

MICROBIOLOGY

Higher secondary- First year

Untouchability is a sin
Untouchability is a crime
Untouchability is inhuman

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and
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Chapter 1

HISTORY OF MICROBIOLOGY

Microbiology

Microbiology is a science that deals with the study of living organisms that can not be seen by the naked eye. These can be seen with the aid of microscopes, which magnify objects. Many scientists contributed to the science of microbiology.

Louis Pasteur (1822-1895)

Louis Pasteur was a French chemist and a crystallographer.

His contribution to microbiology is so great that he is considered to be the “Father of Microbiology”.

Contribution to science as a chemist

He was working with tartaric acid crystals. He could pick up the dextro and levo rotatory crystals by seeing the morphology of the crystals. Later he was called to solve some of the problems in fermentation industry and turned his attention to biological process of fermentation.

Contribution to wine industry

1. He discovered that alcohol production from grape juice was due to Yeast
2. He found out that large amounts of lactic acid production was due to the presence or contamination of rod shaped bacteria.

3. He observed that the process of alcohol production i.e. FERMENTATION took place in the absence of air.
4. He coined the terms **aerobic** to describe those organisms requiring air and **anaerobic** to describe those organisms which do not require air for their growth.

Contribution to modern microbiology

Pasteur disproved the theory of spontaneous generation. The theory proposed that living organisms originated spontaneously, particularly from decaying organic matter. He disproved it.

Pasteur's swan neck flask

Pasteur poured meat infusions into flasks and then drew the top of each flask into a long curved neck that would admit air but not dust. He found that if the infusions were heated, they remained sterile (free from any growth) until they were exposed to dust. He opened them on a dusty road and resealed them and demonstrated the growth of microorganisms in all the flasks. The unopened flasks were sterile.

Thus he disproved the theory of spontaneous generation

Edward Jenner 1796

It was an ancient observation that persons who had suffered from a specific disease such as small pox or mumps, resisted the infection on subsequent exposures. They rarely contracted it second time. Such acquired resistance is specific. Edward Jenner a country doctor in England noted a pustular disease on the hooves of horses called the grease. This was carried by farm workers to the nipples of cows (cow pox). This was again carried by milk

maids. They got inflamed spots on the hands and wrists. The people who got this cow pox were protected from small pox. He reported that 16 farm workers who had recovered from cow pox were resistant to small pox infection.

He took the material from the cow pox and inoculated into the cut of an 8 year old boy on 14 May 1796. Two months later Jenner inoculated the same boy with material taken from small pox patients.

This was a dangerous but accepted procedure of that time and the procedure was called variolation. The boy was protected against small pox. His exposure to the mild disease cow pox had made him immune to the disease small pox.

In this manner Jenner began the science of Immunology, the study of the body's response to foreign substances.

Robert Koch (1843-1910)

Robert Koch was a German physician.

1. For the first time he showed the evidence that a specific germ (Anthrax bacillus) was the cause of a specific disease (splenic fever in sheep)
2. He established that a specific germ can cause a specific disease and introduced scientific approach in Microbiology
3. He discovered Bacillus anthracis (Anthrax bacillus), Mycobacterium tuberculosis, and Vibrio cholerae.
4. He modified Ziehl-Neelsen acid fast staining procedure which was introduced by Ehrlich.
5. He devised the solid medium to grow the microorganism to get single colonies.

6. He introduced Koch's thread method to find out the efficacy of disinfectants
7. He established certain rules that must be followed to establish a cause and effect relationship between a microorganism and a disease. They are known as **Koch's Postulates**
8. He also described the **Koch's Phenomenon**

Koch's Postulates

Robert Koch developed powerful method to isolate the organisms in pure culture from diseased tissue. He also perfected the techniques of identification of the isolated bacteria.

The need for Koch's postulates

In those days there were no perfect techniques to identify the organisms. Solid media and staining techniques were not available. So the etiological role of organisms was not known.

To prove the etiology there were not strict criteria. So there was a need to establish criteria.

Koch's Postulates

1. **The organism should be regularly seen in the lesions of the disease.**
2. **It should be isolated in pure culture on artificial media.**
3. **Inoculation of this culture should produce a similar disease in experimental animals.**
4. **The organism must be recovered from the lesions in these animals.**

Postulate 1

The organism should be found in lesions of the disease

All the causative agents of the disease are seen in the particular diseases. If we take *pneumococci* as example, they are seen in all the pneumonia cases.

Postulate 2

It should be isolated and grown in solid media

Pneumococci are grown in solid media and are isolated from the diseases. Some organisms do not grow on solid media or the solid media are not developed yet.

Example: *Mycobacterium leprae* and
Treponema pallidum

Postulate 3

The organisms should produce the exact disease in experimental animals

Almost all the pathogenic organisms produce the same disease in experimental animals. Usually rats, mice, rabbits or guinea pigs are used as experimental animals.

Pneumococci produce pneumonia in animals. Salmonella species do not produce typhoid fever in rat, mice or rabbit. So chimpanzee is taken as experimental animal and it produces fever in chimpanzee.

Postulate 4

It should be isolated from the diseased animal also

Pneumococci are isolated from the experimental animals also.

Modern addition to Koch's Postulates

Today we recognize additional criteria of causal relation between a microorganism and a disease.

The important one is the demonstration of abnormally high concentration of specific circulating antibodies to the organism in the infected host

Or, the presence of abnormally high degree of specific immunity or hypersensitivity to the infecting agent in a recently recovered host.

Limitations

Some organisms have not yet been grown in artificial culture media

Example: *Mycobacterium leprae* and *Treponema pallidum*.

Usefulness of Koch's Postulates

1. It is useful in determining pathogenic organisms
2. To differentiate the pathogenic and nonpathogenic microorganism
3. For the classification of organisms
4. To detect the susceptibility, resistance of the laboratory animals.

Conclusions

Koch has done a valuable work in the field of Microbiology and has made postulates, which have merits, demerits and limitations with modern omission and addition.

EXERCISE

Points to remember

1. Contribution of Louis Pasteur.
2. Contribution of Edward Jenner
3. Koch's Postulates

Self Evaluation

1. Give a list of contribution of Louis Pasteur to wine industry
2. What is the theory of spontaneous generation?
3. How was spontaneous generation theory disproved?
4. Which is the causative agent of cow pox?
5. Which is causative agent of small pox?
6. Explain the method Edward Jenner used to protect people against small pox?
7. Explain Koch's postulates
8. List two organisms that do not obey Koch's postulates
9. Give the usefulness of Koch's postulates
10. What are the modern additions to Koch's postulates?

Choose the best answer

1. Theory of spontaneous generation was disproved by whom?
a. Robert Koch b. Edward Jenner
c. Louis Pasteur d. All of them
2. Edward Jenner used which of the following to protect the boy against small pox?
a. Cow pox material b. Small pox material
c. Both the above d. Rabbit pox

3. Among the following scientists, who discovered solid medium?
a. Louis Pasteur b. Edward Jenner
c. Robert Koch d. None of them
4. Which of the following organisms does not obey Koch's postulates?
a. cow pox virus b. Small pox virus
c. *Treponema pallidum* d. *M.tuberculosis*
5. Who modified Ziehl-Neelsen staining technique?
a. Louis Pasteur b. Robert Koch
c. Ziehl-Neelsen d. All the above

Chapter 2

THE COMPOUND LIGHT MICROSCOPE

Anton van Leeuwenhoek of Delft, Holland, constructed simple microscopes composed of double convex glass lenses held between two silver plates. His microscopes could magnify around 50 to 300 times. Microbiologists currently use a variety of light microscopes.

Modern microscopes are all compound microscopes. The light microscopy refers to the use of any kind of microscope that uses visible light to make the specimens observable. The most commonly used light microscopes are:

- Bright field microscopes
- Dark-field microscopes
- Phase contrast microscopes
- Fluorescence microscopes

The parts of a modern microscope and its light path are shown in figure. 2.1.

Each type of microscope is adapted for certain type of observations. The standard ordinary light microscope is called a bright-field microscope, because it forms a dark image against a brighter background. A compound microscope with a single eye piece (ocular) is called monocular and with two eye pieces is called binocular.

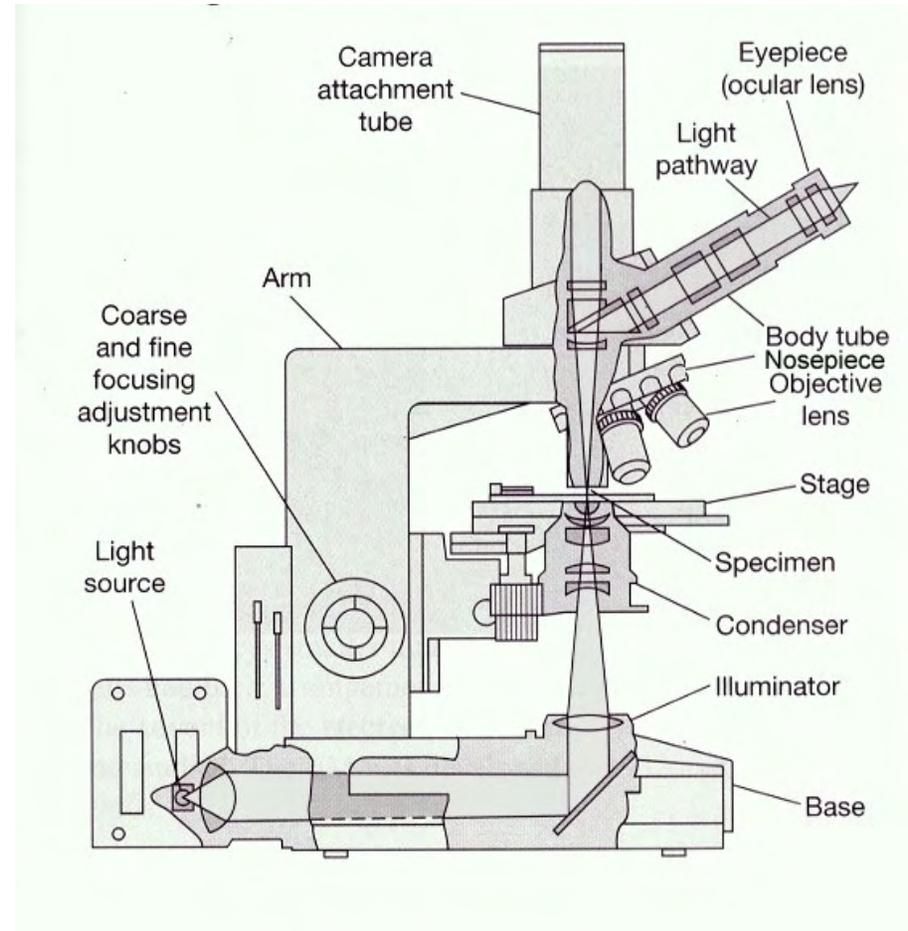


Figure: 2.1

Schematic diagram of a compound light microscope. The arrow indicates the path of light through the microscope

- A mirror or an electric illuminator is a light source which is located in the base of the microscope.
- There are two focusing knobs, the fine and the coarse adjustment knobs which are located on the arm. These are used to move either the stage or the nosepiece to focus the image.
- The mechanical stage is positioned about halfway up the arm, which allows precise contact of moving the slide.
- The sub stage condenser is mounted within or beneath the stage and focuses a cone of light on the slide. In simpler microscopes, its position is fixed whereas in advanced microscopes it can be adjusted vertically.

The upper part of the microscope arm holds the body assembly. The nose piece and one or more eyepieces or oculars are attached to it. The body assembly contains a series of mirrors and prisms so that the barrel holding the eyepiece may be tilted for viewing. Three or five objectives with different magnification power are fixed to the nose piece and can be rotated to the position beneath the body assembly. A microscope should always be par focal, i.e. the image should remain in focus when objectives are changed. Light enters the microscope from the base and passes through a blue filter which filters out the long wavelengths of light, leaving the shorter wavelengths and improving the resolution. The light then goes through the condenser which converges the light beams so that they pass through the specimen. The iris diaphragm controls the amount of light that passes through the specimen and into the objective lens. For higher magnification, greater the amount of light needed to view the specimen clearly. The objective lens magnifies the image before it passes through body tube to the ocular lens in the eyepiece. The ocular of light needed to view the specimen clearly. The objective lens magnifies the image before it passes through body tube to the ocular lens in the eyepiece. The ocular lens further magnifies the image. The total magnification

of the light microscope is calculated by multiplying the magnifying power of the objective lens by the magnifying power of the ocular lens.

Representative magnification values for a 10 X ocular are:

Scanning (4X) x (10X) = 40X magnification

Low power (10X) x (10x) = 100X magnification

High dry (40X) x (10X) = 400X magnification

Oil Immersion (100X) x (10X) = 1000X magnification

Microscope Resolution

Objective is the important part in the microscope which is responsible to produce a clear image. The resolution of the objective is most important. Resolution is the capacity of a lens to separate or distinguish between small objects that are close together. The major factor in the resolution is the wave length of light used. The greatest resolution obtained with light of the shortest wavelength, that is the light at the blue end of the visible spectrum in the range of 450 to 500 nm. The highest resolution possible in a compound light microscope is about 0.2 μm. That means, the two objects closer together than 0.2μm are not resolvable as distinct and separate. The light microscope is equipped with three or four objectives. The working distance of an objective is the distance between the front surface of the lens and the surface of the cover glass or the specimen. Objectives with large numerical apertures and great resolving power have short working distances.

Numerical Aperture

The resolving power of a light microscope depends on the wavelength of light used and the numerical aperture (NA) of the objective lenses.

The numerical aperture of a lens can be increased by

- increasing the size of the lens opening and/or
- increasing the refractive index of the material between the lens and the specimen.

The larger the numerical aperture the better the resolving power. It is important to illuminate the specimens properly to have higher resolution. The concave mirror in the microscope creates a narrow cone of light and has a small numerical aperture. However, the resolution can be improved with a sub stage condenser. A wide cone of light through the slide and into the objective lens increases the numerical aperture there by improves the resolution of the microscope.

Oil immersion

Oil immersion lens is designed to be in direct contact with the oil placed on the cover slip. An oil immersion lens has a short focal length and hence there is a short working distance between the objective lens and the specimen. Immersion oil has a refractive index closer to that of glass than the refractive index of air, so the use of oil increases the cone of light that enters the objective lens.

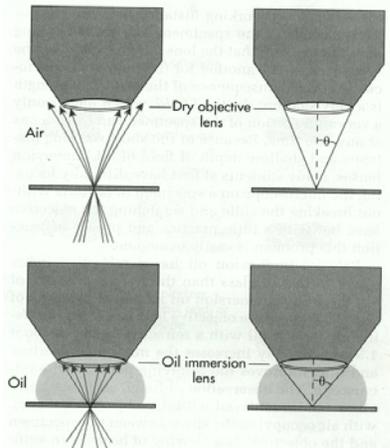


Figure:2-2 The oil immersion objective and resolution

Because of refractive index the light passing from the glass into air makes the light to bend. The light passing from glass through oil does not bend much because the oil has similar refractive index to that of a glass.

The immersion oil with a refractive index of about 1.5 increases the numerical aperture and increases the resolving power of the microscope.

EXERCISE

Points to Remember

1. A Typical compound microscope is composed of a condenser lens which collects light and focuses them on the specimen.
2. An objective lens collects the light coming from the specimen.
3. Ocular lens together with the objective lens magnifies the image.
4. A sub stage condenser focuses a cone of light on the specimen.
5. Microscope should be par focal, which is the image should remain in focus when objectives are changed.
6. Resolution is the ability of a lens to separate or distinguish between small objects that are close together.
7. The resolving power is the minimal distance between two points that can be distinguished by an observer.
8. Working distance of an objective is the distance between the front surface of the lens and the surface of the cover glass or the specimen when it is in sharp focus.
9. There are two types of dyes. They are basic dyes and acidic dyes.
10. Microorganisms can be visualized by simple staining.
11. Differential staining such as Gram staining procedures divide bacteria into two separate groups based on staining properties.
12. Endospore morphology and location can be identified with spore staining methods.

Self evaluation

1. List the parts of a light microscope and their functions.
2. What is the use of sub stage condenser?
3. What is a working distance?
4. Define focal length
5. Define refractive index.
6. What is meant by magnification?
7. How will you find out the magnification of a microscope?
8. How a real image is produced in a light microscope?
9. What is resolution?
10. What factors influence resolution?
11. Define Numerical aperture.
12. What is the function of immersion oil?
13. What is the advantage of using an oil immersion lens to observe bacteria?
14. What is the difference between a simple and differential stain?
15. Why basic dyes are more effective under alkaline conditions?
16. Describe the Gram stain procedure and explain how it works?
17. How to visualize an endospore?
18. What are the uses of the common types of microbial stains?

STAINS AND STAINING REACTIONS

Bacteria are semi-transparent and consist of a clear protoplasmic matter that differs slightly in refractive index from the medium in which they are growing. It is difficult to observe the bacteria in unstained state, except when special methods of illumination are used, to see them in the unstained state.

Stains are useful for the following reasons.

- It makes the microscopic semi-transparent objects visible
- To study the shape and size
- To reveal the presence of various internal and external structures
- To produce specific chemical and physical reaction

The term stain and dye are not the same. A colouring agent that is used for general purposes is called a dye. The one that is used for biological purposes is called a stain. Based on their chemical behavior, the dyes are classified as acidic, basic and neutral.

An acid (or anionic) dye has a negative charge. eg., Eosin, Rose Bengal and Acid fuchsin. The negatively charged groups are carboxyls (-COOH) and Phenolic hydroxyls (-OH). Since they are negatively charged, bind to positively charged cell structures. pH plays an important role in the effectiveness of staining, because the nature and the degree of the charge on cell components change with pH. The anionic dyes stain better under acidic conditions, where the proteins and many other molecules carry a positive charge.

A basic dye (or cationic) carries a positive charge. eg., Methylene Blue, basic fuchsin, crystal violet, malachite green, safranin. Basic dyes bind to negatively charged molecules like nucleic acid and many proteins. Since the bacterial cells surfaces are negatively charged, basic dyes are most often used in Bacteriology. Basic dyes are normally available as chloride salts.

A neutral dye is a complex salt of a dye acid with a dye base.

The dyes used in bacteriology have two features in common.

- They have chromophore groups, groups with double bonds, that give the dye its colour
- They can bind with cells by ionic, covalent or hydrophobic bonding.

Relationship between the type of the dye and its charge when dissociated is summarized.

Dye salt		Dye type
Organic ion (dye base)	Inorganic ion	
Positively charged (Cation)	Negatively charged (anion)	Basic
Negatively charged (anion)	Positively charged (cation)	Acidic

In positive staining procedure, a stain that has a positively charged chromophore (coloured portion of the stain molecule) is attracted to the negatively charged outer surface of the microbial cell. A stain such as Methylene blue has a positively charged blue portion of the molecule that stains the microorganism.

In negative staining procedures, a negatively charged chromophore is repelled by the negatively charged microorganisms, resulting in negative or indirect staining of the microbial cell. Nigrosin and Indian ink are frequently used for negative staining of

microbial cells, and this type of staining is particularly useful for viewing some structures such as capsules that surround some bacterial cells.

Stains are generally prepared largely as aqueous solutions. However in some cases stock solutions are prepared in alcohol, and are diluted with water as needed. Since alcohol removes the stains, pure alcoholic solutions should not be used. Staining solutions are prepared to contain low concentrations of stains rarely exceeding 1%. A very dilute staining solution activity for a long period of time will produce much better results than a more concentrated solution acting for a shorter interval. This procedure has to be followed to reveal internal structure in bacteria.

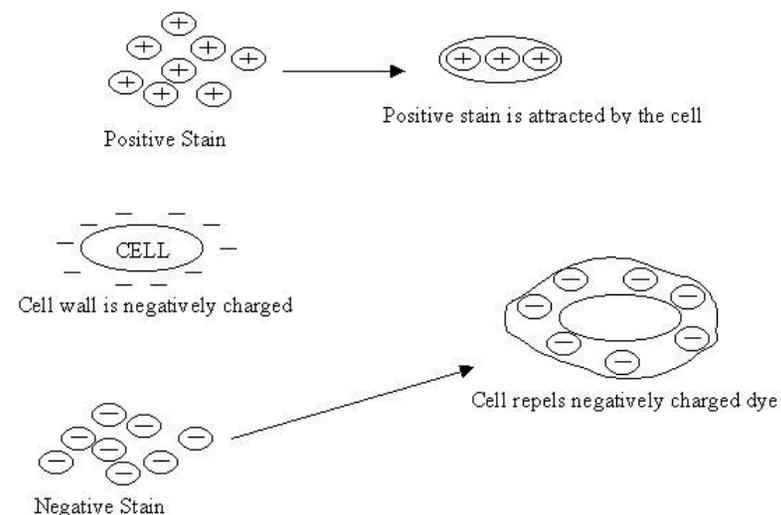


Figure:3.1 The interaction of a cell with negative and positive stain reagents: The outer layer of a cell is negatively charged and a positive stain is attracted to the cell, whereas a negative stain is repelled.

Staining reactions-Interpretation notes

When the pH of the surroundings of the microbial cells is either neutral or alkaline, all microbial cells have a negative charge

on their surface, called the surface charge. Many bacterial cultures produce acids, thereby adding hydrogen ions to a culture medium and decreasing its pH. These hydrogen ions (H^+) interact with the surface of the negative charges on the surface. When this happens, the cell surface no longer strongly attracts positively charged dye ions (basic dyes). Thus the microbes from acidic environments stain poorly with basic dyes. For this reason, the basic dyes are made up as alkaline solutions. For example, potassium hydroxide (KOH) is added to solutions of methylene blue to form the stain called Loeffler's Methylene blue.

Some bacteria excrete alkaline materials during growth and this decreases the number of available hydrogen ions in the culture medium. Under such conditions, the cell surface has a greater negative charge, which is more attractive to basic dyes and therefore, allows greater binding, penetration and internal staining of the microbe. Basic dyes stain microorganisms better under neutral or alkaline conditions.

If the dye base molecule has a negative charge, it is repelled by the cell's negatively charged surface. Thus negatively charged dyes neither bind to the cell's surface nor are they able to penetrate into the cell. These are called acid dyes.

The methodology for using acid dyes are different from basic dyes. An acid dye is mixed with a drop of culture smeared on a microscope slide and allowed to air dry. The negatively charged cells are not stained by the negatively charged dye, and they appear as clear area surrounded by a coloured background. Negatively charged dyes used in this way are called negative stains.

Under neutral or alkaline conditions, the negative stains (acidic dyes) work better, because these conditions allow the surface charge to be more negative. Negative stains are of limited usefulness for those using light microscopes, but they can be used to avoid some of the disadvantages of staining with basic dyes.

Simple staining

A simple staining solution, contains only one stain, which is dissolved in a solvent. It is applied to the microorganism in one application. The microorganisms give the colour characteristic of the staining solution. The purpose of simple staining is to reveal the size and shape of the microorganism. The simple stains that are commonly used by the microbiologists for routine purposes are dilute solution of carbol fuchsin, crystal violet and methylene blue.

Methylene blue is more frequently used than any other stain in bacteriology. It is because of its strong nature and it stains nuclei and nucleic acid granules very intensively. Methylene blue is used for the rapid survey of bacterial population of milk. It is also used for the diagnosis of Diphtheria. This stain is incorporated with Eosin in Lactose agar to distinguish typical *E.coli* in contaminated water.

Differential Staining

In this procedure, more than one dye is employed. Differential staining procedure helps to divide the bacteria into separate groups based on staining characteristics. The two most important differential stains used by bacteriologists are Gram stain and Acid-fast stain.

Gram Staining

The simple staining procedure makes to visualize bacteria clearly, but it does not distinguish between organisms of similar morphology. In 1884, a Danish Physician named, Christian Gram discovered a new technique to differentiate the bacteria of similar morphology. He used two dyes in sequence, each of a different colour. The organisms that retain the colour of the first dye are called Gram positive and those that cannot retain the first dye when

washed with a decolourizing solution, but then take on the colour of the second dye are called Gram negative . In this method, the fixed bacterial smear is subjected to the following staining reagents in the order of sequence listed below:

Crystal violet → Iodine solution → alcohol (decolourizing agent) → Safranin.

Principle

The Gram-positive bacteria will retain the crystal violet and appear deep violet in colour. The Gram-negative bacteria lose the crystal violet on decolorization and are counter stained by the safranin and appear red in colour. Iodine solution is used as a mordant that fixes the primary stain in or on a substrate by combining with the dye to form an insoluble compound-mordant, for the first stain.

The exact mechanism of action of this staining technique is not clearly understood. However, the most plausible explanations for the reactions are associated with the structure and composition of the cell wall.

The cell walls of Gram-negative bacteria are thinner than that of Gram-positive bacteria and contain a higher percentage of lipid content. During the staining of Gram-negative bacteria, the alcohol treatment extracts the lipid. This results in increased porosity or permeability of the cell wall. The crystal violet-iodine (CV-I) complex, thus can be extracted and the Gram-negative bacteria is decolorized. The cells subsequently take up the colour of the counter stain safranin.

The cell walls of Gram-positive bacteria with lower lipid content become dehydrated during alcohol treatment. The pore size decreased, permeability is reduced and the CV-I complex cannot be extracted. Therefore, the Gram-positive cells remain purple-violet.

Endospore Staining

Endospore formation is a distinguishing feature of the family *Bacillaceae*, which includes members of the aerobic genus, *Bacillus* and the anaerobic genus, *Clostridium*. Endospore resists adverse environmental conditions such as dryness, heat and poor nutrient supply. The endospore is a highly retractile body formed within the vegetative bacterial cell at a certain stage of growth. The size, shape, and position of the spore are relatively constant characteristics of a given species and are therefore, of some value in distinguishing the kind of bacillus from another. The position of the spore in the cell may be central, sub terminal or terminal. It may be the same diameter as the cell, smaller, or larger causing a swelling of the cell.

Endospores strongly resist application of simple dyes, but once stained are quite resistant to decolorization. This character suggests one way to make the structure visible. If simple stains are used, the body of the bacillus is deeply colored, whereas the spore is unstained and appears as a clear area in the organism. By vigorous staining procedures the dye can be introduced into the substance of the spore. When thus stained, the spore tends to retain the dye after treatment with decolorizing agents.

To make the distinction clear between the spore and the vegetative portion of the cell, a contrasting counter stain is usually applied in the ordinary fashion and the resulting picture shows the initial stain taken up by the spore and the second stain appear in the cytoplasm. Thus, it makes for a very simple method of distinguishing the endospore from the vegetative cell.

EXERCISE

Points to remember

1. There are two types of dyes. They are basic dyes and acidic dyes.
2. Microorganisms can be visualized by simple staining.

3. Differential staining such as Gram staining procedures divide bacteria into two separate groups based on staining properties.
4. Endospore morphology and location can be identified with spore staining methods.

Self evaluation

1. What is the difference between a simple and differential stain?
2. Why basic dyes are more effective under alkaline conditions?
3. Describe the Gram stain procedure and explain how it works.
4. How to visualize an endospore?
5. What are the uses of the common types of microbial stains?

STERILIZATION

Definition

Sterilization is the freeing of an article from all living organisms, including bacteria and their spores.

Sterilization of culture media, containers and instruments is essential in microbiological work for isolation and maintenance of microbes.

In surgery and medicine, the sterilization of instruments, drugs and other supplies is important for the prevention of infection.

Sterilization can be effected in a variety of ways, which can be conveniently categorized as follows:

I. PHYSICAL METHODS

A. Heat :

1. Dry heat
2. Moist heat

B. Radiations

1. Ultraviolet radiations
2. Ionizing radiations

C. Filtration

II. CHEMICAL METHODS

STERILIZATION BY HEAT

Heat can be applied in two forms.

1. The dry heat
2. Moist heat.

Mechanism of killing by dry heat

- Dry heat kills the organisms by destructive oxidation of essential cell constituents
- Killing of the most resistant spores by dry heat requires a temperature of about 160 °C for 60 minutes
- Dry heat is employed for glassware; syringes, metal instruments and paper wrapped goods, which are not spoiled by high temperatures.
- It is also used for anhydrous fats, oils and powders that are impermeable to moisture.

Mechanism of killing by moist heat

- Moist heat kills the organisms by coagulating and denaturing their enzymes and structural protein.
- Sterilization by moist heat of the most resistant spores generally requires 121 °C for 15-30 minutes.
- Moist heat is used for the sterilization of culture media, and all other materials through which steam can penetrate
- Moist heat is more effective than dry heat
- Sterilization can be done at lower temperatures in a given time at a shorter duration at the same temperature.

FACTORS INFLUENZING STERILIZATION BY HEAT

1. The temperature and time: they are inversely related, shorter time is sufficient at high temperatures.
2. Number of microorganisms and spores: The number of survivors diminished exponentially with the duration of heating
3. Depends on the species, strains and spore forming ability of the microbes.
4. Thermal death point is the lowest temperature to give complete killing in aqueous suspension within 10 minutes
5. Depends on the nature of material: a high content of organic substances generally tends to protect spores and vegetative organisms against heat.
6. Presence of organic or inorganic disinfectants facilitates killing by heat
7. pH also plays an important role in the killing of microorganisms

METHODS OF STERILIZATION BY DRY HEAT

1. RED HEAT

Inoculating wires, points of forceps and searing spatulas are sterilized by holding them in the flame of Bunsen burner until they are seen to be red-hot.

2. FLAMING

This method is used for sterilizing scalpel, mouth of culture tubes, glass slides etc.

It involves passing of an article through Bunsen flame without allowing it to become red-hot.

3. HOT AIR OVEN

This is the main means of sterilization by dry heat.

Exposure at a temperature of 160 °C for 1 hour is generally employed.

4. INFRARED RADIATIONS

Source employed is an electrically heated element, the infra red rays are directed on to the object to be sterilized and temperature of 180 °C can be obtained.

METHODS OF STERILIZATION BY MOIST HEAT

Moist heat can be employed at

1. Temperature below 100 °C
2. Temperature of 100 °C
3. Temperature above 100 °C

MOIST HEAT BELOW 100 °C

EXAMPLES

1. Pasteurization of milk

In Pasteurization of milk the temperature employed is either 63 °C for 30 minutes or 72 °C for 20 seconds. All nonspore-forming pathogens in milk like Salmonellae, M.tuberculosis are killed.

MOIST HEAT ABOVE 100°C

1. Sterilization in an autoclave

- Autoclaving is the most reliable method
- It is the method most widely used for sterilization of culture media and surgical supplies

- When water is boiled within a closed vessel at an increased pressure, the temperature at which it boils and the steam it forms will rise above 100 °C
- This principle is used in the autoclave
- Normally autoclaving is done at 15 lbs. (pounds per sq. inch pressure) and 115 °C for 15 minutes

STERILIZATION BY FILTRATION

When fluids are passed through bacteria stopping filters, they are made free from bacteria.

- It is useful for making preparations of soluble products of bacterial growth such as toxins
- Liquids that would be damaged by heat such as serum and antibiotic solutions can be sterilized by filtration
- Efficient filters should be able to retain *Serratia marcescens*

TYPES OF FILTERS

There are different kinds of filters

1. Earthenware candles - called Berkfield & Chamberland filters
2. Asbestos and asbestos-paper discs filters - called Seitz filters
3. Sintered glass filters
4. Cellulose membrane filters
5. Fibre glass filters.

Berkfield Filters

- Made from Kieselguhr, a fossil diatomaceous earth

- Three grades of porosity are available:
 - a. Veil - coarsest one
 - b. N - normal one
 - c. W- wenig the finest one

Chamberland Filters

- Made from unglazed porcelain
- Four grades are available
 - a. L1- clarifying filters
 - b. L1a-Big
 - c. L2 - normal
 - d. L3- Finest

Seitz filter

- Made up of asbestos pads
- Three grades are available
 - a. K- clarifying filters
 - b. Normal
 - c. Special EK bacteria stopping filters

Sintered glass filters

- Made from sintered glass
- Different grades available
 - Grades 1 to 5
 - Grades 1-2 are for clarifying purpose
 - Grades 3-5 is for sterilization purpose

Membrane filters

- Made up of nitro-cellulose membranes
- Made with different grades of porosity by adjusting the concentration of constituents

MERITS AND DEMERITS OF HEAT STERILIZATION

Advantages of heat sterilization

1. Sterilization is very effective
2. Instruments are standardized to deliver the required effective heat
3. Heat deliver system can be monitored effectively with various controls like pressure gauge, temperature meters etc
4. Established quality control methods available

Disadvantages

1. Steam impermeable materials like fats, oils and powders can not be sterilized by autoclaving.
2. Heat sensitive materials can not be sterilized by heat

Examples:

1. Serum can not be sterilized
 2. Antibiotics
 3. Plastic materials
 4. Vaccines
 5. Rubbers
3. Presence of organic matters interfere with effective sterilization
 4. Dangers of explosion when high pressure is used

EXERCISE

Points to remember

Different methods of sterilization

Importance of sterilization

Self evaluation

1. Define sterilization
2. List the methods of sterilization
3. Explain the methods of sterilization
4. Explain the methods of sterilization by dry heat
5. Explain the methods of sterilization by moist heat
6. Give the mechanisms of killing by dry heat
7. Give the mechanisms of killing by moist heat
8. Describe the method of sterilization by moist heat below 100°C
9. Describe the method of sterilization by moist heat above 100°C
10. Describe autoclaving methods
11. State the principle of sterilization by filtration
12. Classify the types of filters used for sterilization
13. What all the factors that influence sterilization by heat
14. Describe Seitz filter sterilization
15. Describe sintered glass filter sterilization
16. Describe membrane filter sterilization
17. Give the merits and demerits of heat sterilization

PURE CULTURE METHODS

In the natural environments microorganisms exist in mixed cultures. To establish the role of microbial agent to a disease process, it is essential to demonstrate the organisms or its components in the diseased tissues. To accomplish this, the organism must be cultivated from the tissues. Similarly to know the kinds of organism present in the environment it is necessary to grow them in artificial media. Cultivation of the organism is also essential to obtain pure culture of clone of cells derived from a single cell to perform biochemical differentiation tests and **susceptibility tests since mixed cultures give misleading results.**

Artificial culture media

A medium is an environment which supplies the ingredients necessary for the growth of the organism. Various kinds of media have been prepared in the laboratory to isolate, grow and identify an organism. Depending on the need to isolate and identify an organism from a particular sample or environment, different kinds of media are formulated.

Kinds of media

Basal or supportive media

Basal medium is one that contains nutrients that allow the growth of most nonfastidious organism without affording growth advantage to any particular organism over others. Example is *Nutrient agar*, and *Trypticase Soy agar*.

Enrichment medium

Enrichment medium is a liquid medium which enhances the growth of certain bacterial species, while inhibiting the growth or prolonging the lag phase of unwanted organisms thus altering the ratio between the two in favor of the required bacterial species. Example is Selenite F broth for the isolation of Salmonella from stool.

To get a pure culture of the organism, any one of the solid media mentioned above is used. In order to get discrete separate colonies, the surface of the medium must be dry. The material is inoculated on the surface by spreading with a sterile loop in such a way that bacteria are ultimately deposited singly. When the bacteria are at a sufficient distance from each other, the whole progeny of each accumulates locally during growth to form a discrete mass or colony which is readily visible to the naked eye. Each colony is presumed to be a pure culture, consisting exclusively of the descendants of a single cell. It may be picked up with a sterile wire to prepare a pure subculture in a fresh medium.

Growth and colony characteristics of Bacteria

The appearances of growths of bacteria in liquid media are generally not distinctive. There is a uniform turbidity in the liquid and little deposit at the bottom. Colony morphology of the isolated bacteria on the solid media has much more value. Attention is paid to the *size* of the colony (diameter in mm), their *outline*, whether circular and entire or indented, or wavy or rhizoid, their elevation low convex, high convex or flat plateau-like, umbonate or nodular, their *translucency*, whether transparent, translucent, or opaque, their *pigmentation*, colorless, white or otherwise pigmented, and whether they produce any *change in the medium* (haemolysis in a blood-containing medium).

Example: Colony characteristics of *Staphylococcus aureus* on Nutrient agar

After aerobic incubation at 37°C for 24 hours, colonies are 1-3 mm in diameter and have a smooth glistening surface, an entire edge, a soft butyrous consistency and an opaque, pigmented appearance.

Growth characteristics of yeasts

Yeasts are grown on Sabouraud Dextrose agar aerobically. Yeasts grow as typical pasty colonies and give out yeasty odor. The colony morphology varies with different yeasts.

Growth characteristics of filamentous fungi

The most common medium used for the isolation of fungi is Sabouraud Dextrose agar. While observing colony morphology, one must note the colors of the surface and the reverse of the colony, the texture of the surface (powdery, granular, woolly, cottony, velvety or glabrous), the topography (elevation, folding, margins, etc) and the rate of growth.

EXERCISE

Points to remember

1. Existence of organism in nature as mixtures
2. Need to isolate them in pure culture
3. Growth of microbes in different media

Self evaluation

1. Why should the organism be grown in pure culture?
2. Define a culture medium
3. Classify different kinds of culture media and give one example
4. Define basal medium and give one example
5. Define enriched medium and give two examples
6. Define differential medium and give two examples
7. Define selective medium and give one example
8. Define enrichment medium and give one example
9. Describe the growth characteristics of bacteria
10. Describe the growth characteristics of yeast and filamentous fungi.

MICROBIAL NUTRITION AND GROWTH

‘An army marches on its stomach’ said Napoleon Bonapart. This indicates that food is important for any living organism and so also for microbes. Food is any substrate that can be metabolized to provide assimilable material or energy for the cell. Plants synthesize their own food requirements through photosynthesis. Animals ingest the presynthesized food from plants or by devouring other animals. All living organisms, from micro to macroorganisms require nutrients for growth and normal functioning. Animals ingest the food and digest them in their digestive system (Holozoic nutrition) to simpler nutrients which are absorbed by cells for synthesis of all cellular material and derive energy. Plants absorb the nutrients from soil solution (Holophytic nutrition) released by mineralisation of organic matter and grow. Microorganisms particularly fungi derive their nutrients from the extra corporeal digestion by secreting extracellular enzymes. The nutrients are absorbed and cellular materials are synthesized.

All organisms exhibit two universal requirements viz., water and elements. All organisms require energy which they derive from the chemical compounds or radiant energy like light. The elemental components are carbon, nitrogen, phosphorus, sulphur and potassium besides hydrogen and oxygen major ones for synthesizing cellular components. Metal ions like K, Ca, Mg and Fe are required for normal growth. Other metal ions like Zn, Cu, Mn, Mo, Ni, B, Co are often required in low quantities hence known as trace elements. Fe, Mg, Zn, Mo, Mn and Cu are cofactors/coenzymes or prosthetic group of various enzymes. Most bacteria do not require Na but certain marine bacteria, cyanobacteria and photosynthetic bacteria require it. Red extreme halophiles can-

not grow with less than 12 to 15% NaCl which is required to maintain the integrity of cell walls and for the stability and activity of certain enzymes. Silicon is required for the growth of diatoms. Vitamins and vitamin like compounds are also present in living cells. These function either as coenzymes or as building blocks of coenzymes. Some bacteria synthesize their entire requirements of vitamins but some cannot grow unless supplied from external source.

Microorganisms are divided into several types based on the energy source or electron source and carbon assimilation. Those derive energy from the oxidation of chemical compounds are known as ‘chemotrophs’ and others utilizing radiant energy like light are known as ‘phototrophs’. Electrons are required for metabolism and based on the source from which bacteria derive electron they are grouped. Some organisms use reduced inorganic compounds as electron donors and are termed as ‘lithotrophs’ literally meaning rock eating. Others use organic compounds are termed as ‘organotrophs’. Those organisms that derive energy from the chemical compounds (Chemotrophs) and uses inorganic compounds as e^- donors (lithotrophs) are known as chemolithotrophs. Those that derive energy from light (phototrophs) and e^- from inorganic compounds are photolithotrophs. Similarly those chemotrophs that use organic compounds, as e^- donors are chemoorganotrophs and the phototrophs that utilize organic compounds as e^- donors are photoorganotrophs.

Chromatium okenii, a photosynthetic bacterium, uses radiant energy and H_2S as electron donor oxidizing it to elemental sulphur. Some phototrophs use organic compounds such as fatty acids and alcohols as electron donors and hence called photoorganotrophs.

Rhodospirillum rubrum another phototrophic bacterium utilizes succinate as e^- donor converting it to fumarate. A phototrophic bacterium can grow as chemotroph. In the anoxygenic environment (absence of O_2) this bacterium grow as photoorganotroph

but in the presence of oxygen and dark (absence of light) it grows as a chemoorganotroph. Among the chemotrophs some utilize inorganic compounds like NH_4 as e^- donors and hence called chemolithotrophs. *Nitrosomonas* use ammonia for electrons and derive energy by oxidizing ammonia to nitrite. Certain chemotrophs use organic compounds like sugars and amino acids as e^- donor and are called chemoorganotrophs. Some of the chemotrophs can grow either as chemolithotrophs or chemoorganotrophs. *Pseudomonas pseudofulva* can use glucose an organic compound (chemoorganotrophs) or inorganic compound H_2 as e^- source (chemolithotrophs)

Autotrophs and Heterotrophs

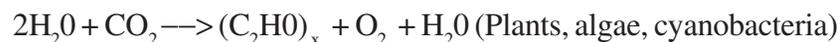
Based on the source of carbon microorganisms are grouped as autotrophs and heterotrophs. Some can use CO_2 as their sole source of carbon like plants and algae are termed as autotrophs. Others like some bacteria, fungi and actinomycetes utilize pre-formed organic compounds as carbon source and hence called heterotrophs. Most organisms that involve in decomposition of organic matter in soil are heterotrophs Fungi are saprophytic and depend on dead organic matter. Some fungi are parasitic on living plants and animals. The saprophytic and parasitic organisms are heterotrophs. Such of these heterotrophs that have elaborate requirements of specific nutrients like vitamins and growth promoting substances are called fastidious heterotrophs as they are not easily pleased or satisfied by ordinary nutrients available in nature.

The source of carbon for microbes is CO_2 or carbohydrates. Autotrophs derive their entire requirement of C from CO_2 while heterotrophs derive the carbon chiefly from carbohydrate. In nature, cellulose, hemicelluloses, starch, pectin, lignin etc serve as carbon sources. Amino acid, purine and pyrimidine bases, protein serve as a source of nitrogen. Phosphorus is obtained from the

nucleotides, phytin etc. For cultivation of microorganisms in laboratory, media containing monosaccharides like glucose and disaccharides like sucrose are used as C sources. Peptone, Tryptone, inorganic salts like ammonium salts, potassium nitrate serve as nitrogen sources. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate are commonly employed to serve as sources of phosphorus and also as a buffering agent.

Autotrophic bacteria have the simplest nutritional requirements as they can grow and reproduce in a mixture of inorganic compounds. They also possess an elaborate capacity to synthesize the carbohydrate, proteins, lipids, nucleic acids, vitamins and other complex substances of living cells. Photosynthesis is a normal autotrophic way of life and this occurs in plants, algae, photosynthetic bacteria and cyanobacteria. In this process, CO_2 is reduced and converted to carbohydrate utilizing light. However, photosynthesis of plants, algae and cyanobacteria perform oxygen evolving photosynthesis by absorbing the reducing power from the photolysis of water. On the other hand, photosynthetic bacteria green and purple bacteria obtain the reducing power from a compound similar to water (H_2O) viz., H_2S i.e. available in anoxygenic environment. The pigments and the light absorption also differ in these organisms.

Over all reaction of photosynthesis is,



Inorganic compounds like H_2 , $\text{H}_2\text{S}_2\text{O}_3$ or the organic compounds lactate, succinate can be the source of reducing power instead of H_2S .

Growth

Living organisms grow and reproduce. The growth indicates that an organism is in active metabolism. In plants and animals one see the increase in height or size. In a butterfly, a small larva hatching from egg grows in size, moults, pupates and become an adult butterfly through metamorphosis. Growth in a common use refers to increase in size but with microorganisms particularly with bacteria, this term refers to changes in total population rather than increase in size or mass of an individual organism. With fungi linear growth of hyphae and radial growth of colony is observed for growth on solid media but a biomass or mycelial dry weight on liquid media. In unicellular fungi like yeast that reproduce by fission or budding the population change is considered as growth.

The change in population in bacteria chiefly involves transverse binary fission in most of the bacteria while budding is observed in *Hyphomicrobium*. In actinomycetes, fragmentation of hyphae and sporulation results in population change. In yeasts, budding and fission are observed that this depends upon the species. In fungi, growth fragmentation asexual and sexual spores serve as propagates for population increase.

The transverse binary fission, an asexual reproductive process is the most common in the growth cycle of bacterial population. A single cell divides after developing a transverse septum (cross wall) and continues to grow by continuous dividing without cell death till it is subjected to stress.

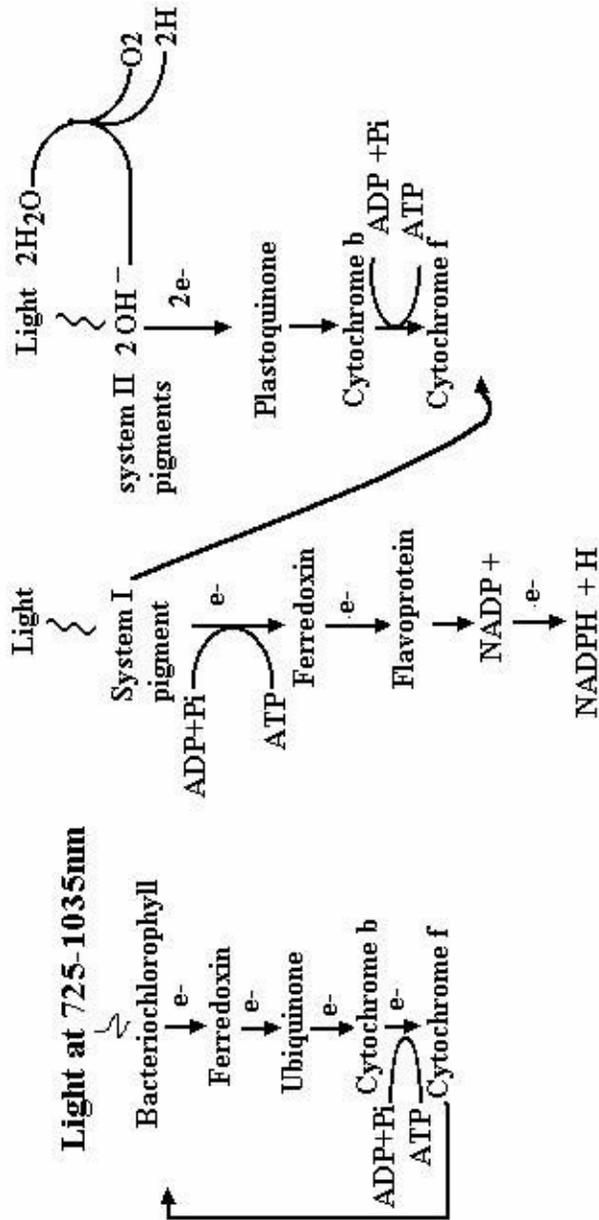
A cell dividing by binary fission is immortal unless subjected to stress by nutrient depletion or environmental stress. Therefore a single bacterium continuously divides. One cell divides providing two cells and two cells divide giving four and so on. Therefore the population increases by geometric progression.

1 2 4 8 16 32

1 2 2² 2³ 2⁴ 2⁵ ——2ⁿ

Comparison of Plant and Bacterial photosynthesis		
	Plant photosynthesis	Bacterial photosynthesis
Pigments	Chlorophyll	Bacterochlorophyll
Light absorption	680 nm	725-1035 nm
Accessory pigments	Xanthophylls, Carotenoids	Carotenoids
Environment	Oxygenic	Anoxygenic
Source of reducing power or electron	Water	H ₂ S, H ₂ , H ₂ S ₂ O ₃ , succinate, lactate
Ultimate element released/accumulates	O ₂	S
Nature of phosphorylation	Non-Cyclic	Cyclic
ATP Synthesized	Two	One
NADP ⁺ reduction	Yes	No

Photosynthesis



Bacterial Photosynthesis

Anoxygenic photosynthesis in photosynthetic bacteria

e^- returns to the bacteriochlorophyll that became +vely charged due to release of e^- initially

e^- starts and returns to the bacteriochlorophyll. Hence cyclic.

ATP is synthesized by phosphorylation of ADP. Cyclic phosphorylation

No NADP synthesis
No external donor

When a single bacterium is incubated into the liquid medium in flask and incubated, bacterium divides by fission and attains a period of rapid growth in which cells multiply at an exponential rate. If the logarithmic number of bacterium versus time is plotted a growth curve is obtained with different phases of growth.

Plant Photosynthesis

Oxygenic photosynthesis in plants, algae, cyanobacteria

A molecule in pigment system II absorbs light energy attain excited state releases e^- .

This e^- transferred to plastoquinone, cyt b, cyt f and finally to pigment system I

Pigment system I absorbs light releases an e^- which is transferred to ferredoxin, flavoprotein, NADP⁺

e^- never returns to its site of origin and non cyclic but e^- are replaced to the pigment by e^- from water

2 ATP is synthesized by phosphorylation of ADP between transfer of e^- from cyt b to cyt f in system II and between system I pigment and ferredoxin

NADP⁺ is reduced in system I

Soon after transfer of an inoculum to a new medium, cells do not immediately multiply and the population remains unchanged. The cells however increase in size synthesizing newer protoplasm and enzyme necessary to the newer environment. The organisms are metabolizing but require more for adjustments to the physical environment around each cell and hence there is a lag for cell division (lag phase).

At the end of lag phase cells divide and there is a gradual increase in the population. When all the cells complete their lag, there is division at regular intervals. The cells divide steadily at a constant rate in the logarithmic or exponential phase and when log number of cells are plotted against time there is a straight line. The population in this phase is almost uniform in chemical composition, metabolic activity and physiological characteristics.

Generation time is the time required for the population to double and this can be determined by the number of generation that occurs at a particular time interval. Not all bacteria have the same generation time. It varies from 15 – 20 minutes for *Escherichia coli* to many hours in others and is also dependent upon the nutrients and physical conditions of the environment. With the growth of the bacterium, there will be a depletion of nutrients. At high concentration of nutrients a small change may not cause significant effect but at low concentration the growth rate decreases significantly.

At the end of the exponential phase growth rate decreases due to exhaustion of some nutrients or due to production of toxic products during growth. The population remains constant due to complete cessation of division or reproduction rate equals to death rate.

The stationary phase is followed by Decline or Death phase as bacteria divide faster than the new cells produced. The depletion of nutrients, accumulation of solubilising products like acids.

The number of viable cells decreases exponentially. G-ve Cocci divide faster but others divide slowly but viable cells may persist for minutes or even years.

Measurement of growth

Growth refers to the magnitude of the population in bacteria. The growth can be measured quantitatively (1) cell count (2) cell mass and (3) cell activity. Cell count shall be made directly by microscopy or using an electronic particle counter. It can also be made indirectly by colony count after serially diluting the sample. Cell mass can be determined directly by weighing a known volume of sample culture broth or by measuring the cell nitrogen. It can also be determined indirectly by finding cell activity, which can be measured by the degree of biochemical activity to the size of population.

Petroff – Hausen counting chamber is used for direct microscopic count. It is a slide accurately ruled into squares of $1/400 \text{ mm}^2$ area over when a cover slip rests at $1/50 \text{ mm}$ above. This gives a volume of $1/20000 \text{ mm}^3$ over one square. The liquid can be placed in the chamber left unstained and counted using a phase contrast microscopy. If 5 cells are present in one square there will be $5 \times 20,000,000$ or 10^8 cells/ml. This method is rapid requires simple equipment. Morphology of cells can be simultaneously obtained but difference of viable or dead cells cannot be made.

In electronic particle counter a bacterial suspension is passed through a tiny orifice of $10\text{-}30 \text{ }\mu\text{m}$ diameter that connects the two compartments of counter containing an electrically conductive solution. The electrical resonance between the two compartments increase momentary when each bacterial cell passes in the orifice creating an electrical signal. The signals are automatically counted. This method is rapid but requires sophisticated electronic equipment.

In plate count method a known volume of bacterial suspension diluted serially if population is dense, is poured in petridishes

and molten agar medium is added and mixed thoroughly. The cells trapped immediately in the gelled medium develop into a colony. The colonies are counted by illumination from below. The number of colonies is multiplied by the dilution factor to detect the population and expressed as number per ml. Only those cells that grow in the medium that show up and get counted under the conditions in which the plates are incubated. If aggregate of cells are formed as in cocci in clusters, chains or pairs the resulting counts will be lower than the individual cells. In such cases counts are referred to as colony – forming units per ml than number per ml. The plate count is used normally for estimation of bacterial population in milk, waste foods, soil and many other materials.

Membrane filter count is used to determine the bacterial numbers in large sample containing very small number of viable cells. Bacteria in large volume of air or wastes shall be collected by filtering in the membrane. The membrane with filtered bacteria is placed on plate containing a pad saturated with medium and incubated. The organisms grow into a colony and counted. Special medium and dyes can be used to detect certain types of organisms.

The bacteria in suspension absorb and scatter light passing through the cell similar to water droplets in fogs absorbing and scattering of light. Because of this phenomenon, a culture of more than 10^7 or 10^8 cells per ml appears turbid to the naked eye.

The cell growth can also be measured by the nitrogen content that forms the process when is a major constituent of cell. Cells are harvested, washed free of medium and nitrogen is analysed by standard method.

The quantitative measurement of a mass of cells is made by the dry weight determination. Very dense suspension of cells can be washed free of extraneous matter and weighed. In cells accumulating β -hydroxy butyrate cell mass may increase without corresponding increase in cell growth. The measurement of acid or

The measurement of acid or any other product of metabolism shall be measured to assess growth.

In case of yeasts, dry weight determination and nitrogen estimation can be done as a measure of growth. In mycelial fungi, mycelial dry weights are determined by filtering the mycelial mat in a previously weighed filter paper drying it in oven at 105°C for 24 hours and weighing it. The mycelial weight is determined by subtracting the weight of filter paper. In agar medium, the linear growth / nodal growth of fungi shall be measured.

EXERCISE

Points to remember

1. Different phases of bacterial growth
2. Different kinds of growth requirements
3. Cultivation and enumeration of bacteria

Self evaluation

1. Describe briefly the nutritional requirement for the growth of microorganisms.
2. Describe briefly different phases of bacterial growth curve of bacteria.
3. Compare and contrast bacterial and plant photosynthesis.
4. What is generation time ? Describe briefly the measurement of growth in bacteria.
5. Differentiate autotrophs and heterotrophs.
6. Give an account of cultivation bacteria in laboratory.
7. Give an account of reproduction in bacteria.
8. Differentiate growth of bacteria from that of fungi.
9. What you understand by plate count ? Describe the method.
10. What do you understand by anoxygenic photosynthesis ?

PROKARYOTIC CELL STRUCTURE

Living organisms are differentiated from nonliving matter by their (1) ability to reproduce (2) ability to ingest or assimilate food and metabolize them for energy and growth (3) ability to excrete waste products (4) ability to react to changes in their environment (irritability) and (5) Susceptibility to mutation. The living organisms include a variety of micro and macro organisms of differing size, shape morphology, and behaviour. They include tiny bacteria, protozoans, worms, plants and animals like man, whale and elephants.

Carlous Linnaeus (1707-1778), the Swedish botanist was the first to introduce nomenclature for plants and animals. Until 18th century only plant and animal kingdoms were recognized. However some organisms are predominately plant like, some animal like and some do not fall in both the groups. Therefore it was felt a third kingdom was necessary. Haeckel (1866), a German zoologist suggested a third kingdom Protista to include those organisms that are not typically plants and animals. Bacteria, algae, fungi and protozoa are cellular organisms placed under protista. Viruses are not cellular organisms and therefore not classified as protists. Bacteria were lower protists while algae, fungi and protozoa were higher protists. A satisfactory criteria to differentiate bacteria, fungi and algae could not be made until the development of electron microscope, which depicted the internal structure of these organisms. The absence of membrane bound internal structures in bacteria and their presence in fungi, algae, protozoa, plants and animal cells was taken as criterion to differentiate prokaryote and eukaryote.

Whittakar (1969) proposed five kingdoms based on three levels of cellular organization and three principal modes of nutrition, photosynthesis, absorption and ingestion. The prokaryotes lacking ingestive mode of nutrition are included in the kingdom. Monera. In the kingdom protista unicellular eukaryotic microorganisms representing all the three modes of nutrition are included. The multicellular green plants and higher algae were placed in the kingdom plantae while multinucleate higher fungi in the kingdom fungi and the multicellular animals in the kingdom Animalia.

Bacteria and cyanobacteria (the blue green algae) of monera, microalgae and protozoa of protists and yeasts molds and fungi represent the microorganisms. Most of them are invisible to the naked eye and requires magnification. The oratically a black dot of 4μ in diameter on a white background can be perceived by retina of human eye but in reality an object of above 30μ in size only will be visible to the eyes and objects lesser than that requires magnification.

Prokaryotes are organisms with primitive type of nucleus lacking a well-defined membrane a less complex nuclear division than mitosis. The nuclear material is a DNA molecule in prokaryotes compared to chromosomes of higher organisms. Eukaryotes are organisms with cells having true nuclei enclosed in a nuclear membrane and are structurally more complex than prokaryotes. A varying degree of localization of cellular functions in distinct membrane bound intracellular organelles like nuclei, mitochondria chloroplasts etc. The cells of living organisms are either prokaryotic or eukaryotic in nature and there is not any intermediate condition. The size, shape, morphology and the internal cellular organizations are different in these two groups.

The size of the microorganisms varies from unicellular tiny bacteria to large brown algae and mushroom. Bacteria are unicellular, small 0.5-1.0mm in diameter, which multiply by binary fission. The algae are photosynthetic simple organism with

unicellular primitive types to aggregates of similar cells and to large brown algae with complex structure. Protozoa are unicellular, most of them living freely in soil and water while a few cause disease of man and animals.

The rigid cell wall of the bacterium confers shape. Bacteria vary in shape from spherical (Coccus) rods (Bacillus) and helically curved rods (Spirillum). Most bacteria possess a constant shape but some exhibit polymorphism (variety of shape).

Bacterial cells are arranged in a characteristic manner in a particular species. In cocci the arrangement is known as diplococci when cells divide in one plane and remain attached in pairs, streptococci when divide in one plane and remain attached to form chains; tetrococci, when they divide in two planes and form group of four cells (tetrads), staphylococci when they divide in three planes and form bundles, sarcinae when they divide in three planes in a regular manner and form a cubical arrangements.

Bacilli are not arranged in such complex form as in cocci. Most of them occur singly or in pairs (diplobacilli), form chains (streptobacilli) form trichomes, similar to chains but with a larger area of contact between cells and lined side by side like match sticks (palisade arrangement) at angles to one another.

Some others form long branched multinucleate filaments called hypha as in fungi. Hyphae ramify and collectively form mycelium. The curved bacteria are vibrioid with less than one twist or turn of helical with one or more complete turns. Rigid helical shape is in Spirilla and is flexible in spirochete.

Cell wall is a very rigid structure that confers shape to the cell. This prevents expansion of cells and bursting due to uptake of water as most bacteria live in environments of higher osmotic pressure than that exists in cells (hypotonic environments). A cell wall is common to almost all bacteria except in mycoplasma that lacks typical cell wall and L-forms of bacteria like *Streptobacillus*

that are having walls but lose them when grown in media containing sub lethal levels of cell wall synthesis inhibiting antibiotics like penicillin. Mycoplasma lack cell wall permanently and hence pleomorphic while L-forms of bacteria can revert back to walled forms. The isolated cell walls without cellular constituents retain the original contour of cells from which they are derived indicating that cell wall confers shape. This is further strengthened as the protoplast derived from any type of cell cocci or bacilli show a spherical shape. Both eubacteria and archaeobacteria are grouped as Gram positive and Gram negative based on the wall thickness. As the chemical composition of both eubacteria and archaeobacteria differ it is only the thickness rather than the chemical composition is the key factor for Gram reaction.

Cell wall constitutes 10-40% of cell. It is essential for growth and division. Cells without walls (protoplasts) cannot grow and divide.

The cell wall in eubacteria consists largely of an insoluble porous, cross-linked polymer of enormous strength and rigidity viz., peptidoglycan (also called murein).

This is a bag shaped macromolecule and surrounds the cytoplasmic membrane and found only in prokaryotes. Although it is tough but in a dynamic state. It is a polymer of N-acetyl glucosamine, N acetyl muramic acid, L-alanine, D-alanine, D-glutamic and a diamino acid (LL or meso diaminopimelic acid, L-lysine, L-orthinine or L-diaminobutyric acid).

The cell wall composition of archaeobacteria is different from eubacteria. Their walls are composed of proteins, glycoproteins or polysaccharides. But in some genera as *Methanobacterium* the cell walls composed of pseudouriein that have some superficial resemblance to peptidoglycan but differs in chemical composition.

The peptidoglycan constitutes about more than 50% of the dry weight of cells in gram-positive eubacteria but only 10% in

gram-negative bacteria. In addition to peptidoglycan other substances like polysaccharides in *Streptococcus pyrogenes* teichoic acids in *Staphylococcus aureus*, lipids as mycolic acids in *Corynebacterium* and *Mycobacterium*. The acid fast cord factor, a mycolic acid derivative is toxic and plays a role in diseases due to *Corynebacterium diphtheriae* and *M. tuberculosis*.

The wall of Gram negative consists of a thin peptidoglycan layer surrounded by an outer membrane rich in lipids. The lipids in the wall constitute 11-12% of the dry weight of the cells, The outer membrane is an impermeable barrier preventing the escape of important enzymes from the periplasmic space between the cytoplasmic membrane and outer membrane. The outer membrane also prevents external chemicals and enzymes that can destroy cells. Lysozyme, which dissolves selectively the peptidoglycan can damage gram positive bacteria.

The outer membrane, a bilayered structure consisting many of phospholipids, proteins, and polysaccharides is anchored to the peptidoglycan layer by means of Braun's lipoprotein. The lipopolysaccharide (LPS) layer has toxic properties and known as endotoxin. This occurs only in outer membrane and is composed of lipid A, core polysaccharide and O antigen. The outer membrane is impermeable to large molecules like protein but allow smaller molecules like monosaccharides peptides and amino acids through channels called porins. Porins span the membrane and are specific for different kinds of small molecules.

There are several structures external to cell wall in bacteria, which vary in structure and composition depending upon the type of bacteria. They are flagella, pili or fimbriae, capsules, sheath, prosthecae and stalk. Flagella are locomotory organs in bacteria, which vary in number and arrangement. Some bacteria do not have flagella.

Flagella are hair like helical appendages 0.01 – 0.02 nm in diameter the flagellar arrangements vary with the organisms. It

may be polar if the flagella are at one or both the ends or lateral if they are arranged on sides. They protrude through the cell wall. A flagellum is composed of a basal body a short hook and a helical filament longer than the cell. The basal body is associated with cytoplasmic membrane and cell wall.

Bacteria swim by rotating their helical flagella similar to cork screw. Bacteria with polar flagella swim in a back and forth fashion. Those with lateral flagella swim in a more complicated manner. Removal of flagella from a flagellate bacterium will not result in death of bacterium and only motility will be affected Spirochetes, the helical bacteria, swim even in viscous media, without any external flagella. They have flagella like structure within the cell located just beneath the cell envelope. They are known as periplasmic flagella (also called endoflagella, axial filament). Spiroplasmas are also helical in shape and swim in viscous media without even periplasmic flagella.

Some bacteria like Cytophaga exhibit a gliding motility, which is a slow sinuous flexing motion. This occurs when the cells come in contact with solid surface.

Pili are short, hollow, non helical and filamentous appendages. They are thinner than flagella but more in number than flagella. They are found in both motile and non motile bacteria and hence not involved in motility.

F pilus (sex pilus), a type of pilus serves as port of entry for genetic material during bacterial mating. Some pili in pathogenic bacteria serve as attachment with host cells in human beings facilitating infection without being washed off easily by mucous.

Capsules

A viscous substance forming a covering layer around the cell is found in some bacteria and is known as capsule. If it is too thin it is called as microcapsule. If it is loose and many cells are embedded in a matrix it is known as slime. The capsular material

is not water soluble in many bacteria but in some it is highly water soluble, thus making the medium in which they grow more viscous. Capsular material is primarily polysaccharide in most bacteria. It may be a homopolysaccharide, made up of a single kind of sugar, synthesized outside the cell from disaccharides. The capsule of *S.mutons* is a glucan (a glucose polymer) synthesized from sucrose. Capsules composing of several kinds of sugars are termed heteropolysaccharides. These are synthesized from sugars within the cell, transported and polymerized outside the cell. The capsule of *Klebsiella pneumonia* is a heteropolysaccharide. The capsule of some bacteria is polypeptides. The capsule of anthrax organism *Bacillus anthracis* is a polymer of D-glutamic acid.

Sheath is a hollow tube that encloses cells in the form of chains or trichomes. This is present in some bacteria living in fresh water and marine environment. The cells some times move out of sheath. In a few cases the sheath is strengthened by deposition of ferric and manganese hydroxides.

Aerobic bacteria in fresh water and marine environment possess prosthecae, which increases the surface area of cells for nutrient absorption from the dilute aquatic environment. They are semirigid extension of cell wall and cytoplasm membrane and smaller than the cell. Some bacteria have single prostheca (*Caulobacter*) and others have more than one (*Stellar* and *Ancalomicrobium*).

Stalks are also found in some bacteria like *Gallionella* or *Planctomyces*. They are non-living ribbon like or tubular appendages that are excreted by cell. These stalks aid in attachments of cells to surface.

The structures internal to cell are cytoplasmic membrane, protoplast, intracellular membranes, the cytoplasm, cytoplasmic inclusions and nuclear material, the DNA.

The cytoplasmic membrane is immediately beneath the cell wall and is about 7.5nm thick. It is made up of phospholipids

(about 20-30 percent) forming a bilayer to which both integral proteins and peripheral proteins are held. The membrane has fluidity owing due to its lipid matrix and this allows components to move laterally.

The phospholipids of eubacteria and archaeobacteria differ in composition. The phospholipids of eubacteria are phosphoglycerides. In this straight chain fatty acids are linked to glycerol by ester linkage. In archaeobacteria, the lipids are polyisoprenoid branched chain lipids. In this phytanols, (long chain branched alcohols) are ether linked glycerols.

The cytoplasmic membrane is a barrier for penetration of most of water soluble molecules. But the small molecules like nutrients and waste products are transported across the membrane by specific proteins. The membrane also contains various enzymes of respiratory metabolism and synthesis of cell wall components and capsule. It is also the site of generation of proton motive force that drives ATP synthesis, nutrition, transport system and flagellar motility. The damage to membrane by physical or chemical agent lead to death of cells.

The cytoplasmic membrane and the cell material bounded by it are called protoplast. The bacterial cell minus the cell wall is the protoplast. Protoplasts of gram positive bacteria can be prepared by dissolving the cell wall by lysozyme or by growing the bacteria in penicillin containing media. Penicillin prevents the synthesis and formation of cell wall. Protoplasts thus prepared have to be suspended in an isotonic medium, other wise the bacteria living in hypotonic environments tend to absorb water and burst.

In Gram-negative bacteria lysozyme treatment may destroy the cell wall. The outer membrane remains with the cytoplasmic membrane enclosing the cell content. Such type of protoplasts with the outer membrane is called as spheroplast.

The bacteria that lack cell wall like mycoplasma are similar to protoplasts but they are parasites of animals, plants or arthropods and hence live in osmotically favourable or isotonic environments.

Bacteria are prokaryotes that do not contain any membrane bound organelles inside the cells. But bacteria have specialized invagination of cytoplasmic membrane that increase the surface area for certain functions. Mesosomes are convoluted tubules or vesicles formed by membranous invagination in bacteria. Central mesosomes are located near the middle of the cell and penetrates deep into the cytoplasm. It seem to be attached to the nuclear material. Peripheral mesosomes shallowly penerate into the cytoplasm and seen to be invalid in export of exocellular enzymes.

The intracellular membrane is extensive in all phototrophic bacteria, chemoautotrophs and in methane oxidizing bacteria. In phototrophic bacteria they are the sites of photosynthesis as the increased surface area increase the light absorbing pigments.

Thylakoids are special intracellular membranes that occur in cyanobacteria but they are separate from cytoplasmic membrane.

The cytoplasmic membrane bound the cytoplasm. the cytoplasm consists of a cytoplasmic area, a chromatinic area and consists of Ribosome. Ribosomes are macromolecular RNA protein bodies and are the sites of protein synthesis. The chromatinic area is rich in DNA. The fluid proteins contain the dissolved substances.

Ribosomes of prokaryotes have a sedimentation coefficient of 70 Svedberg units (70S) and are composed of two subunits of 50S and 30S. On the other hand ribosomes of eukaryotes have a sedimentation coefficient of 80S and are composed of 60S and 40S subunits.

Cytoplasmic inclusions are concentrated deposits of certain substances. Volutin granules or metachromatic granules are polyphosphates deposits. It is a reserve of phosphate. Poly-B-hy-

droxy butyrate is a chloroform soluble lipid like material and serve as carbon and energy source. They are found in aerobic bacteria. Polysaccharide granules viz glycogen is present as inclusion. Elemental sulfur accumulates in certain bacteria growing in environments rich in hydrogen sulphide.

Bacteria in aquatic habitats have gas vacuoles to provide buoyancy. Gas vacuoles have water impermeable boundary but permeable to dissolved gases which fill the cavity. Bacteria do not have a nucleus with a nuclear membrane. The nuclear material is only a single circular DNA molecule. This is called as nucleoid, the chromatin body, the nuclear equivalent or functional chromosome.

EXERCISE

Points to remember

1. Differences between prokaryote and eukaryote
2. Differences in the cell structures
3. Functions and importance of the various cell structures

Self evaluation

1. Define procaryotes. Differentiate procaryotes from eucaryotes.
2. Describe briefly the cell wall structure and composition in eubacteria.
3. Given an account of motility and the arrangements of locomotile appendages in bacteria.
4. Describe briefly the structure of cytoplasmic membrane in bacteria.
5. Give a brief account of the functions of cytoplasmic membrane in bacteria.
6. Give an account of ribosomes and mitochondria in microorganisms.
7. Describe briefly the cytoplasmic inclusions in bacteria.
8. What do you understand by sheath and capsules?
9. Describe the different shape and arrangements of cells in bacteria.
10. Differentiate pili and flagella indicating their functions.

Chapter 8

TAXONOMY

Biodiversity (Biological diversity), the variability among the galaxy of living organizations, includes the diversity of the species between the species and of ecosystems. It is really difficult to estimate the total number of different types of eubacteria, archaeobacteria and virus, as it is very difficult to isolate and recover the organisms from the environment. Further the natural environment pose varying conditions in different ecosystems rendering huge variation among the species existing in main ecosystem. Not all environments have been investigated fully and therefore attempts to estimate total number of species of micro organisms become more difficult. In complete understandings of cultural conditions required by certain obligate parasites add to this problem. Mycoplasma are prokaryotes but have obligate associations with eucaryotic organisms, have remarkable diversity from some infecting insects and some infecting plants. The soil, fresh water and marine ecosystems support a group of diverse organisms on their ecosystem providing luxuriant microbial diversity.

The microorganisms have species that are free living in soil and water, mostly saprophyte in nature, a group that are parasitic on plants and a few others are obligate pathogens of plants, animals and man. Some live in aerobic environment and other living in anaerobic or microaerophilic conditions. Therefore there is a wide diversity.

The advent of DNA techniques like DNA-DNA hybridization, nucleic acid finger printing, RNA sequencing has altered the

microbial diversity. The 16S r RNA sequence and DNA finger printing techniques have enabled to evaluate the genetic relatedness between organisms.

The smallest unit of microbial diversity is a species. Bacteria are defined as a group of similar strains differentiated from other similar groups of strains by genotypic, phenotypic and ecological characters. Bacterial strain is one with approximately 70% or more DNA-DNA relatedness and with 5% or less in thermal stability. A bacterial species is a genomic species based on DNA-DNA relatedness and this concept differs from those of other organisms. There is an estimated total species of 40,000 bacteria, 1,30,000 viruses, 1,50,000 fungi, 60,000 algae compared to the 5000,4,760,6900 and 40,000 known species which constitute 4,12,5 and 67% of known species.

The living organisms were grouped as plants, animals and protista by Haeckel(1866). The protista are primitive organisms including microbe. Based on cell anatomy the bacteria were group as prokaryotes. Whittaker proposed five kingdoms plants, fungi animals, protista and Monera based on cell anatomy and energy yielding systems. Microorganisms are in fungi, protista and monera. Based on the fact that bacteria are distant from plants and animals but not that far away from each other Woese and his coworkers proposed three domains Archaea, Bacteria and Eukarya to cover the microorganisms. The domain bacteria include bacteria, cyanobacteria, actinomyces etc. Archaea includes methanogens, extremely thermophilic organisms extremely halophilic organisms and the Eukarya includes molds, yeasts, algae, protozoa etc.

The microorganisms are named following the Linnaeus method of binomial nomenclature. The taxonomy denotes classification, nomenclature and identification of organisms. The characteristics or properties that are common for a few organisms are grouped together in different groups(taxa). Bacteria were

traditionally based on morphological, biochemical and physiological characteristics. Serological tests and Genetic tools are valuable in identification.

The general methods of classifying bacteria is by (I) intuitive method(ii) numerical taxonomy iii. Genetic Relatedness method.

While identifying a bacteria morphological, physiological, biochemical general and molecular characteristics of organisms are studied. It may be difficult to classify an organism as different microbiologists may consider different characteristics as important. This is the intuitive method.

Numerical taxonomy gives equal weightage for each character of the strain. The percentage similarity of each strain is determined with the following formula

$$\% S = NS / (NS+ND)$$

Where NS: Number of characters for each strain which are similar or dissimilar

ND = Number of characters that are different.,

%S, S = Similarly if it is high to each other placed with groups.

In Genetic relatedness classification is based on relatedness(DNA and RNA) between organisms. The percent G+C determines the relatedness. If two bacteria have a different not % G+C then the species are different and not related. DNA homology (DNA-DNA hybridization) is also determined to assess the relatedness. The DNA from a bacterium is isolated and the two strands are separated and a single strand is mixed with that obtained from another. If the two bacteria are similar the pairing of strands will occur, otherwise no pairing will occur.

Kingdom: Prokaryotae

Division I

Gracilicutes Prokaryotes with thinner cell walls, ordinary Gram negative bacteria

The Spirochetes:

Order: Serochaetales

Spirochaetaceae

Spirochaeta Harmless inhabitants of water, mud and sediments

Cristispira Harmless parasites of molluscs

Treponema inhabitants of mouths, intestinal and genital areas of human and animals, some are pathogenic *T. Pallidum* causes syphilis in man

Borellia Parasites of wild rodents

Leptospiraceae

Leptospira harmless inhabitants of fresh water Environments. *L. interrogans* causes leptospirosis

Aerobic/Microaerophilic, motile helical/vibrioid bacteria.

Aquaspirillum harmless saprophytes in streams and ponds

Azospirillum Nitrogen fixing bacteria

Oceanospirillum harmless saprophytes of main water

Campylobacter inhabitants of intestines, oral cavity and reproductive organs

Bdellovibrio Parasite on gram negative bacteria

Non motile(or rarely motile) curved bacteria

Spirosoma	Yellow pigmented
Runella	Pink pigmented
Flectobacillus	Pink pigmented
Microcystis	intra cellular gas vacuoles present

Aerobic rods and cocci

Pseudomonadaceae

Pseudomonas	inhabitants of soil and water, some are pathogenic to plants, animals, man.
Xanthomonas	Plant pathogens, citrus canker, rice leaf blight
Zoogloea	inhabitant of sewage treatment plants

Azotobacteraceae

Azotobacter free living nitrogen fixer

Rhizobiaceae

Rhizobium	Symbiotic nodule bacteria of legumes
Bradirhizobium	symbiotic nodule bacteria of legumes
Agrobacterium	Plant pathogens causing gall and tumors.

Methylococcaceae

Methylococcus	Obligate methane oxidizers
Methylomonas	Obligate methane oxidizers

Acetobacteraceae

Acetobacter	acetic acid(vinegar) producers
Gluconobacter	sorbose, gluconic acid producer

Legionellaceae

Legionella inhabitant of thermally polluted water
Air conditioning cooling towers

Nisseriaceae

Nisseria Pathogenic to humans, gonorrhoea, meningitis

Facultatively anaerobic bacteria

Enterobacteriaceae

Escherichia Occur in colon of warm blooded animals
Shigella causes bacillary dysentery in human
Salmonella causes typhoid and paratyphoid, salmonellosis
Enterobacter occur in sewage, meat
Erwinia soft rot of vegetables
Serratia occur in soil, water, plant surfaces opportunistic pathogen
Proteus occur in intestine of human, animals; opportunistic pathogen
Yersinia causative agent of plague is *Y. pestis*

Vibrionaceae

Vibrio aquatic habitate, *V. cholerae* causes cholera
Aeromonas aquatic habitate *A. salmonicida* causes furunculosis in salmon fish.

Pasteurella

Pasteurella Parasitic on mucous membranes of upper respiratory tract of mammals.
Haemophilus *H. influenzae* causes meningitis in children
Actinobacillus Occasionally pathogenic to man

Other genera not assigned to any family

Zymomonas	Ferments glucose to ethanol
Chromobacterium	saprophyte of soil and water, infections to human & animals.
Gardnerella	<i>G.vaginalis</i> causes non-specific vaginitis
Streptobacillus	A rat parasite <i>S.moniliformis</i> causes rat bite fever in humans.

Anaerobic curved helical rods

Bacteroidaceae

Bacteroides anaerobic; *B. fragilis* is associated with soft tissue infections

Fusobacterium
Succinomonas
Wolinella
Selenomonas
Anaerovibrio

Dissimilatory sulphate or sulphur reducing bacteria

Desulfuromonas	utilizes elemental sulfur
Desulfovibrio	use sulphate, thiosulphate
Desulfococcus	use sulphate, thiosulphate

Anaerobic cocci

Veillonellaceae

Veillonella inhabitants of oral cavity, respiratory tract, intestinal tract of humans, ruminants. Rodents and pigs

Acidaminococcus inhabitants of oral cavity, respiratory tract intestinal tract of humans, ruminants. Rodents and pigs

Megasphaera inhabitants of oral cavity, respiratory tract, intestinal tract of humans, ruminants. Rodents and pigs

The Rickettsiales

Rickettsiales
Rickettsiaceae
Rickettsia
Rochalima
Bartonellaceae
Bartonella
Anaplasmataceae

Chlamydiales

Chlamydiaceae
Chlamydia *C.trachomatis* cause trachoma; keratoconjunctivitis

The Mycoplasmas

Class Mollicutes

Order: Mycoplasmatales
Mycoplasmataceae

Mycoplasma *M. pneumoniae* causes primary atypical pneumonia in humans

Spiroplasmataceae urethritis in human, pneumonia in cattle

Spiroplasma causes plant disease

Endosymbionts

Endosymbionts of protozoa, ciliate, flagellates, amoeba

Endosymbionts of insects

Endosymbionts of fungi and invertebrates other than Arthropods.

Division II. Fimicutes

(Prokaryotes with thick and strong wall – Gram positive)

Grampositive cocci

Aerobic/Facultatively

Anaerobic cocci

Deinococcaceae

Deinococcus : *D. radiodurans* is a spoilage agent in radiated foods.

Micrococcaceae

Micrococcus harmless saprophytes of soil; found in skin of human and animals

Planococcus harmless saprophytes of soil; found in skin of human and animals

Planococcus harmless saprophytes of marine environments

Staphylococcus Parasites on the skin and mucous membranes of human and warm blooded animals.

Aerotolerant Fermentative cocci

Streptococcus Most are parasites of human and animals some ferments sugars to lactic acid

Leuconostoc harmless saprophytes; forms lactic acid, used in butter and cheese.

Pediococcus saprophytes; form lactic acid

Anaerobic cocci

Peptococcus occur in mud, intestines, respiratory tract

Peptostreptococcus occur in human clinical specimens

Ruminococcus occur in bovine rumen

Caprococcus occur in human feces

Sarcina occur in soil, cereal grain, diseased human stomach

Endospore forming rods /cocci

Bacillus most species are harmless saprophytes of soil and water; *B.anthraxis* causes anthrax of cattle

B.thuringiensis is used as biopesticide to kill insects
Sporosarcina soil inhabitant

Anaerobic spore forming rods

Clostridium distributed in soil, water and sediments botulism, tetanus are caused by species

Desulfotomaculum occur in soil, water, intestines of insects

Non spore forming rods

Lactobacillus saprophytes in fermenting plant and animal products or parasites of mouth and intestines of warm blooded animal.

Listeria *L.monocytogenes* is a pathogen of animals and humans, causes meningitis in adults; pre and post natal disease in infants.

Erysipelothrix parasites of mammals, birds, fish, causes erysipelas in swine, erysipeloid in humans

Brocothrix saprophytes of meat and meat products

Renibacterium parasites of salmonid fishes; cause a kidney disease

Kurthia harmless saprophytes in meat, meat products and animal dung

Caryophanan saprophytes of ruminant dung

Non spore forming irregular shapes

Aerobic / Facultatively anaerobic

Non filamentous rods

Corynebacterium	saprophytes of water, parasites of humans, plant pathogens, <i>C.diphtheriae</i> causes diphtheria in humans
Arthrobacter	saprophytes of soil
Brevibacterium	salt tolerant <i>B.linens</i>
Microbacterium	saprophytes in milk and dairy products
Cellulomonas	cellulose degraders

Aerobic / Facultatively anaerobic branched filamentous rods

Agromyces	soil saprophytes
Arachnia	pathogenic to human and animals causes actinomycoses
Rothia	normal inhabitant of human mouth

Anaerobic nonfilamentous rods

Propionibacterium	occur in dairy products, human skin, intestines
Eubacterium	occur in human oral cavity, intestines of human and animals
Actinomyces	occur in oral cavity of human and animals; <i>A.bovis</i> causes actinomycosis in cattle
Bifidibacterium	occur in intestines of human and animals

The Mycobacteria

Mycobacterium	pathogens, causes leprosy, tuberculosis
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The Nocardioforms

Nocardia	saprophytes of soil and water opportunistic pathogen causing nocardiosis and actinomycetoma in humans and animals
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Pseudonocardia	occur in soil and manures
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III. Tenericutes

Anoxygenic phototrophic bacteria

Rhodospirillales

Rhodospirillaceae

Rhodospseudomonas

Rhodomicrobium

purple non sulfur-bacteria
purple non sulfur-bacteria
purple non sulfur budding
bacteria

Chromatiaceae

Chromatium

Thiocystis

Thiospirillum

Lamprocystis

Thiosarcina

Thiopedia

purple sulfur bacteria
purple sulfur bacteria

Chlorobiaceae

Chlorobium

Prosthecochloris

Green sulphur bacteria
Green sulphur bacteria

Chloroflexuaceae

Chloroflexus

Green non sulphur bacteria

Oxygenic phototrophic bacteria

(Cyanobacteria Blue Green algae)

The Cyanobacteria

Chroococcales

Pleurocapsales

Oscillatoriales

Nostocales

Stigonematales

The Prochlorales

Prochloraceae

Prochloron

unicellular organisms
containing chlorophyll
b lack

Prochlorothrix

-

Gliding fruiting bacteria

Myxobacterales

Stigmatella	degrade cellulose, agar, chitin
Chondromyces	degrade cellulose, agar, chitin

Gliding non fruiting bacteria

Sporocytophaga	forms myxospores without fruiting bodies
Capnocytophaga	occur in oral cavity of humans
Beggiotia	aquatic environments with H ₂ S
Cytophaga	cellulolytic organs
Flexibacter	
Votreoscilla	
Simonsiella	
Saprospira	
Thiothrix	
Herpetosiphon	forms sheath
Flexithrix	forms sheath

The Sheathed bacteria

Sphaerotilus	Sheath surrounds a chain of cells or trichome; iron deposited on sheath
Leptothrix	
Haliscomenobacter	
Streptothrix	
Lieskeella	
Phragmidiothrix	
Crenothrix	
Clonothrix	

Budding and or Appendaged bacteria

Prosthecate Budding bacteria

Hyphomicrobium	soil and aquatic environments
Anclomicrobium	aquatic bacteria form 3-8 prosthec per cell, buds arise from cell

Prosthecate non budding bacteria

Caulobacter	occur in salt water and fresh water
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Non prosthecate budding bacteria

Blastocaulis	
Planctomyces	occurs in all aquatic habitat

Non prosthecate non budding bacteria

Gallinonella	causes clogging in pipelines of water system
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Aerobic chemolithotrophic bacteria

Nitrobacteraceae	
Nitrate oxidizing bacteria	
Nitrobacter	
Nitrococcus	
Nitrospira	

Ammonia oxidizers

Nitrosomonas	
Nitrosococcus	
Nitrosovibrio	
Nitrosolobus	

Sulphur and sulphur compounds metabolizing bacteria

Thiobacillus	occur in soil, water and coal mine drains
Thiomicroscopira	occur in soil, water and coal mine drains

Thiobacterium	
Macromonas	
Thiovulum	
Achromatium	
Thiospira	

Iron or manganese oxidizers

Siderocapsa	deposition of iron or manganese oxides on slime or capsules
Siderococcus	deposition of iron or manganese oxides on slime or capsules
Siderocystis	deposition of iron or manganese oxides on slime or capsules
Naumanniella	deposition of iron or manganese oxides on slime or capsules

Archaeobacteria

Methanogenic archeobacteria

Methanobacteriales

Methanobacteriacea

Methanobacterium	Methane producers
Methenobrevibacter	Methane producers
Methanomicrobium	Methane producers
Methanogenium	Methane producers
Methanogenium	Methane producers

Methanothermaceae

Methanococcales

Methanococcaceae

Methanococcus

Methanomicrobiales

Methanomicrobiaceae

Methanomicrobium

Methanosarcinaceae

Methanosarcina

Methanolobus

Archaeobacterial sulphate Reducers

Archaeoglobales

Archaeoglobaceae

Archaeoglobus

Extremely halophytic archaeobacteria

Halobacteriales

Halobacteriaceae

Halobacterium	require 17-23% NaCl for growth the cells lyse when NaCl falls below 10%
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Halococcus	require 17-23% NaCl for growth cells lyse when NaCl falls below 10%
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Halofere	require 17-23% NaCl for growth cells lyse when NaCl falls below 10%
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Thermoacidiphiles

Cell wall less Archaeobacteria

Thermoplasma	grows at pH2 and optimum temperature is 55-59°C cells lyse at neutral pH.
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Extremely thermophilic sulphate- Metabolizers

Thermococcales

Thermococcaceae

Thermococcus

Thermoproteales

Thermoproteaceae

Thermoproteus

Desulfurococcaceae

Desulfurococcus

Sulfolobales

Sulfolobaceae

Sulfolobus

optimum pH is 2; temperature is 70-87°C

IV. Mendosicutes

Gram positive filamentous bacteria of complex nature

Filamentous bacteria dividing in more than one plate

Dermatophilus *D.congolensis* is a parasite of mammals causing infection

Frankia Nodulating organism in *Casuarina* and *Alnus*

Filamentous bacteria forming true sporangia

Actinoplanes occur in dead plant parts, shed animal hair and soil

Ampullariella occur in dead plant parts, shed animal hair and soil

Spirillospora occur in dead plant parts, shed animal hair and soil

Streptomyces and similar genera

Streptomyces decomposes organic matter streptomycin antibiotic producer

Streptoverticillium

Actinopycnidium

Actinosporoangium

Chainia

Elytroporangium

Kitasatua

Microellobiosporia

Filamentous bacteria of uncertain taxonomic placent

Actinomadura soil saprophytes

Nocardiosis soil saprophytes

Actinopolyspora extreme halophilism is seen

Actinosynnema compact hypha synnemata

Thermomonospora occurs in compost thermophilic and cellulolytic

Thermoactinomyces occur in damp hay, composts and moist grain

Taxonomy of Fungi

The study of fungi is known as Mycology (Gr.mykes= mushroom + logos=discourse). The term fungus denote nucleated, spore bearing achlorophyllous organisms with filamentous branched somatic structure surrounded by cell walls containing cellulose or chitin or both. They reproduce sexually and asexually. However, some true fungi are not filamentous and the filaments of some others do not have cell walls. Some algae lost the chlorophyll in the evolutionary process and achlorophyllous but they are not fungi. The cellular slime molds and net slime molds are also studied by mycologists and are not probably fungi.

The fungi include moulds, white rusts, downy mildews, powdery mildews, sac fungi, black moulds, blue moulds, cup fungi, morels, truffles, rusts, smuts, mushrooms, toadstools, puff balls, stink horn, coral fungi, earth stars, shelf fungi and bird's nest fungi. They are both beneficial and harmful to mankind. The yeasts are used in leavening of bread, alcohol production as food and feed, for single cell protein and several products are prepared from yeasts. The mushrooms and truffles are cultivated and eaten. White rusts, rusts, smuts, wilts, leaf spot, blight, blast are plant diseases caused by fungi. The Irish famine was caused by potato blight and the Bengal famine was caused by *Hemminthosporium* leaf blight in rice. Most of them occur in soil, decomposing litter, animal dung as saprophytes. There is also a beneficial association between fungi and plants in mycorrhiza (Gr.mykes= mushroom + rizha=root) benefiting both the fungus and plants. The mycorrhizal strands formed from the root runs far away from root absorbs water, phosphorus, micronutrients etc and supplied to the plants. The fungus derives its nutrients from the plants. Thus mycorrhiza are cultivated commercially on host roots and given as biofertilizer for inoculating crop plants. Lichen is an association of fungus and alga knit so close and forming a single thallus. The fungus component of lichen is called mycobiont and the algal component as

phycobiont (Gr mykes = fungus phykos=alga bios = life). The lichen fungi are ascomycetes or basidiobycetes while the lichen algae are blue green algae.

The somatic structure of fungus consists of microscopic strands of filaments branching in all directions and spreading over the substratum. The filament known as hypha (pl.hyphae Gr=hypha=web) is a thin, tubular wall filled with a layer of protoplasm. The protoplasm is continuous without any cross wall (septum; L.septum= hedge, partition) in some fungi (aseptate mycelium or coenocytic) and with cross walls (septate). The protoplasm on each side of a septum is connected by living strands passing through a central pore in the septum.

The composition of the cell wall is not the same in all fungi. In some forms cellulose is the chief constituent and in higher fungi chitin is the chief constituent. Callose a lignin like but carbohydrate substances and other organic materials are also present.

In the hypha a true nucleus with nuclear membrane a nucleolus and chromatin strands which organizes to chromosomes during division are seen. In aseptate hypha nucleus are embedded in the cytoplasm uniformly throughout the mass and the condition is called coenocytic (Gr koinos= common + kytos = a hollow vessel). The individual cells of septe hypha may contain 1,2 or many cells. Uninucleate, binucleate, multinucleate cells (most common) occur on fungi. Vacuoles, oil droplets and other inclusions are also present in the mycelium. The mycelium of some higher fungi forms thick strands called rhizomorphs which are resistant to adverse conditions and remain dormant until conditions are favourable. In parasitic fungi the mycelium may grow on surface or inside the host. It may be intercellular (growing between cells) or intra cellular (penetrating into the cell). The food is absorbed through the host cell wall in the former and in the latter a direct contact with protoplasm is established. Obligate plant pathogens growing between cells produce haustoria (haustorium L

hauster= drinker) that penetrate into the host cells through minute pores punctured into the cell walls or as outgrowth of hyphae and obtain nourishment. Fungi parasitising on animal tissue are not known to produce haustoria and fungus grown in culture also do not produce haustoria.

The mycelium of most fungi becomes organized into loosely or compactly woven tissues different from ordinary thallus. The term Plectenchyma (Gr.pleko= I weare + enchyma= infusion – woven tissue) denotes all organized fungal tissues. Prosenchyma (Gr. pros= toward + enchyma= infusion= approaching a tissue) is loosely woven tissue in which hyphae lie more less parallel to one another. Pseudoparenchyma (Gr.pseudo= false + parenchyma=a plant tissue) is closely packed more or less isodiametric or oval cells resembling parenchyma of higher plants. Prosenchyma and the Pseudoparenchyma compose different types of somatic and reproductive structures like stroma and sclerotium.

Two gametangia of opposite sex come in contact and one or more gamete nuclei move from male to female and fuse. There is no fusion of gametangia. The male nuclei enter the female gametangium through a pore developed by the dissolution of gametangial walls at the point of contact. In some fungi fertilization tube is formed for the passage. The antheridium disintegrates after the passage of nucleus but the oogonium develops.

Gametangial copulation occurs due to fusion of the contents of the two contacting gametangia. In holocarpic forms where entire thallus acts as gametangium passage of one gametangial content (male) to the other (female) through a pore developed at the contact point. In others direct fusion of two gametongial cells occur.

Spermatia, uninucleate, spore like male structure, produced by certain fungi are carried by insects, wind or water to the female gametangia or to the receptive or somatic hypha. The contents of

spermatia pass into the receptive structure, female organ, through a pore developing at the contact point.

In many of the higher fungi no sex organs are produced and the somatic cells functions like sex organs as they come in contact and fuse.

The nuclear cycle in fungi generally involve haploid, diploid cycle. The diploid nuclei resulting from karyogamy become haploid after meiosis. Heterokaryosis is a phenomenon wherein nuclei of the genetically similar or different fungi exist in the same cell of hypha. The cells may not contain the same number or same kind of nuclei or the same proportion of each kind in a mixture of nuclei. Each nucleus is independent of all nuclei in the heterokaryotic condition.

The fungi may be classified on the basis of sex as (1) hermaphroditic (2) dioecious and (3) sexually undifferentiated thallus producing morphologically indistinguishable male or female. On the basis of sexual compatibility fungi are grouped as (1) Homothallic (2) heterothallic and (3) secondary homothallic fungi. In homothallism every thallus is self fertile and can reproduce without another thallus. Hermaphroditic thallus are of this type. No dioecious fungi can be homothallic. In heterothallic fungi every thallus is self sterile and requires another compatible thallus of different mating type. Heterothallic fungi may be either bipolar heterothallic or tetrapolar heterothallic. Two mating types differing in their genetic make-up for the compatibility factor occur in the bipolar mating type. Each of the nucleus of one mating type carries the gene A and each of the nucleus of other mating type carries gene a. Those thalli carrying opposite genes of the pair Aa are compatible. Four mating types occur in heterothallism wherein the compatibility is determined by two pair of factors Aa and Bb located in different chromosomes. Those thalli carrying nuclei of opposite genes of both the Mendelian pair Aa and Bb are compatible forming the zygote of AaBb genotype.

Secondary homothallism occur in fungi during the spore formation of bipolar heterothallic fungi. The two nuclei of opposite mating type are present in each spore which upon germination gives a thallus containing both A and a nuclei and then behaves as homothallic.

Some fungi derive the benefits of sexuality through parasexuality without true sexual cycle (Gr.para=beside). In parasexual method plasmogamy, karyogamy and haploidization occur but not at specified points of life cycle. In the Deuteromycetes group parasexuality occurs and no sexual reproduction. Some fungi reproduce sexually and parasexually.

In mycology certain organisms of uncertain affinity like the cellular slime molds and net slime molds are also studied. The cellular slime molds are grouped under order *Acrasiales*. The cells are naked haploid amoeba feeding on bacteria. The cells are not flagellated but cells aggregate together forming pseudoplasmodium. The cells never fuse but remain individually but cooperate. Sorocarps (Gr.soro=heap+karpus=fruit) are fruiting bodies. Cultivated soils are richer in cellular slime molds.

The net slime molds are grouped in the order *Labyrinthulales*. These are aquatic, mostly marine or terrestrial organisms with naked uninucleate spindle or oval shaped cells. These cells become interconnected by slime filaments forming a net along which they glide. The majority of species are marine associated saprobically or parasitically with marine algae *Ulva* or cause a disease in higher plants *Zostera marina*.

The lower fungi includes the true slime molds (Class: Myxomycetes), posteriorly unflagellate fungi (Class: Chytridiomycetes) anteriorly unflagellate fungi (Class: Hyphochytridiomycetes), including water molds, white rusts and downy mildews forming oospores (Class: Oomycetes) endoparasitic slime molds (Class: Plasmodiophoromycetes) and bread molds, fly fungi and animal

traps (Class: Zygomycetes). The higher fungi are the sac fungi (Class: Ascomycetes) including yeasts, leaf curl, black molds, blue molds, perithecial fungi, cup fungi, morels, truffles, parasitic fungi of insects and arachnids, the imperfect fungi (Form Class: Deuteromycetes), smuts, rusts, jelly fungi, mushrooms, puffballs and stinkhorns (Class: Basidiomycetes). Fungi form new individuals by sexual and asexual reproduction. Asexual (somatic or vegetative) reproduction does not involve union of nuclei or sex cells or sex organs whereas sexual reproduction is by union of two nuclei. The fungus is known as holocarpic when the entire thallus is converted into one or more reproductive structures and both somatic and reproductive phases do not occur together on the same individual (Gr:holos= whole + karpos=fruit). In most of the fungi separate reproductive organs arise from a portion of thallus and the remainder remains as somatic structure continuing its activities. Such fungi are known as eucarpic (Gr.eu=good+karpos=fruit). The holocarpic forms are primitive than eucarpic fungi. Stomata (pl.stromata Gr.stroma= mattress) is a compact like a mattress on / in which fungal fructifications are formed. Sclerotia (pl.sclerotia Gr.skelros= hard) is hard resting body resisting unfavourable conditions that remains dormant until favourable conditions returns.

Asexual reproduction results in the production of numerous individuals and important in propagation of species, repeated several times whereas the sexual stage is produced once in a season or year. Asexual reproduction occurs in several ways like (i) fission as in fission yeasts (ii) budding as in budding yeasts (iii) fragmentation of somatic structure each of which giving rise to a new individual (4) spore formation, each spore germinating to form new individuals.

In fragmentation the hypha breaks up into their component cells called oidia (Oidium, Gr.oidion=small egg) or arthrospores (Gr.arthorn=joint+sporos=seed, spore) which function like spores.

In certain cases the cells are enveloped with a thick wall even before they separate and are called chlamydospores (Gr.chlamys=mantle+spores=seed, spores). Fission is division of a cell into two daughter cells by formation of a cross wall in some yeasts. Bud is a small outgrowth from a parent cell and when it is formed the nucleus divides and the daughter nucleus move into the bud. The bud grows in size and breaks and forms a new individual. Budding takes place in majority of yeasts and many other fungi under certain conditions. Production of spores is the common method in many fungi. Spores vary in colour from colourless (hyaline Gr.hyaline= made of glass i.e., colourless) through green, yellow orange, red, brown to black and also vary in size and shape. Some fungi produce only one type of spore whereas others produce more types. Asexual spores are called sporangiospores when they are borne in sporangia a sac like structure (Gr.sporos=seed,spore+angeion=vessel) or called conidia when borne on the tips of hypha (Gr konis=dust, idion, dimin suffix.) The whole content of sporangium may develop into one or more spores. Sporangiospores are of two types zoospores and aplanospores. Zoospores are motile with one or two flagella while aplanospores are non motile. The flagellum may be of whiplash or tinsel type.

Sexual reproduction, the union of two compatible nuclei, consists of three phases viz., plasmogamy, karyogamy and meiosis. Plasmogamy (Gr.plasma= a molded object i.e, a being + gamos= marriage, union) union of two protoplasts occur bringing tow haploid nuclei together in one cell. In karyogamy (Gr.karyon = nut, nucleus + gamos = marriage) fusion of the two nuclei occur into one diploid zygote nuclei. This second phase follows immediately plasmagamy on lower fungi and in higher fungi it is delayed resulting in a dikaryotic (dikaryon di=two + Gr.karyon=nut). the dikaryon = condition may be perpetuated from cell to cell by the division of the associated nuclei and by the separation of the two daughter cells. Meiosis occur sooner or later after the fusion of

two nuclei restoring the haploid condition in the four nuclei formed. In order to effect sexual reproduction some fungal species produce male and female sex organs (gametangia) in each thallus (hermaphroditic species-bisexual (Gr.Hermes = the messenger of the Gods,the symbol of the male sex + Aphrodite = the goddess of love, the symbol of female sex). In other species some thalli produce only male sex organs and others produce only female sex organs (dioecious).

Gametangia, the sex organs, form cells called gametes or may contain one or more nuclei. The morphologically similar but physiologically different male and female gametangia and gametes are called isogametangia and isogametes. If they can be differentiated then they are called as heterogametangia and heterogametes. In heterogametangium the male is designated as antheridium and the female is called as oogonium.

The most common method of plasmogamy are (1) Planogametic copulation (2) gametangial contact (3) gametangial copulation (4) spermatization (5) somatogamy.

In Planogametic copulation two naked gametes, one or both motile fuse together. The gametes may be isogamous or anisogamous. In some cases the female is non motile and male is motile. The male gamete enters the oogonium and fertilizes the egg.

The class Myxomycetes contain the true slime molds. The acellular creeping somatic structure of slime molds are animal like in structure and physiology but the reproductive structures are plant like producing spores. They live in moist shady places dead leaves, on decaying logs or other organic matter. They occur in grasses lawns and develop in bark of trees. They feed on bacteria, protozoa and other minute organisms.

The class chytridiomycetes are coenocytic fungi producing motile cells (zoospores) with a single posterior whiplash flagellum. They occur in aquatic habitat and some parasitize algae.

The class Hyphochytridiomycetes consist of aquatic fungi producing anteriorly unflagellate motile cells with tinsel flagellum. *Rhizidiomyces apophysatus* is parasite on the oogonia of water molds.

The class Oomycetes consists of water molds, white rusts and downy mildews. These fungi produce asexually by means of biflagellate zoospores bearing one forward tinsel flagellum and one backward whiplash flagellum. *Albugo*, *Peronospora*, *Plasmopra*, *Pythium* and *Phytophthora* are parasitic on plants.

The class Plasmodiophoromycetes consist of fungi which are obligate endoparasites of vascular plants, algae and causing hypertrophy. *Plasmodiophora brassicae* cause club root of cabbage. *Spongospora subterranea* causes powdery scab of potatoes.

The class Zygomycetes are characterized by zygospore formation in sexual reproduction and non motile sporangiospores or conidia in asexual reproduction. The majority of them are saprophytic living on dung, decaying plant or animal matter. The common bread mold *Rhizopus stolonifer* is used for the industrial manufacture of fumaric acid and cortisone. A few of them are weak parasites of fruits, soft rot of sweet potatoes. Entomopathogenic fungi like fly fungus *Entomophthora muscae* are found in dead housefly. The zoopagaceous fungi parasitize amoebae, rhizopods and nematodes.

The class Trichomycetes consists of fungi associated with arthropods. They are widely distributed and not parasitic but commensals.

The class Ascomycetes and the class Basidiomycetes are known as higher fungi and produce ascospores and basidiospores respectively. The yeasts, black molds, green molds, the powdery mildews, the cup fungi, the morels and the truffles are ascomycetous fungi. Many are parasitic on plants, some are saprophytic living in soil, decaying logs and leaves. The yeast *Saccharomyces*

cerevisiae are used in bread leavening, production of beverages like beer, wine, alcohol production, in single cell protein production and enzymes. *Chaetomium* is cellulolytic in soil aiding decomposition. The ergot fungus *Claviceps purpurea* cause plant disease in rye, cumbu, etc., the sclerotia of which are deadly to animals when consumed. The *Penicillium* that produces Penicillin antibiotic and the omnipresent *Aspergillus* spp., belong to this class.

Ascus, the sac like structure containing definite number of ascospores are produced by the fungi in sexual reproduction. Eight are usually formed in an ascus but this may vary from 1 to 1000 according to species. The asexual reproduction in ascomycetes is by fission, fragmentation, blastospores (budding) antherospores, chlamydospore or conidia. The fruiting bodies formed in ascomycetes are (1) pycnidium (2) Acervulus and these contain conidiophores bearing conidia. The ascomycetes have two different reproductive phases, the ascus or sexual stage (ascigerous or perfect stage) and the conidial or asexual stage (imperfect stage). Ascomycetes are classified based on the characteristics of perfect stage. But the conidial stages of number of ascomycetes have not been found. Further there are large number of fungi known only by conidial stages and are designated as imperfect fungi (form class Deuteromycetes). These are ascomycetes which have lost their ascus stage in the evolutionary development.

The Ascomycetes are further divided into three subclasses (1) Hemiascomycetidae (2) Euscomycetidae and (3) Loculoascomycetidae. The Hemiascomycetidae includes yeasts (Order: Endomycetales) and leaf curl fungi (Order: Taphrinales). The Euscomycetidae is divided into series (i) Plectomycetes (ii) Pyrenomycetes and (iii) Discomycetes and (iv) Laboul-bemycetes. The Plectomycetes include black molds (*Aspergillus*) green molds and blue molds (*Penicillium*). The series Pyrenomycetes contain those fungi that produce ascus in globose or flask shaped perithecium. The Discomycetes include

cup fungi, earth tongues, the morels and the truffles that are recognized by their cup or disc shaped fruiting bodies produced on the ground. The brown rot of peach and other stone fruits is caused by *Monilinia fructicola* of this group. The truffles (genus *Tuber*) are commercially exploited as food in European countries. The series Laboulbeniomyces include parasites of insects and arachnids. *Herpomyces* parasitize cockroaches. The series Loculoascomycetidae are ascostromatic fungi that produce asci in stromatic locules. These fungi are parasitic to plants and insects. *Elsinoe faucei* causes citrus scab and *E.ampelina* cause anthracnose of grapes. *Myriangium* spp. are parasitic on insects. *Mycosphaerella musicola* causes sigatoka disease of banana. *Venturia indqualis* attacks apple.

The form class Deuteromycetes includes those imperfect fungi that lack a sexual phase or perfect stage. Most of them are saprophytic but many cause diseases of plants, animals and human. It is presumed that the imperfect fungi represent conidial stages of ascomycetes whose perfect stage (ascigerous stage) are rarely formed or not been found or have been lost by these organisms in their evolution. Whenever sexual stages are found in a few species then they are classified under ascomycetes. In certain cases the perfect stage have been found to be similar to those of Basidiomycetes. Hence fungi imperfecti are considered as conidial stages of ascomycetes or rarely as basidiomycetes whose sexual stages have not been found or do not exist. In this group parasexual cycle brings the advantages of sexuality.

Blastospores, conidiospores, chlamydospores, phialospores, antherospores, porospores (conidia produced from the pores of conidiophores) are common in Deuteromycetes. The most important species that cause plant diseases are *Septoria thespisia* (leaf spot of *Thespisia*), *Colletotrichum capsici* (fruit rot of chillies) *C.lindemuthianum* (bean anthracnose), *Helminthosporium oryzae* (sesame leaf spots of rice), *Cercospora personata*, *C. arachidicola*

(tikka leaf spot of groundnut), *Alternaria* (leaf spot), *Rhizoctonia solani* (root rot of groundnut), *Sclerotium rolfsii* (stem rot of rice) and *Fusarium oxysporum* f. *cubense* wilt of banana). *Cercospora apii* was isolated from skin lesions of human.

The class Basidiomycetes includes mushrooms, toad stools, puff balls, bracket fungi, the smuts, rusts and jelly fungi and stink horns. These produce their spores (basidiospores) usually on the outside of the spore producing body called basidium and hence the name Basidiomycetes. Basidiospores are uninucleate and haploid.

The subclass Heterobasidiomycetidae includes jelly fungi, rusts and smuts. Jelly fungi are so called due to the jelly like fruiting bodies (some are waxy and cartilaginous). Some species of *Tremella* is used as food by Chinese people *Septobasidium* parasitizes scale insects. The wheat rust *Puccinia graminis*, Cumbu rust *Puccinia penniseti*, the bean rust *Uromyces appendiculatus* are devastating diseases. Uredia producing uredospores and Telia producing teleutospores are common in rusts. *Puccinia graminis*, the cereal rust has an alternate host barberry in which aecial stage occurs but uredial and telial stages occur in wheat.

The smuts are parasites and produce black dusty spore mass resembling soot or smut. *Tilletia caries* (bunt of wheat) *Uromyces maydis* (corn smut) *Ustilago scitamina* (sugarcane smut), *Sphacelotheca sorghi* (sorghum smut) are very common diseases.

The subclass Homobasidiomycetidae includes mushroom, shelf fungi, coral fungi, puff balls, earthstones, stink horns and bird's nest fungi. *Exobasidium* attacks flowering plants causes abnormal swelling of host tissues. *Poria*, *Fomes*, *Polyporus* and *Ganoderma* are wood rotting fungi. *Agaricus* and *Pleurotus* are edible mushrooms. *Amanita* is poisonous. *A. muscaria*, fly mushroom is used as an insecticide. The species of *Glomus* and *Acaulospora* are mycorrhizal fungi exploited as biofertilizers.

Outline classification of Fungi by Alexopoulos

The fungi are classified with plants under the kingdom Plantae and division Mycota.

Order	:	Acrasiales
Order	:	Labyrinthulales
Division	:	Mycota
Sub-division	:	Myxomycotina
Class	:	Myxomycetes
Sub division	:	Eumycotina
Class	:	Chytridiomycetes
Class	:	Hyphochytridiomycetes
Order	:	Hyphochytriales
Class	:	Oomycetes
Order	:	Saprolegniales Leptomitales Lagenidiales Peronosporales
Class	:	Zygomycetes
Order	:	Mucorales Entomophthorales Zoopagales
Class	:	Trichomycetes
Class	:	Ascomycetes
Subclass	:	Hemiascomycetidae Euascomycetidae Loculoascomycetidae

Subclass : Hemiascomycetidae

Order : Endomycetales
 Ascoidaceae
 Endomycetaceae
 Spermothoraceae
 Saccharomycetaceae
 Taphrinales

Sub class : Euascomycetidae

Series : Plectomycetes

Order : Eurotiales
 Ascosphaeriaceae
 Gymnoascaceae
 Microascales
 Eurotiaceae
 Ophiostomataceae
 Onygenales

Series : Pyrenomycetes

Order : Erysiphales Erysiphaceae
 Meliolales Meliolaceae
 Chaetomiales Chaetomiaceae
 Clavicipitales Clavicipitaceae
 Sphaeriales Sordariaceae,
 Diaporthales Phyllochoraceae
 Diatrypaceae,
 Xylariaceae
 Hypocreales Nectriaceae,
 Coryneliales Hypocreaceae
 Hyphomycetaceae,
 Melamosporaceae
 Coronophorales

Series : Discomycetes

Order : Ostrapales
 Helotiales Phacediaceae
 Sclerotiniaceae
 Perizales Geoglossaceae
 Cyltariaceae
 Tuberales Sarcocyphaceae
 Pezizaceae
 Helvellaceae

Series : Labubenomycetes

Order : Laboulbeniales

Subclass : Loculoascomyctidae

Order : Myriangiales Elsinoeaceae
 Myriangiaceae
 Peiedraiaceae
 Dothideales Dothideaceae
 Pseudosphaeriaceae
 Capriodiaceae
 Order : Pleosporales Venturiaceae
 Pleosporaceae
 Lophiostomataceae
 Microthyriales
 Hysteriales

Form class : Deuteromycetes

Form order : Sphaeropsidales Sphaeropsidaceae
 Zythiaceae
 Melanconiales Melanconiaceae
 Moniliales Gyptococcaceae
 Moniliaceae
 Dematiaceae
 Stilbellaceae
 Tubercularioceae
 Mycelia sterilia

Class	:	Basidiomycetes
Sub class	:	Heterobasidiomycetes
Order	:	Tremellales Ceratobasidiaceae Tulasnellaceae Sirobasidiaceae Dacrymycetaceae Tremellaceae Hyalvriaceae Phleogenaceae Aurocularvaceae Septolasidiaceae Uredinales Puccianiceae Melampsoraceae Coleosporiaceae Ustilanginales Ustilaginaceae Tilletiaceae Graphiolaceae
Family	:	Sporobolomycetacea
Sub class	:	Homobasidiomycetes
Order	:	Exobasidiales
Series	:	Hymenomycetes
Order	:	Polyporales Thelephoraceae Clavariaceae Cantherallaceae Hydnaceae Meruliaceae Polyporaceae Agaricales Agariaceae Boletaceae Paxiellaceae Russalaceae Hygrophoraceae

Series	:	Gasteromycetes
Order	:	Hymenogastrales Lycoperadales Sclerodermatales Sclerodermataceae Astracaceae Tulostomataceae Calostomataceae Phallales Nidulariales Nidulariaceae

Algae

The algae also known as sea weeds, pond scrubs, frog spittle and water mosses (L.alga = sea weeds Gr.phykos – sea weeds) are studied under algology (Gr.phykology phykos-sea weeds+logos- discourse or study. They are simple plants with autotrophic nutrition. They are chlorophyll bearing organisms (and their colourless relatives) which are thalloid and have not differentiated as roots, stems and leaves.

Their occurrence is universal and found in all kinds of habitats in fresh as well as sea waters, soil, on within other plants and even animals, rock stones, in desert sand and snow fields. Based on their habitat they are classified as aquatic growing in fresh and brackish water, terrestrial (edaphophytes), on rocks and stones (lithophytic), halophytic growing in water of high concentrations of salinity, psammon algae of sandy beaches, thermal algae of hot waters near hot springs, cryophytic algae (on ice or snow) forming different colours of the snow. Epiphytic algae occurring within the cells of other plants, Epizoic and endozoic occurring on shells of molluscs, nose of fishes and within *Paramecium*, *Hydra* and molluscs. There are also algae parasitic on plants and animals. Planktonic algae, shell bearing algae and alga of lichens are also algae.

The thallus in alga varies from (1) motile unicellular (*Chlamydomonas*; *Phacus*), (2) motile colonial (*Volvox*; *Eudorina*), (3) Palmelloid (*Chlamydomonas*; *Palmella* dendroid (*Prasinodadus*), Coccoid (*Chlorella*), filamentous (*Spirogyra*; *Nostoc*), heterotrichous (*Fritschella*), siphonaceous (*Botrydium*) uniaxial (*Batrachospermum*), multiaxial (*Polysiphonia*) and parenchymatous (Sargassam).

The size is microscopic (0.5 mm in dia in *Chlamydomonas* to as large as 30 m or even more (*Macrocystis*). Each cell is found by a typical cell wall in all cases except in *Euglena* and *Gymnodinium* where the cytoplasmic membrane called pellicle is present. The cell wall is bilayered composed mainly of cellulose and pectin with substances like pectin, chitin, algin and fucoidon. In a few alga the wall is fortified with calcium, silica and magnesium carbonate.

The motile member, zoospore and gametes of many algae bear flagella consisting of 2 central tubules surrounded by 9 peripheral tubules enclosed in a membrane. Flagella may be equal or unequal in length, inserted apically or laterally and may be tinsel or whiplash.

The cytoplasm contains contractile vacuoles, mitochondria, eyespot, chloroplast, nucleus, pyrenoids, chondriosomes and Golgi bodies. In the prokaryotic blue green algae these are absent.

The pigments in the alage vary with the group. They are chlorophyll a, chlorophyll b, β -carotene and xanthophylls. Phycobilins are present in Rhodophyceae and blue green algae.

The food reserve is in the form of starch. But fats and oils are also present in certain groups. Laminarin and mannitol are in phaeophyceae, Floridian starch, floridoside and mannoglycerate are the chief reserve products in Rhodophyceae.

The reproduction in alga is by (1) vegetative (2) asexual and (3) sexual process. The vegetative propagation is fragmentation, fission, akinete formation, tuber, hormogonia and formation of adventitious thalli.

Asexual reproduction takes place by release of protoplasts in the form of zoospores, synzoospore, aplanospore, hypnospore, autospore, auxospore, carpospore, tetraspore, cyst, etc., These germinate into a new plant. Sexual reproduction takes place by the union of cytoplasm and nuclear material of two gametes of two organisms of the same species on three different ways viz., (1) Isogamy (fusion of morphologically similar gametes) (2) Anisogamy (fusion of morphologically dissimilar gametes) and (3) Oogamy (female is immobile and the male gamete is small and motile).

The algae are important as food (*Chlorella*, *Scenedesmus*, *Laminaria*, *Spirulina*) as medicine (*Chlorella*) as nitrogen fixing fertilizer (blue green algae) as fodder (*Laminaria*, *Sargassum* and *Ficus*) and in the industrial preparations. Agar-agar is produced from *Gelidium* and *Gracillaria*. Iodine is from *Laminaria* and *Macrocystis*, carragenin (from *Chondrus crispus*), alginic acid from *Laminaria* and filter aids (diatomaceous earth) from diatom are industrially important products.

The widely accepted Fritsch classification is outlined below. This algae were divided to (XI) classes (= phyceae) including the prokaryotic blue green algae which is grouped in class Myxophyceae.

Outline Classification of Alage

Myxophyceae	Blue green algae	Chlorophyll a
or Cyanophyceae	Prokaryotic	B-carotene
	Eg. <i>Mosboe</i>	flavicin
	<i>Scytonema</i>	c-phycoyamin
	<i>Anabaena</i>	c-phycoerythrin

Chlorophyceae	Green algae Eg. Chlamydomonas Volvox Chlorella	Chlorophyll a Chlorophyll b α and β carotene Xanthophyls
Xanthophyceae	Yellow green algae Chloramoela Heterochloros Chlorothecaum	Xanthophyls Chlorophyll a, Chlorophyll c, β -carotene
Bacillariophyceae	Diatoms Synedra Baccillaria Surinella	Chlorophyll a Chlorophyll c β -carotene fucoxanthin
Phaeophyceae	Brown algae Macrocystis Ectocarpus	Chlorophyll a Chlorophyll c, β -carotene violaxanthine fucoxanthum
Rhodophyceae	Red algae	Chlorophyll a Chlorophyll b xanthophyll γ -phycoeyarin γ -phycoerythrin

Self evaluation

1. Give an account of biodiversity of microorganisms.
2. Give an outline classification of bacteria.
3. Write briefly on mycoplasma.
4. Give an account of spore forming bacteria.
5. Mention five important human pathogens and indicate their taxonomic grouping.
6. Describe briefly key characteristics of fungi considered for their classification.
7. Give an account of somatic characteristics of fungi.
8. Describe briefly the asexual propagation in fungi.
9. Describe the sexual reproduction in fungi.
10. Give an outline classification of fungi.
11. Indicate the significance of smuts and rusts.
12. Mention five important plant pathogens and indicate their taxonomic grouping.
13. Give an outline classification of algae.
14. Give a brief description of sea weeds.
15. Illustrates different types of spores in fungi.

EXERCISE

Points to remember

1. Biodiversity of the organisms
2. Different types of organisms their respective taxonomical positions

MICROBIOLOGY OF AIR

The air contains gases, dust particles, dried vapor droplets, in addition to these, air also contains more number of microorganisms. The air has vegetative cells and spores of bacteria, fungi and algae and protozoan cyst. In the atmosphere, air mainly acts as dispersal or transport medium for microorganisms. When compared to soil or water, air contains less numbers of microorganisms. The microbiology of air can be studied under two headings such as outdoor and indoor microflora.

Outdoor microflora

The air in the atmosphere, which is found outside the buildings, is referred to as **outside air**. The dominant microflora of outside air are fungi. The two common genera of fungi are *Cladosporium* and *Sporobolomyces*. Besides these two genera, other genera found in air are *Aspergillus*, *Alternaria*, *Phytophthora* and *Erysiphe*. The outdoor air also contains basidiospores, ascospores of yeast, fragments of mycelium and conidia of molds.

Among the bacterial genera *Bacillus* and *Clostridium*, *Sarcina*, *Micrococcus*, *Corynebacterium* and *Achromobacter* are widely found in the outside air. The number and kind of microorganisms may vary from place to place, depending upon the human population densities.

Indoor microflora

The air found inside the building is referred to as Indoor air. The commonest genera of fungi in indoor air are *Penicillium*, *Aspergillus*. The commonest genera of bacteria found in indoor air are *Staphylococci*, *Bacillus* and *Clostridium*. In case of occupants being infected,

Atmospheric air composition

The atmosphere consists of a mixture of gases and variable quantities of water and solid particles. According to Landsberg, air has the following composition:

Element	Volume / percentage
Nitrogen	78.03
Oxygen	20.99
Argon	0.94
Carbondioxide	0.03
Hydrogen	0.01
Neon	traces
Helium	traces
Xenon	traces
Ozone	very variable
Water vapour	very variable
Dust	very variable

The composition shows slight variations with latitude and to a lesser extent with altitude. The ozone owes its existence in the atmosphere to photosynthesis from oxygen under the influence of solar ultraviolet radiations.

Air-quality

The air should be free from pathogenic microorganisms. As per the definition of the World Health Organization air pollution is a "situation which are harmful to people or their environment. The Central Pollution Board, New Delhi has fixed standard for ambient air quality in India under the Air Act, 1981, beyond which an ambient air can be considered polluted in a legal sense.

Ambient air quality standards in India (Concentration μgm^{-3})

Area category	Suspended particulate matters	SO ₂	CO	No
Industrial and mixed	500	120	5000	120
Residential and rural	200	80	2000	50
	100	30	1000	30

The Air Act prescribes emission standards. Thus the air quality standard is a limit on the amount of a given pollutant permitted in the air around us.

Enumeration and assessment of microorganisms in air

There are several methods adopted to enumerate microorganisms in air. They require special devices and design. The most important methods are solid and liquid impingement devices, filtration, sedimentation, centrifugation, electrostatic precipitation. However, none of these devices collects and counts all the microorganisms in the air samples. In general, to assess the microorganisms in air, following methods are adopted.

Impingement in liquids

In this method, the air drawn is through a very small openings or a capillary tube bubbled through the liquid. The organisms get trapped in the liquid medium. Aliquots of the liquid are then plated with medium to determine its microbial content. In this method, living cells which are present in air can be enumerated.

Impingement on solids

In this method, microorganisms are collected or impinged directly on the solid surface of agar medium by gravitational force.

Colonies develop on the surface of the medium after few days of incubation. Several devices are used; of which settling plate technique is the simplest. In this method, cover of the petridish containing agar medium is removed, and the agar surface is exposed to the air sample for several minutes. A certain number of colonies develop on incubation of the petridish. Since the technique does not record the actual volume of sampled, it gives only approximate estimate of microorganisms. However, it gives information about the kind and number of organisms in a particular area.

Sources of contamination

The major sources of contamination in the air are, automobile exhausts like incomplete combustion of fuel, agricultural sources like spraying of pesticides and insecticides, hospitals, industries like tannery industries, distilleries, nuclear power plants and chemical industries. All these industries provide smoke into the air. The smoke comprises of dust particles from the combustion of coal and oil. Automobile engines driven by petrol, produce many different substances in its exhaust. Some of these substances are serious air pollutants. Air pollutants are Co, CO₂, unburnt hydrocarbon NO₂, SO₂ and lead from leaded petrol.

MICROBIOLOGY OF WATER

The drinking water of most communities and municipalities is obtained from surface sources - rivers, streams and lakes. Such natural water supplies are likely to be polluted with domestic and industrial water. Many city dwellers (whose water comes from the rivers) are not aware that a considerable portion of their drinking water may have been used earlier for domestic and industrial purposes. Water is used for bathing, washing clothes, washing utensils and flushing toilets. The domestic water consumption may vary with the availability of water. Most of the water taken into the houses may be returned as waste water through drainage system. All these waste waters contain organic and inorganic waste as suspended or dissolved matter. In addition, these waste waters contain microorganisms, including those of faecal origin and pathogenic nature. As a potential of pathogenic organisms, water can be in danger to health and life. The pathogens most frequently transmitted through water are those which cause infections of the intestinal tract, namely, typhoid and paratyphoid bacteria, dysentery (Bacillary) and cholera bacteria and viruses. The causative organisms of these diseases are present in the feces or urine of an infected person.

Distribution of microorganisms in aquatic environment

Microorganisms occur in all depths. The surface film and bottom sediments have a high concentration of microorganisms. Drifting microbial life of aquatic environment is called *Plankton*. It is composed of Phytoplankton eg. Algae and Zooplankton. The bottom region of the body of water harbours largest number and kinds of microorganisms called benthic microorganisms.

The movement of water by wind, tide and currents affect the distribution of microorganisms up welling occurs in oceans. It is a process in which the bottom water carries with it a rich supply of nutrients and delivers it to the surface region.

Aquatic microorganisms in ponds and lakes

The zonation and stratification of lakes and ponds influence the occurrence of microorganisms. Lakes and ponds of temperate region show thermal stratification, which influences the microbial population in different seasons. In spring and autumn mixing occurs resulting in massive growth of algae called bloom. Lakes and ponds enriched with nutrients show eutrophication. The common microorganisms found in fresh water are *Pseudomonas*, *Flavobacterium*, *Aeromonas* and *Alcaligenes*. Estuary is semi-enclosed coastal water body having connection with the open sea. It receives fresh water with all particulate suspensions through rivers. In areas receiving domestic wastes with organic nutrients contain the following organisms: *Coliforms*, *Faecal Streptococci*, *Bacillus*, *Clostridium*, *Thiothrix* and *Thiobacillus*. Soil bacteria such as *Azotobacter*, *Nitrosomonas* and *Nitrobacter* are also found in water. Very few fungal organisms from the classes *Ascomycetes* *Phycomycetes* and *Fungi-imperfecti* are also present in water.

Aquatic microorganisms in the sea

The sea is the largest natural environment inhabited by microbes. Bacteria, algae, protozoa, molds and yeast are major groups of microorganisms found in the sea. The number of microorganisms is more in coastal waters and it gradually decreases in the open sea.

In sea, phytoplanktons form group of microorganisms which convert radiant energy into chemical energy and which support the entire population of fishes eg. *Diatoms*, *Cyanobacteria*, *Dinoflagellates*, *Chrysomonads* and *Chlamydomonas*.

Importance of aquatic microbes

Aquatic microorganisms, both plants and animals, interact among themselves and between microorganisms. Algae, protozoa and other phytoplankton play key role in the food chain in water and certain organisms perform photosynthesis. They are called primary producers in an aquatic ecosystem. Bacteria and other fungal organisms also play an important role in biogeochemical transformation in soil.

Sources of water pollution

The only source of potable fresh water nature provides is the rain water. This itself gets polluted by the action of nature, as water falls on land and runs as stream, gathering different types of minerals and suspended particles. In this running water, due to anthropogenic activities, more minerals, more chemicals and organic materials are added, that makes the water polluted. There are three main sources of pollution of waters.

1. Sewage or municipal effluents (or) domestic effluents
2. Industrial effluents
3. Agricultural pollution

1. Sewage or Municipal effluents

The quality of water after bath, kitchen work, washing of clothes and animals etc. a large volume of raw sewage discharged into the main stream pollute the river waters. Among the various sources of water pollution, sewage, the domestic waste containing decomposing organic matter is the major source and it accounts for 70 per cent of water pollution. Industrial effluents account for 15 percent of water pollution.

2. Industrial effluents

The industrial effluents are classified under the following heads.

I. Food and drink manufacturing industries

- a. Distilleries and sugar factories.
- b. Food processing units.
- c. Soap and oil manufacturing units.

II. Chemical Industries

- a. Fertilizer and Chemicals, paints
- b. Drug and pharmaceuticals
- c. Insecticides and pesticides

III. Engineering Industries

- a. Metallurgical industries.
- b. Wire making industries.
- c. Rare earths and minerals.

IV. Other industries producing organic effluents

- a. Paper and rayon
- b. Rubber industries
- c. Textiles
- d. Plywood and hardboard industries
- e. Tanneries and leather industries

Potable and contaminated water

Water free from disease causing organisms and free from harmful chemicals is known as *Potable water*. Water contaminated with sewage, domestic or industrial waste with chemicals and pathogenic microorganisms is termed as *contaminated water* or polluted water.

Standards for water

The characteristics of water from various sources depend on rain, nature of substratum on which it is in contact and the effect of other substances added to it. The chemical analysis of water is necessary to ascertain the quality of water. For human consumption, potable water should be used. Any water can be called as potable water, if it is free from undesirable odour, flavour and contains no bacteria capable of producing diseases in man.

Standards for potable water

Sl. No.	Observation	Tolerance	Standard
1.	Appearance	-	Free from any insoluble matter
2.	Colour	-	Colourless (hyaline)
3.	Odour	-	Nil
4.	Taste	-	Nil
5.	pH	6.7-7.9	7.0
6.	Hardness	Carbonate less than 200 ppm	100-150 ppm
7.	Nitrate Nitrogen	Nil	Nil
8.	Chlorides	less than 25 ppm	Nil
9.	Sulphate	Nil	almost Nil
10.	Free ammonia	less than 1.00 ppm	Nil
11.	Fluoride	1.0 ppm	Nil
12.	Coliform organism	less than 5.0% of the samples	Nil
13.	Dissolved oxygen	not less than 6 mg / litre	10 mg
14.	BOD	not more than 5 mg / litre	Nil

Indicator organisms

It is almost impossible to isolate from water the organisms responsible for water-borne diseases. Few organisms are present and they do not multiply in water. The only safe method to prevent water-borne disease is to condemn fecally polluted water as being unfit for human use, as it may contain harmful organisms. Fecal pollution can be determined by examination of water for colon bacilli (*E.coli*). *E.coli* is abundant in feces and not found outside intestinal tract in nature. The *E.coli* in water indicates the presence of pathogenic microorganisms in water, which may be responsible for a number of water-borne diseases. Hence, *E.coli* is known as indicator organism. Water also contains bacteria that resemble *E.coli* but may or may not be of fecal origin. These bacteria also ferment lactose with formation of gas like *E.coli*. The other indicator organisms are *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus bovis*, *Streptococcus equinus* etc., and *Clostridium perfringens*.

EXERCISE (FOR CHAPTERS 9 AND 10)

Points to remember

1. Presence of different kinds of microbes in air
2. Presence of pathogenic microbes in water
3. Know the various sources of contamination of water

Self evaluation

1. What are the outdoor and indoor microflora of air?
2. What is the composition of atmospheric air?
3. What are the standards for good quality of air?
4. What are the methods adopted to enumerate the microorganisms in air?
5. Explain various sources of contamination of water
6. Describe the ways in which microorganism are distributed in water
7. Define pollution
8. Classify industrial effluents
9. Define potable water and contaminated water
10. State the standards for potable water

MICROBIOLOGY OF FOOD

Food is an indispensable item for all living organisms. All food items are associated with microorganisms in one form or other. Foods get contaminated during handling, harvest, transport and storage. Foods also get contaminated due to the methods of food collection, cooking and preparation. Food forms an ideal culture medium for the growth and multiplication of microorganisms. Some of the microorganisms particularly pathogenic forms causing dreadful diseases and food poisoning by their secretions. There are some microorganisms, which are useful for the preparation of different types of food and beverages. The microorganisms themselves play an important role in formation of food (eg.) single cell protein and mushrooms.

Common food items

Foods may be classified as a) fresh foods, b) preserved foods, c) canned foods, d) processed foods, e) fermented food products.

Common food items as follows:

Fruits and vegetables

Milk

egg

Meat

Fish

Poultry

Bread

Pickles

Syrup and juices

Products from milk, vegetables and fruits

Sources of microorganisms in foods

Foods receive the population of microorganisms from soil, plants, cooking vessels, by the use of contaminated water for washing and cooking and also due to unhygienic habitats of food handlers, intestinal tract of humans and animals, animal feeds, animal hides, air and dust.

Factors that influence the growth of the microorganisms

Many factors that influence the growth of the microorganisms in food. Some of the factors are intrinsic and some others are extrinsic.

- a) Intrinsic factors: The intrinsic factors include pH, moisture content, oxidation-reduction potential, nutrient status, antimicrobial constituents and biological structures.
- a) Intrinsic factors: The intrinsic factors include pH, moisture content, oxidation-reduction potential, nutrient status, antimicrobial constituents and biological structures.
 - i) pH : It has been well established that most of the microorganisms grow best at pH values around 7.0, while few grow below 4.0. Bacteria grow at more pH than molds and yeasts.
 - ii) Moisture content: The preservation of foods by drying is a direct consequence of removal of moisture, without which microorganisms do not grow. The water requirements of microorganisms should be defined in terms of the water activity (a_w) in the environment. Water activity is defined by the ratio of the water vapour pressure of food substrate to the vapour pressure of pure water at the same temperature ($a_w = P/p_0$). The a_w of most fresh food is above 0.99. The minimum value of a_w for the growth of the microorganisms in foods should be around 0.86.
 - iii) Oxidation reduction potential: The O/R potential of a substrate may be defined generally as the ease with substrate

gains electrons. When an element or compound loses electrons, the substrate is said to be oxidized, while a substrate that gains electrons becomes reduced.

- iv) **Nutrient content:** In order to grow and function normally, the microorganisms of importance in foods require water, source of energy, source of nitrogen, vitamins and related growth factors and minerals.
- v) **Antimicrobial constituents:** The stability of foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity. Some species contain essential oils that possess antimicrobial activity. Among these are eugenol in cloves, alliin in garlic, cinnamic aldehyde and eugenol in cinamon.
- b) **Extrinsic factors:** These include those properties of the storage environment that affect both the foods and their microorganism. The following extrinsic factors affect the growth of microorganisms: Storage temperature, pH, presence and concentration of gases in the environment.

Causes of food spoilage

Food spoilage refers to the process where the food is made useless, bad and unfit for eating. It alters the chemical proportion appearance, texture, colour, taste, flavour, odour and stability of the food. The altered food is called spoiled food. Food is spoiled by many factors such as a) microorganisms, b) insects, c) rough handling, d) transport, e) improper storage, f) enzyme activity, g) unhygienic conditions.

Causes of food poisoning

Food poisoning refers to the toxicity introduced into food by microorganism and their products. Food poisoning is caused by various factors as follows:

- a) Poisons derived from plant and animal sources.

- b) Such standard chemicals added to the food.
- c) Excess use of preservation in food.
- d) Presence of higher population of microorganisms in food
- e) Toxins produced by various types of microorganisms.

Types of food poisoning

There are two types of food poisoning.

- 1) Food intoxication. eg) Botulism, Staphylococcal food poisoning.
- 2) Food infection. eg) Shigellosis (Bacillary dysentery), Enteropathogen Escherichia, Cholera, Brucellosis.

Food borne diseases

The common food borne diseases are Botulism, Staphylococcal food poisoning, enterococcus food poisoning, Traveller's diarrhoea, Mycotoxicosis, Sligellosis, Enteropathogenic Escherichia, Cholera, Brucellosis, Tuberculosis and Tularemia.

i) Botulism : Botulism is a food borne disease due to exotoxin produced by the bacterium *Clostridium botulinum*. The main sources of this disease are canned food and preserved foods. This disease affects the nervous system so it is called neurotoxin.

ii) Staphylococcal food poisoning or staphylococcal enterotoxemia : The causative organism for the disease is *Staphylococcus aureus*. The main sources for the disease are potato salad, cream-filled bakery goods and dry skim milk. The disease is characterized by sudden nausea, vomiting and diarrhoea.

iii) Enterococcus food poisoning : The causative organism for this disease is *Streptococcus faecalis*. It is frequently found in the intestinal tract of human and animal. The disease is characterized by nausea, frequently vomiting, colicky pain and diarrhoea.

MICROBIOLOGY OF MILK

Milk is the white, fresh clean lateral secretion obtained from female cattle. Milk is used for the nourishment of their younger ones. It is in liquid form without having any colostrum. The milk contains water, fat, protein and lactose. About 80-85% of the proteins is casein protein. Due to moderate pH (6.6), good quality of nutrients, high water contents etc. make milk an excellent nutrient for the microbial growth. It is mainly the udder interior, teats surrounding environment and manual milking process make the source of contamination.

Sources of microorganisms in milk

Milk secreted into the udder is sterile. The first few strippings of milk contain more amount of bacteria and the population of bacteria gradually decreases. It is observed that last strippings of milk from the udder seems to be free from bacteria. This clearly indicates that most of the microorganisms found in the milk are from external source. The different sources of microorganism in milk are from 1) the udder of the cow, 2) skin of the cow, 3) utensils and equipment, 4) feeds, 5) air of the cow shed, 6) milking persons and 7) water.

1) *Udder of the cow* : The milk producing animals should be kept neat and clean. More care should be taken to keep the flanks, udder and teats clean. The interior of the teats of the udder is warm and contains the last remains of the milk which has more microbes which would have entered through opening of teat and multiplied.

2) *Skin of the cow* : Soil, faeces and dirt adhere to the skin and hairs of the cow. Hair, dirt and dust fall in to milking utensils or into the teat cups of milking machines. Most of the organisms from these

sources are gas producers and putrefactive types. Faeces contain enormous quantity of organisms and most of them are pathogenic microorganisms.

3) *Utensils and equipments* : Milking utensils and equipments are the major sources of contamination of milk. They have to be washed properly with detergent. Further the utensils and equipments should be cleaned with hot water, air and steam to remove all the spore forming, fluorescent and coliform microorganisms.

4) *Feeds* : Microorganisms are found everywhere. They are present in abundant in vegetation and soil. Dry feeds have more amount of bacteria and less amount of fungi. These organisms contaminate the milk.

5) *Air of the cow shed* : The air of the cow shed is greatly contaminated by dry dirt and dust. During the mixing of feeds and during the cleaning process of the floor, the air of the cow shed is highly contaminated and it is passed on to the milk.

6) *Milking persons* : Pathogenic microorganisms may enter into the milk through milking persons. They should wear clean clothes and properly wash their hands before milking. Nails should be cleaned and trimmed. Discharge from sneezing, coughing and nose blowing should not reach the atmosphere, equipment or the milk. Some of the organisms may be carriers of diseases.

7) *Water* : Pure water should be used for cleaning purposes. Water exposed to contamination spreads the microorganisms. Water should be free from coliform organisms. Chlorination of water prevents such contamination.

Microbiological standard and grading of milk

The Indian Standard Institute (ISI) has prescribed microbiological standard for quality of milk.

1. Coliform count in *raw* milk is satisfactory if coliforms are absent in 1:100 dilution.

- Coliform count in pasteurized milk is satisfactory if coliforms are absent in 1:10 dilution.

Table. Microbiological quality of milk

	Grade	Methylene blue reduction test in hrs.	Total plate count/ml
1.	Very good	5 & above	Not exceeding 0.2 million
2.	Good	3 - 4	Between 0.2 to 1.0 million
3.	Fair	1 - 2	Between 1-5 million
4.	Very poor	0 . 5	Over 5 million

Grading of milk

The quality of milk is judged by certain standards and it is known as grading milk. Grading of milk is based upon regulations pertaining to production, processing and distribution. This includes sanitation pasteurization holding conditions and microbiological standards. The U.S Public Health Secrine Publication 'Milk Ordinance and code' shows the following chemical, bacteriological and temperature standards for grade A milk and milk products.

Product	Temperature	Bacterial count/ml	Chemical and others
1. Grade A raw milk for pasteurization	Cooled to 50°F and maintained there at until processed.	Individual producer milk should not exceed 100,000/ml prior to combining with other produce of milk	Antibiotics should be less than 0.05 unit/ml
2. Grade A pasteurized milk products	Cooled to 45°F or less	Milk and milk products 20,000/ml, coliform limit not exceeding 10/ml	Phosphates less than 1 mg/ml

EXERCISE (FOR CHAPTERS 11 AND 12)

Points to remember

- Know the organisms causing food spoilage
- Understand the spoilage of milk
- Know the standards for milk

Self evaluation

- What are the important food items?
- Explain the factors that influence the growth of the microorganism on food
- What are the causes of food spoilage?
- Explain the different sources of microorganisms in milk
- What are the standards for good quality of milk?

MICROBIOLOGY OF SOIL

The term soil refers to the outer, loose materials of earth surface. Agriculturally, it is the region supporting plant life and from which plants obtain their mechanical support and required nutrients. The soil contains multitude of organic substances. The soil environment is unique in several ways: it contains bacteria, fungi, actinomycetes, algae and protozoa. It is one of the most dynamic sites of biological interactions in nature. Soil is the region in which occur many of the biochemical reactions concerned in the destruction of organic matter, in the weathering of rocks and in the nutrition's of crops.

The soil is composed of five major components: mineral matter, water, air, organic matter and living organisms. The quantity of these constituents is not the same in all soils but varies with the locality. The inorganic portion of the soil, because its influence on nutrient availability, aeration, and water retention has a marked effect on the microbial inhabitants. The soil is not a dead inert material. Actually it is full of life. One gram of soil contains approximately one million microorganisms. Man depends upon the soil for his food. The soil depends upon the microorganisms for its fertility. The soil is not a static medium. The soil is a tremendous growth medium. The soil has organic matter soil solution and soil air. All these components are affected by the activities of microorganisms. So the soil is constantly changing medium. The soil solution in agricultural soil has ions like K^+ , Na^+ , Mg^{++} , Ca^{++} , Fe^+ , S^- , NO_3^- , SO_4^- , PO_4 and others. These ions are very essential in culture media. In a fertile soil, these elements in mineral form are supplemented by organic compounds derived from the decomposition of animal and plant residues. Thus the soil is an *excellent natural medium* for microorganisms.

Distribution of different types of soil microorganisms

Soils contain five major groups of microorganisms. They are bacteria, actinomycetes, fungi, algae and protozoa. Among the soil microorganisms, bacteria are most dominant group of organisms. All kinds of bacteria are found in the soil. This is because all kinds of organic refuse are disposed off on the soil. Many of the soil bacteria perform useful functions like decomposition of organic matter, conversion of soil constituents into useful materials, production of antibiotics in the soil, and biogeochemical cycling of elements like carbon, nitrogen, phosphorus, iron, sulfur and manganese.

The bacterial population of the soil exceeds the population of all other groups of microorganisms in both number and variety. Direct microscopic counts as high as several billions bacteria per a gram of soil have been reported.

The actinomycetes population as many as millions per gram of soil is present. The most predominant genera present in the soil are *Nocardia*, *Streptomyces* and *Micromonospora*. These organisms are responsible for the characteristic musty or earthy odour soon after the rainfall. This is due to sporulation of actinomycetes. Actinomycetes are capable of degrading many complex organic substances and consequently play an important role in building soil fertility. The actinomycetes have ability to synthesize and excrete antibiotics. Most of the antibiotics are produced by actinomycetes. The presence of antibiotic substances in soil can be detected with great difficulty.

The fungal population ranging from thousands to hundred thousands per gram of soil has been reported. They are aerobic in nature and found more numbers near the earth surface. They exist in the atmosphere as mycelial and spore stage. Fungi are active in decomposing the major constituents of plant tissues, namely, cellulose, hemicellulose, lignin and pectin.

The population of algae in soil is very smaller than that of either bacteria or fungi. The major types present in the soil are the green

algae and diatoms. Their photosynthetic nature accounts for their predominance on the surface or just below the surface layer of soil. In a fertile soil biochemical activities of algae are masked by bacteria and fungi. In certain conditions, algae perform prominent and beneficial changes. For example, on barren and eroded lands they may initiate the accumulation of organic matter because of their ability to carry out photosynthesis and other metabolic activities.

Many soil protozoa are flagellates or amoebas; the population per gram soil ranges from a few hundred to several thousand in moist soils rich in organic matter. Protozoa are of significance since their dominant mode of nutrition involves ingestion of bacteria.

Factors that influencing microbial population

Factors that influencing microbial population include 1) Soil moisture, 2) Aeration, 3) Temperature, 4) pH and 5) Organic and inorganic nutrient supply. In addition to this, cultivation, ploughing, season and depth of soil also influence microbial population in soil.

Soil moisture : Soil moisture governs microbial activity in two ways. Since water is the major component of protoplasm, an adequate supply must be available for vegetative growth and multiplication. But, where moisture becomes excessive, microbial proliferation is suppressed because the over supply of water limits gaseous exchange and lowers the available oxygen supply, creating an anaerobic environment. Moisture is present in the form of film in soil pores. The amount of water increases with increase in porosity of soil. Soil moisture is affected through irrigation, drainage or management practices.

Aeration : The air is essential for the growth of the aerobic organisms. The water logging condition brings about a decrease in the abundance of aerobic organisms. The change from an aerobic to a largely anaerobic flora is effected by the disappearance of free oxygen as a result of its utilization by oxygen-requiring microorganisms, so that only microorganisms tolerant of low oxygen levels complete anaerobiosis are capable of proliferation.

Temperature : Temperature governs all biological processes and it is thus prime factor of concern to the microorganisms. Each microorganism has an optimum temperature for growth. Most microorganisms are mesophilic that can able to grow between 25-35°C. Certain species develop best at temperature below 20°C and they are termed as psychrophiles. Thermophilic microorganisms that grow readily at temperatures of 45°C to 65°C.

pH : The neutral pH is favourable for many types of microorganisms. Highly acidic or alkaline conditions tend to inhibit many common microbes. The greater hydrogen ion concentration, the smaller is the size of the microbial community. Soil-borne fungi are sensitive to high pH.

Organic and inorganic nutrients : These organic and inorganic nutrients are very important for microorganisms as these provide nutrition for growth, activity and survival of microorganisms in soil. The chemical factors are gases, acids, micro and macro elements and clay minerals etc. In the soilsolution, gases and microorganisms are dissolved. However, the dissolved components are in constantly shifting equilibrium with the solid phase. The dead organic materials of plant and animal origin serve as total organic matter, which later is subjected to microbial colonization and decomposition. However, due to incorporation of green manures, crop residues etc., in soil, the community size of microorganisms gets increased. At the same time application of these organic matter alters the composition of soil microflora, microfauna and relative dominance of antagonistic microorganisms. The types of vegetation and its growth stages of plant dominate one or more groups of soil microorganisms. Increased population of microorganisms can be found in the rhizosphere region according to season, growth stages and abundant availability of nutrients.

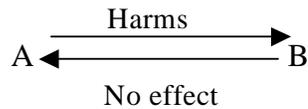
Harmful microbial interactions

Harmful microbial interaction is otherwise described as negative interaction or antagonistic interaction. The composition of the microflora microfauna of any habitat is governed by the biological balance created through interactions and associations of all individuals present

present in a community. Any inhibitory effect of an organism created by any means to the other organisms is known as harmful interactions or antagonistic interaction and the phenomenon of this activity is called antagonism. Harmful interactions have three types. They are amensalism, competition and parasitism.

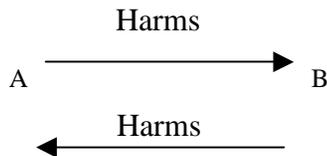
Amensalism

Amensalism is the phenomenon where one microbial species is affected by the other species, whereas the other species is unaffected by the first one. Amensalism is accomplished by secretion of inhibitory substances such as antibiotics. Certain organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances, which affect the normal growth of other organisms. Antagonistic relationships are quite common in nature. For example, *Pseudomonas aeruginosa* is antagonistic towards *Aspergillus terreus*.



Competition

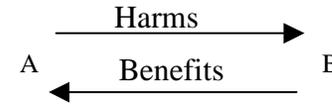
A negative association may result from competition among species for essential nutrients. In such situations the best adapted microbial species will predominate or eliminate other species which are dependent upon the same limited nutrient substance.



Parasitism

Parasitism is defined as a relationship between organisms in which one organism lives in or on another organism. The parasites feed on the cells, tissues or fluids of another organisms, the host, which is harmed

in this process. The parasite depends on the host and lives in intimate physical and metabolic contact with the host. All types of plants and animals are susceptible to attack by microbial parasites.

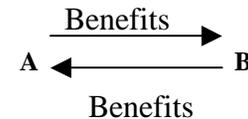


Beneficial Interactions

The beneficial interactions such as symbiosis (mutualism), proto cooperation, and commensalism are found to operate among the soil inhabitants.

Symbiosis (Mutualism)

Mutualism is an example of symbiotic relationship in which each organism benefits from the association. One type of mutualistic association is that involving the exchange of nutrients, between two species, a phenomenon called syntrophisms. Many microorganisms synthesize vitamins and amino acids in excess of their nutritional requirements. Others have a requirement for one or more of these nutrients.



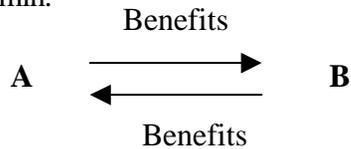
Symbiosis is an obligatory relationship between two populations that benefit both the populations. Both populations are living together for mutual benefit. The relationship between algae and fungi that result in the formation of lichens is a classical example of mutualistic intermicrobial relationship. Lichens are composed of primary producer, the phycosymbiont (algae) and a consumer the mycosymbiont (fungus).

Proto-cooperation

It is an association of mutual benefit between two populations, but not obligatory and only complementary. Both population are

capable of surviving in their natural environment on their own, although when offered, the association offers some mutual advantages.

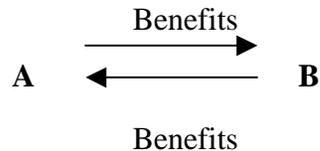
For an example, the mixed culture of *Proteus vulgaris* (produce biotin/requiring nicotinic acid) and *Bacillus polymyxa* (produce nicotinic acid/ requiring biotin). Both grow as partner bacterium synthesizes the missing vitamin.



Commensalism

In a commercial relationship between two microbial populations, one population is benefited and other population remains unaffected. Commensalism is a unidirectional relationship between two populations. The unaffected population does not benefit by the action of second population. For receiving population, the benefit provided may be essential.

In commensalism, the unaffected population modifies the habitat in such a way that another population benefits. For example, a population of facultative anaerobes utilizes oxygen and creates a habitat suitable for the growth of anaerobes. In soil, vitamin and growth factors producing organisms benefit vitamin and growth factors requiring organisms.



Rhizosphere

The region which is adjacent to the root system is called rhizosphere. The microbial population on and around roots system considerably higher than that of root-free soil or non-rhizosphere soil. This may be due to the availability of nutrients from plant roots in the form of root nodules, secretions, lysates mucigel and sloughed off cells.

Plant roots provide shelter to soil microbes in the rhizoplane (root surface) and endorhizosphere (inside root).

Rhizosphere effect

Bacteria predominate in rhizosphere soil and their growth is influenced by nutritional substances released from the plant tissues eg. aminoacids, vitamins and other nutrients; the growth of the plant is influenced by the products of microbial metabolism that are released into the soil. It has been reported that aminoacid - requiring bacteria exist in the rhizosphere in larger numbers than in the root-free soil. It has been demonstrated that the microflora of the rhizosphere is more active physiologically than that of non-rhizosphere soil. The rhizosphere effect improves the physiological conditions of the plant and ultimately result in higher yield. Greater rhizosphere effect is seen with bacteria (R:S ratio ranging from 10-20 times more) than with actinomycetes or fungi.

Phyllosphere

The Dutch Microbiologist Ruinen coined the term phyllosphere. The leaf surface has been termed as phylloplane and the zone on leaves inhabited by the microorganisms as phyllosphere. In forest vegetation, thick microbial epiphytic associations exist on leaves. The dominant and useful microorganisms on the leaf surfaces in the forest, vegetation happened to be nitrogen fixing bacteria such as *Beijerinckia* and *Azotobacter*. Apart from these nitrogen fixing bacteria, other genera such as *Pseudomonas*, *Pseudobacterium*, *Phytomonas* are also encountered on the leaf surface. The quantity and quality of phyllosphere organisms vary with the plant species and its morphological, physiological and environmental factors. The age of plant, its leaf spread, morphology and maturity level and the atmospheric factors greatly influence the phyllosphere microflora.

Spermosphere

The region, which is adjacent to the seed surface is termed as spermosphere. Healthy seeds carry specific bacterial flora in

respect of number and species. There are several reports in the literature on the quantity and quality of microorganisms carried by the seeds of different plant species both externally and internally. Many of the organisms are harmless some may be positively beneficial and very few may be pathogenic under certain favourable conditions. It has been shown that some organisms have beneficial effects on the germinating seed through some biological products, such as growth hormones. It has been reported that the germinating seed excretes some chemicals, which influence the quality and quantity of the microorganisms on the seed. Picci defined the region of such influence around the seed as spermosphere and the phenomenon as spermosphere effect. When the seed is sown in soil, certain interactions between the seed-borne microflora and the soil microorganisms take place, under the influence of chemicals exuded by the germinating seed.

EXERCISE

Points to remember

1. Presence of different kinds of microorganisms in soil
2. presence of useful and harmful microbes in soil

Self evaluation

1. Define soil
2. Explain the characteristics of different types of organisms present in soil
3. What the factors influencing microbial population in soil?
4. Explain harmful microbial interactions and beneficial microbial interactions with suitable example
5. Define the terms Rhizosphere, Phyllosphere and Spermosphere
6. Explain the Rhizosphere effect

MEDICAL MICROBIOLOGY

MICROBIAL DISEASES

Introduction

Thousands of different kinds of microbes are present in all ecological niches. Some are beneficial ones, others are opportunists and some are harmful ones.

Infection is the establishment of the organisms in the tissues resulting in injury or harmful effect to the host. Infections may be endogenous or exogenous. Endogenous infections are contracted from the host himself from the normal flora. Many areas of the body have normal commensal flora. They have many functions. They provide barrier to the infection by competing for nutrition with pathogens. Some produce vitamins which are useful for the host. Some produce colicins to act against pathogens. Generally they do not cause any infection. But there are exceptions. *Streptococcus mitis* is the normal flora of the mouth. It produces infection in the previously damaged heart valves through blood stream after tooth extraction. *Streptococcus faecalis* causes infective endocarditis and the source is the urinary tract and intestine of the host. Exogenous infections are derived from man, animals and soil. Man gets the infections from patients suffering from diseases. Some persons may be carriers for the pathogens and they may transmit the diseases to others without getting affected.

Animals are important sources of infection. Such infections are known as zoonotic diseases. Spread of these diseases is usually from animal to animal. Man may be infected as an end host as in rabies. In some cases the infection may spread from man to man as in pneumonic plague.

Soil has also a role in the transmission of infection. Soil is the reservoir for the spores of *Clostridium* species and *Bacillus anthracis*.

Routes of spread of infection:

There are five main routes by which a host may become infected.

1. The respiratory route
2. The alimentary tract
3. Genital tract
4. The skin and mucous membrane
5. Placenta

Organisms causing respiratory infections are as follows. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycobacterium tuberculosis*, *Bordetella pertussis* are some of the bacterial pathogens. Common cold virus, influenza virus, adenovirus are some of the viruses producing respiratory infections.

The intestinal diseases like cholera, bacillary dysentery, the enteric fever and bovine tuberculosis are contracted when the organisms are ingested. But in the case of enterovirus infections (poliomyelitis) and Hepatitis though the organisms enter through gastrointestinal system, the effects are seen elsewhere in the body.

Organisms may be acquired from the skin as in the case of herpes virus infection or through wounds as in tetanus. Wounds may be formed from trauma or thorn pricks or needle stick injury. Organisms may also be introduced through animal bite as in the case of rabies or by insect bites as in dengue, malaria, filariasis, and yellow fever.

Syphilis, gonorrhoea, hepatitis B and AIDS are some of the sexually transmitted diseases. *Treponema pallidum*, *Neisseria gonorrhoeae*, Hepatitis B virus and Human Immunodeficiency Virus are the etiologic agents respectively.

Bacteria like *T.pallidum*, Viruses like rubella, cytomegalovirus, parasite like *Toxoplasma gondii* are some of the organisms that enter through placenta and cause disease in the newborn.

Interaction between host and microbe

Pathogenicity refers to the ability of the organism to cause disease. Virulence is the quantitative measure of this property. Virulence is not generally attributable to a single property but depends on several parameters related to the organism, the host and their interaction.

Microbes first enter the body, survive then multiply, elaborate many factors and produce the disease.

Virulence Factors

1. Pili

Pili are useful for the attachment of the organisms on the epithelial cells.

2. Capsule

Capsules down regulate the secretion of cytokine. They inhibit leukocyte accumulation. They also induce the suppressor T cells and inhibit lymphoproliferation

3. Intracellular residence

The following microorganisms reside intracellularly and try to avoid host defense mechanisms. They are *M.tuberculosis*, *M.leprae*, *S.typhi*, *T.gondii*, *L.donovani*, *H.capsulatum*.

4. Production of enzymes

Some enzymes like proteases, DNAses, and phospholipases are produced and they help in disruption of cell structures and to hydrolyse host tissues. In *Aspergillus* species proteases help in invasion.

5. Toxins

Bacteria produce both exotoxins and endotoxins which play an important role in the pathogenesis of disease

Exotoxins are produced by some organisms like *C. diphtheriae*, *C. tetani*, *C. botulinum*. The exotoxin produced by *V. cholerae* acts on the intestine and is called enterotoxin. The toxin produced by one type of *Escherichia coli* causes acute gastroenteritis.

Endotoxins are lipopolysaccharide cell wall of gram negative bacteria. They induce production of cytokines by different cells of immune system. Coagulation system and complement system are activated. They also affect various organs like kidney, heart and lungs leading to organ failure.

6. Antigenic variation

Microorganisms evade the host immune responses by changing their surface antigens. *N. gonorrhoeae* very often changes its outer membrane protein. Antigenic drift and shift are common in influenza viruses. *Trypanosoma brucei* are covered with thick protein coats which undergo antigenic change during infection. Some organisms produce surface proteins that are similar to host proteins or coat themselves with host proteins that they are mistaken for part of the host itself

The distinction between the commensal and the organisms associated with disease is subtle. The definition of normal flora or pathogen is derived from the resultant complex interaction between the organism and its host.

EXERCISE

Points to remember

1. Virulence factors of bacteria
2. Toxins of bacteria

Self evaluation

1. List different kinds of microbes
2. Define pathogenicity
3. What are the different routes through which pathogens enter the body?
4. What is the role of capsule in relation to the virulence?
5. Describe the virulence factors of the bacteria
6. Describe the characteristics of bacterial toxins
7. How do bacteria evade host immune response?

Chapter 15

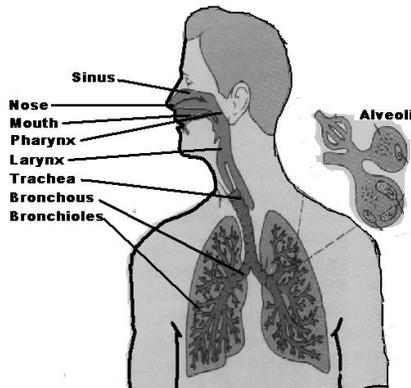
RESPIRATORY TRACT INFECTIONS

Introduction

The lower respiratory tract is sterile. However the upper respiratory tract, the nose and throat are colonized by many organisms.

Normal flora of respiratory tract

These organism are: Staphylococci, Streptococci, Pneumococci, Haemophilus and Neisseria.



Normal defenses against infections

1. Arrangement of nose – there is no direct entry of air
2. Broncho constriction : helps the organisms to be trapped
3. Cough reflex : expels the microbes out side
4. Mucociliary blanket: traps the organisms
5. Mucosal factors: kill the organisms
 - a. Non specific
 - i. Lysozyme : Cell wall of gram positive organism are lysed
 - ii. Influenza virus inhibitors :
 - iii. Resident macrophages : kill the organism

b. Specific

- i. Secretory IgA antibody : gives first line of defense

Predisposing factors for respiratory tract infections

1. Ciliated epithelial cell damage due to
 - a. Viruses
 - b. Chemicals
 - c. Smoking
2. Fluid accumulation
3. Decreased activity of macrophages

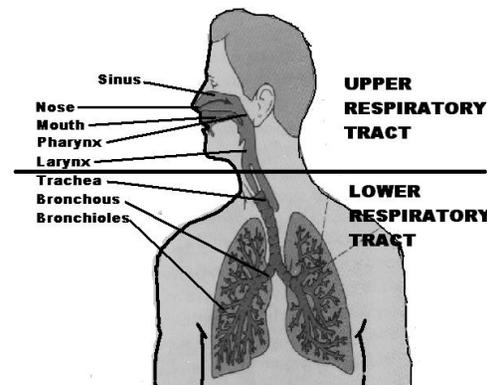
All these factors help in the establishment of the microbes in the respiratory tract.

Alveoli

Strep.pneumoniae ; *M.tuberculosis* ; *Mycoplasma pneumoniae*
Chlamydia pneumoiae

Types of Respiratory infections (Figure 15.3)

Respiratory infections can be conveniently classified into Upper respiratory and Lower respiratory infections.



URI

Sinusitis
Pharyngitis
Laryngitis
Epiglottitis

LRI

Trachiitis
Bronchitis
Tracheo bronchitis
Alveolitis
Pneumonia

Upper respiratory tract infections (URI)

1. Infections of the paranasal sinuses

URI may cause inflammation of the maxillary sinuses. Bacterial infection also occurs in association with obstruction.

The bacteria involved are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*.

2. Otitis media

This is the result of direct spread of pathogens from the throat via the Eustachian tube. In young it may be due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*. In old people it may be due to *Streptococcus pneumoniae*, *Staphylococcus aureus*.

3. Sore Throat

It occurs in the following conditions:

1. Prodromal stages of infectious diseases
2. Diphtheria
3. Vincent's infections
4. Sore throat syndrome

Prodromal stages of infectious diseases

Many viral diseases are acquired via the respiratory tract. Signs and symptoms appear in the mouth and throat. But the target organs may be different.

Example: In measles and chickenpox, the mucosae of the upper respiratory tract and mouth are infected before the target organs show full clinical picture. In measles Koplick's spots appear in buccal mucosa.

Diphtheria

Diphtheria is an acute inflammatory condition of the upper respiratory tract usually the throat. It is caused by *Corynebacterium diphtheriae*. The organisms multiply in the throat and produce a powerful toxin. The toxin acts on myocardium, adrenal glands and nerve endings.

Laboratory diagnosis

Throat swab is collected

1. Smear is stained by Gram and Albert stains
 - a. In positive cases Gram positive bacilli seen
 - b. Albert stain shows bacilli with metachromatic granules
2. Material is inoculated into Blood agar, Loeffler's serum medium and Potassium tellurite agars
3. Suspected colonies are identified by biochemical test using serum sugars
4. Toxigenicity test is done by agar gel precipitation test (Elek's test) and by guinea pig inoculation test

Prophylaxis

- Active immunization is done
- DPT (Diphtheria, Pertusis and Tetanus) immunization should be given in three doses
- 1st dose at third month
- 2nd dose at 6-8 weeks after the first dose
- 3rd dose 4-5 months after the 2 dose
- A booster is given omitting the pertusis at school entry

Treatment

Large dose of anti toxin must be given to confirmed cases. Antibiotics are given to eradicate the organisms .

Vincent's infection

Vincent's spirochetes are *Borrelia*

They are present as normal flora of the mouth

In association with fusiform bacteria they can cause infection

Generally infection occurs during malnutrition

The infection is called **Vincent's angina**

Sore Throat syndrome

Sore throat syndrome is caused by *Streptococcus pyogenes*. Based on the cell wall polysaccharide of beta hemolytic *Streptococci* they are put in different groups. *Streptococcus pyogenes* belongs to Group A. It causes wide range of pyogenic infections in the respiratory tract and skin and life threatening soft tissue infections. Post streptococcal infections may result in adverse immunological reactions leading to rheumatic heart disease or acute glomerulonephritis.

Laboratory diagnosis

For the laboratory diagnosis specimens like throat swab and pus are collected and inoculated in blood agar. The organism is identified by hemolytic property, and serological tests.

Penicillin is the drug of choice for the treatment of streptococcal infections.

Lower respiratory tract infections

Many organisms cause lower respiratory tract infections which you will be studying in later classes.

EXERCISE

Points to remember

1. Structure of respiratory tract
2. Predisposing factors for respiratory tract infection
3. Bacterial cause of respiratory tract infection

Self evaluation

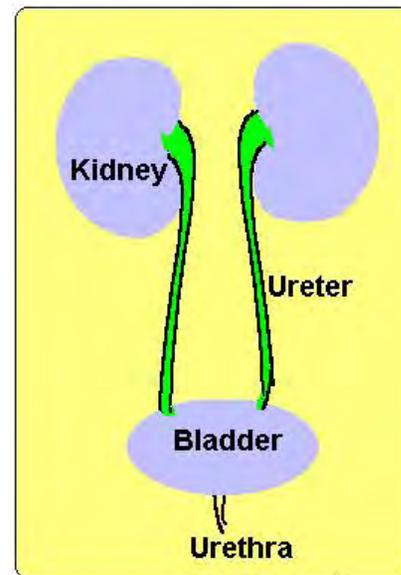
1. Draw the structure of respiratory tract and label the parts
2. Give a list of normal flora of respiratory tract
3. Describe the normal defenses operating in respiratory tract against infection
4. Describe the predisposing factors of respiratory tract infection
5. Give the list organisms commonly involved in respiratory tract infection
6. Describe upper respiratory tract infection
7. Describe sore throat syndrome and its laboratory diagnosis
8. Describe lower respiratory tract infection, its etiology and laboratory diagnosis

URINARY TRACT INFECTIONS

Introduction

Infections of the kidney, ureter and bladder constitute urinary tract infections (UTI). When infections occur in the kidney and ureter it is called upper urinary tract infections and bladder downwards it is called lower urinary tract infections.

Structure Of Urinary Tract (Fig:16)



Urinary tract infection is common in females than in males. The urethra in females are shorter and wider and is less effective in preventing the bacteria entering the bladder. Sexual intercourse is a predisposing factor. High incidence is seen in pregnant women because of hormonal changes and impairment of urine flow due to pressure on

the urinary tract. Other causes of urinary stagnation predispose UTI, such as urethral obstruction, urinary stones, congenital malformation and neurological disorders. Most UTI are caused by organisms originating from the patient's own fecal flora.

Organisms Causing Urinary Tract Infections

For convenience the organisms causing urinary tract infections can be classified into the following:

1. Organisms most commonly involved are: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and other *Proteus* spp, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *S.aureus*
2. Uncommon organisms are: *Enterobacter* species, *Serratia* spp, *Providencia* spp, other nonfermenters
3. Rare organisms are: Streptococci other than group A, *Candida albicans*, *Candida glabrata* etc

Laboratory Diagnosis Of Urinary Tract Infection

Mid stream urine is collected and transported to the laboratory immediately. If there is any delay expected it should be refrigerated. A quantitative culture should be performed to know the number of gram negative organisms present. A count of more than 100,000 (10^5 /ml) organisms per ml of urine in pure culture is indicative of UTI. For the isolated organisms antibiotic susceptibility testing must be done and appropriate treatment should be given

EXERCISE

Points to remember

1. Structure of Urinary tract
2. Organisms causing urinary tract infections

Self evaluation

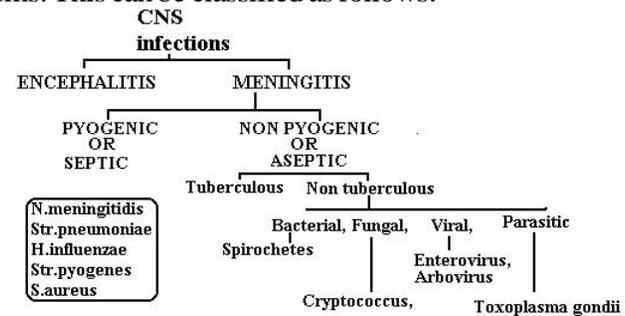
1. Draw the structure of urinary tract and label its parts
2. List the organisms causing urinary tract infections
3. Describe the laboratory diagnosis of urinary tract infection

INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Infecting agents reach the central nervous system (CNS) from the blood or by direct invasion or by ascending through the nerves. The infection of the CNS can be classified as encephalitis and meningitis.

Meningitis- Etiological agents

Meningitis is the inflammation of the membranes covering the brain (meninges). This can be caused by a wide range of micro-organisms. This can be classified as follows:



Acute pyogenic meningitis

This condition is related to the age of the patients. Coliform bacilli and group B streptococci are common cause in neonates. Haemophilus and Neisseria meningitidis are frequently seen in children. *H.influenzae* and *Str.pneumoniae* are commonly found in adults. *N.meningitidis* can cause infections in all age groups. Other organisms involved are *Str.pyogenes*, *S.aureus*, *Salmonella species*, *Listeria*.

Pathogenesis and Epidemiology : Neonatal meningitis

In early days, it was caused by *Str.pyogenes* and *S.aureus*. But in later years coliform bacilli are most commonly found. Coliform men-

ingitis results in congenital deformities and the source of the organisms can be genito-urinary tract, lungs and umbilicus.

Haemophilus meningitis: This occurs mainly in young children between the ages of 3 months to 5 years. Protection before 3 months is given by maternal antibody and after 5 months by acquired immunity. Infection spreads through blood stream.

Meningococcal meningitis (N.meningitidis): The organism enters the body via the naso-pharynx, where they may produce localized inflammatory reaction or remain silent. By two ways they may reach the meninges. 1. They spread directly along the spaces between the sheath and the branches of the olfactory nerve that pierce the cribriform plate. 2. The organisms invade the blood stream, either produce transient bacteremia or multiply in the blood. They cause lesions in the skin, adrenal glands, joints and meninges. There are nine groups of meningococci. Infections spread to one another by close contact.

Pneumococcal meningitis: Subsequent to some conditions like lobar pneumonia, otitis media, or infection of the para nasal sinus, or injury to skull, Streptococcus pneumoniae multiplies in the blood and spread to the meninges.

Meningitis due to other bacteria: Staphylococcus and Streptococcus pyogenes reach the meninges via blood or directly from the exterior after a trauma.

Tuberculous meningitis: This occurs in a severe form in young children and is secondary to a tuberculous focus elsewhere in the body. The bacilli reach the meninges via the blood.

Laboratory Diagnosis

Laboratory diagnosis is made by isolation and identification of specific organism from blood and Cerebrospinal fluid (CSF). Antigen can be detected by counter immunoelectrophoresis or latex agglutination test. Blood cultures are useful in 50% cases. Gram stain and acid fast stains are useful in demonstrating the bacteria in the CSF. Fluorescence microscopy is useful for tuberculous meningitis. Fontana's staining is useful for spirochetes like leptospira, borrelia and treponema.

Once organisms are grown, they are identified by standard biochemical tests. Treatment will depend on the nature of the organisms and the antibiotic susceptibility pattern

Viral meningitis

Viruses are the most frequent cause of meningitis. Most cases are due to enteroviruses, ECHO and Coxsackie viruses. Mumps virus can cause meningitis in children.

Pathogenesis: Viruses enter by oral or respiratory routes. They establish a silent initial focus somewhere in the naso-pharynx or in small bowel. The spread occurs through lymph to the blood. They mainly multiply in lymphoreticular system. The infection is detectable after 5 days. The virus is free in the plasma. They affect the meninges and they may spread to other parts also. This time fever and neck stiffness occur. At this time the virus appears in the throat and gut also. Serum antibody appears about this time.

Diagnosis: Specimens of throat secretions, feces, CSF, are collected during acute phase. During convalescent period feces may be obtained. Enteroviruses can be isolated in tissue cultures. No specific therapy is available.

EXERCISE

Points to remember

1. Types of CNS infections
2. Pyogenic meningitis
3. Viral meningitis

Self evaluation

1. Name two types of CNS infections
2. Name two types of meningitis
3. Define pyogenic meningitis
4. Describe the pathogenesis, epidemiology and laboratory diagnosis of pyogenic meningitis
5. Describe the pathogenesis epidemiology and laboratory diagnosis of viral meningitis

SEXUALLY TRANSMITTED DISEASES

Introduction

Sexually transmitted diseases are the most common communicable diseases after common cold. They are transmitted through sexual contact. They can be classified into the following: 1. Bacterial 2. viral 3. Parasitic 4 and fungal diseases.

Bacterial diseases

The following are some of the sexually transmitted bacterial diseases. 1. Syphilis 2. Gonorrhoea 3. Chancroid 4. Chlamydial diseases

The sexually transmitted viral diseases are 1. AIDS 2. Hepatitis B 3. Warts.

Trichomoniasis and genital candidiasis are the parasitic and fungal diseases respectively.

Syphilis

Treponema pallidum is the organism that causes syphilis. Treponemes are slender spirochetes with fine spirals and pointed or round ends. The pathogenic treponemes have not yet been cultivated in artificial media

Pathogenesis

T. pallidum is transmitted from one partner to another through intact or damaged mucosa. The organisms establish at the point of entrance and multiply within the next 3 months and a chancre appears. This is the primary lesion and may disappear. This stage is called *primary syphilis*

Secondary syphilis occurs in two to 6 months after the primary syphilis. Because of the multiplication of the organisms secondary lesions appear on the skin. Spirochetes are abundant in these lesions. Usually the lesions heal spontaneously.

In few cases *tertiary syphilis* appears later. This causes chronic granulomata known as gummata in the brain, bone, skin and internal organs. Late manifestations are degeneration of brain cells and destruction of nerve fibres. Tertiary lesions contain few spirochetes.

Infection during pregnancy can be transmitted to the fetus. This causes congenital syphilis.

Laboratory diagnosis

Exudates from primary and secondary lesions are collected for examination. They are examined by dark field microscopy for spirochetes and stained by silver staining method to show the spirochetes.

Blood is collected for serological tests to demonstrate antibodies. Serological tests are divided into two groups viz. nonspecific and specific tests.

In the nonspecific test antibodies to cardiolipin which develop during the infection are demonstrated. The test is called VDRL test.

In the specific test, antibodies developed against *T. pallidum* are demonstrated. *Treponema pallidum* immobilization test (TPI), Fluorescent treponemal antibody absorption test (FTA-AB), and *Treponema pallidum* haemagglutination test (TPHA) are some of the specific tests used for the diagnosis of syphilis.

Penicillin is the drug of choice for treatment. For control, all discovered cases must be promptly treated and contacts also must be treated. Sex hygiene, and prophylaxis at the time of exposure are some other control measures. Sexually transmitted diseases can be transmitted simultaneously. Therefore, it is necessary to consider the possibility of syphilis when any other sexually transmitted disease is found.

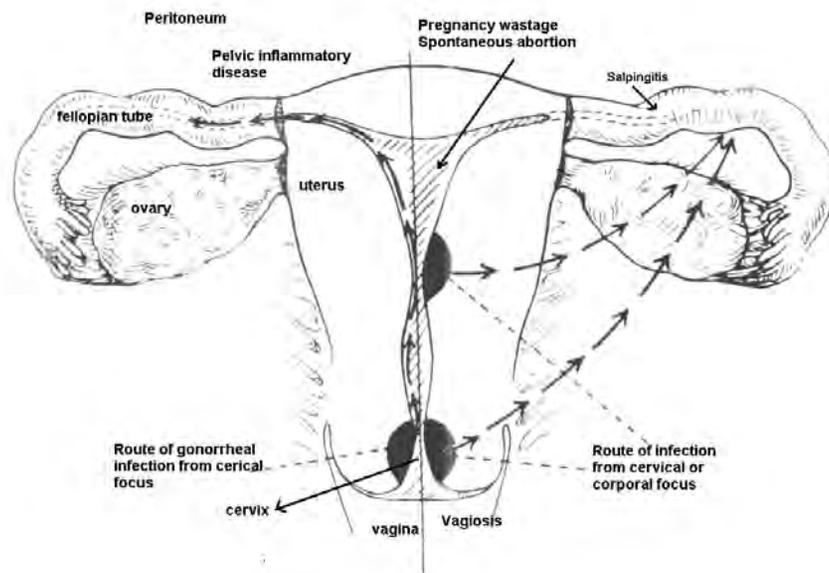
Gonorrhoea

Gonorrhoea is a sexually transmitted infection of columnar and transitional epithelium caused by *Neisseria gonorrhoeae*. The urethra, endocervix, anal canal, pharynx and conjunctivae may be infected directly. Systemic infection may lead to arthritis, tenosynovitis, dermatitis, endocarditis and meningitis.

Organism

Neisseria gonorrhoeae are gram negative diplococci, kidney shaped, the concave side face each other, nonmotile. Grows on enriched medium in presence of 5-10% CO₂...

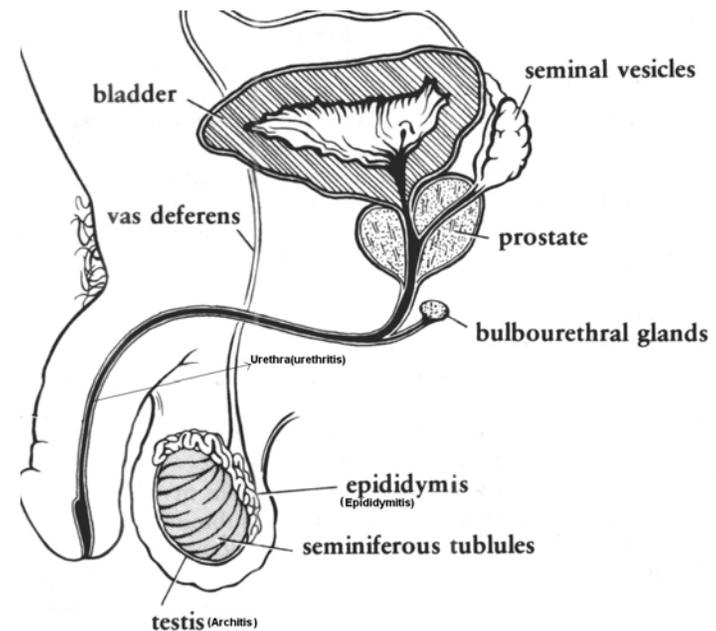
Structure of male and female genital tracts (Figure 18.1)



Pathogenesis

Anterior urethra is mainly affected in men. Anterior urethra and cervix are affected in women. In advanced infection it affects the prostate, seminal vesicles and epididymis in men and uterus and fallopian tubes are affected in women. Rectal infection and throat carriage occur

in both. Gonococcal ophthalmia neonatorum is an infection of the eye of the newborn, is acquired during the passage through infected birth canal. The conjunctivitis progresses and if untreated results in blindness. To prevent this instillation of tetracycline, erythromycin or silver nitrate solution into the conjunctival sac of the new born is compulsory. Gonococcal bacteremia leads to skin lesions on the hands, fore arms, feet, and tenosynovitis and suppurative arthritis.



Male reproductive system.

Laboratory diagnosis

Pus and secretions are taken from urethra, cervix, rectum, conjunctiva, throat or synovial fluid for smear and culture. Cultures are to be done immediately after the collection of specimens.

Treatment: Penicillin is given. If the organisms are resistant, after performing antimicrobial susceptibility testing, appropriate drug is given.

Chancroid

Haemophilus ducreyi causes irregular ulcers in the genitalia. It produces chancroid or soft chancre. This is a venereal disease or sexually transmitted disease. *H. ducreyi* is a gram negative bacilli. Chancroid is treated with sulphonamides. If resistant, erythromycin and cotrimoxazole are used.

Chlamydial disease

There are many serotypes in Chlamydia. Some of them cause genital infections.

Lymphogranuloma venereum is one of chlamydial sexually transmitted diseases. First a vesicle develops and the lesion ulcerates in the genitals. The inguinal lymph nodes enlarge, suppurate and release pus through multiple sinus tracts. If not treated it will lead to other complications. Sulfonamides and tetracycline are used for the treatment.

Trichomoniasis

This is caused by *Trichomonas vaginalis*. Trichomonads are flagellate protozoa with 3-5 anterior flagella, other organelles and an undulating membrane.

In female the infection is limited to vulva, vagina and cervix. It usually does not extend to uterus. The mucosal surface may be painful, inflamed, eroded and covered with a frothy yellow or cream colored discharge. In males the prostate, seminal vesicle and the urethra may be infected.

Trichomoniasis is treated with topical and systemic metronidazole. The patients sexual partner should be examined and treated simultaneously.

Trichomoniasis is treated with topical and systemic metronidazole. The patients sexual partner should be examined and treated simultaneously.

Genital candidiasis

The most common cause of genital candidiasis is due to *Candida albicans*. Generally it is a commensal in the vagina. When infection occurs white membranous patches are produced in the vagina and vulva. Thick or watery vaginal discharge is seen. Gram's stain can identify the yeast like cells. Nystatin or miconazole or ketoconazole are used.

Viral agents

AIDS (Acquired immunodeficiency syndrome)

Human Immunodeficiency Virus (HIV) is the etiologic agent of AIDS. It belongs to the lenti virus sub group which includes slow viruses.

The virus has central nucleoprotein core that contains single stranded RNA genome. The enzyme reverse transcriptase is associated with the viral RNA. This RNA is transcribed into single stranded DNA and then to double stranded DNA.

The virus core is surrounded by a protein shell this is again covered by a lipid bilayer which contains envelope proteins.

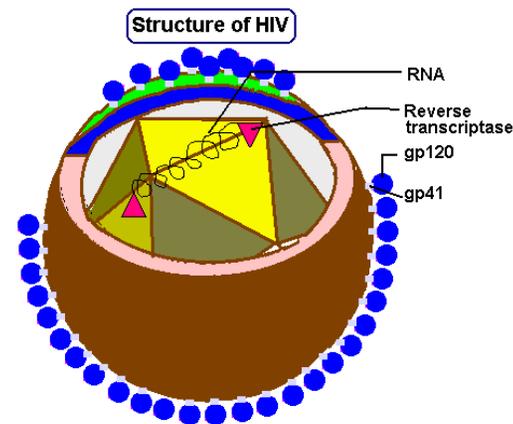


Figure 18.2

Pathogenesis

Transmission is by sexual contact, through blood and blood products- transfusion and injection. After entry it comes in contact with T4 lymphocytes. T4 cells are damaged and are decreased in number and T4:T8 ratio is reversed. Because helper cells are affected, humoral immunity is also affected. AIDS patients are unable to respond to new antigen. Macrophage monocyte functions are affected because of lack of secretion of activation factors.

Within few weeks of infection, low grade fever, malaise, head ache, lymphadenopathy are seen. All persons pass through a period of symptomless infection for several months or years. They show positive antibody tests and are infectious. Lymph nodes are enlarged in some people. Then it leads to other opportunistic infections like oral candidiasis, salmonellosis, tuberculosis. Persons suffer from fatigue, unexplained fever, persistent diarrhea, and weight loss. Finally they reach the stage called AIDS.

Laboratory diagnosis :Immunological tests

Total white blood cell count: usually below 200/cmm

T4 cell count is less

Lowered cell mediated immunity is seen.

Specific tests

Viral antibody detection is performed by ELISA test and confirmed by Western blot test. Virus can be isolated from infected lymphocytes.

Prevention

1. Multipartner sex should be avoided
2. Safer sex should be practiced
3. Blood should be screened before transfusion
4. Sharing of needles should be avoided

Parasitic agent

Trichomonas vaginalis is the organism that is transmitted through sexual contact.

EXERCISE

Points to remember

1. Understand that different kinds of microbes cause sexually transmitted disease in human
2. Know sexually transmitted bacterial disease
3. Sexually transmitted viral disease with special reference to AIDS.

Self Evaluation

1. Give a list of sexually transmitted bacterial diseases
2. Name the agent causing syphilis
3. Describe the pathogenesis of syphilis
4. Describe the laboratory diagnosis of syphilis
5. Name the agent causing gonorrhoea
6. Describe the laboratory diagnosis of gonorrhoea
7. Describe the structure of male genital tract
8. Describe the structure of female genital tract
9. Name the agent causing chancroid
10. What is lymphogranuloma venerum?
11. Name the agent causing Trichomoniasis
12. Name the agent responsible for AIDS
13. Describe the structure of HIV
14. Describe the pathogenesis of AIDS
15. Describe the special tests for the diagnosis of AIDS
16. List the methods of prevention of AIDS

Chapter 19

BACTERIAL SKIN AND WOUND INFECTIONS

Definition

Wound can be defined as any interruption of continuity of external or internal surfaces caused by violence

Wounds may occur following: surgery, trauma or injections

Wound infections may occur mainly after surgical procedures

Wound sepsis is the result of cross infection from human sources and from other outside sources.

Bacteria associated with wound infections

Many bacteria are associated with wound infection.

The normal flora may also cause infection.

The most common bacteria of the skin are: staphylococci, and various streptococci, Sarcina spp, anaerobic Diphtheroids, gram negative rods and others

The nutrition is derived from 1. sweat, 2. aminoacids and peptides from the skin, 3. fatty acids from the sebaceous glands of the skin.

Factors Determining the Ecology of the Skin Bacteria

Four main factors determine the ecology of skin bacteria

1. The climate: The temperature and humidity
2. The effect of free fatty acids
3. Other bacterial inhibitors
4. Maintenance of the flora by products of skin secretions

Defence Against Infection

1. Intact skin. Normal uninterrupted skin provides protection against invasion by bacteria
2. Lysozyme in sweat: The enzyme lysozyme provides protection against gram positive bacteria by lysing the cell wall.
3. Ig A antibodies in the sweat and secretions provide first line of defense against infection
4. Inhibitors like unsaturated fatty acids provide protection against bacteria
5. Bacteriocins produced by the normal flora prevent the establishment of other bacteria

Factors Responsible For Wound Infections

A. Host Factors: The following factors help the organisms to survive and produce the infections

1. Extremes of age
2. Diabetes mellitus
3. Steroid therapy
4. Obesity
5. Malnutrition
6. Immunocompromised individual
7. Presence of remote infection at the time of surgery

B. Exogenous Factors

1. Use of unsterile instruments
2. Surgeons hands / from health workers
3. Air / Hospital environments

C. Endogenous Factors

1. Wound contamination from the patient source: from the normal flora
2. Wound penetrating through structures containing normal flora
3. Surgical procedures involving mucous membranes harboring normal flora
4. Patients carrying pathogens in their nose, throat, axilla etc.

Etiological agents

Ps.aeruginosa

Staph.aureus

Proteus spp

Member of enterobacteriaceae

Anaerobic organisms

Anaerobic cocci

Bacteroides

Post Operative Infections

Gas gangrene organisms

S.aureus

Cl.tetani

Route of entry

Wounds may occur following: surgery, trauma or injections. Wound infections may occur mainly after surgical procedures. Wound sepsis is the result of cross infection from human sources and from other outside sources.

Mechanisms of damage

1. Organisms enter through the skin, multiply there and produce the disease in the skin. For example, impetigo, abscess and cellulitis are caused by Staphylococcus aureus and Streptococcus pyogenes. As soon as the organisms enter the skin they multiple and produce various toxins that kill the cells and produce cellulites. Further damage leads to necrosis and ulcer formation.

2. Organisms multiply in the skin and produce disease in internal organs. For example some group A streptococci multiply in the skin and produce disease known as acute glomerulo nephritis causing damage to the kidneys. Some times C.diphtheriae may multiply in the skin and affect the heart due to the toxin.

3. Some times organism may multiply in the skin and produce the toxin which affect the CNS and the effects are seen. In the case of *Clostridium tetani*, convulsions and paralysis occur due to the production of a powerful toxin

Laboratory diagnosis

Pus and wound swabs are cultured for the aerobic and anaerobic organisms and are identified using appropriate biochemical tests.

EXERCISE

Points to remember

1. What is a wound, how is it infected?
2. Which are the agents that cause wound infections
3. Consequences of wound infection

Self evaluation

1. Define a wound
2. What are the causes of wound?
3. What are the normal flora of the skin
4. State the factors that determine the ecology of skin bacteria
5. State the defenses of skin organisms against the bacterial invasion
6. Describe the factors responsible for wound infection
7. Give the names of the etiologic agents of wound infection
8. Describe the mechanism of damage caused by organisms in wound infection
9. Describe the laboratory diagnosis of wound infections

BACTERIAL INFECTIONS OF GASTRO INTESTINAL TRACT

Many organisms cause gastrointestinal infections. The most important ones are organisms belonging to the genera Salmonella, Shigella, and Vibrio and certain types of *Escherichia coli*. These are acquired by oral route.

Acute gastroenteritis due to Shigella

Organisms belonging to the genus Shigella produce bacillary dysentery. They are gram negative bacilli, nonmotile organisms. There are four species, *Shigella dysenteriae*, *Sh.flexneri*, *Sh.boydii*, and *Sh.sonnei*

Pathogenesis

The infection is limited to the gastrointestinal tract. Blood stream invasion is rare. Infection is communicable. Invasion of the mucosal epithelium leads to the formation of micro-abscesses in the wall of the large intestine and ileum which finally results in necrosis of the mucous membrane, ulceration, bleeding and formation of pseudo membrane on the ulcer area. This consists of fibrin, leukocytes, cell debris, necrotic mucous membrane and bacteria.

Toxins of Shigella

1. Endotoxin: Upon autolysis, the organisms release LPS which causes the irritation of the bowel wall.
2. *Shigella dysenteriae* produces exotoxin which is a heat labile toxin that affects the gut and CNS. It acts as enterotoxin and increases the local concentration of cyclic adenosine mono

mono phosphate (AMP) and results in intense and prolonged hyper secretion of water and chlorides and inhibits the re-absorption of sodium. The gut lumen is distended with fluid, hyper motility and causes diarrhea. As a neurotoxin it acts on CNS and causes meningismus and coma mainly in children.

Shigellae produce an early non bloody, voluminous diarrhea and later dysentery with blood, mucous, and pus in stools.

Laboratory diagnosis

Fresh stool, mucus flek and rectal swabs are collected for culture. Large number of leucocytes, RBC may be seen microscopically. Selective media like Salmonella shigella agar, Deoxycholate citrate agar are used. The organisms are non lactose fermenters and are identified by biochemical test and with specific antisera. Ciprofloxacin is used for the treatment nowadays.

Acute gastroenteritis due to Escherichia coli

Generally *E.coli* is a normal flora of the gut of man. But it may sometimes cause gastrointestinal disease. It may range from mild, self-limiting diarrhea to hemorrhagic colitis. Such stains fall into five groups with specific serotypes and with different **Pathogenic mechanisms**

1. Enteropathogenic *Esch.coli* (EPEC) : These cause infantile enteritis in children
2. Enterotoxigenic *Esch.coli* (ETEC) : These cause community acquired diarrhoeal disease in areas of poor hygiene. It is responsible for traveller's diarrhea.
3. Enteroinvasive *Esch.coli* (EIEC): It causes an illness similar to Shigella dysentery in all age groups
4. Verotoxin producing *Esch.coli* (VTEC): It causes haemorrhagic colitis and haemolytic uremic syndrome
5. Enteroaggrgative *Esch.coli* (EAggEC). It causes chronic diarrhoeal disease.

Contaminated food and water supplies are the most important vehicles of these organisms. Infantile enteritis in hospitals are transmitted through patient to patients, through the hands of attendants and contaminated feeds. VTEC infections are acquired through meat, unpasteurized milk and direct contact with animals.

Laboratory diagnosis

Esch.coli organisms are grown and their pathogenic characteristics are identified by appropriate tests.

Prevention

Avoid exposure to infectious agents. Fluid and electrolyte imbalance must be corrected early. Strict hygiene is essential in hospitals. Food borne infections should be avoided by processing and handling cooked meat products separately from raw materials.

EXERCISE

Points to remember

1. Characteristics of Gastro intestinal infections
2. Important organisms causing Gastro intestinal infections
3. Laboratory diagnosis and prevention of Gastro intestinal infections

Self evaluation

1. Give the characteristics of Gastro intestinal infections
2. List the important organisms causing Gastro intestinal infections
3. Describe the laboratory diagnosis of Gastro intestinal infections
4. Describe the characteristics of shigella infection
5. Describe the laboratory diagnosis of shigella dysentery
6. Name different *Escherichia coli* causing intestinal infections
7. Describe the laboratory diagnosis and prevention of Acute gastro enteritis due to *Escherichia coli*

SUPERFICIAL MYCOSIS AND DERMATOMYCOSIS

Introduction

Thousands of fungi both yeasts and filamentous fungi are present in the environment. About 100 of them cause disease in man and animals. Only very few species like dermatophytes and *Candida* are transmitted from man to man. Generally the fungal infections are classified into superficial, cutaneous, subcutaneous, and systemic mycoses. Infections of skin, hair and nails may be chronic and resistant to treatment but do not affect the general health of the patients.

Superficial mycosis

1. Tinea versicolor

Tinea versicolor is a mild infection of skin. The fungus *Malassezia furfur* grows in the **stratum corneum** of the skin as spherical thick-skinned budding cells and short, bent hyphae. It does not cause any pathological signs.

Fine brownish scales appear on the chest, back, abdomen, neck and upper arm. In brown skinned people it produces white patches and in whites brown patches which are of cosmetic importance only.

2. Tinea Nigra

Tinea nigra is a condition in which blackish macular area appears on the palmar or plantar surface of the skin. This condition is caused by *Exophiala werneckii*. The infected skin can be removed mechanically or chemically.

3 Piedra

Black piedra is a condition in which black nodules are formed around the scalp hair by *Piedraia hartae*.

In white piedra, *Trichosporon cutaneum* causes the formation of soft, white nodules around axillary, pubic, beard and scalp hair

Dermatophytosis

Dermatophytosis is also known as cutaneous mycosis. It is caused by dermatophytes. They infect superficial keratinized tissues such as skin, hair, and nails. They do not invade deeper tissues.

Dermatophytes are closely related fungi and are classified into three genera: 1. *Trichophyton*, 2. *Microsporon* 3. *Epidermophyton*.

These genera are identified by their characteristic morphology of micro and macro conidia produced on Sabouraud's dextrose agar. The infection produced by dermatophytes is called Tinea. In *Tinea corporis* the infection is present in non-hairy, smooth skin.

In *Tinea pedis* infection is present in the inter digital spaces on feet. When the infection is present in the groin it is called *Tinea cruris* whereas when seen on the head it is called *Tinea capitis*. *Tinea barbae* is the infection of the beard hair, and *Tinea unguium* is infection of the nail.

Laboratory diagnosis

Specimens consist of both skin and nail scrapings and hair plucked from the involved areas. Specimens are examined in a drop of 10% KOH. In skin or nails, branching filaments or chains of arthrospores are seen. Specimens are also inoculated on SDA medium and the fungi identified

Fungal skin infections are treated with miconazole cream or with griseofulvin.

EXERCISE

Points to remember

1. Different types of fungal skin infections
2. Superficial mycosis and the agents causing it

Self evaluation

1. What is *Tinea versicolor*? Name the agent causing it
2. What is *Tinea nigra*? Which agent
3. Classify piedra and name the organisms associated
4. Name the genera causing dermatophytosis
5. Describe the laboratory diagnosis of dermatophytosis

AMOEBIASIS

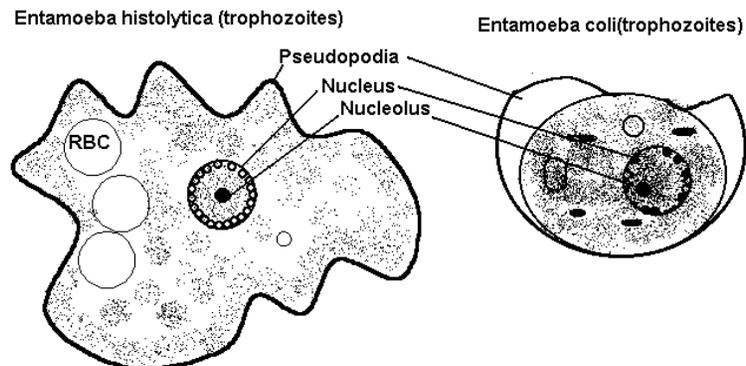
Introduction

- Amoebiasis is an infection due to *E.histolytica*
- It occurs only among human and selected primates
- Commonly *E.histolytica* produce intestinal and extra intestinal infections
- Amoebiasis is transmitted by oral ingestion of materials containing cyst of *E.histolytica*

Morphology of the organism

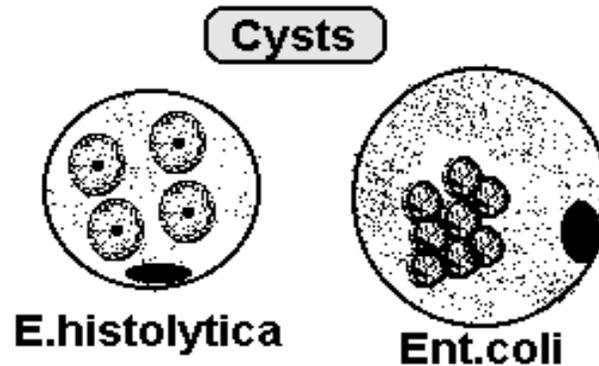
Trophozoites (Figure 22.1)

Entamoeba histolytica and *entamoeba coli* have both trophozoites and cyst stages. The cytoplasm of *E.histolytica* is glossy, contains red cells and spherical vacuoles. The nucleus has small central karyosome and fine regular chromatin granules lining the periphery of nuclear membrane. *Entamoeba coli* has granular cytoplasm which contains bacteria and other inclusions and ellipsoid vacuoles. Nucleus has eccentric nucleolus and coarse beaded chromatin at the periphery.



Cysts

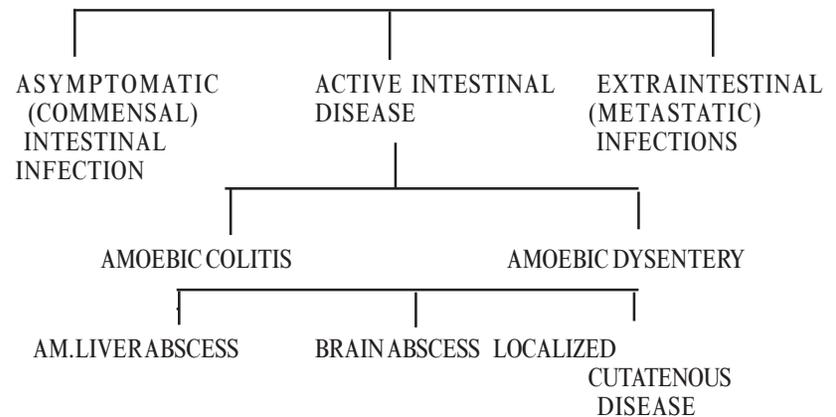
Cysts are round, nuclear morphology is similar to trophozoites. One to four nuclei are seen in *E.histolytica* and eight nuclei are present in *Entamoeba coli* cysts.



Clinical manifestations of the disease

- Both intestinal and extra intestinal amoebiasis have an incubation period of more than one week (several weeks)

TYPES OF CLINICAL MANIFESTATIONS



Asymptomatic Intestinal Infections

- Persons show no symptoms
- They pass cysts in stool

Active Intestinal Disease

- Minority of persons with intestinal infection develop diarrhoeal disease
- They pass red cells and pus in stool
- The symptoms and signs are:
 - Less severe form
 - Fever
 - Abdominal pain
 - Tenesmus
 - More severe form
 - Symptoms similar to ulcerative colitis
 - Peritonitis
 - Secondary intestinal perforations
 - Toxic mega colon

Pathology

- Parasites produce flask-shaped ulcers in which the base is wider than neck at the epithelial surface
- Organisms are present on the edge of the ulcer

Laboratory Diagnosis

DIRECT METHOD

(DEMONSTRATION OF ORGANISM OR COMPONENTS OF THE ORGANISMS)

DEMONSTRATION OF ORGANISMS

INDIRECT METHODS

(DEMONSTRATION OF RESPONSE TO ORGANISMS)

DEMONSTRATION OF COMPONENTS HAVE ORGANISMS

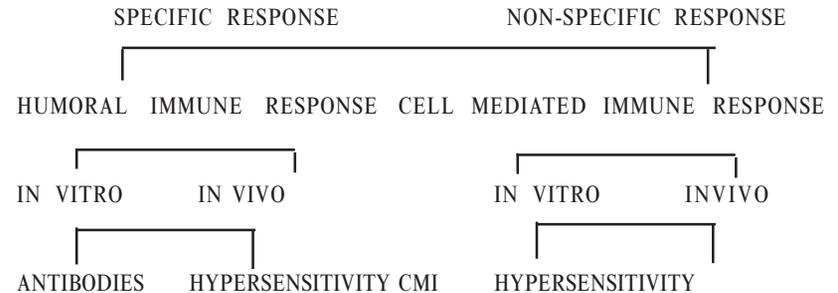
Direct demonstration:

- Wet mount:
 - Saline – Trophozoites, Cysts
 - Iodine – Cysts
 - LCB - Cysts
- Stains : Iodine staining
: Iron haematoxylin stain
: Trichrome stain
: Immuno fluorescence staining
- Culture: non-Axenic and Axenic culture methods are used

Demonstration of antigens from stool (using specific antibodies)
By Counter immuno electrophoresis
By ELISA
Demonstration of DNA by PCR

Indirect Methods

DEMONSTRATION OF RESPONSE TO ORGANISM



Epidemiology, Prevention and control

Cysts are ingested through contaminated food and water

Flies transfer the cysts from infected stools to food

Control measures consist of improving environmental and food sanitation

Carriers must be barred from food handling

Metronidazole is the drug used for symptomatic amoebiasis

Chapter 23

MALARIA

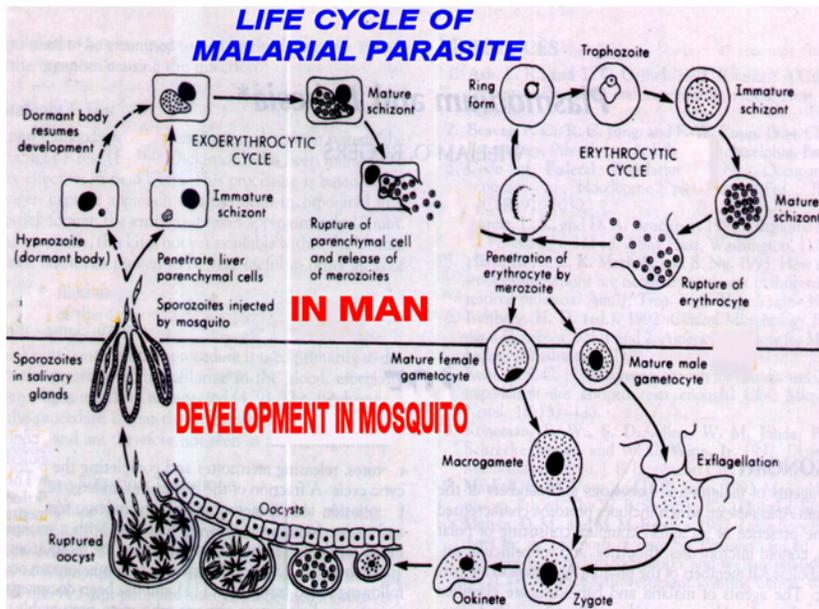
Malaria

Malaria is a mosquito borne parasitic disease characterized by episodes of fever, chills, and rigors which occur typically and periodically every third day.

Etiological agents

Four species of Plasmodium cause Malaria in man. They are *P.vivax*, *P.falciparum*, *P.malariae*, *P.ovale*.

Life cycle of Malarial parasite (Fig: 23.1)



1. Following the bite of an infected mosquito the sporozoites are introduced into the body
2. The parasites first invade the cells of the liver
3. They multiply by the process of schizogony
4. After 6-12 days merozoites are released into the blood
5. The parasites invade the RBC
6. Inside the RBC they continue to multiply and release merozoites.
7. Some parasites transform into macro and micro gametocytes which are taken by the mosquitoes.
8. Inside the mosquitoes further multiplication leads to the production of sporozoites.

Morphological characteristics of developmental stages of malarial parasites

	Rings	Trophozoites	Early schizonts	Mature schizonts	Male gametocyte	Female gametocyte
<i>P.vivax</i>						
<i>P.falciparum</i>						
<i>P.malariae</i>						
<i>P.ovale</i>						

Clinical features

The clinical features of malaria are due to the blood stage parasites. There is fever with rigor, head ache, myalgia, arthralgia, nausea

and abdominal pain seen in malaria. Due to rupture of RBC there is anemia. Mild enlargement of spleen is seen.

Laboratory diagnosis of malaria

Presence of malarial parasites in the blood confirms the diagnosis of malaria. A thickly spread blood film is useful for spotting the parasites. Thinly spread films help in the accurate identification of the species. Blood films are stained by either Giemsa or Leishman stains. Concentration technique like QBC are also available for the diagnosis.

Treatment

Antimalarial drug are used primarily to eliminate the asexual and blood parasites. Treatment also must be given to eliminate liver stage parasites (Hypnozoites) that could give rise to relapse infection

The drug of choice is chloroquine. To prevent relapse primaquine is used.

EXERCISE

Points to remember

1. Morphology of Entamoeba
2. Amoebiasis and its laboratory diagnosis
3. The life cycle of malarial parasite
3. The laboratory diagnosis of malaria and its treatment

Self evaluation

1. What is malaria?
2. List the etiological agent of malaria
3. Describe the life cycle of malarial parasite
4. Describe the morphological characteristics of different stages of malarial parasites
5. Give the clinical features of malaria
6. Describe the laboratory diagnosis of malaria
7. Describe the morphological characteristics of E.histolytica
8. Describe intestinal amoebiasis
9. Describe extraintestinal amoebiasis
10. Describe the laboratory diagnosis of amoebiasis

FILARIASIS

Introduction

Filariasis are vector borne human parasitic diseases with complex life cycle. They are caused by nematodes. In their mature stage they reside in lymphatic system or in connective tissues. Adult parasite itself can provoke inflammatory reactions in tissues. They produce large numbers of larvae called microfilariae .

Human Filarial Species

There are eight filarial species in which humans are the definitive hosts

Species Causing LYMPHATIC FILARIASIS

Wuchereria bancrofti

Brugia malayi

Brugia timori

ONCHOCERCIASIS- RIVERBLINDESS

Onchocerca volvulus

EYE WORM DISEASE

Loa loa

OTHER SPECIES (Generally considered to be non pathogens)

Mansonella ozzardi

- causes pruritus

Mansonella perstans

- causes localized angio edema and pruritus and neurological symptoms

Mansonella streptocerca

- cause pruritus, rash, hypopigmentation

Life Cycle of Human Filarial Worms

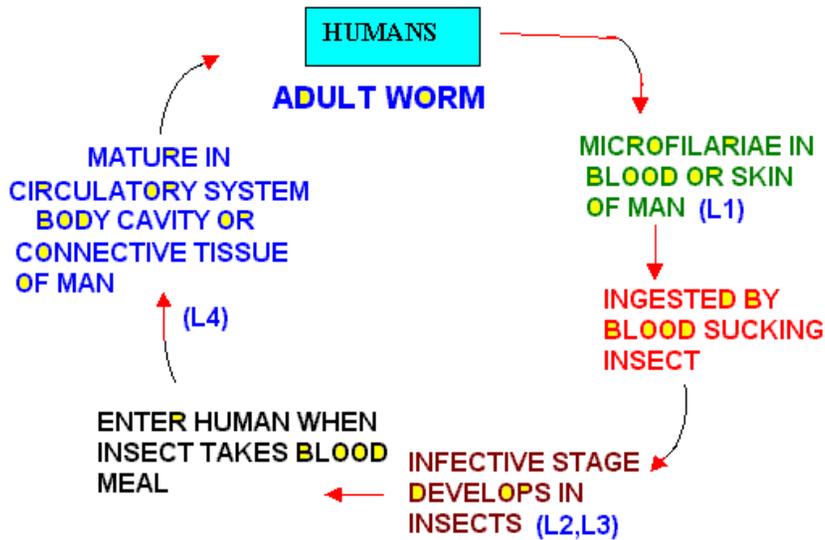


Figure 24

Adult Filarial Worms

- Filarial worms are arthropod-transmitted parasites
- They inhabit lymphatic, subcutaneous, and cutaneous tissues of humans
- Adult male is smaller than females
- Adults are sequestered in the tissues
- All female worms produce primitive larvae called “microfilariae”

Microfilariae

- These microfilariae are found in peripheral blood or in the skin
- They are relatively simple in their organization and structure
- They are vermiform
- In stained preparations they appear to be composed of a column of nuclei

- These nuclei are interrupted along their length by spaces and special cells
- These cells are precursors of body organelles.
- Some species of **mf** are enveloped in sheath others do not have sheath

Filarial Parasites	Location	Disease
<i>Wuchereria bancrofti</i>	Lymphatics	Elephantiasis
<i>Brugia malayi</i>	Lymphatics	Elephantiasis
<i>Brugia timori</i>	Lymphatics	Elephantiasis
<i>Loa Loa</i>	EyeSubcutaneous	Calabar Swelling
<i>Onchocerca volvulus</i>	Subcutaneous	Onchocercomata
<i>Mansonella perstans</i>	Abdominal Cavity	Mild Allergic Reactions
<i>Mansonella ozzardi</i>	Body Cavity	
<i>Mansonella streptocerca</i>	Subcutaneous Fat Subcutaneous	

Lymphatic Filariasis

The following clinical manifestations may be seen

1. Asymptomatic disease
2. Filarial fevers
3. Chronic disease
4. Tropical pulmonary eosinophilia

Asymptomatic Disease

- Commonly seen in endemic areas
- Frequently associated with Bancroftian and Brugian filariasis
- Infected individuals never become symptomatic
- Many chronically infected persons are amicrofilaraemic

Filarial Fevers: Initially

- Acute attacks of fever chills and malaise seen
- It lasts for 3-15 days each
- It is associated with retrograde adenolymphangitis
- This can occur several times a year
- Lymphadenitis affects commonly groin and axilla
- In men infected with *W.bancrofti*
 - Lymph vessels of genitalia are affected
 - This leads to funiculitis, orchitis, epididymitis

Chronic Disease

- Occurs after 10-15 years of filarial fever (6months in non endemic persons)
- Episodes of chronic obstructive disease due to damages to lymph vessels seen
- Reversible lymphedema of legs, scrotum, arms, penes, or breasts present
 - This may progress to elephantiasis
- Chyluria results from rupture of renal lymph vessels
- Hydrocele is due to recurrent orchitis

Tropical Pulmonary Eosinophilia

- It is a well recognized clinical entity in India and Singapore
- It is attributed to occult filarial infection
- It is characterized by paroxysmal nocturnal asthma and cough
- It is associated with high eosinophilia
- Can be relieved by DEC
- There is high titer of antibodies to microfilariae
- Microfilariae have been demonstrated in the lung
- Small eosinophilic granulomas with mf are scattered throughout the lung

- This condition may have resulted from altered host response to parasite, resulting in allergic phenomenon manifested by persistent hyper eosinophilia and pulmonary symptoms

Laboratory Diagnosis Of Filariasis

DEMONSTRATION OF ORGANISMS	
Direct Demonstration	Indirect Demonstration
<ol style="list-style-type: none">1. Demonstration of organisms2. Demonstration of Components of organisms	<p>Demonstration of response to organisms</p> <ol style="list-style-type: none">1. Non specific response2. Specific response

Specimens to be collected

1. Blood : Nocturnal species/area – Night blood 10-12pm
: Diurnal - Day time
2. Chyluric urine:
3. Exudate of lymph varix
4. Hydrocele fluid
5. Lymph node biopsy – not recommended for pure diagnostic purpose
6. Ultrasound examination: shows characteristics filarial dance

Examination of blood and other body fluids

1. By thick smear examination
2. By thin smear examination Giemsa stained smears
3. Saponin lysis and centrifugation- staining
4. Knott concentration method
5. Membrane filter concentration.

Demonstration of Components of the organisms

1. Precipitation using antisera
2. Counterimmuno Electrophoresis
3. Elisa
4. PCR

Demonstration of response to organisms

Responses include Non specific responses and Specific responses

Non specific responses

1. **Filarial lymphangitis**- must be differentiated from bacterial lymphangitis

2. **Chronic lymphedema**-

May also be caused by malignancy, renal and cardiac failure, post operative changes, congenital malformations

3. **Architis and epididymitis**

In sexually transmitted diseases also these may be present

4. **Tropical pulmonary eosinophilia**

May be confused with Loeffler's syndrome, Broncho pulmonary aspergillosis, eosinophilic pneumonia, drug reactions, other parasitic infections(Hook worms, Ascaris, Stongyloides). Typical clinical history, exposure in appropriate areas, response to DEC will help with the diagnosis

Specific responses

1. Humoral immune responses
 - a. Antibody response
 - b. Hypersensitivity response
2. Cell mediated immune responses
 - a. CMI
 - b. CM hypersensitivity responses

Antibody responses

Antibodies can be detected by the following tests

In the past:

1. Gel diffusion
2. Complement fixation
3. Indirect immunofluorescence assays

At present

4. indirect haemagglutination test
5. ELISA test

EXERCISE

Points to remember

1. Different filarial worms
2. Life cycle of filarial parasite
3. Lymphatic filariasis and its consequences

Self Evaluation

1. List human filarial worms
2. Give a list of filarial species that cause lymphatic filariasis
3. Describe the life cycle of W.bancrofti
4. What are the clinical manifestations seen in lymphatic filariasis?
5. Describe the laboratory diagnosis of filariasis
6. What are the different methods adopted for the examination of blood for microfilariae?
7. How are the components of the filarial parasite demonstrated from blood?
8. State the features of tropical pulmonary eosinophilia

POLIOMYELITIS

Introduction

Poliomyelitis is an acute illness with pain and flaccid paralysis affecting mainly lower limbs and is caused by three polio viruses.(poliovirus1,2 and 3)

Polioviruses belong to the family of enteroviruses which multiply in the gut and rarely cause intestinal symptoms.

Characteristics of enteroviruses- Polio virus

These viruses enter the body via ingestion by mouth. They multiply in the lymphoid tissues of the alimentary tract including the pharynx. From the gut they enter the blood (viremia) or shed into the lumen of the intestine.

Virology

Polio virus belongs to Picorna virus (pico = small + RNA).

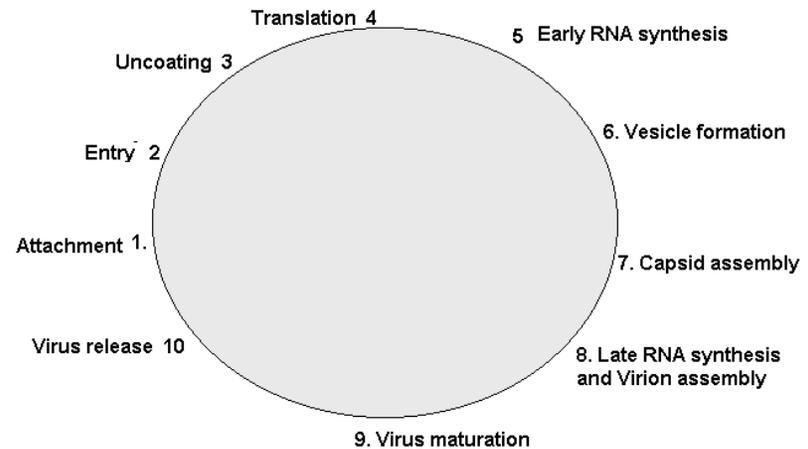
They are RNA viruses, having single stranded positive sense RNA.

Viruses are small roughly spherical particles, 25-30 nm in size, stable at acid pH.

They grow rapidly in tissue cultures and produce cytopathic effects.

Replication of polio virus

1. Polio virus attaches to the specific receptors on the cell surface. If the cell is not possessing the receptor it is not susceptible to infection
2. The virus enters the cell by micropinocytosis
3. Then uncoating of capsid proteins occurs. The viral RNA is released into the cytoplasm



4. Viral RNA is a plus strand and it can act as a messenger RNA. So early translation (protein synthesis) occurs.
5. At the same time Viral RNA synthesis also occurs.
6. During this time a vesicle is formed inside the cells
7. Inside the vesicle assembly of viral components occur
8. Afterwards late viral RNA translation occurs leading to further synthesis of viral proteins
9. Finally maturation of virus occurs
10. The virus is released after the rupture of the cell and the cycle continues

Pathogenesis and Clinical features

Most infections are confined to alimentary tract and are symptomless. A small proportion of infections show fever. Still very few cases progress to aseptic meningitis and to paralysis.

Poliomyelitis is an acute illness with pain and flaccid paralysis affecting mainly the lower legs. Some times the muscles of respiratory tract may be involved. Paralysis is an extension of aseptic meningitis and is therefore accompanied by the signs and symptoms of meningitis

which include fever, head ache with stiffness of neck. Paralysis is due to viral damage to the cells of the anterior horn of the spinal cord with lower motor neurone lesions resulting in flaccid paralysis

Epidemiology

Enterovirus infections are common in children and in conditions of poor hygiene. Infections spread mainly by fecal-oral route.

Laboratory diagnosis and control

Faeces, throat swab and blood are collected for viral isolation. These specimens are inoculated into monkey kidney cell cultures and observed for cytopathic effects. The virus can be identified by neutralization using specific antiserum

Control

Two types of vaccines are available for three polioviruses.

1. Sabin live attenuated virus vaccines. It is administered in three oral doses
2. Salk inactivated virus vaccine: It contains three polio viruses inactivated by formalin and is given in three injections.

EXERCISE

Points to remember

1. What is poliomyelitis and the characteristics of the disease?
2. Spread of poliovirus and its replication
3. Control of poliomyelitis

Self evaluation

1. What is poliomyelitis?
2. Give the characteristics of polio virus
3. Describe the replication of polio virus
4. Describe the pathogenesis of poliomyelitis
5. Give the laboratory diagnosis of poliomyelitis
6. Name the vaccines used for poliomyelitis

INFLUENZA

Influenza is one of the great epidemic diseases. From time to time it becomes pandemic and spreads through out the world.

Influenza virus belongs to the family Orthomyxoviridae. Three immunological types are known. They are influenza type A, B and C viruses. Influenza type A is highly variable antigenically and is responsible for most cases of epidemic influenza, B shows less variation and causes less frequent epidemics, C is antigenically stable and causes only mild infection.

Structure of the virus

Influenza virus is a RNA virus. There are eight separate single stranded negative sense fragments. Each segment is a gene and codes for a different protein viz. haemagglutinin and neuraminidase etc. Viruses are roughly spherical particles, medium size, 80-100 nm, with an envelope. The envelope contains radially projecting spikes of virus haemagglutinin and neuraminidase. Inside the envelope is situated nucleocapsid, nucleic acid surrounded by protein capsomeres. The virus agglutinates the RBCs of many animal species. It grows on monkey kidney cell cultures and in amniotic cavity of developing chick embryo.

Influenza virus replication

The virus attaches to the cell surface sialic acid via the receptor present on the tip of haemagglutinin. Viral particles are then internalized within endosomes. The endosome fuses with the cell membrane leading to uncoating. Viral nucleocapsids are released into the cell cytoplasm. Viral transcription occurs in the nucleus. Viral proteins are synthesized in the cytoplasm. Viral RNA synthesis occurs with the

production of positive strand complete copies of each segment. After viral assembly the mature virus is released by budding off the cell.

Clinical features

Virus enters by inhalation of respiratory secretions from an infected person. The incubation period is 1-4 days. The signs and symptoms include fever, malaise, head ache, generalized aches, sometimes with nasal discharge and sneezing. There may be sore throat and hoarseness. The symptoms last for 4 days but tiredness and weakness persist for long. The virus multiplies in superficial epithelium of the upper and lower respiratory tract. Influenza causes damage to cilia and desquamation of the epithelium. Few patients may develop pneumonia. Secondary bacterial infections may also be seen.

Control

Influenza virus undergoes antigenic variations from time to time. Epidemics are due to the emergence of new virus strains containing new haemagglutinin and neuraminidase. When major change occurs it is called antigenic shift. If minor change occurs it is called antigenic drift. At the time of pandemic, the speed with which new strain spreads makes it difficult to prepare sufficient quantity of vaccine. Current vaccine contains inactivated virus grown in eggs and either purified or disrupted and purified. It is given subcutaneously and the protection is short lived.

EXERCISE

Points to remember

1. What is influenza
- 2 The control of influenza

Self evaluation

1. What is influenza?
2. Describe the structure of influenza virus
3. Describe the replication of influenza virus
4. Give the characteristic features of influenza
5. Describe the methods adopted for the control of influenza

Chapter 27

ZOONOTIC DISEASES

Association between man and animals are there since the beginning of earth. Man's association with animals became more intimate that certain diseases are transmitted from animals to man and vice versa. Zoonotic diseases are transmissible between man and animals. Zoonotic diseases spread by contact with infected animals by handling them or living in their premises. Transmission is through direct contact or aerosol spread. Drinking milk from infected animals is another way of getting zoonotic infections. Insects could transfer mechanically or biologically different zoonotic diseases. These diseases are classified as follows:

Zoonoses	Animals \leftrightarrow	Man
Anthrapozoonoses	Animals \rightarrow	Man (Rabies)
Zooanthroponoses	Man \rightarrow	Animals(Diphtheria)
Cyclozoonoses	Animal \leftrightarrow	Man (Hydatidosis, Taeniosis)
Metazoonoses	Animals \rightarrow	Man (through insect vector: Malaria)
Saprozoonoses	Animals \rightarrow	Man (through soil)

Anthrax

Anthrax and Brucellosis are two of the many bacterial zoonotic diseases. Anthrax is a disease caused by *Bacillus anthracis*. It belongs to the genus *Bacillus*. Organisms belonging to the genus *Bacillus* are gram positive spore forming bacilli, present ubiquitously in all environments. Many of them do not cause disease in man. Anthrax is an important disease of man and animals and it could be used a biological warfare agent.

Bacillus anthracis

The typical organism is a gram positive bacillus, measuring 1x3-4 µm, have square ends and are arranged in long chains. Spores are located in the center of the non motile bacillus. It can grow on ordinary laboratory media like nutrient agar and blood agar. It does not produce haemolysis on blood agar, shows a cut glass appearance, liquefies gelatin.

Anthrax

Anthrax is primarily a disease of sheep, cattle, horse, and many other animals; humans are rarely affected. Infection is acquired by the entry of spores through injured skin or mucous membrane; rarely spores enter the lungs by inhalation. In animals portal of entry is mouth and gastrointestinal tract.

Spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in the formation of edema. Organisms spread through lymphatics to the blood stream and they multiply in the blood and tissues. Inhalation anthrax is also called wool sorter's disease. Infection is acquired by the inhalation of spore present on materials from the animal source. Rapid multiplication of the organisms in the tissue is fatal.

Treatment

Penicillin is the drug of choice. The organism is susceptible to macrolids, aminoglycosides, tetracycline and chloramphenicol. Ciprofloxacin or other fluoroquinolone is recommended for prophylaxis.

Immunization

Live attenuated bacilli were first used by Louis Pasteur in 1881. It gave very good protection in domestic animals. Now the Sterne strain of live spore vaccine is used for animal immunization. Live bacterial vaccine is not safe for human use. Alum precipitated Toxoid has been used to immunize the workers at risk of exposure.

EXERCISE

Points to remember

1. Different types of zoonoses
2. Anthrax and its implications

Self evaluation

1. Define zoonosis
2. Classify different types of zoonosis with example
3. What is anthrax? Give the name of the agent causing it
4. Give the characteristics of B.anthraxis
5. Describe the method of prevention of anthrax in animals

Chapter 28

RABIES

Rabies is a zoonotic disease. Animals act as reservoir of infection. Man gets the infection from the animals

Rabies is a lethal form of encephalitis caused by rabies virus, transmitted through the bite of an infected animal usually a dog.

Properties of the virus

- The virus is bullet shaped
- It is covered by a membranous envelope
- Has protruding spikes 10 nm long
- Spike is composed of a single glycoprotein
- The genome is single stranded RNA- is a minus strand RNA
- Genome contains RNA dependent RNA polymerase
- When freshly isolated from a case, the virus is called “street” virus
 - It can multiply in neural and non-neural tissues
- Serial brain to brain passage in rabbits yields a fixed virus
 - It can not multiply in non-neural tissue

Pathogenesis and Pathology

- Virus is introduced into the tissue by the bite of a rabid animal
 - Canines
 - Bats etc
- Virus multiplies in the muscle or connective tissue

- Infection ascends through the tissue spaces of the sensory nerves to the CNS
- It multiplies in the CNS
- Spreads through peripheral nerves to salivary glands and tissues
- Rabies virus has not been isolated from the blood of an infected person
- The incubation period depends on:
 - Amount of viral inoculum
 - Severity of the bite
 - The distance the virus has to travel from its point of entry to the brain
 - If bitten on the face, higher attack rate and shorter incubation period seen
- There is nerve cell destruction in the cortex, mid brain, basal ganglion, pons and medulla
- It produces cytoplasmic inclusion bodies in nerve cells called Negri bodies.

Clinical features

The incubation period is from 4 to 12 weeks, some times it may be much longer. If the bite wound is in the neck or head the incubation period is shorter

The virus spreads from the wound to the central nervous system through nerves.

Symptoms

The symptoms may be either furious or dumb. In the furious type, the patient shows the symptoms of excitement, with tremor, muscular contractions and convulsions. When there is spasm of muscle of swallowing, patients show fear of water and hence the name hydrophobia is also given to rabies.

In the dumb type of rabies, ascending paralysis is seen involving the muscles of swallowing speech and respiration.

Virus is present in saliva, skin and eyes and brain

The disease is always fatal following convulsions.

Epidemiology

Rabies is a natural infection seen in dogs, cats, bats and carnivorous animals such as foxes and wolves. Man acquires the infection generally from infected dogs. Virus is present in the saliva of the infected dog. Infected dog dies within ten days. If the dog remain healthy for ten days after biting, it can be regarded as being free of the virus at the time of biting. Aerosol infection has been recorded as a result of laboratory accident. Human patients do not seem to be a source of infection.

Diagnosis

Direct demonstration of virus

Specimens such as hair bearing skin from the back of neck, corneal impression smears and brain tissue are examined for virus by immunofluorescence

Intracytoplasmic inclusion body called Negri body can be demonstrated by conventional microscopy

Virus isolation

Attempt to isolate the virus from brain tissue, saliva, CSF and urine from patients can be made by inoculating into mice and the animal is observed for convulsions, paralysis and postmortem examinations for Negri bodies in the brain tissue.

Vaccination

Rabies vaccine was first developed by Pasteur in 1885. It consisted of virus attenuated by drying the spinal cord of infected rabbits for varying length of time over KOH. Wild rabies virus is called street

virus and attenuated virus is called fixed virus. All vaccines prepared for human use contain inactivated virus.

Now human diploid cell vaccine (HDCV) is used for vaccination. It contains inactivated virus grown on WI38 or MRC-5 diploid human embryo lung cells

EXERCISE

Points to remember

1. Rabies is an untreatable disease.
2. The pathogenesis and pathology of rabies.
3. Prevention of rabies.

Self evaluation

1. What is rabies?
2. Give the properties of rabies virus.
3. Describe the pathogenesis and pathology of rabies.
4. State the symptoms of rabies.
5. State the reasons for hydrophobia in rabies.
6. Describe the epidemiology of rabies.
7. Describe the diagnosis of rabies.
8. Describe the prophylaxis against rabies.

STRUCTURE AND DEVELOPMENT OF IMMUNE SYSTEM

Immunity

Introduction

Immune system consists of lymphoid cells (both T and B cells) and lymphoid organs. Cells that make up the immune system are distributed throughout the body. They occur predominantly in lymphoreticular organs. These include: Bone marrow, thymus, lymph nodes, spleen, mucosa associated lymphoid tissues. The cells occupy the intricacies of net works formed by reticular cells and fibers supporting framework. The cells involved in the immune function are: Lymphocytes, monocytes, macrophages of tissues, endothelial cells, mast cells, basophils, eosinophils, neutrophils etc. All cells are derived from pluripotent, self renewing stem cells of the bone marrow.

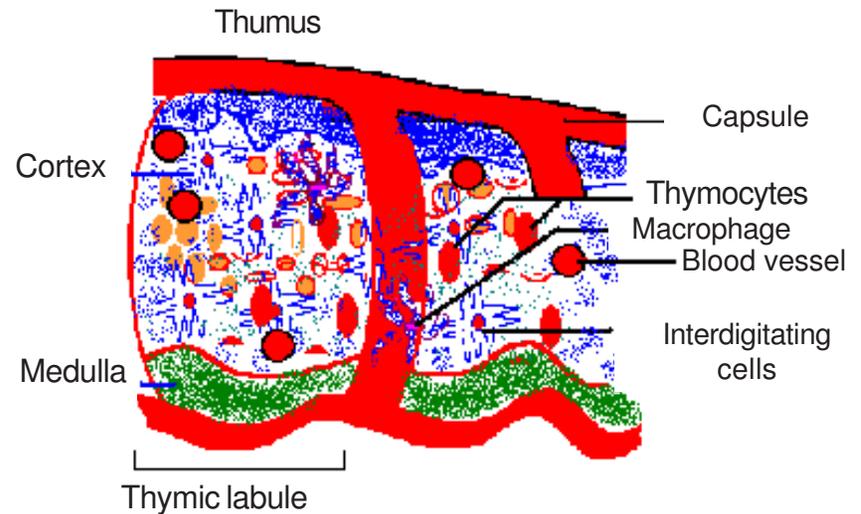
Organs of immune system

Based on the different roles they perform, lymphoid organs can be classified into 1) primary or central lymphoid organs and 2) secondary or peripheral lymphoid organs. Primary lymphoid organs include 1) Thymus, 2) Bone marrow 3) Bursa of Fabricius in birds. In the primary lymphoid organs, the T and B cells mature into antigen recognizing lymphocytes. Secondary lymphoid organs include Lymph nodes, spleen and mucosa associated lymphoid tissue.

Thymus gland

The thymus gland is a bilobed structure. During fetal development the size of the thymus gland increases and reaches its maximum at birth. After birth the thymus begins to decrease in size and undergoes atrophy with aging.

Thymus structure: Fig (29.1)



The thymus gland contains (1) cortex and (2) medulla

Cortex

The cortex contains epithelial cells. It is densely populated with lymphocytes of various sizes. Most of the lymphocytes in thymus are immature.

Medulla

T lymphocytes mature in the cortex and migrate into the medulla. They leave the medulla and enter the peripheral blood circulation. Then they are transported to the secondary lymphoid organs.

Function of the thymus gland

1. The primary function of the thymus is the production of Thymic lymphocytes
2. Thymus is the major site for lymphocyte proliferation in the body

3. One percent of the lymphocytes produced in the thymus leave the thymus, others are destroyed.
4. Lymphocytes produced in the thymus are called T lymphocytes or T cells
5. In the thymus the lymphocytes are educated so that they can produce cell mediated immune (CMI) response against foreign antigens
6. Immature lymphocytes mature in the thymus and acquire different Cell differentiation (CD) molecules on their surface

Effects of thymectomy (removal of thymus)

Removal of thymus is called thymectomy. Thymus can be removed either in the neonates or in adults.

Neonatal thymectomy results in an immediate severe reduction in the quality and quantity of T lymphocytes.

Adult thymectomy could result in deficiency of T cell, after the eventual death of T cells that originally populated the secondary lymphoid organs

Thymectomy affects CMI primarily. It also diminishes antibody response to certain antigens

Bone Marrow

Bursa of Fabricius

It is a primary lymphoid organ of birds which is situated near the cloaca. It consists of lymphoid centers that contain epithelial cells and lymphocytes. These lymphocytes are antibody producing cells. The B cells undergo maturation in this organ. Like thymus the bursa is largest at hatching and undergoes atrophy with maturation.

- Mammals do not have Bursa of Fabricius

- A structure in mammals that functions equivalent to that of birds is the bone marrow

Functions of Bursa of Fabricius

1. It produces B lymphocytes. These are responsible for antibody production and Humoral immunity
2. These B cells mature and go to the peripheral lymphoid organs and are seeded there
3. Following appropriate antigenic stimulation, B lymphocytes transform into plasma cells and produce antibodies

Secondary Lymphoid Organs

Lymph Node

Lymph nodes are small ovoid structures found in various regions through out the body. They form channels called as lymphatic channels through which lymph flows

A Lymph node is surrounded by a fibrous capsule. It has an outer cortex and inner medulla

Cortex

In the cortex the lymphocytes accumulate into primary and secondary follicles. These follicles contain lymphocytes and macrophages

Medulla

In the medulla the lymphocytes are arranged as elongated branching bands or medullary cords. The cortical follicles and medullary cords contain B lymphocytes and constitute bursa/bone marrow dependent areas. Between the cortical follicles and medullary cords, there is a broad, ill-defined intermediate zone called para cortical area. The para cortical area contains T lymphocytes and constitutes the thymus dependent area

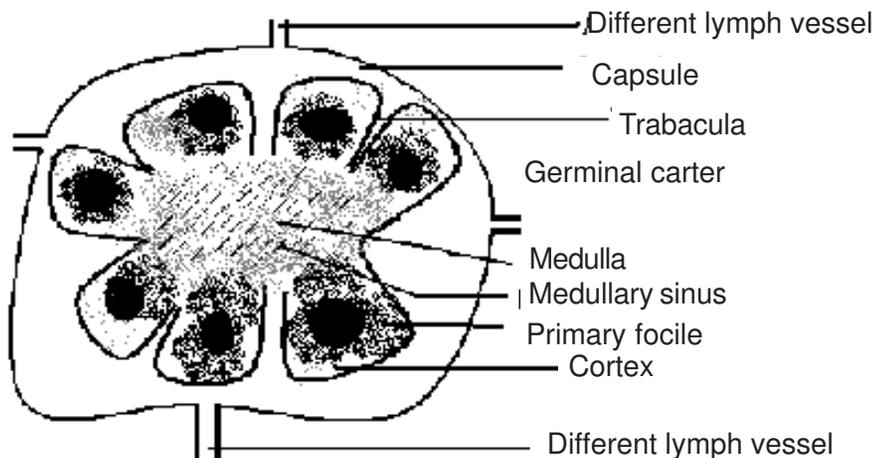


Fig:29.2
Functions of lymph node

1. Lymph nodes act as filter for lymph
2. Phagocytosis of foreign particles takes place in lymph node
3. They help the proliferation and circulation of T and B cells
4. They enlarge following local antigenic stimulus
5. The plasma cells secrete antibodies
6. Lymph node is highly efficient in trapping antigen

Spleen

Spleen is the largest of the lymphoid organs. It has a capsule from which descend trabaculae dividing the organ into several interconnected compartments. Spleen is made up of white pulp and red pulp

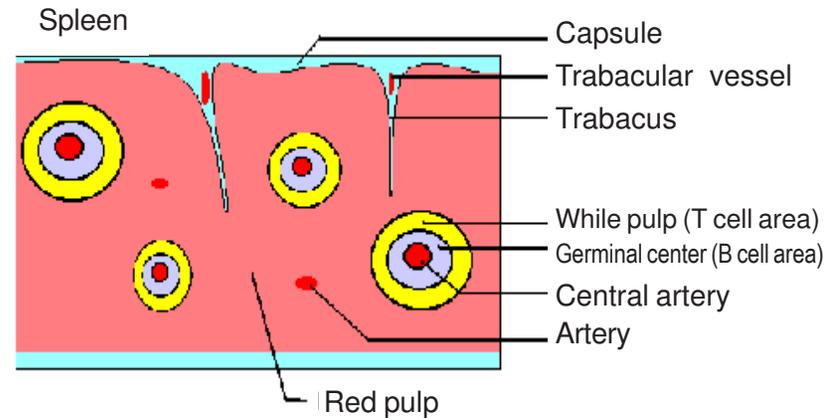


Fig:29.3

White pulp

The splenic artery travels along the trabaculae. On entering the spleen the artery divides into arterioles which are surrounded by lymphoid tissues. This part is called white pulp. White pulp is rich in lymphoid cells.

Red pulp

It is called red pulp because of the red blood cells. Macrophages also are present in this area

Functions of spleen

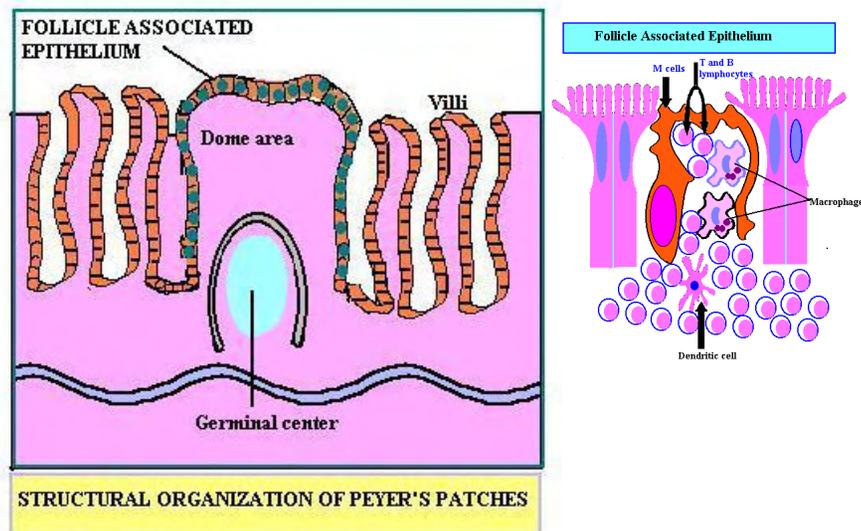
1. The spleen serves as the grave yard for red blood cells
2. The white pulp is rich in T cells and B cells
3. The T cells are seen peripherally and the B cells are seen centrally
4. After an antigenic stimulus the germinal center containing large number of B cells and plasma cells appear in the periarteriolar areas and almost replace T cells.

Mucosa associated lymphoid tissue

The mucosa lining the alimentary, respiratory, genito-urinary and other surfaces is exposed to antigens. So these areas have rich collection of lymphoid cells

These lymphoid cells are called Peyer's patches or scattered isolated lymphoid follicles. These are called as Mucosa associated lymphoid organs (MALT).

Lymphoid tissues in the gut, ie from the adenoids and tonsils up to the follicles of colon are called Gut associated lymphoid tissues (GALT)



Functions of MALT and GALT

1. They contain lymphoid and phagocytic cells and a special cell called M cells
2. Both B and T cells are present
3. Predominant antibody produced is IgA in the mucosal lining. Others such as IgG, IgM, and IgE are also produced.
4. Ig A antibodies provide first line of defense against infectious agents

EXERCISE

Points to remember

1. Different organs of immune system
2. Structure of organs that contribute to immunity
3. Functions of various organs of immune system

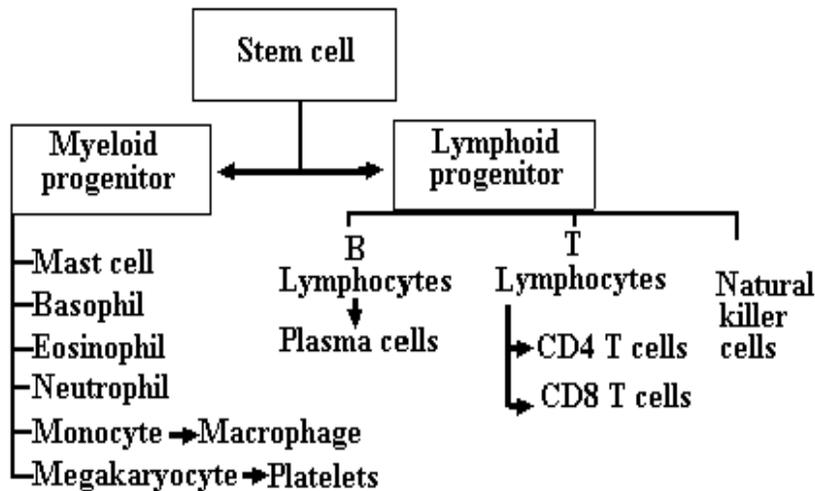
Self evaluation

1. What constitutes immune system?
2. Classify the lymphoid organs
3. Describe the structure of thymus gland
4. State the functions of thymus gland
5. Mention the effects of thymectomy
6. Describe Bursa of Fabricius and its functions
7. Describe the structure of lymph node
8. State the function of lymph node
9. Describe the structure of spleen
10. Describe the structure of Payer's patches
11. Mention the function of MALT

Chapter 30

CELLS OF THE IMMUNE SYSTEM

Immune system consists of many organs and different cell types. The cells of the immune system develop from pluripotent stem cells from bone marrow. The haematopoietic stem cells give rise to red blood cells. White blood cells develop through two different pathways. The lymphoid lineage produces both T and B lymphocytes, and Natural killer (NK) cells. The myeloid lineage produces monocytes, macrophages, granulocytes basophils eosinophils, neutrophils, platelets and also mast cells.



Lymphocytes

T cells:

Origin:

All cells of lymphoid system arise from the pluripotent stem cells from the bone marrow

Maturation:

Maturation of T cells takes place in different stages. Thymus secretes a humoral factor that attracts the T cells to the thymus.

Stage I

Pro T cells or Early thymocytes

They are present in thymic cortex.

These are the earliest recognizable thymocytes. They contain cell differentiation molecules CD2 and CD7. CD3 proteins are present in the cytoplasm. Transferrin molecule is also present

Stage II

Intermediate or common thymocytes

These are present in thymic cortex. They constitute 85% of lymphocytes at this stage. Additional markers like CD1, CD4 and CD8 are also present. They are called double positive cells because they contain both CD4 and CD8 markers. Genes coding for the α chain of TCR2 molecules are rearranged in these thymocytes

Stage III

Mature thymocytes.

This development takes place in thymic medulla. There is loss of CD1, CD 38 and transferrin molecules.

CD3 molecule is present on the cell membrane in association with TCR2 molecules. There is distinction of two cell markers CD4 and CD8. The T cells that possess CD4 are called CD4 T cells and the ones possessing CD8 are called CD8 T cells

Functional sub sets of T cells

Based on their functions the T cells are subdivided into the following:

(1) T helper cell, (2) T suppressor cells, (3) T cytotoxic cells (4) T cells involved in delayed hypersensitivity (DTH) reaction

B cell development

Pre B cells to mature B cells

B cells like other blood cells arise from pluripotent haematopoietic stem cells. In embryogenesis, the first B cell lineage makes μ heavy chain but not light chain. More differentiated cells called pre B cells are detected in the fetal liver about half way through gestation. They appear as large and small lymphocytes with cytoplasmic IgM without surface Immunoglobulin (Ig) molecules. Similar cells are seen in small numbers in adult bone marrow indicating that B cells continue to arise from stem cells throughout the life.

Immature B cells

Pre B cells give rise to immature B cells which have surface IgM (sIgM). They lack several other surface molecules.

Mature B cells

These arise from immature B cells. They contain two or more surface Ig isotypes along with sIgM.. They also have Fc receptors and complement receptors.

They are not easily made tolerant. In the terminal differentiation the mature B cells are stimulated by T dependent antigen to develop into plasma cells which secrete antibody molecules

EXERCISE

Points to remember

1. Know the importance of T cells
2. Know the importance of B cells
3. Understand the different kinds of cells that carry out the immune functions

Self evaluation

4. Give a list of various cells of the immune system and their origin
5. Describe the characteristics of T cells
6. Describe the characteristics of B cells
7. Describe the subsets of T cells and their functions
8. Describe the development of B cells and their functions

Chapter 31

INNATE AND ADAPTIVE IMMUNITY

Introduction

Immunity is a state of protection from infectious diseases. It has two components namely nonspecific and specific immunities. The non-specific component is called *innate immunity*. Innate immunity is defined as disease resistance mechanisms that are not specific to a particular pathogen. The specific component is called *acquired immunity or adaptive immunity*.

Adaptive immunity shows a high degree of specificity and memory and requires more time to develop. Innate immunity provides the **first line of defense** during the critical period just after the host's exposure to a pathogen. A healthy individual when exposed to a variety of microorganisms the innate immunity clears most of them within a few days. During this time the adaptive immune response is not triggered. However if the innate immunity fails and the microorganisms try to invade the tissues the specific immune response of adaptive immunity is triggered.

Innate and adaptive immunity do not operate independently. They function as a highly interactive and cooperative systems.

Innate immunity consists of four types of defensive barriers namely anatomic, physiologic, phagocytic and inflammatory barriers.

Structures contributing to innate immunity

Type	Mechanism
Anatomic Barriers	
Skin	Acts as mechanical barrier. It retards entry of microbes. Acidic environment of sweat (pH 3-5) retards growth of microbes

Mucous membranes	Mucus entraps foreign microorganisms. Cilia propel microbes out of body. Normal flora competes with microbes for attachment sites and nutrients.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.
Low pH	Acidity of stomach contents kills most ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or facilitates phagocytosis
Phagocytic/Endocytic barriers	Various cells internalize (endocytose) and break down foreign macromolecules. Specialized cells (blood monocytes, neutrophils, tissue macrophage) internalize, kill and digest whole microorganisms.
Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into the affected area.

FACTORS CONTRIBUTING TO INNATE IMMUNITY: STRUCTURE AND ROLE PLAYED

Anatomic Barriers

In the first line of defense against infection, physical and anatomic barriers try to prevent the entry of pathogens. The skin and the surface of mucous membranes effectively prevent the entry.

Skin

The skin consists of two distinct layers. A thinner outer layer is called the **epidermis** and a thicker inner layer is called the **dermis**. The epidermis contains many layers of tightly packed epithelial cells. The outermost epidermal layer consists of dead cells and is filled with a hydrophobic protein called keratin. The dermis, which is composed of connective tissue contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called **sebum**. Sebum consists of lactic acid and fatty acids. These acids maintain the pH of the skin between 3 and 5. This low pH inhibits the growth of most microorganisms. Intact skin prevents the entry of pathogens and also its low pH inhibits most bacterial growth. Breaks in the skin, even small ones, result in the entry of pathogens to cause infections. The skin is also penetrated by biting insects (mosquitoes, mites, ticks, fleas, flies); if these insects harbor pathogenic organisms, they can introduce the pathogens into the body as they feed.

Mucous membranes

The conjunctiva and the alimentary, respiratory, urogenital tracts are lined by mucous membranes. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. A number of nonspecific defense mechanisms exist to prevent the entry of pathogens. For example, saliva, tears, and mucous secretions act to wash away potential invaders. They contain antibacterial and antiviral substances. The viscous fluid called mucus, which is secreted by epithelial cells entraps foreign microorganisms. In the respiratory and gastrointestinal tract, the mucus membrane is covered by cilia. The synchronous movement of cilia propels mucus-entrapped microorganisms towards the exterior from these tissues. In addition, nonpathogenic organisms tend to colonize epithelial cells of mucosal surfaces. These normal flora generally compete with pathogens for attachment sites on the epithelial cell surface and for necessary nutrients. Mucus also contains the enzyme lysozyme which lyses the bacterial cell. Mucoproteins present in the mucus also inhibit the haemagglutinins of influenza virus

Many pathogens enter the body by binding to and penetrating mucous membranes, overcoming the protective effects.

Physiologic Barriers

The following act as physiologic barriers of innate immune response:

1. Antibodies against blood group antigens
2. Alternate pathway of complement system
3. Macrophages
4. Interferons
5. $\gamma\delta$ Cells
6. CD5-B Cells

Also, the physiologic barriers that contribute to innate immunity include temperature, pH and various soluble factors. Many animal species are not susceptible to certain diseases simply because their normal body temperature inhibits the growth of the pathogens. Chicken for example have innate immunity to anthrax because their high body temperature inhibits the growth of the bacteria. Gastric acidity acts as physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents. In the mouth, streptococci produce peroxides that compete with bacteria for iron and enhance respiratory activity in neutrophils. Many soluble factors contribute to nonspecific immunity such as the enzyme lysozyme, interferon, and complement.

The flushing action of urine combined with the low pH of the urinogenital tract prevents the pathogens from establishing in the urinogenital tract. Desquamation of epithelial cells from the vaginal wall in adult women provides a substrate for lactobacilli growth. These bacteria produce lactic acid, and also compete with pathogenic bacteria for nutrients and space.

The flushing action of milk in the mammary gland that contains lactenins, bacterial inhibitors, iron binding protein lactoferrin lactoperoxidase, and IgA enhancers also contributes to innate immunity. Phagocytic cells released into the mammary gland caused in response to

irritation due to sucking contributes to phagocytic action, lactoferrins and hydrogen peroxide.

Antimicrobial peptides: Cells of many animals produce antimicrobial substances that act as endogenous natural antibiotics or disinfectants. These micropeptides take many forms.

α-Defensins: There are six known human alpha defensins. Four belong to neutrophils and the other two are present in vagina and cervix

β-Defensins: Large amounts of β-Defensins appear in Henley's loop, distal and collecting tubules of kidney and also in the vagina, cervix, uterus and fallopian tubes. These peptides have broad spectrum, salt-sensitive antibacterial activity and show synergy with lysozyme and lactoferrin.

Cathelicidins: Humans express only one cathelicidin, a prepropeptide that is released after neutrophil elastase action.

Protegrins: They are broad-spectrum antimicrobial peptides found in porcine neutrophils, where they are stored as cathelin containing precursors.

Granulysin: They are found in granules of human cytolytic T lymphocytes and natural killer cells. They act in combination with perforins, gain access to intracellular compartment of microbes and kill them.

Histatins: These are small histidine-rich human salivary proteins that display moderate activity against *Candida albicans* at acidic pH and also have antifungal actions.

Lysozyme: It is a hydrolytic enzyme found in mucus secretions and tears, and is able to cleave the peptidoglycan layer of the bacterial cell wall.

Interferon: comprises a group of proteins produced by virus-infected cells. One of the many functions of the interferons is the ability

to bind to nearby cells. It also induces a generalized antiviral state. There are three different types of interferons IFN- α , IFN- β and IFN- γ . They are synthesized by leucocytes on exposure to viruses, fibroblasts and effector T cells on induction respectively. The second effect of interferons in host defense is to increase expression of the MHC class I complex and TAP transporter proteins, enhancing the ability of virus-infected cells to present viral peptides to CD-8 cells. The third property is the activation of natural killer cells.

Complement is a group of serum proteins that circulate in an inactive state. A variety of specific and nonspecific immunologic mechanisms can convert the inactive forms of complement proteins into active form. When activated complement can cause damage to the membranes of pathogenic organisms, so that they are either destroyed or phagocytosed and cleared

Phagocytic Barriers

Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis. Phagocytosis is a phenomenon in which there is uptake of material by a cell from its environment. In phagocytosis, a cell's plasma membrane expands around the particulate material to form large vesicles called phagosomes. Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages. Phagocytosis may be enhanced by a variety of factors collectively termed as *opsonins* which consist of antibodies and various serum components of complement.

Polymorphonuclear (PMN) leucocytes also referred to as granulocytes include basophils, mast cells, eosinophils and neutrophils. These short-lived phagocytic cells contain lysosomes filled with hydrolytic enzymes. They play a major role in protection against infection.

Macrophages: These cells enter the blood as monocytes and migrate to different tissues. In these tissues they undergo different changes. The monocyte is a small, spherical cell with few projections, abundant

cytoplasm, little endoplasmic reticulum, and many granules. Macrophages of different tissues have different names. In liver they are called Kupffer cells, in lungs - alveolar macrophages, in spleen - splenic macrophages, in brain – microglial cells etc. Macrophages have the following functions: 1.They phagocytose particles from the environment,(2) process antigens and present to T cells,thus function as **antigen-presenting cells**.

Certain cytotoxic or killer cells destroy the target cell not by phagocytosis but by releasing biologically potent molecules. Such killer cells include the cytotoxic T lymphocytes and **Natural killer cells** (NK Cells).

Inflammatory Barriers

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively called as the **inflammatory response**. As early as the first century AD, the Roman physician Celsus described the “five cardinal signs of infection” as *rubor* (redness), *tumor* (swelling), *calor* (heat), *dolor* (pain) and *functio laesa* (loss of function). The cardinal signs of inflammation reflect the major events of an inflammatory response.

1. Vasodilation: An increase in the diameter of blood vessels of nearby capillaries occurs. The vessels that carry blood away from the affected area constrict. This results in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (erythema) and in increase in tissue temperature.
2. An increase in capillary permeability facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates (exudates) has a much higher protein content. Accumulation of fluid contributes to tissue swelling (edema).
3. Influx of phagocytes from the capillaries into the tissue is facilitated by the increased capillary permeability. As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release

lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.

The events in the inflammatory response are initiated by a complex series of events. During the inflammatory response, many chemical mediators are released, in response to tissue damage. They are called **acute-phase proteins**. The concentrations of these proteins increase dramatically in tissue-damaging infections. C-reactive protein is a major acute-phase protein produced by the liver. Another mediator of the inflammatory response is **histamine**, a chemical released by a variety of cells in response to tissue damage. Histamine causes vasodilation and increased permeability. Another important group of inflammatory mediators are called **kinins**. They are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and increased permeability. A particular kinin, called bradykinin, also stimulates pain receptors in the skin. Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of **fibrin**. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection.

Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts and capillaries accumulate, and scar tissue forms.

Collaboration between Innate and Adaptive Immunity

Innate and Adaptive immunity do not operate in total independence of each other. They cooperate in important ways to produce more effective immunity. For example, the encounter between macrophages and microbes can generate antigen presenting cells that stimu-

late and direct adaptive immune responses. This facilitates the participation of the adaptive immune system in the elimination of the pathogen. Macrophages also secrete immunoregulatory hormone-like molecules, called cytokines. The cytokines and other signals generated by innate immunity play important roles in triggering lymphocyte responses.

The adaptive immune system produces signals and components that stimulate and increase the effectiveness of innate responses. There is increase in the ability of macrophages to kill the microbes they have ingested. The production of antibodies against an invading pathogen also has important effects on the recruitment of the complement system to the defense of the host. By binding to the pathogen, antibodies mark it as a target for attack by complement, and the complex of antibody and pathogen is also a potent activator of this attack. Thus, these two systems, nonspecific and specific immunity, form an interactive and mutually supportive network that erects an effective and formidable barrier to infection.

EXERCISE

Points to remember

1. Understand the anatomic barriers that provide natural immunity
2. Understand the physiologic barriers that provide natural immunity
3. Understand the inflammatory barriers that provide natural immunity

Self evaluation

1. What is meant by innate immunity?
2. List anatomic barriers and their functions
3. List physiologic barriers and their functions
4. List inflammatory barriers and their functions
5. Describe the characteristics of skin and its role in innate immunity
6. What are antimicrobial peptides? State their role in innate immunity
7. State the characteristics of interferons
8. Describe the role of phagocytic cells in providing innate immunity

ADAPTIVE IMMUNITY

Introduction

Immunity is the resistance shown by the host towards injury caused by microorganisms and their products. Immunity can be classified into two types. They are innate or natural immunity and acquired or adaptive immunity

Innate immunity is the resistance to infections which individuals possess due to their genetic make up. Innate immunity is again divided into two types. They are specific and non specific.

Acquired or adaptive immunity which occurs after exposure to an infectious agent is specific and is mediated by either antibodies or lymphoid cells. It can be passive or active.

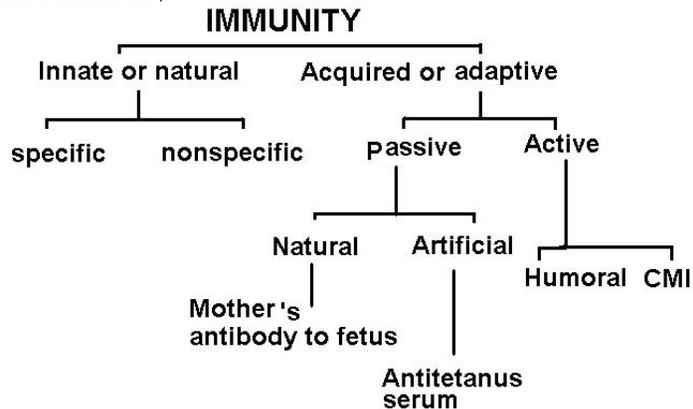
Passive immunity

Passive immunity is transmitted by antibodies preformed in another host. It is of two types. They are natural passive and artificial passive immunity. In natural passive immunity the antibodies which are formed in mother are transferred to foetus. They give protection to the new born babies for three months. In artificial passive immunity the preformed antibodies are administered to the persons. For example anti tetanus serum is used as passive immunization against tetanus. It provides immediate and temporary protection.

Active immunity

It is produced directly by the immune system of the host. Also it is induced by contact with antigen and give long lasting protection. The immune response is of two types. They are humoral immunity and cell mediated immunity.

It is produced directly by the immune system of the host. Also it is induced by contact with antigen and give long lasting protection. The immune response is of two types. They are humoral immunity and cell mediated immunity.



Adaptive or acquired immunity forms the host's second line of defense. When microorganisms overcome or circumvent the innate, nonspecific defense mechanism or deliberately administered as vaccines, they encounter the adaptive immunity

DEFINITION

Adaptive (acquired) immunity, refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen.

This is the immunity one develops throughout life. Six major characteristics of acquired immunity are:

- 1) *Specificity* : It shows exquisite specificity for distinct molecules against which it is induced and does not recognize other molecules
- 2) *Self-non self discrimination or tolerance*: It can discriminate self antigens belonging to the individual's own body and are made tolerant to these self antigens. Under very rare circumstances they react to the self antigens body and are made tolerant to these self antigens.

- 3) *Inducible*
Adaptive immune response can be induced artificially.
- 4) *Diversity*
Different types of microbes induce responses in different ways so that diverse responses are generated
- 5) *Memory*
Adaptive immune response has the ability to remember and respond more vigorously to repeated exposure to the same microbe.
- 6) *Self-limiting*
Adaptive immunity usually improves upon repeated exposure to a given infection.

Adaptive immunity involves the following:

(1) antigen-presenting cells (APCs) such as macrophages and dendritic cells; (2) the activation and proliferation of antigen-specific B-lymphocytes; (3) the activation and proliferation of antigen-specific T-lymphocytes; (4) and the production of antibody molecules, cytotoxic T-lymphocytes (CTLs), activated macrophages and NK cells, and cytokines. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen.

The humoral immune system

B cells develop in bone marrow. These B cells have membrane bound antibody as a receptor for antigen. Each B cell makes one kind of antibody. B cells differentiate into plasma cells, which produce large amounts of soluble antibody.

The cellular immune system

Helper T cells mature in the thymus and contain a single T cell receptor for antigen. Helper T cell activation requires recognition of antigen and a co-stimulatory signal from the innate immune system. Helper T cells contain a surface protein called CD4 and must be activated by the antigen presenting dendritic cells of the innate immune system.

Fate of Antigens

Antigens are of two types namely complete antigens and incomplete antigens. Complete antigens are substances when introduced into the body induce an immune response. Incomplete antigens do not induce immune response but they react with preformed antibodies and are called **haptens**

The mechanisms by which the complete antigens are destroyed depend on factors such as: 1) Physical nature of antigens 2) chemical nature of antigen 3) Dose of antigen 4) Route of entry 5) and antigen presentation.

Physical nature of antigen

Particulate antigens are removed from the circulation by two phases, one by a non immune phase and another by immune phase. In the non immune phase antigen is removed by phagocytic cells and in the immune phase antigen-antibody complexes are formed.

With soluble antigens three phases are seen. In the phase of equilibrium, the antigen is diffused into extravascular spaces. In the metabolic phase the antigen level falls down due to catabolic decay and during immune elimination the antigen is eliminated by antigen antibody reactions

Chemical nature of antigen

Protein antigens are eliminated within weeks. Polysaccharide antigens are eliminated slowly. It takes several months to destroy them.

Dose of antigen

Macrophages modulate the dose of antigens so that they are not too low or too high.

Route of entry

Antigens introduced intravenously are localized in spleen, liver bone marrow, kidneys and lungs and are killed by reticuloendothelial cells mainly tissue macrophages.

Antigens introduced subcutaneously are localized in lymph nodes

Presentation of antigen

Presentation of antigens to the immunocompetent cells is carried out by macrophages and dendritic cells.

Specific memory is the hallmark of the adaptive immune response



Fig. 32-1

Adjuvants

Adjuvants are substances which enhance the immune responses. Immunocompetent B lymphocytes recognize antigens and produce antibodies. For this recognition it requires T cell help. Once B cell contacts its specific antigen, it undergoes clonal proliferation and is converted into plasma cells and secretes antibody.

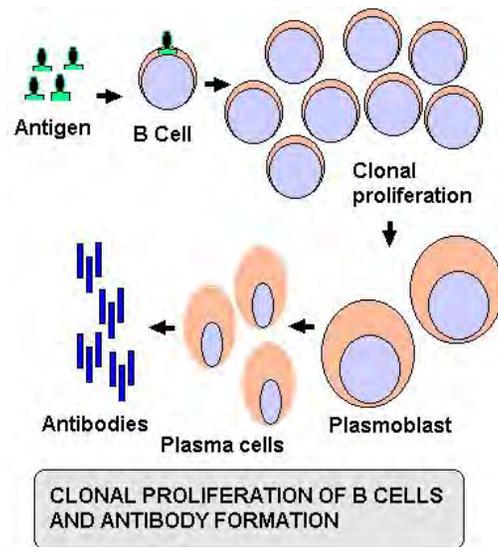


Fig: 32.2

EXERCISE

Points to remember

1. Know the characteristics and importance of adaptive immunity

Self evaluation

1. Define adoptive immunity
2. Give the characteristics of adaptive immunity
3. What all the factors that take part in adaptive immunity
4. What is humoral immunity?
5. What is cell mediated immunity?
6. Describe active immunity
7. State the properties of antigen
8. What is an adjuvant?

ANTIBODIES

Antibodies are glycoprotein molecules which are produced in response to an antigen, and reacts specifically with it in an observable manner

- Tiselius in 1937 analyzed serum by free zone electrophoresis and characterized proteins at pH 8.6

All proteins have negative charge and move towards anode

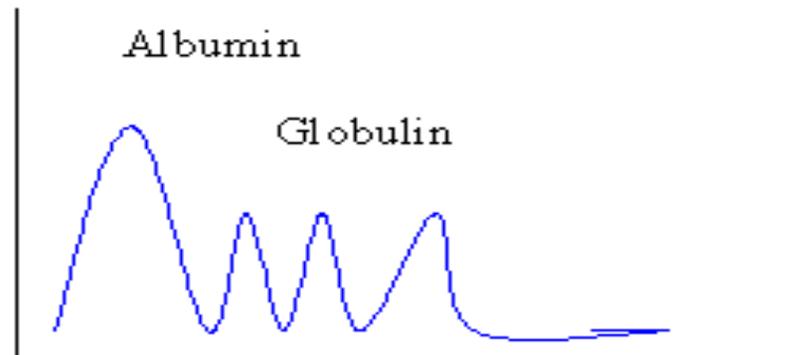


Fig:33.1

- Tiselius and Kabat analyzed rabbit's hyperimmune sera before and after absorption with immunizing agent
 - After absorption there was pronounced decrease in γ globulin
 - Hence antibody activity was traced down to γ globulin

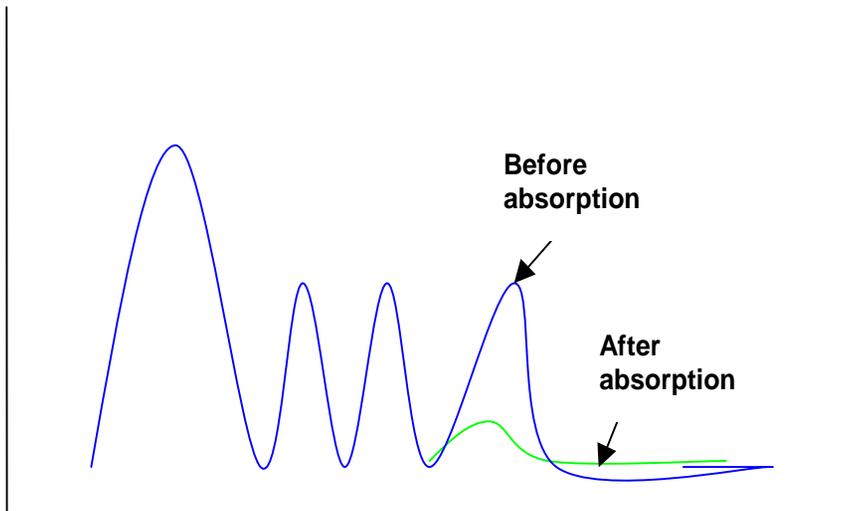


Fig:33.2
Immunoglobulin classes

- γ globulin is not a homogeneous protein
- In 1964 WHO international agreement selected the generic name Immunoglobulin for all antibody containing proteins
- They subdivided immunoglobulin into different classes

Immunoglobulin classes

- In man 5 major classes of Ig are described
 - Ig G : The major serum component
 - Ig M : Macroglobulin
 - Ig A : Present predominantly in secretions
 - Ig D : Important cell membrane receptor form
 - SgE : Antibody involved in hypersensitivity reac-

Characterization of antibodies

- Early physical – chemical studies were done with Ig G from horse, rabbit and human
- Important structural features were predicted even before sophisticated studies were available
- Molecular weight was calculated from sedimentation and diffusion studies
- Asymmetrical and or non globular form by viscosity studies
- Globular domain structure from unique susceptibility to proteolytic enzymes
- Two antigen binding sites by hapten antibody reactions
- Thus early studies predicted three functional domains and have been confirmed

Structure of antibodies

- Before going into the structure of antibody, one must know the structure of proteins
- Proteins are made from amino acids
- Amino acids form poly peptide chains
- Polypeptides form proteins
- Proteins have 3 dimensional structure
 - If any change in the primary sequence of amino acid in polypeptide
 - Or in three dimensional structure there is change in the property

Digestion with enzymes

- Rodney and Porter digested rabbit Ig with the enzyme papain
- It cleaved the molecule and produced two major fractions and a small amount of short peptides

- One fraction (MW 45,000) still possessed antigen binding site and was named as fragment antibody binding (Fab)
- The other fragment could be crystallized, and was called Fragment crystallized (Fc)
- Fab possessed antigen binding site but was monovalent
 - Possessed one reactive site
 - Could not cross link antigen molecule
- When one added up the molecular weights of Fab and Fc fragments, plus the observation that the Fab was monovalent, it appeared that the original antibody contained:
- Two Fab fragments and one Fc fragment

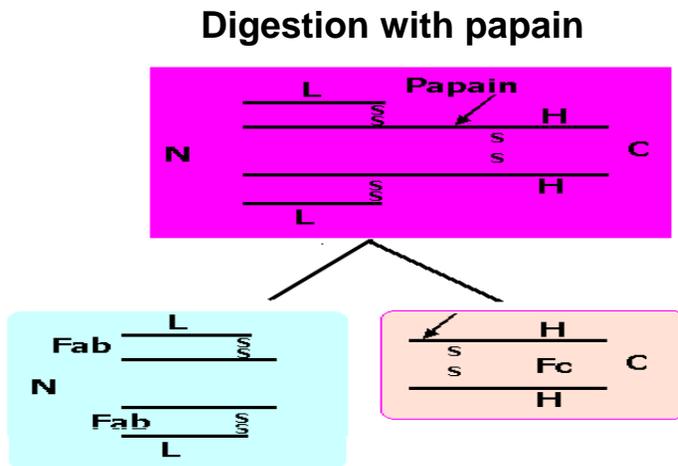


Fig:33.3

General formula for antibody

- The general formula for antibody is $(H_2L_2)_n$
- The immunoglobulins are made of 2 heavy chains and 2 light chains
- These are held together by covalent bonds
- These bonds are interchain disulphide bridges
- Each chain is made of a number of loops
- These loops are known as domains
- Each domain is formed by intrachain disulphide bonds
- There are 2 loop sections per L chain and 4 loop sections per H chain
- There are two terminals in each chain
 - One is called C terminus
 - Other is called N terminus

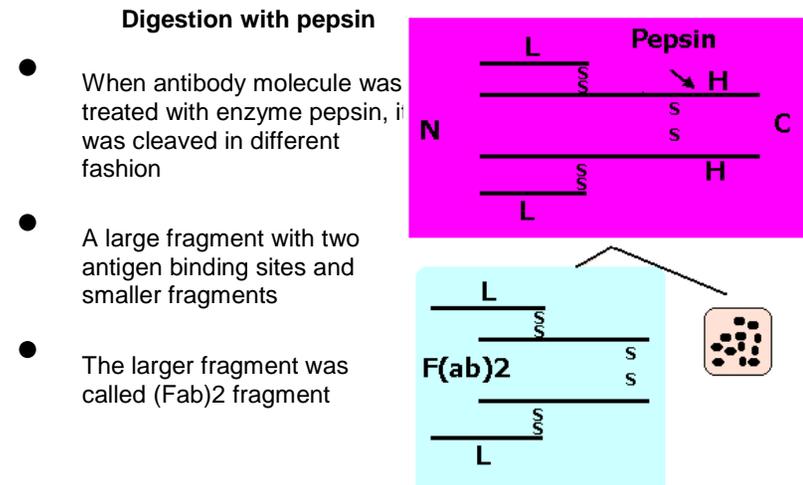


Fig:33.4

Light chain (Fig 33.6)

- C terminus contains the constant region
- N terminus contains the variable region
- L chain is named as Kappa (κ) and lambda (λ)
 - contains two domains
 - domain at N terminus is variable domain of light chain called VL
 - domain at C terminus is constant domain called CL

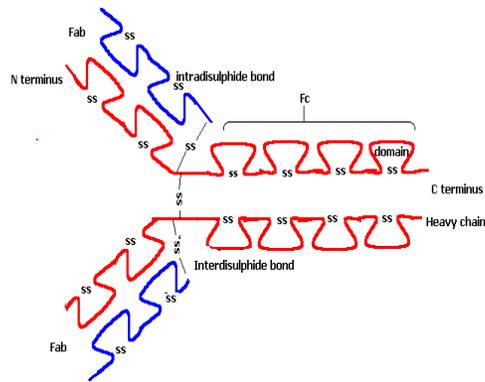


Fig:33.5

Types of heavy chain

- There are 5 different types of H chains
- Based on the type of H chain the classes of antibody is determined
- They are:
 - γ Gamma - Ig G
 - α Alpha - Ig A
 - μ Mu - IgM
 - ξ Epsilon - Ig E
 - δ Delta - IgD

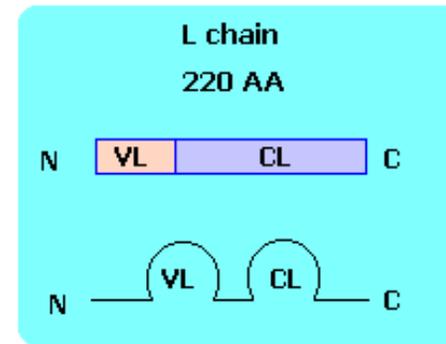


Fig. 33.6

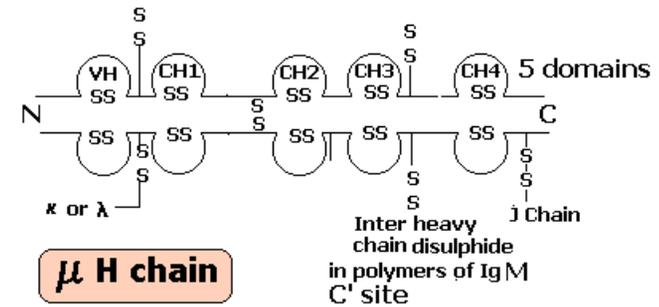


Fig. 33.7

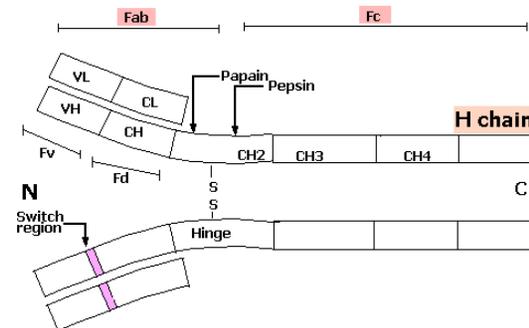


Fig. 33.8

Properties and functions of immunoglobulins

Ig M

- Ig M is the main immunoglobulin produced early in primary immune response
- It is present on the surface of all uncommitted B lymphocytes
- IgM is a pentamer and the valence is 10
- Ig M is the most efficient immunoglobulin in agglutination, complement fixation and other antigen antibody reactions
- It plays an important role in the defense against bacterial and viral diseases
- It does not cross placenta

Ig G

- Ig
- G has two identical antigen binding sites and is bivalent
- There are four subclasses namely Ig G1, Ig G2, IgG3 and IgG4
- Ig G is the predominant antibody in secondary immune response
- It plays an important role in defense against bacteria, viruses
- It also neutralizes toxins
- It crosses placenta and is found in large quantities in newborns

Ig A

- Ig A is found mainly in secretions like milk, tears, saliva and secretions of respiratory, intestinal and genital tracts
- It protects the mucus membranes against microbial attack
- As many microbes enter the body through these mucus membranes, Ig A offers the first line of defense
- Each IgA molecule consists of two H_2L_2 units and a J chain and a secretory component.

- The secretory component is a polypeptide synthesized by epithelial cells and it helps IgA to pass the mucosal surface

IgE

- IgE antibody is present in increased quantities in allergic individuals
- The Fc portions of the molecule binds to mast cells and eosinophils
- When this antibody combines with its antigen on the mast cell surface, it leads to allergic response

IgD

- IgD has no antibody function
- It may act as antigen receptor on cells
- In serum it is present in only trace amounts

EXERCISE

Points to remember

1. General structure of immunoglobulin molecule
2. Characteristics and classes of antibody molecules and their functions

Self evaluation

1. Define an antibody
2. List the classes of Immunoglobulin molecules
3. Describe the general structure of antibody molecules
4. What happens when an antibody molecule is treated with papain?
5. What happens when an antibody molecule is treated with pepsin?
6. Give the structure and characteristics of a H chain
7. Give the structure and characteristics of m H chain
8. List the properties of Ig M antibodies
9. List the properties of Ig G antibodies
10. List the properties of Ig A antibodies
11. List the properties of Ig E antibodies

ANTIGEN-ANTIBODY REACTIONS

Introduction:

ANTIGEN

Antigen is defined as foreign substance capable of inducing a specific immune response, which is also called immunogen.

Antibody

Is a protein produced from plasma cell consisting of two identical heavy chains & light chains that recognizes a particular epitope on an antigen and reacts with it in an observable manner

Antigens and antibodies combine with each other specifically in an observable manner. The reactions between antigens and antibodies are useful in many ways. 1) They form the basis of antibody mediated immunity against infectious diseases. 2) They cause tissue injury in some type of hypersensitivity reactions and autoimmune diseases. 3) In the laboratory, they help (a) in the diagnosis of infections, (b) in epidemiological surveys, (c) in the identification of infectious agents and noninfectious agents such as enzymes. In general these reactions can be used for the identification of either antigens or antibodies. Antigen-Antibody reactions in vitro are known as serological reactions.

Stages Involved in Antigen-antibody Reactions

The reaction between antigen and antibody occurs in three stages.

1. Primary stage
2. Secondary stage
3. Tertiary stage

Primary stage:

The primary stage is the initial reaction between the two, without any visible effects. This reaction is rapid, occurs even at low temperatures and obeys the general law of physical chemistry and thermodynamics. The reaction is reversible, the combination between antigen and antibody molecules being effected by the weaker intramolecular forces such as vander waals forces, ionic bonds and hydrogen bonding rather than by the firmer covalent bonding. The primary reaction can be detected by estimating free and bound antigen or antibody separately in the reaction mixture.

Secondary stage:

The secondary stage leads to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization reactions, complement fixation and enhancement of phagocytosis.

Thus the antigen causing Agglutination was called agglutinin that causing precipitation precipitin. Thus the antibody causing agglutination was called Agglutininogen, that causing precipitin precipitinogen.

Tertiary stage:

Some antigen-antibody reactions occurring in vivo initiate chain reactions that lead to neutralization or destruction of injurious antigens, or to tissue damage. These are the tertiary reactions and include humoral immunity against infectious disease as well as clinical allergy and other immunological diseases.

General Features of Antigen-antibody Reactions

Antigen-Antibody reactions have the following general characteristics:

1. The reaction is specific. An antigen combines only with its homologous antibody and vice versa. The specificity, however is not absolute and cross reaction may occur due to antigenic similarity and relatedness.
2. Entire molecules react and not fragments.

3. There is no denaturation of the antigen or the antibody during the reaction.
4. The combination occurs at the surface.
5. The combination is firm but reversible. The firmness of the union is influenced by the Affinity and the Avidity of the reaction. Affinity refers to the intensity of interaction between the Antigen and Antibody. Avidity is the strength of the bond after the formation of the complexes.
6. Both antigens and antibodies participate in the formation of agglutinates or precipitates.
7. Antigens and Antibodies can combine in varying proportion

Measurement of Antigen and Antibody

Many methods are available for the measurement of antigen and antibody participating in the reactions.

Measurement may be in terms of mass or more commonly as units or titer.

The antibody titer of a serum is the highest dilution of the serum which gives an observable reaction with antigen in the particular reaction

The titer of a serum is influenced by the nature and the type and conditions of the test

There are two important parameters of serological tests. They are sensitivity and specificity. Sensitivity refers to the ability of the test to detect even very minute quantity of the antigen or antibody.

Specificity refers to the ability of the test to detect reactions between homologous antigens and antibodies only.

Types of Antigen-Antibody Reactions

1. Precipitation
2. Agglutination
3. Neutralisation

Agglutination Reaction

When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped together or agglutinated.

Agglutination is more sensitive than precipitation for the detection of antibodies. The same principles govern agglutination and precipitation. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions. The zone phenomenon may be seen when either an antibody or antigen is in excess.

Incomplete or monovalent antibodies do not cause agglutination, though they combine with the antigen. They may act as 'blocking antibodies', inhibiting agglutination by the complete antibody added subsequently.

Types of Agglutination Reactions

1. Slide Agglutination
2. Tube Agglutination
3. Passive Agglutination
4. Hemagglutination

Slide Agglutination

When a drop of the appropriate antiserum is added to a smooth, uniform suspension of a particulate antigen in the drop of a saline on a slide or tile, agglutination takes place. A positive result is indicated by the clumping together of the particles and the clearing of the drop. Mixing the antigen and the antiserum with a loop or gently rocking the slide facilitates the reaction. Depending on the titer of the serum, agglutination may occur instantly or within seconds. Clumping occurring after a minute may be due to drying of the fluid and should be disregarded.

It is essential to have on the same slide a control consisting of the antigen suspension in saline, without the antiserum, to ensure that the

antigen is not agglutinable. Agglutination is usually visible to the naked eye but may sometimes require confirmation under the microscope. Slide agglutination is a routine procedure for the identification of many bacterial isolates from clinical specimens. It is also the method used for blood grouping and cross matching.

Tube Agglutination

This is a standard quantitative method for the measurement of antibodies. When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilution of an antiserum in test tubes, the agglutination titer of the serum can be estimated. Tube agglutination is routinely employed for the serological diagnosis of typhoid, brucellosis and rickettsial fever. Widal agglutination test is done for typhoid, Brucella agglutination test is done for Brucellosis and Weil Felix test is done for rickettsial infections

PRECIPITATION REACTIONS

Introduction

Precipitation Reaction

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature & pH the precipitation of antigen and antibody complex occurs. Precipitation occurs most rapidly and abundantly in which antigen and antibody are present in equivalent proportion. In the preceding tubes in which the antigen is excess & in the latter tube antibody is in excess the precipitation will be weak or even absent.

Quantitative Precipitation Test

Marrack (1934) proposed the lattice hypothesis to explain the mechanism of precipitation. According to this concept, multivalent antigen combines with bivalent antibody in varying proportions. Precipitation results when a layer of lattice is formed consisting of alternating .antigen and antibody molecules. This is possible only at zone of equivalence. In the zones of antigen and antibody, excess, the lattice does not exist.

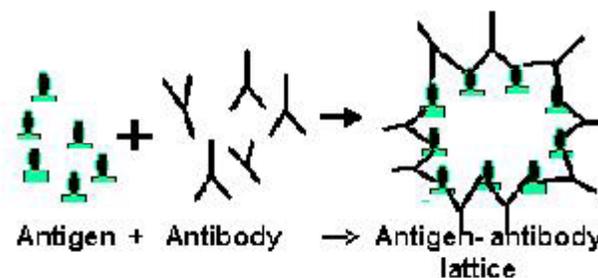


Fig. 34-1

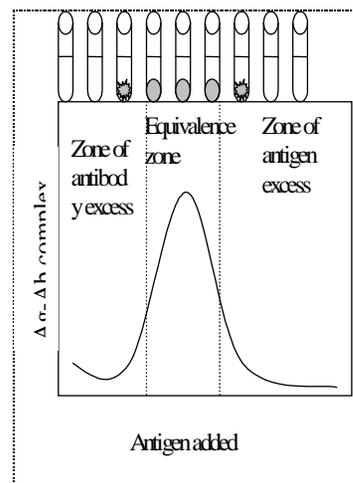


Fig. 34-2

- When increasing concentration of antigen is added to a series of tube containing constant amount of antibody, variable amounts of precipitate forms
- There are three zones obtained
- First is the zone of antibody excess
- Second is equivalence zone in which optimum precipitate is formed
- Third is the zone of antigen excess
- In both antibody and antigen excess zones optimum precipitation does not occur

Application of Precipitation Reaction

The precipitation test may be carried out either as quantitative or as a quantitative test. It is used for detecting antigen and antibody. It is therefore, used in application in the identification of blood and seminal stains and also in testing of food adulteration. The following types of precipitation tests are in common use:

I Ring Test

- 1) **Ascoli test:** This is used for the detection of bacterial antigen Anthrax in tissues /organs of dead animals or man.
- 2) It can be used for Lancefield grouping of Streptococci

II Slide test

In a clean slide a drop of antigen is taken and a drop of antibody is mixed. Floccules appear. VDRL slide test for syphilis is done in this way

III Tube test

Antigen and antibody are allowed to react in the tubes to form precipitates. Khan test for syphilis is done in this way

IV Precipitation test in gels

Immunodiffusion is usually performed in a soft 1% agar gel. Antigen and antibody react in thin gel to form a line of precipitate

Types of Immunodiffusion

IV 1. Single diffusion in one dimension (Oudin Procedure)

Antibody is incorporated in agar gel in a test tube. The antigen solution is layered over it. The antigen diffuses downward through the agar gel forming a line of precipitation

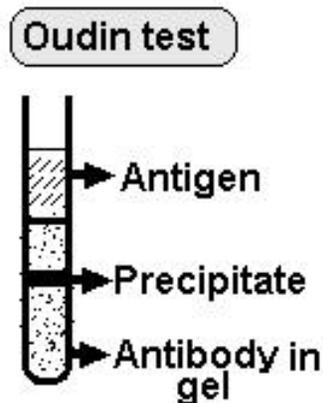


Fig. 34.3

Double diffusion in one dimension (Okley – Fulthorpe Procedure)

Antibody is incorporated in a gel in the tube. Above the antibody a column of plain agar is placed. Antigen is placed on top of this. The antigen and antibody move towards each other in the column of plain agar and form the precipitation line

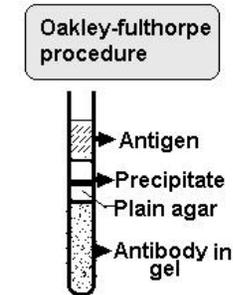


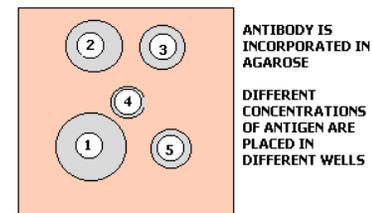
Fig. 34.4

Single diffusion in two dimensions (Radial Immunodiffusion)

In this test the antibody is incorporated in the agar gel. Wells are cut in the gel. Antigen is added to the wells. The antigen diffuses radially from the well and forms ring shaped bands of precipitation concentrically around the well. The diameter of the halo gives the estimate of the concentration of the antigen

It is used to estimate the concentration of the classes of immunoglobulin in the serum

RADIAL IMMUNODIFFUSION TEST



Double diffusion in two dimensions

Agar gel is poured on a slide and wells are cut. Antiserum is placed in the central well different antigens are placed in the surrounding wells. Antigens and antibodies move towards each other and form precipitin lines. Different types of precipitin lines are formed. If the two adjacent antigens are identical, the lines of precipitate will fuse together. If they are not identical the lines will cross each other. If there is partial identity there will be a spur formation as seen in the figure

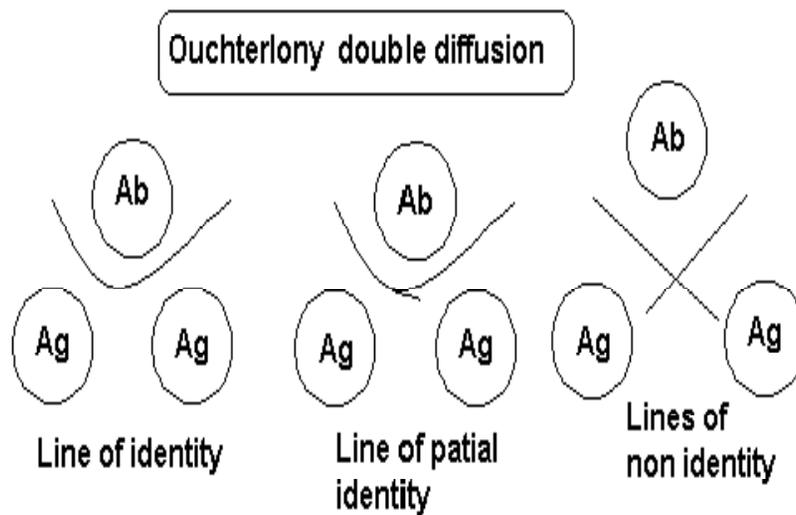


Fig:34.6

This test can be used for comparing different antigen and antibody systems.

Other types of immuno precipitation tests in gel are immuno electrophoresis, electroimmunodiffusion and crossed immunoelectrophoresis etc.

EXERCISE

Points to remember

1. Know the antigen antibody reactions
2. Understand the importance and uses of antigen – antibody reactions

Self evaluation

1. Define an antigen
2. Define an antibody
3. Describe the stages involved in antigen – antibody reactions
4. State the general features of antigen-antibody reactions
5. Describe agglutination reaction
6. Describe precipitation reaction

MICROBIAL GENETICS

DNA as the Genetic Material

The substance that carries the information determines the properties of the organism is called genetic material. This genetic material is responsible for transferring the genetic information from parent to progeny. In all organisms the genetic material is DNA. However, there are some exceptions such as bacteria and numerous plant and animal viruses in which the genetic material is RNA.

Since the DNA is the genetic material that carries the information, it is called the central dogma, i.e., the information contained in DNA is translated to protein structures. Different experiments were carried out to identify DNA as the genetic material.

Griffith Experiment

The concept that nucleic acids are the genetic material is based on the discovery of transformation by Fredrick Griffith in 1927, medical officer in the British Ministry of health, was studying Pneumococcal infection in mice. Different strains of the Bacterium *Diplococcus pneumoniae*, now named as *Streptococcus pneumoniae* were used for his experiments. There are two types of bacterial strains, one virulent and other avirulent. The virulent strains cause pneumoniae in humans and mice, however, avirulent strains do not cause pneumoniae. The virulence is related to the presence of a polysaccharide capsule of the bacterium. The capsulated virulent cells cannot be phagocytosed by the circulatory system, where as non-encapsulated avirulent strains are destroyed by the phagocytic cells.

The capsulated bacteria form smooth shiny surface colony (S) when grown on agar culture plate, whereas non encapsulated strains produce rough colonies (R). Each bacterial strain may be differentiated based on serotype. The specificity of the serotype is due to the chemical structure of the polysaccharide is identified by immunological techniques. They are usually designated by Roman numerals. There are different types such as I, II and III of Pneumococci that have different capsular polysaccharide.

In 1920's, in United States, type I and type II were common in causing pneumoniae. Griffith book type II and type III for his experiments that led him to the concept about genetic material.

The characteristics of bacteria used by Griffith for his original transformation experiment is given below.

Serotype	Colony morphology	Capsule	Virulence
IIR	Rough	Absent	Avirulent
IIIS	Smooth	Present	Virulent

The virulent encapsulated smooth bacteria are effective in killing the mice, whereas if the bacteria is heat killed by heat treatment, they lose their ability to kill the mice. Griffith performed a critical experiment. involving an injection into mice of living RII (Avirulent) cells combined with heat killed IIIS (Virulent) cells. Neither type can cause death in mice when injected. Griffith also expected that the double injection would not kill the mice. However, after five days all mice receiving double injection were dead. The blood analysis of all the dead mice showed a large number of living type III S bacteria. These IIIS bacteria were identified to the IIIS strain from which the heat killed cell preparation was made. A control was maintained, where the mice received only living avirulent II R bacteria that did not develop pneumonia and remained healthy. This experiment ruled out the possibility that the aviru-

lent IIR cells had simply changed to virulent IIIS cells in the absence of the heat killed III S strain.

Some type of interaction was there between living II R and heat killed III S cells. It was concluded by Griffith that the heat killed III S bacteria somehow was responsible for converting live avirulent II R cells and into virulent IIIS cells.

Griffith suggested that some transforming principle from the dead III S cells was responsible for the conversion of II R cells into III S cells. This phenomenon is called transformation. This is called as Griffith effect or more popularly called as Bacterial transformation.

Avery, MacLeod and McCarthy experiment:

Griffith could not understand the cause of bacterial transformation. Oswald T Avery, C.M. MacLeod and M.J. McCarthy identified the transforming principle in 1944. Avery and his coworkers standardized a procedure to extract active power from liquid cultures of type III S virulent cells. From the original 75 liter sample, the procedure yielded 10 to 25 mg of the active factors. The “active factor” that was a fibrous mass was analyzed for its Nitrogen/Phosphorus ratio and was formed to coincide with the ratio of DNA.

The final extracted product was also treated with the proteolytic enzymes trypsin and chymotrypsin and then with an RNA digesting enzyme, ribonuclease. Such treatments destroyed the activity of proteins and RNA, and the transforming activity still remained.

The final confirmation was done by treating the crude samples with DNA digesting enzyme deoxyribonuclease. Digestion with this enzyme destroyed the transforming activity. This proved that the transforming principle was DNA.

Hershey and Chase Experiment:

Another supporting evidence that DNA as the genetic material came from the results of experiments designed by Alfred Hershey and Martha Chase in 1952.

They used T-even Bacteriophages T2. When the Bacteriophages infect a bacterial cell, the following events occur.

- Attachment of phage tail fibers to bacterial wall
- Same components of the phage enters the bacterial cell
- The virus uses the cellular machinery of the host for viral reproduction.
- Viral components accumulate, assembly of mature phages takes place
- Bacterial cell lysed and many new phages released.

Hershey and Chase knew from several experiments, the independent functions of phage protein and nucleic acid in the reproduction process associated with the bacterial cell.

The T2 phage consists of approximately 50% protein and 50% DNA. Hershey and Chase used the radioisotopes ^{32}P and ^{35}P plus DNA contains P but not sulfur. Proteins contain sulfur but not phosphorus. So DNA will be labeled with ^{32}P and the protein with ^{35}P and when infected with T₂ virus. the resulting progeny phages will have either a radioactive labeled DNA core or a radioactively labeled protein coat. The results are summarized below.

DNA Structure

In prokaryotes a chromosome is typically circular and in eukaryotes linear threadlike molecule of DNA. DNA is a long linear polymer called as nucleic acid. This carries the information that can be passed from one generation to the next. DNA consists of a double chain of nucleotides arranged in a helix with the nucleotide base pairs held together by hydrogen bonds (Figure 35.1)

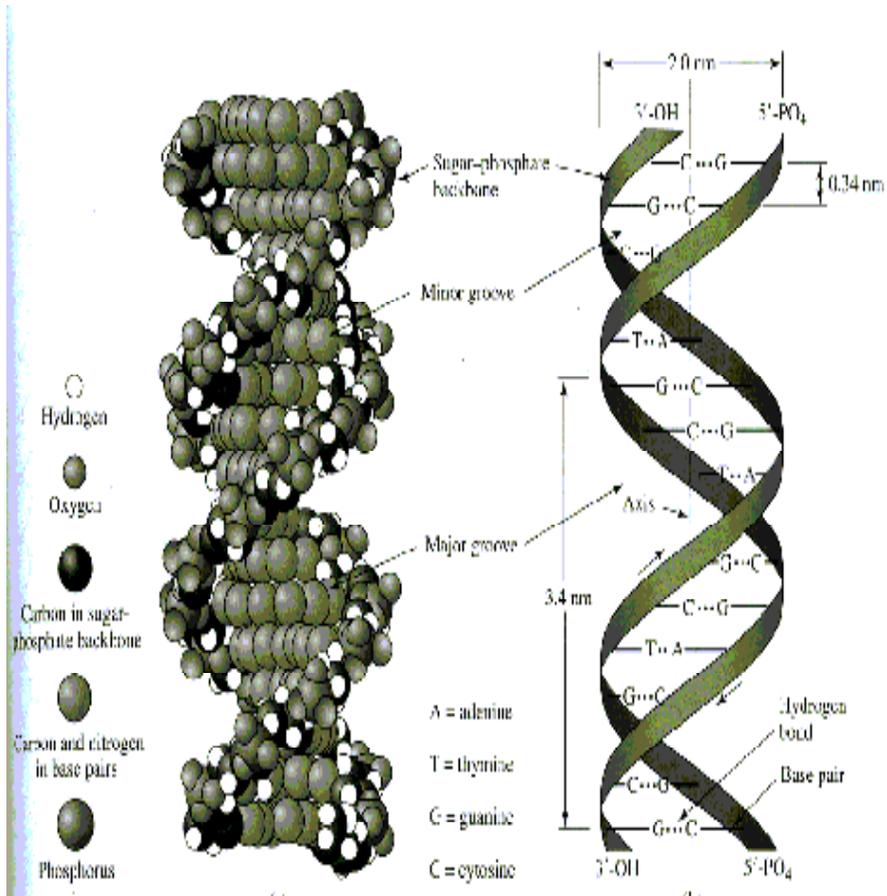


Fig.: 35.1 DNA double helical structure proposed by Watson and Crick.

A nucleotide is composed of three chemical parts (Figure 35.2)

1. an aromatic cyclic compound containing carbon and nitrogen atoms (nitrogenous base)
2. a five carbon carbohydrate in ring form (aldopentose) one, two or three phosphate groups.
3. one, two or three phosphate groups.

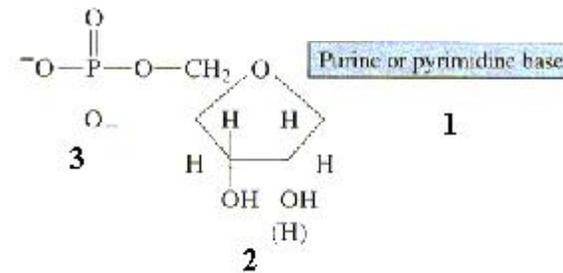


Fig. 35.2

Structure of a nucleotide with the three fundamental units: purine or pyrimidine nitrogenous base(1), ribose or deoxyribose sugar (2) and a phosphate(3).

All the nitrogen bases in DNA and RNA are derivatives of 2-heterocyclic compounds purine and pyrimidine. The purine in DNA are adenine (A) and guanine (G) and pyrimidines are cytosine (C) and Thymine (T). The purine in RNA are adenine (A) and Guanine (G) and the pyrimidines are cytosine (C) and uracil (U) (Figure 35.3)

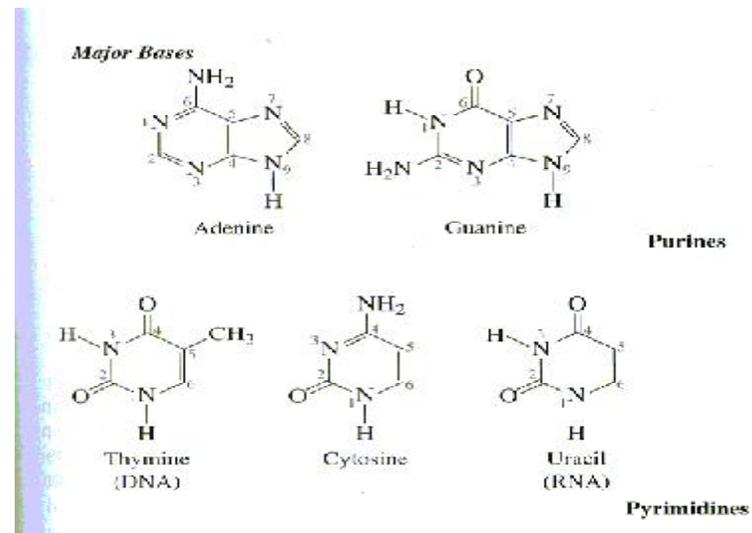


Figure: 35-3 Structure of heterocyclic bases in RNA and DNA

The type of sugar present in DNA is 2-deoxyribose and Ribose sugar in RNA (figure 35.4) The aldopentoses in RNA and DNA.

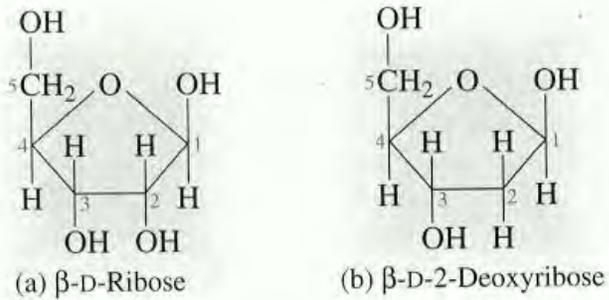
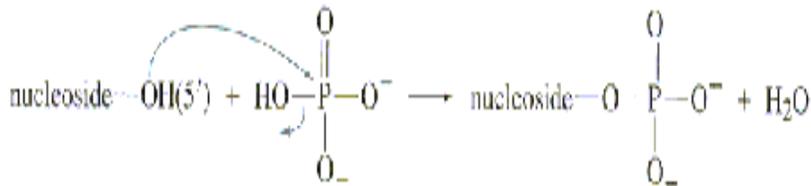


Fig. 35.5

The nitrogen base is linked to position one on the pentose ring by a glycosidic band from N_1 of pyrimidines and N_9 of purines. A base linked to a sugar is called nucleoside Fig. 35.5.



A nucleotide containing purine or pyrimidine linked to a carbohydrate by an N-glycosidic linkage

When a phosphoryl group is linked to a carbohydrate hydroxyl group on a nucleoside, it is called as a nucleotide

Nucleotides form the basis for the construction of nucleic acids. The nucleotides are linked together into a polynucleotide chain by backbone consisting of an alternating series of sugar and phosphate residues (Figure. 35.6)

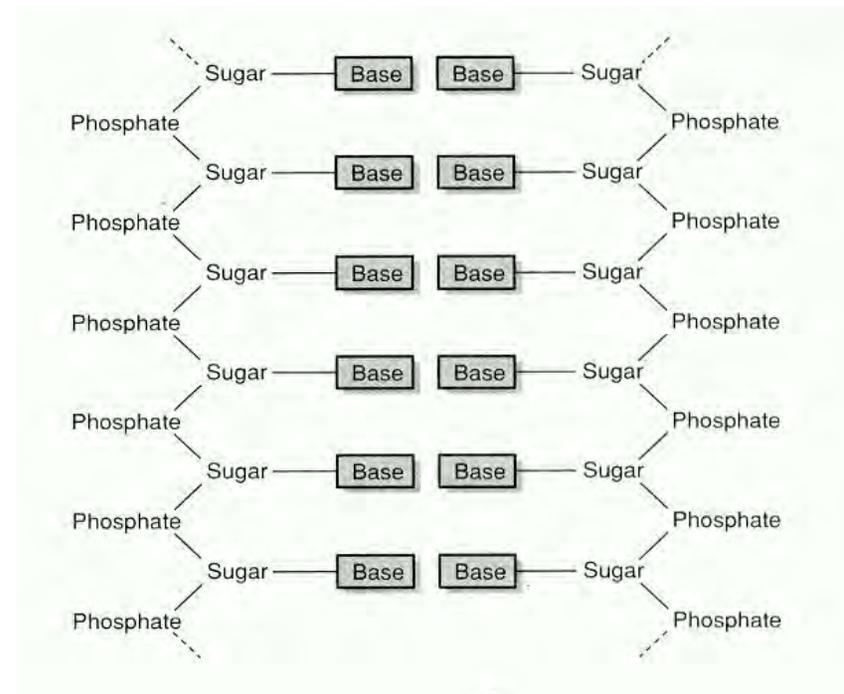


Figure: 35.6 The arrangement of nitrogen, sugar and phosphate groups in a DNA molecule, phosphate forming the backbone of the DNA helix.

X-ray crystallographic studies of DNA molecule by W.T. Astbury, Wilkins and Rosalind Franklin demonstrated that DNA was a helical structure with a diameter of 20Å and has a complete turn of about 34Å. James Watson and Frederic Crick utilized the X-ray crystallographic studies of Franklin and constructed a model for DNA. Watson and Crick published their observation about the structure of DNA in the scientific journal Nature. Wilkins and his colleagues also presented their X-ray evidence for the DNA in the same issue.

Watson and Crick published the DNA structure in 1953. the characteristic feature of the DNA double helix are as follows:

1. The two strands are antiparallel, i.e., they run in opposite directions. One strand has phosphodiester linkage in 3' → 5' direction, while the other strand has phosphodiester linkage in just reverse or 5' → 3' direction.
2. The coiling of the double helix is right handed and a complete turn occurs every 34 Å.
3. The two long polynucleotide chains are coiled around a central axis, forming a right-handed double helix.
4. The adjacent deoxyribonucleotides are joined in a chain by phosphodiester bonds which links the 5' carbon of the deoxyribose of one mononucleotide unit with the 3' carbon of the deoxyribose of the adjacent mononucleotide.

The bases of both chains are flat structures, lying perpendicular to the axis and are 3-4 Å apart. The nitrogenous bases of opposite chains are paired to one another by the formation of the hydrogen bonds.

The helix has two external grooves, alternating larger major grooves and smaller minor grooves, which are apparent along the axis. Both of these grooves are large enough to allow protein molecules to come in contact with the bases.

The base pairing is one of the most genetically significant feature of the model. In 1950, Erwin Chargaff discovered the equivalence rule

which suggested that the amount of A equaled to T and that of G to C. Watson and Crick realized that A pairs with T and G pairs with C. Adenine forms two hydrogen bonds with thymine and guanine forms three hydrogen bonds with cytosine

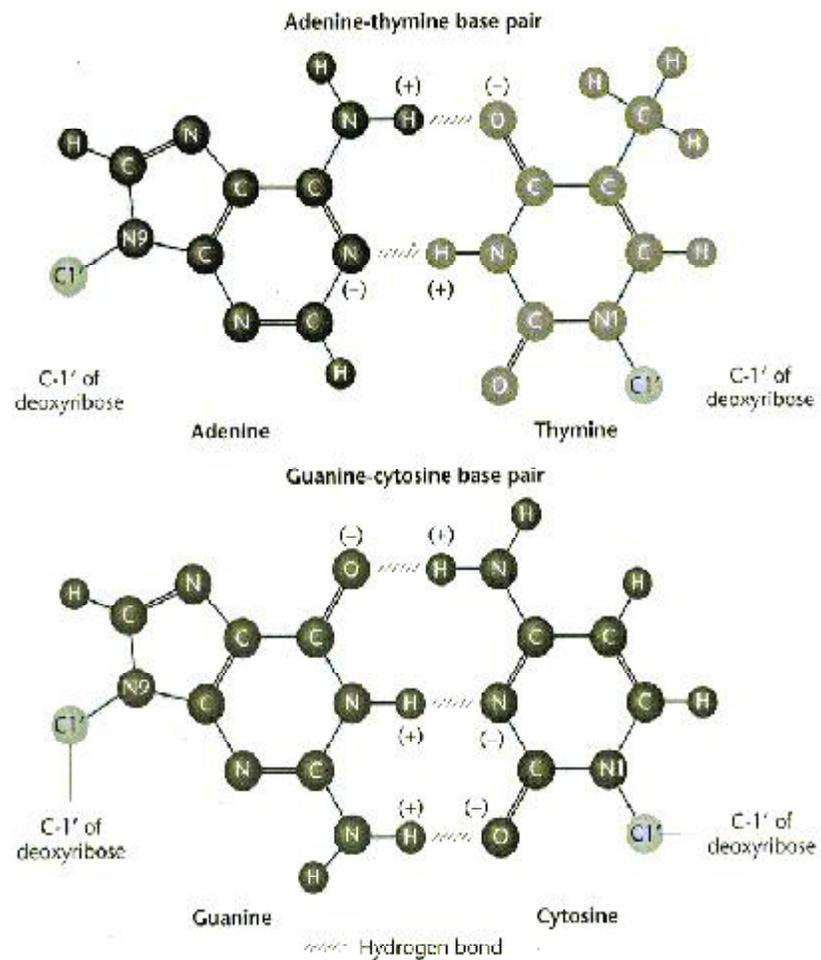


Fig.:35.7 Schematic representation of complementary base pairing involving the formation of two hydrogen bonds between adenine and thymine and three hydrogen bonds between cytosine and guanine.

The hydrogen bond provides chemical stability essential to hold the two chains together. The specific A equal to T and C equal to G base pairing is the basis for the complementarity concept. This complementarity concept is very important in the process of DNA replication and gene expression.

In recognition of their work leading to the double-helix model Nobel prize was awarded in 1962 to Watson, Crick and Wilkins. Nobel prize was not awarded to the contribution of Rosalind Franklin, because, the award is not given posthumously. Since she died in 1958 at the age of 37, making her contributions ineligible for consideration.

Alternative form of DNA

Watson and Crick's model of DNA is called as B-DNA or B-form. However, DNA can exist in other forms also (Figure 35.8). During 1950's DNA studies were based on X-ray diffraction. The recent investigations use single-crystal X-ray analysis. The X-ray diffraction achieved resolution of about 5\AA , but single crystal X-ray analysis at 1\AA , near atomic resolution. Because of this, greater structural detail of DNA is now available.

If water content increases to about 75%, the A form of DNA (A-DNA) occurs. A DNA is more compact with 11 base pairs per turn of the helix and is 23\AA in diameter. The A DNA can occur under experimental conditions.

In 1979, Alexander Rich and his colleagues discovered a left handed helix, called Z DNA. It is called because its backbone formed a zigzag structure. The Z DNA looks like B DNA in which each base was rotated 180 degrees resulting in a zigzag left handed structure.

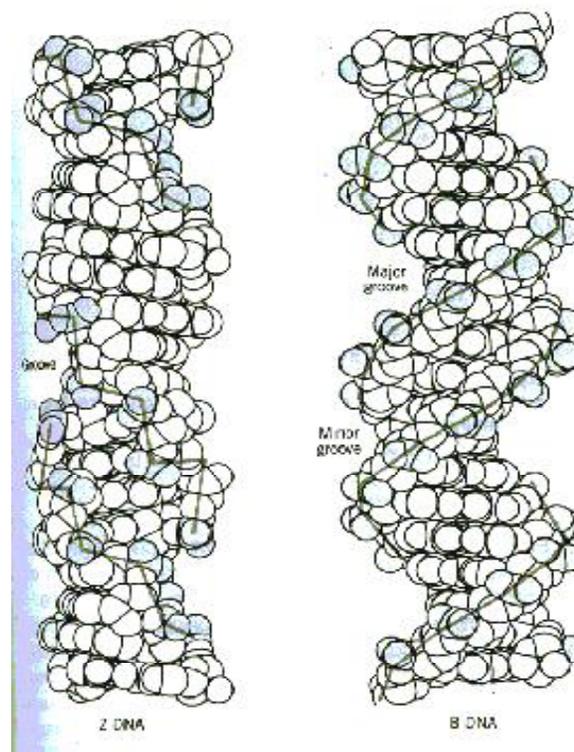


Fig.:35.8 The various forms of DNA. The rotation of bases were indicated by curved arrows.

1. Z DNA has a left handed helical case
2. The phosphate backbone of Z-DNA follows a zigzag course
3. In Z-DNA, the adjacent sequence or residues have opposite orientation.
4. In Z DNA one complete twist (a twist through 360°) has 12 base pairs.
5. The angle of twist in Z-DNA is 60°
6. The Z-DNA one complete turn of helix is 45\AA long
7. The diameter of Z-DNA is 18\AA

After second generation, the two density bands were observed, one at intermediate and one at lighter, which corresponds to the ^{14}N position in the gradient.

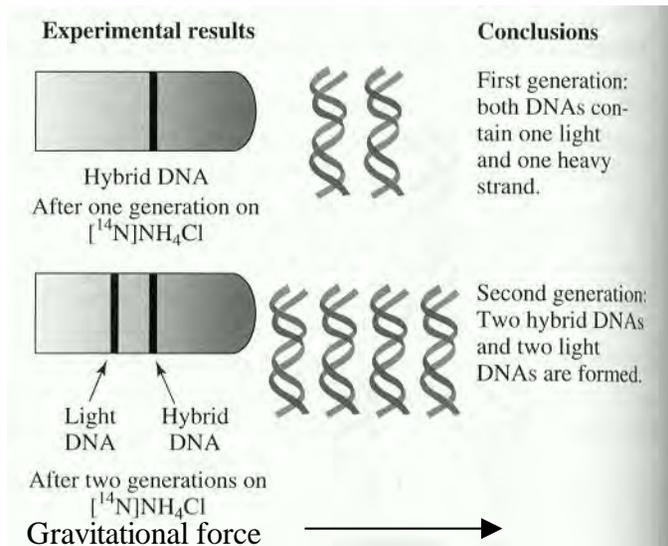


Figure:35.11 The results of semi conservative replication using radio isotopes and gradient centrifugation.

This experimental results and other experiments repeated by Meselson and Stahl with prokaryotes suggested that semi conservative mechanism of replication is universal.

Enzymology of DNA Replication:

Replication of double stranded DNA molecule is a complex process involving a number of enzymes. For DNA replication to occur, the following events should take place

- Temporary separation of the two parental strands.
- Stabilization of the single stranded DNA molecule

- Initiation of daughter strand synthesis.
- Elongation of the daughter strands.
- Termination of the reaction

All the stages are individual enzymatic activities and do not function independently and are contained in a discrete multiprotein structure called the replisome. Enzymes that are able to synthesize new DNA strands on a template strand are called DNA polymerases.

The enzymes that polymerize nucleotides into a growing strand of DNA are called as polymerases. There are three known enzymes in *E.coli*

- DNA Polymerase I
- DNA Polymerase II
- DNA Polymerase III

In a simple model of DNA replication, according to the rule of complementarity, nucleotides will be synthesized on both the strands on the replication fork. During DNA replication polymerization proceeds from 5' to 3' direction. Since both strands are running in opposite direction one new strand has to be replicated in the 5' to 3' direction and the other in the 3' to 5' direction. However, all the known polymerases synthesise nucleotides only in the 5' to 3' direction. Evidence from autoradiography suggests that there are 2 types of replication

- Continuous replication
- Discontinuous replication

The discontinuous form of replication takes place on the complementary strand in short segments in a backward direction. These short segments are called as Okazaki fragments, named after R. Okazaki

who first saw them. The length of Okazaki fragments in prokaryotes is 1500 nucleotides and 150 in eukaryotes. The strand that is synthesized continuously is called as leading strand. The discontinuous strand is called as lagging strand.

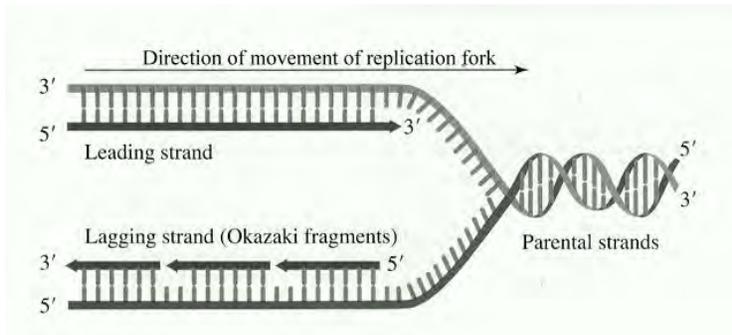
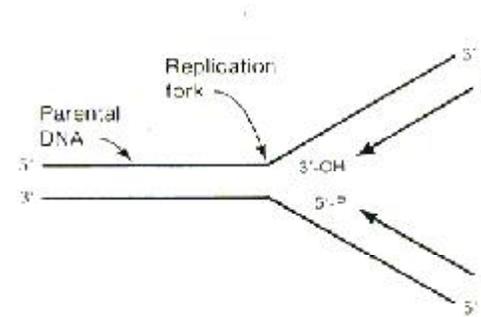


Fig. 35-12 The leading and lagging strands of DNA synthesis

The list of major proteins necessary for DNA replication in *E.coli* is tabulated

Helicase	Starts unwinding of DNA double helix
DNA gyrase	Assists unwinding
SSB Protein	Stabilize single strand of DNA
Primase	Synthesis of RNA primer
DNA Pol III	Elongation of chain by DNA synthesis
DNA Pol I	Removal of RNA primer and fill in gap with DNA
DNA ligase	Closes last phosphodiester gap

The combined effect of helicase and gyrase results in the formation of a replication fork. (Figure 35-13)



The helicase enzyme, (also called as unwinding protein and replication protein) recognizes and binds to the origin of replication and catalyses separation of the two DNA strands by breaking the hydrogen bonds between base pairs. DNA gyrase, a topoisomerase, assists unwinding of DNA strands by inducing supercoiling. The exposed single strands of DNA is stabilized and protected from hydrolytic cleavage of phosphodiester bonds. The SSB proteins (Single Stranded DNA Binding protein) perform this protective role.

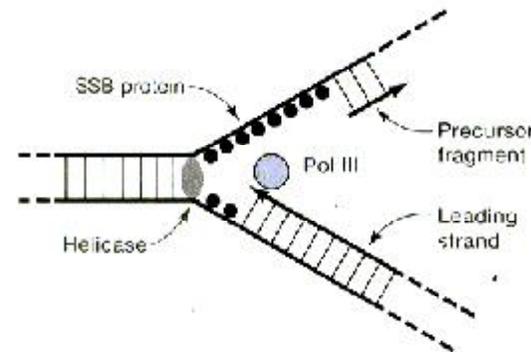


Fig. 35.14

The various events of replication with essential enzymes and proteins

The separated polynucleotide strands are used as templates for the synthesis of complementary strands. The next step is the initiation of DNA synthesis. All of the known DNA polymerases can extend a deoxyribonucleotide chain from a free 3' – OH end, but none can initiate synthesis. The DNA Polymerase III requires primer with a free 3'-hydroxyl end. The primer is short stretch of RNA (4 to 10 nucleotides) complementary to the DNA template.

RNA synthesis is catalysed by an enzyme called primase. The action of primase is required only once for the initiation of the leading strand of DNA where as each okazaki fragment must be initiated by the action of primase.

The DNA synthesis is catalysed by Polymerase III and can proceed from 3'-hydroxyl group. The RNA primer is removed from the DNA by the 5' → 3' nuclease action of DNA Polymerase I and by RNAase H after the second synthesis is completed.

When the primer is removed, there will be a gap. DNA Polymerase I is likely to be involved in filling the gap.

Both leading and lagging strand are extended in the 5' → 3' direction. The leading strand proceed in the direction of the advancing replication fork. Synthesis of the lagging strand continues in the opposite direction until it meets the fragment previously synthesized.

Once the RNA has been removed and replaced, the adjacent Okazaki fragments must be linked together. The 3'-OH end of one fragment will be adjacent to the 5' phosphate end of the previous fragment. The gap is filled by the enzyme DNA ligase by forming the final phosphodiester bond.

Termination of the replication process occurs when the two replication forks meet in the circular *E.coli* chromosome.

EXERCISE

Points to Remember

1. DNA as genetic material came from the studies on transformation by Griffith using *Diplococcus pneumoniae*
2. Avery, McCleod and McCarthy identified the transforming principle in 1944.
3. The supporting evidence for DNA as the genetic material came from the studies of Hershey-Chase in 1952 using T2 phage.
4. The cellular DNA is a double stranded molecule.
5. The structure of DNA molecule was elucidated through the experiments of Astbury, Wilkins and Rosalind using X-ray crystallographic studies.
6. Watson and Crick gave the structure of DNA in 1953.
7. There are four forms of DNA such as B, A, Z and P form with structural variations.
8. DNA replication process is semiconservative and the evidence came from the studies of Meselson and Stahl's experiment.
9. Replication of DNA begins at an initiation site and proceeds on both directions.
10. Unwinding of DNA molecule comes from negative supercoils in DNA by topoisomerases such as gyrase.
11. DNA synthesis leads in both directions as 5' → 3' and 3' → 5' directions. The replication is a continuous process in one strand and called as leading strand and that in the other strand is discontinuous and is called as lagging strand.
12. RNA primer acts as single-stranded DNA template by RNA polymerase and further elongated by DNA polymerase III.
13. Polymerase III aligns the correct nucleotide and then joins it to the 3' end of the growing macromolecule.
14. DNA polymerase I removes and replaces the RNA primer with DNA which is joined to the adjacent polynucleotide by DNA ligase.

15. DNA ligase joins the two ends of the growing DNA molecule to complete the new double stranded DNA molecule that contains one old strand and a new strand.

EVALUATION

1. How does DNA fulfill the requirements of a genetic material?
2. Why were ^{32}P and ^{35}S chosen for use in the Hershey – Chase experiment?
3. Summarize the experiments of Griffith.
4. DNA is not always the genetic material. What are the exceptions?
5. What is a nucleoside?
6. What is a nucleotide?
7. In what compound parts do DNA and RNA differ?
8. Describe the various characteristics of the Watson—Crick double helix model for DNA.
9. In what sense are the two strands of DNA antiparallel?
10. How does ribose and 2'-deoxyribose differ?
11. What are the precursors for DNA synthesis?
12. Name three enzymatic activities of DNA polymerase I.
13. What are the functions of DNA polymerase III in DNA replication?
14. What enzymes are involved in DNA replication in E coli?
15. What is the role of helicase in DNA replication?
16. What is the function of SSB (Single Stranded DNA Binding Protein) in replication fork?
17. What is an Okazaki fragment?
18. What is the length of Okazaki fragment in prokaryotes?
19. Describe the synthesis of Okazaki fragments.

Chapter 36

**XI MICROBIOLOGY PRACTICAL
(50 periods)**

1. Laboratory precautions
2. Cleaning of glass ware appliances
3. Study of microscope and its parts
4. Sterilization
5. Media preparation
6. Isolation of bacteria
7. Isolation of fungi
8. Isolation of actinomycetes
9. Colony characteristics of bacteria
10. Morphological characteristics of algae
11. Preparation of smears and simple staining
12. Differential staining
13. Endospore staining
14. Pure culture technique
15. Blood cell counting (total and differential)
16. Examination of stool sample
17. Methylene blue reduction test (Milk)
18. Isolation of microorganisms from spoiled food
19. Examination of spoiled bread, vegetables and fruits
20. Examination of azolla – anabena symbiosis