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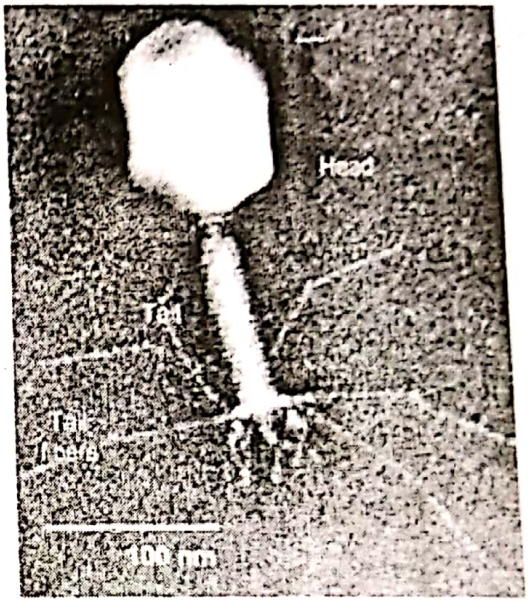
PRACTICAL NO. 1

CLASSIFICATION : ELECTRON MICROGRAPHS/MODELS OF VIRUS - BACTERIOPHAGE, TMV/LYTIC AND LYSOGENIC CYCLE

(1) BACTERIOPHAGE : Bacteriophage is a virus which infects bacteria These viruses are highly host specific. Most phages are tadpole shaped,with head and tail regions. They show complex symmetry.

- **HEAD :** The head is icosahedral in shape. The head contains a tightly packed core of nucleic acid (ds DNA). The head has outer protein coat.
- **TAIL :** The tail is cylindrical and made up of protein and consists of central hollow core or tube surrounded by a contractile sheath. The sheath is connected to a thin disc, called the collar at the upper end and a base plate at lower end. The base plate has six tail fibers at each corner. The long tail fibers which serve for the purpose of attachment of bacteriophage to the host cell.



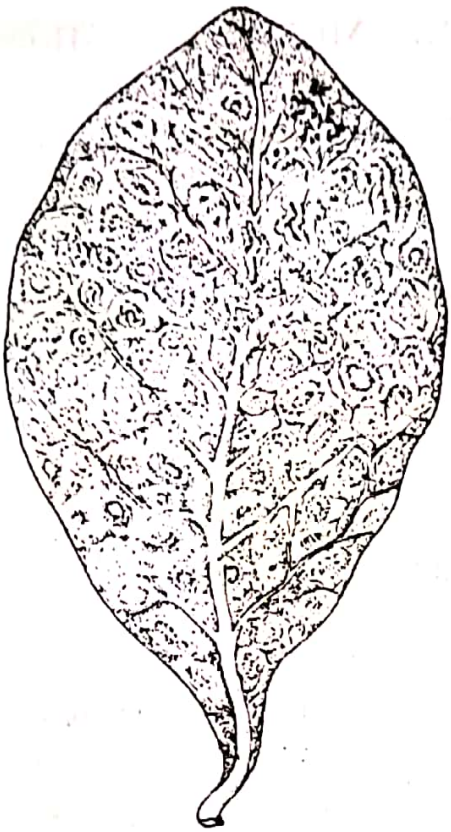
Bacteriophage

(2) TOBACCO MOSAIC VIRUS (TMV)

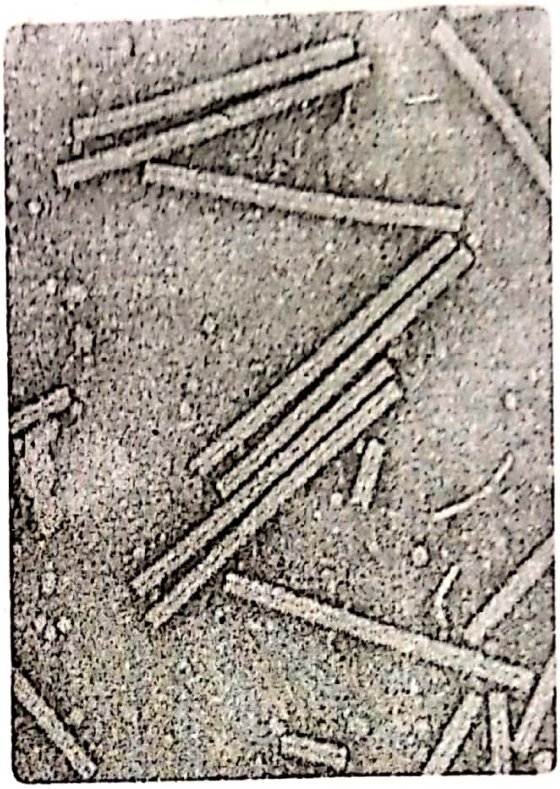
➤ **SYMPTOMS :**

About 200 species of plants belonging to about 36 botanical families are attacked. Solanaceae represents one-third of the recorded hosts. All species of tobacco (Nicotiana) are susceptible.

1. The disease is caused by Tobacco Mosaic Virus.
2. Symptoms exhibited are leaf mottling of light and dark patches, distortion of leaves, unnatural and irregular leaf shape, stunting of the whole plant, variegation, mosaic patterns, necrosis, etc.
3. The first symptoms to appear are clearing of veins, forming mosaic patterns, characteristic mottling and ultimately distortion.
4. The virus is sap transmissible and enters the host through wounds.



(a) Leaf infected with Tobacco Mosaic Virus (TMV)



(b) TMV (Tobacco Mosaic Virus)

TMV is a rod shaped virus which affects the tobacco plant. The virus contains 95% protein and 5%RNA. The capsid is made up of capsomers arranged in a helical manner to form a hollow cylinder. The single stranded RNA is embedded in the furrow of the capsomers. TMV has a wide range of hosts. It forms mosaic symptoms on leaves. The virus is highly stable and heat resistant and spreads through contact.

(3) REPLICATION OF VIRUS :

(A) LYTIC CYCLE : The lytic cycle is divided into following steps.

Adsorption : A virus becomes attached to the surface of host cell. It attaches only at receptor sites containing glycoproteins.

❑ **PENETRATION :**

It takes place by three ways-

1. The entry of virus into host cell. The complete virus enters the host cell ex. TMV
2. The Nucleic acid and protein coat enter the host cell while envelope is left outside ex. Enveloped virus like HIV
3. Only Nucleic acid enters the host cell ex Bacteriophage

- **SYNTHESIS OF VIRAL NUCLEIC ACID AND PROTEIN :** The phage DNA takes the control of the metabolic activities of the host cell. The synthesis of bacterial DNA and protein stops abruptly. Now Viral DNA and Viral protein synthesis is resumed at a higher rate. More number of copies of viral nucleic acid and protein are ready in host cell.

- **ASSEMBLY :** Assembly involves packaging of nucleic acid (core) into protein cover (capsid) to form virus particles. Hundreds of virus particles are assembled . In enveloped viruses, core and capsid are formed and envelope is added later by the host cell at the time of release from host cell.

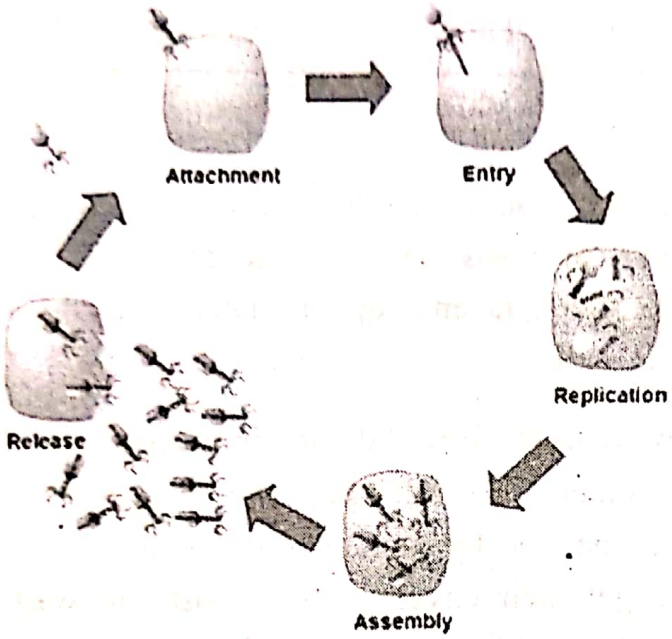
- **RELEASE AND CELL LYSIS :** Many viruses (100-150) are produced within a single host cell and they are released together.. The host cell wall is softened by lysozyme enzymes and phage particles are liberated by rupture of cell wall. The host cell is lysed/ killed. The enveloped virus get their envelope from host cell wall at the time of release. Here, the cell wall makes folding around nucleoprotein particle to form its envelope.The phages which follow the lytic cycle and kill the host cell are called virulent phages.

(B) LYSOGENIC CYCLE :

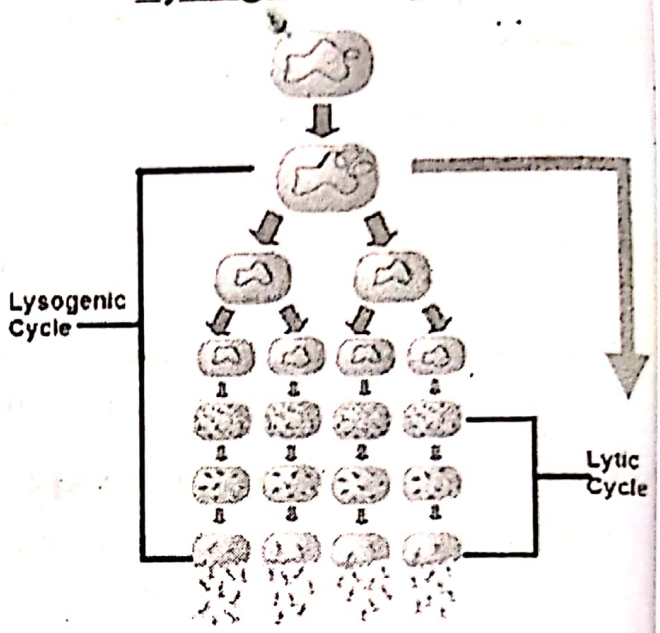
The viruses, which follow lysogenic cycle are called temperate phages and the bacteria in which they occur are called lysogenic strains. The temperate phages are non-virulent. The DNA of phage, becomes integrated with the bacterial genome. The viral genome in the integrated state is called prophage. The new genome replicates as one unit and the daughter genomes are passed on to offsprings. Thus the virus genomes continue multiplying in the daughter lysogenic bacteria indefinitely. Under ordinary condition, the release of phage DNA from bacterial chromosome is a rare event.

Occasionally the association breaks down and the viral genome is separated. This is called induction. Artificially the UV radiation or other agents can induce the cells containing prophage to liberate the phage particle. On release, the viral genome enters the lytic cycle and forms mature phages, which are released by lysis of bacterial cell eg. Lambda phage of E.coli.

Lytic Cycle



Lysogenic Cycle



PRACTICAL NO. 2

STUDY OF BACTERIA

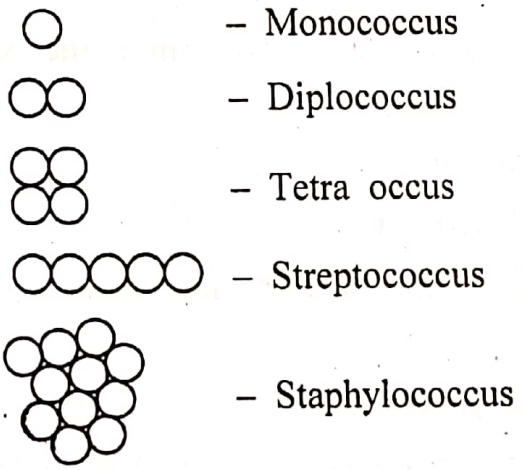
Occurence : Bacteria are omnipresent. They occur everywhere in soil, water and air. Some bacteria are photosynthetic as they have pigments. Other bacteria are either parasite or saprophyte in nature. Parasite bacteria grow in tissue of living organisms like plant, animals and human. Saprophyte bacteria grow on dead organic matter. some Bacteria also live in Symbiosis in root nodules of leguminous plant. Bacteria may be gram +ve or gram –ve based on gram staining. Bacteria may be motile or non based on presence or absence of Flagella.

Size : They are about 0.1 to 1.5 micron in diameter and 0.5 to 5 micron in length.

Shape : Bacteria occur in 3 - 4 shapes :

1. **1. Cocci :** These are spherical bacteria. They occur as single cells - monococcus, pair of cells - diplococcus, in group of four - teracoccus, in long chain - streptococcus, in irregular cluster - staphylococcus.
2. **Bacilli :** These are rod shaped bacteria. They occur singly, in pairs or in chain.
3. **Spirillum :** These are long twisted spiral shaped bacteria.
4. **Vibrio :** These bacteria are curved short rods or comma shaped.
5. **Pleomorphic :** Bactria showing different shape are called Pleomorphic. They are club shaped, square shaped, star shaped and other forms.

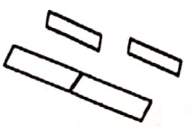
Cocci



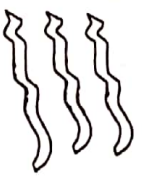
Vibeio

Pleomorphic

Bacilli



Spirillum



PRACTICAL NO. 3

TO STUDY THE STRUCTURE OF PLANT CELL THROUGH TEMPORARY MOUNTS (WITH THE HELP OF EPIDERMAL PEEL MOUNT OF ONION)

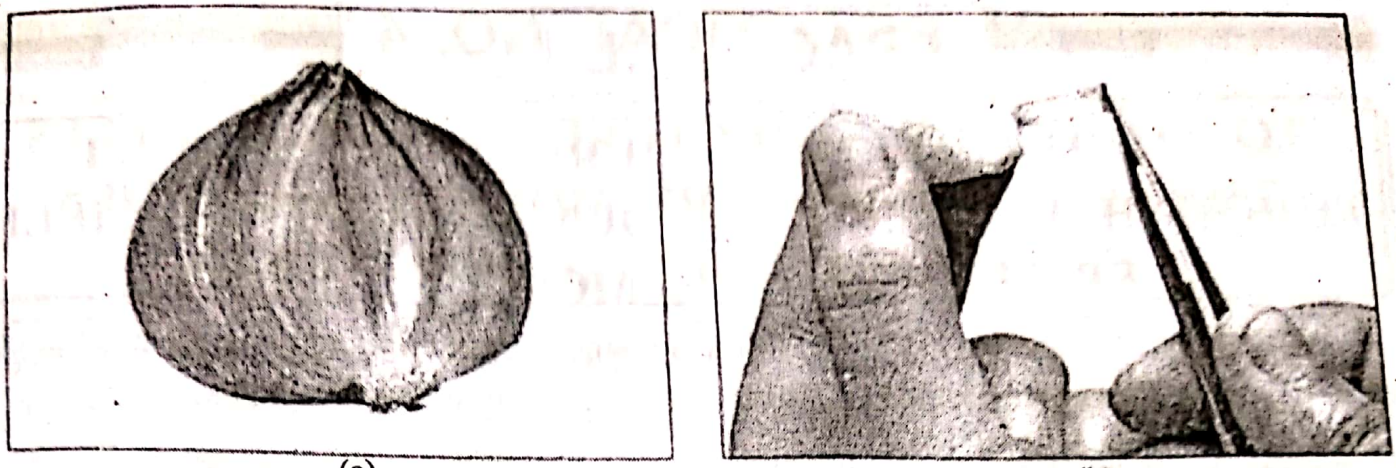
All living organisms are made up of cells. The shape, size and the number of cells vary in different organisms. The major components of plant cell are the Cell wall, Cell membrane, Cytoplasm and nucleus.

- **REQUIREMENT :** An onion bulb, glass slide, watch glass, coverslip, forceps, needles, brush, blade, blotting paper, Safranin, glycerin, dropper, water, compound microscope
- **NOTE :** An onion is made up of layers that are separated by a thin membrane. For this experiment, the thin membrane will be used to observe the onion cells. It can easily be obtained by peeling it from any layer of the onion using tweezers.
- **PROCEDURE :**

1. Take an onion bulb and remove its outer most peel.
2. Now cut a small part from an inner scale leaf with the help of a blade.
3. Separate a thin, transparent peel from the convex surface of the scale leaf with the help of forceps.
4. Using a brush, transfer the peel into the watch glass containing the Safranin solution.
5. Allow to stain for 30 seconds then wash with water.
6. Take a clean slide and put a drop of glycerin in the center of the slide.
7. With the help of a brush and needle, transfer the peel on clean slide. (Glycerin prevents the peel from drying up)
8. Remove excess of glycerin using blotting paper.
9. Observe the slide under compound microscope under 10X and then under 40X magnification

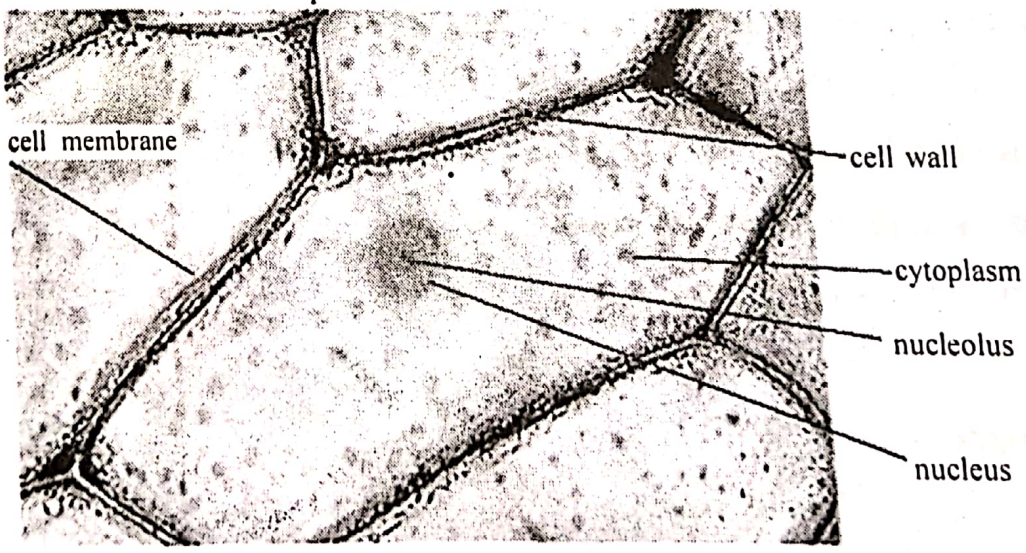
- **OBSERVATIONS :**

- There are a large number of rectangular shaped cells lying side by side. Each cell has a distinct cell wall, cell membrane, nucleus and cytoplasm
- A dark stained distinct nucleus is present in each cell.
- Lightly stained cytoplasm is observed in each cell.



(a)-(b) Methods of separating an onion peel (c) Structure of onion cells as seen under a microscope (450x)

Onion cell epidermis 400x



❑ PRECAUTIONS :

1. Over staining and under staining should be avoided.
2. Folding of the peel should be avoided.
3. Clean and dry glass slide and coverslip should be used.
4. Coverslip should be put carefully avoiding any air bubbles.

PRACTICAL NO. 4

TO STUDY THE STRUCTURE OF ANIMAL CELLS THROUGH TEMPORARY MOUNTS - SQAMOUS EPITHELIAL CELL (CHEEK CELL)

Human cheek cells are made of simple squamous epithelial cells, which are flat cells with a round visible nucleus that cover the inside lining of the cheek.

Cheek cells are easy to obtain and easy to see under a microscope.

Cheek cells are eukaryotic cells (cells that contain a nucleus and other organelles within enclosed in a membrane) that are easily shed from the mouth lining. It is therefore easy to obtain them for observation.

Main parts of a cell include;

1. The cell membrane (outer boundary of the cell)
2. Cytoplasm (the fluid within the cell)
3. A nucleus (at the centre of the cell and controls cell functions)
4. Organelles (e.g. mitochondria-Organelles are cell structures with specific functions)

Using biological stains such as methylene blue, it is possible to clearly observe and differentiate the different parts of a cell. This is because the stain will colour some parts of the cell and not others, allowing them to be clearly observed.

❑ REQUIREMENTS :

- Clean, sterile microscope slides
- Microscope cover slips
- Methylene Blue solution (0.5% to 1%)
- A dropper
- Blotting paper/Tissue paper
- Microscope

❑ HOW TO PREPARE A WET MOUNT OF CHEEK CELLS :

Cheek cells can be easily obtained by gently scraping the inside of the mouth using a clean, sterile cotton swab.

Once the cells have been obtained, the following procedure is used for cheek cell wet mount preparation;

1. Place a drop of physiological saline on a clean microscopic slide (central part of the slide)
2. Smear the cotton swab on to the centre (part containing the saline drop) of the clean slide for about 4 seconds to get the cells on to the centre of the slide
3. Add a drop of methylene blue solution on to the smear and gently place a cover slip on top (to cover the stain and the cells)
4. Any excess solution can be removed by touching one side of the slide with a blotting paper.
5. Observe the slide under microscope 10 x objective to find the cells, then observe under 40X

***Note :** Used cotton swabs and cotton towel should be safely discarded in the trash and not left lying on the working table.

CHEEK CELLS STAINED WITH METHYLENE BLUE

❑ WHY DO WE HAVE TO STAIN THE CELLS ?

Without stains, cells would appear to be almost transparent, making it difficult to differentiate its parts.Methylene blue has a string affinity for both DNA and RNA. When it comes in contact with the two, a darker stain is produced and can be viewed under the microscope.

❑ OBSERVATION :

- Large irregularly shaped cells with distinct cell walls.
- A distinct nucleus staineddark blue in colourat the central part of individual cell ..
- A lightly stained cytoplasm in each cell.

PRACTICAL NO. 5

STUDY OF CELL AND ORGANELLES WITH THE HELP OF ELECTRON MICROGRAPHS

❑ CELL :

Cell is the structural, functional and biological unit of all living organisms.It is the smallest unit of life. Cells are often called the "building blocks of life".

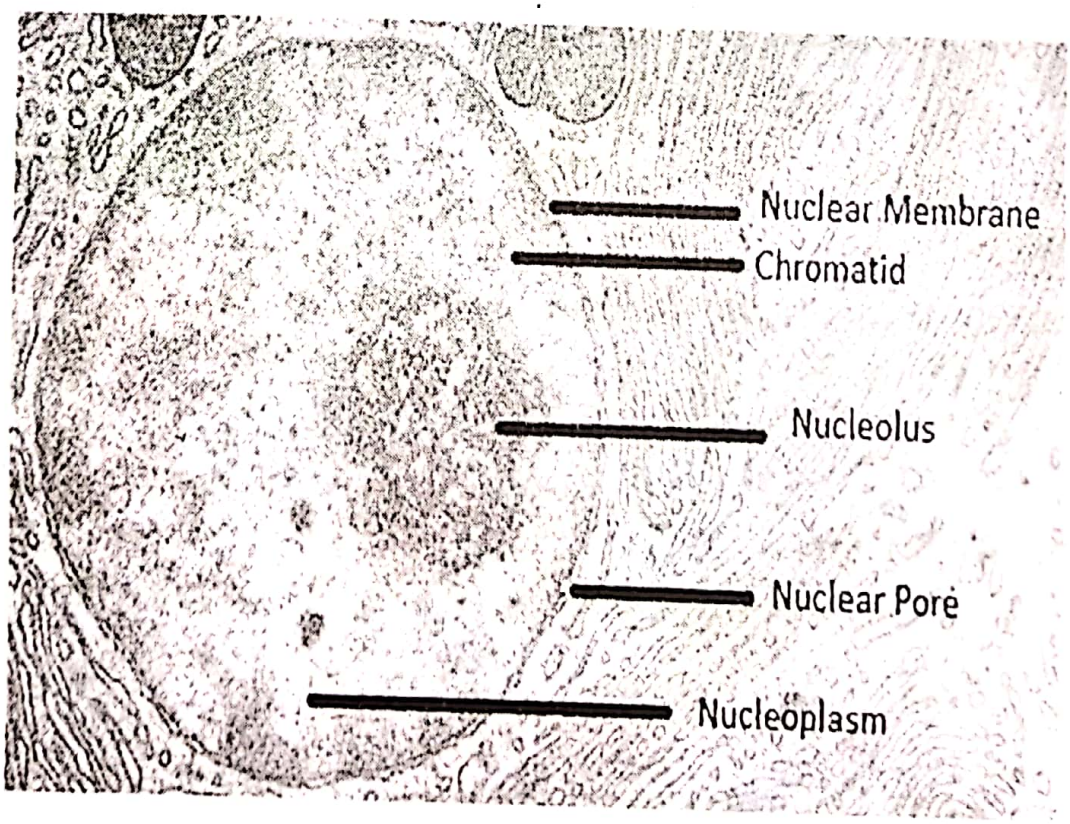
Eukaryotic cells are larger than the prokaryotic cell.The nucleus is enveloped by a nuclear membrane. The eukaryotic cell is composed of plasma membrane,cytoplasam and its organelles viz. Nucleus, Mitochondria, Chlorophyll, Golgi ect.

❑ CELL ORGANELLES :

Cell organelle is a specialized subunit within a cell that has a specific function, which is vital for the cell. Individual organelles are usually separately enclosed within their own membranes.

Eukaryotic cells contain membrane bound organelles in the cytoplasm viz. nucleus, mitochondria, lysosomes, golgi bodies, endoplasmic reticulum, chloroplast (Plastid), ribosomes etc.

1. NUCLEUS :



Nucleus is the most prominent organelle, spherical in shape. It controls all the vital activities of cell. In general, an eukaryotic cell has only one nucleus.. However, some eukaryotic cells are without nucleus, for example, red blood cells (RBCs); whereas, some are multinucleate (consists of two or more nuclei), for example, slime molds.

Nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus and chromosomes.

- **Nuclear Membrane :**

The nuclear membrane is a double-layered structure that encloses the contents of the nucleus.

It separates nucleoplasm from cytoplasm. It encloses a fluid-filled space called perinuclear space present between the two layers of a nuclear membrane.The nucleus communicates with the cell or the cytoplasm through several openings called nuclear pores. Such nuclear pores are the sites for exchange of large molecules (proteins and RNA) between the nucleus and cytoplasm.

- **Nucleoplasm :**

It is also known as karyoplasm. It is the matrix present inside the nucleus. It contains complexes and enzymes necessary for DNA replication and synthesis of RNA molecules

- **Chromatin fibers :**

Chromatin fibers are present in the form of strings of DNA and histones (protein molecules) called chromatin. The chromatin is further classified into heterochromatin and euchromatin based on the functions.

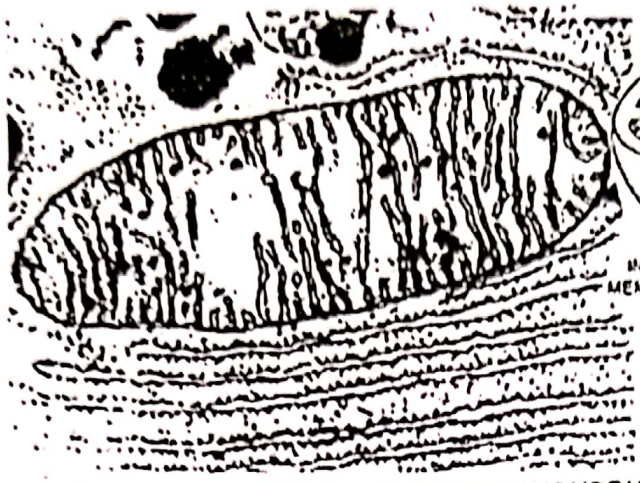
- **Nucleolus :**

The nucleolus (plural nucleoli) is a dense, spherical structure present inside the nucleus. Nucleolus plays an indirect role in protein synthesis by producing ribosomes.

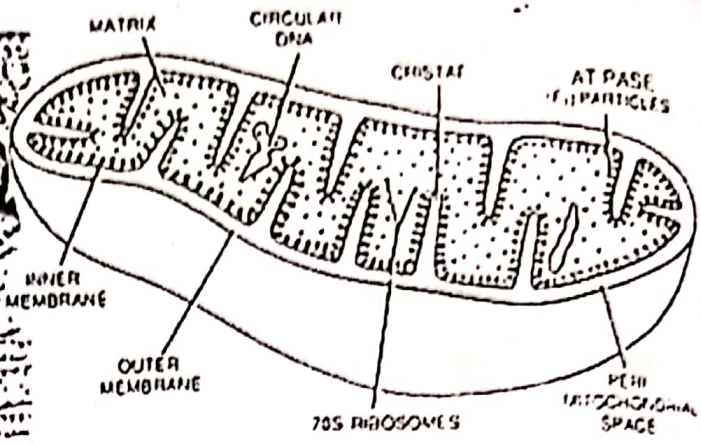
- **Functions :**

Nucleus controls the hereditary characteristics of an organism. It is also responsible for the protein synthesis, cell division, growth and differentiation

2. MITOCHONDRIA :



ELECTRON MICROGRAPH OF MITOCHONDRIA

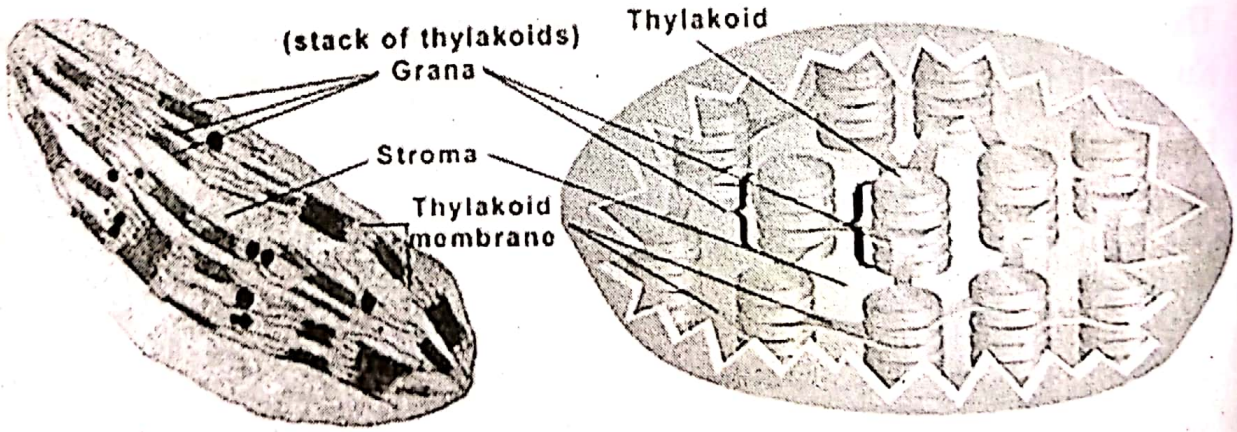


HYPOTHETICAL DETAILED STRUCTURE OF A MITOCHONDRION.

Mitochondria are generally known as power house of cell. They are found in varying shapes depending on physiological conditions. Sometimes they appear rod shaped, filamentous, granular. It is a double membrane bound cell organelle. The membrane is made up of lipids and proteins. The outer and inner membranes are separated from each other by peri mitochondrial space. The outer membrane is smooth and continuous while the inner membrane gives out infoldings or finger like projections called cristae in the lumen or matrix. Inner membrane is covered by stalked particles called F1 particles or oxysomes.

- **Functions :** They are known as power house of cell as they produce ATP molecules (95% of ATP molecules in animal cell), they also perform most important functions like dehydrogenation, oxidation, oxidative phosphorylation, Krebs cycle and respiratory chain of cell

3. CHLOROPLAST :

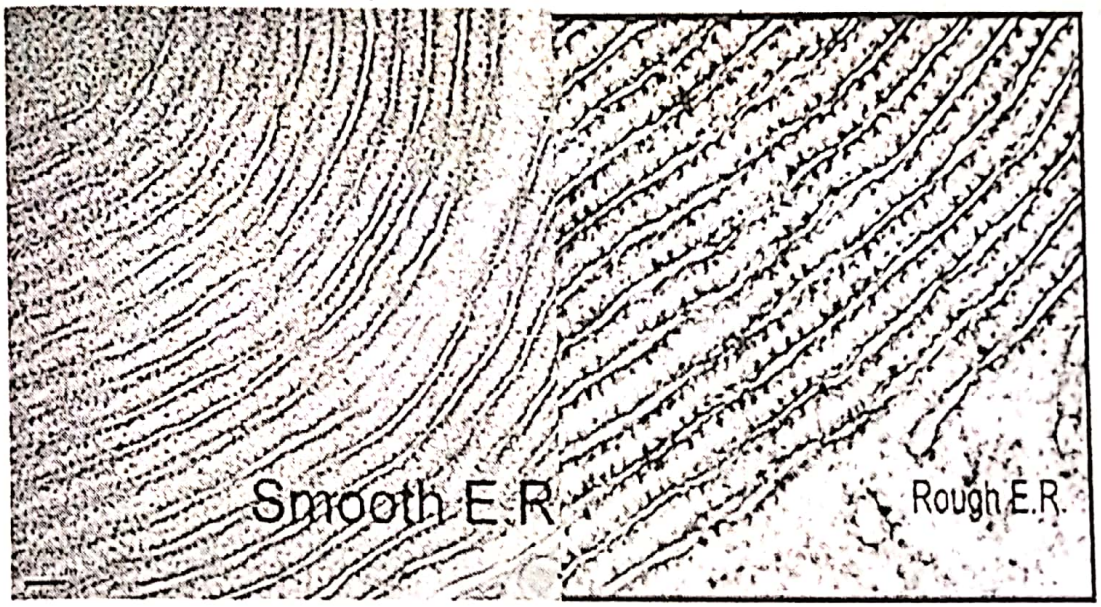


Chloroplasts are found only in algal and plant cells, they produces energy through photosynthesis.

They are oval-shaped and are bound by two membranes. Between the outer and inner membrane is a thin intermembrane space known as peri plastidial space. The space within the inner membrane is known as Stroma, dark reaction of photosynthesis occurs here. Within stroma many small disc-shaped sacs called thylakoids are arranged in the form of pile of coins, termed as Grana. The grana are connected with each other by frets.

- **Functions :** Main function of chloroplasts is to carry out photosynthesis, where the photosynthetic pigments chlorophylls, Xanthophylls, carotenes capture energy from sunlight, convert and store it in the energy-storage molecules ATP and NADPH while liberating oxygen

4. ENDOPLASMIC RETICULUM :



Endoplasmic reticulum is a network of membranous tubules and flattened sacs within the cytoplasm of a eukaryotic cell, continuous with the nuclear membrane that serve a variety of functions in plant and animal cells. Membranes of the ER are continuous with the outer nuclear membrane. It consists of cisternae, vesicles and tubules

- **Cisternae :** Cisternae are long, flattened, sac like unbranchedtubules, they are arranged parallely inbundles or stakes.
- **Vesicles :** Vesicles are oval, membrane bound vacuolar structures, they remain isolated in the cytoplasm
- **Tubules :** Tubules are branched structures forming reticular system along with cisternae and vesicles.

Two types of endoplasmic reticulum are observed in same or different types of cells

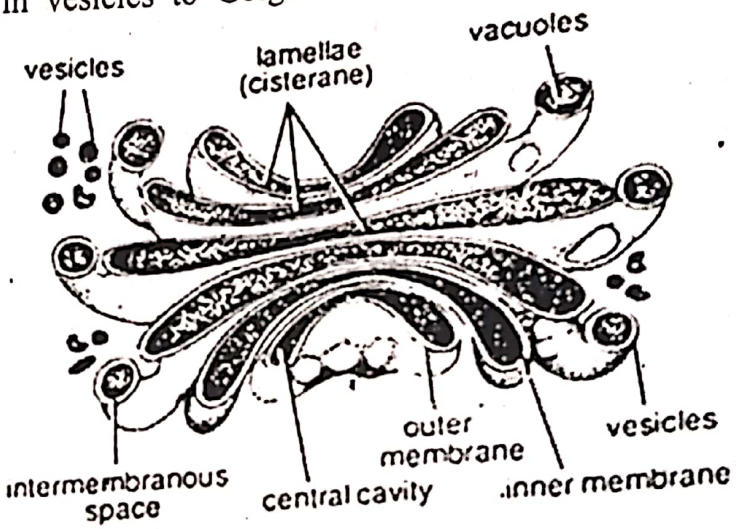
(i) **Agranular or Smooth Endoplasmic Reticulum (SER) :** Smooth Endoplasmi Reticulum has smooth walls as ribosomes are attached to it.SER occurs in those cell which have no role in protein synthesis, cells concerned with lipid synthesis hav well developed SER. It is found in interstitial cells, glycogen storing cells of live spermatocyes, leucocytes, adipose cells

(ii) **Granular or Rough Endoplasmic Reticulum :** Walls of rough endoplasmic reticulur are rough as ribosomes are attached to it. As ribososmes play an active role in protei synthesis they are found in the cells concerned with protein synthesis such as pancreati cells, plasma cells, liver cells, endocrine gland cells

- **Functions of Endoplasmic Reticulum :**

The endoplasmic reticulum serves many general functions. It provides ultra structura skeletal frame work, increased surface area for enzymatic reactions, it also acts a a circulatory or transporting system for various secretory products, it conduct intracellular impulses, folding of protein molecules in cisternae and the transport o synthesized proteins in vesicles to Golgi bodies etc.

5. GOLGI BODY :



GOLGI BODY THREE DIMENTIONAL AND DIAGRAMMATIC MODEL

Shape of Golgi body is pleomorphic, its shape varies with the metabolic state of cell It is normally seen as a convex lamellar structure present near the nucleus. It consist of double membrane bound flattened sacs referred to as cisternae, which are arrange parallel to each other. Cisternae gives out tubules vesicles and vacuoles. It show polarity

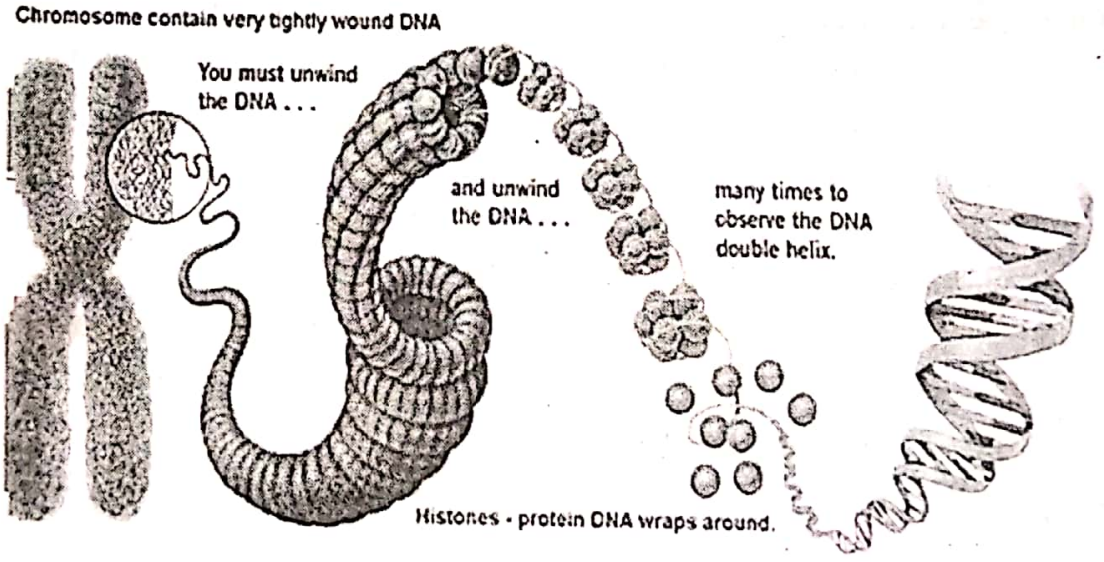
- **Functions :** Packaging of secretory materials, packaging of proteins, secretion o lysosomes, formation of sperm cells

PRACTICAL NO. 6

STRUCTURE OF CHROMOSOME ITS TYPE AND GIANT CHROMOSOMES

Chromosomes (Gr., chroma=colour, soma= body) are the rod shaped, dark stained bodies seen during the metaphase stage of mitosis when cells are stained with a suitable basic dye and viewed under a light microscope. Chromosomes were first described by Strasburger (1815), and the term 'chromosome' was first used by Waldeyer in 1888.

Chromosomes are thread-like structures located inside the nucleus of animal and plant cells. They become visible only during cell division. Each chromosome is made of histone protein and deoxyribonucleic acid (DNA). Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.



Cromosome Structure

❑ CHROMOSOME TYPES :

There are four types of chromosomes based on the position of the centromere:

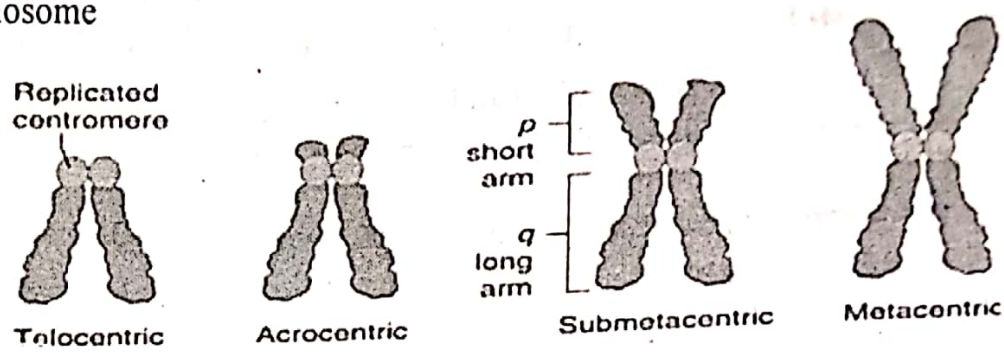
1. Telocentric
2. Acrocentric
3. Submetacentric
4. Metacentric

Telocentric - no p arm; centromere is at end

Acrocentric - very small p arm; centromere is very near end

Submetacentric - p arm just a little smaller than q arm; centromere in middle

Metacentric - p and q arms are exactly the same length; centromere in exact middle of chromosome



❑ FUNCTION OF CHROMOSOME :

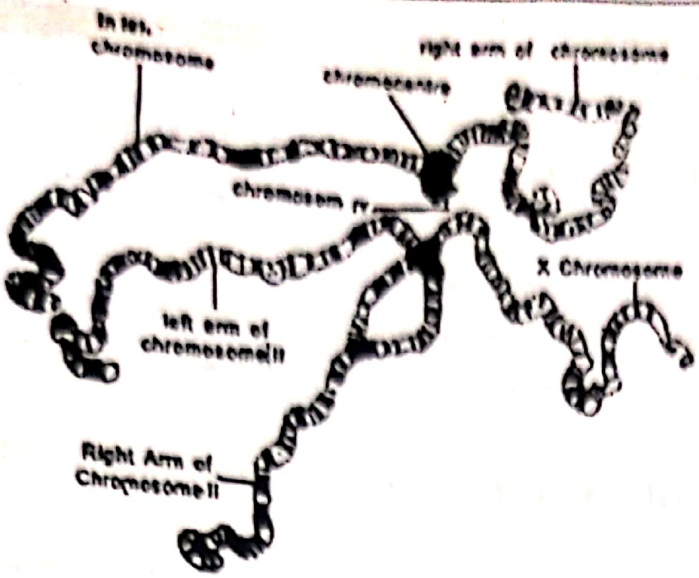
Chromosomes have a unique structure that keeps the DNA in a tightly wrapped position. The DNA strands are placed around a spool-like structure of protein called the histones. If the DNA structure was not tightly wound, it would have been very difficult to accommodate the strands in the cell nucleus. The strands of DNA molecules in a single cell of humans are almost 6 feet long, if unwound. Thus, the strands being wrapped tightly help them to be placed in the tiny, microscopic cell nucleus. Functions of chromosome also include ensuring that during cell division, the DNA is copied exactly as it is and distributed evenly after cell division. If there are any changes in the structure or number of chromosomes, it could lead to serious birth defects in the progeny. Thus, it is absolutely essential for every reproductive cell i.e.the eggs and sperms, to contain the right number and correct structure of chromosomes. Failing this, it will lead to serious complications in the offspring produced.

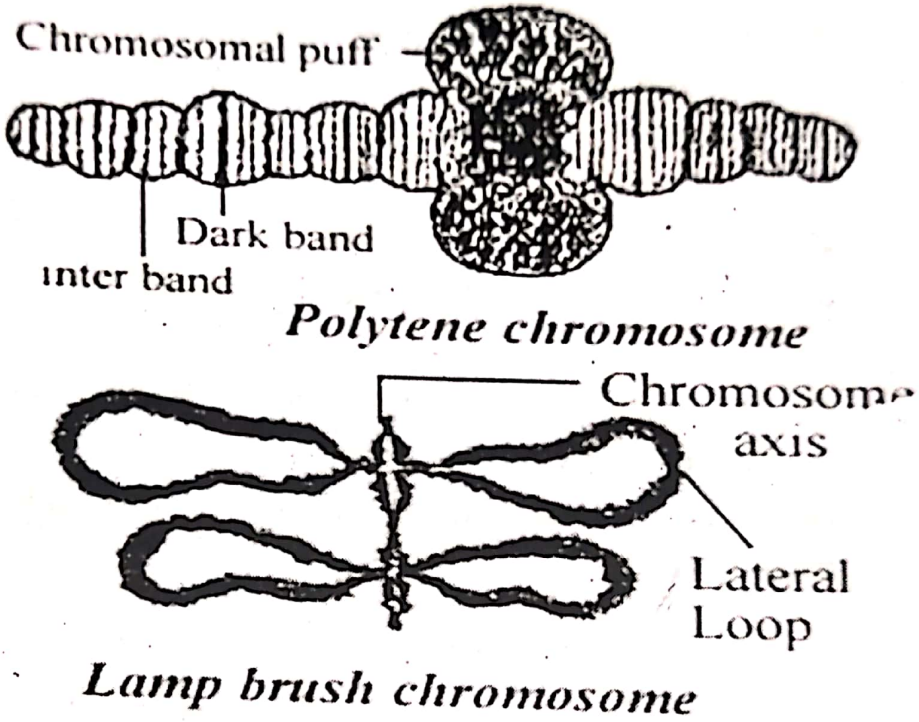
❑ POLYTENE CHROMOSOME :

These are very large size chromosomes also called as Giant chromosomes. They are present in Salivary gland. It was first discovered by Theophilus Painter, Ernst Heitz, and H.Bauer

The name Polytene was suggested by Koller due to the occurrence of many chromonemata.

The Polytene chromosomes are visible during interphase and prophase of mitosis.





❑ LAMBRUSH CHROMOSOMES :

It was first observed in Salamander (amphibian) oocytes in 1882. It is named so because the chromosomes look like brushes which were used for cleaning the glass chimneys of old fashioned paraffin or kerosene lamps.

The lampbrush chromosome occur in the diplotene stage of the meiosis.

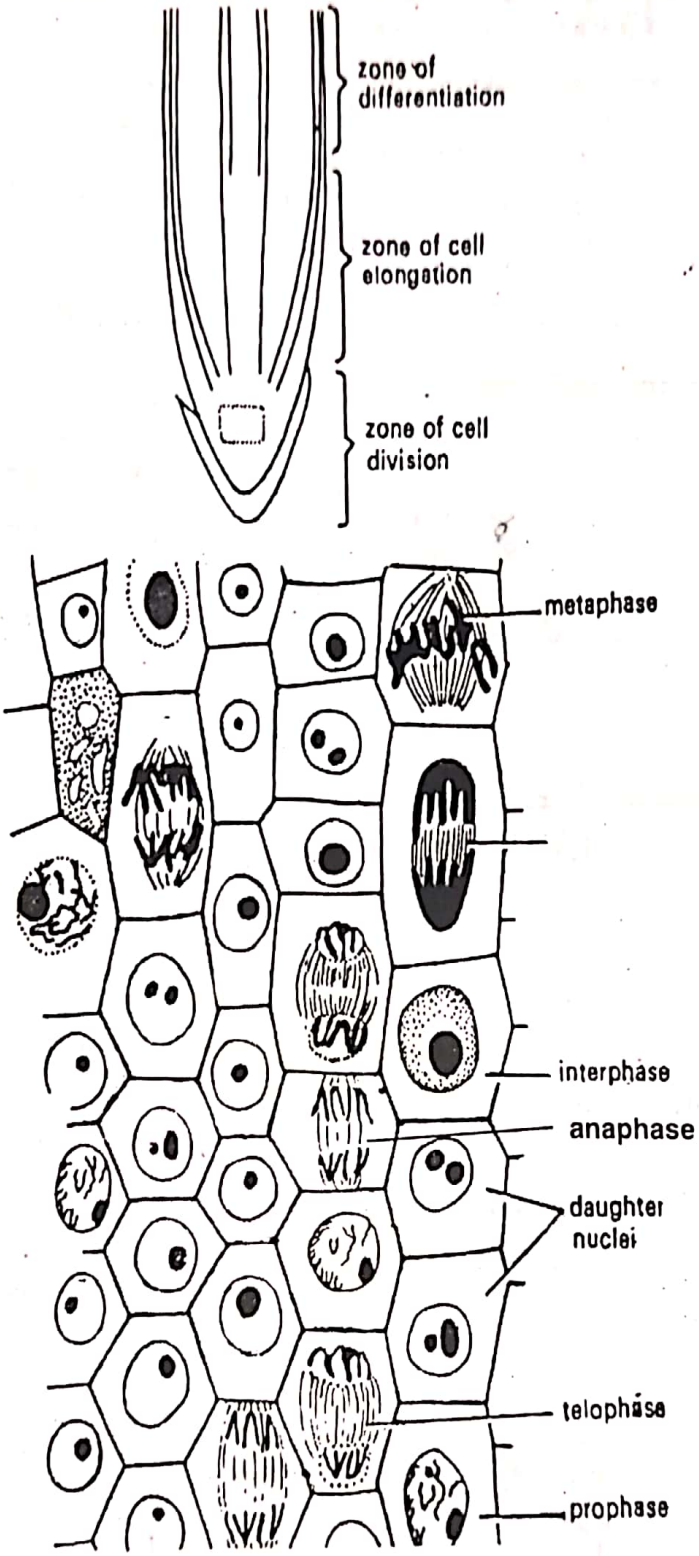
PRACTICAL NO. 7

STUDY OF CELL DIVISION–MITOSIS

Mitosis is a type of cell division which results in the formation of two daughter cells. These cells are identical to parent cell and have same number of chromosomes. It occurs in the somatic or vegetative cells. The chromosome number of both the daughter cells remains the same as that of the parent cell.

➤ **METHOD OF STAINING THE CELL TO OBSERVE DIFFERENT PHASES (Squash Preparation)**

- **REQUIREMENT :** Onion root tip, Acetocarmine stain, slide, cover slip, spirit lamp, watch glass.
- **PROCEDURE :**
  - ✓ Take the fixed root tip (1-2 mm opaque tip) on a clean slide and stain it with acetocarmine.
  - ✓ Place a cover slip over the tip.
  - ✓ Tap gently with the blunt end of the needle and in between pas it over a spirit lamp 2-3 times.
  - ✓ Apply uniform pressure with thumb by keeping the slide between blotting paper.
  - ✓ The squash preparation is ready.
- **Observe various stages under a microscope first under 10X and then under 45X magnification.**



Onion root tip showing different stages of Mitosis

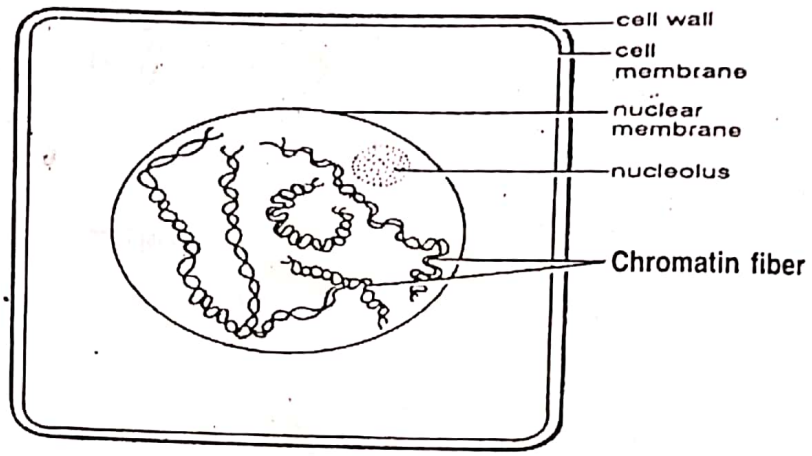
The mitosis shows four stages. (1) Prophase (2) Metaphase (3) Anaphase (4) Telophase

A. INTERPHASE

a. This is a stage prior to actual mitotic cycle.

b. The cell is in resting stage but metabolically very active as DNA replication occurs during this period.

c. The chromosomes are not seen. The chromatin fibers are diffused in small nucle



INTERPHASE

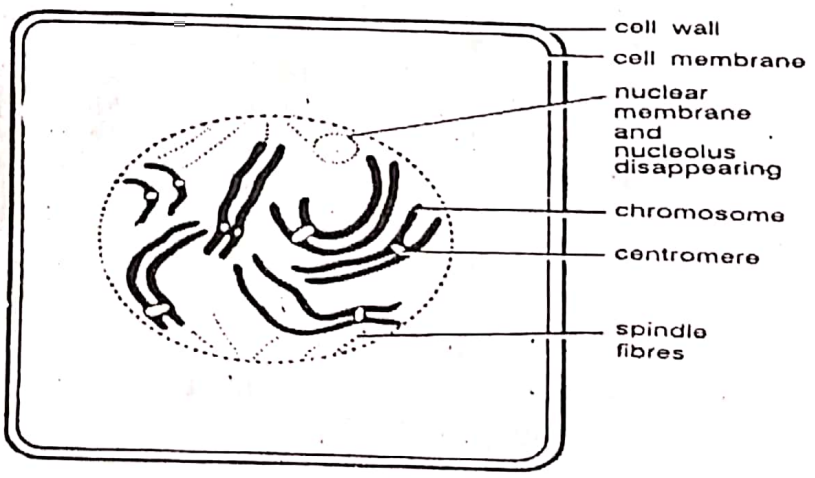
B. PROPHASE : The nuclear membrane and nucleolus start disappearing.

a. The chromosomes become visible due to condensation of chromatin fibers.

b. The chromosomes are with two chromonemata or chromatids.

c. Both remain connected through their centromere and both remain closely associated along their length.

d. The chromosomes become shortened and thickened.

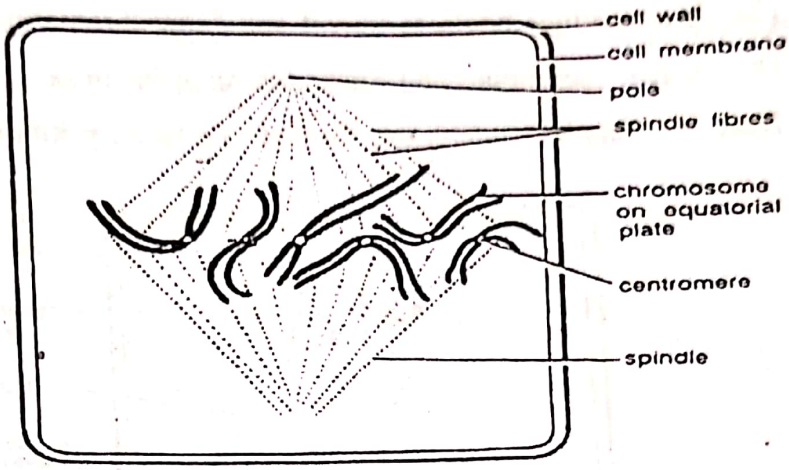


PROPHASE

C. METAPHASE :

a. The chromosomes reach the equator and get arranged themselves at equatorial plane.

b. The spindle fibers are connected to the centromere of chromosomes as well as to opposite poles.



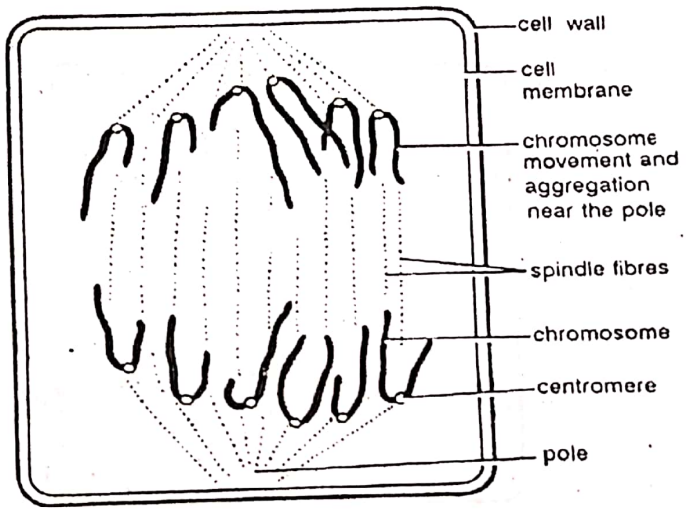
METAPHASE

D. ANAPHASE : The centromere of each chromosome divides into two.

a. The chromatids of each chromosome are separated into two.

b. The chromatids are now referred to as daughter chromosomes.

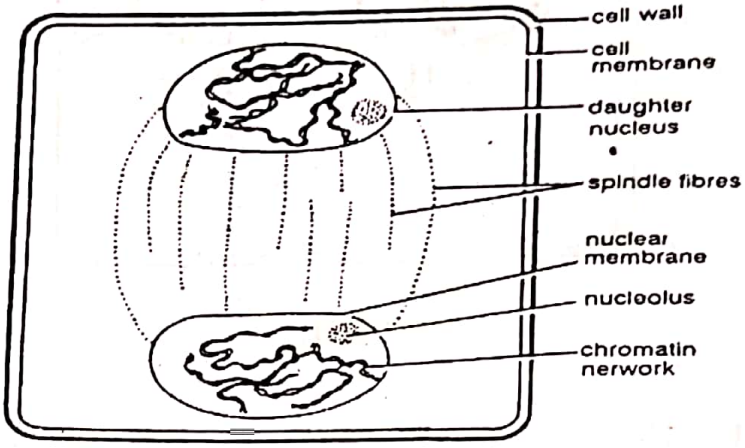
c. The chromosomes become shorter and thicker and migrate towards the opposite poles of the cell.



ANAPHASE

E. TELOPHASE :

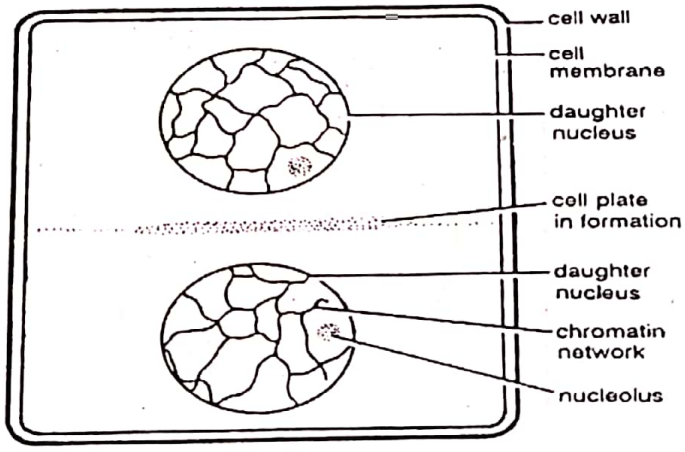
a. It is the final stage of mitosis. It is the reverse process of prophase.
b. The two groups of daughter chromosomes reach to their respective poles in the cell.
c. The chromosomes begin to uncoil and form chromatin network.
d. The nuclear membrane and nucleolus reappear in both daughter nuclei.
e. Thus two daughter nuclei are formed due to karyokinesis.



TELOPHASE

➤ CYTOKINESIS

The division of cytoplasm into two daughter cells is called cytokinesis. The cell plate is formed at the equator by the fragments of E.R. and Golgi, thus two daughter cells are formed.



DAUGHTER CELLS

PRACTICAL NO. 8

STUDY OF CELL DIVISION MEIOSIS

Meiosis is a cell division that is characteristic of organisms which reproduce sexually. During this division, genetic material is duplicated once and nucleus divides twice. As a result four daughter cells are formed. These have half the chromosomes as compared to the parent cells. Meiosis also involves crossing over, i.e. exchange of equal parts of non-sister chromatids of the homologous chromosomes. Therefore, the four daughter cells are genetically different from the parent cells.

Meiosis consists of (1) Meiosis I and (2) Meiosis II.

Meiosis I involves some very characteristic and important stages such as –

(1) Synapsis or pairing of homologous chromosomes,

(2) Recombinations due to crossing over and

(3) Segregation of homologous chromosomes.

The stages included in Meiosis I are Prophase I, Metaphase I, Anaphase I and Telophase I. At the end of meiosis I, two daughter cells are formed. Each cell has half the number of chromosomes compared to parent cell.

Meiosis II is similar to mitosis. It results in the formation of four daughter cells, each having the same chromosome numebr as was present at the end of Meiosis I. Meiosis II is also sub-divided into Prophase II, Metaphase II, Anaphase II and Telophase II.

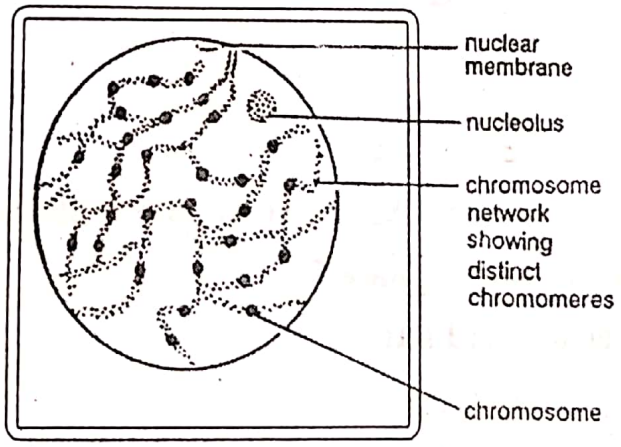
MEIOSIS BY SMEAR PREPARATION

❑ MATERIALS AND TECHNIQUE :

Prepare a smear of young anthers of *Asphodelus* or *Tradescantia* as described earlier.

❑ OBSERVATIONS :

Following stages can be seen in different slides of meiosis –



Meiosis : Cell showing Leptotene of Prophase I

[I] Leptotene (Leptonema) of Prophase I

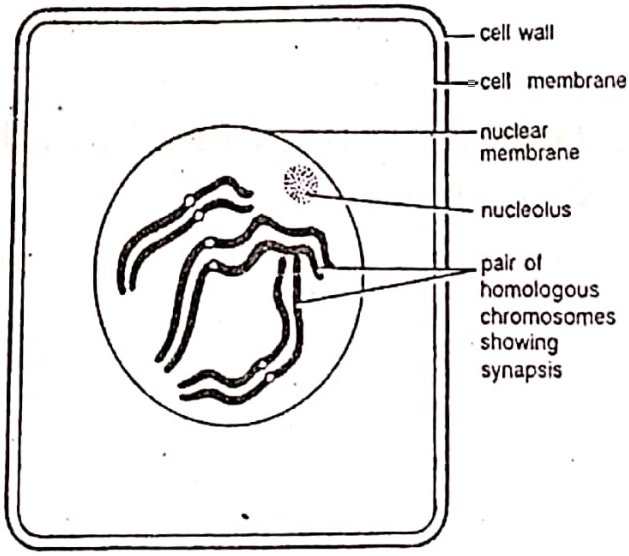
The following characteristics are seen –

1. Nuclear membrane and nucleolus are intact.
2. Chromosomes are long thread-like structures. All the chromosomes are intertwined to form chromatin network.
3. Chromosomes appear beaded due to chromosomes which are distinct at this stage.
4. All the chromosomes finally move towards one part of the nucleus. This stage is known as synizesis or boquet formation.
5. Centrioles are not present. This indicates that it is a dividing plant cell.

[II] Zygotene (Zygonena) of Prophase I

The following characteristics are seen –

1. Nuclear membrane and nucleolus are still very clear.
2. The major character of this stage is synapsis – pairing of homologous chromosomes.
3. Synaptonemal complex is formed as a result of synapsis. This complex is made of two lateral elements and a central region which is bisected by a narrow central component.
4. Synapsis can occur at more than one points along the length of the chromosomes.
5. At each place a pair showing two chromatids is present.



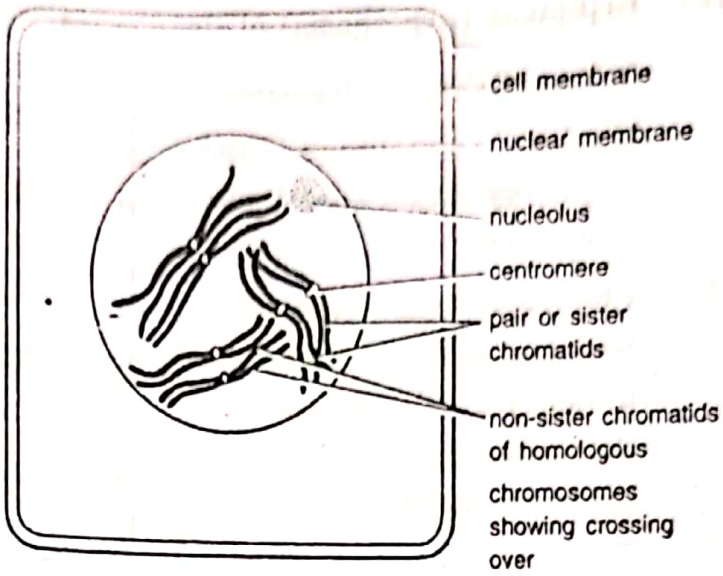
Meiosis : Cell showing Zygotene of Prophase I

[III] Pachytene (Pachyanema) of Prophase I

The following characteristics are seen –

1. Nucleolus and nuclear membrane are distinct.
2. Chromosomes are thickened, coiled and thread-like.

3. Chromosomes are very closely coiled. Each chromosome shows its two chromatids. A pair of homologous chromosomes which is intimately coiled upon one other shows four chromatids together.
4. Pair of homologous chromosomes is called bivalent. It is made of four chromatids and hence known as tetrad.
5. The stage is characterised by crossing over. It is the exchange of equal parts of chromatids of two different but homologous chromosomes.



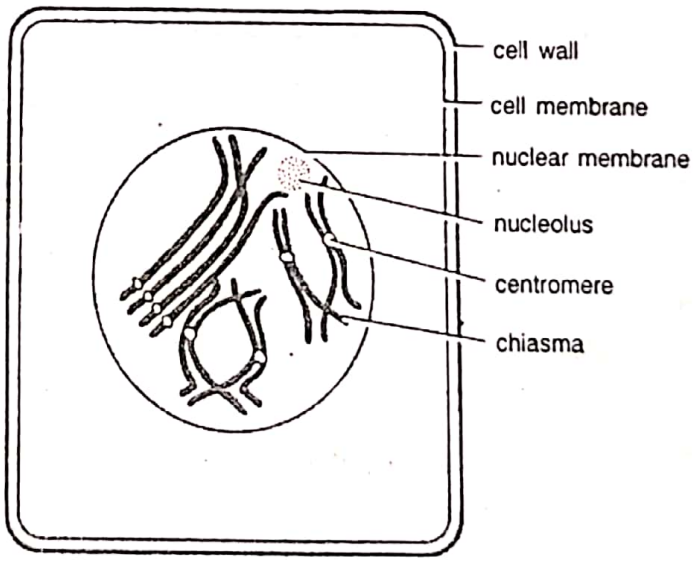
Meiosis : Cell showing Pachytene of Prophase I

6. Nucleolus is distinctly attached to nucleolar organising chromosome.
7. The length of the chromosome being more than that found at metaphase, the chromosome at this stage is also used for the study of morphology.

[IV] Diplotene (Diplonema) of Prophase I

The following characteristics are seen –

1. The nucleolus is disappearing while nuclear membrane is still intact.
2. The close and tight coiling of chromosomes becomes loose and chromosomes appear more clear.
3. Homologous chromosomes still remain in conctact at some points called chiasmata. These are indicators of crossing over having been completed at these points.



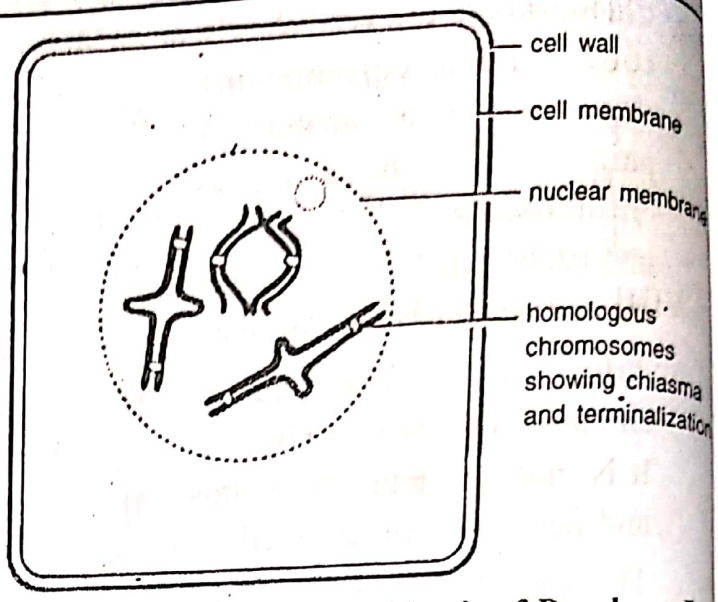
Meiosis : Cell showing Diplotene of Prophase I

4. Chromosomes shortened and thicken. These become still more distinct by the end of this stage.

[V] Diakinesis of Prophase I

It shows following characters –

1. Nuclear membrane and nucleolus have completely disappeared.
2. Chromatids start separating, begining from the centromere towards the end. The chiasmata thus open. This process is known as terminalization.
3. The chromosomes appear almost circular due to continued contraction.
4. Some of the pairs of homologous chromosomes still appear joined with one onother.

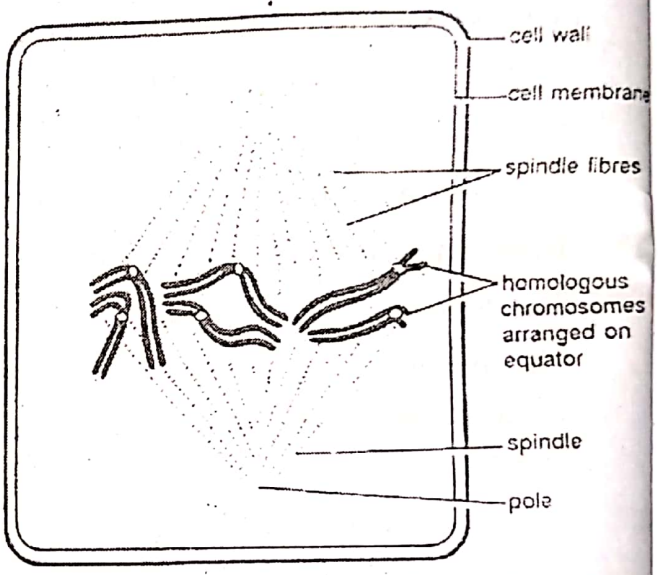


Meiosis : Cell showing Diakinesis of Prophase I

[VI] Metaphase I

The characters observed during Metaphase I are –

1. Nuclear membrane and nucleolus have completely disappeared.
2. Spindle formed by fibres is distinct.
3. Bivalents are arranged on the equatorial plate.
4. Each chromosome of a bivalent is attached to the spindle fibres by its centromere.
5. Centromeres are arranged on both the sides of the equatorial region, almost at equal distance.

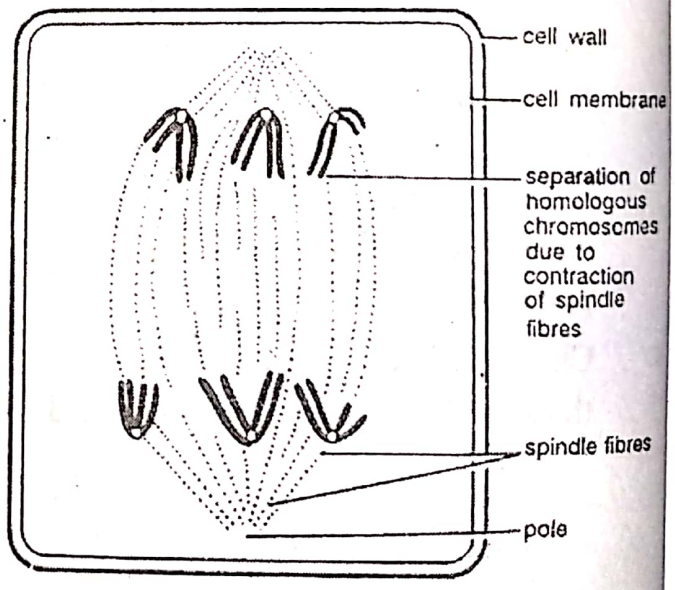


Meiosis : Cell showing Metaphase I

[VII] Anaphase I

The following are characteristics of this stage –

1. Nuclear membrane and nucleolus are completely absent.



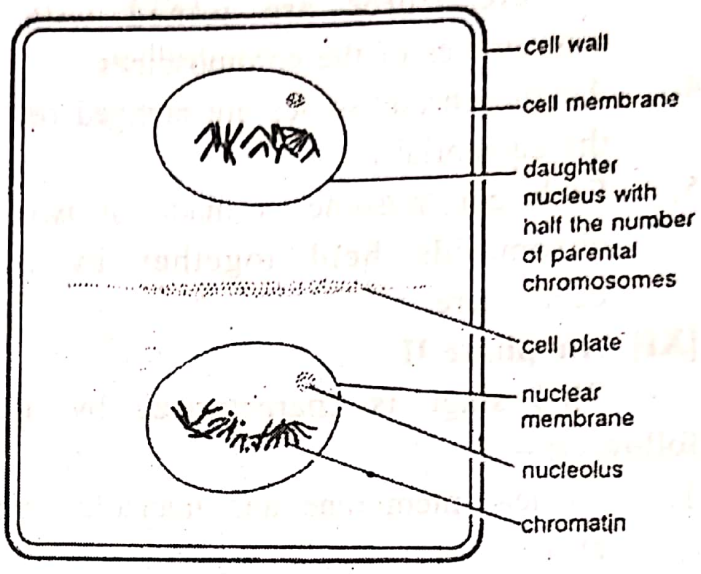
Meiosis : Cell showing Anaphase I

2. The chromosomes separate out of the pair of homologous chromosomes.
3. Spindle fibres contract and pull the centromere alongwith the chromosome to opposite poles.
4. This results in two haploid sets of chromosomes, one at each pole of the cell.
5. Each crhomosome shows characteristic shape during movement.

[VIII] Telophase I

The stage shows following characterisitcs –

1. Nuclear membrane and nucleolus have reappeared and are clearly seen.
2. There are two nuclei on each at the poles of the cell.
3. Each daughter cell has half the number of chromosomes compared to the parent cell. Chromosomes are thin and long. They are intermingled with one another to form a network.
4. Spindle fibres are totally absent.

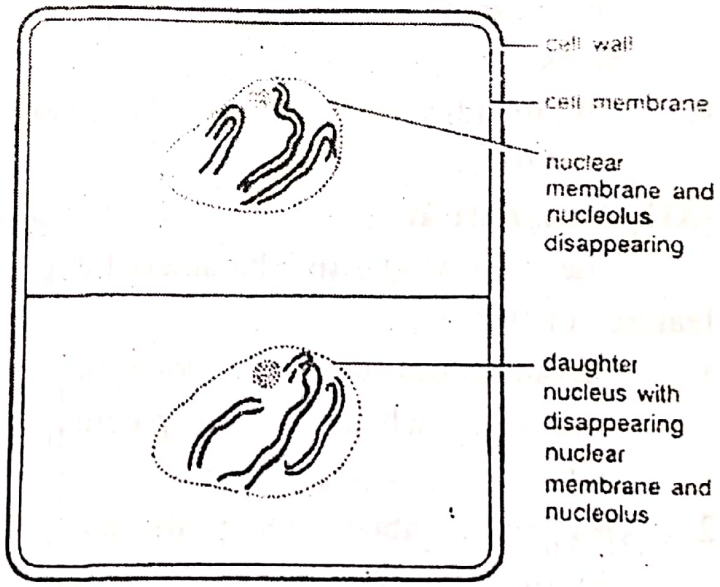


Meiosis : Cell showing Telophase I

[IX] Prophase II

The following characteristics are seen –

1. Nuclear membrane and nucleolus are distinct in the early stages. In late prophase, both these structures disappear gradually.
2. Chromosomes are short and thick.
3. Each chromosome is made of two chromatids bound together by a centromere.
4. The spindle fibres also begin to appear.
5. Chromosomes move towards the equatorial plat which is generally formed at right angles to the plate formed during meiosis I.

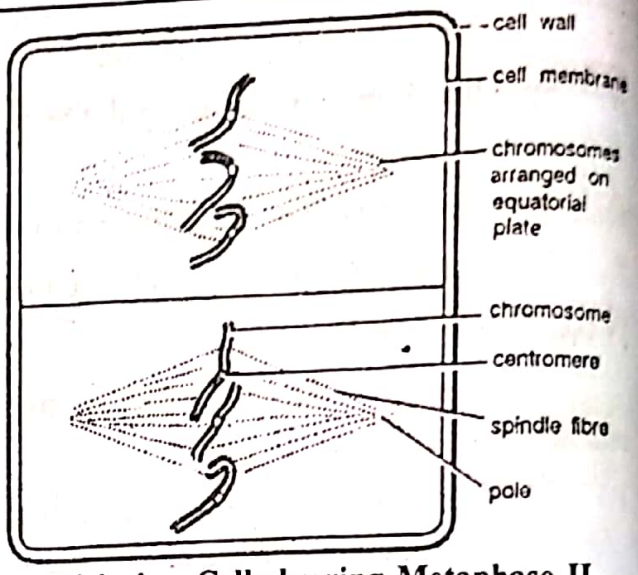


Meiosis : Cell showing Prophase II

[X] Metaphase II

It shows following characteristcs –

1. Nuclear membrane and nucleolus both are absent, having disappeared.
2. Spindle fibres are formed. These are organised into a spindle.
3. Spindle fibres are joined with centromeres of the chromosomes.
4. All the chromosomes are aranged on the equatorial plate.
5. Each chromosome is made of two chromatids held together by a centromere.

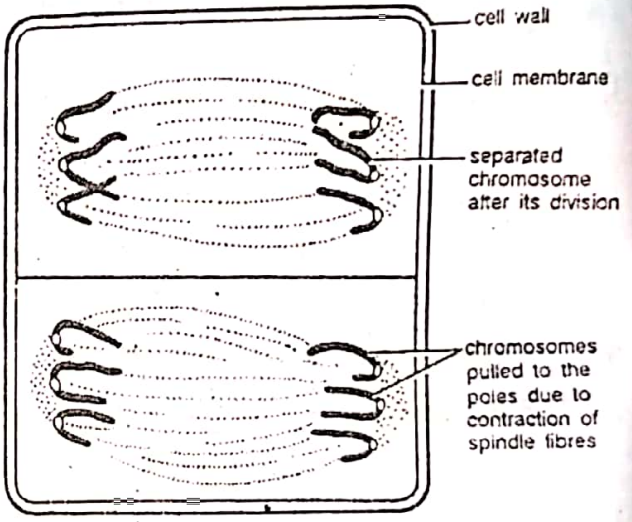


Meiosis : Cell showing Metaphase II

[XI] Anaphase II

This stage is characterised by the following –

1. Nuclear membrane and nucleolus are absent.
2. Centromere that holds two chromatids splits. Each chromatid now has an individual centromere.
3. Spindle fibres contract and each chromosome is now pulled to the opposite poles.
4. Chromatids (now called chromosomes) show characteristic shape during their movement.

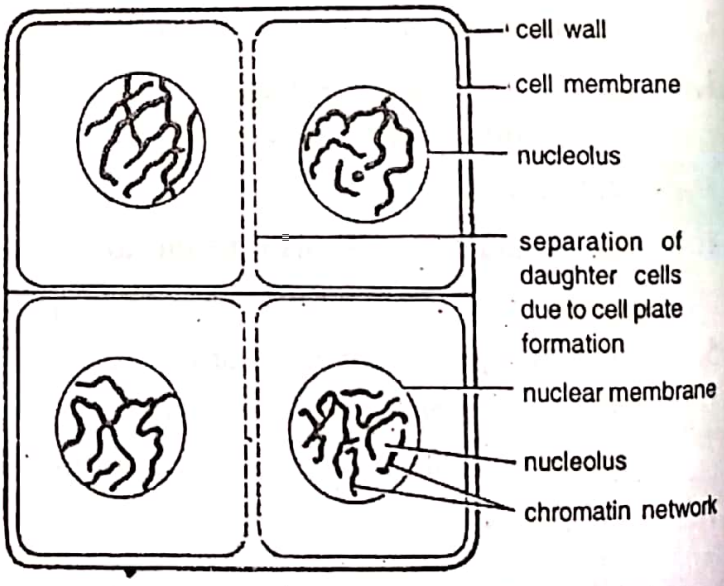


Meiosis : Cell showing Anaphase II

[XII] Telophase II

The following are characteristic features of this stage –

1. Chromosomes are in the form of groups at each end of the parent cell.
2. Nuclear membrane reappears and surrounds the group of chromosomes. This results in the formation of daughter nuclei at the opposite poles of the cells.
3. Spindle fibres disappear completely.



Meiosis : Cell showing Telophase II

PRACTICAL NO. 9

STUDY OF VEGETATIVE/REPRODUCTIVE STRUCTURE OF VOLVOX

❑ VOLVOX

- **Classification :**

Group : Algae

Division : Chlorophyceae (Green algae)

- **Habitat :**
  - *Volvox* is fresh water, free-swimming green alga.
  - It occurs as small green balls in pond water during & after rains.
- **Thallus Structure :**

The thallus is multicellular in form of motile colony. The colonies are mostly spherical, rounded or oval in shape and hollow. The center of colony is filled with gelatinous material. The cells in a colony are arranged in a single layer towards the periphery. The entire colony is surrounded by a gelatinous sheath. The number of cells in colony varies from 500-60,000 according to species. The colony with definite number of cells is called **coenobium.**

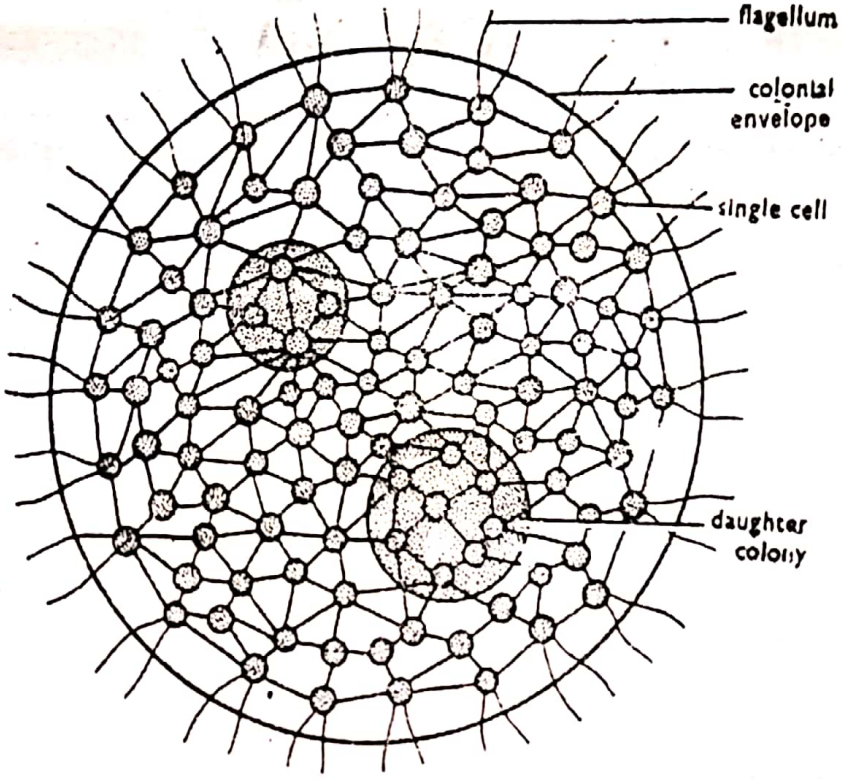
Each cell of the colony is connected with a few of the neighboring cells by delicate cytoplasmic strands. Each cell is enveloped by an individual gelationous sheath. Each vegetative cell is biflagellate, motile and ovoid with 2 to 5 contractile vacuoles, a central nucleus, and cup shaped chloroplast.

➤ REPRODUCTION

Reproduction takes place sexually as well as asexually.

Asexual Reproduction :

- Asexual reproduction takes place by formation of daughter colonies. Many small daughter colonies remain embedded in parent colony. They are similar to parent colony except that they are smaller in size. The daughter colonies are liberated upon gelatinization of parent colony.



Volvox Colony

PRACTICAL NO. 10

STUDY OF VEGETATIVE/REPRODUCTIVE STRUCTURE OF RHIZOPUS

RHIZOPUS (Bread Mould)

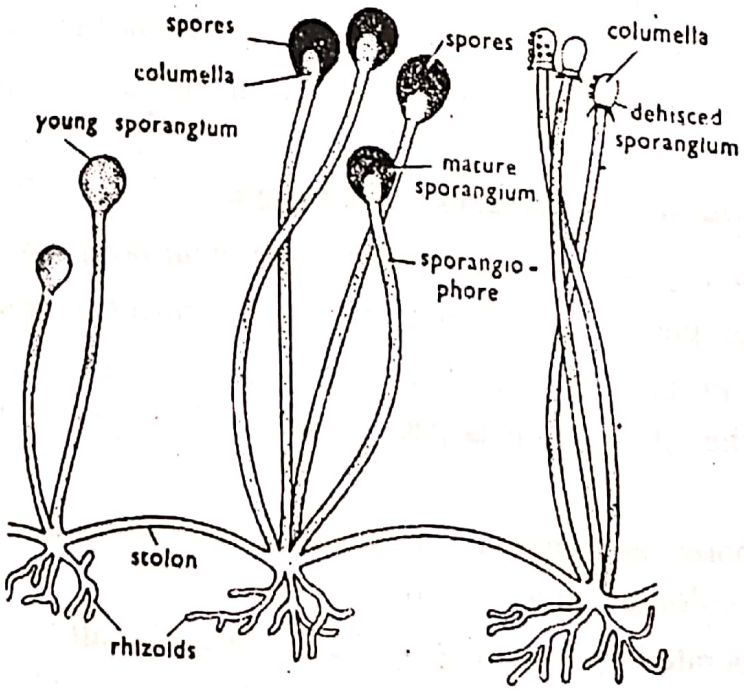
- **CLASSIFICATION :**

Kingdom	:	Mycota
Division	:	Eumycota
Sub-division	:	Zygomycotina
Class	:	Zygomycetes
Order	:	Perponosporales
Family	:	Phthiaceae
Genus	:	*Phytophthora*

(I) HOSTS AND DISEASES :

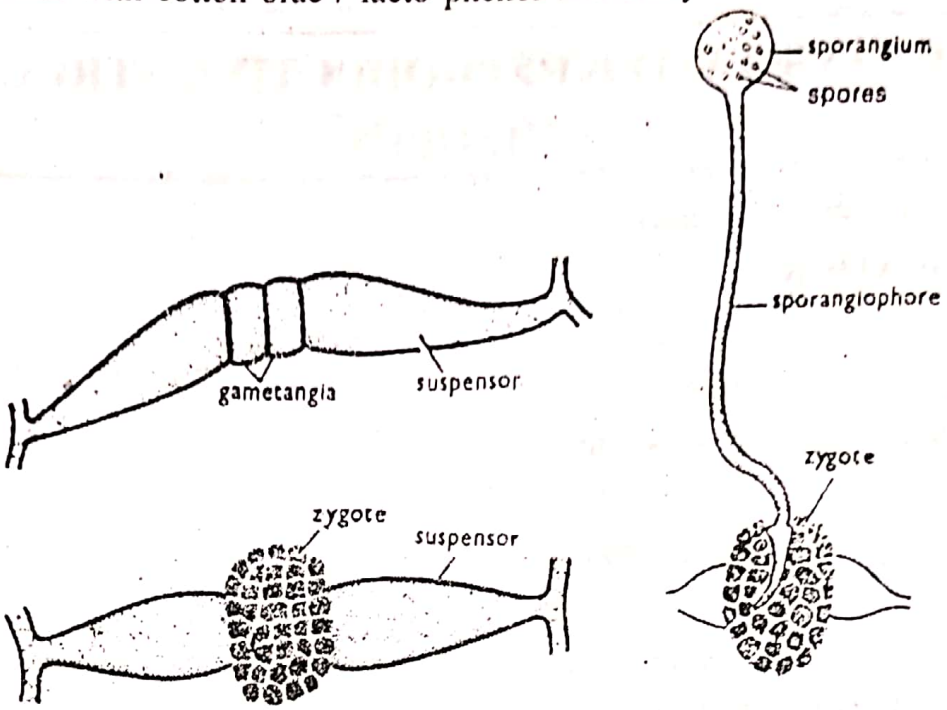
- Most of the species of *Rhizopus* are saprophytic and grow on dead vegetables or animal matter.
- *R. stolonifer* grows so frequently on bread that it is called the 'bread mold'.
- It is also called 'black mould' for its black colored sporangia.
- It also called as 'Pin mould' for small pin-head like sporangia at the tips.
- This fungus causes fruit drop of Jack fruits and apples.

STUDY OF FUNGI



Rhizopus. Mycelium with sporangiophores

- Pick up a few hyphae growing on a slice of bread.
- Stain with cotton blue / lacto phenol and study.



Sexual reproduction in Rhizopus

(III) VEGETATIVE STRUCTURE :

1. The mycelium shows abundant, white cottony growth.
2. The young mycelium is multinucleate, aseptate, with all hyphae alike.
3. In the older mycelium three parts of hyphae can be distinguished
   (a) Branched rhizoids that penetrate the substratum
   (b) Stolon or runner growing horizontally above the substratum for some distance and then bending downward, producing another group of rhizoids and
   (c) The sporangiophores which grow upward in tufts from the point where the stolon form rhizoids.

(IV) Asexual Reproductive Structures - Sporangia

1. The asexual reproductive structures are sporangia borne by the sporangiophores.
2. Each sporangiophore is swollen at the tip and forms sporangium.
3. The sporangium has a columella in the center and the space between columella and wall of the sporangium is packed with aplanospores. This is known as the sporesac.
4. The aplanospores are angular or rounded and multinucleate. The spores are colourless or coloured blue or brown.
5. Spores are liberated by breaking of the sporangial wall.
6. Each spore germinates to form a new mycelium.

PRACTICAL NO. 11

STUDY OF BRYOPHYTES : *RICCIA*

(I) RICCIA

- **CLASSIFICATION :**

  Division : Bryophyta

  Class : Hepaticopsida

  Genus : *Riccia*

- **EXTERNAL FEATURES OF GAMETOPHYTE :**

  (1) The gametophyte plant body is a thallus, which is prostrate, flat, dorsiventral and fleshy.

  (2) It is dichotomously branched and the rosette is formed due to repeated dichotomies of thalli.

  (3) The thallus is linear to wedge shaped with an apical notch at the apex and thickened midrib on the dorsal side, the midrib is transvered by mid-dorsal groove.

  (4) Ventrally, thallus is attached to the soil by means of unicellular rhizoids, which are also useful in absorption.

  (5) The rhizoids are of two types:

  (a) Smooth- walled (b) Tuberculate or Pegged

  (6) On the ventral side, besides the rhizoids there are violet, one celled thick, multicellular scales.

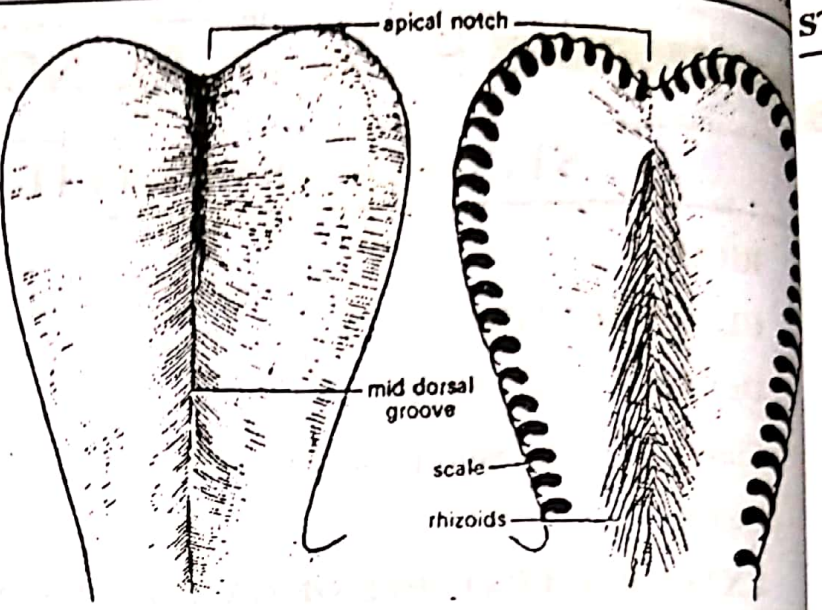
  (7) They anchor the thallus to substratum and protects the growing tip.

  (8) Sex organs are present in the mid-dorsal groove and are embedded in the thallus.

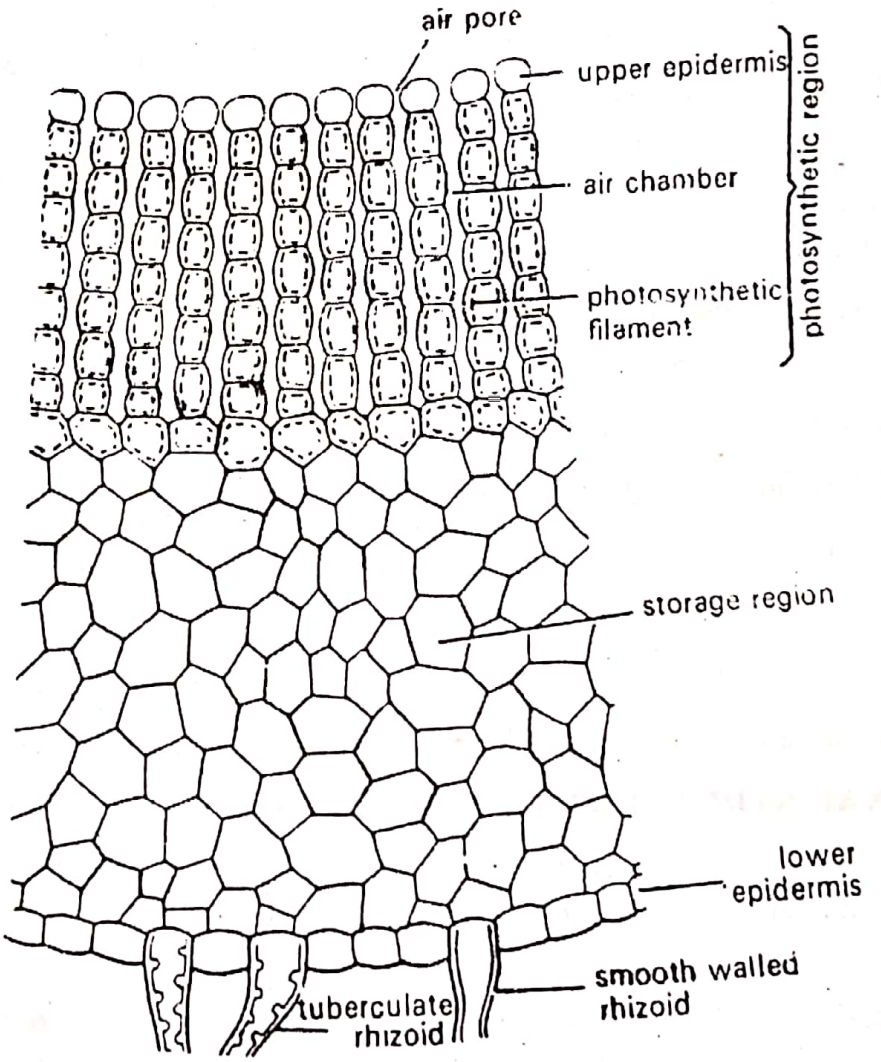
- **INTERNAL STRUCTURE OF GAMETOPHYTE :**

  The thallus shows boat shaped outline. It is several layered thick in the middle and gradually tapers at the margin.

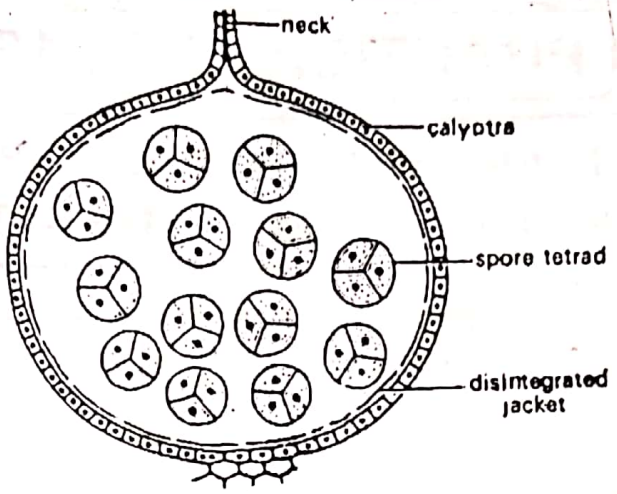
Riccia. Thallus.



Riccia. Dorsal and Ventral view.



V.S. of Riccia thallus



V.L.S. of Riccia Sporophyte

(I) PHOTOSYNTHETIC OR ASSIMILATORY REGION:

(1) It consists of vertical rows of unbranched assimilatory filaments, separated by narrow air chambers.

(2) Each cells of filament possesses numerous chloroplasts.

(3) The uppermost cell of the assimilatory filament is somewhat large, hyaline and lack chloroplast, also known as upper epidemis

(4) The cells are arranged one above another. They synthesize food material by the process of photosynthesis; therefore this zone is called as Photosynthetic region.

(5) The space between two vertical rows of green filaments has characteristic narrow vertical air space called assimilatory chamber or air chamber.

(6) The air chambers communicate with the atmosphere through air pores.

(II) STORAGE REGION :

(1) The lower portion consists of closely packed, colourless, parenchymatous cells without intercellular spaces.

(2) This is useful for storage of starch, which is synthesized in assimilatory region.

(3) The outermost layer of cells on the ventral side, bare the unicellular and multicellular rhizoids.

(4) The main functions of rhizoids are to absorb water and nutrients from soil and provide to gametophyte plant, whereas scales fix the plant to substratum.

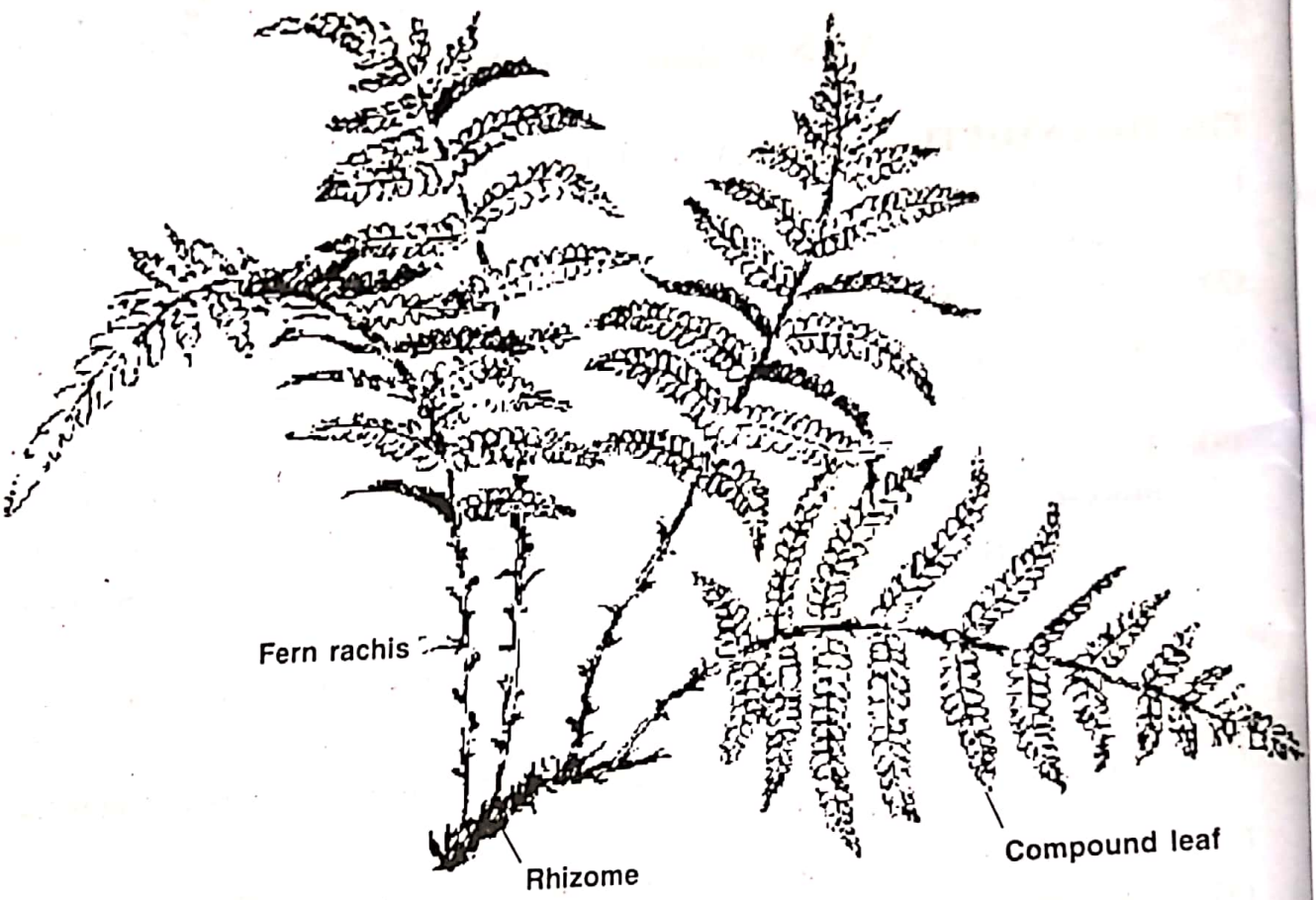
• *RICCIA* SPOROPHYTE :

The sporophyte is embedded in the tissue of gametophyte and it is developed after fertilization. The sporophyte is represented only by capsule, foot and seta are absent. The capsule has outer sterile jacket layer and two layered calyptra. The mature sporophyte has spore tetrads. Each spore is tetrahedral, with 3 layered spore wall.

PRACTICAL NO. 12

STUDY OF PTERIDOPHYIE FERN, NEPHROLEPIS, PLANT MORPHOLOGY, MOUNTING OF SPORANGINA

Fern is a pteridophyte plant. The plant body is differentiated into root, rhizome and leaves. The leaves are unipinnate compound leaves. The leaflets are arranged on both sides of thin, elongated structure, called as rachis.



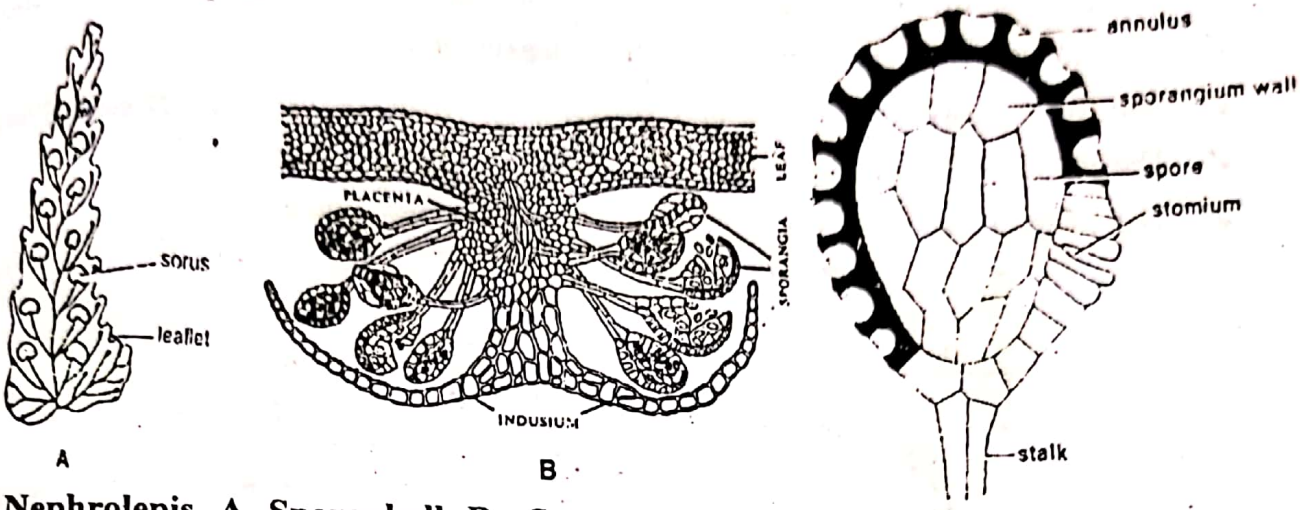
NEPHROLEPIS

Division	:	**Pteridophyta**
Class	:	**Fillicopsida**
Genus	:	**Nephrolepis**

- **STRUCTURE OF SPOROPHYLL, SORUS AND SPORANGIUM :**

Cut a vertical transverse section of the pinnule that has sori on the lower side. Stain in safranin and mount in glycerine.Study the characters of sorus and the sporangium.

1. The leaf bearing sori is called sporophyll.
2. The sporangia occur in groups called sori on the lower or abaxial side of pinnules in form of dark black spots.
3. The sorus is protected by indusium. It is made of upper indusial flap formed by the incurved margins of the pinnule and the lower true indusial flap that is poorly developed.



Nephrolepis. A. Sporophyll. B. Sorus in section C. Sporangium

- **METHOD OF MOUNTING OF FERN SPORANGIA :**

1. Take the fern leaflet and observe the underside of it. Dark colored sori are found arranged on margins of leaflet. Sori(group of sporangia, Sorus- singular, Sori- Plural) are elevated structures, black to brown in color and round in shape.
2. Take clean glass slide and place drop of glycerine on it.
3. Select one sorus, pluck it with the help of forceps and transfer on glass slide.
4. Dissect out the sorus using needle in order to expose the sporangia and then cover with coverslip.
5. Observe under 10X and then in 40 X.

➤ **OBSERVATION :**

1. Many sporangia are seen under microscope
2. Each sporangium has a stalk and a capsule
3. The stalk is long, slender and multicellular
4. The sporangium one celled thick wall made up of thick walled cells and thin walled cells.
5. A ring of thick walled cells is called Annulus.
6. A few thin walled cells form stomium. The sporangium bears spores which are oval or bean shaped.

PRACTICAL NO. 13

STUDY OF CYCAS - MORPHOLOGY, CORLLOID ROOF, LEAFLET, MALE CONE, MEGASPOROPHYLL

❖ **Division :** Gymnosperm

- ✓ Cycas is a gymnosperm plant.
- ✓ The plant is differentiated into erect stem and a crown of leaves. ..
- ✓ The leaves are of two types. (i) Foliage leaves and (ii) Scale leaves.
- ✓ The foliage leaves are compound leaves.
- ✓ The leaflet is thick and leathery.
- ✓ The internal structure of leaflet shows the following details.

CYCAS PLANT MORPHOLOGY

❖ **Cycas Plant Morphology :**

The plants grow under **xerophytic** conditions. Cycas is also cultivated as an ornamental plant in the gardens and parks. Cycas plants live upto hundred years or more. **Cycas is called a living fossil. PLANT BODY**

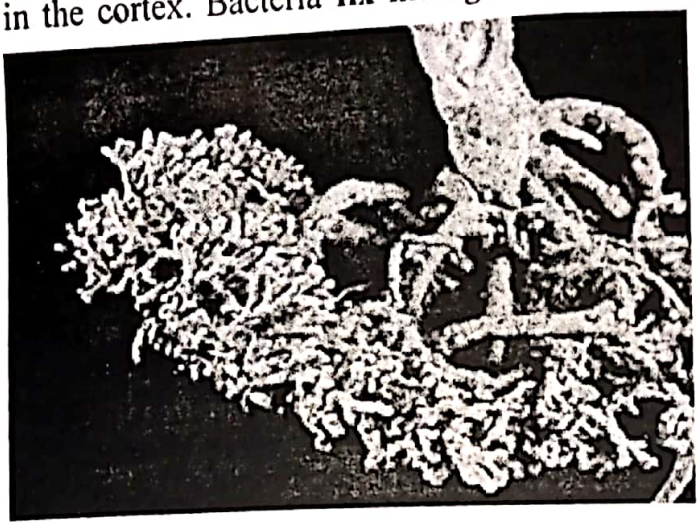
The plant body is a sporophyte. It is differentiated into root, stem and leaves.

- **Stem :** Stem is unbranched. It is covered by thick, woody, persistent leaf bases. It makes the stem rough. The apex of the stem is ensheathed by a group of brown scales. The

lower of stem is covered by pinnate compound leaves. The growth of the stem is very slow. It produces a cluster of leaves each year. Older leaves fall of after two years.

Leaves and scales : The leaves are produced in the **axils of the scales near the apex.** Each leaf is composed of a **petiole, rachis** and lateral **pinnae**. The young leaves show circinnate **vernation. Scales are also produced each year.** Therefore, the clusters of green leaves and scales alternate with each other. **Scales** are also **persistent.** Scales and leaf bases cover the surface of the old stem.

- **Roots :** The primary root persists in Cycas. It becomes **tuberous**. Cycas produces **coralloid roots.**Coralloid roots are short tufts and dichotomously branched roots. These roots contain an **endophytic** alga in the inner part of their cortex. Sometimes, **bacteria** are also present in the cortex. Bacteria **fix nitrogen.**



CORALLOID ROOTS OF CYCAS

❖ **Internal Structure of cycas leaflet**

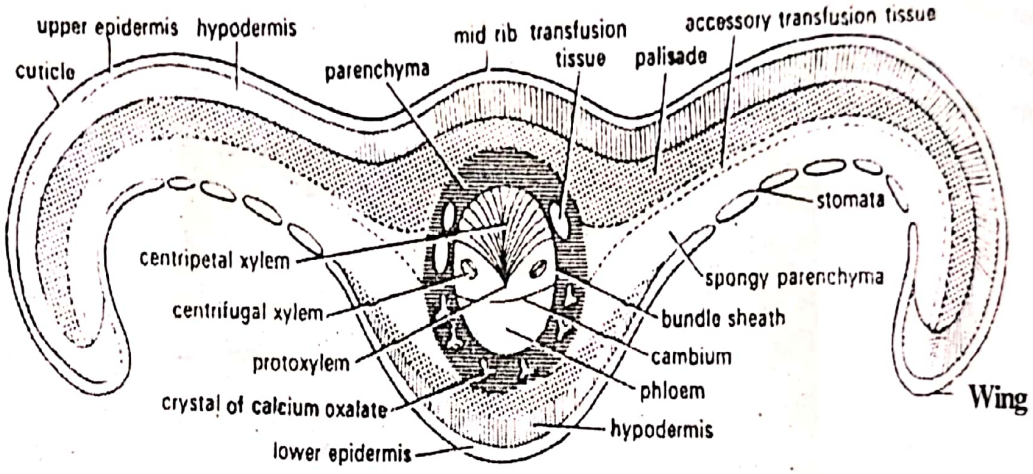
- **Cuticle :**
  The upper surface of leaf is covered with a thick cuticle.
- **Upper Epidermis :**
  A single layered upper epidermis is present.
- **Hypodermis :**
  It is present below the epidermis. It is one or two layered made up of sclerenchyma cells.
- **Mesophyll :**
  Mesophyll lies below the hypodermis & is well developed, it is differentiated into upper palisade tissue & lower layer of spongy parenchyma tissue. Between palisade and spongy cells in the center, elongated living cells are transversely arranged. These cells start from vascular bundle and ends at margin of the leaflet, which conduct water from vascular bundle. They form transfusion tissue.

- **Lower Epidermis:**

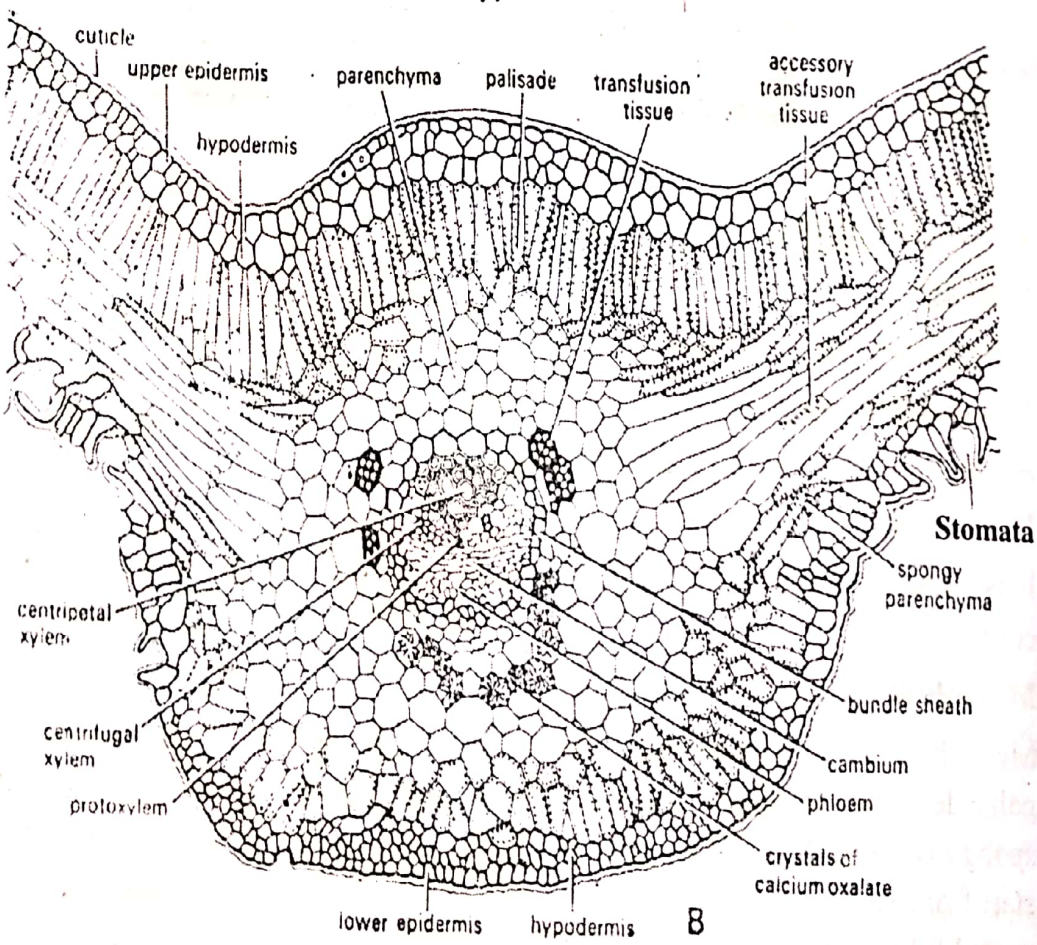
  The lower epidermis is punctured by the sunken stomata.

- **Vascular Bundle :**

  Vascular bundle is present in the mid rib. It has a definite parenchymatous bundle sheath. The vascular bundle is conjoint, collateral, open and diploxylic. It shows a large, V shaped or triangular patch of **centripetal xylem** and two small groups of **centrifugal xylem.** The phloem lies towards lower side. In between xylem and phloem, the cambium is present.



A

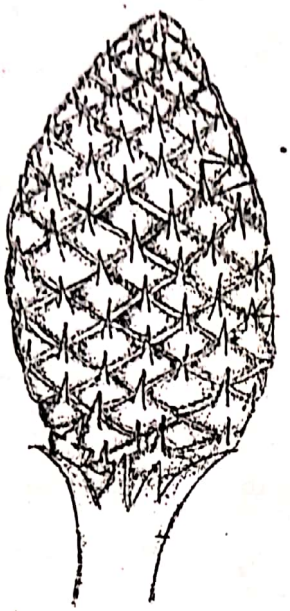


B

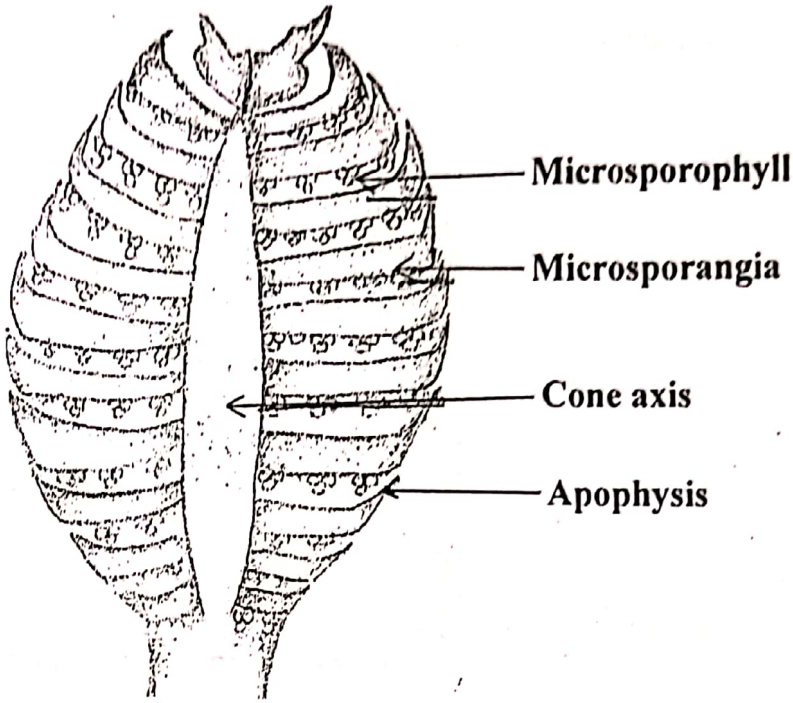
Cycas A. and B. T.S. of leaflet.

➤ **Cycas Male Cone:**

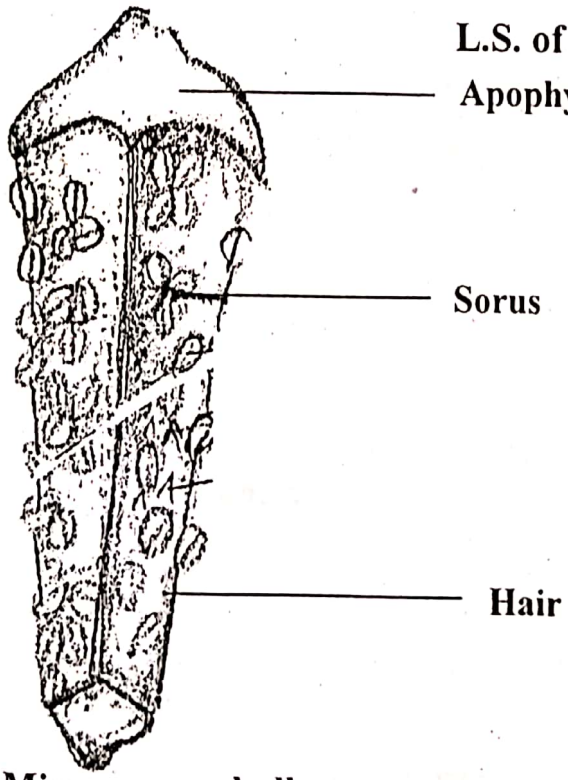
The male cone is terminal, compact, large, oval or conical in shape and consists of central axis and many spirally arranged microsporophylls. Each microsporophyll is woody, flat and triangular in shape. It is differentiated into a fertile and sterile part. The fertile part is wedge-shaped and expanded. The sterile part is distal part of microsporophyll, which tapers into elongated apophysis. The lower surface of the fertile part bears microsporangia in groups of 3-4, forming sori.



L.S. of Cycas Male Cone



L.S. of Cycas Male Cone

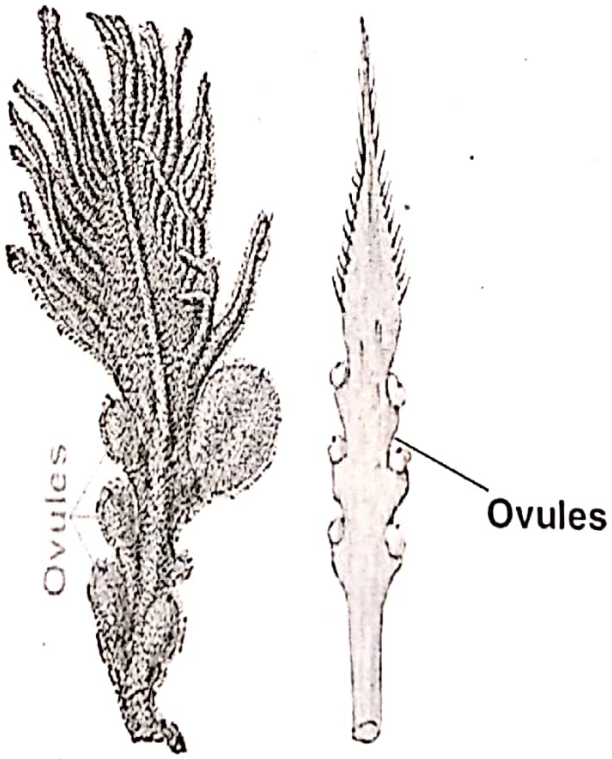


Micro-sporophyll

➤ **Cycas Megasporophyll :**

There is no properly organized female cone. Only a crown of Megasporophylls, is arranged in acropetal succession. Each Megasporophyll is leaf-like, leathery, hairy, with upper portion broadly dissected. It varies in size from 6-12 inches. The lower portion of megasporophyll bears ovules in two rows, on either side. The ovules may be arranged opposite or alternate. The ovules are generally yellow or orange in colour, hard and woody texture. The Cycas ovule is the largest among the ovules of Plant Kingdom.

Cycas Megasporophylls in cluster



C. revoluta C. circinalis

Cycas Megasporophyll

PRACTICAL NO. 14

EXAMPLES BASED ON CHARGAFF'S RULE

❑ **Chargaff rule :**

In DNA there is always equality in quantity between the bases A and T and between the bases G and C. (A is adenine, T is thymine, G is guanine, and C is cytosine). Named after the great Austrian-American biochemist Erwin Chargaff (1905-2002) at Columbia University, who discovered this rule. It is also known as Chargaff's ratio/Chargaff's rule.

According to this rule Adenine is complementary to Thymine and Guanine is complementary to Cytosine. "This observation of complementarity, later called Chargaff's ratios/Chargaff's rule, was essential to the solution of DNA's structure. In hindsight, the complementary pairing of the nucleotides powerfully suggested that a DNA molecule could break into two strands. Only complementary bases could form bonds and line up in place in a new DNA strand."

The rules of base pairing (or nucleotide pairing) are:

- **A** with **T** : the purine **adenine** (A) always pairs with the pyrimidine **thymine** (T)
- **C** with **G** : the pyrimidine **cytosine** (C) always pairs with the purine **guanine** (G)

❑ **Examples :**

1. A segment of DNA has 120 adenine and 120 cytosine bases. The total number of nucleotides present in the segment is.....

Ans : According to Chargaff's rule :

- Adenine (A) is paired to Thymine (T)
- Cytosine (C) is paired to Guanine (G)

Here, Adenine residues =120,

Cytosine residues = 120

[A] = [T]

[A = 120] = [T = 120]

[C] = [G]

[C = 120] = [G = 120]

Therefore total number of nucleotides

= [A] + [T] + [C] + [G]

= 120×4 = **480**

2. In humans, there is approximately 30% adenine. What is the percentage of other nitrogenous bases ?

Ans : According to Chargaff's rule :

- Adenine (A) is paired to Thymine (T)
- Cytosine (C) is paired to Guanine (G)

[A] + [G] = [C] + [T]

[A] + [G] + [C] + [T] = 100%

Here [A] = 30% therefore % of [T] is also 30%

Therefore [G] + [C] = 100 – 60 = 40%

[G] = 20% and [C] = 20%

3. In DNA, guanine is 10%. The content of adenine is,

Ans :

Cytosine = 10%

A + G + C + T = 100%

A + 10% + 10% + T = 100%

A + T + 100% – 20% = 80%

A/T = 80% / 2 = 40%

Ans = 40%

PRACTICAL NO. 15

QUALITATIVE TESTS FOR CARBOHYDRATES-REDUCING SUGARS, NON-REDUCING SUGARS AND STARCH

1. What is reducing Sugar ?

Reducing sugars are carbohydrates that can act as reducing agents due to the presence of free aldehyde group or free ketone group. All monosaccharaides and some disaccharides are reducing sugars.

Reducing sugars can be oxidized by weak oxidizing agents. In aqueous medium, reducing sugars generate one or more compounds containing a free aldehyde group. This is a characteristics property of reducing sugars.

❑ **Examples of reducing sugars :**

Glucose, fructose, galactose (monosaccharaides) and lactose, maltose as (disaccharides

The Benedicts test (and also Fehling's, Cole's test) can be used to identify the presence of a reducing sugar in a given sample. The Cu^{+2} ions in Benedict's reagent are reduced to Cu_2O. The formation of the Cu_2O precipitates indicate the presence of a reducing sugar.

- **Reagents :** Test solution, Benedict's reagent, Iodine solution.
- **Requirements :** Test tubes, Test tube holder, Test tube stand, Burner.
- **Procedure for reducing sugar tests :**

Test No.	Test	Observation	Inference
1.	**Benedict's Test :** • Take 5 drops of test solution • Add 2 ml of benedict's solution • Boil it for 5 minutes.	Formation of precipitations (Green, yellow, orange, red)	Therefore, reducing sugar is present.

Note :

The sugar concentration is shown below as color developed:

Blue : no sugar present

Green : trace conc. of sugar

Yellow : low conc. of sugar

Orange : moderate conc. of sugar

Red : high conc. of sugar

2. What is Non-reducing Sugar ?

Non-reducing sugars are carbohydrates that cannot act as reducing agents due to absence of free aldehyde groups or free ketone groups. Some disaccharides and polysaccharides are non-reducing sugars. In basic aqueous media, non-reducing sug do not generate any compounds containing a free aldehyde group.

Non-reducing sugars do not show a positive result for Fehling's or Benedict te This is because Cu^{+2} in those test solutions cannot be reduced by the sugar.

❑ **Example of Non-reducing sugar :**

Sucrose is a well-known non-reducing sugar. It is a disaccharide.

Test for Non-Reducing sugar :

Test No.	Test	Observation	Inference
1.	**Benedict's Test :** • Take 5 drops of test solution • Add 2 ml of benedict's solution • Boil it for 5 minutes.	**No** Formation of **precipitations** (Green, yellow, orange, red)	Therefore, No reducing sug is present.

3. Starch (Polysaccharide) :

Test No.	Test	Observation	Inference
1.	**Iodine Test :** Take 1 ml of test solution and add 2 drops of iodine solution.	Blue Black color formed.	Therefore, starch is present.

PRACTICAL NO. 16

QUALITATIVE TESTS FOR PROTEIN (HEAT COAGULATION, PRECIPITATION TEST, BIURET TEST, FOLIN'S TEST)

Proteins are polymeric biological molecules consisting of chains of amino acids called polypeptides. A single polypeptide can make a protein, although many proteins consist of multiple polypeptide chains as subunits.Proteins contain carbon, hydrogen, nitrogen and sulphur and some contain phosphorus also.

They are the fundamental constituents of all protoplasm and are involved in the structure of the living cell and in its function. Most of the Enzymes are proteins. Many of the hormones are proteins. The cementing substances and the reticulum which bind or hold the cells as tissues or organs are made up partly of proteins. They execute their activities like transport of oxygen and carbon dioxide as hemoglobin and special enzymes in the red cells. They function in the homostatic control of the volume of the circulating blood and that of the interstitial fluids through the plasma proteins. They are involved in blood clotting as thrombin, fibrinogen and other protein factors. They act as the defense against infections by means of antibodies which are proteins. They perform hereditary transmission by nucleoproteins of the cell nucleus.

Reagent : 10% NaOH, $CuSO_4$, 2% NaOH, Esbach's reagent; $HgNO_3$.

Requirement : Test tubes, Test tube holder, Test tube stand, Burner.

Procedure for qualitative analysis of protein :

- **Color reactions :**
  - **Biuret Test :**

Test No.	Test	Observation	Inference
1.	**Biuret Test :** • Take 2 ml of protein solution • Add 2 ml of 10% NaOH • Add 2 drops of 2% $CuSO_4$	Violet/Pink color is observed.	Therefore, protein is present.

- **Folin's test :**

Test No.	Test	Observation	Inference
1.	**Folin's test :** • Take 3 ml of protein solution • Add 2 drops of Folin's reagent • Add few drops of 2% $CuSO_4$ • Add few drops of 2% NaOH	Blue color is formed.	Therefore, proteins are present.

- **Precipitation reactions :**

Test No.	Test	Observation	Inference
1.	**Alkaloidal test** Take 1 ml of test solution and add 1 ml of Esbach's reagent	Yellow precipitations are observed.	Therefore, proteins are present.
2.	**Heat coagulation test** • Take 5 ml of test solution • Boil it thoroughly.	Jelly like mass of precipitate, sticking to side walls of test tube observed.	Proteins get denatured and adhere to sides of test tube.
3.	**Heavy metal-ion test** Take 3 ml of test solution and add few drops of $HgNO_3$.	White precipitations are observed.	Therefore, proteins are present.

PRACTICAL NO. 17

QUALITATIVE TESTS FOR LIPIDS

Lipids are simple, complex or derived. Simple lipids are esters of fatty acids with various alcohols, e.g., fats (esters of fatty acids with glycerol) and waxes (esters of fatty acids with higher molecular weight of monohydroxy alcohols). Complex lipids are esters of fatty acids containing additional groups in addition to an alcohol and a fatty acid, e.g., phospholipids or glycolipids etc. Derived lipids include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, lipid soluble vitamins, and hormones.

Phospholipids in addition to alcohol and fatty acids contain phosphate and a nitrogenous base like choline, ethanolamine etc. Lecithin and cephalin are representatives of the phospholipids. Similarly glycolipids contain carbohydrates and sulpho-lipids contain sulphate. Lipoproteins are combinations of lipids with proteins.

Requirements : Test tubes, Test tube holder, Test tube stand, Burner.

Reagents : Water, Benzene, Alcohol, Na_2CO_3.

❑ **Procedure :**

Test No.	Test	Observation	Inference
1.	**Grease Spot Test** • Put a drop of oil/grease on paper and gently rub it.	Translucent grease spot appear on paper	Lipid is present
2.	**Solubility test** (a) Take 2 ml of water and add a drop of oil	Insoluble in water	Lipid is present
	(b) Take 2 ml of benzene and add a drop of oil	Soluble in benzene	Lipid is present
	(c) Take 2 ml of alcohol and add a drop of oil	Slightly soluble in cold alcohol and soluble in hot alcohol	Lipid is present
3.	**Saponification test** • Take 4 ml of Na_2CO_3 in a test tube • Add 2 drops of oil • Shake vigorously and boil it	A soapy solution is formed	Lipid is present

PRACTICAL NO. 18

CLASSIFICATION : PROTOZOA, PORIFERA, COELENTERATA

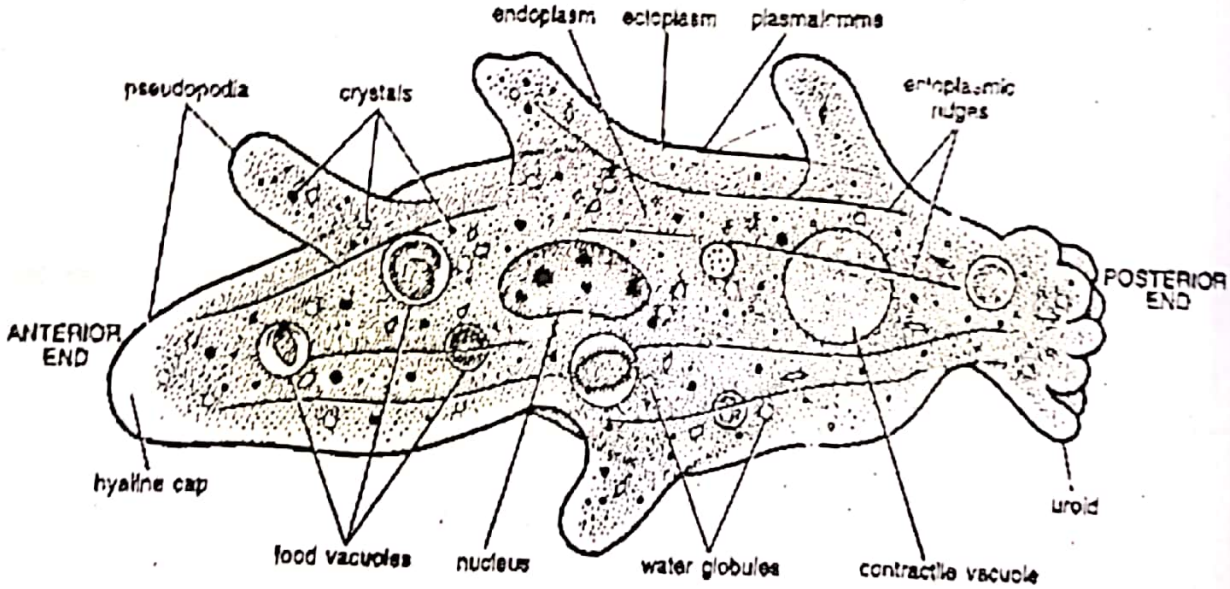
GENERAL CHARACTERS OF PHYLUM - PROTOZOA (PROTO. SIMPLE/ PRIMITIVE/FIRST ZOON-ANIMAL)

1. Protozoa are generally microscopic and acellular animals.
2. Body is either naked or covered by pellicle, and sometimes with Shell (exoskeleton).
3. Body symmetry-asymmetrical, bilaterally, radially or spherically symmetrical
4. Protoplasm is differentiated into Outer ectoplasm and an Inner endoplasm.
5. Locomotory organs are Pseudopodia, Flagella, Cilia or absent.
6. Respiration occurs by diffusion through general body surface.
7. Reproduction is Sexual or Asexual.

STUDY OF PROTOZOANS

[1] Amoeba

PHYLUM : PROTOZOA
SUBPHYLUM : PLASMODROMA
CLASS : RHIZOPODA
TYPE : *Amoeba proteus*



Amoeba proteus

(1) Amoeba is unicellular.

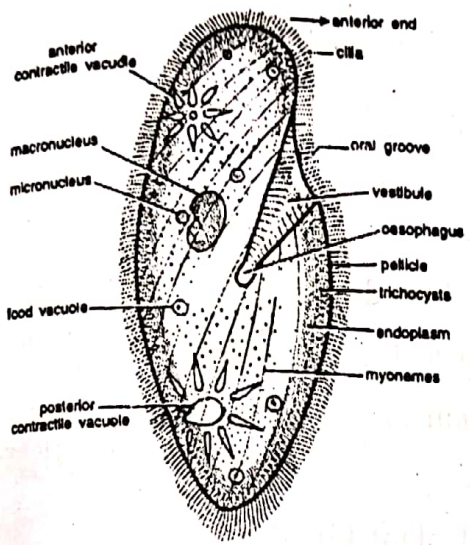
(2) Locomotion and feeding is by Pseudopodia.

(3) Animal is of irregular shape and appears as colourless, transparent and gelatinous mass

(4) Body is covered by a thin, delicate plasma membrane called Plasma lemma.

Identification : As the animal has Irregular body shape and Pseudopodia, it is identified as Amoeba.

[2] Paramoecium



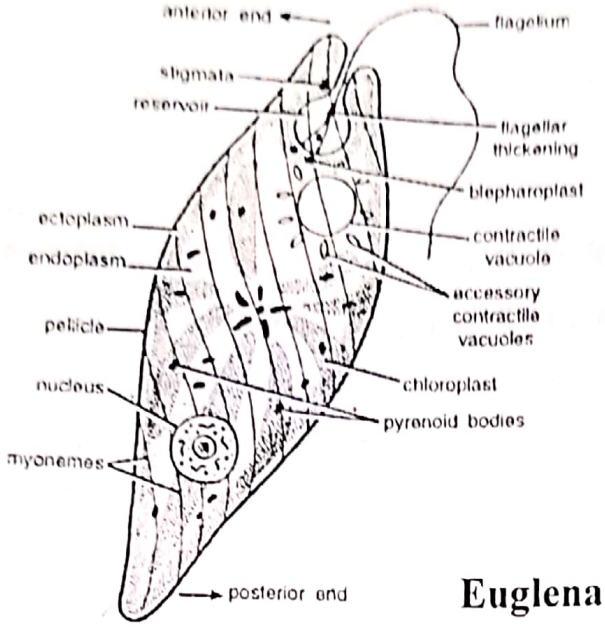
Paramoecium

PHYLUM : PROTOZOA
SUBPHYLUM : CILIOPHORA
CLASS : CILIATA
TYPE : *Paramoecium*

1. Commonly called as Slipper Animalcule.
2. Cilia are present throughout body and life and help in locomotion.
3. Anterior end of the body is rounded, while posterior end is pointed.
4. Body is covered with Pellicle.
5. Endoplasm has Food vacuoles, Micronucleus, Meganucleus and Contractile vacuoles.

Identification : As the animal has Slipper-shaped body and has two Contractile vacuoles, which are star-shaped, it is Paramoecium.

[3] Euglena



Euglena

PHYLUM	:	**PROTOZOA**
SUBPHYLUM	:	**PLASMODROMA**
CLASS	:	**MASTIGOPHORA**
TYPE	:	***Euglena***

1. Euglena is minute, elongated and spindle-shaped.
2. It is pointed at the posterior end and blunt at the anterior end.
3. Body is covered with Pellicle and more than one flagella present.
4. Anterior end of the body has Cytopharynx.
5. It is a connecting link between animal and plant kingdom.

Identification : As the animal bears Cytopharynx, Flagella and Pyrenoid bodies and is spindle shaped it can be identified as Euglena.

GENERAL CHARACTERS OF PHYLUM - PORIFERA

(Pori → pore; fera → bearing)

1. Porifera are aquatic, sessile and sedentary.
2. Body shape is vase-like or cylindrical.
3. Body of sponge is Multicellular comprising of outer ectoderm and inner layer of endoderm with an intermediate layer of Mesenchyme; therefore Diploblastic animals and [Tissue grade of organization is poorly developed.]
4. Sponges have skeleton consisting Spongin fibers and Siliceous spicules
5. Interior space of the body is either hollow or permeated by numerous canals.
6. Reproduction is Asexual and Sexual.

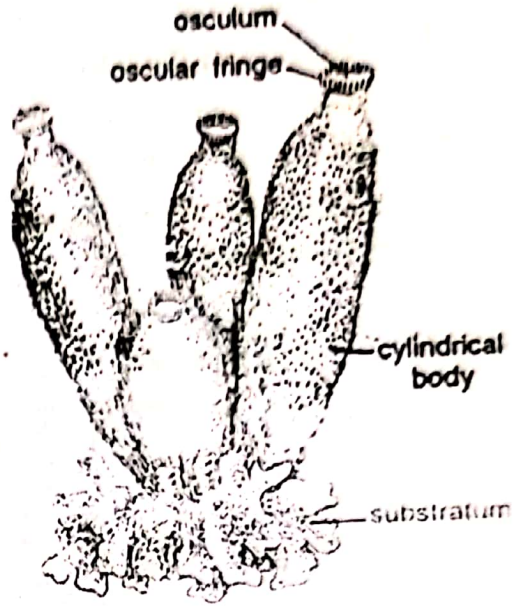
STUDY OF PORIFERANS

[1] Leucosolenia

PHYLUM : PORIFERA
CLASS : CALCAREA
TYPE : *Leucosolenia*

1. Vase-shaped sponge measures 20-25 mm in length and 5-6 mm in diameter.
2. Calcareous spicules present.
3. Body opens to the exterior by osculum.
4. Body wall is thick, through which spicules project.
5. Body is covered by pore-bearing membrane.

Identification : As the animal is vase-shaped white pipe bearing osculum, it is identified as Lecosolenia.



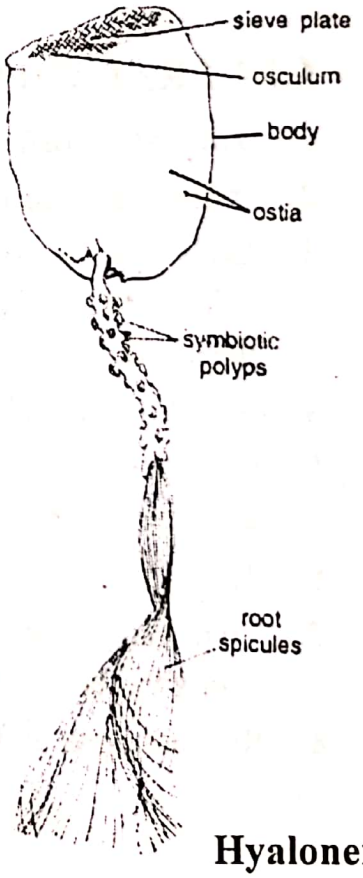
Leucosolenia

[2] Hyalonema

PHYLUM : PORIFERA
CLASS : HEXACTINELLIDA
TYPE : *Hyalonema*

1. Commonly called as Glass Rope Sponge.
2. Glass sponge has siliceous spicules of (Six-ray pointed type)
3. Body is vase-shaped measuring 10-30 cm in height.
4. Spicules are fused to form a lattice-like skeleton giving the sponge a glass-like appearance.
5. Body is elevated from the substratum by stalk-like root-tuft, which is twisted.
6. Root tuft projects above as gastral cone.

Identification : Since the specimen has gastral cone, Root tuft and Root spicules, it is Hyalonema.



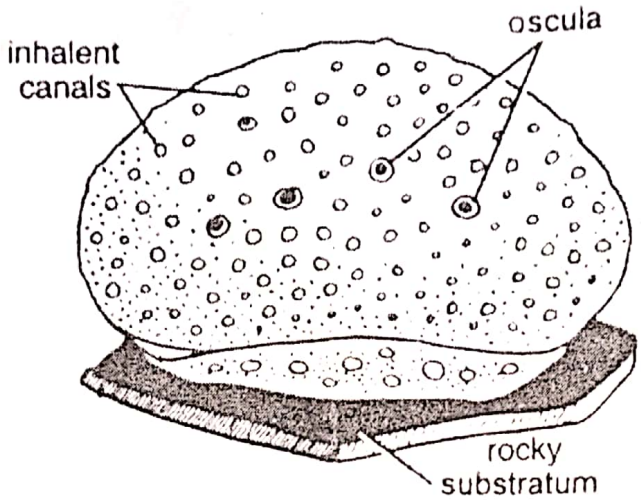
Hyalonema

[3] Euspongia

PHYLUM : PORIFERA
CLASS : DEMOSPONGIA
TYPE : *Euspongia*

1. Commonly called as Bath Sponge.
2. Spongin fibers or siliceous spicules present.
3. Surface of the body contains large openings called as oscula.
4. Bath sponge is used for household use.
5. It is large globular cup-shaped complex and shows infoldings of body wall.

Identification : Since the animal has globose body and oscula, it is known as Euspongia.



Euspongia

GENERAL CHARACTERS OF PHYLUM - COELENTERATA

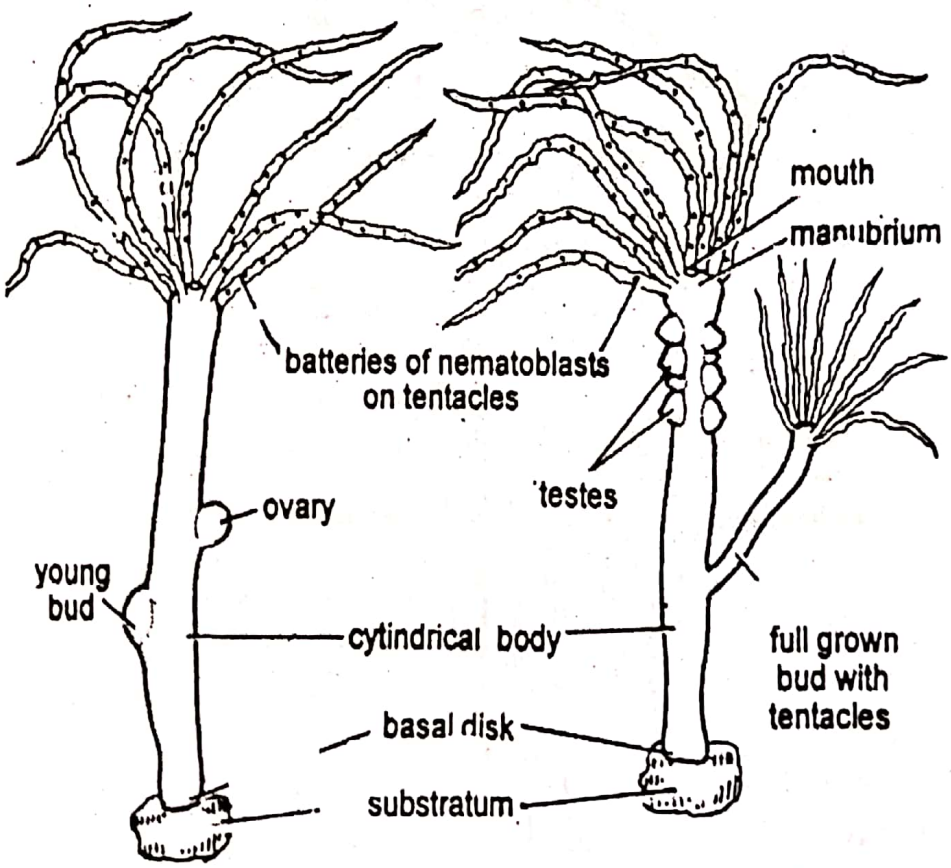
1. They are mostly Marine forms except some freshwater animals.
2. Individuals are radially or biradially symmetrical.
3. Body wall consists of an outer layer of cells called ectoderm and inner layer of cells called endoderm connected by an intermediate layer of non-cellular gelatinous mass known as mesoglea.
4. Acoelomate and diploblastic animals.
5. Coelenterates have specialized celles for locomotion, protection and food capuring known as stinging cells.
6. They exhibit the phenomena of polymorphism.
7. Two main types of structures - polyp and medusa are characteristic of phylum coelenterata
8. Reproduction is both asexual as well as sexual.

STUDY OF COELENTERATES

[1] Hydra

PHYLUM : COELENTERATA
CLASS : HYDROZOA
TYPE : *Hydra*

2. Hydra is tubular, cylindrical and measures about 1 cm in length.
3. Polyp and medusa present.
4. Posterior end acts as a holdfast organ and is called as pedal disc and has gland cells, which secrete adhesive secretion.
5. Oral end is produced into conical projection called hypostome, which has mouth and is surrounded by 6-10 Tentacles
6. Body wall has nematocysts, which are offensive and defensive organs.
7. Identification: As the animal has tubular body and tentacles, it is Hydra.



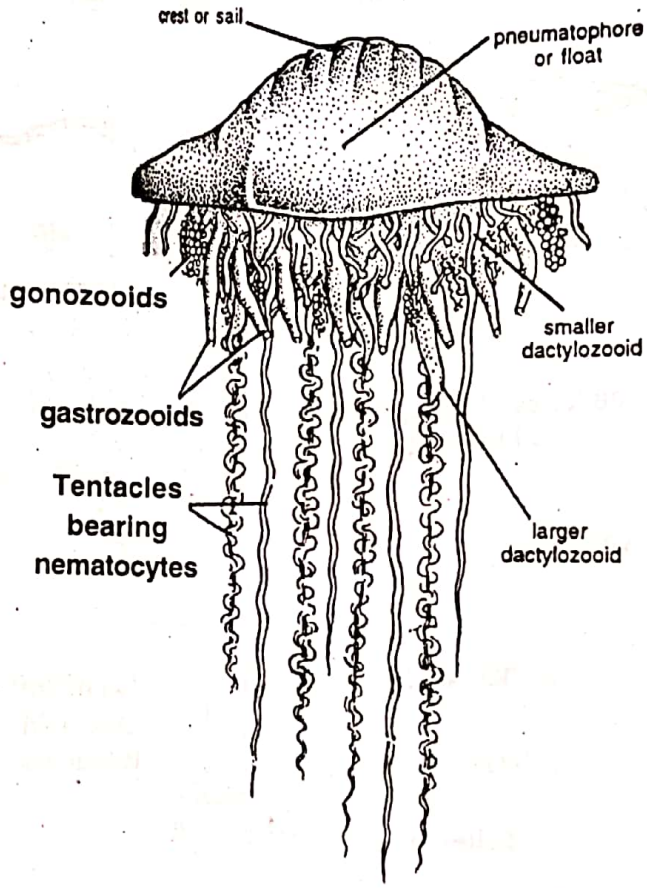
Hydra

[2] Physalia

PHYLUM : **COELENTERATA**
CLASS : **HYDROZOA**
TYPE : ***Physalia***

1. Commonly called as "Portuguese Man of War".
2. Polyp and medusa present.
3. It has pneumatophore or float which contains gas glands, responsible for secreting air (Nitrogen 85-91%, Oxygen 13.5%, 1.5% Argon).
4. Gas fills the body and helps the animal to float and is forced out of pneumatophore, when the animal sinks.
5. Animal is about 10 to 30 cm long and has tentacles.

Identification : The specimen has pneumatophore so it is Physalia.



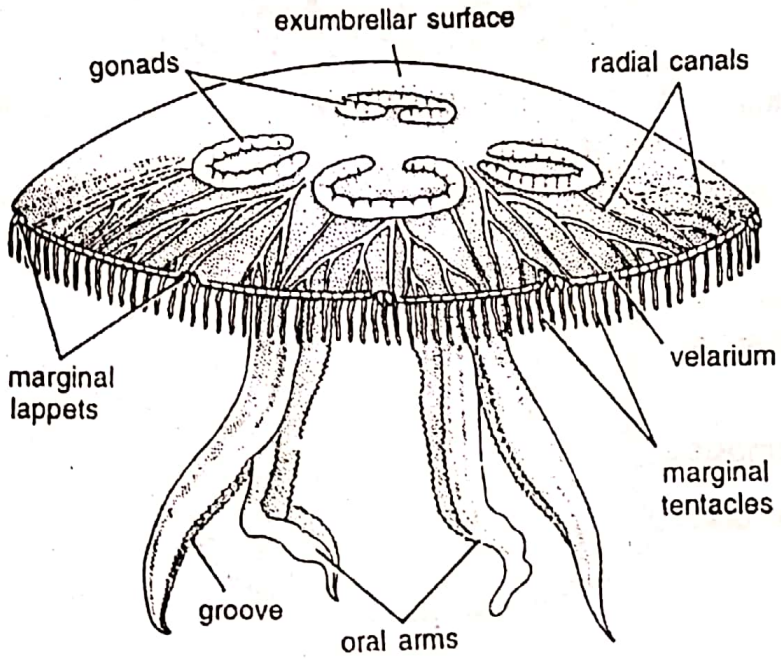
Physalia

[3] Aurelia

PHYLUM : COELENTERATA
CLASS : SCYPHOZOA
TYPE : *Aurelia*

1. Commonly called as Jelly fish.
2. Medusa is saucer-shaped having tetramerous radial symmetry.
3. Body consists of ex-umbrella and sub-umbrella surfaces.
4. Manubrium hangs down form the center and is surrounded by four radial arms.
5. Sub-umbrella region has marginal tentacles having stinging cells and marginal lappets having organs.

Identification : Since the animal has distinct, jelly-like body, small tentacles and ex- and sub-umbrella surfaces, it is Aurelia.



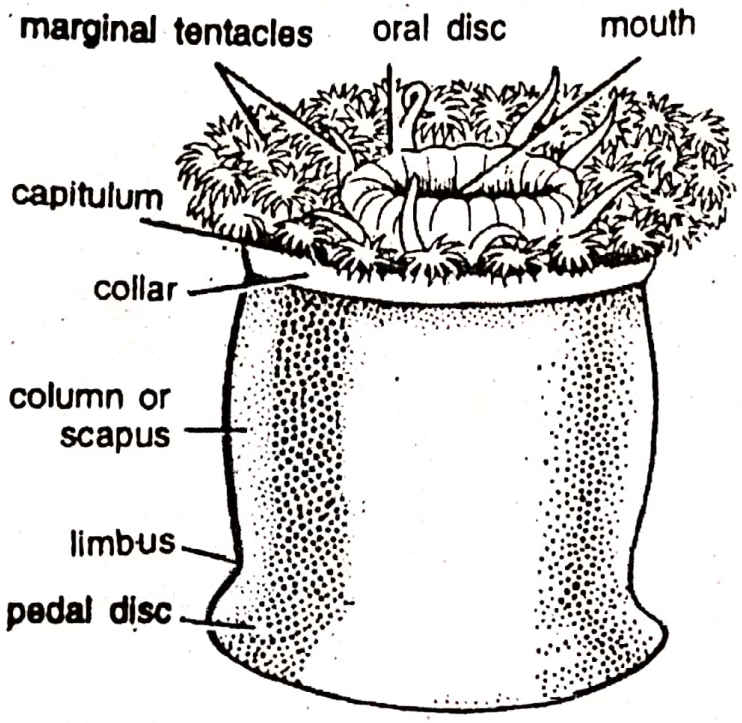
Aurelia

[4] Sea Anemone

PHYLUM : COELENTERATA
CLASS : ANTHOZOA
TYPE : *Metridium*

1. Commonly called as Sea Anemone.
2. Body is short, cylindrical and radially symmetrical and divisible into 3 regions: pedal disc, column and oral disc.
3. Pedal disc is muscular, by which it is attached to the substratum.
4. Oral disc is surrounded by tentacles and opens into gastro-vascular cavity.

Identification : The animal is large & brightly coloured flower-like form hence it is identified as Metridium.



Sea Anemone

PRACTICAL NO. 19

CLASSIFICATION OF HELMINTHES AND ANNELIDA

The Helminthes are worm like parasites. They are classified by external shape and host, Platyhelminths (Plathy = flat) are flat worms eg. Planaria, Liverfluke, tapoorms etc. Nemathelminth are round worms, Nemato means thread, ex. Ascaris, Hook worm.

GENERAL CHARACTERS OF PHYLUM - PLATYHELMINTHES

1. Platyhelminthes are bilaterally symmetrical and dorso-ventrally flattened worms.
2. Body shape generally worm-like but vary from moderately from elongated shape to long flat ribbons and leaf-like.
3. Presence of adhesive organs like hooks and suckers.
4. Triploblastic but parenchyne is present between ectoderm and endoderm.
5. Mostly parasites but some are commensals.
6. Exoskeleton and endoskeleton absent.
7. Respiratory and circulatory systems are absent.
8. Sexes are united i.e. hermaphrodite with few exceptions.

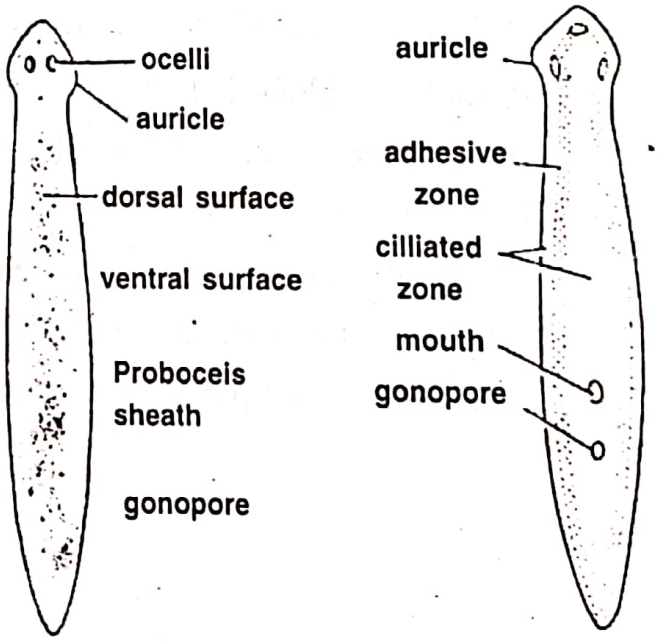
STUDY OF PLATYHELMINTHES

[1] Planaria

PHYLUM : PLATYHELMINTHES
CLASS : TURBELLARIA
TYPE : *Dugesia*

1. Commonly known as **"Planaria".**
2. Epidermis is partly ciliated.
3. Head is triangular containing 2 ear like auricles and 2 semicircular ocelli or eyes.
4. Acoelomate flatworms.
5. Posterior part of the animal is pointed.

Identification : Since the animal has auricles, eyes and dorsoventrally flattened body, it is Dugesia.



DORSAL VIEW **VENTRAL VIEW**

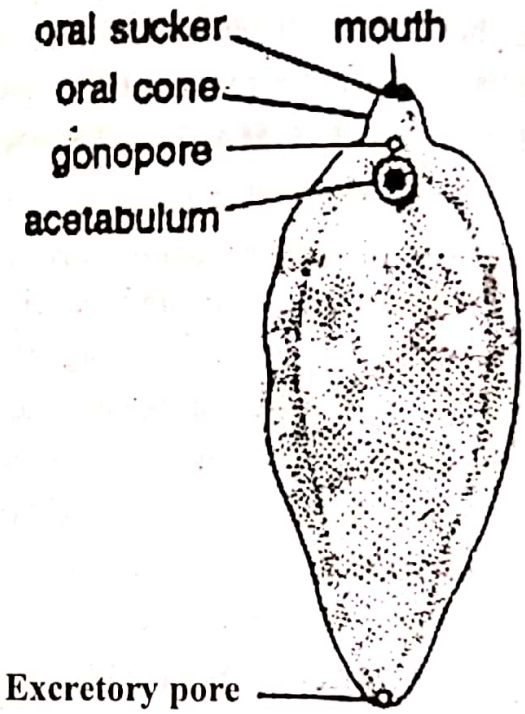
Planaria (Dugesia)

[2] Liver fluke

PHYLUM : PLATYHELMINTHES
CLASS : TREMATODA
TYPE : *Fasciola hepatica*

1. Commonly known as "Liver fluke".
2. Body is leaf-like, dorsoventrally flattened, measuring 18 - 51 mm in length and 4 - 15 mm in breadth.
3. At the anterior end, oral suckers guard mouth.
4. They are endoparasites
5. Ventral sucker or acetabulum is present behind the mouth in between oral sucker and gonopore.
6. It causes anemia, diarrhoea, dysentery and ulcers.
7. Life-cycle is Digenetic.

Identification : As the specimen has leaf-like body and acetabulum, it is identified as Liver fluke.



Liver Fluke

GENERAL CHARACTERS OF PHYLUM - NEMATHELMINTHES

1. Commonly called as Round worms.
2. Cylindrical, elongated, slender worn-like and tapers at bothe end.
3. Body covered with cuticle and unsegmented
4. Bilaterally symmetrical
5. Pseudocoelomate animals Body cavity filled with muscles
6. Sexes separate with sexual dimorphism
7. Free-living, plant-parasitic or animal-parasitic.

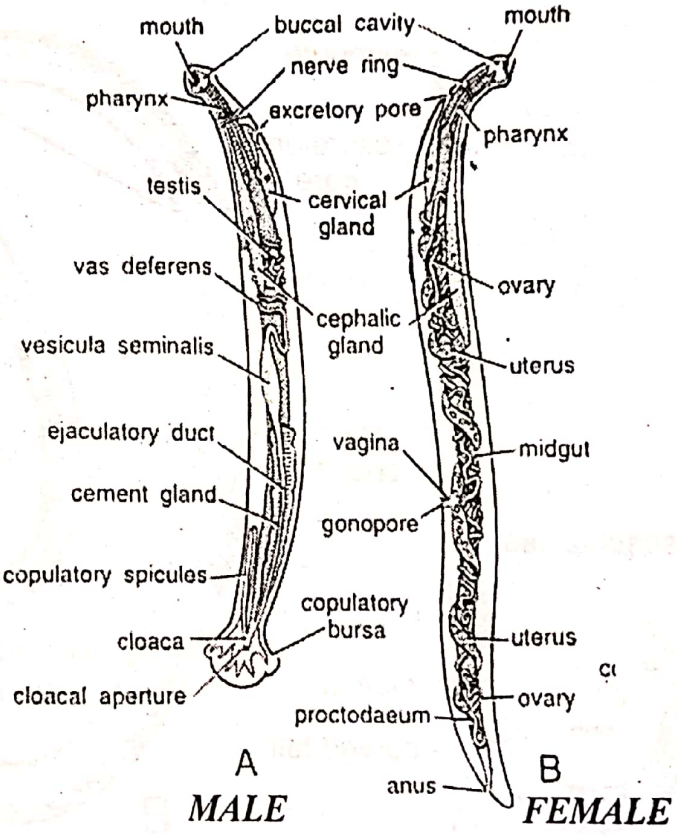
STUDY OF NEMATHELMINTHES

1] Hook worm

PHYLUM : NEMATHEMINTHES

TYPE : *Ancylostoma duodenale*

1. Commonly called as Hookworm.
2. Males are smaller than females.
3. Males measure 8-11 mm in length, while females 10-13 mm.
4. Tail end of female is pointed, while male has copulatory bursa.
5. Anterior end in both sexes is bent dorsally and is provided with large and cup-shaped buccal capsule for attachment with mucous membrane of the intestine.



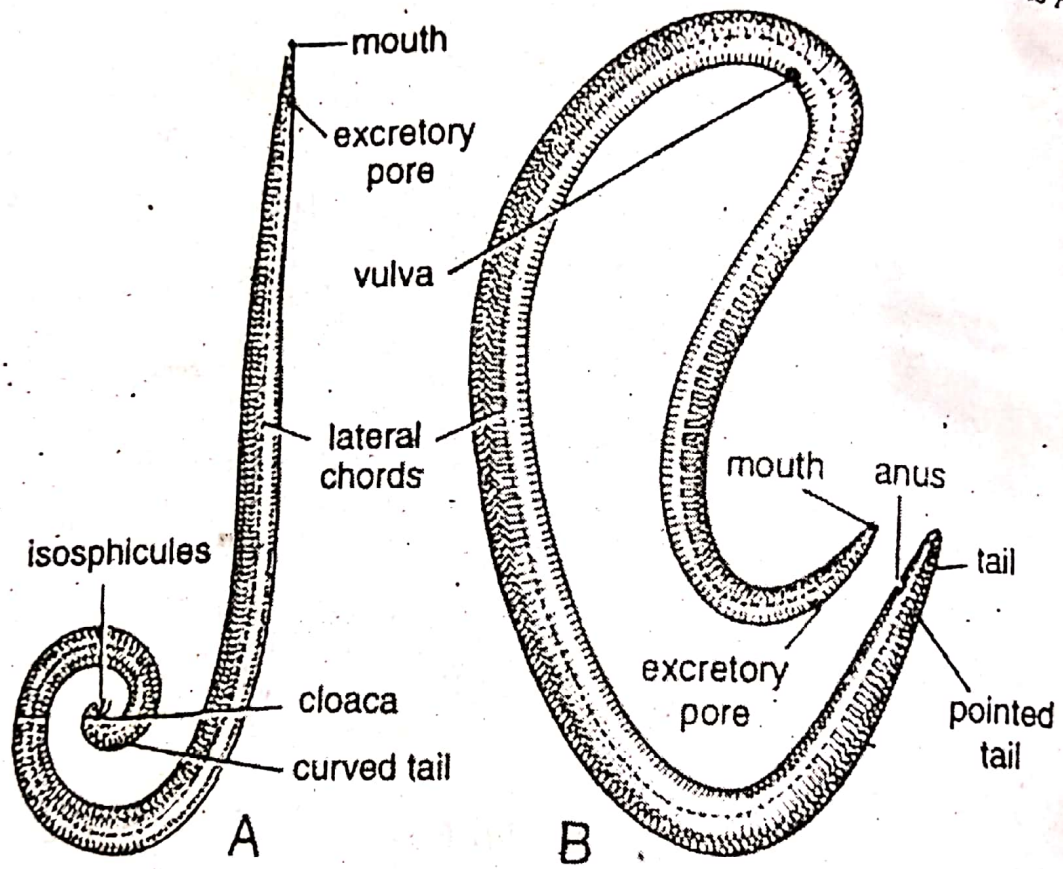
Hook Worm (Ancylostoma)

[2] Ascaris

PHYLUM : NEMANTHELMINTHES
TYPE : *Ascaris lumbricoides*

1. Commonly called as **Roundworm.**
2. Pseudocoelomate and unsegmented nematodes.
3. Causes Ascariasis in man.
4. Tail end of male is ventrally curved containing cloacal aperture and through two equal spicules project.
5. Tail end of female is bluntly pointed.
6. Body is elongated and cylindrical.
7. Excretory pore present at anterior end.

Identification : Body of the animal is unsegmented and cylindrical, so it is Ascaris.



Ascaris. A. Male, B. Female

GENERAL CHARACTERS OF PHYLUM - ANNELIDA

1. Annelida are mostly aquatic, marine or fresh water, burrowing or living in tubes, some free living forms.
2. Body triploblastic, symmentrical and elongated.
3. Body is metamerically segmented, externally by transverse grooves and internally by septa into a division called segment.
4. Presence of true coelom.
5. Locomotory organs are segmentally arranged, paired setae or chaetae.

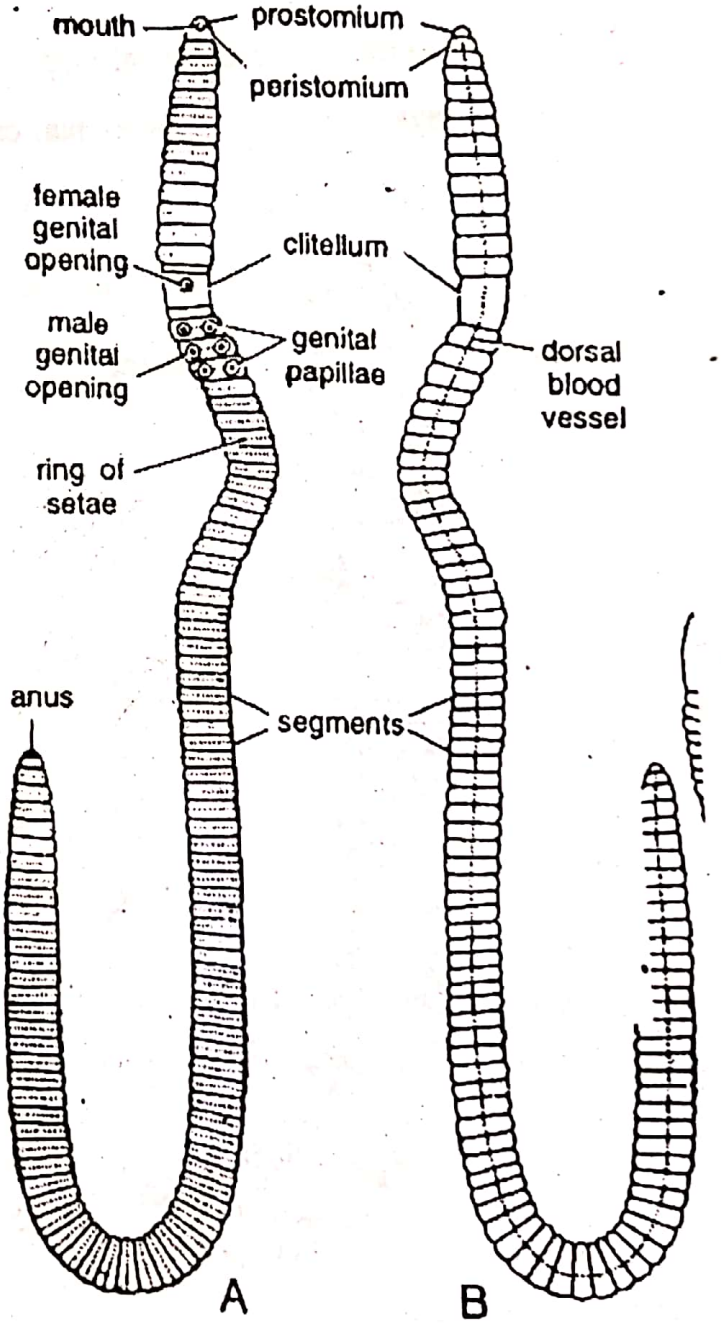
TYPE STUDY OF ANNELIDA

1] Earthworm

PHYLUM : ANNELIDA
CLASS : OLIGOCHAETA
TYPE : *Pheretima posthuma*

1. Commonly Called as "Earthwoarm".
2. Body is cylindrical, elongated and adapted for burrowing life.
3. Setae are present on the body.
4. Body is regenerated and about 100-120 segments are present.
5. Earthworm has clitellum around 14-16 segments.
6. Anterior end is pointed, while posterior end is bluntly pointed.

Identification : Since the specimen contains clitellum in 14-16 segments, it is Pheretima posthuma.



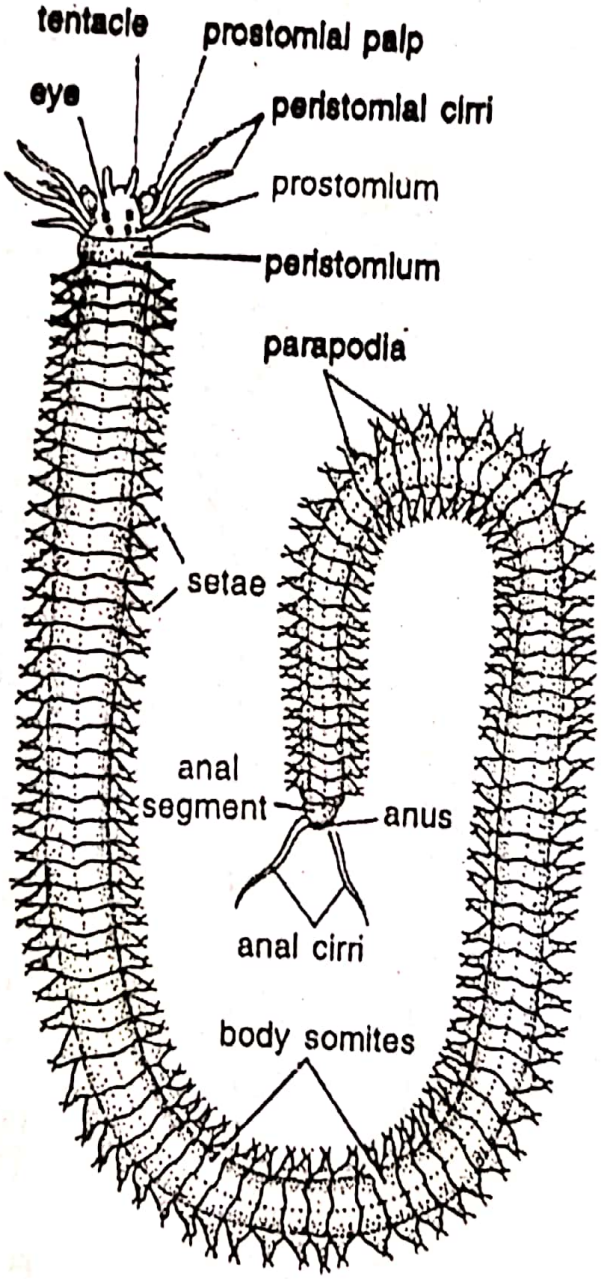
Earthworm (A : Ventral View, B : Dorsal View)

[2] Nereis

PHYLUM : ANNELIDA
CLASS : POLYCHAETA
TYPE : *Nereis virens*

1. Commonly called as "Ragworm or Calmworm."
2. Bodies is divided into metameres or segments and are 200 in number.
3. Parapodia present all over the body.
4. Head is composed of prostomium and peristomial tentacles.
5. Anal segment contains a pair of anal cirri.

Identification : The animal has prostomium, peristomium and parapodium, hence it is Nereis.



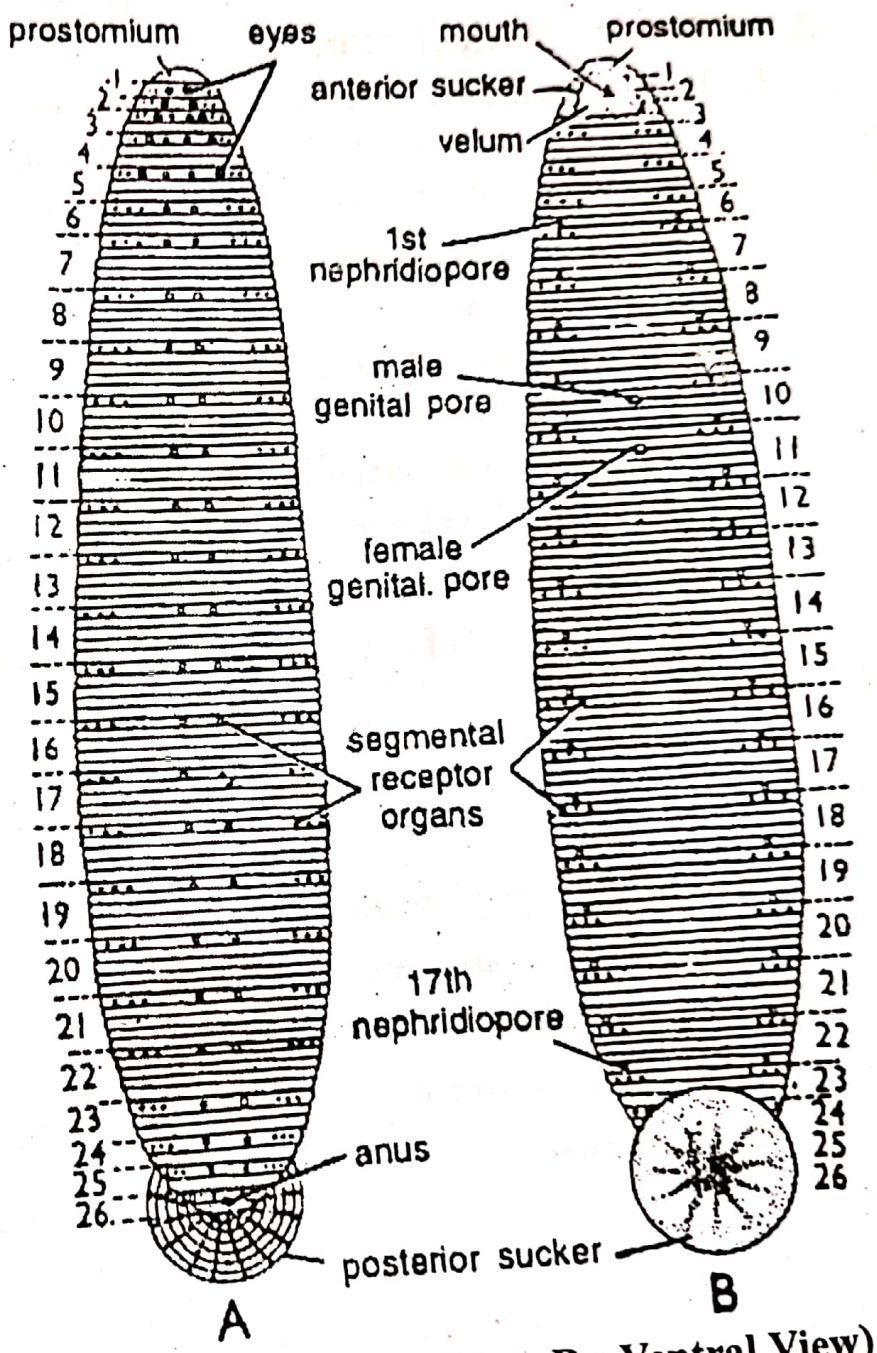
Nereis

[3] Leech

PHYLUM : ANNELIDA
CLASS : HIRUDINEA
TYPE : *Hirudinaria granulosa*

1. Commonly called as "Indian Cattle Leech".
2. Body is soft, elongated dorsoventrally, flattened, measuring 30-35 cm in length.
3. Anterior and posterior suckers are well developed.
4. Five pairs of eyes present.
5. Metamerically segmented and bilaterally symmetrical.
6. Skin is kept moist and slimy due to abundant mucus secretion.
7. Secretes a anticoagulant known as "Hirudin".

Identification : Since the specimen has suckers and 5 pair of eyes it is identified as Leech.



Leech (A : Dorsal View, B : Ventral View)

PRACTICAL NO. 20

CLASSIFICATION OF PHYLUM ARTHROPODA

GENERAL CHARACTERISTICS :

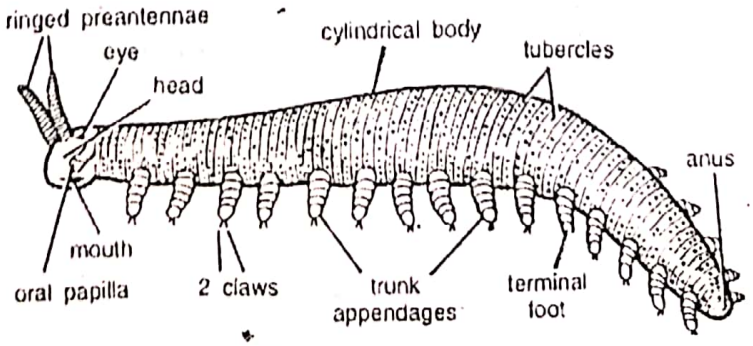
1. Body is bilaterally symmetrical and metamerically segmented.
2. Externally, the body is covered with a thick, tough non-living chitinous and protective cuticle, forming the exoskeleton.
3. Exoskeleton is dead and can't grow.
4. Body is triploblastic.
5. Respiration occurs through general body surface and gills are present in aquatic forms and tracheae or book lungs in terrestrial forms.
6. Excretion occurs by green glands or by malpighian tubules.
7. Compound eyes with mosaic vision have developed.
8. Circulatory System is of open type.

STUDY OF ARTHROPODA

[1] Peripatus

PHYLUM : ARTHROPODA
SUBPHYLUM : ONYCHOPHORA
TYPE : *Peripatus*

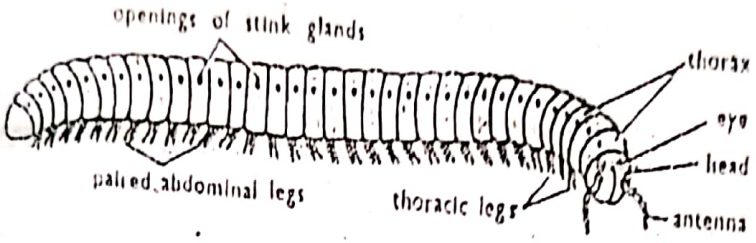
1. Body cylindrically elongated, bilaterally symmetrical and small.
2. Oral papillae and jaws are modified legs.
3. The legs are jointed but their surface bears rings of papillae.
4. A rough cuticle covered with numerous velvety processes.
5. Antennae present.



Peripatus

[2] Millipede

PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : MYRIAPODA
TYPE : *Julus (Millipede)*

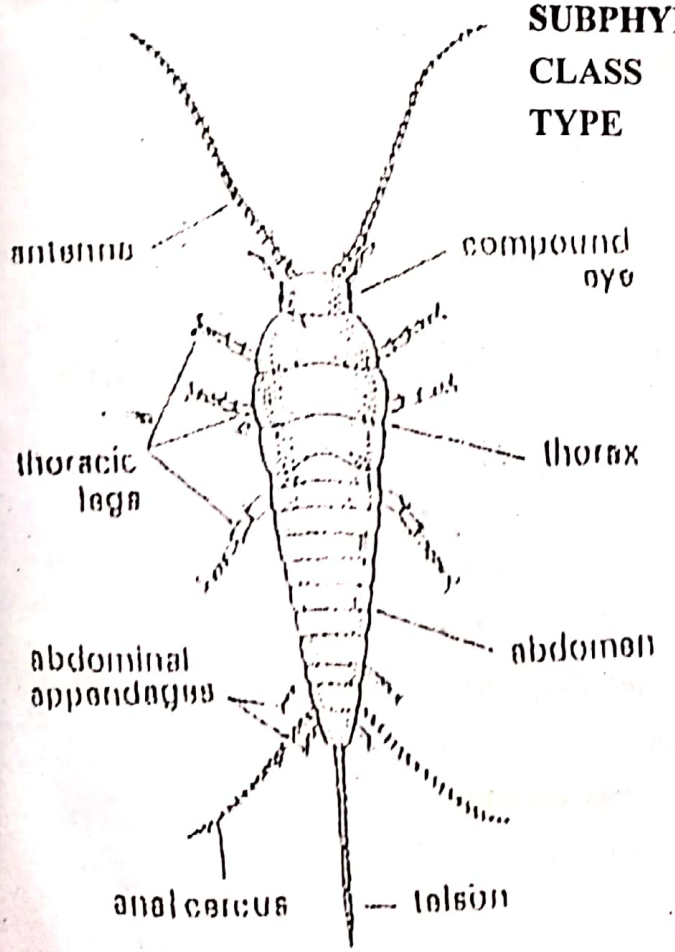


Millipede (Julus)

1. Possess jointed appendages.
2. Head bears a pair of antenna.
3. Body divided into segments.
4. Each body segment bears an opening of the stink gland along the mid dorsal line.
5. Each segment bears two pairs of jointed appendages.

[3] Silverfish

PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : INSECTA
TYPE : *Lepisma (Silver fish)*

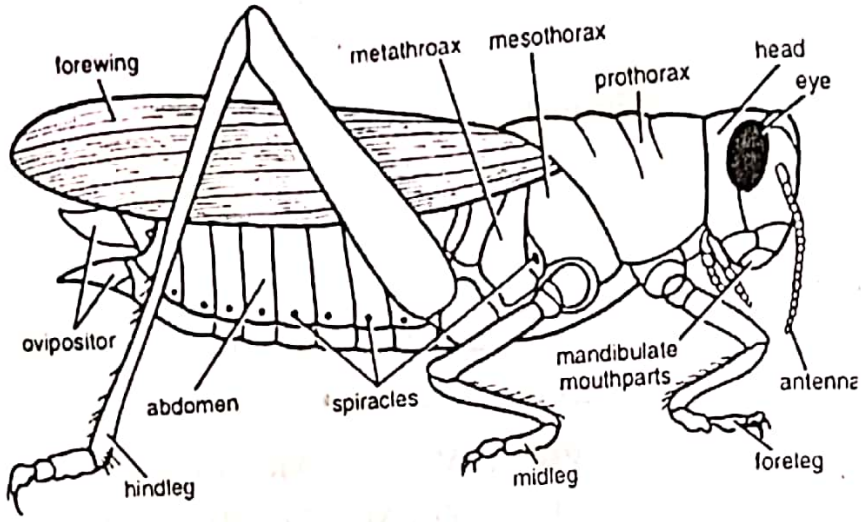


Lepisma (Silverfish)

1. They posses jointed appendages.
2. Body divided into head, thorax and abdomen.
3. Three pairs of jointed appendages.
4. Silver colored.
5. Found in books and clothes.

[4] Grasshopper

PHYLUM : ARTHROPODA
CLASS : INSECTA
TYPE : *Melanopus (Grasshopper)*



Grasshopper

1. They posses jointed appendages.
2. Body divided into head, thorax and abdomen.
3. Green-colored animal.
4. The hind leg is bigger and adapted for jumping.

[5] Cockroach

PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : INSECTA
TYPE : *Periplaneta americana (Cockroach)*

1. Body is elongated, bilaterally symmetrical and dorso-ventrally flattened.
2. Body is metamerically segmented and divided into head, thorax and abdeomen.
3. Body covered by chitinous exoskeleton.
4. Two pairs of wings and three pairs of legs present.
5. Sexes are separate. Exhibit sexual dimorphism.

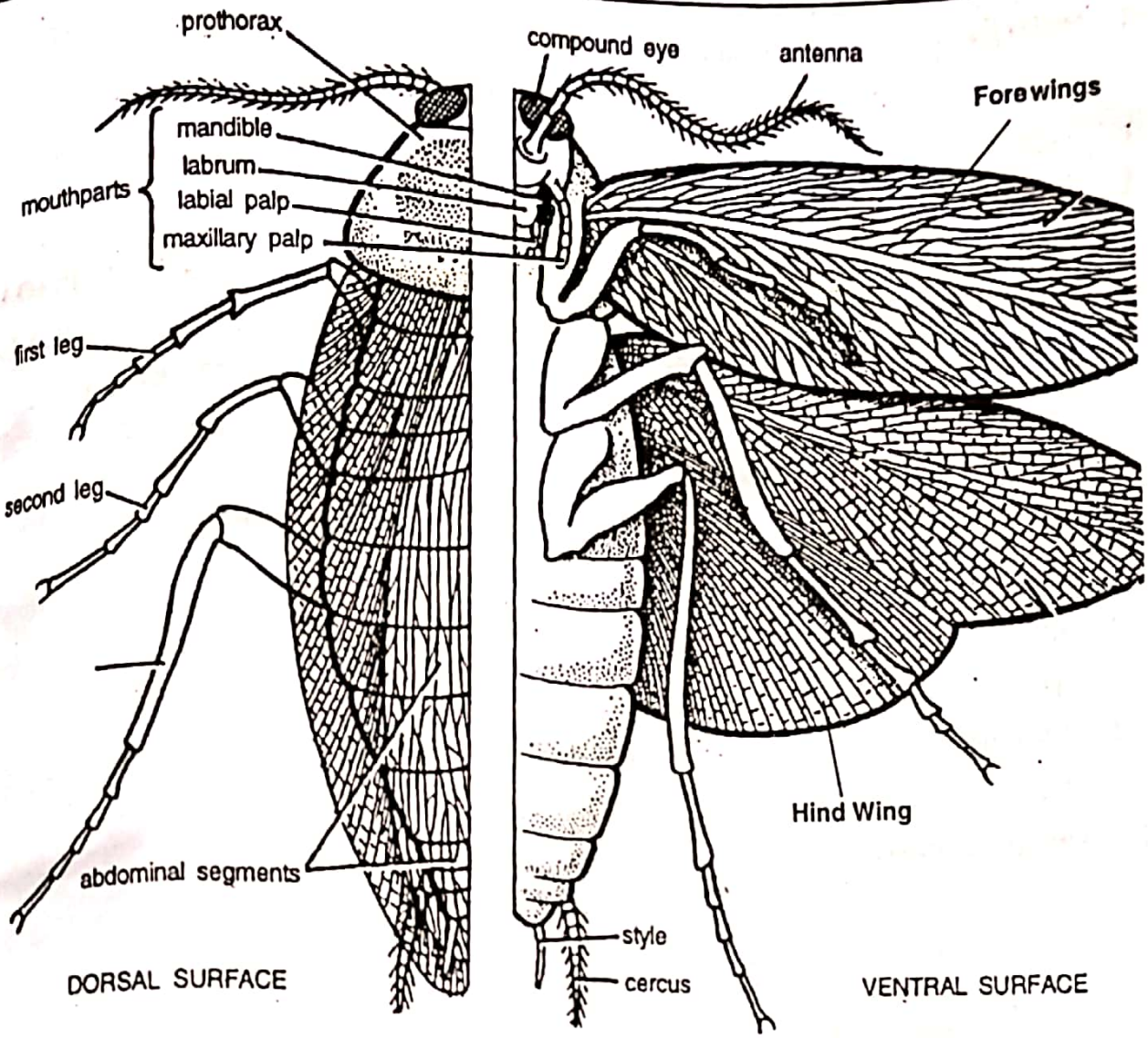
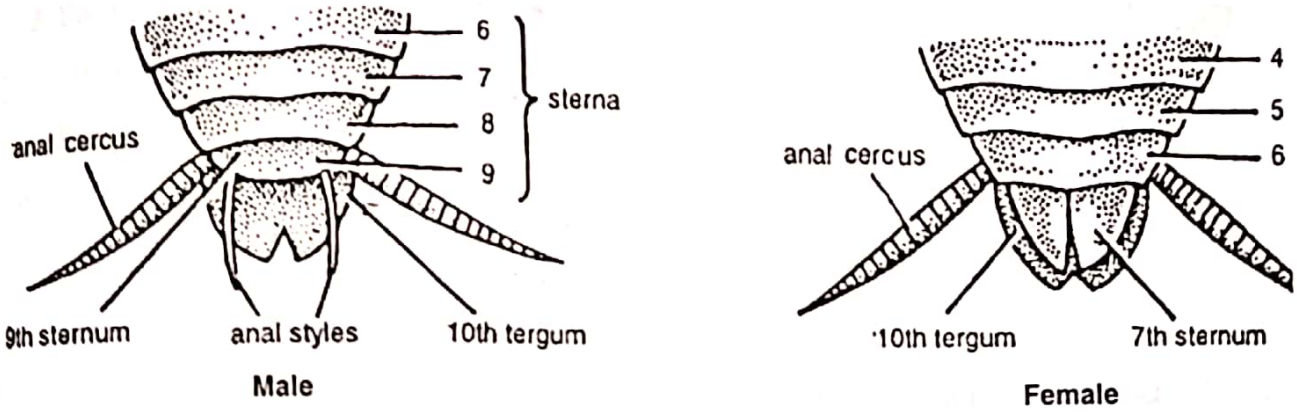
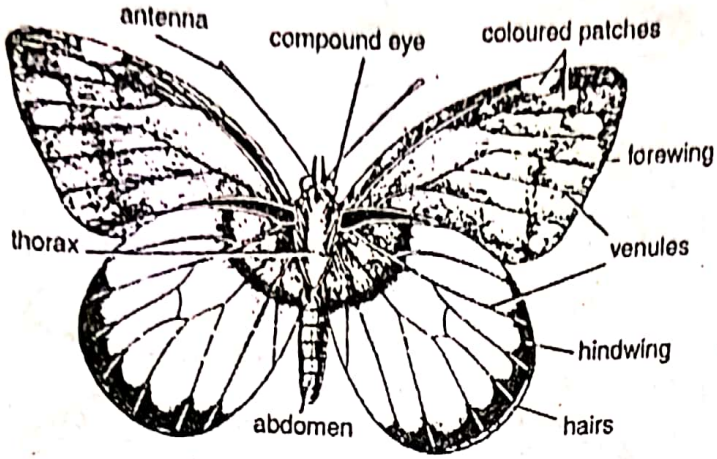


Fig. 26. Cockroach (*Periplaneta americana*). External features of male.



Cockroach (Periplaneta). External Features

[6] Butterfly

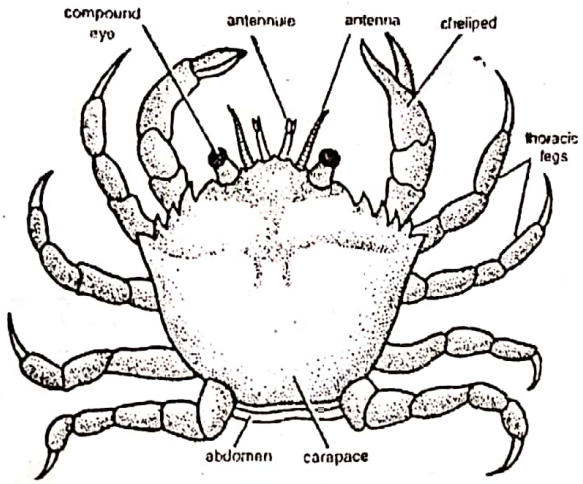


PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : INSECTA
TYPE : *Butterfly*

Butterfly

1. Large beautiful insects.
2. Slender bodies, prominent eyes and long clubate antennae.
3. Their wings are held vertically at rest.
4. Mouthparts form a long sucking tube or proboscis.
5. Development through metamorphosis.

[7] Crab (Carcinus)

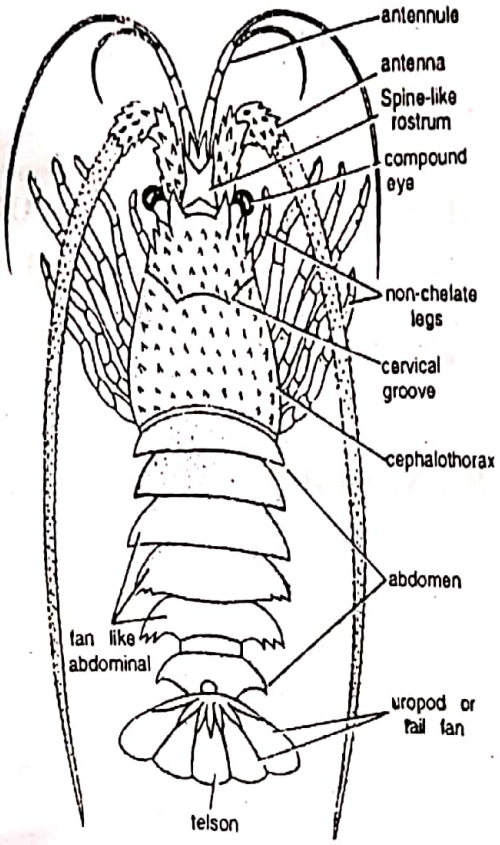


Crab (Carcinus)

PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : CRUSTACEA
TYPE : *Carcinus (Crab)*

1. Essentially aquatic arthropods.
2. Exoskeleton made of thick, protective rigid, chitinous cuticle.
3. Head bears a pair of stalked compound eyes.
4. The cephalothorax is broader, flat and disc-like.
5. The five pairs of thoracic legs are well-developed and clawed.

[8] Lobster



Lobster

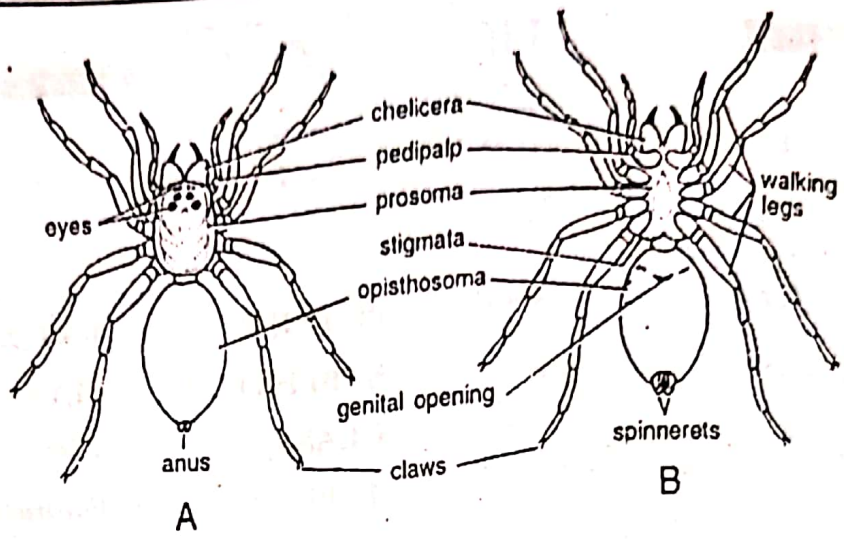
PHYLUM : ARTHROPODA
SUBPHYLUM : MANDIBULATA
CLASS : CRUSTACEA
TYPE : *Palinurus (Lobster)*

1. Triploblastic, metamerically segmented, jointed appendages.
2. Thick exoskeleton, head fused, with the thorax to form cephalothorax.
3. The abdomen consists of six segments and a telson.
4. The cephalothorax is covered with a dorsal shield (carapace) which bears spines of various sizes, some of them arranged in longitudinal rows.
5. Antennules are biramous and antennas are much elongated.

[9] Spider

PHYLUM : ARTHROPODA
SUBPHYLUM : CHELICERATA
CLASS : ARACHNIDA
TYPE : *Aranea (Spider)*

1. Body divided into prosoma and opisthosoma.
2. Opisthosoma unsegmented.
3. The chelicerae are provided with poison glands.
4. Respiration is by means of book-lungs or tracheae or both.
5. Opisthosoma present.



Spider (A : Dorsal View : B : Ventral View)

PRACTICAL NO. 21

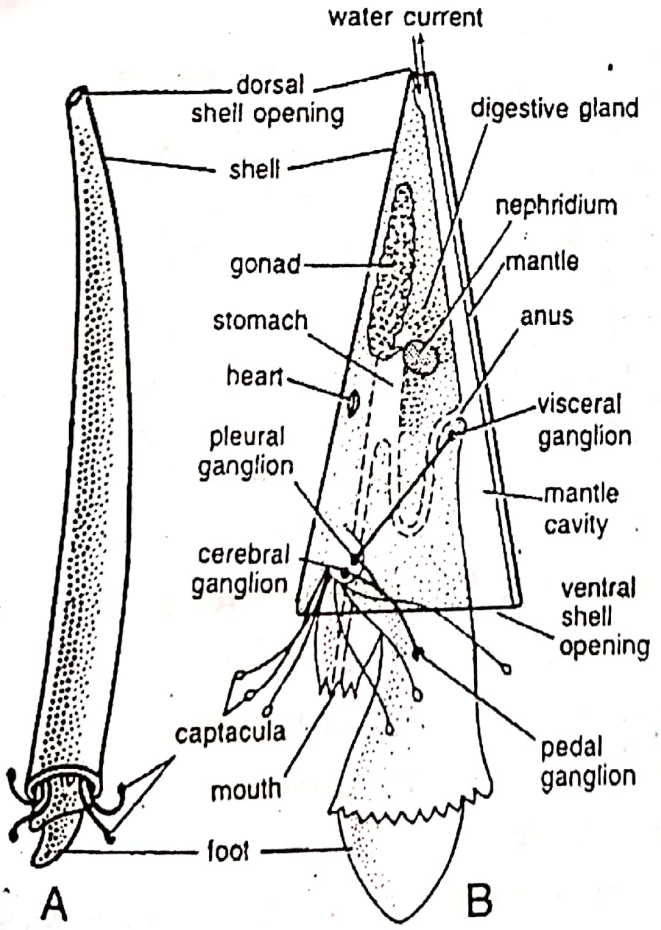
CLASSIFICATION : MOLLUSCA, ECHINODERMATA AND HEMICHORDATA

GENERAL CHARACTERS OF PHYLUM - MOLLUSCA

1. Molluscs are essentially aquatic, marine, few fresh water and some terrestrial forms.
2. Body is soft, unsegmented, bilaterally symmetrical and consists of head, foot, mantle and visceral mass.
3. An exoskeletal calcareous shell secreted by the mantle protects body.
4. Body cavity is haemocoel.
5. Digestive system is simple and circulatory system is open.
6. Sexes are usually separate.

STUDY OF MOLLUSCA

[1] Dentalium



PHYLUM : MOLLUSCA
CLASS : SCAPHOPODA
TYPE : *Dentalium*

Dentalium (A : Shell, B : Animal buried in sand)

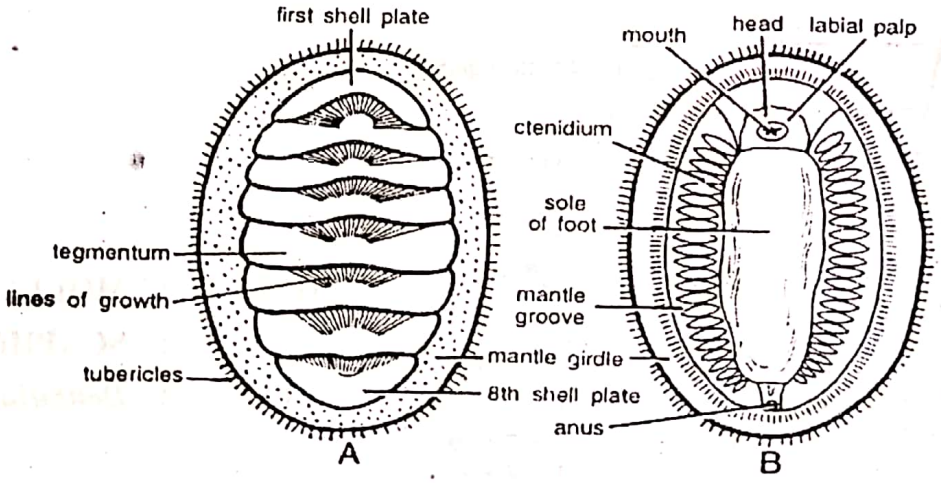
1. Commonly called as "Elephant's Tusk Shell."
2. Head is distinct, shell tubular and opens at both the ends.
3. Mouth is surrounded by leaf like tentacles, called as captacula, having sucker-like tips which are tango receptors and can regenerate.
4. Animal has a vestigial head, which protrudes as proboscis from shell and is without eyes.
5. Used as currency and for ornamental purposes.

Identification : As the animal looks like Elephant Tusk it is identified as Dentalium.

[2] Chiton **PHYLUM : MOLLUSCA**
CLASS : AMPHINEURA
TYPE : *Chiton*

1. Commonly called as Sea Mouse.
2. Head reduced and eyes, tentacles absent.
3. It measures about 1 to 5 cm in length.
4. Mouth and anus are at opposite ends.
5. Dorsal side of mantle has eight calcareous plates.

Identification : As the animal has 8 calcareous plates, it is identified as Chiton.

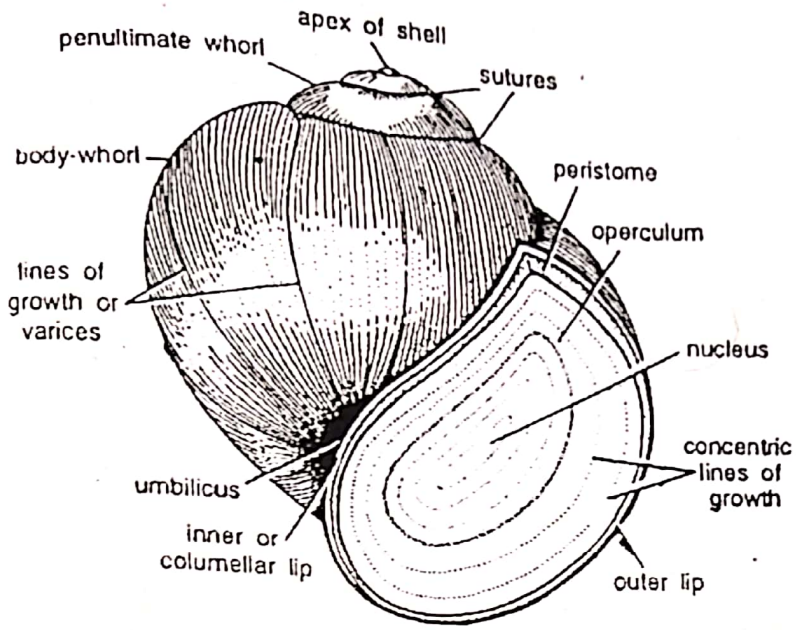


Chiton (A : Dorsal View : B : Ventral View)

[3] Pila **PHYLUM : MOLLUSCA**
CLASS : GASTROPODA
TYPE : *Pila globosa*

1. Commonly called as fresh water "Apple Snail."
2. Body is covered with univalve shell and sutures separate each division.
3. Visceral hump is twisted.

4. Lines of growth are present on the surface of the shell.
5. Coiling is right-handed and is called as dextral.



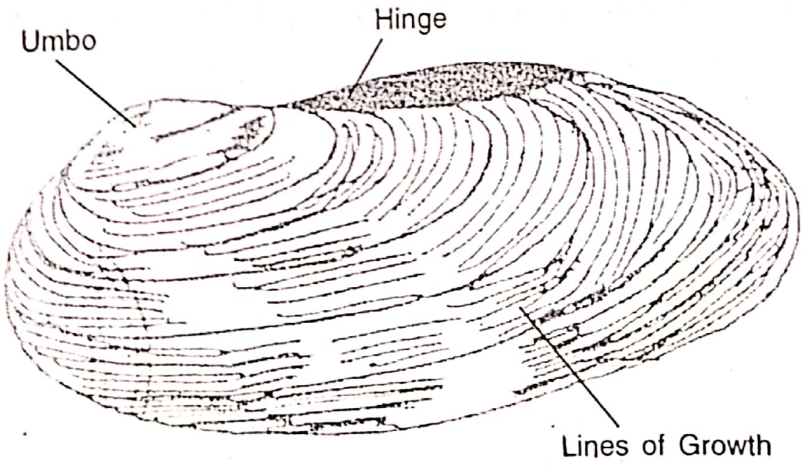
Pila

[4] Unio

PHYLUM : MOLLUSCA
CLASS : PELECYPODA
TYPE : *Unio*

1. Commonly called Freshwater Mussel
2. Bivalve shell.
3. Body is unsegmented, flattened on side and measure about 5-10 cm in length.
4. Two valves are united tighter along the dorsal side.
5. Foot is large and used for burrowing.

Identification : Since the specimen contains umbo and lines of growth on equal valves, hence it is Unio.



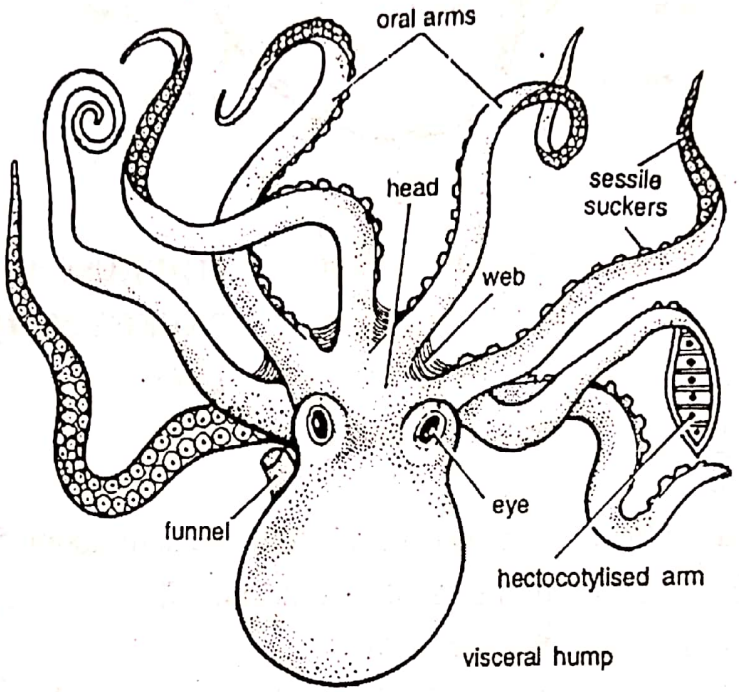
Unio

[5] Octopus

PHYLUM : MOLLUSCA
CLASS : CEPHALOPODA
TYPE : *Octopus*

1. Commonly called as "Devil fish."
2. Head is well developed with eyes and arms and tentacles represent foot.
3. Head has 8 elongated arms and 2 rows of sessile cupped, suctorial suckers towards the inner side.
4. Shell is absent.
5. Rounded body is differentiated into a visceral hump and head.

Identification : Since the body is rounded and the arms 8 contain suckers, hence it is Octopus.



Octopus

❑ GENERAL CHARACTERS OF PHYLUM - ECHINODERMATA

1. Echinoderms are exclusively marine.
2. Symmetry usually radial, nearly always pentamerous.
3. Body has distinct oral and aboral surfaces.
4. Body-shape round to cylindrical or star-like with arms radiating from a central disc.
5. Surface of the body is covered by five symmetrically spaced radiating grooves called ambulacral with five alternating inter-radii.
6. Endoskeleton consists of closely fitted plates forming a shell usually called theca or test.
7. Water Vascular system highly developed.

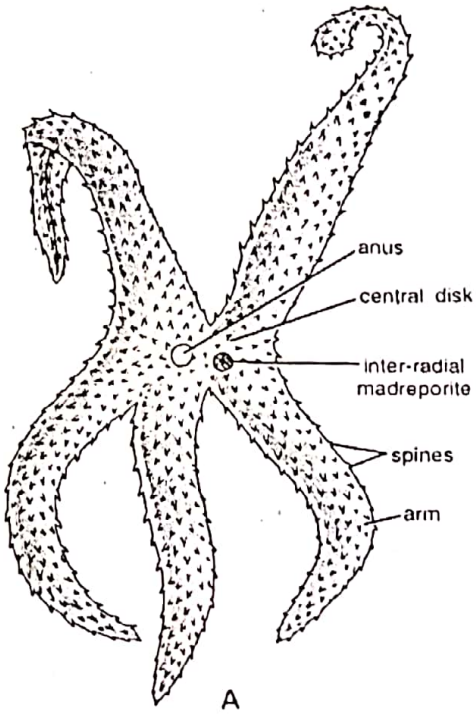
STUDY OF ECHINODERMATA

[1] Asterias

PHYLUM : ECHINODERMATA
CLASS : ASTERODIA
TYPE : *Asterias*

1. Commonly called as "Starfish or Sea Star."
2. Animal has central disc and arms are fused.
3. Five arms are present and contain prolongations of coelom and gonads.
4. Body is star shaped.
5. Skeleton consists of ossicles and plates.

Identification : Since the animal has feet, madreporite and spines all over the body, hence it is Asterias.



A

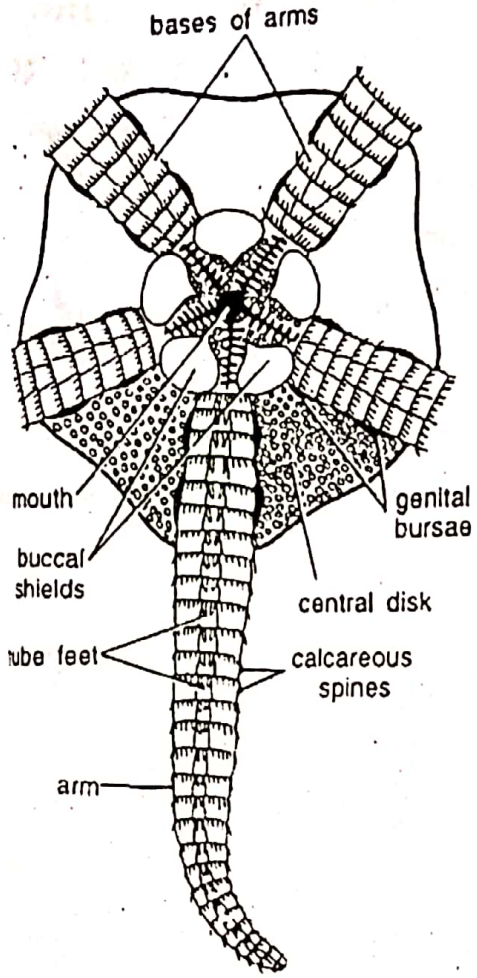
Asteria (Star fish)

[2] Ophioderma

PHYLUM : ECHNODERMATA
CLASS : OPHIUROIDEA
TYPE : *Ophioderma*

1. Commonly called as Brittle Star.
2. Central disc and arms present.
3. Arms are covered with calcareous ossicles and small spines.
4. Skin covers the disc and tube feet lack suckers.
5. Oral surface has star-shaped mouth.

Identification : The specimen contains rounded disc, arms with spine so it is Ophioderma.



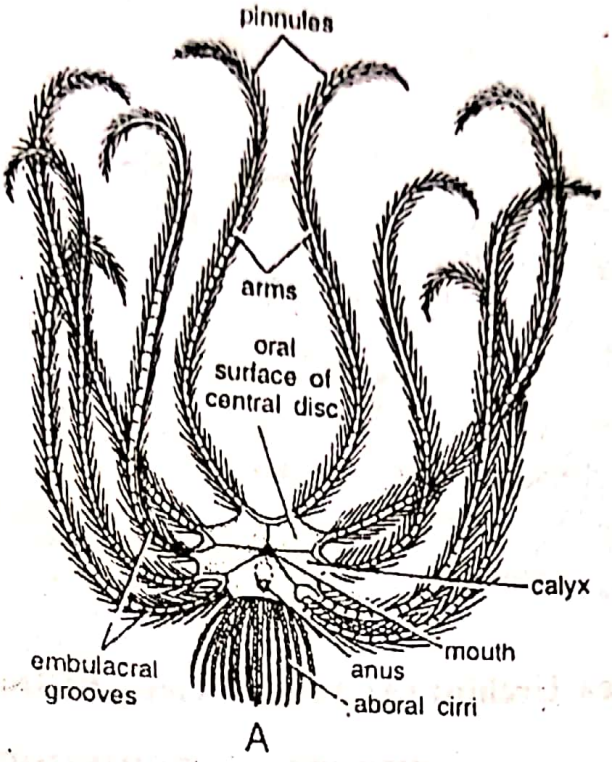
Ophioderma (Brittle Star)

[3] Antedon

PHYLUM : ECHINODERMATA
CLASS : CRINOIDEA
TYPE : *Antedon*

1. Commonly called as "Feather Star."
2. An aboral stalk is attached to the body.
3. A leathery skin in which numerous calcareous plates are embedded covers oral surface.
4. Ten arms are present and have pinnules containing gonads.
5. Anus is on the oral surface.

Identification : Since the animal has aboral cirri, arms with pinnules, hence it is Antedon.



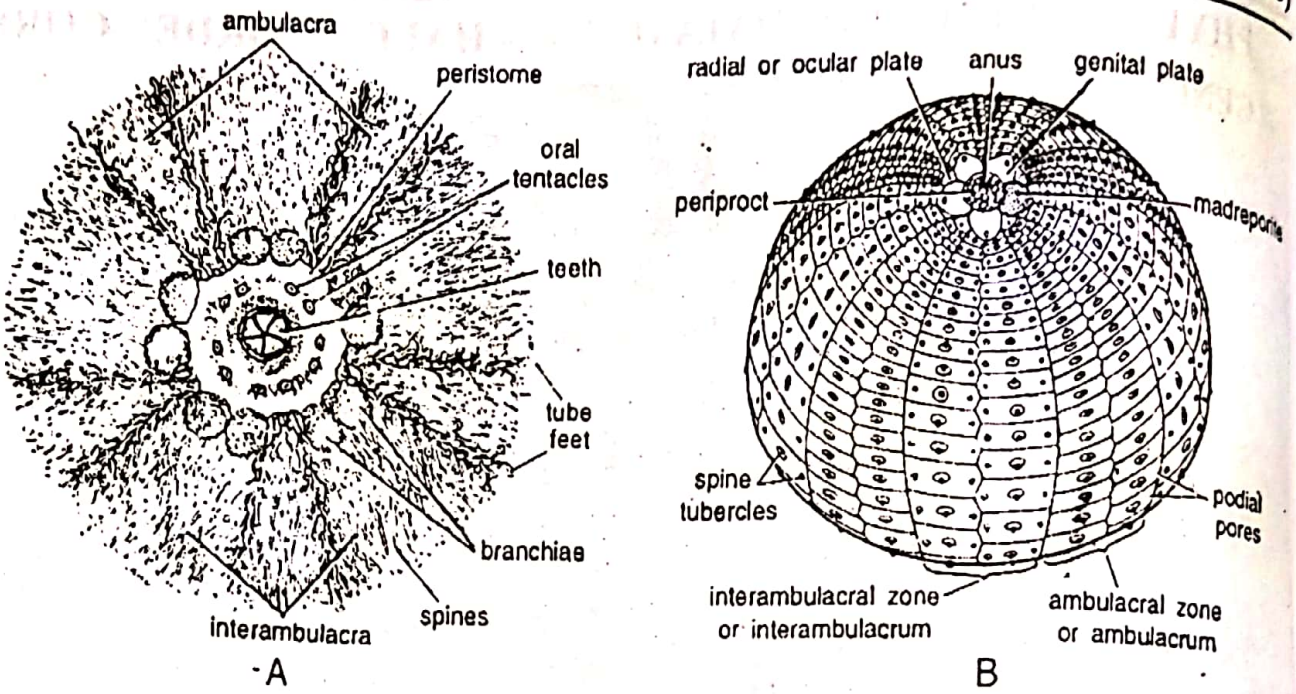
Antedon (Feather Star)

4] Echinus

PHYLUM : ECHINODERMATA
CLASS : ECHINOIDEA
TYPE : *Echinus*

1. Commonly called as ''Sea Urchin.''
2. Body oval or spherical and composed of test.
3. Test is composed of calcareous plates.
4. Madreporite and gonopore are present near anus and aboral in position.
5. Tube feet end in suckers and form five double rows.
6. Test has numerous spines and rows of tube feet.

Identification : As the specimen contains test, spines, madreporite, it is identified as Echinus.



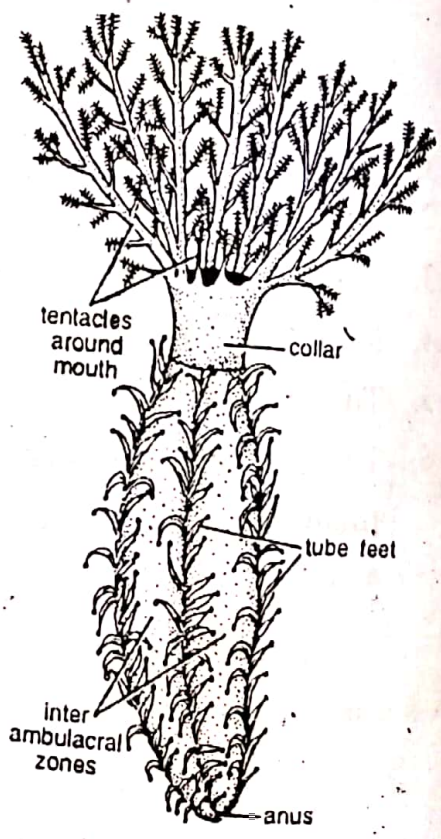
Echinus (Sea Urchin) (A: Ventral View; B: Dorsal View)

[5] Cucumaria

PHYLUM : ECHINODERMATA
CLASS : HOLOTHUROIDEA
TYPE : *Cucumaria*

1. Commonly called as "Sea Cucumber."
2. Body is elongated along oral aboral axis.
3. Tube feet are present and have suckers and are arranged in rows or irregularly scattered.
4. Oral end has 10 dendritic bushy, branched tentacles surrounding the terminal mouth.
5. Anus is present on the aboral side.

Identification : As the specimen bears branched arms, it is Cucumaria.



Cucmaria (Sea Cucumber)

PHYLUM : HEMICHORDATA (HEMI - HALF, CHORDE - CORD)

GENERAL CHARACTERS :

1. Exclusively marine, solitary or colonial, mostly tubiculous.
2. Body soft, fragile, vermiform, unsegmented, bilaterally symmetrical and triploblastic.
3. Body divided into proboscis, collar and trunk.
4. Body-wall of single layered epidermis with mucous glands. No dermis.
5. Digestive tube complete, straight or u-shaped.
6. Circulatory system simple and open gland.
7. Excretion by a single proboscis gland.
8. Nervous system primitive type.
9. Reproduction mainly sexual.

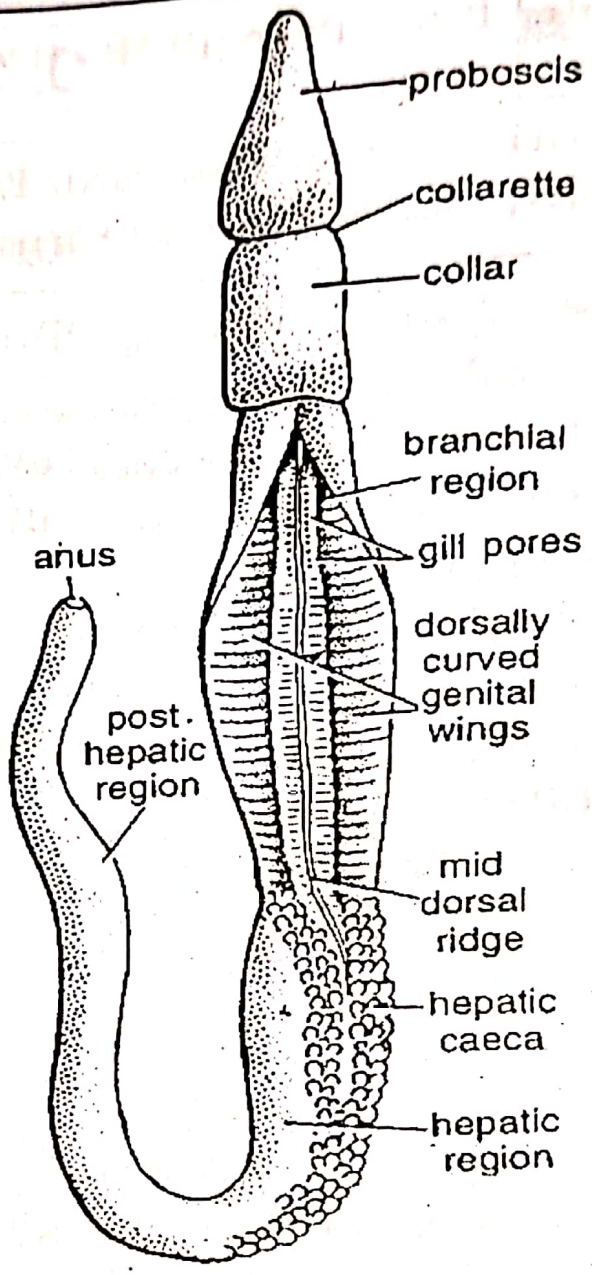
Example : Balanoglosus

(6) CLASSIFICATION :

Phylum : Hemichordata
Class : Enteropneusta
Type : *Balanoglosus*

❖ CHARACTERS :

1. Commonly called as "Acorn Worm."
2. Marine tubicolous and burrowing hemichordate.
3. Body is soft, elongated, worm-like, cylindrical and bilaterally symmetrical.
4. It measures 10-50 cm in length.
5. Color is bright or drab with reddish or orange tints.
6. Body divided into proboscis, collar and trunk.



Balanoglossus

PRACTICAL NO. 22

CLASSIFICATION : LIFE CYCLE AND PATHOGENICITY OF PLASMODIUM VIVAX AND ENTAMOEBA HISTOLYTICA

STUDY OF PLASMODIUM

[A] Plasmodium

PHYLUM	:	**Protozoa**
SUB-PHYLUM	:	**Plasmodroma**
CLASS	:	**Sporozoa**
SUB-CLASS	:	**Telosporidia**
ORDER	:	**Haemosporidia**
GENUS	:	***Plasmodium***

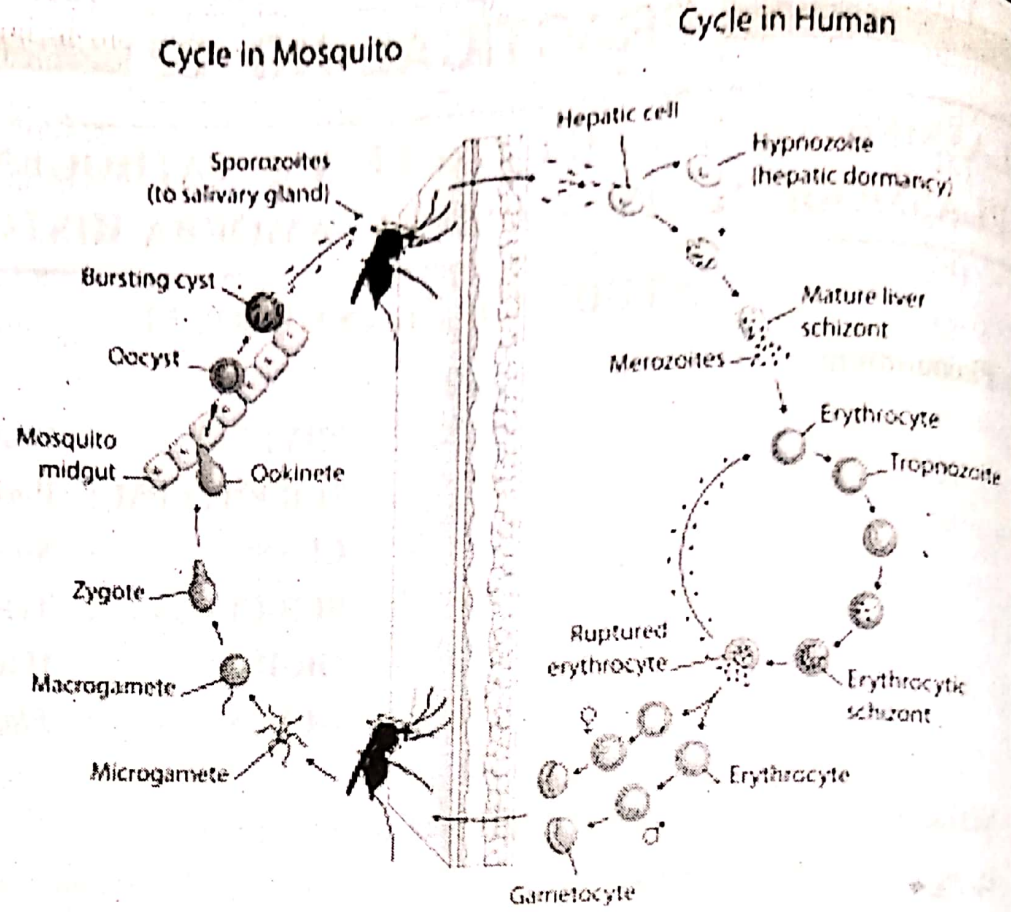
Malaria is one of the serious diseases of human-beings.

It is caused by a species of Plasmodium, transmitted through the bite of female Anopheles mosquito. Man is the principal host while mosquito is the secondary or intermediate host.

Four species of Plasmodium (Fig.) are reported to cause different types of malaria in human-beings, viz., P. vivax, P. ovale, P. malariae and P. falciparum. In human-beings plasmodium attacks the R.B.C. and liver cells and releases a toxic substance 'Haemozoin' which causes malaria. Malaria is identified by periodic attack of high fever.

When a female Anopheles, containing sporozoite stage of Plasmodium in her salivary glands, bites a man, the sporozoites are released into the blood stream of human-beings. After 3 days of enterance these sporozoites start dividing in the parenchyma cells of the liver and attain schizont stage. Now these schizonts attack the R.B.C. and get changed into trophozoites. Now trophozites of each R.B.C. attain schizont stage and divide into 6 to 36 daughter merozoites. Due to the high pressure R.B.C. ruptures and releases the merozoites into the blood plasma. These merozoites attack the other R.B.C. After 10 days the number of parasites becomes large and they produce toxins which cause fever in man.

After schizogony some merozoites attain gametocyte stage and remain in the human blood without any further development. If per chance any female Anopheles bites the person containing gametocytes, the macrogametocyte becomes macrogamete known as female gamete. The microgametocyte forms 6 to 8 microgametes. It is notable that if any stage other than gametocyte is sucked by the mosquito it gets digested in the gut of the mosquito and



Plasmodium life cycle

there is no chance for further infection. After fusion, the zygote is formed in the stomach of mosquito which by penetrating the gut wall reaches outside the gut and gets converted into oocysts. After one week the oocyst is changed into sporozoites which go to the salivary gland of the female mosquito and are transferred to the body of the man by the bite of the mosquito and thus completes its life cycle in man and mosquito.

The parasitised red blood cells accumulate in the sinuses of the spleen, liver and bone marrow. The spleen is typically enlarged, congested, become soft and hemorrhagic in primary stage and further becomes hard in chronic phase. As the quantity of hematin increases, its colour darkens. The liver becomes hypertrophic and congested in acute malaria and its colour index is greater than normal. The bone marrow undergoes similar changes as the spleen but to a lesser degrees. In addition, the production of granulocytes is reduced and erythropoiesis is increased to meet the deficit in functioning red blood cells. The kidneys are congested, the glomerular capillaries become thrombotic with the accumulation of parasitised red blood cells, free hematin and wandering macrophages. The adrenals may be damaged by hemorrhage or affected by general toxemia.

Thus, the release of toxic substances due to the brushing of cells may trigger a response in the temperature regulating mechanism of the body. Anaemia naturally results from the destruction of the blood cells.

Prevention and control : In the prevention of malaria the main objective should be the reduction of the Anopheles below the transmission level and at the same time to save human-beings from mosquito bite. The following measure may be suggested.

(a) Treatment of human infections. Primaquine is very much effective in terminating exo-erythrocytic infection and hence gametocyte production.

(b) Measure against adult mosquitoes. The adequate screening of houses and use of mosquito nets may protect human-beings against, mosquito bite to a greater extent. The use of repellents on exposed parts of the body may protect man for few hours from mosquito bite. The use of pyrethrum and other effective insecticides may kill the adult mosquitoes.

(c) Measures against mosquito breeding. Mosquitoes breed in pools, ponds. irrigation canal, seepage channels, swampy area etc., therefore, it is important to eliminate or keep the minimum aquatic vegetation in which female lays eggs and hatched larvae develop into pupae. Removal of dense vegetation and development of shade because Anopheles requires sun shine. The larvicidal control is universally applicable technique to reduce mosquitoes.)

Treatment : Some of the anti-malarial drugs are :

(a) Cincona products. *Quinine*. Quinine sulfate is administered orally and quinine dihydrochloride is given intravenously to destroy asexual parasites in circulating erythro-cytes.

Totaquine. It is economic and administered orally for the treatment of malaria infected persons.

(b) Acridine compounds. *Quinacrine*. Quinacrine hydrochloride is effective in case of falciparum infection and quartan malaria.

(c) Aminoquinolines. Chloroquine. Chloroquine diphosphate dihydrate is administered orally for the treatment of malaria. It is lethal to asexual stages of Plasmodium in circulating red cells.

Amodiaquin. Dihydrochloride dihydrate is taken orally and is very much effective against asexual stages of Plasmodium in red cells.

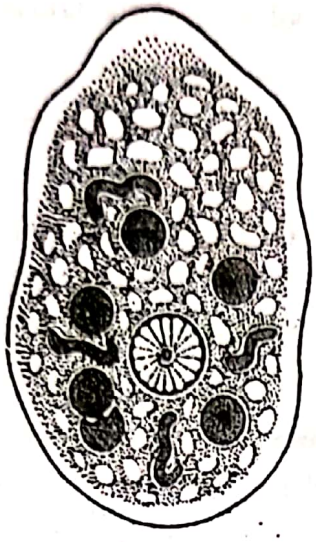
(d) 8-Aminoquinolines. Primaquine sterilises the gametocytes of falciparum malaria.

Pentaquine reduces the relapse rate in vivax infections.

Primäquine is least toxic than others and its action is primarily against fixed tissue stages of vivar infection.

(e) Primidine compounds. *Daraprim* when administered in suppressive doses, it quickens the action of the drug in all the species of malarial parasites.

[B] Entamoeba histolytica :



Entamoeba Histolytica

PHYLUM	:	Protozoa
SUB-PHYLUM	:	Plasmodroma
CLASS	:	Sarcodina
SUB-CLASS	:	Rhizopoda
ORDER	:	Amoebina
GENUS	:	*Entamoeba*
SPECIES	:	*Histolytica*

Entamoeba histolytica is cosmopolitan in distribution, commonly found in epidemic form in tropical and sub-tropical regions. The more epidemic condition of this protozoa is reported from India, China, Philippines, South America, Thailand and Mexico. In India its affect is on higher level in humid climate as compared to dry and cold climates. Allahabad, Agra, Calcutta, Mumbai, Chennai, Delhi, Kanpur and Gorakhpur are comparatively more infested than other places. About Fig. Entamoeba histolytica 40% patients of mental hospital in Georgia are infected by this parasite.

It is unicellular (Fig.) trophozoite of about 25μ which inhabits the lower portion of small and large intestine of man. The protoplasm is not completely. differentiated into ecto-and endoplasm and it contains food vacuoles. The spherical nucleus contains peripheral granules. E. histolytica when observed is found to be very much active moving by single anteriorly directed pseudopodium but after some time, when kept outside the host body, becomes sluggish.

The faecal contamination of drinking water and raw vegetables, or contamination by infected and careless food handling are the mode of transmission of this parasite.

No intermediate host is involved in the life cycle of this parasite. The transmission of parasite from man to man takes place through tetranucleate cysts. Prior to cyst formation the trophozoites become immobile, smaller, discharge the food particles, round off and form the cyst wall. The nucleus is divided into two, then four and ultimately it is called as tetranucleate cyst. These cysts when come out with faecal matter, contaminate the food and water. When man drinks water or eats food, containing cysts, the parasite again reaches the host. The house-flies help in rapid spread of this parasite. The pathology of this parasite has been studied in detail. As man takes food or water contaminated: with tetranucleate cysts, the wall of ingested cyst dissolves in the intestine of man and amoebae come out in the lumen of the intestine. The first site for the colonization of amoebae in the intestine

is in the caecal area but if it does not occur the primary colonization occurs in the lower level of the large intestine. As amoebae enter the intestinal wall, the lesion is formed due to lytic necrosis of the mucosa. In due course of time, the number of amoebae in the colony increases and they proceed in a narrow channel, down to the base of the mucosa, and confined to the epithelial layer. Now amoebae gradually forn a passage through the muscular mucosa into the submucosa where they may spread easily and produce primary lesion. In the primary lesion there is no complication and the tissue reaction does not occur in this stage. From the submucosa, the amoebae may proceed into the muscular coats and make a passage into the serosa and ultimately cause perforation there. Further, they may be carried into the extra intestinal tissue like liver, lungs, brain and skin. Outside the intestinal tract any amoebic lesion, if develops, is without any exception secondary to one or more lesions in the large intestine. The trophozoites of primary bottle-neck lesions are squeezed out with faecal streams due to the peristaltic movements but few trophozoites come in contact with mucosa, produce necrotic ulceration at sigmoidorectal area. Sometimes these trophozoites may be regurgitated into the distal region of the ileum and form colony, due to which majority of the lesions are formed. The primary lesions in large intestine may be maintained for sometime which later on is enlarged. In case where these lesions become cronic, it is invaded by bacteria resulting in microscopic appearance of ulcers. These ulcers rupture, resulting in the discharge of blood and mucus into the lumen of the intestine which passes out with the stool. In the liver, there is a tendency for these lesions to increase in number of which one or very few may become larger and form the so called 'amoebic liver abscess'.

Major diseases caused by E. histolytica are Intestinal amoebiasis, Amoebic hepatitis, Amoebiasis of viscera and Amoebiasis cutis.

Prevention and control :

(1) Amoebiasis has to be accepted as a major health problem, only then serious efforts would be made by concerned persons for its successful prevention. Its infection in individual patient and also on community basis must be detected and noted for official records of the local and national health departments. If it is in cronic stage, a careful survey must be made to know whether water, food material or possibly which source is responsible for the spread of the disease in an epidemic form and then only seriousness would be realized to make practical efforts to remove the causative factors.

(2) Quick and proper disposal of human faeces should be made.

(3) The green vegetable should be washed properly before cooking to ensure that vegetable is free form cysts.

(4) The irrigation of green vegetable with probable contaminated water should be checked.

(5) Chiniofon is an anti-amoebic compound which is administered orally and may be taken in aqueous solution for more effective action. Its proprietary name is yatren.

(6) Diodoquin is used in killing the amoebic trophozoites in the lumen and wall of the intestine and is given orally. Its proprietary name is diodoquin.

(7) Iodochlorhydroxyquine is similar to chiniofon in action and is given orally. The proprietary name of this compound is vioform.

(8) Carbarsone is proved to be the most satisfactory drug in case of intestinal amoebic infection and equally effective in extra intestinal amoebiasis. It is administered orally.

(9) Bismuth glycolylarsanilate is very effective for treating intestinal cases but ineffective in hepatic amoebiasis. It is taken orally and its proprietary name is milibis.

(10) Chloroquine phosphate is the most effective in hepatic amoebiasis but the least effective in intestinal amoebiasis.

PRACTICAL NO. 23

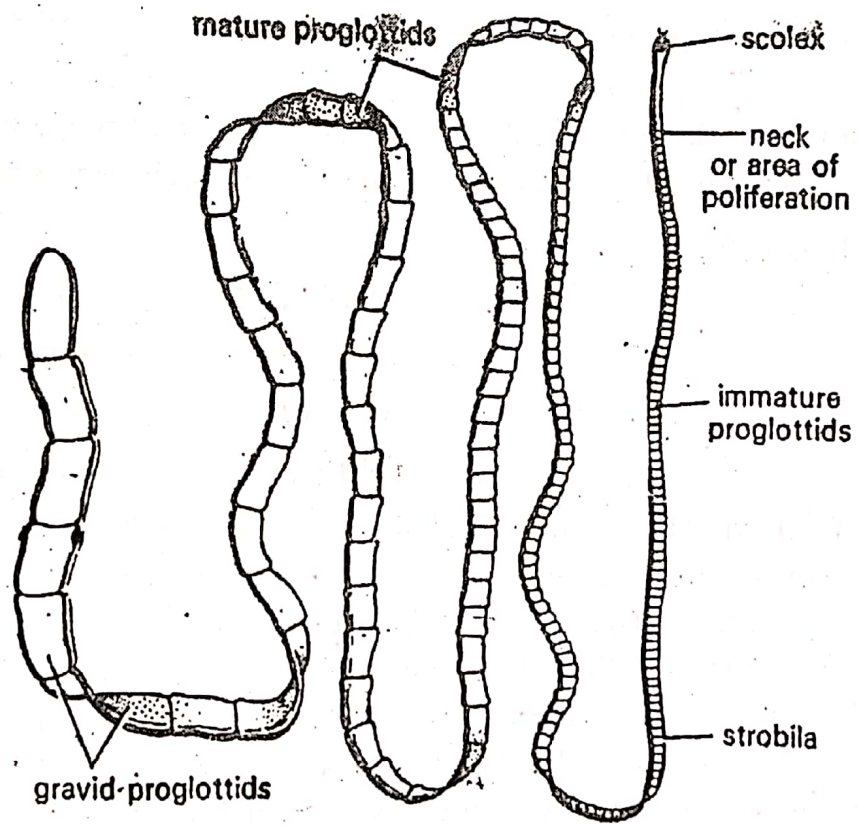
CLASSIFICATION : LIFE CYCLE AND PATHOGENICITY OF TAENIA SOLIUM AND WUCHERERIA BANCROFTI

❖ **Aim : To Study Life Cycle and Pathogenicity of Taenia Solium and Wuchereria Bancrofti**

STUDY OF TAENIA SOLIUM

[A] Taenia Solium

PHYLUM	:	**Platyhelminthes**
CLASS	:	**Cestoda**
ORDER	:	**Taenoidea**
GENUS	:	***Taenia***
SPECIES	:	***Solium***



Taenia Solium

It is commonly called as the **'Pork Tapeworm'** and found in the small intestine of man. It is cosmopolitan in distribution. Countries where pork is consumed much as food, T. *solium* is found in an endemic form (Fig.). Its infection is found in abundance in China,

Germany, India and Yugoslavia. T. solium is opaque white in colour. Its body is elongated, flattened and ribbon-like and differentiated into scolex, neck and strobila.The scolex bears 4 suckers and two rows of retractile hooks. Each proglottid is hermaphrodite. The alimentary canal is absent. The life cycle is complicated and involves a primary host (man) and intermediate host (pig.). With the help of hooks and suckers the worm keeps itself attached with the mucosa of alimentary canal and absorbs the digested food of host's alimentary canal.

Life history. Self-fertilization takes place within the same proglottid and the ripe proglottids containing fertilized eggs get detached from the body of the tapeworm and pass out of the host's body in form of chains with the faeces. These fertilized eggs, if taken by the pigs alongwith the faeces, reach the intestine of.pigs where they are hatched into hooked embryos. They then penetrate the intestinal wall and reach the muscles of the pig through the blood streams. In the muscles they grow and form cysticercus larvae which are still covered with the bladder. If uncooked or undercooked pork is taken by man the cysticercus larvae reach the alimentary canal and develop there. The hooks and suckers on the scolex help them in attaching on the intestine of the man to attain adult stage.

Disease. The infestation caused due to this worm is followed by abdominal pain, nausea, anaemia, indigestion, diarrhoea, eosinophilia etc. The complicated disorders are caused due to the toxins produced by T. solium. Generally one pork tapeworm parasitises a single host at a time.

Prevention and control. The prevention needs the ingestion of properly cooked pork. The taeniasis can be controlled by the use of quinacrine hydrochloride and atabrin.

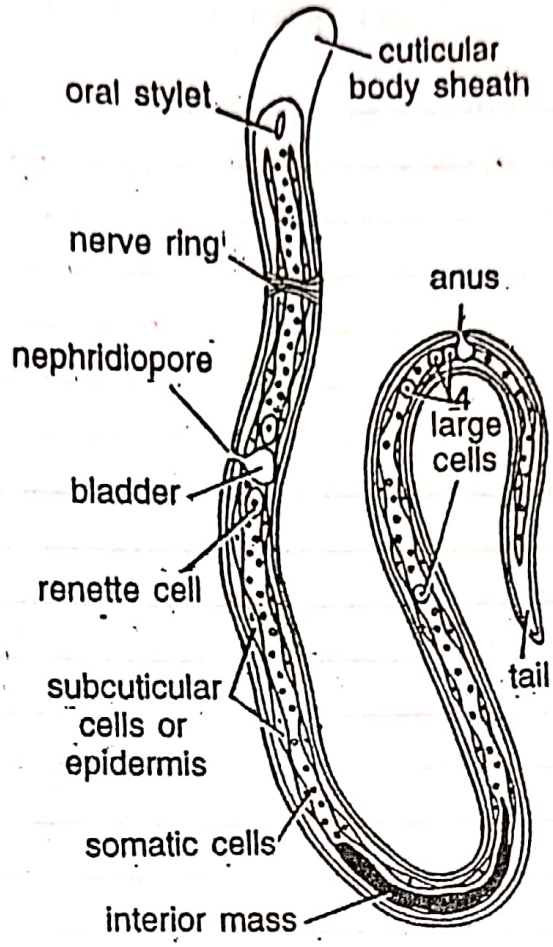
[B] Wuchereria bancrofti

PHYLUM	:	**Nemathelminthes**
ORDER	:	**Filarioidea**
GENUS	:	***Wuchereria***
SPECIES	:	***bancrofti***

It is commonly known as **'Filaria worm'** found in the lymphatic vessels, muscular tissues and glands of man. The worm is distributed in all tropical, sub-tropical and temperate countries and causes filariasis. The female is about 8 cm in length. The body is creamy-white, cylindrical and filiform with blunt ending on the anterior and posterior sides. The posterior end of the male is curved. The mouth is of very simple type without any lip. The intermediate host is a mosquito (Culex fatigans) The adult worms, living in the lymph vessels, copulate when the individuals of both the sexes are present (Fig.).

Life history. The female after fertilization does not lay eggs directly but eggs are retained in the female. The female gives birth to a number of larvae

(microfilariae) which are surrounded by a delicate membranous sheath. These microfilariae live in the lymph vessels or migrate into the blood capillaries underneath the skin.



Wuchereria bancrofti

Microfilariae appear in the skin capillaries during night between 10 P.M. to 2 A.M. and during day time they live in large deep seated blood vessels. If the secondary host (mosquito) bites the man, containing microfilariae, in between 10 P.M. to 2 A.M. the larvae enter into the mosquito's stomach from where they migrate to the thoracic muscles where they grow and attain length of about 1.5 mm in a few weeks. Now, the larvae migrate to the proboscis of intermediate host. In this stage if the mosquito bites a man the larvae are transferred to the blood stream of man from where they go to the lymph glands and lymph passages, and grow into mature stage.

Disease. In the severe infection due to repeated inoculation the adults cause blocking of lymphatic system which tesults in the enlargement of legs, arms, scrotum and mammary glands which is known as elephantiasis. It causes filarial fever and headache.

Prevention and control. The most effective preventive means is to avoid the mosquito bite. Mosquito breeding should also be controlled by all means. - No proper drug has been invented to eradicate the filaria worms. A drug called heterazon and cyanine is effective to a certain extent.