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LABORATORY WORKSHOP OF GENERAL MICROBIOLOGY COURSE

MANUAL FOR INTERNATIONAL STUDENTS

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CONTENS

1. GENERAL LABORATORY RULES:

1. Do not eat or drink in the laboratory, chew gum, or place any object on or near your mouth. All books, backpacks, coats, purses, etc. should be cleared from your table before beginning any procedure. While actively working with microbial cultures, keep your laboratory manual / instructions at a reasonable distance from your work area. Do not wear loose articles of clothing, such as scarves or bulky jackets.
2. Practice good aseptic technique by performing the following at the beginning each class: tie back long hair; wear closed footwear to protect the feet; wear lab coats (preferred) or aprons to protect clothes; clean lab table with an antiseptic wash; wash hands thoroughly before starting any lab exercise; wear disposable gloves when handling BSL-2 organisms; wear safety goggles when conducting procedures that pose a splash or aerosol risk, and when dealing with certain chemicals, such as staining reagents.
3. All cultures, whether test tubes or Petri plates, should be labeled with your name or initials, the date, and the name of the organism. Plates can be written on directly; tape labels should be used for test tubes. Permanent markers designated only for laboratory use must be used to label cultures.
4. Most microbial cultures will be inoculated using a sterile loop or needle; once the transfer is complete the loop or needle should be sterilized again. Liquid cultures should be transferred using a sterile pipette and mechanical pipetting apparatus. No mouth pipetting!
5. All test tubes should be placed in a rack before transporting them from one area of the lab to another. All lids on all test tubes must be secure.
6. All Petri plates should be taped on both sides before carrying to the incubators.
7. All disposable microbiological waste should be discarded in the BIOHAZARD bag. Used test tubes (labels removed) should be placed in the designated pan to be autoclaved and cleaned. Used pipettes should be placed immediately into the provided waste container. Do not place any hazardous or infectious materials in the regular trash. Do not place any hazardous or infectious materials in the sink.
8. All used glass slides and coverslips should be placed in SHARPS boxes. Do not discard slides in the biohazard bags.
9. Practice good aseptic technique by performing the following at the end of each lab exercise:
a) remove gloves inside out and place them in the biohazard bag
b) remove lab coats or aprons, fold them inside out, and place them in the assigned drawer
c) remove goggles (if used) and clean with alcohol wipes before placing in cabinet for sterilization
d) decontaminate your work bench by applying an antiseptic wash
e) wash your hands thoroughly
10. Practice good aseptic technique when making notes or recording results from your experiments in your lab manual -- Do not write in your lab manual or notebook until AFTER you have removed your gloves and apron and washed your hands!
11. If an accident should occur, such as a spill or a broken test tube:
a) inform your instructor immediately
b) soak the area with disinfectant and cover with paper towels
c) dispose of contaminated paper towels in the biohazard bag
d) dispose of contaminated broken glass in the SHARPS container e) wash hands thoroughly
12. If an accident results in bacterial culture coming in contact with eyes, mucous membranes or an open wound: eyes should be flushed immediately at the eye wash station; affected wound should be washed thoroughly with soap and water; the student should contact their personal physician for advice on further treatment.
13. Accident reports must be filed with the Biosafety committee no more than 48 hours following the accident.
14. Students who are pregnant or have a medical condition which could compromise their immune system must have written permission from their attending physician in order to participate in microbiology lab.

2. HISTORY OF MICROBIOLOGY

Discovery of Microbes and the Dawn of Microbiology

- Microbiology is the study of living organisms of microscopic size.
- The term microbiology was given by French chemist Louis Pasteur (1822-95).

Fig. 1. World famous scientists who made discoveries in Microbiology

The Discovery Era

- **Robert Hooke**, a 17th-century English scientist, was the first to use a lens to observe the smallest unit of tissues he called "cells." Soon after, the Dutch amateur biologist **Anton van Leeuwenhoek** observed what he called "animalcules" with the use of his homemade microscopes.
- **Antonie van Leeuwenhoek (1632-1723)** of Delft, Holland (Netherland) was the first person to observe and accurately describe microorganisms (bacteria and protozoa) called 'animalcules' (little animals) in 1676.
- Actually he was a Dutch linen merchant but spent much of his spare time constructing simple microscopes composed of double convex lenses held between two silver plates. He constructed over 250 small powerful microscopes that could magnify around 50-300 times.
- Leeuwenhoek was the first person to produce precise and correct descriptions of bacteria and protozoa using a microscope he made himself. Because of this extraordinary contribution to microbiology, he is considered as the "Father of microbiology".
- Leeuwenhoek is also considered to be the father of bacteriology and protozoology (protistology).
- He wrote over 200 letters which were transmitted as a series of letters from 1674-1723 to Royal Society in London during a 50 years period.

Transition Period

When microorganisms were known to exist, most scientists believed that such simple life forms could surely arise through spontaneous generation. That is to say life was thought to spring spontaneously from mud and lakes or anywhere with sufficient nutrients. This concept was so compelling that it persisted until late into the 19th century.

The main aspects were to solve the controversy over spontaneous generation which includes experimentations mainly of Francesco Redi, John Needham, Lazzaro Spallanzani and Nicolas Appert etc and to know the disease transmission which mainly includes the work of Ignaz Semmelweis and John Snow.

- **Francesco Redi (1626-1697):** The ancient belief in spontaneous generation was first of all challenged by Redi, an Italian physician, who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously.
- **John Needham (1713-1781):** He was probably the greatest supporter of the theory of spontaneous generation. He proposed that tiny organisms the animalcules arose spontaneously on his mutton gravy. He covered the flasks with cork as done by Redi and even heated some flasks. Still the microbes appeared on mutton broth.
- **Lazzaro Spallanzani (1729-1799):** He was an Italian Naturalist who attempted to refute Needham's experiment. He boiled beef broth for longer period, removed the air from the flask and then sealed the container. Followed incubation no growth was observed by him in these flasks. He showed that the heated nutrients could still grow animalcules when exposed to air by simply making a small crack in the neck. Thus Spallanzani disproved the doctrine of spontaneous generation.
- **Nicolas Appert** followed the idea of Spallanzani's work. He was a French wine maker who showed that soups and liquids can be preserved by heating them extensively in thick champagine bottles.
- **Ignaz Semmelweis** and **John Snow** were the two persons who showed a growing awareness of the mode of disease transmission.
- Two German scholars **Schulze (1815-1873)** and **Theodor Schwan (1810-1882)** viewed that air was the source of microbes and sought to prove this by passing air through hot glass tubes or strong chemicals into boiled infusions in flasks. The infusion in both the cases remained free from the microbes.
- **George Schroeder** and **Theodor Von Dusch (1854)** were the first to introduce the idea of using cotton plugs for plugging microbial culture tubes.
- **Darwin (1859)** in his book, 'Origin of the Species' showed that the human body could be conceived as a creature susceptible to the laws of nature. He was of the opinion that disease may be a biological phenomenon, rather than any magic.

The Golden Age

The Golden age of microbiology began with the work of Louis Pasteur and Robert Koch who had their own research institute. More important there was an acceptance of their work by the scientific community throughout the world and a willingness to continue and expand the work. During this period, we see the real beginning of microbiology as a discipline of biology.

- The concept of spontaneous generation was finally put to rest by the French chemist **Louis Pasteur** in an inspired set of experiments involving a goosenecked flask. When he boiled broth in a flask with a straight neck and left it exposed to air, organisms grew. When he did this with his goose-necked flask, nothing grew. The S-shape of this second flask trapped dust particles from the air, preventing them from reaching the broth. By showing that he could allow air to get into the flask but not the particles in the air, Pasteur proved that it was the organisms in the dust that were growing in the broth.
- Pasteur, thus in 1858 finally resolved the controversy of spontaneous generation versus biogenesis and proved that microorganisms are not spontaneously generated from inanimate matter but arise from other microorganisms.
- He also found that fermentation of fruits and grains, resulting in alcohol, was brought about by microbes and also determined that bacteria were responsible for the spoilage of wine during fermentation. Pasteur in 1862 suggested that mild heating at 62.8°C (145°F) for 30 minutes rather than boiling was enough to destroy the undesirable organisms without ruining the taste of the product, the process was called Pasteurization. Pasteurization was introduced into the United States on a commercial basis in 1892. His work led to the development of the germ theory of disease.
- **Louis Pasteur is known as the "Father of Modern Microbiology / Father of Bacteriology.**
- **John Tyndall (1820 – 1893):** An English physicist, deal a final blow to spontaneous generation in 1877. He conducted experiments in an aseptically designed box to prove that dust indeed

carried the germs. He demonstrated that if no dust was present, sterile broth remained free of microbial growth for indefinite period even if it was directly exposed to air. He discovered highly resistant bacterial structure, later known as endospore, in the infusion of hay. Prolonged boiling or intermittent heating was necessary to kill these spores, to make the infusion completely sterilized, a process known as Tyndallisation.

- Around the same time that Pasteur was doing his experiments, a doctor named **Robert Koch** was working on finding the causes of some very nasty animal diseases (first anthrax, and then tuberculosis). He gave the first direct demonstration of the role of bacteria in causing disease. He was a german physician who first of all isolated anthrax bacillus (Bacillus anthracis, the cause of anthrax) in 1876. He perfected the technique of isolating bacteria in pure culture. He also introduced the use of solid culture media in 1881 by using gelatin as a solidifying agent. In 1882 he discovered Mycobacterium tuberculosis. He proposed Koch postulate which were published in 1884 and are the corner stone of the germ theory of diseases and are still in use today to prove the etiology (specific cause) of an infectious disease.

  Koch's four postulates are:

  - The organism causing the disease can be found in sick individuals but not in healthy ones.
  - The organism can be isolated and grown in pure culture.
  - The organism must cause the disease when it is introduced into a healthy animal.
  - The organism must be recovered from the infected animal and shown to be the same as the organism that was introduced.

The combined efforts of many scientists and most importantly Louis Pasteur and Robert Koch established the **Germ theory of disease**. The idea that invisible microorganisms are the cause of disease is called germ theory. This was another of the important contributions of Pasteur to microbiology. It emerged not only from his experiments disproving spontaneous generation but also from his search for the infectious organism (typhoid) that caused the deaths of three of his daughters.

Fanne Eilshemius Hesse (1850 – 1934) one of Koch's assistant first proposed the use of agar in culture media. Agar was superior to gelatin because of its higher melting (i.e. 96°C) and solidifying (i.e. 40-45°C) points than gelatin and was not attacked by most bacteria. Koch's another assistant Richard Petri in 1887 developed the Petri dish (plate), a container used for solid culture media. Thus contribution of Robert Koch, Fannie Hesse and Richard Petri made possible the isolation of pure cultures of microorganisms and directly stimulated progress in all areas of microbiology.

Development in Medicine and Surgery

Once scientists knew that microbes caused disease, it was only a matter of time before medical practices improved dramatically. Surgery used to be as dangerous as not doing anything at all, but once aseptic (sterile) technique was introduced, recovery rates improved dramatically. Hand washing and quarantine of infected patients reduced the spread of disease and made hospitals into a place to get treatment instead of a place to die.

Lord Joseph Lister (1827-1912): A famous English surgeon is known for his notable contribution to the antiseptic treatment for the prevention and cure of wound infections. Lister concluded that wound infections too were due to microorganisms. In 1867, he developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds by the application of phenol on surgical dressings and at times it was sprayed over the surgical areas. He also devised a method to destroy microorganisms in the operation theatre by spraying a fine mist of carbolic acid into the air, thus producing an antiseptic environment. Thus Joseph Lister was the first to introduce aseptic techniques for control of microbes by the use of physical and chemical agents which are still in use today. Because of this notable contribution, Joseph Lister is known as the Father of Antiseptic surgery.

Development of Vaccines

- **Vaccination** was discovered before germ theory, but it wasn't fully understood until the time of Pasteur. In the late 18th century, milkmaids who contracted the nonlethal cowpox sickness from the cows they were milking were spared in deadly smallpox outbreaks that ravaged England

periodically. The physician Edward Jenner used pus from cowpox scabs to vaccinate people against smallpox.

- **Edward Jenner (1749-1823)** an English physician was the first to prevent small pox. He was impressed by the observation that countryside milk maid who contacted cowpox (Cowpox is a milder disease caused by a virus closely related to small pox) while milking were subsequently immune to small pox. On May 14th , 1796 he proved that inoculating people with pus from cowpox lesions provided protection against small pox. Jenner in 1798, published his results on 23 successful vaccinators. Eventually this process was known as vaccination, based on the latin word 'Vacca' meaning cow. Thus the use of cow pox virus to protect small pox disease in humans became popular replacing the risky technique of immunizing with actual small pox material.
- Jenner's experimental significance was realized by Pasteur who next applied this principle to the prevention of anthrax and it worked. He called the attenuated cultures vaccines (Vacca = cow) and the process as vaccination. Encouraged by the successful prevention of anthrax by vaccination, Pasteur marched ahead towards the service of humanity by making a vaccine for hydrophobia or rabies (a disease transmitted to people by bites of dogs and other animals). As with Jenner's vaccination for small pox, principle of the preventive treatment of rabies also worked fully which laid the foundation of modern immunization programme against many dreaded diseases like diphtheria, tetanus, pertussis, polio and measles etc.
- **Elie Metchnikoff (1845-1916)** proposed the phagocytic theory of immunity in 1883. He discovered that some blood leukocytes, white blood cells (WBC) protect against disease by engulfing disease causing bacteria. These cells were called phagocytes and the process phagocytosis. Thus human blood cells also confer immunity, referred to as cellular immunity.

Development of Chemotherapeutics, Antitoxins and Antibiotics

- **Emile Roux (1853-1933)** and **Alexandre Yersin**, the two notable French bacteriologists demonstrated the production of toxin in filtrates of broth cultures of the diphtheria organism. Emil von Behring (1854 -1917) and Shibasaburo Kitasato (1852-1931) both colleagues of Robert Koch, in 1890 discovered tetanus (lock jaw) antitoxin. Only about a week after the announcement of the discovery of tetanus antitoxin, Von Behring in 1890 reorted on immunization against diphtheria by diphtheria antitoxin. The discovery of toxin-antitoxin relationship was very important to the development of science of immunology.
- **Paul Ehrlich (1854-1915)** in 1904 found that the dye Trypan Red was active against the trypanosome that causes African sleeping sickness and could be used therapeutically. This dye with antimicrobial activity was referred to as a 'magic bullet'. Subsequently in 1910, Ehrlich in collaboration with Sakahiro Hata, a japanese physician, introduced the drug Salvarsan (arsenobenzol) as a treatment for syphilis caused by Treponema pallidum. Ehrlich's work had laid important foundations for many of the developments to come and the use of Salvarsen marked the beginning of the eni of chemotherapy and the use of chemicals that selectively inhibit or kill pathogens without causing damage to the patient.
- **Gerhard Domagk** of Germany in 1935 experimented with numerous synthetic dyes and reported that Prontosil, a red dye used for staining leather, was active against pathogenic, *Streptococci* and *Staphylococci* in mice even though it had no effect against that same infectious agent in a test tube. In the same year two French scientists Jacques and Therese Trefonel showed that the compound Prontosil was broken down within the body of the animal to sulfanilamide (Sulfa drug) the true active factor. Domagk was awarded nobel prize in 1939 for the discovery of the first sulpha drug.
- The credit for the discovery of this first 'wonder drug' penicillin in 1929 goes to Sir **Alexander Fleming** of England, a Scottish physician and bacteriologist. Fleming had been actually interested in searching something that would kill pathogens ever since working on wound infections during the first world war (1914-1918).
- Antibiotics were discovered completely by accident in the 1920s, when a solid culture in a Petri dish (called a plate) of bacteria was left to sit around longer than usual. As will happen with any food source left sitting around, it became moldy, growing a patch of fuzzy fungus. The colonies

in the area around the fungal colony were smaller in size and seemed to be growing poorly compared to the bacteria on the rest of the plate. The compound found to be responsible for this antibacterial action was named penicillin. The first antibiotic, penicillin was later used to treat people suffering from a variety of bacterial infections and to prevent bacterial infection in burn victims, among many other applications. In this way, Sir Alexander Fleming in 1929 discovered the first antibiotic penicillin.

- **Waksman** at the Rutgers university, USA discovered another antibiotic, streptomycin produced by two strains of actinomycete, Streptomyces griseus in 1944. Waksman received the noble prize in 1952 for his discovery of Streptomycin used in the treatment of tuberculosis, a bacterial disease caused by Mycobacterium tuberculosis that had been discovered by Robert Koch in 1882. By 1950, three other microorganism were identified that produced antibiotics, such as chloramphenicol (Chloromycetin) from Streptomyces venezuelae by Dr. Paul R. Burkholder in 1947, Aureomycin from S. aureofaciens by Dr. B.M. Dugger in 1948; and Terramycin from S. rimosus by Finlay, Hobby and collaborators in 1950.
- A dramatic turn in microbiology research was signaled by the death of Robert Koch in 1910 and advent of World war I. The Pasteur Institute was closed, and the German laboratories converted for production of blood components used to treat war infections. Thus came to an end what many have called the Golden Age of Microbiology.

Era of Virology

The earliest indications of the biological nature of viruses came from studies in 1892 by the Russian scientist **Dmitry I. Ivanovsky** and in 1898 by the Dutch scientist **Martinus W. Beijerinck**. Beijerinck first surmised that the virus under study was a new kind of infectious agent, which he designated contagium vivum fluidum, meaning that it was a live, reproducing organism that differed from other organisms. Both of these investigators found that a disease of tobacco plants could be transmitted by an agent, later called tobacco mosaic virus, passing through a minute filter that would not allow the passage of bacteria. This virus and those subsequently isolated would not grow on an artificial medium and were not visible under the light microscope. In independent studies in 1915 by the British investigator **Frederick W. Twort** and in 1917 by the French Canadian scientist **Félix H. d'Hérelle**, lesions in cultures of bacteria were discovered and attributed to an agent called bacteriophage ("eater of bacteria"), now known to be viruses that specifically infect bacteria.

The unique nature of these agents meant that new methods and alternative models had to be developed to study and classify them. The study of viruses confined exclusively or largely to humans, however, posed the formidable problem of finding a susceptible animal host. In 1933 the British investigators **Wilson Smith**, **Christopher H. Andrewes**, and **Patrick P. Laidlaw** were able to transmit influenza to ferrets, and the influenza virus was subsequently adapted to mice. In 1941 the American scientist **George K. Hirst** found that influenza virus grown in tissues of the chicken embryo could be detected by its capacity to agglutinate (draw together) red blood cells.

In 20th Century: Era of Molecular Biology

- By the end of 1900, science of microbiology grew up to the adolescence stage and had come to its own as a branch of the more inclusive field of biology.
- In the later years the microorganism were picked up as ideal tools to study various life processes and thus an independent discipline of microbiology, molecular biology was born.
- The relative simplicity of the microorganism, their short life span and the genetic homogeneity provided an authentic simulated model to understand the physiological, biochemical and genetical intricacies of the living organisms.
- The field of molecular biology made great strides in understanding the genetic code, how DNA is regulated, and how RNA is translated into proteins. Until this point, research was focused mainly on plant and animal cells, which are much more complex than bacterial cells. When researchers switched to studying these processes in bacteria, many of the secrets of genes and enzymes started to reveal themselves.

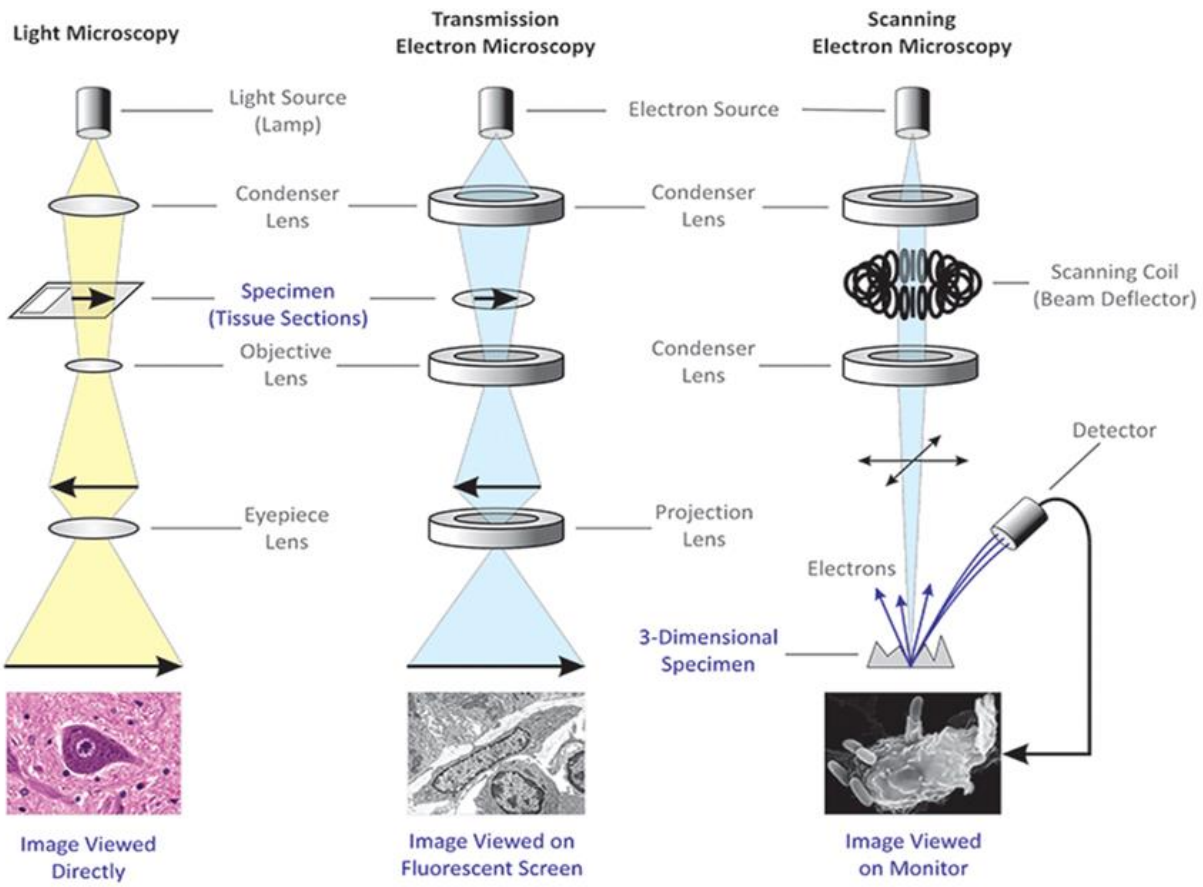


Fig. 2. Schematic representation of types of Microscopy

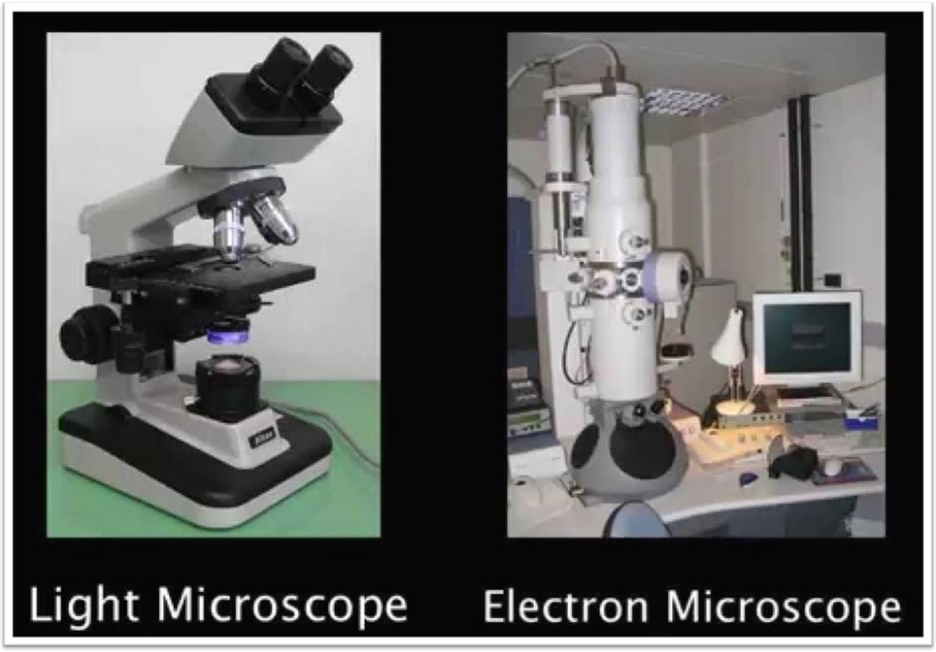


Fig. 3. Comparative view of light and electron microscopes

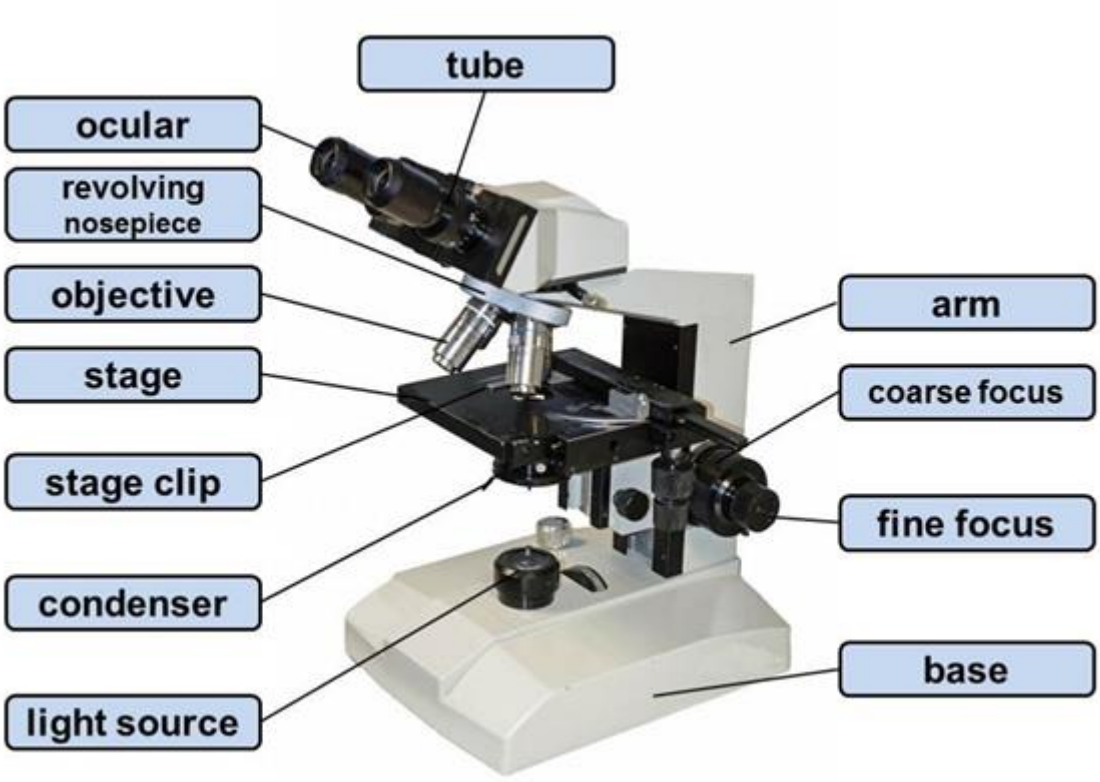


Fig. 4. Light microscope

Types of light microscopy:

1. Immersion light microscopy. Immersion lenses are used to study objects invisible or poorly visible through dry microscope systems.
2. Phase contrast microscopy is designed to obtain images of transparent and colorless objects, invisible when observing the method of light field. The method of interference contrast (interference microscopy) is that each beam bifurcates when entering the microscope. One of the received rays goes through the observed particle, another - past it on the same or additional optical branch of a microscope. In the ocular part of the microscope, both rays are reconnected and interfere with each other. One of the rays, passing through the object, is delayed in phase (acquires a difference in travel compared to the second beam).
3. Polarization microscopy is a method of observation in polarized light for microscopic examination of drugs that include optically anisotropic elements (or consist entirely of such elements).
4. Dark field microscopy. When microscopy by the method of the dark field, the drug is illuminated from the side by oblique beams of rays that do not fall into the lens. Only rays that are deflected by drug particles as a result of reflection, refraction, or diffraction enter the lens. Due to this, microbial cells and other particles appear to glow brightly on a black background (the picture resembles a shimmering starry sky).
5. Luminescent microscopy - a method of observing under a microscope the luminescent glow of microobjects when illuminated by blue-violet light or ultraviolet rays. Luminescent microscopy. The method is based on the ability of some substances to glow under the action of short-wavelength rays of light. The wavelength of the light emitted during luminescence will always be greater than the wavelength of light excited by luminescence. Yes, if you illuminate an object with blue light, it will emit rays of red, orange, yellow and green. Preparations for luminescent microscopy are stained with special luminous luminescent dyes - fluorochromes (acridine orange, fluorescein isothiocyanate, etc.). Rays of light from a strong source (usually a high-pressure mercury lamp) are passed through a blue-violet light filter. Under the action of this short-wave radiation, fluorochrome-stained cells or bacteria begin to

glow red or green. In order for the blue light that caused the luminescence not to interfere with the observation, a closing yellow light filter is placed over the eyepiece, which delays the blue but transmits yellow, red and green rays. As a result, when observed in a fluorescent microscope on a dark background will be visible cells or bacteria that glow yellow, green or red. For example, when stained with acridine orange DNA of the cell (nuclear substance) will glow bright green. The method of fluorescence microscopy allows you to study live non-fixed bacteria, stained with highly diluted fluorochromes that do not harm cell cells. By the nature of the glow can be differentiated individual chemicals that are part of the microbial cell.

Electron microscopy:

An electron microscope (Fig. 3.) is a microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects. A Scanning Electron Microscope (SEM) has achieved better than 50 pm resolutions in annular dark-field imaging mode and magnifications of up to about 10,000,000× whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000×.Electron microscopes use shaped magnetic fields to form electron optical lens systems that are analogous to the glass lenses of an optical light microscope.

The SEM (Fig. 5.) produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated. In the SEM image of an ant shown below and to the right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs.

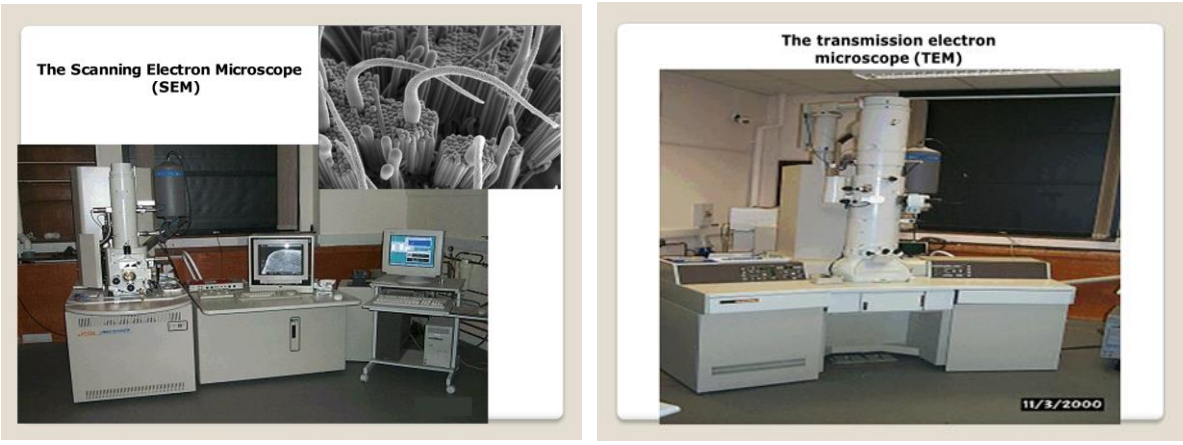


Fig. 5. Types of electron microscopy

The SEM is able to image bulk samples that can fit on its stage and still be maneuvered, including a height less than the working distance being used, often 4 millimeters for high-resolution images. The SEM also has a great depth of field, and so can produce images that are good representations of the three-dimensional surface shape of the sample. Another advantage of SEMs comes with environmental scanning electron microscopes (ESEM) that can produce images of good quality and resolution with hydrated samples or in low, rather than high, vacuum or under chamber gases. This facilitates imaging unfixed biological samples that are unstable in the high vacuum of conventional electron microscopes.

The Transmission Electron Microscope (TEM) (Fig. 5.) uses a high voltage electron beam to illuminate the specimen and create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope.

The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide. Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fiber optic light-guide to the sensor of a digital camera. The image detected by the digital camera may be displayed on a monitor or computer.

4. MICROSCOPY STAINS

Leffler's method (Flagella stain (Fig. 6.))

The fixed smear is stained with an alkaline alcohol-water solution of methylene blue for 4-5 min, dried and microscopically. In this case, the volute grains are stained in dark blue (Fig. 7.), and the cytoplasm in pale blue.

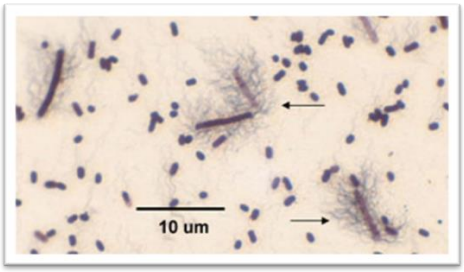


Fig. 6. Flagella stain

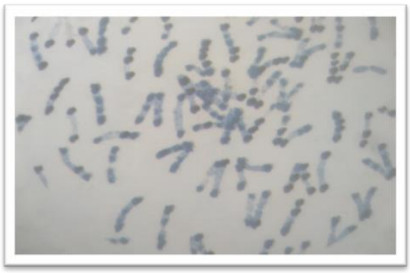


Fig. 7. Volutin granules by Leffler's method

In laboratory practice, the detection of volute grains is of the greatest importance. They were called volute because these inclusions were first discovered in *Spirillum volutans*. They are also called metachromatic because they give the phenomenon of metachromasia - the ability to color in a tone that differs from the main color of the polychrome dye. For example, when dyed with methylene blue, the grains acquire a purple-blue color due to their extremely strong affinity for azures, which are always present in the methylene blue dye. The appearance of a purple hue and due to the ability to give metachromasia. These inclusions are also called Babesch-Ernst grains after the authors who first described them. They are located mainly at the poles of bacteria, less often - along the entire length of the cell. Volutin grains are a characteristic differential feature for the causative agent of diphtheria - *Corynebacterium diphtheriae*.

Neisser's method

Neisser's method belongs to the complex methods of staining. It is performed according to the following algorithm:

1. On a fixed drug is applied acetic acid Neisser's bruise and stained for 1 min, drain the dye and wash with water.
2. Act on the smear with Lugol's solution for 20-30 seconds.
3. Without rinsing the drug with water, apply a solution of vesuvin (or chrysoidin) and stain for 1-3 minutes.
4. The stained smear is washed with water, dried and examined under a microscope.

Microscopic picture: the cytoplasm of bacterial cells is painted in a light yellow-brown hue, metachromatic grains - in dark blue, almost black (Fig. 8.).

Fig. 8. Volutin granules by Neisser's method

Burry-Gin's method

The test material is used to make a negative drug according to the Burry method. The smear is fixed with methanol, washed with water and stained for 3-5 min with phenolic magenta Tsil's, diluted 1:3. Wash with water, dry, microscopy under an immersion lens. On a dark smoky-gray background, unpainted capsules are contracted, inside which are bright red bodies of bacteria (Fig.9.).

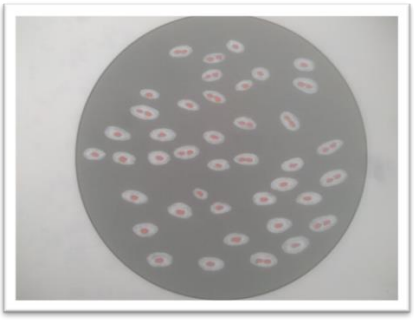


Fig. 9. Bacterial capsules by Burry-Gin's method of staining

Bacterial capsules contain complex heteropolysaccharides and polypeptides with a gel-like consistency. With conventional painting methods, they do not perceive dyes. Only in preparations-prints from the affected tissues and organs, smears from pus, sputum, they are detected by any method of staining in the form of unpainted areas (halos) between the stained bodies of bacteria and the substrate. Various methods have been proposed for staining the capsules themselves.

Ozheshki method (for sporaforming bacteria)

Some bacteria are able to form endospores under adverse conditions. In the study of unstained smears from old agar cultures, spores are found in the form of round or oval formations, which strongly refract light, and look like voids. They are poorly stained with aniline dyes with conventional painting methods.

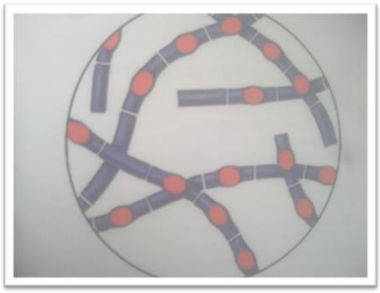


Fig. 10. Sporaforming bacteria by Ozheski method

Ozheshki method. On the prepared dense not fixed smear of a spore-bearing culture of bacteria pour 0,5% solution of hydrochloric acid and heat up 3-4 times before emergence of vapors (pickling). The product is washed with water, dried with filter paper and fixed in the burner flame. Then the

smear is stained by the method of Ziel-Nielsen, washed with water, dried and microscopically. Bacterial bodies are painted blue, spores – red (Fig. 10.).

Zill-Nielsen's method

Acid-resistant microorganisms (pathogens of tuberculosis, leprosy, actinomycetes, etc.) contain a large number of high molecular weight lipids, waxes and mycolic acid. They are very difficult to paint with ordinary aniline dyes. But when stained with concentrated phenolic magenta Tsil with heating firmly hold it and do not discolor with solutions of acids, alkalis and alcohols. Tissue cells, leukocytes, mucus, other bacteria in this treatment easily give off the dye. Therefore, with additional methylene blue staining, all of these elements turn blue after the smears are discolored, and acid-fast bacteria remain red.

Zill-Nielsen's method. The painting technique includes several stages:

1. On a smear of sputum fixed in the flame of the patient put a strip of filter paper, pour on it magenta Tsilya and paint, heating three times to the appearance of vapors (but not bringing to a boil), after which the drug with paint is left for another 1-2 minutes for cooling; drain the dye, remove the paper, wash with water.

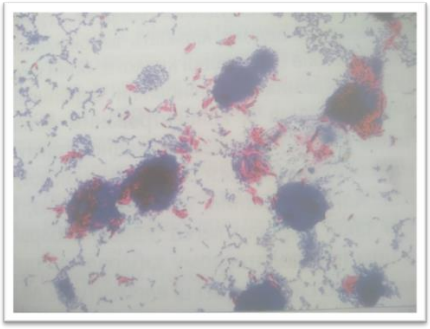


Fig. 11. Mycobacterium tuberculosis by method of Zill-Nielsen's

2. The drug is decolorized with 5% sulfuric or hydrochloric acid until a yellowish tinge (10-30 sec) and washed several times with water.

3. Additionally, the smear is stained with methylene blue Leffler, washed with water, dried and examined under a microscope.

Microscopic picture: on the general blue (blue) background acid-resistant bacteria look ruby-red (Fig. 11.).

Romanowski-Gimza's method

The Romanowski-Gimza's method belongs to the complex methods of painting. According to this method, various structural elements of blood parasites - Plasmodium falciparum, trypanosomes, leishmaniasis - are well stained. It is also often used to detect toxoplasma, spirochetes, rickettsiae, nematode larvae and the like.

Gimza's polychrome dye consists of azure, eosin and methylene blue. Immediately before use, the standard solution is diluted with distilled water of neutral or slightly alkaline reaction (pH 7.0-7.2) at the rate of 1-2 drops of dye per 1 ml of water. Smear preparations are fixed with methanol for 3-5 minutes and dried in air. The prepared solution is applied to the smear, and even better the slide with the smear is lowered into a glass with dye. Staining lasts from 30 minutes to two or more hours. Thick drops of blood are stained for 30 minutes. Then the dye is drained, the drugs are washed with water and air-dried in an upright position. Microscopy is performed using immersion lenses.

Being in a solution of blue-violet polychrome dye Romanovsky-Gimza's stains the cytoplasm in blue, and the nuclei of cells and protozoa, the body of bacteria, their capsules, mucus - in red-violet. In diphtheria rods, the nuclear elements turn dark red-purple, and the volute grains turn cherry red; the cytoplasm has a pink tinge.

Gram's staining method

1. Fixation of clinical materials to the surface of the microscope slide either by heating or by using methanol. (methanol fixation preserves the morphology of host cells, as well as bacteria, and is especially useful for examining bloody specimen material) (Fig. 12.).
2. Application of the primary stain (crystal violet). Crystal violet stains all cells blue/purple.
3. Application of mordant: The iodine solution (mordant) is added to form a crystal violet-iodine (CV-I) complex; all cells continue to appear blue.
4. Decolorization step: The decolorization step distinguishes gram-positive from gram-negative cells.
5. The organic solvent such as acetone or ethanol extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled, gram-positive bacteria. The gram-negative bacteria appear colorless and gram-positive bacteria remain blue.
6. Application of counterstain (safranin): The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue (Fig. 13.).

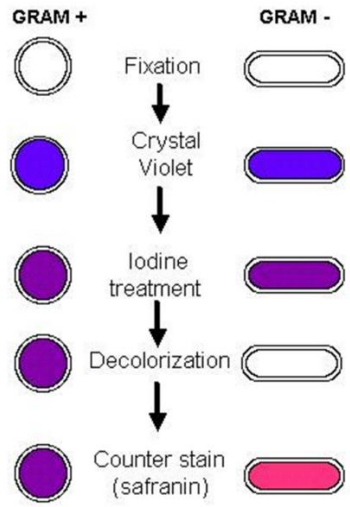


Fig. 12. Gram stain procedure

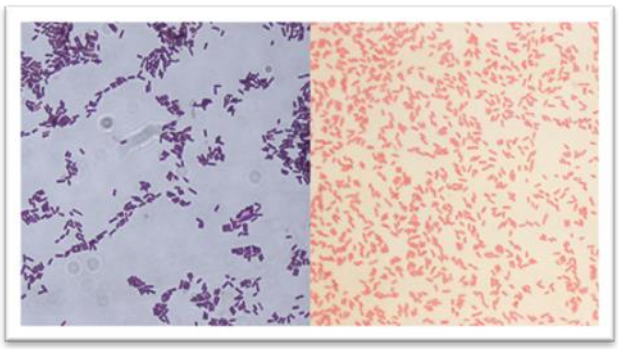


Fig. 13. Results of Gram stain method for Gram-positive and Gram-negative bacteria

5. MORPHOLOGY OF BACTERIA: SHAPE AND ARRANGEMENT OF MAIN TYPES OF BACTERIA

Bacteria with different shapes present different physical features to the outside world, and these features help cells cope with and adapt to external conditions.

It has been observed that bacterial shape contributes a measure of survival value in the face of nutrient acquisition, cell division, predators, attachment to surfaces, passive dispersal, active motility, and internal or external differentiation.

The common categories of bacteria based on their shapes are:

- **Cocci**. The bacteria that are oval or spherical in shape are included called cocci bacteria. These may either remain single or attached to one another in groups. They appear flattened when placed in groups. It is assumed that coccoid forms were derived from rod-shaped organisms through evolutionary time.
- **Bacilli** (Rod-shaped). These are rod-shaped cells that also like cocci, remain either single or attached to other cells. Bacilli bacteria are among the first bacteria to have arisen, and this shape is said to be not as advantageous as other shapes. This has been assumed upon the observation of

the behavior of filamentous *E. coli* cells which, though motile and chemotactic, move slowly and cannot tumble to change direction.

- **Spiral.** This group includes bacteria that are either helical-shaped or curved (comma-shaped). The bacteria can range from slightly curved to corkscrew-like spiral.

Arrangements of Cocci

Cocci bacteria can be arranged either singly, in pairs, in groups of four, in chains, in clusters or cubes consisting of eight cells. These cells remain attached during cell division.

Coccus

 - This group includes bacteria that are present as a single cell.

Diplococci

 - This arrangement results when two bacterial cells occur as a pair (joined together). Some of the cells in this arrangement might remain spherical while some might appear flattened, elongated, or bean-shaped. Examples: *Streptococcus pneumonia, Moraxella catarrhalis, Enterococcus spp, Neisseria gonorrhea.*

Tetrad

 - Tetrad bacteria are arranged in a group of four cells that remain attached and grow in the attachment after cell division. This arrangement results when the cells divide into two planes. Examples: A*erococcus, Pediococcus, and Tetragenococcus.*

Sarcina

 - In this arrangement, the bacterial cells form a group of eight cells. This happens when the cells divide in a perpendicular plane. The common characteristic associated with these organisms is being strict anaerobe. Examples: *Sarcina aurantiaca, Sarcina lutea, Sarcina ventriculi*.

Streptococci

 - Here, the bacteria are arranged in long chains. These bacteria are present in family Streptococcaceae, which is characterized by a lack of motility and Gram-positive bacteria. Examples: *Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus mutans*.

Staphylococci

 - This type includes bacteria that are arranged in grape-like clusters. This results from cell division in both the planes and are characterized by organisms which are immotile and Gram-positive. Examples: *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus aureus, Staphylococcus capitis.*

Arrangement of Bacilli

Bacillus

 - Bacilli are the bacteria which are rod-shaped and are present as single cells. These bacteria can form endospores and are facultative anaerobes. Examples: *Salmonella enterica subsp, Bacillus cereus, and Salmonella choleraesuis.*

Diplobacilli

 - As in Diplococci, Diplobacilli also exists in pairs. After cell division, the two cells do not divide and grow in an attached arrangement. Examples: *Coxiella burnetii, Klebsiella rhinoscleromatis, Moraxella bovis.*

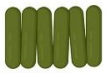
Streptobacilli

 - In this group, bacteria are arranged in chains. This results from cell division in a single chain. Examples: *Streptobacillus moniliformis, Streptobacillus levaditi, Streptobacillus felis, Streptobacillus hongkongensis.*

Coccobacilli

 - As the name suggests, coccobacilli resemble both cocci as well as bacilli. These are shorter in size and thus, appear stumpy. Examples: *Chlamydia trachomatis, Haemophilus influenza, Gardnerella vaginalis.*

Pallisades

 - Pallisades are the type of bacilli bacteria that resemble a picket fence structure as a result of the bent at the point of division during cell division. They appear similar to Chinese letters. Example: *Corynebacterium diphtheria* that causes diphtheria.

Arrangement of Spiral

Vibrio

 - These are the slightly curved bacteria resembling a comma shape. Examples: *Vibrio mytili, Vibrio anguillarum, Vibrio parahaemolyticus, Vibrio cholera.*

Spirochetes

 - Spirochetes are spiral bacteria having a helical shape. These are flexible and have an axial filament which helps in motility. These filaments are essential distinguishing character between spirochetes and other bacteria. These filaments run throughout the length of the bacteria and thus, help in twisting the motion of the bacteria. Examples: *Leptospiraspecies (Leptospira interrogans), Treponema pallidum, Borrelia recurrentis.*

Spirilla (Helical-shaped/Corkscrew form)

 - These bacteria are similar in structure with spirochetes but are more rigid. They, too, have a flagellum but lack the endoflagella like in spirochetes. Examples: *Campylobacter jejuni, Helicobacter pylori, Spirillum winogradskyi.*

Other Shapes and Arrangements

Appendaged Bacteria. The bacteria that produce a unique structure like pillus or fimbriae are called appendaged bacteria. These bacteria are more virulent than other bacteria that do not form these appendages. Example: *Neisseria gonorrheae*, the agent of Gonorrhea.

Box-shaped/ Rectangular Bacteria. Box-shaped bacteria are rectangular in shape and resemble a box. Example: *Haloarcula marismortui*.

Club-shaped Rod Bacteria. These bacteria are thinner on one side than the other. One of the classic examples of this group is *Corynebacterium*.

Filamentous Bacteria. These are bacteria that are long, thin, and filament-shaped. They, sometimes, divide to form branches resembling strands of hair or spaghetti called mycelium. Example: *Actinomycetes*.

Triangular-shaped Bacteria. This group includes bacteria that are triangular in shape. Example: *Haloarcula.*

Pleomorphic Bacteria. The bacteria that do not have a specified shape are included in this group. They can change their shape, but in pure culture, they appear to have a definite form. Examples: *Mycoplasma pneumoniae, M. genitalium.*

Stalked Bacteria. These are the bacteria that possess a stalk on one end of the cell. Examples: *Caulobacter crescentus.*

Star-shaped Bacteria. The bacteria that look like stars or are star-shaped are included in this group. Examples: *Stella humosa.*

6. TAXONOMIC CLASSIFICATION OF MICROORGANISMS

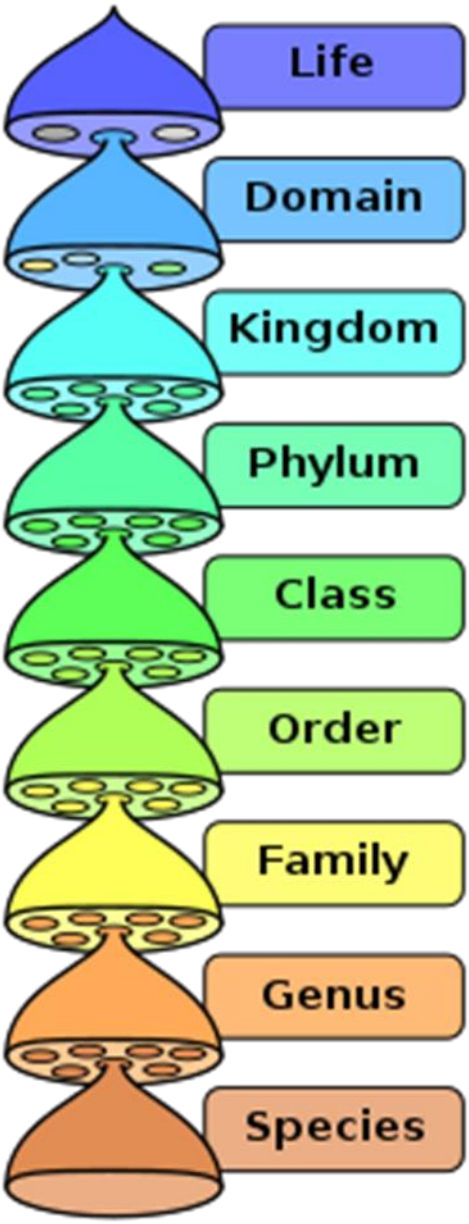


Fig. 14. The principle of taxonomic classification

TAXONOMY is the science of microorganisms classification. Classification is the assignment of organisms (species) into anorganised scheme of naming idealy these schemes are based on evolutionary relationships (i.e the more similar the name, the closer the evolutionary relationships). Thus, classification is concerned with:-

1. The establishment of criteria for identifying organisms & assignment to groups (what belongs where) (Fig. 14.).
2. The arrangement of organisms into groups of organism of organism (e.g. At what level of diversity should a single species be split in to two or more species).
3. Consideration of how evolution resulted in the formation these groups.

TAXON:- a group or category of related organisms.

Two key characteristics of taxa are:

-Members of lower level taxa (e.g. Species) are more similar to each other than are members of higher level taxa (eg.Kingdom or domain).

-Member of specific taxa are more similar to each other than any are to members of different specific taxa found at the same hierarchical level (eg. Humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, Escherichia coli). Thus once you know that two individuals are member of the sametaxon, you can inter certain similarities between the two organisms NOTE that taxa are dynamic, changing as our knowledge of organism and evolutionary relationships change.

Bacterial taxonomy, however, is important due to following reasons:

1. Bacterial taxonomy senses to be a library catalogue that helps easily access large number of books. Taxonomy therefore helps classifying and arranging bewildering diversity of bacteria into groups or taxa on the basis of their mutual similarity or evolutionary relatedness.

BINOMIAL NOMENCLATURE is a formal system of naming species of living things by giving each a name composed of two parts, both of which use Latin grammatical forms, although they can be based on words from other languages. Such a name is called a binomial name (which may be shortened to just "binomial"), a binomen or a scientific name; more informally it is also called a Latin name.

Many species are named after people, either the discoverer or a famous person in the field of microbiology, for example *Salmonella* is after D.E. Salmon, who discovered it (albeit as "*Bacillus typhi*"). For the generic epithet, all names derived from people must be in the female nominative case,

either by changing the ending to -a or to the diminutive -ella, depending on the name. For the specific epithet, the names can be converted into either adjectival form (adding -nus (m.), -na (f.), -num (n.) according to the gender of the genus name) or the genitive of the latinised name.

Many species (the specific epithet) are named after the place they are present or found (e.g. *Borrelia burgdorferi*). Their names are created by forming an adjective by joining the locality's name with the ending -ensis (m. or f.) or ense (n.) in agreement with the gender of the genus name, unless a classical Latin adjective exists for the place. However, names of places should not be used as nouns in the genitive case.

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all bacterial taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. arizonae at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar *Typhimurium*. After the first use, the serovar may also be given without a species name: *Salmonella typhimurium*, S. typhimurium, or *Salmonella* serovar *typhimurium*.

7. UNICELLULAR PARASITES

Groups of unicellular parasites:

1.Rickettsia

- these are polymorphic microorganisms that live and multiply only in cells (cytoplasm and nucleus) of tissues of animals, humans and vectors. Rickettsia do not form spores and capsules, are immobile, are well colored according to Romanowsky - Gims, Tsell - Nelsen, gram-negative.
- Rickettsia reproduce by dividing cocoid and rod-shaped forms with the formation of homogeneous populations, as well as a result of fragmentation of filamentous forms with the subsequent development of cocoid and rod-shaped formations.
- Pathogenic rickettsia from the family Rickettsiaceae are divided into three genera:
  - Rickettsia,
  - Coxiella,
  - Rochalimae,

they affect different species of animals and humans.

- The bacterial genus Rickettsia was named after Howard Taylor Ricketts, in honor of his pioneering work on tick-borne spotted fever. Diseases caused by rickettsia are called rickettsioses.

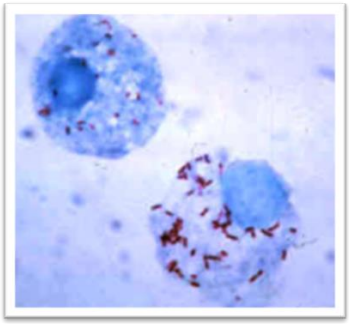


Fig. 15. Rickettsia in cells

Fig. 16. Ixodes tick

2.Chlamydia

The genus *Chlamydia*, family *Chlamydiaceae* includes pathogens of trachoma, conjunctivitis (blenorrhea with inclusions), inguinal lymphogranulomatosis (venereal lymphogranulomatosis), ornithosis. *Chlamydia* have a similar cycle of development, a common group antigen, the same chemical composition. They contain DNA and RNA, nucleoproteins, lipids and carbohydrates.

There are three stages in the development cycle of *Chlamydia*:

- In the first stage, these are small (0.2–0.4 μm) elementary bodies surrounded by a three-layer shell that contain the genetic material of the nucleoid and ribosome in a compact stage;
- In the second stage, these are primary large (0.8–1.5 μm) bodies with nucleoid fibrils and ribosomal elements covered with a thin shell; reproduce by division; daughter cells are reorganized into elementary bodies that can be contained extracellularly and penetrate into other cells.
- The third stage of development of chlamydia is intermediate (transistor) between primary and elementary bodies. Small (elementary) bodies have infectious properties, large (primary) bodies perform a vegetative function.

The growth, reproduction and maturation of chlamydia are completed within 40 hours.

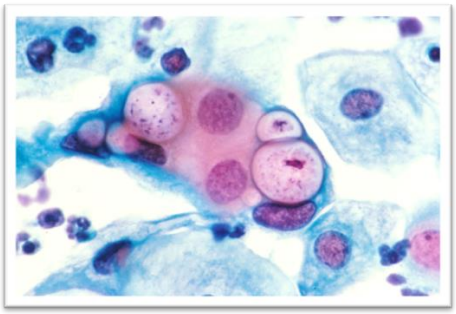


Fig. 17. *Chlamydia trachomatis*

Fig. 18. *Mycoplasma hominis*

3. Mycoplasma

Mycoplasmas belong to the class Mollicutes, families *Mycoplasmataceae, Acholoplasmataceae, Spiroplasmaceae*.

Morphology. Mycoplasmas are small polymorphic bacteria 0.3–0.8 μm in size, do not form spores, and are immobile and gram-negative. Their shape can be ovoid, cocobacilli, filamentous, branched. In the late phase of growth, chains of coccoid bodies are formed. Mycoplasmas do not have a cell wall covered with a three-layer cytoplasmic membrane 7.5–10 nm thick; the cytoplasm contains DNA and RNA, ribosomes and other cellular components in which cholesterol is present. Mycoplasmas are better stained by Romanowsky - Gims.

Enzymatic properties. Metabolic processes in mycoplasmas are very variable. They do not have proteolytic properties, although several species are known to dilute gelatin and clot blood serum; most strains ferment glucose, some of them form arginase, phosphatase.

Antigenic structure. Mycoplasmas have species specificity. The family *Mycoplasmataceae* includes two genera (*Mycoplasma, Ureaplasma*), which include 50 species, of which the most important medical importance are *M. pneumoniae, M. hominis* and *M. urealyticum*.

Toxin formation. Hemolysin and thermostable endotoxin were isolated from mycoplasmas.

Resistance. Most strains of mycoplasmas die at a temperature of 45-55 ° C within 15 minutes. *Mycoplasmas* are very sensitive to all disinfectants, to drying, ultrasound and other physical acts, resistant to penicillin, ampicillin, methicillin, cephalosporins, sensitive to erythromycin and other macrolides.

Cultivation. Most species of mycoplasmas are facultative anaerobes. Their growth requires proteins, sterols, phospholipids, glycoproteins (mucin), as well as purine and pyrimidine bases. On dense media grow in the form of characteristic colonies with a compacted, growing in the environment, the center and a delicate openwork edge; After 3-5 days of incubation, they sometimes become large (1.5–2 μm), but they are often difficult to see with the naked eye. There is a zone of

hemolysis on the blood agar around the colonies. In the broth mycoplasmas develop with the formation of turbidity and fine-grained sediment.

Mycoplasmas are cultured at a temperature of 36–37 ° C (extreme limits of 22–41 ° C) on nutrient media with a pH of 7 that contain blood serum, with the formation of very small colonies.

Addition to the nutrient medium of cholesterol or other sterols, yeast extracts accelerate the growth of mycoplasma. They can be cultured in environments without serum, but in the presence of 0.02% hemoglobin and 0.01% cysteine. Mycoplasmas multiply well in the chorioallantois membrane of the chicken embryo.

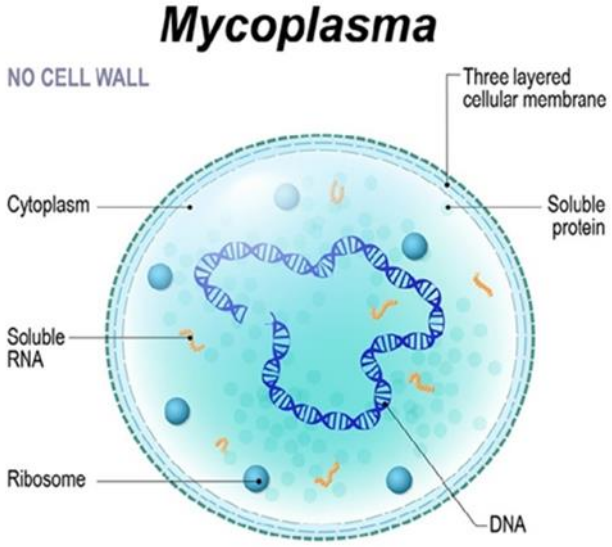


Fig. 19. Structure of Mycoplasma

4. Spirochetes

The families *Spirochetaceae* and *Leptospiraceae* include saprophytes and pathogenic bacterial species. Saprophytes include *Spirochaeta* and *Cristispira*, which are large cells 200–500 μm in size with pointed or blunt ends; some of them have crypts (wavy ridges).

Pathogenic spirochetes live on dead substrates, in polluted water bodies, in the intestines of cold-blooded animals; nonpathogenic *Leptospira* also live in natural conditions. According to Romanowsky-Gims, these bacteria staining blue.

Pathogenic spirochetes include three genera: *Treponema*, *Borrelia*, *Leptospira*.

Fig. 20. *Treponema*

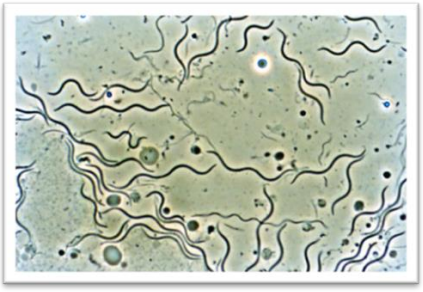


Fig. 21. *Borrelia*

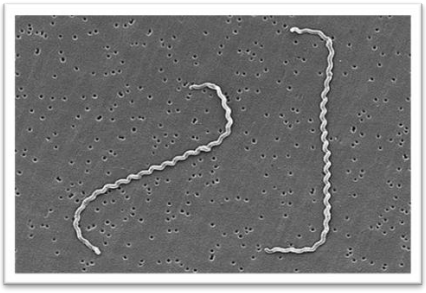


Fig. 22. *Leptospira*

Treponema. Morphology. *T. pallidum* are thin spiral-shaped cells with 12 to 14 curls. They do not have a visible axial filament or axial ridge. The ends of the treponemes are pointed or rounded. Their length is 10–13 μm and their width is 0.1–0.18 μm.

At electron microscopy of longitudinal and cross ultrathin sections of the activator of a syphilis the three-layer external membrane under which basal dark field of a microscope, a body are located is well visible; filamentous formations — fibrils with a diameter of 17 nm — are attached to them. There are three fibrils at each end of the cell. In the cytoplasm of bacteria there are ribosomes, nucleoid vacuoles and mesosomes. Bacteria multiply by transverse division.

Treponemes are mobile (they are characterized by rotational, translational, bending and wavy movements), poorly perceive dyes, gram-negative. According to the Romanowski-Gims method, they turn pale pink, which is explained by the low content of nucleoproteins. Under the influence of environmental factors and drugs, treponema in some cases coagulate into balls, forming cysts covered with an impermeable mucin-like shell. Cysts can be in the patient's body for a long time in a latent state, under favorable conditions, they turn into grains, and then into typical spiral treponema. Cyst formation is one of the forms of treponema protection, which allows them to resist the action of drugs prescribed to patients with syphilis. Pale treponemes can form L- shapes. Cysts and L-forms of treponemes are more resistant to external and internal factors.

Cultivation. Pale treponema is a very demanding microorganism. It does not grow in normal environments, develops at a temperature of 35 ° C under anaerobic conditions in environments containing kidney or brain tissue; extreme temperature limits of growth - 34-40 ° C. Pale treponema develops well on the chorioallantois tissue of the chicken embryo, in the serum of rabbits with the addition of pieces of brain tissue under a layer of Vaseline oil.

Resistance. At low temperatures (in the cold) pale treponema persists for a long time in the homogenates of the affected tissues. From the action of a temperature of 45-48 ° C, it dies in 1 hour, 55 ° C in 15 minutes. Sensitive to heavy metals (mercury, bismuth, arsenic), acids, disinfectants and drying.

Borrelia species are members of the family *Spirochaetaceae*. Morphology. Borrelia species have an outer membrane that contains a substance similar to lipopolysaccharides, an inner membrane, and a layer of peptidoglycan in a periplasmic space, which classifies them as Gram-negative. They are typically 20–30 μm long and 0.2–0.3 μm wide. Spirochetes move using axial filaments called endoflagella in their periplasmic space. The filaments rotate in this space, between the outer membrane and the peptidoglycan layer, propelling the bacterium forward in a corkscrew-like motion.The outer membrane of Borrelia species contains outer surface proteins (Osp) that play a role in their virulence.

Borrelia species – causes Relapsing fever, Lyme disease, also called Lyme borreliosis, a zoonotic, vector-borne disease transmitted primarily by ticks and by lice, depending on the species of bacteria. Diagnosis. Direct tests include culture of *Borrelia* from skin, blood, or cerebrospinal fluid (CSF), and detection of genetic material by polymerase chain reaction in skin, blood, or synovial fluid. Two-tiered serological testing is performed for differential diagnosis of *Borrelia* infection. The first-tier tests detect specific antibodies (IgM and IgG together or separately) and include enzyme-linked immunoassays (e.g. ELISAs) and immunofluorescent assays. Positive results for first-tier tests are confirmed using second-tier testing. The second tier consists of standardized immunoblotting, either by using Western blots or blots striped with diagnostically important purified antigens. Positive results for second-tier tests are confirmatory for the presence of *Borrelia* infection. Spirochetes can also be seen using Wright-stained blood smears.

Leptospira belong to the family *Leptospiraceae*. Leptospirosis is caused by *Leptospira interrogans*. Morphology. Leptospira are microorganisms with 12-18 small primary curls that are close to each other. Reminiscent of a spring with curved and thickened ends. At the ends of the *Leptospira* are secondary curls that give them an S- or C-shape. There are also hookless strains of *Leptospira*. The length of the *Leptospira* is 7–14 (sometimes 20–30) μm, and the thickness is 0.06–0.15 (0.25–0.3) μm. They are mobile, make rotational and translational movements. Leptospira are gram-negative, according to Romanowsky - Gims they turn pale pink.

Cultivation. *Leptospira*, chemoorganotrophs and obligate aerobes, grow at a temperature of 28 to 29 ° C in liquid and semi-liquid nutrient media (Fairworth-Volyrat serum-free medium). The growth of *Leptospira* on liquid nutrient media is detected on the 7th-10th day, and it is detected when viewing a drop of medium in a dark field: the medium does not become turbid.

Resistance. *Leptospira* survive in water for 5-10 days, and in soil for 2 days. In food (milk, butter, bread, etc.), the viability of *Leptospira* does not exceed a few hours. *Leptospira* are stored for a long time at low temperatures (minus 70 - minus 90 ° C), are very sensitive to drying, the action of acids, at a temperature of 56 ° C die after 30 minutes. Quickly soluble in bile acids.

5. Protozoa

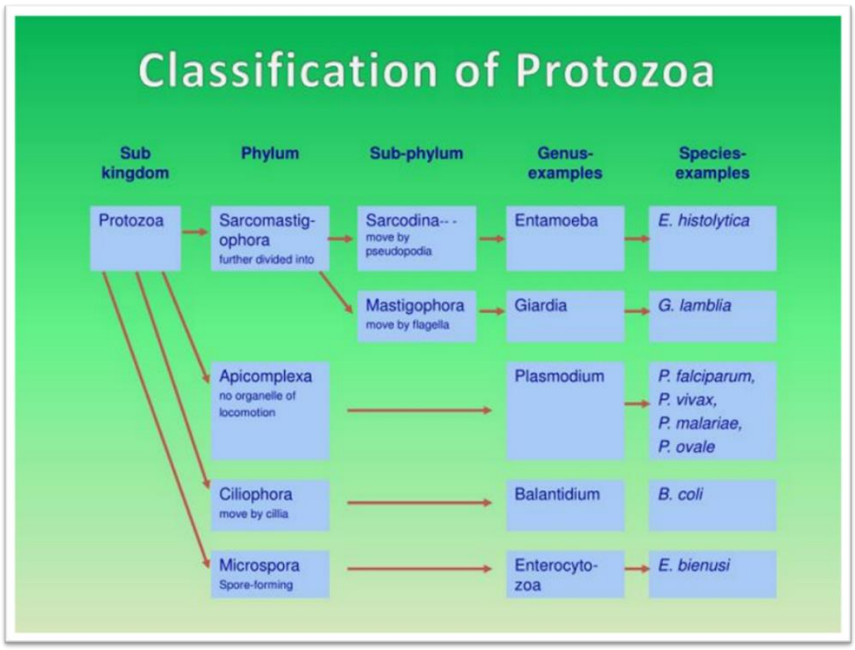


Fig. 23. Classification of Protozoa

Protozoa are one-celled animals found worldwide in most habitats. Most species are free living, but all higher animals are infected with one or more species of protozoa. Infections range from asymptomatic to life threatening, depending on the species and strain of the parasite and the resistance of the host.

Structure. 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.

Classification. On the basis of light and electron microscopic morphology, the protozoa are currently classified into six phyla. Most species causing human disease are members of the phyla Sacromastigophora and Apicomplexa.

Life Cycle Stages. The stages of parasitic protozoa that actively feed and multiply are frequently called trophozoites; in some protozoa, other terms are used for these stages. Cysts are stages with a protective membrane or thickened wall. Protozoan cysts that must survive outside the host usually have more resistant walls than cysts that form in tissues.

Reproduction. Binary fission, the most common form of reproduction, is asexual; multiple asexual division occurs in some forms. Both sexual and asexual reproduction occur in the Apicomplexa.

Nutrition. All parasitic protozoa require preformed organic substances—that is, nutrition is holozoic as in higher animals.

6. Fungi

- The fungi include diverse saprotrophic eukaryotic organisms with chitin cell walls.
- Fungi can be unicellular or multicellular; some (like yeast) and fungal spores are microscopic, whereas some are large and conspicuous.
- Reproductive types are important in distinguishing fungal groups.
- Medically important species exist in the four fungal groups Zygomycota, Ascomycota, Basidiomycota, and Microsporidia.
- Members of Zygomycota, Ascomycota, and Basidiomycota produce deadly toxins.
- Important differences in fungal cells, such as ergosterols in fungal membranes, can be targets for antifungal medications, but similarities between human and fungal cells make it difficult to find targets for medications and these medications often have toxic adverse effects.

The fungi comprise a diverse group of organisms that are heterotrophic and typically saprozoic. In addition to the well-known macroscopic fungi (such as mushrooms and molds), many unicellular yeasts and spores of macroscopic fungi are microscopic. For this reason, fungi are included within the field of microbiology.

Fungi are important to humans in a variety of ways. Both microscopic and macroscopic fungi have medical relevance, with some pathogenic species that can cause mycoses (illnesses caused by fungi). Some pathogenic fungi are opportunistic, meaning that they mainly cause infections when the host's immune defenses are compromised and do not normally cause illness in healthy individuals. Fungi are important in other ways. They act as decomposers in the environment, and they are critical for the production of certain foods such as cheeses. Fungi are also major sources of antibiotics, such as penicillin from the fungus *Penicillium.*

The structure of fungi is similar to algae, they have a differentiated nucleus (one or more), cell wall and cytoplasmic membrane. The cytoplasm in young cultures is homogeneous, in mature - granular, in the cytoplasm there are mitochondria, Golgi complex, vacuoles, various inclusions (glycogen, volute, lipids, crystals of organic salts, pigments).

Cultivation is carried out under aerobic conditions at a temperature of 22-37 °C on nutrient media, the pH is 6.0-6.5, but pathogenic fungi can grow at a wider pH range from 3.0 to 10.0. Pathogenic fungi require various growth factors (vitamins, amino acids), minerals and trace elements (zinc, cobalt, salts of iron, sodium, magnesium, copper, phosphorus).

By the nature of growth on agar nutrient media pathogenic fungi are divided into several types: leathery, smooth, firm consistency; fluffy, loose, cotton-like consistency; velvety-hairy, covered with a very short dense mycelium.

Characteristics of Fungi. Fungi have well-defined characteristics that set them apart from other organisms. Most multicellular fungal bodies, commonly called molds, are made up of filaments called hyphae. Hyphae can form a tangled network called a mycelium and form the thallus (body) of fleshy fungi. Hyphae that have walls between the cells are called septate hyphae; hyphae that lack walls and cell membranes between the cells are called nonseptate or coenocytic hyphae) (Fig.24.).

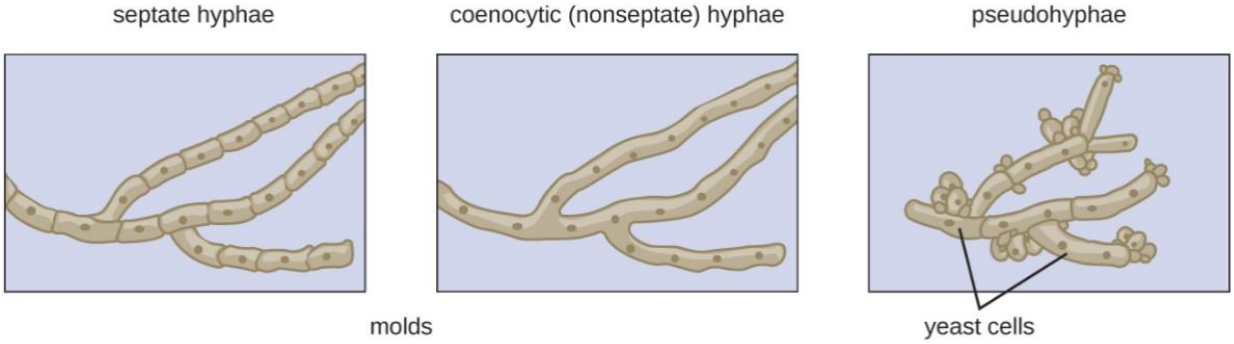


Fig. 24. Multicellular fungi (molds) from hyphae, which may be septate or nonseptate. Unicellular fungi (yeasts) cells from pseudohyphae from individual yeast cell.

In contrast to molds, yeasts are unicellular fungi. The budding yeasts reproduce asexually by budding off a smaller daughter cell; the resulting cells may sometimes stick together as a short chain or pseudohypha (Fig. 23.). *Candida albicans* is a common yeast that forms pseudohyphae; it is associated with various infections in humans, including vaginal yeast infections, oral thrush, and candidiasis of the skin.

Some fungi are dimorphic, having more than one appearance during their life cycle. These dimorphic fungi may be able to appear as yeasts or molds, which can be important for infectivity. They are capable of changing their appearance in response to environmental changes such as nutrient availability or fluctuations in temperature, growing as a mold, for example, at 25 °C, and as yeast cells at 37 °C. This ability helps dimorphic fungi to survive in diverse environments. Histoplasma capsulatum, the pathogen that causes histoplasmosis, a lung infection, is an example of a dimorphic fungus.

7. Viruses

Viruses are typically described as obligate intracellular parasites, acellular infectious agents that require the presence of a host cell in order to multiply. Viruses that have been found to infect all types of cells – humans, animals, plants, bacteria, yeast, archaea, protozoa…some scientists even claim they have found a virus that infects other viruses! But that is not going to happen without some cellular help.

A virus is a small collection of genetic code, either DNA or RNA, surrounded by a protein coat. A virus cannot replicate alone. Viruses must infect cells and use components of the host cell to make copies of themselves. Often, they kill the host cell in the process, and cause damage to the host organism. Viruses have been found everywhere on Earth. Researchers estimate that viruses outnumber bacteria by 10 to 1. Because viruses don't have the same components as bacteria, they cannot be killed by antibiotics; only antiviral medications or vaccines can eliminate or reduce the severity of viral diseases, including AIDS, COVID-19, measles and smallpox.

Virus Characteristics (Fig. 25.). Viruses can be extremely simple in design, consisting of nucleic acid surrounded by a protein coat known as a **capsid**. The capsid is composed of smaller protein components referred to as **capsomers**. The capsid+genome combination is called a **nucleocapsid.**

Viruses can also possess additional components, with the most common being an additional membranous layer that surrounds the nucleocapsid, called an **envelope**. The envelope is actually acquired from the nuclear or plasma membrane of the infected host cell, and then modified with viral proteins called **peplomers**. Some viruses contain viral enzymes that are necessary for infection of a host cell and coded for within the viral genome. A complete virus, with all the components needed for host cell infection, is referred to as a **virion.**

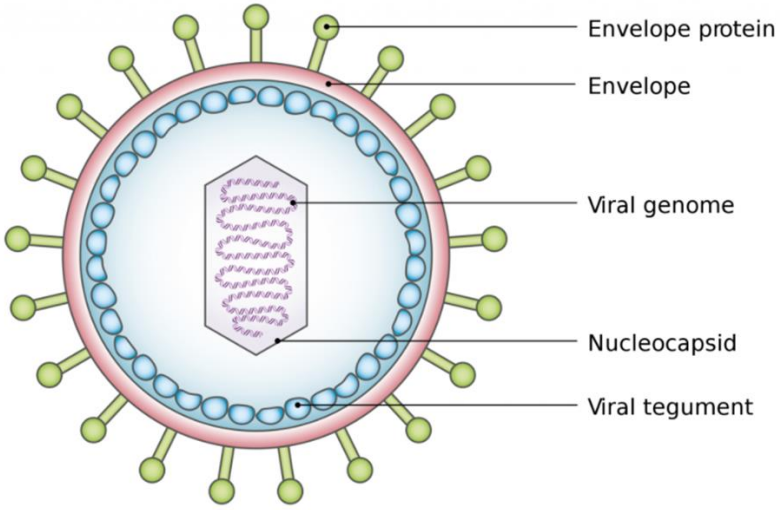


Fig. 25. Virus Characteristics

Virus Genome. While cells contain double-stranded DNA for their genome, viruses are not limited to this form. While there are dsDNA viruses, there are also viruses with single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), and single-stranded RNA (ssRNA). In this last category, the ssRNA can either positive-sense (+ssRNA, meaning it can transcribe a message, like mRNA) or it can be negative-sense (-ssRNA, indicating that it is complementary to mRNA). Some viruses even start with one form of nucleic acid in the nucleocapsid and then convert it to a different form during replication.

Virus Structure. Viral nucleocapsids come in two basic shapes, although the overall appearance of a virus can be altered by the presence of an envelope, if present. Helical viruses have an elongated tube-like structure, with the capsomers arranged helically around the coiled genome. Icosahedral viruses have a spherical shape, with icosahedral symmetry consisting of 20 triangular faces. The simplest icosahedral capsid has 3 capsomers per triangular face, resulting in 60 capsomers for the entire virus. Some viruses do not neatly fit into either of the two previous categories because

they are so unusual in design or components, so there is a third category known as complex viruses. Examples include the poxvirus with a brick-shaped exterior and a complicated internal structure, as well as bacteriophage with tail fibers attached to an icosahedral head.

Virus Replication Cycle. While the replication cycle of viruses can vary from virus to virus, there is a general pattern that can be described, consisting of five steps:

1. **Attachment** – the virion attaches to the correct host cell.
2. **Penetration or Viral Entry** – the virus or viral nucleic acid gains entrance into the cell.
3. **Synthesis** – the viral proteins and nucleic acid copies are manufactured by the cells' machinery.
4. **Assembly** – viruses are produced from the viral components.
5. **Release** – newly formed virions are released from the cell.

Bacteriophage. Viruses that infect bacteria are known as bacteriophage or **phage**. **A virulent phage** is one that always lyses the host cell at the end of replication, after following the five steps of replication described above. This is called the **lytic cycle** of replication.

There are also **temperate phage**, viruses that have two options regarding their replication. Option 1 is to mimic a virulent phage, following the five steps of replication and lysing the host cell at the end, referred to as the lytic cycle. But temperate phage differ from virulent phage in that they have another choice: Option 2, where they remain within the host cell without destroying it. This process is known as **lysogeny** or the **lysogenic cycle** of replication (Fig. 26.).

A phage employing lysogeny still undergoes the first two steps of a typical replication cycle, attachment and penetration. Once the viral DNA has been inserted into the cell it integrates with the host DNA, forming a prophage. The infected bacterium is referred to as a lysogen or lysogenic bacterium. In this state, the virus enjoys a stable relationship with its host, where it does not interfere with host cell metabolism or reproduction. The host cell enjoys immunity from reinfection from the same virus (Fig. 26.).

Exposure of the host cell to stressful conditions (i.e. UV irradiation) causes induction, where the viral DNA excises from the host cell DNA. This event triggers the remaining steps of the lytic cycle, synthesis, maturation, and release, leading to lysis of the host cell and release of newly formed virions.

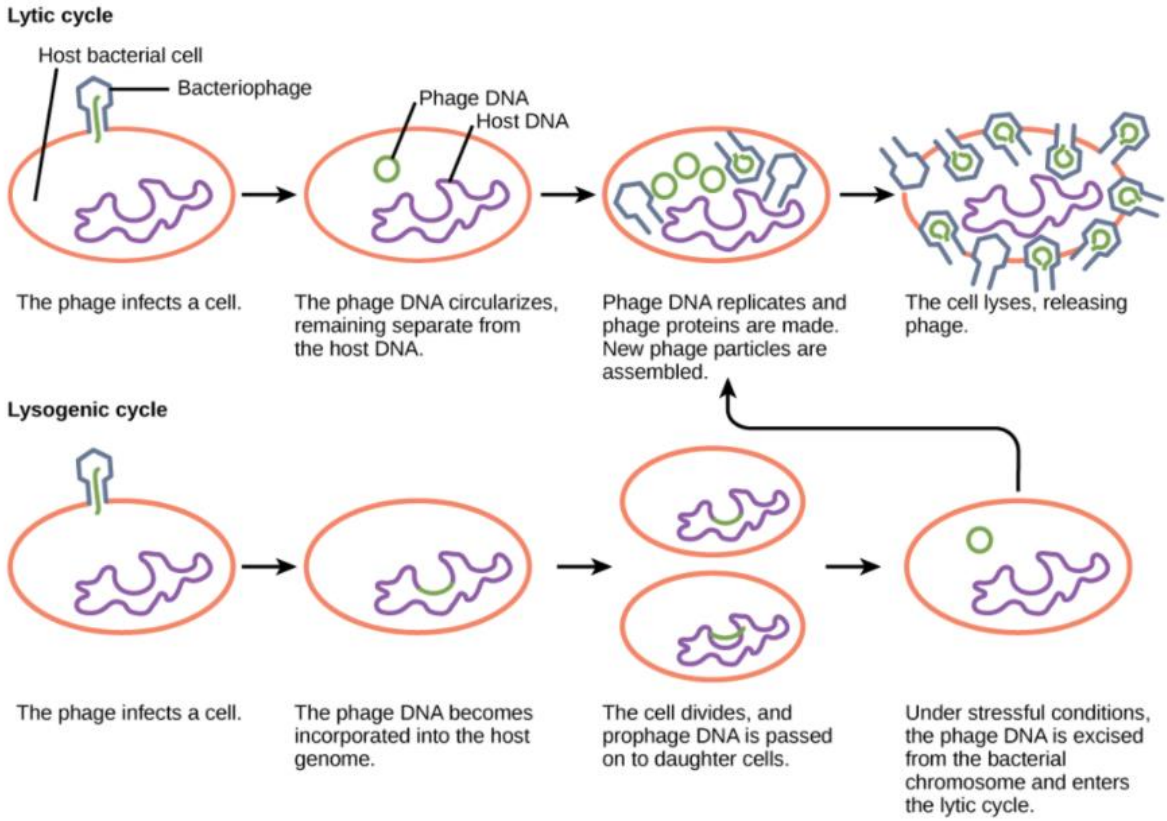


Fig. 26. Lytic and Lysogenic cycles

8. STRUCTURE OF BACTERIA

The most significant sign of prokaryotes is the absence of a nucleus. This role is played by a nucleoid - a nuclear substance that is diffusely located in the cytoplasm and is not separated from it by a karyolema. The nucleoid of the cell consists of a single strand of DNA closed in a ring, histone-like proteins and the nucleolus are absent. Bacteria do not have such organelles as mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, microbodies (Fig.27.). However, they have mesosomes whose function is similar to mitochondrial. The sedimentation constant of microbial ribosomes is 70S, while in eukaryotes - 80S. There are also significant differences in the structure of flagella, the presence of vacuoles and so on.

Despite such fundamental differences in the structure of cells of different systems, the general plan of their structure remains similar. The prokaryotic organism contains almost all cellular elements: a cover, cytoplasm, the nucleoid, inclusions (Fig. 28.).

COMPARISON of PROKARYOTIC and EUKARYOTIC CELLS

CELL PART	FUNCTION	PROKARYOTIC CELL*	EUKARYOTIC CELL
Cell Wall	Support/Protection	Present*	_____ only
Plasma membrane	Homeostasis/ Transport	Present	Present
Nucleus	Control Center	Absent	Present
Genetic Material	Cell Function/Properties	Circular	_____
Histones	Gene regulation/organization	Absent	Present
Ribosomes	Protein Synthesis	Present (_____)	Present (_____)
_____	Internal Structure	Absent	Present
Mitochondrion	Energy Production	Absent	Present
Chloroplast	Photosynthesis	Absent*	Present*
Endoplasmic Reticulum	Intracellular transport/ chemical reactions	Absent	Present
Golgi Apparatus	Protein packaging/transport	Absent	Present
_____	Digests Material	Absent	Present in some
Vacuole	Storage	Absent	Present

Fig. 27. Comparison of procaryotic and eucaryotic cells

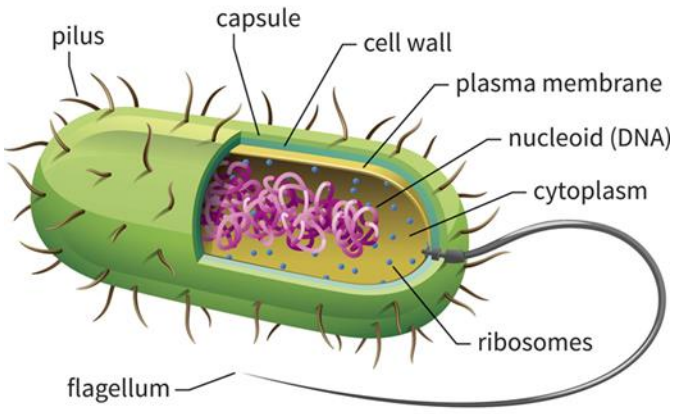


Fig. 28. Structure of procaryotic bacteria

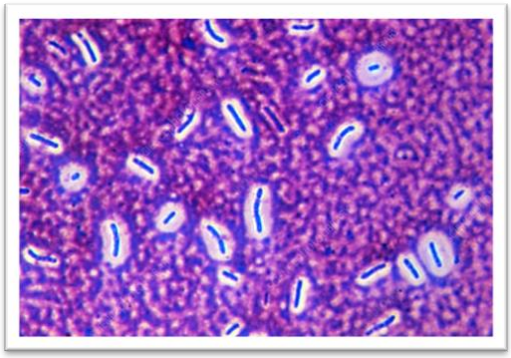


Fig. 29. Bacteria capsules by Burry-Gin's method

CAPSULE

Externally, the bacterial cell may be covered with a mucous substance - capsule. It is not life-sustaining for the microbe, but protects it from adverse environmental factors, provides resistance to phagocytosis, protects against the penetration of bacteriophages, provides virulent properties of pathogens. According to its chemical structure, the capsule belongs to the polysaccharide substances and in B. anthracis - to protein. Characteristic of the capsule is the presence of a large amount of water.

The capsule can be viewed under a normal light microscope if stained with native drugs in a simple way. However, the Burry-Gin's method is more often used to detect capsules (Fig. 29.), in which the background of the drug is created with mascara, and the microorganism is additionally stained with magenta. In such cases, on a dark background you can see a red stick, which is surrounded by a light rim - a capsule.

CELL WALL

The cell wall creates a protective layer that balances the high internal osmotic pressure of bacteria (5-20 atmospheres). This strength is provided by a substance - peptidoglycan (murein).

The structure created from peptidoglycan serves as a support framework, giving shape to the microbial cell, in addition, other substances bind to it.

According to the structure of the microbial peptidoglycan skeleton and the content of some substances in the cell wall, we can distinguish the so-called gram-positive bacteria from negative. Their division into these two groups was proposed in 1884 by Christian Gram, who drew attention to the peculiarities of microbial staining.

In gram-positive bacteria, the layer of peptidoglycan is 30-70% of the mass of the cell wall, forming up to 40 layers. An essential feature is the presence of special teichoic acids (Fig. 30.).

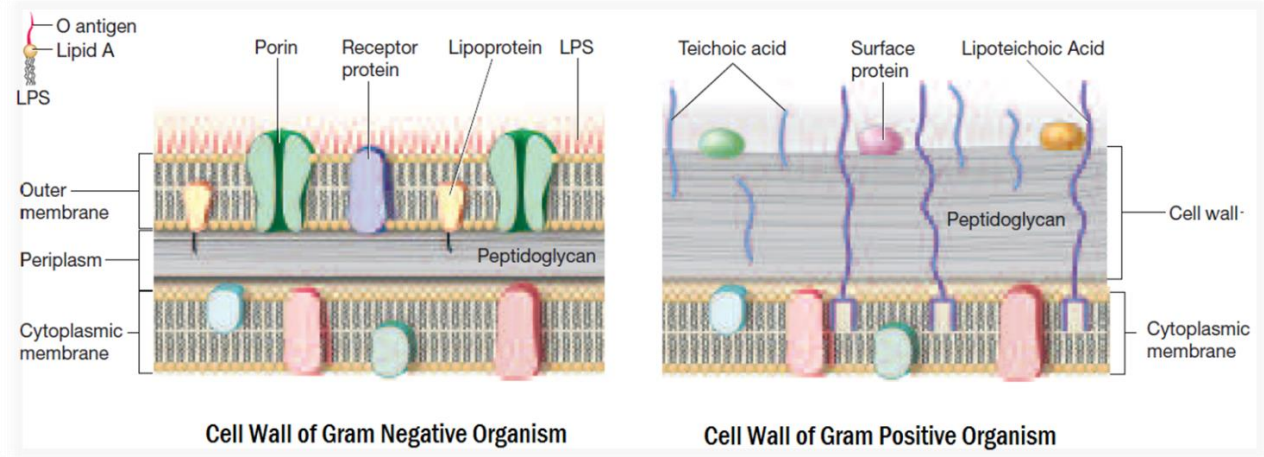


Fig. 30. Comparison structure of cell wall Gram-Negative and Gram-Positive bacteria

Under the influence of various substances, the cell wall can be destroyed. Thus, under the action of lysozyme on a suspension of gram-positive micrococci there is their rapid lysis and enlightenment of the environment. A similar effect is caused by penicillin.

Lysozyme breaks glycosidic bonds in murein, and penicillin prevents the formation of peptidoglycan, which is accompanied by destruction of the cell wall. At the same time round cells sensitive to osmotic conditions are formed - protoplasts in which the cell wall is completely lost. Under the action of these drugs on gram-negative bacteria, cells are formed that retain the remnants of the cell wall. They are called spheroplasts.

CYTOPLASMIC MEMBRANE

The contents of the cell are separated from the environment by a cytoplasmic membrane (CPM) - a soft, plastic formation. The membrane is a necessary structural component of the microbial cell, without which they die. In terms of chemical composition, it is a protein-lipid complex with a

small amount of carbohydrates. Forming only 8-15% of the cell mass, the membrane contains up to 70-90% of its lipid substances.

Studies under an electron microscope have shown that the membrane is a multilayered formation. It consists of a double layer of phospholipid molecules (Fig. 31.). Their hydrophobic ends (phospholipids and triglycerides) are directed inwards, and their hydrophilic "heads" are directed outwards. This type of arrangement stabilizes the membrane. Integral proteins are embedded in this layer, which permeate it through. Some groups of proteins attach to the surface of the membrane, so they are called peripheral. Sometimes the membrane is covered with another special type of protein - surface.

The functions of the membrane complex are diverse: it provides selective permeability and transport of various substances from the outside to the inside and vice versa due to the existence of special proteins-enzymes of permeases; carries out transport of electrons and oxidative phosphorylation of substrates; generates electrochemical energy of transmembrane potential; secretes hydrolytic enzymes; exhibits biosynthetic activity; is the place of attachment of flagella.

Some surfactants and antibiotics (polymyxins) can destroy the membrane and cause cell death. It is used in search of optimal ways to combat pathogens of infectious diseases.

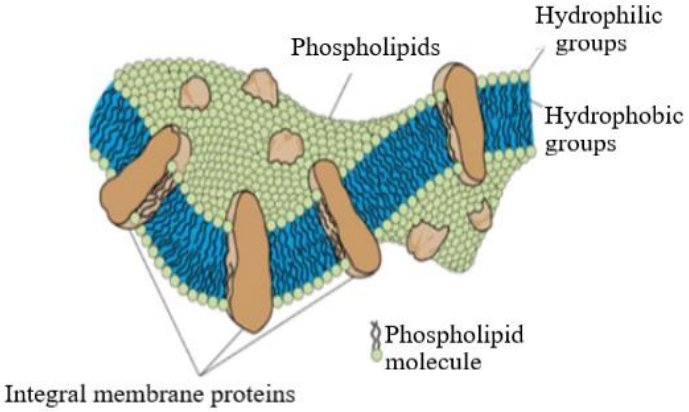


Fig. 31. Structure of cytoplasmic membrane of the microbial cell

CYTOPLASM

The cytoplasm of bacterial cells has a liquid consistency, transparent, homogeneous, separated from the environment by the cytoplasmic membrane. It is a kind of colloidal system consisting of various molecules of proteins, lipids, water, DNA and RNA, carbohydrates, polysaccharides and other compounds. Its viscosity is 800-8000 times higher than that of water.

The structure and consistency of the cytoplasm depends on the age of the microbe - homogeneous in young cells, it gradually turns into a fine-grained structure in the old, taking the form of seals. Vacuoles and fibrous formations appear in it, its density increases, and it resembles a gel in consistency.

Ultracentrifugation of the cytoplasm can produce a "soluble" fraction, which includes various enzymes, and a fraction of "particles" from membranes and ribosomes.

Ribosomes act as a factory for protein synthesis, their size reaches 16x18 nm. They consist of two protein subunits 30S-50S. The cell can contain up to 5000-50000 ribosomes, their number increases with active protein synthesis. Sometimes ribosomes gather in clusters called polyribosomes.

Differences between ribosomes of eukaryotic and prokaryotic organisms are crucial in finding ways to control the latter as pathogens of infectious diseases. It is known that some antibiotics are able to partially or completely stop protein synthesis on 70S ribosomes, leaving intact 80S ribosomes.

NUCLEOID. PLASMIDS

Nucleoid. The nuclear apparatus of a bacterial cell occupies its central part, has an irregular shape and is not separated from the cytoplasm by a shell, combines with the cytoplasmic membrane and mesosome.

It consists of one supercoiled double strand of DNA up to 2 nm in diameter, closed in a ring, integrated with RNA, RNA polymerase and protein in a ratio of 1:1:3. The length of this giant molecule can reach 1.5-3 mm. The molecular weight of the nucleoid is (1-3) $x10^9$ daltons, and it contains up to 8x106 pairs of nucleic bases. The content of base pairs A + T and G + C in the molecule of each cell is constant for a particular species of bacteria, and the share of G + C in the total molecular weight is 23-75 %.

Typically, a nucleoid is represented by a single copy in a cell, but the number of copies may increase to 2-9 during cell division. Quite often, the bacteria next to the chromosomal contain extrachromosomal DNA of much smaller size, also twisted into a ring and localized in the cytoplasm. Such elements are called plasmids (Fig. 32.).

A **plasmid** is an extra-chromosomal DNA molecule separate from the chromosomal DNA, which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids usually occur naturally in bacteria, but are sometimes found in eucaryotic organisms.

Plasmids determine the synthesis of certain substances, enzymes, ensure the resistance of bacteria to antibiotics, and therefore give them certain selective advantages.

The nuclear substance of microbes can be detected in ultrathin sections by examining them in an electron microscope using an immunofluorescent.

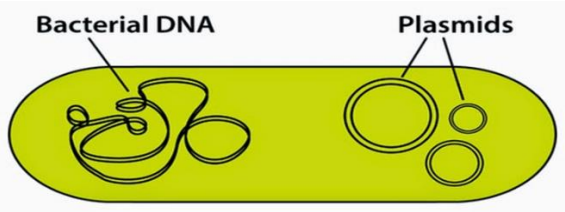


Fig. 32. Morphology of bacterial DNA and plasmids

Types of Bacterial Plasmids

Based on their function, there five main classes:

- **F-plasmids (Fertility)** – they are capable of conjugation or mating.
- **R-plasmids (Resistance)** – containing antibiotic or drug resistant gene(s). Also know as R-factors, before the nature of plasmids was understood.
- **Col-plasmids** – contain genes that code for colicines, proteins that can kill other bacteria.
- **Degrative plasmids** – enable digestion of substances, e.g., toluene or salicylic acid.
- **Virulence plasmids** – turn the bacterium into a pathogen.

  Plasmids can belong to more than one of these functional groups.

REPLICATION OF BACTERIA CHROMOSOME

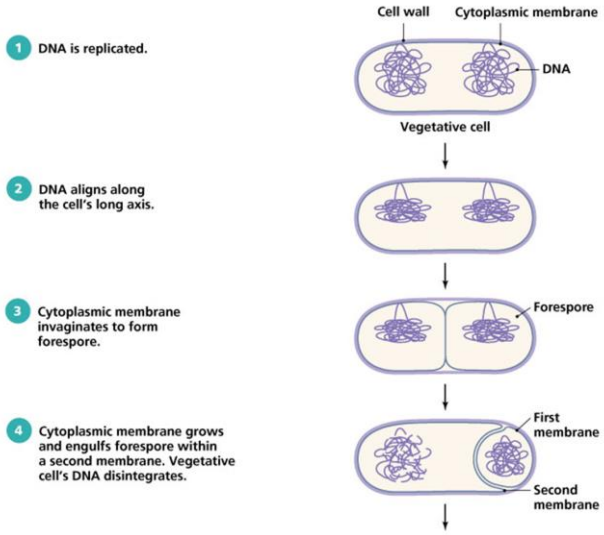


Fig. 33. Replication of bacterial chromosome

VOLUTIN GRANULES (methachromatic inclusions)

In the course of life of microorganisms in the cytoplasm there are morphologically differentiated parts, which are called **inclusions**. They build different for their nature and choose different functions.

Reserve substances of prokaryotes are polysaccharides, lipids, polypeptides, polyphosphates, sulfur. As polysaccharides starch, glycogen, granulosa are deposited. Under adverse conditions, they create a cell with carbon and energy.

A common type of nutrient is polyphosphates. They can coalesce into granules called volutin granules and use cells as a source of phosphorus. In addition, they have macroenergic phosphate bonds, provide cell energy consumption.

Volutin granules is also called **metachromatic inclusions**, so they have fun in a color that is not typical of the main dye. For example, methylene blue entertains them in a dark purple color, while the cytoplasm of the cell - in blue. The inclusions of this type was first found in *Spirillum volutans*, whence they supported this name. The presence of green volutin character for nutrients and, in particular, for the causative agent of diphtheria (Fig. 34.).

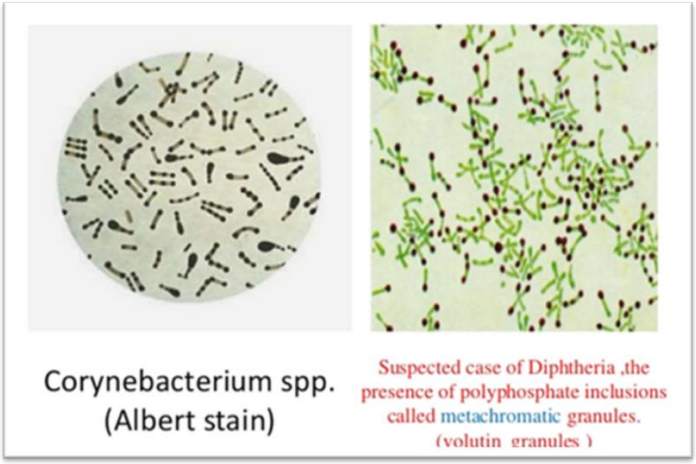
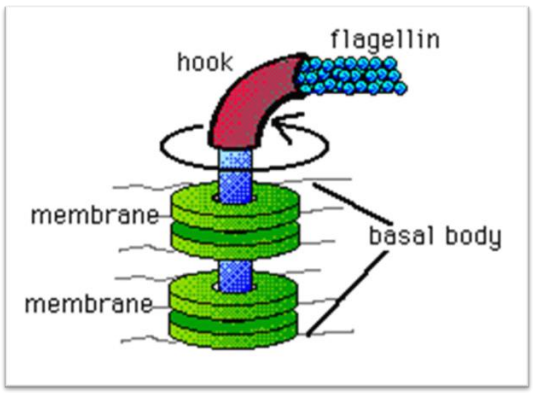


Fig. 34. Volutin granules in the causative agent of diphtheria – *Corynebacterium spp.*

FLAGELLA

The surface of the body of microorganisms can be covered with special growths, called flagella, which provide locomotor function. Their number, method of placement, length are constant signs for a certain type of bacteria, which is taken into account when conducting taxonomy of prokaryotic organisms.

The length of the flagella reaches 20 μm, while the thickness is only 12-18 nm, which is beyond the resolution of the microscope. The flagella of bacteria consist of spirally twisted filaments of a special flagellin protein that forms a spiral around the internal hollow space. There are three main parts: a spiral thread, a hook and a basal body (two to four special rings with a central rod) (Fig. 35.), by means of which the flagellum is fixed in the cytoplasmic membrane and cell wall).



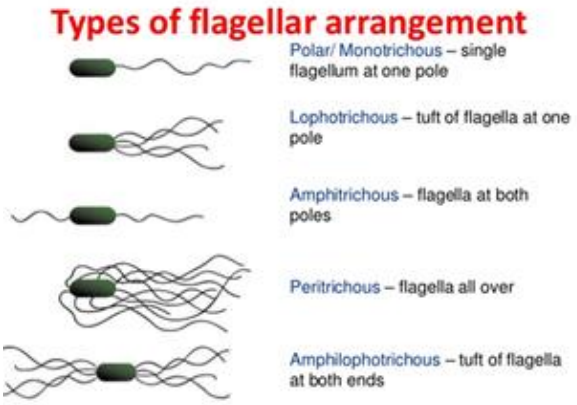


Fig. 35. Structure of the bacterial flagella

SPORES

Spore formation. At a certain stage of their development, when nutrient reserves are depleted, bacteria form a round spores (endospores) inside.

They differ from vegetative forms by the suppression of the functioning of the genetic apparatus, almost complete lack of metabolism, low amount of free water, increased concentration of calcium ions, the appearance of dipicolinic acid, which is associated with heat resistance of spores. They are characterized by the appearance of additional shells that prevent diffusion and penetration of substances from the outside, higher resistance to environmental damages and the ability to maintain its viability for a long time. Spores form two genera of gram-positive rods - *Bacillus* (spores smaller than the diameter of the rod) and *Clostridium* (spores larger than the size of the rod) and one genus of gram-positive cocci (*Sporosarcina*).

Spores are formed only in the external environment, in animals and humans the process of sporulation does not occur. They have evolutionary significance, ensuring the preservation of the species, rather than performing the function of reproduction.

Spore formation begins when nitrogen and carbon sources disappear in the cell environment.

Initially, a special terminal nucleus is secreted in the cell during invagination of the membrane, which contains one cellular genome, components of the protein synthesis apparatus and its own energy system. It is covered by its own membrane and the membrane of the mother cell, which form the wall of the spores. It consists of normal peptidoglycan. The wall is surrounded by a crust that contains an unusual peptidoglycan with a small number of lumbar joints and is sensitive to lysozyme (its autolysis plays a crucial role in the germination of spores).

The shell of the spores consists of keratin-like protein and causes poor permeability and resistance to chemicals. Exosporium surrounds the entire spores and consists of lipoproteins.

Induction of spore formation occurs within a few hours. There are several stages: preparatory, prespores, shell formation and maturation (Fig. 36.).

The presence of spores in bacteria can be of diagnostic value, and also leads to the choice of tactics for disinfection of surgical instruments and dressings.

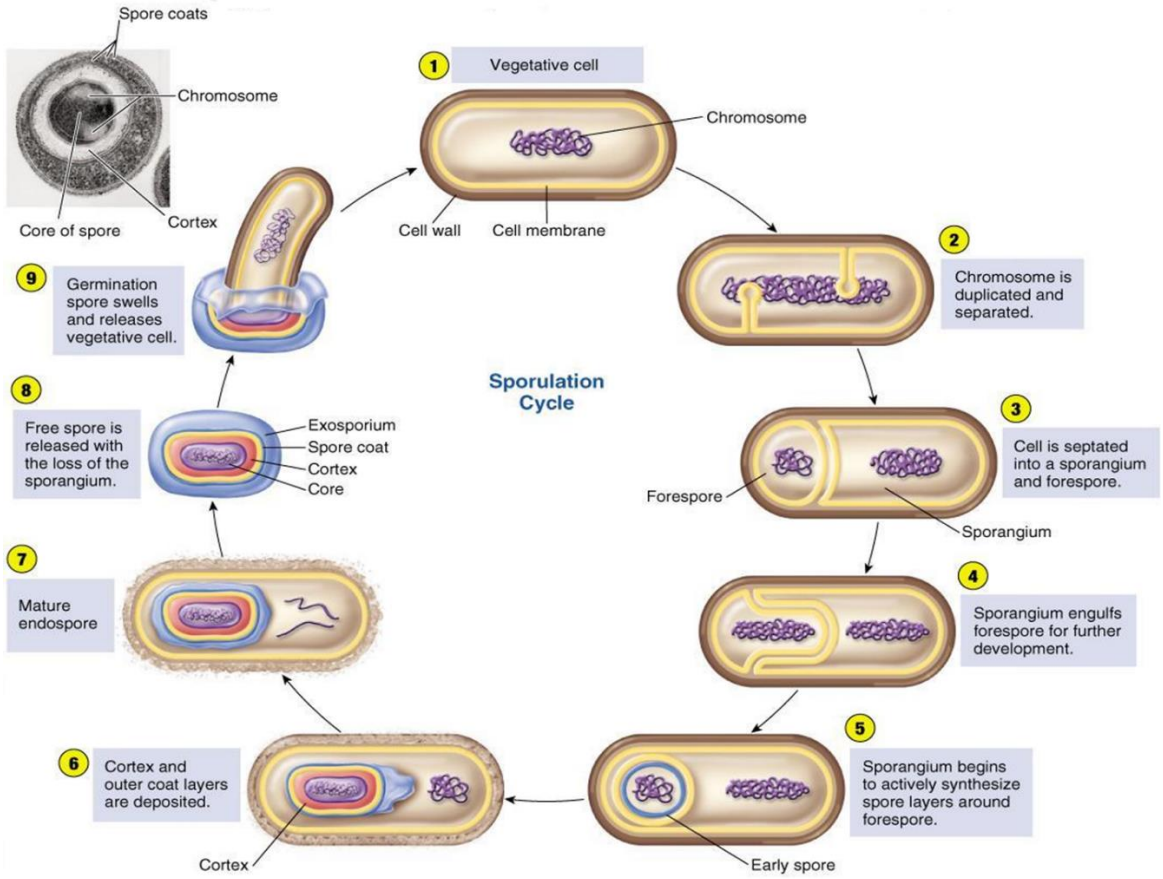


Fig.36. Spore formation process for prokaryotic bacteria

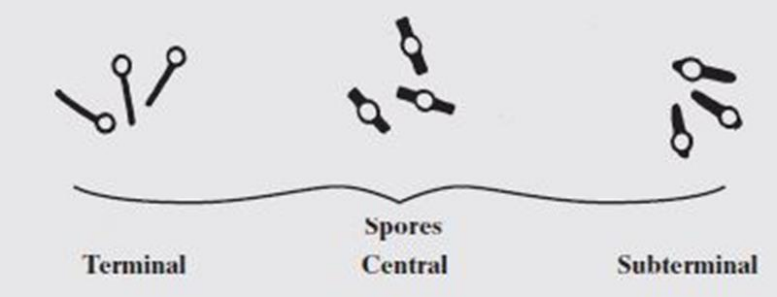


Fig. 37. The location of the spores inside the bacterial cell


Fig. 38. *Clostridium tetani*

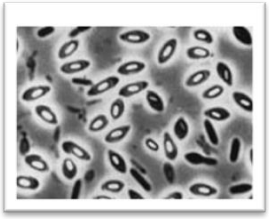


Fig.39. *Bacillus subtilis*

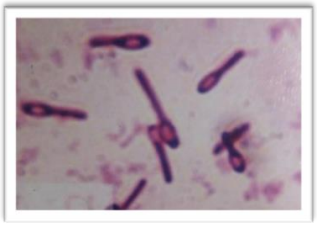


Fig. 40. *Clostridium botulinum*

9. MEDIA

Classification based on Nutritional Components:

1. **Simple media**. Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. It is also called as basal media. Eg: Nutrient Broth (NB), Nutrient Agar (NA). NB consist of peptone, yeast extract and NaCl. When 2% of agar is added to Nutrient Broth it forms Nutrient agar.
2. **Complex media**. Media other than basal media are called complex media. They have special ingredients in them for the growth of microorganisms. These special ingredients like yeast extracts or casein hydrolysate, which consists of a mixture of many chemicals in an unknown proportion.
3. **Synthetic media/ Chemically defined media**. Specially prepared media for research purposes where the composition of every component is well known. It is prepared from pure chemical substances. Eg: peptone water (1% peptone + 0.5% NaCl in water).

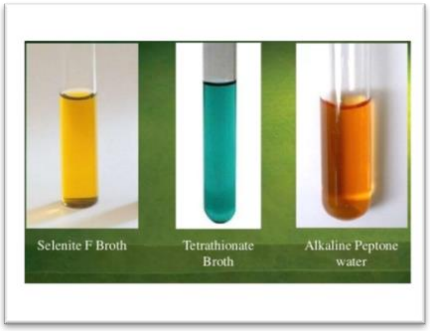


Fig. 41. Liquid media


Fig. 42. Solid media


Fig. 43. Semi-solid media

Classification based on consistency:

1. **Liquid media** (Fig. 41.). These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as "broth" (e.g. nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. No agar is added. Mostly used for inoculums preparation.
2. **Solid media** (Fig. 42.). An agar plate is a Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms. 2% of agar is added. Agar is the most commonly

used solidifying agent. Colony morphology, pigmentation, hemolysis can be appreciated. Examples include Nutrient agar and Blood agar.

3. **Semi-solid media** (Fig. 43.). Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains. Examples of Semi-solid media (Hugh&Leifson's oxidation fermentation). 0.5% agar is added.

Fig. 44. *Bacillus cereus* on the Selective Media

Fig. 45. *S. epidermi*dis and *S. aureus* on the Manittol solt agar

Selective media – media designed to inhibit the growth of some organisms while encouraging the growth of others (Fig. 44.)

Differential media – media that contains substances (indicators) that reveal differences between organisms (Fig. 45.).

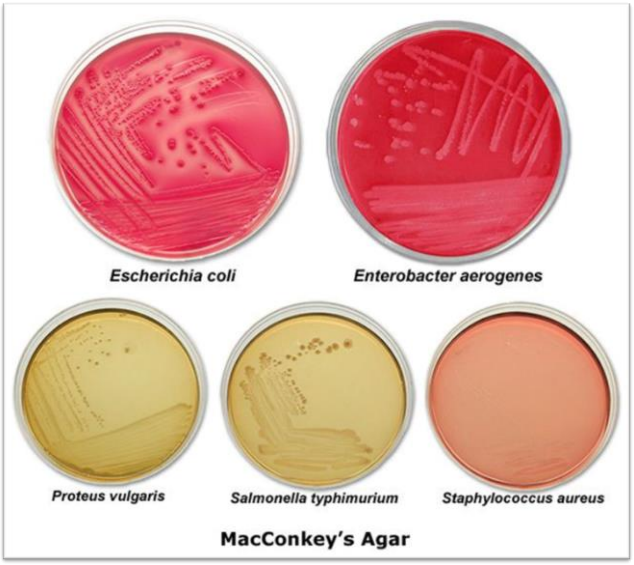


Fig. 46. Growth of different microorganisms on the MacConkey's Agar

Media can be both selective and differential. Example on the MacConkey's agar (Fig. 46.) selets for enteric bacteria and differentiates between lactose positive and lactose negative enteric bacteria.

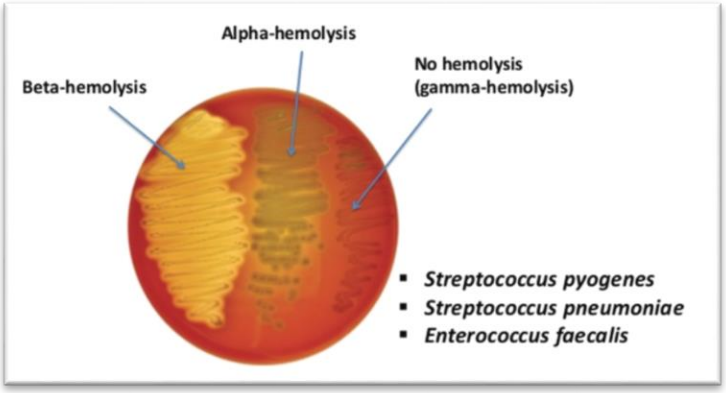


Fig. 47. Hemolysis on the Blood agar

Enriched, selective, and differential media help establish the presents of pathogens. Blood agar plate is an enriched and differential medium which is usually used to detect hemolytic activity (Fig. 47.).

Fig. 48. Transport media

Transport media (Fig. 48.) are essentially solutions of buffers with carbohydrates, peptones and other nutrients (excluding growth factors) designed to preserve the viability of bacteria during transport without allowing their multiplication.

10. GROWTH OF BACTERIA

- An orderly increase in the quantity of all the cellular constituents (Fig. 49.)
- The growth of microorganisms in influenced by various physical and chemical factors of their environment
- **Physical factors:** temperature (Fig. 50.), pH, osmotic pressure, hydrostatic pressure and radiation
- **Chemical factors:** oxygen, carbon, nitrogen, phosphorus, sulfur, etc.

BACTERIAL GROWTH

Time (minutes)	Number of Bacteria
0	1
20	2
40	4
60	8
80	16
100	32
120	64

Fig. 49. Bacterial growth

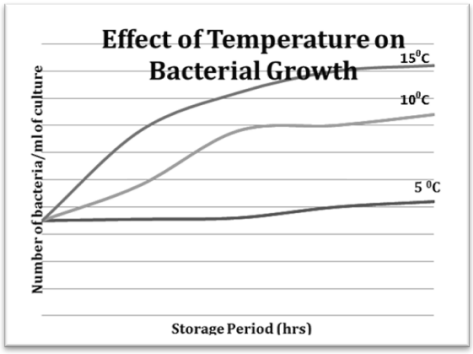


Fig. 50. Effect of temperature on the bacterial growth

TEMPERATURE:

- Temperature is the most important factor that determines the rate of growth, multiplication, cultivar, and death of all living organisms;
- High temperatures damage microbes by denaturing enzymes, transport carriers, and other proteins;
- Microbial membrane are disrupted by temperature extremes;
- At very low temperatures membranes also solidify and enzymes also do not function property;

Types of temperature:

- **Minimum growth temperature** – the lowest temperature at which organisms grow is minimum growth temperature;
- **Optimum growth temperature** – the temperature at which the most rapid rate of multiplication occurs;

- **Maximum growth temperature** – the highest temperature at which growth occurs. A temperature only slightly above this point frequently kills the microorganisms by inactivating critical enzymes.

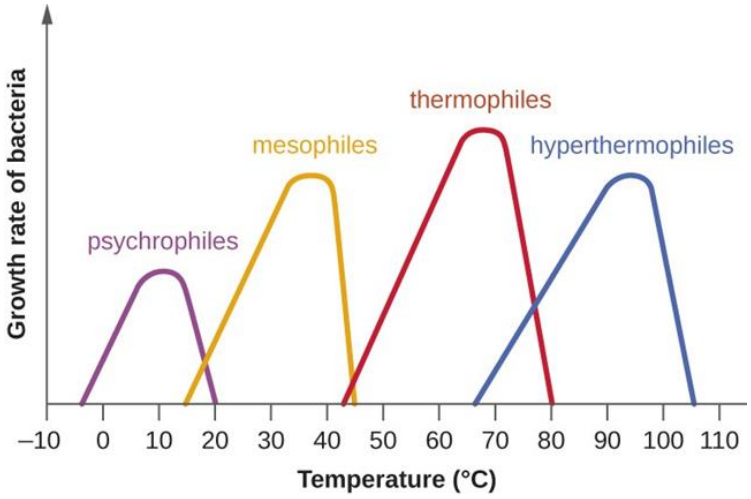


Fig. 51. Growth rate of bacteria

Growth rate of bacteria (Fig. 51.):

- **Psychrophilic** bacteria are defined as cold-loving bacteria. Specifically, their cardinal temperatures are 20 °C for maximal growth, 15 °C or lower for optimal growth, and 0 °C or lower for minimum growth (Morita, 1975), and this definition is accepted by most microbiologists.
- **Mesophilic** bacteria are those in which optimum growth occurs between 20 and 45 °C, although they usually can survive and grow in temperatures between 10 and 50 °C. Animal pathogens are mesophiles.
- **Thermophilic** bacteria refer to a type of extremophiles that thrives at relatively high temperatures while the mesophilic bacteria refer to a bacteria that grow best in moderate temperatures. Growth Temperature Thermophilic bacteria live at 45-122 °C while mesophilic bacteria live at 20-45 °C. Optimum Temperature.

pH

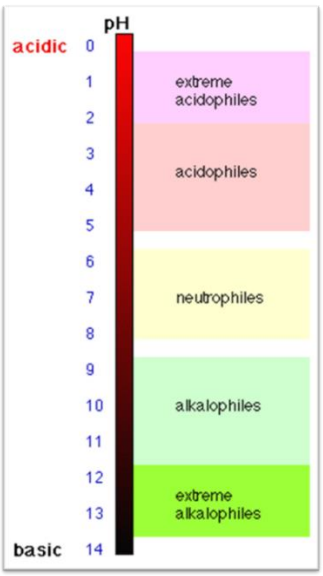


Fig. 52. Factors affecting Microbial Growth Nutrition

Classification of Bacteria on the Basis of pH of Growth:

- **Acidophiles:** These bacteria grow best at an acidic pH (Fig. 52.). The cytoplasm of these bacteria are acidic in nature. Some acidopiles are thermophilic in nature, such bacteria are

called Thermoacidophiles. Examples: *Thiobacillus thioxidans, Thiobacillus ferroxidans, Thermoplasma sulfolobus.*

- **Alkaliphiles:**These bacteria grow best at an alkaline pH (Fig. 52.). Example: *Vibrio cholerae* optimum ph of growth is 8.2.
- **Neutrophiles:** These bacteria grow best at neutral pH (6.5-7.5). Most of the bacteria grow at neutral pH. Example*: Escherichia coli (E. coli).*

Classification of Bacteria on the Basis of Osmotic Pressure Requirement:

- **Halophiles:** Require moderate to large salt concentrations. Cell membrane of halophilic bacteria is made up of glycoprotein with high content of negatively charged glutamic acid and aspartic acids. So high concentration of Na+ ion concentration is required to shield the –ve charge. Ocean water contains 3.5% salt. Most such bacteria are present in the oceans. Archeobacteria, Halobacterium, Halococcus.
- **Extreme or Obligate Halophiles:** Require a very high salt concentrations (20 to 30%). Bacteria in Dead Sea, brine vats.
- **Facultative Halophiles:** Do not require high salt concentrations for growth, but tolerate upto 2% salt or more.

Classification of Bacteria on the Basis of Hydrostatic pressure:

- **Barotolerant –** Does not get affected by increased pressure.
- **Barophiles/Piezophiles** – Bacteria which grow at moderately high hydrostatic pressures. Example: *Halomonas salaria*.

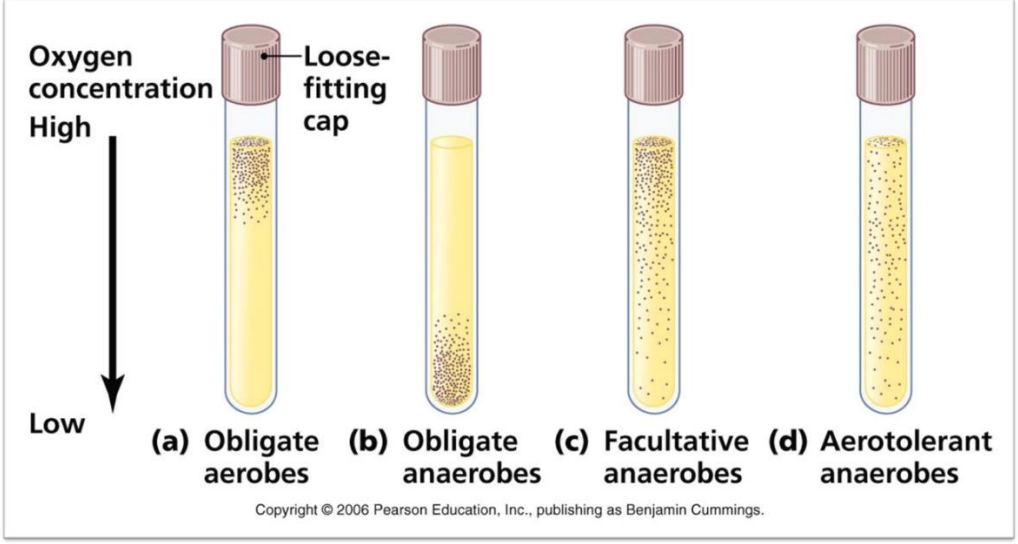


Fig. 53. Bacterial growth depends of oxygen requirement

Classification of Bacteria on the Basis of Oxygen Requirement:

- Obligate Aerobes: Require oxygen to live (Fig. 53.). Example: *Pseudomonas,* common nosocomial pathogen.
- Facultative Anaerobes: Can use oxygen, but can grow in its absence (Fig. 53.). They have complex set of enzymes. Examples: *E. coli, Staphylococcus*, yeasts, and many intestinal bacteria.
- Obligate Anaerobes: Cannot use oxygen and are harmed by the presence of toxic forms of oxygen. Examples: *Clostridium* bacteria that cause tetanus and botulism.
- Aerotolerant Anaerobes: Cannot use oxygen, but tolerate its presence. Can break down toxic forms of oxygen (Fig. 53.). Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.
- Microaerophiles: Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen. Example: *Campylobacter.*

11. BIOFILM OF BACTERIA

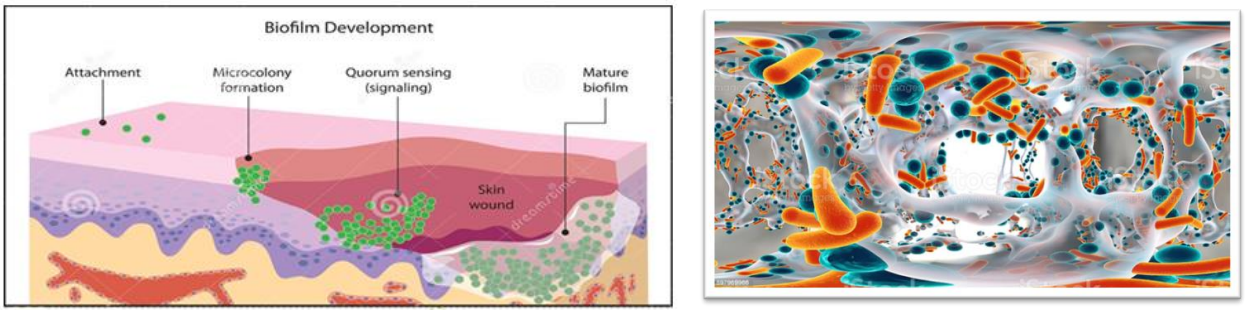


Fig. 54. Biofilm development of bacteria

The popular image of bacteria depicts single cells floating around, releasing toxins and damaging the host. However, most bacteria do not exist in this planktonic form in the human body, but rather in sessile communities called biofilms. To form a biofilm, bacteria first adhere to a surface and then generate a polysaccharide matrix that also sequesters calcium, magnesium, iron, or whatever minerals are available.

Within a biofilm, one or more types of bacteria and/or fungi share nutrients and DNA and undergo changes to evade the immune system. Since it requires less oxygen and fewer nutrients and alters the pH at the core, the biofilm is a hostile community for most antibiotics. In addition, the biofilm forms a physical barrier that keeps most immune cells from detecting the pathogenic bacteria.

A number of problems make biofilms difficult to detect.

- First, bacteria within the biofilm are tucked away in the matrix. Therefore, swabs and cultures often show up negative. Stool samples usually do not contain the biofilm bacteria, either.
- Second, biofilm samples within the GI tract are difficult to obtain. The procedure would require an invasive endoscope and foreknowledge of where the biofilm is located. What's more, no current procedure to remove biofilm from the lining of the GI tract exists.
- Third, biofilm bacteria are not easily cultured. Therefore, even if you are able to obtain a sample, it may again test negative because of the microbes' adapted lower nutrient requirements, rendering normal culture techniques null.
- Fourth, biofilms might also play a role in the healthy gut, making it difficult to distinguish between pathogenic and healthy communities.
- Although a culture might come back negative, the microbes in a biofilm could still be pumping out toxins that cause illness. Some clinicians look for mycotoxins in the urine to identify biofilms. Because the bacteria sequester minerals from the host, mineral deficiency is probably associated with the presence of biofilms, although mineral deficiencies are all too common in the general population to use this alone as a diagnostic criterion.

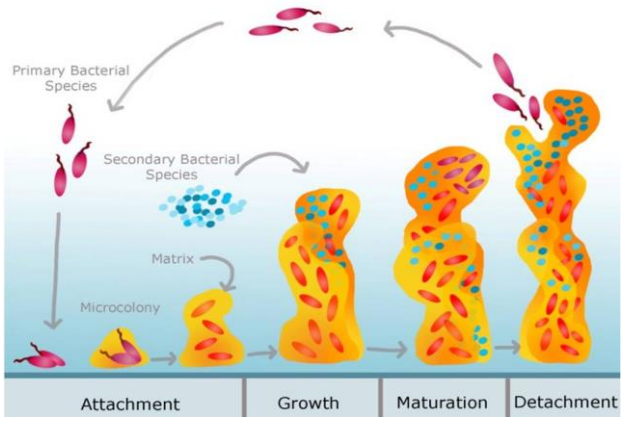


Fig. 55. Formation of bacterial biofilm

12. THE GROWTH CURVE

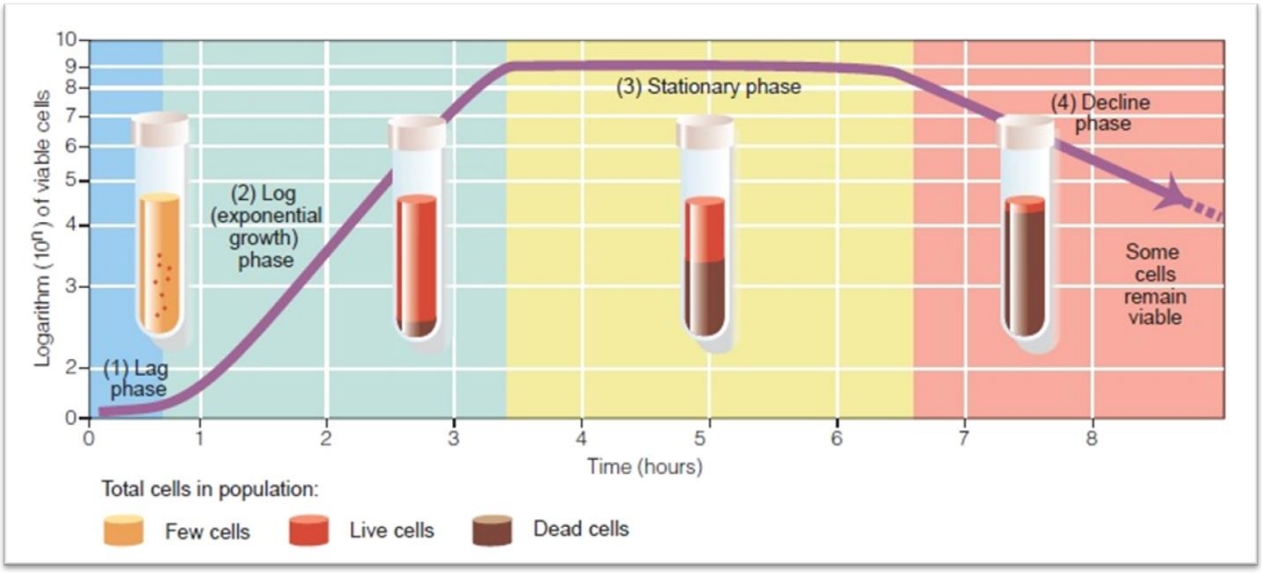


Fig. 56. The growth curve of bacteria

The Lag Phase

The lag phase represents a period during which the cells (Fig. 56.), depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume. If the cells are taken from an entirely different medium, it often happens that they are genetically incapable of growth in the new medium. In such cases a long lag may occur, representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to be apparent.

The Exponential Phase

The mathematics of which has already been discussed, the cells are in a steady state (Fig. 56.). New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in the medium become exhausted, or toxic metabolic products accumulate and inhibit growth. For aerobic organisms, the nutrient that becomes limiting is usually oxygen.

The Maximum Stationary Phase

Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely (Fig. 56.). In most cases, however, cell turnover takes place in the stationary phase: There is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. When this occurs, the total cell count slowly increases although the viable count stays constant.

The Death Phase

After a period of time in the stationary phase, which varies with the organism and with the culture conditions, the death rate increases until it reaches a steady level (Fig. 56.). The mathematics of steady-state death is discussed below. In most cases the rate of cell death is much slower than that of exponential growth. Frequently, after the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die and lyse.

13. PURE CULTURE

- In laboratory bacteria are isolated and grown in pure cultutr in order to study the functions of particular specie.
- A pure culture is a population of cells or growing in the absence of other species or types. A pure culture may originate from a single cell or singl organism, in which case the cells are genetic clones of one another.
- For isolation pure culture we can use two methods – the Streak Pleate Isolation Method (Fig. 57.) and Spead Plate Technique

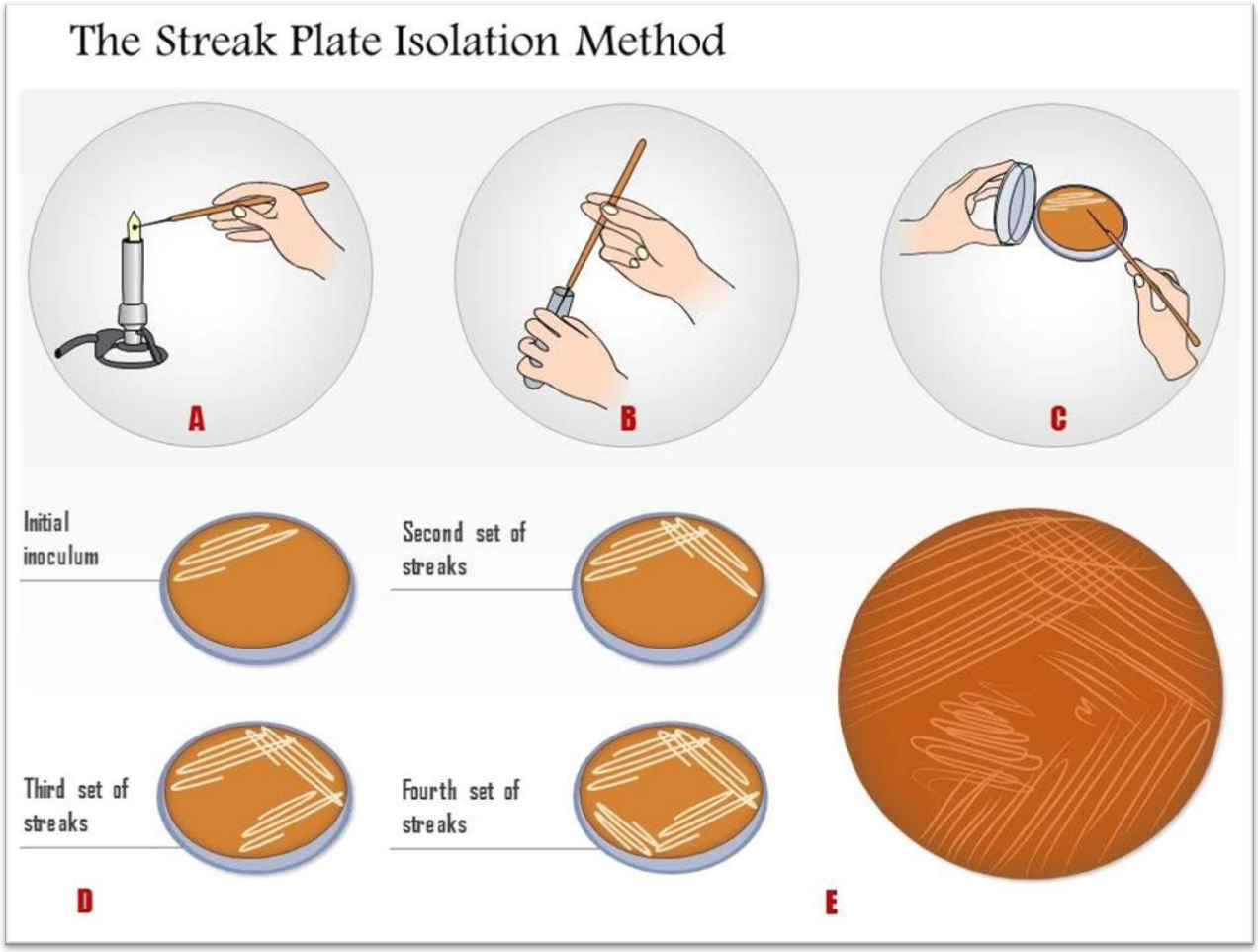


Fig. 57. The Streak Plate Isolation Method

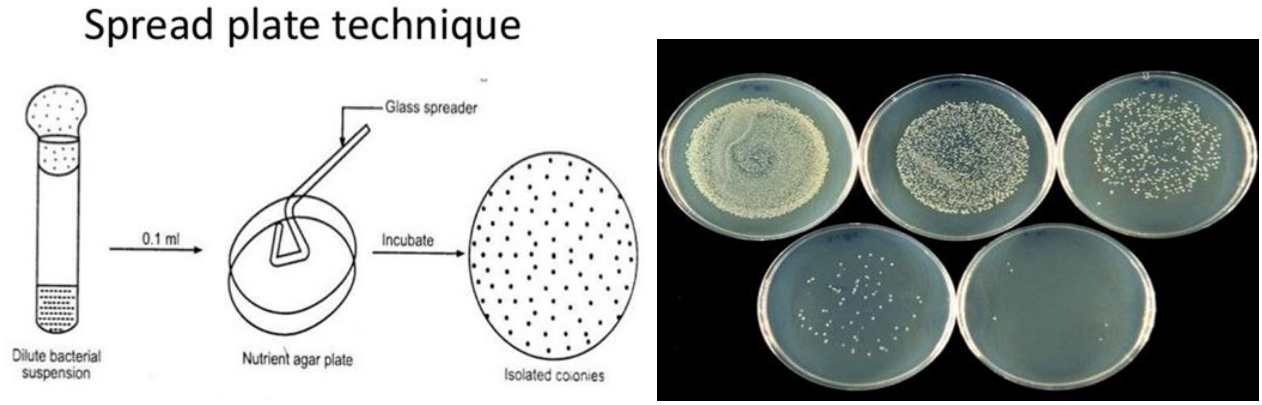


Fig. 58. Spread plate technique

14. METHODS OF CULTIVATION OF ANAEROBIC BACTERIA

1.Anaerobic jar is a heavy- walled jar with a gas tight seal within which tubes, plates, or other containers to be incubated are placed along with H_2 and CO_2 generating system (GasPak system). After the jar is sealed oxygen present in the atmosphere inside jar and dissolved in the culture medium, is gradually used up through reaction with the hydrogen in the presence of catalyst. The air in the jar is replaced with a mixture of H_2 and CO_2, thus leading to anoxic conditions (Fig. 59.).

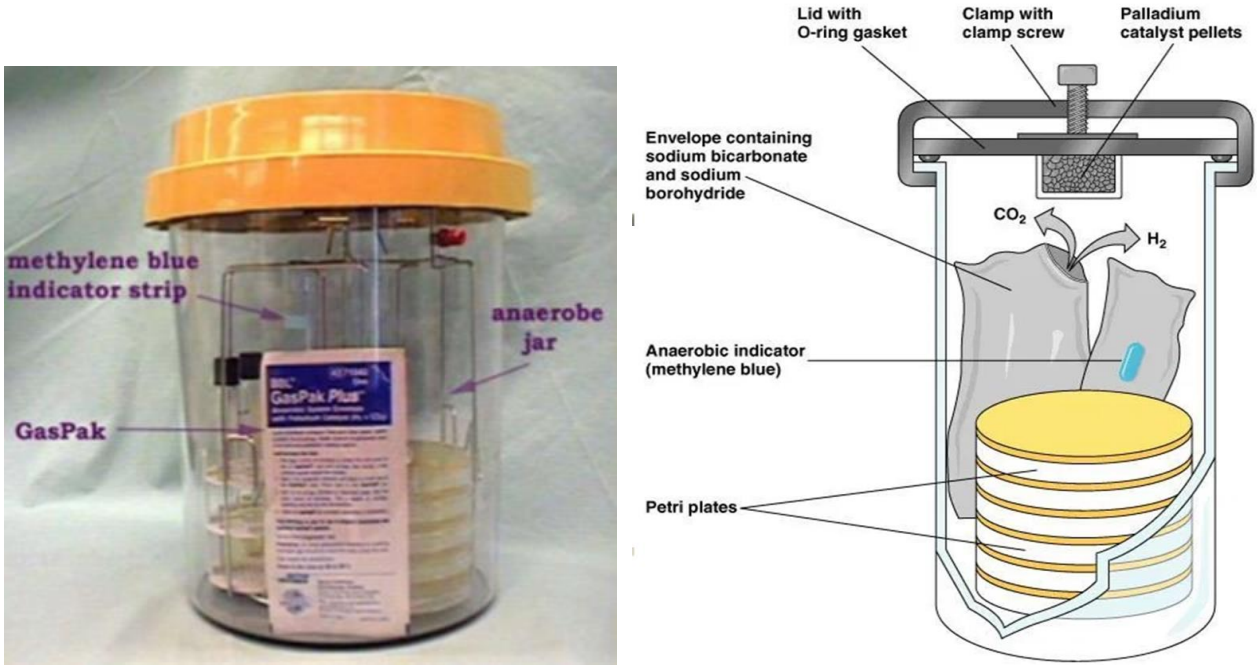


Fig. 59. Absorption of O_2 by Chemical method (Anaerobic Jar)

2. Paraffin method / Preservation by Overlaying Cultures with Mineral Oil

- Simple, most economical method
- Agar slants are inoculated & incubated
- Then covered with sterile mineral oil to a depth of 1 cm above the tip of start surface (Fig. 60.)
- Transfers are made by removing a loop full of grow-touching the tip to the glass surface to drain excess oil-inoculating a fresh medium preserving the initial stock culture
- Functions-providing anaerobic condition, prevents the dehydration of the medium and decreases the metabolic rate of the microorganisms.

Fig. 60. Preservation by Overlaying Cultures with Mineral Oil

15. STERILIZATION AND DISINFECTION

Antisepsis: A process involving the destruction or inhibition of microorganisms in living tissue thereby limiting or preventing the harmful effects of infection.

Antiseptic: Typically an antiseptic is a chemical agent that is applied to living tissue to kill microbes. Note that not all disinfectants are antiseptics because an antiseptic additionally must not be so harsh that it damages living tissue. Antiseptics are less toxic than disinfectants used on inanimate objects. Due to the lower toxicity, antiseptics can be less active in the destruction of normal and any pathogenic flora present.

Decontamination: The killing of organisms or removal of contamination after use, with no quantitative implication, generally referring to procedures for making items safe before disposal.

Disinfectant: A germicide that inactivates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms. They may not be effective against bacterial spores.

Disinfection: A procedure of treatment that eliminates many or all pathogenic microorganisms with the exception of bacterial spores.

Deratisation: rodent control.

Sterilization: The complete elimination or destruction of all forms of life by a chemical or physical means (Fig. 61.). This is an absolute not a relative term.

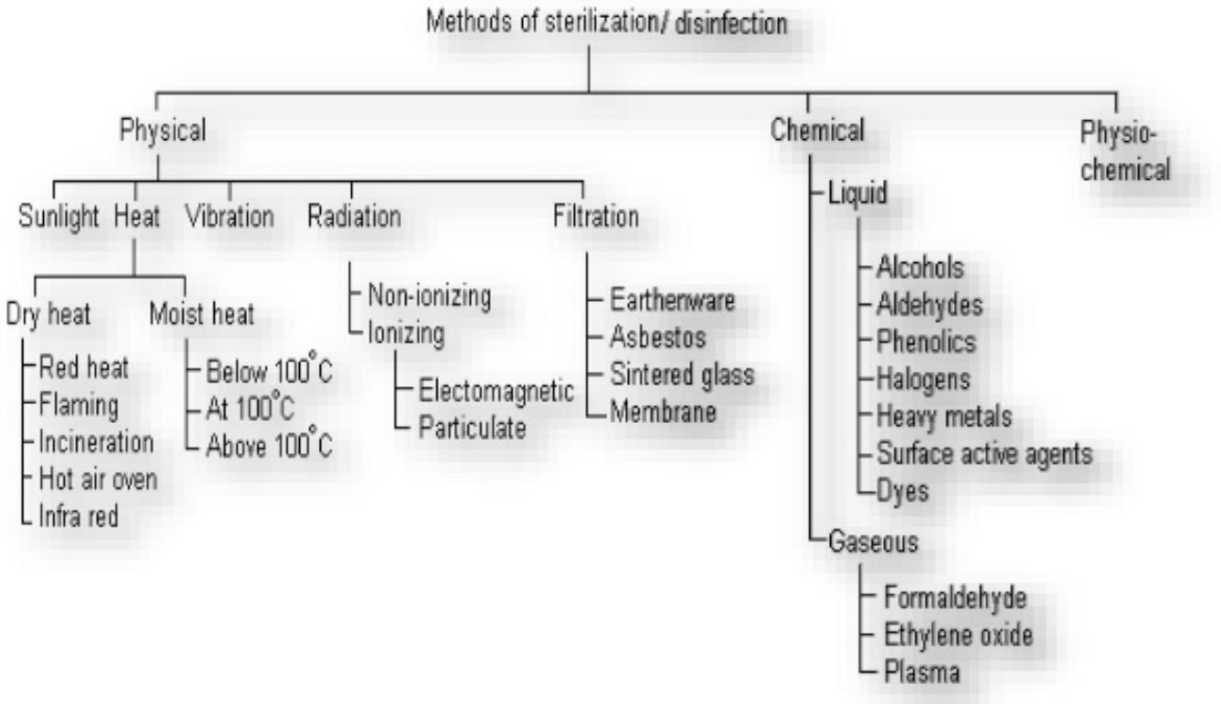


Fig. 61. Schematic representation of sterilization methods

Heat Sterilization

- Heat sterilization is the most effective and widely used method of sterilization, where the bactericidal activity results through the destruction of enzymes and other essential cell constituents.
- The effects of heat sterilization occur more rapidly in a fully hydrated state, as it requires a lower heat input, with low temperature and less time, under high humidity conditions where the denaturation and hydrolysis reactions are predominant, rather than in the dry state where oxidative changes take place.
- Under circumstances where thermal degradation of a product is possible, it can usually be minimized by adopting a higher temperature range, as the shorter exposure times generally result in a lower partial degradation.This method of sterilization is applicable to thermostable products. Still, it can be applied to both moisture-sensitive and moisture-resistant products, for which dry (160–180°C) and moist (121–134°C) heat sterilization procedures are respectively used.

Moist Heat Sterilization

- Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.
- Moist heat sterilization usually involves the use of steam at temperatures in the range 121–134°C.
- High pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.
- High pressure also facilitates the rapid penetration of heat into deeper parts of material and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.
- The high temperature-short time cycles not only often result in lower fractional degradation, but they also provide the advantage of achieving higher levels of sterility assurance due to more significant inactivation factors.
- The most commonly used standard temperature-time cycles for clinical porous specimens (e.g. surgical dressings) and bottled fluids are 134°C for 3 minutes and 121°C for 15 minutes, respectively.
- An autoclave is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.
- In this method, the microorganisms are killed by coagulating their proteins, and this method is much more effective than dry heat sterilization where microbes are killed through oxidation.
- In the pharmaceutical and medical sectors, it is used in the sterilization of dressings, sheets, surgical and diagnostic equipment, containers, and aqueous injections, ophthalmic preparations, and irrigation fluids, in addition to the processing of soiled and contaminated items.

Pasteurization

- Pasteurization is a process (Fig. 62.) in which packaged and non-packaged foods (such as milk and fruit juice) are treated with mild heat, usually to less than 100 °C, to eliminate pathogens and extend shelf life.
- The process is intended to destroy or deactivate organisms and enzymes that contribute to spoilage or risk of disease, including vegetative bacteria, but not bacterial spores. Since pasteurization is not sterilization, and does not kill spores, a second "double" pasteurization will extend the quality by killing spores that have germinated.

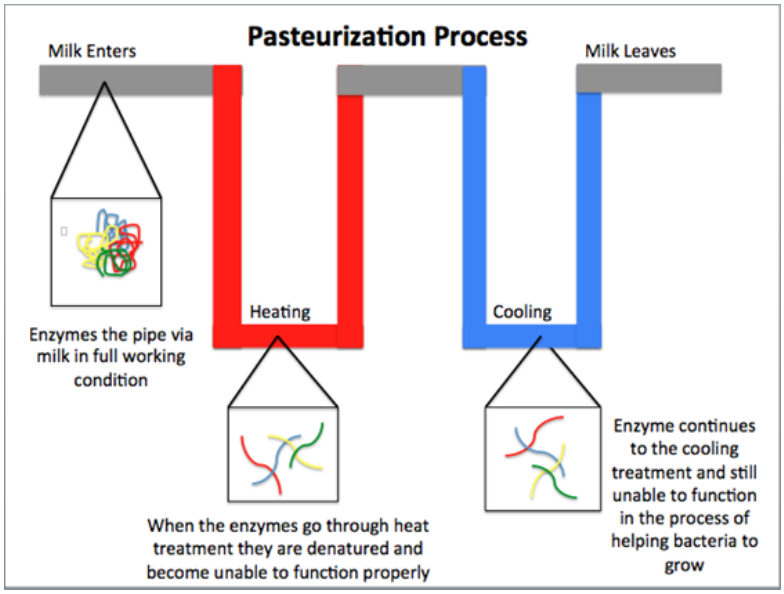


Fig. 62. Scheme of the pasteurization process

- The process was named after the French microbiologist, Louis Pasteur, whose research in the 1880s demonstrated that thermal processing would inactivate unwanted microorganisms in wine. Spoilage enzymes are also inactivated during pasteurization. Today, pasteurization is used widely in the dairy industry and other food processing industries to achieve food preservation and food safety.
- Most liquid products are heat treated in a continuous system where heat can be applied using a plate heat exchanger or the direct or indirect use of hot water and steam. Due to the mild heat, there are minor changes to the nutritional quality and sensory characteristics of the treated foods. Pasterization or high pressure processing (HPP) and pulsed electric field (PEF) are non-thermal processes that are also used to pasteurize foods.

Tyndallization

- Tyndallization is a process dating from the nineteenth century for sterilizing substances, usually food, named after its inventor, scientist John Tyndall, that can be used to kill heat-resistant endospores. Although considered old-fashioned, it is still occasionally used.
- A simple and effective sterilizing method commonly used today is autoclaving: heating the substance being sterilized to 121 °C for 15 minutes in a pressured system. If autoclaving is not possible because of lack of equipment, or the need to sterilize something that will not withstand the higher temperature, unpressurized heating for a prolonged period at a temperature of up to 100 °C, the boiling point of water, may be used. The heat will kill the bacterial cells; however, bacterial spores capable of later germinating into bacterial cells may survive. Tyndallization can be used to destroy the spores.
- Tyndallization essentially consists of heating the substance to boiling point (or just a little below boiling point) and holding it there for 15 minutes, three days in succession. After each heating, the resting period will allow spores that have survived to germinate into bacterial cells; these cells will be killed by the next day's heating. During the resting periods the substance being sterilized is kept in a moist environment at a warm room temperature, conducive to germination of the spores. When the environment is favourable for bacteria, it is conducive to the germination of cells from spores, and spores do not form from cells in this environment (see bacterial spores).
- The Tyndallization process is usually effective in practice. But it is not considered totally reliable—some spores may survive and later germinate and multiply. It is not often used today, but is used for sterilizing some things that cannot withstand pressurized heating, such as plant seeds.

Steam Sterilization: Autoclave (Fig. 63.), the process of sterilization by the use of heated steam under pressure to kill vegetative microorganisms and directly exposed spores. Common temperature and pressure for being effective is 121°C at 15 psi (pounds per square inch) over pressure for 15 minutes. Special cases may require a variation of the steam temperature and pressure used.

Fig. 63. Autoclave

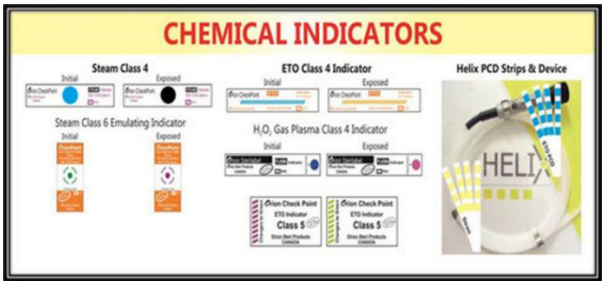


Fig. 64. Chemical indicators to check the correct sterilization in autoclave

Dry heat sterilization

- Dry sterilization is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.
- heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration
- Dry heat sterilization (Fig. 65.) is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons.
- Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.
- Similarly, laboratory equipment like Petri dishes and pipettes are challenging to sterilize by moist heat due to the penetration problem.
- The lethal effects of dry heat on microorganisms are primarily due to oxidative processes which are less effective when compared to the hydrolytic damage that results from exposure to steam in moist heat sterilization.
- Thus, in dry heat sterilization usually higher temperatures in the range 160–180°C are employed and also require exposure times of up to 2 hours depending upon the temperature employed.
- This principle is used in instruments like hot air oven and incineration, which generates very hot moisture-free air.
- The primary industrial application of dry heat sterilization is in the sterilization of glass bottles which are to be filled aseptically.
- In addition to the fact that this method achieves an adequate sterility assurance level, this method also destroys bacterial endotoxins (which are the products of Gram-negative bacteria also called pyrogens, which cause fever when injected into the body) which are difficult to eliminate through other sterilization techniques.
- For the purposes of depyrogenation of glass, temperatures of approximately 250°C are used.

Fig. 65. Dry heat sterilization

Irradiation

- Irradiation is the process of exposing surfaces and objects to different kinds of radiation for sterilization (Fig.66).

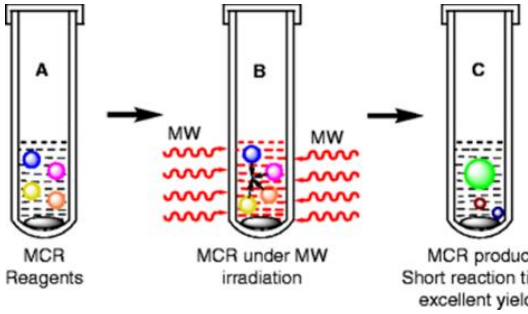


Fig. 66. Principle of operation of radiation for sterilization.

- Mainly electromagnetic radiation is used for sterilization.
- The major target for these radiations is considered to be microbial DNA, where damage occurs as a result of ionization and free radical production (gamma-rays and electrons) or excitation (UV light).

Ultraviolet (non-ionizing) radiation

- Ultraviolet radiation includes light rays from 150-3900 Å, of which 2600 Å has the highest bactericidal effect.
- Non-ionizing waves have a very little penetration power, so microorganisms only on the surface are killed.
- Upon exposure, these waves are absorbed by many materials, particularly nucleic acids.
- The waves, as a result, cause the formation of pyrimidine dimers which bring error in DNA replication and cause the death of microbes by mutation.
- UV radiation owing to its poor penetrability of conventional packaging materials is unsuitable for sterilization of pharmaceutical dosage forms.
- It is, however, applied in the sterilization of air, for the surface sterilization of aseptic work areas, and the treatment of manufacturing-grade water.

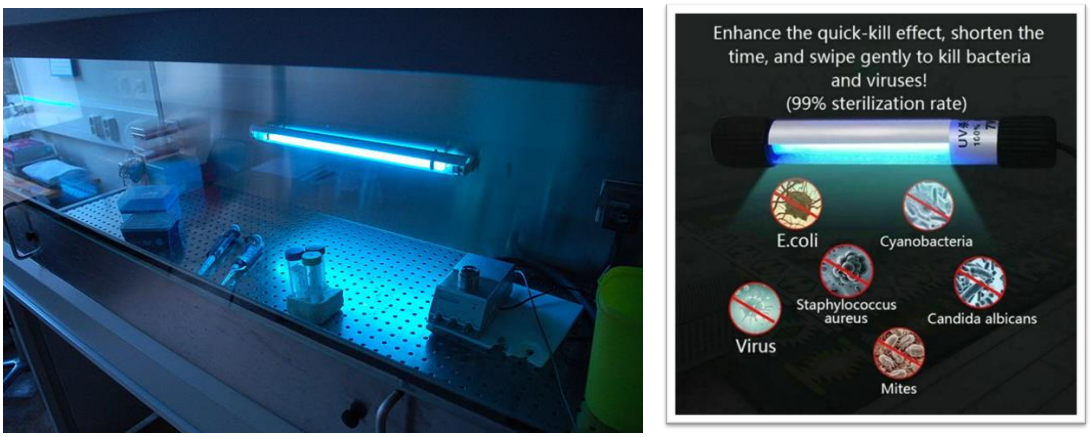


Fig. 67. Ultraviolet (non-ionizing) radiation

Ionizing Radiation

- X-ray and gamma rays are the commonly used ionizing radiation for sterilization.

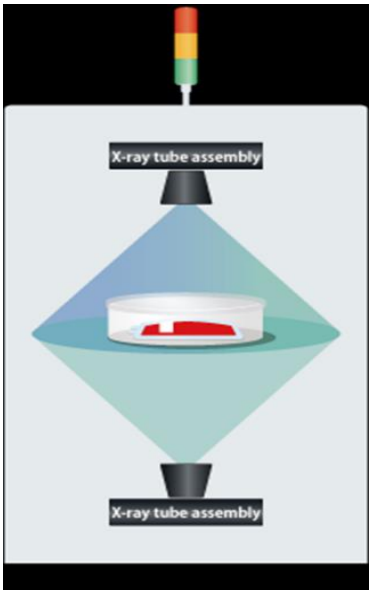


Fig. 68. Principle of operation of Ionizinf Radiation

- These are high energy radiation which causes ionization of various substances along with water.
- The ionization results in the formation of a large number of toxic O_2 metabolites like hydroxyl radical, superoxide ion, and H_2O_2 through ionization of water.
- These metabolites are highly oxidizing agents and kill microorganisms by oxidizing various cellular components.
- With ionizing radiation, microbial resistance decreases with the presence of moisture or dissolved oxygen (as a result of increased free radical production) and also with elevated temperatures.
- Radiation sterilization is generally exposed to items in the dried state which include surgical instruments, sutures, prostheses, unit-dose ointments, plastic syringes, and dry pharmaceutical products.

Filtration

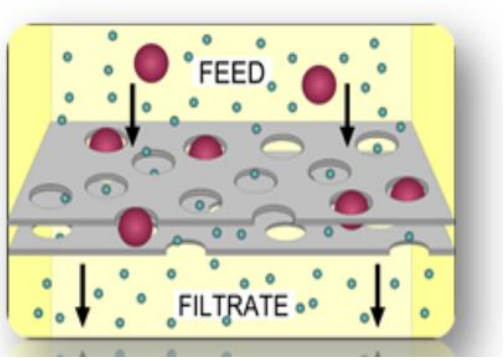


Fig. 69. The principle of operation of the filtration process

- The process of filtration is unique among sterilization techniques in that it removes, rather than destroys, microorganisms (Fig. 69.).
- Further, it is capable of preventing the passage of both viable and nonviable particles and can thus be used for both the clarification and sterilization of liquids and gases.
- The primary mechanisms involved in filtration are sieving, adsorption, and trapping within the matrix of the filter material.
- Filtration uses membranous filters that have tiny pores that let the liquid pass through but prevent bigger particles such as bacteria from passing through the filter. Therefore, the smaller the pore, the more likely the filter is to stop more things from going through it.
- Certain types of filter (membrane filters) also have an essential role in sterility testing, where they can be employed to trap and concentrate contaminating organisms from solutions under test.
- These filters are then placed in a liquid nutrient medium and incubated to encourage growth and turbidity.
- The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections and ophthalmic solutions, biological products, air, and other gases for supply to aseptic areas.
- They may also be required in industrial applications where they become part of venting systems on fermenters, centrifuges, autoclaves, and freeze dryers.

Chemical Agents for sterilization

ALCOHOLS

Ethyl alcohol, isopropyl alcohol, and n-propanol exhibit rapid, broad-spectrum antimicrobial activity against vegetative bacteria, viruses, and fungi but are not sporicidal. Activity is optimal when they are diluted to a concentration of 60–90% with water.

ALDEHYDES

Glutaraldehyde is used for low-temperature disinfection and sterilization of endoscopes and surgical equipment. It is normally used as a 2% solution to achieve sporicidal activity. Formaldehyde is bactericidal, sporicidal, and virucidal.

HEAVY METAL DERIVATIVES

Silver sulfadiazine, a combination of two antibacterial agents, Ag+ and sulfadiazine, has a broad spectrum of activity. Binding to cell components such as DNA may be responsible for its inhibitory properties.

ORGANIC ACIDS

Organic acids are used as preservatives in the pharmaceutical and food industries. Benzoic acid is fungistatic; propionic acid is both bacteriostatic and fungistatic.

PEROXYGENS

Hydrogen peroxide has broad-spectrum activity against viruses, bacteria, yeasts, and bacterial spores. Sporicidal activity requires higher concentrations (10–30%) of H2O2 and longer contact times.

PHENOLS

Phenol and many phenolic compounds have antiseptic, disinfectant, or preservative properties.

16. METABOLISM

Metabolism - is the collection of controlled biochemical reactions that take place within a microorganism (Fig. 70.). Ultimate function of metabolism is to reproduce the organism two major classes of metabolic reactions - Catabolism and Anabolism.

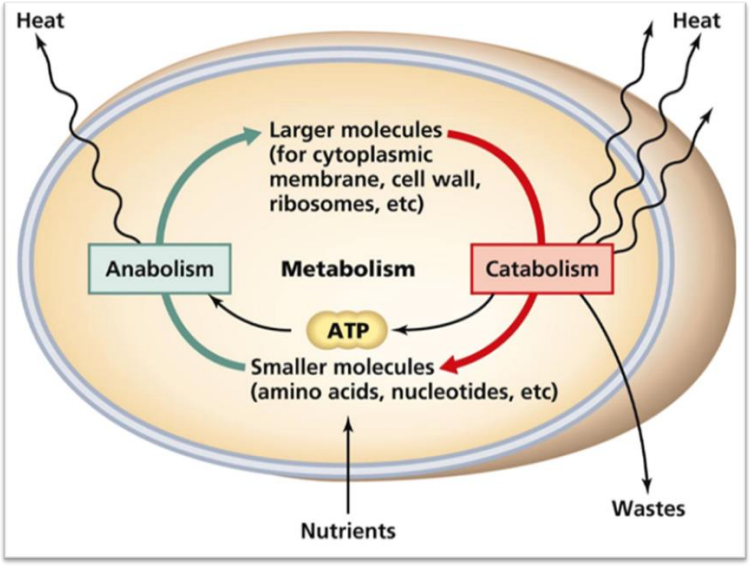


Fig. 70. Collection of controlled biochemical reactions that take place within a microorganism

Catabolic reaction - enzymes are involved in the breakdown of complex organic molecules in order to extract energy and form simpler end products (Fig. 71.).

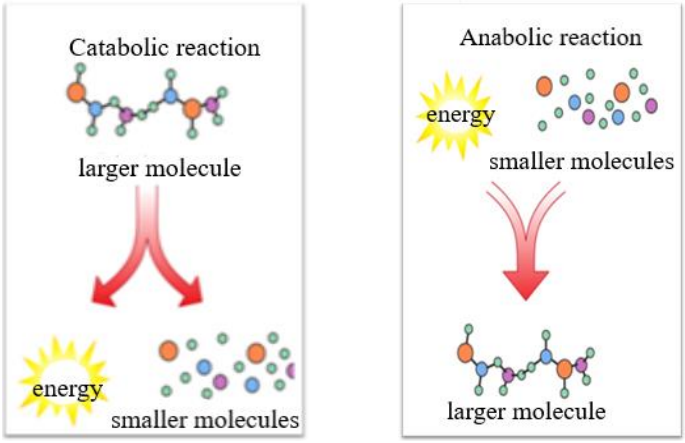


Fig. 71. Catabolic and Anabolic reactions

Anabolic reaction - enzymes are involved in the use of energy from catabolism in order to synthesize macromolecules and cell structures from precursors (simpler products) (Fig. 71.).

17. ROLES AND REGULATION OF BACTERIAL ENZYMES

Bacteria exhibit great diversity in their physiological activities. The energy necessary for carrying on cell activity and the building materials needed for the formation of new cells during multiplication is secured in a variety of ways. The acquisition of energy and materials, in turn, is related in large measure to the different enzymes produced by various bacteria (Fig. 72.).

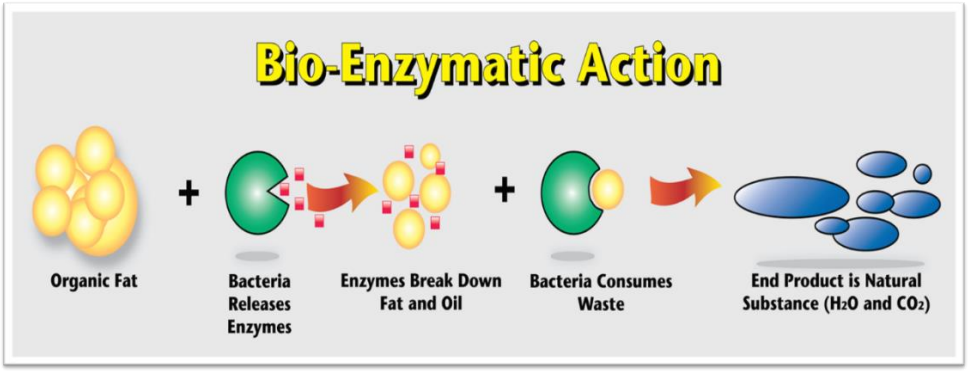


Fig. 72. Bio-Enzymatic Action

Conditions Affecting Enzyme Formation in Bacteria:

- **Constitutive enzymes** are always produced by cells independently of the composition of the medium in which the cells are grown.
- **Inducible enzymes** are produced ("turned on") in cells in response to a particular substrate; they are produced only when needed. The substrate, or a compound structurally similar to the substrate, evokes formation of the enzyme and is sometimes called an **inducer.**
- **A repressible enzyme** is one whose synthesis is downregulated or "turned off" by the presence of (for example) the end product of a pathway that the enzyme normally participates in. In this case, the end product is called a corepressor of the enzyme.

Many enzymes are discharged from the cells that produce them and, therefore, function outside the living cells ("extra cellular"). For example, the secretions of the digestive tract of animals contain many such extra cellular enzymes. All of the enzymes of the digestive tract act to convert the complex molecules of food into smaller, simpler molecules which are easier to take into the bacterial cell. The process of degradation is called hydrolysis. This degradation, which involves the conversion of solids into water soluble substances, and of large water-soluble molecules into smaller ones, is the essence of the process of digestion.

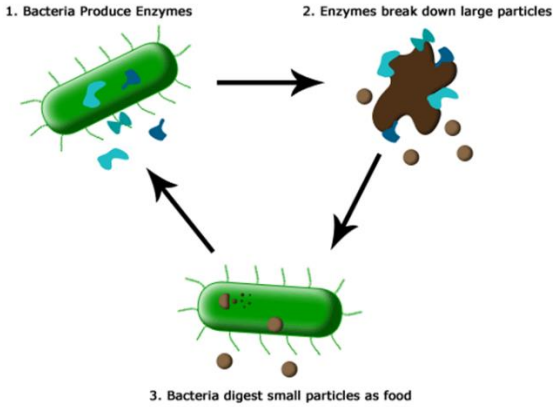


Fig. 73. Example of Enzyme Action

The difference between endoenzyme and exoenzyme is that endoenzyme is (enzyme) any enzyme that functions within the cell in which it was generated while exoenzyme is (enzyme) any enzyme, generated by a cell, that functions outside of that cell.

Endoenzyme is (enzyme) any enzyme that functions within the cell in which it was generated.

Exoenzyme is (enzyme) any enzyme, generated by a cell, that functions outside of that cell.

18. CLASSIFICATION OF BACTERIA ON THE BASIS OF NUTRITION

- Nutrition is substances used in biosynthesis and energy production and therefore are required for all living things.
- Bacteria, like all living cells, require energy and nutrients to build proteins and structural membranes and drive biochemical processes.
- Bacteria require sources of carbon, nitrogen, phosphorous, iron and a large number of other molecules.
- Carbon, nitrogen, and water are used in the highest quantities.
- The nutritional requirements for bacteria can be grouped according to the carbon source and the energy source.
- Some types of bacteria must consume pre-formed organic molecules to obtain energy, while other bacteria can generate their own energy from inorganic sources.

On the basis of carbon source bacteria may be:

- All organisms require carbon in some form for use in synthesizing cell components.
- All organisms require at least a small amount of CO_2.
- However, some can use CO_2 as their major or even sole source of carbon; such organisms are termed as Autotrophs (Autotrophic bacteria).
- Others require organic compounds as their carbon source and are known as Heterotrophs (Heterotrophic bacteria).

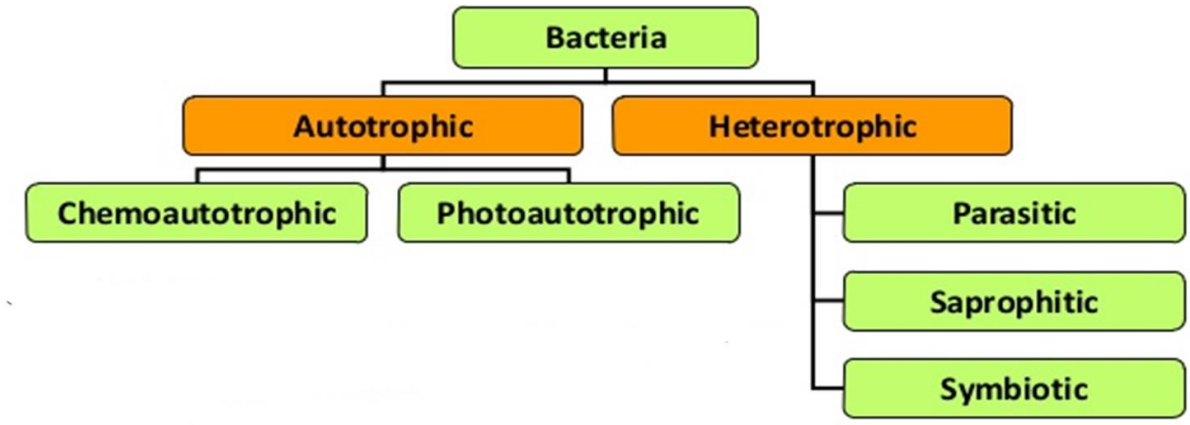


Fig. 74. Classification of Bacteria Nutrition

Autotrophs:

- They are able to synthesize their own organic food frominorganic substances.
- Can use CO_2 as a sole carbon source (Carbon fixation).

Heterotrophs:

- They are unable to manufacture their own organic food and hence are dependent on external source.
- Can not use CO_2 as a carbon sourse.

On the basis of energy source organisms are designated as:

- **Phototrophs:** the organisms which can utilize light as an energy source are known as phototrophs. These bacteria gain energy from light.
- **Chemotrophs:** these bacteria gain energy from chemical compounds. They cannot carry out photosynthesis.

On the basis of electron source organisms are designated as:

- **Lithotrophs:** some organisms can use reduced organic compounds as electron donors and are termed as Lithotrophs. They can be Chemolithotrophs and Photolithotrophs.
- **Organotrophs:** some organisms can use organic compounds as electron donors and are termed as organotrophs. Some can be Chemoorganotrophs and Photoorganotrophs.

Photo-lithotrops: These bacteria gain energy from light and use reduced inorganic compounds such as H_2S as a source of electrons. eg: *Chromatium okeinii.*

Photo-organotrophs: These bacteria gain energy from light an d use organic compounds such as Succinate as a source of electrons.eg; *Rhodospirillum*.

Chemo-lithotrophs: These bacteria gain energy from reduced inorganic compounds such as NH_3 as a source of electron eg; *Nitrosomonas*.

Chemo-organotrophs: These bacteria gain energy from organic compounds such as glucose and ammino acids as a source of electrons.eg; *Pseudomonas pseudoflora*.

Some bacteria can live ether chemo-lithotrophs or chemo-organotrophs like *Pseudomonas pseudoflora* as they can use either glucose or H_2S as electron source.

1) Saprophytic bacteria - obtain their food from the dead and organic decaying matter such as leaves, fruits, vegetables, meat, animal feces, leather, humus etc.

- These bacteria secrete enzymes to digest the food and absorb it.
- The enzymes secreted to break down the complex compounds such as carbohydrate and protein, into simpler soluble compounds, which are easily absorbed.

Examples are *Bacillus mycoides, B. ramosus, Acetobacter* etc.

2) Parasitic bacteria. These bacteria obtain their nutrition from the tissues of the hosts on which they grow.

- They may be harmless or may cause serious diseases.
- Parasitic bacteria which cause various diseases in plants and animals are known as pathogens, e.g., *Bacillus typhosus, B. anthracis, B. tetani. B. diplheriae, B. tuberculosis, B. pneumoniae, Vibrio cholerae, Pseudomonas citri* etc.

3) Symbiotic bacteria - live in close association with other organisms as symbionts.

- They are beneficial to the organisms.
- The common examples are the nitrogen-fixing bacteria, e.g., *Bacillus radicicola, B. azotobacter, Rhizobium, Clostridium, Rhizobium spp*.
- These bacteria live inside the roots of leguminous plants.
- These bacteria fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria.

19. SOLUTE TRANSPORT MECHANISMS IN BACTERIA

Bacteria require several nutrients for their growth. Structural components such as cell wall and cell membrane restrict the entry of several molecules inside the cell. Therefore, the specific mechanism for nutrient uptake is highly important for the bacterial cell.

Passive Diffusion.

Some of the molecules such as glycerol can pass the plasma membrane by Passive Diffusion. This is a process by which molecules present at a higher concentration move towards the lower concentration level. The rate of passive diffusion is dependent on the difference of size of the gradient present inside or outside of the cell. Molecule transport by passive diffusion requires a fairly large concentration gradient outside the cell while the concentration of the gradient inside the cell needs to be low. Small molecules such as water, oxygen, carbon dioxide, etc. can move across the plasma membrane by passive diffusion (Fig. 75.).

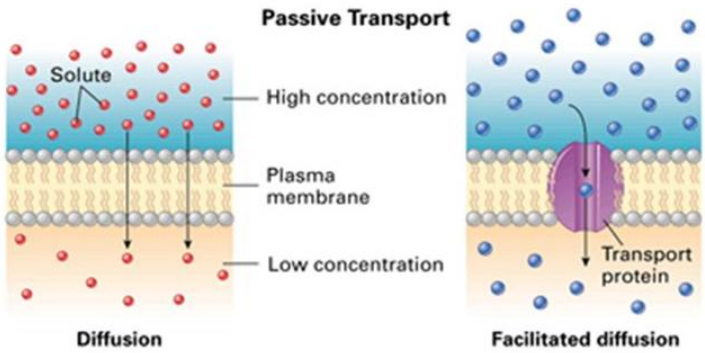


Fig. 75. Both Diffusion and Facilitated Diffusion are forms of passive transport, as neither process requires the cell to expend energy. In Facilitated Diffusion, solute particles pass through a channel in a transport

Facilitated Diffusion. This process requires carrier proteins such as permease to transport the solute across the membrane (Fig. 75 – 76). Due to the involvement of the carrier proteins, the rate of diffusion is higher than Passive Diffusion. The rate of diffusion increases with the concentration gradient much more rapidly and at a lower concentration of diffusing molecule than that of passive diffusion each of the carrier proteins involved in transporting specific molecules. Though this process requires carrier proteins for movement of molecules, but the movement depends on the concentration of the gradient, no extra energy is required for the process. In this process, the carrier protein complex spans the membrane. After the attachment of the solute at the outside of the carrier, the protein changes its conformation and releases the solute inside of the cell. At the end of this step, the carrier again changes to its previous conformation to carry more molecules. As the process is dependent on the gradient concentration, the solute can come out of the cell if the concentration inside the cell is higher than outside. Glycerol is often transported inside a bacterial cell by facilitated diffusion. The process is also found in different Eukaryotes.

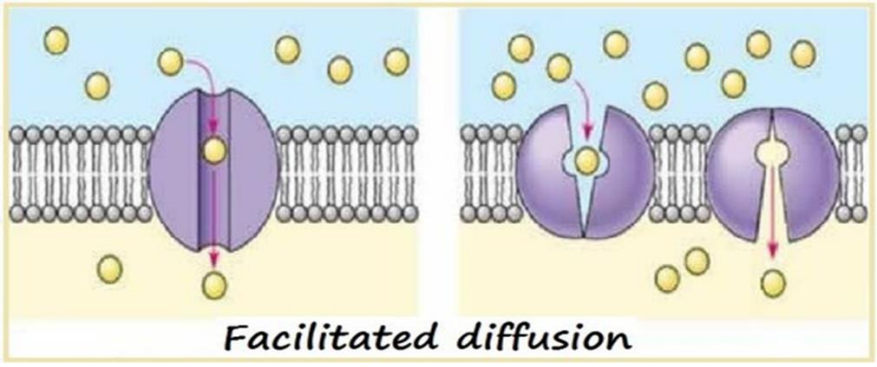


Fig. 76. Facilitated Diffusion

Active transport is a process (Fig. 77.) to transport the solute at a higher concentration, i.e., against the gradient concentration. Microorganisms often live in environments that lack nutrients, therefore, this process plays an important role to overcome those situations. Moreover, this process requires energy to carry forward the nutrient uptake. ATP binding cassette transporters (ABC) are the important examples of active transport system which is present in bacteria, Achaea and eukaryotes. These transporters consist of two hydrophobic membrane domains along with two ATP binding domains. ABC transporters facilitate the involvement of special substrate-binding proteins which binds with the solute and interacts with the membrane transport protein to transport the solute inside the cell. ABC transporters also involve in pumping out antibiotics in several antibiotic-resistant bacteria. Molecules entering the gram-negative bacteria need to pass through the outer membrane before ABC transporters and active transport systems can take action. An example of this movement is the transport of phosphate molecules in *E. coli*. The inorganic phosphate molecules cross the outer membrane by the involvement of the porin protein channel.

Electron transport during the energy-conserving process generates a proton gradient in prokaryotes; the protons are at higher concentration outside the cell. The transport process can be described by using the example of lactose uptake by E. coli. Lactose permease is a single protein that transports lactose molecule inward as a proton simultaneously enters the cell. This linked transport of two substances is called symport. Here the energy in form of a proton gradient drives transport. Transport proteins are present as outward and inward-facing conformations when proton and lactose bind to the specific binding proteins, those proteins alters the conformations to uptake the sugar and proton.

Apart from this, the proton gradient can indirectly involve in active transport through the formation of sodium ion gradient. In E. coli, the sodium transport system pumps sodium outside of the cell when the protons move inside. This type of transport is known as antiport. The proton antiport system facilitates the uptake of sugars or amino acids. In this case, sodium ions attache to the carrier protein and the protein alters its shape. Then the carrier binds with the sugars or amino acids and orients the binding sites towards the interior of the cell. Due to low intracellular sodium concentration, the sodium ion dissociates from the carrier.

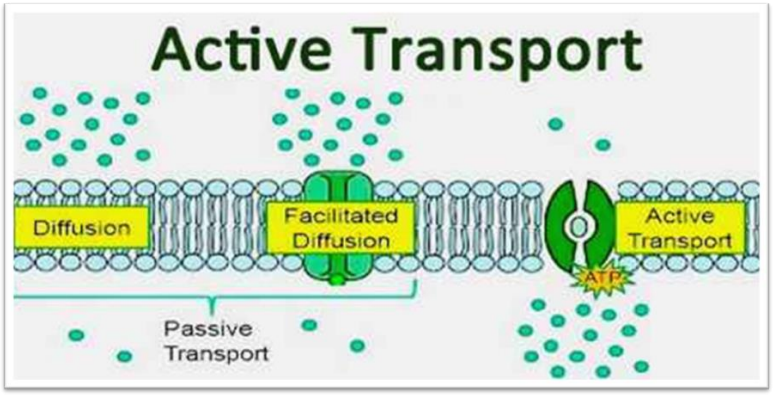


Fig. 77. Active transport

Group Translocation. In this case, the solute is chemically modified when it is transported inside the cell. It is also a type of active transport as metabolic energy is used during the nutrient uptake. The process can be described by the sugar-phosphate transferase system (PTS). This system helps to transport many sugars by phosphorylating them using phosphoenolpyruvate (PEP). PEP is used for ATP synthesis, but, in PTS the energy present in PEP is used to energize the uptake molecule. The transfer of phosphate from PEP requires different proteins. In E. coli and Salmonella, two enzymes (Enzyme I and Enzyme II) and one low molecular weight heat-stable protein (HPr) is connected with PTS. Enzyme II is made up of three domains: EII A (cytoplasmic and soluble), EII B (hydrophilic), EII C (hydrophobic). Phosphate is transferred from PEP to EII by the help of EI and HPr. Then a sugar molecule is phosphorylated as it carries across the membrane by EII. EII transport is specific for sugars and varies in each PTS, but EI and HPr are the same in different PTs systems.

Osmosis

Diffusion of water through a permeable but selective membrane. Water moves toward the higher solute concentrated areas: Isotonic, Hypotonic, Hypertonic (Fig. 78.).

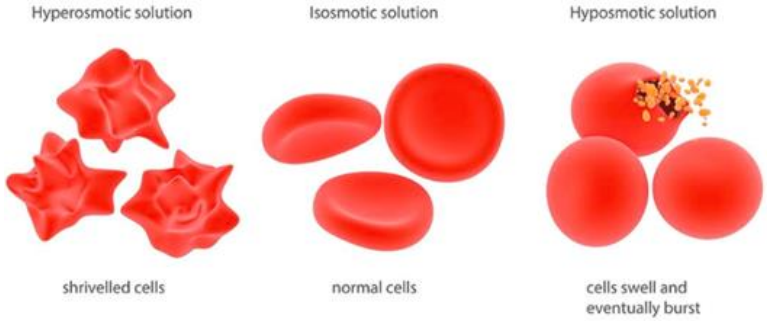


Fig. 78. Diffusion of water through a permeable but selective membrane.

20. MICROFLORA OF THE WATER, SOIL, AIR, FOOD AND METHODS OF RESEARCH

Environmental microbiology. A feature of sanitary-microbiological assessment of the environment is the simultaneous accounting of both quantitative indicators of the microflora and indicators that characterize its condition. When sanitary and microbiological studies is limited to the potential danger of an object for people and animals, it's possible presence of pathogens, not their direct detection. This is one of the differences between sanitary and microbiological research from diagnosis.

In this regard, it is necessary to know the biological properties of microorganisms that cause environmental pollution, food spoilage, methods of microbiological and virological studies of the environment (soil, water, air), as well as methods for assessing ways and degrees of infection by animals and humans environment by pathogenic bacteria and viruses; normal exchange of microflora between animals, humans, the environment; industrial and domestic processes that cause violations of natural self-cleaning and disinfection of the environment.

Types of interaction between microorganisms: mutualism, commensalism, synergy, parasitism, antagonism.

Mutualism - a type of coexistence of different species, from which they benefit from each other.

Commensalism - is a type of relationship between two living microorganisms in which one organism benefits from the other without harming it, but without benefiting it.

Synergy - is a summing effect, which consists in the fact that when two or more factors interact, their action significantly outweighs the effect of each individual component.

Parasitism - the kind of relationships between different types of microorganisms, in which one of these (parasite) uses another (the host) as a power source and habitat.

Antagonism of microorganisms is a type of relationship between microorganisms in which one strain completely suppresses or slows down the growth of another. As a rule, antagonism occurs when a microorganism releases chemicals with antibiotic properties that suppress the growth and vital activity of other microorganisms.

Sanitary-indicative microorganisms must meet certain requirements:

- constantly live in the body of humans and animals and in large quantities to be released into the environment;
- maintain viability in the environment for a period close to the survival of pathogenic microorganisms that are released in the same way;
- should not reproduce in the environment;
- should be easily isolated from objects and not suppressed by saprophytes;
- should not change their biological properties;
- must not have any other natural reservoir than the human and animal body;
- must be more resistant to physical and chemical environmental factors than pathogenic microorganisms;
- indication, identification and quantification should be carried out by modern, simple, easily accessible and economical microbiological methods.

WATER

Natural waters, like soil, are the natural habitat of many microorganisms, where they are able to live, multiply, participate in the cycle of carbon, nitrogen, sulfur, iron and other elements. Quantitative and qualitative composition of the microflora of natural waters is diverse.

Groundwater. The composition of the microflora of groundwater (artesian, key, groundwater) depends mainly on the depth of the aquifer, its protection from contamination from the outside. Artesian waters at great depths contain very few microorganisms. Groundwater extracted through conventional wells from shallow aquifers, where surface contaminants can penetrate, contains a significant number of bacteria, among which may be pathogenic. The closer the groundwater is to the surface, the more abundant their microflora.

Surface waters. These are waters of open reservoirs (rivers, lakes, reservoirs, etc.). They have a great variety of the composition of their microflora depending on the chemical composition of the water, the nature of the use of the reservoir, the population of coastal areas, seasons, meteorological and other conditions. In addition to their own aquatic microorganisms, many microorganisms from outside enter open water bodies.

In a river, for example, flowing in the area of large settlements or industrial enterprises, water can contain hundreds of thousands and millions of bacteria in 1 cm3, and above these points - only hundreds or thousands of bacteria.

There are more microorganisms in the water of the coastal zone of reservoirs, especially standing ones, than in the distance from the shores. More microorganisms are also contained in the surface layers of water, but especially many of them in the sludge, mainly in its upper layer, where it is formed as if a film of bacteria, which plays an important role in the conversion of substances in the reservoir. The number of bacteria in open water during the spring floods or after heavy rains increases significantly.

Bacteriology of water. The main processes of microbial treatment of water bodies are rot and fermentation. Rot - the decomposition of nitrogen-containing organic compounds under the influence of microbes. Rot is caused by: bacilli (*Bacillus mesentericus, B. subtilis*), enterobacteria (*Proteus, Escherichia, Klebsiella,* etc.), clostridium (*Clostridium botulinum, C. sporogenes,* etc.). The end products of protein breakdown: skatole, indole, fatty acids, ammonia, hydrogen sulfide. Fermentation - the decomposition of organic matter that does not contain nitrogen, under the influence of microbes. Representatives of various physiological groups, bacteriophages, bacteria-predators (*Bdellovibno bacteriovs),* protozoa, worms take part in processes of clearing of reservoirs of microorganisms. The main source of microbial contamination of water bodies is wastewater. With sewage, the inhabitants of the intestines of humans and animals - representatives of normal, opportunistic flora and pathogens (pathogens of intestinal infections, yersiniosis, leptospirosis, hepatitis A and E viruses, poliomyelitis, etc.) enter the reservoirs.

According to the degree of microbial contamination, there are four categories (Fig. 79.) of water - **saprobic zones of the reservoir:**

1. **Polysaprobic zone** – the water most polluted with organic compounds, it has little oxygen. The number of bacteria in 1 ml of water - from 1 million and more. Among them are many anaerobic bacteria, actinomycetes, fungi. Under the influence of these bacteria, complex organic compounds decompose into simple substances with the formation of ammonia, hydrogen sulfide, carbon dioxide, indole, skatole, methane.
2. **Mesosaprobic zone** - a zone of moderate pollution, characterized by the mineralization of organic matter with oxidation and nitrification. In 1 ml of water to hundreds of thousands of microbes. Qualitative composition is diverse. These are mainly nitrifying bacteria - gram-negative rods, cocci, which are obligate aerobes.
3. **Oligosaprobic zone** – it's zone of pure water; characterized by the fact that the processes of self-cleaning end. The number of microbes in 1 ml - from 10 to 1000. Water has a high degree of purity, Escherichia coli is absent. It's a drinking water.
4. **Cathartic zone** - a zone of very pure water, which occurs in reservoirs in the autumn-winter period, far from settlements and shores.

Fig. 79. Saprobic zones of the reservoir

The living microworld of man and our environment has undergone great changes. Intense microbial corrosion of the earth, colonization of various objects of external environment and foodstuff by the changed bacteria, fungi, viruses proceeds. From year to year, the carrier of pathogenic bacteria among medical staff and various dysbacteriosis in relatively healthy and sick people are growing. Hospital infections caused by pathogenic and opportunistic microorganisms are becoming more common. All this requires the development of new and improvement of existing methods of laboratory research of the microflora of water, air, soil, other objects of the environment, food. It is important to determine human dysbacteriosis, carriers of Staphylococcus aureus, meningococci, pathogens of diphtheria, cholera, typhoid fever, dysentery. A doctor of any profile must know how to properly take the test material, deliver it to the profile laboratory, conduct a microbiological study, properly evaluate its results.

The sanitary state of environmental objects is regulated by various physical-chemical and sanitary-microbiological indicators.

Microbial contamination of water is controlled according to 4 main indicators:

1) microbial number of water;
2) coli-titer and coli-index;
3) by detecting pathogenic bacteria of the intestinal group;
4) the number of thermophiles in the soil.

The microbial number (total bacterial count) - (number of microorganisms in 1 ml of the test water) is determined by quantitative seeding of water diluted 10, 100 and 1000 times in Petri dishes with meetpeptone agar, followed by counting the number of grown colonies.

For good water - not more like 100 CFU (colony forming unit).

For soil – not more like 1000.

Coli index is determined by the number of microbes— normal inhabitants of the human intestine (mainly *Escherichia coli*)— per one liter of water. For pure water – not more like 3.

Coli titer is the smallest amount of test water, which contains at least one living of colibacillus *(Escherichia coli),*. For pure water – 333 ml.

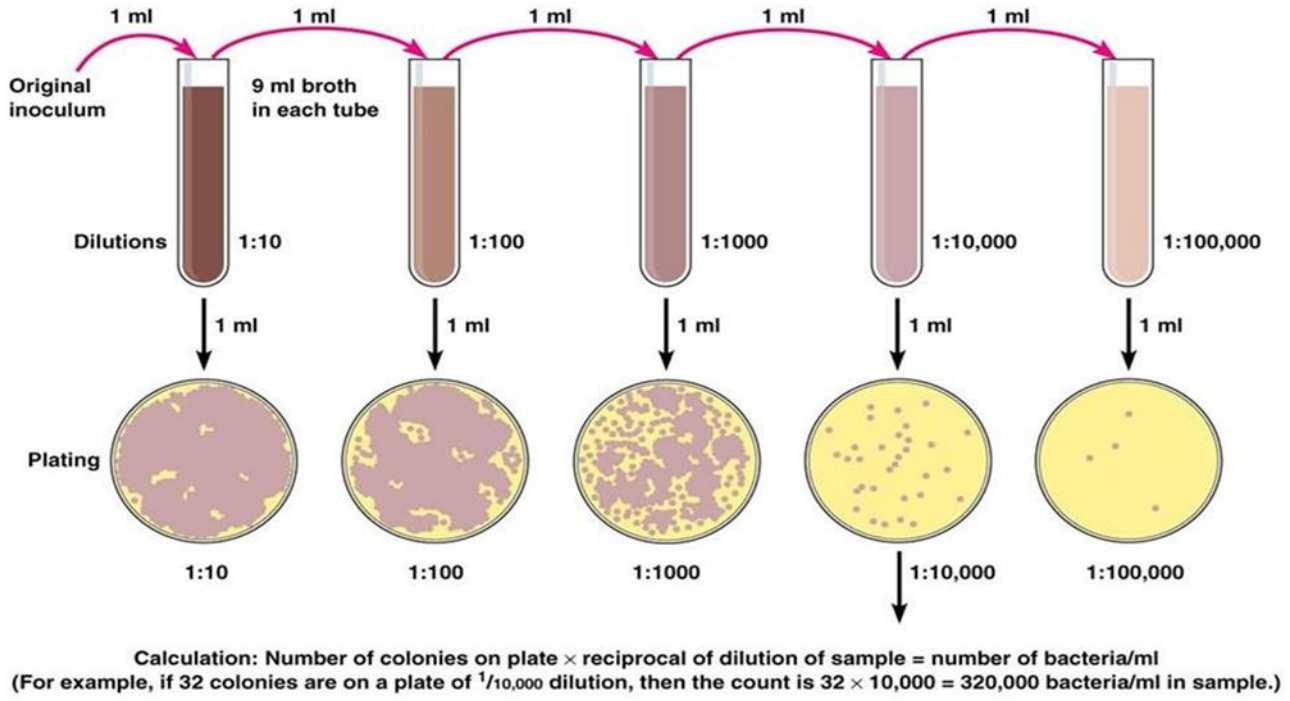


Fig. 80. Serial dilutions Method

Bacteriology of soil

Soil is a natural habitat for microorganisms. They find in the soil all the conditions necessary for their development, food, moisture, protection from the harmful effects of direct sunlight and drying.

The microflora of the soil in quantitative and species composition varies significantly depending on the chemical composition of the soil, its physical properties, reaction (pH), moisture content, degree of aeration. Climatic conditions, time of year, methods of agricultural tillage, the nature of vegetation and other factors also have a significant impact.

Unequally distributed microorganisms on the soil horizons. The smallest of them is usually contained in the surface layer of the soil with a thickness of several millimeters, where microorganisms are exposed to the adverse effects of sunlight and drying. The next layer of soil up to 5 10 cm thick is especially abundantly inhabited by microorganisms. As it deepens, the number of microorganisms decreases. At a depth of 25-30 cm, their number is 10-20 times less than in the surface layer 1-2 cm thick.

The composition of the microflora also changes with depth. In the upper layers of the soil, which contain a lot of organic matter and are well aerated, dominated by aerobic saprophytes, capable of decomposing complex organic compounds. The deeper the soil horizons, the poorer the organic matter; air access to them is difficult, so there is a predominance of anaerobic bacteria.

The soil microflora is represented by various species of bacteria, actinomycetes, fungi, algae and protozoa.

The permanent inhabitants of the soil include various putrefactive, mainly spore-bearing, aerobic (*Bacillus subtilis*, *B. cereus* var. *Mycoides*, *B. megaterium*) and anaerobic (*Clostridium sporogenes, C. putrificum*) bacteria, as well as bacteria that decompose, nitrifying fiber , nitrogen-fixing, gray and iron bacteria.

The activity of soil microorganisms plays an important role in creating soil fertility. Consistently changing each other, microorganisms carry out processes of circular mouth substances in the soil. Organic substances that enter the soil in the form of plant remains, animal carcasses and other contaminants, are gradually mineralized. Compounds of carbon, nitrogen, phosphorus and other elements from forms inaccessible to plants are converted into digestible substances.

Along with the usual inhabitants of the soil, there are also pathogenic microorganisms, mainly spore-forming bacteria, such as pathogens of tetanus, gas gangrene, botulism, and others. Therefore, contamination of food with soil is a danger.

The number of microorganisms in the soil depends on the extent of contamination with faecas and urine, and also on the nature of treating and fertizing the soil. Saprophytic spores (*B. cereus*, *B. megaterium*, etc. (Fig. 81.)) survive for long periods in the soil. Pathogenic bacteria which do not produce spores due to lack of essential nutrients, and also as a result of the lethal activity of light, drying, antagonistic microbes, and phages do not live long in the soil (from a few days to a few months).

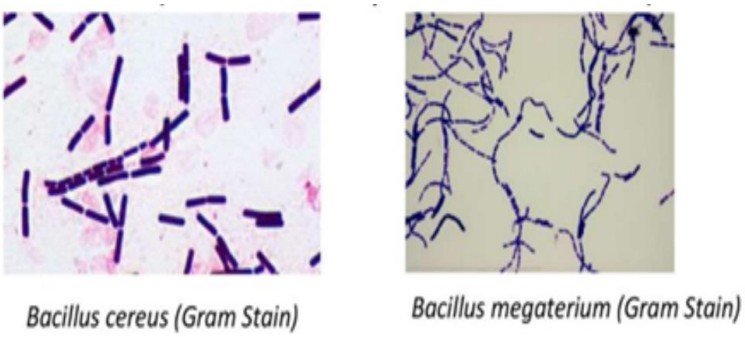
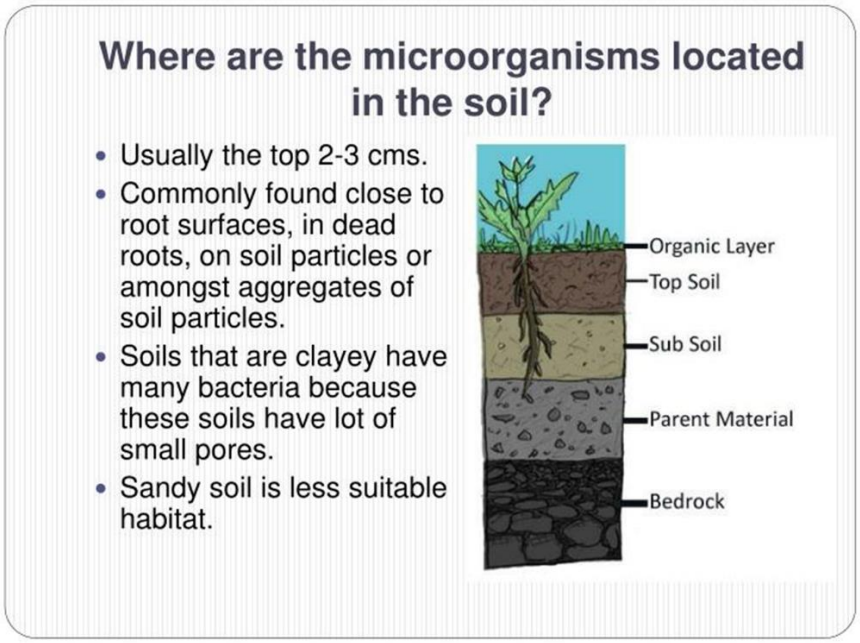


Fig. 81. An example of saprophytic microorganisms

Coli-index for soil – amount of *E. coli* in 1g of soil.
Coli-titre for soil – smallest amount of soil where present 1 *E. coli*.

An important criterion for the sanitary condition of the soil and its ability to self-clean is the **Perfringens-titer** (the minimum amount of soil in which *Clostridium perfringens* is still determined). At fecal pollution of soil in 4-5 months *Escherichia coli* disappear, and clostridia find in titer of 0,01 g.

Fresh fecal contamination is indicated by the detection of enterococci, a large number of *Escherichia coli* bacteria in the absence of nitrifying bacteria and thermophiles, a relatively high content of vegetative forms of clostridia. Determination of thermophilic bacteria helps to assess soil contamination with manure, compost or wastewater and the stage of decomposition of their organic substrate. The appearance of nitrifying bacteria indicates the development of self-cleaning processes. For a more complete assessment of the self-cleaning process, groups of microorganisms that quickly destroy the organic substrate are identified: bacilli, actinomycetes, fungi. Low titers of *E. coli* bacteria indicate that self-cleaning is complete, the soil is free of pathogenic enterobacteria and organic contaminants.



Bacteriology of air

Microorganisms get into atmospheric air mainly from soil, and also from plants, from animals, people. Usually microorganisms are contained in the air together with dust particles and in the smallest droplets of moisture that are in a suspended state.

Air is not a favorable environment for the development of microorganisms, as it lacks drip-liquid water. In the air, microorganisms can retain their viability only temporarily, and many more or less quickly die under the influence of drying and solar radiation.

The air microflora is diverse in number and composition and can vary significantly depending on climatic conditions, seasons, sanitary conditions and other factors. Above the seas, mountains, and icy fields of the Arctic, the air contains very few microbes (units in 1 m3). Much more of them in the air of populated areas, especially large industrial cities.

As you move away from populated areas, the number of microorganisms in the air decreases markedly, but viable microorganisms are found even in the stratosphere, although there are very few. In winter, the air germs are much less than in summer.

Green plantations are of great importance for reducing the number of microbes in the air.

The main sources of the spread of pathogenic microorganisms in the environment are humans and warm-blooded animals (patients, carriers). The release of microorganisms into the environment occurs mainly by fecal and airborne routes.

It is extremely difficult to directly detect pathogenic microorganisms in the environment. This is due to their low concentration, fluctuations in content (presence during the epidemic and absence in the inter-epidemic period.

Standards for bacterial contamination and the content of sanitary-indicative microorganisms in indoor air must be justified taking into account the type, purpose of the room and the class of cleanliness.

It is especially important to conduct sanitary-microbiological studies of air in surgical and pediatric wards of hospitals, operating rooms, maternity hospitals - where various microorganisms (pathogenic, opportunistic and some saprophytes) can cause nosocomial infections or serious complications after surgery, childbirth in humans. with low body resistance.

In the air of hospital rooms, in addition to the usual indicators, determine the presence of pathogenic staphylococci, as well as *Pseudomonas aeruginosa* and other gram-negative opportunistic bacteria, depending on **the class of cleanliness of the room:**

• **especially clean (A)** - operating rooms for childbirth, aseptic boxes;

• **clean (B)** - procedural, intensive care units and wards, children's wards, assistant and packing pharmacies, premises of bacteriological and clinical laboratories;

• **conditionally clean (B)** - wards of surgical departments, corridors leading to operating rooms, maternity wards, boxes and wards of infectious wards, offices, etc .;

• **dirty (D)** - corridors and rooms of administrative buildings, sanitary rooms, toilets, rooms for dirty linen, etc. For indoor air that is classified as dirty (class D), sanitary-microbiological indicators are not normalized.

The main indicators of the sanitary condition of the air:

1. The total microbial count "-number of units m / o forming (CFU), in 1 m3 in the air.
2. The presence of Staphylococcus aureus - air is inoculated on yolk-salt agar, where grow characteristic colonies of gram-positive cocci having the enzyme.
3. The presence of beta-hemolytic streptococci.

Quantitative and qualitative composition of air microflora is studied by different methods. The simplest method of determining air microflora is sedimentation. The aspiration method developed by Yu.A. Krotov is much more exact.

Sedimentation method of air examination

The sedimentation method was proposed by Robert Koch and consists in the ability of microorganisms under the action of gravity and under the influence of air movement (along with dust particles and aerosol droplets) to settle on the surface of the nutrient medium in open Petri dishes. Cups are installed at sampling points on a horizontal surface. When determining the total microbial contamination, the plates with meat peptone agar are left open for 5-10 minutes or longer depending on the degree of suspected bacterial contamination. After exposure, all cups are closed, placed in a thermostat for 24 hours for cultivation (Fig. 82.).

Fig. 82. Sedimentation method of air examination

Investigation of air microflora by aspiration (the method of Yu. A. Krotov's)

In sterile Petri dishes pour molten sterilized simple nutrient medium and leave for 5-10 minutes to solidify. After that, the Petri dishes with nutrient medium are fixed on the movable disk of the Krotov's apparatus, and the device is turned on.

Due to the rotation of the centrifugal fan, air is drawn into the apparatus through a wedge-shaped slit and enters the surface of the solid nutrient medium in the Petri dish. The rotation of the cup ensures uniform inoculation of microbes. The amount of air that passes through the device, learn from the manometer.

The air flow rate can be adjusted in the range of 20-50 liters per 1 minute. The inoculated media are incubated at 37 ° C for 2 days. The number of microbial colonies grown on the nutrient medium is calculated and analyzed visually and microscopically.

Knowing the amount of air passed and the time of sampling, determine the total volume of air from which to sow. The number of colonies grown in a Petri dish determines the number of bacteria in 1 cubic meter of air.

To quantify microorganisms in the air, the following calculations are performed:

Example of calculation: missed 100 liters of air (25 liters per minute), the number of colonies that have grown, 80 in 1 cubic meter of air contains:

$$X = \frac{80 \times 1000}{100} = 800. \quad (1)$$

Bacteriology od Food

Food poisoning is an acute digestive disease that occurs when eating food, massively inoculated with microorganisms or containing toxic substances of bacterial or nonbacterial nature.

Unlike intestinal infections, food poisoning is not contagious, ie it is not transmitted from a sick person to a healthier person, only those who ate poor quality food.

These diseases occur suddenly and usually end quickly, hence the name 'outbreaks'. Outbreaks appear to be exacerbated within a few days after the product or food product that caused the illness

Food poisoning can be divided into the following groups:

- the first - food poisoning of microbial etiology;
- the second - food poisoning of nonbacterial etiology;
- the third - of an unspecified etiology.

Poisoning of microbial etiology, in turn, is divided into toxic infections and toxicosis (bacteriotoxicosis, mycotoxicosis), **nonbacterial etiology - into three subgroups:**

1) poisoning by products that are toxic in nature;

2) poisoning by products of plant and animal origin, which acquire toxic properties under certain conditions;

3) food poisoning.

Microorganisms that cause food poisoning are characterized by moderate pathogenicity to humans. Therefore, the disease occurs when the pathogen has previously multiplied in the food product and entered the human body in large quantities. The name 'toxicoinfection' is explained by the fact that poisoning is caused by the action of living microorganisms (infection), and its sudden onset and rapid short-term course resembles poisoning by toxins of these microorganisms (toxicosis). The causative agents of toxicoinfections are microorganisms of the genus Salmonella (*S. typhimurium*, *S. enteritidis*), some species of *Escherichia coli*, *Proteus*, *Streptococci*, *Clostridium perfringens* and *Bacillus cereus.*

Methods of sanitary-bacteriological research are direct bacterioscopy, bacterial culture on pathogenic and conditionally pathogenic flora on nutrient medium, biological test.

21. HUMAN MICROBIOME

All the microorganisms are not harmful for example, our body surface contains different varieties of microorganisms in a large number, which are provides benefits to our body.

The normal microbiota of a human body is not static. They are started to colonize in our body after birth, and they constantly change with the human ages.

When a new-born comes in contact with the environment, microorganisms instantly started to colonize in the body surface. After that, the number of microorganisms is started to increase constantly with the human age.

Types of Normal Microflora

1.Bacteria. The initial studies show that an individual contains thousands of bacteria on their body surface and vital organs. The mouth and the gut portion of the human body contains a large number of bacteria, which is greater than the bacterial population of Skin and vaginal sites. There are present different species of bacteria that are found in the human body, which are vary based on the type of organs.

2.Archaea. Archaea mainly found in the gut portion of the human body. The species of archaea is much more limited in our body.

3.Fungi. They are mainly found at the gut portion and skin of the human body.

4.Viruses. Viruses, including bacterial viruses (bacteriophages), are colonize in our various body parts, such as skin, gut, lung, oral cavity, etc.

Microorganisms are present in different parts of our body such as skin, eye, nose, mouth, gastrointestinal tract, etc. (Fig. 83.). We discussed the normal flora of each body part in below.

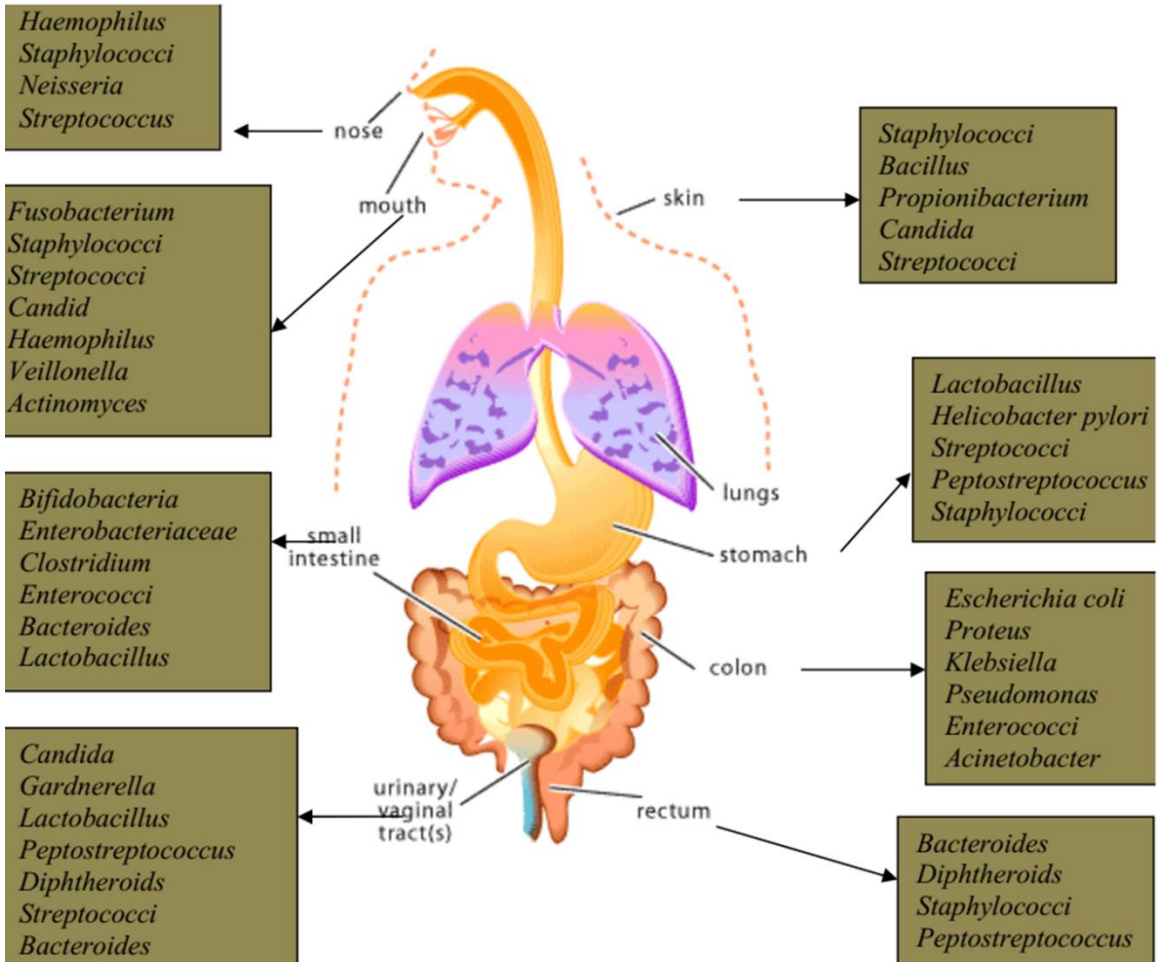


Fig. 83. Normal flora of the human body

TYPES OF NORMAL FLORA

1. **Resident Flora**
    - Microbes that are always present
    - Consists of relatively fixed types of microorganisms regularly found in a given area at a given age
    - If disturbed, it promptly reestablishes itself
2. **Transient Flora**
    - Microbes that live in or on your body for a period of time (hours, days, weeks, months) then move on or die off
    - Consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, or days

Beneficial Functions of Normal Flora

1. Protect our organs and systems that are in direct contact with the outer environment from invading pathogens. Some normal flora produces substances that kills pathogens and others complete for with them for nutrients (Fig. 84.).
2. In newborns, normal flora stimulates the development of immune systems.
3. Normal flora of the gut provides important nutrients such as Vitamin K which aid in digestion and absorption of nutrients.

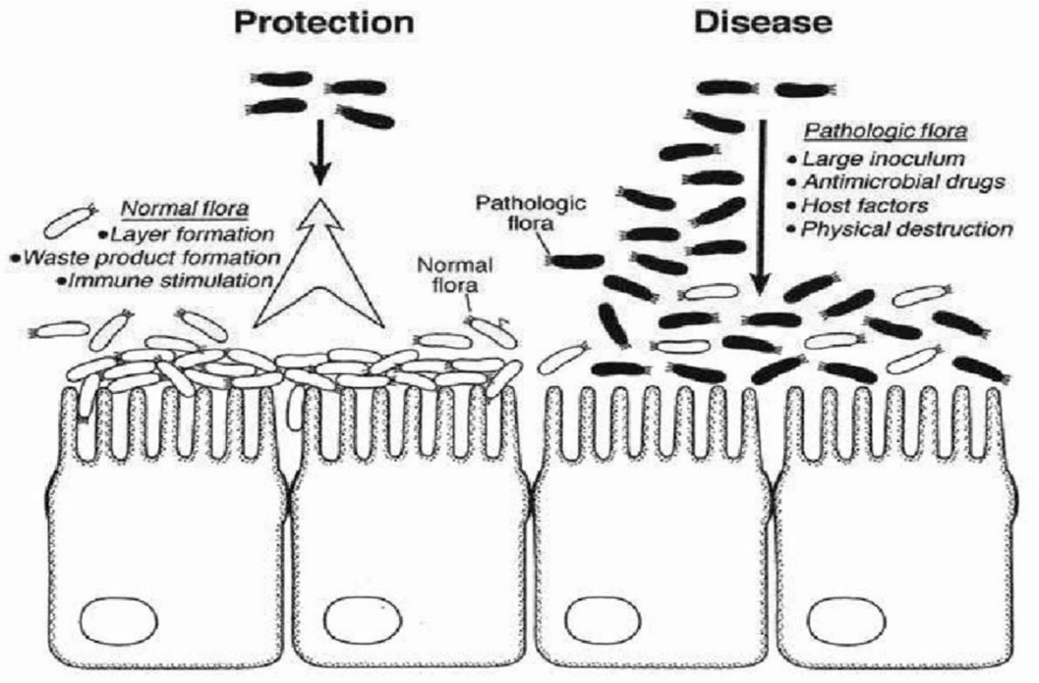


Fig. 84. Function of Normal Flora and Pathologic flora

MICROBIOME OF THE HUMAN SKIN

There are present different types of bacteria on our skin surface. The diversity of bacteria is vary based on the different locations of our skin. Our skin is divided into three parts: the dry portion, the moist portion, and sebaceous (containing sebum).

The dry portions of our skin include forearm, buttocks, hands, etc. This portion contains the greatest diversity of gram-negative and gram-positive bacteria, such as *Actinobacteria*, *Bacteroides*, *Firmicutes*, and *Proteobacteria*.

The moist areas of our skin include umbilicus, underarm, inguinal and gluteal creases, and inside the elbow, etc. These portions of the skin exhibit less diversity of microorganisms. It contains mostly some firmicutes and actinobacteria such as *Staphylococcus* and *Corynebacterium spp*.

The oily sebaceous sites contain the lowest diversity of bacteria including most members of actinobacteria (*Propionibacterium spp*.). The oily portions of our skin include the forehead, behind the ear, and the back.

Some occasionally pathogenic bacteria are present in our skin surface such as:

Staphylococcus epidermidis, S. aureus, S. warneri, Streptococcus pyogenes, S. mitis, Cutibacterium acnes, Corynebacterium spp., Acinetobacter johnsonii, Pseudomonas aeruginosa.

Except bacteria, our skin also contains more than 14 different genera of fungi, including: dermatophytes (skin living fungi) such as *Microsporum gypseum*, and *Trichophyton rubrum* and nondermatophyte fungi (opportunistic fungi that can live in the skin) such as *Rhizopus stolonifer, Trichosporon cutaneum, Fusarium, Scopulariopsis brevicaulis, Curvularia, Alternaria alternata, Paecilomyces, Aspergillus flavus* and *Penicillium* species.

More microorganisms on areas of skin covered with hair. All microorganisms feed on dead epithelial cells, sebaceous and sweat glands, and so on. The breakdown products of sweat under the influence of bacteria give it an unpleasant odor. In addition to the characteristic **automicroflora** of the skin, **transit microorganisms** can be detected, which quickly disappear under the influence of bactericidal properties of the skin. Upon contact with the soil, the skin is contaminated with spores of various microorganisms, especially the hands. In such cases, *Clostridium tetani, Escherichia coli, Staphylococci, Streptococci, Fungi* are found on the skin.

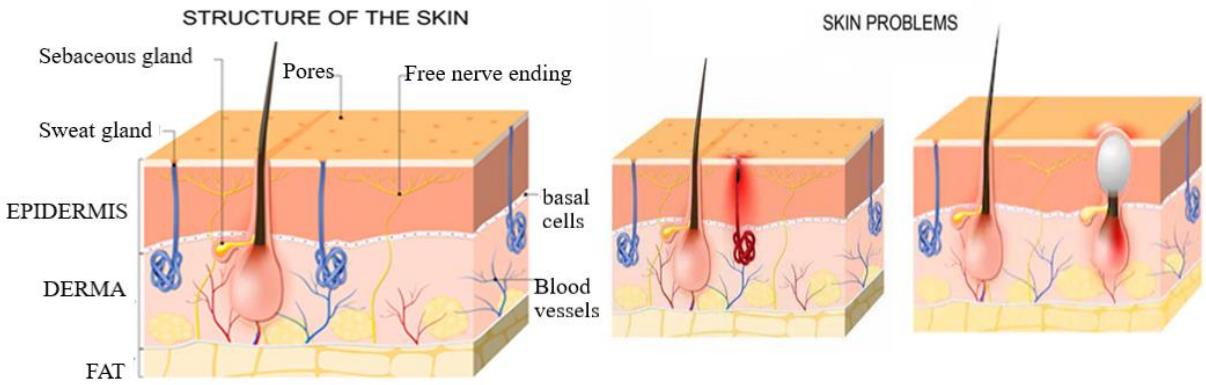


Fig. 85. Structure of the skin and example of skin problems

The skin (epidermis) is not a very good place for the existence of microorganisms.

This is due to a number of factors:

1) The skin is washed periodically. Lack of moisture leads to the transition of microorganisms in the resting phase. However, in some places (head, ear, urogenital and anal areas) moisture is sufficient for the development of microorganisms.

2) The skin has a weakly acidic pH, which is created by organic acids produced by normal staphylococci, secretions of sweat and sebaceous glands (pH 4 ÷ 6) (Fig. 85.).

3) Sweat contains high concentrations of NaCl. This makes the skin surface hyperosmotic and causes osmotic stress in most microorganisms.

4) Certain antibacterial substances in the skin help control colonization, growth and infections caused by transiting microorganisms. For example, the sweat glands secrete lysozyme, which lyses Staphylococcus epidermidis and other gram-positive bacteria.

METHODS OF EXAMINATION SKIN

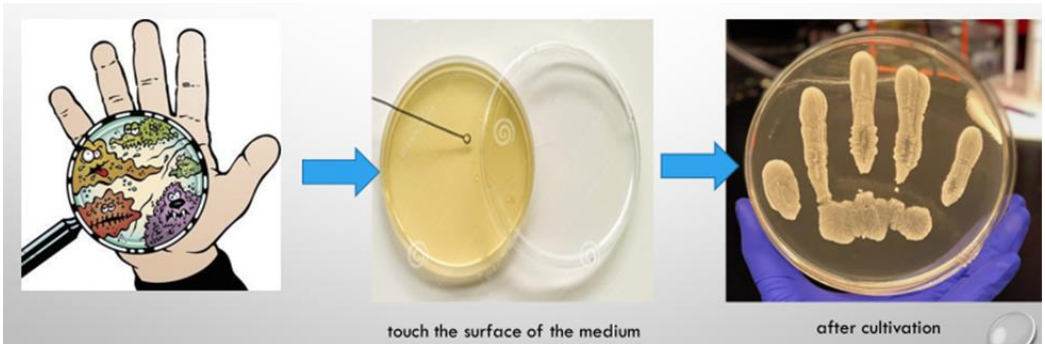


Fig. 86. Print method of examination skin

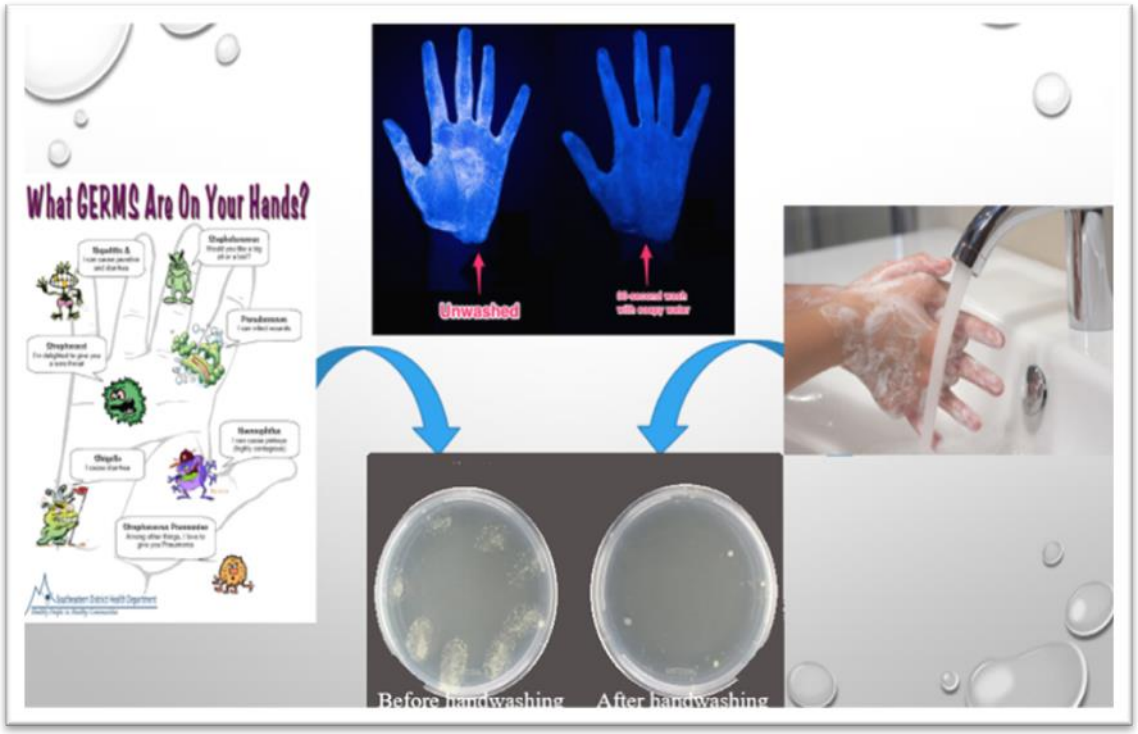


Fig.87. Results of examination skin by Print method

Material for the study of healthy or pathologically altered skin is often taken with a sterile cotton swab, for a more accurate study of the skin microflora using the method of scrubbing Williams and Kligman. In response to the doctor indicate the total microbial contamination of the studied area of the body: high - more than 10^6 medium - 10^3-10^5, low - less than 10^3 CFU / cm^2; types of isolated microorganisms; state of microbiocenosis (eubiosis, dysbacteriosis).

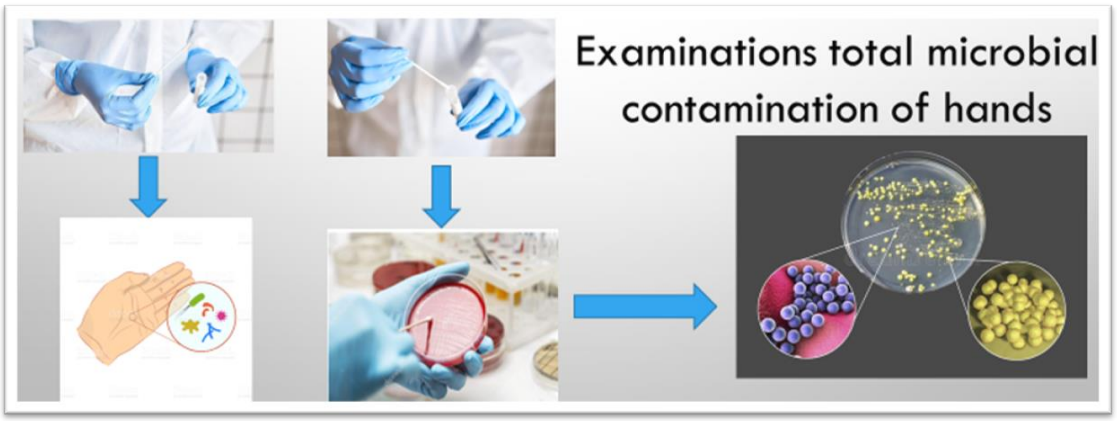


Fig. 88. Examinations total microbial contamination of hands

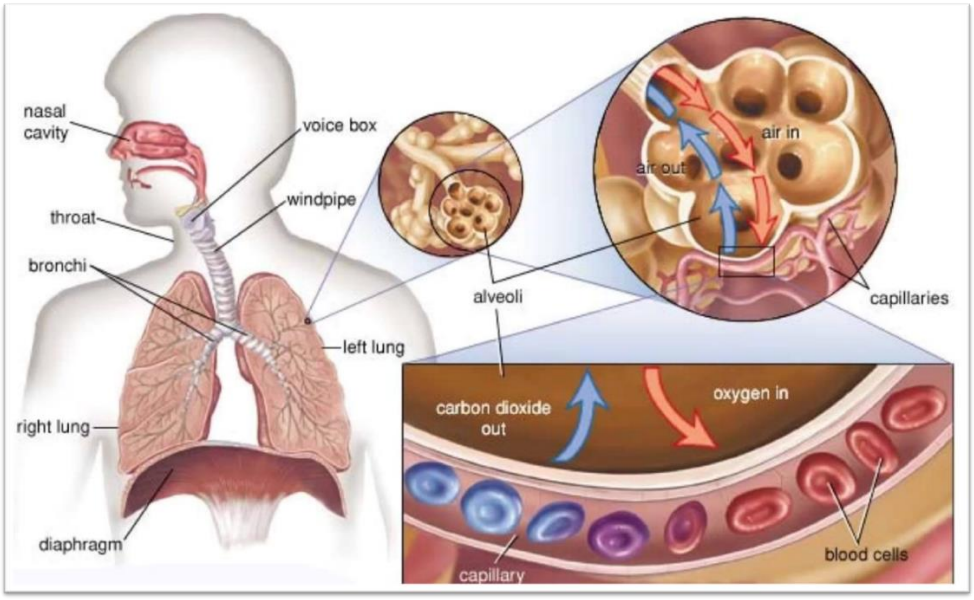


Fig. 89. Respiratory system

Normal Microflora of Nose, and Pharynx. The nasopharynx is a part of the pharynx lying above the level of the soft palate. The normal flora of Pharynx is including some potential pathogenic bacteria such as *Streptococcus pneumonia*, *Neisseria meningitides*, and *Haemophilus influenzae*. Also, a large number of non-pathogenic Gram-positive bacteria are commonly found in both the nose and nasopharynx.

Normal flora of Mouth. The mouth has an optimum environment (water, nutrients, neutral pH, and moderate temperature) which will help in the growth of Microorganisms. The mouth portion of the human body contains mostly the genera of some *Streptococcus*, *Neisseria*, *Actinomyces*, *Veillonella*, and *Lactobacillus*, as well as some yeasts.

Normal flora of Throat. The microbial flora of throat is including non-hemolytic and alpha-hemolytic streptococci, some *Neisseria* species, *Staphylococci*, diphtheria and Hemophilus organisms, pneumococci, yeasts, and Gram-negative rods.

Normal flora of Respiratory Tract. Our lower and upper portion of the respiratory tract is free of microorganisms because there are three important reasons such as; A continuous stream of mucus entraps the microorganisms, and the ciliated epithelial cells continually move the entrapped microorganisms out of the respiratory tract. Alveolar macrophages destroy the microorganisms. A bactericidal effect is exerted by the enzyme lysozyme, present in the nasal mucus. The enzyme lysozyme (nasal mucus) shows a bactericidal effect that kills all the bacteria.

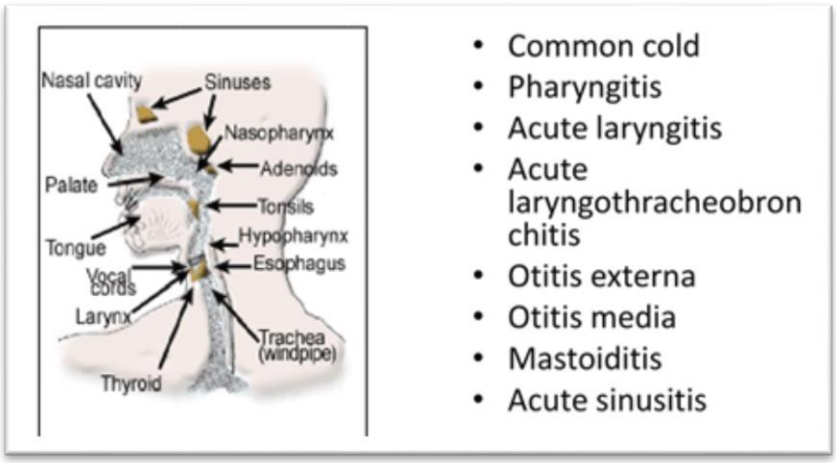


Fig. 90. Upper Respiratory Tract Infections

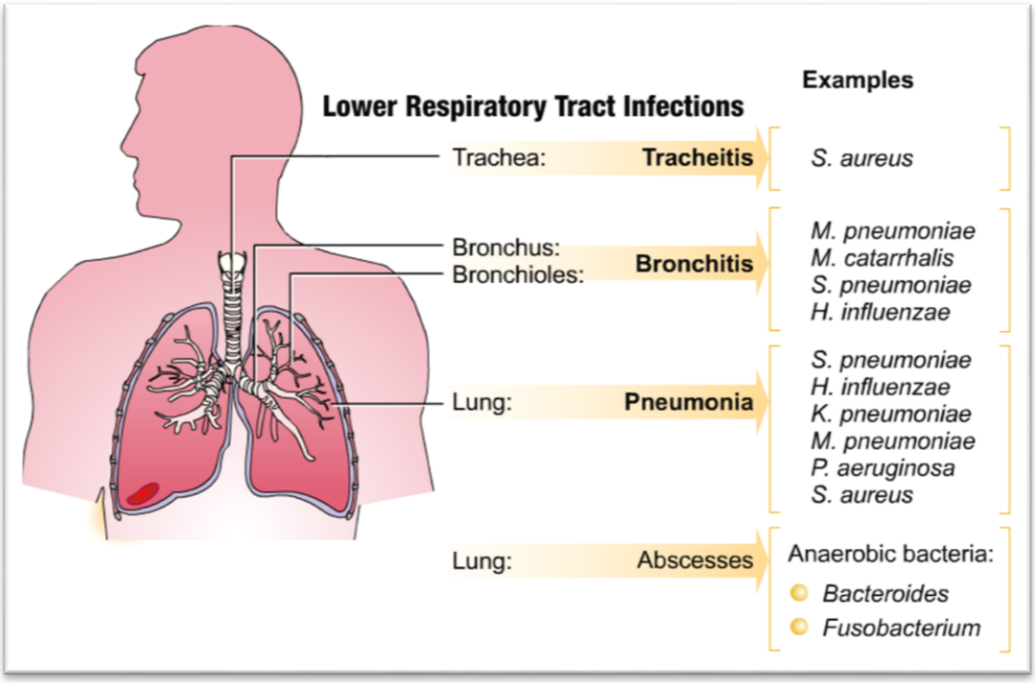


Fig. 91. Lower Respiratory Tract Infections

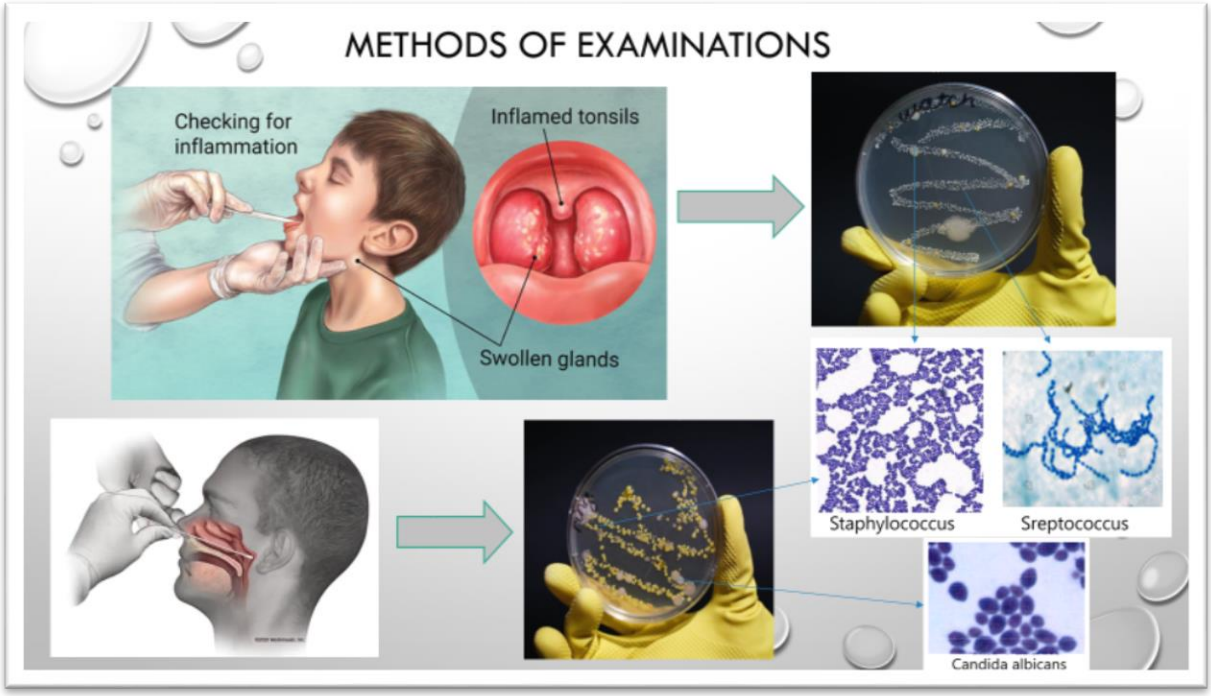


Fig. 92. Methods of examinations of Upper Respiratory Tract Bacterial Infections

The microflora of the oral cavity includes bacteria: *Actinomycetes*, *Fungi*, *Protozoa*, *Spirochetes*, *Rickettsia*, *Viruses*. It should be noted that a significant part of the microorganisms of the oral cavity of adults are anaerobic species.

The largest group of bacteria that live permanently in the oral cavity are cocci - 85-90% of all species. They have significant biochemical activity, decompose carbohydrates, break down proteins to form hydrogen sulfide.

Streptococci are the main inhabitants of the oral cavity: *S. mutans*, *S. mitis*, *S. sanguinis*. Most of them are facultative anaerobes, but there are also obligate anaerobes (*Peptococci*). With significant enzymatic activity, *Streptococci* ferment carbohydrates by type of lactic acid fermentation with the formation of a significant amount of lactic and some other organic acids. Acids produced by

streptococci inhibit the growth of some putrefactive microorganisms that enter the oral cavity from the environment.

In plaque and on the gums of healthy people there are also *Staphylococci - S. epidermis*, sometimes *S. aureus*.

Rod-shaped *Lactobacilli* also constantly vegetate in a certain amount in a healthy oral cavity. Like *Streptococci*, they produce lactic acid. Under aerobic conditions, *Lactobacilli* multiply much worse than in anaerobic ones, because they emit hydrogen peroxide, but not catalase. Lactic acid formed by them in the course of life delays growth of other microorganisms: *Staphylococci*, intestinal, typhoid and dysenteric sticks. The number of *Lactobacilli* in the oral cavity in dental caries increases significantly in proportion to the size of carious lesions.

In the mouth of healthy people in 40-50% of cases there are yeast-like fungi of the genus *Candida* (*C. albicans, C. tropicalis, C. krusei*). They have the appearance of oval or elongated cells with a size of 7-10 μm, often with a new branching cell. Pathogenic properties are most pronounced in *C. albicans*. Yeast-like fungi, multiplying intensively, can cause candidiasis or local lesions of the oral cavity (in children it is called thrush). These diseases are endogenous in nature and occur as a result of uncontrolled self-medication with broad-spectrum antibiotics. In this case, the growth of normal bacterial microflora is inhibited, and the growth of fungi of the genus *Candida* is sharply enhanced.

Spirochetes inhabit the oral cavity from the moment of appearance of temporary teeth in a child and since then become its permanent residents. *Spirochetes* are very mobile, perform bending, rotational, rectilinear and contractile movements. They are easiest to detect by microscopy of the native drug in a dark field. *Spirochetes* are strict anaerobes. They constantly multiply in the oral cavity with a significant reproduction of other anaerobic microorganisms and cause pathological processes in association with some strains of *Fusobacteria, Vibrio*. Many *Spirochetes* are found in ulcerative-necrotic lesions of the mucous membrane (ulcerative stomatitis, Vincent's sore throat), in pathological gingival pockets in severe forms of periodontitis, in carious foci and necrotized pulp.

In 50% of healthy people in the mouth are *Entamoeba gingivalis, Trichomonas*. They are localized mainly in dental plaque, crypts of tonsils, in purulent contents of periodontal pockets, intensively multiplying at unhygienic contents of an oral cavity.

Diseases Caused by the Resident Oral Microflora

Disease	Important microorganisms
Plaque-induced diseases	
Periodontal diseases	Gram-negative rods, spirochaetes
Dental caries	*Streptococcus mutans, Lactobacillus* spp.
Denture-induced stomatitis	*Candida albicans*
Opportunistic oral infections	
Pulpitis	
Periapical abscess	
Periodontal abscess	Mixtures of several facultative and obligate anaerobes
Pericoronitis	
Wound infection	
Opportunistic infections in other organs	
Aspiration pneumonia	
Lung abscess	Mixtures of facultative and obligate anaerobes, or single isolates comprising streptococci, actinomyces, Gram-negative rods
Liver abscess	
Brain abscess	
Infective endocarditis	

CONJUNCTIVA

A small number of bacteria are present in eye including: *Corynebacterium* species, *Moraxella spp, Neisseria spp, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridians.*

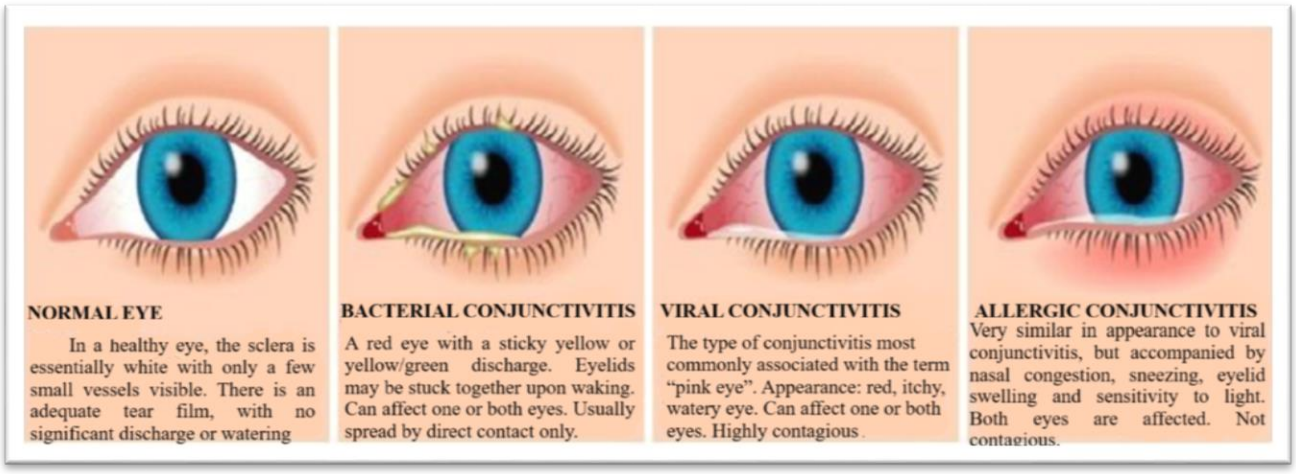


Fig. 93. Comparison of different types of conjunctivitis

GASTROINTESTINAL TRACT

Due to the presence of very low acidic pH (2 or 3), the **stomach** contains few bacteria including *Streptococcus, Staphylococcus, Lactobacillus, Peptostreptococcus spp*.

The **small intestine** also contains several bacteria such as: *Enterococcus faecalis, Lactobacilli, Diphtheroid*, and the yeast *Candida albicans* are occasionally found.

The **large intestine** contains a huge population of microorganisms. More than 400 species of bacteria were isolated from human feces. Most of them are anaerobic, Gram-negative bacteria, and Gram-positive rods. Al those bacteria belong to the genera of *Bacteroides, Clostridium, Faecalibacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus,* and *Bifidobacterium*. There also present several fungi such as *Candida, Saccharomyces, Aspergillus, Penicillium, Rhodotorula, Trametes, Pleospora, Sclerotinia, Bullera*, and *Galactomyces*.

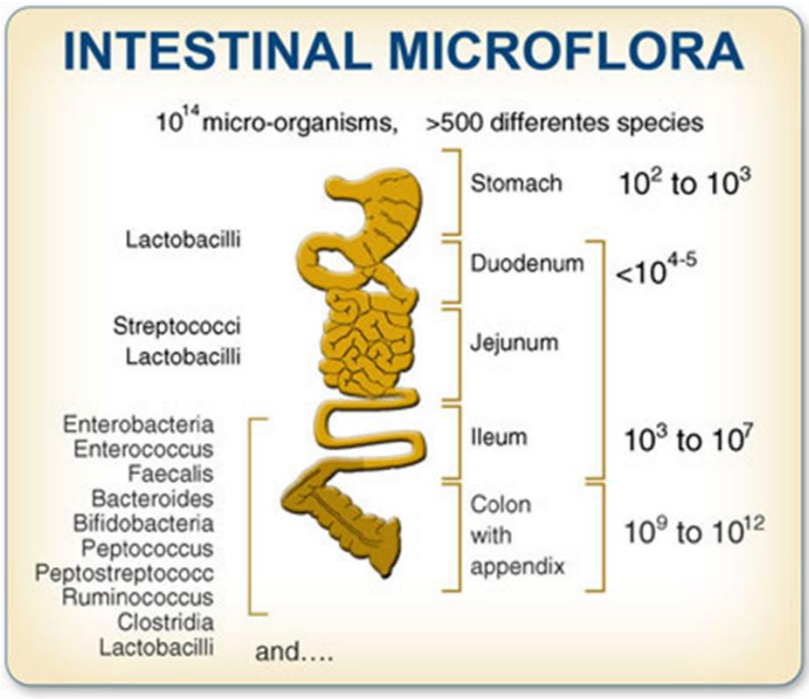


Fig. 94. Intestinal microflora

Beneficial flora are *Bifidobacterium* and *Lactobacilli*, which are responsible for the normal functioning of the intestines. Also, these beneficial bacteria protect the human body from the penetration of pathogenic foreign microbes and toxins, and, accordingly, promote the absorption of vitamins, digestive processes, as well as strengthen the immune system.

If the gastrointestinal tract is functioning normally, the intestinal microflora has a balance of pathogenic and beneficial microbes and bacteria. There are not many bacteria in the human stomach, as it has an acidic environment, their number is 103 species, the largest number of bacteria is located in the large intestine, their number is about 1013 species. If the balance of beneficial and pathogenic bacteria is disturbed, it leads to dysbacteriosis and other diseases.

Fig. 95. Example of Intestinal Microflora

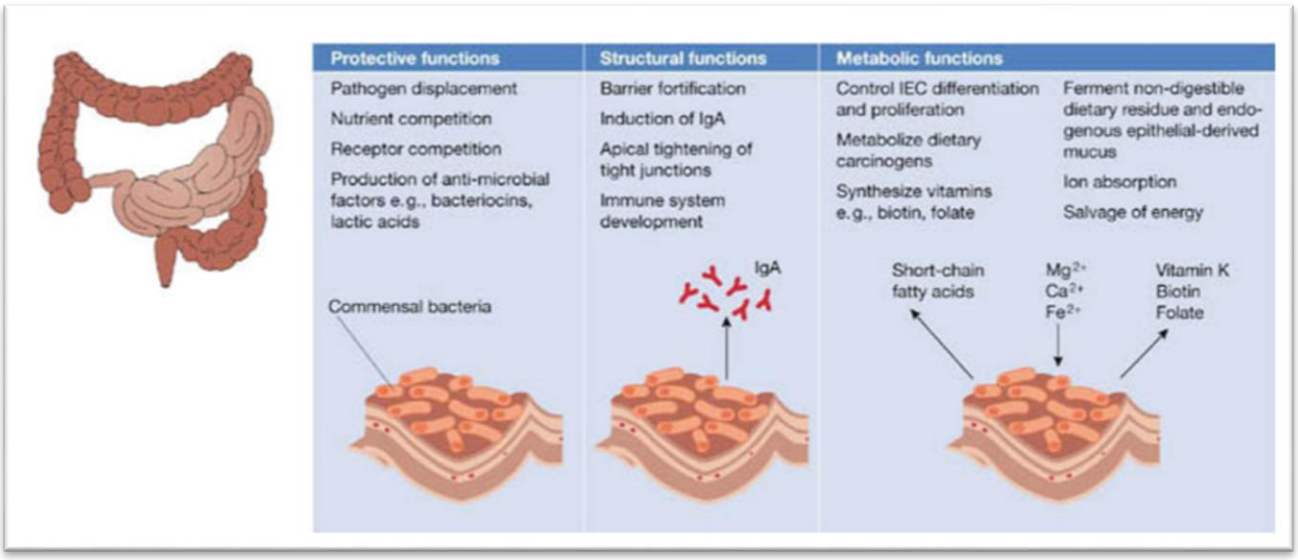


Fig. 96. Roles of commensal bacteria

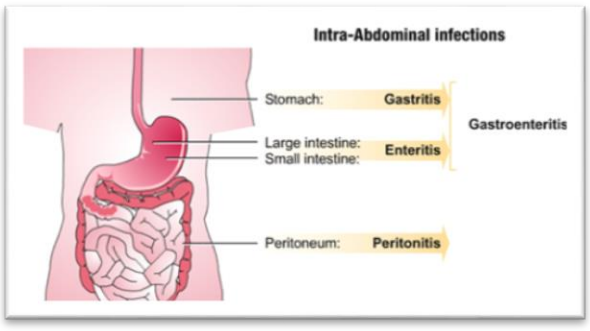


Fig. 97. Intra-Abdominal Infections

Normal Flora of the Urogenital Tract. Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms have problems gaining access and becoming established. The flora of the anterior urethra, as indicated principally by urine cultures, suggests that the area may be inhabited by a relatively consistent normal flora consisting of *Staphylococcus epidermidis*, *Enterococcus faecalis* and some alpha-hemolytic *Streptococci*. Their numbers are not plentiful, however. In addition, some enteric bacteria (e.g. *E. coli*, *Proteus*) and *Corynebacterium*, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.

The vagina becomes colonized soon after birth with *Corynebacterium*, *Staphylococci*, *Streptococci*, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). During reproductive life, from puberty to menopause, the vaginal epithelium contains glycogen due to the actions of circulating estrogens. Doderlein's bacillus predominates, being able to metabolize the glycogen to lactic acid. The lactic acid and other products of metabolism inhibit colonization by all except this *Lactobacillus* and a select number of lactic acid bacteria. The resulting low pH of the vaginal epithelium prevents establishment by most other bacteria as well as the potentially-pathogenic yeast, *Candida albicans*. This is a striking example of the protective effect of the normal bacterial flora for their human host.

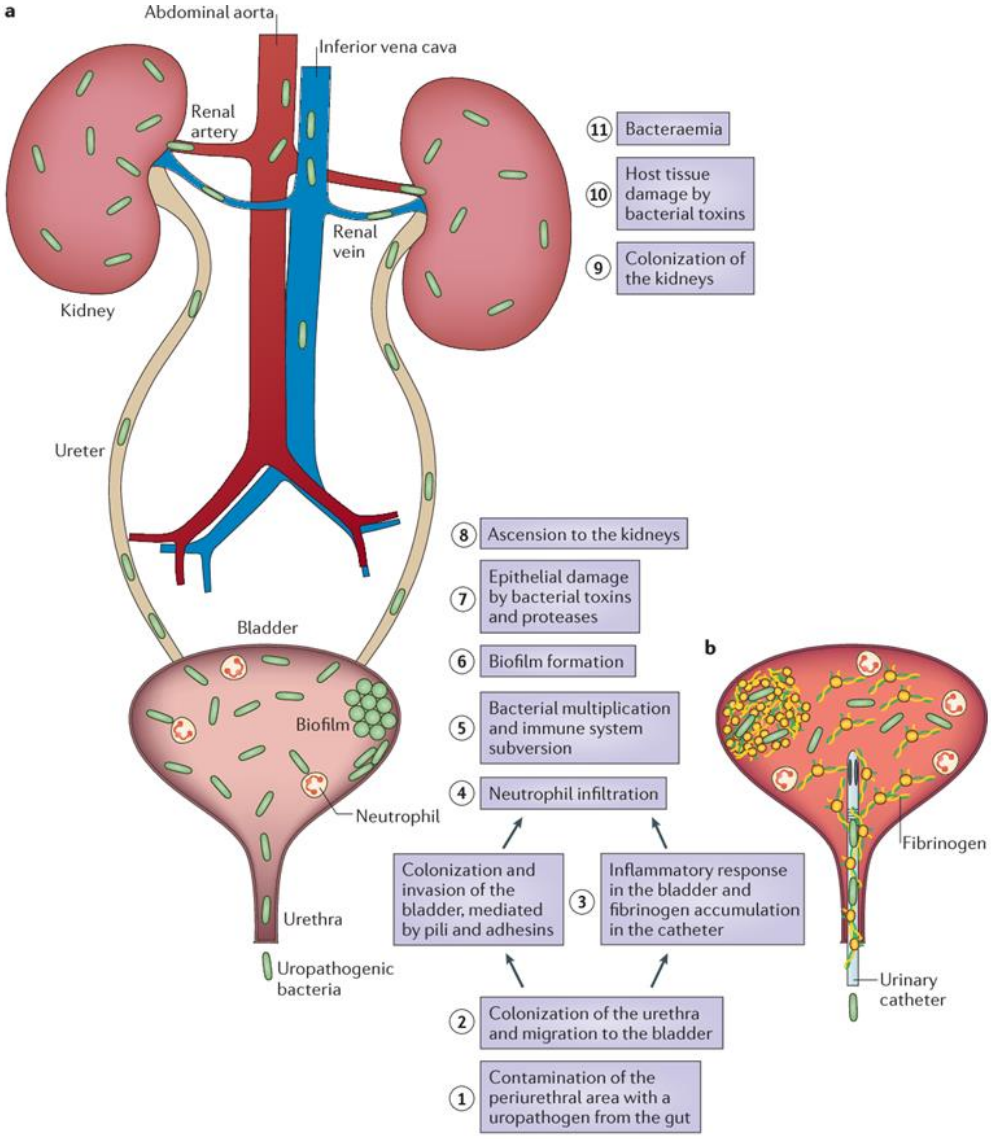


Fig. 98. Spread of Urinary tract infections

Urinary tract infections are caused by microorganisms — usually bacteria — that enter the urethra and bladder, causing inflammation and infection. Though a UTI most commonly happens in the urethra and bladder, bacteria can also travel up the ureters and infect kidneys. More than 90% of bladder infection (cystitis) cases are caused by *E. coli*, a bacterium normally found in the intestines.

PROBIOTICS and PREBIOTICS

Probiotics are live bacteria and yeasts that are good for you, especially your digestive system. We usually think of these as germs that cause diseases. But your body is full of bacteria, both good and bad. Probiotics are often called "good" or "helpful" bacteria because they help keep your gut healthy. Most often, probiotics contain such useful - "friendly" bacteria as *Lactobacilli* and *Bifidobacterium*.

Prebiotics are compounds in food that induce the growth or activity of beneficial microorganisms such as bacteria and fungi. The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

When to use probiotics?

- When using antibiotics, which in one way or another inhibit the activity of beneficial intestinal bacteria.
- After the transferred diseases for the purpose of improvement of resistance of an organism. Since immune cells are formed in the intestine, the general state of immunity directly depends on the state of the microbiocenosis.
- With manifestations of dysbacteriosis (dysbiosis), such as diarrhea, constipation, bloating, flatulence (adults and children).
- In the complex treatment of intestinal infections to eliminate intoxication and diarrhea.
- For other needs, including a change in diet.
- When traveling, especially in exotic countries (lack of quality drinking water, stress, unsanitary conditions)

DYSBIOSIS

Dysbiosis is a disruption of the normal balance of microflora in the organs. In other words, in case of dysbacteriosis abnormal ratio of useful and opportunistic bacteria is observed.

Signs and symptoms of Gut Dysbiosis:

- Abdominal discomfort or indigestion that worsens after eating
- Bloating and/or distention
- Abdominal pain
- Excessive gas
- Inconsistent bowel movement patterns — e.g. diarrhea, constipation, or mixed patterns
- Acid reflux

Correction of dysbiosis disorders is carried out with the help of replacement bacteriotherapy based on the use of probiotics (Fig. 98.). Among the drugs in this area there are 3 main groups. The **first group - probiotics**, which contain live, specially selected strains of microorganisms of one or more cultures of lactic acid bacteria from the genera *Lactobacillus* or *Bifidobacterium*. The **second group - prebiotics** - drugs of non-microbial origin, which contain specific substances that stimulate the growth and development of normal intestinal microflora. **The third group - symbiotics,** which contain live microorganisms in a mixture with food additives and metabolites of these bacteria, which provides them with a symbiotic effect. Among probiotics, special attention should be paid to multiprobiotics - new generation drugs.

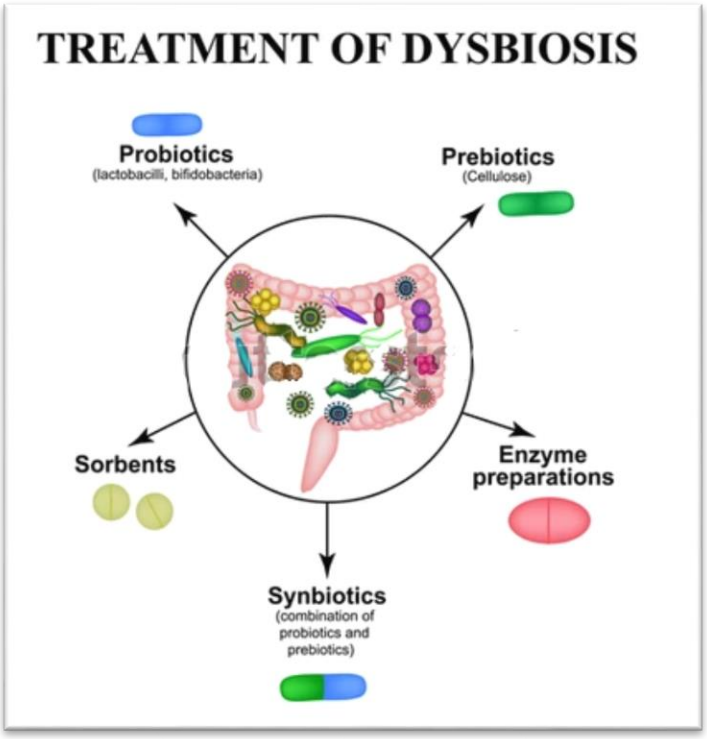


Fig. 99. Treatment of dysbiosis

22. MICROBIAL GENETIC

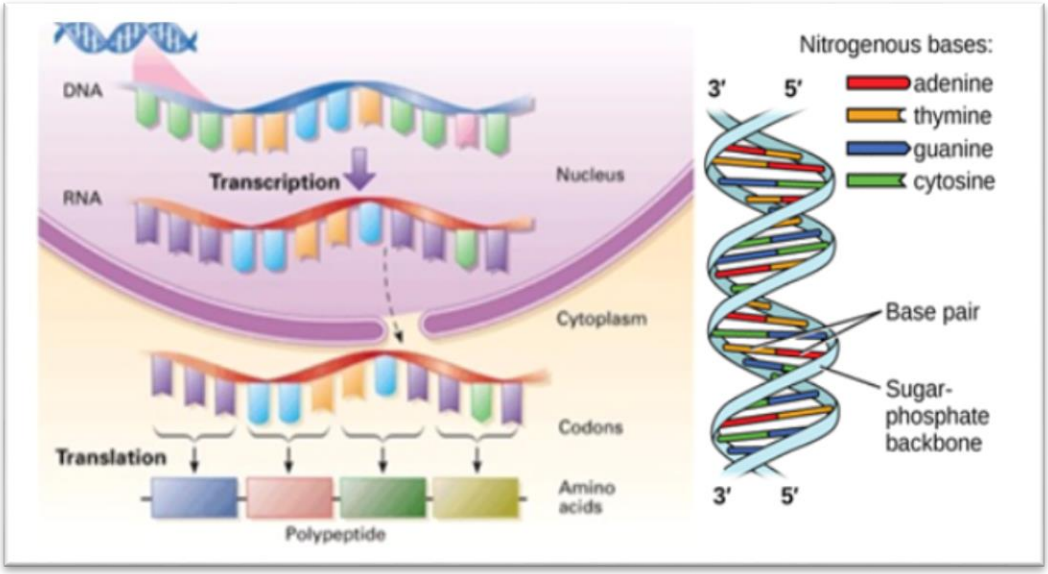


Fig. 100. DNA replication Transcription and Translation

Bacterial cells do not have a nucleus. Their nucleus is found in the cytoplasm so, the process of transcription and translation are coupled and occur in the cytoplasm. While in eukaryotes, DNA is confined to the nucleus and the process of transcription occurs in the nucleus only. DNA moves to the cytoplasm for the process of translation.

In prokaryotes, transcription and translation can occur simultaneously in the cytoplasm of the cell, whereas in eukaryotes transcription occurs in the nucleus and translation occurs in the cytoplasm. There is only one type of prokaryotic RNA polymerase whereas eukaryotes have 3 types.

Bacterial transcription is the process in which a segment of bacterial DNA is copied into a newly synthesized strand of messenger RNA (mRNA) with use of the enzyme RNA polymerase. The process occurs in 3 main steps: initiation, elongation, and termination; and the end result is a strand of mRNA that is complimentary to single strand of DNA (Fig. 101.).

Unlike in eukaryotes, bacterial transcription and translation can occur simultaneously in the cytoplasm. This is impossible in eukaryotes, where transcription occurs in a membrane-bound nucleus while translation occurs outside the nucleus in the cytoplasm.

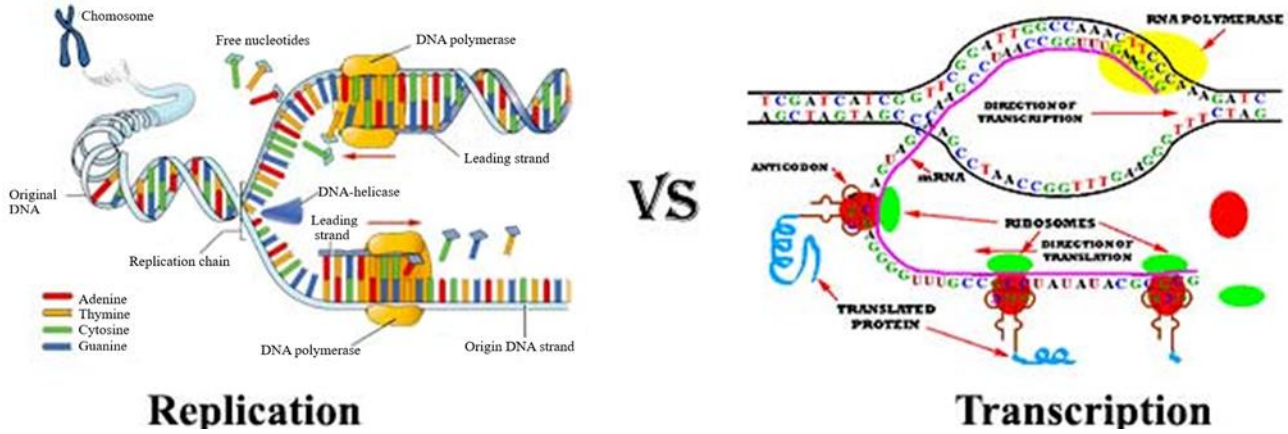


Fig. 101. Difference Between Replication and Transcription

Changes in the genetic code: mutations and recombination

Mutation, an alteration in the genetic material (the genome) of a cell of a living organism or of a virus that is more or less permanent and that can be transmitted to the cell's or the virus's descendants. (The genomes of organisms are all composed of DNA, whereas viral genomes can be of DNA or RNA; see heredity: The physical basis of heredity.) Mutation in the DNA of a body cell of a multicellular organism (somatic mutation) may be transmitted to descendant cells by DNA replication and hence result in a sector or patch of cells having abnormal function, an example being cancer. Mutations in egg or sperm cells (germinal mutations) may result in an individual offspring all of whose cells carry the mutation, which often confers some serious malfunction, as in the case of a human genetic disease such as cystic fibrosis. Mutations result either from accidents during the normal chemical transactions of DNA, often during replication, or from exposure to high-energy electromagnetic radiation (e.g., ultraviolet light or X-rays) or particle radiation or to highly reactive chemicals in the environment. Because mutations are random changes, they are expected to be mostly deleterious, but some may be beneficial in certain environments. In general, mutation is the main source of genetic variation, which is the raw material for evolution by natural selection.

Mutations in DNA occur for different reasons. For example, environmental factors, such as exposure to ultraviolet radiation or certain chemicals, can induce changes in the DNA sequence. Mutations can also occur because of hereditary factors.

Mutation hotspots – are segments of DNA that are especially prone to genetic alteration. The increased susceptibility of these areas of DNA to mutation is attributed to interactions between mutation-inducing factors, the structure and function of the DNA sequence, and enzymes involved in DNA repair, replication, and modification.

Changes within genes are called **point mutations**. The simplest kinds are changes to single base pairs, called base-pair substitutions. Many of these substitutes an incorrect amino acid in the corresponding position in the encoded protein, and of these a large proportion result in altered protein function. Some base-pair substitutions produce a stop codon. Normally, when a stop codon occurs at the end of a gene, it stops protein synthesis, but, when it occurs in an abnormal position, it can result in a truncated and nonfunctional protein. Another type of simple change, the **deletion** or **insertion** of single base pairs, generally has a profound effect on the protein because the protein's synthesis, which is carried out by the reading of triplet codons in a linear fashion from one end of the gene to the other,

is thrown off. This change leads to a frameshift in reading the gene such that all amino acids are incorrect from the mutation onward. More-complex combinations of base substitutions, insertions, and deletions can also be observed in some mutant genes.

Mutations that span more than one gene are called chromosomal mutations because they affect the structure, function, and inheritance of whole DNA molecules (microscopically visible in a coiled state as chromosomes). Often these chromosome mutations result from one or more coincident breaks in the DNA molecules of the genome (possibly from exposure to energetic radiation), followed in some cases by faulty rejoining. Some outcomes are large-scale deletions, duplications, inversions, and translocations (Fig. 102.).

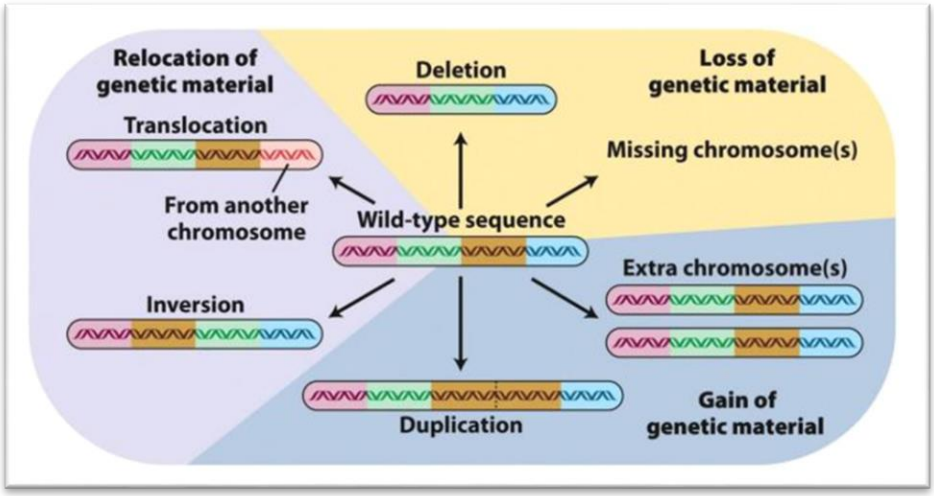


Fig. 102. Types of Chromosome Mutations

Genetic Recombination in Bacteria

This is a process where genetic materials, contained in two separate genomes, are brought together within one unit. In bacteria the recombination takes place by (1) transformation, (2) transduction and (3) conjugation.

TRANSFORMATION

The genetic transfer in bacteria also occurs by transformation in which the DNA molecule of the donor cell, when liberated by its disintegration, is taken up by another recipient cell and its offspring inherit some characters of the donor cell. When different strains of bacteria are found in a mixed stage either in culture or in nature, some of the resultant offspring possess a combination of characters of the parent strains. This phenomenon is known as **recombination.**

The phenomenon of transformation was first recorded by Griffith (1928). Avery, Macleod and McCarty (1944) demonstrated that the transforming principle being DNA in the sequence of events in bacterial transformation.

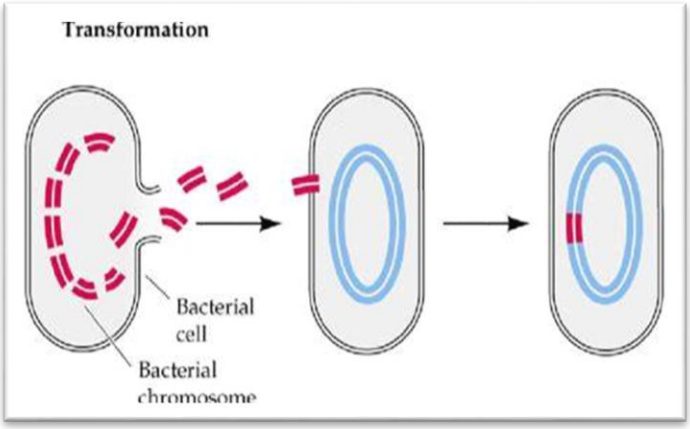


Fig. 103. Transformation process

Frederick Griffith, established that there was a transforming principle in bacterial genetics in a ground-breaking experiment, performed in 1928.

He postulated that information could somehow be transferred between different strains of bacteria. This was long before the discovery of DNA and was an inspired piece of scientific detective work.

For this study, Griffith used two strains of Pneumococcus bacteria (Fig. 104.), type III-S and type II-R.

There is one major difference between these two types; the III-S strain has a smooth polysaccharide coat which makes it resistant to the immune system of mice, whereas the II-R strain lacks this coat and so will be destroyed by the immune system of the host.

For the first stage of the transforming principle experiment, Griffith showed that mice injected with III-S died but when injected with II-R lived and showed few symptoms.

The next stage showed that if the mice were injected with type III-S that had been killed by heat, the mice all lived, indicating that the bacteria had been rendered ineffective.

The interesting results came with the third part of the experiment, where mice were injected with a mixture of heat killed III-S and live II-R.

Interestingly enough, the mice all died, indicating that some sort on information had been passed from the dead type III-S to the live type II-R. Blood sampling showed that the blood of the dead mice contained both live type III-S and live type II-R bacteria.

Somehow the type III-S had been transformed into the type III-R strain, a process he christened the transforming principle.

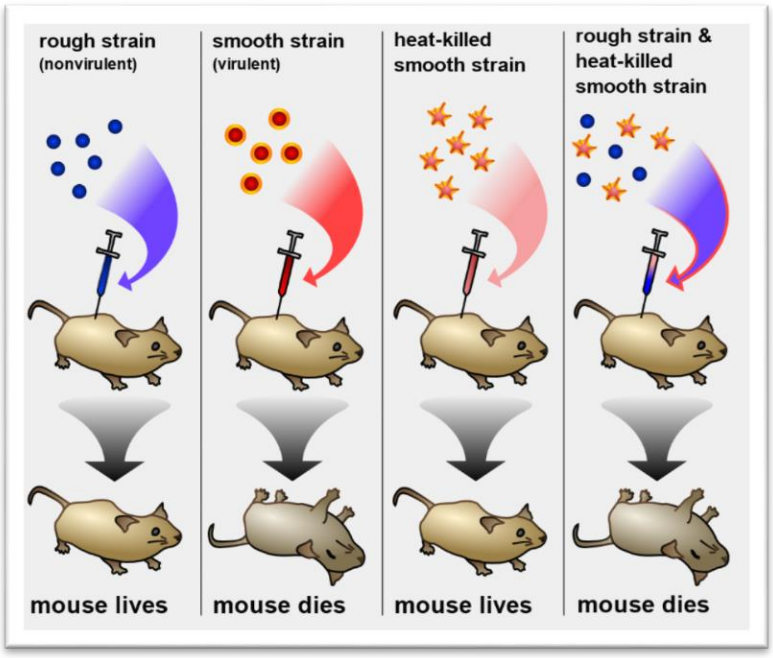


Fig. 104. Griffith's experiment

TRANSDUCTION

The genetic transfer in bacteria is achieved by a process known as transduction (Fig. 105.). Laderberg and Zinder's (1952) experiment in U-tube Salmonella typhimurium indicated that bacterial viruses or phages are responsible for the transfer of genetic material from one to the other lysogenic and lytic phages. Thus the host acquires a new genotype. Transduction has been demonstrated in many bacteria.

In this process, the DNA molecule that carries the hereditary characters of the donor bacterium is being transferred to the recipient cell through the agency of the phage particle.

When a bacterial cell is being infected with a temperate virus either lytic-cycle or lysogeny starts. Thereafter, host DNA breaks down into small fragments along with the multiplication of virus. Some of these DNA fragments are incorporated with the virus particles becoming transducing one.

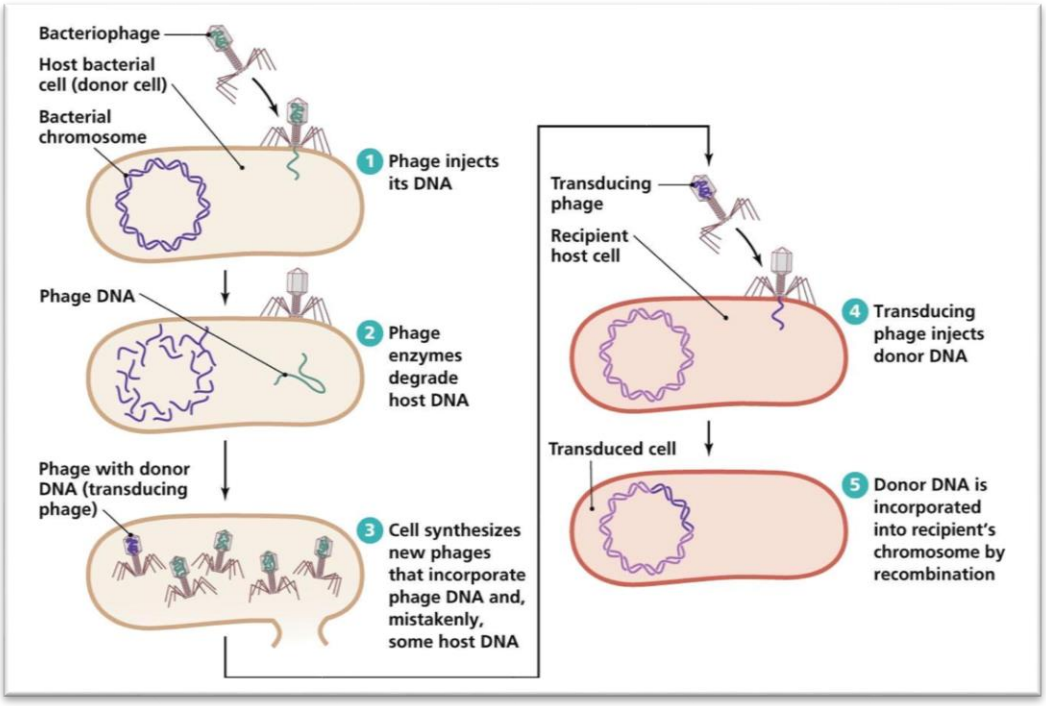


Fig. 105. Transduction process

When bacteria lyse these particles along with normal virus particles are released when this mixture of transducing and normal virus particles is allowed to infect the population of recipient cells, most of the bacteria are infected with normal virus particles and with the result lysogeny or lytic-cycle occurs again. A few bacteria are infected with transducing particles, transduction takes place and the DNA of virus particles undergo genetic Recombination with the bacterial DNA.

CONJUGATION

Wollman and Jacob (1956) have described conjugation in which two bacteria lie side by side for as much as half an hour. During this period of time a portion of genetic material is slowly passed from one bacterium which is designated as a male to a recipient designated as a female. This was established that the male material entered the female in a liner series (Fig. 106.).

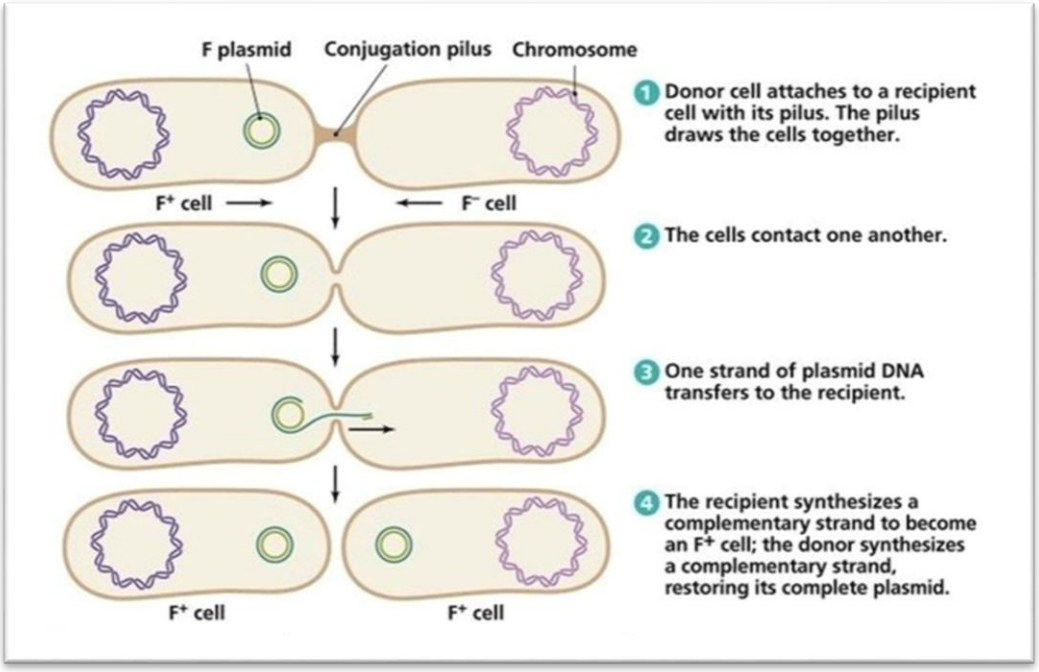


Fig. 106. Bacterial Conjugation process

The genetic recombination between donor and recipient cells takes place as follows: The Hfr DNA after leaving apart in fragment to recipient cell again reforms in circular manner. In F-strain genetic recombination takes place between donor fragment and recipient DNA. Gene transfer is a sequential process and a given Hfr strain always donates genes in a specific order. A single stranded donor DNA (F factor) is integrated into the host chromosome with the help of nuclease enzyme.

Plasmids as extrachromosomal DNA

Plasmids are small pieces of circular extrachromosomal DNA in bacteria (Fig. 107.). They are able to copy themselves inside bacterial cells independently from the chromosome (self-replicating) and exist in multiple copies. Some can integrate into the chromosome. They can transfer from one cell to another via cell-to-cell contact.

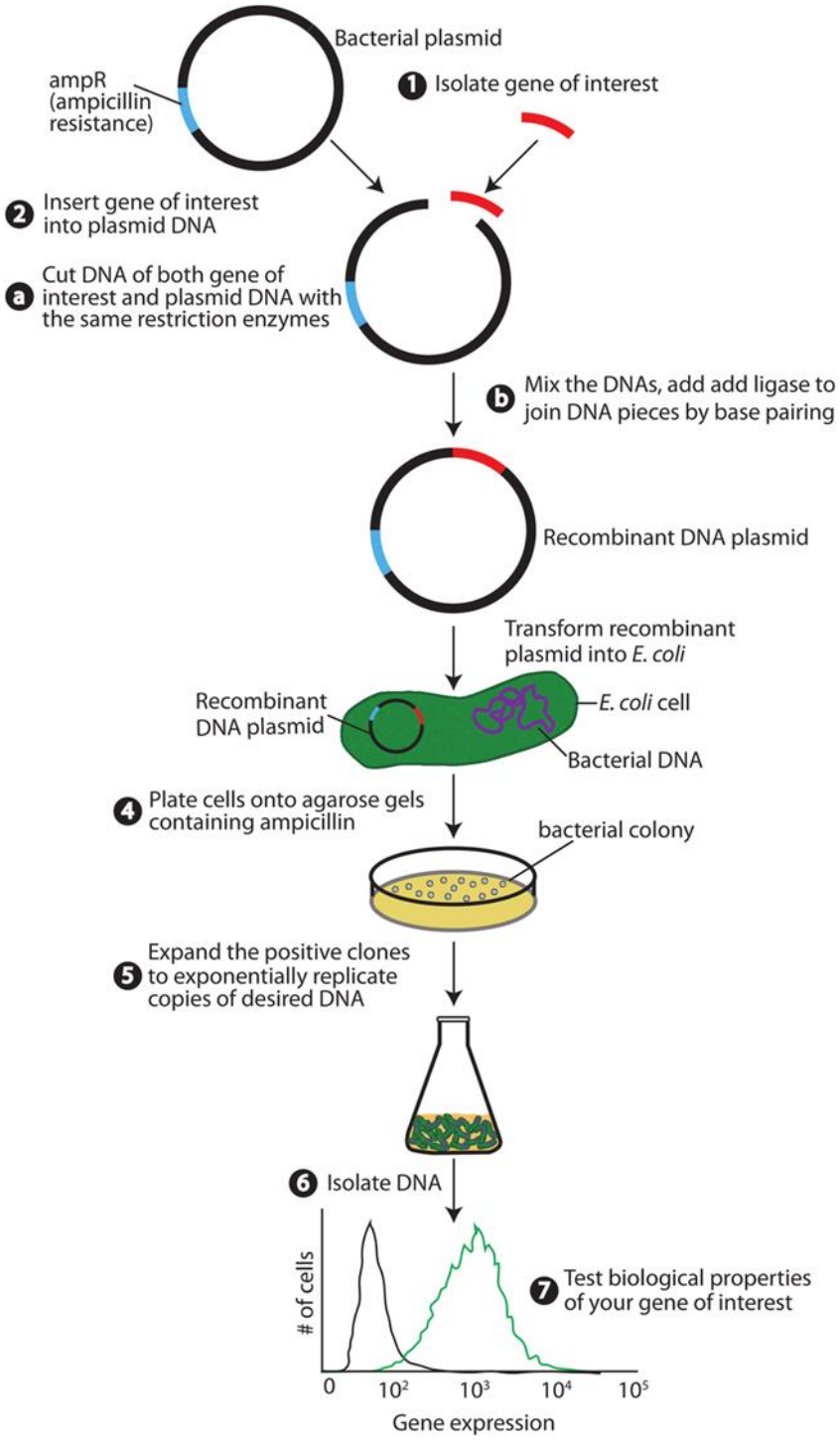


Fig. 107. Plasmids as extrachromosomal DNA

Basic elements and applications of genetic engineering

Genetic engineering, also called genetic modification or genetic manipulation, is the direct manipulation of an organism's genes using biotechnology. It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. New DNA is obtained by either isolating and copying the genetic material of interest using recombinant DNA methods or by artificially synthesising the DNA.

Medical applications of DNA technology:

1. Basic research – understanding of structure and functions of DNA and proteins.
2. Diagnosis of diseases – genetic and microbial.

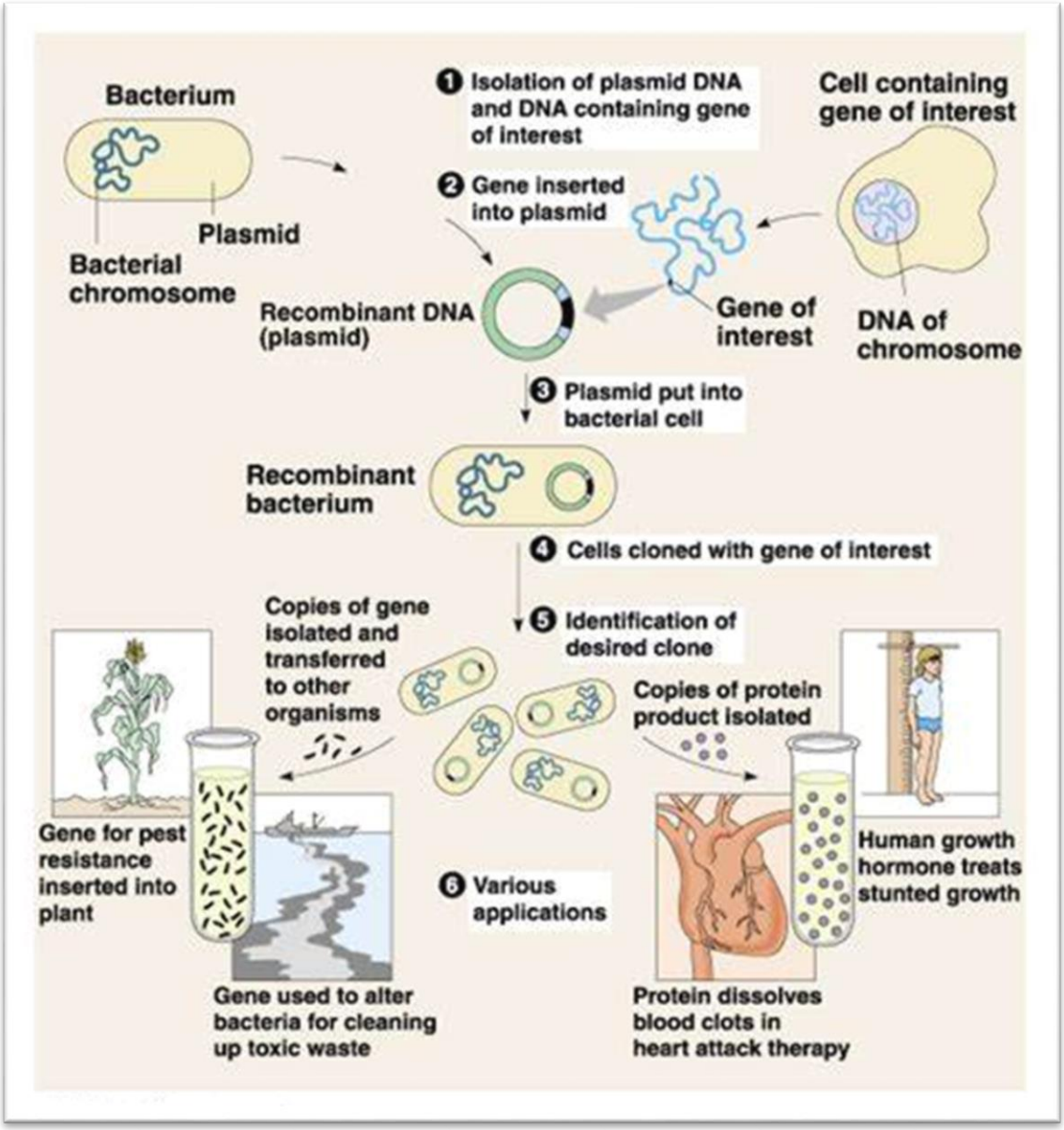


Fig. 108. Example of Genetic Ingineering

Genetic engineering has been applied in numerous fields including research, medicine, industrial biotechnology and agriculture. In research Genetically Modified Organisms (GMO) are used to study gene function and expression through loss of function, gain of function, tracking and expression experiments. By knocking out genes responsible for certain conditions it is possible to create animal model organisms of human diseases. As well as producing hormones, vaccines and other drugs, genetic engineering has the potential to cure genetic diseases through gene therapy.

23. THE ELEMENTS OF CHEMOTHERAPY

The path to drug approval involves drug discovery and drug development. In drug discovery, compounds are screened for favorable biological activity against a target(s) and lead compounds are chosen to be moved forward through drug development. Drug development evaluates these candidates for toxicity and ADME properties. An important part of preclinical drug development is to define the dose and schedule for Phase I clinical trials (First In Man).

Before conducting in vivo PK and ADME studies to determine the dosing schedule, the dose has to be defined. There are typically five options for defining a dose: Maximum Tolerated Dose (MTD), Maximum Feasible Dose (MFD), limit dose (1000mg/kg), exposure saturation, and dose providing a 50-fold margin of exposure. The most common of these, the **Maximum Tolerated Dose** (MTD) – is defined as the highest dose of a drug that does not cause unacceptable side effects or overt toxicity in a specific period of time. These side effects can range from mild effects such as reduced weight gain, moderate effects such as weight loss up to 20% or substantial effects such as unresponsiveness. The MTD can be determined by acute toxicity studies, short duration dose escalation studies and dose ranging studies. These studies are designed with a minimum number of animals and include toxicological endpoints such as clinical observations and clinical pathology, for example blood tests for liver function. This maximum tolerated dose is then used for longer-term safety assessments. The rationale for using the MTD in long term studies is to maximize the likelihood of detecting any chronic disease effects or other hazards of a drug candidate. It is also more humane to determine the MTD before conducting any PK or ADME studies to minimize animal morbidity. Maximum tolerated dose studies are not designed to cause mortality, therefore death is not an appropriate end point.

Chemotherapeutical index – the ratio of the minimal effective dose of a chemotherapeutic agent to the Maximum Tolerated Dose (MTD).

Chemotherapeutical index - The ratio of the toxicity of a drug, expressed as the maximum tolerated dose per kilogram of body weight to the minimal curative dose per kilogram of body weight. This index is used in judging the safety and effectiveness of drugs.

The concept **of minimal infective dose** (MID) has traditionally been used for bacteria that contaminate foods that cause infection in or from the digestive tract. MID was defined as the number of bacteria ingested (the dose) from which a pathology is observed in the consumer. Examples such as this are to cause gastrointestinal disorders, the food must contain more than 100,000 Salmonella per gram. However, in such a formulation, we immediately see an inaccuracy: to know the dose ingested, concentration is not enough.

It is also necessary to know the mass of the portion:

$$\boxed{D=c*m}$$

where:D= number of bacteria i.e. dose;

c= concentration of bacteria;

m= mass.

24. CHEMICAL AGENTS FOR MICROBIAL CONTROL.

Chemical agents for microbial control:

- Alcohol
- Phenol and phenolic compounds
- Halogen compounds
- Heavy metals and their compounds
- Aldehydes
- Gaseous agents
- Detergents
- Antibiotics

ALCOHOL

- Alcohols are antimicrobial agents. Germicidal action of alcohol increases with increase in molecular weight of alcohol.
- Ethanol is the most commonly used alcohol for controlling microorganisms.
- Ethanol between concentration of 50-90% are effective against vegetative cell. for practical purposes 70% ethanol is used.
- Alcohol causes death of organism by denaturing the cellular proteins.
- Alcohol is a lipid solvent that damages the lipid bilayer of cell membrane and cell wall. It is also a dehydrating agent and causes loss of water from cell.
- lcohol is commonly used as sanitizer on skin, disinfectant to clinical instruments, thermometers and surgical instruments.
- Concentration above 60% is effective in killing viruses.

Mode of action:

- When alcohol is used as disinfectant, it solubilizes the lipid bilayer of cell wall and membrane and creates pores. The remaining alcohol enters into the cytoplasm through the pore and denature the cellular proteins killing the bacteria.
- 70% alcohol is more effective than absolute (100%) alcohol because absolute alcohol only brings bacteriostasis.
- With increase in concentration of alcohol, both denaturing and lipid solubilizing power and dehydration power increases and are counter to each other. Absolute alcohol causes extreme dehydration resulting in shrinking of cell, hence further alcohol cannot enter the cell. Therefore it only brings bacteriostasis.
- However, 70% alcohol is very effective in dehydration and denaturation, almost equilibrium manner causing bacteriocidal effects.

PHENOL AND PHENOLIC COMPOUNDS

- Phenol have wide spectrum of antimicrobial action.
- Vegetative cell are more and rapidly killed by concentrated aqueous solution of phenol whereas bacterial spore are resistant.
- Usually 2-5% aqueous solution of phenol is used as disinfectant.
- Phenol has limited application because it is absorbed by skin and mucus membrane and causes toxicity.

Mode of action:

Phenol and phenolic compounds kills the microorganisms by varities of effects such as disruption of cell, precipitation of cellular protein, inactivation of enzymes and leakage of cellular materials.

HALOGEN COMPOUNDS:

1. Iodine:

- Iodine is used in many forms such as aqueous solution, tincture of iodine and iodophor.
- Aqueous iodine and tincture of iodine have some side effects such as staining and irritation. So, now a days Iodophore is as replacement because it has less side effects.

Uses:

- Iodine is effective against all kind of bacteria. it also possess sporicidal activity.
- Iodine is highly fungicidal and to some extent virucidal.
- Iodophore are widely used for antisepsis of skin, mucus membrane and wound.
- Iodine preparation can also be used for other purposes such as disinfection of water, air and sanitization of food utensils.

Mode of action:

- Iodine is powerful oxidizing agents and irreversibly oxidises the cellular materials.
- Iodine also brings halogenation of tyrosine residue of protein and enzymes and inactivates it.

2. Chlorine:

- Chlorine in the form such as Hypochlorite and chloramine is used as disinfectant. Free gaseous chlorine is difficult to handle, as it is corrosive and toxic.
- Calcium hypochlorite and sodium hypochlorite are commonly used.
- Aqueous solution of sodium hypochlorite (5.25%) is called house hold bleach.
- Chloramine is more stable than hypochlorite, so it is more effective germicidal than hypochlorites.

Uses:

- Cholrine is one of the commonly used water disinfectant.
- Calcium hypochlorite is used as sanitizer for cooking utensils.
- 1% bleach is used for personal hygiene eg. bathing water
- Higher concentration (5-12%) bleach is used in swimming pool, house hold purposes.

Mode of action:

- when hypochlorite or chloramine is added in water, free chlorine is releases which form hypochlorous acid (HClO).
- Hypochlorous acid decomposes to release nascent oxygen which is powerful oxidizing agent and kills the microorganisms by oxidizing the cellular components.
- chlorine and chlorine compounds also inactivates the proteins and enzymes by direct chlorination.

HEAVY METALS AND THEIR COMPOUNDS:

- Most of the heavy metals have antimicrobial action.
- Most effective and commonly use are Mercucry (Hg), Silver (Ag) and Cupper (Cu).

Mode of action:

- Heavy metals and their compounds combines directly with cellular proteins and enzymes and inactivates them.
- High concentration of heavy metals salts also coagulates and precipitates the cellular proteins and kills the microorganisms.

Some commonly used metal compounds are:

1. HgCl, HgCl2: used in ointments as antiseptic.
2. AgNO3: it is bacteriostatic as well as bactericidal. it is used in eye-drops to prevent Ophthalmia neonatarum in children.
3. Cupper suphate: it is widely used against algae and mold in swimming pool.

ALDEHYDES:

Formaldehyde and Gluteraldehyde are commonly used aldehydes. Both are highly microbicidal including sporicidal.

1. Formaldehyde:

- Formaldehyde is stable only in higher concentration and higher temperature. At room temperature, it polymerized to form para-formaldehyde.
- Formaldehyde is used in two form- gaseous formaldehyde and formalin (40%solution of formaldehyde).

Uses:

- Formaldehyde vapour either from formalin or paraformaldehdye is used for disinfection and sterilization of closed room, such as Operation theater.
- Formaldehyde vapour is also used to disinfect woolen blanket, wools and footwares of fungal infected person.
- Formalin is used for preservation of biological specimens.

2. Gluteraldehyde:

- Gluteraldehye is used in 2% solution.
- like formaldehyde it is effective against bacteria, fungi, spores and viruses.
- Gluterladehyde is used to sterilize Urological instruments and respiratory therapy instruments.

GASEOUS AGENTS:

Ethylene oxide, β-propiolactone and formaldehyde are commonly used gaseous sterilizing agents.

1. Ethylene oxide:

- It is gaseous above 10.8°C.
- Ethylene oxide have high antimicrobial activity, it kills even endospores.
- It is used for sterilization of heat sensitive materials such as spices, oils, plastics etc.
- Ethylene oxide is used in formulation with CO2 as Freon (CClFe).

2. β-Propiolactone:

- It is gas above 15.5°C.
- Penetration power of β-propiolactone is less than ethylene oxide but it is more active in killing microorganisms.
- Due to its carcinogenic effects, it is not commonly used.

DETERGENTS:

- Detergents are used primarily for leaning purposes but it has also antimicrobial properties.
- There are three types of detergents- Cationic detergent, anionic detergent and non-ionic detergent.
- Cationic detergents is more significant germicidal agent than other two.
- For example: Quaternary ammonium compound is a cationic detergent having germicidal action. It is more effective against Gram positive bacteria.
- Detergents are used as disinfectants, sanitizers and antiseptic. They are also used to disinfect hospital floor.

Mode of action:

- Detergents kills the microorganisms by denaturing proteins and enzymes and interfering with glycolysis
- Detergents also damages cell wall and cell membrane.

ANTIBIOTICS:

- Antibiotics are secondary metabolites produced by certain microorganisms which inhibits the growth of other microorganisms.
- Different groups of antibiotics have different mode of actions

Mode of action antibiotics:

- **Inhibits cell wall peptidoglycan synthesis:** Penicillin, Cephalosporin, Bacitracin, Vancomycin, Cycloserine;
- **Inhibit cell membrane biosynthesis:** Polymyxin, Polyenes, Gramicidin;
- **Inhibits protein synthesis:** Aminoglycosides, Macrolides, Azalides, Lincosamides, Tetracycline, Chloramphenical;
- **Reacts with nucleic acids:** Rifampin, Quinolone;
- **Inhibits folic acid synthesis**: Sulfonamide, Trimethoprim.

25. CLASSIFICATION OF ANTIBIOTICS

Antibiotics are the chemical therapeutic agents of microbial or synthetic or semi-synthetic origin which in lower concentration inhibit the growth of other microorganisms.

Classification of antibiotics:

- On the basis of chemical structure
- On the basis of origin
- On the basis of range of activity (spectrum of activity)
- On the basis of mode of action
- On the basis of effects of their activity
- On the basis of route of administration

Classification of antibiotics on the basis of chemical structure:

- Carbohydrate containing Antibiotics:
- Pure saccharides antibiotics: examples; Streptozotocin
- Aminoglycosides: examples; Streptomycin
- N/O glycosides: eg. Chromomycin
- Other: eg; Lincomycin
- Macrocyclic lactone antibiotics: eg. Erythromycin
- Quinolones antibiotics; eg. Fluroquinolone
- N-containing heterocyclic antibiotics: eg. Beta-lactum
- O-containing heterocyclic antibiotics: eg. Cycloserine
- Alicyclic antibiotics: eg. Cycloheximide
- Aromatic antibiotics (Nitrobenzene): eg. Chloramphenicol
- Aliphatic amine antibiotics: eg. Spermidine
- Peptide antibiotics: eg. Polymyxin, Bacitracin, Gramicidin

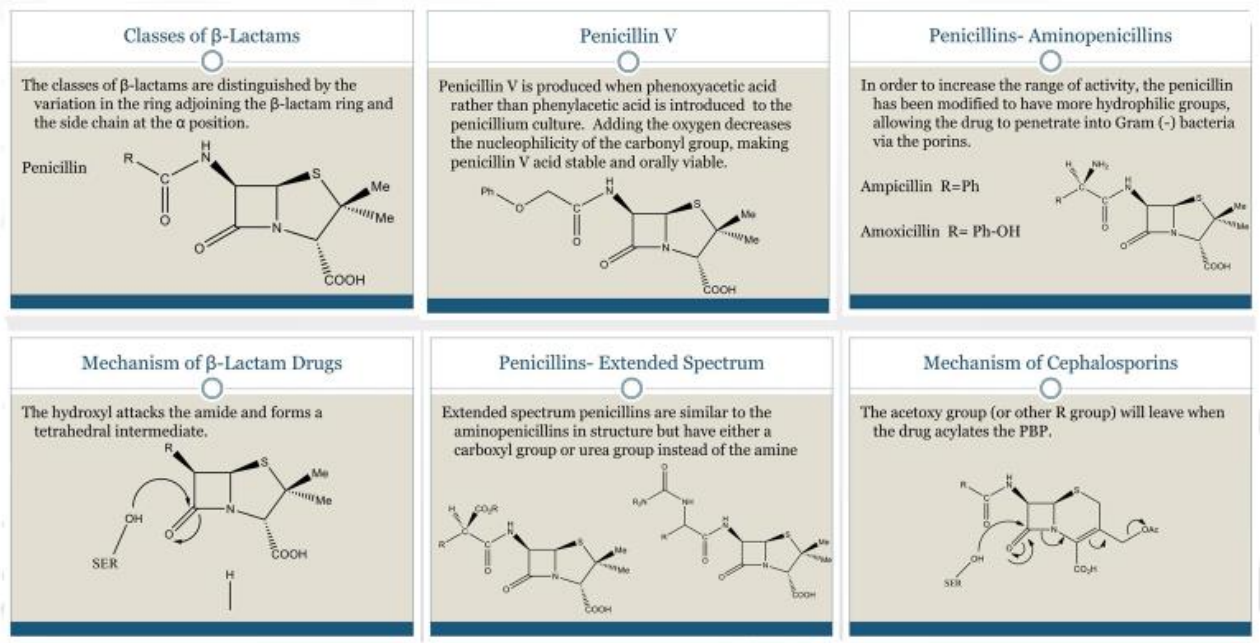


Fig. 109. Exemples of antibiotics on the basis of chemical structure

Classification of antibiotics on the basis of origin:

1.Microbial origin:

1). <u>Bacterial origin:</u>

- Bacillus polymyxa: Polymyxin
- Chromobacter violaceum: Bacitracin
- Micromonospora spp: Gentamycin

2). <u>Fungal origin:</u>

- Penicillium notatum: Penicillin
- Cephalosporin spp: Cephalosporin

3). <u>Actimomycetes origin:</u>

- Streptomyces griseus: Streptomycin
- S. venezuelue: Chloramphenicol
- S. erythreus: Erythromycin
- S. mediterranae: Rifampicin
- S. venezuelae: Chloramphenicol

2.Plant origin (Fig. 110.): Garlic, Echinacea, Eucalipt, Cabbag

Semi-synthetic antibiotics: Examples: Amoxycillin, Ampicillin, Doxycycline, Tigecycline, Sulfonamide etc

Synthetic antibiotics: Examples: Chloramphenicol (* it was extracted from Streptomyces venzuelae but now produced synthetically), 4-quinolones, Sulfonamide

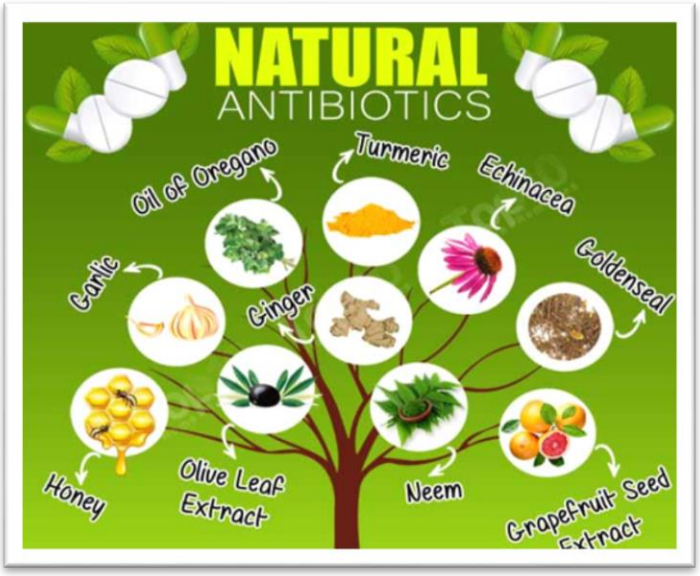


Fig.110. Examples of antibiotics on the basis of plant origin

Classification of antibiotics on the basis of Mode of action:

1. **Inhibitor of cell wall synthesis/Peptidoglycan Inhibitors**: Beta-lactum, Penicillin, Bacitracin, Cycloserine, Phosphomycin, Cephalosporin, Vancomycin.
2. **Inhibitor of protein synthesis**: Streptomycin, Aminoglycosides, Fusidic acid, Tetracycline, Mupirocin, Chloramphenicol, Macrolides.
3. **Inhibitor of Nucleic acid synthesis:** Quinolones, Ciprofloxacin, Nalidixic acid, Metronidazole, Nitrofurantoin.
4. **Inhibitor of folic acid synthesis (Folate antagonistic) :** Sulfonamide; Trimethoprim.
5. **Inhibitor of cytoplasmic membrane**: Polymyxin; Colistin.

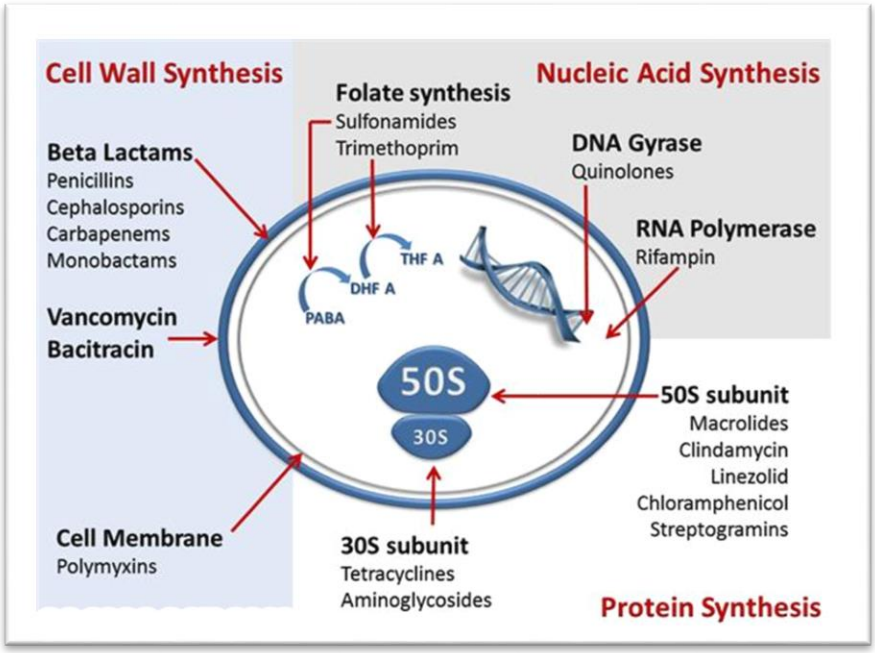


Fig. 111. Examples of antibiotics on the basis of Mode of action

Classification of antibiotics on the basis of effects of their activity:

1. Bactericidal: Kills bacteria

Examples: Aminoglycosides, Penicillin, Cephalosporin

2. Bacteriostatic: Inhibits the growth of bacteria

Examples: Sulfonamide, tetracycline,
chloramphenicol, trimethoprim, macrolides, Lincosamide

Classification of antibiotics on the basis of Route of administration:

1. Oral antibiotics: Acid stable antibiotics,

Examples; Penicillin V

2. Parenteral route: Intravenous administration

Examples; Penicillin G

Resistance to antimicrobial drugs, its acquisition by microorganisms.

- Antibiotics exert selective pressure on bacterial populations, killing susceptible bacteria while allowing strains with resistance to that particular antibiotic to survive and multiply. Traits for such resistance are then vertically passed on to daughter cells, subsequently creating a resistant population which can then spread and be further sources of resistance genes for other strains.
- Because resistance traits are not naturally eliminated or reversed, resistance to a variety of antibiotics may be accumulated over time. This can lead to strains with multiple drug resistance, which are more difficult to kill due to reduced treatment options.

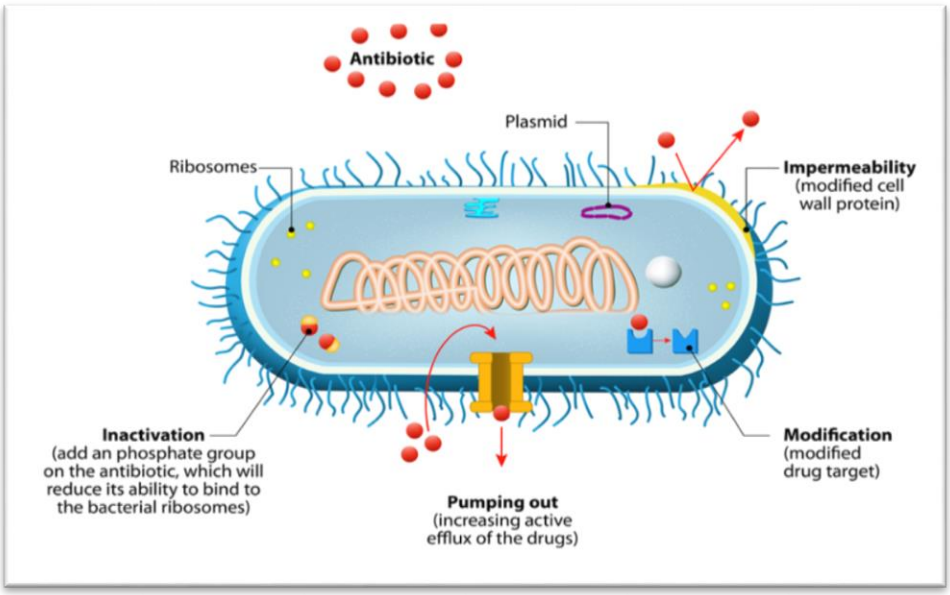


Fig. 112. Mehanisms of antimicrobial resistense

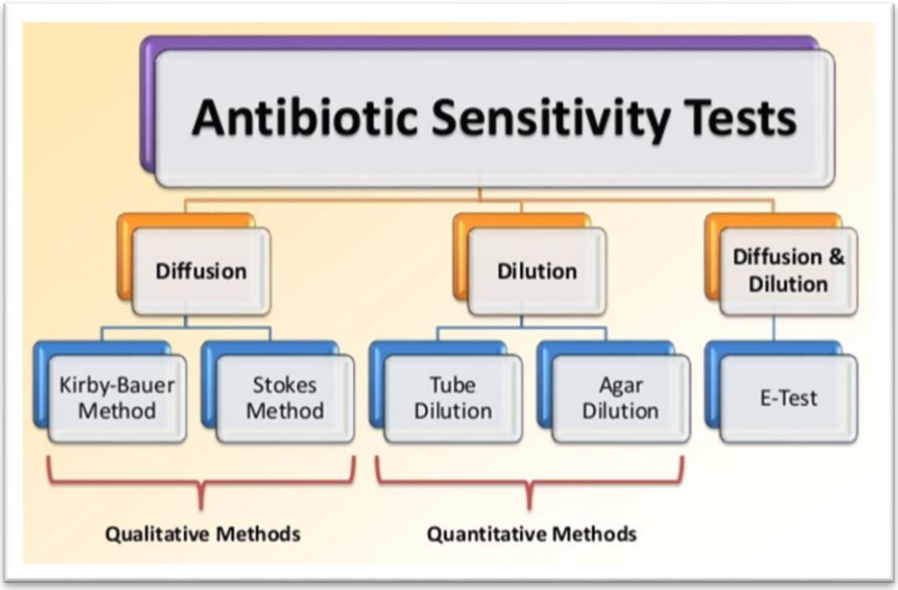


Fig. 113. Classification of Antibiotic Sesitivity Test

Antibiotic sensitivity testing: disk-diffusion

Antibiotic integrated filter paper discs are placed on spesific agar plates, which have been inoculated with the bacteria to be tested. If the microorganism is sensitive to the antibiotic it will not be able to grow in a zone around the antibiotic,called the zone of inhibition. Resistant bacteria will be able to grow close to the disc. Because resistance is usually relavite it is necessary to measure the zone diameter to seeif it is large enough to correspond physiologycally achiavable concentrations of antibiotic. This method takes 24-48 hourse for cultivation (Fig. 114 - 115.).

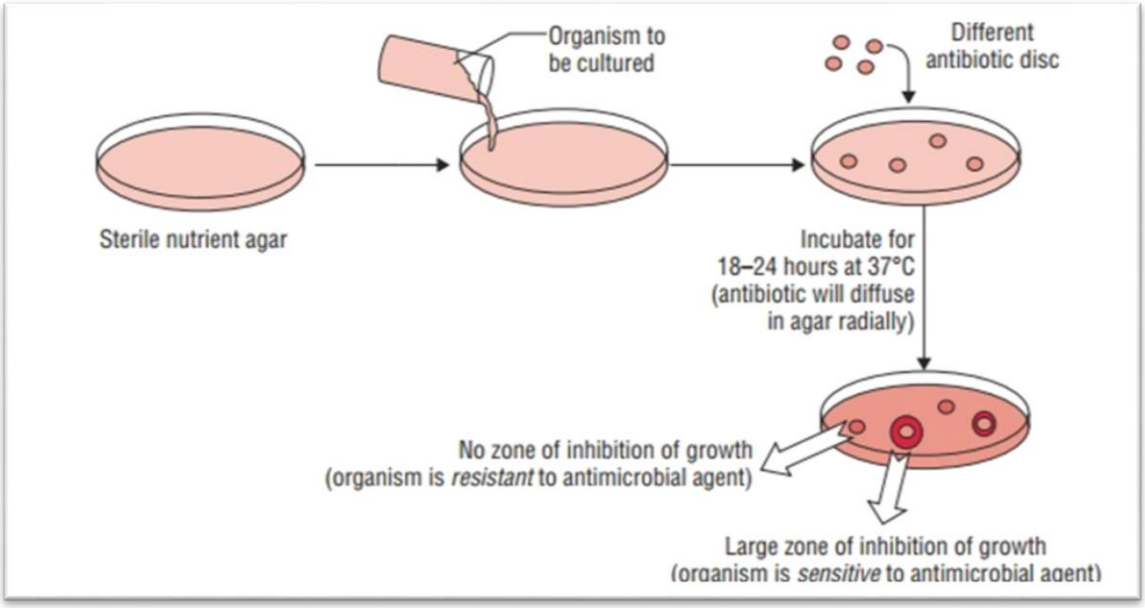


Fig. 114. Schematic diagram showing the performance of antibiotics sensitivity testing by disc diffusion method

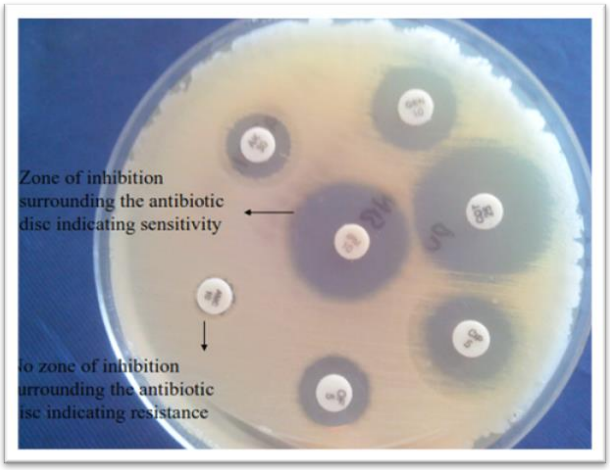


Fig. 115. Results of Disk-diffusion method

Broth dilution method for measuring minimum inhibitory concentration of antibiotics

Minimum inhibitory concentration (MIC) is determined when a patient does not respond to treatment thought to be adequate, relapses while being treated or when there is immunosuppression.

Antibiotic stock solution can be prepared by commercially available antimicrobial powders (with given potency).

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 µg/mL (example: 1280 µg/mL) or 10 times the highest concentration to be tested, whichever is greater.

Preparation of inoculum.

Prepare the inoculum by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour agar plate (use a non-selective medium, such as blood agar).

Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland turbidity standard. This results in a suspension containing approximately 1 to 2 x 10^8 colony forming units (CFU)/mL. Compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL.Note: This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10^6 CFU/mL. The subsequent 1:2 dilution in step 3 brings the final inoculum to 5 x 10^5 CFU/mL.

Inoculation.

Within 15 minutes after the inoculum has been standardized as described above, add 1 mL of the adjusted inoculum to each tube containing 1 mL of antimicrobial agent in the dilution series (and a positive control tube containing only broth), and mix.

This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculums.

Incubation.

Incubate the inoculated tubes at 35 ± 2 ºC for 16 to 20 hours in an ambient air incubator. To maintain the same incubation temperature for all cultures, do not stack microdilution trays more than four high.

Interpretation.

Compare the amount of growth in the wells or tubes containing the antimicrobial agent with the amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set of tests when determining the growth end points. For a test to be considered valid, acceptable growth (≥ 2 mm button or definite turbidity) must occur in the growth-control well (Fig. 116.).

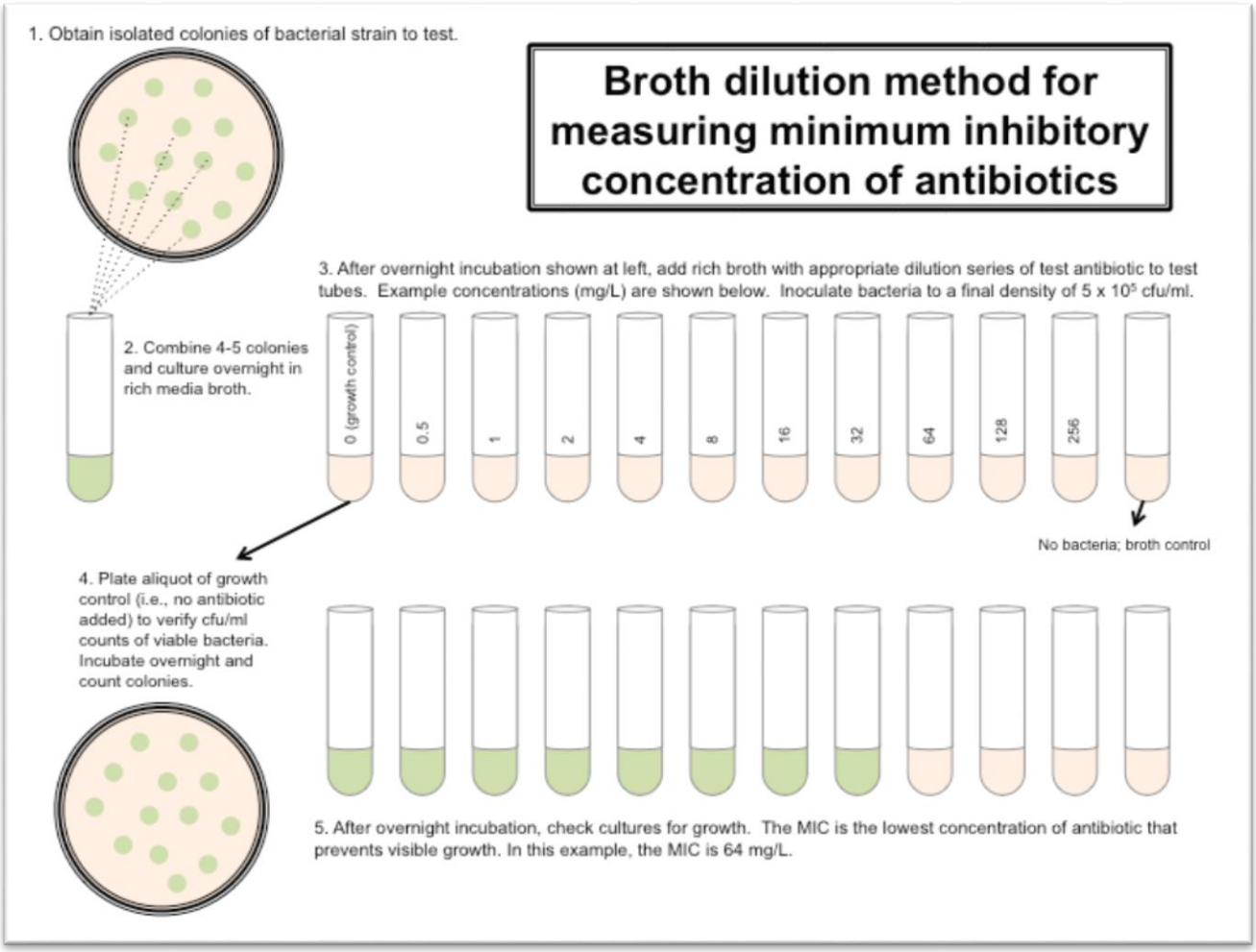


Fig. 116. Broth dilution method for measuring minimum inhibitory concentration of antibiotics

27. INFECTION

Koch's postulates:

1. A specific microorganism is always associated with a given disease.
2. The microorganism can be isolated from the diseased animal and grown in pure culture in the laboratory.
3. The cultured microbe will cause disease when transferred to a healthy animal.
4. The same type of microorganism can be isolated from the newly infected animal.

Major factors in the development of an infection

- Human health is affected by physical, chemical and biological harmful factors: natural (natural) and anthropogenic (caused by human activities).
- Chemically dangerous and harmful factors are most often toxic substances for technological processes, household chemicals, agricultural fertilizers, drugs (in violation of the instructions for use), alcohol and its surrogates.
- Biologically dangerous and harmful factors are various pathogenic microorganisms: viruses, bacteria, protozoa and others, as well as plants and animals.
- Physical factors that disrupt health include magnetic and electromagnetic fields, temperature effects, infrasound and ultrasound, ionizing radiation, changes in barometric pressure, mechanical vibrations and influences.
- Chemical, physical and biological harmful and dangerous factors can worsen the living conditions of man (indirect action), as well as have a pathogenic effect on himself (direct action).

Infection is a complex pathological process that occurs due to the interaction of pathogenic microbes with a macroorganism in certain environmental conditions and manifests itself in the form of an infectious disease or microcarrier.

The dynamics of the development of protective and adaptive reactions of the macroorganism to the reproduction and activity of microorganisms - is called an **infectious process.** The form of infection with a certain clinical picture is an **infectious disease**.

Infectious are all diseases caused by microorganisms that parasitize in animals and humans. Conditions of occurrence and development of an infectious disease are:

1. pathogenic microorganism.
2. susceptible macroorganism.
3. transmission factor.

Pathogenicity and virulence. Units of virulence measurement: DLm, DcL, LD 50.

Pathogenicity - the ability of microorganisms to cause disease.

The degree of pathogenicity - a species trait - is denoted by the term virulence. Pathogenic is a microorganism that multiplies in animals and suppresses protective devices and secretes toxins. Virulence is not a constant factor, but a variable, it can be artificially increased or decreased. The change in virulence can occur naturally. The unit of lethal dose is LD 100. The foot-and-mouth disease virus can cause disease at a dilution of 1: 100,000,000. The microorganism acts with toxins.

Virulence is a degree of pathogenicity that can be numerical. In medical microbiology, its value is determined by three methods:

Dlm - the minimum lethal dose of a microorganism (or its toxin), which causes the death of a laboratory animal for a certain period of time.

Dcl is definitely a lethal dose that causes the death of any animal.

Dcl 50 is the statistically most reliable method for determining virulence. Characterizes the dose that kills 50% of infected animals.

Highly virulent microorganisms can cause disease in animals or humans in the smallest doses. For example, it is known that 2-3 mycobacteria of tuberculosis when injected into the trachea cause fatal tuberculosis in guinea pigs. Virulent strains of anthrax bacilli in the amount of 1-2 cells can cause death in guinea pigs, white mice and even large animals.

In the same microorganism, the virulence can vary significantly. It depends on a number of biological, physical and chemical factors that affect the microorganism. The virulence of the microorganism can be increased or decreased by artificial means.

Prolonged cultivation of crops outside the body on normal nutrient media, growing crops at maximum temperature, adding antiseptic substances to crops weaken the virulence of microorganisms.

Virulence factors: adhesion, colonization, invasion, toxigenesis. Toxins. Enzymes.

Virulence factors are the adaptive mechanisms of infectious disease agents to the changing conditions of the macroorganism, synthesized in the form of specialized structural or functional molecules through which they participate in the implementation of the infectious process.

According to their functional significance, they are divided into four groups:

1) microbial enzymes that depolymerize structures that prevent the penetration and spread of the pathogen in the macroorganism;

2) surface structures of bacteria that promote their fixation in the macroorganism;

3) surface structures of bacteria with antiphagocytic action;

4) pathogenicity factors with toxic function.

Hence, there are two broad qualities of pathogenic bacteria that underlie the means by which they cause disease: invasiveness and toxigenesis.

Toxigenesis is the ability to produce toxins. Toxic substances produced by bacteria, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth.

Toxins. Many bacteria are able to produce poisonous substances called toxins. Toxins act on the body's cells, tissues, and organs and interfere with important body processes, thereby interrupting normal body functions. Those microorganisms that produce toxins are said to be toxigenic. The condition in which toxins are produced is called **toxemia**.

Two important types of toxins are exotoxins and endotoxins. **Exotoxins** are proteins produced by bacteria during their growth (Fig. 117.) and liberated into their surrounding environment. **Exotoxins** are produced chiefly by Gram-positive bacteria, and the genes for this production are carried primarily on the plasmids.

Various types of **exotoxins** exist. Neurotoxins interfere with the nervous system, while **enterotoxins** interfere with activities of the gastrointestinal tract. In response to toxins, the body produces special antibodies called **antitoxins**, which unite with and neutralize the toxins, providing defense against disease.

t is possible to immunize against the effects of exotoxins by injecting toxoids into individuals. Toxoids are preparations of exotoxins chemically treated to destroy their toxigenicity but retain their ability to elicit antibody formation in the body. Toxoids are currently available to protect against diphtheria and tetanus (the DT injection).

Endotoxins are portions of the cell wall of Gram-negative bacteria. They consist primarily of lipopolysaccharides and are released when bacteria break apart during the process of lysis. Since lysis occurs during antibiotic therapy, the effects of endotoxins can bring about a worsening of symptoms during the recovery period. This condition is called endotoxin shock. It is accompanied by fever, chills, aches, and cardiovascular collapse.

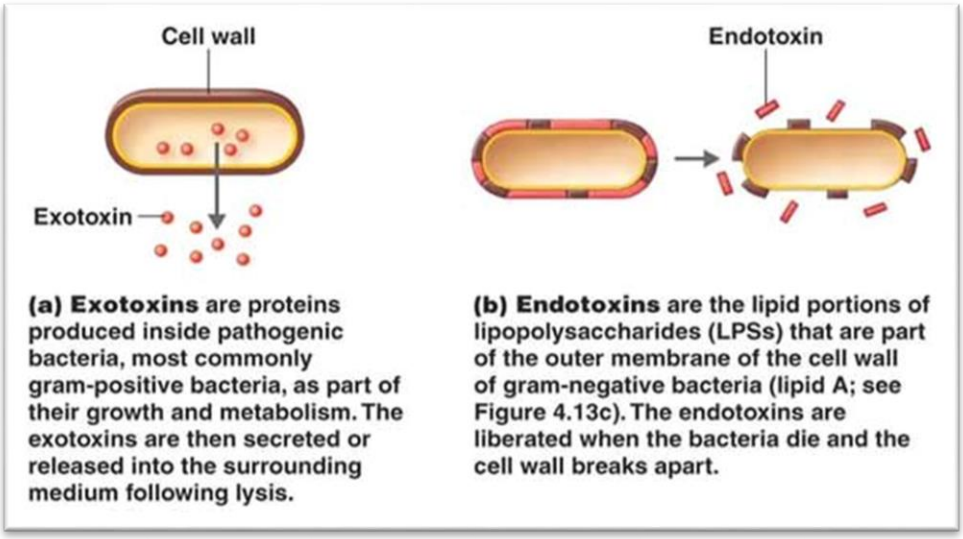


Fig. 117. Differences Between Exotoxins and Endotoxins

Invasiveness is the ability of a pathogen to invade tissues. Invasiveness encompasses (1) mechanisms for colonization (adherence and initial multiplication), (2) production of extracellular substances ("invasins"), that promote the immediate invasion of tissues and (3) ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process. This chapter deals with the first two aspects of of invasiveness: colonization and invasion.

Invasion - the ability of a microbe to overcome the body's protective barriers, penetrate organs, tissues and cavities, multiply in them and suppress the body's defenses. Invasive properties of pathogenic bacteria are provided by microbial enzymes (hyaluronidase), capsules and other chemical components of microbes.

The first stage of microbial infection is **Colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed

tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

Bacterial Adherence to Mucosal Surfaces. In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a receptor and a ligand. The receptors so far defined are usually specific carbohydrate or peptide residues on the eucaryotic cell surface. The bacterial ligand, called an adhesin, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion with specificity comparable to enzyme-substrate relationships and antigen-antibody reactions.

Adhesion - the phenomenon where bacterial proteins and carbohydrates bind to host structure to promote bacterial adherence.

- Adhesion is an essential step in bacterial pathogenesis or infection, required for colonizing a new host.
- Fimbriae are believed to be involved in attachment to solid surfaces or to other cells and are essential for the virulence of some bacterial pathogens.

Adhesion of bacteria to host surfaces is a key element in the formation of biofilms, i.e. matrix-enclosed microbial assemblies that can adhere to biological or nonbiological surfaces. Biofilm formation (Fig. 118.) constitutes a protected mode of growth that allows bacteria to survive in hostile environment. In the context of infectious diseases, biofilms may be critical as matrix-embedded bacterial aggregates are more resistant to host defenses or antibiotic treatments.

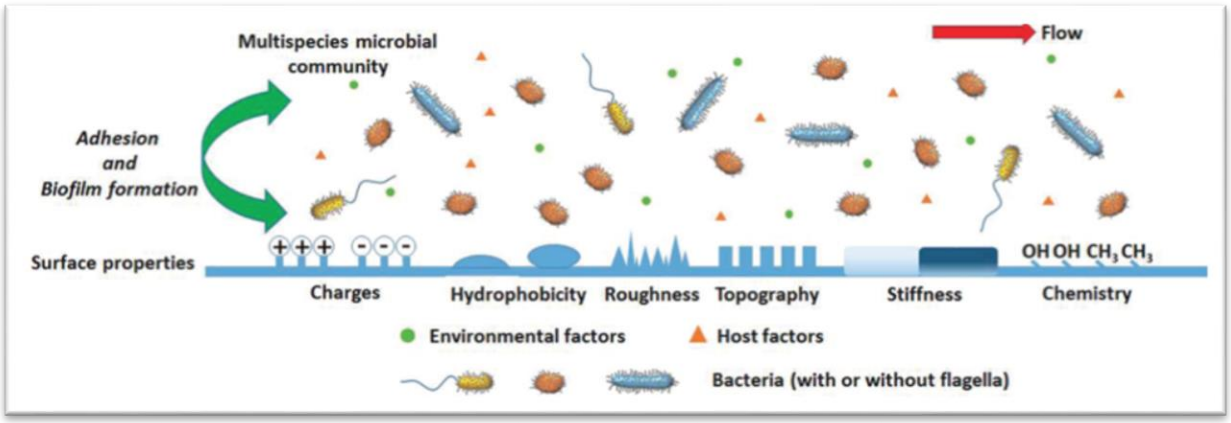


Fig. 118. Schematic illustration of bacterial adhesion and the effects of material properties in complex environments

Enzymes. Many pathogens produce a series of enzymes to help overcome body defenses and establish themselves in the host. One example is **leukocidins,** a group of enzymes that destroy white blood cells. This destruction lessens the body's ability to perform phagocytosis.

Other bacterial enzymes are **hemolysins**. These enzymes destroy red blood cells. *Streptococci*, *Staphylococci*, and certain *Clostridium* species produce hemolysins.

Coagulases are bacterial enzymes that clot the blood. These enzymes convert fibrinogen into fibrin, which forms the threads of a blood clot. The clot helps *Staphylococci* avoid the body's phagocytes and contributes to its pathogenicity.

Other important enzymes are streptokinase and hyaluronidase. **Streptokinase** is a streptococcal enzyme that dissolves blood clots. This activity helps the organism escape the body's attempt to wall off an infection. **Hyaluronidase** destroys hyaluronic acid, a polysaccharide that "cements" cells together in a tissue. Hyaluronidase thus permits organisms to spread through tissues and establish themselves at sites distant from that of the intial infection. Another enzyme, called **collagenase,** breaks down collagen in the connective tissues of muscles. It thereby encourages the spread of infection.

Stages of pathogenesis of infection.

Infection occurs when an organism, such as a virus or bacterium, invades the body. The infectious agent rapidly multiplies in the body's tissues. Although not all infections result in disease, some can trigger the immune system, causing symptoms of illness.

There are five stages of infection:

1. **Incubation stage.** The incubation stage includes the time from exposure to an infectious agent until the onset of symptoms. Viral or bacterial particles replicate during the incubation stage. This stage can range from hours for some infections to days, weeks, or even years for other infections.
2. **Prodromal stage.** The prodromal stage refers to the period after incubation and before the characteristic symptoms of infection occur. People can also transmit infections during the prodromal stage. During this stage, the infectious agent continues replicating, which triggers the body's immune response and mild, nonspecific symptoms. These symptoms can include: low-grade fever, fatigue.
3. **Illness or clinical disease.** This stage includes the time when a person shows apparent symptoms of an infectious disease: fever, fatigue, headache, muscle aches, swollen lymph nodes.
4. **Decline stage.** During the decline stage, the immune system mounts a successful defense against the pathogens, and the number of infectious particles decreases. Symptoms will gradually improve. However, a person can develop secondary infections during this stage if the primary infection has weakened their immune system. During this stage, the virus can still transmit to other people. The decline stage occurs when the number of infectious microbes declines and symptoms resolve.
5. **Convalescence stage.** During this stage, symptoms disappear, and the body starts to recover. Depending on the severity of the infection, some people may have permanent damage even after the infection resolves.

Essentials of epidemiology: modes of transmission and reservoirs of infections.

An individual capable of transmitting a pathogen without displaying symptoms is referred to as a **carrier.** A **passive carrier** is contaminated with the pathogen and can mechanically transmit it to another host; however, a passive carrier is not infected. For example, a health-care professional who fails to wash his hands after seeing a patient harboring an infectious agent could become a passive carrier, transmitting the pathogen to another patient who becomes infected.

By contrast, an **active carrier** is an infected individual who can transmit the disease to others. An active carrier may or may not exhibit signs or symptoms of infection. For example, active carriers may transmit the disease during the incubation period (before they show signs and symptoms) or the period of convalescence (after symptoms have subsided). Active carriers who do not present signs or symptoms of disease despite infection are called asymptomatic carriers. Pathogens such as hepatitis B virus, herpes simplex virus, and HIV are frequently transmitted by asymptomatic carriers.

Transmission

Regardless of the reservoir, transmission must occur for an infection to spread. First, transmission from the reservoir to the individual must occur. Then, the individual must transmit the infectious agent to other susceptible individuals, either directly or indirectly. Pathogenic microorganisms employ diverse transmission mechanisms.

Contact Transmission

Contact transmission includes direct contact or indirect contact. Person-to-person transmission is a form of **direct contact transmission**. Here the agent is transmitted by physical contact between two individuals through actions such as touching, kissing, sexual intercourse, or droplet sprays. Direct contact can be categorized as vertical, horizontal, or droplet transmission. **Vertical direct contact** transmission occurs when pathogens are transmitted from mother to child during pregnancy, birth, or breastfeeding. Other kinds of direct contact transmission are called **horizontal direct contact** transmission. Often, contact between mucous membranes is required for entry of the pathogen into the new host, although skin-to-skin contact can lead to mucous membrane contact if the new host subsequently touches a mucous membrane. Contact transmission may also be

site-specific; for example, some diseases can be transmitted by sexual contact but not by other forms of contact.

When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected. This leads to direct droplet transmission**,** which refers to **droplet transmission** of a pathogen to a new host over distances of one meter or less. A wide variety of diseases are transmitted by droplets, including influenza and many forms of pneumonia. Transmission over distances greater than one meter is called **airborne transmission**.

Indirect contact transmission involves inanimate objects called fomites that become contaminated by pathogens from an infected individual or reservoir. For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections.

Vehicle Transmission

The term vehicle transmission refers to the transmission of pathogens through vehicles such as water, food, and air. Water contamination through poor sanitation methods leads to waterborne transmission of disease. Waterborne disease remains a serious problem in many regions throughout the world. The World Health Organization (WHO) estimates that contaminated drinking water is responsible for more than 500,000 deaths each year. Similarly, food contaminated through poor handling or storage can lead to foodborne transmission of disease.

Dust and fine particles known as **aerosols,** which can float in the air, can carry pathogens and facilitate the **airborne transmission** of disease. Although droplet transmission over short distances is considered contact transmission as discussed above, longer distance transmission of droplets through the air is considered vehicle transmission. Unlike larger particles that drop quickly out of the air column, fine mucus droplets produced by coughs or sneezes can remain suspended for long periods of time, traveling considerable distances. In certain conditions, droplets desiccate quickly to produce a droplet nucleus that is capable of transmitting pathogens; air temperature and humidity can have an impact on effectiveness of airborne transmission.

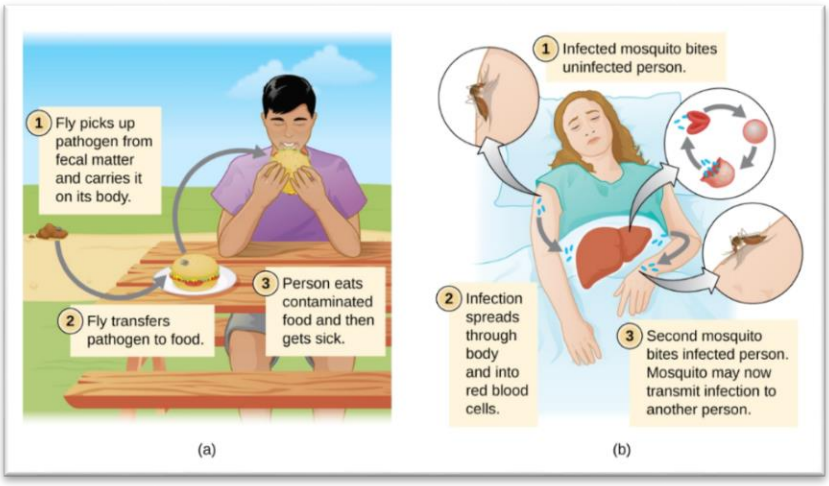


Fig. 119. (a) A mechanical vector carries a pathogen on its body from one host to another, not as an infection. (b) A biologycal vector carries a pathogen from one host to another after becoming infected itself

Vector Transmission

Diseases can also be transmitted by a mechanical or biological **vector**, an animal (typically an arthropod) that carries the disease from one host to another. **Mechanical transmission** is facilitated by a **mechanical vector**, an animal that carries a pathogen from one host to another without being infected itself. For example, a fly may land on fecal matter and later transmit bacteria from the feces

to food that it lands on; a human eating the food may then become infected by the bacteria, resulting in a case of diarrhea or dysentery (Fig. 119.).

Biological transmission occurs when the pathogen reproduces within a **biological vector** that transmits the pathogen from one host to another (Fig. 119.). Arthropods are the main vectors responsible for biological transmission. Most arthropod vectors transmit the pathogen by biting the host, creating a wound that serves as a portal of entry. The pathogen may go through part of its reproductive cycle in the gut or salivary glands of the arthropod to facilitate its transmission through the bite.

Biological insect vectors include mosquitoes, which transmit malaria and other diseases, and lice, which transmit typhus. Other arthropod vectors can include arachnids, primarily ticks, which transmit Lyme disease and other diseases, and mites, which transmit scrub typhus and rickettsial pox. Biological transmission, because it involves survival and reproduction within a parasitized vector, complicates the biology of the pathogen and its transmission. There are also important non-arthropod vectors of disease, including mammals and birds. Various species of mammals can transmit rabies to humans, usually by means of a bite that transmits the rabies virus. Chickens and other domestic poultry can transmit avian influenza to humans through direct or indirect contact with avian influenza virus A shed in the birds' saliva, mucous, and feces.

FORMS OF INFECTION OCCURRENCE

Depending on intensity and extent occurrence of infectious diseases can be distinguished accordingly:

Sporadic – isolated cases of disease without obvious epidemiological connections;

Endemic infection – occurrence of the disease which is bound to a centain territory without time limitation i.e. tick encephalitis, tularemia or where transmitter (vector) is bound to certain climatic condidion (yellow fever, malaria).

Edipemical – mass occurrence of infectious disease in mutual epidemiological connection taking place in a limited territory for a certain amount of time. When two or more cases of disease occur in one family or household we speak about family occurrence.

Pandemic – mass occurrence of infectious disease affecting the whole continent i.e. without territorial limits.

Endogenous infection - is a disease arising from an infectious agent already present in the body (normally resident microflora) but previously asymptomatic.

Exogenous infection – that caused by organisms not normally present in the body but which have gained entrance from the environment.

Latent form of infection - a form of carrier in which the pathogen is in the macroorganism for a long time, without contributing to severe clinical symptoms and is not released into the environment.

Monoinfection - when the infection is caused by one type of microorganism.

Mixed infection – is infection with more than one kind of organism at the same time.

Primary infection is the first time you are exposed to and infected by a pathogen. During a primary infection, your body has no innate defenses against the organism, such as antibodies . Antibodies take time to develop after you have been exposed to an infectious organism, although they can help to prevent future infections with the same disease.

Secondary infection - is infection by a pathogen following an infection by a pathogen of another kind.

Reinfection - re-infection with the same type of microorganism after the disease (gonorrhea, syphilis).

Relapse - return of symptoms of the same disease (malaria, typhoid fever).

Vector-borne infection – is infection caused by microorganisms transmitted from one host to another by a carrier, such as a mosquito, louse, fly, or tick.

Superinfection - infection with the same microorganism, which has not yet ended the underlying disease.

Sepsis - the presence of pus-forming bacteria or their toxins in the blood or tissues.

Septicemia – is a life-threatening bloodstream infection that occurs when bacteria from another infection spread throughout the blood. It may also be called blood poisoning and is considered a medical emergency. Septicemia can rapidly progress to sepsis if left untreated.

Septicopyemia – is a septic process that develops with the formation of purulent foci of infection in various organs and tissues.

Bacteremia - is when there are bacteria present in bloodstream of the human.

Viremia – is a medical condition where viruses enter the bloodstream and hence have access to the rest of the body.

Toxinemia – toxins present in bloodstream.

Healthcare-Associated (Nosocomial) Infections - infections acquired in health-care facilities, including hospitals, are called nosocomial infections or healthcare-associated infections (HAI). HAIs are often connected with surgery or other invasive procedures that provide the pathogen with access to the portal of infection.

Opportunistic infection - any infection caused by a microorganism that does not normally cause disease in humans; occurs in persons with abnormally functioning immune systems (as AIDS patients or transplant patients receiving immunosuppressive drugs).

28. IMMUNE SYSTEM

The immune system protects human body from outside invaders, such as bacteria, viruses, fungi, and toxins (chemicals produced by microbes). It is made up of different organs, cells, and proteins that work together.

There are two main parts of the immune system:

- The innate immune system, which you are born with.
- The adaptive immune system, which you develop when your body is exposed to microbes or chemicals released by microbes.

These two immune systems work together.

The innate immune system

This is human body rapid response system. It patrols human body and is the first to respond when it finds an invader. The innate immune system is inherited and is active from the moment child is born. When this system recognizes an invader, it goes into action immediately. The cells of this immune system surround and engulf the invader. The invader is killed inside the immune system cells. These cells are called phagocytes.

The acquired immune system

The acquired immune system, with help from the innate system, produces cells (antibodies) to protect human body from a specific invader. These antibodies are developed by cells called B - lymphocytes after the body has been exposed to the invader. The antibodies stay in human body. It can take several days for antibodies to develop. But after the first exposure, the immune system will recognize the invader and defend against it. The acquired immune system changes throughout human's life. Immunizations train human's immune system to make antibodies to protect him or her from harmful diseases.

Primary and secondary lymphatic organs.

Primary lymphoid organs (Fig. 120.) refer to immune system organs in which lymphocytes form and mature while secondary immune organs refer to immune system organs that maintain mature naive lymphocytes and initiate an adaptive immune response. This is the major difference between lymphoid organs of primary and secondary origin.

• **Primary lymphoid organs**—include bone marrow and thymus—are sites where immune cells develop from immature precursors.

• **Secondary lymphoid organs**—include spleen, lymph nodes, and specialized sites in the gut and other mucosal tissues—sites where the mature antigen-specific lymphocytes first encounter antigen and begin their differentiation into effector and memory cells.

• Two circulatory systems—blood and lymphatic vessels—connect these organs, uniting them into a functional whole.

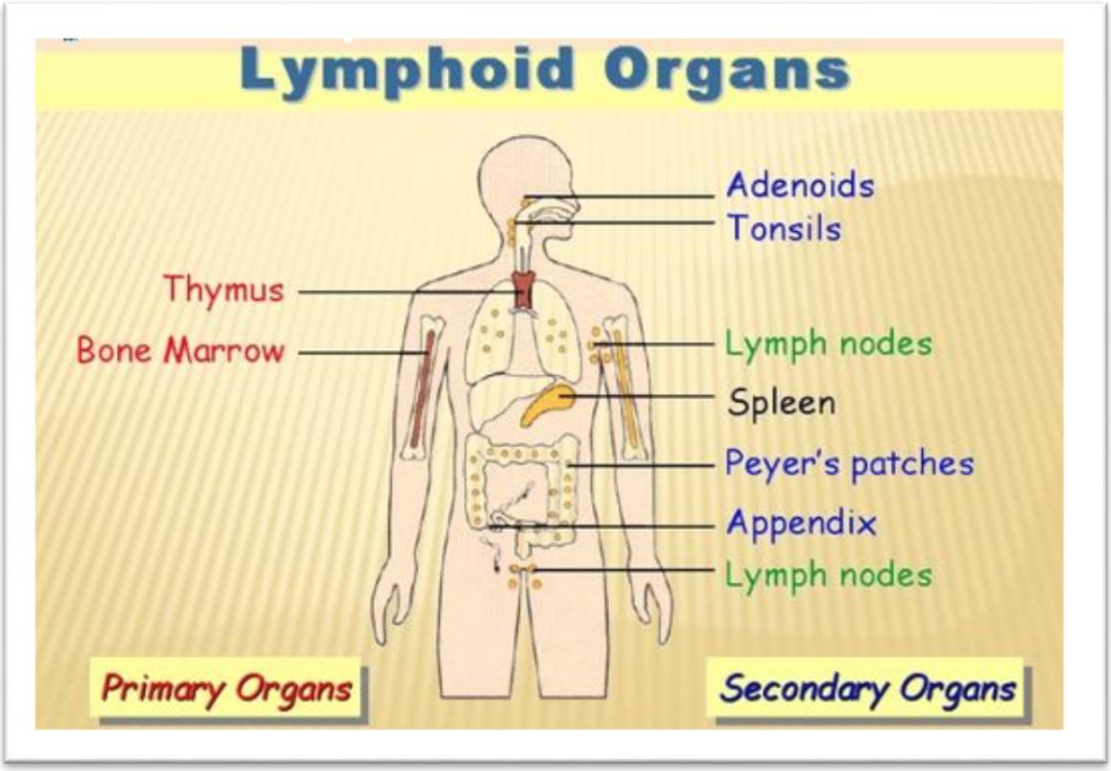


Fig. 120. Primary and secondary lymphatic organs

29. INFLAMMATION

OVERVIEW OF HOST DEFENSE MECHANISMS

The Lines of Defense

In the constantly shifting battle for dominance between invading microorganisms and the host, our bodies have developed a plethora of physical and chemical defense mechanisms. Hierarchically, the body's mechanisms for handling infectious agents can be divided into three primary lines of defense (Fig. 121.):

1. **First line** of defense: innate, nonspecific barriers against microorganisms – includes any barrier that blocks invasion at the portal of entry. Access to the internal environment is prevented.

2. **Second line** of defense: innate, nonspecific, more internalized system of protective cells and fluids including responses such as inflammation and phagocytosis. Acts rapidly at local and systemic levels once the first line of defense has been broached.

3. **Third line** of defense: acquired, specific immunity = highly specialized response wherein the full capabilities of the immune system are brought to bear on individual invading microbes. Utilizes specific lymphocytes and antibodies to chemically attack specific microorganisms.

Barriers at the Portal of Entry: An Inborn First Line of Defence

I. The First Line of Defence

In addition to the physical barriers at the portals of entry covered in chapter 13, there are other nonspecific methods for preventing infections including chemical and genetic barriers.

Physical barriers:

- intact skin
- flushing motion of tears, saliva, mucous

- ciliary movement (clearing the lungs)

Nonspecific Chemical barriers:

- Sebaceous secretions from oil (sebaceous) glands.
- Lysozyme (in tears and saliva), an enzyme that destroys peptidoglycan.
- Perspiration with high sodium chloride, potassium, urea and lactic acid content.
- Acidic pH of skin secretions.
- Hydrochloric acid barrier of stomach.
- Acidic pH of vagina

Genetic barriers: certain potential infectious agents never get started since they need to bind to specific protein receptors (e.g. viruses); those receptors are specific for each host. Put another way: your pet cat cannot contract your mumps infection anymore than you can catch their distemper.

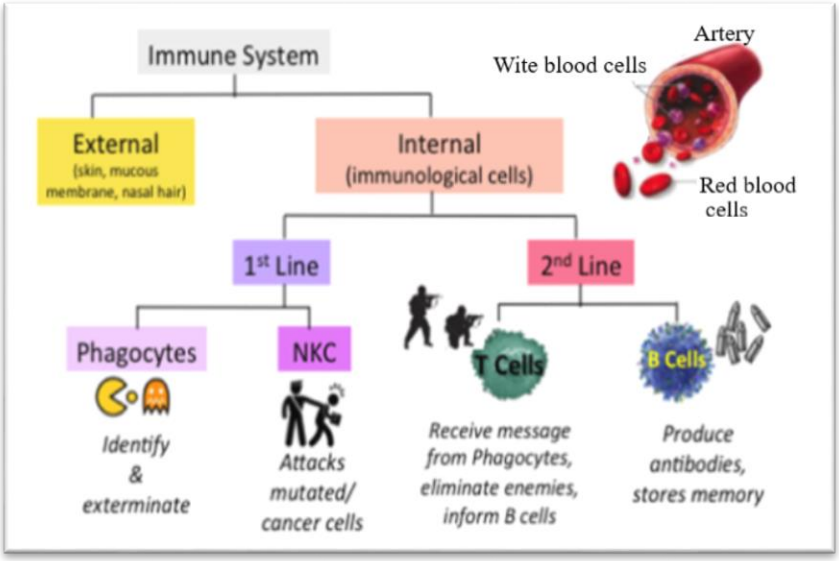


Fig. 121. The Immune System Our Ultimate Line of Defence

STRUCTURE AND FUNCTION OF THE ORGANS OF DEFENSE AND IMMUNITY

Immunology is the study of the body's second and third line of defense. A healthy immune system encompasses:

1. Surveillance of the organs and tissues
2. Recognizing foreign pathogens
3. Attack and destroy foreign invaders

White blood cells (wbc) / leukocytes constantly survey the body and they should be able to recognize self from **nonself.**

How Do White Blood Cells Carry Out Recognition and Surveillance?

White blood cells display special molecules on their membranes known as pattern **recognition receptors (PRRs),** which "feel" for pathogens, for example the **toll-like receptors (TLRs).** These white blood cells are able to detect **pathogen-associated molecular patterns, PAMPs**, that are unique to microbes. These PAMPs are like "red-flags" for the white blood cells.

Compartments and Connections of the Immune System

We will focus on the bloodstream.

Origin, Composition, and Functions of the Blood

Blood circulates in the arteries, veins and capillaries as **whole blood**. Blood is composed of **blood cells** (formed elements) and **plasma.** When settled or spun in a test tube, the formed elements, blood cells, are at the bottom, and the liquid plasma is on top. **Serum** is the clear fluid of plasma with the clotting factors removed.

PLASMA 92% water, plus proteins such as albumin (which helps suspend antibodies) and globulins (including antibodies), immunochemicals such as cytokines, fibrinogen and other clotting factors, hormones, nutrients, ions, dissolved gases and waste products (urea). All these substances

support the normal physiological functions of nutrition, development, homeostasis, and immunity. Serum is plasma minus the clotting factors.

A SURVEY OF BLOOD CELLS

Hemopoiesis / hematopoiesis is the production of blood cells, eventually by the red bone marrow. The primary blood cell is called stem cells, which eventually differentiate in to the individual blood cells.

The leukocytes (white blood cells)

Identified when the blood is stained, they may or may not contain granules in the cytoplasm; and they have different shaped nucleus. The white blood cells are divided into two (2) groups: granulocytes and agranulocytes (Fig. 122.).

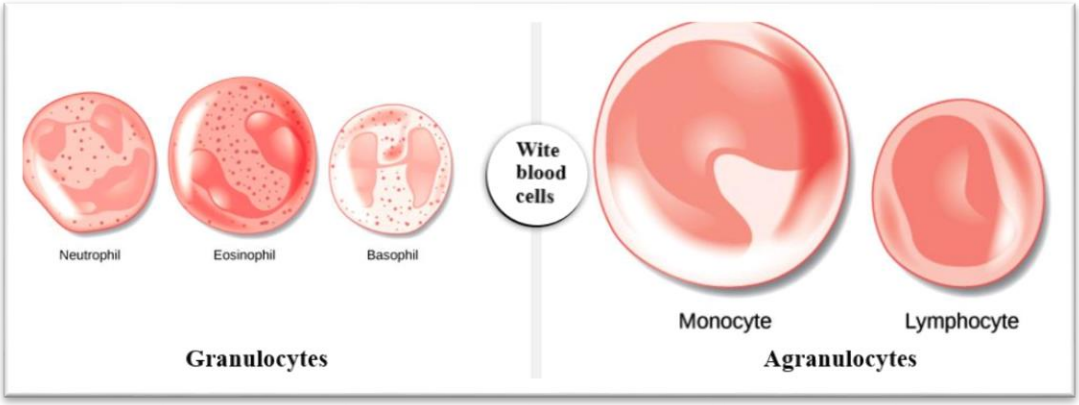


Fig. 122. The white blood cells are divided into two groups: granulocytes and agranulocytes.

1. Granulocytes: have granules in the cytoplasm and a lobed nucleus.

- neutrophils – have fine pale lavender granules and lobed nucleus, also called polymorphonuclear neutrophils (PMNs), ~55% - 90% of circulating leukocytes; main work is phagocytosis.
- eosinophils – have orange granules and bilobed nucleus, ~1% - 3% in circulating blood; destroy large eukaryotic pathogens, involved in allergic reactions.
- basophils – have deep blue granules and constricted nuclei, less than ~0.5% in circulating blood; help to attract other white blood cells to an injury site.

2.Agranulocytes: lack prominent cytoplasmic granules and have nonlobed nuclei.

- Lymphocytes – second most common wbc, ~20% - 35% in circulating blood, there are two (2) types:
  - **B-lymphocytes (B cells) –** contribute to humoral immunity, form antibodies.
  - **T- lymphocytes (T cells)** – mature in the thymus, function in cell-mediated immunity.
- Monocytes – largest of the wbcs, ~3% - 7% in circulating blood, kidney shaped nucleus, eventually differentiate into **macrophages** and **dendritic cells**.

Erythrocyte and Platelet Lines

Erythrocytes are red blood cells (rbc), loose their nucleus just before entering the blood stream, have a biconcave shape, function is gas transport of O_2 and CO_2. They do not have an immune function.

Platelets formed elements that are not whole cells, they function in clotting.

SECOND LINE DEFENSES: INFLAMMATION

The Second Line of Defense

The Inflammatory Response: A Complex Concert of Reactions to Injury

Inflammation is a nonspecific defensive response by the body to an injury in the tissue. It develops after a mechanical injury (cut or bruise) or from exposure to a chemical agent (bee venom), physical agent (burn), or biological organism (parasite).

The four characteristic signs of inflammation:

1. *rubor (erythema*) = red color from blood accumulation

2. *calor* = warmth from the heat of the blood. Erythema and warmth are the result of vasodilation.

3. *tumor (edema)* = swelling from accumulation of fluid

4. *dolor* = pain from injury to the local nerves. Pain may be the result of pressure against the nerves from fluid accumulation, or prostaglandins.

The Stages of Inflammation

Vascular Changes: Early Inflammatory Events

These signs match with the physiological response taking place at the site of injury:

1. Initially, blood vessels narrow (vasoconstriction) to cut off blood flow. After clot formation they dilate (expand) in response to chemicals called cytokines1 and other chemical mediators released by the injured tissues and white blood cells.

2. Edema, the buildup of extracellular fluid in one location, swells the tissues and keeps infection from spreading; neutrophils are attracted to phagocytose debris and microbes.

3. White blood cells, microbes (living and dead), cellular debris, leukocytes and fluid accumulate to form pus. If pus becomes enclosed in a wall of fibrin, it can form an abscess or boil. When several abscesses accumulate, an enlarged structure called a carbuncle results (often seen in *Staphylococcus* infections).

4. Macrophages clean up the remaining residue and lymphocytes carry out immune reactions such as antibody formation.

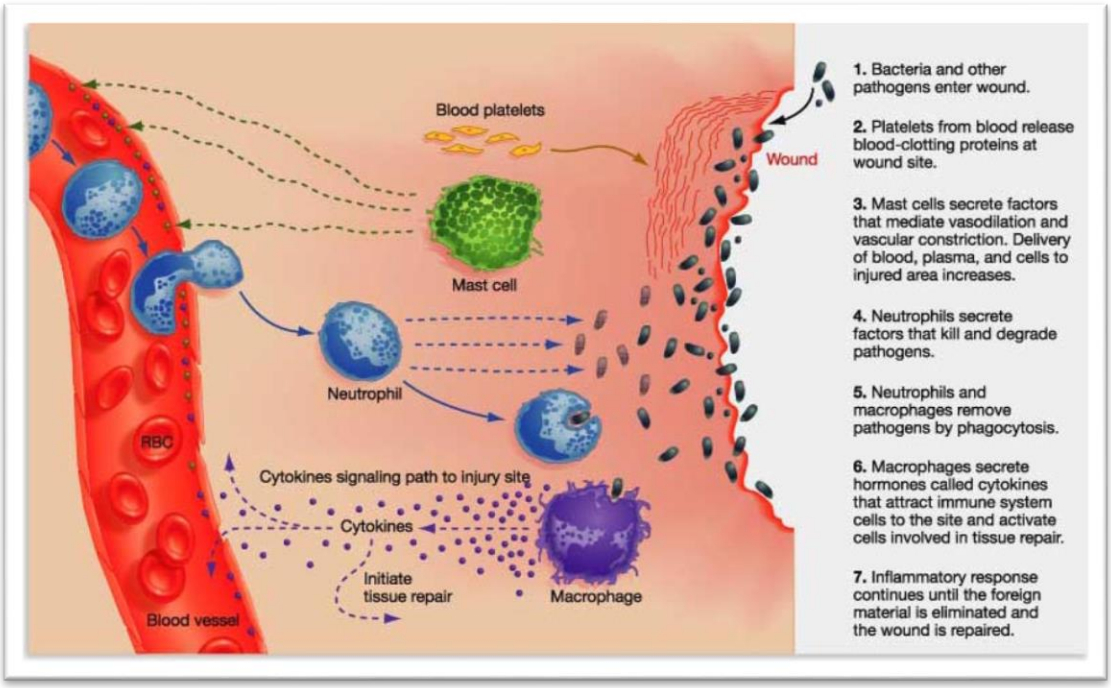


Fig. 123. Inflammation InterActive Health

Fever: An Adjunct to Inflammation

Fever is an abnormally high body temperature that may provide nonspecific mechanisms for defense.

Normal: 37 C (98.6 F) 1 F

Low: 37.7 -38.3 C (100 -101 F)

Moderate: 38.8 -39.4 C (102 -103 F)

High: 40.0 -41.1 C (104 -106 F)

A temperature above 45ºC (113 F) leads to convulsions and death.

30. PHAGOCYTOSIS, INTERFERON, AND COMPLEMENT

Phagocytosis: Partner to Inflammation and Immunity

Phagocytosis is a process in which solid particles are taken into the cell (Fig. 124.). While most cells can perform phagocytosis, three types of immune cells specialize in it; they are: neutrophils, monocytes, and macrophages.

Two types of phagocytotic cells:

- **Neutrophils** are "general-purpose" phagocytes that react during the early inflammatory response; a common sign of bacterial infection is a high neutrophil count.
- **Macrophages** are the predominant phagocytes and migrate through tissues (diapedesis) to sites of infection by chemotaxis - a chemical attraction between the macrophage and pathogen. Certain macrophages reside permanently in specific tissues (e.g. Kupffer cells in the liver.)

Phagocytosis (Fig. 124.) begins with an invagination and pinching of the cell membrane to form a phagocytic vesicle (phagosome). The phagosome fuses with a lysosome, an organelle that contributes digestive enzymes, lysozyme, and an acidic pH to the digestion process. When the process is completed, waste materials are excreted from the phagocyte.

Note: The presence of a capsule on a bacterium and the presence of specific cell wall components can prevent phagocytosis. Also, some bacteria can live and grow inside of our macrophages (e.g. *R. rickettsi*).

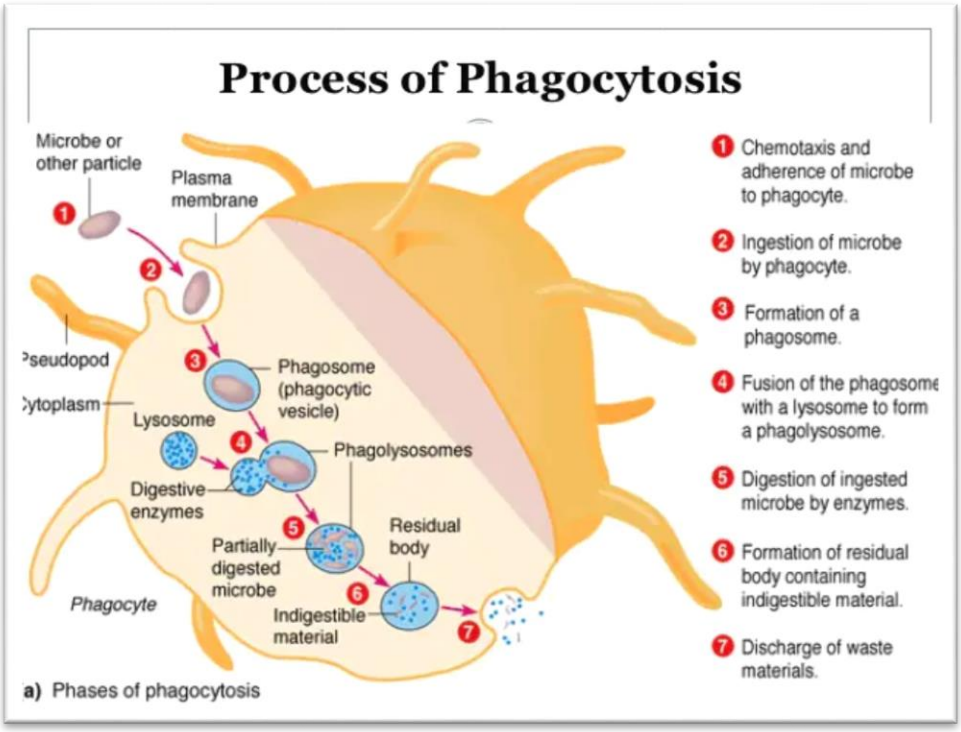


Fig. 124. Process of Phagocytosis

Interferon: Antiviral Cytokines and Immune Stimlulants

Interferon (INF) is a small protein produced by white blood cells in response to (predominantly) viral attacks.

After a cell is infected, it produces INF, which is then secreted and diffuses to neighboring cells where it binds to surface receptors. A biochemical cascade is initiated inside the second cell that results in gene activation and the production of antiviral proteins. This response is non-specific for individual viruses and helps prevent the spread of the virus.

IFN-alpha is produced in the leukocytes infected with virus, while **IFN-beta** is from fibroblasts infected with virus. **IFN-gamma** is induced by the stimulation of sensitized lymphocytes with antigen or non-sensitized lymphocytes with mitogens (Table 1.).

Table 1. Types of interferons

Properties	Alpha	Beta	Gamma
Current Nomenclature	IFN-α	IFN-β	IFN-γ
Former Designation	Leukocyte	Fibroblast	Immune Interferon
Type Designation	Type I	Type I	Type II
No. Of Genes that code for Family	≥20	1	1
Principal Cell Source	Most Cell Types	Most cell Types	Lymphocytes
Inducing Agent	Viruses; dsRNA	Viruses; dsRNA	Mitogens
Stability at pH 2.0	Stable	Stable	Labile
Homology with IFN-α	80-95%	30%	<10%
Chromosomal location of genes	9	9	12
Size of secreted protein (Number of amino acids)	165	166	143
IFN receptors	IFNAR	IFNAR	IFNGR
Chromosomal location of IFN receptor genes	21	21	6

COMPLEMENT: A VERSATILE BACKUP SYSTEM

The **complement system** consists of a chain reaction that takes place when the body has recognized any microbe. In a series of steps, small proteins (numbered C1-C9) initially bind to, then digest holes in the cell membranes of pathogens, thus destroying them (Fig. 125.), outlines the steps of complement proteins attacking a membrane. The classical pathway is initiated by antibodies, but alternate pathways do not rely on antibodies; therefore the cascade is considered a non-specific response.

Classical Pathway

This pathway involves complement components C1, C2 and C4. The pathway is triggered by antibody-antigen complexes binding to C1, which itself has three subcomponents C1q, C1r and C1s. The pathway forms a C3 convertase, C4b2a, which splits C3 into two fragments; the large fragment, C3b, can covalently attach to the surface of microbial pathogens and opsonise them; the small fragment, C3a, activates mast cells, causing the release of vasoactive mediators such as histamine.

Alternative Pathway

This pathway involves various factors, B, D, H & I, which interact with each other, and with C3b, to form a C3 convertase, C3bBb, that can activate more C3, hence the pathway is sometimes called 'the amplification loop'. Activation of the loop is promoted in the presence of bacterial and fungal cell walls, but is inhibited by molecules on the surface of normal mammalian cells.

Mannose-binding **Lectin Pathway**

This pathway is activated by the binding of mannose-binding lectin (MBL) to mannose residues on the pathogen surface. This in turn activates the MBL-associated serine proteases, MASP-1 and MASP-2, which activate C4 and C2, to form the C3 convertase, C4b2a.

Lytic Pathway (Terminal)

This pathway is initiated by the splitting of C5, and attachment of C5b to a target. C6, C7, C8 and C9 unite with C5b, and this membrane-attack complex (MAC), when inserted into the outer membrane of some bacteria, can contribute to their death by lysis. Red cells which have antibody bound to the cell surface can also activate the classical and lytic pathways, and become susceptible to lysis.

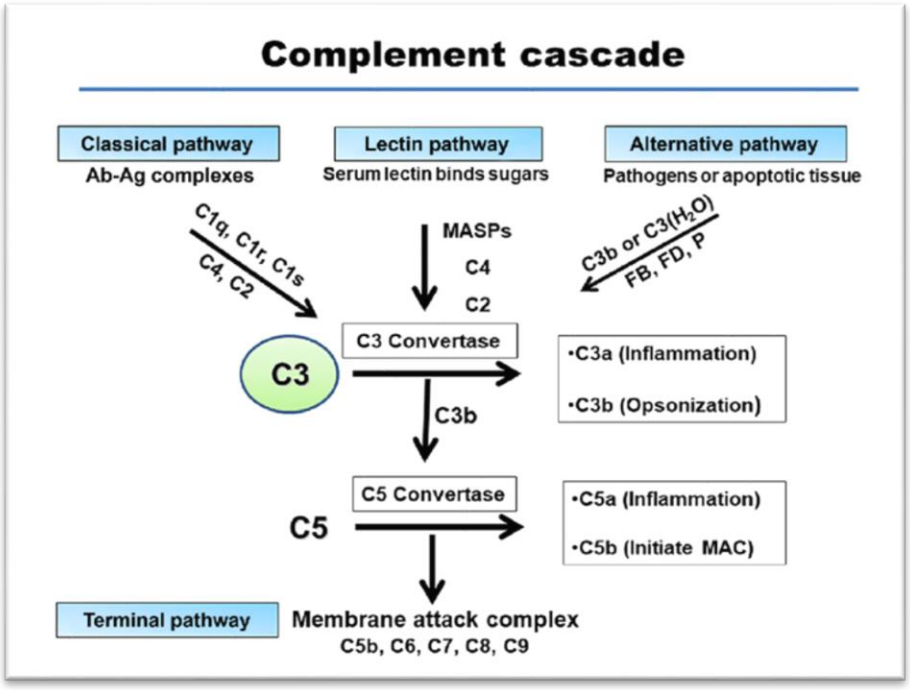


Fig. 125. Complement System

Role of Complement in Disease

The complement system plays a critical role in inflammation and defence against some bacterial infections. Complement may also be activated during reactions against incompatible blood transfusions, and during the damaging immune responses that accompany autoimmune disease. Deficiencies of individual complement components or inhibitors of the system can lead to a variety of diseases, which gives some indication of their role in protection against disease.

31. ADAPTIVE (ACQUIRED) IMMUNITY

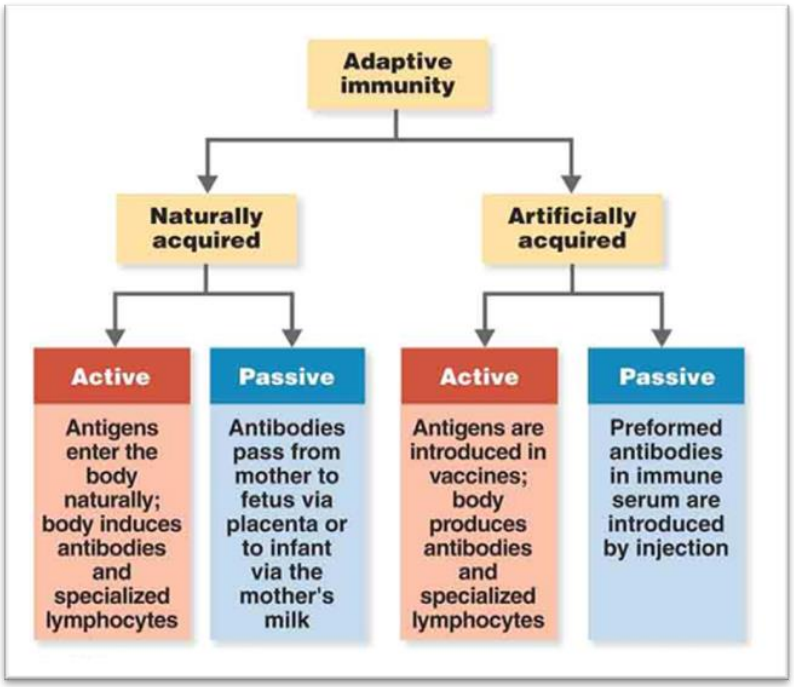


Fig. 126. Adaptive Immunity

The adaptive immune system (Fig.126.) takes over if the innate immune system is not able to destroy the germs. It specifically targets the type of germ that is causing the infection. But to do that

it first needs to identify the germ. This means that it is slower to respond than the innate immune system, but when it does it is more accurate. It also has the advantage of being able to "remember" germs, so the next time a known germ is encountered, the adaptive immune system can respond faster.

This memory is also the reason why there are some illnesses you can only get once in your life, because afterwards your body becomes “immune.” It may take a few days for the adaptive immune system to respond the first time it comes into contact with the germ, but the next time the body can react immediately. The second infection is then usually not even noticed, or is at least milder.

The adaptive immune system is made up of:

- T lymphocytes in the tissue between the body's cells
- B lymphocytes, also found in the tissue between the body's cells
- Antibodies in the blood and other bodily fluids

The two types of **adaptive immunity**, **humoral immunity** and **cell-mediated immunity**, are mediated by different cells and molecules and provide defense against extracellular microbes and intracellular microbes (Fig. 127.). **Humoral immunity** is mediated by proteins called **antibodies,** which are produced by cells called **B lymphocytes. Antibodies** are secreted into the circulation and mucosal fluids, and they neutral- ize and eliminate microbes and microbial toxins that are present outside of host cells, in the blood and in the lumens of mucosal organs, such as the gastrointestinal and respiratory tracts. One of the most important functions of antibodies is to stop microbes that are present at mucosal surfaces and in the blood from gaining access to and colonizing host cells and connective tissues. In this way, antibodies prevent infections from ever being established. Antibodies cannot gain access to microbes that live and divide inside infected cells. Defense against such intracellular microbes is called **cell-mediated immunity** because it is mediated by cells, which are called **T lymphocytes**.

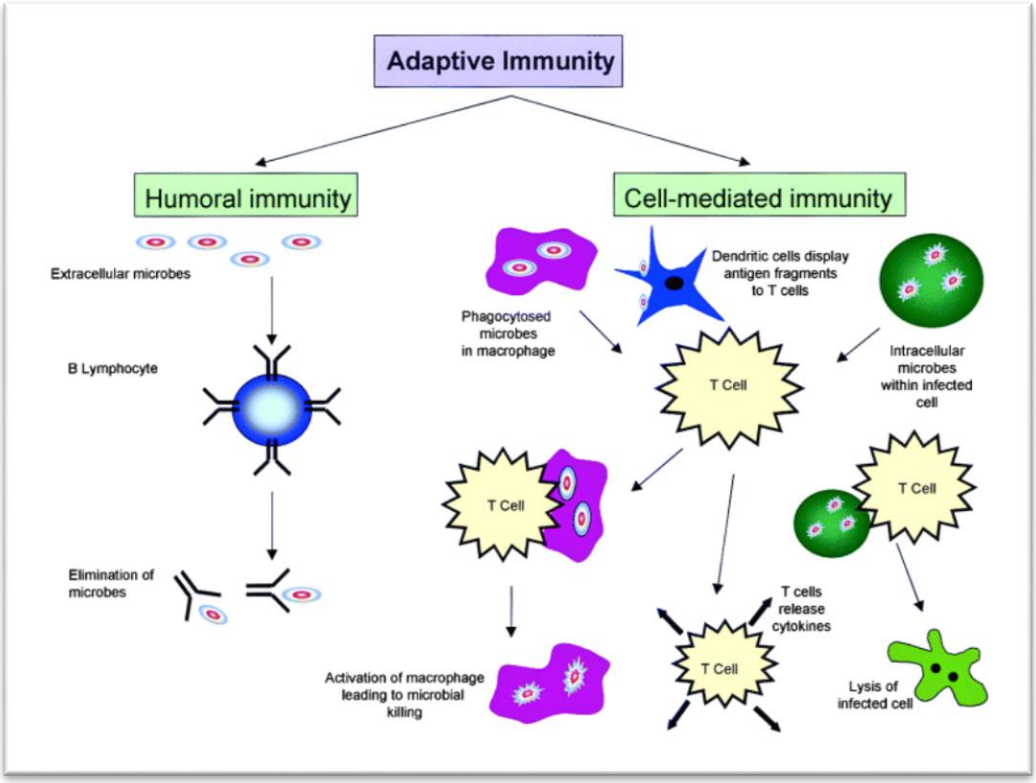


Fig. 127. Adaptive Immunity

Some T lymphocytes activate phagocytes to destroy microbes that have been ingested by the phagocytes into intracellular vesicles. Other T lymphocytes kill any type of host cells that are harboring infectious microbes in the cytoplasm. Thus, the antibodies produced by B lymphocytes recognize extracellular microbial antigens, whereas T lymphocytes recognize antigens produced by intracellular microbes. Another important difference between B and T lymphocytes is that most T

cells recognize only protein antigens, whereas B cells and antibodies are able to recognize many different types of molecules, including proteins, carbohydrates, nucleic acids, and lipids. Immunity may be induced in an individual by infection or vaccination (active immunity) or conferred on an individual by transfer of antibodies or lymphocytes from an actively immunized individual (passive immunity). In active immunity, an individual exposed to the antigens of a microbe mounts an active response to eradicate the infection and develops resistance to later infection by that microbe.

Such an individual is said to be immune to that microbe, in contrast with a naive individual, not previously exposed to that microbe's antigens. We are concerned mainly with the mechanisms of active immunity. In passive immunity, a naive individual receives antibodies or cells (e.g., lymphocytes, feasible only in genetically identical [inbred] animals) from another individual already immune to an infection; for the lifetime of the transferred antibodies or cells, the recipient is able to combat the infection. Passive immunity is therefore useful for rapidly conferring immunity even before the individual is able to mount an active response, but it does not induce longlived resistance to the infection. The only physiologic example of passive immunity is seen in newborns, whose immune systems are not mature enough to respond to many pathogens but who are protected against infections by acquiring antibodies from their mothers through the placenta and breast milk.

32. STRUCTURE AND CLASSIFICATION OF IMMUNOGLOBULINS.

Immunoglobulins are glycoproteins comprises of four polypeptide chain: two identical light (L) and two identical heavy (H) chains (Fig. 128.). Further, L and H chains are subdivided into variable and constant regions. The terms light and heavy refer to molecular weight. The heavy chains are longer whereas light chains are shorter. Light chains have a molecular weight of about 25,000 Da whereas heavy chains have a molecular weight of 50-70,000 Da.

The simplest antibody molecule has a 'Y' or 'T' shape structure which is the most widely recognizable feature of immunoglobulin structure. All antibody molecules share the same basic structural characteristics but display remarkable variability in the regions that bind antigens. Because the core structural unit of each antibody molecule contains two heavy chains and two light chains, every antibody molecule has at least two antigen-binding sites.

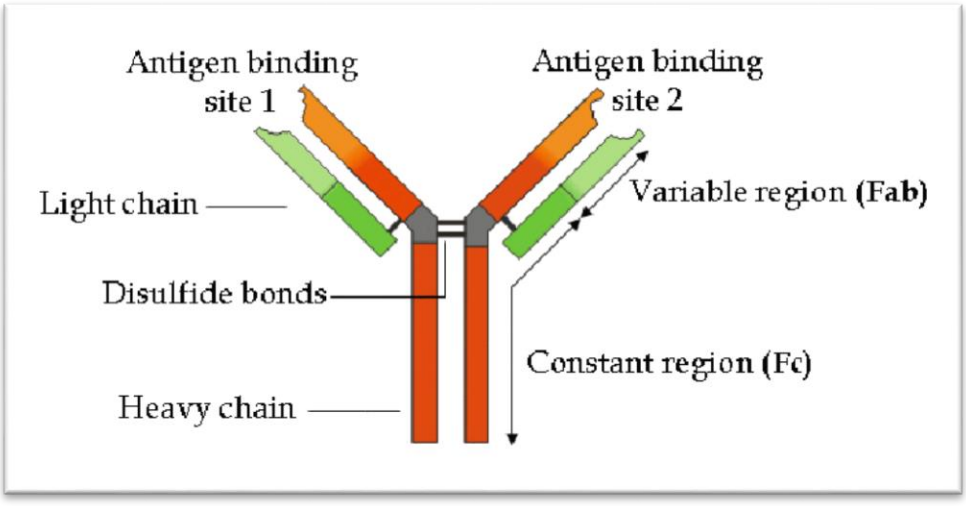


Fig. 128. Structure of immunoglobulin

Both the **light chains** and the **heavy chains** contain a series of repeating, homologous units. Each unit is about 110 amino acid residues long, that fold independently in a globular motif that is called an Ig domain. An Ig domain contains two layers of a β-pleated sheet, each layer composed of three to five strands of the antiparallel polypeptide chain. However, the number of strands per sheet varies among individual proteins.

Within the strands, hydrophobic and hydrophilic amino acids alternate and their side chains are oriented perpendicular to the plane of the sheet.

Heavy chains of immunoglobulins. An immunoglobulin molecule has two heavy chains. Each heavy chain comprises 420–440 amino acids. Also, each heavy chain binds to a light chain by a disulfide bond and by noncovalent bonds.

The sequences of the heavy-chain constant regions fall into five basic patterns. These five basic sequences named with Greek letters and these are:

- μ (mu)
- δ (delta)
- γ (gamma)
- ε (epsilon)
- α(alpha).

The heavy chains of a given antibody molecule determine the class of that antibody. For example, IgM contains μ (mu), IgG contains γ (gamma), IgA contains α(alpha), IgD contains δ (delta), and IgE contains ε (epsilon). heavy chains. These heavy chains are structurally and antigenically distinct for each class of immunoglobulin.

In addition, heavy chains exist in two forms that differ at their carboxyl-terminal ends. One form of the heavy chain anchors membrane-bound antibodies in the plasma membranes of B lymphocytes whereas the other form is secreted when associated with Ig light chains.

Light chain. An immunoglobulin molecule has two light chains. Each light chain comprises 220–240 amino acids. Further, the light chain attaches to the heavy chain by a disulfide bond. Unlike heavy chains, the light chains are structurally and chemically similar in all classes of immunoglobulins. They are of two types:

- K (kappa), and
- λ (lambda).

Each immunoglobulin has either two K (kappa) or two λ (lambda) chains but never both. In humans, about 60% of antibody molecules have K (kappa) light chains and about 40% have λ light chains. The K (kappa) and λ (lambda) chains are present in human serum in a ratio of 2:1.

Each light chain comprises of one V (variable) region Ig domain and one C (constant) region Ig domain. Variable regions distinguish the antibodies made by one clone of B cells from the antibodies made by other clones.

Variable and constant region. Each polypeptide chain of an immunoglobulin molecule contains an amino-terminal part and a carboxy-terminal part. The amino terminal part is called the variable region (V region) whereas the carboxy-terminal part is called the constant region (C region).

The variable regions of both the light and heavy chain are responsible for antigen binding whereas the constant region of the heavy chain is responsible for various biologic functions. For example, complement activation and binding to cell surface receptors.

Constant region. The constant (C) region is the carboxyl-terminal of the molecule. It consists of two basic amino acid sequences. The constant region of the light chain does not have any biological function whereas the constant region of the heavy chain is responsible for activation of the complement, binding to cell surface receptors, placental transfer, and many other biological activities.

Moreover, the C region domains are separate from the antigen-binding site and do not participate in antigen recognition.

Variable region. The variable region consists of 100-110 amino acids at amino-terminal end. This region is different for each class of immunoglobulins. Basically, the variable regions of both L and H chains have three extremely variable (hypervariable) amino acid sequences at the amino-terminal end that form the antigen-binding site.

The hypervariable loops are like fingers protruding from each variable domain, three fingers from the heavy chain and three fingers from the light chain coming together to form an antigen-binding site.

These antigen-binding sites are responsible for specific binding of antibodies with antigens.

Fab fragments (fragment antigen-binding)

It is a region on an antibody that binds to antigens. It comprises one constant and one variable domain of each of the heavy and the light chain. These domains shape the antigen-binding site, at the amino terminal end of the monomer.

Fc fragments (fragment crystallizable)

Fc region is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. This property allows antibodies to activate the immune system.

IgM (Immunoglobulin M)

IgM makes up approximately 13% of the serum antibodies and is the first antibody produced during an immune response. IgM is found mainly in the bloodstream rather than in the intracellular spaces of tissues where it can control infections in the blood. IgM has a half-life of about 5 days. IgM is a pentamer and has 10 epitope-binding sites (Fig. 129.).

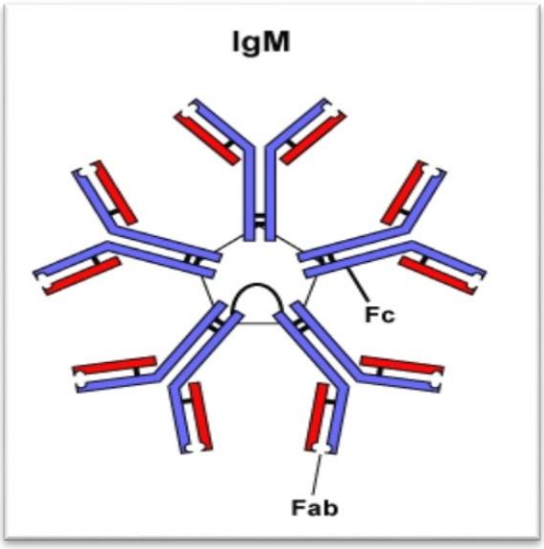


Fig. 129. IgM is a pentamer and, therefore, has 10 Fab sites.

The Fc portions of IgM are able to activate the classical complement pathway. IgM is the most efficient class of antibody for activating the classical complement pathway. Monomeric forms of IgM are found on the surface of B-lymphocytes as B-cell receptors.

IgA (Immunoglobulin A; 2 subclasses, IgA1-2)

IgA makes up approximately 6% of the serum antibodies where it has a half-life of approximately 6 days. IgA is found mainly in body secretions (saliva, mucous, tears, colostrum and milk) as secretory IgA (sIgA) where it protects internal body surfaces exposed to the environment by blocking the attachment of bacteria and viruses to mucous membranes. While only 6% of the antibodies in the serum are IgA, secretory IgA is the most immunoglobulin produced. IgA is made primarily in the mucosal-associated lymphoid tissues (MALT). IgA appears as a dimer of 2 "Y"-shaped molecules and has 4 epitope-binding sites and a secretory component to protect it from digestive enzymes in the secretions (Fig. 130.).

The Fc portion of secretory IgA binds to components of mucous and contributes to the ability of mucous to trap microbes. The Fc portion of secretory IgA can bind to macrophages and neutrophils for enhanced attachment (opsonization). IgA can activate the lectin complement pathway and the alternative complement pathway.

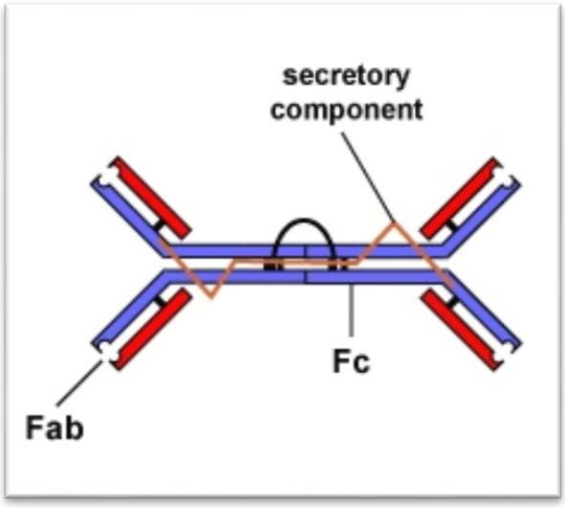


Fig.130. Secretory *IgA* is a dimer and has 4 Fab sites. A secretory component helps protect it from digestion in body secretions.

IgD: (Immunoglobulin D)

IgD makes up approximately 0.2% of the serum antibodies. IgD is a monomer and has 2 epitope-binding sites and is found on the surface of B-lymphocytes (along with monomeric IgM) as a B-cell receptor where it may control of B-lymphocyte activation and suppression. IgD may play a role in eliminating B-lymphocytes generating self-reactive autoantibodies.

IgE (Immunoglobulin E)

IgE makes up about 0.002% of the serum antibodies with a half-life of 2 days. Most IgE is tightly bound to basophils and mast cells via its Fc region . IgE is a monomer and has 2 epitope-binding sites. IgE is made in response to parasitic worms (helminths) and arthropods. It is also often made in response to allergens(allergens are antigens causing allergic reactions). IgE may protect external mucosal surfaces by promoting inflammation, enabling IgG, complement proteins, and leukocytes to enter the tissues, as well as by triggering coughing, sneezing, and vomiting for mechanical removal of microbes and toxins. .

The Fc portion of IgE can bind to mast cells and basophils where it mediates many allergic reactions. Cross linking of cell-bound IgE by antigen triggers the release of vasodilators for an inflammatory response. The Fc portion of IgE made against parasitic worms and arthropods can bind to eosinophils enabling opsonization. This is a major defense against parasitic worms and arthropods.

33. STRUCTURE AND CLASSIFICATION OF ANTIGENS

In immunology, an antigen is a substance that evokes an immune response. Formally they are defined as a substance that causes the production of antibodies specific to that antigen, however they also cause T cell mediated immune responses, and may lead to an inflammatory response. The substance may be from the external environment or formed within the body. The immune system will try to destroy or neutralize any antigen that is recognized as a foreign and potentially harmful invader.”Self” antigens are usually tolerated by the immune system; whereas “non-self” antigens can be identified as invaders and can be attacked by the immune system.

Molecular Structure of Antigens

At the molecular level, an antigen is characterized by its ability to be “bound” at the antigen-binding site of an antibody. Antibodies tend to discriminate between the specific molecular structures presented on the surface of the antigen. Antigens are usually either proteins, peptides, or polysaccharides. This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides.

Antigens have several structural components of interaction that may be bound by different classes of antibodies. Each of these distinct structural components is considered to be an **epitope**, also called an antigenic determinent. Therefore, most antigens have the potential to be bound by several distinct antibodies, each of which is specific to a particular epitope. The antigen binding receptor on an antibody is called a paratope, and is specific to the epitope of the antigen. Using the "lock and key" metaphor, the antigen itself can be seen as a string of keys – any epitope being a "key" – each of which can match a different lock.

Types of Antigens

Antigens are categorized into broad classes of antigens based on their origin. So many different molecules can function as an antigen in the body, and there is considerable diversity even within these categories.

These are the main classes of antigens that are involved in immune system activation. Their diversity is analogous to the immense diversity of the diseases that the immune system works to overcome.

Exogenous Antigens. Exogenous antigens are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. Exogenous antigens are the most common kinds of antigens, and includes pollen or foods that may cause allergies, as well as the molecular components of bacteria and other pathogens that could cause an infection.

Endogenous Antigens. Endogenous antigens are that have been generated within previously-normal cells as a result of normal cell metabolism or because of viral or intracellular bacterial infection (which both change cells from the inside in order to reproduce). The fragments are then presented on the surface of the infected cells in the complex with MHC class I molecules.

Complete Antigens and Haptens

Haptens are molecules that create an immune response when attached to proteins.

Antigens are basic molecules that induce an immune response when detected by immune system cells. Antigens may be either complete or incomplete based on the nuances of their molecule structure.

Haptens

A hapten is essentially an incomplete antigen. These small molecules can elicit an immune response only when attached to a large carrier such as a protein; the carrier typically does not illicit an immune response by itself. Many hapten carriers are normal molecules that circulate through the body. When haptens and carriers combine, the resulting molecule is called an adduct, the combination of two or more molecules. Haptens cannot independently bind to MHC complexes, so they cannot be presented to T cells.Some haptens induce autoimmune disease.

Complete Antigens

A complete antigen is essentially a hapten-carrier adduct (Fig. 131.). Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but will usually not initiate an immune response.

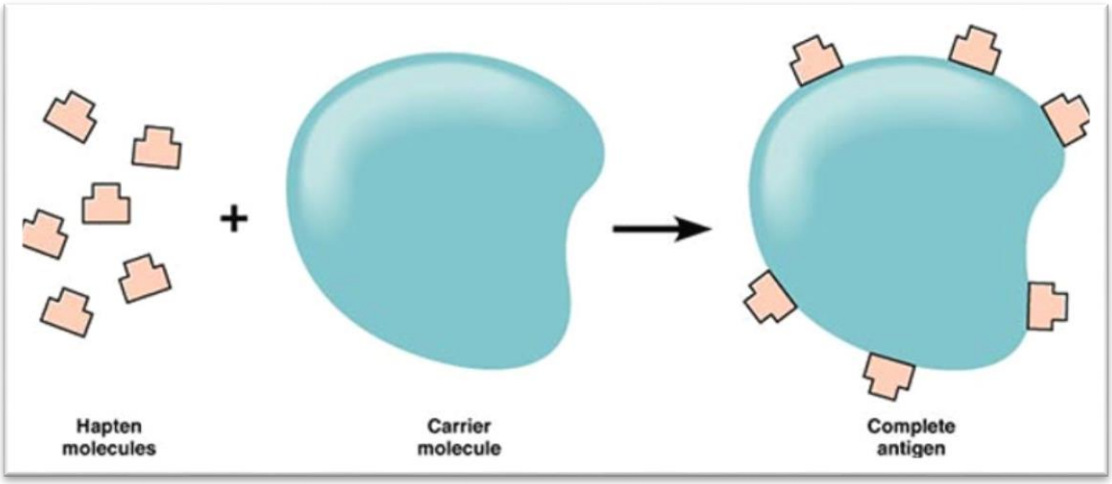


Fig. 131. Complete antigen

34. ANTIGENICITY AND IMMUNOGENICITY. AFFINITY, AVIDITY, CROSS REACTIONS

Immunogenicity: the difference in the ability of foreign molecules tostimulate an immune response

Antigenicity: the ability of a molecule to be recognized by antibodies orlymphocytes.

- Immunogenicity is the ability to induce a humoral and/or cell-mediated immune response.

B cells + antigen = effector B cells + memory B cells
T cells + antigen = effector T cells + memory T cells

- Antigenicity is the ability to combine specifically with the final products of immune response (i.e. secreted antibodies and/or surface receptors on T-cells).
- Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true.

Factors that influence antigenicity:

1. Molecular size: large molecules are better antigens then small molecules.

Very small molecules may, however, bind to large proteins, and the resulting complexesmay provoke an immune respons.

2. Complexity: the more complex an antigen is the better.
3. Structural stability: in order to recognize a molecule as a foreign the immune system mustrecognize its shape.
4. Degradability: not all foreign molecules are capable of stimulating an immuneresponse.e.g. Stainless steel pins and plastic joints are implanted in the bodywithout triggering an immune response.The lack of antigenicity of the large, inert organic polymers is due totheir molecular uniformity and their inertness. Foreign molecules that are unstable and destroyed very rapidly may notprovide sufficient antigen to stimulate an immune response.
5. Foreignness: the suppression of cells that react with normal body components (self-antigens) occur because these cells are exposed to self-antigen whenimmature (usually early in fetal life) and consequently eliminated(selectively killed or otherwise suppressed).

Autoantigens: In some situations (and not always abnormal ones) an animal may mount immuneresponses against normal body components. These are called autoimmuneresponses. Antigens that induce autoimmunity are called **autoantigens.**

Not all antibodies bind with the same strength, specificity, and stability. In fact, antibodies exhibit different affinities (attraction) depending on the molecular complementarity between antigen and antibody molecules. An antibody with a higher affinity for a particular antigen would bind more strongly and stably, and thus would be expected to present a more challenging defense against the pathogen corresponding to the specific antigen. **Affinity** refers to the strength of single interaction between antigen and antibody (Fig. 132.), while avidity refers to the strength of all interactions combined.

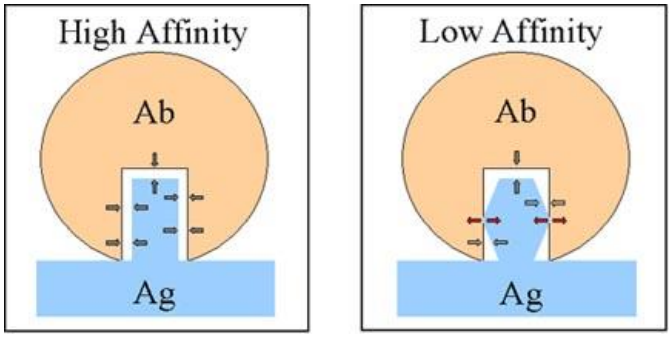


Fig. 132. Affinity refers to the strength of single interaction between antigen and antibody

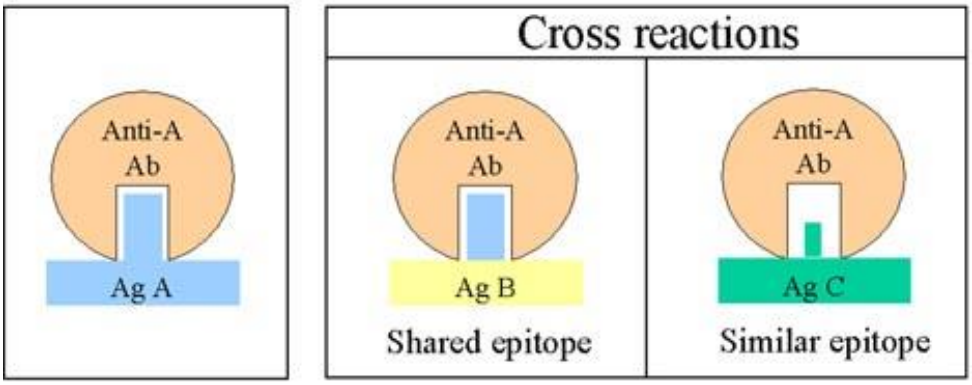


Fig. 133. Cross reactions

Antibodies secreted after binding to one epitope on an antigen may exhibit **cross reactivity** for the same or similar epitopes on different antigens (Fig. 133.). Because an epitope corresponds to such a small region (the surface area of about four to six amino acids), it is possible for different macromolecules to exhibit the same molecular identities and orientations over short regions. Cross reactivity describes when an antibody binds not to the antigen that elicited its synthesis and secretion, but to a different antigen.

Cross reactivity can be beneficial if an individual develops immunity to several related pathogens despite having only been exposed to or vaccinated against one of them. For instance, antibody cross reactivity may occur against the similar surface structures of various Gram-negative bacteria. Conversely, antibodies raised against pathogenic molecular components that resemble self molecules may incorrectly mark host cells for destruction and cause autoimmune damage. Patients who develop systemic lupus erythematosus (SLE) commonly exhibit antibodies that react with their own DNA. These antibodies may have been initially raised against the nucleic acid of microorganisms but later cross-reacted with self-antigens. This phenomenon is also called molecular mimicry.

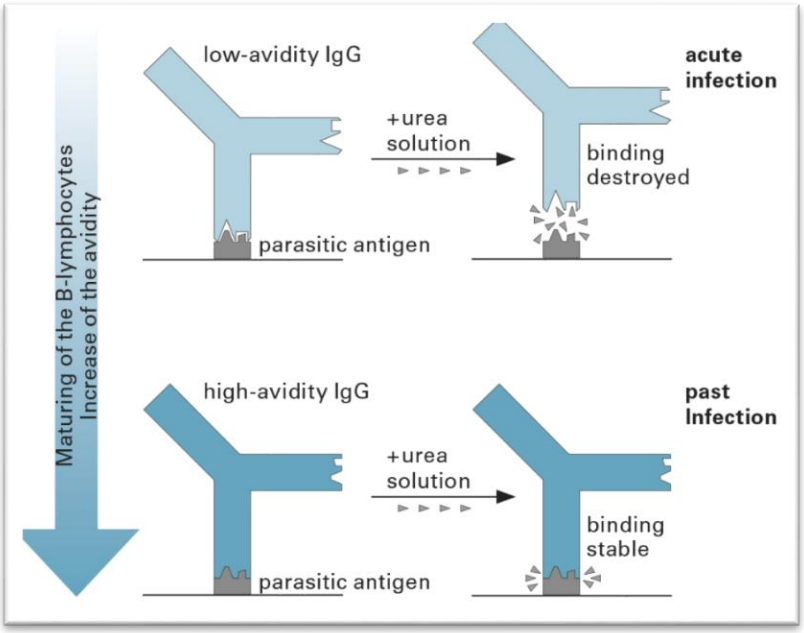


Fig. 134. Maturing of the B-lymphocytes increase of the avidity

The term **avidity** describes binding by antibody classes that are secreted as joined, multivalent structures (such as IgM and IgA) (Fig. 134.). Although avidity measures the strength of binding, just as affinity does, the avidity is not simply the sum of the affinities of the antibodies in a multimeric structure. The avidity depends on the number of identical binding sites on the antigen being detected, as well as other physical and chemical factors. Typically, multimeric antibodies, such as pentameric IgM, are classified as having lower affinity than monomeric antibodies, but high avidity. Essentially,

the fact that multimeric antibodies can bind many antigens simultaneously balances their slightly lower binding strength for each antibody/antigen interaction.

35. MAJOR HISTOCOMPATIBILITY COMPLEX 1ST AND 2ND TYPES, ANTIGENIC PRESENTATION.

The major histocompatibility complex can be defined as a tightly linked cluster of genes whose products play an important role in intercellular recognition and in discrimination between self and non-self. The term 'histo' stands for tissue and 'compatibility' refers to 'getting along or agreeable'. On the other hand, the term 'complex' refers to the 'genes that are localized to a large genetic region containing multiple loci'. These genes code for antigens which involve in the determination of the compatibility of the transplanted tissue. The compatible tissues will be accepted by the immune system while the histo-incompatible ones are rejected. The rejection of foreign tissue leads to an immune response to cell surface molecules. The concept was first identified by Peter Gorer and George Snell. The main function of MHC molecules is to bring antigen to the cell surface for recognition by T cells. In humans, the genes coding for MHC molecules are found in the short arm of chromosome 6.

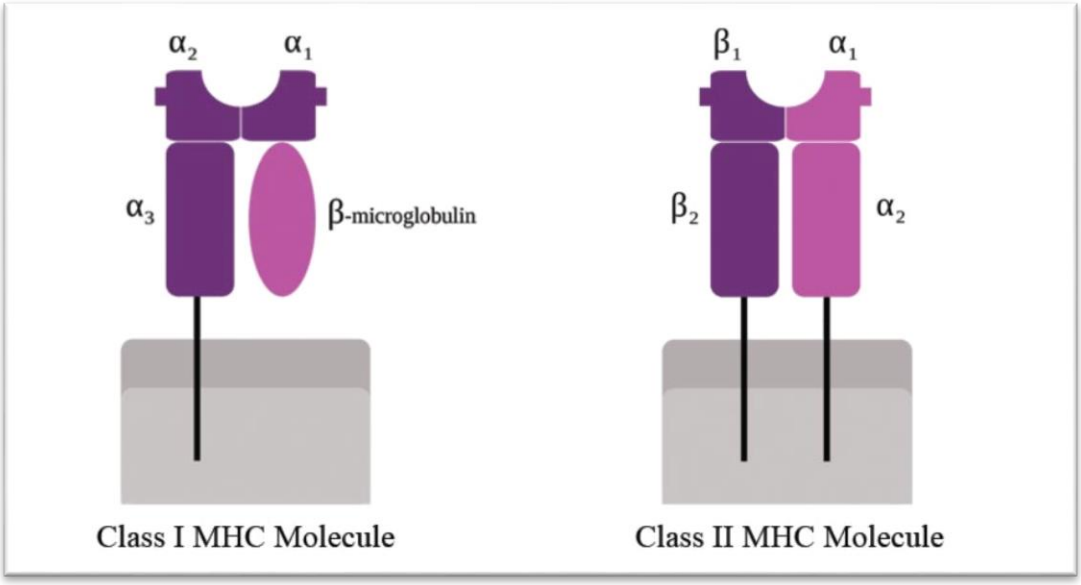


Fig. 135. Major Histocompatibility Complex (MHC) Types

Major Histocompatibility Complex (MHC) Types

In humans, the MHC molecules are divided into three types, Class I, Class II and Class III. Class I MHC molecules are coded from three different locations called A, B and C and these molecules are expressed in all nucleated cells. Class II MHC genes are located in the D region and there are several loci such as DR, DQ and DP and these molecules are expressed only in antigen-presenting cells. Class III MHC genes are coded in the region between Class I and Class II genes. Class III MHC genes codes for cytokines and complement proteins which play an important role during the immune response.

Antigen Presentation and Processing

The T cells can recognize the foreign antigen when the antigen is attached to the MHC molecules as an MHC peptide complex. The formation of the MHC-peptide complex requires the degradation of protein antigen by several steps. The degradation process is known as antigen processing. These degraded proteins are then attached to the MHC molecules inside the cell and then the MHC molecules transported to the membrane to present the antigen with the T cell.

Antigen Presentation Pathway: Class I MHC molecules (Cytosolic pathway):

- Class I MHC molecules involve in presenting intracellular or endogenous pathogens or antigens. Intracellular pathogens refer to those organisms which live and replicates inside the host cell. An example of this type of pathogen is a virus.
- Under normal condition the MHC class I molecules forms a complex with the self-peptides or self-antigens. While, in case of any viral infection, the MHC class I molecules present the peptide derived from the virus which is then further recognized by T cells.
- Cell components such as a nucleus, endoplasmic reticulum and Golgi apparatus play an important role in antigen processing and presentation.
- When a virus infected a normal cell, the viral DNA moves inside the cell and produce viral proteins with the help of the host cell mechanisms. The viral proteins are synthesized in the cytosol.
- The cytoplasm also contains a cylindrical protein complex called the proteasome. The main function of the proteosome is to degrade the unwanted or damaged protein into smaller peptides. At the time of viral infection, the viral proteins interacted with the proteosomes present in the cytoplasm. The processing took place in the cytosol and as a result, the proteins are degraded into smaller peptides (8-15 amino acid long).
- In the next step, these fragmented peptides are transported into the endoplasmic reticulum. The transport took place due to a peptide delivery system called the transporter associated with antigen processing (TAP). TAP is made up of two domains or subunits called TAP 1 and TAP 2.
- Inside the endoplasmic reticulum the α and β chains of MHC class I molecules are synthesized and by the help of a group of chaperone proteins, the MHC class I molecule is formed and moves towards the TAP. As a result, the peptides bind at the peptide-binding site of the class I MHC molecule inside the endoplasmic reticulum and forms the MHC class I-peptide complex.
- In the next step, the MHC class I- peptide complex moves to the surface of the Golgi apparatus and by the help of secretory vesicle, it moves towards the surface of the plasma membrane.
- Once the MHC class I-peptide complex reaches the cell surface, the T cell receptors recognize the antigen peptide complex. Moreover, the co-receptor CD8 of the T cell attaches with the α3 domain of the MHC class I molecule. Hence, the antigen is presented to the T cell.

Antigen Presentation Pathway: Class II MHC molecules (Endocytic Pathway)

- MHC class II molecules are responsible for presenting exogenous or extracellular pathogen or antigen. The extracellular pathogen refers to the organisms which can grow and reproduce outside of the host cell. Bacteria, exotoxins, parasites are examples of extracellular antigens. These antigens are taken up by the cell by endocytosis or phagocytosis.
- Only the antigen-presenting cells involved in antigen processing and presentation by MHC class II molecules. These cells include B cells, macrophages, and dendritic cells. The pathway took place only after the engulfment of the antigen by the antigen-presenting cells.
- Inside the cell, the antigen carries a covering called an endosome. The endosome is fused with the lysosome present in the cytoplasm and forms endolysosomes. As a result, the foreign protein is degraded by the proteolytic enzyme present inside the lysosome and small peptides are formed.
- The class II MHC molecules are synthesized and formed in the endoplasmic reticulum. The α and β chain of the molecule is also associated with the invariant chain. This association helps to restrict the binding of self-antigen with the class II MHC molecule. The invariant chain-MHC complex is then transported from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to another vesicle. Inside the vesicle, the invariant chain is digested and only a small fragment (Class II-associated invariant chain polypeptide: CLIP) is attached with the molecule.

- In the next step, the vesicle containing the MHC class II molecule is then fused with the vesicle containing fragmented peptides. The fragmented peptide is then bound with the MHC class II molecule by displacing the CLIP. This newly formed MHC class II-peptide complex is then transported to the surface of the cell.
- Once at the cell surface, the antigen is presented to the T cells. The T cell recognizes the peptide bound with the MHC class II molecule by the help of the T cell receptor and the CD4 co-receptor binds with the β_2 domain of the class II MHC molecule.

36. TYPES OF IMMUNITY: ACTIVE, PASSIVE, HERD AND LOCAL

Apart from active and passive immunity, there's herd immunity which is achieved through active or passive ones. Herd immunity means when most of a population is immune to an infectious disease, this provides population immunity- to those who are not immune to the disease. An example for the same could be if 80 percent of a particular population is immune to polio, four out of every five people who encounter someone with the disease won't get sick and won't spread the disease any further. Thankfully, due to this, the spread of infectious diseases is kept under control.

Depending on how contagious infection is, usually, 50 percent to 90 percent of the population needs immunity before infection rates start to decline. This is why it is important to get as many people as possible vaccinated against a particular disease provided that the vaccine for it is available. Once herd immunity has been established for a while, and the ability of the disease to spread is hindered, the disease can eventually be eliminated.

Local immunity is a set of certain cells and substances in a certain place that has contact with the environment, protects this place from the penetration of pathogens (bacteria, viruses, foreign substances). For example, local immunity of the oral cavity, intestinal cavity, bronchi. As you can see, all these surfaces are in contact with the environment and are most prone to disease. The system of local immunity is diverse. It includes immunoglobulin A, contained in saliva, intestinal mucus, bronchial sputum. And the cells that carry out phagocytosis, and lysozyme and much more. Secretory immunoglobulins that can neutralize the virus (bacteria) where it tries to enter the body.

Active and passive immunity difference can be understood with the help of a tabular representation as follows (Table 2).

Table 2. Active and passive immunity

Characteristics	Passive Immunity	Active Immunity
Length of protection	Short-term protection	Long-lasting protection
Production of Abs	The host receives antibodies from another source.	The host's own body will make antibodies when exposed to pathogens; lymphocytes form memory cells.
Immunological memory	It doesn't generate immunological memory.	It generates immunological memory.
Speed of response	It has a faster response.	It has a slower response.
Side-effects	It may consist of certain side-effects when it is given externally.	It has no side-effects and doesn't cause chemical reactions.

Active Immunity - it is the immunity where the body produces its own antibodies when a body is exposed to that disease. It allows an immune system to recognize a disease which will then trigger our body to fight against it. It is often long-lasting and may sometimes give us life-long protection against diseases.

Passive Immunity - it is a type of immunity that is provided when a person is given antibodies from outside. It provides immediate protection but doesn't guarantee long-term protection like active immunity.

Below are a few examples of active and passive immunity that can make us understand both categories in a better manner (Table 3).

Table 3. Examples of Active and Passive Immunity.

Passive Immunity	Active Immunity
Examples- • Baby receiving antibodies (IgG) from the mother during the third trimester of pregnancy. • Breast Milk that contains IgA antibodies in addition to other Abs. • Immunoglobulin injection, for example, is given after rabies exposure.	Examples- • When a body is exposed to the pathogens in everyday life, passive immunity is triggered. • Exposure of the body to some part of the pathogen in a vaccine such as: • Live attenuated vaccine (MMR) • Deactivated toxins from a pathogen (Corynebacterium diphtheria toxin, Clostridium tetani toxin) • Part of the bacterial cell (Bordetella pertussis and streptococcus pneumonia)

37. SEROLOGICAL REACTIONS

1. Precipitation Test:

When soluble antigens and its homologous antibody molecules react, they sometimes form large polymeric macromolecules terminating into visible precipitate (Fig. 136.).

The precipitation occurs in two stages: first, the antibodies bind to antigens forming antibody-antigen-complex within a few seconds or minutes, then the "constant regions" of antibodies of the complexes bind to each other within some hours resulting in the formation of visible precipitate.

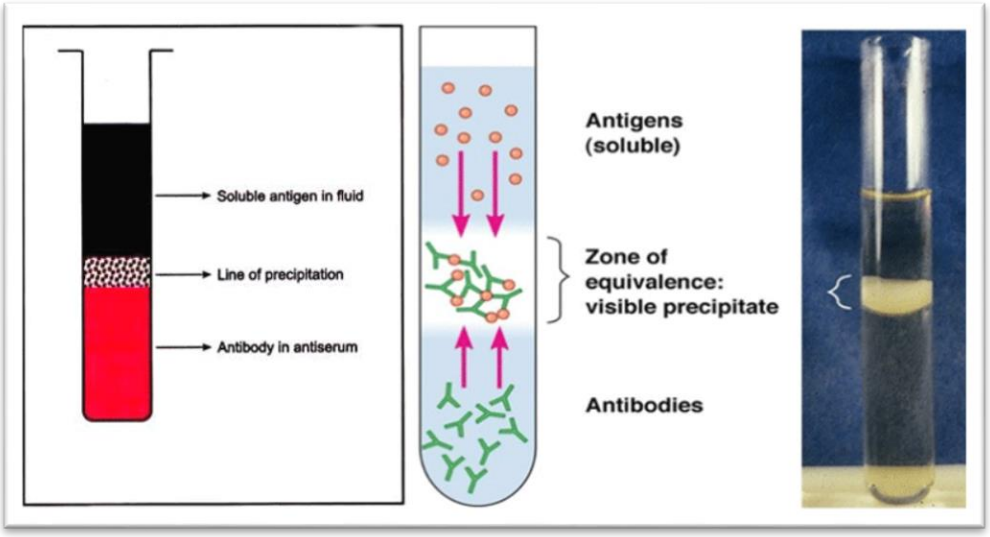


Fig. 136. Ring precipitation test

The formation of precipitate is dependent on the proper relative concentrations of the antigen and antibody molecules in a specific region called the zone of equivalence or zone of precipitation, i.e., the zone of equivalence (or precipitation) defines the region wherein the concentrations of antigen and antibody molecules reach almost equivalence.

Precipitation-tests are performed either in fluid-fluid precipitation, or gel-gel precipitation (Fig. 137-138.). In the former, the solutions of antigen and antibody are layered over each other in a thin tube, whereas the diffusion of antigen and antibody takes place through a semisolid gel (such as agarose).

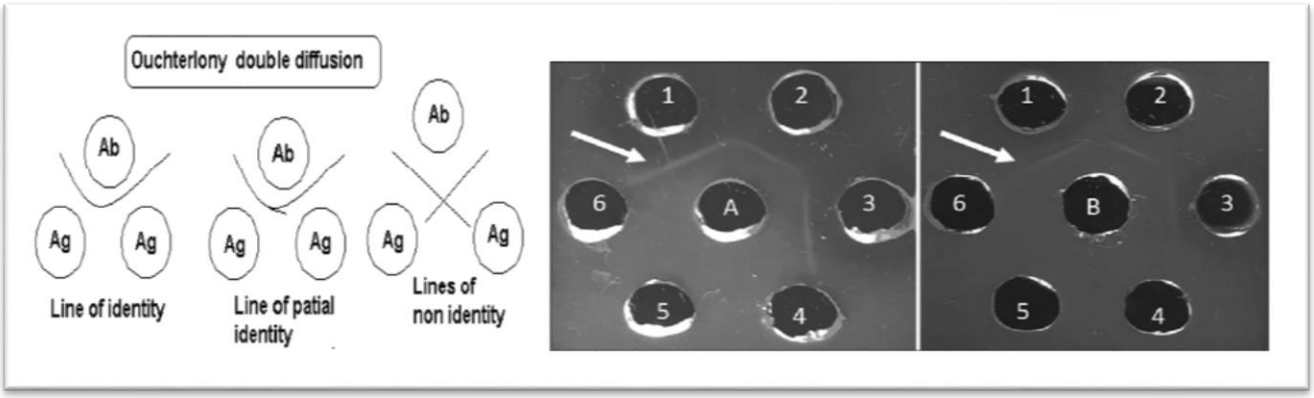


Fig. 137. Double diffusion Precipitation or Ouchterlony test

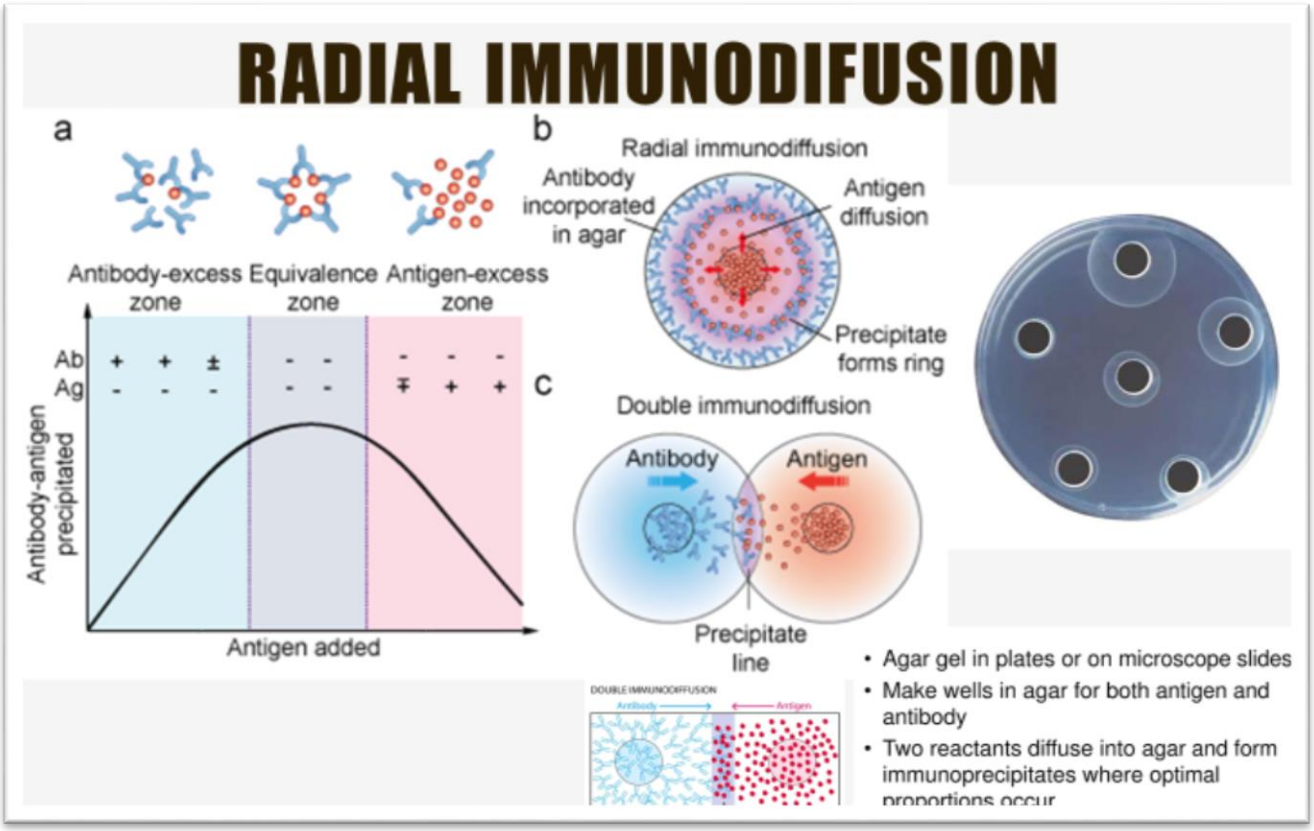


Fig. 138. Radial Immunodifusion

2. Agglutination Test:

Agglutination-test is that in which visible clumping or aggregation of cells or particles takes place due to the reaction of surface-bound antigens of such cells or particles with homologous antibodies (Fig. 139.).

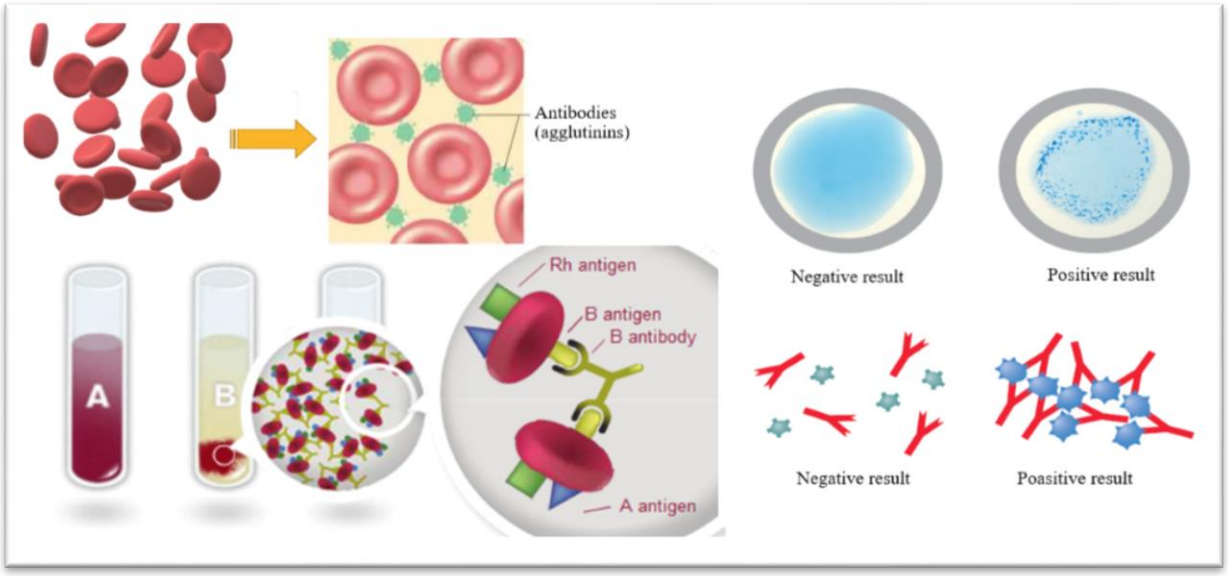


Fig. 139. Agglutination-test

Pathogens causing many diseases like typhoid fever (Salmonella typhi), gonorrhoea (Neisseria gonorrhoeae), rickettsial diseases are detected by agglutination-tests; it is probably best known for its use in human blood typing (haemagglutination).

Four types of human blood (A, B, AB, O) are recognized on the basis that the human red blood cells (RBCs) possess either type A or type B polysaccharide antigens, or both type A and type B polysaccharide antigens, or neither of these two antigens on their surface, respectively.

Blood types are determined by mixing known antisera (anti-A and anti-B antibodies) with a blood sample. An agglutination reaction indicates the presence of the corresponding antigen.

Type A blood possesses type A but not type B antigens on the surface of RBCs; type B blood has the type B but not type A antigens on the surface of RBCs; type AB blood possesses both type A and type B antigens on the surface of RBCs; and, type O blood has neither type A nor type B antigens on the surface of RBCs.

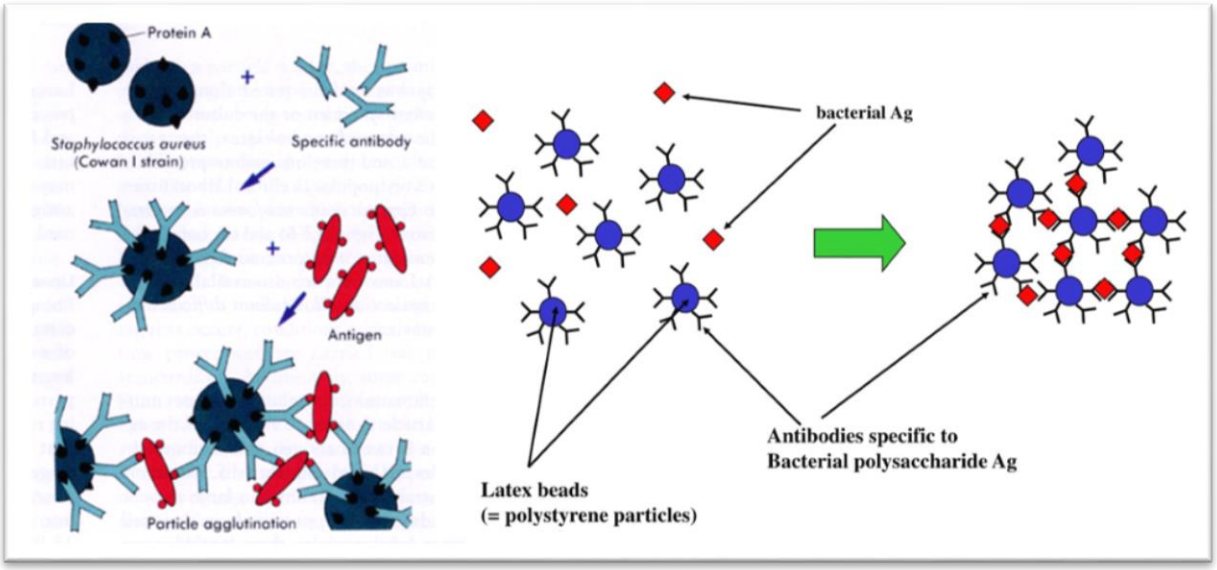


Fig. 140. Passive agglutination (Latex agglutination test)

In those cases where the antigens are not present or cell on particle surfaces and remain free in soluble- state, the direct agglutination-tests normally fail. For detection of such antigens, passive agglutination-test is employed.

In passive agglutination-test (Fig. 140.), the soluble antigens are taken out, are first attached to the surface of one of the carriers like latex beads, polystyrene, particle, red blood cells, and then

mixed with patient's serum. Homologous antibodies present in serum attach with antigens present on the surface of the carrier forming antigen-antibody-complexes that agglutinate.An excellent example of passive agglutination-test using latex beads as carrier is one of the modern pregnancy test; other examples are the detection of pathogens like Haemophilus influenzae (meningitis), Streptococcus pneumoniae (pneumonia), Neisseria meningitis (meningococcal meningitis), Treponema pallidum (Syphilis), Rubella virus (German measles), etc.

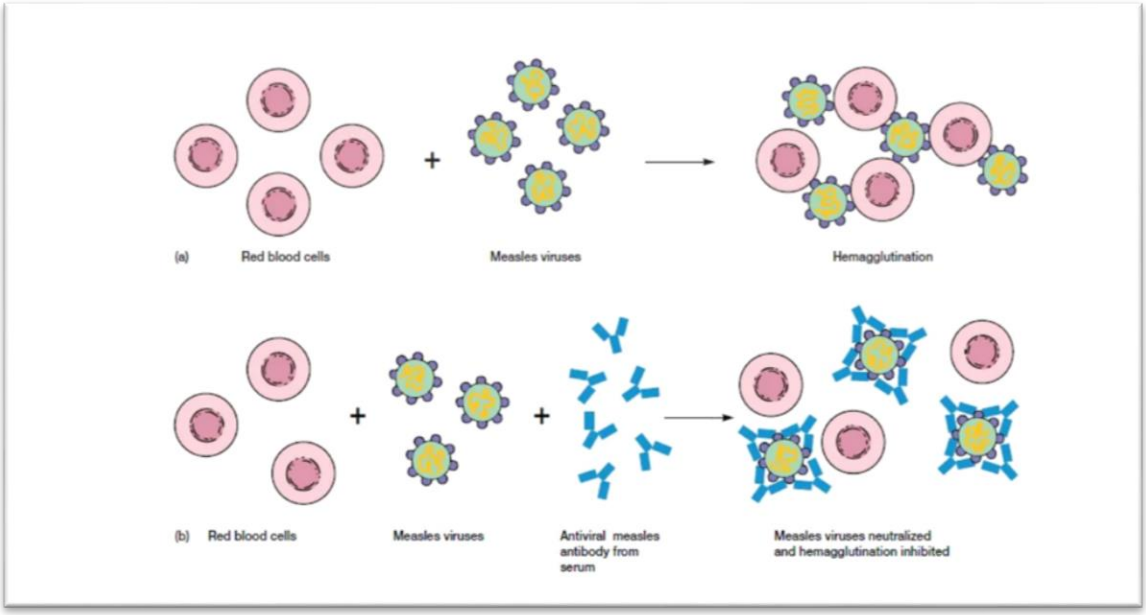


Fig. 141. Hemagglutinations inhibition test

3. Radioimmunoassay (RIA):

Radioimmunoassay (RIA) is a widely accepted and highly sensitive serological test in which one of the reactants—antibody, antigen or hapten—is radiolabeled with radioactive isotopes of elements like iodine (125I) or hydrogen (3H) are detected in situ by radioautography.

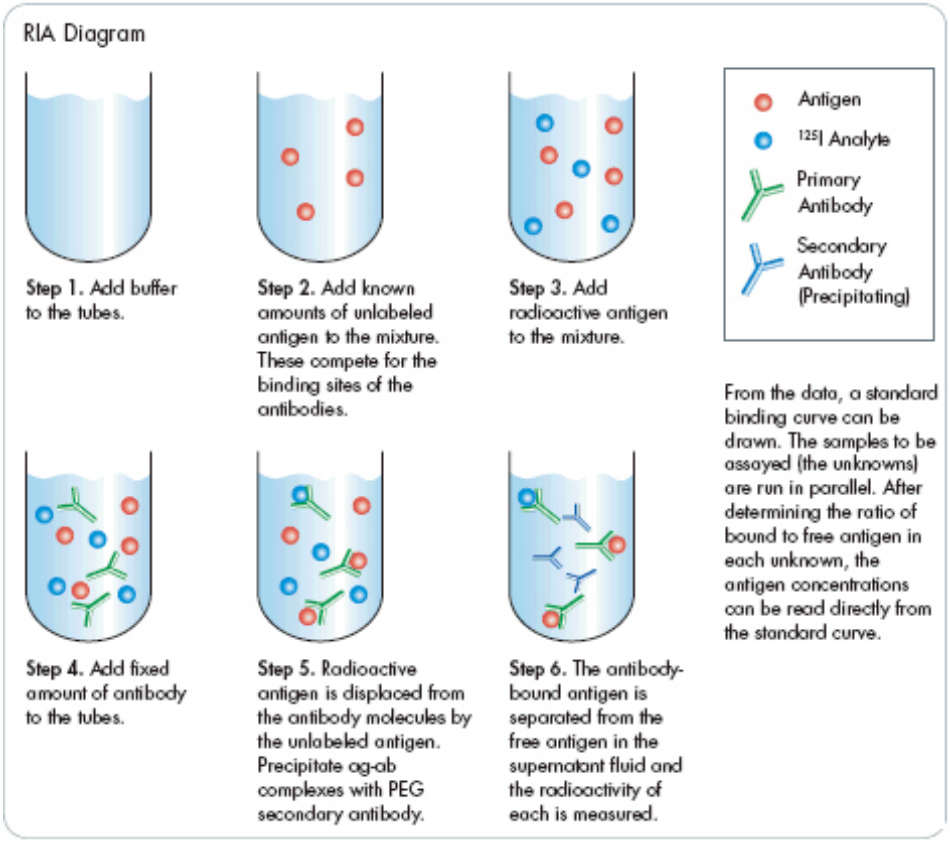


Fig. 142. Radioimmunoassay (RIA)

This technique was developed in 1960s to detect hormones such as insulin, and is now used to quantify very low levels of polypeptides, steroids, thyroid hormones, vitamin B12, and viruses.

The steps involved in radioimmunoassay are:

- A sample containing an unknown quantity of antigenic substance and its specific antibodies that react to form antigen-antibody-complex, is taken.
- A known amount of radiolabeled antigenic substance is added which combines with unreacted antibody molecules in the sample forming radiolabeled antigen-antibody complex,
- The radiolabeled antigen-antibody-complex is separated from the sample and its amount is determined,
- The concentration of the unknown antigenic substance in the sample is calculated (Fig.. 142.).

The basis of radioimmunoassay is the competition between a known amount of an antigenic substance that is radiolabelled and an unknown amount of the same antigenic substance that is non-radiolabelled for the same antibody.

The relative amounts of radiolabeled and non-radiolabelled antigenic substance that bind with the antibody molecules indicates the levels of the antigenic substance in the sample. High levels of antigen- antibody-complex (radiolabelled) indicates a low level of unknown antigenic substance whereas low level of radiolabelled antigen-antibody-complex indicates a high level of unknown antigenic substance in the sample.

4.Complement fixation test

Complement fixation refers to the ability of antigen-antibody complex to bind complement so that the latter becomes “fixed” and “used up” (Fig. 143.). The complement fixation is used in complement fixation test (CFT), which is very versatile and sensitive and can be used to detect extremely small ; mount of an antibody (as little as 0.04 μg) for a suspect microorganism in an individual’s serum.

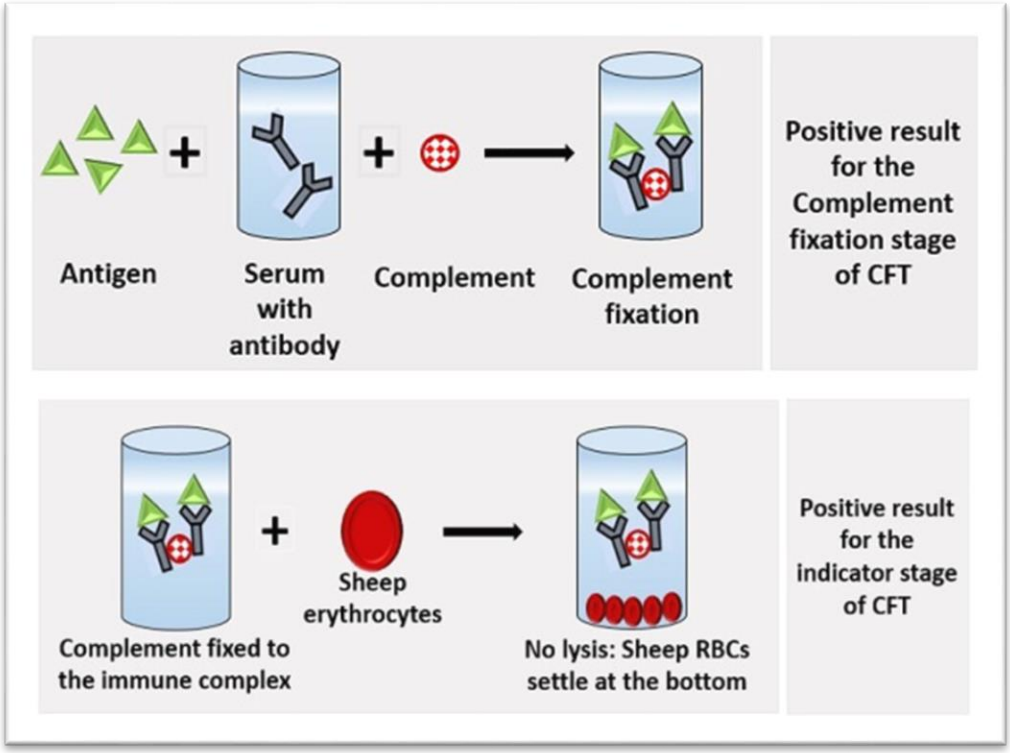


Fig. 143. Complement Fixation test (Positive test)

Complement fixation test is performed in two stages. In stage 1, test serum (for the detection of antibody) and the antigen are mixed carefully in a test tube. If the test serum contains antibody then antigen-antibody complexes (immune complexes) are formed. Now a measured amount of

complement is added to the mixture and the latter is incubated at 37°C for one hour. The complement is fixed and used by the immune complexes.

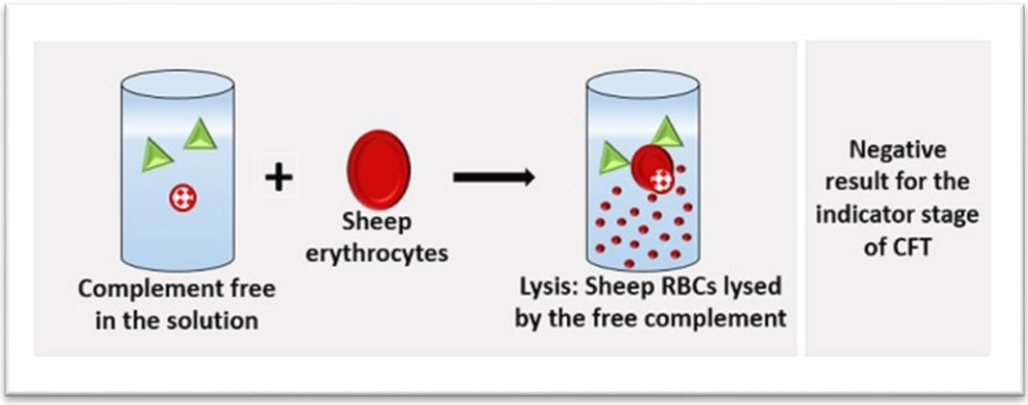


Fig. 144. Complement Fixation test (Negative test)

In stage 2, sensitized indicator cells, usually sheep red blood cells previously coated with complement-fixing antibodies, are added to the mixture; the indicator cells help determining whether the complement has been fixed and consumed in stage 1 reaction or not.

One of the two, positive and negative, tests are found at this stage. If the complement has been fixed and consumed during stage I reaction, insufficient amount of complement will be available to lyse the indicator cells and; therefore, a positive test is obtained (Fig. 143.).

On the other hand, if the complement has not been fixed and consumed due to absence of antibodies during stage 1 reaction, the unused complement results in lysis of the indicator cells and, therefore, a negative test is obtained. (Fig. 144.). Absence of lysis of indicator cells (positive test) shows that specific antibodies are present in the test serum, whereas presence of lysis (negative test) shows that specific antibodies are absent in the test serum.

Complement fixation test was once used in the diagnosis of syphilis and is currently employed in the diagnosis of certain viral, rickettsial, chlamydial, protozoan, and fungal diseases.

5. Enzyme-Linked Immunosorbant Assay (ELISA):

ELISA has been pioneered by two groups of scientists, one in Sweden by Engvall and Perlmann, and the other in Holland by Van Weeman and Schurs in 1972, and developed by Clark and Adams in 1977. ELISA is based on, as the name suggests, enzyme-linked antibodies adsorbed on some solid surface.

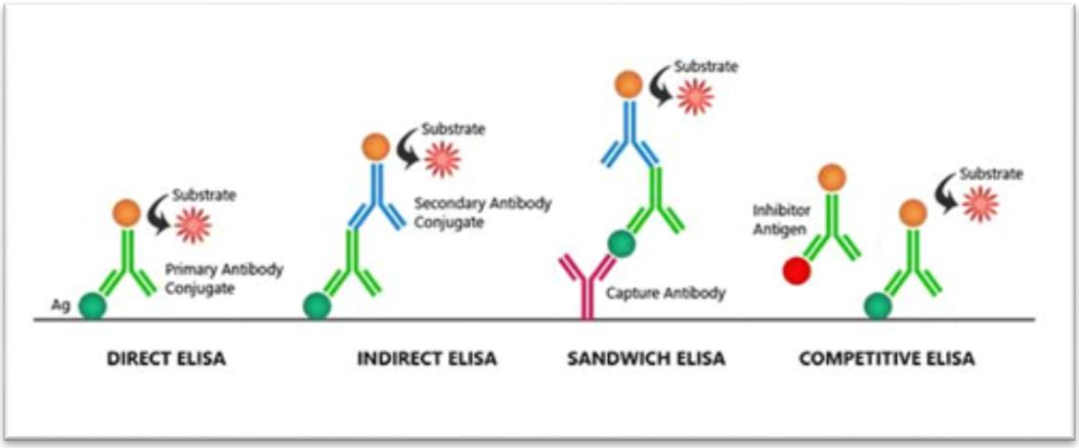


Fig. 145. Types of ELISA

The most commonly employed enzyme is alkaline phosphatase (other enzymes used are peroxidase, glucose oxidase, p-galactosidase, malate dehydrogenase, etc.) and the solid surface is that of micro-ELISA plates (Fig. 146.). having shallow walls (depressions, capacity 0.4 ml) and made up of polystyrene which has the property to bind with antigen or antibody covalently.

Fig. 146. Microplates for ELISA

There are two method of ELISA: indirect- ELISA used for the detection and measurement of antibody and direct-ELISA used for the detection of antigen.

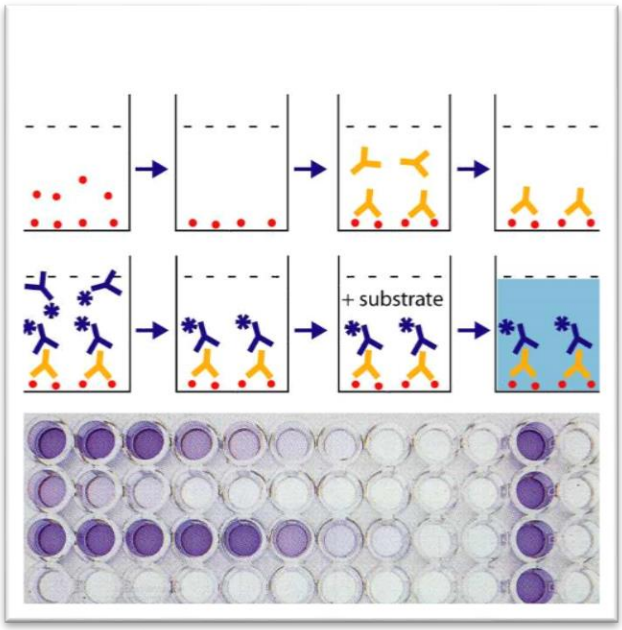


Fig. 147. Inderect ELISA

1. Indirect-ELISA (Fig. 147.):

Indirect-ELISA is per-formed involving the following steps:

- The wells (depressions) of the micro-ELISA plates are first filled with antigen which adsorb to the well surface, the wells are then emptied and washed so that free antigens are removed.
- Test-antiserum is added to these walls and allowed to incubate; if the antibodies in the antiserum are homologous, they bind with adsorbed antigens forming antigen- antibody complex. The wells are again washed to remove free antibodies, if any.
- Enzyme- conjugated (labelled) antibodies are now added which link to the antigen-antibody-complex formed in the previous step. Unlinked enzyme-conjugated antibodies are washed away.
- A substrate that reacts with the enzyme is added. The substrate is degraded (hydrolysed) as a result of its reaction with enzyme and a coloured product is resulted in.
- The concentration of the antibody present in the test antiserum can be estimated by measuring the intensity of the colour using spectrophotometer. It is because the intensity of the colour is associated with amount of substrate degradation which is directly proportional to the amount of enzyme-linked antibody which, in turn, is proportional to the amount of antibody present in the test-antiserum.

2. Direct-ELISA or Double-Antibody Sandwich ELISA (Fig. 148.):

Direct-ELISA (or double-antibody sandwich ELISA) is performed involving the following steps:

- The wells of the micro-ELISA plate are filled with antiserum, the antibodies present in the antiserum adhere to the surface of each well. The wells are then emptied and washed so that free antibodies are removed.
- The test-antiserum is added and, if the antigens are homologous, they bind with absorbed antibodies forming antigen-antibody-complex. The wells are again washed to remove free antigens, if any.
- Enzyme-conjugated (labelled) antibodies specific to the antigen are then added. These antibodies link to the antigen already fixed by the first antibody; this results in an antibody (with enzyme)-antigen-antibody "sandwich". Washing helps removing unbind enzyme-conjugated antibodies at this stage.
- Enzyme substrate is added which is degraded (hydrolysed) as a result of its action with the enzyme, and results in colour change that can be visualized or measured using spectrophotometer.
- The intensity of colour is proportional to the enzyme action, which is directly proportional to the quantity of enzyme-conjugated antibodies present, and that in turn is proportional to the amount of the test-antigen.

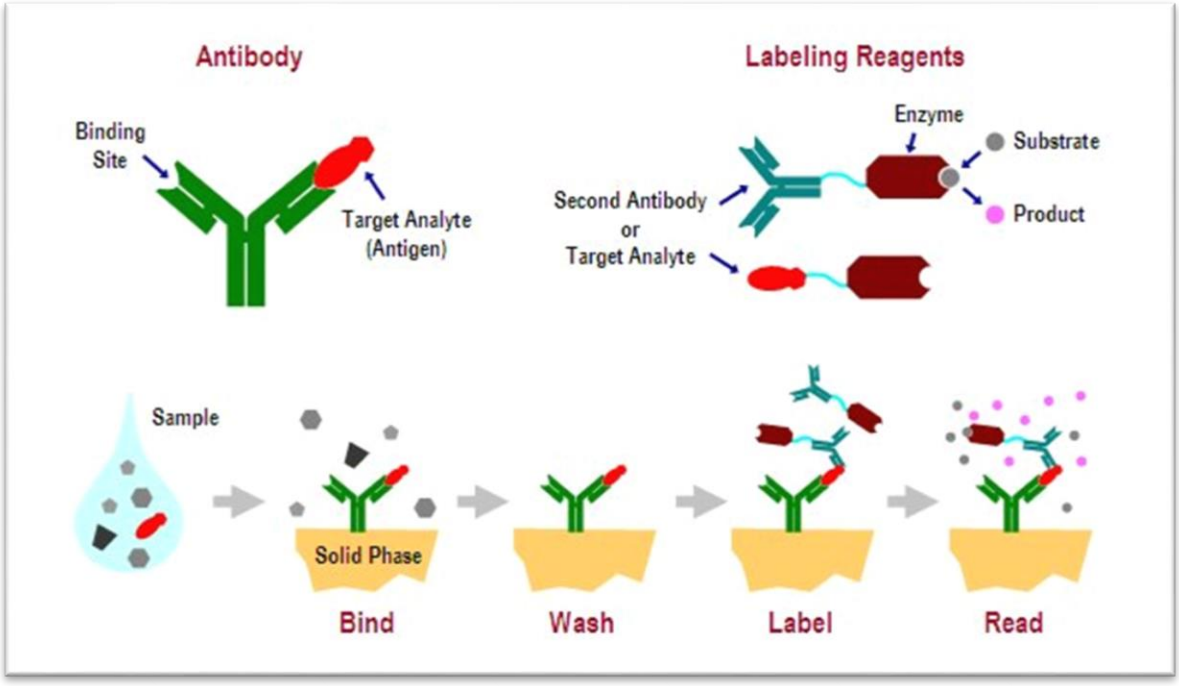


Fig. 148. Direct-ELISA or Double-Antibody Sandwich ELISA

ELISA is advantageous over other methods of serology because of its simplicity, less expensiveness, sensitivity, and accuracy similar to that of radioimmunoassay (antigens and antibodies detectable at levels of about 10-10 g/ml or part in ten billion), stability of reagents (reagents remain fully functional both immunologically and enzymatically throughout the process), and most importantly, the lack of radiation hazards as the radioisotopes are not used. ELISA, however, is a time saving device and can be completed within hours even in laboratories with rudimentary facilities if prepared enzyme-conjugated antibodies are available.

38. ALLERGY

An allergy is a reaction by your immune system to something that does not bother most other people. People who have allergies often are sensitive to more than one thing.

Substances that often cause reactions are:

- Pollen
- Dust mites
- Mold spores
- Pet dander
- Food
- Insect stings
- Medicines

Normally, your immune system fights germs. It is your body's defense system. In most allergic reactions, however, it is responding to a false alarm. Genes and the environment probably both play a role.

Allergies can cause a variety of symptoms such as a runny nose, sneezing, itching, rashes, swelling, or asthma. Allergies can range from minor to severe. Anaphylaxis is a severe reaction that can be life-threatening. Doctors use skin and blood tests to diagnose allergies. Treatments include medicines, allergy shots, and avoiding the substances that cause the reactions.

Allergies often bring to mind sneezing, a runny nose or watery eyes. While these are symptoms of some types of allergic disease, it’s important to understand that an allergic reaction is actually a result of a chain reaction that begins in your genes and is expressed by your immune system.

Allergy symptoms, which depend on the substance involved, can affect your airways, sinuses and nasal passages, skin, and digestive system. Allergic reactions can range from mild to severe. In some severe cases, allergies can trigger a life-threatening reaction known as anaphylaxis.

Anaphylaxis is a serious, life-threatening allergic reaction. The most common anaphylactic reactions are to foods, insect stings, medications and latex. Anaphylaxis typically affects more than one part of the body at the same time. Symptoms include a feeling of warmth, flushing, a red, itchy rash, feelings of light-headedness, shortness of breath, throat tightness, anxiety, pain/cramps and/or vomiting and diarrhea. In severe cases, you may experience a drop in blood pressure that results in a loss of consciousness and shock.

Anaphylaxis requires immediate medical treatment, including an injection of epinephrine and a trip to a hospital emergency room. If it is not treated properly, anaphylaxis can be fatal.

<u>Hay fever, also called allergic rhinitis, can cause:</u>

- Sneezing
- Itching of the nose, eyes or roof of the mouth
- Runny, stuffy nose
- Watery, red or swollen eyes (conjunctivitis)

<u>A food allergy can cause:</u>

- Tingling in the mouth
- Swelling of the lips, tongue, face or throat
- Hives
- Anaphylaxis

<u>An insect sting allergy can cause:</u>

- A large area of swelling (edema) at the sting site
- Itching or hives all over the body
- Cough, chest tightness, wheezing or shortness of breath
- Anaphylaxis

<u>A drug allergy can cause:</u>

- Hives
- Itchy skin

- Rash
- Facial swelling
- Wheezing
- Anaphylaxis

Atopic dermatitis, an allergic skin condition also called eczema, can cause skin to:

- Itch
- Redden
- Flake or peel

Antihistamines are medications used mainly Trusted Sourcefor hay fever and other allergies. They counter the effects of histamines, substances that the body produces to help the immune system fight invaders such as viruses.

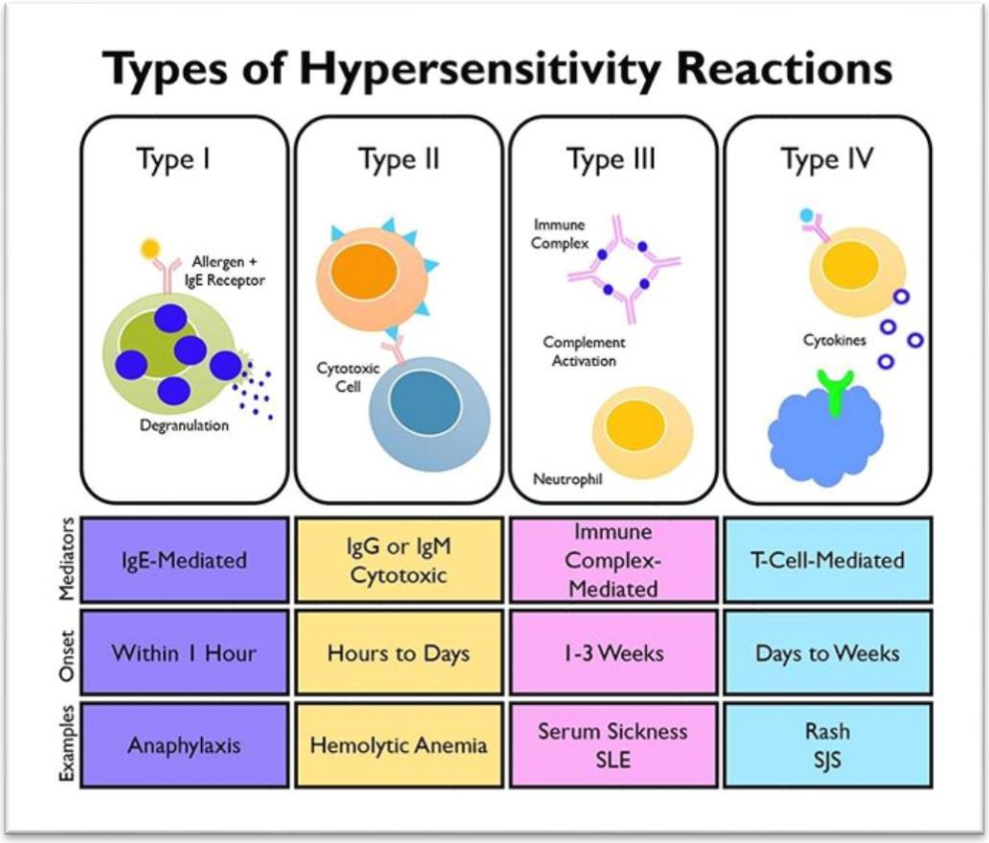


Fig. 149. Types of Hypersensitivity Reactions

Hypersensitivity reactions (Fig. 149.) are exaggerated immune responses to allergens. There are four types of hypersensitivity reactions. Types I through III are mediated by antibodies, while type IV is mediated by T cell lymphocytes.

Type I hypersensitivities involve IgE antibodies that initially sensitize an individual to an allergen and provoke a quick inflammatory response upon subsequent exposure. Allergies and hay fever are both type I.

Type II hypersensitivities involve the binding of IgG and IgM antibodies to antigens on cell surfaces. This induces a cascade of events that leads to cell death. Hemolytic transfusion reactions and hemolytic disease of newborns are type II reactions.

Type III hypersensitivities result from the formation of antigen-antibody complexes that settle on tissues and organs. In an attempt to remove these complexes, underlying tissue is also damaged. Serum sickness and rheumatoid arthritis are examples of type III reactions.

Type IV hypersensitivities are regulated by T cells and are delayed reactions to antigens associated with cells. Tuberculin reactions, chronic asthma, and contact dermatitis are examples of type IV reactions.

39. VACCINES AND IMMUNE SERUMS.

The first human vaccines against viruses were based using weaker or attenuated viruses to generate immunity. The smallpox vaccine used cowpox, a poxvirus that was similar enough to smallpox to protect against it but usually didn't cause serious illness. Rabies was the first virus attenuated in a lab to create a vaccine for humans.

Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

Vaccine Types. There are several different types of vaccines. Each type is designed to teach your immune system how to fight off certain kinds of germs—and the serious diseases they cause.

There are several types of vaccines, including:

- Inactivated vaccines
- Live-attenuated vaccines
- Messenger RNA (mRNA) vaccines
- Subunit, recombinant, polysaccharide, and conjugate vaccines
- Toxoid vaccines
- Viral vector vaccines

Inactivated vaccines

Inactivated vaccines use the killed version of the germ that causes a disease. Inactivated vaccines usually don't provide immunity (protection) that's as strong as live vaccines. So you may need several doses over time (booster shots) in order to get ongoing immunity against diseases.

Inactivated vaccines are used to protect against:

- Hepatitis A
- Flu (shot only)
- Polio (shot only)
- Rabies

Live-attenuated vaccines

Live vaccines use a weakened (or attenuated) form of the germ that causes a disease. Because these vaccines are so similar to the natural infection that they help prevent, they create a strong and long-lasting immune response. Just 1 or 2 doses of most live vaccines can give you a lifetime of protection against a germ and the disease it causes.

But live vaccines also have some limitations. For example: because they contain a small amount of the weakened live virus, some people should talk to their health care provider before receiving them, such as people with weakened immune systems, long-term health problems, or people who've had an organ transplant.

They need to be kept cool, so they don't travel well. That means they can't be used in countries with limited access to refrigerators.

Live vaccines are used to protect against:

- Measles, mumps, rubella (MMR combined vaccine)
- Rotavirus
- Smallpox
- Chickenpox
- Yellow fever

Messenger RNA vaccines—also called mRNA vaccines

Researchers have been studying and working with mRNA vaccines for decades and this technology was used to make some of the COVID-19 vaccines. mRNA vaccines make proteins in order to trigger an immune response. mRNA vaccines have several benefits compared to other types

of vaccines, including shorter manufacturing times and, because they do not contain a live virus, no risk of causing disease in the person getting vaccinated.

mRNA vaccines are used to protect against:

- COVID-19

Subunit, recombinant, polysaccharide, and conjugate vaccines

Subunit, recombinant, polysaccharide, and conjugate vaccines use specific pieces of the germ—like its protein, sugar, or capsid (a casing around the germ). Because these vaccines use only specific pieces of the germ, they give a very strong immune response that's targeted to key parts of the germ. They can also be used on almost everyone who needs them, including people with weakened immune systems and long-term health problems.

One limitation of these vaccines is that you may need booster shots to get ongoing protection against diseases.

These vaccines are used to protect against:

- Hib (Haemophilus influenzae type b) disease
- Hepatitis B
- HPV (Human papillomavirus)
- Whooping cough (part of the DTaP combined vaccine)
- Pneumococcal disease
- Meningococcal disease
- Shingles

Toxoid vaccines

Toxoid vaccines use a toxin (harmful product) made by the germ that causes a disease. They create immunity to the parts of the germ that cause a disease instead of the germ itself. That means the immune response is targeted to the toxin instead of the whole germ.

Like some other types of vaccines, you may need booster shots to get ongoing protection against diseases.

Toxoid vaccines are used to protect against:

- Diphtheria
- Tetanus

Viral vector vaccines

For decades, scientists studied viral vector vaccines. Some vaccines recently used for Ebola outbreaks have used viral vector technology, and a number of studies have focused on viral vector vaccines against other infectious diseases such as Zika, flu, and HIV. Scientists used this technology to make COVID-19 vaccines as well.

Viral vector vaccines use a modified version of a different virus as a vector to deliver protection. Several different viruses have been used as vectors, including influenza, vesicular stomatitis virus (VSV), measles virus, and adenovirus, which causes the common cold. Adenovirus is one of the viral vectors used in some COVID-19 vaccines being studied in clinical trials.

Viral vector vaccines are used to protect against:

- COVID-19

Vaccine (immunology) - a substance given to stimulate the body's production of antibodies and provide immunity against a disease without causing the disease itself in the treatment, prepared from the agent that causes the disease, or a synthetic substitute

Serum - blood serum from the tissues of immunized animals, containing antibodies and used to transfer immunity to another individual, called antiserum. Used for therapeutic and prophylactic purposes, when an infection of the body is expected or occurred and you need to quickly create immunity.

Blood serum from persons or animals whose bodies have built up antibodies is called antiserum or immune serum. Inoculation with such an antiserum provides temporary, or passive, immunity against the disease, and is used when a person has already been exposed to or has contracted the disease. Diseases in which passive immunization is sometimes used include diphtheria, tetanus, botulism, and gas gangrene.

40. IMMUNE DISORDERS AND AUTOIMMUNITY

Let us first briefly regard what is immune system before looking at the difference between autoimmune disease and immune deficiency. The immune system is the defense system of the body which helps to protect self-tissues from harmful external agents. **Autoimmune diseases** are caused by overactive immune system leading to damage of self-tissues and organs in the absence of a harmful stimulus. **Immune deficiency** is a disease where the immune system is not capable of mounting an immune response against foreign material, organisms due to a single or multiple defects in the immune system. This is the key difference between autoimmune disease and immune deficiency.

A healthy immune system defends the body against disease and infection. But if the immune system malfunctions, it mistakenly attacks healthy cells, tissues, and organs. Called **autoimmune disease**, these attacks can affect any part of the body, weakening bodily function and even turning life-threatening.

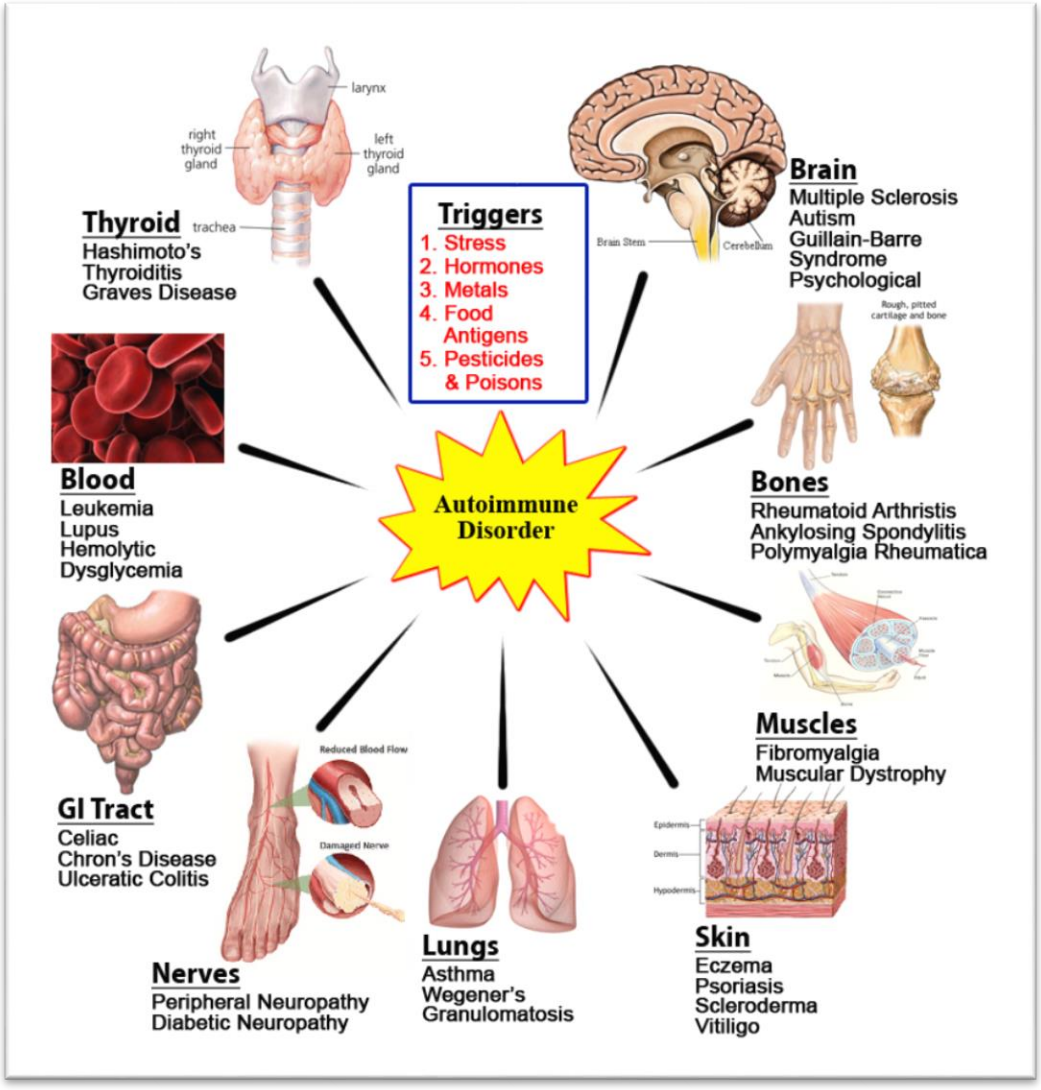


Fig. 150. Tissues of the Body Affected By Autoimmune Attack

Scientists know about more than 80 autoimmune diseases. Some are well known, such as type 1 diabetes, multiple sclerosis, lupus, and rheumatoid arthritis, while others are rare and difficult to diagnose. With unusual autoimmune diseases, patients may suffer years before getting a proper diagnosis. Most of these diseases have no cure. Some require lifelong treatment to ease symptoms.

Autoimmune diseases are affecting more people for reasons unknown. Likewise, the causes of these diseases remain a mystery.

Studies indicate these diseases likely result from interactions between genetic and environmental factors. Gender, race, and ethnicity characteristics are linked to a likelihood of developing an autoimmune disease.

Immune deficiency is the lack of single or multiple components of the immune system. Therefore, these patients are not capable of mounting an effective immune response against certain pathogens depending on the missing component. For example, these defects can be in cellular immunity, humoral immunity or in the complement system. Immune deficiency can be inherited or acquired immunity. This can occur due to some disease like diabetes, HIV or drugs such as immune suppressants. Typically, these patients suffer from recurrent or atypical infections. Diagnosis is based on the detection of the missing component of the immune system by laboratory assays. Treatment is mainly by prevention of infections by immunization, prophylactic antibiotics as well as by replacement of the missing component of the immune system in certain cases. These patients will have poor quality lifestyle due to recurrent infections. A permanent cure is not usually possible, and some cases can be treated with stem cell transplantation. These patients need lifelong follow-up and care.