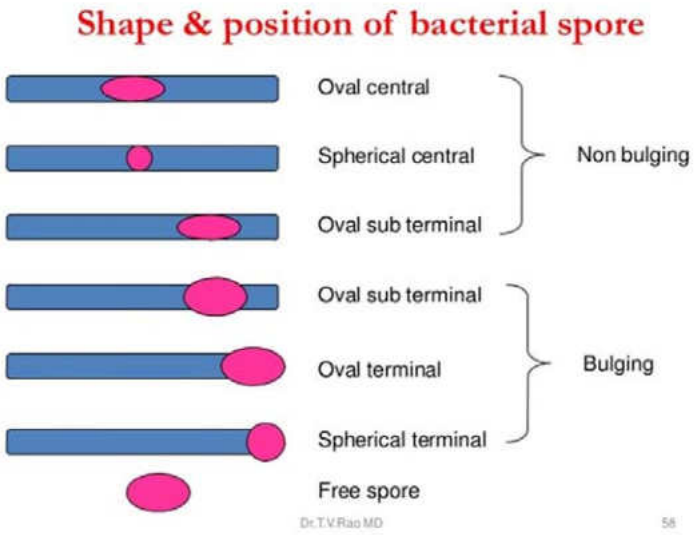
2. Terminally (at the end of the rod – C. tetani); **3. Subterminally** (towards the end – C. botulinum, C. perfringens).

In some species of sporulating micro-organisms, the diameter of spore is greater than the within of the bacterial cell. If the spore is located subterminally, the microbes take on the form of a spindle (closter). In tetanus clostridia the spore diameter is also greater than the width of the vegetative cell, but the spore is located terminally and hence the drum – stick appearance. This property of sporulation is important in characterizing and identifying spore-forming microbes, and also when selecting methods of decontaminating objects, housings, foodstuffs, and other substances.

The microbe may lose its ability to sporulation by frequent cultivation on fresh media or by subjecting it to high temperatures (table).

Table : Summery of important features of bacterial spores.

1. They are highly resistant to heating, i.e., they are not killed by boiling at 100c. However, they are killed by raising the temperature to 121°c in an autoclave.
2. They are highly resistant to many disinfectants. The resistance to chemicals and to heat is attributed to their thick keratin like coats and to the absence of water.
3. They are produced only by members of two genera of bacteria of medical importance: Bacillus and Clostridium, both of which are gram (+) rods.
4. They are produced under conditions of nutritional deprivation, i.e., when carbon or nitrogen sources are lacking. When nutritional sources are restored, the spores germinate to form vegetative bacterial cells.
5. They can survive for many years, especially in soil.
6. They exhibit no measurable metabolic activity.
7. They contain dipicolinic acid is a calcium chelator that is found virtually nowhere else in the biological world.



THE MORPHOLOGY OF BACTERIA

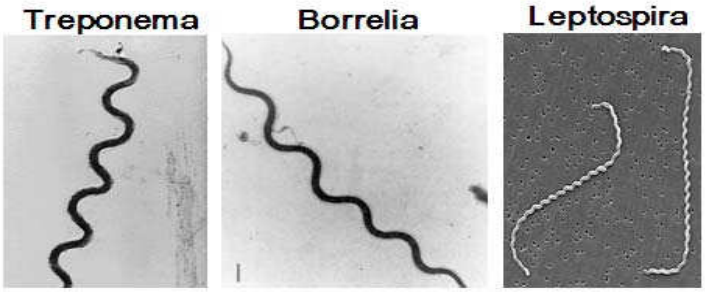
Spirochetes - elongated, motile, flexible bacteria. The body of Spirochete consists from the hollow coil which enclosed by the cytoplasmic membrane, the outer membrane is the thin cell wall. All spirochetes are actively motile and achieve their motility by means of two or more axial filaments. The axial filaments are enclosed in the space between an outer sheath and the body of the cell. One end of each axial filament is attached near a pole of the cell on the disks-blepharoplasts. By rotating its axial filament, the cell rotates in the opposite direction to move through liquids like a corkscrew. Generally spirochetes are free living saprophytes. They are found in contaminated water in swage, soil, and decaying organic material, and within the bodies of humans and animals (Treponema orale, Treponema denticola, Treponema refringens). Spirochetes belong to the order Spirochaetales, genus Spirochaeta, and family Spirochaetaceae. Spirochaetes vary in size from 5-500μm in length. Three pathogenic genera belong to this family: genus Treponema, which includes Treponema pallidum, the cause of syphilis; genus Borrelia the cause of relapsing fever and Lyme disease; genus Leptospira the cause of leptospirosis. These three genuses are differentiated of the staining properties, the number of twists.

Genus Borrelia – their cells have large, obtuse-angled, irregular spirals, the number of which varies from 3 to 10 (distance between turn 2 to 4 μm) and they stain blue-violet with the Romanowsky-Giemsa stain. They measure 8-30 μm in length and 0.2-0.5 μm in width. Borrelia is microaerophilic. Optimum temperature for growth is 28-30º C. These may be grown in fluid media containing blood, serum or tissue, and on chorioallantoic membrane of chick embryo. Pathogenic for man Borrelia are: Borrelia recurrentis (transmitted by the bite of louse), the causative agent of epidemic relapsing fever; Borrelia burgdorferi (transmitted by the bite of small ticks), the causative agent of Lyme disease.

Genus Treponema – (Greek-trepin turn, nema-thread) exhibits thin, delicate, flexible cells with 8-12 twists (distance between turn 1 μm) and tapering ends. Besides the typical form, may be treponemas seen as granules, L-forms and other structures. In unfavorable conditions they form **cyst.** Spirochetes stain pale-pink with the Romanowsky-Giemsa stain. A typical representative is the causative agent of syphilis - Treponema pallidum. Conditionally pathogenic treponemas (T. orale, T. denticola, T. buccalis, T. refringens), stains more intensive and they are large and thick organisms.

Genus Leptospira – (GK – leptos thin, speira coil) are characterized by very thin cell structure. The Leptospira form 12 to 18 coils wound close to each other, shaping small primary spirals. The organisms have two paired axial filaments attached at opposite ends (basal bodies) of the cell and directed toward each other. Due to the presence of the two pairs of axial filaments the leptospirae are capable of quite complex and active movement. They have secondary spirals which give them an S-like appearance. They stain pinkish with the Romanowsky-Giemsa stain. Some serotypes which are pathogenic for animals and

man cause leptospirosis. Leptospirae grow best under aerobic conditions at 28-30° C in protein rich semisolid media, where they produce round colonies 1-3 mm in diameter in 6-10 days. Leptospirae also grow on chorioallantoic membrane of embryonated eggs.

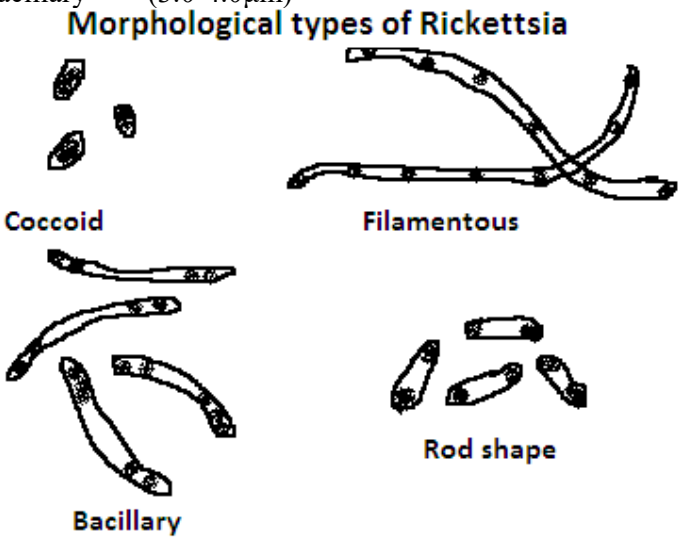


Spirochetes can be observed by following methods: in native preparations in **dark-field or phase contrast microscopy**; staining by **silver impregnation methods (Morozov method);** staining by **Romanowsky-Giemsa method.**

Rickettsia and Chlamydia – both are obligate intracellular parasites, which mean that they can reproduce only within a host cell. In this respect, they are similar to viruses. However, they resemble bacteria and are therefore classified as such.

Rickettsia – are rod-shaped bacteria or cocobacilli that have a high degree of polymorphism (appearing either as short rods, 600X300nm in size, or as cocci, and they occur singly, in pairs, in short chains, or in filaments). By shape they can be:

- coccoid (0.5-0.7μm)
- rod shape (1.0 -1.5μm)
- filamentous (30-40μm)
- bacillary (3.0-4.0μm)

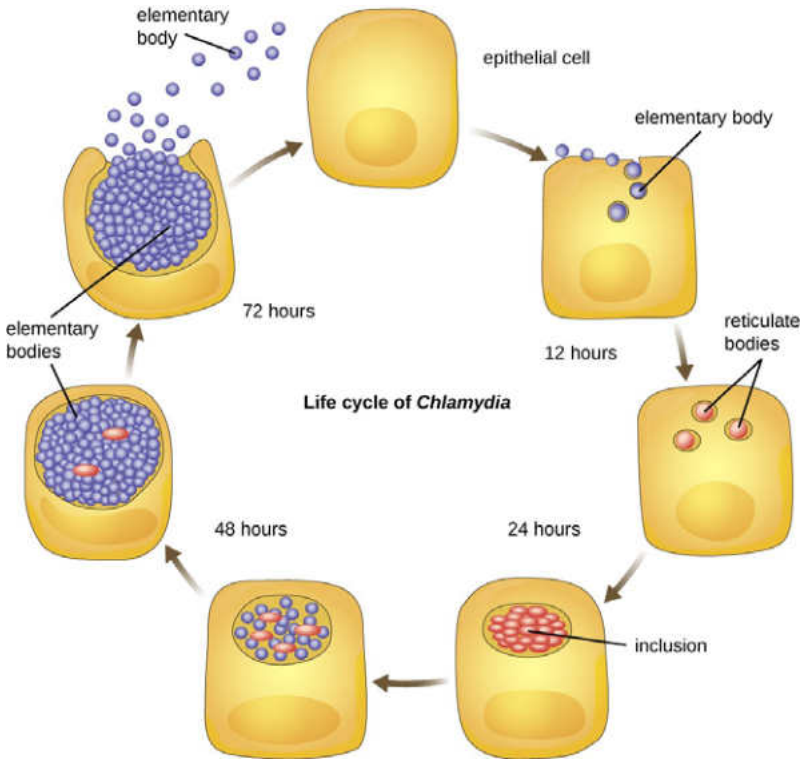


They are gram negative (purified rickettsia contain both RNA and DNA in ratio similar to that bacteria - 3,5:1), and divided by binary fission. They live and multiply only within the cells (in the cytoplasm and nucleus) of the tissues of humans, animals and vectors. Rickettsia are non-motile, do not produce spores and capsules and stain well by the Giemsa's stain (they appear blue) and Zdradovsky stain (they appear red). The diseases caused by rickettsia are known rickettsioses. A typical representative is *Rickettsia prowazekii* (the name was given in honor of the scientists, the American Howard Ricketts and the Czech Stanislaus Prowazek) the causative agent of Epidemic typhus fever.

Chlamydia – they are in genus Chlamydia, family Chlamydiaceae, order Chlamydiales. They are the agents of trachoma, conjunctivitis, lymphogranuloma venerum, ornithosis, and other infections. Chlamydiae are obligate intracellular parasite. Chlamydia are small in size and measured 0,2-1,5μm.

Reproduction occurs only in the cytoplasm of the cells of the vertebrates. The organisms are characterized by low metabolic activity and are cultivated at 33°-41° C in the yolk sac of a chick embryo. Chlamydia has a replicative cycle different from that of all other bacteria. The cycle begins when the extracellular, metabolically inert, "spore-like" **elementary body** enters the cell and reorganizes into a larger, metabolically active **reticulate** body. The latter undergoes repeated binary fission to form daughter elementary bodies, which are released from the cell. Within cells, the site of replication appears as an inclusion body, which can be stained and visualized microscopically. These inclusions are useful in the identification of these organisms in the clinical laboratory. Growth, reproduction and maturation of Chlamydia organisms are completed in 40-72 hours.

Life cycle of Chlamydia:



Actinomycetes – are true bacteria (related to Corynebacteria and Mycobacteria), but they form long, branching filaments that resemble the hyphae of fungi. They are gram-positive, but the peptidoglycan of Actinomycetes contain arabinose, galactose, which are absent in bacteria, but some are also acid-fast. They grow and reproduce by spores, fragmentation of hyphae or by budding. In infected tissues they form **druze** (accumulation of mycelium). In artificial nutrient media they form air and substrate mycelium. There are two medically important genus *Streptomyces* and *Nocardia.* Nocardia are common in soil. Some species, such as Nocardia asterioides, occasionally, cause chronic, difficult-to-treat pulmonary Nocardiosis. Species of Streptomyces are valuable because they produce most of our commercial antibiotics. Most of them participate in synthesis of vitamin B.

They do not require nutrient media; they grow on simple nutrient media. On solid media they form small **S** or **R** type colored colonies (with blue, red, green pigments). Pathogenic actinomycetes possess high protein lysing and sugar lysing activities. The virulence factors are: polysaccharide capsule, adhesive factors, and enzymes of aggression. Antigenicity depends on polysaccharides of the cell wall. They don't produce exotoxin.

Mycoplasmas are pathogens of the respiratory and genitourinary tracts and joints. They are the smallest free-living organisms. Their most striking feature is the absence of a cell wall. Consequently, Mycoplasma stain poorly with Gram stain, and antibiotics that inhibit cell wall synthesis, e.g., penicillin and cephalosporin, are ineffective. Their outer surface is a flexible three-layer cell membrane; hence, these organisms can assume a variety of shapes. They can be grown in the laboratory on artificial media, but they have complex nutritional requirements, including several lipids. They grow slowly and require at

least 1 week forming a visible colony. The colony frequently has a characteristic “fried-egg” shape, with a raised center and thinner outer edge. There are two medically important genus Mycoplasma and Ureoplasma.

The main characteristics of Mycoplasma:

1. The smallest mycoplasmas are 125-250 nm in size**.**

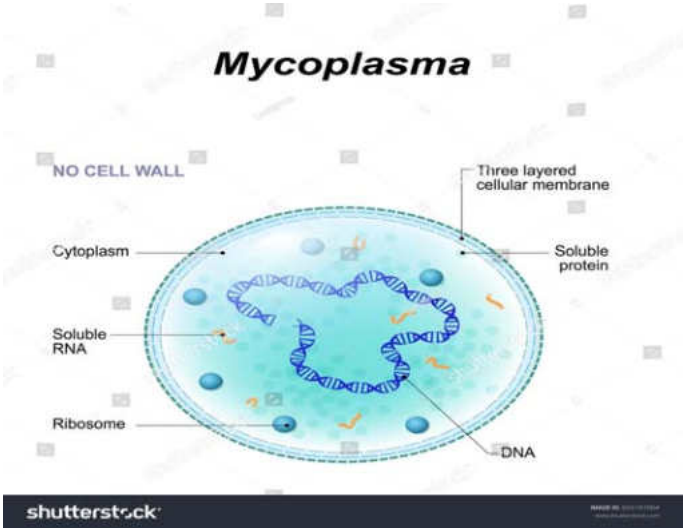
2. They are highly polymorphic because they lack a rigid cell wall and instead are bounded by a triple-layered “unit membrane” that contains a sterol (mycoplasmas, but not all mollicutes, require sterols for growth).

3. They are completely resistance to penicillin because they lack the cell wall structure, where penicillin acts, but they are inhibited by tetracycline or erythromycin**.**

4. They can reproduce in cell-free media; on agar, the center of the whole colony is characteristically embedded beneath the surface (“fried-egg”) shape.

5. Growth is inhibited by specific antibody.

6. Mycoplasmas have an affinity for mammalian cell membrane.



V I R U S E S

MORPHOLOGY AND ULTRASTRUCTURE OF VIRUSES
CLASSIFICATION OF VIRUSES
INTERACTION BETWEEN VIRUSES AND HOST

In contrast to Eukaryotic and Prokaryotic cells Viruses are not cells. Viruses form the kingdom Vira, which is classified into two subkingdoms by the type of nucleic acid: **DNA** viruses and **RNA** viruses.

Subkingdoms consists from families - DNA viruses united into six families, RNA viruses are united into 14 families

Viruses are characterized by the following **features**:

1. They haven't cell structure (do not have nucleus, and do not have organelles such as ribosome, mitochondria and etc.).

2. They contain only one kind of nucleic acid either *DNA or RNA.*

3. Viruses replicate in a manner different from that of cells; i.e., viruses do not undergo binary fission or mitosis. The type of replication is reproduction (disjunctive way), and they are not capable to reproduce independently. Viruses must replicate within cells, because they cannot generate energy to synthesize proteins. Because they can reproduce only within cells, viruses are **obligate intracellular parasites**.

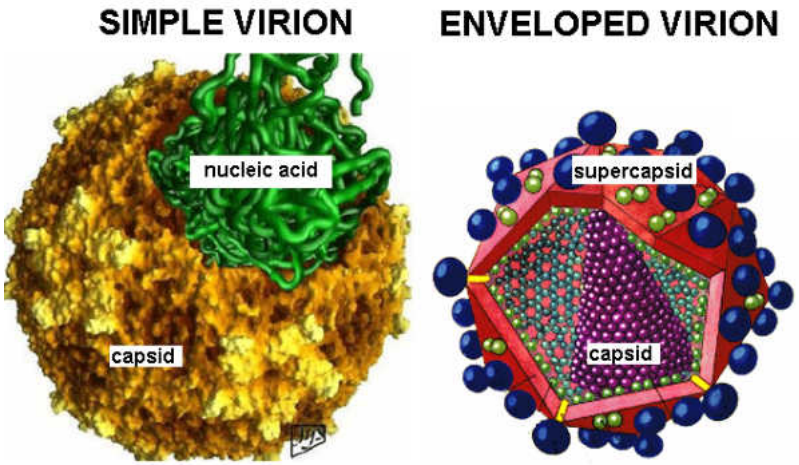
4. Viruses are smaller, they are measured in nanometers (nm) which is the 1/1000th part of micrometer /μm/ and cannot be seen in the light microscope.

Table: Properties of Prokaryotes and Viruses

	Bacteria	Mycoplasma	Rickettsia	Chlamydia	Viruses
Cellular organization	+	+	+	+	-
Growth on inanimate media	+	+	-	-	-
Binary fission	+	+	+	+	-
Both DNA and RNA	+	+	+	+	-
Ribosomes	+	+	+	+	-
Sensitivity to antibacterial antibiotics	+	+	+	+	-
Sensitivity to interferon	-	-	-	+	+

The intracellular forms are named **viruses**, extra-cellular forms – **virion**.

The virion consists essentially of a nucleic acid core surrounded by a protein coat called a **capsid.** The capsid is composed of repeating protein units called **capsomers.** Each capsomer consists of one or several proteins. Viral proteins are divided into structural and functional. **The structural proteins** are in the capsid composition. Their major purpose is to facilitate transfer of the viral nucleic acid from one host cell to another. They serve protective function, participate in the attachment of the virus particle to a susceptible cell, and provide the structural symmetry of the virus particle**. Functional proteins** are enzymes, which are present in very small amounts and are probably not important in the structure of the virus particles; however, they are essential for the initiation of the viral replicative cycle when the virion enters a host cell. The capsid with the enclosed nucleic acid is known as the **nucleocapsid**. These viruses are called *simple* viruses.



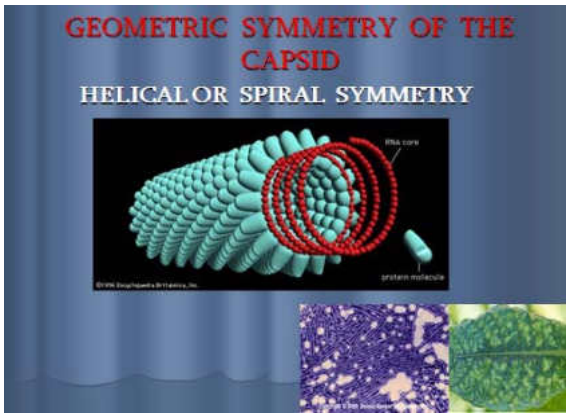
The arrangement of capsomers gives the virus structure its geometric symmetry. There are following types of symmetry in virus capsids.

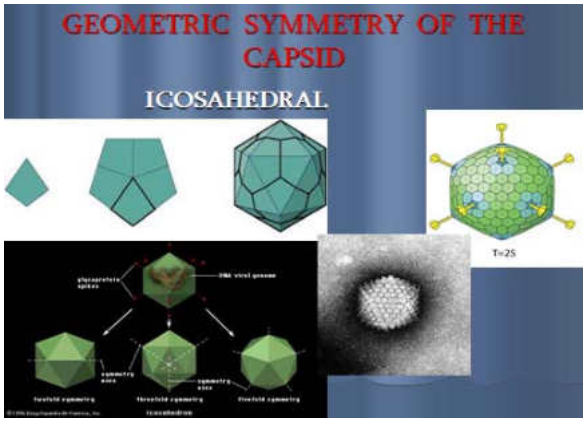
1. Icosahedral, in which the capsomers are arranged in 20 triangles that form a symmetric figure (an icosahedron) with the approximate outline of a sphere /cubic symmetry/.

2. Helikal, in which the capsomers are arranged in a hollow coil that appears rod-shape (spiral symmetry).

3. Complex structure: *Some virus particles do not exhibit simple cubic or helical symmetry but are more complicated in structure (poxviruses are brick-shaped with ridges on the external surface and a core and lateral bodies inside).*

Symmetry of the capsid





The functions of nucleic acid:

- are carriers of genetic information
- infectivity

The functions of capsid are:

- protective (protection of nucleic acid)
- adhesive and invasive (make attachment to the host cell and invasion)
- antigenicity

Some viruses have **supercapsid** membrane (enveloped viruses). The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane and protein that is virus specific. Furthermore, there are frequently glycoproteins in the form of spike-like projections on the surface, which attach to host cell receptors during the entry of the virus into the cell.

The **functions of supercapsid** are the same as the capsid's: adhesive and invasive, protective, and they are the principal antigens against which the host mounts its immune response to viruses. In general, the presence of an envelope makes the virus more sensitive to heat, detergents, and lipid solvents such as alcohol and ether than are non-enveloped (nucleocapsid) viruses.

THE CLASSIFICATION OF VIRUSES

The classification of viruses is based on the following properties:

1.The type of nucleic acid **– either DNA or RNA** (but not both), the **number of strands** in nucleic acid. Viral nucleic acid (genome) is located internally and can be either single or double strand DNA or single or double strand RNA. Only viruses have genetic material composed of single stranded DNA or of single stranded or double stranded RNA. The functions of the nucleic acid are: they carried genetic information and secure infectious properties. **Polarity (sense)**-single stranded RNA can be single stranded RNA (+); single stranded RNA(-). **Nuclear weight of the nucleic acid,** its **percentage in** the virion, **number of capsomers in the capsid**.

2. According to **nucleocapsid** symmetry (**icosahedra**l, **helical)**

3. According to the structure: presence or absence of the second membrane **/supercapsid/** -simple virus, enveloped virus.

4**.** According **to the size** the viruses fall into 3 groups:

- Small-size viruses – 30-40 nm (poliomyelitis virus)
- Middle-size viruses – 80-150 nm (viruses of influenza, AIDS)
- Large-size viruses – from 200 and over till 400 nm (Paramyxoviruses, virus of parotitis)

The **size of viruses** can be determined by: **a)** Filtration through colloid membranes, **b)** Centrifugation in high speed centrifuges, **c)** Electron microscopy, d) Diffusion in gel.

5. According to their **shape:**

- Spherical form (viruses of influenza, AIDS)
- Rod-shaped form (causative agents of tobacco mosaic disease)
- Spermatozoid form (phages)
- Bullet-shaped (rabies)
- Star shaped
- Crown shaped

6. According **to the host** which they can affect:

- plants (causative agent of tobacco mosaic disease)
- animals and humans (animal viruses)
- bacteria (bacteriophages)

7. **A**ccording **to the tropism**. By this classification they may be neurotropic (rabies virus); pneumotropic-affect the respiratory system (Orthomixoviruses, Paramixoviruses); enterotropic (Epidemic poliomyelitis virus, Hepatitis virus A).

8.According **to the formation of inclusion bodies** (intranuclear, cytoplasmic)

9.According **to the antigenic** structure: there are internal core nucleocapsid "**S" antigen** and superficial viral "**V" antigen.**

THE MECHANISM OF THE INTERACTION OF THE VIRUS WITH THE HOST CELL

Viruses are known to be intracellular obligate parasites. The mechanism of the interaction of the virus with the susceptible cell is rather a complex cycle. A single virus can give rise to several or even thousands of similar viruses in a single host cell. This process can drastically change the host cell and can even cause its death.

In the result of this interaction following forms of infection develop:

1. Productive – when reproduction of viruses takes place (formation of new virion).

2. Abortive form – death of virions or cession of their reproduction.

3. Integrative – mutual adaptation of the virus and the cell (virogeny) or the development of a neoplastic process in which intensive growth and reproduction of cells are observed (oncogenic function of viruses).

There are **three types** of persistent viral infections of clinical importance:

- **Chronic - carrier infection**: Some patients who have been infected with certain viruses continue to produce significant amount of the virus for long periods. This **carrier state** can follow an asymptomatic infection as well as the actual disease and it can itself be either asymptomatic or result in chronic illness. Important clinical examples are chronic hepatitis, which occurs in hepatitis B and hepatitis C virus in carriers, in which carriers can produce virus for years.
- **Latent infections:** Best of all these infections are illustrated by the herpes virus group, the patient recovers from the initial infection and virus production stops.
  Subsequently, the symptoms may recur, accompanied by the production of virus. The molecular nature of the latent state is unknown.
- **Slow Virus infections:** The term "slow virus" refers to the **prolonged period** between the initial infections and the onset of disease, which usually last for years. The incubation period and the progress of the disease are prolonged (e.g. subacute sclerosing panencephalitis, which follows several years after measles virus infections).

Infected cells during viral infections frequently contain **inclusion bodies**, which are discrete areas containing viral proteins or viral particles. They have either intranuclear (in herpes, chickenpox), or intracytoplasmic location (Guarnieri's inclusion bodies in small pox; Babes-Negri bodies in rabies), or in the nucleus and cytoplasm (in smallpox). Some inclusion bodies serve as a diagnostic character.

Thus, the multiplication cycle of viruses can be divided into following **stages**:

1.Attachment (adsorption) - adsorption takes place only if there is an affinity between virions and host cells. This attachment is a chemical interaction in which weak bonds are formed between the attachment and receptor sites. The cell surface should contain specific receptor sites to which the virus can gain attachment. However, the receptor sites of animal cells are proteins and glycoproteins of the plasma membrane. The attachment sites of animal viruses are distributed over the surface of the virus. The sites themselves vary from one group of viruses to another. In icosahedral viruses, the attachment sites are small fibers at the corners of the icosahedron. In many of the envelope viruses, the attachment sites are spikes located on the surface of the envelope. As soon as one spike attaches to a host receptor, additional receptor sites on the same migrate to the virus. Attachment is completed when many sites are bound.

2. Penetration – occurs by several ways: **endocytosis or viropexis** – penetration of envelope animal viruses occurs by endocytosis, an active cellular process by which nutrients and other molecules are brought into a cell. A cell plasma membrane continuously folds inward to form vesicles. These vesicles contain elements that originate outside the cell and are brought into the interior of the cell to be digested. If a virion attaches to a small out-folding on the plasma membrane of a potential host cell, the host cell will enfold the virion into a fold of plasma membrane, forming a vesicle. Once the virion is enclosed within the vesicle, its viral envelope is destroyed. Penetration sometimes occurs by an alternative method called **fusion,** in which the viral envelope fuses with the plasma membrane and released the capsid into the cell cytoplasm.

3. Uncoating – is the process of viral stripping from outer layers and capsid so the nucleic acid is released into the cell. It occurred by the proteolytic enzymes.

4. Eclipse period (the time during which no virus is found inside the cell). – from the stage of penetration till the appearance of mature daughter virions, the virus cannot be demonstrated inside the host cell. This period during which the virus seems to disappear or go "underground" is known as the eclipse phase. In this step the synthesis of the virus components begins, so reproduction begins.

5. Biosynthesis –The first step in viral gene expression is mRNA synthesis. It is at this point that viruses follow different pathway depending on the nature of their nucleic acid and the part of the cell in which they replicate.

Steps in biosynthesis:

1.Transcription of messenger RNA (mRNA) from the viral nucleic acid

2.Translation of the mRNA into "early proteins". These early or non structural proteins are enzymes which initiate and maintain the synthesis of virus components. They may also induce shutdown of host protein and nucleic acid synthesis.

3.Replication of viral nucleic acid.

4.Synthesis of "late" or structural proteins, which are the components of daughter virion capsid.

- **DNA viruses** – generally, DNA containing viruses replicate their DNA in the **nucleus** of the host cell by using the host cell DNA-dependent RNA polymerase to synthesize their mRNA. The genome of all

DNA viruses consists of double-stranded DNA, except for the parvoviruses, which have a single-stranded DNA genome. The reproduction of DNA viruses takes place in the classic way:

DNA – mRNA – viral proteins

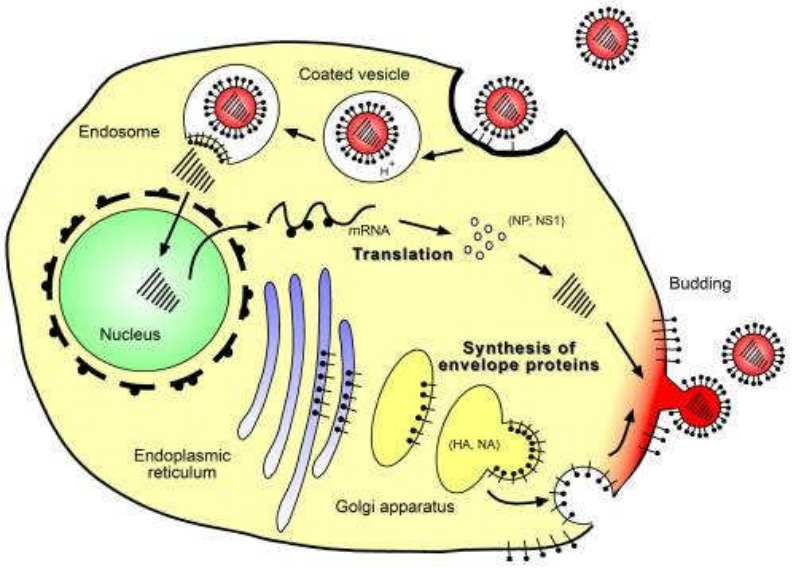
DNA viruses that replicate in the nucleus generally use host cell DNA and RNA polymerases and processing enzymes. The poxviruses are the exception because they replicate in the cytoplasm, where they do not have access to the host cell RNA polymerase. They therefore carry their own polymerase within the virus particle.

- **RNA viruses** – Most RNA viruses undergo their entire replicative cycle in the cytoplasm (the two exceptions are retroviruses, influenza viruses). RNA viruses fall into following groups with quite different strategies for mRNA synthesizing: **a)** The simplest strategy is the viruses, which has **single-stranded RNA** of **positive polarity** as its genetic material. These viruses use their RNA genome directly as mRNA (poliovirus): **RNA(+)—viral proteins**
- **b)** The second group has **single-stranded RNA** of **negative polarity** as its genetic material. An mRNA must be transcribed by using the negative strand capable of using RNA as a template; the virus carries its own RNA-dependent RNA polymerase (Paramyxoviruses, Rabdoviruses):
- **RNA(-) – mRNA – viral proteins**
- **c)** The third group has **double-stranded RNA** as its genetic material. Because the cell has no enzyme capable of transcribing this RNA into mRNA, the virus carries its own polymerase (Reovirus).
- **d)** The fourth group, exemplified by r**etroviruses**, has single – stranded RNA of positive polarity that is transcribed into double-stranded DNA by the RNA-dependent DNA polymerase (**reverse transcriptase**, so called because it carries out the reaction: RNA-DNA that is exactly the reverse of the familiar transcription of DNA-RNA) carried by the virus. This DNA copy is then transcribed into viral mRNA by the regular host cell RNA polymerase (polymerase II), before transcription the viral DNA must be integrated into the DNA of a host cell chromosome. In this integrated state, the viral DNA is called a provirus: **RNA – DNA(copy) – RNA(+) – viral proteins**

6. Assembly and Release - the first step is an assembly of the protein capsid. The progeny particles are assembled by packaging the viral nucleic acid within the capsid proteins.

Virus particles are released from the cell by either of the two processes. One is the **rupture** of the cell membrane and the release of the mature particles; this usually occurs with non-enveloped viruses. The other, which occurs with enveloped viruses, is the release of viruses by **"budding"** through the outer cell membrane. Budding does not immediately kill the host cell, and in some cases the host cell survives.

INTERACTION OF THE VIRUS WITH THE HOST CELL



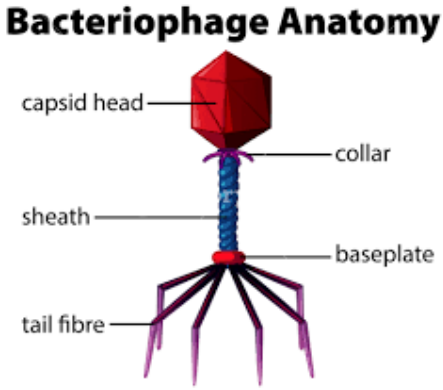
Viral enzymes – in contrast of Prokaryotes and other organism viruses deprived of enzymes, which are capable to participate into metabolic processes. But many viruses in their capsid structure contain one or two groups of enzymes: **The first group** contains virus-specific enzymes which are the replication and transcription enzymes and the enzymes which contribute to penetration of viruses into the host cell and virus particles released from the host cell (RNA dependent RNA polymerase, reverse transcriptase, neuraminidase, lysozyme). **The second group** contains virus- induced enzymes. The structure of these enzymes is encoded in the virus genome but they are synthesized in the host ribosomes (RNA-polymerase, DNA-polymerase).

B A C T E R I O P H A G E S

Bacteriophages are viruses that infect bacteria. The F.D'Herelle (French scientist) was the first who discovered bacteriophages.

The phages have very complicated structure and are called **complex viruses**. They are tadpole or spermatozoid shaped. They consist **of *head, neck*, *tail*** and ***basal plate*** from each angle of which six teeth and filaments going out. Due to these, phage is adsorbed on the bacteria cell.

The nucleic acid DNA or RNA is contained in the hexagonal head. The head is surrounded with protein membrane or capsid. The neck is free of this membrane. The tail is composed of a hollow core surrounded with protein membrane and formed the cylindrical symmetry and the free filament of nucleic acid is hanged in this cylinder. The lysozyme enzyme is contained in the basal plate. Due to this lysozyme phage breaks down a portion of the bacterial cell wall and penetrates through the cell wall of bacteria.



The interaction of phage and bacteria is manifested in the following phases occurring in succession:

- Adsorption
- Penetration into the cell
- Reproduction: biosynthesis, maturation
- Release

1. Adsorption. After a chance collision between phage particles and bacteria, attachment, or adsorption, occurs. During this process, the attachment site on the virus attaches to a complimentary receptor site on the bacterial cell. This attachment is a chemical interaction in which weak bonds are formed between the attachment and receptor sites. The complementary receptor sites are on the bacterial cell wall.

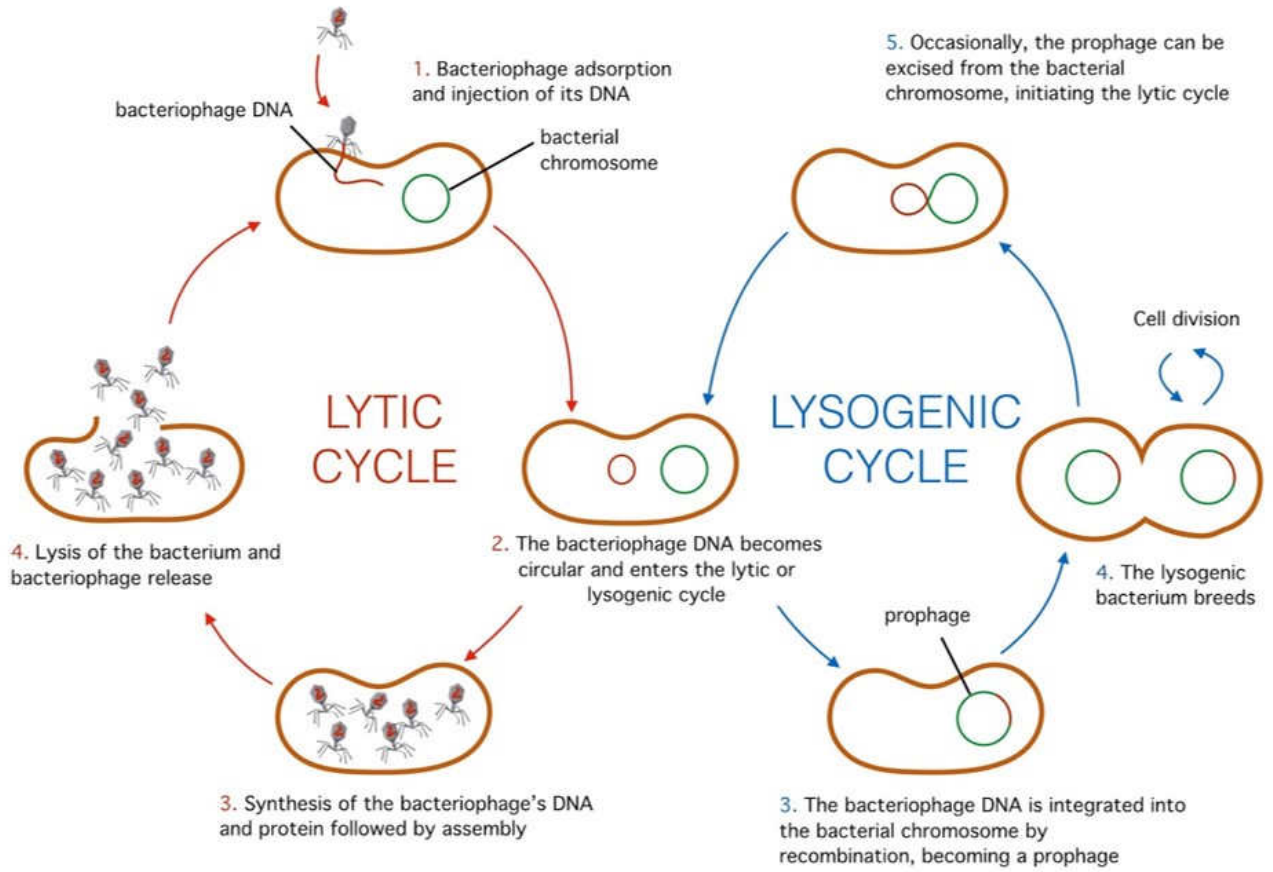
2. Penetration. After the attachment the bacteriophage injects its nucleic acid into the bacterium. To do this, bacteriophage's tail releases an enzyme, *phage lysozyme*, which breaks down a portion of the bacterial cell wall. During the process of penetration the tail sheath of the phage contracts and the tail core is driven through the cell wall. When the tip of the core reaches the plasma membrane, the DNA from the bacteriophage's head passes through the tail core and through the plasma membrane and enters the bacterial cell.

3. Biosynthesis. Is the process when the nucleic acid is integrated in the bacterial genetic material and the synthesis of substrates necessary for the phage takes place. Initially, the phage uses the host cell's

nucleotides and several of its enzymes to synthesize many copies of phage DNA. Soon after, the biosynthesis of the viral proteins begins.

4. Maturation and Release. In the next sequence of events, maturation occurs. In this process, bacteriophage DNA and capsids are assembled into complete virions. The phage heads and tails are separately assembled from protein subunits and the head is filled with phage DNA and attached to the tail. After self-collection the phages are released from the bacterial cell and affect that cell. These viruses are termed **virulent** phages. In contrast to virulent bacteriophage some viruses do not cause lysis and death of host cell when they multiply. These ones called **temperate phages** penetrate into the bacterial cell then integrate in the genetic material of the bacteria (bacterial chromosome). The integrated into nucleic acid phage is known as a prophage. The prophage behaves like a segment of the host chromosome and replicates with it. This phenomenon is called **lysogeny**, and a bacterium that carries a prophage within its genome is called **lysogenic bacterium.**

LIFE CYCLE OF BACTERIOPHAGE



The lysogenic bacterium gets new properties. This phenomenon is known as lysogenic conversion or phage conversion. The result of lysogeny is that the host cell may exhibit new properties. For example, the bacterium Corynebacterium diphtheriae the causative agent of diphtheria, is a pathogen whose disease – producing properties are related to synthesis of a toxin. The organism can produce toxin only when it carries a temperate phage, because the prophage carries the gene coding for the toxin. Streptococci carrying a temperate phage are capable of producing the toxin causing scarlet fever. The toxin produced by Clostridium botulinum which is causes botulism is encoded by a prophage gene.

Phages are used in **prophylaxis** and **medical treatment** against infectious diseases, and are used in the **diagnoses** of certain infectious diseases.

CULTIVATION OF VIRUSES

As viruses are obligate intracellular parasites, they cannot be grown on any animate culture medium. Three methods are employed for the cultivation of viruses: **1.** Infection of animals, **2.** Embryonated eggs, **3.** Cell (tissue) cultures.

Detection of virus growth in embryonated egg: 1. death of embryo. 2.hemagglutination

Detection of virus growth in cell culture: virus growth in cell cultures can be detected by the following methods.

1. Cytopathic effect - many viruses cause morphological changes in cultured cells in which they grow. These changes can be readily observed by microscope examination of the cultures. These changes are known as "cytopathic effects" **(CPE)** and the viruses causing CPE are called "cytopathogenic viruses''. This cytopathic effect may be manifested by the degeneration of the cells (enteroviruses). Viruses can produce **syncytium** formation (measles etc.), in which infected cells fuse with neighboring infected or uninfected cells to form giant cells containing several (up to 100) nuclei, herpes virus causes discrete focal degeneration and etc.

2. Metabolic inhibition. In normal cell cultures, the medium turns acid due to cellular metabolism. When viruses grow in the cell cultures, cell metabolism is inhibited and there is no acid production. This can be made out by the color of the indication (phenol red) incorporated in the medium.

3. Hemadsorption. When hemagglutinating viruses grow in cell cultures, their presence can be indicated by the addition erythrocytes to the cultures. If the viruses are multiplying in the culture, the erythrocytes will adsorb onto the surface of cells. This is known as "hemadsorption".

4. Interference. The growth of a noncytopathogenic virus in cell culture can be tested by subsequent challenge with a known cytopathogenic virus. The growth of the first will inhibit the infection by the second virus due to interference.

5. Transformation. Tumors forming (oncogenic) viruses induce cell "transformation" and loss of contact inhibition, so that growth appears in a piled-up fusion producing "microtumors".

6. Immunofluorescence. Cells from virus infected cultures can be stained by fluorescent conjugated anticrum and examined by microscope for the presence of virus antigen. This gives positive results earlier than other methods and, therefore application in diagnostic virology.

7. Formation of inclusion bodies (revealing by Romanovsky-Giemza staining method).

8. Formation of patches.

9. Hemagglutination

PRIONS

Prions are very unusual agents that are responsible for at least six neurodegenerative diseases in humans and animals; these are always fatal. Prions are small proteinaceous infectious agents with properties distinct from other infectious agents, particularly in their resistance, appearance only by protein, without any nucleic acid. They do not contain nucleic acid genome that codes for their progeny. However, viruses, viriods, bacteria, fungi and parasites all have nucleic acid genomes that code for their progeny. There are two types of prions **normal** and **pathogenic.** The cellular form of **normal prion protein (PrPc) is contained in human organism and has** regulator function. These normal prions participate in growing old of brain and nerve system.

The mechanism of reproduction of prions is not known. One hypothesis is that PrPsc forms a heterodimer with PrPc, serving as a template which alters the folding of the latter to that of PrPsc. In contrast to PrPc, PrPsc is highly resistant to proteolysis. Because of their resistance to digestion, these abnormal isoforms accumulate. Prions are lack nucleic acid. Their infectivity is very resistant to inactivation by a variety of physical and chemical treatments that inactivate viruses, including nucleases, UV-irradiation, formalin, mild proteolysis, and even boiling. They do not elicit an immune response in the host.

Spongiform encephalopathy of humans and animals

Prions produce slow infections with long incubation periods (months to years), followed by progressive disease, which leads to death from a degenerative condition of the brain characterized by a spongiform appearance. These include Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker (GSS) syndrome, and fatal familial insomnia (FFI) in humans. Prions diseases of animals are **scrapie** of sheep and goats, bovine **spongiform encephalopathy** (BSE) of cattle, mink and feline encephalopathy and wasting disease of deer and elk (table).

Characteristics of prion diseases: Following are the characteristics of diseases caused by prions:

1. The prion diseases are infectious, inherited and sporadic disorders.
2. They are confined to the nervous system.
3. The basic lesion is a progressive vacuolation in neurons, an extensive astroglial hypertrophy and proliferation, and spongiform change in the grey matter.
4. Prion protein accumulates within the central nervous system both as diffuse deposits and in the form of amyloid plaques.
5. Long incubation periods (months to decades) precede the onset of clinical illness and are followed by chronic progressive pathology (weeks to years).
6. Diseases are always fatal.
7. Host shows no inflammatory response.
8. Prions do not appear to be antigenic, therefore, host shows no immune response.
9. Interferon is not produced.
10. There is no effect on host B or T cell function.
11. Between 10 and 15% cases of prion diseases are inherited whilst the remaining cases are sporadic.

Table: Prion diseases (spongiform encephalopathy) of humans and animals

Diseases of humans	Diseases of animals
• Kuru	• Scrapie of sheep and goats
• Creutzeldt-Jakob diseases	• Bovine spongiform encephalopathy of cattle
• Gerstmann-Straussler-Scheinker syndrome	• Mink and feline encephalopathy
• Fatal familial insomnia	• Wasting diseases of deer and elk

VIROIDS

Unusual agents which contain super spiral shape of RNA, lack proteins and are pathogenic for plants. Subviral agents characterized by the apparent absence of an extracellular dormant phase (virion) and by a genome much similar than those of known virus. The infective agent is a protein –free low molecular weight RNA resistant to heat and organic solvents but sensitive to nucleases.

M I C R O B I A L G E N E T I C S

The science of genetics defines and analyzes heredity, or constancy and change in the vast array of physiologic functions that form the properties of organisms. The unit of heredity is the gene, a segment of DNA that carries in its nucleotide sequence information for a specific biochemical or physiologic property. The traditional approach to genetics has been to identify genes on the basis of their contribution to **phenotype**, or the collective structural and physiologic properties of a cell or an organism. A phenotypic property, be it eye color in a human or resistance to an antibiotic in a bacterium, is generally observed at the level of the organism. The chemical basis for variation in phenotype is change in genotype, or alteration in the sequence of DNA within a gene or in the organization of genes.

Genetic information is stored as a sequence of bases in deoxyribonucleic acid (DNA). Most prokaryotic genes are carried on the bacterial chromosome, a single circular DNA molecule with a molecular weight about $2x10^{9}$ and composed of approximately 5x106 base pairs. Bacteria are haploid, since they have a single chromosome, in contrast to human cells, which are diploid. Genes essential for bacterial growth are carried on the chromosome, and plasmids carry genes associated with specialized functions. **Plasmids, transposons, Is** (insertion sequences) are heredity extrachromosomal factors, which are the molecules of DNA with different molecular weight (kilobase pairs-kbp). Extrachromosomal factors are not genetic elements and do not carry the genetic information.

Plasmids were identified as small genetic elements capable of independent replication in bacteria and yeasts. The introduction of a DNA restriction fragment into plasmid allows the fragment to be amplified many times. There are: **F-plasmids** or fertility factor; **R-plasmids** resistance factor to antibacterial drugs, **bacteriocinogene** plasmids-they regulate synthesis of bacteriocines (eg, E. coli by synthesizing of colicines suppress the activity of other pathogens), plasmids of pathogenicity, etc.

Transposons are genetic elements that contain several kbp of DNA, including the information necessary for their migration from one genetic locus to another. Simple transposons, insertion sequences, carry only this genetic information. Complex transposons carry genes for specialized functions such as antibiotic resistance and are flanked by insertion sequences. Unlike plasmids, transposons do not contain genetic information necessary for their own replication.

Is – insertion sequences are transposon elements. The functions of Is are: **1.** Coordination of interaction between transposons, plasmids and temperate phages, ensuring their recombination. **2.** Inactivation of genes. **3.** Induction of mutation by deletions, insertions type.

MUTATIONS. SPONTANEOUS MUTATIONS

Mutation – is a change in the base sequence of DNA that usually results in insertion of a different amino acid into a protein and the appearance of an altered phenotype. Mutations result from three types of molecular changes:

The most common type of mutation involving single base pairs is **base substitution or point mutation**, in which a single base at one point in the DNA is replaced with different base. Then, when DNA replicates, the result is a substituted base pair. For example C-G might be substituted for G-C. If the base substitution results in an aminoacid substitution in the synthesized protein, this change in the DNA is known as a **missense mutation. Nonsense mutations** terminate synthesis of proteins and thus result in a protein truncated at the site of mutation. The gene products of nonsense mutations are usually inactive (Nonsense mutations almost always destroy protein function).

The second type of mutation is the **frame shift mutation.** This occurs when one or more base pairs are added or deleted, which shifts the reading frame on the ribosome and results in incorporation of the wrong amino acids “downstream” from the mutation and in the production of an inactive protein.

The third type of mutation occurs when transposons or insertion sequences are integrated into the DNA. These newly inserted pieces of DNA can cause profound changes in the genes into which they insert and in adjacent genes.

Mutation can be caused by **mutagens** (chemicals, radiation, viruses)**.**

The frequency of mutation is greatly enhanced by exposure of cells to mutagens. Ultraviolet light is a physical mutagen that damages DNA by linking neighboring thymine bases to form dimmers. Sequence errors can be introduced during enzymatic repair of this genetic damage. Chemical mutagens may act by altering either the chemical or the physical structure of DNA. Reactive chemicals alter the structure of bases in DNA.

Genetic recombination refers to the exchange of genes between two DNA molecules to form new combinations of genes on a chromosome. The three major forms of prokaryotic genetic exchange are distinguished by the form of the donor DNA: **conjugation. transduction, transformation.**

Transformation: Direct uptake of donor DNA by recipient cells depend on their competence for transformation. Natural occurrence of this property is unusual among bacteria, and some of these strains are transformable only in the presence of competence factors, produced only at a specific point in the growth cycle. The competent condition is in the logarithmic phase of growth. Transformation falls into **three phases**:

1. Adsorption of donor's DNA on the surface of recipient cell
2. Penetration DNA into the recipient cell
3. Integration DNA into the chromosome and recombination

Transduction: Transduction is phage – mediated genetic recombination in bacteria. There are **three types** of transduction:

- generalized
- specialized
- abortive

The generalized type occurs when the virus carries a segment from any part of the bacterial chromosome. **The specialized type** occurs when the bacterial virus DNA that has integrated into the cell DNA is excised and carries with it an adjacent part of the cell DNA. **Abortive type,** when phage carries a segment from the bacterial chromosome but it do not integrated into the recipient DNA, it is in the recipient's cell cytoplasm.

Conjugation: Conjugation is the mating of two bacterial cells during which DNA is transferred from the donor to the recipient cell. The mating process is controlled by an **F (fertility) plasmid** (F factor), which carries the genes for the proteins required for conjugation. One of the most important proteins is pilin, which from the sex pilus (conjugation tube). Mating begins when the pilus of the donor male bacterium carrying the F factor **(F+)** attaches to a recipient on the surface of the recipient female bacterium, which does not contain an F factor **(F-).** The cells are then drawn into direct contact by "reeling in" the pilus. After an enzymatic cleavage of the F factor DNA, one strand is transferred across the **conjugal bridge** into the recipient cell. The process is completed by synthesis of the complementary strand to form a double- stranded F factor plasmid in both the donor and recipient cells. The recipient is now an F+ male cell that is capable of transmitting the plasmid further. In this instance only F factor, and not chromosome, has been transferred.

Some F+ cells have their F plasmid integrated into the bacterial DNA and thereby acquire the capability of transferring the chromosome into another cell. These cells are called **Hfr (high – frequency recombination)** cells. During this transfer, the single strand of DNA that enters the recipient F-cell contains a piece of the F factor at the leading end followed by the bacterial chromosome and then by the remainder of the F factor. The time required for complete transfer of the bacterial DNA is approximately 100 minutes. Most mating result in the transfer of only a portion of the donor chromosome, because the attachment between the two cells can break the donor cell genes that are transferred vary, since the F plasmid can integrate at several different sites in the bacterial DNA. The bacterial genes adjacent to the leading piece of the F factor are the first and therefore the most frequently transferred. The

newly acquired DNA can recombine into the recipient's DNA and become a stable component of its genetic material.

Once the DNA is transferred from the donor to the recipient cell by one of the three processes just described, it can integrate into host cell chromosome by recombination. There are **two types of recombination**:

1. Homologous recombination, in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage reunion.

2. Nonhomologous recombination, in which little, if any, homology is necessary.

Different genetic loci govern these two types, and so it is presumed that different enzymes are involved. Although it is known that a variety of endonucleases and ligases are involved, the precise sequence.

ECOLOGY OF MICROORGANISMS
MICRO FLORA OF SOIL, WATER, AIR
NORMAL MICROFLORA OF THE HUMAN BODY

Microbes are distributed everywhere in the environment surrounding us. They are found in the soil, water, air, in plants, animals, food products, in the human body and on the surface of the human body.

The relationship of micro-organism with the environment has been named **ecology** (GK. Oikos - home, native land; logos - idea, science). This is an adaptive relationship. Micro-organisms have a remarkable ability to adapt themselves to certain environmental conditions.

In Ecological microbiology common concepts and ecological categories are used:

Biosphere is the living membrane of the earth

Biotop –is the limited area of the biosphere with the relatively constant conditions.

In nature micro-organisms constitute a component of the **biocoenosis** (a community of plants and animals living in a part of the habitat with more or less homogenous conditions of life).

Population -is the group of the same species of microorganisms in the same biotope which are described by heterogenicity.

Ecosystem: biotop and biocoenosis together form ecosystem.

Microbes are found in nature in associations among which there is a constant struggle for existence.

Among various groups of microbes there are several types of relationships: symbiosis, metabiosis, satellism, synergism, antagonism.

Symbiosis (living together) represents intimate mutually beneficial relation-organisms of different species. They developed together better than separately. Sometimes the adaptation of two organisms becomes so profound that they lose their ability to exist separately (e.g., symbiosis of the fungus and blue-green algae, nitrogen - fixing bacteria and cellulose - decomposing bacteria).

Metabiosis is that type of relationship in which one organism continues the process caused by another organism, liberating it from the products of life activities, and thus creating conditions for its further development (nitrifying and ammonizing bacteria).

Satellism during which one of the symbionts known as the favorable microbe incites the growth of the other (some yeasts and Sarcinae producing amino acids, vitamins, enhance the growth of microbes more strict in relation to nutrient media).

Synergism is characterized by the increase in the physiological functions of the microbial associations (Yeasts, Lactobacilli, Fusobacteria, and Borrelia organisms).

Antagonism during which there is a struggle for oxygen, nutrients, and habitat. On the mechanism of antagonistic relationships based the action of antibiotics: the products of the life activities of one bacterium have bactericidal action on another.

Commensalism: In the symbiotic relationship called commensalisms, one of the organisms is benefited and the other is unaffected. Many of the microorganisms that make up our normal microbiota are commensals. These bacteria live on secretions and sloughed of cells, and they bring no apparent benefit or harm to the host (for example, Corynebacteria that inhabit the surface of the eye).

Mutualism: Mutualism is a type of symbiosis that benefits both organisms. For example, the large intestine contains bacteria, such as E. coli, that synthesize vitamin K and some B vitamins. These vitamins are absorbed into the blood–stream and distributed for use body cells. In exchange, the large intestine provides nutrients for the bacteria so that they can survive.

Parasitism: Is a type of symbiosis that one organism is benefited at the expense of the other. Many disease – causing bacteria are parasites.

Human microflora is the result of a mutual adaptation of micro and macro-organisms in the process of evolution. Most bacteria of the normal and constant microflora of the human body have adapted themselves to life in certain parts of the body.

Microflora of the skin: The skin and mucous membranes always harbor a variety of microorganisms that can be arranged into two groups: **1. The resident flora** consists of relatively fixed types of microorganisms regularly found in a given area at given age. **2. The transient flora** consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks; it is derived from the environment and does not establish itself permanently on the surface.

The predominant resident microorganisms of the skin are Staphylococci (S. epidermidis, occasionally S. aureus); Streptococci (peptostreptococcus species, alpha hemolytic streptococci, enterococci); moulds, yeasts, diphtheroids; acid-fast, non-pathogenic Mycobacteria occur in areas rich in sebaceous secretions (genitalia, external ear).

Most frequently the exposed parts of the human body are infected, e.g. the hands on the surface of which different kinds of bacteria are found.

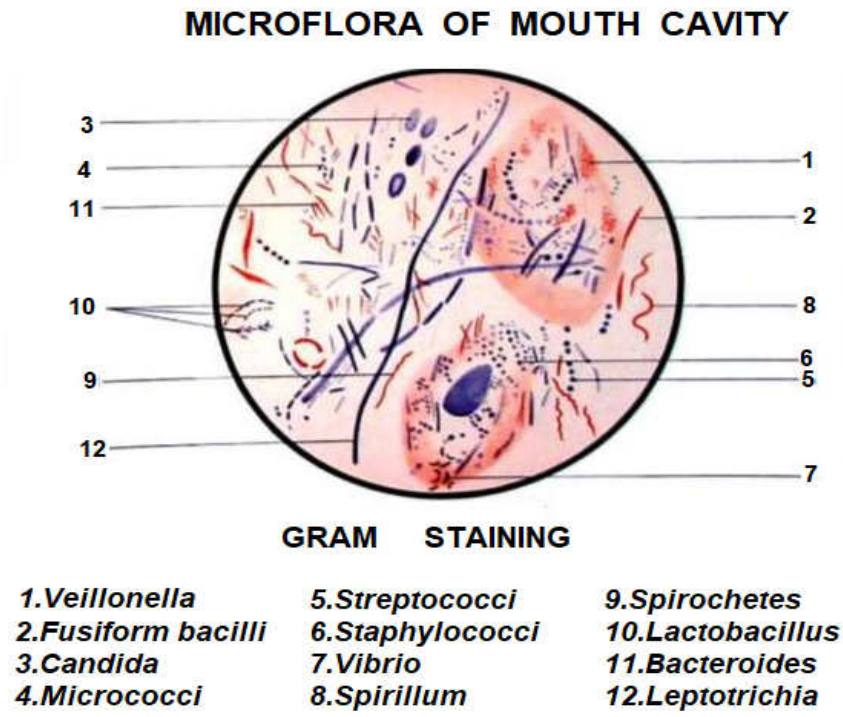
Among the factors that may be important in eliminating non-resident microorganisms from the skin are low pH, the fatty acids in sebaceous secretions, and presence of lysozyme.

Microbes of the mouth cavity: The mouth cavity is a favorable medium for many microbes; it has an optimal temperature, a sufficient amount of food substances, and has a weakly alkaline reaction.

In the mouth cavity there are more than 100 species of microbes. There are the natural inhabitants (acidophilic bacillus, Treponema microdentium, Streptococcus salivarius, Streptococcus mutans, Entamoeba gingivalis, etc.). Besides, in the mouth cavity there are foreign microbes or those which have been carried in from the environment together with food, water, and air.

Pathogenic and conditionally pathogenic microbes (Staphylococci, Streptococci, Diphtheria bacillius, Diphtheroids, Borrelia organisms, Protozoa: Amoebae and Trichomonads) are found on the mucous membrane of the mouth.

The greatest amount of microbes can be found at the necks of the teeth and in the spaces between teeth. The presence of carious teeth is a condition for increasing the microflora in the mouth cavity, for the appearance of decaying processes and unpleasant odors.



Microflora of the respiratory tract: People breathe in a large number of dust particles and adsorbed microorganisms. Experimentally, it has been established that the amount of microbes in inspired air is 200-500 times greater than in expired air. Most of them are trapped in the nasal cavity and only a small amount enters the bronchi. The pulmonary alveoli and the terminal branches of bronchi are usually sterile. The upper respiratory tract (nasopharynx, pharynx) contains relatively constant species (Staphylococcus albus, streptococci, diphtheroids).

Normal flora of the genitourinary tract: Soon after birth, aerobic lactobacilli appear in the vagina and persist as long as the pH remains acid (several weeks). When the pH becomes neutral a mixed flora of enterococci, streptococci, Staphylococci and Corynebacteria is present. After sexual maturation pH is low: 4.0-4.2 and Doderlain rods appear.

There are four categories of the vaginal flora: **I.** pH is acidic, presence of Doderlein rods, other type of microbes don't indicate. **II-III** categories: pH is low acidic the quantity of Doderlein rods is decreased,

Streptococci, Staphylococci are appear. **IV.** pH is alkaline, there are high amount of Streptococci, Staphylococci, vaginal flora often includes also Bacteriodes, Enterobacter. Leukocytes are indicated too.

Normal flora of the eye (conjunctiva): The predominant organisms of the conjunctiva are diphtheroids (Corynebacterium xerosis); Staphylococcus epidermidis, Neisseria and gram-negative bacilli resembling Hemophilus are also frequently present. The conjunctival flora is normally held in check by the flow of tears, which contain antibacterial lysozyme.

The microflora of the gastro-intestinal tract: When the stomach functions normally, it is almost devoid of microflora due to the marked bactericidal properties of gastric juice. The gastric juice is considered to be a reliable defense barrier against the penetration of pathogenic and conditionally pathogenic microbes into intestine.

The small intestine usually contains small numbers of streptococci, lactobacilli, yeasts, particularly Candida albicans.

In the large intestine there are large amount of microorganisms. Almost 1/3 of the dry weight of the feces of certain animal species is made up of microbes. More than 100 distinct types of organisms occur regularly in normal fecal flora.

The intestinal microflora undergoes essential changes with age of man. The intestinal tract of the new-born baby in the first hours of the life is sterile. During the first days it becomes inhabited by temporary microflora from the environment, mainly from breast milk. Later on, in the intestine of the new born baby a specific bacterial flora established consisting of lactic acid bacteria (biphidobacteria, acidophilic bacillus). It has antagonistic properties in relation to many microbes capable of causing intestinal disorders in breast – fed children, and remains during the whole period of breast feeding. However, on the 3-th/5-th day of the life, in the intestine of breast -fed children E. coli and enterococci can be found, the amount of which sharply increases with the change to mixed feeding. After breast feeding is stopped the microflora of the child's intestine is completely replaced by microflora typical of adults (E. coli, Clostridium perfringens, Clostridium sporogeneses, Streptococcus feacalis, Proteus vulgaris, etc.). In the normal adult colon, 96-99% of the resident bacterial flora consists of anaerobes Bacteriodes, Fusobacterium species; anaerobic lactobacilli, e.g. Biphidobacterium, Cl. perfringens, anaerobic gram-positive cocci: Peptostreptococcus. Only 1-4% is facultative aerobes.

Now it has been established that such a constant inhabitant of the intestine of human as Cl. perfringens is capable of secreting digestive enzymes. The colibacillus and other species of microbes in the intestine produce the vitamins essential for the human body (B1, B2, B12, K, D). Intestinal bacteria are important in antagonism to microbial pathogens (acidophilic, lactobacillus, E. coli cells produce bacteriocins, proteins that inhibit the growth of other bacteria, such as pathogenic Salmonella and Shigella, etc.). The normal flora of the intestinal tract plays a significant role in extraintestinal diseases. For example, E. coli is leading cause of urinary tract infections, Enterococcus feacalis (S. feacalis)) causes urinary tract infections and endocarditis, Pseudomonans aeruginosa, which can cause various infections, particularly in hospitalized patients with decreased host defense. P. aeruginosa is present in 10% of normal stool.

Antibiotic therapy can suppress the predominant normal microflora, thereby allowing a rare organism such as the toxin – producing Clostridium. difficile to overgrow and cause a severe colitis.

Soil micro-biota: The soil is of the main reservoirs of microbial life. The most numerous organisms in soil are bacteria. Typical garden soil has millions of bacteria in each gram. The population is highest in the top few centimeters of the soil and declines rapidly with depth.

For vegetative forms of bacteria soil is unfavorable environment. Human pathogens, which are mostly parasites generally, find the soil an alien, hostile environment. Even relatively resistant enteric pathogens, such as Salmonella species, have been observed to survive for only a few weeks or months when introduced into soil. Most human pathogens that can survive in soil are endospore-forming bacteria. For example, endospores of Bacillus anthracis, which causes anthrax in animals, can survive in certain soils for decades before finally germinating when ingested by grazing animals. Disposing of the body of animal infected with anthrax requires care so that the soil is not seeded with the endospores from the dead animal.

Clostridium tetani (the causative agent of tetanus), Clostridium botulinum (the causative agent of botulism), Clostridium perfringens (the causative agent of gas gangrene) are all endospore-forming pathogens whose normal habitat is the soil. From soil they are introduced into foods or wounds, where they grow and produce toxins. Soil is the source of infections: wound infections, enteric infections, and food-toxinfections by contaminating vegetables, fruits.

Existence of **E. coli, Clostridium perfringens, Enterococcus feacalis** in the soil indicate the fecal contamination of the soil. E**. coli and Enterococcus fecalis (S. feacalis**) are indicators of **fresh** contamination; **Enterobacter and Citrobacter** are indicators of **not fresh** contamination. **C.perfringens** is the indicator of **old** contamination. Indicators of exact valuation (appreciation) soil microbial contamination are Coli (perfringens) titer, Coli (perfringens) index and microbial number.

Coli (perfringens) index is the quantity of microorganisms in one gram of soil.

Coli (perfringens) titer is the mass of soil (in grams) in which one microorganism is revealed.

Microbial number: quantity of saprophytes, thermophiles and nitrifying bacteria in one gram of soil.

Soil contamination depends on type and structure of the soil, concentration of mineral and organic substances, temperature, pH, humidity concentration of carbon dioxide, osmotic pressure, physical-chemical condition.

Aquatic microorganisms: Large numbers of microorganisms in water generally indicate high nutrient levels in the water. Water contaminated by inflows from sewage systems from biodegradable industrial organic wastes is relatively high in bacterial counts. Some pathogens can transmit through water: Typhoid fever, cholera, which is caused by bacteria, hepatitis A which is caused by a virus. Water is unfavorable for pathogenic and conditionally pathogenic bacteria and survival period depend on their type contaminated dose, temperature of the water, presence of organic substances and the composition of saprophytes. Endospores of Bacillus anthracis can survive during years; Salmonella, Leptospira, Hepatitis A virus-months; Shigella (causative agent of dysentery), Vibrio cholera (causative agent of cholera), Brucella (causative agent of brucellosis)-days, weeks.

Sanitary microbiological condition of the water is appreciated by:

Microbial number: quantity of mezophilic chemoorganotrophes in one milliliter of water (till 100).

Coli-titer: the minimum quantity of water which contains one E. coli (>300ml).

Coli index: quantity of E. coli in one liter of water (<3).

Air microorganisms- Air is not favorable for microorganisms (absence of nutrients, sufficient humidity), but in closed areas microbes can survive and air can play the role of transmission of the infections (droplet transmission).

Sanitary microbiological condition of the air is appreciated by:

- microbial number: quantity of microorganisms in $1m^3$ of air .
- sanitary demonstrating microorganisms of the air: **α (S. pneumonia) and β (S. pyogenes) hemolytic streptococcus, Staphylococcus aureus (golden staphylococcus).**

THE ROLE OF NORMAL MICROFLORA

Non-specific defense factor	*Provides several vital processes*	*Stimulation of formation of immune system*
Antagonism against pathogenic microorganisms.	Participate in digestion. Contribute to absorption of Ca, Fe, vitamin D.	Stimulation of functional activity.
Inhibition of synthesizing of thermolabile exotoxin by enteropathogenic E. coli.	Participate in neutralization of endogenous and exogenous toxic products.	
Blockade the receptors of intestinal membranes.	Participate in synthesizing of amino acids proteins vitamins, nicotinic acid.	

NORMAL MICROFLORA OF LARGE INTESTINE

Mucous flora	Lumen flora
Bifidum bacteria, Lactobacteria	Non-spore forming anaerobes (96-99%): gram positive Bifidobacterium species, gram negative Bacteriodaceae family.
	Facultative anaerobes (1-4%): gram negative E. coli, gram positive Enterococci and non- spore forming Lactobaccilus species.
	Residual flora (0.01-0.001%): Staphylococci, Proteus, Candida, Clostridium, Pseudomonas.
	Different species of Enterobacteriacea family: Salmonella, Shigella, Enterobacter, which can cause intestinal infections.

P H Y S I O L O G Y
O F B A C T E R I A

NUTRITION OF BACTERIA

The bacterial cell has the same general chemical pattern as the cell of the organisms. The principal constituent of bacterial cells is water, which represents about 80 per cent of the total weight. Proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds make up rest.

A constant exchange of compounds with the surrounding environment is inherent in all organisms. To carry out the processes of nutrition and reproduction certain conditions are necessary: the presence of food material from which microbes synthesize the component parts of their cell, and, by oxidation of different substances receive the required energy.

Bacterial metabolism also is closely similar to that of the other organisms, exemplifying the "unity of biochemistry". There are, however, some differences which are exploited in selective toxicity and chemotherapy. The main peculiarities of bacterial nutrition are the following:

1. microorganisms haven't digestive organs.
2. anabolism and catabolism are very intensive.
3. the whole surface of the bacterium cell participates in the nutrition process.
4. bacteria have an expressed adaptation to the nutritive conditions of the environment

All organisms, including microbes, can be classified metabolically according to their nutritional pattern – that is, their source of energy and their source of carbon and nitrogen. Considering the energy source bacteria generally classified as ***phototrophs or chemotrophs.***

Bacteria which derive their energy from sunlight (solar energy) are called **Phototrophs**.

Chemotrophs obtain energy from chemical reactions (oxidation-reduction reactions of inorganic or organic compounds).

For growth and multiplication of bacteria the minimum nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts. Water is the vehicle for the entry of all nutrients into the cell and for the elimination of all waste products. It participates in the metabolic reactions and also forms an integral part of the protoplasm.

Bacteria by their principal carbon and nitrogen source are subdivided into (table 1):

Autotrophs (self-feeders): Some organisms can utilize very simple inorganic compounds, such as carbon dioxide as carbon source and ammonium salts as nitrogen source.

Heterotrophs (feeders on others; GK-heteros-another) are unable to synthesize their own metabolites and depend on preformed organic compounds. They require an organic source of carbon, such as glucose, and obtain energy by oxidizing or fermenting organic substances. Often, the same substance (for example glucose)is used as both the carbon source and energy source. All bacteria that inhabit the human body fall into the heterotrophic group.

Autotrophs are also referred to ***lithotroph****s* (rock eating; GK-lithos-rock, stone; trophe-nutrition), and heterotrophs are referred to ***organotrophs.*** Based on the combination of the energy and carbon sources microrganisms are classified into:

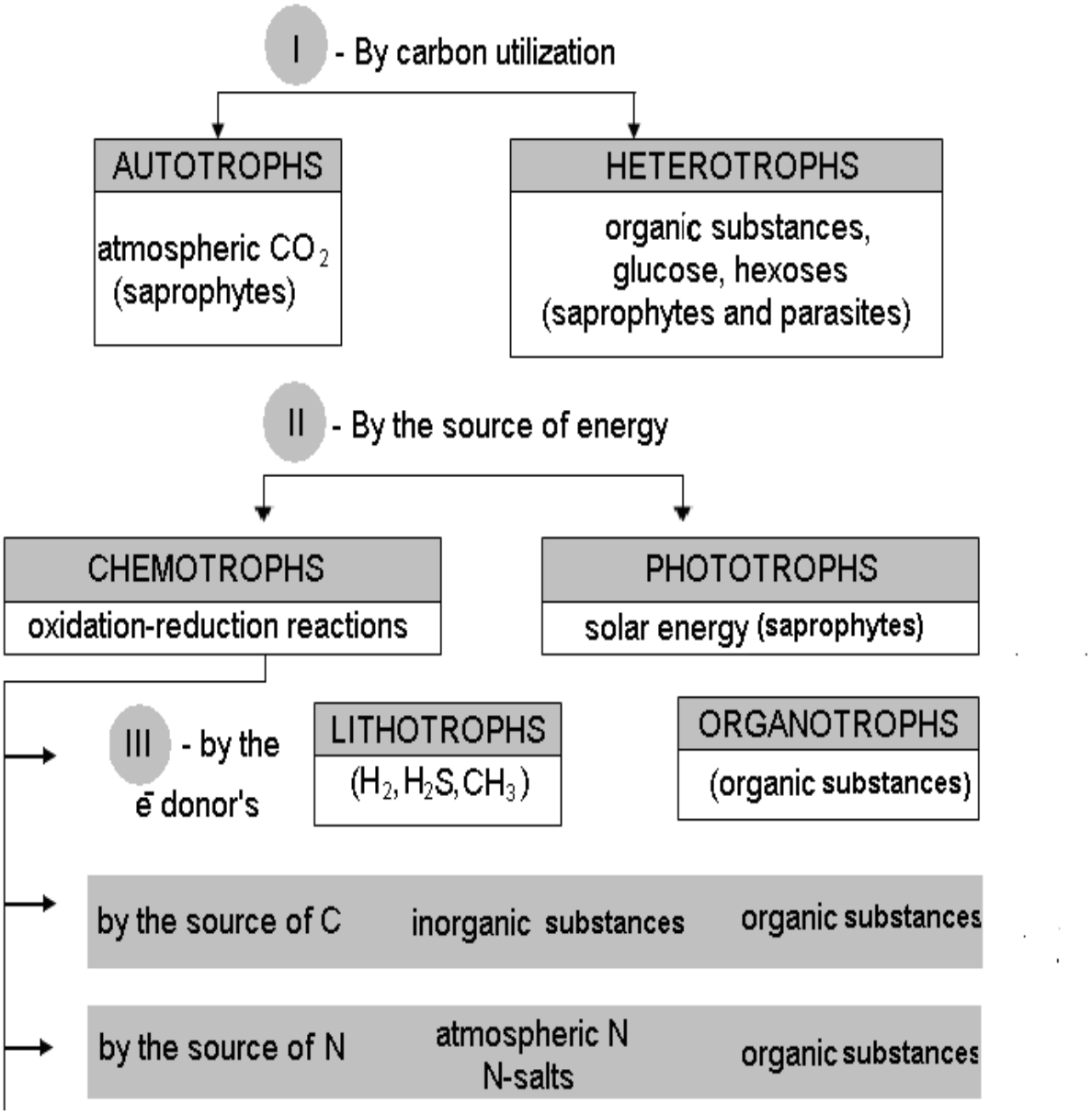
Photoautotrophs- the source of energy is solar energy, the source of carbon and nitrogen are inorganic compounds-CO_2, NO_2.

Photoheterotrophs – the source of energy the same, the source of carbon and nitrogen are organic compounds.

Chemoautotrophs- they receive energy from the oxidation of inorganic substances, the source of carbon is CO_2.

Chemoheterotrophs –the source of energy and carbon are organic compounds. Almost all of the medically important microorganisms are chemoheterotrophs. Typically, infectious organisms catabolize substances obtained from the host.

Table 1



Growth factors – beside peptones, carbohydrates, fatty and inorganic elements bacteria require the special substances **growth factors,** which act as catalyst in the biochemical cellular processes and are structural units for the production of certain enzymes. Some microbes do not require a supplement of growth factors to the nutrient medium as they are able to synthesize these compounds themselves. These bacteria are named ***prototrophs*.** Others grow poorly on growth factors free media; they are unable to synthesize growth factors themselves. These bacteria are named ***auxotrophs.***

Growth factors are:

- vitamins
- amino acids
- purines, pyrimidines
- lipids (cholesterol and other sterols)
- ferroporphyrins (Fe-porphyrins)

THE MECHANISMS OF NUTRITION

Entrance of nutrients into bacterial cell is a complex of physical-chemical processes and several factors promote these processes: different nutrient's concentrations, molecule size, their dissolution in water and lipids, pH of environment, penetration through membranes, etc.

There are **four mechanisms** of nutrient penetration into the cell:

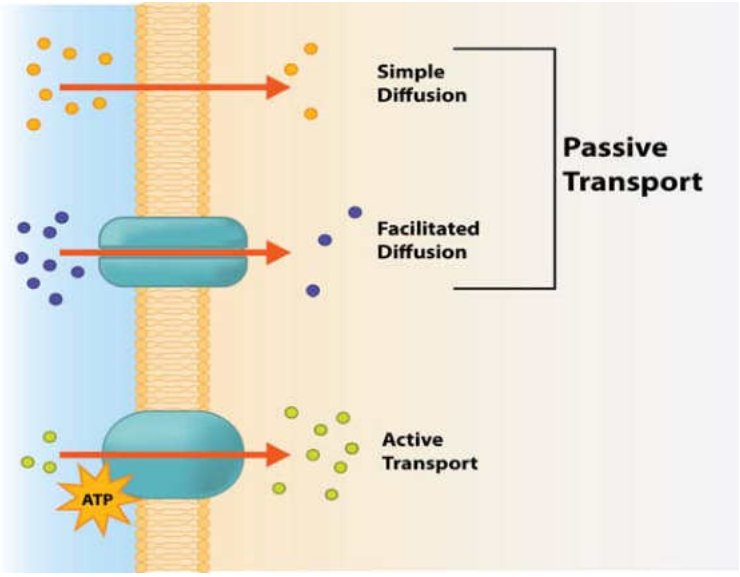
1.Passive diffusion. Penetration of substances occurs due to the difference in nutrient concentrations inside and outside the cell. Energy expenditure is absent because it is realized from large concentration to the low. Water, oxygen, CO_2, penetrate by this mechanism.

2.Light diffusion (facile). Penetration occurs due to nutrient large concentration out of the cell. Membrane enzymes-translocases take part in these processes. At this time energy expenditure is absent, as the nutrients pass from the environment having high nutrient concentration to the one having low concentration (penetration over the gradient).

3.Active diffusion. It occurs when the nutrient concentration is low in the environment and the molecule transfer is realized from the environment having low concentration into the one having a high concentration (i.e. in the opposite direction). Enzymes (permease) take part in these processes too. Due to this the active diffusion is accompanied with energy expenditure and the cell use ATP, which is accumulated by the oxidation – reduction reactions.

4.Translocation of radicals. Is an active process and it is accompanied with energy expenditure and enzymes participate in this process. Chemically changed molecules, that can't pass through the membrane in their unchanged structure, can also pass in the condition of active diffusion.

The mechanisms of nutrition



ENZYMES AND THEIR ROLE IN METABOLISM

Enzymes organic catalysts of a highly molecular structure are produced by the living cell. They are of protein nature, are strictly specific in action, and play an important role in the metabolism of micro-organisms. Their specificity is associated with active center formed by a group of amino acids. The names of enzymes usually end in – ase. The bacterial enzymes can be grouped into six classes; according to the type of chemical reaction they catalyze (table1).

Table 1. Enzyme classification based on type of chemical reaction catalyzed.

CLASS	TYPE OF CHEMICAL REACTION CATALYZED	EXAMPLE
1.Oxidoreductase	Oxidation-reduction in which oxygen and hydrogen are gained or lost	Cytochrome oxidase, lactate dehydrogenase
2. Transferase	Transfer of functional groups such as an amino group, acetyl group, or phosphate group	Acetate kinase, alanine deaminase
3. Hydrolase	Hydrolysis addition of water	Lipase
4. Lyese	Removal of groups of atoms without hydrolyses	Oxalate decarboxylase
5. Isomerase	Re-arrangement of atoms within a molecule	Glucose-phosphate isomerase alanine racemase
6. Ligase	Joining of two molecules using energy usually derived from breakdown of ATP	Acetyl–CoA synthetase, DNA ligase

Bacterial enzymes are subdivided into following groups:

- **Exo-enzymes** are excreted by the cell into the environment by breaking down complex colloid nutrient materials (hydrolase).
- **Endo-enzymes** are contained inside the cell (catalase). Some of the endoenzymes in the bacterial cell act separately (monoenzymes); other enzymes are closely linked to each other (multienzymatic system) and provide a sequence of metabolic processes (enzymes of respiratory system).

Depending on the conditions of origin of enzymes there are:

- **Constitutive enzymes** which are constantly found in the cell irrespective of the presence of a catalyzing substrate (enzymes which take part in metabolic processes). These include the main enzymes of cellular metabolism (lipase, carbohydrase, oxidase, and catalase).
- **Adaptive enzymes** occur only in the presence of the corresponding substrate (β-lactamase, which break down penicillin, decarboxylase and etc.).

Bacteria possess virulence providing enzymes which promote bacteria penetration into the host cell and spreading (hyaluronidase, neuraminidase, plasmocoagulase, fibrinolysin).

Functional activity of enzymes depends on environmental conditions (temperature, pH, substrate concentration). The rate of most chemical reactions increases as the temperature increases. Molecules move more slowly at low than at higher temperatures and may not have enough energy to cause a chemical reaction. Most enzymes have a pH optimum at which their activity is characteristically maximal. Enzyme activity and therefore the reaction rate declines
above or below this pH value. Extreme changes in pH can cause denaturation. There is a maximum rate at which a certain amount of enzyme can catalyze a specific reaction. Only when
the concentration of substrate(s) is extremely high can this maximum rate be attained.

Bacteria vary in their requirement of **temperature** for growth. For each species, there is a temperature range and grow does not occur above the maximum or below the minimum of this range. Microorganisms are divided into three groups on the basis of their preferred range of temperature:

Mesophiles (moderate-temperature-loving microbes). Most of pathogenic bacteria grow within 36º - 37º C. For pathogenic microorganisms the optimum temperature is 37º C.

Psychrophiles (cold-loving) are those that grow best at temperatures below 20º C. They are soil and water saprophytes, though not of direct medical importance, may cause spoilage of refrigerated food.

Thermophiles (heat loving) – grow best at high temperatures 55-80º C (they can cause spoilage of food).

Bacteria are sensitive to variations in **pH.** Each species has a pH range, above or below which it cannot survive, and an optimum pH at which it grows best.

The majority of pathogenic bacteria grow best at neutral or slightly alkaline reaction (pH (7.2 to 7.6). Very few bacteria (for example M. tuberculosis) grow at an acidic pH below about 4.0. Some of bacteria like Vibrio cholera grow at alkaline pH about 8.5-9.0. They are very sensitive to acids.

RESPIRATION IN BACTERIA

Respiration in bacteria is a complex process which is accompanied with the liberation of energy required by the microorganism for the synthesis of different organic compounds.

All microbes according to type of respiration can be subdivided into:

1. **Obligate aerobes, w**hich will grow in the presence of oxygen, develop well in an atmosphere containing 21 per cent of oxygen. They require oxygen to grow because their ATP-generating system is dependent on oxygen as the hydrogen accepter. They grow on the surfaces of liquid (they form pellicle) and solid nutrient media (brucellae, tubercle bacilli, etc.).
2. **Facultative anaerobes.** They utilize oxygen to generate energy by respiration if it is present**,** and can be reproduced even in the absence of molecular oxygen (the majority of pathogenic and saprophytic microbes). They may act in both ways.
3. **Obligate anaerobes** for which the presence of molecular oxygen is a harmful growth-inhibiting factor, because they lack either superoxide dismutase or catalase, or both. Obligate anaerobes vary in their response to oxygen exposure; some can survive but are not able to grow, whereas others are killed rapidly, because of the result of accumulating of hydrogen peroxide. The catalase which splits hydrogen peroxide is absents in anaerobes and hydrogen peroxide kills the bacteria (causative agents of tetanus, botulism, gas gangrene anaerobic infections, etc.). Anaerobic bacteria use as electron acceptors compounds such as **nitrates** (facultative anaerobes-for example E coli) or **sulphates** instead of oxygen (anaerobic respiration).
4. **Microaerophiles-** microorganisms which can develop well in a presence of one per cent of oxygen.
5. **Aerotolerant organisms** such as Clostridium histolyticum; can survive shortly but not multiply in oxygen conditions.

6. Capnophilic (Helicobacter) are microorganisms that metabolically active (thrive) in the presence of high concentrations of carbon dioxide (CO2)

GROWTH AND MULTIPLICATION OF BACTERIA

Bacteria normally reproduce by binary fission. The first step in division is cell elongation and duplication of the chromosomal DNA. The cell wall and cell membrane then begins to grow inward from all sides at a point between the two regions of the chromosomal DNA. Eventually, the in-growing cell walls meet, and two individual cells are formed, each of which is essentially identical to parent cells. Because one cell gives rise to two progeny cells, bacteria are said to undergo exponential growth (logarithmic growth).

The concept of exponential growth can be illustrated by the following relationship:

Number of cells	-	1	2	4	8	16
Exponential	-	2^0	2^1	2^2	2^3	2^4

The time required for a cell to divide (and its population to double) under optimum conditions is called the **generation time or population doubling time**. It varies considerably among organisms and with environmental conditions such as temperature, the pH and other. Most medically important bacteria have generation time 20-30 minutes. Some bacteria are slow-growing; the generation time is 15- 48 hours (e.g. M. tuberculosis). When pathogenic bacteria multiply in host tissues, the situation may be intermediate between a **batch** cultures (nutrient are not renewed, nor are waste products removed. Under these conditions, the cell population increases in number in a predictable fashion and then eventually declines) and a **continuous** culture (nutrients must be continuously added and waste products removed); the source of nutrients may be inexhaustible but the parasite has to contend with the defense mechanisms of the body. Bacteria growing on solid media form colonies. **Colony** is visible accumulation of generation of one mother cell on solid nutrient media. Each colony represents a clone of cells derived from single parent cell. **Clone** is a population derived by binary fission from a single cell. Description of colonies by size, form, consistency, surface, pigments, the form of ends, transparency has differential diagnostic meaning.

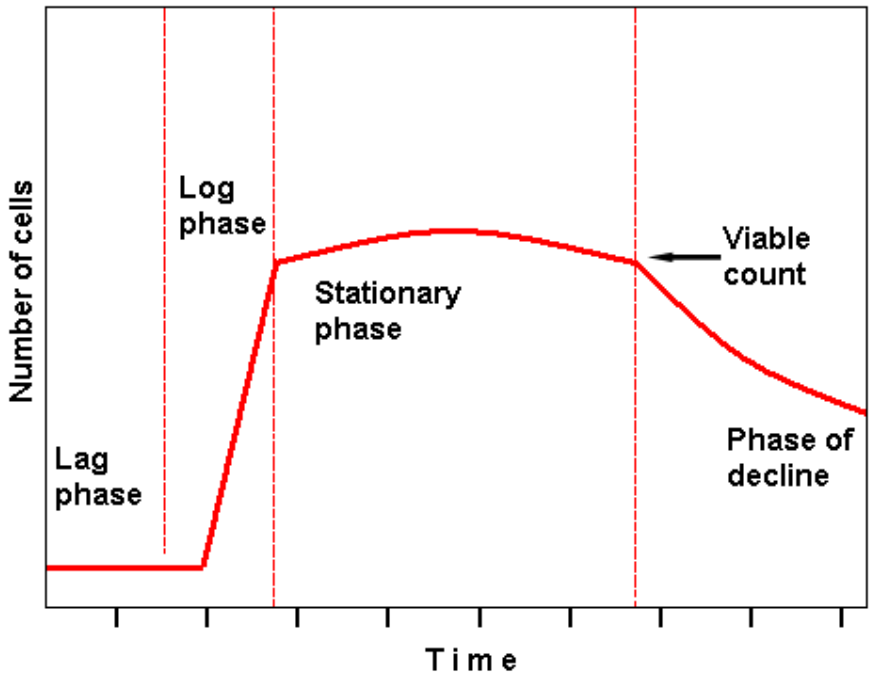
The **growth** of microorganisms represents the increase of the mass of bacterial cytoplasm as a result of the synthesis of cellular material. Bacteria generally **multiply** by the process of **binary fission (**in some cases by budding; viruses by reproduction). After a bacterial cell has increased in size and doubled all of its parts, it

divides. One cell divides into two; those two divide to become four, and so on. In other words, the increase in cell numbers is exponential. DNA replication is an important condition in the process of amitotic binary fission of bacteria; the hydrogen bonds are ruptured and two DNA strands are formed, each one is contained in the daughter cells. The single stranded DNA is eventually linked by means of hydrogen bonds and again forms double-chain DNA responsible for genetic information.

In liquid media growth is diffuse. When a few bacteria are inoculated into a liquid growth medium and population is counted at intervals, it is possible to plot a bacterial growth curve that shows the growth of cells over time.

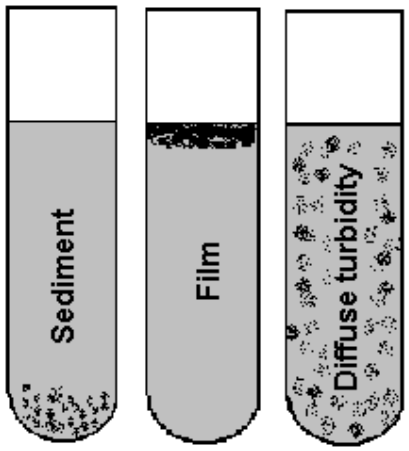
There are four basic phases of growth:

- **Lag phase:** when bacteria are seeded into fresh medium during which the number of cells are changed very little because the cells do not immediately reproduce in a new medium. During this period the organisms adapt themselves to growth in fresh medium and increase in size and metabolic activity (it can last for an hour or several days). During this time, however, the cells are not dormant. The microbial population is undergoing a period of intense metabolic activity involving, in particular, DNA and enzyme synthesis.
- **Log phase or exponential phase.** Eventually, the cells begin to divide and enter a period of growth multiplying at their maximum rate and their number is increased exponentially or by geometric progression with time. Cellular reproduction is most active during this period, and a generation time reaches a constant minimum. The log phase is the time when cells are most active metabolically. Beta-lactam drugs, such as penicillin, act during this phase because the drugs are effective when cells are making peptidoglycan, i.e., when they are dividing. Exponential phase is off limited duration because of: **1.** Nutrients exhaustion. **2.** Accumulation of toxic metabolic end products. **3.** Rise in cell density. **4.** Change in pH. **5.** Decrease in oxygen tension (in case of aerobic organisms).
- **Stationary phase:** this phase occurs when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die, resulting in a steady state. Cells grown in a special apparatus called a thermostat**,** into which fresh nutrients are added and from which waste products are removed continuously, can remain in the log phase and do not enter the stationary phase.
- **Death phase (phase of decline) –** usually, the number of deaths soon exceeds the number of new cells formed, and the population enters the death or logarithmic decline phase. This phase continues until the population is diminished to a tiny fraction of the number of cells in the previous phase, or the population might die out entirely. Finally, after a variable period, all the cells die and culture becomes sterile.



In liquid media the growth and multiplication of bacteria expressed by:

- Diffuse turbidity
- Pellicle (film) formation
- Sediment formation



Growth and multiplication of bacteria in broth

A few bacterial species reproduce by **budding** (Mycoplasma), that is, they form a small initial outgrowth that enlarges until its size approaches that of the parent cell, and then it separates. Some filamentous bacteria (certain Actinomycetes) reproduce by producing chains of spores carried externally at the tips of the filaments. A few filamentous species simply fragment, and the fragments initiate the growth of new cells.

I N F E C T I O N

THE STUDY OF INFECTION, INFECTIOUS PROCESS. PATHOGENICITY. VIRULENCE, TOXIGENICITY. MICROBIAL TOXINS. FORMS OF INFECTIONS AND CHARACTERISTICS. DEVELOPMENT OF THE INFECTIOUS PROCESS. PECULIARITIES OF VIRAL INFECTIONS.

The term infection (lat.- infectio to infect) signifies the sum of biological processes which take place in the macro-organism (host organism) upon the penetration of pathogenic microorganism into it that is interaction between the animal body (host) and the infecting microorganism.

Based on their relationship to their hosts, microorganisms can be classified into:

SAPROPHYTES (Sapros decayed, phiton plant), and ***PARASITES*.**

Saprophytes are free-living microbes. They are found in soil and water and play important role in the degradation of organic materials in nature. Saprophytes live on dead organic matter. *Parasites* are microbes that can establish themselves and multiply in hosts, derive nutrients from a living host. Parasitic microbes may be either *pathogens (*Greek pathos suffering; and gene produce, that is, disease-producing) or *commensals (*Latin – com with; and mensa -living together). **Commensals** live in complete harmony with the host without causing any damage to it. The normal bacterial flora of the body consists largely of commensals. Many commensals behave as facultative pathogens in that they can produce disease when the host resistance is lowered. **Pathogens** are microorganisms that are capable of producing disease in host. They are to types: opportunists and primary pathogens. **Opportunistic** pathogens rarely cause disease in individuals with intact immunological and anatomical defenses. In immunocompromised hosts these bacteria are able to cause disease. For example, E. coli is normally carried in human intestine. If they enter into urinary tract they lead to urinary tract infection. **Primary pathogens** are organisms which are capable of causing disease in previously healthy individuals with intact immunity.

For the development of infectious processes it is necessary presence of three sections: *pathogenic microorganism*, *susceptible macroorganism (host)* and *corresponding environment*.

For the development of infectious processes very important the ***infectious dose*** – the minimum quantity of microbe's cells which can cause infectious process. The infectious dose is varies greatly among the pathogenic bacteria and it depends on the type of bacteria, virulence, etc. (E.g., the infectious dose for S. typhi is 10^5; for the V. cholerae - $10^{11)}$.

Pathogens can gain entrance to the human body and other hosts through several avenues (routs), which are called ***portals of entry***. Many pathogens have a preferred portal of entry that is a prerequisite to their being able to cause disease. If they gain access to the body by another portal, disease might not occur. For example, the bacteria of typhoid fever (Salmonella typhi) produces all the sings and symptoms of the disease when swallowed (preferred route), but if the same bacteria are rubbed on the skin, no reaction occurs, or Streptococci that are inhaled (preferred route) can cause pneumonia; those that are swallowed generally do not produce sign or symptoms. Some pathogens, such as Yersinia pestis (plague), Staphylococci, the microorganism can initiate disease from more than one portal of entry.

The most important *portals of entry* for pathogens are mucous membranes, skin, respiration tract, gastrointestinal tract, genital tract.

In nature infectious diseases are subdivided into *exogenous* and *endogenous*.

E**xogenous infections**-the causative agent penetrates into the macro-organism from the environment (from patients, carriers, from foodstuffs, water, air, soil and etc.).

Endogenous diseases (auto-infections) originate as a result of the activation of the indigenous microbes of the body due to disturbances of the internal medium of the macro-organism as a result of external factors and social conditions. The state of autoinfection is quite a wide spread phenomenon.

When infection occurs with one species of causative agent we speak about ***mono*** infection. When infection occurs not with one species of causative agent, but with two or more, this is ***mixed*** infection.

In some cases infection causes a weakening of the body which then becomes susceptible to other diseases. Thus e.g., after influenza or measles pneumonia occurs. This is known as *secondary infection*. There are also ***local*** and ***generalized*** infections. For example, during infection with staphylococcus, the

infectious process causes furunculous (local infection), and if the causative agent penetrates into the blood sepsis will develop (generalized infection).

Re-infection is a repeated infection by the same species of microbe responsible for the disease which terminated in convalescence (gonorrhea, syphilis, etc.). ***Super-infection*** is a fresh infection (with the same bacteria) of the body in which the main disease has not ended. ***Relapse*** is a return of the symptoms of the same disease (relapsing fever, paratyphoid fevers, etc.). Of certain significance in the occurrences of relapses is the low level of immunological activity of the organism during illness and convalescence.

According to the extent of spread infectious diseases may be ***sporodic*** (separate diseases observed in a given area during a certain length of time). A considerable increase in the level of sporadic incidence of a given disease is known as an epidemic, when the epidemic reaches an unusually large size in some country or spreads over many countries or even continents, it is called a ***pandemic***. Besides, a special form of spread of infectious diseases exist known as an ***endemic,*** in which infectious diseases are refined for a long time in some locality (yellow fever, tularemia; etc.).

In some cases infected individuals do not develop disease symptomology. Such individuals are called asymptomatic carriers or are simply referred to as **carriers**. Although they do not become sick themselves, carriers are an important reservoir of infectious agents. Carrier state with duration of 3 months is considered *acute*, while carrier state for longer periods is considered *chronic.*

Another useful way of defining the scope of a disease is in terms of its severity or duration. **An acute disease** is one that develops rapidly but lasts only a short time (for example influenza). **A chronic** disease develops more slowly, and the body's reactions may be less severe, but the disease is likely to be continual or recurrent for long, periods (tuberculosis, infectious mononucleosis, syphilis).

A disease that is intermediate between acute and chronic is described as a **subacute disease** (subacute sclerosing panencephalitis).

A latent disease is one in which the causative agent remains inactive for a time but then becomes active to produce symptoms of the disease (shingles, one of the diseases caused by the varicella-zoster virus).

The transmission of disease involves the movement of pathogens from a source to the appropriate portal entry. The source of infections agents is known as the **reservoir.** In some cases, the reservoir of human pathogens is animals. Diseases for which animals are the reservoirs are called **zoonoses (**brucellosis, tularemia, anthrax). The principal living reservoir of human disease is the human body itself. Many people harbor pathogens and transmit them directly or indirectly to others. These diseases are called **anthroponose** (cholera, gonorrhea, syphilis). The two major nonliving reservoirs of infectious disease are soil and water. Diseases for which environment is the reservoir are called **sapronose.**

PECULIARITIES OF INFECTIOUS DISEASES. TYPICAL STAGES OF INFECTIOUS DISEASES

Infectious diseases differ from somatic diseases, which are expressed in the following features:

1. Specificity - each infectious disease caused by the specific (concrete) bacteria – presence of specific etiological agent –pathogenic microorganism. **2.** Contagious – indicates that a pathogen will move with ease from one infected individual to the next. **3.** Cyclic trend obligatory presence of incubation period. **4.** Each infection has specific symptoms. **5.** Formation of immunity during infectious process.

A typical acute infectious disease has **four stages**:

1.The incubation period, which is the time between the acquisition of the microorganism (or toxin) and the beginning of a symptoms. This time varies from hours to days to weeks depending on the microorganism, virulence and quantity of microorganism, and the resistance of the host. E.g. the incubation period for the botulism is 6-12 hours, for cholera 7 days, for AIDS months and years. As a rule discharges of microbes is not detected.

2.The prodrome period is a relatively short period (duration 3-5days) that follows the period of incubation. In this period intensive growth and multiplication of microbes is occur, they colonize tissues and they begin to synthesize toxins and enzymes. The prodromal period is characterized by early, mild symptoms (nonspecific symptoms) such as general aches, fever, malaise and loss of appetites. Only in measles very specific symptom such as Koplik's spots appears.

3.The specific - illness period, during which the disease most acute. The person exhibits overt is detected characteristic signs and symptoms of the disease. These depend on intensive growth and multiplication of microorganisms, synthesizing of enzymes and toxins, destruction of tissues and accumulation of toxic metabolites. In this period appearance of specific antibacterial and antitoxic antibodies and increasing of the titer of immunoglobulins is detected. As a rule discharging of microbes is detected. These persons are high infective for others.

4.The recovery period (convalescence), during which the illness abates and the patient returns to the healthy state. Duration of this period is depends on state of the organism, quality of treatment and rehabilitation measures.

THE CAUSATIVE AGENTS OF INFECTIONS AND THEIR FEATURES

Infections are caused by pathogenic micro-organisms which are capable attaching (adherence) to host tissues, colonizing defeated parts.

Pathogenicity – is the potential capacity of certain species of microbes to cause an infectious process in susceptible organism. Pathogenicity is a **specific** character of pathogenic microbes (depends on **genotype)**. This is **polydeterminant** property which depends on biological active substances (lipids, proteins, carbohydrates) which present in the structure of bacterial cell and depends on enzymes and toxins which they produce. Pathogenic microbes are characterized by a specific action. Each species is capable of giving rise to a definite infectious process.

Virulence – signifies the degree of pathogenicity of the given culture (**phenotypic property**). Virulence is a quantitative measure of pathogenicity and it measured by the number of organisms required to cause disease. The virulence of microbe is often expressed as the **LD50** (lethal dose for 50 % of hosts), the number of microbes in a dose that will kill 50 % of inoculated test animals under normal conditions; **DLM** (dosis letalis minima lethal dose for 95 % of hosts).

THE FACTORS OF VIRULENCE ARE:

Adherence – (attachment) is an important step in pathogenicity. The attachment between pathogen and host is accomplished by means of surface molecules on the pathogen called adhesions or ligands that bind specifically to complementary surface receptors on the cells of certain host tissues. Adhesins may be located on a microbe's glycocalyx or on the other microbial surface structures, such as pili (fimbriae). The majority of adhesins on the microorganisms are glycoproteins, lipoproteins, lipoteichoich acid, fimbriae and capsule.. The receptors on host cells are typically sugars, such as mannose. Adhesins on different strains of the same species of pathogen can vary in structure. Different cells of the same host can also have different receptors that vary in structure. If adhesins, receptors, or both can be altered to interfere with adherence, infection can often be prevented (or at least controlled). Receptors of the host are following: **native, inductive and acquired.**

1.native – are on the epithelial cells and participate in adhesion of specific bacteria

2.inductive can appear only after absorption of viruses on susceptible cells

3.acquired – appear during special conditions. These receptors are bridges between epithelial cells and bacteria. The role of bridges plays immunoglobulin, components of complement system.

Kolonization – the growth and reproduction of bacteria.

Invasion and penetration: Invasion when the microorganism penetrates through mucous and connective barrier (Staphylococcus, Streptococcus). Several enzymes secreted by invasive bacteria play role in pathogenesis. Among the most prominent are: hyaluronidase, neuraminidase, collagenase, which degrade collagen and hyaluronic acid, respectively, thereby allowing the bacteria to spread through subcutaneous tissue. **Penetration** – when the microorganism penetrates into epithelial cells, leukocytes and lymphocytes (Shigalla, E.coli).

Aggression – several virulence factors contribute to invasiveness by limiting the ability of the host defense mechanisms, especially phagocytes, to operate effectively.

The most important of these antiphagocytic factors is the capsule external to the cell wall of several important pathogens such as S. pneumoniae and Neisseria meningitidis.

A second group of antiphagocytic factors are the cell wall proteins of the gram-positive cocci, such as the **M protein** of S. pyogenes and protein A of S. aureus. The M and A proteins are antiphagocytic, they bind to Fc fragment of IgG and prevents the activation of complement and inhibit phagocytosis.

The third group factors which suppress the host defense are **enzymes**: a) protease, which degrades IgA, allowing the organism to adhere to mucous membranes and is produced chiefly by N. Gonorrhea, S. pneumonia and etc. b) coagulase, which is produced by S. aureus and accelerates the formation of a fibrin clot from its precursor, fibrinogen (this clot may protect the bacteria from phagocytosis by walling off the infected area and by coating the organisms with a layer of fibrin) c) leukocidins, which can destroy both neutrophilic leukocytes and macrophages.

T O X I N P R O D U C T I O N

The second major mechanism by which bacteria cause disease is the production of toxins. The capacity of microorganisms to produce toxins is called **toxigenicity.** The term toxemia refers to symptoms caused by toxins in blood. According to the nature of production, microbial toxins are subdivided into: **protein toxins (exotoxins)** and **lipopolysaccharide toxins (endotoxins).**

Exotoxins are produced inside some bacteria as part of their growth and metabolism and are released into the surrounding medium. There are 80 bacterial exotoxins, identification of these toxins by the mass, chemical structure, by the target cells and by the biological activity. Exotoxins are proteins. They consist off two different polypeptides, designated A (active) and B (binding). Although only part **A** causes symptoms in the host, whereas part be binds to surface receptors on the host cell and causes the transport of the entire protein across the plasma membrane into the cell. Most bacteria that produce exotoxins are gram-positive. Because exotoxins are soluble in body fluids, they can easily diffuse into the blood and are rapidly transported throughout the body. Exotoxins work by destroying particular parts of the host's cells or by inhibiting certain metabolic functions.

The main properties of exotoxins:

1.Exotoxins are proteins and they are thermolabile toxins.
2.Specific action
3.They possess high antigenic and immunogenic properties
4.Because exotoxins are soluble in body fluids, they can easily diffuse into the blood and are rapidly transported throughout the body.
5. Most bacteria that produce exotoxins are gram-positive.
6.Exotoxins are used for preparation of anatoxins.

Exotoxins may be grouped into following principal types, based on their mode of action:

1. **Cytotoxins** – which kill host cells or affect their functions. They are inhibits protein synthesis in Eukaryotic cells (Diphtheria toxin).
   a)antielongaters -they inhibit protein synthesis in Eukaryotic cells: antielongaters, which inhibit transferase II enzyme (e.g., Diphtheria histotoxin).
   b)enterotoxins (S. aureus, C. perfringens)
   c)dermonecrotoxins (S. pyogenes, B. pertussis, B.anthracis, P. aeruginosa)
2. **Membrane toxins** – hemolysins and leukocydins. These toxins raise the permeability of membranes.
3. **Functional blockaters** – which are subdivided into several groups:
   a) *Enterotoxins (thermostable and thermolabile)* – thermolabile enterotoxin induces the formation of cyclic AMP from ATP in the cytoplasm. As a result, epithelial cells discharge large amounts of fluids and electrolytes (ions). Normal muscular contractions are disturbed, leading to severe diarrhea that may be accompanied by vomiting (Cholera toxin). Thermostable enterotoxin activate guanilate cyclase (Y. enterocilitica)
   b) *Neurotoxins* - prevents the transmission of impulses from the nerve cell (Botulinum toxin-blocks transmission of nerve signals to the muscles by preventing the release of acetyicholine; Tetanus toxin-blocks the action of inhibitory neurons by preventing the release of neurotransmitters).
   c) *Toxicoblokaters* – they render the opposite action of the enterotoxins. These toxins cause accumulation of cAMP in tissues and development of oedema (plague, anthrax).

4. Erythrogenic toxin and Exfoliatin toxin. S. pyogenes produces erythrogenic toxins which damage blood capillaries under the skin and produce a red skin rash. S. aureus produces exfoliatin toxin which damage intracellular bonds and cause impetigo in the new–born.

The body produces antibodies called antitoxins that provide immunity to exotoxins (antitoxic immunity). When exotoxins are inactivated by heat and formaldehyde, or other chemicals, they no longer cause the disease but are still able to stimulate antitoxin production so that immunity is produced to disease. Diphtheria and tetanus can be prevented by toxoid vaccination (anatoxins).

Protein toxins are classified into three classes:

1.Class A - fully secretion of the toxin into environment (histotoxin)

2.Class B - partial secretion of this toxin (tetanospasmin)

3.Class C – (unrealizable) liberation of this toxin can occur after lysis of bacteria (plague's mice toxin)

Endotoxins differ from exototoxins in several ways. Endotoxins are components of the outer portion of the cell wall of gram-negative bacteria. Gram-negative bacteria have an outer membrane surrounding the peptidoglycan layer of the cell wall. This outer membrane consists of lipoproteins, phospholipids, and lipopolysaccharides (LPS).The lipid portion of LPS, called **lipid A,** is the endotoxin. Thus, endotoxins are lipopolysaccharids, whereas exotoxins are proteins.

Endotoxins exert their effect when gram-negative bacteria die and their cell wall undergo lysis, thus liberating the endotoxin. Antibiotics used to treat diseases caused by gram-negative bacteria can lyses the bacterial cells; this reactions releases endotoxin and may lead immediate worsening of the symptoms, but condition usually improves as the endotoxin break down. All endotoxins produce the same sign and symptoms, regardless of the species of microorganism, although not to the same degree. Responses by the host include chills, fever, weakness, generalized aches, and, in some cases, shock and even death. Endotoxins can also induce miscarriage and prevent blood from clotting.

Shock caused by gram-negative bacteria is called **septic (**or endotoxic**) shock.**

Endotoxins do not promote the formation of effective antitoxins. Endotoxins are weakly antigenic; they induce protective antibodies so poorly. Antibodies are produced, but they tend not the counter the effect of the toxin; sometimes, in fact, they actually enhance its effect. No toxoids have been produced from endotoxins. Endotoxins are not used as antigens in any available vaccine. Representative microorganisms that produce endotoxins are Salmonella typhi (the causative agent of typhoid fever, Neisseria meningitides (the causative agents of meningococcal meningitis).

Table 4. Main features of exotoxins and endotoxin. Comparison of properties

Property	Exotoxin	Endotoxin
1. Source	Certain species of some gram-positive and gram-negative bacteria	Cell wall of gram-negative bacteria
2. Secreted from cell	Yes	No
3. Chemistry	Polypeptide	Lipopolysaccharide (LPS)
4. Toxicity	High (fatal dose on the order of 1mg)	Low/fatal dose on the order of hundreds of microbes
5. Clinical effects	Various effects	Fever, shock
6. Antigenicity	Induces high titer antibodies called antitoxins (high antigenicity)	Poorly antigenic
7. Vaccines	Toxoids (anatoxin) used as vaccines	No toxoids formed and no Vaccine available
8. Heat stability	Destroyed rapidly at 60ºc	Stable at 100º c for 1 hour
9. Typical disease	Tetanus, botulism, diphtheria	Meningococcemia, sepsis by gram-negative rods

THE MAIN PROPERTIES OF VIRAL INFECTIONS

Interaction between viruses and host lies in the base of viral infections. Viral diseases arise when the causative agents enter into the portals. So the first stage of the viral infection is adsorption, the next stage is entrance into defeated organism like microbes, but all the following stages are different and due to viral nucleic acid and for the viral infections **the main properties** are:

1.Virogeny, when the viral nucleic acid becomes integrated into the host cell chromosome (hepatitis B virus, AIDS). During virogeny the reproduction, maturation and release is absent. The cell with integrative viral genome (provirus) can save their functions. But sometimes neoplastic process can develop in which intensive growth and reproduction of cells (oncogenic function of viruses) is observed.

2.Viraemia – virus enters into the bloodstream and is transported.

3.Defeating of immune system cells - B-cells; T-cells (AIDS, hepatitis B virus).

4.Inclusion bodies formation (intranuclear, intracytoplasmic).

THE TYPES OF INFECTIONS

Sign	Name of the type of infection
1. Nature of the pathogenic agent	Bacterial, viral, fungal, protozoa
2. The origin	Exogenous, endogenous, autoinfection
3. Localization of causative agent in the host organism	Focal, generalized, bacteremia, viremia, septicopyemia, sepsis, toxico-septic shock
4. Number of the types of the causative agents	Mono-infection, mixed infection
5. Repeated manifestations of the diseases caused by the same agents or others one	Secondary infection, re-infection, super-infection, relapse
6. Duration of the interaction between the causative agent and host	Acute, chronic, carrier state
7. Manifestation	Manifest, asymptomatic
8. The sources of infections:	
Human	Anthroponose
Animal	Zoonose
Environment	Sopronose

MICROBIOLOGICAL AND IMMUNOLOGICAL CHARACTERISTIC OF PERIODS OF INFECTIOUS DISEASES

Period of infectious disease	Behavior of causative agent	Discharges of causative agent into environment	Immune responses
1. Incubation	Adhesion on the specific receptors of susceptible cells.	As a rule discharges is not detected.	Antibodies are not detected.
2. Prodrome period	Colonization of the susceptible cells. Manifestation of the first non-specific symptoms.	The same.	The same.
3. The specific illness period	Intensive reproduction of causative agent. Manifestation of specific symptoms.	Discharged.	Appearance of immunoglobulins class M, at the end of the period changes IgM into IgG and IgA is occurring.
4. Convalescence (recovery)	Cessation (stopping) of the reproduction and death of causative agent. Normalization of functions of the patient.	Stopping of the discharging of the causative agent or carrier state can occur.	Increasing of the titer of IgG, IgA. In some cases immediate type of hypersensitivity can develop.

I M M U N O L O G Y

Immunology is the study of immunity. Immunity is a complex of physiological defence reactions which determine the relative constancy of the internal medium of the macro-organism, hinder the development of the infectious process or intoxication, and are capable of restoring the impaired functions of the organism.

Immunity involves a specific defensive response when a host is invaded by foreign organisms or other foreign substances. The immune system of the body recognizes these substances as not belonging itself and it develops an immune response against them. There are two sections in modern immunology: infectious and non-infectious.

- **Non-infectious immunity –studies:** immunogenetics, vaccinology, transplantation, ontogenesis, tolerance, antitumor immunity, immunopathology.
- **Infectious immunity – studies**: defense mechanisms against infectious diseases (humeral and cellular), structure and synthesizing of Igs and the mechanisms of specific interaction between antigens and immunoglobulins, diagnostic and prophylactic measures.

Host defences are composed of two, complementary, frequently interacting systems:

1. **Non-specific defence (**species, innate, hereditary, natural) is constantly active. Innate immunity is resistant, not acquired through contact with an antigen. Innate immunity does not improve after exposure to the antigen, in contrast to acquired immunity which does. Natural immune process has no memory.
2. **Specific, acquired immunity** occurs after exposure to an antigen improves upon repeated exposure, and specific. It is mediated by antibody and by T lymphocytes. The cells responsible for acquired immunity have long –term memory for specific antigen.

N O N – S P E C I F I C D E F E N C E

The **non-specific defence factors** are classified into four major categories (table 1).

Table1

External barrier	Internal barrier	Cellular factors	Humoral factor
1. Normal microflora of the human body	Lymph nodes	Phagocytes	Lysozyme. Proteins of acute-phase response.
2. Skin	Tissue and cell barrier	Natural killers	Complement Interferon.
3. Mucous membrane			Other cytokines: C-reactive protein, serum amyloid A,P

- Inflammation, fever, secretory function of the organism

External barrier

Skin: 1.mechanical barrier
2.biochemical barrier

Intact skin is the first line of defence against many organisms. In addition to the physical(mechanical) barrier presented by skin, the fatty acids secreted by sebaceous glands in the skin, high concentration of salt in drying sweat have antibacterial and antifungal activity.

A second important defence is the ***mucous membrane*** of the respiratory tract, which is lined with cilia and covered with mucus. The coordinated beating of the cilia drives the mucus up to the nose and mouth, where the trapped bacteria can be expelled.

Other protective mechanisms of the respiratory tract involve **alveolar macrophages**, **lysozyme** in tears and mucus, hairs in the nose, and the **cough reflex,** which prevents aspiration into the lungs.

The non-specific protection in the **gastrointestinal tract** includes hydrolytic enzymes in saliva, acid in the stomach, and various enzymes and macrophages in the small intestine.

The vagina of adult women is protected by the low pH generated by lactobacilli that are part of the normal flora.

Normal microflora of the body is stimulator of immune system: natural non-specific stimulator of immunogenesis is muramyldipeptide, which formed from bacterial peptidoglycane under the influence of intestinal lysozyme and other lytic enzymes. It results in abundant saturation of intestinal tissue with lymphocytes and macrophages, so, in norm intestine is in chronic inflammation like conditions. The important function of normal microflora is participation in colonizative resistance (antiadhesive factor - occupy the receptors of human organism preventing pathogens from multiplying in these sites). Members of normal microflora are antagonists for pathogenic microorganisms. When antimicrobial therapy suppresses these beneficial organisms, pathogens such as Candida albicans (which presence in our microflora), can to cause diseases (table2)

.

Table2

The role of normal microflora of human organism

Non-specific defense factor	Provides several vital processes	Stimulation of formation of immune system
Antagonism against pathogenic microorganisms.	Participate in digestion. Contribute to absorption of Ca, Fe, vitamin D.	Stimulation of functional activity.
Inhibition of synthesizing of thermolabile exotoxin by enteropathogenic E. coli.	Participate in neutralization of endogenous and exogenous toxic products.	
Blockade the receptors of intestinal membrane	Participate in synthesizing of amino acids proteins vitamins, nicotinic acid.	

Internal barrier

Lymph nodes are small nodular aggregates of secondary lymphoid tissue found along the lymphatic channels of the body. They are designed to initiate immune responses to tissue-borne antigens. Lymph nodes act as a filter for lymph, each group of nodes draining a specific part of the body. They phagocytose foreign materials including microorganisms. They participate in proliferation and circulation of T and B cells. They enlarge following local antigenic stimulation.

Cellular factors

Phagocytosis (from Greek words for eat and cell) is the ingestion of a microorganism or any particulate matter by a cell. The human cells that perform this function are collectively called *phagocytes.* The phagocytic cells are the mononuclear macrophages (of blood and tissue) and polymorphonuclear microphages.

Microphages (which are granulocytes) are the polymorphonuclear leukocytes of the blood: neutrophils, eosinophils and basophils. **Neutrophil**s are actively phagocytic and form the predominant cell type in acute inflammation. Do not appear to have any role in specific immune processes. **Eosinophils** are found in large numbers of in allergic inflammation, parasitic infection and around antigen and antibody complexes. **Basophils** are found in the blood and tissues (mast cells). Degranulation of mast cells, with release of pharmacologically active agents, constitutes the effector mechanism in anaphylactic and atopic allergy.

Monocytes are lack granules in their cytoplasm and are not actively phagocytic until they leave circulating blood, enter body tissues, and mature into macrophages, with morphological and functional features characteristic of the tissues. Macrophages have different function to perform and are named according to tissue location: alveolar macrophages, histiocytes, Kupffer cells, osteoclasts and mesangial cells.

The process of phagocytosis can be divided into following steps:

- **chemotaxis**
- **adherence**
- **ingestion (endocytosis)**
- **phagosome formation**
- **phago-lysosome formation**
- **digestion**

1. Chemotaxis - is the chemical attraction of phagocytes to macro-organisms.

2. Adherence – is the attachment of the phagocyte's plasma membrane to the surface of the micro-organism or other foreign material.

The receptors on the macrophages membrane are:

- **MFR** (mannose fucose) which recognizes superficial carbohydrate structures of microorganisms.
- **FcR-**receptors for IgG Fc fragment
- **C3R-**receptors for C3b fragment of complement system
- **IFN R -** receptors for γ- interferon

There are MHC antigens (major histocompatibility complex proteins)-MHC-I and MHC-II antigens on the superficial membrane. T helper cell can recognize only antigens that are displayed together with class MHC II molecules on the surface of macrophages (APCs).

The phagocytosis by FcR and C3R receptors is more effective and is called **immune phagocytosis,** for it proceeds in presence of specific immunoglobulins and activation of complement system.

3.Endocytosis by two pathways:

a)phagocytosis (0.1μm)

b)pinocytosis (<0.1μm)

4.Phagosome formation-during the processes of ingestion, the plasma membrane of the phagocyte extends projections called pseudopods that engulf the microorganism is surrounded, the pseudopods meet and fuse, surrounding the micro-organism with a sac called a phagosome or phagocytic vesicle.

5.Phago-lysosome formation - upon contact, the phagosome and lysosome membranes fuse, to form a single, larger structure called a *phagolysosome.*

6. Digestion – in this phase the phagosome pinches off from plasma membrane and enters the cytoplasm. Within the cytoplasm it contacts lysosome that contains digestive enzymes and bactericidal substances

The contents of the phago-lysosome take only 10-30 minutes to kill most types of bacteria. Intracellular digestion occurs in phagolysosome by two pathways:

- **Oxygen-dependent:** the mechanism of destroying of microorganisms by respiratory burst, during which assimilation of oxygen and glucose is very intensive and the result of which is the excretion of biologically active products H_2O_2; OH^-, which leave bactericidal action.
- **Oxygen-independent**: it occurs under the action lysosomal enzymes.

Phagocytosis can be:

a)complete

b)incomplete

Complete phagocytosis - complete digestion and destruction of microbial cells in phagocytes.

Incomplete phagocytosis – is observed in certain diseases (gonorrhoea, tuberculosis, leprosy) in which microorganisms are absorbed by phagocytes, but do not perish, are not digested and in some cases are reproduced.

There are 3 mechanisms of ***incomplete*** phagocytosis

- microorganism inhibits phagolysosome formation (M. tuberculosis, M. lepreae)
- microorganism is stable to lysosomal enzymes (gonococcus)
- after endocytosis the microorganism can leave phagosome before the formation of phagolysosome (rickettsia).

The main functions of phagocytosis are:

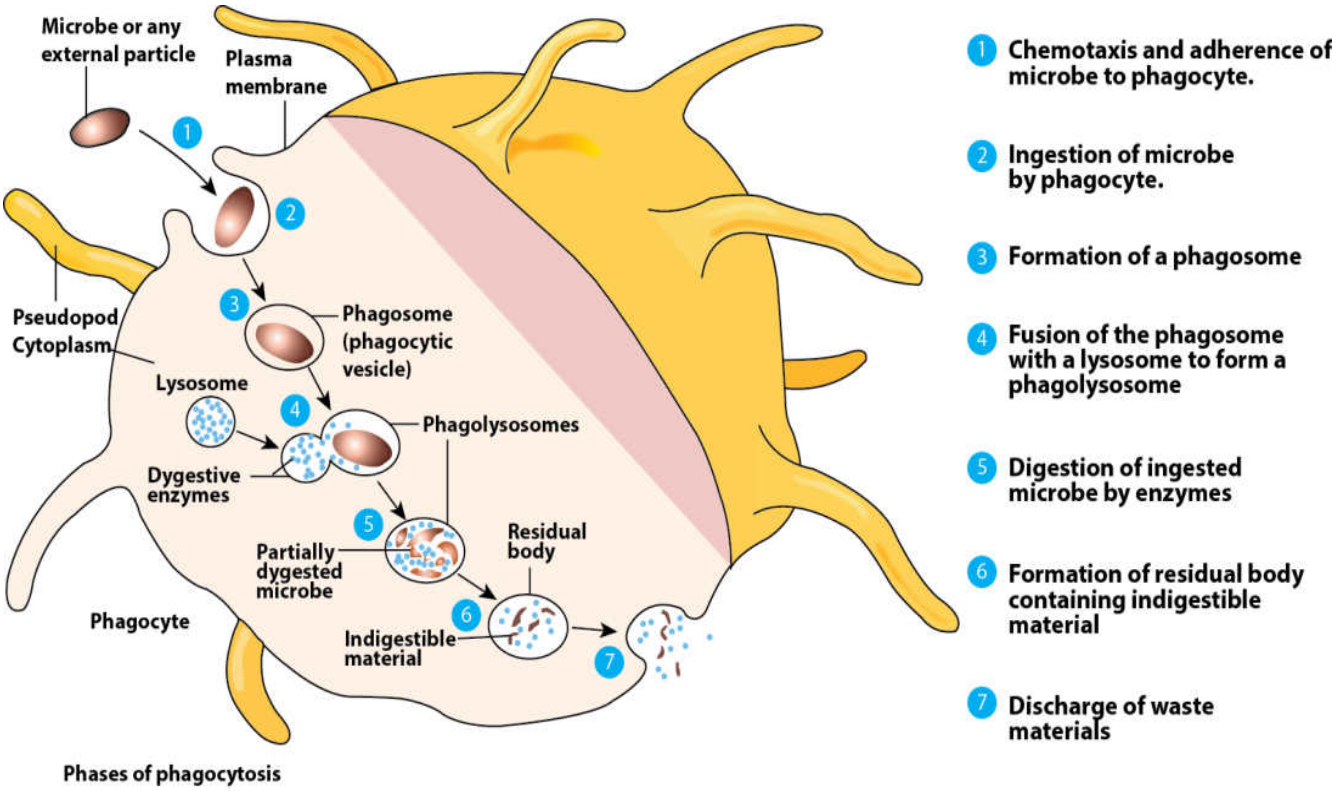
1.Phagocytosis (protective) – macrophages ingest bacteria, viruses, and other foreign particles. They have surface Fc receptors that interact with the Fc portion of immunoglobulins, thereby enhancing the uptake of opsonized organisms.

Macrophages have receptors for complement also.

2.Antigen presentation – foreign material is ingested and degraded, and fragments of antigen are presented on the macrophage cell surface (in conjunction with class II MHC molecules) for interaction with the TCR (T cell receptor).

3.Secretory (cytokine production) – macrophage produces several cytokines): **IL-1**(monokine), tumour necrosis factor **(TNF), lysozyme, Gamma interferon,** etc. IL-1 plays an essential role in the activation of helper T cells, and TNF is an important inflammatory mediator.

Phases of phagocytosis:



Phases of phagocytosis

Natural killer cells (NK) – play an important role in the innate host defences. NK cells are lymphocytes (they contain 5-10 % of peripheral lymphocytes) with some T cell markers, but they do not have to pass through the thymus in order to mature. NK cells are capable of destroying especially virus-infected cells and tumour cells by secreting cytotoxins (perforins, granzymes). In contrast to cytotoxic T cells, they do not seem to be immunologically specific; that is, they do not need to be stimulated by an antigen. They are not need phagocytic but must contact the target cell to lyse it. The functions of natural killer cells are to attack and destroy target cells. They can kill without antibody, but antibody enhances their effectiveness a process called antibody-dependent cell-mediated cytotoxicity. They have no immunologic memory and, unlike cytotoxic T cells, have no T cell receptors also, killing does not require recognition of MHC proteins. NK cells have receptors that detect the presence of class IMHC proteins on the cell surface. If a cell displays sufficient class I MHC proteins, that cell is not killed by the NK cells. Virus infected cells and tumour cells display a significantly reduced amount of class I MHC proteins, and it is those cells that are recognized and killed by the NK cells.

Humaral factors

Complement – the body produces certain antimicrobial substances. Among the most important of these are the proteins of the complement system. Complement is the defensive system consists of approximately 20 proteins that are present in normal human (and other animal) serum and nine of them **C1-C9** are the main fractions. The term "complement" refers to the ability of these proteins to complement, i.e., augment, the effects of other components of the immune system, eg, antibody. Complement is an important component of our innate host defences. Complement proteins are synthesized mainly by the

liver and mononuclear phagocytes. Proteins of the complement system make up about 5-10% of the serum proteins.

Complement heat – labile; it is inactivated by heating serum at 56º C for 30 minutes.

Activation of complement system occurs by three pathways:

1.Classical pathway - activation initiated by antigen-antibody complex (**Ag+IgM; Ag+IgG** -the system can be activated by an immune reaction).

2.Alternative pathway – activation complement system can be initiated by a variety of non-immunologic molecules (endotoxin, the particles of viruses or bacteria and etc.) and formation of antigen-antibody complexes (**Ag+IgA**; **Ag+IgE)**

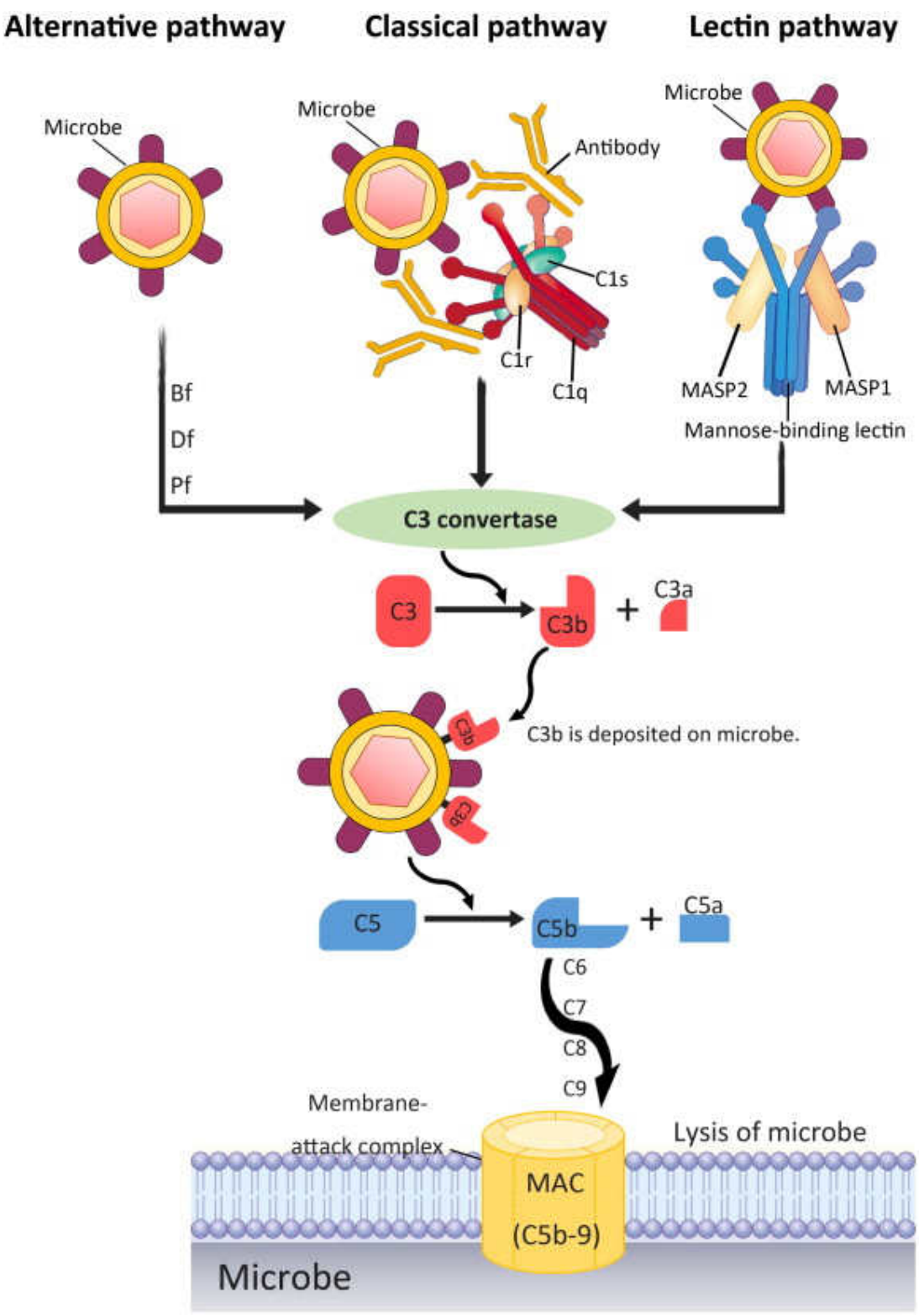
3.Lectin pathway – activation by the lectin pathway requires mannose-binding lectins (MBLs) without participation of imunoglobulins. Blood specific proteins can bind mannose which is in the bacterial membrane. This interaction activate C4 fraction after it activation by classical pathway.

Classical pathway – in the classical pathway antigen - antibody complexes activate C1 to form a protease, which cleaves C2 and C4 to form a C4b2a complex. The latter is C3 convertase, which cleaves C3 molecules into two fragments C3a and C3b. C3a, is an anaphylatoxin. C3b forms a complex with C4b2a, producing a new enzyme, C5 convertase, which cleaves C5 to form C5a and C5b. C5a, is an anaphylatoxin and a chemotactic factor. C5b binds to C6 and C7 to form a complex that interacts with C8 and C9 to produce the "**membrane attack"** complex – **C5b - C6 - C7 - C8 – C9**, which causes cytolysis. C5a fragment is split off and has other activities (e.g.C5a can contribute to the development of acute inflammation; C5a is also chemotactic factor that attracts phagocytes to the site of complement).

Alternative pathway – This pathway does not require the presence of specific antibodies. A wide range of chemically unrelated substances are known to activate alternative pathway: certain polysaccharides and lipopolysaccharides, the proteins of bacterial cells superficial structures of viruses, Immune complexes including IgA and IgE. During this processes factors B, D, P and Mg^{2+} ions take place. Factor D in active form is protease which split the factor B into Bb which plays role of C3-convertase in alternative pathway. Role of P (properdin) is activation and stabilisation of C3a and Bb.

Factors B, D, P react with C3 to produce C3b in the serum. The alternative pathway is initiated when C3b, factor B, factor D, and factor P combine with certain polysaccharides. After, activation of "**membrane attack**" complex **– C5b – C6 – C7 – C8 – C9** is occurs, like in classic pathway. Note that this pathway does not involve C1, C2 and C4.

The alternative pathway is of particular importance in combating enteric gram-negative bacteria. The outer membrane of the bacterial cell wall contains a lipopolysaccharide that is an endotoxin (lipid A), triggering the alternative pathway.



The main effects of complement:

1. **Cytolysis** – insertion of the C5b and C6 - C9 complex into the cell membrane leads to killing or lysis of many types of cells including erythrocytes, bacteria, and tumour cells. Cytolysis is not an enzymatic process; rather, it appears that insertion of the complex results in disruption of the membrane and the entry of water and electrolytes into the cell.

The utilization of the complement components in this process is called complement fixation; it forms the basis of an important clinical laboratory test.

2. **Enhancement of antibody production** – the binding of C3b to its receptors on the surface of activated B cells greatly enhances antibody production compared with that by B cells that are activated by antigen alone.

3. **Chemotaxis** – C5a and C567 complex attract neutrophils. They migrate especially well toward C5a. C5a also enhances the adhesiveness of neutrophils to the endothelium.

4. **Opsonization** – cells, antigen-antibody complexes, and viruses are phagocytized much better in the presence of C3b because of the presence of C3b receptors on the surface of many phagocytes. When bound to surface of a microorganism, C3b can interact with special receptors on phagocytes to promote phagocytosis. This phenomenon is called opsonization or immune adherence. In the process, C3b functions as an opsonin by coating the microorganism and promoting attachment of the phagocyte to the microbe.

5. **Anaphylatoxin** – C3a, C5a bind to mast cells, basophils, and blood platelets to trigger the release of mediators, e.g., histamine, which increases blood vessel permeability and smooth muscle contraction of the bronchioles leading of bronchospasm. C5a also functions as a powerful chemotactic factor that attracts phagocytes to the site of complement fixation.

Cytokines are soluble chemical messengers by which cells of the immune system communicate with each other. The most important cytokines are: ***interferon, interleukins (Il), cytotoxins (tumour necrosis factor).*** Cytokines which regulate communication between leukocytes and other cells named Interleukin - a name chosen to describe their function of communication between white cells.

CONFORMITY OF CYTOKINE REGULATION

- The same cytokine can be synthesized by different cells, the same cell can synthesize different cytokines
- The same cytokine can stimulate and suppress the target cell activity
- The simultaneous effect of several cytokines on the target cell can be both synergistic and antagonistic
- Cytokines can interact with the receptors released out of the cell, thus inhibiting the contact of cytokines with the target
- Cytokines act in low concentrations 0,001 microgram/milliliter
- For action of cytokines it is enough to be combined with 10 per cent of cell receptors

Classification of cytokines:

1. By the source: **Lymphokines** (Lymphocytes) **Monokines** (Monocytes, Macrophages)

2. By the participation in immune mechanisms:

Promotion of inflammation	**Anti-inflammatory**
IL-1; IL-6; IL8; ά THF, Interferon-stimulation of innate non-specific defense, inflammation and development of specific immune reactions	IL-4; IL-13: They inhibited non-specific and specific immune reactions

Interferons are a class of similar antiviral proteins produced by certain animal cells after viral stimulation (or after exposure to other inducers). One of the principal functions of interferons is to interfere with viral multiplication. The next feature of interferons is that they are host-cell-specific but not virus specific. This means that interferon produced by human cells protects human cells, but not other cell. Other animals cannot be used as a source of interferons for human therapy. Rather, the genes for human interferons have been cloned and material for medical trials is now produced by genetic

engineering techniques. However, the interferon of a species is active against a number of different viruses.

Human interferons are of **three types**:

- Alpha interferon **(ά)**
- Beta interferon **(β)**
- Gamma interferon **(γ)**

ά interferon is produced by leukocytes and possess mainly antiviral effect and after that anticancer (inhibit cancer cells), antiproliferative effect**.**

β interferon, produced by the fibroblasts in connective tissue, possess mainly anticancer effect and then antiviral effect.

γ interferon produced by lymphocytes and have high immunomodulative effect and faint (poor) antiviral effect.

All interferons are small proteins, with molecular weights between 15000and 30000. They are quite stable at low pH and fairly resistant to heat. Produced by virus-infected host cells only in very small quantities, interferon diffuses to uninfected neighbouring cells. It reacts with plasma or nuclear membrane receptors, inducing the uninfected cells to manufacture mRNA for the synthesis of antiviral proteins (AVPs). These proteins are enzymes that disrupt various stages of viral multiplication. For example, one AVP inhibits translation of viral mRNA by blocking initiation of protein synthesis. Another inhibits polypeptide elongation. Still another is involved in destroying viral mRNA before translation. The low concentrations at which interferon inhibit viral multiplication are non toxic to uninfected cells. Interferon is effective for only short periods. It typically plays a major role in infections that are acute and short term, such as cold and influenza. Another problem is that it has no effect on viral multiplication in cells already infected. They inhibit the growth of viruses by blocking the translation of viral proteins. Because interferons are produced within a few hours of the initiation of viral replication, they may act in the early phase of viral diseases to limit the spread of virus.

Interferon used to treat a variety of blood cancers, and solid tumours. For rising antiviral protection we use interferogenes which raise the production of interferons.

The main peculiarities of interferons are:

- Universality- are not virus specific (**IFN** are active against all viruses)
- Expressed species activity
- Biological activity of **IFN** is depends on polypeptide component
- Species specificity is depends on saccharide portion
- Residual affection of **IFN** is detected after washing of preparations
- Absence of toxic action
- High effective action (minimal doses of **IFN** -s can leave antiviral action).

Cytotoxins - macrophages produce tumour necrosis factor **(TNF)** which is an important inflammatory mediator. Low concentrations of it is activates neutrophils and increases their adhesion to endothelial cells. High concentration mediates septic shock, acts as cachectin, causes necrosis of tumours.

Acute-phase proteins are also produced early in inflammation, mainly by the liver at action of cytokines such as interleukin-1 (IL- 1), IL-6, tumour necrosis factor (TNF). These proteins take place in antimicrobial action, promote phagocytosis, activate complement system, they also promote formation and liquidation of inflammation centre. The best known of these are *C-reactive protein, mannose-binding protein*, *serum amyloids A and P* which bind to the surface of bacteria and enhance the activation of the phagocytosis and alternative pathway of complement; *lipopolysaccharide (endotoxin)-binding protein,* which produced in response to gram-negative bacteria *Fe-binding protein, blood coagulating factors ,transferrin, inhibitors of proteas, components of complement system*.

Lysozyme is thermostable protein, which produced by blood monocytes, tissue macrophages, neutrophils; and are present in all biological secretions (tear, saliva, mucus). This is an enzyme (muramidase) with proteolytic activity. The mechanism of action is **hydrolysis of β-glycosyl bonds** of the cell wall, thereby contributing to the natural resistance of the host to microbial infection. However, if the lysozyme-treated cells are in a solution with the same osmotic pressure as that of the bacterial interior, they will survive as spherical forms, called protoplasts, spheroplasts, L-forms surrounded only by a cytoplasmic membrane.

Fever –infection causes a rise in the body temperature that is attributed to endogenous pyrogen (Interleukin-1) released from macrophages, which act on the hypothalamic temperature –regulatory centre. Fever may be a protective response, since a variety of bacteria and viruses grow more slowly at elevated temperatures. 38-40ºC is optimal temperature for activation of macrophages. High temperature is unfavourable for intracellular reproduction of majority of viruses. The higher body temperature may directly inactivate the virus particles, particularly enveloped viruses, which are more heat-sensitive than non-enveloped viruses. Replication of some viruses is reduced at higher temperatures; therefore, fever may inhibit replication.

Inflammatory response – the presence of foreign bodies, such as bacteria within the body provokes a protective inflammatory response. This response is characterized by clinical findings of redness, swelling, warmth and pain at the site of infection.

Secretary systems of the organism are nonspecific defense factors: genitourinary tract, gastro-intestinal tract, sebaceous and sudoriferous glands.

THE CHARACTERISTIC, TYPES AND FORMS OF IMMUNITY

The term **immunity** (Latina immunis free from) usually means resistance of the body to pathogenic microbes, their toxins or to other kinds of foreign substances.

In the process of evolution, organisms have developed the property of distinguishing "self" and "non-self" very accurately, which is just what protects them from being penetrated by foreign proteins (pathogenic micro-organisms, heterogenic transplants and other foreign substances).

Modern classification subdivides immunity into two types according resistance:

1.Innate, hereditary, **species** (non-specific) defence

2.Acquired immunity (specific)

Innate defence is protection against micro-organism in general. The underlying factors of the mechanisms of species immunity (hereditary resistance) to infectious diseases are the absence in the organism's cells of receptors and substrates necessary for the adsorption and reproduction of the causative agent.

Species immunity is insusceptibility of certain species of animals to diseases which attack other species. An example of species immunity is insusceptibility of man to chicken cholera, or insusceptibility animals to syphilis, scarlet fever. It is transmitted by heredity from one generation to the next (named hereditary). The innate immunity mediated by non-specific factors (external barrier, internal barrier, cell factors, humoral factors).

Acquired immunity refers to the protection an animal develops against certain types of microbes or foreign substances. Acquired immunity is developed during an individual's lifetime.

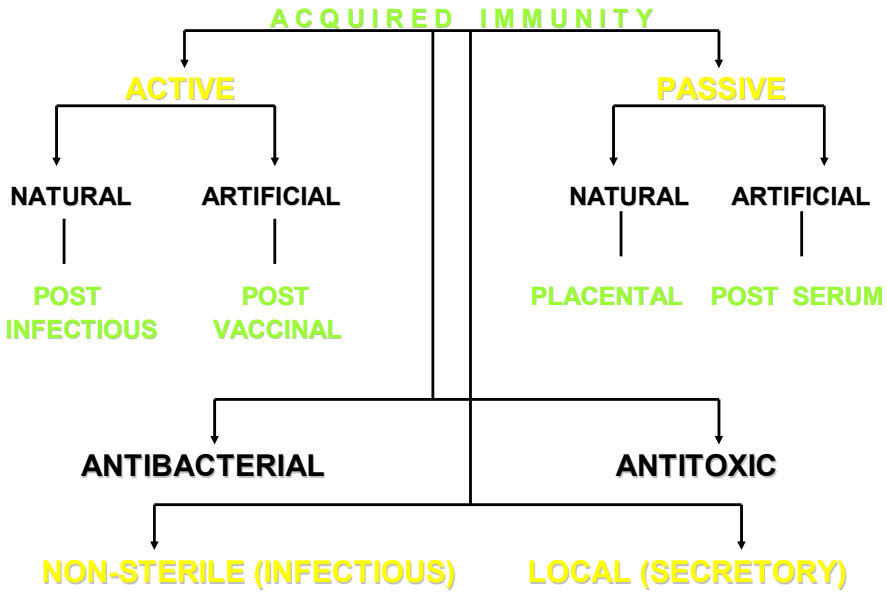
Acquired immunity is subdivided into: **Active** and **Passive.**

Acquired immunity in turn is divided into: **Natural** and **Artificial**

Naturally acquired active immunity is obtained when a person is exposed to antigens in the course of daily life, e.g. post infectious immunity. This is an *acquired active natural* immunity. Immunity after vaccination is acquired active too, but *artificially.* These types of immunity are active, because a person actively produced antibodies against the antigen himself.

Artificial acquired passive immunity involves the introduction of antibodies into the body. This type of immunity is considered passive because the recipient does not actively synthesize the antibodies.

Naturally acquired passive immunity involves the natural transfer of antibodies from a mother to her infant. These antibodies pass from placenta *(placental immunity);* certain antibodies are also passed from the mother to her nursing infant in breast milk. The duration of passive acquired immunity is short; it lasts only as long as the antibodies are present in the recipient. In most cases it continued 15-20 days, sometimes 6 months. After about six months this immune state disappears and children become susceptible to many infections.



A N T I G E N

Normally the immune system recognizes components of the body it protects as "self" and foreign matter as "non-self". Antigens (immunogens) provoke highly specific immune response in an organism.

The name **antigen** (GK. anti against, genos genus) is given to substances (independent of chemical nature) which upon injection into the body recognized by immune system of the organism as a "non-self" and induce formation of immune response:

1. production of specific antibodies and reacting specifically with them
2. proliferation and accumulation of sensitive lymphocytes
3. immune memory
4. immune tolerance

Antigens are biopolymers which have animal, plant, bacterial, synthetic origin:

Antigens are proteins and different protein complexes in combination with lipids or polysaccharides(lipoproteins, glycoproteins). Complex polysaccharides and lipopolysaccharides.

Antigens, consequently, are characterized by the following main properties:

1. ***Antigenicity***
2. ***Specificity***
3. ***Foreignness***
4. ***Immunogenicity and tolerance***

ANTIGENICITY:

A. Chemical nature

B. Molecular mass **(**most potent antigens are proteins with high molecular weights above 10.000)

C. Colloid structure - antigenic substances must have colloid structure, must be soluble in the body fluids.

Some proteins which have high molecular weight but cannot be in colloid solution, are non immunogenic-natural silk, catgut keratin. These proteins can be used in clinical practice for restoring of tissues, organs for stitching and when inflammation develops on the site of stitches- this is not immunological process.

D. Sensitivity to catabolic destruction -polypeptides, which contain L-aminoacids are antigens. Polypeptides, which contain D-aminoacids are not antigens, because they can't be destroyed completely by the enzymes of the organism.

E. Type of immunized animal

F. Dosage, route of antigen administration.

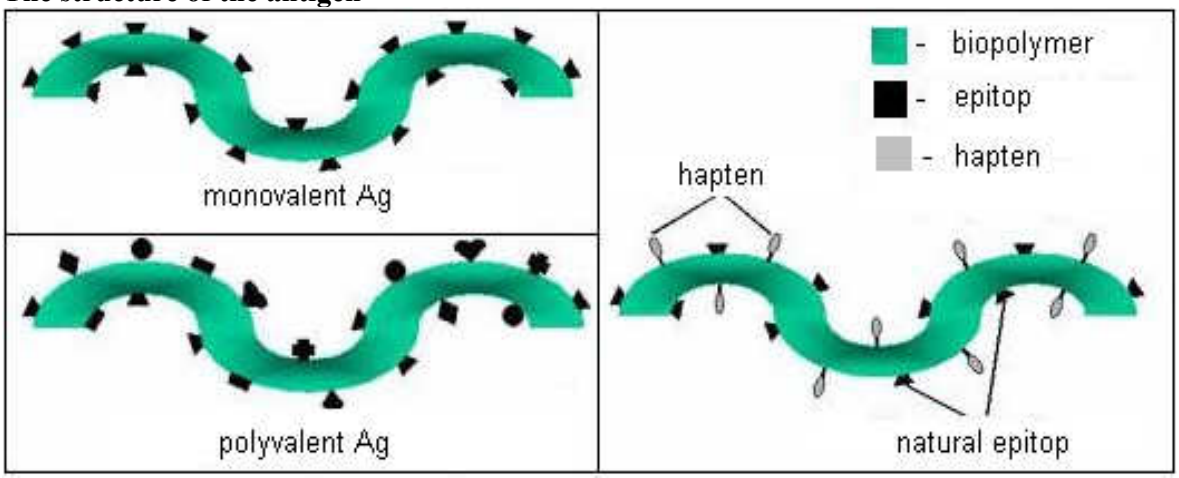
ANTGENIC SPECIFITY-is mediated by antigenic determinants **(epitopes).** Epitopes are small chemical groups on the antigen molecule that can elicit and react with antibody. Generally, antibodies recognize and interact with these specific regions antigenic determinants –epitopes.

The nature of this interaction depends on the size, shape, and chemical structure of the binding site on the antibody molecule. An antigen can have one or more determinants. Most antigens have many determinants; i.e., they are multivalent (specificity is depends on surface amino-acid groups only, not all molecule of biopolymer).

Because different determinants are recognized by different antibodies, the immune system may produce several distinct antibodies against a single antigen. Antibodies which synthesized for one epitope are named monoclonal.

Antigens have an immunogenic property, especially microbial antigens which can induce strained immunity.

The structure of the antigen



FOREIGNNESS (heterogenicity for the body). In general, molecules recognized as "self" are not immunogenic; i.e, we are tolerant to those self-molecules. To be immunogenic, molecules must be recognized as "non-self", i.e., foreign.

IMMUNOGENICITY: The ability of antigenic substrates that induce positive immune response: synthesizing of immunoglobulin, accumulation of sensitized lymphocytes, immune memory.

TOLERANCE: Tolerance is specific immunologic unresponsiveness; i.e., an immune response to a certain antigen does not occur, although the immune system is otherwise functioning normally. In general, antigens that are present during embryonic life are considered "self" and do not stimulate an immunologic response. These are known as **complete antigens -** substances which cause the production of antibodies in the body, react with them in vivo as well as in vitro (foreign proteins, sera, bacteria, toxins, viruses and cellular elements).

Molecules with molecular weight below 10.000 are weakly immunogenic, and very small ones are non-immunogenic (eg. an amino acid, lipids), they are known as **haptens** (partial antigens) which is a molecule that is not immunogenic by itself but can react with specific antibody. Haptens are not immunogenic, because they can't activate **T_h cells,** as they are unable to bind to **MHC** proteins; they can't bind because they aren't polypeptides and only polypeptides can be presented by MHC protein. Furthermore, haptens are univalent and therefore cannot activate B cells by themselves. Haptens interact with our immune system, because many haptens, such as drugs (eg. Penicillin) and poison oak oil, bind to our normal proteins, to which we are tolerant. The hapten-protein combination now becomes immunogenic; i.e., the hapten modifies the protein sufficiently such that when the hapten-peptide combination is presented by the MHC protein, it is recognized as foreign. The addition of proteins to haptens even in a small amount gives them the properties of complete antigens. In these cases the protein carries out the function of a **carrier protein.**

The interaction of antigen and antibody is highly specific, and this characteristic is frequently used in the diagnostic laboratory to identify microorganisms.

Adjuvants enhance the immune response to an immunogen. They are chemically unrelated to the immunogen and may act by non-specifically stimulating the immunoreactive cells or by releasing the immunogen slowly.

Some human vaccines contain adjuvants such as aluminium hydroxide or lipids.

Adjuvants promote creating of antigenic depot, which stimulates phagocytosis and they help in production of cytokines.

Superantigens (**SAgs**) are a class of antigens that result in excessive activation of the immune system. SAgs are produced by some pathogenic viruses and bacteria most likely as a defence mechanism against the immune system. In contrast to the usual antigen, which activates one or a few helper T cell (0.0001-0.001% of the body's T-cells are activated), these SAgs are capable of activating up to 20% of the body's T-cells. the large number of activated T-cells secrete large amounts of cytokines, the most important of which is gamma Interferon. This excess amount of IFN-gamma in turn activates the macrophages. The activated macrophages, in turn, over-produce proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha. TNF-alpha is particularly important as a part of the body's inflammatory response. In normal circumstances it is released locally in low levels and helps the immune system defeat pathogens. However, when it is systemically released in the blood and in high levels (due to mass T-cell activation resulting from the SAg binding), it can cause severe and life-threatening symptoms, including shock and multiple organ failure.

ANTIGENS OF HUMAN ORGANISM

Isoantigens: are those substances which have antigenic properties and are contained in some individuals of a given species. They have been found in the erythrocytes of animals and man. And on the basis of antigenic structure the erythrocytes of all people can be subdivided into four groups (O; A; B; AB). These data are taken into account during blood transfusion.

Autoantigens: are substances capable of immunizing the body from which they are obtained. Thus, they become modified and are capable of bearing an antigenic function. These substances include the eye lens, spermatozoids, liver, lungs and other tissues. Under ordinary conditions they do not come in contact with the immunizing system of the body, therefore antibodies are not produced against such cells and tissues. However, if these tissues are injured, then autoantigens may be absorbed, and may cause the production of antibodies which have a toxic effect on the corresponding cells. The origination of autoantigens is possible under the influence of cooling, radiation, drugs, virus infections, bacterial proteins and toxins of streptococci, staphylococci, tubercule bacilli and other factors. The production of autoantigens is the result of the disturbance of species specificity which provides for the antigenicity of a number of substances found in the given body.

Alloantigens: Histocompatibility antigens which subdivided into three groups:

Class I MHC (Major Histocompatibiliti Complex) proteins – these are glycoproteins found on the surface of virtually all nucleated cells. They play role of transplant antigen.

The main biological role of MHC I is recognition of self and non-self. Cytotoxic T cells respond to antigen in association with class I MHC proteins.

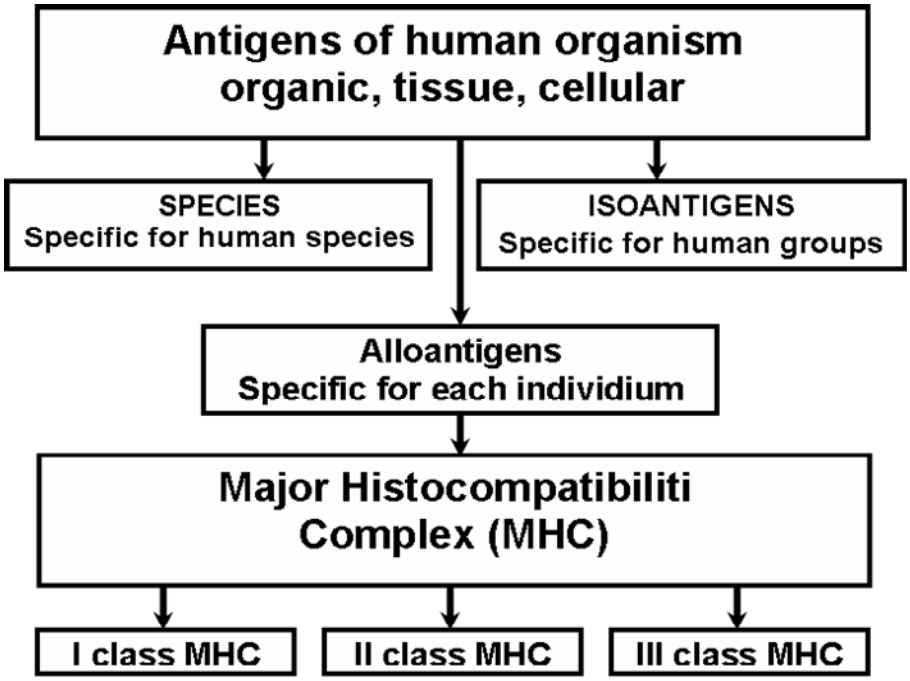
Class II MHC proteins – These are glycoproteins found on the surface of certain cells (professional APC), including macrophages, B cells, dendritic cells of the spleen, and Langerhans cells of the skin. Helper T-cells recognize class II proteins.

Class III MHC proteins - include some components of the complement system and a few others cytokines.

MHC genes and proteins are also important that many autoimmune diseases occur in people who carry certain MHC genes and that the success of organ transplants is, in large part, determined by the compatibility of the MHC genes of the donor and recipient.

Tumour antigens – are result of malignant transformation of cells.

Singen antigens –antigens of different individuals, which are not differ.



ANTIGENIC STRUCTURE OF THE MICROBIAL CELL

Bacteria are a complex of antigens, which include highly molecular compounds of a protein nature and biologically active specific polysaccharide.

Bacterial antigens are:

- **H-antigen** (flagellar) which thermo labile and are destroyed at a temperature of 56-80° C. Only flagellated organisms have H antigen (E. coli, S. typhi).
- **O-antigens** (somatic) the cell wall antigen. It is the outer polysaccharide portion of the lipopolysaccharide. O antigen is the basis for the serologic typing of many enteric rods. Somatic antigens are thermo resistant and withstand heating to 80-100° C.
- **Vi-antigens-**a relatively thermolabile antigen was isolated from virulent strains of the typhoid bacillus. Possess high virulence property and named virulence antigen.
- **K-antigen:** capsular or polysaccharide antigen. They are formed at the expense of the O-antigens and are located on the surface of the cells. The K antigen contain thermo-labile L-and B-antigens and thermo-resistant A antigen. K antigen located superficial to O antigen, masking O antigen. K antigen contains acidic polysaccharides such as glucoronic acid, galacturonic acid.
- **Protective** antigens have been found in exudates of animals suffering from anthrax.

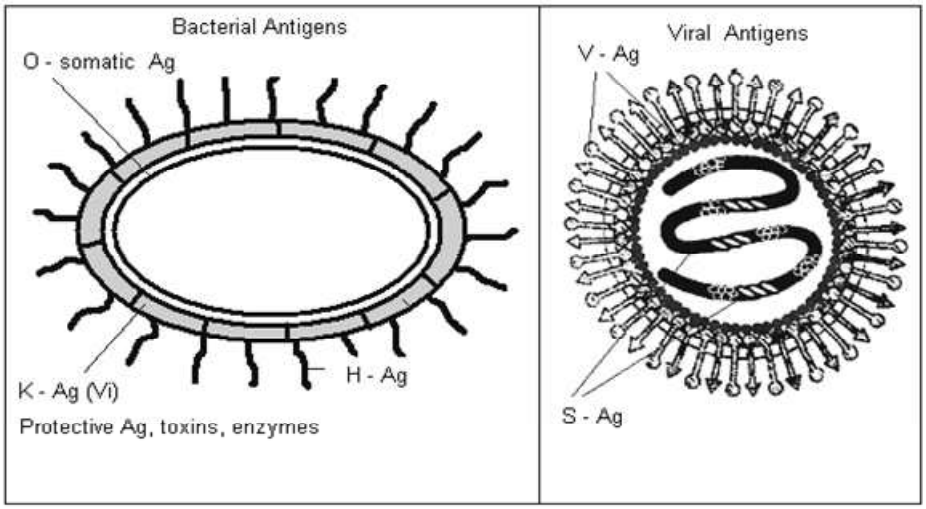
Microbial **toxins** also have antigenic properties. Rendered harmless by formalin and heat treatment, exotoxins lose their toxic properties and almost completely retain their antigenic functions. They are known as anatoxins, and are widely used in immunizing people against diphtheria and tetanus. Bacterial **enzymes** are complete antigens too.

Heterogenic antigen (Forssman antigen): There are antigens (haptens) found in different species of animals (eg, heterogenic antigens are contained in the protein structure of organs of guinea pigs, in the erythrocytes of sheep and in salmonellas etc.).

When antigenic structures of the host (human organism) are similar to those of the causative agent, the micro-organism is incapable of producing immunity, as the result of which the disease follows a graver course. Such a condition is called **antigenic mimicry** (eg, human erythrocytes have antigens common with staphylococci, streptococci and other causative agents of infectious disease).

Cross reacting antigen(CRA)-discovers in microorganisms and in human tissues (haemolytic streptococci of A group contains CRA which is common with autoantigens of myocardium and kidney glomeruli and they can cause myocarditis and glomerulonephritis).

Antigenic structure of viruses: For simple viruses antigenic structure connected with nucleocapsid. By chemical structure they are ribonucleoprotein either deoxyribonucleoprotein which are soluble and signified **S-antigen** (solutio). At complex (enveloped) viruses antigenic structure connected with nucleocapsid and glycoproteins of external membrane. Many viruses contain peculiar (specific) superficial (surface**) V (viral)-antigens** which are hemagglutinin and neuraminidase.



THE IMMUNE SYSTEM OF HUMAN ORGANISM

Immune system consists of lymphoreticular system.

The lymphoreticular system is a complex organization of cells of diverse morphology distributed widely in different organs and tissues of the body. This system is responsible for specific immunity.

The lymphoid system consists of the lymphoid cells (lymphocytes and plasma cells) and lymphoid organs. Based on the different roles they perform, **lymphoid organs** can be classified into the central (primary) and the peripheral lymphoid organs.

Central organs are bone marrow, thymus, fetal liver, Peyer's patches (Fabricius bursa in birds). In central organs immune cells formation and maturation take place.

Peripheral organs are lymphoid tissue, lymph nodes, spleen, tonsils, blood etc. Peripheral organs contain mature lymphocytes. Here after antigen influence proliferation and differentiation of lymphocytes take place.

Cells of immune system subdivided into three groups:

1. Immunocompetent cells (T and B lymphocytes).

2. Antigen-presenting cells (macrophages, Langerhans cells, dendritic cells, B-cells, etc.).

3. Antigen-non-specific response cells (granulocytes: eosinophils, basophils, neutrophils; NK cells, mast cells, monocytes, macrophages).

IMMUNOCOMPETENT CELLS

Lymphocytes: Lymphoid stem cells differentiate into main lymphocyte populations: T-lymphocytes and B-lymphocytes. The ratio of T-cells to B-cells is approximately 3:1. Of the lymphocytes in peripheral circulation, about 55-75 per cent is T cells and about 15-30 per cent are B cells. B cells take part in humoral immune response. T cells take part in cell-mediated (cellular) immune response and in regulation in both forms of immune response.

T-cell precursors formed from the bone marrow cells, which entered into thymus. Within the thymus, within the outer cortical epithelial cells (nurse cells) T cell progenitors differentiate under the influence of thymic hormones (thymosin and thymopoietins) into T cell subpopulations. They form 80% of blood lymphocytes. These cells are characterized by certain surface glycoproteins, eg, CD2, CD3, CD4, and CD8 (CD-cluster of differentiation). CD2 are adhesive molecules, they responsible for contact of T-lymphocytes to other cells. All T cells have CD3 proteins on their surface in association with antigen receptors (they determine cell contact with specific antigens). From cortex these CD4; CD8 cells

migrate into the medulla and the complete maturation into the $CD4^+$; $CD8^+$ cells takes place here. Later lymphocytes migrate into the blood, lymph, into the immune peripheral organs.

T cells are subdivided into two major categories on the basis of whether they have CD4 or CD8 proteins on their surface. Mature T cells have either CD4 or CD8 proteins but not both.

CD4-positive, CD8-positive cells, bearing antigen receptors for "self" proteins, are killed (clonal selection) by a process of "programmed cell death" called apoptosis. The removal of these self- reactive cells, a process called **negative selection**, results in **tolerance** to our own proteins, i.e., self-tolerance, and prevents autoimmune reactions.

IMMUNOLOGICAL FUNCTIONS OF IMMUNE SYSTEM CELLS (T AND B-LYMPHOCYTES)

$CD4^+$ lymphocytes perform **helper functions**:

1. They help B cells develop into antibody – producing plasma cells.
2. They help CD8 T cells to become activated cytotoxic T cells
3. They help macrophages effect delayed hypersensitivity.

These functions are performed by two subpopulations of $CD4^+$ cells (under the antigen influence CD4 cells subdivided into Th-1 and Th-2 subpopulations). **Th-1** (T helper) cells help activate cytotoxic T-cells by producing IL-2 and help initiate the delayed hypersensitivity response by producing primarily IL-2 and gamma interferon. **Th-2** cells perform the B cell helper function by producing primarily IL-4 and IL-5. Important regulator of the balance between Th-1 cells and Th-2 cells is IL-12 which is produced by Th-1cells. IL-12 increases the number of Th-1 cells, thereby enhancing host defences against organisms that are controlled by a delayed hypersensitivity response. Another important regulator is gamma interferon which inhibits the production of Th-2 cells. CD4 cells make up about 65per cent of peripheral T cells and predominate in the thymic medulla, tonsils, and blood. Th1 cells are activated mainly by intracellular parasites (eg, tubercule bacilli, bacteria) and mediate cellular immune response. Activation of Th2 cells is promoted by allergens, toxins, Helminths and they mediate mainly humoral immune response. To mount a protective immune response against a specific microbe requires that the appropriate subpopulation, i.e., either Th-1 or Th-2 cells, play a dominant role in the response. For example, if individuals are infected with M. tuberculosis and Th-2 cells are the major responders, and then humoral immunity is stimulated rather than cell-mediated immunity, it will not be protective against M. tuberculosis and the patient will suffer severe tuberculosis. Similarly, if an individual is infected with Streptococcus pneumoniae and Th-1 cells are the major responders instead of humoral immunity the patient will have severe pneumococcal disease. Precisely which component of a microbe activates either Th-1 or Th-2 cells is unknown.

$CD8^+$ lymphocytes perform **cytotoxic functions**; that is, they kill virus – infected, tumour cells. They kill by either of two mechanisms, namely the release of perforans, which destroy cell membranes, or the induction of programmed cell death (apoptosis). CD8 cells predominate in human bone marrow and gut lymphoid tissue. They make up 22-24 % of lymphocytes and the ratio of $CD8^+$ and $CD4^+$ lymphocytes is 1:1,9 – 1:2,4.

T cells recognize only polypeptide antigens. They recognize those polypeptides only when they are presented in association with MHC proteins. Helper T cells ($CD4^+$) recognize antigen in association with class II MHC proteins. Class II MHC proteins are on the surface of antigen-presenting cells, eg, macrophages, dendritic cells, etc. Within the cytoplasm of macrophage, the foreign protein is cleaved into small peptides that associate with the class II MHC proteins. The complex is transported to the surface of the macrophage, where the antigen, in association with a class II MHC protein, is presented to the receptor on the CD4-positive helper cell.

Cytotoxic $CD8^+$ cells recognize antigen in association with class I MHC proteins

Natural killer (NK) cells are lymphocytes too but they do not pass through the thymus, do not have an antigen receptor, and do not bear CD4, CD8 proteins. They recognize and kill target cells (virus-infected cells and tumour cells) without the requirement that the antigens be presented in association with class I or class II MHC proteins.

B-lymphocytes: B cells perform two important functions: **1.** They differentiate into plasma cells and produce antibodies. **2.** They are antigen-presenting cells (APCs).

B cell precursors differentiate into immunocompetent B cells in the bone marrow; they do not pass through the thymus for maturation. The maturation of B cells has two phases: a) T– independent phase consists of stem cells, pre-B cells, and B cells. b) T-dependent phase consists of the cells that arise subsequent to the interaction of antigen with the B cells. B cells differentiated into plasma cells which produce large amounts of immunoglobulins. Plasma cells secrete thousands of antibody molecules per second for a few days and then die (one million molecules in an hour). **T cell-independent response, primarily IgM is made.** This indicates that lymphokines produced by the helper T cells are needed for class switching. The T cell - dependent response generate memory B cells, whereas the T cell – independent response does not; therefore, a secondary antibody response does not occur in the later. The T-cell independent response is the main response to bacterial capsular polysaccharides, because these molecules are not effectively processed and presented by APCs and hence do not activate helper T cells. The most likely reason for this is that polysaccharides do not bind to class II MHC proteins whereas peptide antigens do.

ANTIGEN PRESENTING CELLS

Macrophages - in contrast to T cells, B cells, and NK cells, which differentiated from lymphoid stem cells, macrophages arises from myeloid precursors. Macrophages have three main functions: **a) phagocytosis, b) antigen presentation, c)cytokine production**. They take part in immune response, due to macrophages foreign material is ingested and degraded, and fragments of antigen are presented on the macrophage cell surface (in conjunction with class II MHC molecules) for interaction with the TCR (T cell receptor).

Dendritic cells they are close to macrophages, but they haven't phagocytic property. They possess II MHC molecules and they have antigen fixation property. They formed antigen-MHC complex and present it to T lymphocytes. They have I MHC molecules too and can present antigen to the CD8 cells and initiate cytotoxic reaction.

B lymphocytes are antigen presenting cells, they interact with antigen over the specific receptors, after antigen undergo an endocytosis. After few hours antigen expressed on the surface of B cell in complex with II MCH cells further B- lymphocytes enter into contact with T cells and activate them.

APCs are endothelial cells, fibroblasts, Langerhans cells, etc.

INTERACTION (COOPERATION) CELLS DURING THE DIFFERENT IMMUNE RESPONSE

Immune response to an antigen is of following types:

- Humoral or antibody mediated immunity which is mediated by antibodies produced by plasma cells
- Cell-mediated immunity which is mediated directly by sensitized lymphocytes
- Immune memory
- Immune tolerance -specific immunologic unresponsiveness

Humoral immune response is characterized by specific antibody production.

Antibody synthesis typically involves the cooperation of three cells **macrophages, helper T cells, and B cells.** After processing by a macrophage, fragments of antigen appear on the surface of the macrophage in association with class II MHC proteins. The antigen and class II MHC protein complex binds to specific receptors on the surface of a helper T cell, which then produces interleukins such as interleukin-2 (T cell growth factor, stimulates the helper T cell to multiply into a clone of antigen-specific helper T cells. Most cells of this clone perform effector and regulatory function, but some become **"memory"** cells, which are capable of being rapidly activated upon exposure to antigen at a later time.), interleukin-4 (B cell growth factor), and interleukin-5 (B cell differentiation factor). These factors activate the B cell capable of producing antibodies specific for that antigen. The activated B cell proliferates and differentiates to form many plasma cells that secrete large amounts of immunoglobulins (antibodies).

Cell-mediated (cellular) immunity: In the cell-mediated response, the initial events are similar to those described above for antibody production. The constituents of the cell-mediated immune response include: **macrophages,** which present the antigen to T cells; **helper T cells**, which participate in antigen recognition and in regulation (helper and suppressor) functions; **cytotoxic T cells**, which can kill virus-infected cells with or without antibody. The antigen is processed by macrophages, is fragmented, and is presented in conjunction with class II MHC molecules on the surface. These interact with the receptor on the helper T cell, which is then stimulated to produce lymphokines such as IL-2 (T cell growth factor), which stimulates the specific helper and cytotoxic T (CD8 cells) cells to grow.

Tolerance: Tolerance is specific immunologic unresponsiveness; although the immune system is otherwise functioning normally. In general, antigens that are present during embryonic life are considered "self" and do not stimulate an immunologic response.

Tolerance determined:

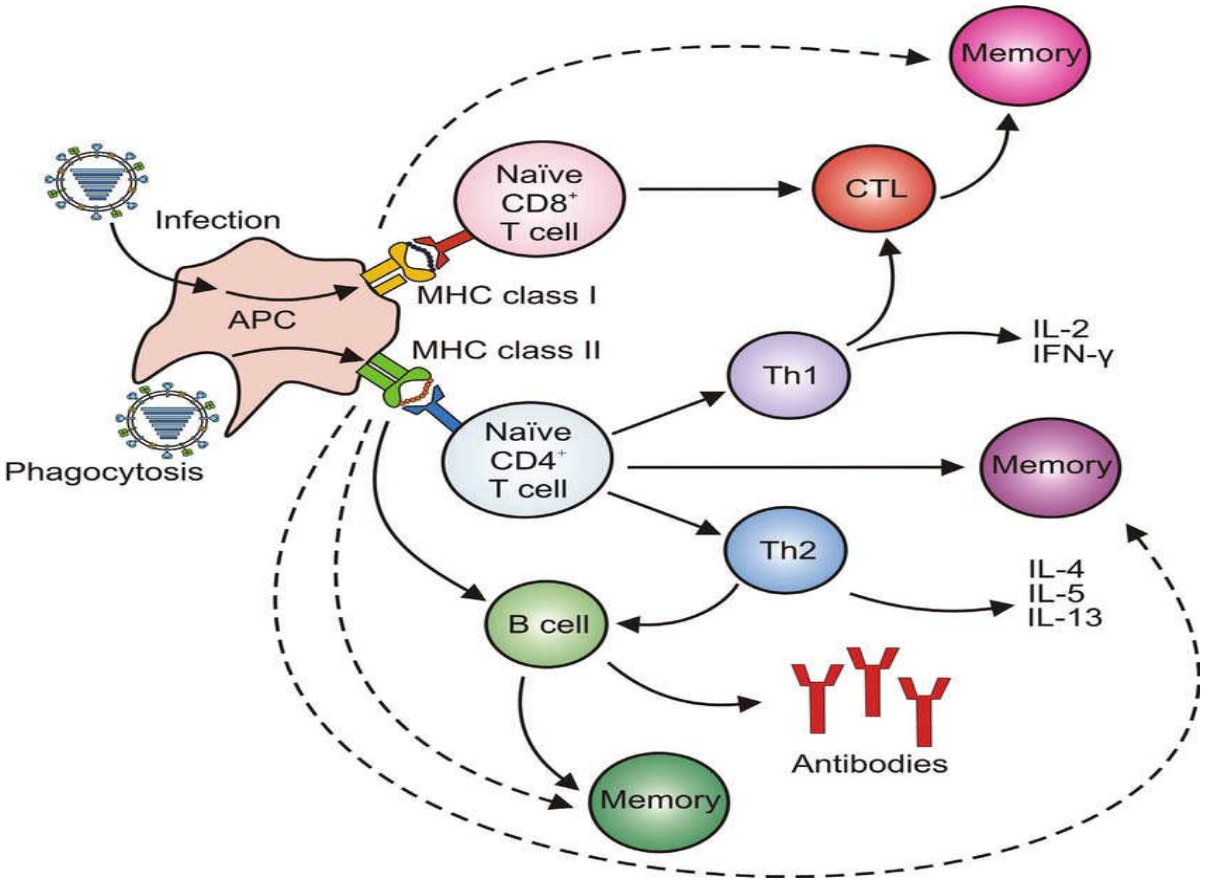
1. The immunologic maturity of the host; eg, neonatal animals are immunologically immature and do not respond too well to foreign antigens

2. The structure and dose of the antigen; eg, a very simple molecule induced tolerance more readily than a complex one, and very high low doses of antigen may result in tolerance instead of an immune response

3. Administration of immunosuppressive drugs enhances tolerance, eg, transplants.

4. Administration of a cross-reacting antigen tends to terminate tolerance.

Development of humoral and cellular immune responses.



ANTIBODIES-IMMUNOGLOBULINS

Antibodies are gamma globulin (by their electrophoretic migration rate) proteins (immunoglobulins) that react specifically with the antigen that stimulated their production. They make up about 20 % of the protein in blood plasma. There are five classes of antibodies: IgG, IgM, IgA, IgD, and IgE.

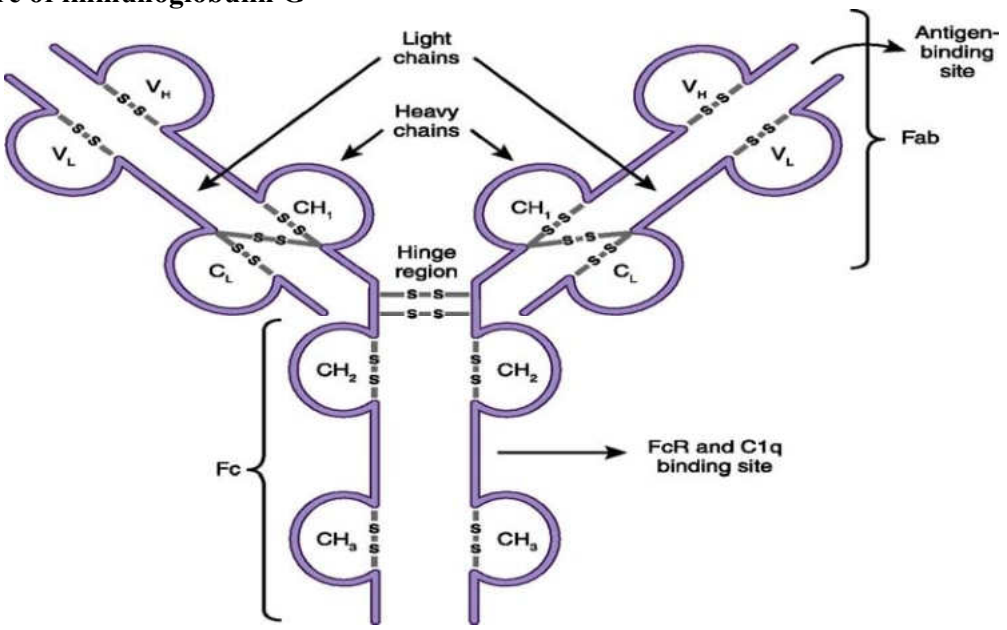
Immunoglobulins are glycoproteins made up of light (**L**) and heavy **(H)** polypeptide chains. The term light and heavy refer to molecular weight; light chains have a molecular weight of about 25000, heavy chains have molecular weight 50.000-70.000. The simplest antibody molecule has a **Y shape** (Y-shaped molecule is flexible) and consists of four polypeptide chains: two H chains and two L chains. These chains are linked by disulphide bonds. The H chains are structurally and antigenically distinct for each classes and are designated by the Greek letter corresponding to the immunoglobulin class: **alpha** (IgA); **gamma(**IgG); **delta** (IgD); **epsilon** (IgE); **mu** (IgM).

The L chains are similar in all classes of immunoglobulins. There are two types of light chains: **kappa** and **lambda**. Both types occur in all classes of immunoglobulins, but each immunoglobulin molecule contains only one type of L chain: either kappa or lambda, but not both together (in humans the ratio of immunoglobulins containing kappa chains to those containing lambda chains is approximately 2:1).

L and H chains are subdivided into variable and constant regions. The regions are composed of three-dimensionally folded, repeating segments called domains. An L chain consists of one variable (VL) and one constant (CL) domain. Most H chains consist of one variable (VH) and three constant (CH) domains. Each domain is approximately 110 amino acids long. The variable regions are responsible for antigen-binding. The constant region is responsible for various biologic functions, eg, complement activation and binding to cell surface receptors.

If an antibody molecule is treated with a proteolytic enzyme such as papain, peptide bonds in the "hinge" region are broken, producing two identical **Fab** fragments, which carry the antigen-binding sites, and one **Fc** fragment, which is involved in placental transfer, complement fixation, attachment site for various cells, and other biologic activities.

Structure of immunoglobulin G



C L A S S E S OF I M M U N O G L O B U L I N S

The basis of the structure of all classes of antibodies is the monomer consisting of two heavy and two light chains that form di and polymers. The classes of immunoglobulins differ in the number of monomers, valency (the number of active antigen binding centers- monovalent bivalent, polyvalent), avidity, ability to pass through a placental barrier. Monovalent antibodies are considered **incomplete.**

IgM-antibodies are the first antibodies to appear in response to initial exposure to an antigen. The main immunoglobulin produced early in the primary response. IgM has a pentamer structure consisting of five monomers. Because the pentamer has 10 antigen-binding sites (this is observed only with small haptens. With larger antigens, the effective valency falls to five); it is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody reactions and is important in defence against bacteria and viruses. IgM antibodies are relatively short lived, disappearing earlier than IgG; their demonstration in serum indicates recent infection. It has the highest avidity of immunoglobulins. It is the earliest immunoglobulin to be synthesised by the fetus, beginning by about 20 weeks of age. It is not transported across the placenta. They make up 5-13 % of the antibodies in serum. IgM can activate complement. Monomeric IgM is the major antibody receptor on the surface of B lymphocytes for antigen recognition.

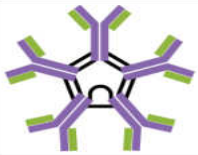
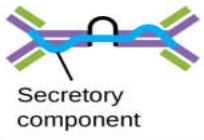
IgG - It is divalent because it has two identical antigen-binding sites (two L chains, two H chains). IgG antibodies account for about 80% of all antibodies. IgG is the predominant antibody in the secondary response and constitutes an important defence against bacteria and viruses, neutralize bacterial toxins. IgG is the only antibody to cross the placenta and confer passive immunity to a fetus; only its Fc portion binds to receptors on the surface of placental cells. It is therefore the most abundant immunoglobulin in newborns. IgG can activate complement, and when bound to antigens, enhance the effectiveness of phagocytic cells. IgG subdivided into four classes IgG1-IgG4, based on antigenic differences in the H chains and on the number and location of disulfide bonds. IgG1 makes up most (65%) of the total IgG. IgG2 antibody is directed against polysaccharide antigens and is an important host defence against encapsulated bacteria.

IgA - accounts about 10-15% of the antibodies in serum. IgA is the main immunoglobulin in secretions such as colostrum (its presence in colostrum probably helps to protect infants from gastrointestinal infections especially), saliva tears, and respiratory, intestinal and genital tract secretions. It consists of two parts: Serum and secretory. The main function of secretory IgA is to prevent attachment of micro-organisms eg, bacteria and viruses, to mucous membranes. It ensured local immunity. SIgA can activate complement by alternative pathway, which stimulated local phagocytic response. Serum IgA circulates in the serum mostly as a monomer.

IgD – is monomer, makes up only about 0.2 % of the total serum antibodies. This immunoglobulin has no known antibody function but may function as an antigen receptor; it is present (with IgM) on the surface of many B lymphocytes and serve as recognition receptors for antigens.

IgE – they constitute 0.002 % of the total serum antibodies IgE mediates immediate (anaphylactic) hypersensitivity; it participates in host defences against certain parasites (Helminths). The Fc region of IgE binds to the surface of mast cells and basophils. Bound IgE serves as a receptor for antigen (allergen), and this antigen – antibody complex triggers allergic responses of the immediate (anaphylactic) type through the release of mediators. Persons with allergic reactivity have greatly increased amounts, and IgE may appear in external secretions. IgE does not fix complement and does not cross the placenta.

IgE is the main host defence against certain important Helminth infections, such as Trichinella, Ascaris, and the hookworms. The serum IgE level is usually increased in these infections. Because worms are too large to be ingested by phagocytes, they are killed by eosinophils that release worm destroying enzymes. IgE specific for worm proteins bind to receptors on eosinophils, triggering the antibody-dependent cellular cytotoxicity response.

The Five Immunoglobulin (Ig) Classes					
Properties	**IgG monomer**	**IgM pentamer**	**Secretory IgA dimer**	**IgD monomer**	**IgE monomer**
Structure					
Heavy chains	γ	μ	α	δ	ε
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Because immunoglobulins are proteins, they are antigenic, and that property allows them to be subdivided into isotypes, allotypes and idiotypes.

Isotypes are defined by antigenic (amino-acid) differences in their constant regions. For example, IgG and IgM are different isotypes; the constant region of their H chains is different antigenically (the five immunoglobulin classes – IgG, IgM, IgA, IgE, IgD- are different isotypes; their H chains are antigenically different.

Allotypes are additional antigenic features of immunoglobulins that vary among individuals. They vary because the genes that code for the L and H chains are polymorphic, and individuals can have different alleles. Each individual inherits different allelic genes that code for one or another amino-acid.

Idiotypes are the antigenic determinants formed by the specific amino acids in the hypervariable region. Each idiotype is unique for the immunoglobulin produced by a specific clone of antibody-producing cells. Anti-idiotype antibody reacts only with the hypervariable region of the specific immunoglobulin molecule that induced it.

Although antibody formation usually involves helper T cells, certain antigens, eg, bacterial polysaccharides can activate B cells directly, without the help of T cells, and are called T cell-independent antigens.

Dynamics of antibody formation. When an individual encounters an antigen for the first time, antibody to that antigen is detectable in the serum within days or weeks, depending on the nature and dose of the antigen and the route of administration (eg, oral, parenteral). The antibody synthesis subdivided into **Primary** and **Secondary.** *Primary* response - when an antigen is first encountered. *Secondary* response – when there is a second encounter with the same antigen or a closely related (or cross-reacting) one, months or years after the primary response (Secondary response is also called memory response).

The kinetics of antibody synthesis in primary and secondary response passed several stages: latent (lag), logarithmic (log), plateau, and decline.

A lag phase - during which no antibody is detected. During this phase takes place microbial (antigen) distraction and its presentation to immune competent cells.

A log phase when the antibody titre increases logarithmically. Antibody is detected in lymph and blood.

Plateau phase during which the antibody titre stabilizes. The antibody level during this phase is arriving at maximum.

A decline phase, during which the antibody is cleared or catabolized.

An examination of the responses following primary and secondary antigenic challenge shows that the responses differ in four major respects:

Time course- Secondary response has a shorter lag phase (hours or 1-2 days) and an extend plateau and decline. In a primary response lag period is longer 3-5 days.

Antibody titre – The plateau levels of antibody are much greater in the secondary response, typically 10-fold or more than plateau levels in the primary response.

Antibody class – IgM antibodies form a major proportion of the primary response, whereas the secondary response consists almost entirely of IgG, with very little IgM.

Antibody affinity- The affinity of the antibodies in the secondary response is usually much higher. This is referred to as 'affinity maturation'.

Memory cells (T and B), as the name implies, endow our host defences with the ability to respond rapidly and vigorously for many years after the initial exposure to a microbe or other foreign material. This memory response to a specific antigen is due to several features: **1.** many memory cells are produced, so that the secondary response is greater than the primary response, in which very few cells respond; **2.** memory cells live for many years or have the capacity to produce themselves; **3.** memory cells are activated by a smaller amounts of antigen and require less co-stimulation than do naive, inactivated T cells; **4.** activated memory cells produce greater amounts of interleukins than do naive T cells when they are first activated.

Immunology was originally considered a protective process, helping the body to overcome infectious agents and their toxins. Immune response sometimes can give rise to an excessive or inappropriate reaction. This is referred to as immunodeficiency, autoimmune diseases, hypersensitivity.

Autoimmunity is a condition in which structural or functional damage is produced by the action of immunologically competent cells or antibodies against normal components of the body. The adult host usually exhibits tolerance to tissue antigens present during fetal life that are recognized as "self". However, in certain circumstances tolerance may be lost and immune reactions to host antigens may develop, resulting in autoimmune diseases. The most important step in the production of autoimmune disease is the activation of self-reactive helper (CD4) T cells. These self-reactive Th-1 or Th-2 cells can induce either cell-mediated or antibody-mediated autoimmune reactions, respectively.

The following three main mechanisms for autoimmunity have been proposed.

1. Molecular mimicry: Various bacteria and viruses are implicated as the source of cross-reacting antigens that trigger the activation of autoreactive T cells or B cells (e.g. relationship between the M protein of S. pyogenes and myosin of cardiac muscle and antibodies against certain M proteins cross-react with cardiac myosin leading the rheumatic fever).

2. Alteration of normal proteins: Drugs can bind to normal proteins and make them immunogenic.

3. Release of sequestered antigens: Certain tissues, eg, sperm, central nervous system, and the lens are sequestered so that their antigens are not exposed to the immune system. When such antigens enter the circulation accidentally, e.g., after damage, they elicit both humoral and cellular responses, producing aspermatogenesis, encephalitis and endophthalmitis, respectively.

A L L E R G Y
(IMMEDIATE HYPERSENSITIVITY, AND DELAYED HYPERSENSITIVITY)

Allergy is an altered reactivity of the body, which manifests itself in the disturbance of the usual course of general or local reactions, often during repeated entrance into the body of substances known as *allergens.* For indication of hypersensitivity reactions, the host should have had contact with the antigen (allergen). The initial contact sensitizes the immune system, leading to the priming of the appropriate B and T lymphocytes. This is known as the "sensitizing" dose. Subsequent contact with the allergen causes manifestations of hypersensitivity. This is known as the "shocking" dose.

- Type I (anaphylaxia, atopy) is mediated by **IgE**
- Type II (cytotoxic reaction) is mediated by **IgG** and **IgM**
- Type III (Immune complex hypersensitivity) is mediated by **IgG** and **IgM**
- Type IV (delayed hypersensitivity) is cell-mediated (mediated by **T-lymphocytes)**

C. Pirquet gave the name allergy (GK. allos other, ergein to work) to the altered reactivity of the body under the effect of pathogenic microbes, toxins, medicines and other substances.

Hypersensitivity reactions can be subdivided into two groups:

1. Immediate hypersensitivity

2. Delayed hypersensitivity

Allergic reactions of immediate action subdivided into I, II, and III types, which are antibody-mediated. Type I reactions are mediated by **IgE.** Types II, III are mediated by **IgG** and **IgM.**

Allergic reactions of delayed action is included into the type IV, it cell-mediated (mediated by T-lymphocytes).

Type I or Immediate Hypersensitivity (anaphylactic, IgE or reagin dependent) are mediated by **IgE.** Anaphylaxia (ana-without, phylaxis-protection) is characterized by the production of IgE antibodies against foreign proteins that are commonly present in the environment, for example pollens, animal dander or dust mites. These antibodies bind specifically to a high affinity receptor on mast cells and basophils, which are the only human cells that contain histamine. These two cells are similar in morphology and in their contribution to allergic reactions. Mast cells are especially prevalent in the connective tissue of the skin and respiratory tract and in surrounding blood vessels. Basophils circulate in the bloodstream, where they contribute less than 1% of the leukocytes. Both are packed with granules containing a variety of chemicals called mediators. The Fc region of the IgE antibody attaches to specific receptor site on the mast cell or the basophil, leaving two antigen combining sites free. Subsequent exposure to the same antigen will lead to rapid release of histamine, and more gradual release of other mediators including leukotrienes and cytokines (IL4,IL5,IL13),which participate in switching of Igs into formation of IgE. IL4 activates proliferation of mast cells which are important for development of anaphylactic reaction. The pharmacological effects of histamin are to increase the permeability and dilation of blood capillaries, resulting in edema (swelling) and erythema (redness). Other effects include increased mucus secretion (a runny nose), and smooth - muscle contraction, which in the respiratory bronchi results in breathing difficulty. The conditions that are associated with Type I hypersensitivity include eczema, rhinitis and conjunctivitis (known as hay fever), and asthma.

Which clinical manifestation occurs depends in large part on the location of the mast cells bearing the IgE specific for the allergen. For example, patients who respond to an allergen with urticaria have the allergen specific IgE on mast cells in the skin, whereas those who respond with rhinitis have the allergen-specific mast cells in the nose (localized anaphylaxis). Common clinical manifestations include hay fever, asthma, eczema, and urticaria. Asthma is an allergic reaction that affects mainly the lower respiratory system. Such symptoms as wheezing and shortness of breath are caused by the constriction of smooth muscles in the bronchial tubes Many sufferers give immediate type reactions to skin tests (injection, patch, scratch, etc) using the offending antigen. The most severe form of type I hypersensitivity is **Systemic anaphylaxis** (anaphylactic shock) in which **severe broncho-constriction and hypotension (shock)** can be life-threatening (systemic anaphylaxis can be fatal within few minutes) and **atopy**. Atopic disorders are immediate hypersensitivity reactions that exhibit a strong familial predisposition and are associated with elevated IgE levels. Predisposition to atopy is clearly genetic, but symptoms are induced by exposure to specific allergens (eg, respiratory allergy to pollens, or house dust) or food (eg, intestinal allergy to shellfish or nuts). Production of IgE is opposite to production of IgA.

Deficiency of IgA is risk factor in development of allergic reactions (especially atopic reactions). Distribution of lymphocytes capable of synthesizing IgA and IgE is closely parallel, especially in the submucosa. In normal individuals, the inhalant and ingestant antigens are dealt with by IgA lining the respiratory and intestinal mucosa and therefore they do not come into contact with potential IgE producing cells. When IgA is deficient, the antigens cause massive stimulation of IgE forming cells, leading to overproduction of IgE.

Prevention of anaphylactic reactions is by desensitization. This procedure consists of a series of dosages of the antigen carefully injected beneath the skin. The objective is to cause the production of IgG antibodies rather than those of the IgE class, in the hope that the circulating IgG antibodies will act as blocking antibodies to intercept and neutralize the antigens before they can react with cell-bound IgE. Recent evidence indicates that desensitization might also induce the production of suppressor T cells.

Type II or Cytotoxic hypersensitivity: Type II reactions generally involve the activation of complement by the combination of IgG and IgM (rarely) antibodies with antigenic determinants on the surface of the cells leading to cytotoxic or cytolytic effects. Cytotoxic hypersensitivity occurs when antibody directed at antigens of the cell membrane activates complement. This generates a membrane attack complex which damages the cell membrane. The antibody (IgG or IgM) attaches to the antigen via the Fab region and acts as a bridge to complement via the Fc region. As a result, there may be complement-mediated lyses, as occurs in haemolytic anaemia, ABO transfusion reactions (When a transfusion is incompatible, as when type B blood is transfused into a person with type A blood, the antigens on the type B blood cells will react with anti-B antibodies in the recipient's serum. This antigen-antibody reaction activates complement, which in turn causes lysis of the donor's red blood cells as they enter the recipient's system), or Rh haemolytic disease (The roughly 85% of the population whose cells possess Rh antigen are called Rh+; those lacking this antigen (about 15%) are Rh-. Antibodies that react with the Rh antigen do not occur naturally in the serum of Rh- individuals, but exposure to this antigen can sensitize their immune system to produce anti-Rh antibodies. If blood from an Rh+ donor is given to an Rh- recipient, the donor's red blood cells stimulate the production of anti-Rh antibodies in the recipient. If the recipient then receives Rah+ red blood cells in a subsequent transfusion, a rapid, serious haemolytic reaction will develop).

Drugs such as penicillin, phenacetin can attach to surface proteins on red blood cells and initiate antibody formation. Such autoimmune antibodies (IgG) may then combine with the cell surface, with resulting hemolysis. Other drugs (eg.quinine) can attach to platelets and induce autoantibodies that lyses the platelets, producing thrombocytopenia and, as a consequence, a bleeding tendency. Certain infections eg., rheumatic fever, can induce antibodies that cross-react with cardiac tissue.

Type III or Immune complex hypersensitivity: When antibody combines with its specific antigen, immune complexes are formed. Normally, they are promptly removed by the reticuloendothelial system, but occasionally they persist and are deposited in tissues, resulting in several disorders. In persistent microbial or viral infections, immune complexes may be deposited in organs (eg, kidneys), resulting in dysfunction. In autoimmune disorders, "self" antigens may elicit antibodies that bind to organ antigens or are deposited in organs as complexes, especially in joints (arthritis), Kidneys (nephritis), or blood vessels (vasculitis).

Wherever, immune complexes are deposited, they activate the complement system; and polymorphonuclear cells are attracted to the site, where they cause inflammation and tissue injury. A typical type III hypersensitivity reaction is serum sickness, immune complex disease.

Serum sickness: Following the injection of foreign serum (or certain drugs) the antigen is slowly cleared from the circulation and antibody production begins. Simultaneous presence of antigen and antibody leads to production of immune complexes, which may circulate or may be deposited at various sites. Typical serum sickness results in fever, urticaria, arthralgia, lymphadenopathy, and splenomegaly a few days to 2 weeks after injection of the foreign serum. Symptoms improve as the immune elimination of antigen continues and subside when it is complete.

Nowadays, serum sickness is caused more commonly by drugs, eg, penicillin, than by foreign serum because foreign serum is used so infrequently.

Immune complex disease: Many clinical disorders associated with immune complex have been described, though the antigen often cannot be identified. A representative example is glomerulonephritis. Acute post-streptococcal glomerulonephritis is a well-known immune complex disease. Its onset occurs

several weeks after β-haemolytic streptococcal infection, particularly of the skin, and often occurs in infection due to nephritogenic types of streptococci. The complement level is typically low, suggesting an antigen–antibody reaction. Lumpy deposits of immunoglobulin and C3 are seen along glomerular basement membranes stained by immunofluorescence, suggesting antigen-antibody complexes; streptococcal antigens have been rarely demonstrated, however. It is assumed that streptococcal antigen-antibody complexes are filtered out by glomeruli and that they fix complement, attract polymorphs, and start the inflammatory process.

Type IV or Cell-mediated (delayed) Hypersensitivity: Cell-mediated hypersensitivity is a function of T lymphocytes (CD4-helper T lymphocytes), not of antibody. It can be transferred by immunologically committed (sensitized) T cells but not by serum. The response is delayed - i.e., it starts hours (or days) after contact with the antigen and often lasts for days. It consists mainly of mononuclear cell infiltration (macrophages and helper-CD4 T cells) and tissue indurations, as typified by the tuberculin skin test.

1. Contact Hypersensitivity: This manifestation of cell-mediated hypersensitivity occurs after sensitization with simple chemicals (eg, nickel, formaldehyde), plant materials (poison ivy, poison oak) topically applied drugs (eg, antibiotics), some cosmetics, soaps, and other substances. In all cases, small molecules enter the skin and then, acting as haptens, attach to body proteins to serve as complete antigen. Cell-mediated hypersensitivity is induced, particularly in skin. When the skin again comes in contact with the offending agent, the sensitized person develops erythema, itching, vesication, eczema, or necrosis of skin within 12-48 hours. Patch testing on a small area of skin can sometimes identify the offending antigen.

Subsequent avoidance of the material will prevent recurrences. The antigen-presenting cell in contact sensitivity is probably the Langerhans cell in the epidermis.

2. Tuberculin-type hypersensitivity: Delayed hypersensitivity to antigens of microorganisms occurs in many infectious diseases and has been used as an aid in diagnosis. It is typified by the tuberculin reaction. When a small amount of tuberculin is injected into the epidermis of a patient previously exposed to Mycobacterium tuberculosis, there is little immediate reaction, gradually, however, indurations and redness develop and reach a peak in 48-72 hours. A positive skin test indicates that the person has been infected with the agent, but it implies no current disease. However, a skin test that changes from negative to positive suggests recent infection and possible current activity.

A positive skin test (Mantuo, Pirquet, Burne, Mitzuda skin allergic tests) response assists in diagnosis and provides support for chemoprophylaxis or chemotherapy.

Table

Immediate type of hypersensitivity	Delayed type of hypersensitivity
1.reaction occurs in sensitized organism during repeated entrance of antigen into the body after 15-30minutes	Development of the reaction after 6-8 till 48 and more hours
2. reaction more frequently develops in blood stream, in smooth muscles, and in organs which are rich of blood vessels	reaction more frequently develops after antigen's prolonged contact with skin
3. in blood stream IgE is present	in bloodstream immunoglobulins are absent
4.passive transmission of hypersensitivity by sensitized blood serum is possible	passive transmission of hypersensitivity by the cells of lymphoid organs and by leukocytes is possible

Diseases of hypersensitivity

Type of allergy	Mechanisms of immunological reaction	Manifestation

Type-I Anaphylactic shock	Production of cytophilic IgE. Reaction of antigen-antibody induces realization of mediators(e.g. histamine)	Anaphylactic shock (medicamentous), atopy, Quinke oedema, atopic dermatitis, urticaria, eczema of new born, bronchial asthma, conjunctivitis, rhinitis
Type-II Cytotoxic	Production of IgG and IgM against antigens which are involved in the cell membrane composition. Reaction antigen and antibody through the activation of complement	Cytotoxic reactions in drug allergy, hemolytic anemia, autoimmune diseases
Type-III Immune complexes	Production of precipitant IgM and IgG, abundance of antigen, pathogenic reactions, initiation by immune complexes through the activation of complement and leukocytes	Arthuse's reaction, systemic diseases, serum diseases, relapsing aphthous stomatitis, paradontopathia
Type-IV Cellular	Accumulation of sensitized T-lymphocytes, reaction between antigen and sensitized T-lymphocytes-effectors by production of lymphokines and cytotoxic reactions in presence of macrophages	Allergic manifestations in infectious diseases-tuberculosis, brucellosis, tularaemia; and in autoimmune diseases, contact allergy, drug stomatitis, parodontopathia, ulceronecrotic stomatitis, relapsing aphthous stomatitis,

PECULIARITIES OF IMMUNITY OF VIRAL INFECTIONS

Host defense against viruses fall into two major categories: **Non-specific** – (interferon, phagocytes, fever, serum inhibitors), **Specific** is including both humoral and cell-mediated immunity (T and B lymphocytes).

Non-specific defense:

Interferon: inhibit the intracellular replication of a wide variety of both DNA and RNA viruses but have little effect on the metabolism of normal cells; i.e., they exhibit a remarkable degree of selective inhibition. Because interferons are produced within a few hours of the initiations of viral replication, they may act in the early phase of viral diseases to limit the spread of virus. In contrast, antibodies begin to appear in the blood several days after infection.

Phagocytosis: Macrophages, particularly fixed macrophages of the reticuloendothelial system and alveolar macrophages are the important cell types in limiting virus infection. In contrast, polymorphonuclear leukocytes are the predominant cellular defense in bacteria infections.

Fever: Elevated body temperature may play a role in host defense, but its importance is uncertain. Fever may act in two ways: a) the high temperature of the body may directly inactivate the virus particles, particularly enveloped viruses, which are more heat-sensitive than non-enveloped viruses. b) Replication of some viruses is reduced at higher temperatures; therefore fever may inhibit replication.

Mucocilliary clearance: The mucocilliary clearance mechanism of the respiratory tract may protect the host. Its damage, eg, from smoking, results in an increased frequency of viral respiratory tract infections, especially influenza.

Specific defense:

There is evidence for natural resistance to some viruses in certain species, which is probably based on the absence of receptors on the cell of the resistant species. However, acquired immunity is far the most important type of defense, which is either actively acquired by exposure to the virus or passively acquired by the transfer of immune serum. Active immunity can be elicited by contracting the actual disease, by having an inapparent infection, or by vaccination.

The main role depends on T and B lymphocytes, production of M and G immunoglobulins. sIgA confers protection against viruses which enter through the respiratory and gastrointestinal mucosa, and IgG and IgM protect against viruses which enter or are spread through the blood.

V AC C I N S

One of the important directions of applied immunology is the creation of effective preparations for immunotherapy and immunoprophylaxis of infectious diseases. These kinds of preparations are:

1.Vaccins and different types of prophylactic preparations and preparations for treatment prepared from microbes and from substances of their metabolic activities (anatoxins, phages, eubiotics).

2.Immune serums

3.Immunomodulators

4.Diagnostic preparations

Immunoprophylaxis is the prevention of individuals or in groups of people from the infections which can be organized by two pathways:

- Active immunization is the injection of microbial antigens (vaccines) into organism, which forms active immunity
- Passive immunization is the injection of specific antibodies (immune sera, immunoglobulins), which forms passive immunity

Preparations, which are used against microorganisms and against their toxins for development of acquired **active immunity**, are named vaccines.

Conformities to vaccines:

- must be high immunogenic because they must form stable and prolonged immunity
- must be non-dangerous
- must be non-reactogenic and mustn't develop side effects
- must save antigenic and immunogenic properties during transportation and preservation

By the antigens containing in the structure of vaccines they can be:

a)monovaccines which contain one serotypic antigens

b)polyvaccines which contain antigens of different serotypes

c)complex, combinative or associated vaccines which contain different types of antigens

Routes of injection of vaccines are different (subcutaneous, intracutanous, intramuscular, intranasal, per os); they depend on peculiarities of microorganisms.

According to the origin and structure all vaccines are classified into:

ALIVE attenuated (weakened) vaccines - are obtained by passages of microorganisms, are derived from strains with low or lack virulence but with preserved antigenic and immunogenic properties.

These strains are able to grow in vaccinated organisms and cause latent infection. They develop acquired artificial immunity. This immunity is similar to the acquired natural immunity; can be stable, tense, prolonged; and are used for prevention of viral and bacterial infections (eg., BCG vaccine, vaccines against tularemia, anthrax, brucellosis).

These vaccines can leave different side effects:

- sensitization of the organism
- can cause development of classical infection
- some antiviral vaccinal strains can cause development of persistency

KILLED or inactivated vaccines –do not produce development of infectious process, but they are less immunogenic. These vaccines are obtained from pathogenic microorganisms which are cultivated by chemicals or temperature and are used against bacterial and viral infections. These vaccines can leave side effects also:

- sensitization of the organism
- loading of immune system
- reactogenicity
- toxigenicity which depends on presence of lipids and other chemical compounds.

SUBUNIT, COMPONENT vaccines –are free of unimportant antigens, which do not produce loading of immune system (vaccines against hepatitis B, typhoid fever, cholera).

ANATOXINS are component vaccines which are prepared from exotoxins by cultivation of them with formalin under the temperature.

LIPOSOMIC VACCINES – are complexes of antigens and carriers of liposome, which protect antigenic epitopes for introduction of antigens to immunocompetent cells and activate synthesis of immunoglobulins; they play role of adjutants.

SYNTHETIC VACCINS- prepared by chemical synthesis.
RECOMBINATIVE, GENE ENGINEERING VACCINS – introduced by none-pathogenic strains of microorganisms which get property to synthesize immunogenic antigens (Hepatitis B, tetanus) by gene engineering methods.
ANTIIDIOTYPIC VACCINS – explained by the similarity between antigenic determinates and active centers (Fab fragmant) of anti-idiotypic antibodies.

Vaccines in general are used

a)by plan-specific prophylaxis:
b)vaccines which are obligate to endemic foci for specific professional groups
c)vaccines, which are used depending on epidemiological conditions
In some bacterial and viral infections specific immunoglobulines are used for prevention of the organism –**seroprophylaxis.**
Immune serums are used in

- diagnosis of infections
- treatment of infections
- prophylaxis of infections

Immune serums can be prepared by immunization of animals. These preparations are heterogenic and can cause allergic reactions. Nowadays human homological immune serums are used, which are obtained from donors or placental pools. Normal pooled human immunoglobulin contains enough antibodies against common infections for a dose of 100-400mg of IgG to protect hypogammaglobulinaemic patients for month. Over 1000 donors are used for each pool, and the sera must be screened for HIV and hepatitis B and C.
Immunoglobulins- are perpetrations which form in organism passive humoral immunity and capable to protect organism from intoxication or infections.
For treatment and prophylaxis of infections **immunostimulators and immunomodulators** are used too. These are obtained from living organisms or by chemical synthesis, which are able to regulate (stimulate or inhibit) immune response.
By the origin immunemodulators are subdivided into **homological and heterological.**

- Homological are endogens substances, which are synthesized in human organism-cytokines, interferon, interleukins, TNF.
- Heterological immunomodulators are prepared by chemical synthesis-levamyzol, cyclosporine A and can be obtained from bacteria - polysaccharides, LPS, muramilpeptide, pyrogenal.

Immunodeficiency disease results from the absence, or failure of normal function, of one or more elements of immune system. The deficiencies can be either **congenital** (primary) or **acquired** (secondary).
Primary immunodeficiency

Defective antibody response result in increased susceptibility to pyogenic infections and are due to failure of B cell function, such as in X-linked agammaglobulinaemia, or from failure of proper T-cell signals to B cell such as occurs in hyper-IgM syndrome, common variable immunodeficiency and transient hypogammaglobulinaemia of infancy.

Defective cell-mediated immunity results in increased susceptibility to opportunistic infections and is due to failure of T-cell function such as occur in severe combined immunodeficiency, MHC class II deficiency, etc.

Hereditary complement component defects are found in a number of clinical syndromes, the most common of which is that of the C1 inhibitor, which result in hereditary angioedema.

Hereditary complement deficiencies of the terminal complement components (C5, C6, C7, and C8) and the alternative pathway proteins (factor D, factor B, properdin) lead to extraordinary susceptibility to infections with two Neisseria species: N. gonorrhoeae and N. meningitidis.

Defects in phagocytes the resulting persistence of bacterial products in phagocytes leads to abscesses or granulomas depending on the pathogen.

Leucocyte adhesion deficiency is associated with a persistent leucocytosis because phagocytic cells with defective integrin molecules cannot migrate through the vascular endothelium from the blood stream into the tissues.

Secondary immunodeficiency

Immunomodulatory drugs can severely depress immune functions.

Steroids affect cell traffic, induce leukocytopenia and inhibit cytokine synthesis.

Cyclophosphamide, azathioprine and mycophenolate mofetil act directly on DNA or its synthesis.

Severe protein-energy malnutrition (PEM) reduces the efficacy of the immune system. Malnutrition increases the risk of infant mortality from infection through reduction in cell-mediated immunity, reduced CD4 helper cells, reduced T-cell help and reduction of secretory IgA.

Trace elements, iron, selenium, copper and zinc are important in immunity. Lack of these elements can lead to diminished neutrophil killing of bacteria and fungi, susceptibility to viral infections and diminished antibody response.

Vitamins A, B_6, C, E and also folic acid are important in overall resistance to infection. Carotenoids are antioxidants like vitamin C and E enhance NK cell activity, stimulate the production of cytokines and increase the activity of phagocytic cells.

Diet and nutrition are powerful innovative tools to reduce illness and death caused by infection.

AIDS is caused by human immunodeficiency virus (HIV), which is a single-stranded RNA retrovirus that binds to CD4 and depletes CD4+ T cells. Severe CD4 depletion results from a variety of mechanisms, with drastic functional impairment of cell-mediated immunity and death from opportunistic infections.

Combination therapy for AIDS with reverse transcriptase and protease inhibitors is reasonably successful, but costly.

Successful vaccines for HIV have not yet been identified.

THE CHEMOTHERAPEUTIC AGENTS.
ANTIBIOTICS

The most important problem of medicine is treatment of infectious diseases. Drugs have been used for treatment of infectious diseases since the 17th century (e.g., quinine for malaria); however, chemotherapy as a science is associated with P. Ehrlich. In 1910 Ehrlich established fact that the various chemical substances, which contain arsenic, had antimicrobial action. He used this property for treatment protozoa infections. He synthesized the preparations such as salvarsan, neosalvarsan, which had bactericidal action on Spirochetes and Protozoa. Ehrlich formulated the principles of selective toxicity and recognized the specific chemical relationships between microbial pathogens and drugs, the development of drug resistance, and the role of combined therapy. **Selective toxicity** - this term implies that a drug is harmful to a parasite without being harmful to the host. Selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical events essential to the organism but not to the host.

The current era in chemotherapy began in 1935, with the discovery of the sulfanilamide. Domagk (German chemist) who worked with aniline dyes noticed that the prontozile dye had bactericidal action on staphylococci in vivo, but in vitro this action was not detected. He studied the mechanism of action of this preparation and became to conclusion that in vivo the prontozile splinted into sulfanilamide, which is the analogy of the paraaminobenzoic acid (PABA). The microorganisms need the PABA to synthesize folic acid. And when sulfanilamide is in the environment the microorganisms bind these substances instead of PABA and that lead to destroying them.

This discovery led to the rapid development of a number of related drugs-sulfonamides, or sulfa drugs, which are still used today.

These drugs are:

- Sulfanilamides
- DNA-tropic nitrofurans
- Quinolon's group which inhibit replication and transcription
- Oxiquinolon's

Sulfanilamides are antimetabolites. In the basis of action of these drugs is structural similarity to metabolites which ensure vital processes in host organism. These drugs can be equivalent harmful for bacterial cell and for the cells of macroorganism.

A N T I B I O T I C S

Antibiotics (anti-against, bios-life) are chemical substances excreted by some micro-organisms, which inhibit the growth and development of other microbes that is based on antagonistic interrelations between microorganisms of various species. The study of antibiotics began in 1929, when A. Flaming proved that the filtrate of a broth culture of the fungus Penicillium notatum had antibacterial properties.

Further development of this problem is associated with the works of various scientists: R. Dubos isolated gramicidin and tyrocydin from the cultural liquid of S. brevis; S. Waksman and coworkers devised a method of producing streptomycin. B. Tokin discovered antimicrobial substances from plants – phytoncides, and others who enriched modern medical practice with numerous preparations widely used for treating infectious diseases.

Criteria for evaluating of antimicrobial drugs:

1.SELECTIVE TOXICITY (absence of toxic influence, which determined by chemotherapeutic index).

Chemotherapeutic index = min therapeutic dose / maximal enduring (tolerant) dose <1

2. ANTIMICROBIAL INFLUENCE

- bacteriostatic inhibition of growth and multiplication of microorganisms
- bactericidal –destroying of microorganisms
- selective action on microorganisms, which determined by the spectrum of action (narrow and broad)

- their long-term application should not promote the appearance of resistant forms to antibacterial drugs
- must be soluble in body fluids
- should not produce hypersensitivity(allergy)
- for expression of the influence must penetrate into the cell interact with the target and must save the structure

Classification of antibiotics

The antibiotics are classified according to the:

- **Chemical structure of the drug:**

1. β-lactamates
    - penicillin
    - cephalosporin
    - cycloserine
2. Poliens -nystatin, levorin, amphotericin B, etc.
3. Polymyxins –polymyxin A, polymyxin B
4. Aminoglicozides -streptomycin, neomycin, monomycin, kanamycin, gentamycin, etc.
5. Tetracycline – tetracycline, oxytetracyclin, chlortetracycline, rondomycine, etc.
6. Macrolides – erythromycin, oleandamycine
7. Rifamicin – rifamicin, rifampicin

- **Origin:** Antibiotics produced by

1. fungi. Penicilium and Cephalosporium are used for this purpose.
2. actinomycetes – frequently genus Streptomyces (streptomycin), is used which has a good therapeutic action on Mycobacterium tuberculosis, F. tularensis, on causative agent of whooping cough, etc. Erythromycin, nystatin are the products of Streptomycin. 80 per cent of antibiotics are produced by Actinomycetes.
3. bacteria- for this purpose genus Bacillus and genus Pseudomonas are used (polymixins are prepared from bacteria).
4. tissues (animal origin**): lysozyme,** which discovered in saliva, tear. Lysozyme has bactericidal action on bacteria they are break the glycoside bonds in bacterial cell wall. The next antibiotics are **ecmoline, ectericid**, which are synthesized by **fish tissues**. **Interferons (α, β)** are glycoproteins produced by human and other animal cells after viral infection. They inhibit the reproduction of viruses by blocking the translation of viral proteins. Interferons are universal, are not virus specific (**IFN** are active against all viruses).
5. plants – volatile plant substances phytoncides have bactericidal properties, which cause a lethal effect and they are used for treatment of infectious diseases. Onion, garlic, tomato are contain volatile and are bactericidal products.

- **the way of reception:**

1. biological synthesizes – (natural antibiotics) during this method high productive stem is used which is cultivated on special media (penicillin).
2. chemical – synthesizing of antibiotics from chemical substances (synthetic antibiotics-levorin).
3. combined (complex) method: here two methods are used: complex of biological and chemical methods. By the biological method the main ring of antibiotic is obtained than chemicals are added and the new preparation is prepared (e.g. cephalosporin, semisynthetic penicillin). These preparations are used in the treatment of diseases induced by penicillin-resistant staphylococci and other causative agents.

THE MECHANISMS OF ACTION OF ANTIBIOTICS AND ANTIMICROBIAL SPECTRUM

1. Antibacterial antibiotics. The numerous groups. In this group antibiotics subdivided are into broad (large) spectrum and narrow spectrum. Antibiotics of broad spectrum act on the whole groups of bacteria: gram negative, gram-positive (Gracilicutes, Firmicutes, Tenericutes). Narrow

spectrum antibiotics acted on the few (small) groups of bacteria (e.g. only gram negative or only gram positive).

2. Antifungal antibiotics. There are few preparations in this group - nystatin, levorin the drugs against genus Candida. Amphotericin B, which has large spectrum and act on Candida genus and the other genesis (e. g. Blastomycosis, Aspergiliuosis, etc.)

3. Antiprotozoal group. There are few antibiotics in this group in major narrow spectrum (Fumagilin).

4. Antiviral group: Of interest is the very difficult of chemotherapy of viral diseases. At present there are no effective drugs against viral infections. This is due to the biological peculiarities of viruses as obligate intracellular parasites.

The mechanisms of action of antibiotics various due to this they are subdivided into 4 groups**:**

1. Antibiotics, which inhibits the synthesis of the bacterial cell wall (cephalosporin, penicillin, cycloserine).

2. Antibiotics, which antimicrobial action through inhibition of cell membrane function (Polymyxine, Poliens, Amphotericin B).

3. The group of antibiotics, which inhibits protein biosynthesis in bacteria on ribosomal level (aminoglicozides, Macrolides, Tetracyclines).

4. Antibiotics, which action through inhibition of nucleic acid synthesis. To this group belong antitumour antibiotics, which inhibit RNA synthesis (Actinomycin, Rifampin) and inhibitors of DNA synthesis (Rubomycin, all quinolones and ftouroquinolones). A number of inhibitors of nucleic acid synthesis are sufficiently selective to serve as antiviral drugs.

SIDE EFFECTS OF ANTIBIOTICS ON MACROORGANISMS (DANGERS OF INDISCRIMINATE USE)

1. Direct drug toxicity. The toxic action is associated with the quality of preparation, doses, with the kind of employment and the state of patients. Antibiotics can be hepatotoxic (tetracyclines), nephrotoxic (aminoglycosides), ototoxic (streptomycin, gentamycin). It has been established that high doses of penicillin and streptomycin have a neurotoxic action. Levomycetin has toxic effect on the haematopoetic organs. Cephalosporins suppress the synthesis of vitamins and can cause bleeding. Antibiotics has a toxic effect on fetus - **teratotoxic action** (anzamycin, levomycetin)

2. Changes in the normal flora of the body. Antimicrobial drugs affect not only the infecting microorganisms but also susceptible members of the normal microbial flora of the body. An imbalance appears which cause profound disturbances of symbiotic relationships among normal microflora resulting in disbacteriosis. The condition of disbacteriosis enhances intensive multiplication and spread of some of the co members of the intestinal, mucosal and skin biocoenosis and their transformation from a saprophyte state into conditionally pathogenic and pathogenic forms. As a result local and general lesions develop. The oppressing of normal flora with antibiotics resulting in the oppressing of antagonism and it promote the development of infectious disease, or development of secondary infections. To prevent these complications we use the following methods: **1.** If it is possible using antibiotics of narrow spectrum, **2.** With antibiotics it is necessary to use antifungal preparations, **3.** To restore the normal microflora it is necessary to prescribe eubiotics.

3. Influence on immune system: Widespread sensitization of the population, those result in **hypersensitivity,** anaphylaxis, rashes, fever, blood disorders, cholestatic hepatitis, and collagen-vascular diseases. The development of these reactions coherent of the quality of preparation, the way of injection, from the individual felt of patient. Quite frequently allergic reactions arise during local application of antibiotics. During these reactions can develop allergic rash, contact dermatitis, anaphylactic reactions. In order to avoid the allergic reactions before the using of antibiotics Bezredka test is used.

Immunodepression can occur during antibiotic therapy; this can be used in transplantation (e.g., cyclosporine which is produced as an antifungal preparation, but immune depressive function is higher and now it used in transplantation).

Bacteria and bacterial cell can lose the main antigenic structures during antibiotic therapy and due to this cannot be presented complete antigenic functions, due to this, immune response is not complete and infection can transform into chronic form, relapse or re-infection.

To prevent these complications immune- antibiotic- therapy can be used. It means parallel using of antibiotics and vaccines. Under the action of antibiotics microorganism is killed, under the action of vaccines immune system is stimulated.

SIDE EFFECTS OF ANTIBIOTICS ON MICROORGANISM

During antibiotic therapy changes in structure of the bacterial cell, in biochemical activity (e.g., L-forms can form. It leads to difficulties in diagnosis) can occur.

During antibiotic therapy resistance to antimicrobial drugs can occur. There are many different mechanisms by which microorganisms might exhibit resistance to drugs: Innate and acquired. Example of innate resistance is mycoplasma. This bacteria is without cell wall (it is innate property), and penicillin cannot act on this bacteria.

Acquired drug resistance is subdivided into genetic (chromosomal) and biochemical (extrachromosomal) resistance.

Genetic origin of drug resistance: Most drug resistant microbes emerge as a result of genetic change and subsequent selection processes by antimicrobial drugs.

Chromosomal (genetic) resistance: This develops as a result of spontaneous mutation in a locus that controls susceptibility to a given antimicrobial drug. Chromosomal resistance to antibiotic develops only to single drug.

Extrachromosomal resistance: Bacteria often contain extrachromosomal genetic elements called plasmids (e.g., R-factor). R factors are a class of plasmids that carry genes for resistance to one- and often several-antimicrobial drugs. Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs.

Thus, plasmids determine resistance to penicillin and cephalosporin by carrying genes for the formation of β-lactamases. Microorganisms produce enzymes that destroy the drug. Examples: Staphylococci are resistant to penicillin by producing β-lactamase enzyme that destroys the drug.

Genetic material and plasmids can be transferred by the transduction, transformation, and conjugation.

Besides, changes in permeability of membranes (against tetracycline), changes in target cells (e.g., during streptomycin therapy changes in ribosomal proteins on which this drug is binds occur).

There are three cases by which antibiotic can leave bactericidal or bacteriostatic action:

1. Antibacterial drug must enter into the cell,
2. Antibacterial drug must interact with target cell,
3. Antibiotic during this interaction must save primary structure.

In order to avoid complications of antibiotics, the following principles should be applied:

1. Microbiological: A specific etiologic diagnosis must be formulated. Antibiotic must be used only during infection where participates microorganism. It is necessary to isolate pure culture; it is preferable to obtain a representative specimen before giving antimicrobial drugs. Before giving this drug it is necessary measurement of antimicrobial activity. For measurement of antimicrobial activity we'll use two principal methods: dilution or diffusion.

2. Pharmacologic principle: Before giving antimicrobial drug it is necessary to define correct dose, the intervals, route of injection. It is necessary to know drug combinations.

3. Clinical principles: Before giving these drugs it is necessary to take into consideration the state of the patient, age, sex, the condition of immune status, pregnancy, attendant diseases.

4. Epidemiological principle: By avoiding resistance it is necessary limiting its use especially in hospitals.

5. Pharmaceutics principal: For saving the antimicrobial drugs it is necessary favorable environmental conditions: pH, temperature, humidity, because in unfavorable conditions toxic product are formed which can damage the organism.

ANTIVIRAL PREPARATIONS

Unlike viruses, bacteria and protozoa do not rely on host cellular machinery for replication, so processes specific to these organisms provide ready targets for the development of antibacterial and antiprotozoal drugs. Because viruses are obligate intracellular parasites, antiviral agents must be capable of selectively inhibiting viral functions without damaging the host. Furthermore, an ideal drug would reduce disease symptoms without modifying the viral infection so much as to prevent an immune response in the host. Molecular virology studies have succeeded in identifying virus-specific functions that can serve as realistic targets for inhibition. Theoretically, any stage in the viral replication cycle could be target for antiviral therapy. The most amenable stages to target in viral infections include the stages of attachment of virus to host cells; of uncoating of the viral genome; of reverse transcription; of replication of viral nucleic acid; of translation of viral proteins; and of assembly, maturation, and release of progeny virus particles. In reality, it has been very difficult to develop antivirals that can distinguish viral from host replicative processes.

Compounds have been found that are of value in treatment of some viral diseases. However, most antivirals are of use in only a limited number of situations and may be toxic to the host.

Antiviral preparations subdivided into following groups:

- **Virulocydal preparations:** these preparations suppressed extra cellular viruses. **Oxolin -** clinically, is used to treat pneumonia, bronchitis, bronchi-pneumonia caused by paramyxoviruses, rinoviruses. **Tetrofein -** used to treat infections caused by herpes viruses and adenoviruses (pharyngitis, laryngitis, and herpes).
- **Preparations, which blockade adsorption of viruses on specific receptors -** these, are the analogs of viral receptors they are adsorbed on the host cell receptors (they occupy all receptors) and when virus entered into the host organism there aren't receptors for them. Nowadays, there is one kind of analogs only CD4 receptors on which AIDS virus is adsorbed.
- **Preparations, which participate in blocking of uncoating** – Amantadine, a synthetic amine, specifically inhibits influenza A viruses by blocking uncoating. When administered prophylactically, it has a significant protective effect in humans against influenza A strains but not against influenza B or other viruses. Rimantadine is a derivative of amantadine with the same spectrum of antiviral activity, but it is less toxic and associated with fewer side effects.
- **Preparations, which are inhibit the stage of assembly of viruses** – Metisazon during smallpox.
- **Preparations, which are inhibit the stage of releasing of viruses** – Tamiflu (**Oseltamivir** inhibits the neuraminidase enzyme, which is expressed on the viral surface. The enzyme promotes release of virus from infected cells and facilitates viral movement within the respiratory tract).
- **Inhibitors of replication:** these preparations integrated into the viral RNA or DNA and blockaded the functions of polymerases. For example: Vidarabine is a purine analog used as an ophthalmic antiviral drug. It blocks viral DNA synthesis by inhibiting viral DNA-polymerase. Zalcitabine inhibits the reverse transcriptase of HIV and, after phosphorylation in the cell, blocks the synthesis of proviral DNA.
- **Interferons:** They are host-coded proteins of the large cytokine family that inhibit viral replication (IFN-α; IFN-β). Interferons fall into three general groups, designated IFN-α; IFN-β; IFN-γ**.** Inteferons are produced by all vertebrate species. Normal cells do not generally synthesize interferon until they are induced to do so. Infection with viruses is a potent insult leading to induction: RNA viruses are stronger inducers of interferons than DNA viruses.
- IFH-γ is not produced in response to most viruses but is induced by mitogen stimulation.