# YOUR SYLLABUS

## MICROBIOLOGY

**History and development of microbiology:** **1-51 & 137-158**

Pasteur's experiments, concept of sterilization, methods of sterilization (dry heat, wet heat, radiation, chemical and filtration), microscopy (optical, TEM and SEM), concept of microbial species and strains, growth curve, various forms of microorganism (bacteria, fungi , viruses, protozoa, PPLOs), nature of microbial cell surfaces, gram positive and gram negative bacteria, kinds of flagella, serotypes, nutritional classification of microorganism.

**Genetic homogeneity in colonel populations:-** **52-82**

Isolation of auxotrophs (replica plating technique and analysis of mutation in biochemical pathways), microbial assay for vitamins and antibiotics, strain improvement by selection.

**Microbial agents of diseases:** **83-130**

Bacterial, viral, fungal and protozoan

**Microbes in extreme environments:**

The thermophiles and alkalophiles, pathogenic microorganism, defense mechanism against microorganism, symbiosis and antibiosis among microbial population, nitrogen f..ing microbes in agriculture and forestry.

**Industrial microbuses and their uses:** **131-136**

Production of food (dairy and SCP) and antibiotics (with reference to penicillin and streptomycin), ferment centurion product, a survey of product from microorganism.

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CHAPTER 1

# History and Development of Microbiology

## LONG ANSWER QUESTIONS

**Q.1. Comment upon Serotypes.**

**Ans.** **Serotypes**

**Serotype** or **serovar** refers to distinct variations within a species of bacteria or varuses or among immune cells of different individuals. These microorganism, viruses, or cells are classified together based on their cell surface antigens, allowing the pidemiologic classification of organisms to the sub-species level. A group of serovars with common antigens is called a **serogroup**.

Serotyping often plays an essential role in determining species and subspecies. The Salmonella genus of bacteria, for example, has been determined to have over 4400 serotypes, including Salmonella enterica serovar Typhimurim, S. enterica serovar Typhi, and S. enterica serovar Dublin. Vibrio cholerae, the species of bacteria that causes cholera, has over 200 serotypes, based on cell antigens. Only two of them have been observed to produce the potent enterotoxin that results in cholera : 0 : 1 and 0 : 139. Serotypes were discovered by the American microbiologist Rebecca Lancefield in 1993.

**Role in Organ Transplantation**

The immune system is capable of discerning a cell as being 'self' or 'non-self' according to that cell's serotype. In humans, that serotype is largely determined by humna leukocyte antigen (HLA), the human version of the major histocompatibility complex. Cells determined to be non-self are usually recognized by the immune system as foreign, causing an immune response, such as hemagglutination. Serotypes differ widely between individuals; therefore, if cells from one human (or animal) are introduced into another random human, those cells are often determined to be non-self because they do not match the self-serotype. For this reason, transplants between genetically non-identical humans often induce a problematic immune response in the recipient, leading to transplant rejection. In some situations this effect can be reduced by serotyping both recipient and potential donors to determine the closest HLA match.

Agglutination of HLA-A3 positive red blood cells (RBCs) with anti-A3 alloreactive antisera containing Anti-A3 lgM. ●

**Q.2. Write an essay on isolation and cultivation of micro-organisms.**

**Ans.** For microbiological studies it is necessary that we take pure cultures of micro-organisms. A pure culture is a collection of cells that have arisen through a single cell. In nature, micro-organisms exist in mixed populations. Whenever it is possible to isolate a given type of organisms through using the enrichment culture technique. A variety of these methods are available by which a selective group of organisms can be encouraged to grow. Once the culture is enriched, pure culture isolation can be made by the following methods :

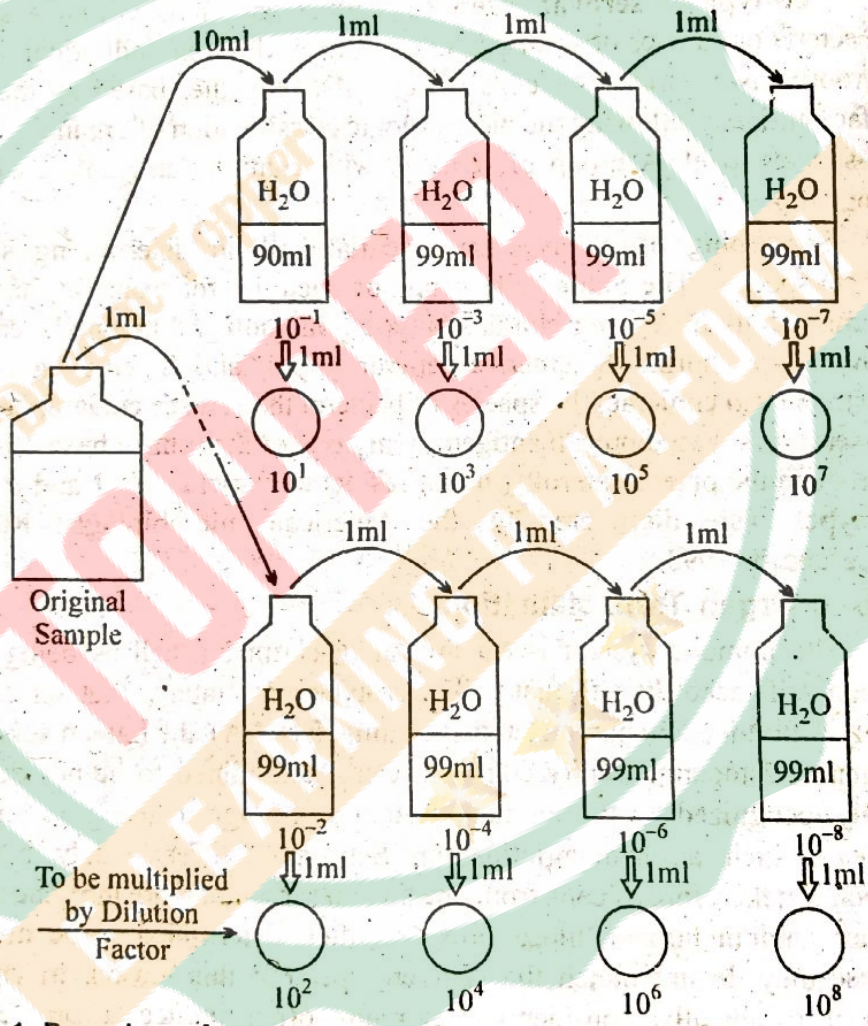



**Fig. 1. Procedure of preparing different dilutions of an original sample of a micro-organism and colony count.**

## (I) Pour Plate Technique

The agar medium is maintained in molten state at 45°C 1 ml of each dilution is added to each sterile petridish to which is then pourned 9 ml of sterile cool agar medium. The contents are mixed and allowed to solidify. The

dishes are incubated at favourable temperature. After a few days, different types of microbes grow as separate colonies. (Fig. 1.)

### (II) Serial Dilution Technique

A culture can be purified through serially diluting the mixed culture sample in a sterile liquid medium, such that final dilution will consist one or none of the micro-organisms. Growth in the last tube of the dilution series may be presumed to have originated from a single cell.

Suppose we have a bottle of any sample consisting 10 ml of the soil sample in sterile water and it consist 1,000 micro-organisms i.e., 100 microbes per ml. It we have out one ml of it and mix it with 9 ml of sterile water then each ml would consist a single micro-organism. When we use one ml of this final dilution to inoculate some sterile medium and we obtain growth, this presumably comes from one cell and is pure.

### (III) Special Methods

The above methods are used for majority of microbes. There are some special methods which are used only for several special types of microbes.

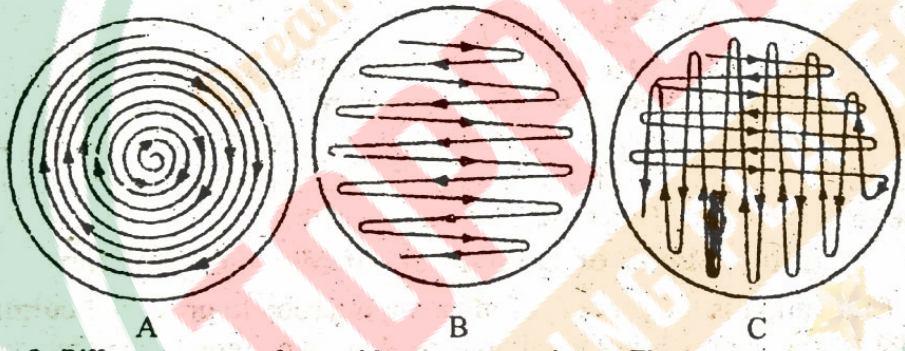



**Fig. 2. Different ways of streaking on agar plates. The arrows indicate the direction of streaking needle.**

**1. Enrichment culture :** Enrichment culture method is used for microbes which are in small number and have a slower growth than other species of microbes found. The enrichment of culture nutrients and culture conditions are controlled in this way that only the given species grows in that medium. e.g., pure colonies of *Nitrobacter* bacteria can be obtained if a medium with salt solution with $NaNO_2$ at *p*H 8.5 is inoculated with a sample of garden soil and incubated in dark in the presence of oxygen at 25–30°C.

**2. Single cell isolation :** In single cell isolation method droplets consisting individual bacterial cells are deposited on a cover glass in a special chamber. The droplets are then transferred by sterile micropipettes to suitable nutrient media.

**3. Selective media :** Selective media consist specific chemicals which encourage the growth of the bacterium to be isolated but discourage the

growths of other bacteria. e.g. a specific concentration of sodium azide isolates lactic acid bacteria.

**4. Differential media :** Differential media contain dyes or chemicals which divided between different types of bacterial colonies developed after incubation, e.g., when raw sewage is streaked on eosin methylene blue agar, some bacteria produce brilliant green colonies and others produce pink colonies with dark centres.

### (IV) Streak Plate Method

In streak plate method a small amount of sample is transferred onto the surface of a favourable, solid agar method medium either by loop or transfer

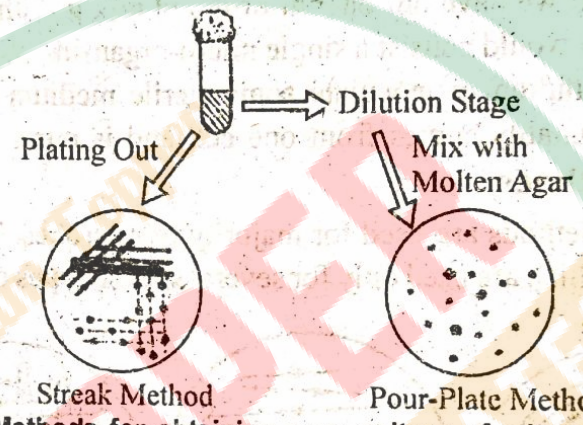



**Fig. 3. Methods for obtaining pure culture of micro-organisms.**

needle. This is then streaked in such a way as to provide successive dilutions and finally to have well-isolated colonies. The sample becomes progressively diluted and at the end of streak one would expect the well isolated colonies.

## Culture Media or Cultivation of Micro-organisms

Cultivation is the process of inducing microbe to grow and **culture** is employed for a growth in the laboratory. A pure culture is one that consist only a single kind of microbe. To culture microbes in the laboratory, we require the preparation of substances which they can use as food. These nutrient preparations which micro-organisms can use as food are known as **culture media.**

Different micro-organisms wants different nutrient materials. Thus culture media vary in form and composition, depending on the species to be cultivated. There are three types of culture media :

1. Natural or empirical culture media
2. Synthetic or defined culture media
3. Living media

### 1. Natural or Empirical Culture Media

Natural or empirical media include milk, urine, diluted blood, vegetable juices, meat extracts and infusions etc. Mostly empirical media consist either only a major ingredient, peptone. Peptone sources are animals or vegetables. Peptones provide insoluble and assimilable form of all the phosphorous,

sulphur and essential mineral content of living material as well as organic carbon and nitrogen sources.

Among meat extracts and infusions, beef extract and an aqueous meat infusion made through infusing fresh, ground meat in water are common ingredients of media useful for several species. Beef infusions are rich in minerals, organic micro nutrients, proteins and carbohydrates. They are often supplemented with 1% peptone or yeast extract. Culture fluids made from beef infusions are commonly known as **infusion broth**, whereas those made from beef extract as **extract broth.** All these empirical media including simple solutions of peptones or yeast extracts, are known as **nutrient broth** or **nutrient solution.** To any of the empirical media, different substances may by added for different purposes. e.g., blood carbohydrates, esters, alcohols, glucosides etc.) is added to an infusion broth, which is now known as **blood-infusion broth.** Lactose may be added to broth and is called as **lactose-broth.**

## 2. Synthetic or Defined Culture Media

Synthetic culture media contains a dilute, reproducible solution of chemically pure, known inorganic and/or organic compounds. Artificial media of exactly known, reproducible composition are known as **synthetic** or **chemically defined media**. The formulation and use of these media requires an exact knowledge of the nutritional acquirements of the micro-organisms to be cultivated. A simplest inorganic synthetic culture medium common, for sulphur-oxidising, soil bacterium, *Thiobacillus thio-oxidans* is as :

| | |
|---|---|
| $(NH_4)_2SO_4$ | 2.2g |
| $MgSO_4 . 7H_2O$ | 0.5g |
| $KH_2PO_4$ | 3.0g |
| $CaCl_2$ | 0.25g |
| S (Powder) | 10.0g |
| Distilled water | To make 1 litre |

Commercially available, portable, dehydrated, powdered, bottled mixtures of a great variety of dehydrated media are used. These are prepared simply through adding weighed portions of the ingredients to the required amount of water.

The synthetic media are as :

(i) **Selective media** used for a selected microbe,

(ii) **General purpose media** used for majority of microbes or

(iii) **Differential media** used for differential isolation of microbes in the same sample.

## 3. Living Culture Media

These are groups of living cells as tissues or cells or an organ used for growing viruses, rickettsias etc. Chick embryos are used for cultivation of viruses. Yeasts moulds and bacteria when enter as contaminants, then are able to multiply in the fluids of the chick in a culture tube.

**Q.3. What are different growth stages of bacteria in liquid medium ? In which stages antibiotics are produced?**

**Ans.** In bacteria growth involves increase of cell numbers. Bacteria cells multiply through **binary fission.** At first cell size doubles and the cell than divides into two daughter cells those have approximately the same size as the **original mother cell.** The time required for a doubling of cell numbers is called as the **generation time** while the time required for doubling of cell mass is referred to as the **doubling time.**

## Bacterial Growth in Batch Culture

When bacteria are inoculated into a nutrient solution and incubated under favourable conditions, they generally continue to multiply until one necessary factor approaches exhaustion and becomes growth limiting. When during this period nutrient are added or waste products removed the growth in such a closed system is known as **batch culture.**

Growth of a bacterial culture can be shown graphically through plotting the logarithms of cell numbers against time. A typical growth curve of this type is sigmoidal in shape and can be divided into a **number of growth phases.** These are :

**(a) Lag phase :** Lag phase occupies the time between inoculation and attainment of the maximum division rate. Actual duration of lag phage depends on the age of the culture and the composition of the nutrient medium. If the inoculum is derived from an old culture i.e. one in the stationary phase, the cells will require to adapt themselves to active growth in the new environment. It means the cells will need to synthesise RNA ribosomes, enzymes etc. to adapt to the new growth conditions. Hence, lag phase will be long if the inoculum cells are old, damaged or grown previously in a different medium.

**(b) Exponential or logarithmic phase :** This phase is characterised through a constant doubling rate. This depends on the particular organism and the growth conditions. Enterobacteria can grow with a doubling time of 15-30 minutes. *E. coli* can grow at 37°C with a doubling time of 20 minutes. In case of other bacteria, the generation time may be much longer.

Some other bacteria, cell size and protein content are constant in the log phase. In many cases the cells undergo changes in size or composition in batch culture because the medium is also undergoing continuous change. The substrate concentration decreases, the cell concentration rises and metabolic products may accumulate while, the biomass and cell number remains constant. This depends largely on the initial substrate concentration and on the organisms growth yield. It is the mass of cells produced per unit of subsfrate converted. It is often related to the efficiency of growth on a particular substrate.

**(c) Stationary phase :** In many microbial processes that are aimed at the formation of antibiotics like penicillin, the **stationary phase** is the real production phase. The bacterial mass that synthesised when the stationary phase is reached is called the yield.

**(d) Death phase :** In this phase the cells begin to die and the viable count decreases exponentially until no cells remain alive. In some cases lysis occurs due to cellular enzymes.

### Bacterial Growth in Continuous Culture

In case of batch culture, the culture conditions undergo continuous change the bacterial concentration rises and the substrate concentration diminishes. To keep the cells growing at a constant exponential rate, frequent transfer of the cell population to fresh nutrient medium is done. This is achieved through constant addition of new growth medium to a growing cell population and concomitant withdrawal of the equal volumes of the bacterial culture.

**Q.4. How will you proceed for Gram's Staining of bacteria? Give some details about differences between wall structure of G+ive and G-ive Cells.**

**Ans.**

### Gram Stain Technique

Gram stain was discovered by Danish physician, **Christian Gram,** in 1884. In this staining, a group of bacteria was observed to retain the stain even after decolourisation with alcohol. This stain is known as **Gram stain.** Such organisms were termed as **Gram positive.** The bacteria which lose the stain after treatment with alcohol were termed as **Gram negative.** The staining technique involves following steps :

(i) A bacterial smear is prepared through spreading a drop of the culture suspension of bacteria on a glass slide followed by gentle heating.

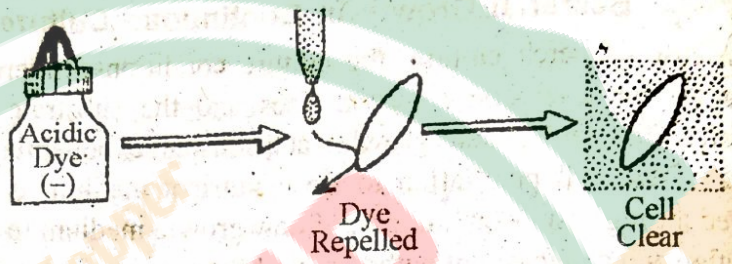
(ii) For his smear, a drop of crystal violet solution is applied.

(iii) After a few seconds, crystal violet is removed. At this stage, the bacteria appears violet in colour.

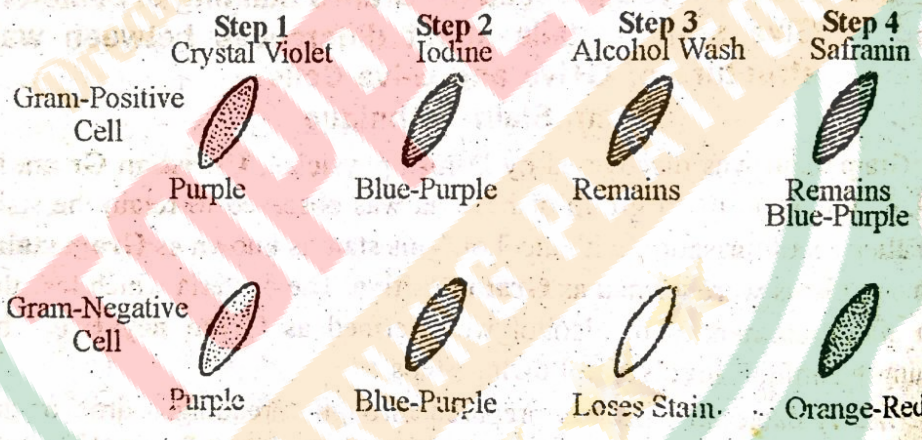
(iv) For this smear, a few drops of iodine-potassium iodide are applied. The bacteria now become deep violet or deep purple in colour.

(v) This smear is covered with 70% alcohol for decolourisation. The Gram-positive bacteria retain the stain and appear deep violet in colour, while the Gram-negative ones get decolourised. It is the preliminary test of Gram reaction. The confirmatory test for Gram-positive and Gram-negative is carried out through counter staining above preparation with safranin. With safranin the Gram-positive bacteria remain deep violet, whereas Gram-negative bacteria are stained red.


(A) Simple Stain Technique




(B) Negative Stain Technique




(C) Gram Stain Technique

**Fig. A Staining techniques.**

**Mechanism of differential staining :** The difference in staining reaction is because of the changes in the lipid content in the cell wall of Gram-positive and Gram-negative bacteria. The lipid content of Gram-positive bacteria is lower than that of Gram-negative bacterial. Lipid, being alcohol soluble, gets extracted in decolourisation. This solubilisation of lipids from bacterial cell wall results in increased porosity and permeability with the result that alcohol penetrates the cytoplasm and decolourises the crystal violet solution. Due to this action, the Gram-negative bacteria lose colour. In the Gram-positive bacteria, the lipid content is very small and with alcohol, the cells get dehydrolysed and become more tough. This decreases their pore size as well as membrane permeability. Because of decreased permeability, alcohol does not reach the cytoplasm and colour is not lost. It has been observed that when cell wall of Gram-positive bacteria is removed

through lysozyme treatment, the stained protoplasm can be decolourised by alcohol. This confirms that staining of bacterial cell depends upon the lipid content of the cell wall.

**Differences in cell walls of two groups of bacteria**

| S. No. | Gram-negative | Gram-positive |
|---|---|---|
| 1. | Less homogeneous, more complex and multi-layered. | More homogeneous, amorphous, single layered. |
| 2. | Thinner, 10-15 nm thick, may be as many as 5-layered. 10-20% of the total dry weight of cell. | Thicker, 25-30 nm thick, 20-40% of total dry weight of cell. |
| 3. | Peptidoglycans-5-15% of total cell wall; Phospho-lipids-35%; Proteins-15%; Lipopolysaccharides-50%. | Peptidoglycans-20-80%, Others : *i.e.*, rest, proteins, polysaccharides and teichoic acids. |
| 4. | Lipopolysaccharides are the principal surface antigens. | Teichoic acids are the principal surface antigens. |

☛ **Q.5. What is Electrophoresis ? Explain the technique of electrophoresis.**

**Ans. Electrophoresis :** Electrophoresis is defined as the migration of charge particle in the solution under the influence of electric field. The rate of migration is directly proposal to the number of charge present on the component. Protein are colloidal particle and have charged either +ve or −ve, which depend on the *p*H of the solution.

In acidic medium it act as cation and in alkaline medium as anion. If uncharged particle are charged to charged particle. Then they can be separated. If a potential difference is applied across them, current will flow and cation move towards cathode and anion towards anode. Migration depend upon :

(i) *p*H of Buffer
(ii) Net charge of atmospheric particle
(iii) Temp
(iv) Voltage and current.

**Types of Electrophoresis**

(1) Immuno electrophoresis :

(a) Counter immuno electrophoresis
(b) Crossed immuno electrophoresis

(2) Agarase or polyacrylamide gel electrophoresis.
(3) PFGE (Pulsed field gel electrophoresis)
(4) CHEFE (Contour Clamped Homogenous electric field electrophoresis)

Different size of DNA can be separated by Agarose or poly acrylamide gel electrophoresis. PFGE (Pulsed field gel electrophoresis) technique used for separation of large size of DNA molecules. CHEFE technique is used for mapping of DNA sequence on special chromosome.

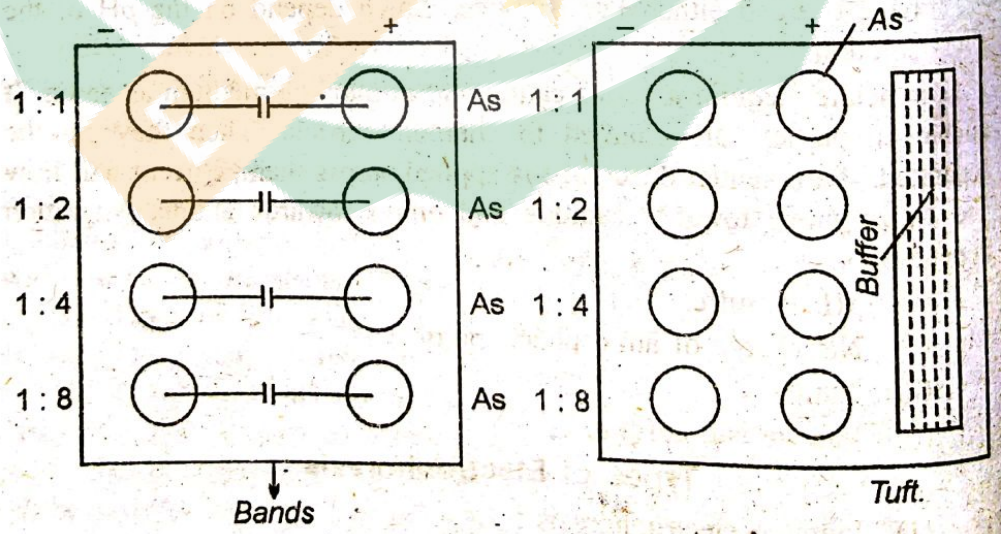
**Immunoelectrophoresis**

In electrophoresis carbohydrate is always move positive to negative but protein move negative to positive when electric current are apply. First we take a slide of glass and in the middle of slide take a small drops of antigen. On the left side of antigen we have polysaccharide and on the right side we have protein or spread the protein below having a antibodies, than this slide put into the most chamber than precipitation band are present.

Precipitation Band showing following Characters :

(i) Major antigen is protein or carbohydrate.
(ii) When maximum number of Band than maximum number of Antigen.
(iii) A broad idea of antigen, low or high molecular weight.
(iv) Higher molecular weight away from the antigen and low molecular weight towards the antigens. Protein are antigen and go further.

**Counter Immunoelectrophoresis :** This method released the movement of Antigen towards the cathode or anode during the passage of



electric current through Agar. The melting of antigen and antibodies is greatly accelerated and made visible in 30-60 minute. This has been applied to detection of bacteria polysaccharide. Bands are appear after 90 minutes.

**AP Garg and Miller 1993 modified this technique**

Counter immunoelectrophoresis technique is modified by Dr. A.P. Garg and Miller in 1993. In this technique they used a tuft near the antibodies and filled with Buffer. In which it acts as electrophoresis.

The ions are present in the Buffer at the surface of antibodies the potential (electrochemical potential) it improved the hypersensitivity. ●

☛ **Q.6. Describe the composition, principle of working and uses of an Electron Microscope.**

**Or**

**Describe the principles and structure and different types of electron microscope.**

**Ans.** **Electron Microscope (EM)**

(i) The electron microscope was designed by **Knoll** and **Ruska** of Germany in 1932, by **Marton** of Beligium in 1934 and **Prebus** and **Miller** in 1934 in Canada.

(ii) The present limit of resolution of EM is about 3Å *i.e.* 3 × 1/10,000 of a micron ( μ )

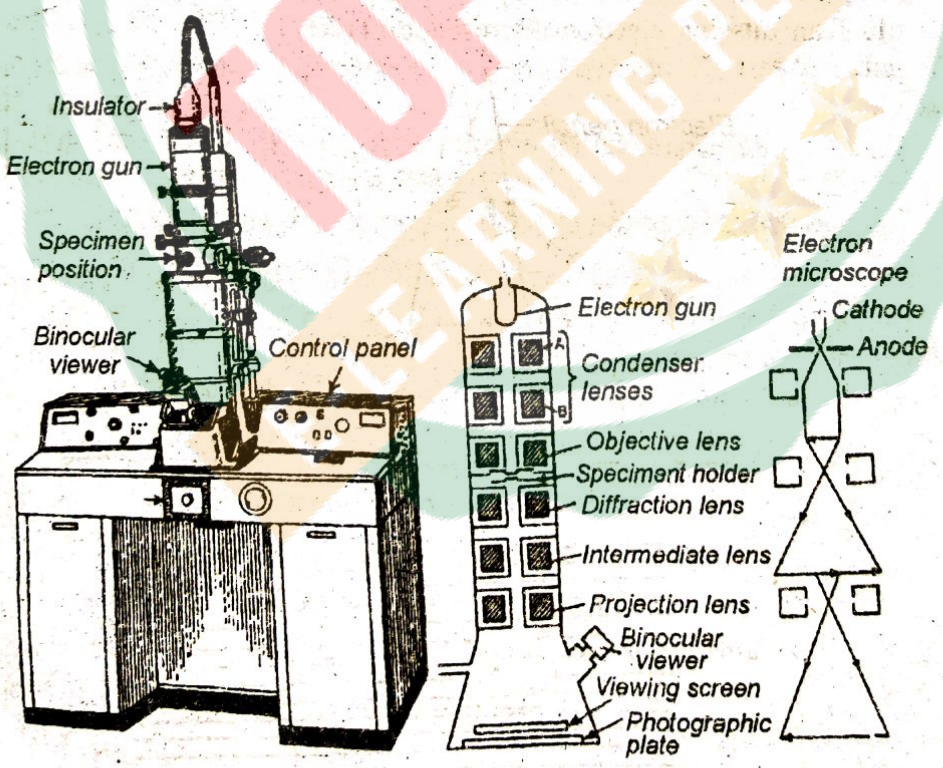



**Fig. Electron Microscope.**

(iii) The **illuminating agent** used are electrons of short wavelength ( $\lambda = 0.50Å$ )

(iv) The electromagnetic condensers act as lenses.

(v) The image is formed on photographic screen or photographic film.

(vi) The image formed is called electron micrograph.

(vii) The electron microscope is enclosed in vacuum chamber, because electrons travel in vacuum.

**Structure :** Electron microscope contains following parts :

(i) **Filament** or **cathode** which emits stream of electrons.

(ii) **Cathode ray tube,** through which electrons travel and reach the condenser lens.

(iii) **Condenser lens** is the magnetic coil which focuses or condenses the electron beam in the plane of the object.

(iv) **Objective lens** is also an electromagnetic coil which produces first magnified image of the object.

(v) **Ocular or projector lens** is another electromagnetic coil which magnifies the first image formed by the objective lens.

(vi) **Fluorescent screen** or **photographic plate** receives the final image of the object.

**Types of electron microscopes :** There are three types of them :

1. Transmission electron microscope (TEM)

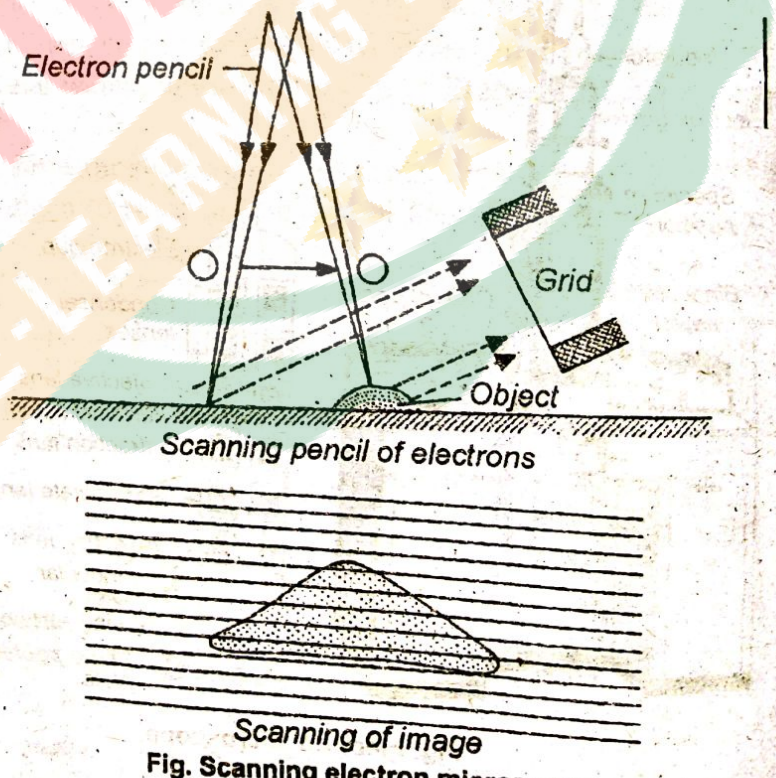



**Fig. Scanning electron microscopy.**

2. Scanning electron microscope (SEM)
3. High voltage electron microscope (HVEM)

**1. Transmission Electron Microscope (TEM)**

(i) The electrons pass through the stained specimen or are absorbed or scattered by atoms of metallic stain.

(ii) Image is formed by the electrons that pass through the object as bright areas.

(iii) The electrons that are absorbed *i.e.* are not transmitted by specimen leave dark regions on the viewing plate.

(iv) Image formed by TEM is a pattern of bright and dark areas.

(v) Its resolving power is about 0.1-0.2 nm.

**2. Scanning Electron Microscope (SEM) :** SEM is used for examining the surface of a specimen, because the image or photograph is formed by the electrons reflected from the surface of an object. A very fine pencil of electrons (about 20 nanometer in diameter) is focussed on the specimen and then scanned over the surface. Some of them may be reflected from the surface or may excite emission of secondary electrons from the surface of specimen at the point where the beam is scanned over the surface. Some of them may be reflected from the surface or may excite emission of secondary electrons from the surface of specimen at the point where the beam is scanning. These secondary electrons are collected by the positively charged grid. The collector gives rise to a flash of light in a solid scintillator. The light output is amplified in a **photo multiplier or video amplifier.** The signal from the grid is transferred to a television tube, which scans and forms the image on the screen. The resolving power of SEM is comparatively less than that of transmission microscope.

**Q.7. Describe the various types of Centrifuges.**

**Or**

**What is Centrifuges ? Write the types of Centrifuges.**

**Ans.**

**Principle of Centrifugation**

Sedimentation of the solute at the base of the tube is a function of a series of complex interacting factors joined to the properties of the solute and the system. The centrifugal force applied to the sample is equal to gravitational force and is determined in gravities ($G$)

$$G = \omega^2 r$$

where

$r$ = is the radius of rotation in centimeters

$\omega$ = is angular velocity in radians per second

It is more usual to monitor the speed of a centrifuge in terms of revolutions per minute than in radians per second

Thus

$$RCF = G = \frac{(2\pi rpm)^2}{60} r \times r^{-1}$$

where

$2\pi$ = one revolution of the rotor

$RCF$ = relative centrifugal force

g = radians per second

For a particle to sediment, it must displace the equal volume of the solvent from beneath it. It can only be achieved by centrifugation when the mass of the particle is greater than the mass of the solvent displaced.

Net force = $(M_p - M_s)\ \omega^2 r - fv$

where $f_v$ = friction

$M^p$ = mass of particle

$M_s$ = mass of the equal volume of solvent

It is easy to classify particles by their rate of sedimentation per unit centrifugal field, a parameter called sedimentation coefficient (s),

A Swedberg unit (S) is defined as sedimentation coefficient of $1 \times 10^{13}$ seconds,

## Type of Centrifuges

Different types of centrifuges are available now for different purposes. Generally they are divided into four groups : high speed refrigerated the bench centrifuges, large capacity refrigerated centrifuges, and ultracentrifuges.

## Small bench centrifuge

Simplest centrifuges that are used to separate erythrocytes, coarse precipitates and cells are called as bench or laboratory centrifuges. They have a speed ranging from 4000 to 6000 RPM and a relative centrifugal force of 3000 to 7000 g. They can be operated at ambient temperature.

Small samples are sedimented nowadays with microfuges that after a speed of 8000-13000 RPM and relative RCF of approximately 10,000 g. They sediment small volumes of material in 1 or 2 min. Blood samples are sedimented in routine using these microfuges.

## Large refrigerated centrifuge

Such instruments can work at a speed of 6000 RPM and produce a maximum relative centrifugal field of 6500 g. Their rotor chamber is refrigerated. Swinging type or fixed angle rotors can be interchanged that can accommodate 10,50 and 100 $cm^3$ tubes. They are normally used to collect substances that sediment rapidly, erythrocytes, nuclei, chloroplast, yeast cell and bulky precipitates.

**High speed refrigerated centrifuge**

Since the name suggests, these centrifuges can attain a speed of 25,000 RPM and a centrifugal field of 6000 g. Interchangeable rotor heads can be used to accommodate desired volume of the sample. They are generally used to collect micro-organisms, cellular debris, large cellular organelles and precipitated proteins. While, viruses and smaller organelles *viz* : ribosomes can not be separated by these centrifuges.

**Ultracentrifugation**

Such instruments operate at a speed of 60,000 to 100,000 RPM. Despite the high speed, technique is very gentle and gives a valuable method for the separation of large labile molecules or particles. ●

☛ **Q.8. Describe in brief photo-micrography.**

**Ans.** The unit is derived for performing photomicrography easily and conveniently using single lense reflex camera. Such cameras are called SLR cameras.

The photo micro unit is composed of outer and inner hood bauales and tighting clamp ring which can be used on any biological or mellallógical scope, having an eye piece sleeve with 25 mm outer diameter P.M.G., is possible with or without eyepiece of microscope.

(i) **Attaching on two microscope :** With tightning knob of inner band or accessory = clamp ring.

(ii) **Photomicrographic Magnification :** With tightning knob of inner band or accessory = clamp ring.

(iii) **Details of Apparatus :** Any biological microscope with eyepiece sleeve with 25 mm outer diameter.

(iv) **Attaching on two cameras :** With bayonet ring of outer barrel.

**Connection when Shooting with Eye Piece of Microscope :**

(i) In that condition joined the inner barrel to the microscope sleeve and fix it with the tightning knob with the inner barrel.

(ii) Pull off the eye piece of microscope get it into inner barrel.

(iii) Remove the inner barrel from outer barrel by loosening the tightning screw on outer barrel.

(iv) Slide the outer barrel joined camera over inner barrel which is connected with microscope and fixed with tightning screw on outer barrel.

**Connection when shooting without eyepiece of microscope :**

(a) Separate the outer and inner hood barrels

(b) Put the accessory Sharpe barrel instead of eye piece into inner barrel. The successing assembly procedures are performed in exactly the same manner as outline in earlier procedure.

**Precautions in Photomicrography :** In case of shooting with high magnification special and much care is to be taking about vibrations,

exposure and focusing. Following precautions should be taken for failure proof photomicrography.

(i) **Prevent Camera Shake :** Following precautions are found to prevent any image blurs due to cameras shake which easily occurs as the camera mechanism functions.

- The stop down liver which is locked in metering should remain in that position. The mirror should also be locked in the raised position after focusing and metering.
- Set the shutter speed of 1/2 second or slower or faster and control exposure conditions to get proper exposure conditions to obtain proper exposure by changing illumination and film speed by regulating apertures.
- Shutter release always use cable release never press the shutter release button directly.

(ii) **Avoid vibration :** It is most important to eliminate vibrations completely avoid places close to motor driver machinery and use antivibration rubber base whenever possible.

(iii) **Lighting :** For colour films : There are two types of films according to colour temperature they are the (a) day light (b) Tungston type in order to get a reliable colour balance it is necessary to get light by from a light source that suits the film used.

**Table**

| Film type | Lights source | Filter to be used on light source |
|---|---|---|
| Tungsten | Tungsten flood light Day light-Blue flood light | CCA 12 |
| Day light | Daylight blue flood light Tung. Flood light | CCB-12 |

**For black and white fills :** There are no light source restrictions if using black and white films since in photomicrography the brightness of the subject is ordinarily lower that in general photography use films with light e.g. copy films or Kodak paratomic.

If using an cromatic microscope objective. Cover the light source with a G1 colour balancing filter as the objective usually has an acromatic correct for greenish, yellowish light, the G1 filter is necessary for obtaining more highly contrasting images and more detailed structure of the subject.

**Lighting Method :** Photomicrography both direct and indirect illumination can be used normally Kohler illumination method for most uniform distribution is recommended.

**Deciding Proper Exposure :**

(i) Set the film speed on camera.
(ii) Push the stop down lever.
(iii) While looking into view finder term the shutter speed dial to align. The mt. needle with the index mark the shutter speed shows on shutter speed dial whenever needle in aligned with index mark is proper exposure value.

**Focusing :** With the high magnification, the light beam becomes extremely narrow, as focusing with split image or micro poison range finder is very difficult under these circumstances focus by means of surrounding field viewing screen instead. ●

☛ **Q.9. Describe the structure and function of light microscope.**

**Ans.** Our eyes are unable to resolve structures beyond one millimeter in size. Microscope (Gr. micros, small : *scopien,* watch) is used to observe structures below this range. **Microscope** *represents combination of magnifying lenses, especially designed for the study of objects too small to be seen with the naked eye.*

The first operational light microscope was constructed by JANSSENS. Since then, a number of optical devices have been evolved to ensure higher magnification. Those which are more commonly used are summarised here :

1. Light microscope, 2. Electron microscope, 3. Phase contrast microscope, 4. X-ray diffraction microscope, 5. Interference microscope, 6. Polarized light microscope, 7. Ultraviolet and Fluorescent microscope.

## LIGHT MICROSCOPE

The following types of light microscopes are normally used by undergraduates in the laboratory work—(i) simple dissecting micro-scope, (ii) binocular dissecting microscope and (iii) compound microscope.

### 1. Simple Dissecting Microscope

The simple microscope has a single lens system. It is composed of a single lens through which upturned image of the object is seen. In other words, the magnifying lens is in a sense a simple microscope. In its working form, a simple dissecting microscope is composed of (i) a simple convex lens mounted on a vertical stand through a folded arm, (ii) a short **limb** on the stand fitted with a screw for focusing, (iii) **stand,** (iv) **basal foot** (v) **reflecting mirror,** (vi) glass stage. The magnification of simple microscope ranges from 4 to 40 times depending upon the magnification of the lens. A 10 × lens provides an image ten times the size of the object.

### Operation of Dissecting Microscope

While observing from the dissecting microscope, place the eye close above the lens and then bring the object towards its focussing range on the glass stage. Turn the focussing screw up or down as the need may be to get a sharp and distinct image of the object. ●

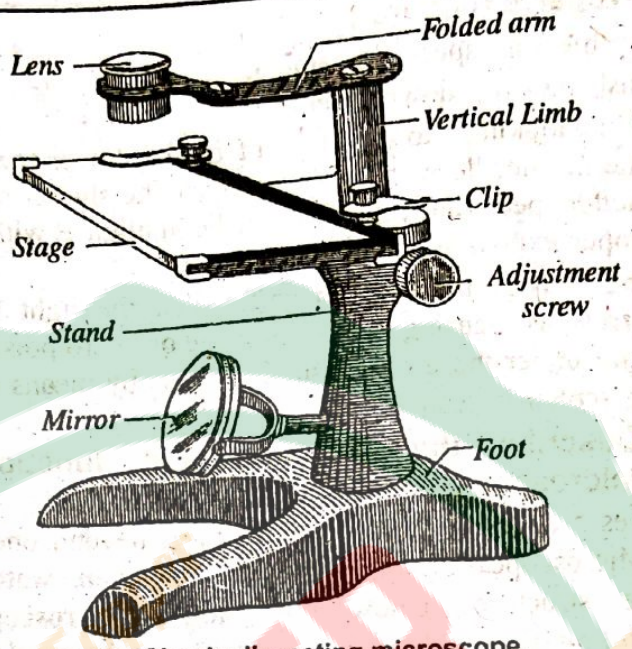



Fig. Simple dissecting microscope.

☛ **Q.10. Describe the principles structure and use of compound microscope ?**

**Ans.** **Compound Microscope**

The compound microscope is used to study details of minute organisms the sections of different organisms and their parts and the histological detail of different organs. It consists of a combination of two lens systems. The len system close to the object is known as objective and that which is close to th

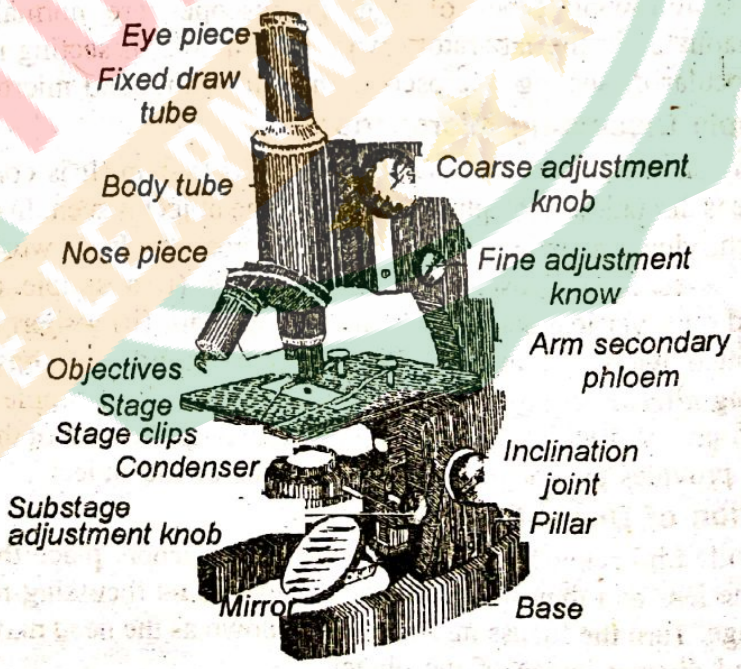



Fig. Compound microscope.

eye is eye piece or ocular lens. The two lens systems are placed one above the other in a vertical tube (body tube) at a proper calculated distance so that the image formed by one lens system is magnified again by the second lens system. Its structure can be studied under two headings :

| S. No. | Parts | Function |
|---|---|---|
| 1. | **Stand or Base** | Rests on the table |
| 2. | **Arms or limb** | Body tube is attached to its upper end by rack and pinion mechanism; can be moved up and down by two knobs. |
| 3. | **Body tube** | Carries eye piece attached to its upper end and a nose piece attached to its lower end. |
| 4. | **Nose piece (a circular disc)** | It attached to the lower end of body tube; bears 2 or 3 holes for the attachment of objectives of different magnifying powers; can be rotated to bring desired objective in position. |
| 5. | **Objective** | (i) Low power objective 10X (ii) High power objective : 45X<br>Consists of concave-convex lens, forms image of the object. |
| 6. | **Eye piece** | Consists of Plano-convex lens; magnifies image formed by the objective. |
| 7. | **Coarse adjustment (Large knob)** | Moves the body tube up and down for proper focussing while examining under low magnification. |
| 8. | **Fine adjustment (Small knob)** | Used for more accurate focussing while examining under high magnification. |
| 9. | **Stage** | For placing the slide. |
| 10. | **Stage clips** | For fixing the slide on the stage while examining under the microscope. |
| 11. | **Condenser and Substage** | Lies below the stage; condenses the light rays on the object. |
| 12. | **Diaphragm** | Attached to substage, used to control the amount of light falling on the object. |
| 13. | **Mirror (Plano-concave)** | (i) Plane side focusses sunlight rays on the object through condenser.<br>(ii) Concave mirror is used to focus light rays from the lamp. |

## Use of Compound Microscope

### Setting the Microscope

1. Take out the microscope from the box and place it gently on the working table, keeping the arm towards you and stage away. The base should be several inches away from the edge of the table.
2. Locate the various parts and check the damage or loss of part if any.
3. Clean the body and stage of microscope with some cloth- piece and the lenses with lens paper.
4. Using the coarse adjustment raise the body tube about an inch above the stage.
5. Rotate the nose piece of low power objective and hear the click. This indicates that the objective is brought in line with the body tube. While rotating, be careful that the objective is not in contact with the stage.
6. Open the diaphragm of the condenser fully.
7. Look through the eye piece with your left eye but keep both eyes open. Hold the edge of the mirror below the stage and tilt and turn the mirror towards the light source and adjust its position so as to make the circular field in the microscope illuminated uniformly.

### Focussing of Object Under the Microscope

Focussing under low magnification

1. Place the slide to be examined on the stage of the microscope and move it till the object comes in the centre of the hole of the stage.
2. Lower the body tube with the coarse adjustment until the low power objective comes close to the cover glass or until it cannot be moved down.
3. Look through the eye piece and raise the objective slowly and gently with coarse adjustment until the object is visible.
4. Make the focus sharp with fine adjustment of microscope.

### Focussing Under High Magnification

If the object is to be viewed under high power, do not try to focus it directly under high power but first focus it under low power and then change the high power objective and use fine adjustment to get sharp image.

While focussing under high power never use coarse adjustment.

### Care and Precautions

Microscope is a delicate instrument. It should be handled with all care and precautions. Some of them are being listed here :

1. Take out the microscope from its case only when it is to be used and replace it immediately afterwards.

2. For lifting or removing from the box, hold the arm of the microscope with one hand and support the stand or base with the other. **Do not lift the microscope carelessly with one hand.**
3. Place the microscope on the working table gently and at least $2\frac{1}{2}$" away from the table edge in order to prevent its accidental fall.
4. The arm of the microscope should always be towards you and the stage away from you.
5. You are supposed to know all parts of microscope. **Check** all its parts before use. In case of damage or absence of any part immediately inform the teacher-in-charge.
6. Clean different parts of the microscope before use.
7. Avoid tilting of the microscope, keep it in **upright position.**
8. While focussing light on the object do not **allow the direct sun-rays to strike the mirror.**
9. Use plain mirror for natural daylight and concave for artificial light.
10. The stage should always be dry and clean.
11. **Do not put wet slide on the stage and never observe any object dry and uncovered.** The material to be observed should be properly mounted and covered with cover glass.
12. The lenses should never be touched or cleaned with hand. Always wipe them with lens paper or muslin cloth.
13. Clean the objective lens with xylol after use.
14. After use, make the objective free and lower the body piece. Transfer it into its box and keep it in its position.
15. While observing through microscope, keep both eyes open.

☛ **Q.11. Systematize the microbes into various groups giving their salient features. How are the microbes useful for human life ?**

**Ans.** **Position of Micro-organisms**

**Plantae and Animalia**

In the 19th century, the micro-organisms were kept into the traditional two kingdoms of organisms, the **Animalia** and **Plantae.** This assignment of the microbes to either kingdom was made on the basis of basic differences between animals and plants :

(1) Ability to photosynthesise.

(2) Ability of active movement.

**Algae** and **fungi** were grouped under plants. Microscopic motile forms were put together in one group, the **Infusoria** under animals.

Infusoria represents a heterogeneous group in terms of their cellular organisation. Thus, the simpler, unicellular forms were subdivided into two groups : the **protozoa** with large and complex cells and the **bacteria** with much smaller and simple cells. The old Infusoria was therefore split into three categories.

1. Bacteria that were put with plants,
2. Protozoa or unicellular animals and
3. Metazoa or multicellular invertebrate animals.

But, the distribution of micro-organisms into plants and animals gives several inconsistencies. With more information about micro-organisms hardly justified their inclusion in either kingdoms. For example unlike plants fungi and many bacteria are non-photosynthetic. Many bacteria are motile and some fungi and algae have motile spores. Hence the division of living world into these two kingdoms can no longer be kept.

## Protists

**E. Haeckel** in 1886 gave a third kingdom, the **Protists.** It consists of protozoa, algae, fungi and bacteria. It included both photosynthetic and non-photosynthetic forms. The **simple biological organisation** distinguished all protists from plants and animals.

## Eukaryotes and Prokaryotes

When the development of electron microscope occurs, fine structure of cells was revealed and it was observed that two radically different kinds of cells exist in the living world :

(1) the less complex **prokaryotic cell** as observed in bacteria and cyanobacteria or blue-green bacteria.

(2) the more complex, **eukaryotic cell** as observed in plants metazoan animals, protozoa, fungi and algae and

Micro-organisms hence belong to both these groups. hence algae, fungi and protozoa are eukaryotes and all bacteria and cyanobacteria are prokaryotes. The eukaryotic cells consist a nucleus with a nuclear membrane and multiple chromosomes. The nucleus of a prokaryotic cell is a single circular chromosome, without a nuclear membrane. Prokaryotic cells also lack membrane-bound organelles. Some prokaryotes are able to fix nitrogen.

## Monera

In 1969, **R. H. Whittaker** gave a five kingdom system of classification. He proposed a separate kingdom. **Monera,** for the true prokaryotes : the bacteria and cyanobacteria. Algae and protozoa we grouped under kingdom **Protista** and **Fungi** were given an independent kingdom. The two other kingdoms were the **Plantae** and **Animalia** for plants and animals respectively.

1. **Fungi :** Includes zygomycetes, ascomycetes, basidiomycetes and deuteromycetes.
2. **Protista :** This includes algae, protozoa, slime moulds and water moulds. Slime moulds and water moulds are usually considered as fungi but phylogenetically they are said to be more related to protists.
3. **Monera :** This includes Eubacteria, rickettsiae, chlamydiae, mycoplasma, archaebacteria and cyanobacteria or blue green bacteria.
4. **Animalia :** Includes invertebrates and vertebrates.
5. **Plantae :** Includes bryophytes and tracheophytes.

## Main Groups of Micro-organism

### A. Bacteria

These are true prokaryotes. This group includes the true bacteria archaebacteria, blue-green bacteria, mycoplasmas, rickettsias and chlamydiae.

**1. Eubacteria :** They have some characteristics given as follows :

(i) Growth occurs over a wide range of temperature.
(ii) Nutrition by diverse methods.
(iii) They are prokaryotes and occur in every terrestrial and aquatic environment.
(iv) Many can live both in oxygen rich and oxygen-free environments.
(v) Reproduce by simple fission.

**2. Archaebacteria :** They differ from other bacteria in following respects :

(i) Their wall does not contain peptidoglycan.
(ii) Their ribosomes are insensitive to chloramphenicol.
(iii) Cell membrane is of a single layer of glycero-hydro-carbon-glycerol chains instead of a bi-layer of phospholipids.
(iv) They inhabit extreme environments. They include the methanogens, the extreme red halophiles, salt-loving bacteria of saturated brine and salted fish and the thermoacidophile, observed in hot sulphur springs. They shows a very ancient group which diverged from the eubacteria very early in the evolutionary process.
(v) Their 16S rRNA differs greatly from those of other bacteria and from eukaryotes.

**3. Myxobacteria** from fruit bodies, specialised multicellular structures like slime moulds. Some cells at tips of fruit body gives cysts. They are most complex in behaviour.

**4. Blue-green bacteria** are typical prokaryotes. They evolve oxygen during photosynthesis and have **chlorophyll,** a pigment also observed in all algae. They are perhaps major primary producers in the world's oceans and many are important as fixers of atmospheric nitrogen.

**5. Rickettsiae** are tiny rods that are transmitted by arthropods and multiply mere in living cells. The chlamydiae are among the smallest bacteria which were formerly considered viruses. Mycoplasmas are the smallest organisms which can be cultivated outside living tissues. Some are involved in lung disorders.

### B. Protozoa

These are a group of unicellular non-photosynthetic eukaryotic micro-organisms. These obtain their food through phagocytosis and do not possess cell wall. Some are involved in blood and tissue diseases. Important characteristics are as follows :

1. Food taken by phagocytosis.

2. Locomotory organs may be pseudopodia, flagella or cilia. On the basis of locomotive movement, protozoa have been divided into :

(a) Protozoans with ciliary movement-ciliates e.g. *Paramecium.*

(b) Protozoans with flagellar movement-flagellate protozoa.

(c) Protozoans with amoeboid movement-*Amoeba.*

(3) Degree of specialisation in single cells as in *Paramecium.*

(4) Life-cycle less complicated, exception being forms like *Plasmodium.*

(5) Cell wall is lacking.

**Slime Moulds :** Some authors also study slime moulds along with protozoa. For example fungi, they form fruiting bodies. Their feeding phase is amoeboid. They live on decaying vegetation. There are two main groups : (a) *a cellular slime moulds* and (b) the **cellular slime moulds,** whose vegetative stage is single amoeboid cells that may aggregate to form a *pseudo-plasmodium* as in *Dictyostelium.* In this case a single amoeba gives multinucleate plasmodium of indefinite size and shape which moves on the surface of the substratum engulfing food particles.

### C. Actinomycetes

They are Gram-positive prokaryotes, a large group of filamentous bacteria which represents branching patterns just like those of fungi, to give rise a spreading mycelium. They, like fungi, produce spores if grown in culture. Their main characteristics are :

(i) Cell wall with a very large number of other peptidoglycan types.

(ii) Mycelial, Gram-positive prokaryotes.

(iii) These produce antibiotics like **streptomycin, tetracyclines** and chloromycetin.

(iv) Some develop were in mycelial state and reproduce by forming spores as in *Streptomyces, Micromonospora, Actinoplanes.* Thermoactinomyces. Some reproduce through fragmentation into rod-shaped cells as in *Bifidobacterium, Actinomyces, Mycobacterium, Nocardia,* etc. Some other are unicellular bacteria which do not form endospores and have a regular shape as in *Leuconostoc, Pediococcus, Streptococcus, Lactobacillus, Sarcina* and *Staphylococcus.*

**D. Fungi**

Chief characteristics are as follows :

(1) Life cycle may be simple or complex. In some, large fruit bodies are formed.
(2) Mycelia may be coenocytic or septette.
(3) Motile stage absent, never possess flagella.
(4) Heterotrophic eukarytic microbes. Take their food in soluble form.
(5) Asexual reproduction by a variety of spores.
(6) Mycelium made of filaments, the **hyphae.** Yeasts are unicellular.
(7) Cell wall thick, made of polysaccharides and chitin.

**E. Algae**

Pigmented eukaryotes ranging in size from microscopic forms to giant keeps of marine waters. Main characteristics are as follows :

(1) Small aquatic algae form part of plankton.
(2) Cell wall made up of pectin, cellulose or xylan. The cell wall of red seaweeds contain the agar gel.
(3) Exhibit a wide range of morphological types.
(4) Reproduce asexually and sexually.
(5) Many are motile, usually by flagella.
(6) Produce their food by photosynthesis.

**Q.12. Describe the main characteristics of Virus.**

**Ans. Characteristics of the Viruses :** Viruses are ultramicroscopic, obligate, intracellular parasites, self replicating, non-cellular organisms. Their detailed studies have been made by electron microscope. The mature virus particle is also designated as **virion** and the terms are sometimes used interchangeably. **Virion** or **infection particle** of virus, always consists of a nucleic acid core of genetic material surrounded by a protein coat and is termed as nucleoprotein. The virions of most bacteriophages contain DNA; a few contain RNA. Virions of some animal viruses contain DNA and others have RNA. Cauliflower mosaic virus has double stranded DNA. Different virions also exhibit following normal characters alongwith their specific characters :

(1) These are ultramicroscopic and vary from 20—350 nm.
(2) These are obligate, intracellular parasites and non-cultivable on inanimate culture media. Easily propagated in living culture media.
(3) These are non-filterable through molecular filters.
(4) These are made up of nucleoproteins only.
(5) The outer shell or capsid is mostly of proteins which ranges from 60-95% of total virual mass. Lipoviruses have additional covering of lipids or lipoproteins.
(6) These have only one kind of nucleic acid either DNA or RNA but never both.
(7) Nucleic acid is a single molecule *i.e.*, one replicon, which may be single or double stranded.
(8) These contain no metabolic enzymes or proteins synthesizing machinery of their own and use host machinery for synthesis of their proteins.
(9) These replicate but do not grow, but their nucleic acid directs the host cell to make various parts of virus and then to assemble these parts into complete, infectious particles, virions.
(10) These can be precipitated and crystallized.
(11) Viruses have host specificity (particularly animal and bacterial viruses). Plant viruses are not so specific.
(12) Generally antibiotics have no effects on viruses since they do not have their own metabolic machinery.
(13) They are transmissible from diseases to healthy plants.
(14) They posses the capacity of variations and adaptations.
(15) They are highly resistant to acids, alkalies, salts and high temperature.
(16) There is no direct effect of sunlight on viruses. They only have a single type of nucleic acid either DNA or RNA.

☛ **Q.13. Describe in short the history of Virology.**

**Ans.** The study of viruses is called **Virology.** The term virus is derived from latin, meaning **"venom or poisonous fluid"** and name was given to the causative agents of infectious diseases by **Pasteur.** Yellow fever (first viral disease of man) and small pox like diseases of virus are known since early days of human history but their cause was known quite late. **Adolf Meyer** (1886) observed mottling in tobacco plant leaves and named it **Mosaikkrankhet** *i.e., Mosaic.* He also showed the infectious nature of filtrate of diseased leaves. But due to the loss of disease causing capacity by heating at 80°C, he concluded that bacteria was the cause of the disease.

**Ivanowski** (1892) suggested that the cause of Tobacco Mosaic disease is filterable organism. He passed the sap of infected tobacco leaves through bacteria proof filter and sprayed the filtrate on healthy leaves of tobacco plant. After some days he observed reappearance of the disease. He concluded that the disease causing agents are filterable entities and smaller than bacteria. Therefore he is considered as discoverer of Viruses.

**M.W. Beijerinck** (1888) introduced the theory of **"contagium vivum fluidum"** *i.e.*, **living infectious fluid,** when they observed that the filterable, invisible and non-cultivable infectious principle would diffuse through the agar gel, like a fluid. Viral diseases of vertebrates also were well known by 1892, since **Pasteur** had been studying canine rabies for sometime. He indicated the cause of this diseases as virus. **Loeffer** and **Frosch** (1898) demonstrated that foot and mouth disease of cattle (first known viral disease of vertebrates) is caused by filtrate apparently free from any bacteria and concluded that if the agent of this disease is particulate, it must be smaller than the diameter of the smallest known bacteria. In 1900 **Walter Reed** and his associates discovered the virus of yellow fever.

Viruses which attack bacteria were first described by **F.W. Twort** (1915). These were also independently observed and more fully studied by **F.D. Herelle** (1917). He also coined the term **Bacteriophage** for such viruses. The Stainley (1935) was first to crystallize virus (T.M.V.) and showed that crystals consisted largely of proteins.

Tobacco Mosaic Virus (T.M.V.) was the first that was studied under Electron microscope by Takahashi and Rawlins in 1933. Bawden and Pirie (1937) and Darlington (1944) suggested that all Viruses consists of protein coat called 'Capsid' over a nucleic acid core (RNA or DNA). Hasshey and Chase (1952) suggested that protein is non infective and nucleic acid is infective part of the bacteriophage respectively.

☛ **Q.14. Describe the contribution of the some Virologist.**

**Ans.** M.W. Beijerinck : M.W. Beijerinck (1888) introduced the theory of **"contagium vivum fluidum"** *i.e.*, **living infectious fluid,** when they observed that the filterable, invisible and non-cultivable infectious principle would diffuse through the agar gel, like a fluid. Viral diseases of vertebrates also were well known by 1892, since **Pasteur** had been studying canine rabies for some time. He indicated the cause of this disease as virus. **Loeffer** and **Frosch** (1898) demonstrated that foot and mouth disease of cattle (first known viral disease of vertebrates) is caused by filtrate apparently free from any bacteria and concluded that if the agent of this disease is particulate, it must be smaller than the diameter of the smallest known bacteria. In 1900 **Walter Reed** and his associates discovered the virus of yellow fever.

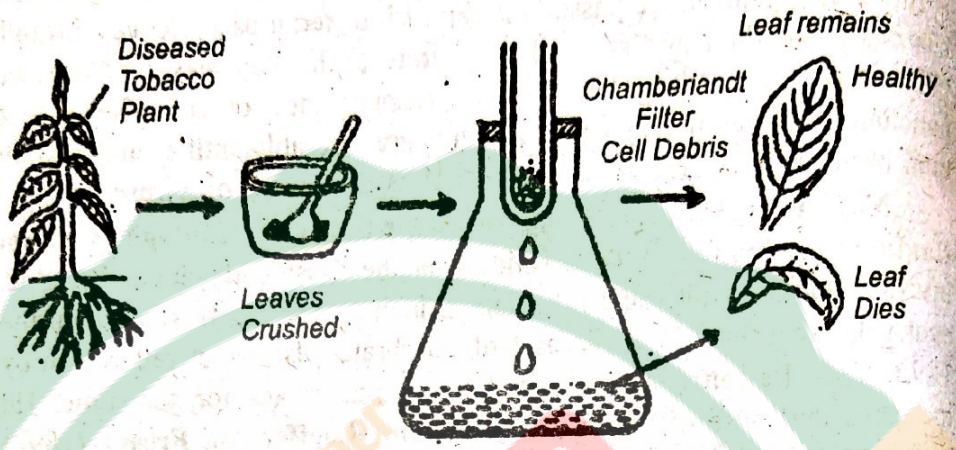



**Fig. Ivanowski's Experiment**

**Stanley :** He is 1935 crystallize the TMV virus for the first time. He was awarded Nobel Prize for this discovery. He obtained these crystals by treated the juice of infected tobacco leaves with ammonium sulphate.

**Ivanowski :** Ivanowski (1892) suggested that the cause of Tobacco Mosaic disease is filterable organism. He passed the sap of infected tobacco leaves through bacteria proof filter and sprayed the filtrate on healthy leaves of tobacco plant. After some days he observed reappearance of the disease. He concluded that the disease causing agents are filterable entities and smaller than bacteria. Therefore he is considered as discoverer of Viruses.

**Anton Von Leeuwenhock :** He was of Holland. Credit goes to him for having seen bacteria for the first time in the year 1675 by his crude microscope which had magnification of 150 times only. He observed hair fibers, crystals, blood etc. with the help of this microscope. He called tiny microbes of pond water as **"animalcules."**

**Christian gram :** He introduced differential or gram stain in 1884 for identification of bacteria. Firstly staining the bacteria with basic dye crystal violet solution followed by 0·5% iodine. It is then treated by 95% absolute alcohol Gram positive bacteria turn while the Gram negative become colourless and can be stained red by sarfranin Gram$^+$ e.g. *Bacillus, Staphyloccocus* and *Streptococcus*, Gram$^-$ e.g. *Escherichia, Solmonella, Spirillum* etc.

**Winogradsky :** Russian Bacteriologist, **Winogradsky** described autotrophic bacteria that are capable of bringing about nitrification. He was the first to discover anaerobic bacteria in 1894 which is capable of fixing atmospheric nitrogen into ammonia and named it closteridium.

☛ **Q.15.Describe the short history of bacteriology.**

**Ans.** Although the use of microbes like yeasts is known from ancient times, their study started from **Leeuwenhock** known as "father of

microbiology" in 1675 when he studied several types of microbes with the help of self designed microscope and called them as **animalcules** (little animals). He observed these little animals (Fig.) in rain and pond water, in material of his own teeth and in several other objects. **Linneaus** (1738) also studied several microbes and also regarded them as little animals. Genus **Vermes** was established by him for these microbes. **Muller** (1773, 1786) coined the term **Vibrio** and **Monas** and first of all classified Bacteria into these two groups. However, the term **Bacterium** was coined by **Ehrenberg** (1828). In 1938, he (1857) suggested the name **schizomycetes** (the fission fungi) for microbes which he studied. **Pasteur** (1864) made a series of experiments and affer extensive studies established that there is no spontaneous origin of microorganisms. Several other important discoveries in the field of microbiology like fermentation by microbes, aerobic and anaerobic microbes were also made by him. It was **Cohn** (1872) who established that bacteria belong to plant kingdom. **Koch** (1876) observed that microbes cause disease and presented his germ theory of disease which is commonly known or **Koch's postulates.** In 1888 **Bejirink** isolated and cultivated first $N_2$ fixing bacteria from root nodules and only after it, large scale studies were carried out on different aspects of microbes by several workers which helped in establishing microbiology as an important branch of biology.

**Important Bacteriologists :**

**(1) Louis Pasteur (1822-1895) of France :** In 1862 he was the first to draw attention to many of the microbial activities.

(i) **Fermentation :** The end product of sugar fermentation is not alcohol if some microbes (bacteria) are present (not yeast).

(ii) Milk is soured by the presence of bacteria.

(iii) Microbes come from some other microbes.

(iv) **Pasteur** further demonstrated that not only fermentation but similar process as purification, infection and souring are also caused by germs or microbes.

(v) He also discovered the principle of immunization, *i.e.*, the ability of hosts body to prevent invasion by disease causing bacteria.

**(2) Anton Von Leeuwenhock of Holand :** Credit goes to him for having seen bacteria for the first time in the year 1675 by his crude microscope which had magnification of 150 times only.

(3) Russian Bacteriologist, **Winogradsky** described autotrophic bacteria that are capable of bringing about nitrification. He was the first to discover anaerobic bacteria in 1894 which is capable of fixing atmospheric nitrogen into ammonia and named it closteridium.

**(4) Ehrenberg** (1829) was the first to establish genus bacterium.

**(5) Robert Koch (1843-1910) of Germany :** He was first to isolate some of disease causing bacteria such as Anthrax bacillus in 1876, tuberculosis bacillus in 1882 and comma bacillus of Asiatic Cholera in 1883.

**(6) Christian Gram** introduced differential or gram stain in 1884 for identification of bacteria. Firstly staining the bacteria with basic dye crystal violet solution followed by 0·5% iodine. It is then treated by 95% absolute alcohol Gram positive bacteria turn while the Gram negative become colourless and can be stained red by sarfranin Gram$^+$ e.g. *Bacillus, Staphyloccocus* and *Streptococcus,* Gram$^-$ e.g. *Escherchia, Solmonella, Spirillum* etc.

**(7) Elie Metchnikoff** (1805-1960) of Russia Humen leucocytes eat disease causing bacteria.

**(8) Beizerinck** 1888 established the role of symbotic bacteria *Rhizobium* in fixing nitrogen in leguminous plants he also discovered in 1901 aerobic bacteria *Azotobacter* fixing atmospheric Nitrogen in soil. ●

☛ **Q.16. Describe the classification of bacteria based on their structure.**

**Ans.** A detailed scheme of classification of micro-organisms was presented in 7th edition of **Bergey's Mannual of Determinative Bacteriology** (1957). A separate and new division **Protophyta** was recognised in the plant kingdom to include the more primitive of the plant like microorganisms. This was further divided into three classes **viz., Schizophyceae** (Blue green algae), **Schizomycetes** (Bacteria and related forms) and **Microtatobiotes** (Rickettsiae and Viruses). Detailed outline of this classification of microorganisms.

The classification given in 8th edition (1974) of **Bergey's Manual** or **Determinative Bacteriology** has been accepted as the official document bacteriologists. In this system, Bacteria constitute as one of the divisions of the kingdom Prokaryotae. Classification of Bacteria as per **Bergey's Mannual of Determinative Bacteriology**, 8th Edition, 1974 is as follows :

**Kingdom Procaryote**

**Division I. Cyanobacteria :**

Photosynthetic prokaryotes, photosynthetic process similar to that of higher plants, photosynthetic pigments chlorophyl and phycobiliproteins, simple or branched chain of cells, reproducing by binary fission, spores or fragmentation.

**Division II. Bacteria :**

Unicellular or simple arrangements, cell wall rigid, of peptidoglycans, photosynthetic process, amoxygenic (electron donor other than water) bacteriochlorophylls as photosynthetic pigments.

The division bacteria has been further divided into 19 groups on the basis of their photosynthetic ability, type of movement and responses to Gram stain etc. Important characters of these groups are as follows :

**Group 1. Phototrophic Bacteria :**

Photosynthetic bacteria, characterized by the presence of bacteriochlorophyll, occur mostly in aquatic environments, motile of non-motile, include a single order with 3 families and 18 genera.

**Group 2. Gliding Bacteria :**

Produce slime and show gliding movement, some forms produce brightly coloured macroscopic fruiting bodies, occur abundantly in soil, decomposing plant matter and aquatic environment, include 2 orders and 8 families, representing 21 genera of uncertain affiliation.

**Group 3. Sheathed Bacteria :**

Rod-shaped or filamentous bacteria, surrounded by a sheath of insoluble compounds of iron and manganese, motile or non-motile, Gramnegative, occur in aquatic environments, sludge etc. represented by 7 genera.

**Q.17.Write a small essay on Rickettsiae.**

**Ans.** The fundamental differences from ordinary bacteria are (i) a smaller size with smaller genome providing fewer enzymes (ii) a longer generation time and (iii) a requirement for an exogenous energy supply for growth. They infect ordinarily non-phagocytic cells and are protected from lysosomal degradation by envelopment in a vesicle of the host cell membrane.

**Structure**

The rickettsiae (sing, rickettsia) measure about 0·3-0·5 μm in diameter and 0·3-0·4 μm in length and usually appear as rods with rounded edges, a form known as the *Coccobacillus*. They also exist in alternate shapes and hence pleomorphic. They are hardly visible under the light microscope. They have no flagella, pili, capsules or spores.

The cell wall is chemically similar to that of Gram-negative bacteria and the cytoplasm contains both DNA and RNA as well as many of the enzymes. Reproduction is by binary fission.

Except for the organism of trench fever, they do not grow on artificial laboratory media. Living tissues as vertebrate cell cultures, fertilised chicken egg or live animals are used for their growth. They are, therefore, obligate intracellular parasites. They are closely associated with arthropods, particularly with arachnids (ticks, mites) and insects (lice, fleas).

Rickettsiae infect both humans and arthropods, the latter serving as **vectors.** Arthropod is a primary host and the human, a secondary host. Ticks and mites pass the rickettsiae to their young through the eggs in a process called **transovarian infection** (Fig.).

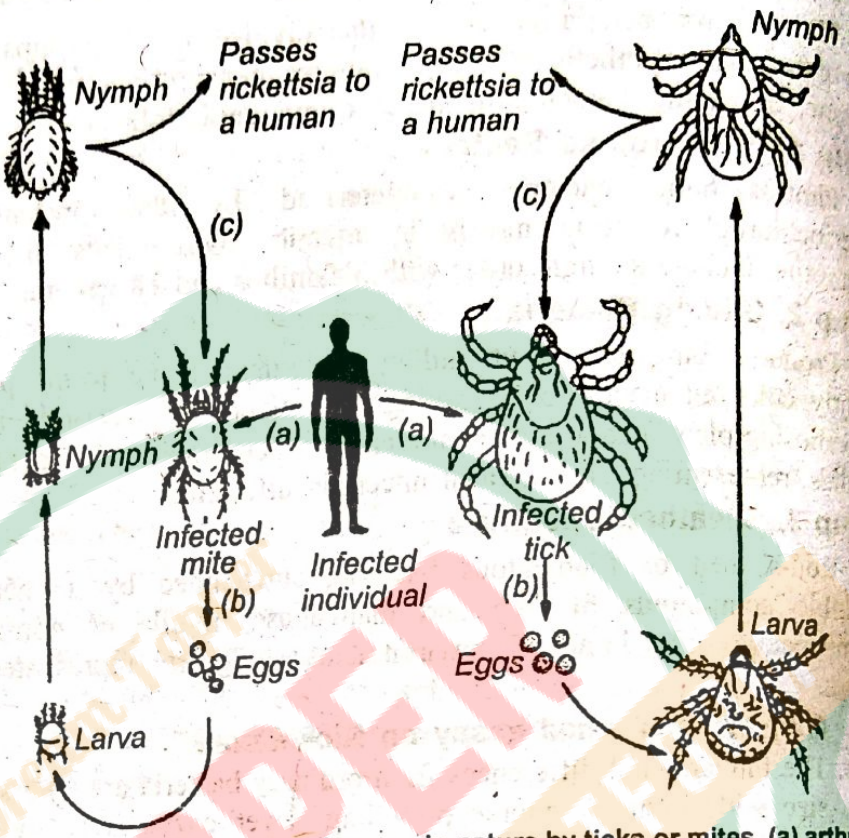



Fig. Maintenance of rickettsiae diseases in nature by ticks or mites. (a) arthropo absorbs the blood of infected man and acquires rickettsiae. (b) it passes t organisms to its eggs in fertilisation. (c) the eggs develop into infected lar nymph and adult stages, after which the cycle repeats itself. The mite or tick ma pass the infection to another man during a blood meal at any of these stages.

A summary of Rickettsial diseases is presented in Table.

**Table : A summary of rickettsial diseases.**

| Disease | Causative agent | Symptoms | Wel-Felix reaction | Vector | Treatment |
|---|---|---|---|---|---|
| Rocky Mountain spotted fever | *Rickettsia rickettsii* | Fever | + | Tick | Tetracycline |
| Epidemic typhus (Typhus fever) | *R. prowazecki* | Fever<br>Rash | + | Louse | Tetracycline |
| Endemic typhus (Murine typhus) | *R. typhi* | Fever<br>Rash | + | Flea | Tetracycline |

| | | | | | |
|---|---|---|---|---|---|
| Scrub typhus (Tsutsuga mushi) | *Rickettsia tsutsugamushi* | Fever<br>Rash<br>Eschar | + | Mite | |
| Rickettsial pox | *R. akari* | Fever<br>Rash<br>Eschar | – | Mite | |
| Q fever | *Coxiella burnetti* | Fever<br>Bronchitis | – | Ticks<br>Dairy products<br>Airborne dust, Droplets | |
| French fever | *Rochalimaea quintana* | Fever<br>Rash | + | Louse | |
| Tickborne fevers | *R. conorii* | Fever<br>Rash | + | Tick | |

## SHORT ANSWER QUESTIONS

**Q.1. In microbiology describe the contribution of Robert Koch.**

**Ans. Robert Koch :** The German doctor, Robert Koch is credited for his following main contributions to development of microbiology :

(a) Final proofs that bacteria could be isolated and shown to cause disease, while working on **anthrax** disease of animals. Thus he presented his **germ theory of disease.**

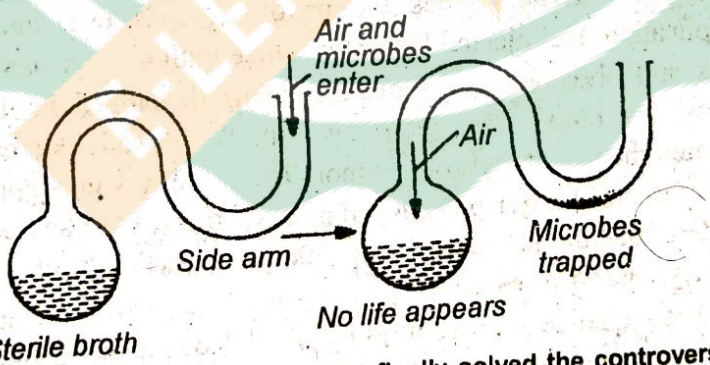



**Fig. Swan-necked flask of Pasteur that finally solved the controversy of spontaneous generation.**

(b) Developing the series of procedure—**Koch's postulates,** by which a specific organism could be related to a specific disease.

(c) Development of pure culture techniques.

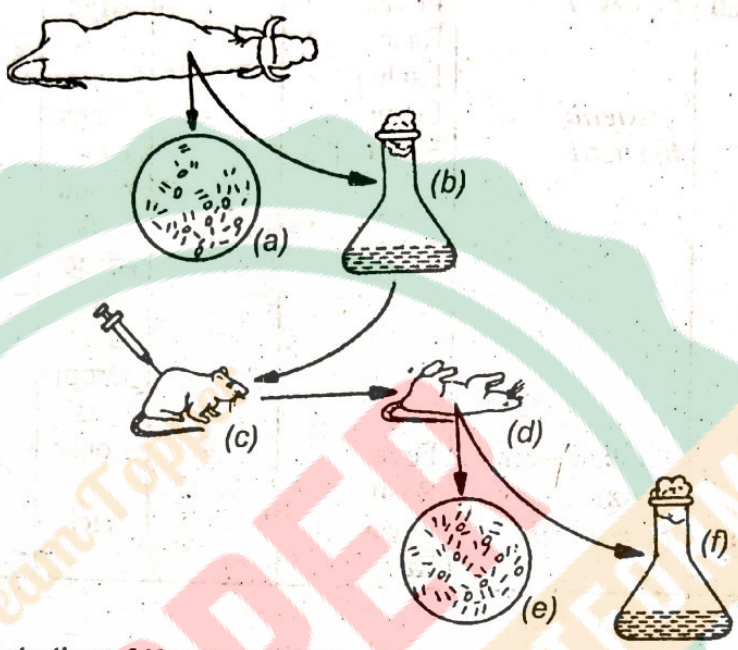



**Fig. Demonstration of Koch's postulates. (a) organisms from a dead animal are observed in tissue specimens and (b) isolated in pure culture. (c) a sample of the pure culture is inoculated to a healthy animal and (d) the animal is observed for symptoms of disease, (e) identical organisms are then observed in tissue samples of the dead organism and (f) reisolated in pure culture.**

Koch had observed thread-like organisms in the blood of animals that had died of **anthrax,** a disease that was serious threat to farmers killing their sheep and cattle herds periodically. He injected laboratory animals with the bacteria- ladden blood of dead sheep. He then performed autopsies and noted how the same symptoms appeared regularly. Koch isolated a few of the bacilli in the clear sterile vitrous humor of an ox's eye and watched their multiplication. He injected laboratory mice with a silver of wood containing spores and observed as anthrax symptoms developed in the animals. On autopsy, the blood was swarming with the thread like bacteria, and he reisolated them in the vitrous humor. The cycle was now complete. In 1867 Koch presented his **germ theory of disease.** Ironically, van Leeuwenhoek had first described the microorganisms 200 years earlier. Koch had now proved that they caused disease.

The sequence of procedures by which Koch established his germ theory of disease came to be known as **Koch's postulates.** Figure demonstrates their use. These procedures became a guide for relating a specific organism to a single disease.

**Q.2. Write major contributions of Louis Pasteur.**

**Ans.** Louis Pasteur in 1860-61 supported the doctrine of **spontaneous generation or abiogenesis.** However, later in 1864 he proved that microbes can only be produced by earlier microbes. The main contributions of this French scientist are :

(a) **Fermentation process :** Pasteur's experiments proved that yeasts and bacteria brought important chemical changes in fermentation of grape juice to wine. If care was taken to eliminate bacteria, the wine would not become sour. He reported :

(i) Grape Juice + Yeasts + Bacteria.....Incubation ..... Sour wine

(ii) Grape Juice + Yeasts + Bacteria ..... Boil ..... Cool and add yeast ..... Incubation ..... Good wine.

(b) **Disease control :** He believed that bacteria were in the soil and air and thus their spread could be controlled. In 1880, he separated bacteria of cholera and invented the vaccine of cholera.

(c) He also discovered the principle of immunization, *i.e.,* the ability of hosts body to prevent invasion by disease causing bacteria.

**Q. 3. Write a short note on Archaebacteria.**

**Ans.** Archaebacteria have following features (in common) whereby they differ from other bacteria :

(1) Their 16s rRNA molecules are similar to each other, but differ greatly from those of other bacteria and from eukaryotes.

(2) Their walls do not contain peptidoglycan, but a range of other unique polysaccharides.

(3) Their cell membrane is of a single layer of glycero-hydro-carbon-glycerol chains instead of a bi-layer of phospholipids arranged tail to tail.

(4) Their ribosomes are insensitive to chloramphenicol.

(5) They inhabit extreme environments. They include the methanogens (the methane-generating bacteria of anaerobic muds); the extreme red halophiles; the salt-loving bacteria of saturated brine and salted fish; and the thermoacidophiles, found in hot sulphur spring or smouldering coal wastes. It is suggested that they represent a very ancient lineage which diverged from the eubacteria very early in evolutionary process and have survived only in these specialised ecological niches.

**Q.4. Write a note on methanogenic bacteria.**

**Ans.** Methanogenic bacteria may have been among the earliest livin organisms on earth because they can grow autotrophicall (chemoautotrophs) on hydrogen and carbon dioxide under anaerobi conditions and it is presumed that the early atmosphere of the eart would have permitted the growth of just such organisms. Methanogen can be differentiated on the basis of relatively few morphological an physiological features (Table 1).

**Table : The methanogenic bacteria**

| | | |
|---|---|---|
| **Order** | **Methanobacteriales** | Cells short rods, lancet- shaped cocci to long filaments; cells appear Gram-positive but lack murein; strict anaerobes; oxidise hydrogen; reduce $CO_2$ to $CH_4$. |
| **Family** | **Methanobacteriaceae** | |
| **Genera :** | ***Methanobacterium*** | Slender rods often forming filaments. |
| | ***Methanobrevibacter*** | Short rods or lancet- shaped cocci often in pairs or chains. |
| **Order** | **Methanococcales** | Cells cocci; oxidise hydrogen or formate; reduce $CO_2$ to $CH_4$; Gram-negative, with protein units external to cell membrane. |
| **Family** | **Methanococcaceae** | |
| **Genus :** | ***Methanococcus*** | |
| **Order** | **Methano microbiales** | Cocci single, in pairs or clumps. Cells cocci to rods; Gram-negative or Gram-positive; motile or non-motile; strict anaerobes; oxidise hydrogen or formate; reduce $CO_2$ to $CH_4$ or form $CH_4$ via fermentation of methanol and related compounds. |
| **Family** | **Methanomicrobiaceae** | Gram-negative cocci to rods. |
| **Genera :** | ***Methanomicrobium*** | Short motile rods |
| | ***Methanogenium*** | Irregular coccoid cells |
| | ***Methanospirillum*** | Curved, slender motile rods often forming filaments. |
| **Family** | **Methanosarcinaceae** | Gram-positive coccoid cells occurring in packets. |
| **Genus :** | ***Methanosarcina*** | |

**Q.5. Write a short note on slow viruses.**

**Ans. Slow viruses (Prions) :** Prions are described as proteinaceous particles thought to cause a number of diseases including the slow virus diseases. Prions were named by Stanley B. Prusiner. Prions can survive heat, radiation and chemical treatments that normally inactivate viruses. They appear to be composed of only proteins. The viruses cause in their hosts a range of infections viz. acute inapparent, chronic, persistent, latent, slow progressive and tumorigenic infections. The so-called slow viruses are involved in slow progressive diseases. The agents which are presumed responsible for slow progressive diseases have been misnamed **slow viruses** or also as **prions**. Many of these affect the central nervous system and one of the best known is the **scrapie agent** of sheep and goat which causes the animal to scratch itself against obstacles. This disease attacks central nervous system and may take two forms. (1) sometimes an extreme sleepiness and (2) more frequently an intense irritation that causes the sheep to rub itself against a gate post or similar object, generally removing some of its wool in the process. It is from this habit the name **scrapie** was derived. There is a long incubation period of 1-4 years.

**Q.6. Differentiate the structure of Phytoplasma and yeast cell.**

**Ans.**

| | Character | Phytoplasma | Yeast cell |
|---|---|---|---|
| 1. | Size of cell | 2-3 µm | > 5 µm |
| 2. | Nucleus | Very diffuse | Prominent |
| 3. | Cell wall | Absent | Present |
| 4. | Shape | Pleomorphic | Definite shape |
| 5. | **Cytoplasmic Organelles :** | | |
| | (a) Endoplasmic reticulum | Absent | Present |
| | (b) Golgi apparatus | Absent | Present |
| | (c) Mitochondria | Absent | Present |
| | (d) Chloroplasts | Absent | Present |
| | (e) Vacuoles | Absent | Present |
| | (f) Microtubular system | Absent | Present |
| | (g) Ribosomes | Present (70S type) | Present (80S type) |

☛ **Q.7. Write a note on single cell proteins.**

**Ans. Single Cell Protein :** The name-single cell protein (SCP) is given to those microbial products which are produced by fermentation. Now-a-days proteins are manufactured from hydrocarbon wastes of petroleum industries. Yeast, fungi, bacteria and algae are grown on these hydrocarbon wastes and their new formed cells are good source of proteins. The SCP are used in food of man and in fodder of animals. Basically SCP have proteins, fats, carbohydrates, ash-ingredients, water and other elements like phosphorus and potassium etc.

**Table : Some examples of substrate materials that support the growth of microorganisms in the production of SCP.**

| Substrates | Micro-organisms |
|---|---|
| $CO_2$ and sunlight | *Chorella pyrenoidosa, scenedesmus quadricauda spriulina maxima* |
| n-Alkanes, kerosene | *Candida intermedia, C. lipolytica, C. tropicalis Nocardia sp.* |
| Methane | *Methylomonas sp. (Methanomonas) Methylococcus capsulatus sp. Trichoderma* |
| $H_2$ and $CO_2$ | *Alcaligenes eutrophus (Hydrogenomonas eutropha)* |
| Gas oil | *Acinetobacter calcoaceticus (Micrococcus cerificans) Candida lipolytica* |
| Methanol | *Methylomonas methanica (Methano-monnas methanica)* |
| Ethanol | *Candida utilis, Acinetobacter calcoaceticus* |
| Sulfite liquor wastes | *Candida utillis* |
| Cellulose | *Cellulomonas sp., Trichoderma viride* |
| Starches | *Endomycopsis fibuligera* |
| Sugars | *Saccharomyces cerevisiae, Candida sp., Kluyuveromyces fragilis* |

☛ **Q.8. What is difference between Prokaryotic and Eukaryotic Cell ?**

**Ans.**

| | Characters | Prokaryotic Cell (A Bacterial cell) | Eukaryotic Cell (A Yeast cell) |
|---|---|---|---|
| 1. | **Size** | Single celled 1-2 × 1-4μm or less | More than 5 μm |
| 2. | **Nucleus** | Diffuse or Incipient | Prominant |

| | | | |
|---|---|---|---|
| 3. | **Cell wall** | Think, composed of mucopoly-saccharides and other compounds | Thick, chiefly composed of cellulose |
| 4. | **Peptidoglycan** | Usually present | Absent |
| 5. | **Cytoplasmic organelles** | | |
| | (a) Endoplasmic reticulum | Absent | Present |
| | (b) Golgi apparatus | Absent | Present |
| | (c) Mitochondria | Absent | Present |
| | (d) Chloroplasts | Absent | Present |
| | (e) Lysosomes | Absent | Present |
| | (f) Vacuoles | Absent | Present |
| | (g) Microtubular system | Absent | 70S (Organelles) |
| | (h) Ribosomes | 70S | 80S (Organelles) |
| 6. | **Genetic Organisation** | | |
| | (a) Nuclear membrane | Absent | Present |
| | (b) Nucleolus | Absent | Present |
| | (c) Hereditary Material | DNA | DNA |
| | (d) Chromosome number | One | More than one |
| | (e) Histones in chromosome | Absent | Present |
| | (f) DNA in organelles | Absent | Present |
| | (g) Mitosis/ Meiosis | Absent | Present |
| | (h) DNA | Circular | Linear |
| 7. | **Genetic Recombination** | Rare, incomplete | Common, complete (Sexual Reproduction) |

| | | | |
|---|---|---|---|
| | (a) Fusion of gametes | Absent | Present |
| | (b) Formation of partial diploids | Absent | Present |
| 8. | **Flagella** | Submicroscopic and simple without 9 + 2 fibrillar arrangement. | Microscopic and complex with 9 + 2 arrangement (9 + 2 microtubules and membrane) |
| 9. | **Functional Attributes** | | |
| | Endo and Exocytosis like Phagocytosis, Pinocytosis, Intracellular digestion etc. | Absent | Present |
| 10. | **Amoeboid Movement** | No | Yes |
| 11. | **Size of Oxidative Phosphorylation** | Cell membrane | Mitochondrion |
| 12. | **Site of Photosynthesis** | Cell membrane | Chloroplast |
| 13. | **Sterols** | Present | Absent |

**Q.9. Write a short note on endotoxins.**

**Ans. Lipopolysaccharide toxins (Endotoxins)**

The name given to these toxins is equated with the LPS component of the Gram-negative eubacterial cell wall. Although all Gram-negative eubacteria have LPS in their cell walls, LPS is not toxic unless it is released from the outer layer of the cell. When these bacteria die, their cell walls disintegrate and release the toxin. Some Gram-negative bacteria produce LPS while growing and their LPS may have toxic effect on host. Toxicity is associated with the lipid portion of the LPS molecule, termed lipid A (composed of fatty acids, such as β-hydroxy myristic). This same structure is found in all eubacterial Gram-negative LPS. The physiological effects of LPS toxins include fever, circulatory changes and other general symptoms like weakness and nonlocalised aches. The effects of LPS toxins are generally the same for all species of Gram-negative bacteria because of the common nature

of lipid A. Thus there is no specific characteristic symptoms associated with the endotoxin of a particular species of bacterium. Species of *Salmonella* and *Shigella*, besides LPS toxins, also produce protein toxins (exotoxins) and these are exotoxins that are largely responsible for their pathogenicity. *Shigella* produces protein toxins that act on nerve cells, (neurotoxins). ●

☛ **Q.10. What is Bioweedicides ?**

**Ans.** **Bioweedicides**

Several fungi have been found very useful in the control of troublesome weeds of crop fields. Registered products are available in market for use in several countries.

Some fungi registered for commercial use as fungal weedicides are as follows :

| Fungus used as weedicide | Registered product, if any | Target weed |
|---|---|---|
| *Colletotrichum gloeosporioides f. sp. aeschynomene* | COLLEGO | *Aeschynomene virginica* (Northern jointvetch) |
| *Phytophthora palmivora* | DE VINE | *Morrenia odorata* (Milkweed vine) |

●

☛ **Q.11. Write a short note on Mycoplasmal viruses.**

**Ans.** **Mycoplasmal viruses :** Excellent review on viruses of mycoplasmas and spiroplasmas is presented by Maniloff *et al* (1977). The first virus infecting a mycoplasma was reported by Gourlay (1970) and since then more than 75 viruses on mycoplasmas have been reported. They have been morphologically and serologically classified into three groups : Group 1 consists of naked bullet-shaped particles; group 2 roughly spherical enveloped viruses and Group 3 the polyhedral particles with tails. Most viruses have been isolated from *Acholeplasma laidlawil.* All three groups are DNA viruses in group 1 the DNA is single-stranded covalently closed circular molecule; group 2 DNA is shown to be double-stranded and that of group 3 probably also double-stranded in an electron micrograph. ●

☛ **Q.12. What is Satellite viruses ?**

**Ans.** **Satellite viruses (Incomplete viruses) :** Sometimes one virus may depend upon the assistance of another virus in the same cell to help it perform a necessary function of its existence. It has been shown in some viruses of both, plants and animals. One form of such assistance is concerned with vectors, when one virus in a plant may depend upon

the presence of another virus to enable it to be transmitted by the aphid vector a phenomenon called **dependent transmission.**

The form of dependence in satellite viruses is concerned even more fundamental because it deals with the vital function of replication. In other words a situation exists in which one virus lacks the power of multiplication without the presence of a second virus to supply the necessary aid. Such a situation is known as **satellitism** and the incomplete virus is a **satellite virus.** The virus that allows the satellite virus to infect and multiply is termed **activator** and the whole phenomenon as **activation.**

☛ **Q.13. Write a short note on weil-felix test.**

**Ans. Weil-Felix test :** This test is used to identify the presence of rickettsiae without specifying a particular species. A sample of patients' blood is first obtained and the clean serum is separated from the red blood cells. A sample of serum is mixed with the cells of the bacterium, *Proteus Ox19.*

The bacteria will clump together if the serum contains antibodies against the ricketsiae, indicating that a person has the disease or once had it. If no clumping takes place, rickettsial antibodies are probably not present. The Weil-Felix test works because rickettsiae possess antigens, also located in *Proteus* cells and therefore, both organisms stimulate the immune system to produce the same antibodies.

☛ **Q.14. Describe the nature of Viruses.**

**Ans.** The nature of viruses is a vexed question. The opinions differ whether they are living or non-living. There are two major thoughts about their nature. These are as follows :

**(1) Antimate Theory :** On the basis of following characters viruses are supposed to be living organisms :

(i) Growth and reproduction are chief distinctive characteristics of living things. Viruses possess both. These are smallest living organisms and contain all information for their multiplication. They grow, reproduce and undergo mutation. They occur indefinite races each with its specific characters.

(ii) They possess genetic continuity and occur in different races and strains, each with specific character.

(iii) They are obligate parasites and grow and reproduce only in living tissues of hosts.

Besides above mentioned characters, there are other properties of viruses also in favour of living nature of these organisms.

**(2) Inanimate Theory :** Some of the characters which show non-living nature of viruses, are as follows :

(i) Viruses do not have cellular organisation.

(ii) They show no metabolic activity except multiplication with in host.

(iii) Viruses can be extracted and crystallized like chemicals.

(iv) They are lifeless when present outside the host.

(v) They behave like non-living chemicals and seems to be inert in free state (crude sap) showing no sign of life.

Thus, viruses are neither living nor non-living cells. They represent simplest form of life known as present. They are subcellular and stand at the threshold of life. ●

☛ **Q.15. Differentiate between viruses and other organisms.**

**Ans.** (1) Viruses possess both living and non-living properties.

(2) Viruses lack cellular organelles like ribosomes, mitochondria. They make use of the ribosomes of the host cells.

(3) Viruses contain only one type of nucleic acid *i.e.*, either DNA or RNA, whereas cells of other organisms contain both.

(4) Viruses can not multiply if they are separated from the host cells as these are molecular obligate parasites, whereas cells of bacteria and other microorganisms can do so.

(5) Viruses do not multiply by fission or mitosis as other cells do. They show replication of genetic material only (for which they are dependent upon the host cell machinery).

(6) Viruses do not have energy yielding system of their own and are lacking in metabolic system characteristic of living cells.

(7) Viruses do not contain chemical compounds which are characteristically found in the cell walls of algae, bacteria or other organisms *e.g.* Polysaccharides. ●

☛ **Q.16. Explain the analysis of different growth stages of Bacteria in Liquid Medium. At what stages Bacteria produce antibiotics ?**

**Ans.** In bacteria growth involves increase of cell numbers. Bacteria multiply from binary fission. The time required for a doubling of cell numbers is called the generation time while the time required for doubling of cell mass is referred as the doubling time.

When bacteria are inoculated into a nutrient solution and incubated under suitable conditions they usually essential factor aproachs exhaustion and becomes growth limiting. Growth of bacterial culture can be indicated graphically by plotting the logarithms of cell number against time. A typical growth curve of this type is sigmoidal in shape and can be divided into a number of growth phases. These phases are as follows :

**(1) Stationary Phase produced :** At this phase the rate of growth of bacterial cells decline. In many microbial processes that are aimed to the

formation of antibiotic like penicillin, the static phase is the real production phase.

**(2) Log Phase :** This phase is characterized by a constant doubling rate. In this phase growth of bacterial cells occur very rapidly. It depends on the particular organism and the growth conditions. Enterobacteria can grow with a doubling time of 15-30° C with a doubling time of 20 minutes. For other bacteria, the generation time may be much longer.

**(3) Lag Phase :** This occupies the time interval between inoculation and attainment of the maximum division rate. Duration of lag phase depends on the age of the culture and the composition and suitability of the nutrient medium. When the inoculum is derived from an old culture the cells will need to adapt themselves to active growth in the new environment. In such phase bacterial growth occurs gradually at initial stage.

**(4) Death Phase :** In this phase the cells begin to die and the viable count decreases exponentially until no cells remain alive. In some cases lysis occurs due to cellular enzymes.

☛ **Q.17.How solid culture medium is prepared ?**

**Ans.** **Preparation of Solid Culture Media**

Solid culture media for bacteria and other microorganism's prepared from using agar-agar. It is complex polysaccharide consisting of 3, 6 anhydro L-galactose and D-galacto-pyranose. It liquifies on heating to 95°C but solidifies into jelly if cooled to 40-45/C.

Liquid culture media is mixed with agar-agar and heated to 96°C. Such liquid is transferred into petridishes or tubes and it is allowed to cool and solidify. These are autoclaved to make these sterile. These agar plates or tubes are used for inoculation of required microoorganisms for prepared their culture.

☛ **Q.18.What is Antibodies or Immunoglobulins ? Describe the usages of antibodies in Cell Biology.**

**Ans.** **Antibodies**

Antibodies are proteins produced from the lymphocytes of vertebrates as a defense against microbial infection or against any foreign protein that happens to enter the body Antibodies are made in million various forms, each with a different binding site that helps in specially recognizing that particular type of protein molecule that induced its production.

**Uses of Antibodies in Cell Biology :** The precise antigen specificity of antibodies make them powerful tools for cell biologists. These are used as probes after being labelled with various substances.

**(1) As biochemical Tools :** Antibodies are used as biochemical tools to defect and quantify molecules in cell extracts and to identify specific proteins after they have been fractioned from electrophoresis in polyacrylamide gels.

**(2) Labelled with Electron dense Particles :** Antibodies labelled with electron dense particles such as colloidal gold spheres are used for locating specific protein molecules at high resolution by electron microscope. This is known immunocytochemical localisation of protein molecules.

**(3) Labelled with Fluorescent Dyes :** Antibodies labelled with electron dense are used to identify specific protein molecules in cells by flourescent microscopy.

**(4) In Affinity Chromatography :** Antibodies are coupled to an inert matrix to produce an affinity column, which is then used either to isolate in purified form a specific molecule from a crude cell extract or to pickout types of living cells from a heterogenous cell population. ●

☛ **Q.19. Write some important point on phase contrast microscope.**

**Ans.**

## Phase Contrast Microscope

1. Phase contrast microscope works on the principle that different cell organelle have different refractive indices and refractive index of the cell and its surrounding medium are also different.

2. The light rays passing through a cell will emerge in the same phase if they encounter the same refractive index and thickness of the medium.

3. If a light ray passes through medium of different thickness or different refractive indices, it becomes out of phase due to phase retardation. It means one light ray passes through cell organella and the other one passes through the ground substance, the first light ray is retarded and emerges slightly out of the phase.

4. The phase contrast microscope selectively retards the out of phase light wave with respect to other.

5. The image formed by phase contrast microscope appears in the shades of gray.

### Uses of Phase Contrast Microscope

1. It is used to observe living cells and tissues.

2. It is used in the observation of cells cultured *in vitro*.

3. It is also used to study the effect of different chemical and physical agents on the living cells and to examine the artifacts introduced by different methods of fixation and staining.

4. By using time-lapse motion picture, the following phenomena can be studied under phase contrast microscope.

(i) Different nuclear and cytoplasmic changes occurring during cell division and cell movement.

(ii) Formation of fibrillar expansions and fine membranes.

(iii) Formation of water vacuoles by the process of pinocytosis. ●

**Q.20. Write a short note on determination of Serum proteins.**

**Ans. Determination of Serum protein's and A/G Ratio**

Serum protein estimation yield most useful information in chromic liver disease. The liver is the site of albumin, fibrinogen and some of $\alpha$ and $\beta$ globins synthesis.

The normal Serum protein level is 6·0-8·0 g%

albumin level is 3·5 to 5·5 g%

Globulin level is 2·0—3·5 g%

The normal A/G ratio is 1·2 : 1

In advanced liver disease, the albumin is decreased and globulin is increased to that Albumin. Globulin ratio is reversed.

Serum proteins are decreased in mal nutritions liver damage.

Low Serum albumin is found in severe liver damage due to the impaired ability of the liver to form albumin.

**Q.21. Write a short note on Alkaline Phasphatase.**

**Ans. Estimation of Serum alkaline Phasphatase**

Normal Level is 3·12 King-Arm strong units or 3-4 Bodansky unit. Increased level of alkaline Phasphatase is found in past necrotic disease cirrohosis, carcinoma of liver, obstructive jaundice, hepato cellular jaundice, bone disease and may go upto 200 KA units.

Serum alkaline phosphatase activity is high in abstructive jaundice but remain unchanged in hemalytic jaundice. So estimation of this activity may be of help to identify the type of jaundice.

**Q.22. Write a short note on Serum lipase.**

**Ans. Determination of Serum lipase**

Serum lipase hydrolyses the ester of long chain fatty acids containing 8 to 18 carbon atoms.

Serum lipase parallels change in Amylase but rises later and lasts longer. The increase in more pronounced. Serum lipase value in elevated in all conditions in which amylose is elevated Serum lipase value is more informative than amylase in pancreatic cancer.

**Q.23. Defined the Cytochemistry.**

**Ans.** **Cytochemistry** : Cytochemistry involves the identification and localization of specific chemical component of the cell. This can be done by staining the cells with suitable specific types of dyes, which can bind a particular type of substance *e.g.* Feulgen reaction for demonstrating presence of DNA in cell, million reaction for protein detection, Schiff's reagent for aldehydes, Sudan for lipid, PAS (periodic acid schiff) reaction for starch, cellulase, hemicellulose and pectin in plant cell and in animal cell it is used to detect chitin hyaluronic acid and mucoproteins.

Similarly, under the suitable condition, substrates can be used to localize the distribution of enzyme. This is possible since under suitable conditions, the enzyme with substrate can produce insoluble product visible through the Microscope. For example phosphoric ester of glycerol are used as the substrate to demonstrate the enzyme alkaline phosphatase other hydralytic enzyme such as estrase, lipase, sulfatase β glucuronidase can be delected with this method by changing the condition substrate. ●

☛ **Q.24. What is Microspectro photometry ?**

**Ans.** Micro spectro photometry : It is a device to estimate the quantitative aspect since the amount of dye taken up may be directly proportional to the amount of stained components. ●

☛ **Q.25. Write a short note on flow cytophotometry or density centrifugation.**

**Ans.** **Cytophotometry as density centrifugation**

The transfer of whole chromosome, the chromosome are first isolated from metaphase cell by hypotonic lysis and may be fractionated using density centrifugation or flow cytometry. Individual chromosome or fragment thus isolated and than incubated with whole cell for incorporation of chromosome into nuclei. ●

☛ **Q.25. What is Monera ? Write its characteristics.**

**Ans.** **Monera**

Kingdom Monera includes all the microscopic prokaryotic and simplest forms of life. They are taken as the most ancient and primitive group of organisms.

Monera includes different types of bacteria. They are found every where : in hot springs, under ice, in deep ocean floor, in deserts and on or within the bodies of plants and animals.

**Characteristics of Monera are as follows :**

(1) Presence of a rigid cell wall.

(2) An organised nucleus is; absent, A double stranded DNA (nucleoid) is found lying in the cytoplasm.

(3) They are prokaryotic organisms.

(4) Some, like cyanobacteria, help in fixation of free nitrogen of the atmosphere.

(5) Process of nutrition **autotrophic** or **heterotrophic.**

**Classification**

Monerans are divided into two groups given as follows :

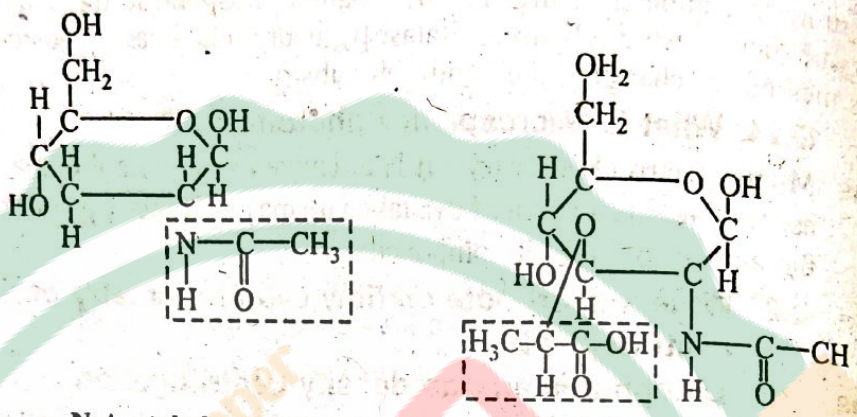
1. Archaebacteria.
2. Eubacteria.

Kingdom Monera includes two major groups, **Eubacteria** and **Archaebacteria.** Eubacteria include cyanobacteria or blue green algae or true bacteria. ●

**Q.27. Define bacterial cell wall.**

**Ans.** **Bacterial Cell Wall**

The bacterial cell wall is a strong, dense binding layer outside the cell membrane.



N-Acetyl glucosamine N-Acetylmuramic acid

**Fig. Sugar components of bacterial cell wall.**

(a) It preserves the shape of cell.
(b) It forms a mesh or molecular sieve and prevents large molecules to pass through.
(c) It gives rigidity or structural firmness to cell.
(d) It preserves the identity and integrity of the cell.

The structure of bacterial cell wall was described by **Salton** and **Horne.** It is formed of strong fibres composed of heteropolymers generally known as

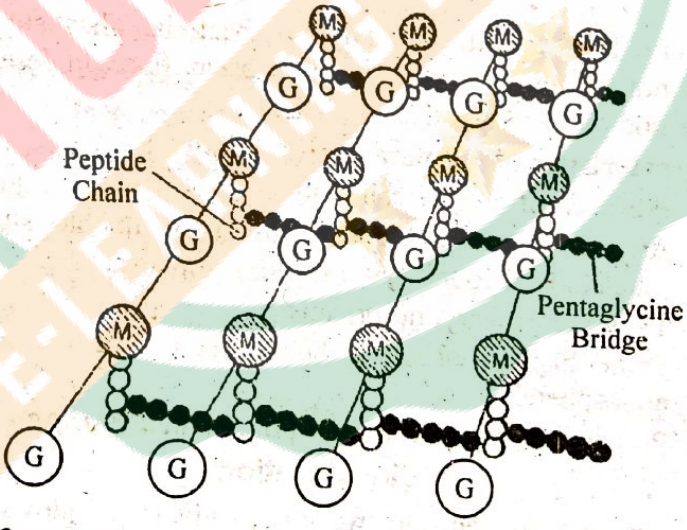



**Fig. Composition of bacterial cell wall showing peptidoglycans.**

**peptidoglycans** or **mucopeptides.** These are formed of two types of sugar units arranged alternately. These are as follows :

1. **N-acetyl-muramic acid** which is a lactic acid derivative of glucosamine. These molecules are connected through β-1, 4-linkage.

2. **N-acetyl-glycosamine** in which an amino acid is atached to a sugar molecule.

Linked with each muramic acid molecule is a short peptide made of 3-4 amino acids. These amino acids are alanine glycine, glutamic acid, and lysine. These short chains of peptide units are linked with the long chain polysaccharide units in such a way that they form a three-dimensional configuration to form glycopeptides or peptidoglycans gives structural rigidity. ●

☛ **Q.28. Write an account of cyanobacteria.**

**Or**

**What are chief characteristics of cyanobacteria.**

**Ans.** Cyanobacteria form a group of ancient Gram negative, photosynthetic prokaryotes. Some prefer to call them blue-green algae. They have survived successfully for about 3 billion years. They found like individual cells, as small groups of cells or colonies of cells or as long filamentous chains of cells. They lack flagella.

Some of the cyanobacteria are unicellular, some may be colonial and some filamentous.

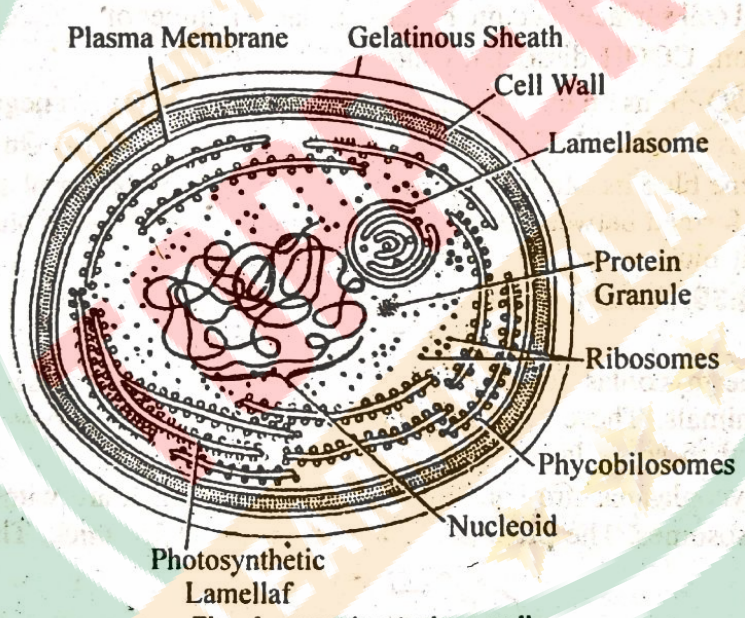



**Fig. A cyanobacterium cell.**

## Ultra structure

The cyanobacterial cell is bigger than a bacterial cell. Like a bacterial cell, it consists of a tiny mass of protoplast surrounded by the cell wall. A typical cell of cyanobacteria consists of various structures given as follows :

1. **Plasma Membrane :** It is formed of unit membrane.

2. **Cell Wall :** It resembles the cell wall of bacteria and is formed of lipoproteins, lipopolysaccharides and mucoproteins.

3. **Slimy Layer or Gelatinous Sheath :** It is the outer most covering present outside the cell wall. It is gelatinous in nature.

**4. Nucleoid or Genophore :** It lacks a definite nucleus. The nuclear material consists of a single chromosome made up of naked strand of DNA helix which lies in the centre. DNA is not associated with histone proteins.

**5. Cytoplasm :** It lacks ER, Golgi complex mitochondria and lysosomes. It contains numerous ribosomes (70S). They form **polyribosomes** in protein synthesis.

Cytoplasm also have blue pigment **phycocyanin** and red pigment **phycoerythrin.** These two pigments are collectively known as **phycobilin** and are found inside small granules known **cyanosomes** or **phycobilisomes.** These granules are about 4 in diameter. The reserve food in the form of oil and fat droplets and proteinacous granules. ●

☛ **Q.29. What do you mean by simple staining ? Explain it with reference to methylene blue.**

**Ans. Simple staining is monochrome staining :** In this were single dye is used to colour the microorganism or bacteria or living cell.

Methylene blue is a basic dye. Its stains bacterial cell in alkaline medium. It is used to stain cell surfaces that have negative charge. e.g. bacterial cell surface is acidic because of large number of –COOH groups. On ionization, COOH dissociates into $COO^-$ and $H^+$.

$COO^-$ ions on the cell surface of bacterial cells impart negative charge. It attracts $Na^+$ and $K^+$ ions from the alkaline medium. On addition of methylene blue its $Mb^+$ are exchanged with $Na^+$ on bacterial surface and a bond is formed between bacterial cell surface and methylene blue molecules giving it blue colour. ●

☛ **Q.30. Write a detailed note on PPLO.**

**Ans. Mycoplasmas or PPLO**

The mycoplasmas or PPLO results a number of diseases in man and other animals. These are the smallest and simplest prokaryotes. These are smaller than some larger viruses.

Mycoplasmas are bacteria-like organisms which are without cell wall and mesosomes. The **plasma membrane** is about 75 thick. The cytoplasm

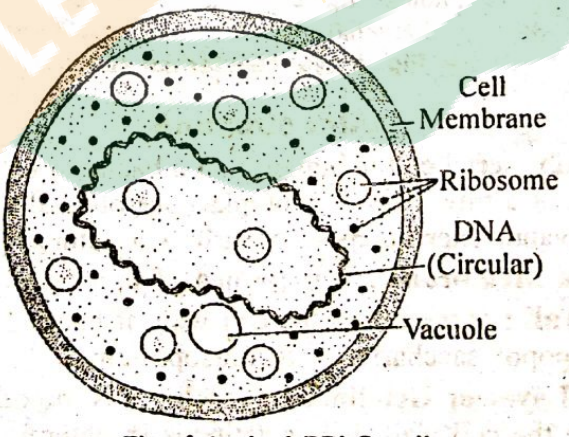



**Fig. A typical PPLO cell.**

contains enzymes for protein synthesis and for DNA replication. Within the cell there are numerous ribosomes. Nucleus is absent. The nuclear area contains double-stranded DNA molecule that may be circular or in the form of fibrils.

The smallest PPLO, *Mycoplasma ldilawi,* is about 0.1μ is diameter and *M. gallisepticum* is about 25 μ is diameter. Thus the mycoplasma are close to the minimum size and complexity required for independent cellular life capable of free existence and reproduction.

**Q.31. How will you isolate a fungus from the natural sources and cultivate it in PDA medium ? What are the precautions to be taken in such method ?**

**Ans.**

### Isolation of Fungus

The isolation of fungi from a mixed population of fungi and bacteria can be made easily through incorporating antibiotics in the growth medium to which the bacteria are sensitive. A variety of methods are available through which a selective groups of organisms can be encouraged to grow.

### Cultivation of Fungus in PDA Medium

For preparing PDA medium, boil 200g peeled, sliced potato in one litre water for one hour. Filter and make volume 1 litre through adding distilled water. Add 20g D-glucose and 50g agar. Now this PDA medium is inoculated with the pure culture of the fungus.

**Precautions**

(1) Sterilisation of culture medium and glassware by autoclave is the most suitable method.

(2) The design of the vessel should be of a type that it is impossible for microbes to enter from outside.

(3) The growth medium should be properly sterilised so that no living organisms exist at the start of the experiment.

(4) All the glass ware should be throughly cleaned before they are used for culture purpose.

(5) After cooling the sterilised medium, inoculation of micro-organisms is done. Inoculation is the method through which micro-organisms are transferred from any source to the medium for their cultivation in laboratory. For it following precautions are essential :

(a) Space should be free from micro-organisms. There condition can be achieved by burning 4 or 5 gas burners around the inoculating table.

(b) Space where the inoculation is performed should be closed enough to avoid entrance of foreign agents of soil and air.

(c) The inoculation needle should be sterilised after each inoculation.