МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

MORPHOLOGY OF BACTERIA, VIRUSES AND PROTOZOA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

МОРФОЛОГІЯ БАКТЕРІЙ, ВІРУСІВ І НАЙПРОСТІШИХ

Методичні вказівки з дисципліни «Мікробіологія, вірусологія та імунологія» для студентів II і III курсів медичного та стоматологічного факультетів з англійською мовою викладання

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Learning guide is related to the program of Ministry of Health of Ukraine and is recommended to students of medical and dentistry faculties of high medical schools of III-IV level accreditation.

Learning guide includes sections of taxonomy, morphology and ultrastructure of bacteria, viruses and pathogenic protozoa. The most modern information on methods of microscopic examination is represented.

Морфологія бактерій, вірусів і найпростіших : метод. вказівки з дисципліни «Мікробіологія, вірусологія та імунологія» для студентів II і III курсів мед. та стомат. фак-тів з англ. мовою викладання / упоряд. Н. І. Коваленко. – Харків : ХНМУ, 2017. – 76 с.

Упорядник Н. І. Коваленко

MORPHOLOGY OF COCCI. MICROSCOPIC EXAMINATION. SIMPLE TECHNIQUES OF STAINING

Actuality of the theme.

Infections may be caused by bacteria, viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the patient's indigenous flora. Endogenous infections can occur when the microorganism is aspirated from the upper to the lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal. Although it is important to establish the cause of an infection, the differential diagnosis is based on a careful history, physical examination, and appropriate radiographic and laboratory studies, including the selection of appropriate specimens for microbiologic examination. Results of the history, physical examination, and radiographic and laboratory studies allow the physician to request tests for the microorganisms most likely to be the cause of the infection.

Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, or more than 100 other viruses.

Most often, therefore, it is necessary to use microbiologic laboratory methods to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology laboratory is to test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified.

Direct examination of specimens frequently provides the most rapid indication of microbial infection. A variety of microscopic, immunologic, and hybridization techniques have been developed for rapid diagnosis. The sensitivity of a technique usually depends on the number of microorganisms in the specimen. Its specificity depends on how morphologically unique a specific microorganism appears microscopically.

Goal: Studying of morphology of cocci by microscopic examination.

Concrete goals:

1. Study of the rules of work in a bacteriological laboratory.
2. Study of arrangement of cocci in microslide.

3. Study of simple method of staining of microslide.
4. Study of structure of immersion microscope.

Students should be able to:

1. Perform simple method of staining of microslide.
2. Using a microscope, identify different bacterial shapes and arrangements.

Equipment: museum cultures of bacteria, bacteriological loops, gas burners, basic days, immersion microscopes, immersion oil, tables, atlas.

Rules of work in bacteriological laboratories. 1. The personnel working at laboratories is supplied with medical coats and kerchiefs or caps. Special clothes protect the worker and also prevent contamination of the material to be studied with foreign microflora.

2. Eating and smoking in the laboratory are strictly forbidden.

3. Unnecessary walking about the laboratory, sharp movements, and irrelevant conversations should be discouraged. It is also necessary to avoid rubbing one's eyes or nose, scratching one's head, biting nails, pencils, etc.

4. In the process of examination the working place should be kept clean and tidy. Bacteriological loops are rendered harmless by burning them in the burner's flame; used spatulas, glass slides, pipettes, and other instruments are placed into jars with disinfectant solution.

5. Upon the completion of work the nutrient media with inoculated cultures are placed into an incubator; museum cultures, into safe-refrigerators; devices and apparatuses are set up in places specially intended for them. Wipe tables with disinfectant solution and thoroughly wash the hands.

6. If the material to be analyzed or the culture of microorganisms is accidentally spilt onto the hands, table, coat, or shoes, they should be immediately treated with disinfectant.

Students must observe the principles of hygiene. They must disinfect and wash their hands always after contaminating them with a biological material and before leaving the hall. For disinfecting hands, 0.5 % chloramine is used for 2 minutes. Then the hands are to be rinsed with warm water and washed with soap.

Morphology of cocci

Bacteria (Gk bakterion small staff) are, for the most part, unicellular organisms lacking chlorophyll. Their biological properties and predominant reproduction relates them to prokaryotes. Bacteria divide by **binary fission**, a process by which one bacterium splits into two. The size of bacteria is measured in micrometres (mcm). Most pathogenic bacteria measure 0.2 to 10 mcm.

Morphologically, bacteria possess three main forms. They are either spherical (cocci), rod-shaped (bacteria, bacilli, and clostridia) or spiral-shaped (vibrios, spirilla, and spirochetes).

Cocci (Gk. chokes berry). These forms of bacteria are spherical, ellipsoidal, bean-shaped, and lancelet. Cocci are approximately **0.5 micrometer (µm)** in diameter and may be seen, based on their planes of division and tendency to remain attached after replication, in one of the following arrangements (*Fig. 1*):

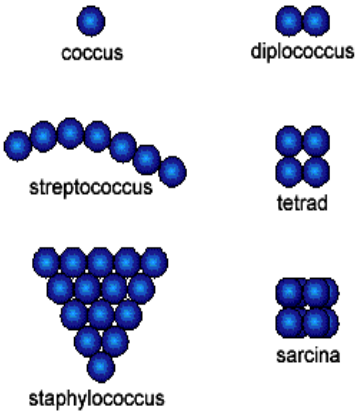


Fig. 1. Arrangements of cocci

1. ***Micrococci.*** The cells are arranged singly or irregularly They are saprophytes, and live in water and in air (Micrococcus agilis, M. roseus, M. luteus, etc).

2. ***Diplococci*** (Gk. diplos double) divide in one plane and remain attached in pairs. These include Neisseria meningitidis or meningoccus (*Fig. 2*), causative agent of epidemic cerebrospinal meningitis, N.gonorrhoeae or gonococcus, causative agent of gonorrhoea and blennorrhoea, and Streptococcus pneumoniae, causative agent of respiratory infections, meningitis etc. (*Fig. 3*).

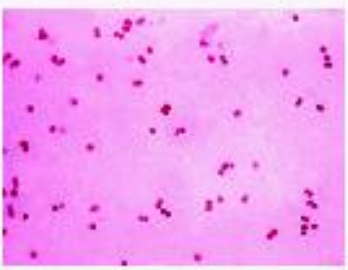


Fig. 2. Neisseria meningitidis

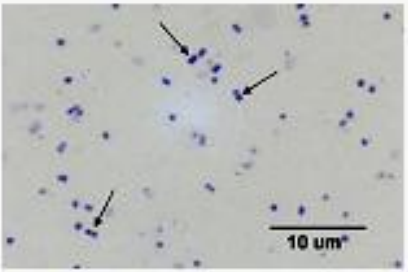


Fig. 3. Streptococcus pneumoniae

3. ***Streptococci*** (Gk. streptos curved, kokkos berry) divide in one plane and are arranged in chains of different length. Some streptococci are pathogenic for humans and are responsible for various diseases (Streptococcus pyogenes) (*Fig. 4*).

4. ***Tetracocci*** (Gk. tetra four) divide in two planes at right angles to one another and form groups of 4 cells (*Fig. 5*). They very rarely produce diseases in humans.

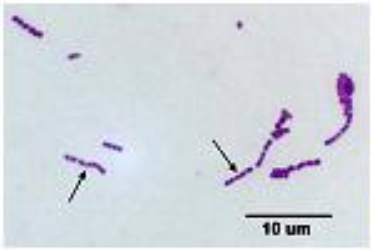


Fig. 4. Streptococcus pyogenes

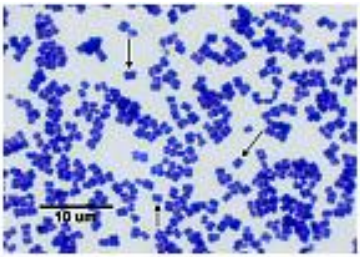


Fig. 5. Tetracoccus arrangement

5. ***Sarcinae*** (L sarcio to tie) divide in three planes at right angles to one another and resemble packets of 8, 16 or more cells (*Fig. 6*). They are frequently found in the air. Virulent species have not been encountered.

6. ***Staphylococci*** (Gk. staphyle cluster of grapes) divide in several planes resulting in irregular bunches of cells, sometimes resembling clusters of grapes (*Fig. 7*). Some species of Staphylococci cause diseases in man and animals (Staphylococcus aureus, S. epidermidis, S. saprophyticus).

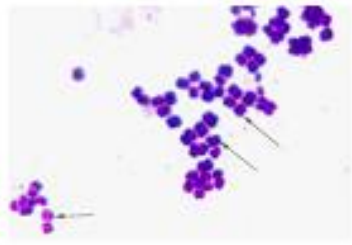


Fig. 6. Sarcina arrangement

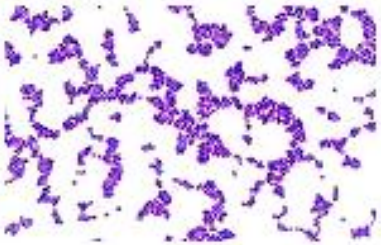


Fig. 7. Staphylococcus aureus

As you observe these different cocci, keep in mind that **the procedures used in slide preparation may cause some arrangements to break apart or clump together**. The correct form, however, should predominate. Also remember that each coccus in an arrangement represents a complete, single, one-celled organism.

Principal microbiological procedures. A complex of bacterioscopic, bacteriological, serological, allergological, and biological techniques is used in the microbiological diagnosis of bacterial infections. Depending on the nature of the given infectious disease, one of these methods is used as the main one, while the others are supplementary. Such biological substances as blood, faeces, urine, cerebrospinal fluid, bile, etc. serve as the material for microbiological diagnosis.

Microscopic examination. Light microscopy. A light microscope is fitted with dry and immersion objectives. A dry objective with a relatively large focal distance and weak magnification power is ordinarily utilized for studying large biological and

histological objects. In examining microorganisms, the immersion objective with a small focal distance and a higher resolving power is predominantly employed (*Fig. 8*).

Reason for using immersion oil. Normally, when light waves travel from one medium into another, they bend. Therefore, as the light travels from the glass slide to the air, the light waves bend and are scattered (the "bent pencil" effect when a pencil is placed in a glass of water). The microscope magnifies this distortion effect. Also, if high magnification is to be used, more light is needed.

In microscopic examination with the help of an immersion objective the latter is immersed in oil (cedar, peachy, "immersiol", etc.) that has the same refractive index as glass and, therefore, provides an optically homogeneous path between the slide and the lens of the objective. Light waves thus travel from the glass slide, into glass-like oil, into the glass lens without being scattered or distorting the image. When such a medium is used, a beam of light emerging from the slide is not diffused and the rays arrive at the objective without changing their direction (*Fig. 9*). In other words, the immersion oil "traps" the light and prevents the distortion effect that is seen as a result of the bending of the light waves. **The resolving power of the immersion objective is about 0.2 mcm.**

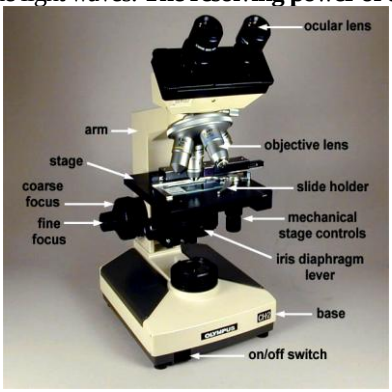


Fig. 8. A light microscope

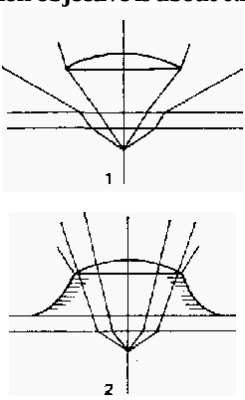


Fig. 9. The course of rays in the dry (1) and oil-immersion (2) systems

Preparation of a smear. Before making a preparation, glass slides are flamed to ensure their additional degreasing.

In preparing a *smear from bacterial culture grown on a solid medium*, a drop of isotonic saline or water is transferred onto the precooled glass. A test tube with the culture is taken by the thumb and the index finger of the left hand. The loop is sterilized in the flame. A cotton-wool plug is pinched by a small finger of the right hand, removed from the test tube, and left in this position. The edges of the test tube are flamed and then the loop is introduced into the test tube through the flame. Having cooled the loop against the inner wall of the tube, the loop is touched to the nutrient medium where it meets with the glass

wall (if the loop is not sufficiently cooled, it induces cracking and melts the medium). Then the loop is touched to the culture of the microorganisms on the surface of the medium. Then the loop is withdrawn, the edges of the test tube are quickly flamed, the tube is closed with a stopper passed through the flame, and then replaced into the test tube rack. All the above described procedures are made above the flame. The culture sample is placed with the loop into a drop of water on the glass slide and spread uniformly with circular movements on an area of 1-1.5 cm in diameter then the loop is flamed.

Drying and fixation of the smear. The dried smears are flamed to kill and fix the bacteria on the glass slide, preventing thereby their washing off during staining. The dead microorganisms are more receptive to dyes and present no danger for the personnel working with them. The glass slide is grasped with a forceps or with the thumb and index finger of the right hand, the smear being in the upside position, and passed three times through the hottest part of the burner's flame.

Staining of a smear. Staining of bacteria is a complex physicochemical process. Interaction of the dye with the cell substances results in the formation of salts ensuring stability of staining. **Relationship between various types of microorganisms and dyes is called a tinctorial property.**

The following dyes are employed most extensively: (1) red (basic fuchsine, acid fuchsine, safranine, neutral red, Congo red); (2) blue (methylene blue, toluidine blue, trypan blue, etc.); (3) violet (gentian, methyl or crystal); and (4) yellow-brown (vesuvin, chrysoidine).

Stains are generally salts in which one of the ions is colored. (A salt is a compound composed of a positively charged ion and a negatively charged ion.) For example, the dye methylene blue is actually the salt methylene blue chloride, which will dissociate in water into a positively charged methylene blue ion which is blue in color and a negatively charged chloride ion which is colorless.

Dyes or stains may be divided into two groups: basic and acidic. If the color portion of the dye resides in the **positive ion**, as in the above case, it is called a **basic dye** (examples: methylene blue, crystal violet, safranin). If the color portion is in the **negatively charged ion**, it is called an **acidic dye** (examples: nigrosin, congo red).

Because of their chemical nature, **the cytoplasm of all bacterial cells have a slight negative charge** when growing in a medium of near neutral pH. Therefore, when using a **basic dye**, the positively charged color portion of the stain combines with the negatively charged bacterial cytoplasm (opposite charges attract) and the organism becomes **directly stained** (*Fig. 10*). An **acidic dye**, due to its chemical nature, reacts differently. Since the color portion of the dye is on the negative ion, it will not readily combine with the negatively charged bacterial cytoplasm (like charges repel). Instead, it forms a **deposit around the organism**, leaving the organism itself colorless (*Fig. 10*). Since the organism is seen indirectly, this type of staining is called **indirect** or **negative,** and is used to get a more accurate view of bacterial size, shapes, and arrangements.

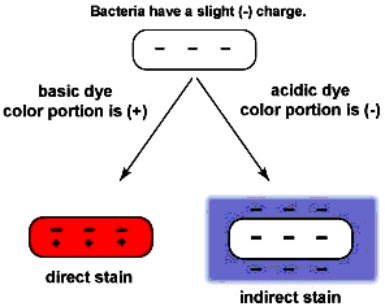


Fig. 10. Direct staining and indirect staining

Simple techniques of staining make use of only one dye and demonstrate the form of bacteria. The fixed preparation is placed, the smear upward, on the support. A dye solution is pipetted onto the entire surface of the smear. With fuchsine the staining lasts 1-2 min, with alkaline solution of Loeffler's methylene blue or water-alcoholic solution of methylene blue, 3-5 min. Following the staining procedure the dye is dispensed, the preparation is washed with water, dried between sheets of filter paper, and then examined under the oil-immersion objective.

Introduction to staining. Bacterial morphology (form and structure) may be examined in two ways: by observing living unstained organisms (wet mount), or by observing killed stained organisms.

Since bacteria are almost colorless and therefore show little contrast with the broth in which they are suspended, they are difficult to observe when unstained. Staining microorganisms enables one to: see greater contrast between the organism and the background, differentiate various morphological types (by shape, arrangement, gram reaction, etc.), observe certain structures (flagella, capsules, endospores, etc.).

Before staining bacteria, you must first understand how to **"fix" the organisms to the glass slide**. If the preparation is not fixed, the organisms will be washed off the slide during staining. A simple method is that of air drying and heat fixing. The organisms are heat fixed by passing an air-dried smear of the organisms through the flame of a gas burner. The heat coagulates the organisms' proteins causing the bacteria to stick to the slide.

The **procedure for heat fixation** is as follows:

1. If the culture is taken from an **agar medium**:

Step 1. Using the dropper bottle of distilled water found in your staining rack, place **1/2 a drop of water** on a clean slide by touching the dropper to the slide (*Fig. 11*).

Step 2. Aseptically remove a **small amount** of the culture from the agar surface and **just touch it several times** to the drop of water until it just turns cloudy (*Fig. 12*).

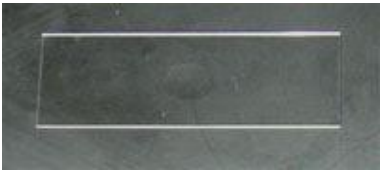


Fig. 11. Preparing the slide for staining: Step 1.

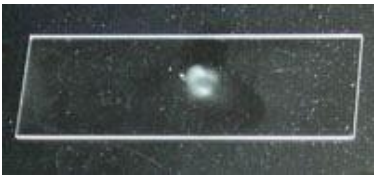


Fig. 12. Preparing the slide for staining: Step 2.

Step 3. **Burn the remaining bacteria off of the loop.** (If too much culture is added to the water, you will not see stained individual bacteria.)

Step 4. Using the loop **spread the suspension over the entire slide** to form a thin film (*Fig. 13*).

Step 5. Allow this thin suspension to **completely air dry** (*Fig. 14*).

Fig. 13. Preparing the slide for staining: Step 4.

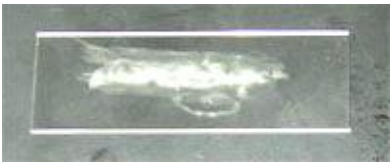


Fig. 14. Preparing the slide for staining: Step 5.

Step 6. Pass the slide (film-side up) through the flame of the bunsen burner 3 or 4 times to heat-fix (*Fig. 15*). Caution: Too much heat might distort the organism and, in the case of the gram stain, may cause gram-positive organisms to stain gram-negatively. The slide should feel very warm but not too hot to hold.

Fig. 15. Preparing the slide for staining: Step 6.

2. If the organism is taken from a **broth culture:**

a. Aseptically place 2 or 3 loops of the culture on a clean slide. **Do not use water**.

b. Using the loop **spread the suspension over the entire slide** to form a thin film.

c. Allow this thin suspension to **completely air dry**.

d. Pass the slide (film-side up) through the flame of the gas burner 3 or 4 times to heat-fix.

The **procedure for staining** is as follows:

Step 1. Place the slide on a staining tray and cover the entire film with **carbol fuchsin**. Stain for **one minute**.

Step 2. Pick up the slide by one end and hold it at an angle over the staining tray. Using the wash bottle on the bench top, gently **wash off the excess carbol fuchsin** from the slide (*Fig. 16*). Also wash off any stain that got on the **bottom** of the slide as well.

Fig. 16. Washing with water.

Step 3. Use a book of blotting paper to **blot the slide dry**. Observe using oil immersion microscopy.

The rules of work with immersion system of a microscope.

Positioning the slide. Place the slide specimen-side-up on the stage so that the specimen lies over the opening for the light in the middle of the stage. Secure the slide **between** (not under) the arms of the mechanical stage. The slide can now be moved from place to place using the 2 control knobs located on the right of the stage. Place a rounded drop of **immersion oil** on the area to be observed (*Fig. 17*).

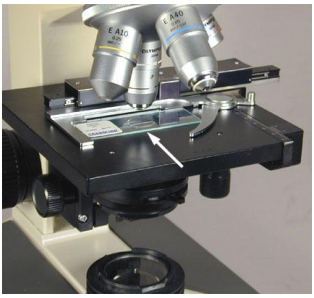


Fig. 17. The slide goes between the two arms of the slide holder of the mechanical stage

Adjusting the illumination. Adjust the **total light available** by turning the flat mirror. Adjust the **amount of light coming through the condenser** using the **iris diaphragm lever** located below and to the front of the stage. Light adjustment using the iris diaphragm lever is critical to obtaining proper contrast. For oil immersion microscopy (900X), the iris diaphragm lever should be set almost all the way open (to your left for maximum light).

Focusing with oil immersion microscope (*Fig. 18*)**.**

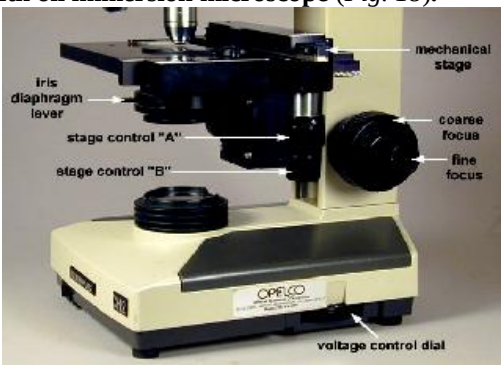


Fig. 18. Focusing of microscope: iris diaphragm lever: moving the lever to the left increases the light; moving the lever to the right decreases the light;
stage control "A": moves the mechanical stage holding the slide forward and backward;
stage control "B": moves the mechanical stage holding the slide left and right;
coarse focus: turning the knob away from you raises the stage; turning the knob towards you lowers the stage;
fine focus: turning the knob away from you raises the stage; turning the knob towards you lowers the stage.

Obtaining different magnifications. The final magnification is a product of the 2 lenses being used. The **eyepiece or ocular lens** magnifies **7×**. The **objective lenses 90×** (**oil immersion lens)**. Final magnifications are as follows:

Ocular lens	×	**Objective lens**	=	**Total magnification**
7×	×	90×	=	630×

Watching the slide and objective lens carefully from the front of the microscope, lower the oil immersion objective into the oil by raising the stage until the lens **just touches** the slide (*Fig. 19*). Do this by turning the **coarse focus** (larger knob; *Fig. 18*) **away from you** until the spring-loaded objective lens **just begins to spring upward**.

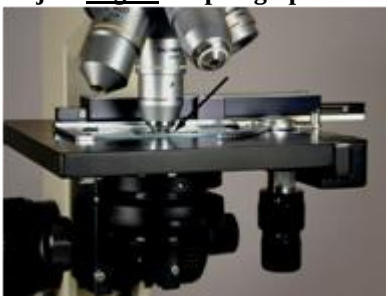


Fig. 19. Lowering the oil immersion objective into the oil

While looking through the eyepieces, turn the **fine focus** (smaller knob; *Fig. 18*) **towards you at a slow steady speed** until the specimen comes into focus. (If the specimen does not come into focus within a few complete turns of the fine focus control and the lens is starting to come out of the oil, you missed the specimen when it went through focus. Simply reverse direction and start turning the fine focus away from you.) Using the **iris diaphragm lever, adjust the light** to obtain optimum contrast (*Fig. 18*).

Cleaning the microscope. Clean the exterior lenses of the eyepiece and objective before and after each lab using **lens paper** only. (Paper towel or kim-wipes may scratch the lens.) **Remove any immersion oil from the oil immersion lens before putting the microscope away.**

Practical tasks, being carried out during practical classes:

1. Studying different bacterial shapes and arrangements (in microslides and atlas).
2. Prepare microslide from S. epidermidis, perform simple method of staining.
3. Recognize a direct stain preparation when it is observed through an oil immersion microscope and state the shape and arrangement of the bacteria.

Therminology: micrococci, diplococci, streptococci, staphylococci.

Theoretical questions for control:

1. State three basic shapes of bacteria.
2. State and describe six different arrangements of cocci.
3. Define the following: acidic dye, basic dye, direct stain, and indirect stain.
4. State in chemical and physical terms the principle behind direct staining.
5. Describe procedures of fixing and staining ob bacteria by simple method.

Test tasks for control:

1. Gram-negative bean-shaped diplococcus inside and outside of leucocytes were detected on bacteriological examination of the purulent exudates from the cervix of the uterus. Name the causative agent of purulent inflammation of the cervix of the uterus.

A. Neisseria meningitidis
B. Streptococcus pneumoniae
C. Staphylococcus aureus
D. Streptococcus pyogenes
E. Neisseria gonorrhoeae

2. Coccus-shaped bacteria were isolated from the nasopharynx of a boy that suffered from chronic tonsillitis. In the smear they occurred in chains. What kind of bacteria are they?

A. Streptococcus
B. Sarcina
C. Staphylococcus
D. Tetrad
E. Diplococcus

3. Immersion microscope differs from light microscope in:

A. Using of additional light source
B. Using of immersion oil
C. Using of a beam of electrons
D. Using of a special condensor
E. Using of a special ocular

MORPHOLOGY OF ROD-SHAPED BACTERIA. STRUCTURE OF BACTERIAL CELL. GRAM'S METHOD

Goal: Studying of morphology of rod-shaped bacteria by microscopic examination.

Concrete goals:

1. Study of arrangement of rod-shaped bacteria in microslides.
2. Study of principle and mechanism of Gram's method of staining of microslide.
3. Study of structure of bacterial cell.

Students should be able to:

1. Perform Gram's method of staining of microslide.
2. Using a microscope, identify different bacterial shapes and arrangements.

Equipment: museum cultures of bacteria, bacteriological loops, gas burners, gentian violet, fuchsine, alcohol, water, immersion microscopes, immersion oil, tables, atlas.

Rods. Rod-shaped or cylindrical forms are subdivided into bacteria, bacilli, and clostridia. Bacteria include those microorganisms which, as a rule, do not produce spores (Escherichia coli, and Salmonella typhi - organism responsible for enteric fever, paratyphoids, Shigella dysentery, Corynebacterium diphtheria, Mycobacterium tuberculosis, etc.) Bacilli and clostridia include organisms the majority of which produce spores (Bacillus anthracis that causes anthrax, Clostridium tetani causes tetanus, causative agents of anaerobic infections).

Rod-shaped bacteria exhibit differences in form. Some are short (Escherichia coli), others are long (Bacillus anthracis), the majority has blunted ends, and others have tapered ends (fusobacteria). A single bacillus is typically **0.5–1.0 μm wide** and from **1–4 μm long**. Small bacilli or bacilli that have just divided by binary fission may at first glance be confused for cocci so they must be **observed carefully**.

According to their arrangement, cylindrical forms can be subdivided into three groups (1) diplobacilli occurring in pairs (Klebsiella pneumoniae) (*Fig. 20*); (2) streptobacilli occurring in chains of different length (causative agents of anthrax) (*Fig. 21*), (3) bacilli which are not arranged in a regular pattern (these comprise the majority of the rod-shaped forms – Escherichia coli (*Fig. 22*), Salmonella, Shigella). Some rod-shaped bacteria have pin-head thickenings at the ends arranged like Chinees or Latin letters (Corynebacterium diphtheriae – causative agents of diphtheria) (*Fig. 23*); others form lateral branchings (Mycobacterium tuberculosis and leprosy).

Cellular organization: prokaryotic and eukaryotic cells. The cell is the basic unit of life. Based on the organization of their cellular structures, all living cells can be divided into two groups: prokaryotic and eukaryotic. Animals, plants, fungi, protozoans, and algae all possess eukaryotic cell types. Only bacteria have prokaryotic cell types.

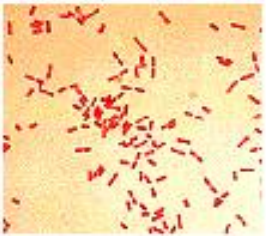


Fig. 20. Klebsiella pneumoniae

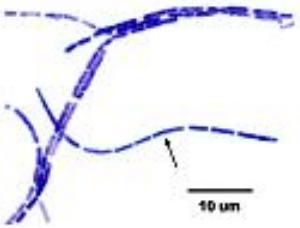


Fig. 21. Bacillus anthracis

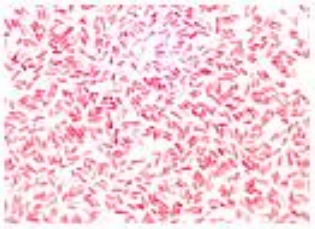


Fig. 22. Escherichia coli

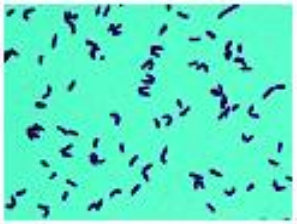


Fig. 23. Corynebacterium diphtheriae

Prokaryotic cells are generally much smaller and more simple than eukaryotic. Prokaryotic cells are, in fact, able to be structurally simpler because of their small size. The smaller a cell, the greater is its surface-to-volume ratio (the surface area of a cell compared to its volume). For example, a spherical cell 2 micrometers (μm) in diameter has a surface-to-volume ratio of approximately 3 : 1, while a spherical cell having a diameter of 20 μm has a surface-to-volume ratio of around 0.3 : 1. A large surface-to-volume ratio, as seen in smaller prokaryotic cells, means that nutrients can easily and rapidly reach any part of the cells interior. However, in the larger eukaryotic cell, the limited surface area when compared to its volume means nutrients cannot rapidly diffuse to all interior parts of the cell. That is why eukaryotic cells require a variety of specialized internal organelles to carry out metabolism, provide energy, and transport chemicals throughout the cell. Both, however, must carry out the same life processes. Some features distinguishing prokaryotic and eukaryotic cells are shown in detail later.

1. Nuclear body

eukaryotic cell

a. Bounded by a nuclear membrane having pores connecting it with the endoplasmic reticulum (*Fig. 24*).

b. Contains one or more aired, linear chromosomes composed of deoxyribonucleic acid (DNA) associated with histone proteins.

c. Nucleolus present.

d. Nuclear body is called a nucleus.

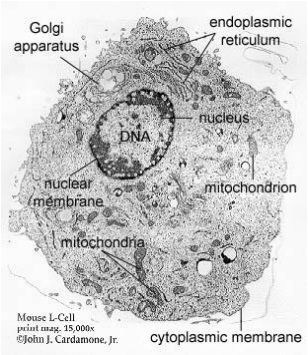


Fig. 24. Eukaryotic cell structure

prokaryotic cell

a. Not bounded by a nuclear membrane (*Fig. 25*).

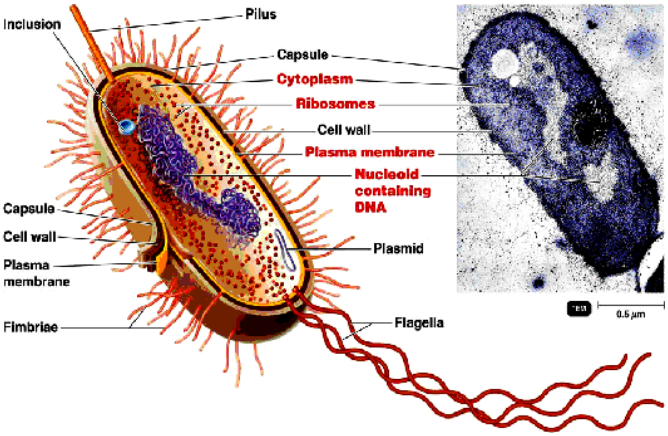


Fig. 25. Prokaryotic cell structure

b. Usually contains one circular chromosome composed of deoxyribonucleic acid (DNA) associated with histone-like proteins.

c. No nucleolus.

d. Nuclear body is called a nucleoid.

e. **Plasmids** are small circular DNA molecules that can be thought of as carrying extra genes that can be used for special situations.

2. Cytoplasmic membrane (cell membrane, plasma membrane)

eukaryotic cell

a. Cytoplasmic membrane is a fluid phospholipid bilayer containing sterols as well as carbohydrates.

b. Capable of endocytosis (phagocytosis and pinocytosis) and exocytosis.

prokaryotic cell.

a. Cytoplasmic membrane; is a fluid phospholipid bilayer without carbohydrates and usually lacking sterols. Many bacteria do contain sterol-like molecules called hopanoids.

b. Incapable of endocytosis and exocytosis.

3. Cytoplasmic structures

eukaryotic cell

a. Ribosomes composed of a 60S and a 40S subunit.

b. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are present (*Fig. 24*).

prokaryotic cell

a. 70S ribosomes composed of a 50S and a 30S subunit.

b. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are absent (*Fig. 25*).

4. Respiratory enzymes and electron transport chains

eukaryotic cell

Located in the mitochondria.

prokaryotic cell

Located in the cytoplasmic membrane (mesosomes).

5. Cell wall

eukaryotic cell

a. Plant cells, algae, and fungi have cell walls, usually composed of cellulose or chitin but never containing peptidoglycan.

b. Animal cells and protozoans lack cell walls.

prokaryotic cell

a. Most Eubacteria have cell walls composed of peptidoglycan.

b. The Archaebacteria have cell walls composed of protein, a complex carbohydrate, or unique molecules resembling but not the same as peptidoglycan.

6. Locomotor organelles

eukaryotic cell

may have flagella or cilia. Flagella and cilia are organelles involved in locomotion and in eukaryotic cells consist of a distinct arrangement of sliding microtubules surrounded by a membrane. The microtubule arrangement is referred to as a $2 \times 9 + 2$ (*Fig. 26*).

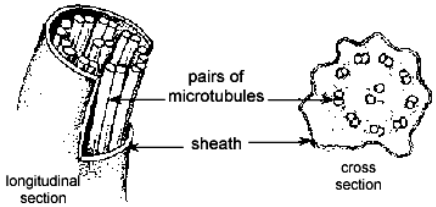


Fig. 26. Structure of eukaryotic flagellum

prokaryotic cell

Some have flagella, each composed of a single, rotating fibril and not surrounded by a membrane (*Fig. 27*). No cilia.

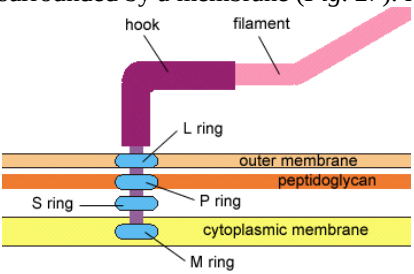


Fig. 27. Insertion structure of bacterial flagellum

Bacteria often store reserve materials in the form of insoluble cytoplasmic granules, which are deposited as osmotically inert, neutral polymers. Many bacteria accumulate reserves of inorganic phosphate as granules of polymerized metaphosphate, called volutin. Volutin granules are also called metachromatic granules because they stain red with a blue dye. They are characteristic features of corynebacteria.

The bacterial cell wall is a unique structure which surrounds the cell membrane. Structurally, the wall is necessary for:

- Maintaining the cell's characteristic shape – the rigid wall compensates for the flexibility of the phospholipid membrane and keeps the cell from assuming a spherical shape.
- Countering the effects of osmotic pressure – the strength of the wall is responsible for keeping the cell from bursting when the intracellular osmolarity is much greater than the extracellular osmolarity.

Gram's method of staining. An important taxonomic characteristic of bacteria is their response to Gram's stain. The Gram's stain is the most widely used staining procedure in bacteriology. It is called a **differential stain** since it differentiates between Gram-positive and Gram-negative bacteria. Bacteria, which stain **purple** with the Gram staining procedure are termed **Gram-positive;** those which stain **pink** are said to be **Gram-negative**. The terms positive and negative have nothing to do with electrical charge, but simply designate two distinct morphological groups of bacteria.

The Gram-staining property appears to be a fundamental one, since the Gram reaction is correlated with many other morphologic properties in phylogenetically related forms.

Gram-positive and Gram-negative bacteria stain differently because of fundamental differences in the structure of their cell walls. The bacterial cell wall serves to give the organism its size and shape as well as to prevent osmotic lysis. The material in the bacterial cell wall which confers rigidity is peptidoglycan.

The Gram-staining procedure begins with the application of a basic dye, crystal (gentian) violet. A solution of iodine is then applied; all bacteria will be stained blue at this point in the procedure. The cells are then treated with alcohol. Gram-positive cells retain the crystal violet-iodine complex, remaining

blue; Gram-negative cells are completely decolorized by alcohol. As a last step, a counter stain such as the red dye fuchsine is applied so that the decolorized Gram-negative cells will take on a contrasting pink color; the Gram-positive cells now appear purple.

The cell walls of all bacteria are not identical. In fact, cell wall composition is one of the most important factors in bacterial species analysis and differentiation. There are two major types of walls: Gram-positive and Gram-negative. The cell wall of Gram-positive bacteria consists of many polymer layers of peptidoglycan connected by amino acid bridges. The peptidoglycan polymer is composed of an alternating sequence of N-acetylglucosamine and N-acetyl-muraminic acid. Each peptidoglycan layer is connected, or crosslinked, to the other by a bridge made of amino acids and amino acid derivatives. Also, 90 % of the Gram-positive cell wall is comprised of peptidoglycan. In electron micrographs, the Gram-positive cell wall appears as a broad, dense wall 20–80 nm thick and consisting of numerous interconnecting layers of peptidoglycan (*Figs. 28*). Interwoven in the cell wall of Gram-positive are teichoic acids. Teichoic acids, which extend through and beyond the rest of the cell wall, are composed of polymers of glycerol, phosphates, and the sugar alcohol ribitol. Some have a lipid attached (lipoteichoic acid) (*Fig. 30*). The outer surface of the peptidoglycan is studded with proteins that differ with the strain and species of the bacterium.

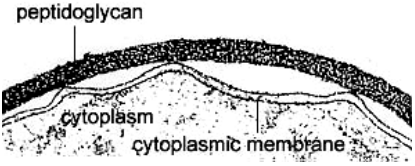


Fig. 28. Electron micrograph of a Gram-positive cell wall

Common Gram-positive bacteria of medical importance include Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Bacillus anthracis, and Clostridium species.

The **Gram-negative cell wall**, on the other hand, contains only 2–3 layers of peptidoglycan (*Fig. 29*). The cell wall of Gram-negative bacteria is much thinner, being comprised of only 20 % peptidoglycan. Gram-negative bacteria also have two unique regions which surround the outer plasma membrane: the periplasmic space and the lipopolysaccharide layer (*Fig. 31*). The periplasmic space separates the outer plasma membrane from the peptidoglycan layer. It contains binding proteins and proteins which destroy potentially dangerous foreign matter present in this space. The lipopolysaccharide (LPS) layer is located adjacent to the exterior peptidoglycan layer. It is a phospholipid bilayer construction similar to that in the cell membrane and is attached to the peptidoglycan by lipoproteins. The lipid portion of the LPS contains a toxic

substance, which is responsible for most of the pathogenic affects associated with harmful Gram-negative bacteria. The LPS, lipoproteins, and the associated polysaccharides together form what is known as the outer membrane.

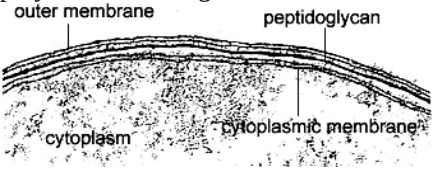


Fig. 29. Electron micrograph of a Gram-negative cell wall

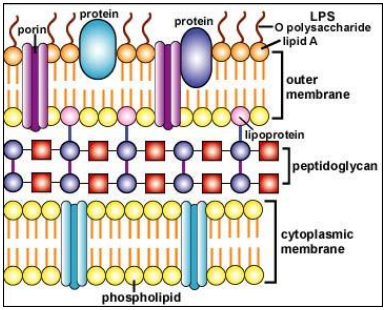


Fig. 30. Structure of a Gram-positive cell wall

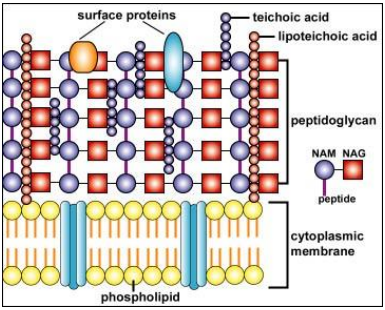


Fig. 31. Structure of a Gram-negative cell wall

Common Gram-negative bacteria of medical importance include Salmonella species, Shigella species, Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Proteus species, Yersinia pestis, and Pseudomonas aeruginosa.

Gram-staining Procedure. Gram-staining is a four part procedure which uses certain dyes to make a bacterial cell stand out against its background. The specimen should be mounted and heat fixed on a slide before you precede stain it. The reagents you will need to successfully perform this operation are:

- Crystal Violet (the Primary Stain)
- Iodine Solution (the Mordant)
- Decolorizer (ethanol)
- Fuchsine (the Counterstain)
- Water

STEP 1: Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds (*Fig. 32*). When the time has elapsed, wash your slide for 5 seconds with the water bottle. Shake off the excess water but **do not blot dry between steps**. The specimen should appear blue-violet when observed with the naked eye.

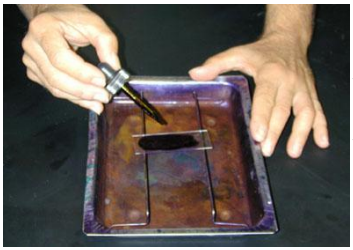


Fig. 32. Gram stain: staining with crystal violet

STEP 2: Now, flood your slide with the iodine solution. Let it stand about a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately precede step three. At this point, the specimen should still be blue-violet.

STEP 3: This step involves addition of the decolorizer, ethanol. Step 3 is somewhat subjective because using too much decolorizer could result in a false Gram-negative result. Likewise, not using enough decolorizer may yield false Gram-positive results. To be safe, add the ethanol dropwise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinse with the water for 5 seconds.

STEP 4: The final step involves applying the counterstain fuchsine. Flood the slide with the dye as you did in steps 1 and 2. Let this stand for about a minute to allow the bacteria to incorporate the fuchsine. Gram-positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram-negative bacteria, however, take on a pink color and are easily distinguishable from the Gram-positives. Again, rinse with water for 5 seconds to remove any excess of dye.

After you have completed steps 1 through 4, you should dry the slide with bibulous paper or allow it to air dry before viewing it under the microscope.

The Gram-positive bacteria. As previously mentioned, Gram-positive bacteria are characterized by their blue-violet color reaction in the Gram-staining procedure. The blue-violet color reaction is caused by crystal-violet, the primary Gram-stain dye, complexing with the iodine mordant. When the decolorizer is applied, a slow dehydration of the crystal-violet/iodine complex is observed due to the closing of pores running through the cell wall. Because the crystal-violet is still present in the cell, the counter stain is not incorporated, thus maintaining the cell's blue-violet color (*Fig. 33*).

The Gram-negative bacteria. Unlike Gram-positive bacteria, which assume a violet color in Gram staining, Gram negative bacteria incorporate the counter stain rather than the primary stain, and bacteria become pink (*Fig. 34*). Because the cell wall of Gram-negative bacteria is high in lipid content and low in peptidiglycan content, the primary crystal-violet escapes from the cell when the decolorizer is added. This is because primary stains like to bind with peptidoglycan - something the Gram-negative cell lacks. The pathogenic nature of Gram-negative bacteria is usually associated with certain components of their cell walls, particularly the lipopolysaccharide (endotoxin) layer.

Bacteria that lack of cell wall. The ß-1,4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine is specifically cleaved by the bacteriolytic enzyme lysozyme. Widely distributed in nature, this enzyme is present in human tissues and secretions and can cause complete digestion of the

peptidoglycan walls of sensitive organisms. When lysozyme is allowed to digest the cell wall of Gram-positive bacteria suspended in an osmotic stabilizer (such as sucrose), **protoplasts** are formed. These protoplasts are able to survive and continue to grow on suitable media in the wall-less state. Gram-negative bacteria treated similarly produce **spheroplasts**, which retain much of the outer membrane structure. The dependence of bacterial shape on the peptidoglycan is shown by the transformation of rod-shaped bacteria to spherical protoplasts (spheroplasts) after enzymatic breakdown of the peptidoglycan. The mechanical protection afforded by the wall peptidoglycan layer is evident in the osmotic fragility of both protoplasts and spheroplasts.

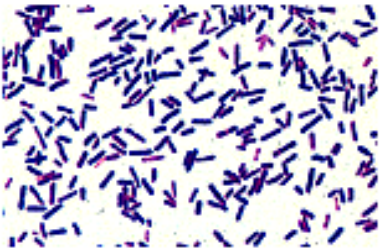


Fig. 33. Gram-positive bacteria

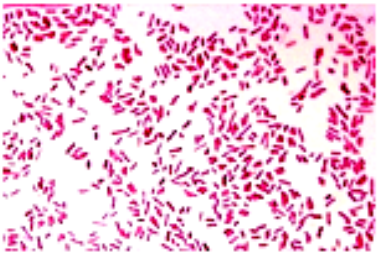


Fig. 34. Gram-negative bacteria

There are two groups of bacteria that lack the protective cell wall peptidoglycan structure, the **Mycoplasma species**, one of which causes atypical pneumonia and some genitourinary tract infections and the **L-forms**, which originate from Gram-positive or Gram-negative bacteria and are so designated because of their discovery and description at the Lister Institute, London. The mycoplasmas and L-forms are all Gram-negative and insensitive to penicillin and are bounded by a surface membrane structure. L-forms arising "spontaneously" in cultures or isolated from infections are structurally related to protoplasts and spheroplasts; all three forms (protoplasts, spheroplasts, and L-forms) revert infrequently and only under special conditions.

Practical tasks, being carried out during practical classes:

1. Studying different bacterial shapes and arrangements (in microslides and atlas).
2. Prepare microslide from E. coli, B. anthracoides, and mixture of bacteria, perform Gram's method of staining.
3. Recognize Gram's stain preparation when it is observed through an oil immersion microscope and state the shape, color and arrangement of the bacteria.

Therminology: nucleoid, plasmid, ribosome, cell wall, cell membrane, cytoplasm, fimbria, flagella, capsule, coccobacilli, diplobacilli, streptobacilli, Gram-positive and Gram-negative bacteria.

Theoretical questions for control:

1. State structure of bacterial cell.
2. State and describe different arrangements of rod-shaped bacteria.
3. Describe procedures of fixing and staining of bacteria by Gram's method.
4. State why the gram stain is said to be a differential stain. Describe the differences between a Gram-positive and a Gram-negative cell wall.
5. Describe a theory as to why gram-positive bacteria retain the crystal violet-iodine complex while gram-negatives become decolorized.
6. Describe three conditions that may result in a gram-positive organism staining gram-negatively.

Tests tasks for control

1. A recently hired laboratory technologist forgets the iodine-fixation step while performing a Gram's stain of Staphylococcus. The most likely result is that the organisms would

A. Appear pink *C. Be colorless* *E. Lyse*
B. Appear blue *D. Wash off the slide*

2. The difference between Gram positive and Gram negative bacteria is shown to reside in the:

A. Cell wall *C. Cell membrane* *E. Flagella*
B. Nucleus *D. Mesosomes.*

MORPHOLOGY OF SPIRAL BACTERIA. METHODS OF EXAMINATION OF FLAGELLA

Goal: Studying of morphology of spiral bacteria by microscopic examination.

Concrete goals:

1. Study of arrangement of spiral bacteria in microslides.
2. Study of method of detection of motility of bacteria.
3. Study of structure of bacterial flagella.

Students should be able to:

1. Perform Gram's method of staining of microslide.
2. Using a microscope, identify different bacterial shapes and arrangements.

Equipment: museum cultures of bacteria, bacteriological loops, gas burners, gentian violet, fuchsine, alcohol, water, immersion microscopes, immersion oil, tables, atlas.

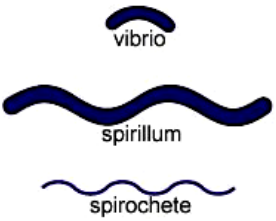


Fig. 35. Spiral forms of bacteria

Spiral-shaped bacteria. Vibrios, spirilla and spirochetes belong to this group of bacteria (*Fig. 35*).

1**. Vibrios** (L. vibrio to vibrate) are cells which resemble a comma in appearance. Typical representatives of this group are Vibrio cholera (*Fig. 36*), the causative agent of cholera, and aquatic vibrios which are widely distributed in fresh water reservoirs.

2. **Spirilla** (L. spira coil) are coiled forms of bacteria exhibiting twists with one or more turns (*Fig. 37*). Pathogenic species is Helicobacter pylori which is responsible for gastritis, duodenum and stomach ulcer and cancer.


Fig. 36. Vibrio cholera


Fig. 37. Spirilla

3. **Spirochaetes.** Genetically spirochaetes (L. spira curve, Gk. chaite cock, mane) differ from bacteria and fungi in structure with a corkscrew spiral shape. Their size varies considerably (from 0.3 to 1 5 mcm in width and from 7 to 500 mcm in length). The body of the spirochaete consists of an axial filament and cytoplasm wound spirally around the filament. No special membrane separates the nucleoid from the cytoplasm. Spirochaetes have a three-layer outer membrane. As demonstrated by electron microscopy, they possess a fine cytoplasmic membrane

enclosing the cytoplasm. The spirochaetes do not possess the cell wall characteristic of bacteria, but electron microscopy has revealed that they have a thin cell wall (periplast) which encloses the cytoplasm.

In spite of the absence of flagella, spirochaetes are actively motile due to the distinct flexibility of their bodies. Spirochaetes have a rotating motion which is performed axially, a translational motion forwards and backwards, an undulating motion along the whole body of the microorganism, and a bending motion when the body bends at a certain angle.

Classification of Spirochaetes. The order Spirochaetales, family Spirochaetaceae includes two pathogenic genera (**Borrelia, Treponema),** and one belong to family Leptospiraceae (**Leptospira)** (*Fig. 38*).

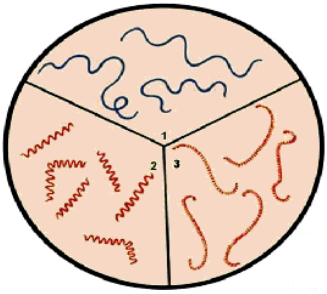


Fig. 38. Morphology of spirochaetes

The organisms of genus **Borrelia** differ from spirochaetes in that their cells have large, obtuse-angled, irregular spirals, the number of which varies from 3 to 10 (*Fig. 38*). Pathogenic for man are the causative agents of relapsing fever transmitted by lice (Borrelia hispanica), and by ticks (Borrelia persica, etc.), causative agent of Lime disease (B. burgdorferi). These stain blue-violet with the Romanowsky–Giemsa stain (*Fig. 39*).

The genus **Treponema** (Gk. trepein turn, nema thread) exhibits thin, flexible cells with 6–14 twists (*Fig. 38*). The microorganisms do not appear to have a visible axial filament or an axial crest when viewed under the microscope. The ends of treponemas are either tapered or rounded, some species have thin elongated threads on the poles. The organisms stain pale-pink with the Romanowsky–Giemsa stain (*Fig. 40*). Burry stain with Indian ink (negative staining) is more suitable (*Fig. 41*). A typical representative is the causative agent of syphilis Treponema pallidum.

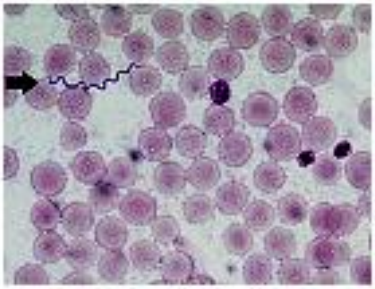


Fig. 39. Borrelia in blood. Romanowsky–Giemsa stain

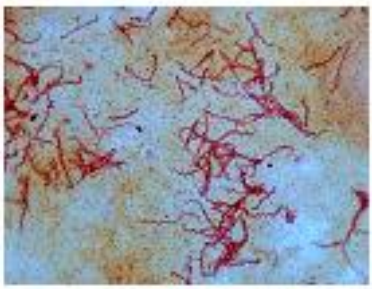


Fig. 40. Treponema pallidum. Romanowsky–Giemsa stain

Organisms of the genus **Leptospira** (Gk. leptos thin, speira coil) are characterized by very thin cell structure. The leptospirae form 12 to 18 coils wound close to each other, shaping small primary spirals (*Fig. 38*). Due to the presence of the two pairs of axial filaments the leptospirae are capable of quite complexand active movement. During movement the ends of the organisms rotate rapidly at a right angle to the main part of their body. At rest the ends are hooked while during rapid rotary motion they resemble buttonholes. Secondary spirals give the leptospirae the appearance of brackets or the letter S or C. The cytoplasm is weakly refractive. They stain pinkish with the Romanowsky-Giemsa stain so other method such as Morosov stain is used (*Fig. 42*). Some serotypes which are pathogenic for animals and man cause leptospirosis.

In a negative method of living bacteria staining by Burry technique, which is used to detect spirochetes, the bacteria remain unstained against a dark background (*Fig. 41*). In a drop of Indian ink diluted with distilled water 1 to 10 the culture to be tested is introduced and spread uniformly with a loop or the edge of a glass slide. The smear is air dried.

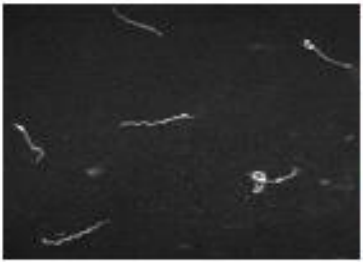


Fig. 41. Treponema. Burry stain

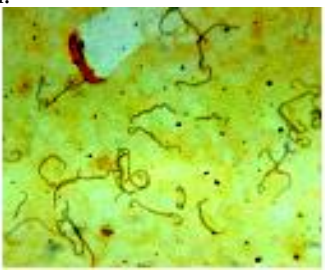


Fig. 42. Leptospira. Morosov stain

Flagella. Motile bacteria possess flagella, thin hair-like cytoplasmic appendages measuring 0.02 to 0.05 mcm in thickness and from 6 to 9 mcm in length. In some spirilla they reach a length of 80 to 90 mcm. Investigations have confirmed that the flagella are made up of proteins the composition of which differs considerably from that of the bacterial cell proteins (keratin, myosin, fibrinogen).

According to a pattern in the attachment of flagella motile microbes can be divided into 4 groups (*Fig. 43*): (1) monotrichates, bacteria having a single flagellum at one pole of the cell (Vibrio cholera), (2) amphitrichates, bacteria with two polar flagella or with a tuft of flagella at both poles, (3) lophotrichates, bacteria with a tuft of flagella at one pole (Helicobacter pylori), (4) peritrichates, bacteria having flagella distributed over the whole surface of their bodies (E. coli, salmonellae of enteric fever and paratyphoids A and B, Proteus).

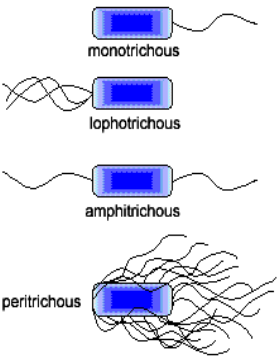


Fig. 43. Bacterial flagella arrangements

A bacterial flagellum has 3 basic parts: a filament, a hook, and a basal body.

1) The **filament** is the rigid, helical structure that extends from the cell surface. It is composed of the protein **flagellin** arranged in helical chains so as to form a hollow core. During synthesis of the flagellar filament, flagellin molecules coming off of the ribosomes are thought to be transported through the hollow core of the filament where they attach to the growing tip of the filament causing it to lengthen. With the exception of a few bacteria such as *Vibrio cholerae*, the flagellar filament is not surrounded by a sheath (*Fig. 44*).

2) The **hook** is a **flexible coupling between the filament and the basal body** (*Fig. 44*).

3) The **basal body** consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane (*Fig. 44*). Gram-positive bacteria have only the inner pair of rings. Unlike eukaryotic flagella, the bacterial flagellum has no internal fibrils and does not flex. Instead, the basal body **acts as a molecular motor, enabling the flagellum to rotate** and propell the bacterium through the surrounding fluid. In fact, the flagellar motor rotates very rapidly. (The motor of *E. coli* rotates 270 revolutions per second!)

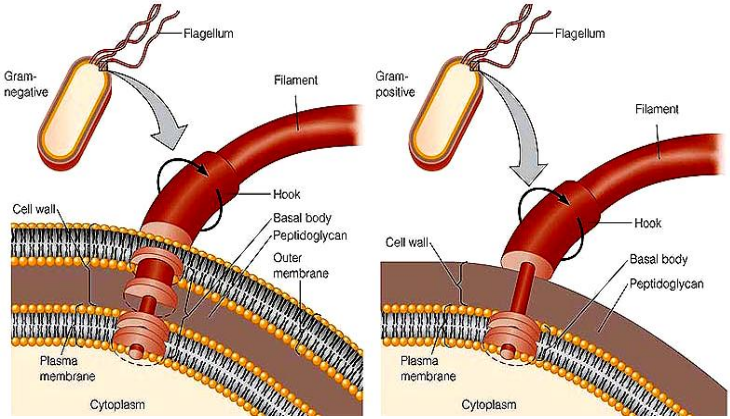


Fig. 44. Insertion structure of bacterial flagellum in Gram-negative and Gram-positive bacteria

One group of bacteria, the **spirochetes**, has internally-located axial filaments or endoflagella. Axial filaments are composed of from two to over a hundred axial fibrils (or endoflagella) that extend from both ends of the bacterium between the outer membrane and the cell wall, often overlapping in the center of the cell (*Fig. 45*). A popular theory as to the mechanism behind spirochete motility presumes that as the endoflagella rotate in the periplasmic space between the cytoplasmic membrane and the cell wall, this could cause the corkscrew-shaped outer membrane of the spirochete to rotate and propell the bacterium through the surrounding fluid.

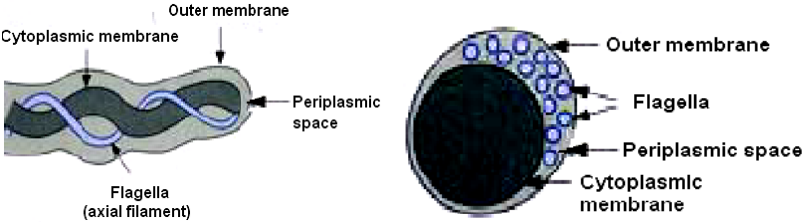


Fig. 45. Spirochete axial filaments

Function: Flagella are the organelles of **locomotion** for most of the bacteria that are capable of motility. Two proteins in the flagellar motor, called MotA and MotB, form a proton channel through the cytoplasmic membrane and **rotation of the flagellum is driven by a proton gradient**. This driving proton motive force occurs as protons accumulating in the space between the cytoplasmic membrane and the cell wall as a result of the electron transport system travel through the channel back into the bacterium's cytoplasm.

The bacterial flagellum can rotate both counterclockwise and clockwise (*Fig. 46*). This is controlled by a protein switch in the molecular motor of the basal body. Clockwise rotation results in a tumbling motion and changes the direction of bacterial movement. On the other hand, counterclockwise rotation leads to long, straight or curved runs without a change in direction. During a run, that lasts about one second, the bacterium moves 10–20 times its length before it stops. In the case of a tumble, the movement lasts only about one-tenth of a second and no real forward progress is made.

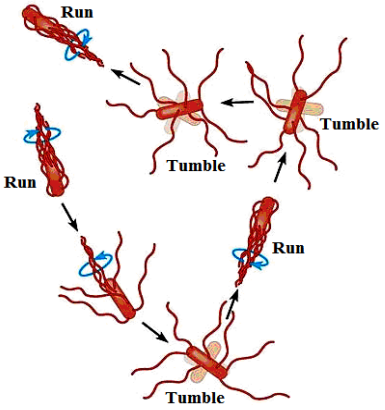


Fig. 46. Flagella and bacterial motility. Notice that direction of flagellar rotation determines which of these movements occurs. Arrows indicate direction of movement

Around half of all known bacteria are motile. Motility serves to keep bacteria in an optimum environment via **taxis**. **Taxis is a motile response to an environmental stimulus**. Bacteria can respond to chemicals (chemotaxis), light (phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis).

Chemotaxis is a response to a chemical gradient of attractant or repellent molecules in the bacterium's environment. In an environment that lacks such a gradient, the bacterium moves randomly. It travels in a straight line, or runs, for a few seconds, then stops, tumbles, and runs in a different direction. However, when the bacterium is

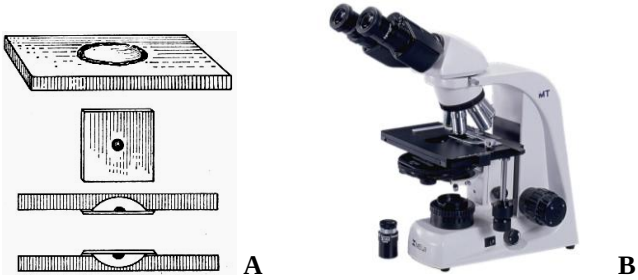
exposed to a chemical gradient of, for example, an attractant, it tumbles less frequently (has longer runs) as it moves up the gradient, but tumbles at the normal rate if it travels down the gradient. In this way, the net movement is towards a more optimum environment. Chemotaxis is regulated by **chemoreceptors** located in the cytoplasmic membrane or periplasm of the bacterium bind chemical attractants or repellents. An increasing concentration of attractant or decreasing concentration of repellent (both conditions beneficial) causes less tumbling and longer runs; a decreasing concentration of attractant or increasing concentration of repellent (both conditions harmful) causes normal tumbling and a greater chance of reorienting in a "better" direction. As a result, the organism's net movement is toward the optimum environment.

Motility and chemotaxis probably help some intestinal pathogens to move through the mucous layer so they can attach to the epithelial cells of the mucous membranes. They also enable spirochetes to move through viscous environments and penetrate cell membranes.

The flagella can be observed by the following three methods: 1) direct observation by means of special-purpose microscopes (phase-contrast and dark-field), and by electron microscopy, 2) motility media, 3) flagella staining by special methods involving treatment with mordants, adsorption of various substances and dyes on their surfaces.

1. Direct observation of motility using special-purpose microscopes.

Hanging drop technique. To prepare this kind of preparation, special glass slides with an impression (well) in the centre are utilized. A small drop of the test material is put in the middle of the cover slip. The edges of the well are ringed with petrolatum. The glass slide is placed onto the cover slip so that the drop is in the centre of the well. Then it is carefully inverted and the drop hangs in the centre of the sealed well, which prevents it from drying. The prepared specimens are examined by phase-contrast (*Fig. 47*) or dark-field microscopes.



A **B**

Fig. 47. A – "hanging drop" specimen. B – phase-contrast microscope

Occasionally, molecular (Brownian) motility is mistaken for the motility of microorganisms. To avoid this error, it should be borne in mind that microorganisms propelled by flagella may traverse the entire microscopic field and make circular and rotatory movements.

Phase-contrast microscope uses special phase-contrast objectives and a condenser assembly to control illumination and give an optical effect of direct staining. The special optics convert slight variations in specimen thickness into corresponding visible variation in brightness. Thus, the bacterium and its structures appear darker than the background. Phase-contrast microscope is based on the fact that the optical length of the light travelling in any substance depends on its refractive index. Light waves transversing through optically denser sites of the object lag in their phase behind the light waves which do not have to pass through these sites. The intensity of light in this case remains unaltered but the phase of fluctuation, detected by neither eye nor photoplate, is changed. To increase resolution of the image, the objective is fitted with a special semi-transparent phase plate to create difference in the wave length between the rays of the background and the object. If this difference reaches one-fourth of the wave length, a visually tangible effect occurs when a dark object is clearly seen against a light background (positive contrast) or vice versa (negative contrast) depending on the structure of the phase plate.

Phase-contrast microscopy does not enhance the resolving power of the optical system but helps to elucidate new details of the structure of living microorganisms and to study different stages of their development, the effect on them of chemical agents, antibiotics, and other factors.

Dark-field microscopy belongs to ultramicroscopic methods. A dark-field microscope uses a special condenser to direct light away from the objective lens. Living objects 0.02–0.06 mcm in size are visualized in lateral illumination in a dark field of vision. In order to achieve bright lateral illumination, the usual condenser is replaced by a special parabolic condenser in which the central part of the lower lens is darkened, while the lateral surface is mirror (*Fig. 48*). This condenser intercepts the central portion of the parallel beam of rays forming a dark field of vision. The marginal rays pass through the circular slit, fall on the lateral mirror surface of the condenser, are reflected from it, and concentrate in the focus. On encountering in their path the cells of microorganisms or other optically non-homogeneous structures, the ray of light is reflected from them and gets into the objective. The organism will appear bright against the dark background (*Fig. 49*). An electrical illuminator serves as a source of artificial light. To achieve lateral illumination, one needs a parallel beam of light which is created by means of a flat mirror of the microscope.

In dark-field microscopic examination a dry system is typically employed (objective 40). A small drop of the studied material is placed on the slide and covered with a cover-slip, taking care to prevent the formation of air bubbles. A drop of immersion oil is pipetted on the upper lens of the condenser. This oil should fill the space between the condenser and the slide.

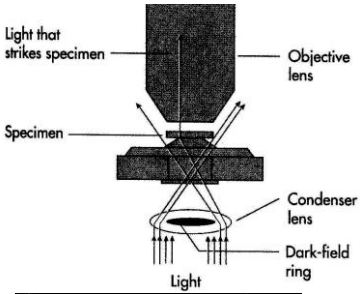


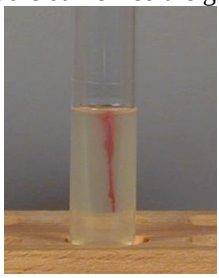
Fig. 48. Diagram of a dark-field microscope shows the path of light. The dark-field ring in the condenser blocks the direct passage of light through the specimen and into the objective lens. Only light that is reflected off a specimen will enter the objective lens and be seen

Fig. 49. Dark-field microscope micrograph of *Leptospira*

Dark-field microscopy is employed for detecting unstained causative agents of syphilis, recurrent typhoid fever, leptospirosis, and other illnesses, as well as for investigating the motility of microorganisms.

2. Motility test medium.

Semi-solid motility test medium may also be used to detect motility. The agar concentration (0.3 %) is sufficient to form a soft gel without hindering motility. When a nonmotile organism is stabbed into motility test medium, **growth occurs only along the line of inoculation**. Growth along the stab line is very sharp and defined (*Fig. 50.A*). When motile organisms are stabbed into the soft agar, they swim away from the stab line. Growth occurs throughout the tube rather than being concentrated along the line of inoculation. Growth along the stab line appears much more cloud-like as it moves away from the stab (*Fig. 50.B*). A tetrazolium salt (TTC) is incorporated into the medium. Bacterial metabolism reduces the TTC producing formazan, which is red in color. The more bacteria present at any location, the darker red the growth appears.


A

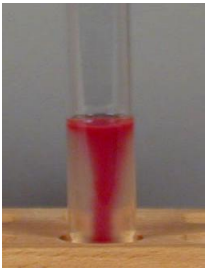

B

Fig. 50. Motility test medium. The red color indicates growth of bacteria.
A. Nonmotile bacteria do not move away from the stab line.
Heavy growth appears only along the stab.
B. Motile bacteria swim away from the stab line.
The growth around the stab line appears more diffuse or cloud-like

3. Flagella staining (Loeffler stain) can be used indirectly to denote bacterial motility. Since flagella are very thin (20–28 nm in diameter), they are below the resolution limits of a normal light microscope and cannot be seen unless one first treats them with special dyes and mordants which build up as layers of precipitate along the length of the flagella, making them microscopically visible. This is a delicate staining procedure.

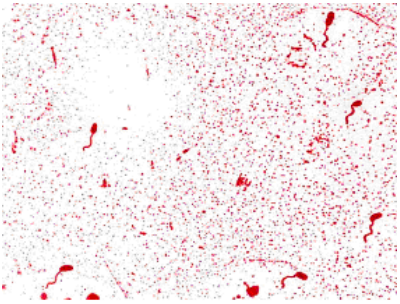


Fig. 51. Vibrio cholerae. Loeffler stain

Practical tasks, being carried out during practical classes:

1. Studying different bacterial shapes and arrangements (in microslides and atlas).
2. Prepare microslide from V. cholerae, perform Gram's method of staining.
3. Recognize a direct stain preparation when it is observed through an oil immersion microscope and state the shape and arrangement of the bacteria.
4. Observe the phase-contrast microscopy demonstration of motile Vibrio.
5. Observe the flagella stain demonstrations of a *Vibrio* species, *Proteus vulgaris.*

Therminology: spirochetes, spirilla, vibrio, flagella, dark-field microscope, phase-contrast microscope, hanging drop specimen.

Theoretical questions for control:

1. State three basic shapes of spiral bacteria.
2. Define the following: Giemsa stain, Burry stain, Morosov stain, mordants.
3. State in the principle of dark-field microscope and phase-contrast microscope.
4. Describe procedures of hanging drop preparation.
5. Interpret the results of motility test medium.
6. Recognize monotrichous, lophotrichous, amphitrichous, and peritrichous flagellar arrangements.

Test tasks for control:

1. Bacterioscopic examination of chancre material revealed some mobile, long, convoluted microorganisms with 8-12 regular coils. These features are typical for:

A. Vibrios *C. Leptospira* *E. Treponema*
B. Borrellia *D. Campylobacter*

2. Negative staining is especially useful in demonstration:

A. Vibrios *C. Neisseria* *E. Staphylococcus*
B. Spirochetes *D. Clostridia*

SPORES. METHODS OF SPORE STATINING

Goal: Studying of morphology of spore-forming bacteria by microscopic examination.

Concrete goals:

1. Study of structure and arrangement of bacterial spores in microslides.
2. Study of methods of bacterial spores detection.

Students should be able to:

1. Perform Ozeshko method of staining.
2. Using a microscope, identify bacterial shapes and spore arrangements.

Equipment: museum cultures of bacteria, bacteriological loops, gas burners, carbol fuchsin, 5 % H_2SO_4, methylene blue, water, immersion microscopes, immersion oil, tables, atlas.

Endospores. Endospores are highly heat-resistant, dehydrated resting cells formed intracellularly in members of the genera Bacillus and Clostridium. Endospores are small spherical or oval bodies formed within the cell. These include the causative agents of anthrax (Bacillus anthracis), tetanus (Clostridium tetani), anaerobic infections-gas gangrene (C.perfringens, C.septicum, C.hystolyticum, C.novyi), botulism (C.botulinum), pseudomembranous colitis (C.difficile) and also saprophytic species living in the soil, water and bodies of animals. Spore formation only rarely occurs in cocci (*Sarcina lutea, Sarcina ureae)* and in spiral forms *(Desulfovibrio desulfuricans).* Sporulation occurs in the environment (in soil and on nutrient media), and is not observed in human or animal tissues.

In bacteria spores are located (1) *centrally*, in the centre of the cell (causative agent of anthrax) (*Fig. 52*); (2) *terminally*, at the ends of the rod (causative agent of tetanus) (*Fig. 53*); (3) *subterminally*, towards the ends (causative agents of botulism, anaerobic infections, etc.) (*Fig. 54*).

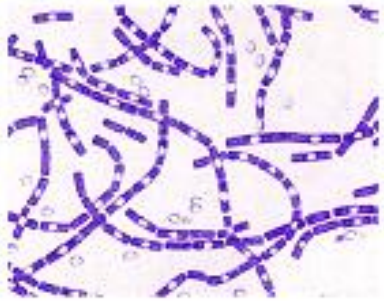


Fig. 52. Central spore of *B. anthracis.* Note the endospores within the streptobacillus

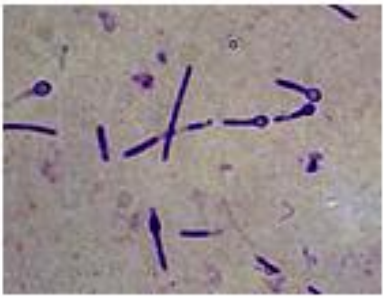


Fig. 53. Terminal spore of *C.tetani*

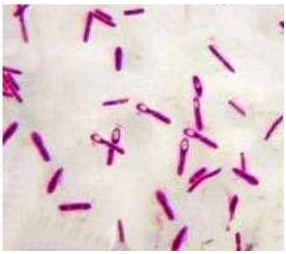


Fig. 54. Subterminal spore of C. botulinum

In some species of microorganisms, the spore diameter is greater than the width of the bacterial cell. If the spore is located subterminally, the microbes take on the form of a spindle (closter). In Clostridia tetani 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 (*Fig. 53*). Cells of Clostridium botulinum with spores have tennis racket appearance (*Fig. 54*).

Sporulation: The sporulation process begins when nutritional conditions become unfavorable, depletion of the nitrogen or carbon source (or both) being the most significant factor. Sporulation involves the production of many new structures, enzymes, and metabolites along with the disappearance of many vegetative cell components. The series of biochemical and morphologic changes that occur during sporulation represent true differentiation within the cycle of the bacterial cell. The cell then undergoes a highly complex, well-defined sequence of morphologic and biochemical events that ultimately lead to the formation of mature endospores. As many as seven distinct stages have been recognized by morphologic and biochemical studies of sporulating.

Endospore formation (sporulation) occurs through a complex series of events. One spore is produced within each vegetative bacterium. Once the endospore is formed, the vegetative portion of the bacterium is degraded and the dormant endospore is released.

Spore formation, **sporogenesis or sporulation**, normally begins when growth ceases due to lack of nutrients (*Fig. 55*). An axial filament of nuclear material forms (stage 1), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the **forespore** septum (stage II). The membrane continues to grow and engulfs the immature spore in a second membrane (stage III). Next, **cortex** is laid down in the space between the two membranes, and both calcium and **dipicolinic acid** are accumulated (stage IV). **Protein coats** then are formed around the cortex (stage V), and maturation of the spore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage VII). Sporulation requires only about 10 hours.

When newly formed, endospores appear as round, highly refractile cells within the vegetative cell wall, or sporangium (*Fig. 56*). Some strains produce autolysins that digest the walls and liberate free endospores. During maturation, the spore protoplast dehydrates and the spore becomes refractile and resistant to heat, radiation, pressure, desiccation, and chemicals. The spores of certain bacilli are capable of withstanding boiling and high concentrations of disinfectants. They are killed in an autoclave exposed to saturated steam, at a temperature of 115–125 °C, and also at a temperature of 150–170 °C in a Pasteur hot-air oven.

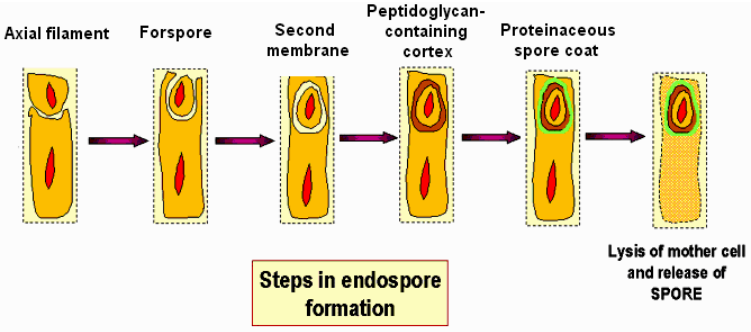


Fig. 55. Stages of sporulation

The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals. The heat resistance of endospores is due to a variety of factors: a) calcium-dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA. As much as 15 % of the spore's dry weight consists of dipicolinic acid complexes with calcium ions; b) specialized DNA-binding proteins saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation; c) the cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation; d) DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

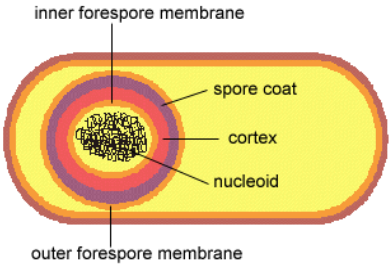


Fig. 56. Endospore within a vegetative cell

Since one vegetative cell gives rise to one endospore, sporulation is obviously not a means of cell reproduction. Spores make survival of bacteria possible under unfavourable condition.

Structure and properties of endospores.

1. **Core.** The spore protoplast, or core contains a complete nucleoid (chromosome), ribosomes, and energy generating components that are enclosed within a modified cytoplasmic membrane. A number of vegetative cell enzymes are increased in amount (eg, alanine racemase), and a number of unique enzymes are formed (eg, dipicolinic acid synthetase). The energy for germination is stored as 3-phosphoglycerate rather than as ATP. The heat resistance of spores is due in pan to their dehydrated state and in part to the presence of large amounts (5–15 % of the spore dry weight) of calcium dipicolinate, which is formed from an intermediate of the lysine biosynthetic

pathway. In some way not yet understood, these properties result in the stabilization of the spore enzymes, most of which exhibit normal heat lability when isolated in soluble form.

2. **Spore wall.** The innermost layer surrounding the inner spore membrane is called the spore wall. It contains normal peptidoglycan and becomes the cell wall of the germinating vegetative cell.

3. **Cortex** is the thickest layer of the spore envelope. It contains an unusual type of peptidoglycan, with many fewer cross-links than are found in cell wall peptidoglycan. Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a key role in spore germination.

4. **Coat** is composed of a keratin-like protein containing many intramolecular disulfide bonds. The impermeability of this layer confers on spores their relative resistance to antibacterial chemical agents.

5. **Exosporium** is a lipoprotein membrane containing some carbohydrate.

Germination is a transformation of spore into vegetative cell.

The germination process occurs in 3 stages (*Fig. 57*):

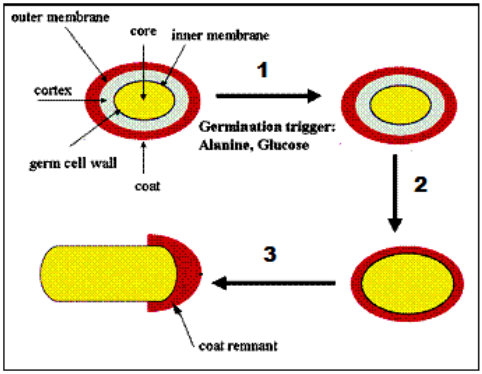


Fig. 57. Germination of spore

1. **Activation**-Even when placed in an environment that favors germination (eg, a nutritionally rich medium), bacterial spores will not germinate unless first activated by one or another agent that damages the spore coat. Among the agents that can overcome spore dormancy are heat (60 to 70 °C for a few minutes), abrasion, acidity, and compounds containing free sulfhydryl groups.

2. **Initiation**-Once activated, a spore will initiate germination if the environmental conditions are favorable. Initiation is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and stress, release of spore component, increase in metabolic activity. Species of bacteria after getting activated recognize effectors as signalling a rich medium. Many normal metabolic nutrients (e.g. amino acids and sugars) can trigger germination after activation. Binding of the effector substance to spore coat activates an autolysin, which destroys peptidoglycan of the cortex. After this

water in taken up and calcium dipicolinic acid is released. Thus a number of hydrolytic enzymes degrade the spore constituent.

3. **Outgrowth**. Now active biosynthesis takes place producing an outgrowth. The spore protoplast makes new components from the remains of the spore coat, and develops again active bacterium. Spore coat breaks open and single germ cell comes out and starts growing to new vegetative cell (consisting of spore protoplast with its surrounding wall). Outgrowth is a stage right from germination to the formation of first vegetative cells just before first cell division. Outgrowth requires supply of all nutrients essential for cell growth.

Endospores can remain in the dormant state for long periods (at least as long as five hundreds years). Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology. This is because it is essential to be able to sterilize solutions and solid objects. Endospores often survive boiling for about 6 hours, therefore autoclaves must be used to sterilize many materials.

Some spore-forming bacteria can produce deadly toxins. Therefore, it is very important that foods that may contain these spores be processed adequately to insure that endospores are killed. Many people have died of botulism after ingesting improperly canned food that contained spores of botulism. In such improperly processed food, the heat-resistant spores germinated into vegetative cells under the appropriate conditions. These cells then synthesized the toxin, which when ingested causes botulism. However, since the toxin is destroyed by heat, boiling contaminated food for 30 minutes will destroy the toxin, although such treatment will not kill the endospores.

Due to the resistant nature of the endospore coats, endospores are difficult to stain. Strong dyes and vigorous staining conditions such as heat are needed. Once stained, however, endospores are equally hard to decolorize. Since few bacterial genera produce endospores, the endospore stain is a good diagnostic test for species of Bacillus and Clostridium. Because spores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue, and other simple stains, or Gram-staining. Special spore stains are used to make them clearly visible: (1) Modified Ziehl-Neelsen method, (2) Ozheshko stain, (3) Gansen stain, (4) Moller stain.

Procedure of Ozheshko stain.

1. Heat-fix a smear of Bacillus subtillis as follows:

a. Using the dropper bottle of distilled water found in your staining rack, place **a small drop of water** on a clean slide by touching the dropper to the slide.

b. Aseptically remove a **small amount** of the culture from **the edge of the growth** on the agar surface and **generously** mix it with the drop of water until the water turns cloudy.

c. **Burn the remaining bacteria off of the loop.**

d. Using the loop spread the suspension over the entire slide to form a thin film.

e. Allow this thin suspension to **completely air dry**.

f. Pass the slide (film-side up) through the flame of the burner 3 or 4 times to heat-fix.

2. Place a piece of blotting paper over the smear and saturate with carbol fuchsin.

3. Let the carbol fuchsin sits on the slide for **one minute** and proceed to the next step.

4. Holding the slide with forceps, carefully heat the slide in the flame of a bunsen burner until the stain **just begins to steam**. Remove the slide from the heat until steaming stops; then gently reheat. Continue steaming the smear in this manner for **five minutes**. **As the carbol fuchsin evaporates, continually add more. Do not let the paper dry out.**

5. After five minutes of steaming, **wash** the excess stain and blotting paper off the slide **with water**. Don't forget to wash of any dye that got onto the **bottom** of the slide.

6. Blot the slide dry.

7. Now flood the smear with methylen blue and stain for **one minute**.

8. Wash off the excess methylen blue with water, blot dry, and observe using oil immersion microscopy. With this endospore staining procedure, endospores will stain red while vegetative bacteria will stain blue (*Fig. 58*).

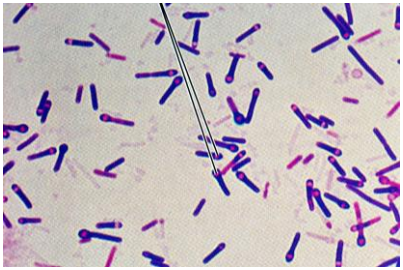


Fig. 58. Endospore of Bacillus subtillis. Ozheshko stain

Practical tasks, being carried out during practical classes:

1. Observe different bacterial spores shapes and arrangements (in microslides).
2. Prepare microslide from B.subtillis, perform Ozheshko method of staining.
3. Recognize endospores as the structures observed in an endospore stain preparation.
4. Identify bacteria as an endospore-containing Clostridia by their appearance.

Therminology: spore, cortex, exosporium, central, subterminal and terminal location.

Theoretical questions for control:

1. State three basic types of bacterial spores arrangement.
2. State the function of bacterial endospores.
3. State in the stages of sporulation and germination.
4. Define the following: Ozheshko stain, Gansen stain, Ziehl-Neelsen stain.

Test tasks for control:

1. Microscopic examination of a microbal culture revealed fusiform spore-forming microorganisms that get violet-blue Gram's stain. What microorganisms were revealed?

A. Actinomycete *C. Diplococci* *E. Spirochaete*
B. Streptococci *D. Clostridia*

2. Quite often, the soil may contain a number of pathogenic microorganisms. The causative agents of the following disease may stay viable in the soil for a long time:

A. Cholera *C. Diphtheria* *E. Dysentery*
B. Anthrax *D. Viral hepatitis*

3. In the surgical ward, the dressing material was undergoing sterilization in an autoclave. Through an oversight of a nurse the mode of sterilization was changed and the temperature in the autoclave reached only 100 °C instead of the due 120 °C. What microorganisms can stay viable under these conditions?

A. Yeast fungi *C. Staphylococci* *E. Mycobacteria*
B. Bacilli *D. Salmonella*

4. There are areas where humans or animals are exposed to the constant risk of contracting certain types of bacteria. What feature of these bacteria is responsible for their long viability in the soil?

A. No cell wall *C. Plasmids* *E. Capsule formation*
B. Spore formation *D. Thick cell wall*

5. A smear of streptobacillus preparation stained by Ozheshko method has been studied microscopically with oil immersion. What structural feature of the bacteria has been studied?

A. Spores *C. Inclusions* *E. Flagella*
B. Capsule *D. Cell wall*

CAPSULES OF BACTERIA. METHODS OF DETECTION OF CAPSULE

Goal: Studying of morphology of capsule-producing bacteria by microscopic examination.

Concrete goals:

1. Study of arrangement of capsule-producing bacteria in microslides.
2. Study of method of detection of bacterial capsule.
3. Study of structure of bacterial capsule.

Students should be able to:

1. Perform Burri-Gins method of staining of microslide.
2. Using a microscope, identify different bacterial shapes and arrangements.

Capsules and Loose Slime

Some bacteria form capsules, which constitute the outermost layer of the bacterial cell and surround it with a relatively thick layer of viscous gel. Capsules may be up to 10 μm thick. Some organisms lack a well-defined capsule but have loose, amorphous slime layers external to the cell wall or cell envelope. The hemolytic Streptococcus mutans, the primary organism found in dental plaque is able to synthesis a large extracellular mucoid glucans from sucrose. Not all bacterial species produce capsules; however, the capsules of encapsulated pathogens are often important determinants of virulence. Encapsulated species are found among both Gram-positive and Gram-negative bacteria. In both groups, most capsules are composed of highmolecular-weight viscous polysaccharides that are retained as a thick gel outside the cell wall or envelope (*Table 1*). The capsule of Bacillus anthracis (the causal agent of anthrax) is unusual in that it is composed of a g-glutamyl polypeptide. Table presents the various capsular substances formed by a selection of Gram-positive and Gram-negative bacteria. A plasma membrane stage is involved in the biosynthesis and assembly of the capsular substances, which are extruded or secreted through the outer wall or envelope structures. Mutational loss of enzymes involved in the biosynthesis of the capsular polysaccharides can result in loss of virulence of bacteria.

The capsule is not essential for viability. Viability is not affected when capsular polysaccharides are removed enzymatically from the cell surface. The exact functions of capsules are not fully understood, but they do confer resistance to phagocytosis and hence provide the bacterial cell with protection against host defenses to invasion. In soil and water, capsules help prevent bacteria from being engulfed by protozoans. Capsules also help many bacteria to adhere to surfaces and thus resist flushing.

Capsules are usually demonstrated by the negative staining procedure with Indian ink (Burri-Gins stain). A smear is prepared from pure culture of bacteria from nutrient medium, after staining capsule is colorless, bacteria cell – pink, background – black (*Fig. 59*). To detect capsule in a smear-imprint from fresh tissue simple method of staining with methylene blue is used (*Fig. 60*).

Table 1. Nature of capsular substances formed by various bacteria

Species	Capsular substance
S. pneumoniae	Polysaccharides
K. pneumoniae	Polysaccharides
N. meningitidis	Polysaccharides
N. gonorrhoeae	Polysaccharides
S. aureus	Polysaccharides
B. anthracis	Polypeptide
S. pyogenes	Hyaluronic acid

Procedure of Burri-Gins stain

1. Aseptically remove a **small amount** of the culture from **the edge of the growth** on the agar surface and **generously** mix it with the drop of Indian ink on a slide.
2. Using your loop spread it out over the entire slide to form a thin film.
3. Let it completely air dry. **Do not heat fix**. Capsules stick well to glass, and heat may destroy the capsule.
4. Stain with **fuchsin** for **one minute**.
5. Wash off the excess stain with water.
6. **Blot dry** and observe using oil immersion microscopy. The organisms on the slide will be pink while the capsule will remain colorless (Fig. 59).

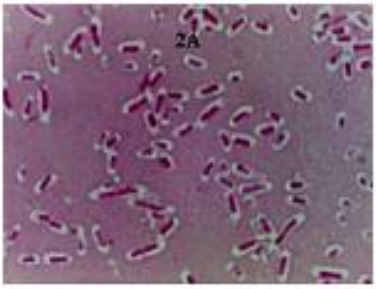


Fig. 59. Capsule of K. pneumoniae by Burri-Gins stain

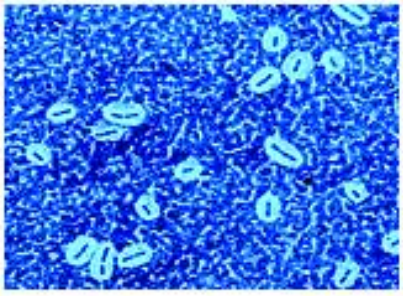


Fig. 60. Capsule of K. pneumonia with methylene blue stain

Practical tasks, being carried out during practical classes:

1. Observe the demonstration of capsule stain of Streptococcus pneumoniae, an encapsulated bacterium often with a diplococcus arrangement.
2. Observe the demonstration capsule stain of Klebsiella pneumoniae.
3. Prepare microslide from K.pneumoniae, perform Burri-Gins method of staining.

Therminology: glycocalix, Burri-Gins stain, negative staining, Indian ink.

Theoretical questions for control:

1. State the chemical nature and major functions of bacterial capsules.
2. Recognize capsules as the structures observed when microscopically viewing a capsule stain preparation.
3. Define the following: Burri-Gins stain, methylene blue stain.

Test tasks for control:

1. During examination of the sputum of a patient suffering from pneumonia a lot of Gram-negative rod-shaped capsule-producing bacteria were detected. What microorganisms are revealed in the sputum?

A. E. coli *C. S. aureus* *E. N. meningitides*
B. N. gonorrhoeae *D. K. pneumoniae*

2. A smear of streptobacillus preparation stained by Burry-Gins method has been studied microscopically with oil immersion. What color of capsule was observed?

A. Red *C. Blue* *E. Yellow*
B. Colorless *D. Pink*

3. A patient has a suspected pneumonia. In his sputum there were revealed capsulated Gram-positive diplococci, prolonged with the slightly pointed opposite ends. What microorganisms are revealed in the sputum?

A. S. pneumoniae *C. S. aureus* *E. N. meningitides*
B. N. gonorrhoeae *D. K. pneumoniae*

4. A patient was admitted to an infectious department with complaints of headache, fatigue, cough and high temperature. In the smears from the patient's sputum Gram-positive capsular elongated diplococci with lanceolate shape were revealed. What pathogen was found in the specimen?

A. S. pneumoniae *C. S. aureus* *E. N. meningitides*
B. N. gonorrhoeae *D. K. pneumoniae*

5. For the microscopic examination of sputum of patients with suspected pulmonary disease Burry-Gins method was used. What kind of paint was used in the first stage?

A. Carbol fuchsin *C. Gentian violet* *E. Fuchsin*
B. Methylene blue *D. Indian ink*

6. Gram-positive cocci of lanceolate form arranged in pairs and short chains with the capsule were isolated from patient nasopharynx. Carrier of Streptococcus pneumonia was suspected. What method of staining should be used to identify capsule?

A. Gram *C. Ozheshko* *E. Giemsa*
B. Burri-Gins *D. Gansen*

MORPHOLOGY of RICKETTSIA, CHLAMYDIA AND MYCOPLASMA

Goal: Studying of morphology of rickettsia, chlamydia and mycoplasma by microscopic examination.

Concrete goals:

1. Study of rickettsia, chlamydia and mycoplasma in microslides.
2. Study of method of detection of rickettsia, chlamydia and mycoplasma.
3. Study of life cycle of chlamydia.

Students should be able to:

1. Perform Romanowsky-Giemsa method of staining of microslide.
2. Using a microscope, identify different bacterial shapes and arrangements.

Order Rickettsiales. The name of the bacteria was given in 1916 in honor of the American bacteriologist H. Ricketts and Czech – P. Provachek, who died while studying the causative agent of typhus. The rickettsia are a diverse collection of obligately intracellular Gram-negative bacteria found in ticks, lice, fleas, mites, chiggers, and mammals (they are obligate intracellular parasites of eukaryotic cells). They include the genera Rickettsia, Ehrlichia, Orientia.

Almost all members of the order Rickettsiales are rod-shaped, coccoid, or pleomorphic bacteria with typical Gram-negative walls (*Fig. 61*). Rickettsia are non-motile, do not produce spores and capsules and stain well by the Romanowsky-Giemsa stain and the Ziehl-Neelsen stain (*Fig. 62*), according to them, bacteria are bluish purple. According to Zdrodovsky, they are painted red.

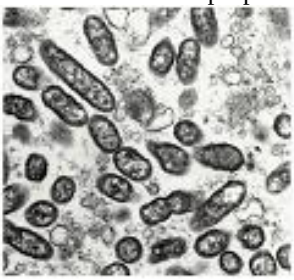


Fig. 61. Morphology of rickettsia

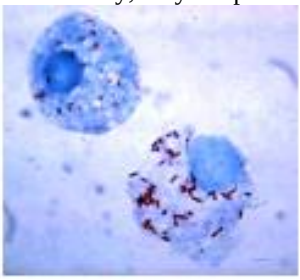


Fig. 62. Rickettsia. Romanowsky–Giemsa stain

All are parasitic or mutualistic. The parasitic forms grow in vertebrate erythtocytes, reticuliendothelial cells, and vascular endothelial cells. Often they also live in blood-sucking arthropods such as fleas, ticks, mires, or lice, which serve as vectors or primary host.

These bacteria resemble viruses in their intracellular existence. They differ from viruses in having both DNA and RNA, a plasma membrane, functioning ribosomes, enzymes, participating in metabolic pathway, reproduction by binary fission and also by the breaking down of the filamentous forms giving rise to coccoid and rod-shaped entities.

Rickettsia are transmitted to humans by the bite of infected ticks and mites and by the feces of infected lice and fleas. They enter via the skin and spread through the bloodstream to infect vascular endothelium in the skin, brain, lungs, heart, kidneys, liver, gastrointestinal tract, and other organs.

One advantage of living intracellularly is that the host cell supplies materials that the bacteria would otherwise have to synthesize for themselves. In contrast with chlamydia, all rickettsia can synthesize ATP.

Rickettsia species cause Rocky Mountain spotted fever, rickettsialpox, other spotted fevers, epidemic typhus, and murine typhus (*Table 2*). Orientia (formerly Rickettsia) tsutsugamushi causes scrub typhus. Patients present with febrile exanthems and visceral involvement; symptoms may include nausea, vomiting, abdominal pain, encephalitis, hypotension, acute renal failure, and respiratory distress. Ehrlichia species cause ehrlichioses that vary in severity from a life-threatening febrile disease.

Table 2. Medically important rickettsia

Species	Disease	Transmission (vector)
Rickettsia typhi	Murine typhus fever	Fleas
Rickettsia provazekii	Epidemic typhus fever Brill-Zinsser disease	Lice
Rickettsia rickettsii	Rocky Mountain spotted fever	Ticks
Rickettsia akari	Rickettsial pox	Mouse mite
Ehrlichia sp.	Erhlichioses	Ticks
Orientia tsutsugamushi	Scrub typhus	Chigger mite

R. provazekii causes epidemic typhus. Between epidemics R prowazekii persists as a latent human infection. Years later, when immunity is diminished, some persons suffer recrudescent typhus fever (Brill-Zinsser disease). These milder sporadic cases can ignite further epidemics in a susceptible louse-infested population. Rickettsiae typhi is associated with rats and fleas, particularly the oriental rat flea, although other ecologic cycles (e.g., opossums and cat fleas) have been implicated. Fleas are infected by transovarian transmission or by feeding on an animal with rickettsiae circulating in the blood. Rickettsiae are shed from fleas in the feces, from which humans acquire the infection through the skin, respiratory tract, or conjunctiva.

Order Chlamydiales

The order Chlamydiales has only one family, Chlamydiaceae, with two genera, Chlamydia and Chlamydophylla, with three species that cause human diseases (Table 3). The name chlamydia is derived from the characteristic appearance of inclusion bodies produced by these agents, which are seen enclosing the nuclei of infected cells as cloak or mantle (“chlamys” means mantle).

Table 3. Medically Important Chlamydia

Species	Type	Disease
Chlamydia trachomatis	A, B, Ba, C	Trachoma, a serious eye infection.
	D, F, G, H, I, J, K	– Nongonococcal urethritis and genital tract disease. – Reiter's syndrome (urethritis, conjunctivitis, arthritis). – Conjunctivitis and pneumonia of newborn
	L_{1-3}	Lymphogranuloma venereum
Chlamydophylla psittaci		Pneumonia ("psittacosis", "ornitosis" or "parrot fever"), bronchitis, upper respiratory infection
Chlamydophylla pneumoniae		Pneumonia, atherosclerosis

All three species cause disease in humans. C. psittaci infects a wide variety of birds and a number of mammals, whereas C. trachomatis is limited largely to humans. C. pneumoniae has been found only in humans. Human diseases caused by chlamydia can be divided into two types: (1) chlamydial agents transmitted by direct contact (C. trachomatis genital and ocular infections) and (2) chlamydial agents that are transmitted by the respiratory route (C psittaci and C. pneumoniae.)

The spread of C. trachomatis from person to person may cause trachoma, inclusion conjunctivitis, or lymphogranuloma venereum. Transmission of C. trachomatis from the urogenital tract to the eyes and vice versa occurs via contaminated fingers, towels, or other fomites and, in neonates, by passage through an infected birth canal. These diseases appear in an epidemic form in populations with low standards of hygiene. C. trachomatis genital infections are sexually transmitted. C. psittaci is transmitted from infected birds or animals to humans through the respiratory tract. C. pneumoniae spreads from infected individuals by respiratory tract infections.

Chlamydia are nonmotile, cocoid, Gram-negative bacteria, ranging in size from 0.2 to 1.5 μm. They can reproduce only within cytoplasmic vesicles of host cells by a unique developmental cycle involving the formation of elementary bodies and reticulate bodies. Chlamydia are extremely limited metabolically and are obligately intracellular parasites of mammals and birds, and were once considered to be viruses. However, they contain DNA, RNA and ribosomes and make their own proteins and nucleic acids and are now considered to be true bacteria. They possess an inner and outer membrane similar Gram-negative bacteria and a lipopolysaccharide but do not have a peptidoglycan (*Fig. 63*). Although they synthesize most of their metabolic intermediates, they are unable to make their own ATP and thus are energy parasites.

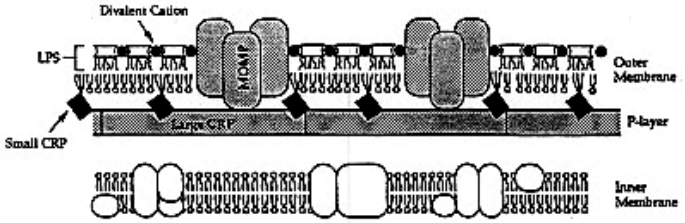


Fig. 63. Structure of chlamydial cell wall

The chlamydia exist in nature in two forms: (1) a nonreplicating, infectious particle called the elementary body (EB), 0.25 to 0.3 μm in diameter, that is released from ruptured infected cells and can be transmitted from one individual to another (C trachomatis, C pneumoniae) or from infected birds to humans (C psittaci), and (2) an intracytoplasmic form called the reticulate body (RB), 0.5 to 0.6 μm in diameter, that engages in replication and growth (Fig. 64).

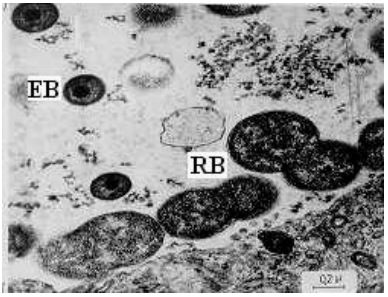


Fig. 64. Micrograph of elementary and reticulate body of chlamydia

The elementary body, which is covered by a rigid cell wall, contains a DNA genome. Ribosomes and ribosomal subunits are present in the elementary bodies. They possess a rigid outer membrane that is extensively cross-linked by disulfide bonds. Because of their rigid outer membrane the elementary bodies are resistant to harsh environmental conditions encountered when the chlamydia are outside of their eukaryotic host cells. The elementary bodies bind to receptors on host cells and initiate infection. Most chlamydia infect columnar epithelial cells but some can also infect macrophages. Throughout the developmental cycle, the DNA genome, proteins, and ribosomes are retained in the membrane-bound prokaryotic cell (reticulate body). Reticulate bodies are the metabolically active replicating form of the chlamydia.

Chlamydial reproduction begins with the attachment of EB to receptors on susceptible cells then EB are internalized by endocytosis and/or by phagocytosis (*Fig. 65*). The host cell phagocytes the EB, which then prevents the fusion of lysosomes with the phagosome and begins to reorganize itself to form a RB or initial body. The RB is specialized for reproduction rather than infection. About 8 to 10 hours after infection, the RB begins to divide by binary fission to form particles, which, after synthesis of the outer cell wall, develop into new infectious EB progeny. The yield of chlamydial elementary bodies is maximal 36 to 50 hours after infection. A chlamydia-filled vacuole or inclusion can be big enough to be seen in a light microscope and the host cytoplasm (*Fig. 66*).

The resulting inclusions may contain 100–500 progeny EB reproduction continues until the host cell dies.

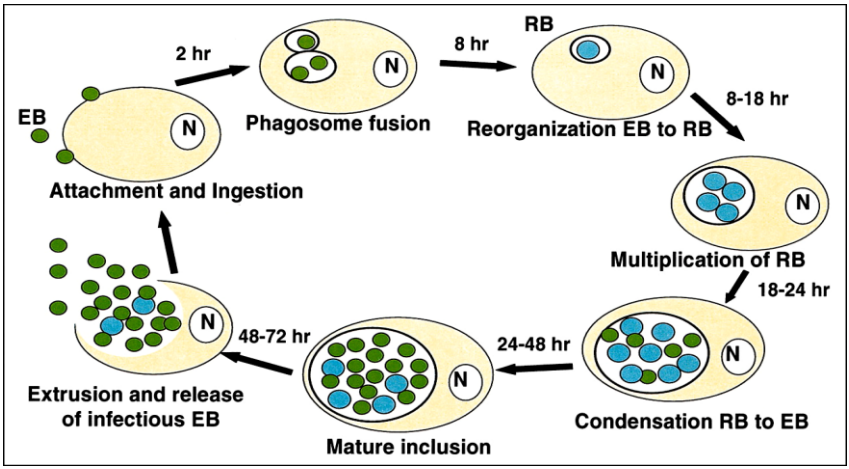


Fig. 65. Developmental cycle of the chlamydia

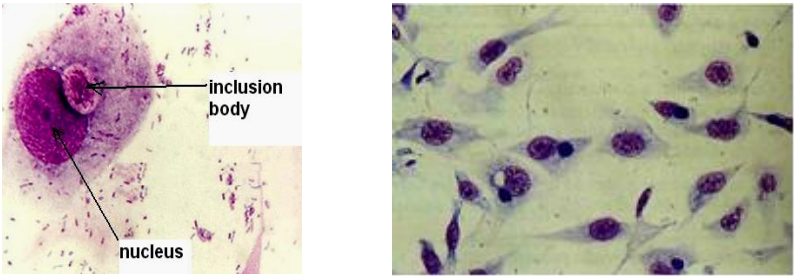


Fig. 66. Inclusion of chlamydia in host cell

Mycoplasma and Ureaplasma

The family Mycoplasmataceae contains two genera that infect humans: Mycoplasma and Ureaplasma. Although there are many species of mycoplasmas, only four are recognized as human pathogens: Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum (*Table 4*). All mycoplasmas cultivated and identified thus far are parasites of humans, animals, plants, or arthropods. The primary habitats of human and animal mycoplasmas are the mucous surfaces of the respiratory and urogenital tracts and the joints in some animals. Although some mycoplasmas belong to the normal flora, many species are pathogens, causing various diseases that tend to run a chronic course.

Table 4. Medically important mycoplasma

Organism	Habitat	Disease
M. pneumoniae	Human respiratory tract	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia, pericarditis, meningitis, polyartritis
M. hominis	Human respiratory and genital tract	Pyelonephritis, postpartum fever, pelvic inflammatory disease
M. genitalium	Human respiratory and genital tract	Nongonococcal urethritis
U. urealyticum	Human genitourinary tract	Nongonococcal urethritis
M. fermentans	Human genitourinary tract	Pyelonephritis, arthritis
M. orale	Human mouth	Periodontitis
M. salivarium	Human mouth	Periodontitis
M. arthritidis	Sinovial liquid	Arthritis

Mycoplasmas are the smallest and simplest self-replicating bacteria. They range from 0.2–0.8 mcm. They have the smallest genome size. The mycoplasma cell contains the minimum set of organelles essential for growth and replication: a plasma membrane, ribosomes, and a genome consisting of a double-stranded circular DNA molecule (*Fig. 67*).

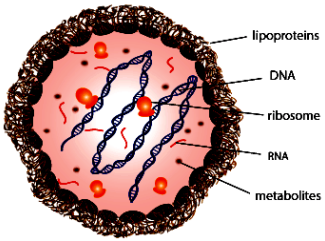


Fig. 67. Structure of mycoplasma cell

A characteristic feature that distinguishes the mycoplasmas from other bacteria is **the lack of a cell wall.** Thus, they can assume multiple shapes including round, pear shaped and even filamentous (*Fig. 68*). They are consequently placed in a separate class Mollicutes (mollis, soft; cutis, skin).

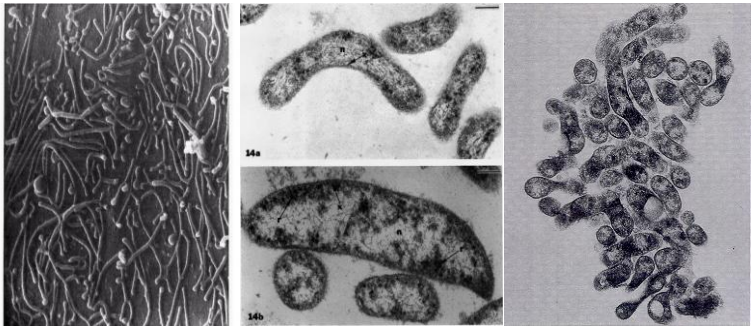


Fig. 68. Various shapes of mycoplasma cells

In most mycoplasma cultures, elongated or filamentous forms (up to 100 μm long and about 0.4 μm thick) also occur. The filaments tend to produce truly branched mycelioid structures, hence the name mycoplasma (myces, a fungus; plasma, a form). Mycoplasmas reproduce by binary fission, but cytoplasmic division frequently may lag behind genome replication, resulting in formation of multinuclear filaments.

Practical tasks, being carried out during practical classes:

1. Observe the demonstration of chlamydia, rickettsia and mycoplasma.

Therminology: elementary body, reticulate body.

Theoretical questions for control:

1. State the stages of life cycle of clamydia.
2. Recognize mycoplasma and rickettsia observed when microscopically viewing Romanowsky-Giemsa stain.
3. Recognize inclusion of chlamydia inclusion observed when microscopically viewing Romanowsky-Giemsa stain.
4. Define the following: Romanowsky-Giemsa stain, Ziehl-Neelsen stain.

Test task for control:

1. While microscopy of the scraping from urethra cytoplasmic inclusions were identified in epithelial cells in the form of characteristic "cap" over the nucleus of the cell. Which of the following disease can be assumed?

A. Chlamydiosis *C. Gonorrhea* *E. Syphilis*
B. Candidiasis *D. Mycoplasmosis*

STRUCTURE AND CHEMICAL COMPOSITION OF VIRUSES

Goal: Studying morphology of viruses and virus-induced damage to host cell.

Concrete goals:

1. Study of structure of virion.
2. Study of virus-induced damage to host cell and detection by microscopic examination.
3. Study of type of reproduction of viruses.
4. Study of structure of bacteriophages.

Students should be able to:

1. Recognize virus-induced damage to host cell by microscopic examination.

Viruses are the smallest infectious agents (20–300 nm in diameter), containing one kind of nucleic acid (RNA or DNA) as their genome, usually as single molecule. The nucleic acid is encased in a protein shell (capsid), some viruses have additional outer envelope (supercapsid) (*Fig. 69*). The entire extracellular infectious unit is termed **a virion**.

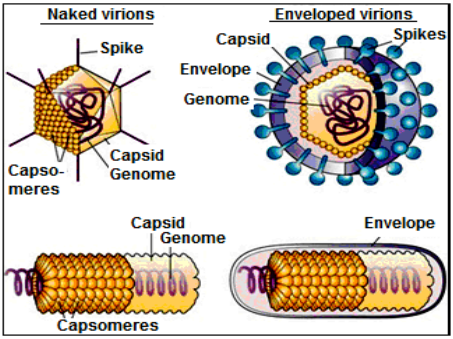


Fig. 69. Structure of viral particle

Viruses replicate only in living cells. They are **obligatory intracellular parasite**. The viral nucleic acid contains information necessary for programming the infected host cell to synthesize a number of virus-specific macromolecules required for the production of virus progeny. During the replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat protein assemble together to form the capsid, which encases and stabilizes the viral nucleic acid against the extracellular environment (nucleases) and facilitates the attachment and perhaps penetration of the virus upon contact with new susceptible cells.

Some useful definitions in virology

Capsid: The symmetric protein shell that encloses the nucleic acid genome.

Capsomeres: Morphologic units seen in the electron microscope on the surface of virus particles. Capsomeres represent clusters of polypeptides, which when completely assembled form the capsid.

Nucleocapsid: The capsid together with the enclosed nucleic acid.

In many viruses, the capsid is the only outer coat and the virion is termed a **naked virus**.

Outer envelope (supercapsid) consists of lipid bilyer and surface spikes of glycoproteins. Viruses that have an additional outer envelope are called **enveloped viruses.**

Virion: The complete infective virus particle, which in some instances (adenoviruses, papovaviruses, picornaviruses) may be identical with the nucleocapsid. In more complex virions (herpesviruses, myxoviruses), this includes the nucleocapsid plus a surrounding envelope. Virion is able to establish infection.

Defective virus: A virus particle that is functionally deficient in some aspect of replication. Defective virus may interfere with the replication of normal virus.

Symmetry and size of viruses

Virus architecture can be grouped into 3 types based on the arrangement of morphologic subunits. (1) those with helical symmetry, eg, paramyxo- and orthomyxovimses (*Fig. 70.A*), (2) those with cubic symmetry, eg, adenoviruses (*Fig. 70.B*), and (3) those with complex structures, eg, poxviruses (*Fig. 70.C*).

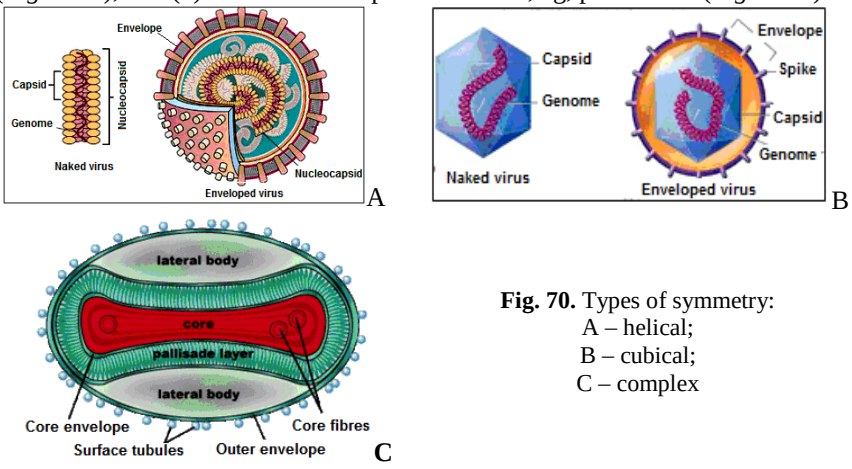


Fig. 70. Types of symmetry:
A – helical;
B – cubical;
C – complex

Helical symmetry. In the replication of viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path (*Fig. 70.A*).

Cubical symmetry. The nucleic acid is packaged inside the capsid shell and protected from the environment by the capsid *(Fig. 70.B*). All cubic symmetry observed with animal viruses to date is of the icosahedral pattern. The icosahedron has 20 faces (each an equilateral triangle), 12 vertices, and 5-fold, 3-fold, and 2-fold axes of rotational symmetry.

Chemical composition of viruses

Viral protein. The structural proteins protect the viral genome against inactivation by nucleases, participate in the attachment of the virus particle to a susceptible cell, and are responsible for the structural symmetry of the virus particle. Also, the proteins determine the antigenic characteristics of the virus.

Viral nucleic acid. Viruses contain a single kind of nucleic acid, either DNA or RNA that encodes the genetic information necessary for the replication of the virus.

RNA of viruses can be:
(-) genome and must be converted to RNA or DNA before translation;
(+) genome is ready to be translated into proteins.
Viruses can have many genome arrangements (*Fig. 71*).

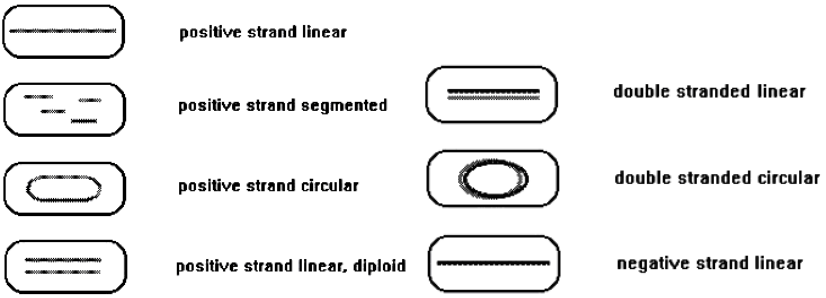


Fig. 71. Possible viral genome arrangements

Viral lipids. A number of different viruses contain lipids as part of their structure.
Viral carbohydrates. Virus envelopes contain glycoproteins.
Classification of viruses (*Fig. 72*)

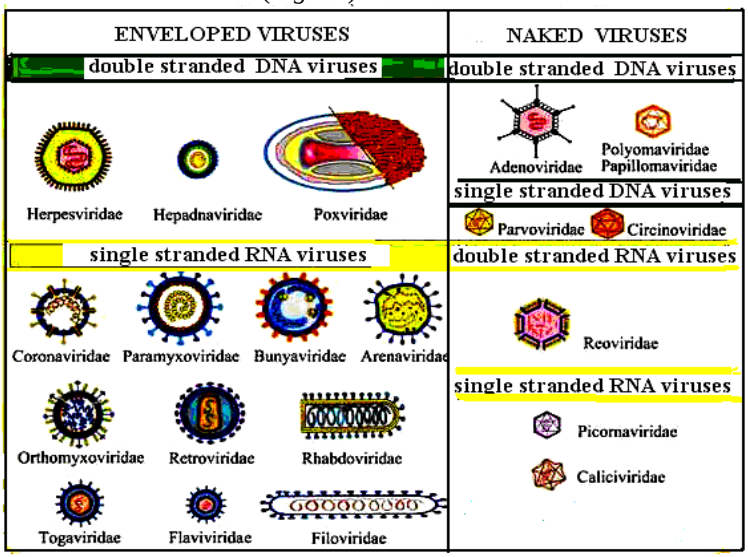


Fig. 72. Classification of viruses

The following properties, listed in the order of preference or importance, have been used as a basis for the classification of viruses.

(1) Nucleic acid type: RNA or DNA; single-stranded or double-stranded; strategy of replication.

(2) Size and morphology, including type of symmetry, number of capsomeres, and presence of envelope.
(3) Presence of specific enzymes, particularly RNA and DNA polymerases concerned with genome (HIV), and neuraminidase necessary for release of certain virus particles (influenza) from the cells in which they were formed.
(4) Susceptibility to physical and chemical agents, especially ether.
(5) Immunologic properties.
(6) Natural methods of transmission.
(7) Host, tissue, and cell tropisms.

Pathology; inclusion body formation.

Measuring the size of viruses

Direct observation in the electron microscope is used for determining the sizes of viruses and their components.

As compared with the light microscope, the electron microscope uses electrons rather than light waves and electromagnetic lenses rather than glass lenses. The electron beam obtained has a much shorter wavelength than that of light, so that objects much smaller than the wavelength of visible or ultraviolet light can be visualized. Viruses can be visualized in preparations from tissue extracts and in ultrathin sections of infected cells. Electron microscopy is the most widely used method for estimating particle size.

Quantification of virus

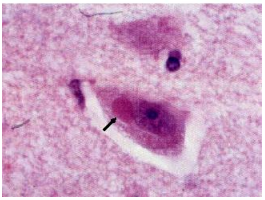
Cytopathic effect – virus-induced damage to host cell (*Fig. 73*):

1. Changes in size and shape.
2. Cytoplasmic inclusion body.
3. Nuclear inclusion body.
4. Cells fuse to form multinucleated cells.
5. Cell lysis.
6. Alter DNA.
7. Transform cells into cancerous cell.

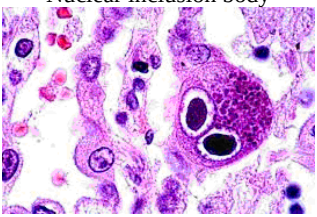
Inclusion body formation. In the course of virus multiplication within cells, virus-specific structures called inclusion bodies may be produced. They become far larger than the individual virus particle and often have an affinity for acid dyes (eg, eosin). They may be situated in the nucleus (herpesvirus), in the cytoplasm (rabies virus, influenza virus, pox virus), or in both (measles virus). In many viral infections, the inclusion bodies are the site of development of the virions (the virus factories).

The presence of inclusion bodies may be of considerable diagnostic aid. The intracytoplasmic inclusion in nerve cells, the Babesh-Negri body (*Fig. 73*), is pathognomonic for rabies. Guaenieri body is inclusion of smallpox virus in cytoplasm of epithelial cells.

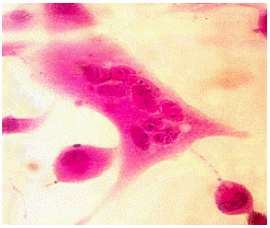
Cytoplasmic inclusion body



Nuclear inclusion body



Cells fuse to form multinucleated cells



Changes in size and shape (rounding of cells)

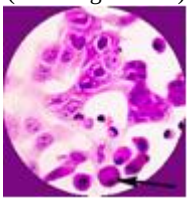


Fig. 73. Cytopathic effect of viruses

Viruses of bacteria (bacteriophages)

Bacteria are host to a special group of viruses called bacteriophage, or «phage», which pass through fine-porous filters and develop at the expense of reproducing bacteria.

Morphology. A typical phage particle consists of a "head" and a "tail" (*Fig. 74*). The head represents a tightly packed core of nucleic acid surrounded by a protein coat, or capsid. The protein capsid of the head is made up of identical subunits, packed to form a prismatic structure, usually hexagonal in cross section. The smallest known phage has a head diameter of 25 nm; others range from 55 × 40 nm up to 100 × 70 nm.

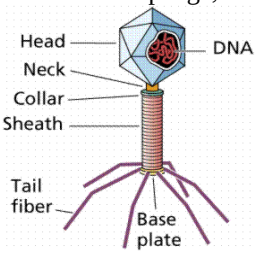


Fig. 74. Structure of bacteriophage

The phage tail varies tremendously in its complexity from one phage to another. The most complex tail is found in phage T2 and in a number of other coli and typhoid phages. In these phages, the tail consists of at least 3 parts: a hollow core, a contractile sheath, and a terminal base-plate, hexagonal in shape, to which may be attached prongs, tail fibers, or both.

A number of other tail morphologies have been reported. In some of these, sheaths are visible but the contracted state has not been observed; and in one case no sheath can be seen. The phages also vary with respect to the terminal structure of the tail: some have base-plates, some have «knobs», and some appear to lack specific terminal structures (*Fig. 75*).

The phage tail is the adsorption organ for those phages that possess them. Some phages lack tails altogether; in the RNA phages, for example, the capsid is a simple icosahedron.

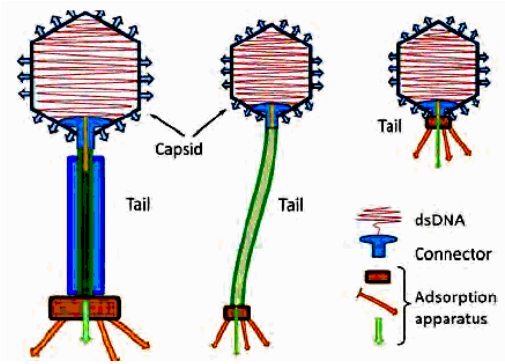


Fig. 75. Various types of phage tails

Although most phages have the head-and-tail structure described above, some **filamentous phages** have been discovered that possess a very different morphology. One of these, called «fd», has been characterized in some detail. It is a rod-shaped structure measuring 6 nm in diameter and 800 nm in length. It contains DNA and protein.

Phage reproduction

Lytic or virulent phages are phages, which can only multiply on bacteria and kill the cell by lysis at the end of the life cycle.

The lytic life cycle of phage consists of the following steps (*Fig. 76*):

1. Adsorption. The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers and it is reversible. The tail fibers attach to specific receptors on the bacterial cell and the host specificity of the phage (i.e. the bacteria that it is able to infect) is usually determined by the type of tail fibers that a phage has.

2. Penetration. A bacteriophage enzyme "drills" a hole in the bacterial cell wall and the bacteriophage injects its genome into the bacterium.

Sheath Contraction: The irreversible binding of the phage to the bacterium results in the contraction of the sheath (for those phages which have a sheath) and the hollow tail fiber is pushed through the bacterial envelope. Phages that do not have contractile sheaths use other mechanisms to get the phage particle through the bacterial envelope. Some phages have enzymes that digest various components of the bacterial envelope.

Nucleic Acid Injection: When the phage has gotten through the bacterial envelope the nucleic acid from the head passes through the hollow tail and enters the bacterial cell.

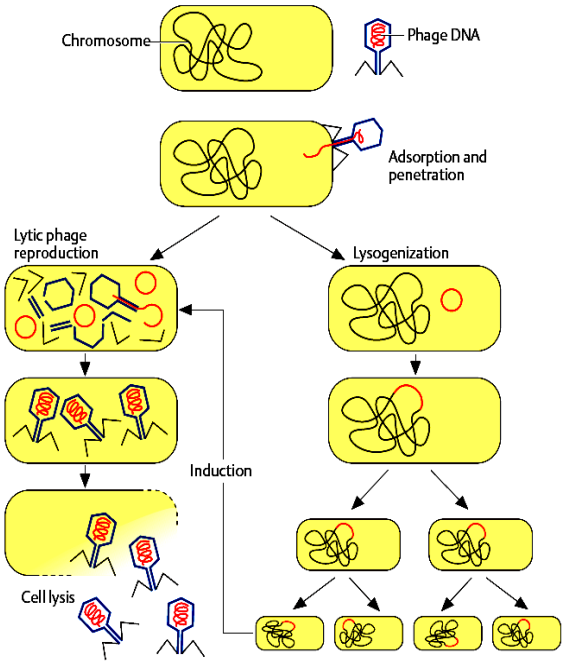


Fig. 76. Lytic and lysogenic life cycles of phages

3. Replication. Enzymes coded by the bacteriophage genome shut down the bacterium's macromolecular (protein, RNA, DNA) synthesis. The bacteriophage genome replicates and the bacterium's metabolic machinery is used to synthesize bacteriophage enzymes, structural proteins and nucleic acid.

During the replication of any viruses, whether they are bacterial, animal, or plant, the viral protein and nucleic acid components replicate separately from one another.

4. Maturation. The bacteriophage parts assemble around the genome and infectious phage particles accumulate within the cell.

5. Release. A bacteriophage-coded lysozyme breaks down the bacterial peptidoglycan causing osmotic lysis of the bacterium and release of the intact bacteriophages.

Temperate Phages

Some bacteriophages replicate by the lysogenic life cycle and are called temperate bacteriophages (*Fig. 76*). When a temperate bacteriophage infects a bacterium, it can either 1) replicate by the lytic life cycle and cause lysis of the host bacterium, or it can 2) incorporate its DNA into the bacterium's DNA and assume a noninfectious state. In the latter case, the cycle begins by the bacteriophage adsorbing to the host bacterium and injecting its genome, as in

the lytic cycle. However, the bacteriophage does not shut down the host bacterium. Instead, the bacteriophage DNA inserts or integrates into the host bacterium's DNA. At this stage, the virus is called a prophage. Expression of the bacteriophage genes controlling bacteriophage replication is repressed by a repressor protein and the bacteriophage DNA replicates as a part of the bacterial nucleoid and passed on to the daughter cells. The cell harboring a prophage is not adversely affected by the presence of the prophage and the lysogenic state may persist indefinitely.

Lysogenic conversion – when a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell. This process is called lysogenic or phage conversion. This can be of significance clinically. e.g. Toxin production by Corynebacterium diphtheriae is mediated by a gene carried by a phage. Only those strains that have been converted by lysogeny are pathogenic.

Distribution of phages in nature. Phages are wide-spread in nature. Wherever bacteria are found – in the animal body, in body secretions, in water, drainage waters and in museum cultures, conditions may be created for the development of phages. Specific phages have been found in the intestine of animals, birds, humans, and also in galls of plants. and in nodules and legumes. Phage has been isolated from milk, vegetables and fruits.

Practical importance of the phage in medicine. Arising from the data obtained on the mechanism of phage activity, phages have been used in prophylaxis and medical treatment against dysentery, enteric fever, paratyphoid, cholera, plague, anaerobic. staphylococcal, streptococcal, and other diseases. Bacteriophagia is used in the diagnosis of certain infectious diseases. With the help of special phages the species and types of isolated bacteria of the typhoid-dysentery group, staphylococci. causative agents of plague, cholera, etc. are determined.

Practical tasks, being carried out during practical classes:

1. Observe the demonstration of smallpox virus in microslide.
2. Observe the demonstration of cytopathic effect of viruses in microslides.

Therminology: capsid, capsomere, envelope, helical symmetry, cubical symmetry, complex symmetry, bacteriophage, temperate phage, lysogenic convertion.

Theoretical questions for control:

1. Define the following: structure of viruses and bacteriophages, chemical composition of virion.
2. Recognize cytopathic effect of viruses when microscopically viewing Romanowsky-Giemsa stain.
3. State the stages of lytic and lysogenic life cycles of phages.

Test task for control:

1. Which of the following is found in all infectious virions, regardless of the type of the virus?

A. Nucleocapsid *C. DNA genome* *E. Envelope*
B. Matrix protein *D. One enzyme*

2. Every virion contains a genome surrounded by capsid, which consists of:

A. Lipid *C. Lipoprotein* *E. Protein*
B. Polysacharide *D. Nucleic acid*

3. The following statements regarding viruses are correct, except:

A. Have icosahedral symmetry *D. Have helical symmetry*
B. They are unicellular *E. They possess DNA or RNA*
C. They are acellular

4. Viruses possess all, except:

A. Contain DNA or RNA *D. Have genome*
B. Divide by binary fission *E. Lack enzymes*
C. Have symmetrical structure *for protein synthesis*

5. Morphology of viruses is studied by which of the following methods:

A. Immersion microscopy *D. Electrophoresis*
B. Electron microscopy *E. Phase-contrast microscopy*
C. Dark-field microscopy

MORPHOLOGY OF PATHOGENIC PROTOZOA

Goal: Studying of morphology of pathogenic protozoa by microscopic examination.

Concrete goals:

1. Study of classification of pathogenic protozoa.
2. Study of morphology of pathogenic protozoa in microslides.
3. Study of method of staining of pathogenic protozoa.
4. Study of structure of protozoan cell.

Students should be able to:

1. Using a microscope, identify pathogenic protozoa.

Equipment: immersion microscopes, immersion oil, tables, atlas.

Structure of protozoan cell

Protozoa are microscopic unicellular eukaryotes that have a relatively complex internal structure and carry out complex metabolic activities. Some protozoa have structures for propulsion or other types of movement. Most parasitic protozoa in humans are less than 50 μm in size. The smallest (mainly intracellular forms) are 1 to 10 μm long, but Balantidium coli may measure 150 μm. As in all eukaryotes, the nucleus is enclosed in a membrane. In protozoa other than ciliates, the nucleus is vesicular, with scattered chromatin giving a diffuse appearance to the nucleus. The ciliates have both a micronucleus and macronucleus, which appear quite homogeneous in composition.

The organelles of protozoa have functions similar to the organs of higher animals. The plasma membrane enclosing the cytoplasm also covers the projecting locomotory structures such as pseudopodia, cilia, and flagella. The outer surface layer of some protozoa, termed a pellicle, is sufficiently rigid to maintain a distinctive shape, as in the trypanosomes and Giardia. However, these organisms can readily twist and bend when moving through their environment. In most protozoa the cytoplasm is differentiated into ectoplasm (the outer, transparent layer) and endoplasm (the inner layer containing organelles); the structure of the cytoplasm is most easily seen in species with projecting pseudopodia, such as the amebas. Some protozoa have a cytosome or cell "mouth" for ingesting fluids or solid particles. Contractile vacuoles for osmoregulation occur in some, such as Balantidium. Many protozoa have subpellicular microtubules; in the Apicomplexa, which have no external organelles for locomotion, these provide a means for slow movement. The trichomonads and trypanosomes have a distinctive undulating membrane between the body wall and a flagellum. Many other structures occur in parasitic protozoa, including the Golgi apparatus, mitochondria, lysosomes, food vacuoles, conoids in the Apicomplexa, and other specialized structures.

MALARIA

Etiology and epidemiology: There are an estimated 200 million global cases of malaria leading a mortality of more than one million people per year. P. falciparum (malignant tertian malaria) and P. malariae (quartan malaria) are the most common species of malarial parasite and are found in Asia and Africa. P. vivax (benign tertian malaria) predominates in Latin America, India and Pakistan, whereas, P. ovale (ovale tertian malaria) is almost exclusively found in Africa.

Morphology (*Fig. 77*): The micro-metacryptozoite feeds on erythrocytes, a vacuole appears in it, the nucleus is pushed to one side, and the micro-metacryptozoite is changed into what is called as the ring-shaped trophozoite, the signet ring stage. Malarial parasite trophozoites are generally ring shaped, 1–2 microns in size, although other forms (ameboid and band) may also exist. The full grown trophozoite is known as a schizont. The schizont undergoes multiple fission to form oval-shaped merozoites. The sexual forms of the parasite (gametocytes) are much larger and 7–14 microns in size. P. falciparum is the largest and is banana shaped while others are smaller and round. P. vivax and P. ovale cause stippling of infected red cells.

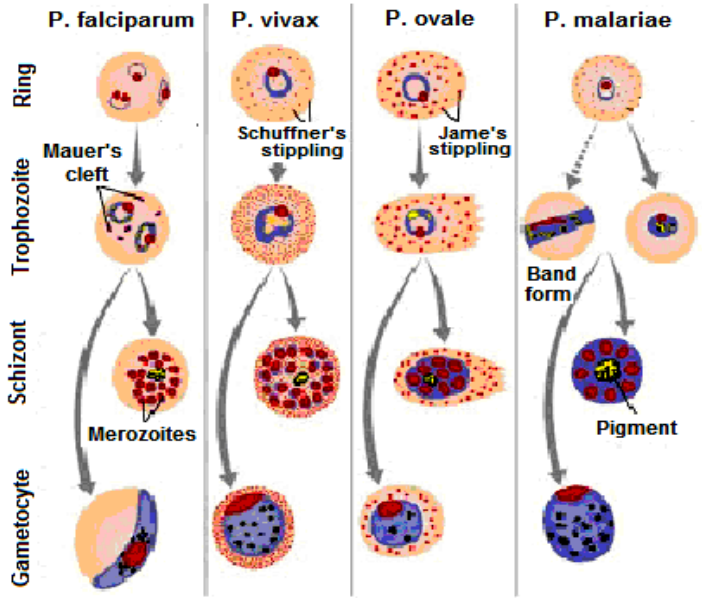


Fig. 77. Plasmodium species differentiation

Life cycle (*Fig. 78*): The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito (definitive host) inoculates sporozoites into the human (intermediate host).

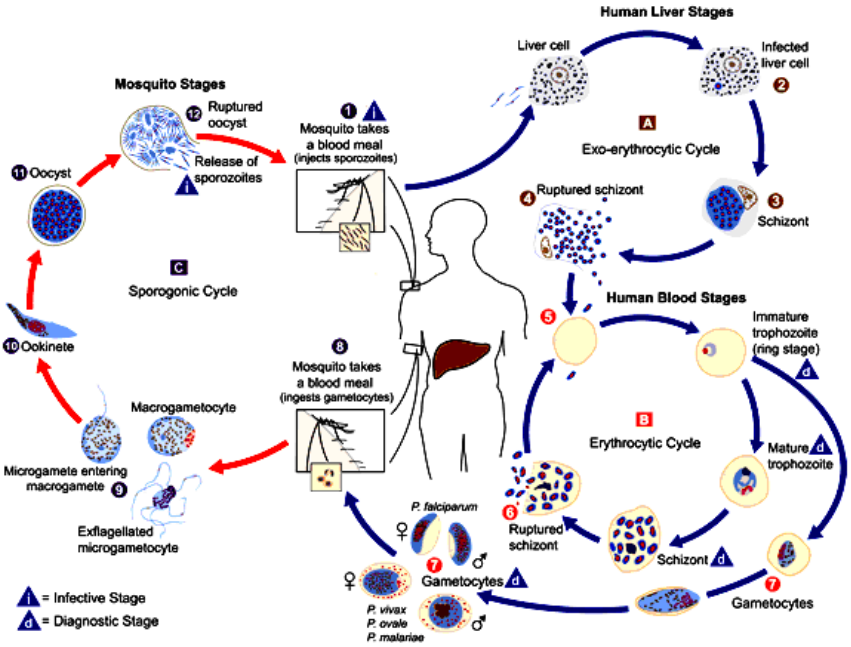


Fig. 78. Plasmodium life cycles

Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in P. vivax and P. ovale a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later). After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

TOXOPLASMOSIS

Etiology: Toxoplasma gondii.

Morphology: Toxoplasma may exist in three infectious stages of the organism: the rapidly multiplying forms called **tachyzoites** (*Fig. 79*), the quiescent **bradyzoites** that occupy cysts in infected tissue (*Fig. 80*), and the **oocysts** shed in feces. The intracellular parasites (tachyzoite) are 3x6 microns, pear-shaped organisms that are enclosed in a parasite membrane to form a cyst measuring 10–100 µm in size. Cysts in cat feces (oocysts) are 10–13 µm in diameter. The bradyzoites, which are a slow multiplying stage, penetrate the epithelial cells of the small intestine and initiate the formation of numerous asexual generations before the sexual cycle (gametogony, the production of gametes) begins. After the male gamete fertilizes the female gamete, two walls are laid down around the fertilized zygote to form the oocyst, which is excreted in the feces in an unsporulated stage. Each sporulated oocyst contains two sporocysts and each sporocyst contains four sporozoites (*Fig. 81*).

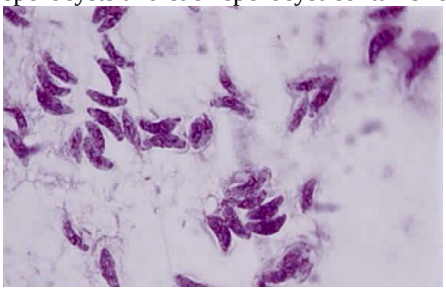


Fig. 79. Tachyzoites of T. gondii

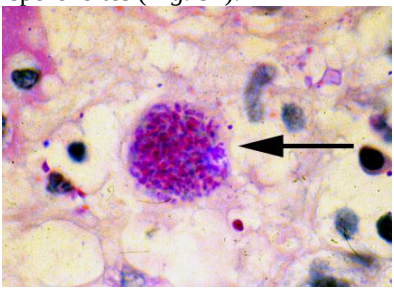


Fig. 80. Tissue cysts of T. gondii

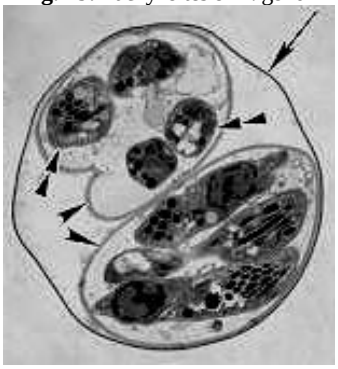


Fig. 81. Oocysts of T. gondii

Sporulated oocysts are remarkably resistant and can survive in soil for several months. The oocysts are infectious to most mammals and birds. Toxoplasma can be transmitted to intermediate hosts through oocysts, by carnivorism, or transplacentally. Other bradyzoites penetrate the lamina propria and begin to multiply as tachyzoites.

Tachyzoites can enter almost any type of host cell and multiply until the host cell is filled with parasites and dies. Tissue cysts are formed most commonly in the brain, liver, and muscles. Cysts in neural tissues are up to 60 μm in diameter and contain 50–5 000 bradyzoites in a thin membrane (*Fig. 80*). Tissue cysts usually cause no host reaction and may remain for the life of the host.

Life cycle (*Fig. 82*): The natural life cycle of T. gondii occurs in cats and small rodents, although the parasite can grow in the organs (brain, eye, skeletal muscle, etc.) of any mammal or birds. Cats get infected by ingestion of cysts in flesh. Decystation occurs in the small intestine, and the organisms penetrate the submucosal epithelial cells where they undergo several generations of mitosis, finally resulting in the development of micro- (male) and macro- (female) gametocytes. Fertilized macrogametocytes develop into oocysts that are discharged into the gut lumen and excreted.

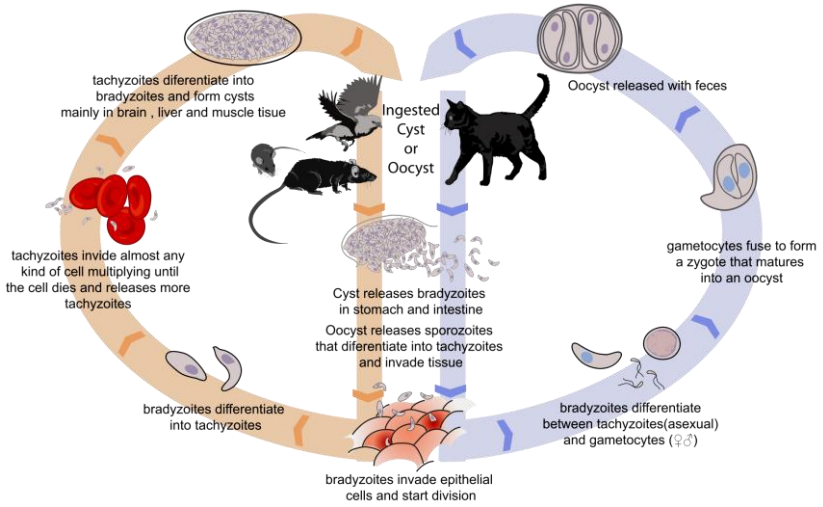


Fig. 82. Toxoplasma life cycle

Oocysts sporulate in the warm environment and are infectious to a variety of animals including rodents and man. Sporozoites released from the oocyst in the small intestine penetrate the intestinal mucosa and find their way into macrophages where they divide very rapidly (hence the name tachyzoites) and form a cyst which may occupy the whole cell. The infected cells ultimately burst and release the tachyzoites to enter other cells, including muscle and nerve cells, where they are protected from the host immune system and multiply slowly (bradyzoites). These cysts are infectious to carnivores (including man). Unless man is eaten by a cat, it is a dead-end host.

In nonfeline intermediate hosts, such as humans or mice, the extraintestinal cycle of T. gondii is similar to the cycle in cats. However, sexual stages are produced only in the feline definitive hosts.

TRYPANOSOMIASIS

African trypanosomiasis (Sleeping sickness)

Etiology: There are two clinical forms of African trypanosomiasis: 1) a slowly developing disease caused by Trypanosoma brucei gambiense and 2) a rapidly progressing disease caused by T. brucei rhodesiense.

Morphology: T. b. gambiense and T. b. rhodesiense are similar in appearance: The organism measures 10–30 mcm × 1–3 mcm. It has a single central nucleus and a single flagellum originating at the kinetoplast and joined to the body by an undulating membrane. The outer surface of the organism is densely coated with a layer of glycoprotein, the variable surface glycoprotein (*Fig. 83*).

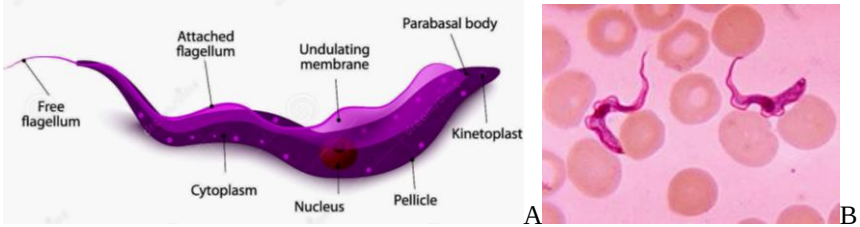


Fig. 83. Morphology of Trypanosoma cell: A – structure of cell; B – micrograph

Life cycle (*Fig. 84*): The infective, metacyclic form of the trypanosome is injected into the primary host during a bite by the vector, the tsetse fly. The organism transforms into a dividing trypanosomal (trypomastigote) blood form as it enters the draining lymphatic and blood stream. The trypanosomal form enters the vector during the blood meal and travels through the alimentary canal to the salivary gland where it proliferates as the crithidial form (epimastigote) and matures to infectious metacyclic forms. Trypomastigotes can traverse the walls of blood and lymph capillaries into the connective tissues and, at a later stage, cross the choroid plexus into the brain and cerebrospinal fluid. The organism can be transmitted through blood transfusion.

American trypanosomiasis (Chagas' disease)

Etiology: Trypanosoma cruzi.

Morphology: Depending on its host environment, the organism occurs in three different forms (*Fig. 85*). The trypanosomal (trypomastigote) form, found in mammalian blood, is 15 to 20 microns long and morphologically similar to African trypanosomes. The crithidial (epimastigote) form is found in the insect intestine. The leishmanial (amastigote) form, found intracellularly or in pseudocysts in mammalian viscera (particularly in myocardium and brain), is round or oval in shape, measures 2–4 microns and lacks a prominent flagellum.

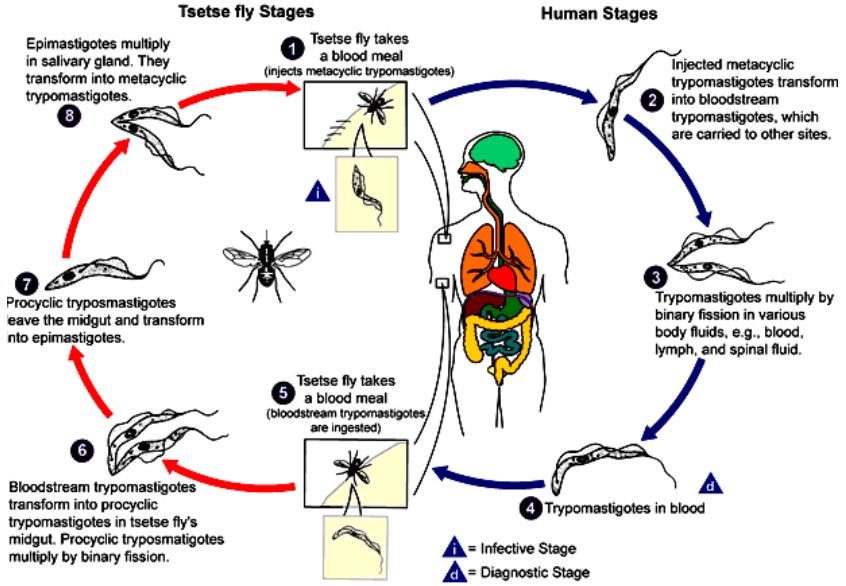


Fig. 84. Life cycle of Trypanosoma brucei

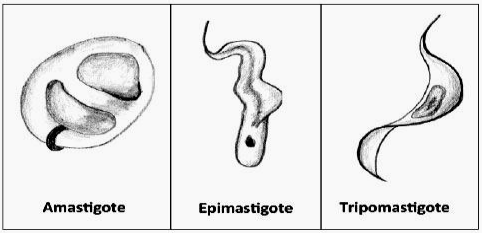


Fig. 85. Forms of Trypanosoma cruzi

Life cycle (*Fig. 86*): The organism is transmitted to mammalian host by many species of kissing (riduvid) bug, most prominently by Triatoma infestans, T. sordida, Panstrongylus megistus and Rhodnius prolixus. Transmission takes place during the feeding of the bug, which normally bites in the facial area (hence the name, kissing bug) and has the habit of defecating during feeding. The metacyclic trypamastigotes, contained in the fecal material, gain access to the mammalian tissue through the wound, which is often rubbed by the individual that is bitten. Subsequently, they enter various cells, including macrophages, where they differentiate into amastigotes and multiply by binary fission. The amastigotes differentiate into non-replicating trypomastigotes and the cells rupture to release them into the bloodstream. Additional host cells, of a variety of types, can become infected and the trypomastigotes once again form amastigotes inside these cells. Transmission may also occur from man to man by blood transfusion and by the transplacental route.

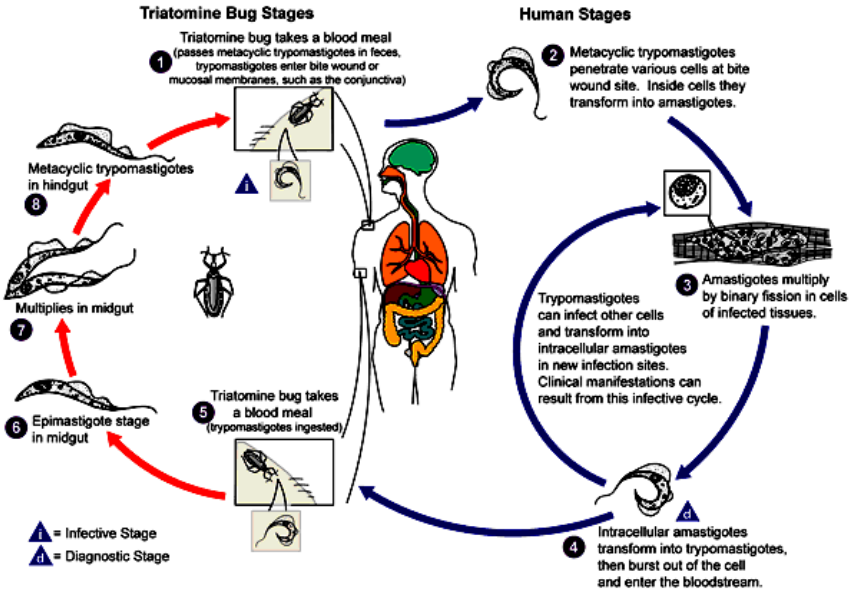


Fig. 86. Life cycle of Trypanosoma cruzi

LEISHMANIASIS

Etiology: Several species of Leishmania are pathogenic for man: L. donovani causes visceral leishmaniasis (Kala-azar, black disease, dumdum fever); L.tropica (L. t. major, L. t. minor and L. ethiopica) cause cutaneous leishmaniasis (oriental sore, Delhi ulcer, Aleppo, Delhi or Baghdad boil); and L.braziliensis (also, L. mexicana and L. peruviana) are etiologic agents of mucocutaneous leishmaniasis (espundia, Uta, chiclero ulcer).

Morphology: All species of Leishmania in man are morphologically similar and appear as intracellular amastigotes. Amastigote (leishmanial form) is oval and measures 2 to 6 µm long by 1 to 3 µm in diameter (*Fig. 87.A*). Promastigotes (leptomonad) are elongated with single flagellum, develop in the intestine of the sand fly and measure 14–20 µm (*Fig. 87.B*).

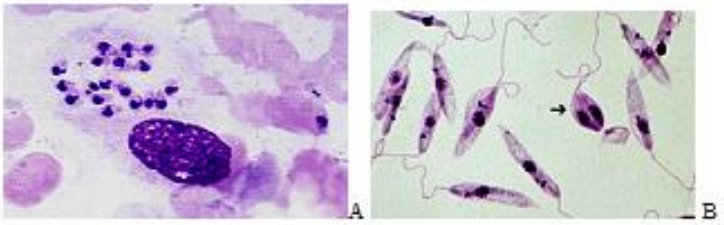


Fig. 87. Forms of Leishmania: A – amastigote; B – promastigote

Life cycle (*Fig. 88*): The organism is transmitted by the bite of several species of blood-feeding sand flies (Phlebotomus) which carry the promastigote in the anterior gut and pharynx. The parasites gain access to mononuclear phagocytes where they transform into amastigotes and divide until the infected cell ruptures. The released organisms infect other cells. Dogs and rodents are common reservoirs.

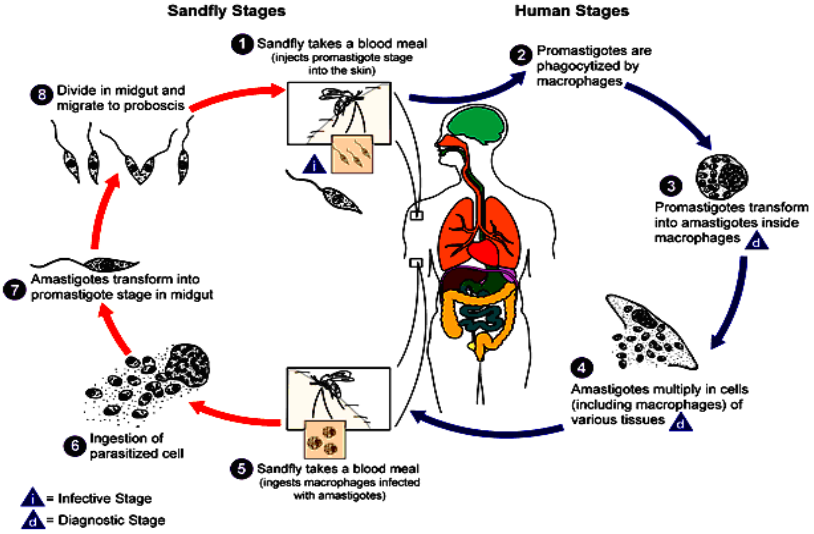


Fig. 88. Life cycle of Leishmania

GIARDIASIS (lambliasis)

Etiology: Giardia lamblia.

Morphology. Trophozoite (*Fig. 89.A*): Giardia is a 12 to 15 µm, half pear-shaped organism with 8 flagella and 2 axostyles arranged in a bilateral symmetry. There are two anteriorly located large suction discs. The cytoplasm contains two nuclei and two parabasal bodies. **Cyst** (*Fig. 89.B*): Giardia cysts are 9 to 12 µm ellipsoidal cells with a smooth well-defined wall. The cytoplasm contains four nuclei and many of the structures seen in the trophozoite.

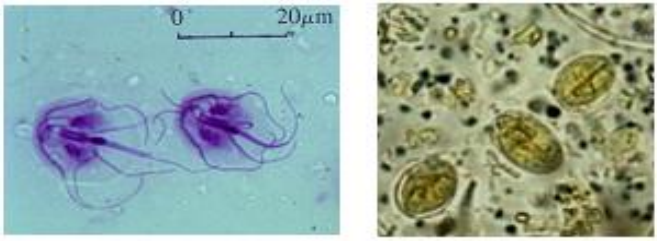


Fig. 89. Morphology of Giardia lamblia: A – trophozoite; B – cyst

Life cycle (*Fig. 90*): Infection occurs by ingestion of cysts, usually in contaminated water. Decystation occurs in the duodenum and trophozoites (trophs) colonize the upper small intestine where they may swim freely or attach to the sub-mucosal epithelium via the ventral suction disc. The free trophozoites encyst as they move down stream and mitosis takes place during the encystment. The cysts are passed in the stool. Man is the primary host although beavers, pigs and monkeys are also infected and serve as reservoirs.

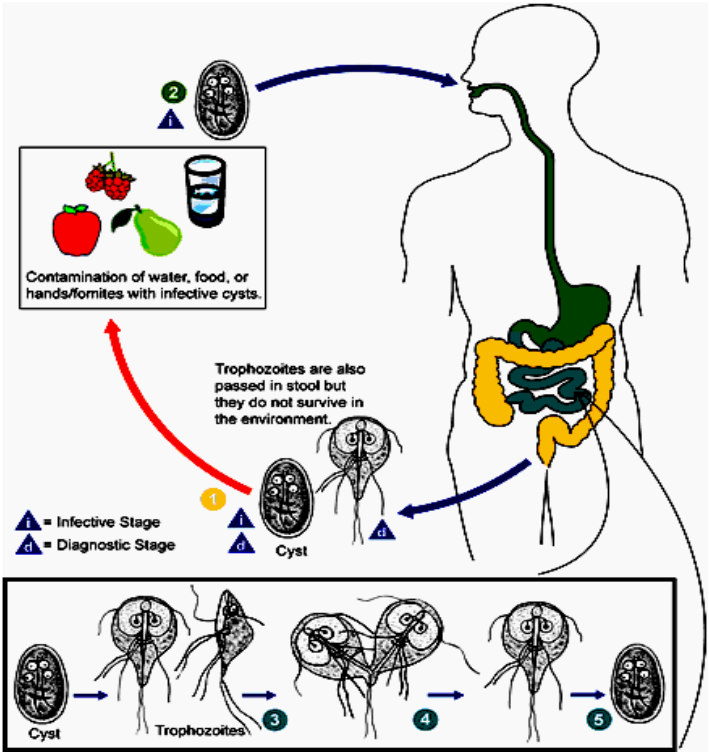


Fig. 90. Life cycle of G. lamblia

TRICHOMONIASIS

Etiology: Trichomonas vaginalis.

Morphology: The trophozoite form is 15 to 18 μm in diameter and is half pear shaped with a single nucleus, four anterior flagella and a lateral flagellum attached by an undulating membrane. Two axostyles are arranged asymmetrically (*Fig. 91*). The organism does not encyst.

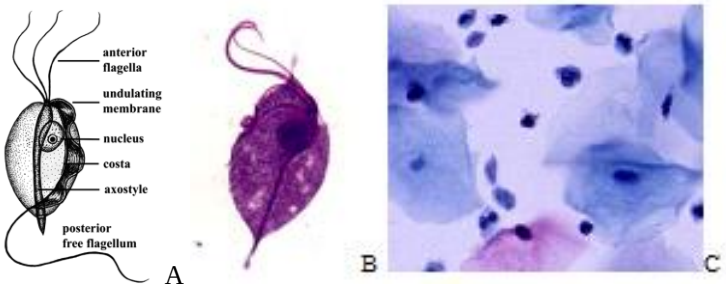


Fig. 91. Morphology of T. vaginalis:
A – cell structure, B – micrograph of cell, C – T. vaginalis in vaginal scrapping

Life cycle: T. vaginalis colonizes the vagina of women and the urethra (sometimes prostate) of men. Infection occurs primarily via sexual contact, although non-venereal infections are possible. The organism does not encyst and divides by binary fission, which is favored by low acidity (pH > 5.9; the normal pH is 3.5 to 4.5). There is no non-human reservoir.

AMEBIASIS (amebic dysentery, amebic hepatitis)

Etiology: Entamoeba histolytica.

Morphology. Trophozoite: This form has an ameboid appearance and is usually 15–30 μm in diameter, although more invasive strains tend to be larger. The organism has a single nucleus with a distinctive small central karyosome (*Fig. 92*). The fine granular endoplasm may contain ingested erythrocytes (*Fig. 93A*). The nuclear chromatin is evenly distributed along the periphery of the nucleus. **Cyst** (*Fig. 93B*): is spherical, with a refractile wall; the cytoplasm contains dark staining chromatoidal bodies and 1 to 4 nuclei with a central karyosome and evenly distributed peripheral chromatin.

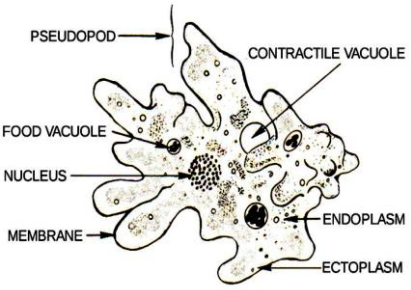


Fig. 92. Structure of E. histolytica cell

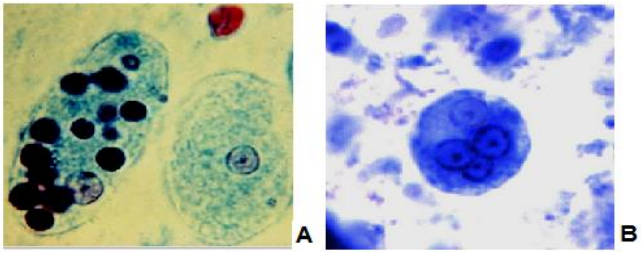


Fig. 93. Morphology of E. histolytica: A – trophozoites; B – cyst.

Life cycle: Infection occurs by ingestion of cysts on fecally contaminated food, water, or hands (*Fig. 94*). The cyst is resistant to the gastric environment and passes into small intestine where it decysts. The metacyst divides into four and then eight amoebae which move to the large intestine. The majority of the organisms are passed out of the body with the feces but, with larger bolus of infection, some amoebae attach to and invade the mucosal tissue forming "flask-shaped" lesions (bomb craters). The organisms encyst for mitosis and are passed through with feces. In some patients the trophozoites invade the intestinal mucosa, or, through the bloodstream, extraintestinal sites such as the liver, brain, and lungs, with resultant pathologic manifestations.

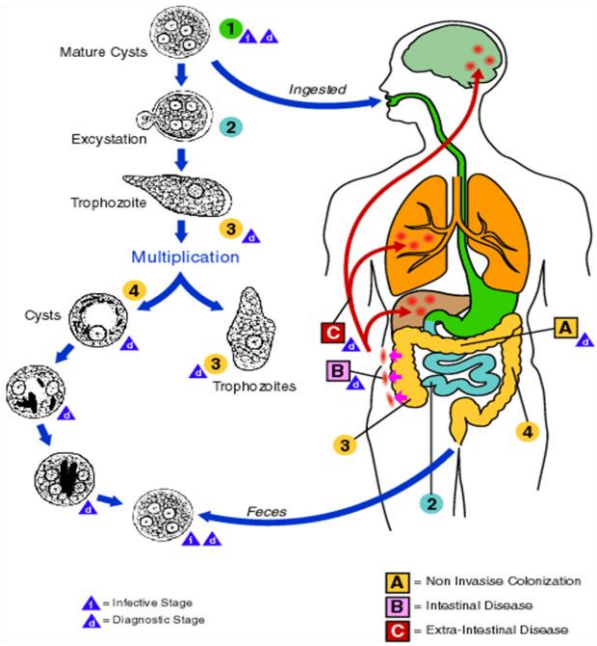


Fig. 94. Life cycle of E. histolytica

BALANTIDIASIS

Etiology: Balantidium coli.

Morphology: The organism is a large (100 × 60 micrometer) ciliate with a macro- and a micro-nucleus (*Fig. 95, 96.A*). Macronucleus is typically elongate and kidney-shaped; micronucleus is spherical. Conspicuous vestibulum leads into a large cytostome; opposite of which a cytopyge lies. There are 2 prominent contractive vacuoles; food vacuoles in the cytoplasm contain debries, bacteria, RBCs, and fragments of host epithelium.

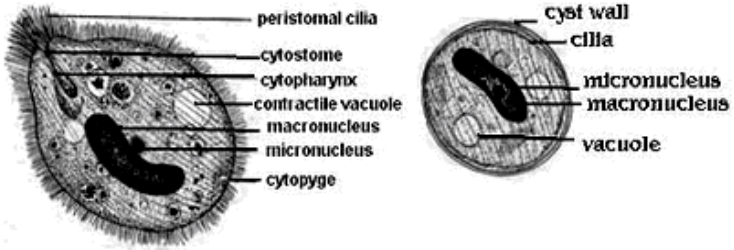


Fig. 95. Structure of B. coli trophozoite and cyst

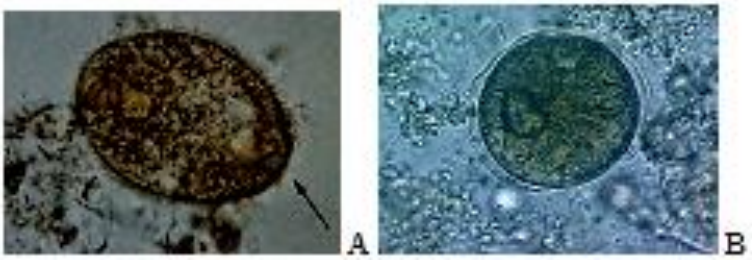


Fig. 96. Micrograph of Balantidium coli: A – trophozoite; B – cyst

Life cycle: This is a parasite primarily of cows, pigs and horses. Cysts are the parasite stage responsible for transmission of balantidiasis. The host most often acquires the cyst through ingestion of contaminated food or water. Following ingestion, excystation occurs in the small intestine, and the trophozoites colonize the large intestine. The trophozoites reside in the lumen of the large intestine of humans and animals, where they replicate by binary fission (*Fig. 97*), during which conjugation may occur. Trophozoites undergo encystation to produce infective cysts. Some trophozoites invade the wall of the colon and multiply. Some return to lumen and disintegrate. Mature cysts are passed with feces (*Fig. 98*).

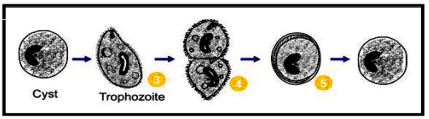


Fig. 97. Replication of B. coli

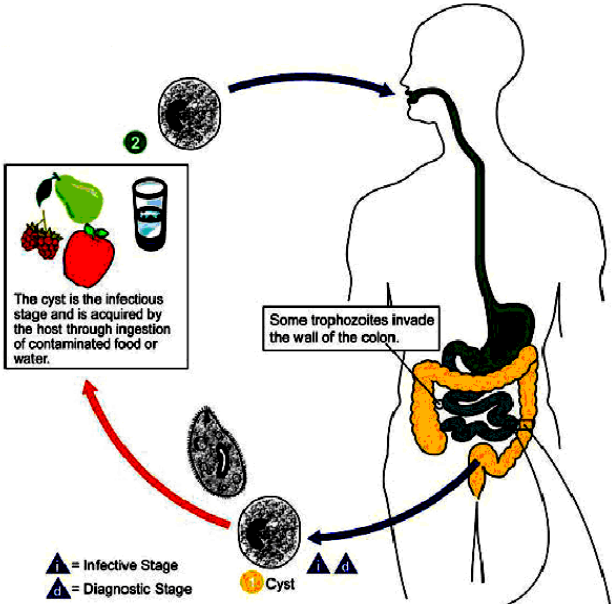


Fig. 98. Life cycle of B. coli

Practical tasks, being carried out during practical classes:

1. Observe the demonstration of pathogenic protozoa in microslide.

Therminology:

Theoretical questions for control:

1. Define the cell structure of pathogenic protozoa.
2. Recognize pathogenic protozoa when microscopically viewing Romanowsky-Giemsa stain.
3. State the life cycles of intestinal and blood-born protozoa.

Test task for control:

1. Microscopy of dental plaque revealed unicellular organisms. Their cytoplasm had two distinct layers, barely visible core, wide pseudopodia. The patient is most likely to have:

A. Entamoeba gingivalis
B. Entamoeba histolytica
C. Trichomonas tenax
D. Lamblia
E. Entamoeba coli

2. A 13 year old child complains about poor appetite, pain in the right subcostal area. Microscopical examination of duodenal contents revealed big pyriform cells with two nuclei. What microorganism was revealed?

A. Lamblia
B. Trypanosoma
C. Trichomonas
D. Toxoplasma
E. Amoeba

3. While examining a blood smear taken form a patient and stained by Romanovsky's method a doctor revealed some protozoa and diagnosed the patient with Chagas disease. What protozoan is the causative agent of this disease?

A. Leishmania donovani
B. Trypanosoma cruzi
C. Trypanosoma brucei
D. Toxoplasma gondii
E. Leishmania tropica

4. A smear from frothy and purulent vaginal discharges of a 42 y.o. woman was stained by Romanovsky-Giemsa method. Its analysis revealed some microorganisms of flagellates class. What microorganism were the most probably revealed?

A. Leishmania donovani
B. Trypanosoma cruzi
C. Trihomonas vaginalis
D. Trypanosoma brucei
E. Lamblia intestinalis

5. Finding intraerythrocytic trophozoite, schizont, and gametocytes stages in stained thin blood smears during febrile periods is the usual means for diagnosis of which of the following?

A. African trypanosomiasis
B. Malaria
C. American trypanosomiasis
D. Toxoplasmosis
E. Visceral leishmaniasis

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CONTENTS

Навчальне видання

МОРФОЛОГІЯ БАКТЕРІЙ, ВІРУСІВ І НАЙПРОСТІШИХ

Методичні вказівки
для студентів II і III курсів
медичного та стоматологічного факультетів
з англійською мовою викладання

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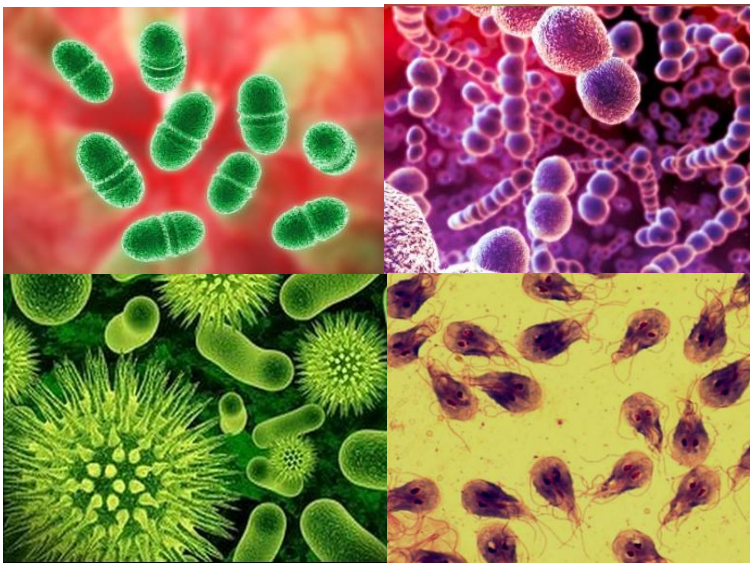

MORPHOLOGY OF BACTERIA, VIRUSES AND PROTOZOA

Learning guide for the 2nd and 3rd year English media students of the Faculty of Medicine and the Faculty of Dentistry (Microbiology, virology and immunology)

МОРФОЛОГІЯ БАКТЕРІЙ, ВІРУСІВ І НАЙПРОСТІШИХ

Методичні вказівки з дисципліни «Мікробіологія, вірусологія та імунологія» для студентів II і III курсів медичного та стоматологічного факультетів з англійською мовою викладання