

BBYEL-142
CELL AND MOLECULAR
BIOLOGY: LABORATORY

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CELL AND MOLECULAR BIOLOGY : LABORATORY

The laboratory course BBYEL-142 is the elective course in Life Sciences. In this 2 credit course you will be introduced to a set of simple experiments that you will perform on your own. The experiments are designed in such a way that will improve your observation, analysis and interpretation skills.

Modern cell biology combines three important facets: classical cytology devoted to the study of visualization and description of cellular structure both at the light and the ultrastructural level, biochemistry which highlights the development of techniques for studying the molecules and cellular components in order to understand the cellular function and genetics which encompasses the knowledge of regulation of cellular functioning at the gene level.

Experiments in this course are oriented towards the study of cell structure, cell organelles, ultrastructural profiles of organelles, cell division along with a study of special chromosomes and selected human karyotypes representing various genetic makeups.

We begin with the study of electron micrographs of prokaryotic and eukaryotic cells in order to identify their characteristic features. In addition, structure of plant virus (TMV), animal virus (Adenovirus), and bacteriophage (T4) will also be studied with the help of photographs. This is followed by a description of organelles present in eukaryotes. The ultrastructure of these organelles will be studied with emphasis on the role played by them in overall functioning of the cell. Exercise 3 on micrometry has been designed to enable you to understand the usage of ocular and stage micrometer, in order to measure the dimensions of microscopic objects like epidermal cells of leaves, or the length of a *Spirogyra* filament.

Exercises 4 to 6 are devoted to a study of plant and animal cells by preparing temporary mounts. The structure of nerve cells, striated muscles, epithelial and epidermal cells have been explained with the help of photographs. You will perform vital staining of mitochondria with Janus Green in the cheek epithelium and plant epidermal cells in Exercise 7. Exercises 8 and 9 are exclusively based on the study of cell division where you will learn the procedure for squash preparation to study mitosis and meiosis. Photographs of various stages of cell division supplement these exercises.

You will perform experiment 10 to study the effect of organic solvents like alcohol and benzene on the permeability of cell membrane using beet root cylinders. The experiments 11 and 12 will be based on plasmolysis and dialysis. These experiments will certainly enhance your abilities of handling the biological material and laboratory equipment.

Exercises 13-15 are related to Genetics and Molecular Biology in Exercise 13; you will study the structure of the nuclear pore complex with the help of electron micrographs and 3-D diagrams. Exercise 14 presents detailed information on polytene and lamp brush chromosomes. The Exercise 15 on human karyotypes includes some mind stimulating information on karyotypes and ideograms. You will be preparing normal and abnormal karyotypes by cutting the images of chromosomes from photographs provided to you.



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EXPERIMENT 1

TO STUDY VIRUSES, PROKARYOTIC AND EUKARYOTIC CELLS BY ELECTRON MICROGRAPHS

Structure

- | | | | |
|-----|--------------------|-----|---|
| 1.1 | Introduction | 1.5 | Observations |
| | Objectives | | Prokaryotic cell (Bacteria) |
| 1.2 | Study Guide | | Eukaryotic cell (Plant and Animal Cell) |
| 1.3 | Materials Required | | Viruses |
| 1.4 | Procedure | 1.6 | Terminal Questions |
| | | 1.7 | Answers |

1.1 INTRODUCTION

The diverse forms of life exhibit hierarchy in biological organization. These range from simple unicellular organisms among the prokaryotes to more complex multicellular eukaryotes, including plants and animals. Viruses occupy a unique position as they are acellular, obligate parasitic entities existing at the threshold of life. Each of these shows a characteristic structural organization. The cell is a fundamental unit of life. You have studied about structure of cells-in the Unit 4 of paper BBYET-141 Cell and Molecular Biology.

The structure of the cell can be studied with the help of electron micrographs. An electron micrograph is a magnified image of the specimen taken through an electron microscope. In the present exercise you will be studying electron micrographs of various cells. This will make you familiar with characteristic features of various cells including prokaryotic and eukaryotic cells (both plant and animal), viruses and bacteriophages. This will help you to understand their structural organization at the sub-cellular level.

Objectives

After going through these electron micrographs you would be able to:

- ❖ differentiate between various types of cells;
- ❖ distinguish between the structural organization of cells; and
- ❖ explain the unique features of each cell type with reference to its size, shape, nature of the cell wall, cellular organelles.

1.2 STUDY GUIDE

For doing satisfactory work you must study the Unit 4 of Paper BBYET-141 Cell and Molecular Biology.

1.3 MATERIALS REQUIRED

You will be provided with electron micrographs of the following cells:

- i) Prokaryotic cell
- ii) Plant cell
- iii) Animal cell
- iv) Plant virus (TMV)
- v) Animal virus (Adenovirus)
- vi) Bacteriophage (T4)

1.4 PROCEDURE

Carefully observe the electron micrographs provided to you. Note down the structural details of each cell type. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) with the help of a sharp pencil in your notebook and write comments. It is advised that figures should be drawn after observing the actual electron micrographs and not copied from the book.

1.5 OBSERVATIONS

You have been provided with electron micrographs of different cell types such as prokaryotes, eukaryotes, Plant cell, Animal cell, Plant virus (TMV), Animal virus (Adenovirus) and Bacteriophage (T4). Observe each one of them carefully and note down the characteristic features of each one of them.

1.5.1 Prokaryotic Cell (Bacteria)

You will observe the following features in a bacterial cell (Figs 1.1 and 1.2):

Capsule: A thick polysaccharide slimy layer which protects the cell from desiccation and phagocytosis;

Cell wall: A thick rigid outer covering which helps to maintain shape and prevents desiccation;

Flagella: Long, slender projections emerging from the cell wall, containing a motor protein that enables movement of the organism;

Pili: Hair-like extensions that enable adherence to surface (attachment pili) or mediate bacterial conjugation (sex pili);

Cell membrane: Semi-permeable and selective barrier surrounding the cell;

Cytoplasm: Internal fluid component of the cell;

Nucleoid: Region of the cytoplasm where the DNA is located (DNA strand is circular and called a genophore in cyanobacteria);

Plasmids: Autonomous circular DNA molecules that may be transferred between bacteria (horizontal gene transfer); and

Ribosomes: Complexes of RNA and protein that are responsible for polypeptide synthesis (prokaryote ribosome = 70S).

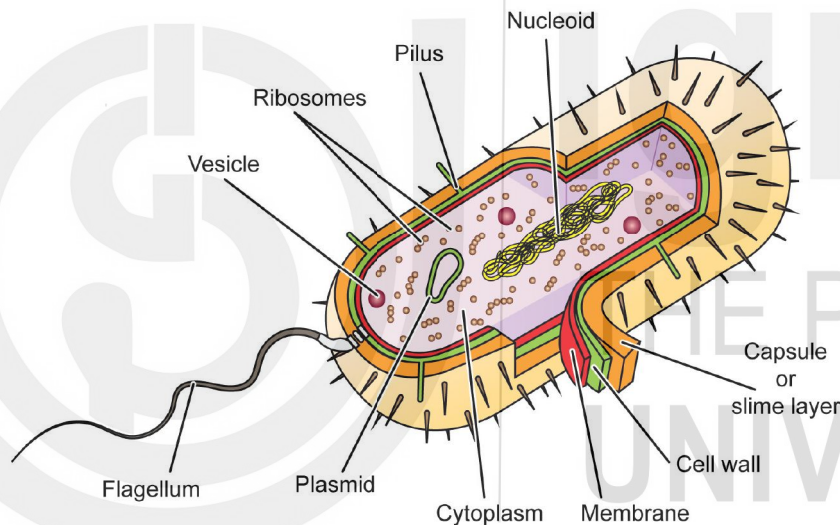


Fig.1.1: Diagrammatic representation based on the ultrastructure of a rod-shaped bacterium showing cell wall, pili, flagella, nucleoid with naked DNA and 70S ribosomes.

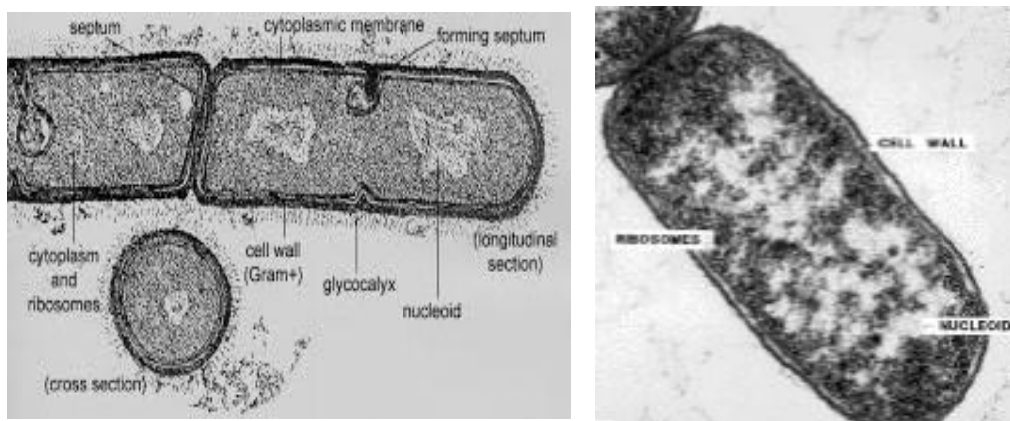


Fig. 1.2: Electron micrographs of a bacterial cell in longitudinal and cross section showing internal details.

1.5.2 Eukaryotic Cell (Plant and Animal Cell)

Ultrastructure of a plant and animal cell based on the electron micrographs provided to you should show the compartmentalized structure of a typical eukaryotic cell. The cell shows the presence of cell wall, a plasma membrane surrounding the cytoplasm, well organized nucleus, and membrane bounded organelles such as mitochondria, chloroplasts, endoplasmic reticulum, Golgi bodies, vacuoles and peroxisomes. In addition, microtubules and free 80S ribosomes are also observed. You will be studying about the structures of these organelles in detail in the Exercise 2.

Plant cell

Plant cells are generally rectangular in shape and surrounded by a rigid cell wall. They also possess a cell membrane and specifically contain organelles like central vacuole, chloroplasts, nucleus, mitochondria, Golgi bodies and ribosomes in the cytoplasm (Fig. 1.3).

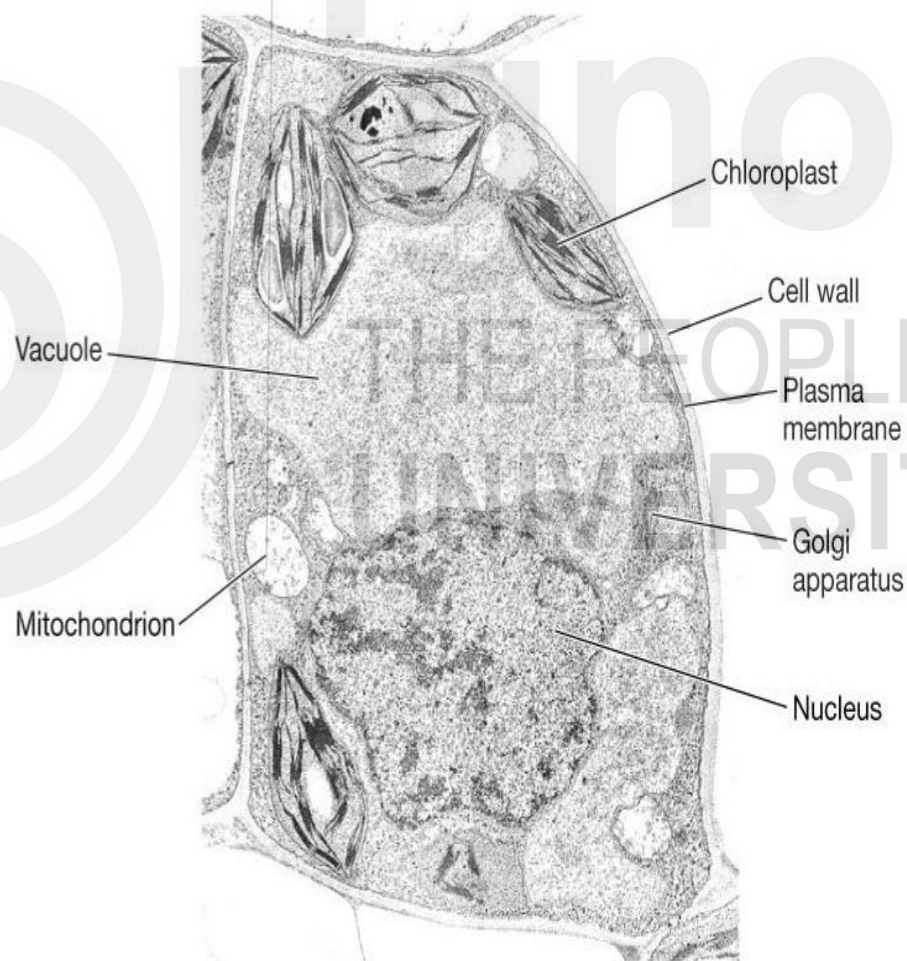


Fig. 1.3: Electron micrograph of a plant cell.

Animal cell

Animal cell is generally round in shape and does not possess a cell wall. The plastids and vacuoles are also absent in animal cell. Animal cells specifically contain centrosome, centrioles, and lysosomes (Fig. 1.4).

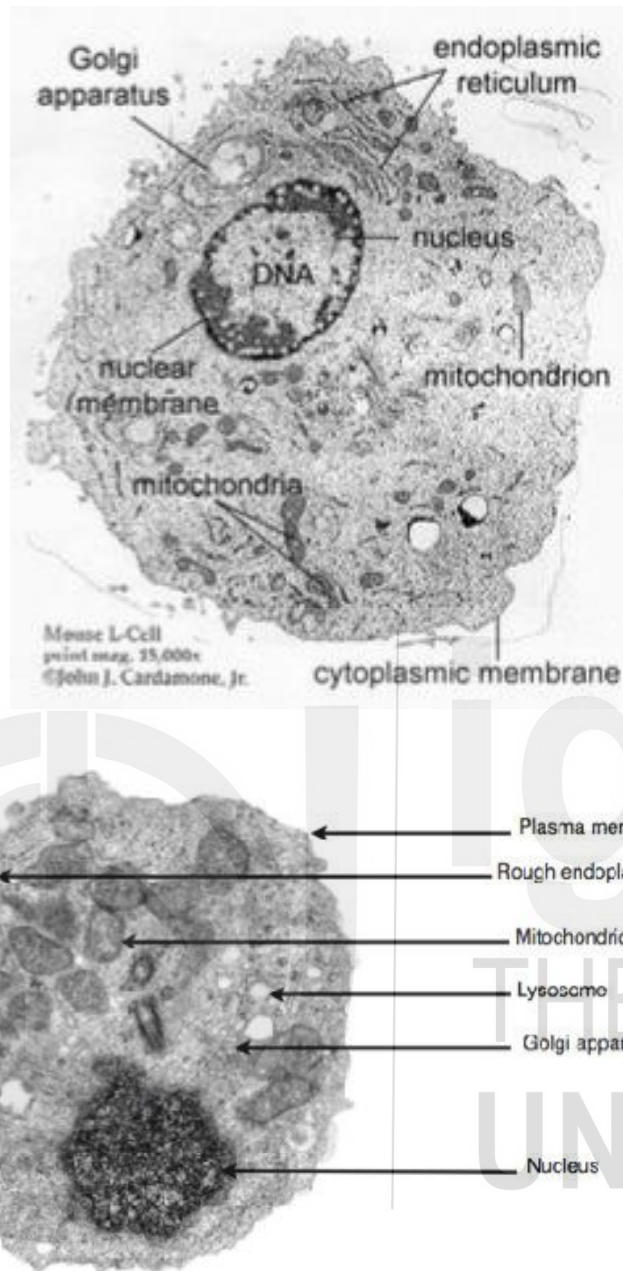


Fig.1.4: Electron micrographs showing animal cell.

1.5.3 Viruses

Viruses are minute infectious agents that multiply only in living cells of animals, or plants. All viruses contain nucleic acid in the form of either DNA or RNA. Proteins form a shell (called a capsid) around the nucleic acid in most of the viruses. Certain viruses also have proteins present internal to the capsid.

Plant virus

Tobacco Mosaic Virus (TMV):

TMV is a simple rod-shaped helical virus consisting of centrally located single-stranded RNA enveloped by a protein capsid or coat (Fig.1.5 a, b).

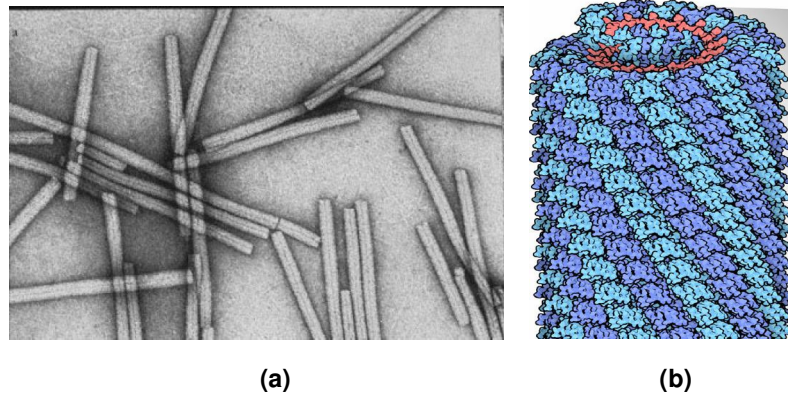


Fig.1.5: a) TEM of Tobacco Mosaic Virus; b) Diagrammatic view of TMV showing molecular organization.

The rod are about 300nm (3,000 Å) in length and about 16 -18nm (160-180 Å) in diameter (Fig.1.6 a). Each rod shaped structure in TMV is composed of 2,130 identical elliptical protein sub-units or capsomeres (Fig 1.6.b). The capsomeres are compactly arranged in the form of a helical hollow cylinder with a core (Fig. 1.6 c). RNA is a single stranded helical molecule. The RNA helix extends to the whole length of the TMV rod and is deeply embedded within the capsomeres. Each capsomere consists of about 158 amino acids. Each helical turn of the capsid has 16 capsomeres making about 130 turns per rod of TMV. The RNA molecule which is about 33nm (3300 Å) long, consists of about 6500 nucleotides and has a molecular weight of about 25 kD.

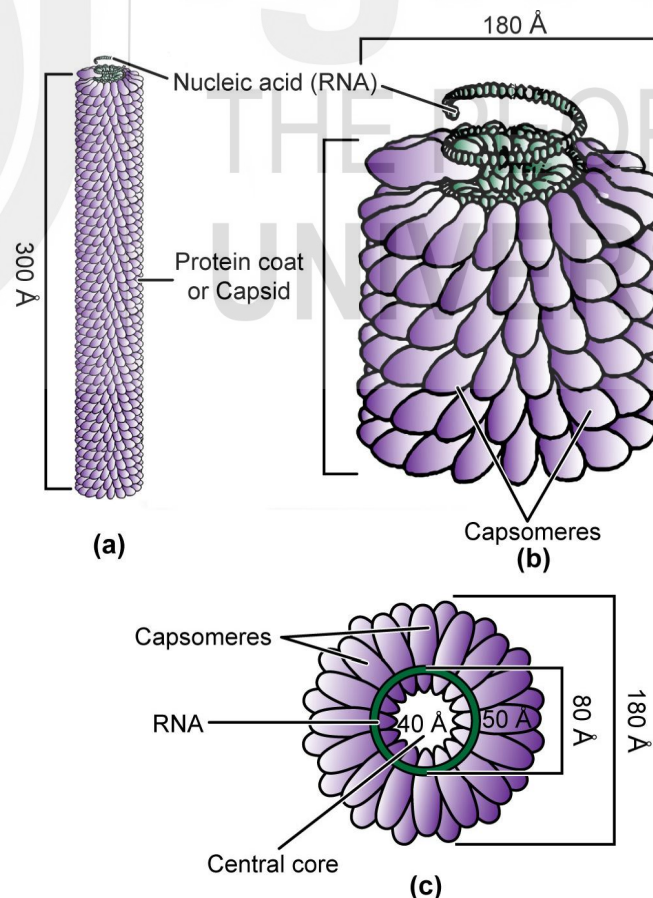


Fig. 1.6: Architecture of TMV a) Surface view of TMV; b) RNA-capsomere arrangement; and c) Transverse section of TMV.

Animal virus (Adenovirus)

Adenoviruses are DNA-containing viruses that cause cold-like infections of the upper respiratory tract (Fig 1.7a). The virus particles are 70-90 nm in diameter (Fig1.7 b).

Adenovirus is a large virus, composed of a complex protein capsid which surrounds its double stranded DNA genome and core proteins (Fig.1.7 b). The capsid is in the form of an icosahedron. This twenty-sided symmetry comprises a total of 252 capsomeres each composed of three identical proteins and 12 pentons (5 units) present on the vertices, each composed of five protein chains. A long fiber extends from each vertex. It is composed of three identical chains that forms a knob at the end (Fig. 1.7c). These are unique cell binding fibers. The adenovirus also reproduces through a lytic cycle.

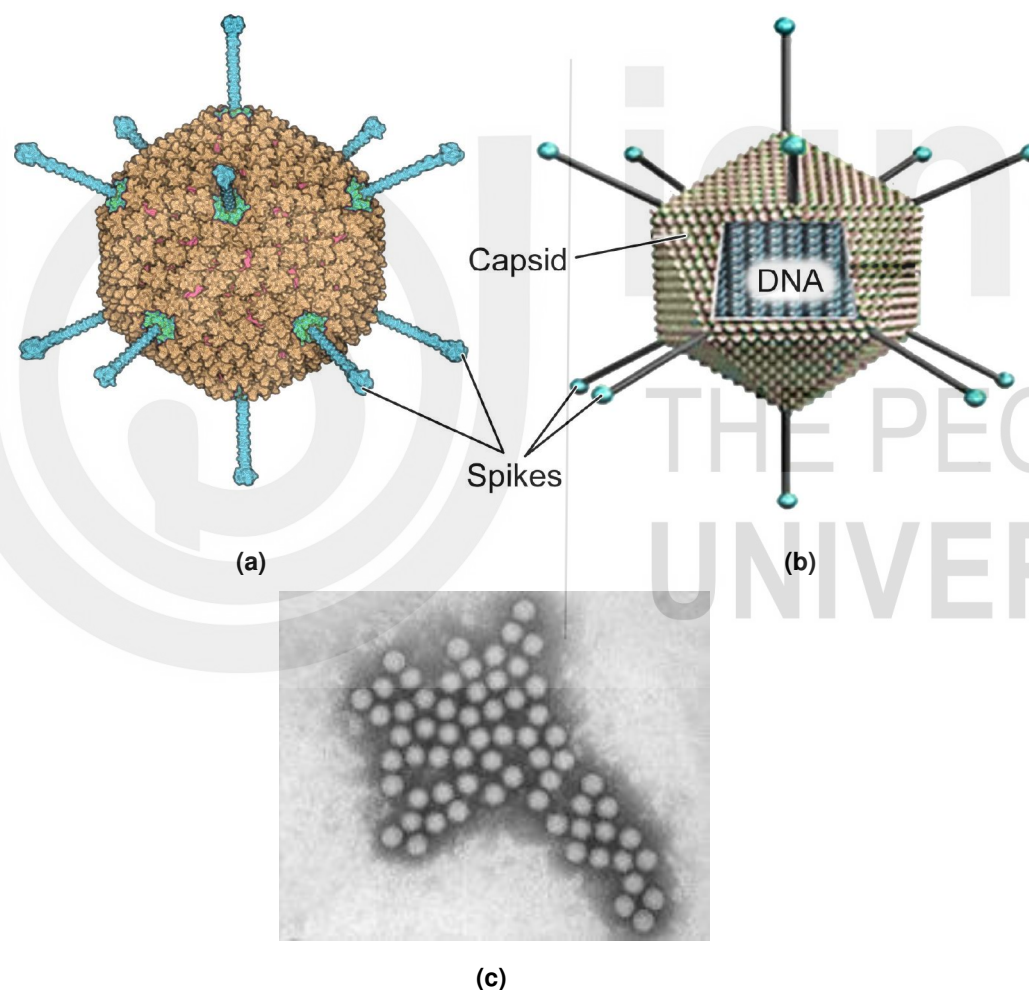


Fig. 1.7: (a, b) 3-dimensional view of an Adenovirus; c) Transmission electron micrograph of an Adenovirus

Bacteriophage (T4)

In the end you will study the fine structure of a T4 bacteriophage. T4 bacteriophage is a complex tadpole-shaped virus (Fig.1.8 a) that parasitizes the bacterial cells. It belongs to the category of T-even phages and infects the colon bacteria *Escherichia coli*. T stands for type. The terminology of T-even

and T-odd is based on the nature of DNA. T-even bacteriophages possess double stranded DNA, whereas single-stranded DNA is present in the T-odd ones. In addition, the T-even phages have a head and tail and are more complex.

The proteinaceous protective coat or Capsid comprises of 2000 subunits or **capsomeres**. Each capsomere is made up of structural units in the form of one or more polypeptide chains. The specific arrangement of capsomeres in the T4 bacteriophage gives its head a typical **Icosahedral** (20 sided) symmetry (Fig. 1.8 a, b). The bacteriophage head is about 1250Å long and 850 Å wide.

The head rests on a short **neck** with a **collar** and is connected to a long helical **tail**. The tail comprises a thick, hollow **mid-piece** which is covered by a Sheath, a hexagonal **baseplate** (**end-plate**) and the attached **spikes** (six) with six long **tail fibres** (Fig 1.8 b).

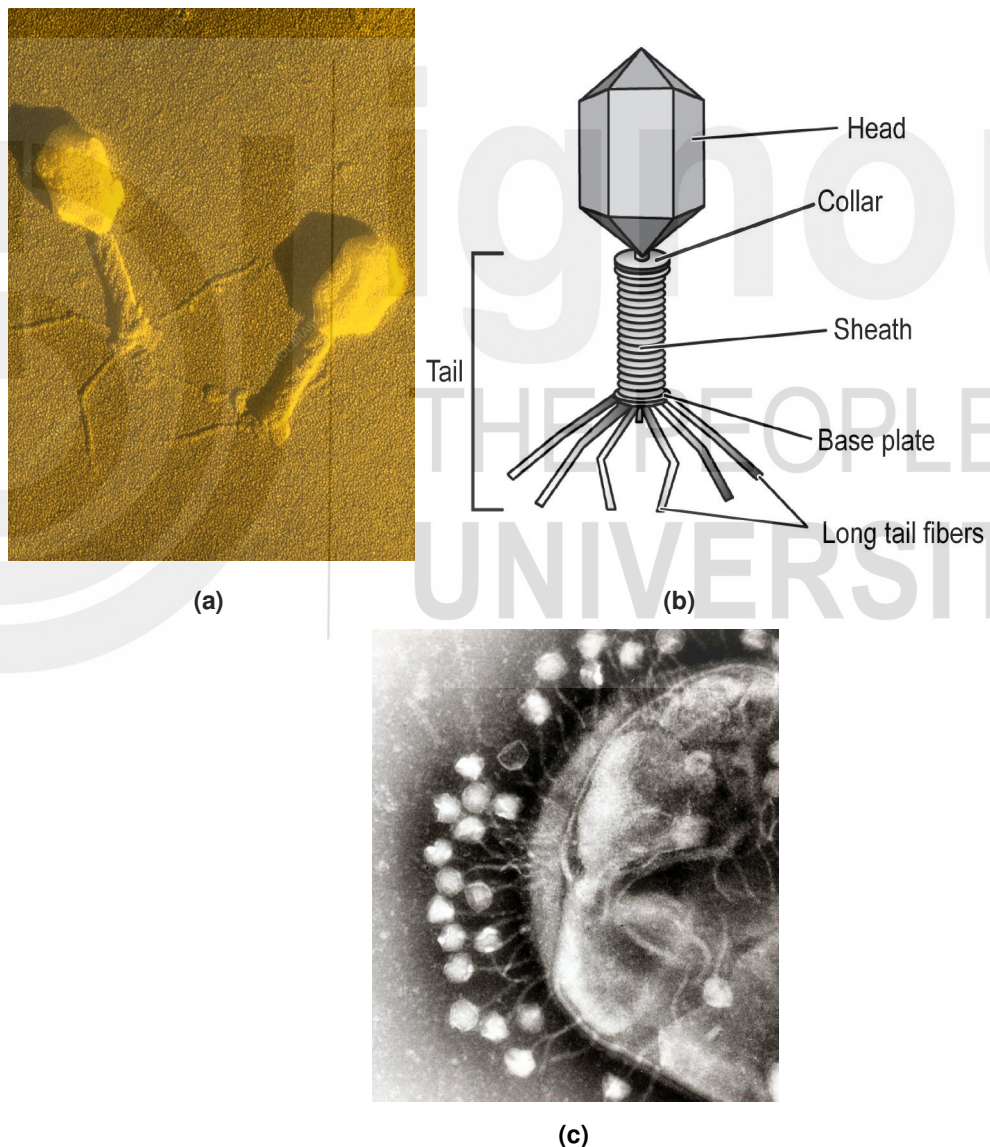


Fig. 1.8: a) Transmission electron micrograph of T4 bacteriophage; b) A diagrammatic sketch of T4 bacteriophage with structural details; c) Electron micrograph of T4 phages attached to the host bacterial cell wall prior to injection.

A single DNA molecule (1,66, 000 bp coding for more than 200 different proteins) is present tightly packed within the head is the viral genome. T4 bacteriophage is a virulent phage and exhibits a lytic cycle. In the first phase, the phage gets attached to the surface of the cell wall of the host bacterium with the help of its long tail fibers (Fig. 1.8 c). The viral genome gets injected into the host cell. After a certain eclipse period, the replicated phage DNA gets assembled into a complete phage, which gets released through the lysis of the host cell.

1.6 TERMINAL QUESTIONS

1. Enlist the organelles present in a typical eukaryotic cell.
2. How is animal cell different from plant cell?
3. Make a list of different structures seen in a T4 bacteriophage.

1.7 ANSWERS

1. Mitochondria, chloroplasts, endoplasmic reticulum, Golgi bodies, vacuoles, peroxisomes. Nucleus and ribosomes are the major organelles found in a eukaryotic cell.
2. Plant cells are generally rectangular in shape. They are surrounded by a rigid cell wall and a cell membrane. They possess a large central vacuole. Animal cell is generally round in shape and does not possess a cell wall. The plastids and a vacuoles are also absent in animal cell. Animal cells specifically contain centrosome, centrioles, and lysosomes.
3. Refer to Subsection 1.5.3.

Acknowledgements

Fig. 1.1 : Source: <https://qph.fs.quoracdn.net/main-qimg-2213c2b207d36592d120aad93e71f>

Fig. 1.2 : https://lh3.googleusercontent.com/proxy/qlUPv03Bru6FvTFbAvP2nCCCObIWofl6Wlz0obgRcre2jddvsOOM5DJlcl5OYQYReN3FJ8EPWKuzn3XtDwO_Oyw-DZ5M

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Fig. 1.3 : Source: <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fipinimg.com%2Foriginals%2Fef%2F2b%2F22%2Fef2b228648e0f63174ce70644cc80029.jpg&imgrefurl>

Fig. 1.3 : Source: <https://slideplayer.com/slide/8940273/27/images/28/Animal+Cell+Ultrastructure+as+Seen+with+an+Electron+Microscope.jpg>

Fig. 1.4 : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fibguides.com%2Fimages%2F2.3.2.png&imgrefurl=https%3A%2F%2Fibguides.com%2Fbiology%2Fnotes%2F2.3-eukaryotic-cells%2F>

Fig. 1.5 : Source: <https://cns1.rc.fas.harvard.edu/wp-content/uploads/2016/09/tmv.jpg>
https://cdn.rcsb.org/pdb101/motm/images/2tmv_composite.jpg

Fig.1.6 : Source:
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Fig. 1.7 : Source:

- a) https://cdn.rcsb.org/pdb101/motm/images/132-Adenovirus_adenovirus.jpg
- b) <https://micro.magnet.fsu.edu/cells/viruses/images/virus.jpg>
- c) <https://iiif.wellcomecollection.org/image/B0001486.jpg/full/1010%2C0/default.jpg>

Fig. 1.8 : Source:

- a) <https://media.sciencephoto.com/image/m0900045/800wm>
- b) <https://mk0morgridgeorgkfioq.kinstacdn.com/wp-content/uploads/bacteriophage.png>
- c) <https://upload.wikimedia.org/wikipedia/commons/5/52/Phage.jpg>

EXPERIMENT 2

CELL STRUCTURE – STUDY OF PHOTOGRAPHS AND ELECTRON MICROGRAPHS OF CELL ORGANELLES

Structure

2.1	Introduction	Lysosomes and Microbodies
	Objectives	Peroxisomes
2.2	Study Guide	Microtubules
2.3	Materials Required	Centrosome
2.4	Observations	Cilia and Flagella
	Cell Wall	Nucleus
	Plasma Membrane	Nucleolus
	Chloroplasts	Ribosomes
	Mitochondria	2.5 Terminal Questions
	Endoplasmic Reticulum	2.6 Answers
	Golgi Bodies	

2.1 INTRODUCTION

You have been made familiar with the structure of prokaryotic and eukaryotic cells in the previous exercise. You have also studied the comparison between an animal and plant cell. Eukaryotic cell is highly compartmentalized with several organelles. Each organelle has a specific structure and performs specific function. Yet you will appreciate that these organelles do not function alone but are integrated in the overall functioning of the cell as a single composite unit. This exercise will make you familiar with the structure of the major organelles seen in a eukaryotic cell. You will be able to appreciate the fine structure of these organelles with the help of electron micrographs. You

have already studied about the structure and function of these organelles in theory units 5, 6 and 7 of BBYET-141.

Objectives

After performing this experiment you would be able to:

- ❖ describe the structure of various organelles present within a eukaryotic cell; and
- ❖ correlate the structure with the role these organelles play in the functioning of the cell.

2.2 STUDY GUIDE

For doing satisfactory work you must study Units 5, 6 and 7 of UGC (CBCS) Course - Paper Cell and Molecular Biology (BBYET-141), before coming to the laboratory.

2.3 MATERIALS REQUIRED

You are provided with the light and electron micrographs of various structures and organelles seen within a eukaryotic cell.

2.4 PROCEDURE

Observe the photographs and electron micrographs carefully. Note down the structural details of each organelle and their subcellular structure. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the structural details) in your notebook and write comments. It is advised that figures should not be copied from the book but should be drawn after observing the photographs provided to you.

2.5 OBSERVATIONS

Observe each structure and organelle shown in the light and electron micrographs and make a well labelled sketch along with a brief description of each component.

2.5.1 Cell Wall

- Plant cells have a rigid wall surrounding the cell (plasma) membrane (Fig. 2.1a,b).
- It is an extracellular structure. Cell wall is a semi-rigid structure that prevents excessive uptake of water.
- Cell wall provides protection to the cell.
- It is much thicker than the cell membrane. Dimensions may range from 0.1 μ m to several micrometers.

- It is composed of cellulose microfibrils which are embedded in a matrix made up of polysaccharides and in some cases proteins.
- In young cells, a thin, flexible **primary cell wall** is formed around the protoplast.
- The primary walls of two adjacent cells are joined by a pectinaceous thin layer acting as a cementing material called the **middle lamella**.
- In some plants a **secondary wall** is formed between cell membrane and primary wall. It is rigid, inelastic and has lignin and other components which provide mechanical strength to a plant.
- Adjacent plant cells are interconnected by perforations called **plasmodesmata**.

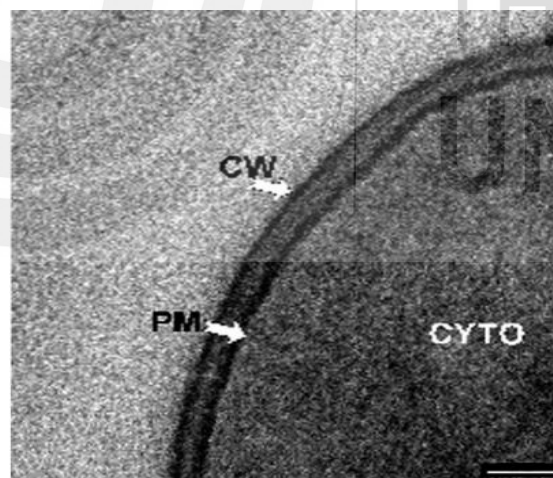
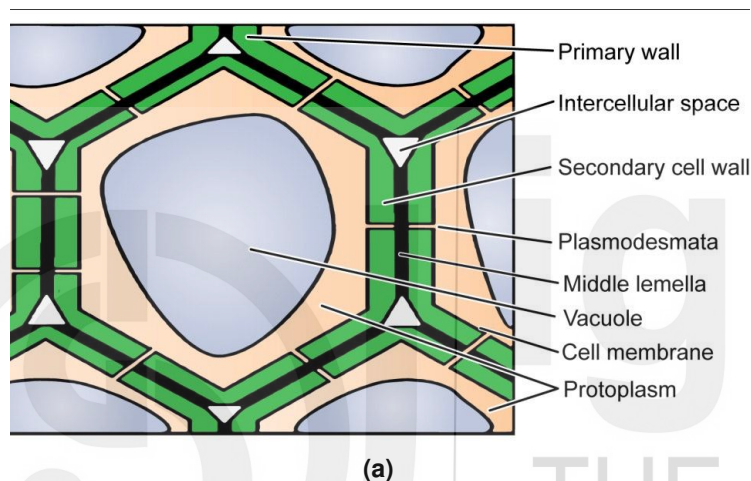
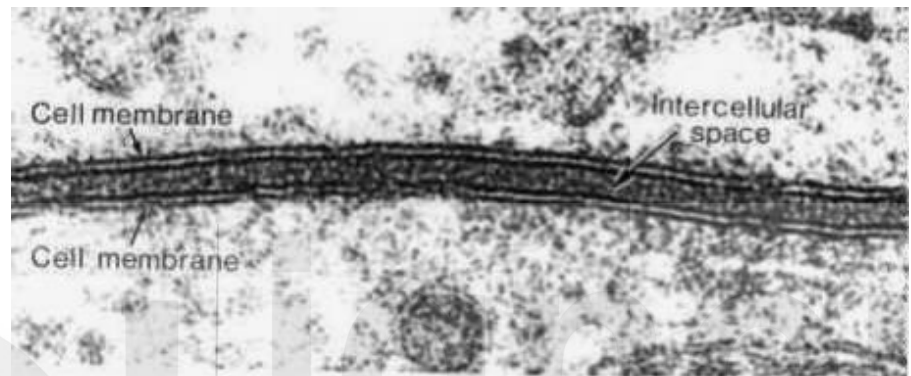


Fig. 2.1: a) Diagram showing layers of cell wall; b) EM graph of plant cell wall.
[CW=cell wall; PM=plasma membrane, and CYTO=cytoplasm]

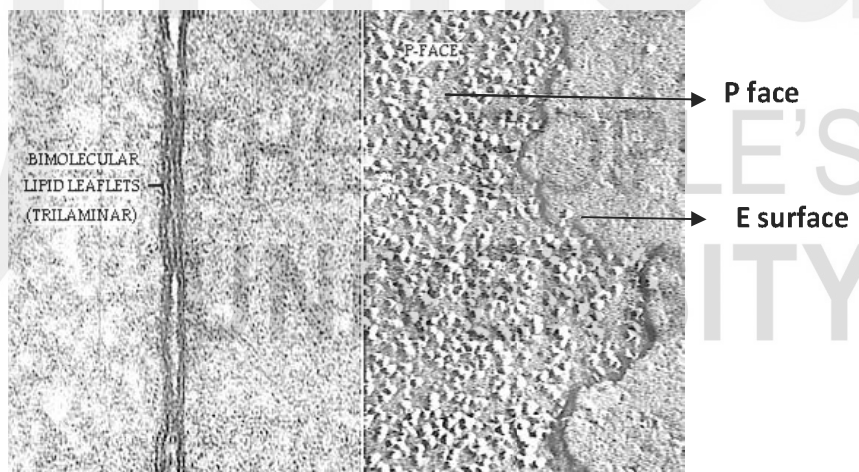
2.5.2 Cell Membrane

- Cell (plasma) membrane represents the thin boundary which separates the living portion of the cell from its nonliving environment (Fig. 2.2).
- It is a thin film of about 8nm thickness and is made of a fluid mosaic of lipids and proteins.

- The plasma membrane is selectively permeable and controls the influx and efflux of water and substances across it.
- In addition to acting as a selective permeability barrier, the membrane also acts as pumps and gates for regulating the uptake of nutrients and the elimination of wastes through its channels.
- The membrane also forms the envelope of many organelles and is a seat of various important biochemical transitions. Many enzymes and signaling molecules are also membrane bounded. It is also a site for various receptors.



(a)



(b)

Fig. 2.2: Ultrastructure of cell membrane. a) TEM of cell membrane showing a trilaminar appearance; b) A comparison between a section and a freeze-fracture preparation of membrane highlighting its different faces [P (protoplasm) face and E (external) surface].

2.5.3 Chloroplasts

- These organelles are the sites for carrying out photosynthesis. They are also called as kitchens of a cell.
- Chloroplasts are the green plastids containing mainly chlorophylls and a low amount of carotene and xanthophylls.
- Chloroplasts may be lens-shaped, oval, spherical, discoid or ribbon-like.

- Chloroplast is surrounded by a double-walled permeable membrane (Fig. 2.3). Each membrane is 40-60 Å thick. The space of about 100-300 Å wide enclosed between the two membranes is called periplastidial space.
- Many flat membranous structures called thylakoids occur in the form of stacks of coins called grana, whose number is about 40-100 in a chloroplast.
- The double membrane covering, encloses a colourless matrix called stroma. The stroma contains a small, circular, double-helical DNA, ribosomes, and several enzymes. It is also a site for Dark reaction.
- The grana are interconnected by stroma lamellae.
- Light reaction of photosynthesis takes place in the thylakoids.

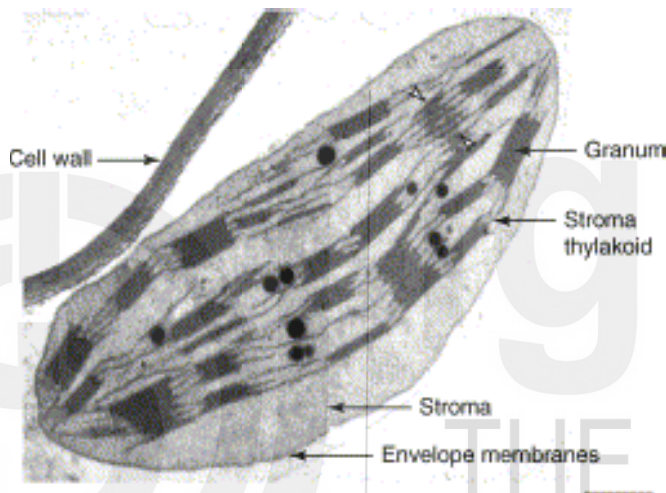


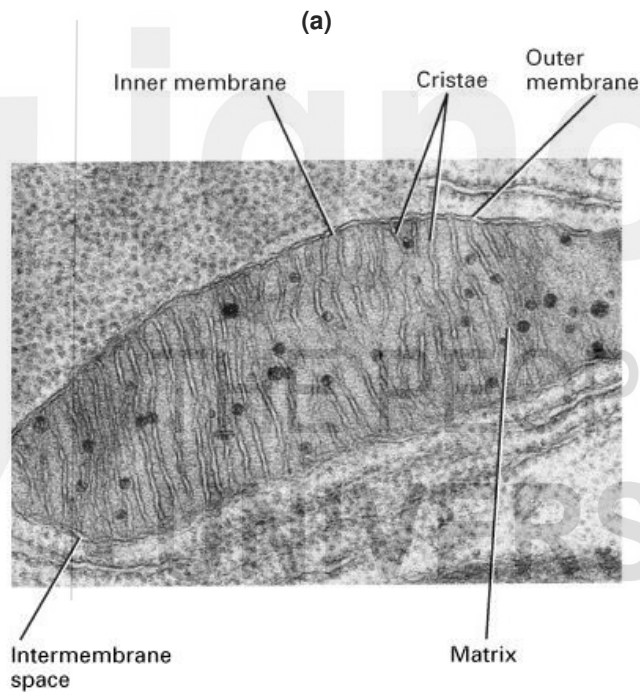
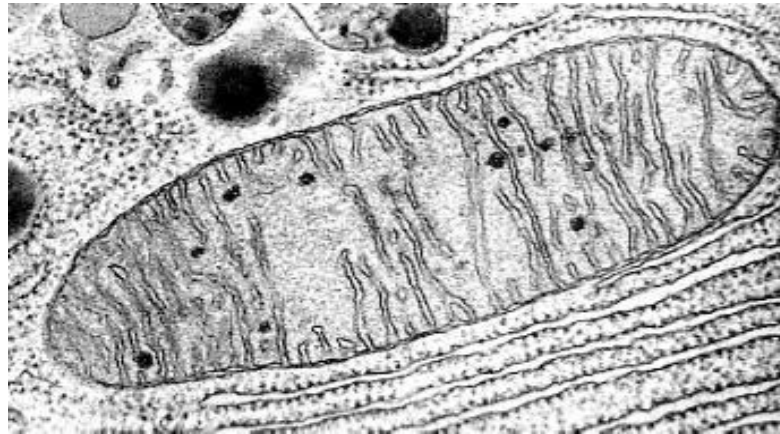
Fig. 2.3: Electron micrograph showing structural details of a chloroplast.

Chloroplasts contain DNA, RNA, and ribosomes. Chloroplasts arise from preexisting proplastids, do not arise *de novo*, and show semi-genetic autonomy.

2.5.4 Mitochondria

- Mitochondria (singular mitochondrion) are cytoplasmic organelles found in almost all eukaryotic cells, both plants and animals. These are absent in prokaryotic cells.
- Mitochondria are called the powerhouse of the cell, as these are associated with generation of energy (ATP) necessary for cellular function. In plant cells they break down carbohydrates and sugar molecules to provide energy.
- Mitochondria are oblong shaped organelles found in the cytoplasm of all eukaryotic cells. These are double-membrane bounded, sausage-shaped semi-autonomous organelles (Fig. 2.4 a). They may be rod-shaped, granular, or filamentous.
- Size varies from 0.5µm to 2.0 µm. Number of mitochondria depends on the functional state of the cell.

- The electron micrograph shows that the boundary membranes are separated by a fluid filled space called outer compartment. The outer membrane is smooth but the inner membrane projects in the form of many folds called cristae into the matrix (Fig. 2.4b).



(b)
Fig. 2.4: a, b) Electron micrographs showing structure of mitochondria.

- The mitochondrial matrix contains protein, lipids, DNA, ribosomes, and calcium phosphate granules. Mitochondrial ribosomes are smaller than the cytoplasmic ribosomes and are like prokaryotic ribosomes in terms of the size and composition of subunits.
- Like the chloroplasts, the mitochondria also do not arise *de novo*, and show semi-autonomous nature.

2.5.5 Endoplasmic Reticulum

- The endoplasmic reticulum (ER) is a network of sac that helps in the manufacture, processing and transport of chemical compounds inside and outside of the cell.

- It is connected to the double layered nuclear envelope, thus acting as a channel between the nucleus and the cytoplasm. In plants the ER also connects adjacent cells via the plasmodesmata.
- ER is an irregular network of double- membraneous tubules enclosing a fluid-filled space (Figs. 2.5 and 2.6). Electron microscopy reveals the presence of a fine reticulum of tubules in the cytoplasm.
- ER is found in all eukaryotic cells except mammalian RBCs. Prokaryotes lack this organelle.
- ER occurs in three different forms, viz. cisternae, vesicles, and tubules. The cisternae are long, flattened, unbranched lamellae arranged in parallel rows. The vesicles are usually round or ovoid sacs, ranging from 25 to 500Å in diameter. The tubules are irregularly branched, tube-like structures, varying in diameter from 20 to 100Å.
- ER is of two types: i) Rough or granular endoplasmic reticulum (RER) containing ribosomes on its surface (Fig. 2.5); and ii) smooth or agranular endoplasmic reticulum (SER) which lacks ribosomes on its surface (Fig. 2.6).

i) **Rough endoplasmic reticulum**

- It has ribosomes on its outer surface (Fig. 2.5). It consists mainly of cisternae and a few tubules.
- Its membrane is continuous with that of SER of nuclear membrane.
- It is present more in protein/enzyme secreting cells.

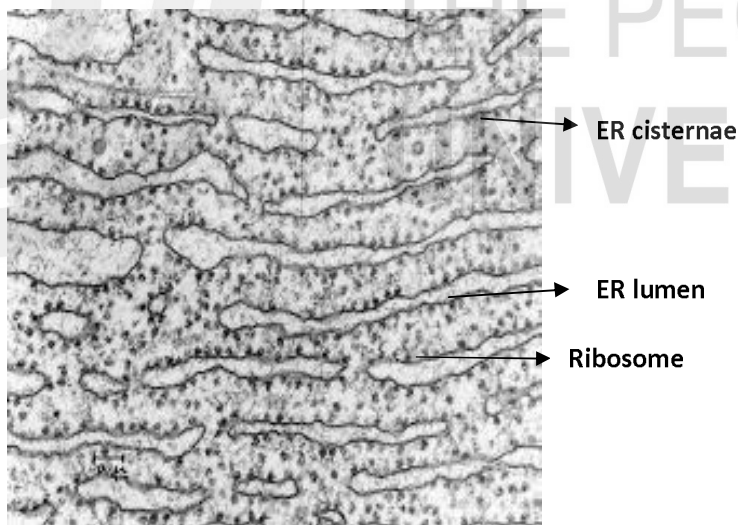


Fig. 2.5: Electron micrograph showing rough endoplasmic reticulum (RER).

ii) **Smooth endoplasmic reticulum**

- It has no ribosomes attached to it (Fig. 2.6).
- It consists mainly of tubules and vesicles. Its membrane is continuous with Golgi cisternae and of plasma membrane.
- It is predominantly present in cells that synthesize lipids and sterols.

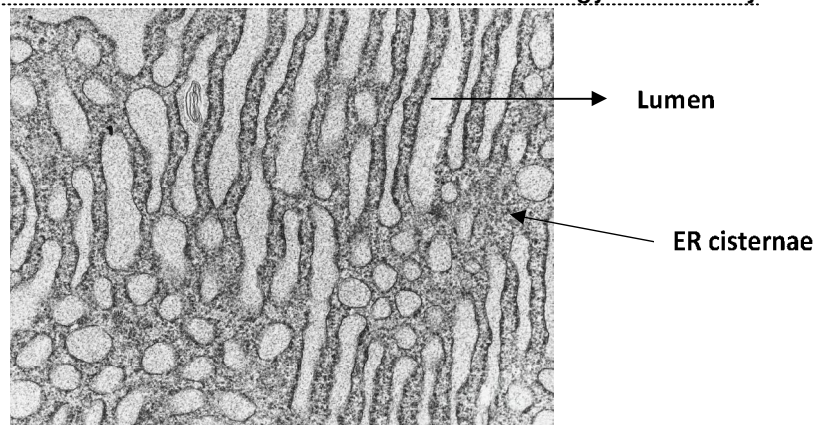


Fig. 2.6: Electron micrograph showing smooth endoplasmic reticulum (SER).

2.5.6 Golgi Bodies

- Golgi bodies are found in all eukaryotic cells, except fungi, male gametophytes of bryophytes and pteridophytes, mature sieve tubes of angiosperms and mammalian RBCs. Prokaryotic cells lack Golgi apparatus.
- The Golgi apparatus modifies proteins and fats synthesized in the ER and prepare them for export as outside of the cells.
- Golgi complex consists of one or more stacks of flat, expanded interconnected cisternae, with some vesicles and vacuoles (Fig. 2.7 a). The Golgi stacks are found between endoplasmic reticulum and plasma membrane.
- In plant cells, Golgi apparatus consists of a single stack, and it is called a dictyosome. Tubules arise from the periphery of the cisternae and form a highly branched network. Vesicles are rounded structures discharged from the cisternae.

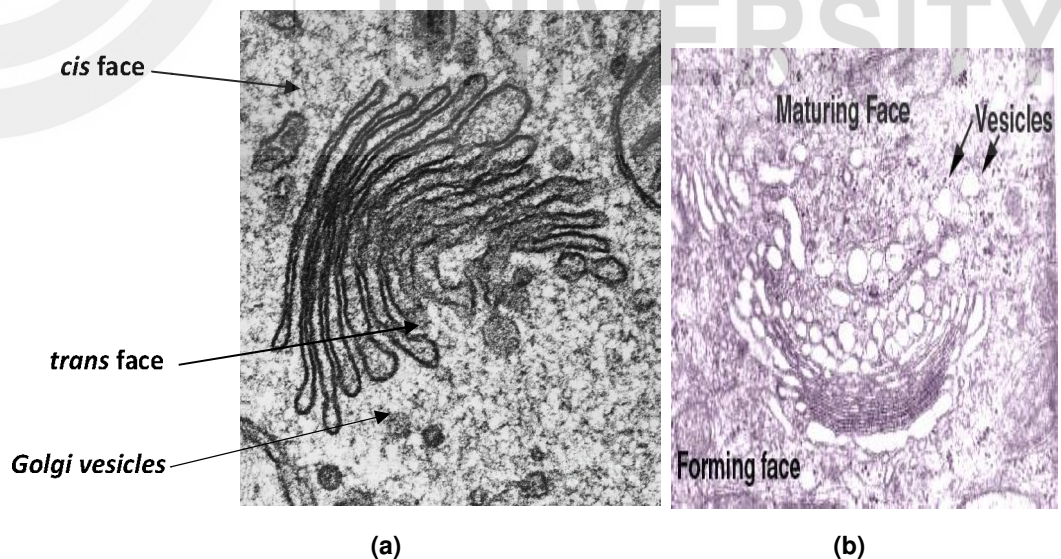


Fig. 2.7: Electron micrographs showing a) structure of Golgi bodies b) different faces in Golgi complex.

- Each cisterna is a fluid-filled space enclosed by a single unit membrane and is curved like a shallow bowl. The convex face of the curve is called the **forming face** (directed towards the ER) whereas the concave one is

termed as the **maturing face** which is usually directed towards the plasma membrane (Fig. 2.7 b).

- The lumen of Golgi cisternae has continuities with one another and with the lumen of SER.
- Golgi apparatus is rich in proteins (60%) and phospholipids (40%).

2.5.7 Lysosomes

- Lysosomes are single membrane bounded sacs which contain hydrolytic enzymes (Fig. 2.8). These enzymes are synthesized by the rough ER and transferred to the Golgi apparatus and after further processing are pinched off from the *trans* face of the Golgi.
- Lysosomes are scattered in the cytoplasm and carry out intracellular digestion of various organelles as a part of recycling of cell's organic components. Lysosomes carry out autophagy as well as heterophagy.

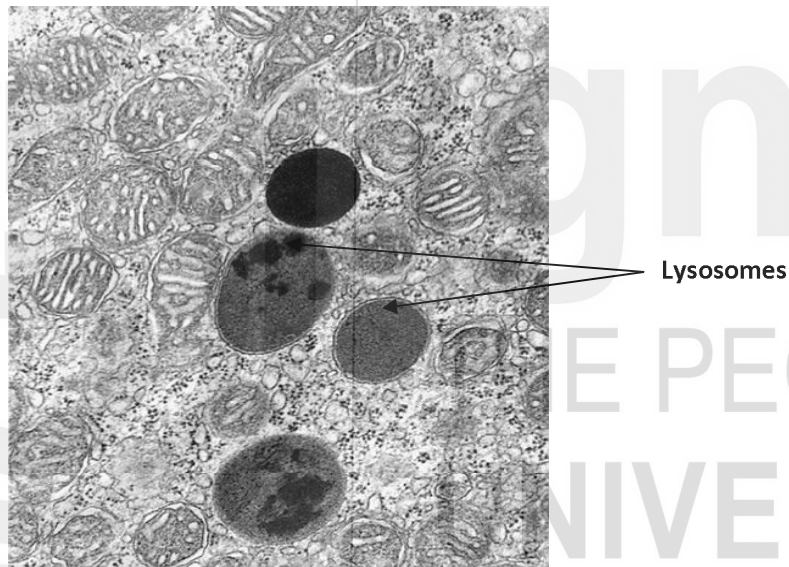


Fig. 2.8: Electron micrograph showing Lysosomes.

2.5.8 Peroxisomes

- Peroxisomes are specialized, single membrane bounded bodies present in close contact with the chloroplasts (Fig. 2.9).
- The roughly spherical bodies have a crystalline core which is an aggregate of enzyme molecules.
- These bodies are rich in *catalase* enzyme which breaks down H_2O_2 into O_2 .
- Some peroxisomes break fatty acids into smaller molecules which are then transported to mitochondria.
- Specialized peroxisomes called **glyoxisomes** are found in fat-storing tissues of seeds which convert fatty acids to sugars as a prerequisite to seed germination, e.g., seeds of castor and mustard.

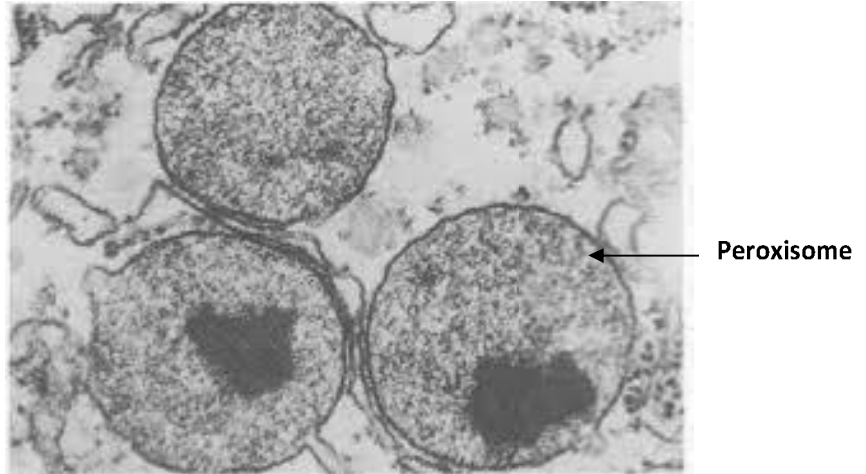


Fig. 2.9: Electron micrograph showing structure of Peroxisomes.

2.5.8 Microtubules

- Microtubules are hollow cylindrical rods present in the cytoplasm of all eukaryotic cells (Fig. 2.10).
- Each cylinder measures about 25nm (250μm) in diameter and up to 200nm in length. The microtubule wall is composed of a globular protein tubulin. Each tubulin is in the form of a dimer consisting of two slightly different polypeptides (α tubulin and β tubulin). These two subunits are arranged in a helical manner and joined by several MAPs (microtubule associated proteins).
- Cytoplasmic microtubules can be assembled and disassembled at various sites in a cell. Microtubules are responsible for maintaining the shape of a cell. Microtubules are an integral part of the network of fibers extending throughout the cytoplasm-the **cytoskeleton**. They also serve as tracks along which the selected organelles can move. They also guide the vesicles from the Golgi apparatus to the cell membrane. Microtubules play an important role in separation of chromosomes during cell division.

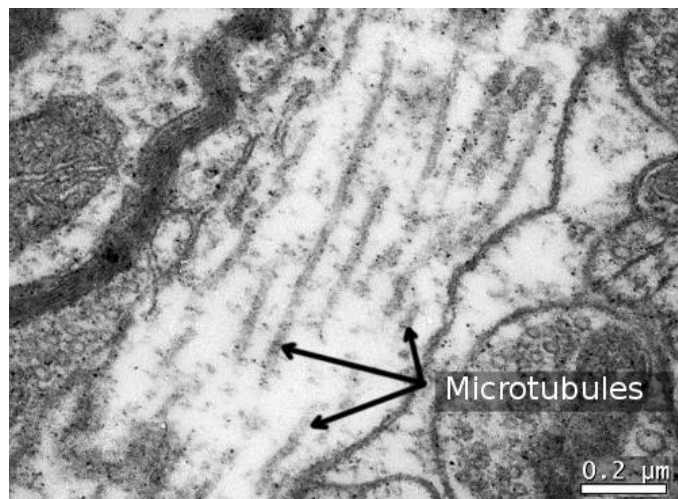


Fig. 2.10: Electron micrograph depicting microtubules.

- Microtubules form the structural basis of cilia and flagella.

2.5.9 Centrosome

- Centrosome is a small region situated close to the nucleus in animal cells. Centrosome is absent in plant cells. Some lower plants like *Chlamydomonas* possess centrioles at the base of flagella. The microtubules grow out of this region and are therefore, considered as a “microtubule organizing center”.
- Each centriole is composed of nine sets of triplet microtubules arranged in a ring (Fig. 2.11a).
- In plant cells, polar caps perform the function of centrosome.
- During cell division, the centrioles develop several radiating threads or rays. The whole structure (Centriole and rays) is then called an aster. Centrosome helps in the formation of spindle fibers during cell division (Fig. 2.11b).
- Centriole takes part in the formation of cilia and flagella.
- Centrioles are absent in higher plants. Instead, polar caps perform the function of centrosome. These caps are formed during preprophase of cell division near prophase nucleus and poles. The microtubules get reorganized during the G2 phase in the form of a ring, called as the preprophase band. This band encircles the nucleus and helps in an oriented cell division in plants.

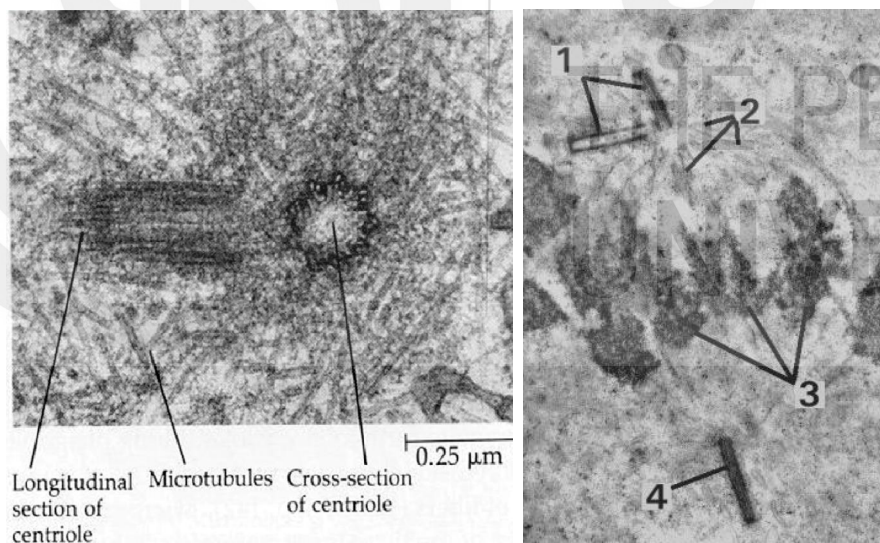


Fig. 2.11: a) Electron micrograph showing centriole. Nine sets of triplet microtubules arranged in a ring are seen in the cross section view; b) Centrosome showing 1 and 4= centrioles, 2=spindle fibers, and 3= chromosomes

2.5.10 Cilia and Flagella

- Cilia and flagella are hair-like outgrowths of the cell membrane which are responsible for cell motility (Fig. 2.12). Cilia are small structures which work like oars with sweeping movements of either the cell or the surrounding fluid. Flagella are comparatively longer, fewer in number and responsible for cell movements by undulating movements.

- The prokaryotic bacteria also possess flagella, but these are structurally different from that of eukaryotic cell. The electron microscopic study of a cilium or the flagellum shows that they have a core of microtubules called **axoneme** which is covered by a sheath of plasma membrane. The axoneme possesses several microtubules running parallel to the long axis.
- The axoneme usually has a ring of nine doublets of radially arranged peripheral microtubules which encircle two single centrally located microtubules. Such an arrangement of axonemal microtubules is referred to as the “9 (2) + 2” pattern.

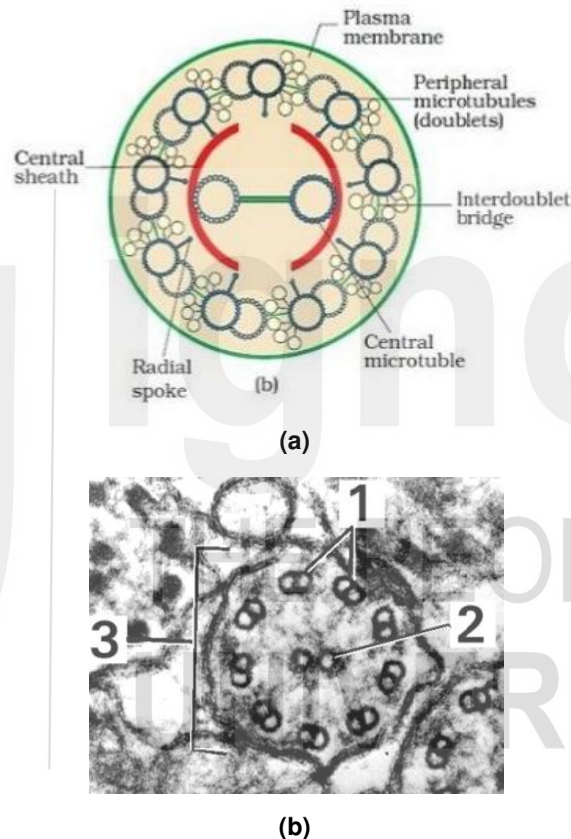


Fig. 2.12: a) Diagram of axoneme showing arrangement of microtubules; b) electron micrograph showing axoneme structure of a cilia. 1=Peripheral microtubules doublet, 2= central microtubules pair, and 3=cilium membrane.

- The central tubules are connected by bridges. It is also enclosed by a central sheath which is connected to one the tubules of each peripheral doublets by a radial spoke (cross linking proteins). Thus, there are nine radial spokes. The peripheral doublets are also interconnected by linkers. Each outer doublet has pairs of side-arms which connect to the neighboring doublet in the rim. These motor proteins are in the form of Dynein arms which give the bending movements to cilia and flagella .In a transverse section these organelles look like a cartwheel.
- Both the cilia and flagella emerge from centriole like structure called the basal bodies.

2.5.11 Vacuole

- Vacuoles are membrane bound organelles present in the cytoplasmic matrix of eukaryotic cells (Fig. 2.13). In plant cells there is a single central vacuole enveloped by a single cell membrane called the **tonoplast**.
- In plant cells, vacuoles behave as lysosomes and carry out hydrolysis. Vacuoles are derived from the ER and Golgi apparatus. The tonoplast is also selectively permeable and helps the vacuole to store cell sap. Vacuole acts as a storage site for proteins, inorganic ions like potassium and chloride, metabolic wastes.

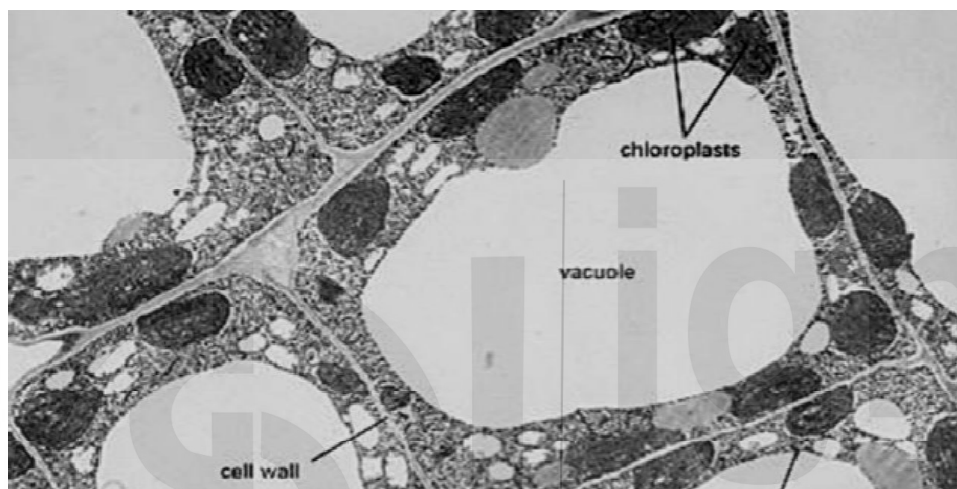


Fig. 2.13: Electron micrograph a plant cell showing presence of a large vacuole.

2.5.12 Nucleus

- Nucleus is the largest and most prominent organelle present in the cell (Fig. 2.14 a). Nucleus contains genetic material. The nucleus stores the cell's hereditary material or DNA as it coordinates metabolism, protein synthesis and reproduction (Cell division).
- Nucleus is the site of DNA replication and RNA synthesis. The translation of RNA into protein takes place on ribosomes in the cytoplasm.
- The interphase nucleus has highly extended elaborate nucleoprotein fibers called chromatin, nuclear matrix, one or more spherical bodies called nucleoli (single nucleolus).
- Transmission Electron microscopy has revealed that the nuclear envelope which consists of 2 parallel membranes with a space between (10-50 nm) called the perinuclear space, form a barrier between the materials present inside the nucleus that of the cytoplasm. (Fig. 2.14 b).
- The outer membrane usually remains continuous with ER and bears ribosomes. At number of places the nuclear envelope is interrupted by minute pores, which are formed by the fusion of two membranes.

- The nuclear matrix or the nucleoplasm contains nucleolus chromatin. The nucleoli are spherical structure present in the nucleoplasm. The content of nucleolus is continuous with the rest of the nucleoplasm as it is not membrane bounded structure. It is a site for active ribosomal RNA synthesis. Larger more numerous nucleoli are present in cells actively carrying out protein synthesis. You will study the fine structure of the nuclear pore complex in Exercise 13 of this laboratory course.

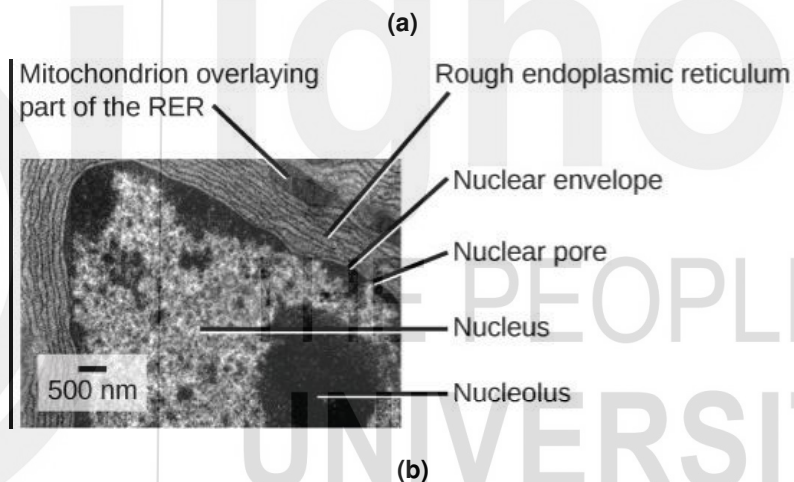
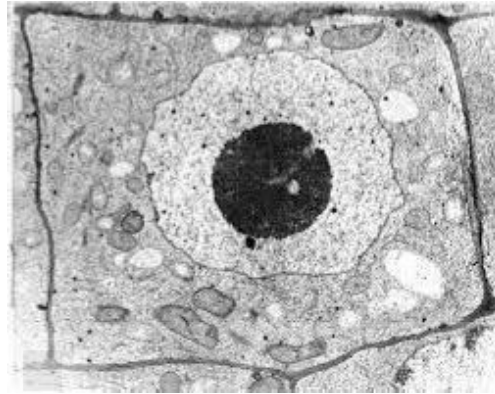


Fig. 2.14: a) Electron micrograph of a plant cell showing presence of nucleus; b) Nucleus showing presence of nucleolus, nuclear pores and nuclear envelope.

2.5.13 Nucleolus

- Nucleolus is a prominent spherical, colloidal body present within the nucleus (Fig. 2.15).
- When observed under the electron microscope, nucleolus appears as a mass of densely stained granules and fibers which seem to be associated with the chromatin (nucleolar organizing chromosome).
- The nucleolus exhibits a tripartite organization: i) a **fibrillar center** where the rRNA genes for NOR (Nucleolar organizing region) are located; ii) the **dense fibrillar component** which surrounds the fibrillar center. RNA synthesis takes place in this region; and iii) the outermost region, **cortical granular component** which is the site of processing and maturation of pre-ribosomes.

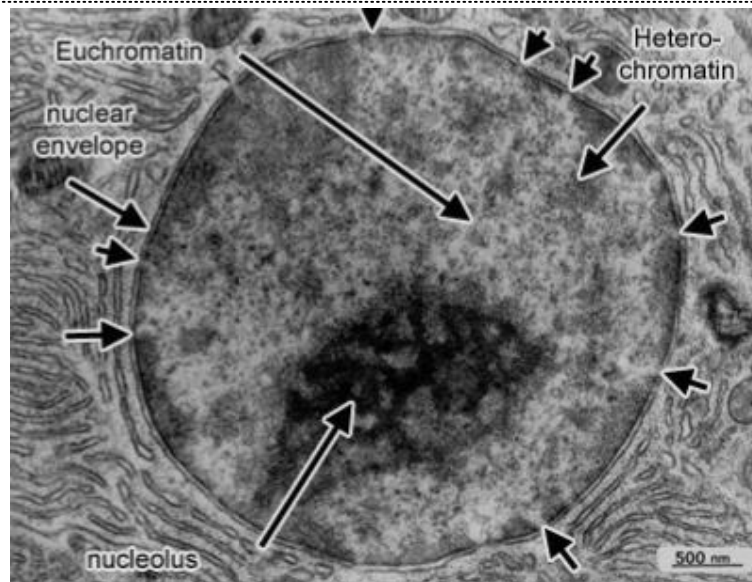


Fig. 2.15: Electron micrograph of nucleus showing Nuclear envelope, Nucleolus, Heterochromatin, and Euchromatin.

2.5.14 Ribosomes

- All living cells contain very small granular structures called ribosomes (Fig. 2.16). They are popularly called as the protein factories of the cell.

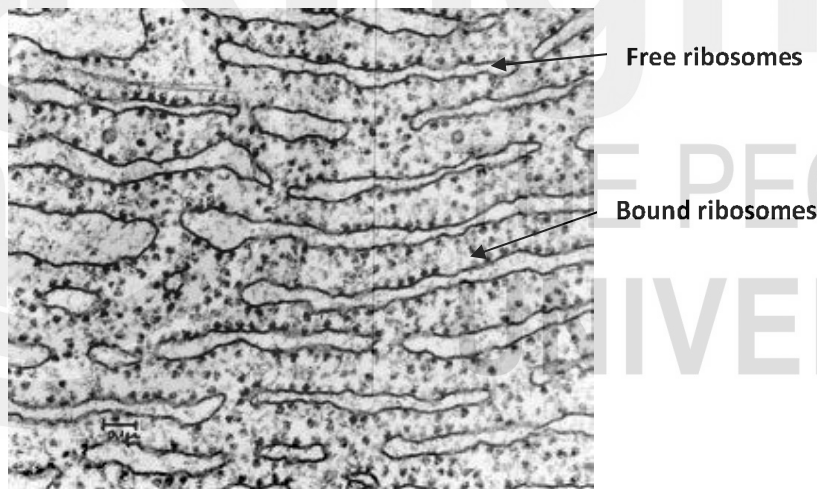


Fig. 2.16: High resolution electron micrograph showing free and bound ribosomes.

- They provide for the sites of protein synthesis. Ribosomes do not possess a membrane and are in fact composed of rRNA and proteins.
- Ribosomes are composed of two subunits: a larger and a smaller one. Both the subunits are synthesized in the nucleolus. They come out into the cytoplasm from the nuclear pores and assemble into fully functional ribosomes there.
- Eukaryotes have 80S ribosomes (60S and 40S subunits) while the prokaryotic ribosomes sediment at 70S (50S and 30 S subunits).
- The ribosomes assemble proteins in two different locations: i) **Free ribosomes** that occur in the cytoplasm, and ii) **bound ribosomes** which are attached to the outside of the ER giving the latter the name rough

ER or the outer face of the nuclear envelope (Fig. 2.16). Both ribosomes are structurally identical. Tiny organelles are composed of approximately 60% RNA and 40% protein. In eukaryotes ribosomes are made of four strands of RNA while in prokaryotes three strands of RNA are found.

2.6 TERMINAL QUESTIONS

1. What is the function of lysosomes?
2. How are the microtubules arranged in a cilium?
3. What are the main functions of *cis* and *trans* faces of Golgi bodies?
4. Name five structures found in mitochondria.
5. Differentiate between the functions of RER and SER.
6. What is the function of peroxisomes?
7. What is euchromatin and where is it found in the nucleus?

2.7 ANSWERS

1. Refer to Subsection 2.5.7.
2. In cilia, a core of microtubules forms a structure called axoneme which is covered by a sheath of plasma membrane. The axoneme possesses several microtubules running parallel to the long axis. In an axoneme usually a ring of nine doublets of radially arranged peripheral microtubules encircle two single centrally located microtubules such arrangement of microtubules is referred to as the "9 (2) + 2" pattern.
3. *cis* face of Golgi bodies receives the proteins from the endoplasmic reticulum for packaging, while *trans* face of Golgi bodies releases the modified proteins into the cytoplasm.
4. Cristae
Matrix
Outer membrane
Inner membrane
Intermembrane space
5. Rough endoplasmic reticulum (RER) has ribosomes attached to its surface where as ribosomes are not found on the surface of smooth endoplasmic reticulum (SER).
6. Refer to Subsection 2.5.8.
7. Euchromatin is the part of the chromatin material that is lightly packed enriched in genes and actively takes part in the transcription. Euchromatin is present in the inner body of the nucleus.

Acknowledgements

Fig. 2.1 : a) [https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.glycopedia.eu%2FIMG%2Fpng%2Fplantcellwal.png&imgrefurl=https%3A%2F%2Fwww.glycopedia.eu%](https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.glycopedia.eu%2FIMG%2Fpng%2Fplantcellwal.png&imgrefurl=https%3A%2F%2Fwww.glycopedia.eu%2F)

b) <https://www.researchgate.net/profile/Ahmed-Touhami/publication/8556471/figure/fig1/AS:280943182008324@1443993483009/High-magnification-TEM-of-a-thin-section-of-S-aureus-D-2-H-showing-the-cell-wall-CW.png>

Fig. 2.2 : a) https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fcytochemistry.net%2F_Media%2Fmembr1_med_hr.jpeg&imgrefurl=http%3A%2F%2Fcytochemistry.net%2Fcell-biology%2Fmembrane.htm

b) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww1.udel.edu%2Fbiology%2FWags%2Fhistopage%2Fempage%2Fecu%2Fecu14.gif&imgrefurl=https%3A%2F%2Fwww1.udel.edu>

Fig. 2.4 : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.biologypages.info%2FM%2Fmitochondrion.jpg&imgrefurl=https%3A%2F%2Fwww.biologypages.info%2FC%2FCellularRespiration.html>

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Fig. 2.5 : <https://image.slidesharecdn.com/biok1-140817011307-phpapp01/95/bioknowledgy-12-ultrastructure-of-cells-17-638.jpg?cb=1409870160>

Fig. 2.6 : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fimages.fineartamerica.com%2Fimages-medium-large-5%2Fendoplasmic-reticulum-tem-david-m-phillips.jpg&imgrefurl>

Fig. 2.7 : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fi.pinimg.com%2Foriginals%2Fcb%2Fbb%2F5a%2Fcbbb5a3453d85fafa027cb34b4f5a774.jpg&imgrefurl=https>

b) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.doctorc.net%2FLabs%2FLab3%2FIMAGES%2FORG16.JPG&imgrefurl>

Fig. 2.8 : <https://www.google.co.in/imgres?imgurl=http%3A%2F%2F1.bp.blogspot.com%2F>

Fig. 2.9 : [https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fperoxisomes.weebly.com%2Fuploads%](https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fperoxisomes.weebly.com%2Fuploads%2F)

- Fig. 2.10** : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fmegias.webs.uvigo.es%2F02-english%2F5-celulas%2Fimágenes-e%2F7-microtubulos.png>
- Fig. 2.11** : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fslideplayer.com%2Fslide%2F12727241%2F76%2Fimages%2F50%2FThis%2Belectron%2Bmicrograph%2F>
b) <http://www.histoweb.co.za/em/img/em-010.jpg>
- Fig. 2.12** : b) <http://www.histoweb.co.za/em/img/em-012.jpg>
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- Fig. 2.14** : <https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fbotit.botany.wisc.edu%2FResources%2FBotany%2FPlant%2520Cell%2FElectron%2520Micrographs%2FGeneral%2520Cell%2FYoung-Cell.jpg&https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fs3-us-west-2.amazonaws.com%2Fcourses-images%2Fwp-content%2Fuploads%2F>
- Fig. 2.15** : <https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fhistology.leeds.ac.uk%2Fcell%2Fassets%2Fnucleus2.gif&imgrefurl=http%3A%2F%2Fhistology.leeds.ac.uk%2Fcell%2Fnucleus.php&>
- Fig. 2.16** : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fslideplayer.com%2Fslide%2F5815406%2F19%2Fimages%2F10%2FVisualized%2Bon%2Ban%2BElectron%2BMicrograph.jpg>

EXPERIMENT 3

MICROMETRY|

Structure

3.1	Introduction	3.5	Micrometric Measurements
	Objectives	3.6	Measuring Microscopic Objects
3.2	Study Guide	3.7	Terminal Questions
3.3	Materials Required	3.8	Answers
3.4	Procedure		

3.1 INTRODUCTION

Micrometry is a technique to measure the size of objects viewed under a microscope. You can measure the diameter, height, length and breadth of the cells using micrometry technique. This technique can be used to measure diameter of red blood cells, cell nuclei, pollen grains, stomatal pore, and height of the columnar epithelial cells or dimensions of the epidermal cells, guard cells of a leaf, stomatal index and stomatal frequency. In this exercise, you will be explained the principle behind micrometry and how to make use of a micrometer scale to measure the size of microscopic objects.

Objectives

After performing this experiment you would be able to:

- ❖ distinguish between ocular micrometer and stage micrometer;
- ❖ calibrate an ocular micrometer with reference to a stage micrometer; and
- ❖ measure the size of the objects using the ocular micrometer.

3.2 STUDY GUIDE

For doing satisfactory work you must study the Unit 4 of the paper BBYET-141 before coming to the laboratory.

3.3 MATERIALS REQUIRED

1. A compound microscope

2. A 15 cm transparent plastic ruler (with millimeter divisions),
3. An ocular micrometer,
4. A stage micrometer,
5. A few prepared slides of epidermal peels of dicot leaves (mesophytes)
6. Sections of tissues showing cells and their nuclei distinctly.

3.4 PROCEDURE

Prior to the commencement of the experiment, record the name and number (if any) of the microscope, the magnification power of the ocular (eye piece) and objective lens with which you are measuring in your notebook. For example,

Make: Olympus

No. : (if provided)

Ocular: 10X

Objective: 10X

This information is necessary since any calibration and measurement you make is valid and specific for that particular microscope and its magnification. Change in microscope or magnification would require recalibration of the microscope. In other words, there will be differences in calibrated values between any two microscopes. This is also true when the magnifications are changed.

Before calibrating the microscope with micrometer, you may use a thin plastic ruler to find out the approximate field diameter of the microscope. Place the ruler on the stage of the microscope and position it in such a way that you could measure the maximum distance across the circular field. First bring the 10X objective lens to position, focus the ruler and record the measurement in your observation notebook. Now, find out the field diameter with the high-power objective (45X) in position. Record your observations. Is there any difference in the measurements made under different magnifications? If the answer is yes, then how do you account for this difference?

Microscope Make

No. :

S. No.	Ocular	Objective	Field Diameter (mm)
1.	10X	10X	
2.	10X	40X	

3.5 MICROMETRIC MEASUREMENTS

To measure an object under a microscope, two types of micrometers are required.

1. Ocular Micrometer
2. Stage Micrometer

Ocular Micrometer: Ocular micrometer is a small glass disc in the centre of which there is a line etched and divided into 100 divisions (Fig. 3.1).

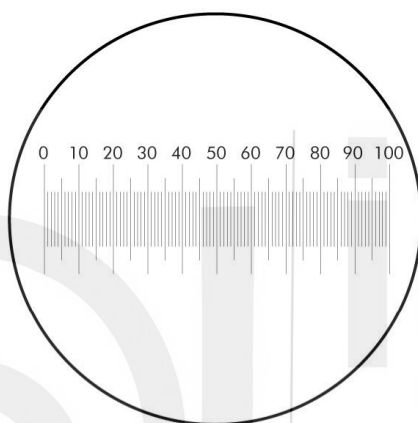
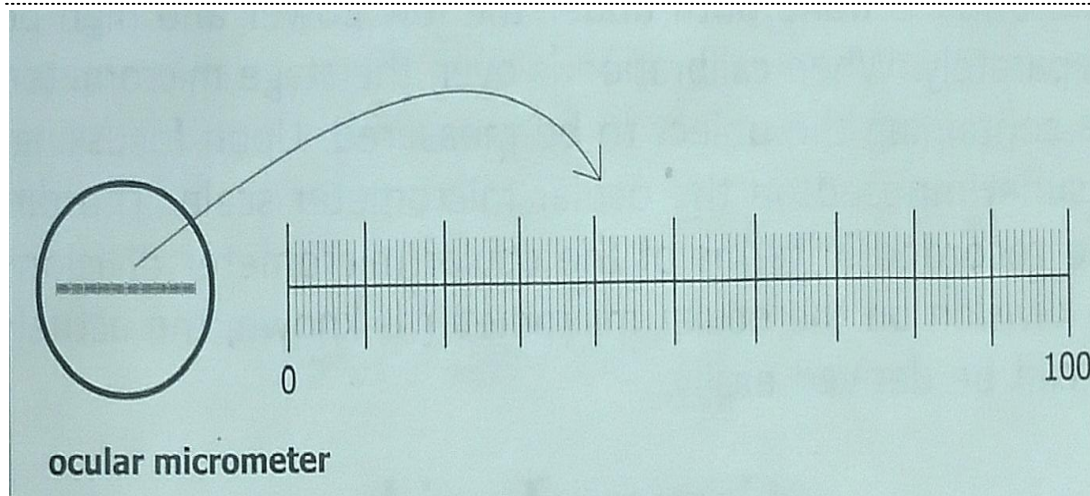
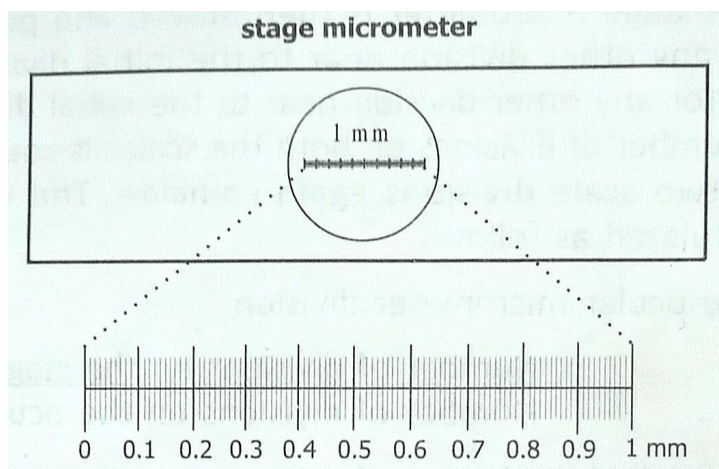
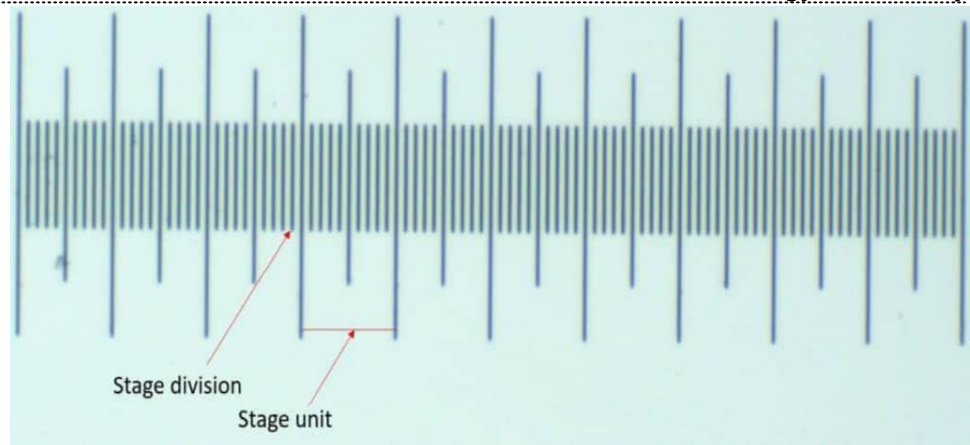


Fig. 3.1: An ocular micrometer with 10 divisions divided into 10 sub-divisions each. Dimensions are uncalibrated.

The value of each division is not known and must be calibrated in relation to stage micrometer at every magnification of the objective lens. The ocular micrometer can be fitted conveniently into the ocular lens of the microscope. Ocular lens etched with ocular micrometer is also available.

Stage Micrometer: A stage micrometer is a glass microslide in the centre of which a one-millimeter line is etched and divided into 100 divisions. Each division in a stage micrometer corresponds to 0.01 mm (10 μ m) (Fig. 3.2).





(b)

Fig. 3.2: a) A stage micrometer with 1 mm scale divided into 100 sub-divisions. Each small division is equal to $1/100=0.01\text{mm}$ ($10\text{ }\mu\text{m}$); b) scale showing stage division.

The stage micrometer is placed on the stage of the microscope and since it has known dimensions of its division, is used to calibrate the micrometer under different magnifications.

The procedure to measure the microscopic object is as follows:

1. Turn the nosepiece of the microscope to bring the 10X objective to position and place the stage micrometer under it. Fit the ocular micrometer into the ocular lens.
2. Bring into focus the 1 mm line of the stage micrometer.
3. Now, when you observe under the microscope, you should be able to see two sets of lines, one of stage micrometer and the other of ocular micrometer.
4. Looking through the microscope adjust the ocular lens and the stage micrometer appropriately so that the zero of the two scales coincide as shown in Fig. 3.3.

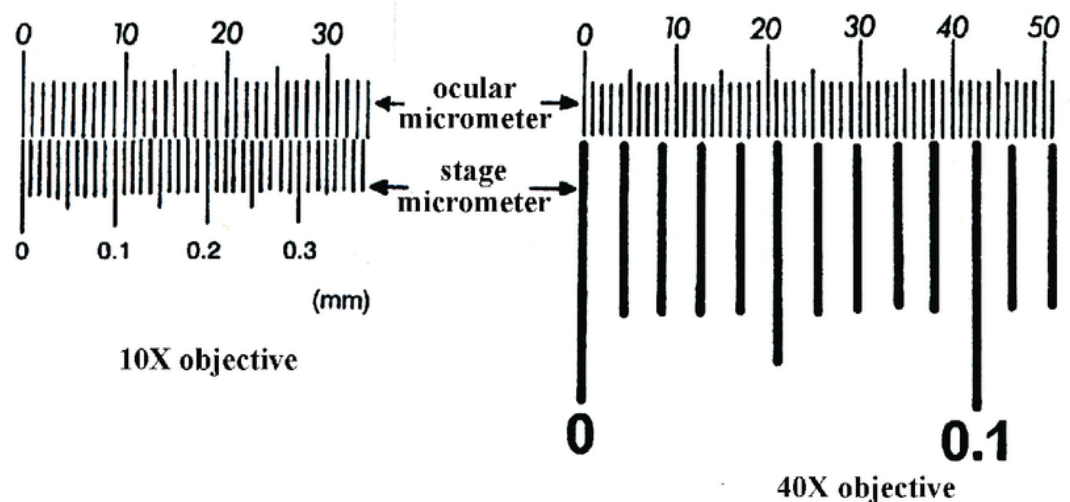


Fig. 3.3: Ocular and stage micrometer scales with the zero of each of them coinciding with the other.

5. Starting from zero position, move your eyes towards the right look for and read lines of coincidence across the field of the microscope. For instance, 20th division in the stage micrometer (SM) may coincide with 18th division in the ocular micrometer (OM). You may record such lines of coincidence in the table provided. Record at least five readings from left to the right of the field. You may now remove the stage micrometer and carefully replace it in the box. Do not remove the ocular micrometer.

Calculate as follows and determine the value of one OM division.

$$\text{Value of one OM division (microns)} = \frac{\text{SM reading}}{\text{OM reading}} \times 10$$

Where SM and OM readings refers to numbers relating to the lines of coincidence between the stage and the ocular micrometers

10 refer to the value of 1 SM division.

If you have recorded five readings, you may have calculated 5 different OM values. Obtain the mean of the five values. This mean value is the value of one OM division at the magnification of 10 X for the given microscope. As pointed out earlier, with the change in magnification, the value of one OM division also changes.

You may check this by calibrating the microscope with a 40X objective. Record your results and calculate the value of one OM division.

Ocular: 10X

Objective: 40X

S. No	OM Reading	SM Reading	$\frac{1}{V} \times 10$
1			
2			
3			
4			
5			

Ocular: 10X

Objective: 40X

S. No	OM Reading	SM Reading	$\frac{1}{V} \times 10$
1			
2			
3			
4			
5			

3.6 MEASURING THE MICROSCOPIC OBJECTS

Having calibrated the microscope, you may measure the size of various preparations. You may measure the length and breadth of a *Spirogyra* filament, diameter of cells of onion peel, human blood cells. All these measurements are made with the ocular micrometer and denote the number of OM divisions. The number of OM division that you have obtained for each measurement has to be multiplied by the value on one OM division you have obtained by calibrating the microscope.

For example, the length of *Spirogyra* cell

= Value of one OM division $\times$ No. of OM divisions

S. No	Magnification	Parameter measured	No. of OM Division	Value of one OM Division	Actual Value of measurement
1	10 $\times$ 10X	Length of <i>Spirogyra</i> cell	10	10.3 μm	103 μm
2	10 $\times$ 40X	Diameter of the nucleus of onion cell	5	2.5 μm	12.5 μm (Area = πr^2 diameter = 12.5 μm $r = \frac{d}{2} = \frac{12.5}{2} = 6.25$ Area = $\pi r^2 = 3.14 \times 6.25 \times 6.25 = 122.65 \mu\text{m}^2$
3					
4					
5					

3.7 TERMINAL QUESTIONS

1. Describe a stage micrometer and an ocular micrometer.
2. Calibrations of a microscope using a 10 $\times$ 10X magnification showed that one ocular division is equal to 9.8 μm . Measurements of the length of onion root tip cells showed that they are an average eight ocular micrometer divisions long. What is the actual length of the onion root tip cells?

3.8 ANSWERS

1. Refer to Section 3.5.
2. $9.8 \times 8 = 78.4 \mu\text{m}$.

Acknowledgements

Fig. 3.1 : <https://lh3.googleusercontent.com/-Re1aSSeXrEs/XIfcovJbell/AAAAAAAAApg/xcyYh5w6qLofVjJCGRspXhpTu4AjNP09wCLcBGAsYHQ/s1600/1582816413702268-1.png>

Fig. 3.2 : a) https://lh3.googleusercontent.com/-2bcd_gMlmeQ/XIfcquD0xI/AAAAAAAAApk/6iQCIGVqRG4SQ7dafxPpD-IYNLQ2fUlwgCLcBGAsYHQ/s1600/1582816420444645-0.png
b) <https://microscopeclarity.com/wp-content/uploads/2020/12/Measurement-terms.jpg>

Fig. 3.3 : <https://www.researchgate.net/profile/Jonathan-Eisenback/publication/234111617/figure/fig8/AS:649634728906753@1531896397110/Ocular-micrometer-calibrated-with-a-stage-micrometer-With-the-10X-objective-the-tenth.png>



EXPERIMENT 4

TO STUDY PLANT CELLS BY TEMPORARY MOUNTS

Structure

4.1	Introduction	4.6	Observations
	Objectives	4.7	Precautions
4.2	Study Guide	4.8	Terminal Questions
4.3	Materials Required	4.9	Answers
4.4	Procedure		
4.5	Preparation of Temporary Mount of a Plant Cell		

4.1 INTRODUCTION

In the Unit 1 of BBYET-141 (Cell and Molecular Biology) you have learnt about the parts of compound microscope. You have also studied micrometry and to measure the size of microscopic objects in the previous exercise. In this exercise, you will be introduced to the technique of preparation of temporary mounts of plant cells and how to study them under a compound microscope.

This exercise will familiarize you with the general structure of plant cell. The study of the animal tissues follows in the next exercise. Both these exercises will make you acquainted with the basic similarities and differences between these cells.

Objectives

After performing this experiment you would be able to:

- ❖ make temporary mounts of plant cells;
- ❖ describe the structure of plant cells; and
- ❖ list the characteristic features found specifically in plant cells.

4.2 STUDY GUIDE

For satisfactory work you must read Unit 8 of Block II of course BBYET-141 Cell and Molecular Biology.

4.3 MATERIALS REQUIRED

1. Compound Microscope
2. Slide and coverslips
3. Dissecting needles
4. Pasteur pipettes or droppers
5. Stain - Methylene blue
6. Glycerine
7. Filter paper
8. Onion bulb (fresh)
9. Scalpel or blade
10. Brush (No. 6)

4.4 PROCEDURE

To examine a material under microscope it is necessary to process the material first. In many cases you have to view the object as a wet mount. A wet mount or a temporary mount is prepared by placing a drop of liquid containing the material to be studied on a slide or in case of the dry material, by placing it directly on the slide and adding a drop of water or stain or glycerine to it. The mount is then covered by a coverslip as illustrated in Fig. 4.1.

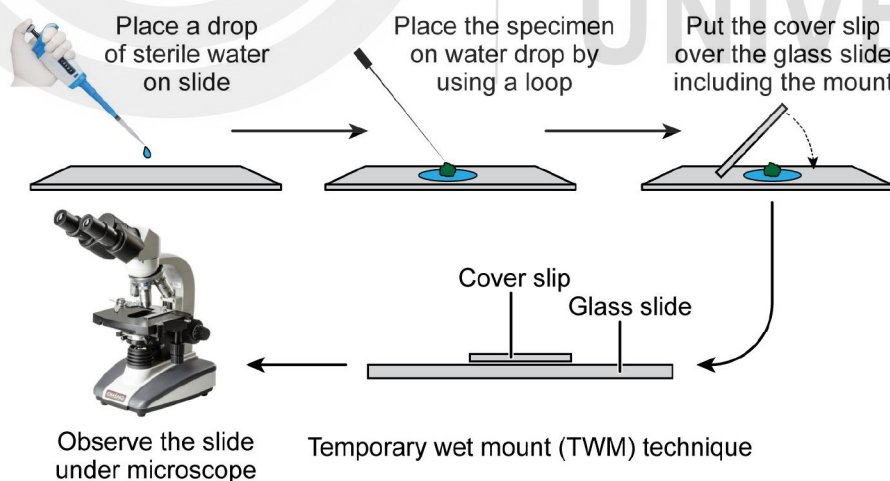


Fig. 4.1: An outline of the procedure for preparation of a slide for microscopic examination.

As shown in the figure, while placing the coverslip, place a needle under the coverslip and slowly let it down on the drop of the liquid. If you allow the coverslip to fall on the slide, it will trap air bubbles along with the specimen which will interfere with your observation.

4.5 PREPARATION OF TEMPORARY MOUNT OF A PLANT CELL

You can make a temporary mount of plant cells with epidermal cells obtained from onion by the following procedure:

1. Cut an onion into quarters and separate the thick modified leaves which make up the bulb. Note that inner and outer surfaces of each leaf are covered by a delicate membrane. The membrane on the inner or concave surface can be stripped off readily.
2. With a scalpel or blade strip off a thin layer from inside of the onion leaf.
3. Gently place it flat on the slide, cut a small suitable square and discard the rest.
4. Quickly add a drop of water and a drop of the stain with the help of Pasteur pipette.
5. Remove the excess stain using the edge of filter paper and add a drop of glycerine.
6. Place a coverslip and press it gently with the back of a pencil to spread the peel and remove the air bubbles if any.
7. Examine the slide under the microscope.

You may also use methylene green or iodine to stain the cells.

4.6 OBSERVATIONS

In the **wet whole mount** of onion epidermal cells you can see rectangular shaped cells lying side by side with distinct cell walls. You can see the nucleus near the cell wall in every cell (see Fig. 4.2).

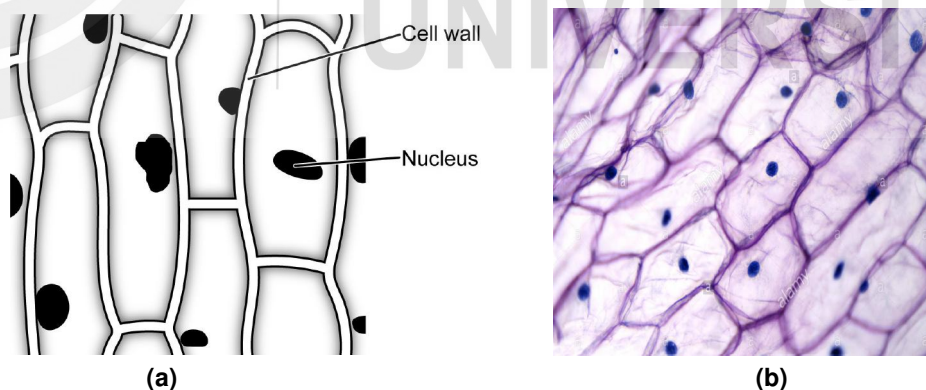


Fig. 4.2: Whole mount (WM) of onion epidermal cells. a) Diagrammatic representation; b) Microscopic image.

Examine these wet mounts under higher magnification and record your observations.

4.7 PRECAUTIONS

You should take the following precautions while making temporary preparations.

1. Slides and coverslips to be used should be clean and free from dust and fingerprints.
2. The material and fluid should be placed centrally on the slide.
3. The amount of fluid should be appropriate. With too much of fluid the coverslip will move around and with too little of fluid you will have air bubbles trapped inside.
4. The excess mounting fluid should be removed carefully from the sides of the coverslip using the edge of a filter paper. Filter paper moistened with water will help remove excess glycerine.
5. Place the coverslip gently to prevent trapping of air bubbles.
6. Do not let the specimen dry out. Use a drop of water to keep it moist.

4.8 TERMINAL QUESTIONS

1. What is the advantage of using stains while preparing the temporary mounts?
2. Refer to Exercise 3 and measure the size of the cells and nucleus in the plant cell. Record your results in your observation notebook in the following format.

Measurement		
Plant	Cell Size	Nucleus size

4.9 ANSWERS

1. Stains help in better visualization of the cells and its components.
2. The size of the plant cell generally varies between 10-100µm. The size of nucleus varies between 2 to 10 µm. You can take out few plant cells and measure the size to verify these facts.

Acknowledgement

Fig.4.1 : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fmicrobiologynote.com%2Fwp-content%2Fuploads%2F2021%2F01%2FTemporary-Wet-Mount-TWM-technique.jpg&imgrefurl>

Fig. 4.2 : <https://c8.alamy.com/comp/HWA476/onion-epidermis-with-large-cells-under-light-microscope-clear-epidermal-HWA476.jpg>

EXPERIMENT 5

STUDY OF ANIMAL CELLS BY TEMPORARY MOUNTS OF SQUAMOUS EPITHELIUM AND PHOTOGRAPH OF NERVE CELL

Structure

5.1	Introduction	5.5	Observations
	Objectives	5.6	Nerve cells
5.2	Study Guide	5.7	Precautions
5.3	Materials Required	5.8	Terminal questions
5.4	Preparation of Temporary Mount of Squamous Epithelial Cells	5.9	Answers

5.1 INTRODUCTION

In previous exercise you have learnt about plant cells and methods to prepare them for microscopic observations. In this exercise we will be studying the structure of some of the cells found in animals. You will be examining epithelial cells obtained from the human buccal cavity. Epithelial tissues are usually arranged in a single or multilayered sheet. They cover the internal and external surfaces of the body of an organism. The lower ends of the epithelial cells are attached to a basement membrane. The main function of the epithelial tissues is to cover other tissues and protect them from injury through abrasion, pressure, or infection. The free surfaces of the epithelial cells are highly modified to perform specific functions such as absorption, secretion and excretion. Depending upon their shape and functions, epithelial cells are classified into five categories-squamous epithelial cells, cuboidal epithelial cells, columnar epithelial cells, ciliated epithelial cells and glandular epithelial cells.

These cells are found in the lining of the nephrons, the alveoli of the lungs and walls of the blood capillaries. These cells also provide smooth lining to hollow

structures such as blood vessels and chambers of the heart. Squamous epithelial cells permit diffusion of materials because of their thinness. In blood vessels and heart, the cells allow a friction free flow of blood.

In addition, you will also study the structure of a human nerve cell with the help of photographs provided to you. Nerve cells or neurons are specialized cells with properties of responding to stimuli, conductivity and communication. The brain and spinal cord for example, are composed of a network of nervous tissue. The neurons or nerve cells are the units of the nerve tissue.

Objectives

After performing this experiment you would be able to:

- ❖ describe the structure of squamous epithelial cells; and
- ❖ study the different parts of the nerve cells.

5.2 STUDY GUIDE

For satisfactory work you must study the course BZYCT-135 (Physiology and Biochemistry) before coming to the laboratory.

5.3 MATERIALS REQUIRED

1. Compound microscope
2. Slide and coverslips
3. Pasteur pipettes or droppers
4. Stain - Methylene blue
5. Glycerine
6. Filter paper
7. 0.9% NaCl solution
8. Photographs of human nerve cells

5.4 PREPARATION OF TEMPORARY MOUNT OF SQUAMOUS EPITHELIAL CELLS

Let us learn the procedure for making a temporary mount of buccal (cheek) squamous epithelial cells.

1. Rinse your mouth well with water.
2. Gently scrap the inside of your cheek with the broad end of a clean toothpick. Discard this material.
3. Scrape again and spread these cells gently on a clean slide. Add a drop of 0.9% NaCl or physiological saline and a drop of methylene blue with the help of a dropper.

4. After two minutes, remove the excess stain and saline using the edge of a filter paper and add a drop of glycerine on the cells.
5. Mount with a coverslip. Gently press the coverslip with the back of a pencil to spread the cells.
6. Finally examine the slide under the microscope.

You can also make the wet mount of human epithelial cells without using glycerine. In such a case spread the cells on a clean, dry slide. Add a drop of 0.9% NaCl solution and mount a coverslip. The cells are stained by adding a drop of the stain at the periphery of the coverslip. The excess saline and stain can be dried from the slide by using the edge of a filter paper. You can also directly stain the specimen on the slide before covering it with the coverslip..

5.5 OBSERVATIONS

In the wet mount of cheek epithelial cells, you can see irregular shaped cells flat cells with a thin membrane. The nucleus stained dark blue is seen as a central round body in each cell (Fig. 5.1) with little cytoplasm enclosing it. When viewed from the surface, these cells present a mosaic appearance.

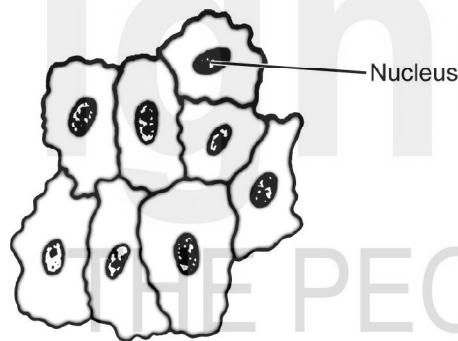
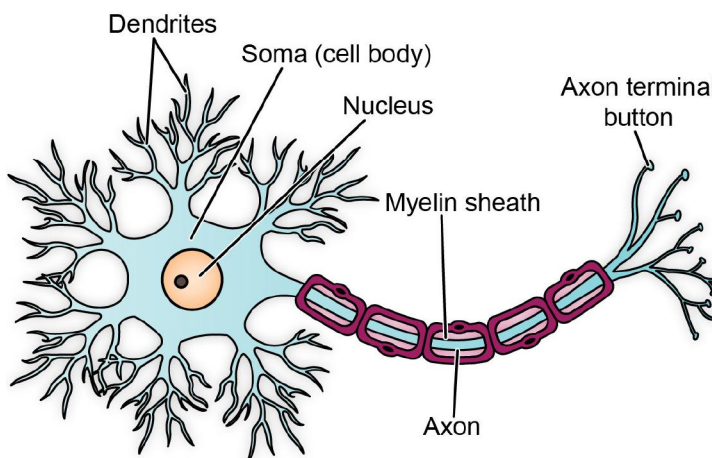


Fig. 5.1: Whole mount (WM) of epithelial cells from inner lining of the cheek.

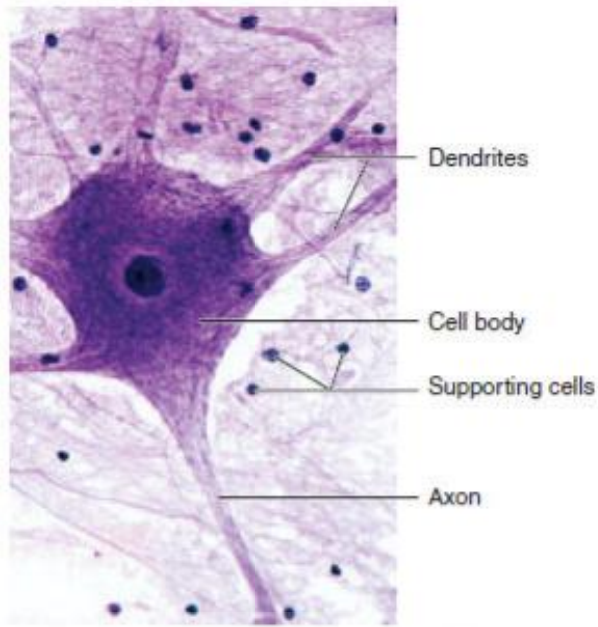
5.6 NERVE CELLS

Observe the photographs of these nerve cells and make neat and labelled sketches of the same in your observation notebook.

Typically, each neuron has three parts (Fig. 5.2 a).



(a)



(b)

Fig. 5.2: a) Structure of a neuron; b) Structure of nerve cell as observed under the microscope.

1. Soma (cyton), the cell body of the neuron.
2. dendron, one or more short processes arising out of the cyton.
3. axon, a single long process forming fine terminal branches ending in an effector organ or another neuron (Fig. 5.2 b).

A cyton has a centrally placed nucleus and the cytoplasm has fine granules called Nissl bodies. A cyton may have a single dendron in which case the neuron is known as the unipolar neuron. Presence of a dendron and an axon makes a neuron bipolar one while presence of several dendrons make it a multipolar neuron (Fig. 5.3).

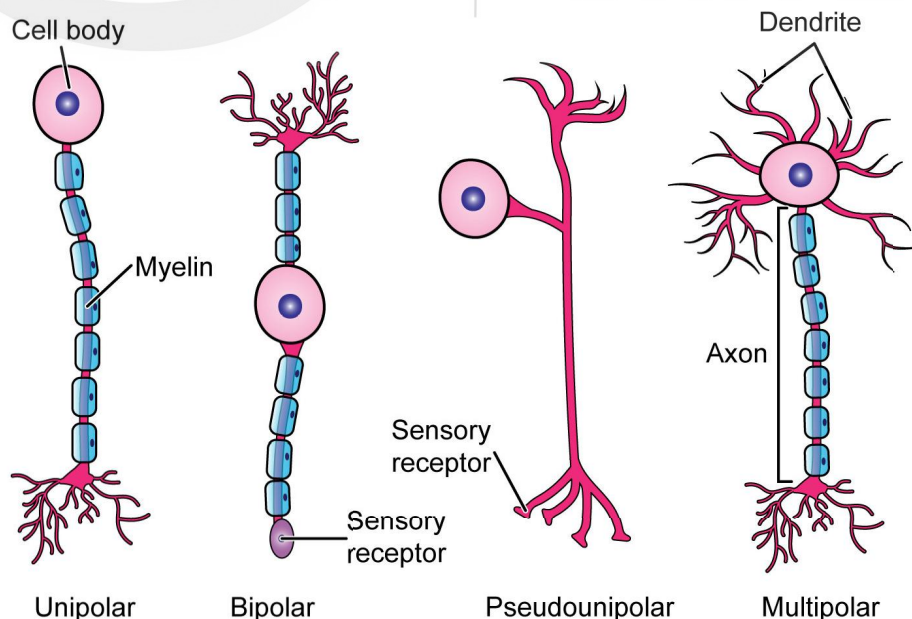


Fig. 5.3: Types of nerve cells.

An axon may be surrounded by a myelin sheath and come to be known as a medullary fiber and in the absence of the myelin sheath it is a non-medullary fiber. Both medullary and non-medullary axons are surrounded by a membrane the neurilemma. The axons conduct the electrical signals away from the cyton. These signals are a result of a flux of ions across the nerve cell membrane.

5.7 PRECAUTIONS

You should take the following precautions while making temporary preparations.

1. Slides and coverslips to be used should be clean and free from dust and fingerprints.
2. The material and fluid should be placed centrally on the slide.
3. The amount of fluid should be appropriate. With too much of fluid the coverslip will move around and with too little of fluid you will have air bubbles trapped inside.
4. The excess mounting fluid should be removed carefully from the sides of the coverslip using the edge of a filter paper. Filter paper moistened with water will help remove excess glycerine.
5. Place the coverslip gently to prevent trapping of air bubbles.
6. Do not let the specimen dry out. Use a drop of water to keep it moist.

5.8 TERMINAL QUESTIONS

1. Which are the five categories of epithelial cells?
2. Enlist the different parts of a neuron.
3. Find out the function of nerve cell.
4. Describe the characteristics of epithelial cells.

5.9 ANSWERS

1. Five types of epithelial cells are categories-squamous epithelial cells, cuboidal epithelial cells, columnar epithelial cells, ciliated epithelial cells glandular epithelial cells.
2. Neurons have three parts: a cell body (cyton/soma), an axon, and dendrites. Cell body is the neuron's core and contains nucleus and specialized organelles. Axon is a long, tail-like structure which joins the cell body at a specialized junction called the axon hillock. Axons are insulated with a fatty substance called myelin. Dendrites are fibrous roots that branch out from the cell body.
3. The nerve cell or neuron transmits messages to the brain and also takes messages from the brain and send it to the receptor organs.

4. Epithelial tissues are continuous layer of cells forming a sheet. These cells form covering for all the outer surfaces of the body such as skin. They also form the lining of respiratory, digestive, reproductive and excretory tracts. One surface of the epithelial tissue is exposed to the external environment or the body fluid, while the other surface is attached to tissue by a membrane, which consists of fibers and polysaccharides secreted by epithelial cells.

Acknowledgements

- Fig. 5.2** : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Ftrustudies-app-public.s3.ap-south-1.amazonaws.com%2Ftypes-of-neurons-QBI.jpg&imgrefurl=https%3A%2F%2Fqbi.uq.edu.au%2Ffiles%2F27928%2Ftypes-of-neurons-QBI.jpg>
- b) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Ftrustudies-app-public.s3.ap-south-1.amazonaws.com%2Ftypes-of-neurons-QBI.jpg&imgrefurl=https%3A%2F%2Fqbi.uq.edu.au%2Ffiles%2F27928%2Ftypes-of-neurons-QBI.jpg>
- c) <https://i.pinimg.com/originals/62/7a/25/627a257cdaa9a90da83533a0d284516c.png>



EXPERIMENT 6

STUDY OF STRIATED MUSCLE FIBERS THROUGH PHOTOGRAPHS

Structure

6.1	Introduction	6.5	Observations
	Objectives		Skeletal Muscles
6.2	Study Guide		Cardiac Muscles
6.3	Materials Required	6.6	Terminal Questions
6.4	Procedure	6.7	Answers

6.1 INTRODUCTION

Muscles are responsible for locomotion and movement of internal organs. Contractility is the characteristic property of all muscle cells. They produce the mechanical force by their contraction. Muscular or contractile tissue cells are of three types viz. skeletal muscles, smooth muscles, and cardiac muscles. The three types of muscles differ in their structure. Striated muscles are mainly responsible for creating force and contraction. The striated muscles are of two types: i) Skeletal muscles which include skeletal muscle fibers, blood vessels, nerve fibers, and connective tissue which are responsible for posture, body movements and breathing, and ii) Cardiac muscles, constituting the heart connected with pumping of blood throughout the body.

The contractions of striated muscle pumps blood throughout the body (cardiac muscles) or powers breathing, movement or posture (skeletal muscles).

In this exercise you will be studying the internal structure of the striated muscles in the form of skeletal and cardiac muscles.

Objectives

After going through these photographs, you will be able to:

- ❖ describe the structure of skeletal and cardiac muscles, and
- ❖ identify the tissues by identifying the characteristic structural features of striated muscles.

6.2 STUDY GUIDE

For having good comprehension of this exercise, you must go through the theory units before coming to the laboratory.

6.3 MATERIALS REQUIRED

You will be provided with the photographs of:

1. Skeletal Muscles
2. Cardiac Muscles

6.4 PROCEDURE

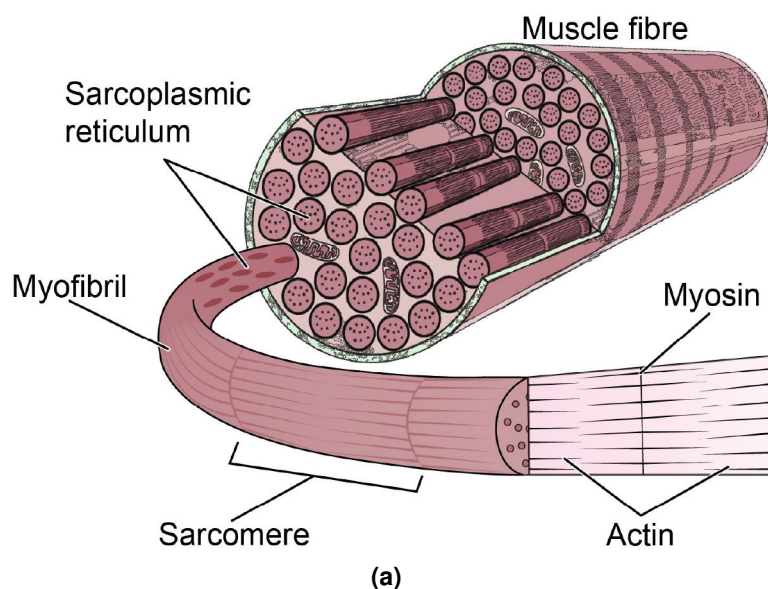
You have been provided with photomicrographs of striated muscles of various organs. Observe each one of them carefully and note down the characteristic features of each one of them.

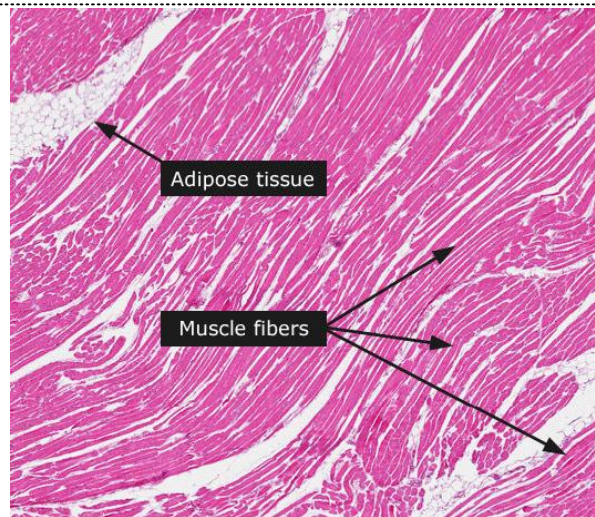
6.5 OBSERVATIONS

You have been provided with photomicrographs of striated muscles of various organs. Observe each one of them carefully and note down the characteristic features of each one of them.

6.5.1 Skeletal Muscles

These muscles are also referred to as voluntary or striated muscles (Fig. 6.1). Each muscle is a bundle of muscle fibers each of which has a very large multinucleate cell. Usually, these cells are very long and cylindrical enclosed by a thin membrane called sarcolemma. Each fiber has several longitudinal myofibrils embedded in cytoplasm called sarcoplasm. Each myofibril is made of alternating light and dark bands or cross striations. Skeletal muscles help in the movement of joints by their strong and rapid contraction. Since the contractions are under the control of our will they are also called as voluntary muscles.



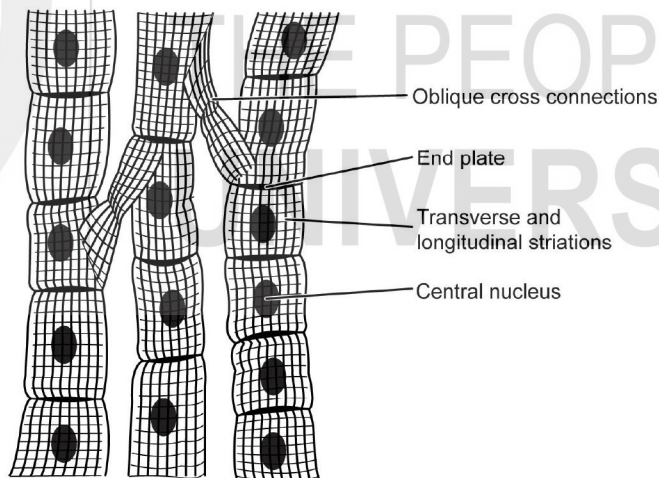


(b)

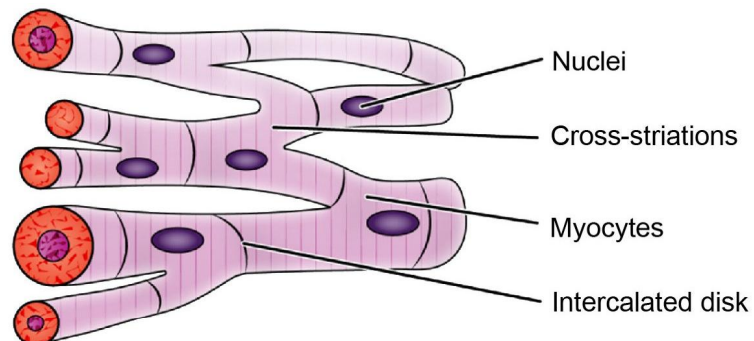
Fig. 6.1: a) Diagram showing structure of skeletal muscle; b) Microscopic image of skeletal muscles.

6.5.2 Cardiac Muscles

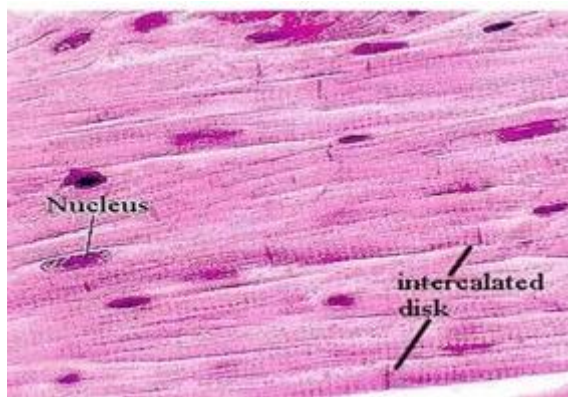
Structurally the cardiac muscles are intermediate between skeletal and smooth muscles (Fig. 6.2). Heart is made up of cardiac muscles. Like the striated muscles they are also branched, and the branches are connected to each other. There is a sarcolemma surrounding the muscle fibers and sarcoplasm with the longitudinal myofibrils of alternating light and dark bands. There is only a single nucleus in each fiber.



(a)



(b)



(c)

Fig. 6.2: a) Structure of a cardiac muscle; b) Cross section of cardiac muscles; c) Microscopic image of cardiac muscles.

6.6 TERMINAL QUESTIONS

1. List major differences between striated and smooth muscles.
2. What are the characteristic features of cardiac muscles?
3. Enlist the functions of Skeletal Muscles.
4. Enlist the functions of Cardiac Muscles.

6.7 ANSWERS

1.

Striated muscle	Smooth muscle
Striated muscle is composed of long and cylindrical muscle fibers.	Smooth muscles are spindle shaped.
These muscles are voluntary in nature.	These muscles are involuntary in nature.
Striated muscles have alternative dark and light bands due to actin and myosin.	Smooth muscles do not have any bands.
2. Cardiac muscles are, striated, branched, and single nucleated. Cardiac muscles are involuntary and intrinsically controlled muscles found only in the heart. These muscles are specialized to pump blood from the heart to the different parts of the body. Cardiac muscle appears striated due to the presence of sarcomeres.
3. Skeletal muscles help in the movement of bones and joints. They protect the internal body organs (abdominal cavity) by acting as an external barrier. Skeletal muscles contribute to the maintenance of homeostasis in the body by generating heat.
4. Cardiac muscles regulate the functioning of the heart by the relaxation and contraction of the heart muscles. The cardiac muscles also play a role in movement or the locomotion.

Acknowledgements

Fig. 6.1 : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.online-sciences.com%2Fwp-content%2Fuploads%2F2018%2F05%2FStructure-of-skeletal-muscle-3-300x251.jpg>

b) https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fv15.proteinatlas.org%2Fimages_dictionary%2Fskeletal_muscle__1__example_1__overview.jpg&imgrefurl=https%3A%2F%2Fv15.proteinatlas.org%2Fimages_dictionary%2Fskeletal_muscle__1__example_1__overview.jpg

Fig. 6.2 : b) https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fhaygot.s3.amazonaws.com%2Fquestions%2F636902_519917_ans.JPG&imgrefurl=https%3A%2F%2Fwww.toppr.com

c) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.assignmentpoint.com%2Fwp-content%2Fuploads%2F2017%2F08%2FCardiac-Muscle.jpg&imgrefurl=https%3A%2F%2Fwww.assignmentpoint.com%2Fwp-content%2Fuploads%2F2017%2F08%2FCardiac-Muscle.jpg>



EXPERIMENT 7

VITAL STAINING OF MITOCHONDRIA WITH JANUS GREEN B IN BUCCAL (CHEEK) EPITHELIUM AND PLANT EPIDERMAL CELLS

Structure

7.1	Introduction	7.5	Observations
	Objectives		Buccal Epithelial Cells
7.2	Study Guide		Leaf Epidermal Cells
7.3	Materials Required	7.6	Precautions
7.4	Procedure	7.7	Terminal Questions
	Preparation of Dye	7.8	Answers
	Vital Staining in the Buccal Epithelial Cells		
	Vital Staining in the Leaf Epidermal Cells		

7.1 INTRODUCTION

Vital staining is a method for the demonstration of details of live tissues and cells using certain specific dyes. A vital dye is one which indicates the living nature of a cell. It shows whether the cells are alive. Such stains can either indicate the functioning of enzymes within certain organelles indicating that they are alive (e.g., Janus Green B and Triphenyl tetrazolium chloride-TTC). Vital dyes are also helpful for studying the cell and tissue permeability phenomenon related to absorption, transport, accumulation, and excretion of substances (e.g., Fluorescein diacetate). Vital stains either belong to acidic or basic type. Janus Green B is a basic dye.

L. Michaelis selectively stained mitochondria with Janus Green B in living cells. The *cytochrome oxidase* enzyme present in the mitochondrial electron

transport chain reacts with this dye to give a blue green colour. Other components of the cytoplasm are not stained. Michaelis proposed that mitochondria were the organelles with oxidizing reactions.

Objectives

After doing this exercise you should be able you to:

- ❖ make temporary mounts of the buccal epithelial cells and leaf epidermal cells;
- ❖ demonstrate whether cheek cells and plant epidermal cells are living; and
- ❖ distinguish between the vital and non-vital dyes.

7.2 STUDY GUIDE

For doing satisfactory work you must read the Unit III Cell Structure and Function Zoology course BZYCT 135 before coming to the laboratory.

7.3 MATERIALS REQUIRED

You will be provided with a compound microscope and the following materials:

1. Slides and coverglass
2. Dissecting needles
3. Pasteur pipettes or droppers
4. Filter paper
5. Toothpicks
6. Cotton wool
7. Scalpel or razor blade
8. Camel hair brush (No.6)
9. Measuring cylinder (100mL)
10. *Calendula* leaves (fresh)/Onion bulbs
11. 0.2% Janus Green B

7.4 PROCEDURE

7.4.1 Preparation of Dye

You will be provided with the Janus Green B dye ($C_{30}H_{31}ClN_6$) for the experiment.

The dye is prepared in the following manner:

- a) 0.2% Janus Green B in 0.9% saline solution (Dissolve 0.9g NaCl in 100 mL distilled water).
- b) Dissolve 200mg Janus Green B powder in some 0.9% saline and make the final volume to 100mL with saline.

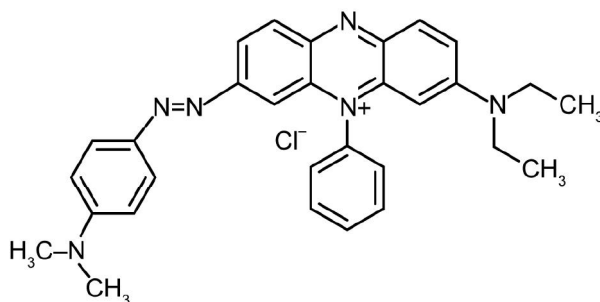


Fig. 7.1: Chemical structure of the dye, Janus Green.

7.4.2 Vital Staining in the Buccal Epithelial Cells

1. Rinse your mouth well with water.
2. Using a spatula or the broad side of a tooth pick scrape the inner side of your cheek. Discard the first scraping.
3. Repeat this step and gently spread the scraping on a clean slide.
4. Pour 3-4 drops of freshly prepared Janus Green B stain.
5. Keep the slide for 10 min. Ensure that the stain does not dry up. Replenish the stain if necessary.
6. Place a cover slip on the preparation. Deliberately allow the entry of some air bubbles inside the cover slip.
7. Observe the slides carefully under the compound microscope both under low and high power.

7.4.3 Vital Staining in the Leaf Epidermal Cells

1. Take out the epidermal peel from the upper surface of *Calendula* leaf.
2. Place the epidermal peel in freshly prepared Janus Green B stain in a watch glass.
3. Incubate for 20 min at room temperature.
4. Remove the epidermal peel and mount it on a glass slide in the same stain.

7.5 OBSERVATIONS

Observe the slides carefully under the compound microscope both under low and high power. Note the structural details of each type of cells. Make neat, well- labelled diagrams (both outline and a portion enlarged to show the cellular details) in your note book and write comments. It is advised that figures should not be copied from the book but should be drawn after observing the slides under the microscope.

7.5.1 Buccal Epithelial Cells

In the wet mount of buccal (cheek) epithelial cells you can see irregular shaped cells with a thin membrane (Fig. 7.2 a, b).

Many rod-shaped and spherical bodies are stained blue/blue green in the cytoplasm which represent the mitochondria.

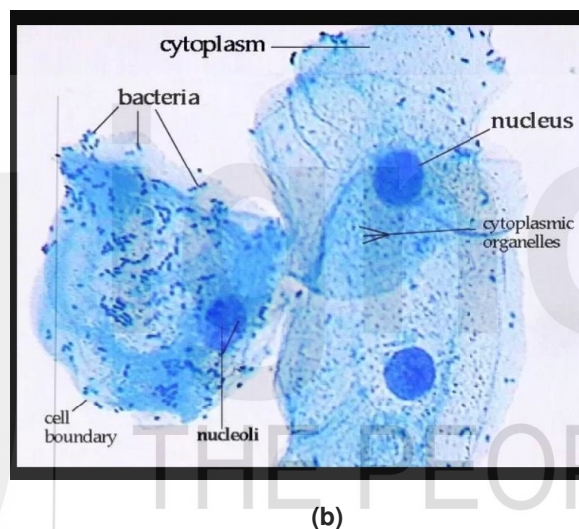
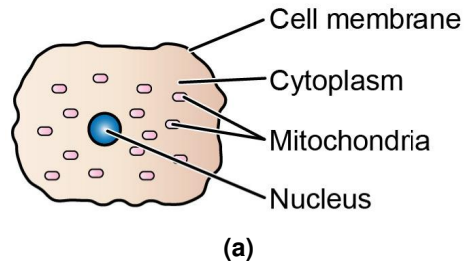


Fig.7.2: a) Structure of cheek cells; b) Janus Green-stained cheek cells as observed under the microscope.

7.5.2 Leaf Epidermal Cells

Similarly several randomly distributed green coloured rod-shaped and spherical bodies representing the mitochondria are observed in the cytoplasm of the epidermal cells.

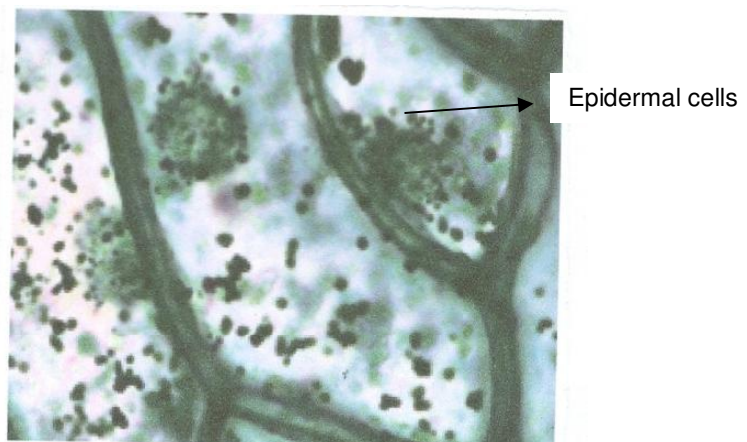


Fig. 7.3: Structure of epidermal cells as observed under microscope.

7.6 PRECAUTIONS

1. Freshly prepared Janus Green dye should be used.
2. Both the scraped Buccal cavity and the epidermal cells must be freshly taken and must be immediately incubated in the stain.
3. Ensure that some air bubbles enter beneath the cover slip. This will create an aerobic environment for the proper functioning of the mitochondria.

7.7 TERMINAL QUESTIONS

1. Name some more vital dyes.
2. Why are the air bubbles encouraged in the slide preparations?

7.8 ANSWERS

1. Rhodamine and Fluorescein diacetate (FDA),
2. The bubbles help in creating an aerobic environment for the functioning of the mitochondria.

Acknowledgements

Fig. 7.1 : [https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.sigmaaldrich.com%2Fdeepweb%](https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.sigmaaldrich.com%2Fdeepweb%2F)

Fig. 7.2 : a) <https://www.google.co.in/imgres?imgurl=x-raw-image%3A%2F%2F%2F4bc2a2cd7dcd2a3247213e5db572a28e4df0a93ba3b76cc2eee6e5b9d9a1f7b5&imgrefurl>
b) [https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fqph.fs.quoracdn.net%2Fmain-qimg-3daab99f6967306bb17dfc25b923eb8e.webp&imgrefurl=http%3A%2F%2Fwww.quora.com%](https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fqph.fs.quoracdn.net%2Fmain-qimg-3daab99f6967306bb17dfc25b923eb8e.webp&imgrefurl=http%3A%2F%2Fwww.quora.com%2F)

Fig. 7.3 : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fphotos.smugmug.com%2FFullCatalogs%2FPlants%2FPlantTissues%2FFi-vqTbj43%2F3%2Fdc70a42b%2FL%2FPlantTissue-132TradescanEpiderm100X-L.jpg>

EXPERIMENT 8

TO STUDY MITOSIS USING ONION ROOT TIPS

Structure

8.1	Introduction	8.4	Procedure
	Objectives	8.5	Observations
8.2	Study Guide	8.6	Terminal Questions
8.3	Materials Required	8.7	Answers

8.1 INTRODUCTION

Squash technique is one of the simple techniques widely used for the study of chromosomes. The technique consists of applying a gentle pressure on a small piece of previously stained tissue to flatten the cells and spread the chromosomes. The technique can be performed rapidly and is an efficient one for the study of dividing cells. The method helps to study a single layer of large cells in their totality.

Mitosis is an equational division that is normally associated with the division of somatic cells. This type of cell division is typical of meristems. The process of mitosis plays an important role in growth, regeneration and healing of wounds.

Objectives

After doing this exercise you should be able to:

- ❖ make squash preparations of plant material such as onion root tip and observe and interpret mitotic stages; and
- ❖ compare the processes of mitosis and meiosis.

8.2 STUDY GUIDE

For doing satisfactory work you must study the Unit 8 of course BBYET-141 Cell and Molecular Biology before coming to laboratory.

8.3 MATERIALS REQUIRED

You will be provided with the following material for the present exercise.

1. Compound microscope
2. Onion root tips
3. Acetic alcohol
4. Acetocarmine or acetoorcein
5. 2 N Hydrochloric acid (HCl)
6. Slides
7. Pasteur pipettes
8. Coverslips
9. Spirit lamp
10. Watch glass
11. Filter paper
12. Dissection kit
13. Nail polish or DPX mountant

8.4 PROCEDURE

Onion root tip is an ideal material for the preparation of mitotic stages because of its easy availability. The chromosomes found in the material are relatively large and few in number. The onion roots can be grown by placing onion bulbs with their root side down in contact with water in a beaker or a conical flask.

After 3 days you can see the roots grow to a length of 2 to 3 cm. remove the onion bulbs, cut the root tips, and transfer them to a solution of acetic alcohol (acetic acid 1 part and ethyl alcohol 3 parts v/v). The root tips should be kept in the acetic alcohol solution for 12 to 24 h. At the end of the fixation period the material can be transferred to and stored in 70% alcohol until it is used for squash preparation.

Now, follow the procedure given below for squash preparation:

1. Transfer the root from the fixative (acetic alcohol) or storage solution (70% alcohol) onto a watch glass and wash extensively with water.
2. Drain off the water with a Pasteur pipette and add a few drops of 2N HCl to the watch glass. The tissues can be hydrolyzed in 2N HCl for 10 min at room temperature or for a minute over a spirit lamp flame. While hydrolyzing over the flame, make sure that you move the watch glass over the flame. This would prevent damage to the tissue by overheating.
3. After hydrolysis drain off the HCl and wash the root tips in water.

4. Remove water and add 1% acetocarmine or acetoorcein to the root tips for staining. Keep the slide for staining for 10 to 15 min.
5. At the end of the staining process, transfer 2-3 root tips onto a slide, cut and retain only the meristematic region and remove the debris from the slide.
6. Place a drop of 45% acetic acid on the material and carefully place a coverslip over it.
7. Remove the excess acetic acid from the sides of coverslip by using filter paper.
8. Place the slide between the filter paper and gently press the coverslip down. Using the flat bottom of a pencil slowly tap on the filter paper to obtain a uniform spread of the material. The tapping flattens the cells and spreads the chromosomes.
9. Put a coverslip and seal the edges of coverslip by applying nail polish so that the evaporation of fluid is minimized.
10. Observe the slide under the compound microscope.

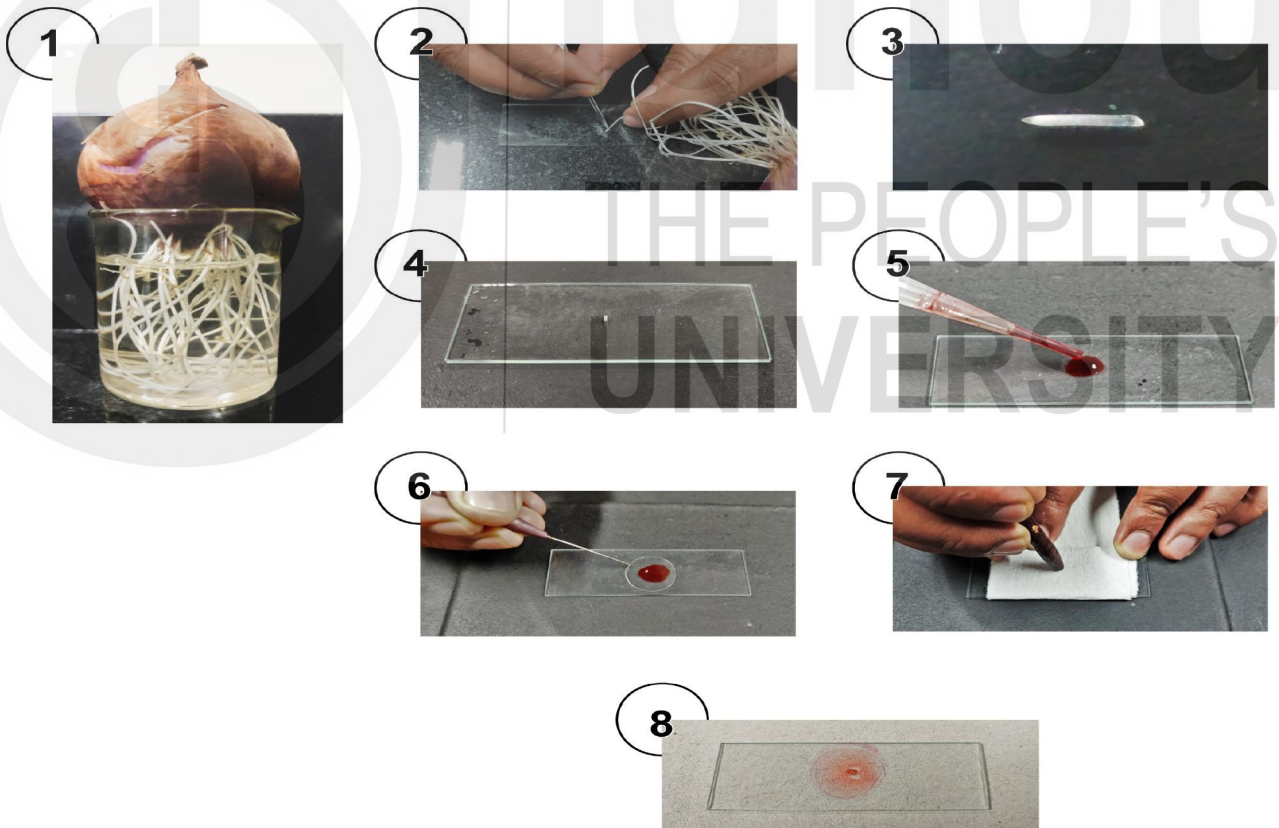


Fig 8.1: Various steps in the squash preparation of onion root tip cells for study of mitosis. 1) onion roots coming from bulb; 2) separation of roots from bulb; 3) a portion of onion root tip; 4) onion root tip placed on a slide; 5) acetocarmine stain on the root tip; 6) squash the root tip to get separation of cells; 7) placing slides between folds of filter paper to remove excess stain; 8) slide of squash preparation with coverslip placed on it.

8.5 OBSERVATIONS

You are already familiar with the concept of cell cycle and know that cells can be observed in either dividing or non-dividing phase of the cycle (Fig. 8.2). First observe the slide under low magnification and look for a region which is comparatively more darkly stained than the rest of the slide. Without changing the settings, switch to high power and look for the various stages of mitosis.

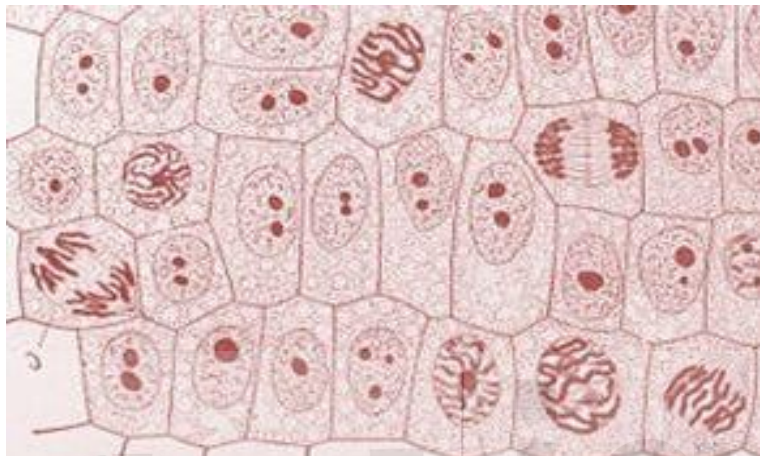


Fig. 8.2: Squash preparation of onion root tip cells showing dividing and non-dividing cells.

In a squash preparation you will find that majority of the cells are in interphase (non-dividing cells). The interphase nucleus will appear as a darkly stained body with acetocarmine or acetoorcein. The nuclear membrane is intact and the chromatin is in the form of a network.

We now provide you a brief description of each stage and you should sketch the stages that you observe under the microscope in the record notebook. It is advised that figures should not be copied from the photographs (given only for reference and identification) but should be drawn after observing the actual slides.

Stages of Mitosis

Interphase

- This stage is prior to actual mitotic cycle.
- In this stage, cell appears to be inactive but metabolically is the most active stage in cell cycle.
- Nuclear membrane and nucleolus are very distinct.
- Chromosomes appear double stranded.

Prophase

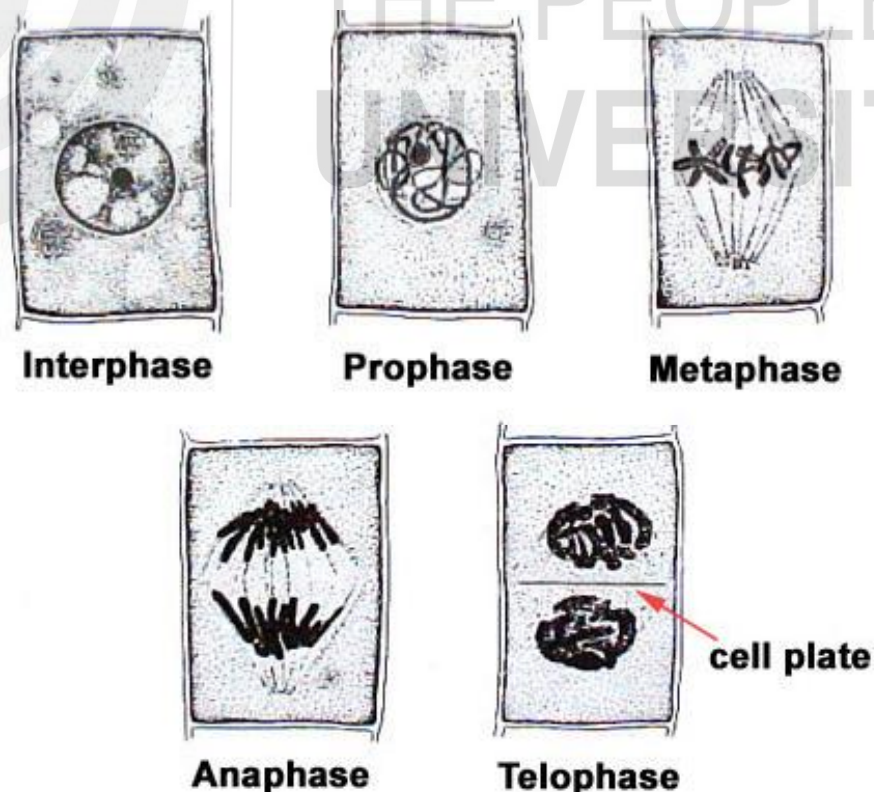
- The first stage in the division process is characterized by the unwinding of the chromatin.
- Each chromosome begins to appear as two chromatids with a single centromere.
- The nuclear membrane slowly disappears.

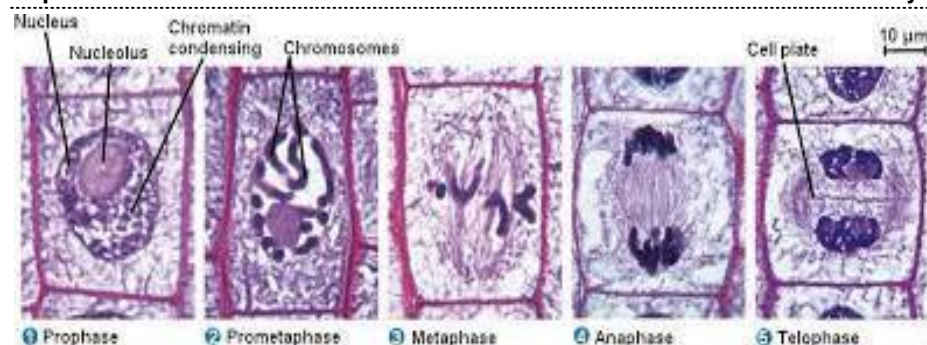
Metaphase

- Nuclear membrane and nucleolus disorganizes completely.
- The chromosomes tend to arrange themselves in the middle of the cell called the equatorial plate.
- It is possible to count the number of chromosomes at the metaphase stage. When observed carefully, the sister chromatids of the chromosomes can be seen.

Anaphase

- Anaphase is marked by the movement of the chromosomes to the opposite poles of the cell (Fig. 8.3).
- Essentially the centromere splits and a single chromosome with two chromatids becomes two independent chromosomes each with a centromere.
- Each chromosome appears in characteristic shape, depending on the position of the centromere.
- One can observe the anaphase in various stages such as early anaphase, middle anaphase and late anaphase depending on the position of the chromosomes during their movement.
- The spindle fibers which are attached to the centromere of the chromosomes may not be distinctly visible with the stain you have used.





(b)

Fig. 8.3: a) Diagrammatic representation of various stages of mitosis b) Stages of mitosis as observed under the microscope.

Telophase

- In telophase two daughter nuclei each with the same number of chromosomes as the parent nucleus is formed (Fig.8.3).
- The chromosomes undergo coiling and become thread like.
- The nuclear membrane reappears and the nuclear division is completed.
- Spindle fibers appear to disassemble.

Cytokinesis

Once the nuclear division is complete, cytokinesis, i.e., the division of a cell into two daughter cells occurs. The two daughter cells formed at the end of mitosis get separated by a wall formed between them.

8.6 TERMINAL QUESTIONS

1. Name the stage where
 - a) The chromosomes get arranged at the equatorial plate.
 - b) The nuclear division is complete and cell divides into two daughter cells.
 - c) The chromosomes appear double stranded.
2. Why mitosis is also called equational division?

8.7 ANSWERS

1.
 - a) Metaphase
 - b) Cytokinesis
 - c) Interphase
2. Mitosis is the process of cell division in which the chromosomes replicate and gets equally distributed into two daughter cells. The chromosome number in each daughter cell is equal to that in the parent cell. Hence, mitosis is known as equational division.

Acknowledgments

Fig. 8.2 : [https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fi.pini mg.com%2F600x315%2Fa5%2F76%2Fd1%2Fa576d16c8342f7 d5c3e6e8650d596557.jpg&imgrefurl=https%3A%2F%2Fin.pinterest.com%](https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fi.pini mg.com%2F600x315%2Fa5%2F76%2Fd1%2Fa576d16c8342f7 d5c3e6e8650d596557.jpg&imgrefurl=https%3A%2F%2Fin.pinterest.com%2F600x315%2Fa5%2F76%2Fd1%2Fa576d16c8342f7d5c3e6e8650d596557.jpg&imgrefurl=https%3A%2F%2Fin.pinterest.com%2F600x315%2Fa5%2F76%2Fd1%2Fa576d16c8342f7d5c3e6e8650d596557.jpg)

Fig. 8.3 : <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fimage.slideserve.com%2F440537%2Fstages-of-mitosis-in-onion-root-tip-cells-l.jpg>



EXPERIMENT 9

STUDY OF MEIOSIS WITH THE HELP OF PERMANENT SLIDES

Structure

9.1	Introduction	9.5	Observations
	Objectives		Meiosis I
9.2	Study Guide		Meiosis II
9.3	Materials Required	9.6	Terminal questions
9.4	Procedure	9.7	Answers

9.1 INTRODUCTION

Meiosis is a special type of cell division which is involved in the formation of gametes during sexual reproduction of eukaryotes. In this type of nuclear division the chromosome number gets reduced to half. A diploid ($2N$) nucleus of the reproductive cells after meiosis gives rise to haploid (N) gametes with half the chromosome number. Meiosis is therefore called the reduction division. The chromosome number is restored to diploid at syngamy (fertilization). In addition, the process of crossing over during meiosis results in genetic recombination or genetic reshuffling which is the source of variation in sexually reproducing organisms. Variation is the driving force of evolution.

You will be studying various stages of Meiosis I and II in this exercise.

Objectives

After going through these prepared slides of meiosis you will be able to:

- ❖ describe various stages of meiosis by noting down the stage;
- ❖ know the characteristic features of each substages of prophase I; and
- ❖ differentiate between mitosis and meiosis with reference to the behaviour of chromosomes.

9.2 STUDY GUIDE

For doing satisfactory work you must read the Unit 8 of course BBYET-141 (Cell and Molecular Biology) before coming to the laboratory.

9.3 MATERIALS REQUIRED

You will be provided with a compound microscope and permanent slides of squash preparations of anthers of *Allium cepa* and *Tradescantia* showing different stages of meiosis.

9.4 PROCEDURE

Observe the permanent slides provided carefully under the compound microscope both under low and high power. Make neat, well- labelled diagrams in your note book and write comments. It is advised that figures should not be copied from the book or the photographs given in this unit but should be drawn after observing the slides under the microscope.

9.5 OBSERVATIONS

You have been provided with permanent slides of meiosis. Observe each one of them carefully and note down the characteristic features of each one of them.

A description of different stages of meiosis is given below.

Meiosis actually consists of two division processes: meiosis I and meiosis II. Meiosis I constitute the reduction division where the chromosomal number is reduced by half. Meiosis II resembles mitosis. At the end of the two divisions four cells are formed, each with half the number of chromosomes as the parent cell.

9.5.1 Meiosis I

1. Prophase-I

The prophase-I is a lengthy phase and consists of five different stages. They are leptotene, zygotene, pachytene, diplotene and diakinesis.

During these stages the uncoiling of the chromatin material, condensation of the chromosomes, synapsing of the homologous chromosomes, crossing over and chiasma formation and recombination occurs. If observed carefully the different stages of Prophase-I can be identified in the squash preparations of anthers of different ages. You may identify only one or two meiotic stages in each anther since the various meiotic events are highly synchronized in plant tissues. Draw your observations in your notebook and compare them with the photographs provided (Fig. 9.1)

Prophase usually begins with the earliest recognizable changes in the nuclear contents.

Leptotene

- Nuclear membrane and nucleolus are intact.
- At this stage the reticulum of the meiotic nucleus opens up and the chromosomes start shortening and are seen as slender threads.
- The chromosomal threads are mostly irregularly arranged or sometimes may tangle in the form of a Synizetic knot.

Zygotene

- At this stage the homologous chromosomes pair up i.e., become intimately associated (but not fuse).
- This selective pairing between chromosomes (one from male and the other from female parent) is called Synapsis (**Syndesis** or **Synizesis**).
- The selective pairing or synapsis is seen to get initiated at several points.
- The synapsed chromosomes result in the formation of a **Bivalent** (Actually a tetrad as each chromosome has already replicated into two chromatids).
- The bivalents shorten and thicken by coiling and can be visualized in the form of a **Synaptonemal complex**.

Pachytene

- It is the longest substage of meiotic prophase I.
- This stage begins with the completion of synapsis. During pachytene the synaptonemal complex is formed and towards the end also starts breaking down.
- Crossing over (breakage and reunion) between the two non- sister chromatids gets initiated.
- The chromosomes start thickening.

Diplotene

- This substage is characterized by complete dissolution of the synaptonemal complex.
- Crossing over is completed and the chromosomes have started to separate.
- During the separation there remain certain points which still remain in contact. These are the **chiasmata** (*singular- Chiasma*).
- Repulsion between the homologous continues and the chiasmata begin to displace along the length of the chromosome.
- The terminal chiasma may slip off from the chromosome pair followed by the next one and so on. This process is called **terminalization**.

Diakinesis

- During this substage the chromosomes become more condensed.
- The meiotic spindle is assembled and the homologous chromosomes (bivalents) start migrating towards the poles.
- By the end of this substage the nucleolus disorganizes completely. The nuclear membrane also gets broken down.
- The bivalents are fully condensed.

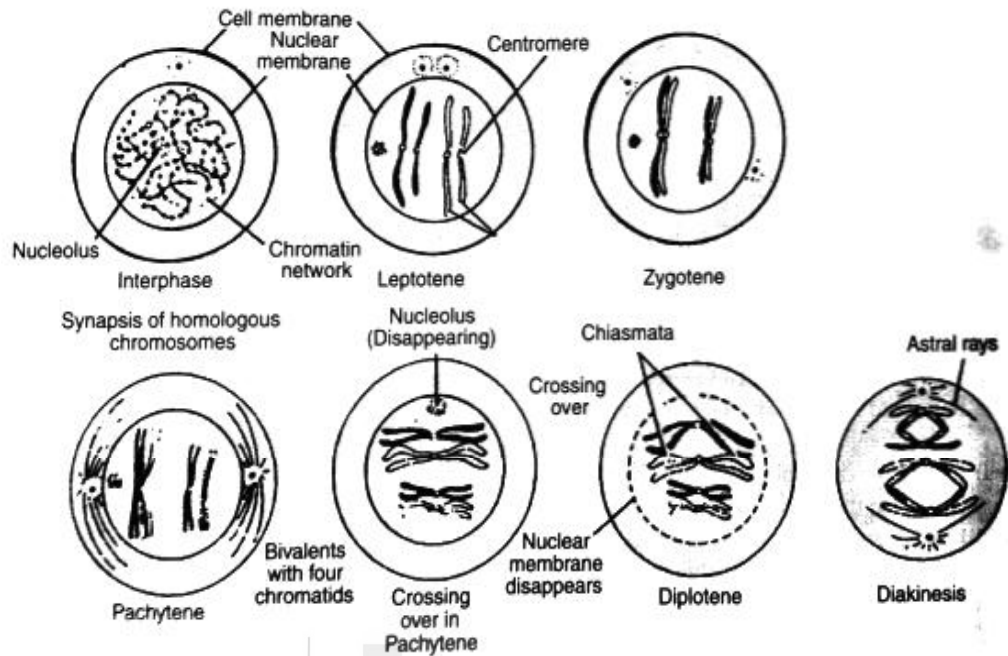


Fig. 9.1: Different stages in prophase- I of meiosis I.

2. Metaphase-I

Metaphase 1 shows the arrangement of the chromosomes at the equatorial plate. Metaphase-1 is the most suitable stage for counting the number of chromosomes. Since the number of chromosomes differ in different species, count and record the number of chromosome in the species you have used for your studies. In the anthers of the onion buds (*Allium cepa*) the number of chromosomal bivalents present is 7. The chromosomes become ready to separate as the repulsive forces between the homologous chromosomes increase.

3. Anaphase-I

Anaphase-I is a stage at which the chromosomal number is reduced by half and unlike the mitotic anaphase the centromere does not split and the entire chromosome moves to one pole with the centromere first. This process is called **disjunction**. As a result each nucleus receives only half the number of **chromosomes, i.e., one haploid set each**. Anaphase-I shows the chromosomes of either maternal or paternal origin moving to the opposite poles of the cell. Since these chromosomes have undergone crossing over they are genetically different from the parental ones (genetic variation).

4. Telophase-I

In telophase-I once the haploid set of chromosomes arrive at their respective poles, the two nuclei are reassembled (Fig. 9.2). The nuclear membrane is reorganized, and the nucleoli reappear. The chromosomes begin to uncoil and decondense.

Cytokinesis follows karyokinesis and two daughter cells with half the number of chromosomes are formed.

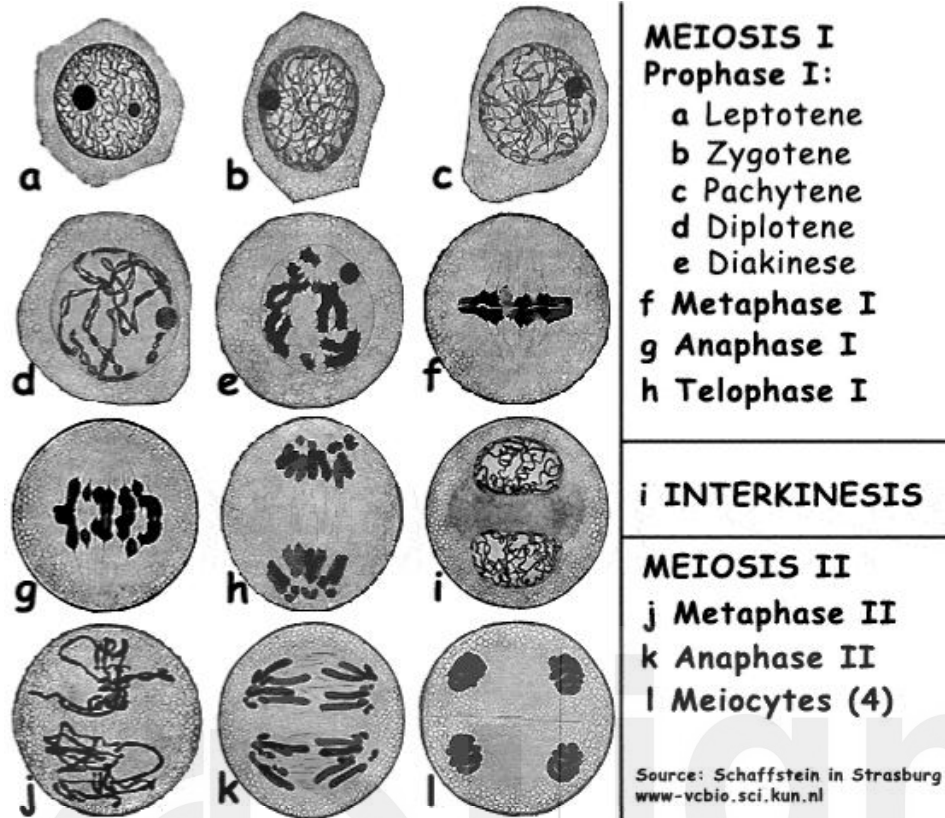


Fig.9.2: Various stages of Meiosis I and Meiosis II.

Meiosis II

Meiosis II depicts the different division stages similar to those of mitosis.

You will note that the cells which undergo meiosis II are relatively smaller than the cells which undergo meiosis I. Also, they carry only half the numbers of chromosomes like the meiosis-I cells. This fact can be verified by actually counting the number of chromosomes during meiosis II.

1. Prophase-II

The nuclear membrane and nucleoli disorganize. The chromosomes with two chromatids start to shorten and thicken. The spindle fibers (microtubules) get arranged at right angles to the spindle of the first meiosis.

2. Metaphase-II

The chromosomes align separately on the equator of the spindle. The centromere divides into two. Each chromosome gets divided into two daughter chromosomes or monads.

3. Anaphase-II

The chromosomes split at the centromere to form sister chromatids that migrate to opposite poles along the spindle fibers.

4. Telophase-II

The chromosomes uncoil, elongate and become indistinct. Nuclear membrane and the nucleolus are reformed. Each nucleus now possesses a haploid set (half the number) of chromosomes. Subsequent cytokinesis produces four haploid daughter cells.

9.6 TERMINAL QUESTIONS

1. Name the stage of meiosis in which crossing over of chromosomes takes place.
2. What is cytokinesis?
3. What is the difference between Meiosis II and Mitosis?
4. What is the significance of meiosis?
5. List out the differences between prophase of mitosis and prophase-1 of meiosis.
6. How is metaphase-I of meiosis different from metaphase of mitosis?

9.7 ANSWERS

1. Crossing over (breakage and reunion) between the two non- sister chromatids takes place at Pachytene stage of Meiosis I.
2. *Cytokinesis* is the process in which cytoplasm of a parental cell into two daughter cells. This takes place after telophase phase of cell division.
3. **Meiosis II occurs in haploid cells formed after meiosis I** whereas the mitosis mainly occurs in diploid cells. Meiosis II leads to production of gametes in sexual reproduction while mitosis occurs in asexual reproduction. Mitosis results in the formation of two daughter cells, though four daughter cells are formed after meiosis.
4. Meiosis generates genetic diversity by recombination. Recombination at meiosis plays an important role in the repair of genetic defects in germ line cells.
5. The prophase of mitosis is smaller while prophase I of meiosis is longer. The prophase I of meiosis has five stages. In prophase I of meiosis, synapsis and crossing over take place while these events are not found in prophase of mitosis.
6. During metaphase of mitosis, the centromere divides and lies towards the equatorial plate while during meiosis I, the centromere does not divide and lies towards the opposite poles. In both the stages chromosomes lie at the equatorial plate

Acknowledgements

Fig. 9.1 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.sarthaks.com%2F%3Fqa%3Dblob%26qa_blobid%3D2702670654210178227&imgrefurl=https%3A%2F%2Fwww.sarthaks.com%2F%3Fqa%3Dblob%26qa_blobid%3D2702670654210178227

Fig. 9.2 : <https://www.google.com/imgres?imgurl=https%3A%2F%2Fwww.vcbio.science.ru.nl%2Fimages%2Fcellcycle%2Fmaloemeiosis-zoom.gif&imgrefurl=https%3A%2F%2Fwww.vcbio.science.ru.nl%2Fimages%2Fcellcycle%2Fmaloemeiosis-zoom.gif>

EXPERIMENT 10

TO STUDY THE EFFECT OF ORGANIC SOLVENTS AND TEMPERATURE ON THE PERMEABILITY OF CELL MEMBRANE

Structure

10.1	Introduction	10.5	Effect of Temperature
	Objectives		Procedure
10.2	Study Guide		Observations
10.3	Materials Required		Results and Discussion
10.4	Effect of Concentrations of Alcohol	10.6	Precautions
	Procedure	10.7	Terminal Questions
	Observations	10.8	Answers
	Results and Discussion		

10.1 INTRODUCTION

All living biological membranes are selectively permeable. They regulate the movement of water and solutes across cells. This movement is however, influenced by various internal as well as external factors. Most of the internal organelles are surrounded by membranes; hence permeability is a very important for the influx and efflux of water and various solutes. As the membranes are made of lipoprotein complexes, hence all the factors which influence the stability of the membrane structure also affect its permeability. Membrane permeability is affected by factors like temperature, organic solvents, pH, radiation, and electric currents. The protein component of the membranes acts as solute carriers and control solute movement whereas lipids give more permeability to many non-polar and lipid- like solutes. In this experiment you will be made familiar with two factors that affect the permeability of cell membranes i.e. organic solvents and temperature.

Different organic solvents have varying effects on membrane permeability. These dissolve phospholipids of the membrane and alter both the fluidity and permeability properties of the membrane. Alcohols have been shown to damage the weak hydrogen bonds that hold together the proteins of the cell membrane thus resulting in their denaturation.

Temperature affects the membrane permeability by interacting with the protein part of the cell membrane. At higher temperatures, proteins get denatured and membrane permeability is altered resulting in release of betacyanin pigments from within the cell into its immediate environment/ medium. Moreover, high temperature induces phase transition of the phospholipid bilayer from a highly ordered gel like to a more mobile fluid state over a short temperature range.

Objectives

After performing this experiment you would be able to:

- ❖ explain the effect of various organic solvents on the permeability of the beet root membranes; and
- ❖ study the effect of varying temperature on the degree of denaturation of the membrane thus affecting its permeability.

10.2 STUDY GUIDE

For doing satisfactory work you must read the Units 2, 3 of BBYCT-137 before coming to the laboratory.

10.3 MATERIALS REQUIRED

1. Beet root (*Beta vulgaris*) (Family – Chenopodiaceae)
2. Ethanol (alcohol) [concentrations (20%, 40%, 60%, 80%)],
3. Benzene
4. Distilled water
5. Boiling tubes
6. Petri dish
7. Glass rod
8. Cork borer
9. Blunt forceps
10. Blade
11. Scale and graph paper
12. Colorimeter or spectrophotometer
13. Thermometer

You will perform two set of experiments: a) effect of concentration of alcohol and b) effect of temperature.

10.4 EFFECT OF CONCENTRATIONS OF ALCOHOL

You will be using beetroot slices for this experiment. Beetroot contains beet sugar as well as a reddish water soluble betacyanin pigments. These pigments are synthesized in the cytoplasm, and later transported to the vacuole via the tonoplast and get confined to vacuolar sap. (Fig. 10.1) In a healthy state these pigments remain within the vacuole but will leak out if the membrane permeability is altered.

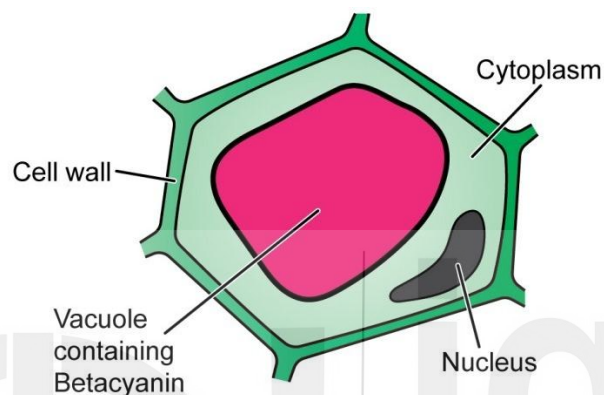


Fig.10.1: Accumulation of betacyanins in the vacuole.

10.4.1 Procedure

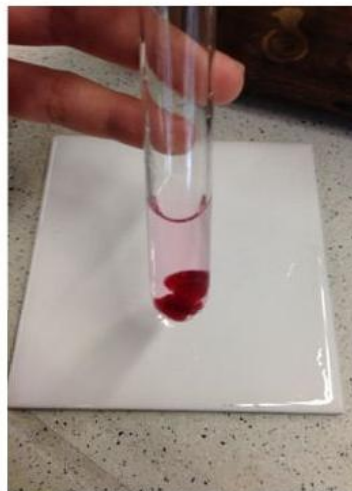
- Prepare four different alcohol concentrations (20%, 40%, 60%, 80%) from absolute alcohol as shown in the dilution table (Table 1).
- Cut beet root cylinders (each of 1 cm length) with the help of cork borer (Fig. 10.2) and transfer two of these into boiling tubes having 10 mL of different concentrations of alcohol.
- Keep one tube as a control for comparison. Leave the tubes undisturbed for 5 minutes; discard the cylinders using blunt forceps and note down absorbance at 540 nm.
- In another set of experiment, immerse two beet root cylinders in benzene for 5 minutes. After that transfer the cylinders in 10 mL of distilled water and observe the change.



(a)



(b)



(c)

Fig. 10.2: a) Beetroot (*Beta vulgaris*); b) Beetroot cylinders cut into slices; c) Beetroot slices kept in alcohol.

Table 1: Dilutions for various concentrations of alcohol.

S. No.	Concentration of Absolute alcohol (%)	Amount of Absolute Alcohol (mL)	Amount of Distilled water (mL)	Total amount (mL)
1.	20%	2	8	10
2.	40%	4	6	10
3.	60%	6	4	10
4.	80%	8	2	10
5.	100%	10	0	10

10.4.2 Observations

You must record the observations in the form of absorbance in the following observation table (Table 2):

Table 2: Absorbance values for various alcohol concentrations measured after a reaction time of 5 min.

S. No	Concentration of Absolute Alcohol (%)	Absorbance (540 nm)
1	Control (distilled water)	
2	20	
3	40	
4	60	
5	80	
6	100	

Draw a graph on the basis of your observations (an example has been provided to you in the Fig.10.3 for better understanding).

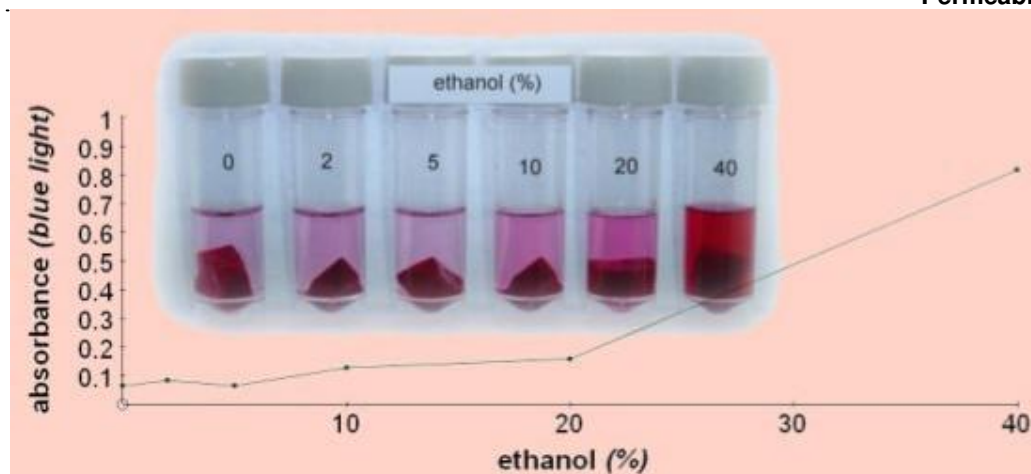


Fig. 10.3: Graph representing the effect of different alcohol concentrations on membrane permeability. (vials with beet root slices showing the amount of pigment released).

10.4.3 Result and Discussion

You will observe that with an increase in concentration of organic solvent (alcohol), absorbance of the solution increases due to more and more release of betacyanin pigments. This is because at higher concentrations, alcohol damages the membranes and alters their permeability by dissolving their phospholipid components. However, at 100% alcohol, the solution shows less absorbance as compared to 80% alcohol as less water is available in the external medium and betacyanin pigments are water-soluble. You can record your observations by taking pictures of the tubes, as shown in the example in Fig.10.4.



Fig. 10.4: Effect of different alcohol concentrations on the intensity of pigment released.

In another set, with benzene solution, water immediately turns dark pink due to loss in permeability of the membrane.

10.5 EFFECT OF TEMPERATURE

Since membrane permeability gets affected by rise in temperature, it is worth while now to study the effect of temperature on the intensity of the pigment released into the medium.

10.5.1 Procedure

- Take out cylinders of beetroot (each of 1 cm length) using the cork borer. Wash the beetroot slices for 10 minutes with distilled water.
- Transfer two of them into a boiling tube having 10 mL distilled water maintained at 35° C. The solution is kept for 5 minutes.
- Repeat the same procedure for 50° C, 65° C, 80° C and 100° C.
- One boiling tube is kept at room temperature. This tube is set as a control.
- Gently mix the contents of the tubes by inverting, decanting after 5 minutes. Arrange the tubes according to intensity of colour obtained via measurement of absorbance spectrophotometrically at 540 nm.

10.5.2 Observations

You must record the observations in the form of absorbance in the following observation table (Table 3).

Table 3: Observation table showing readings for the effect of temperature on membrane permeability measured after a reaction time of 5 min.

S. No.	Temperature (° C)	Absorbance (540 nm)
1	17 (Room temp)	
2	35	
3	50	
4	65	
5	80	
6	100	

Present the results of absorbance versus temperature in a graphic form. An example of this graph has been presented here for your reference (Fig. 10.5).

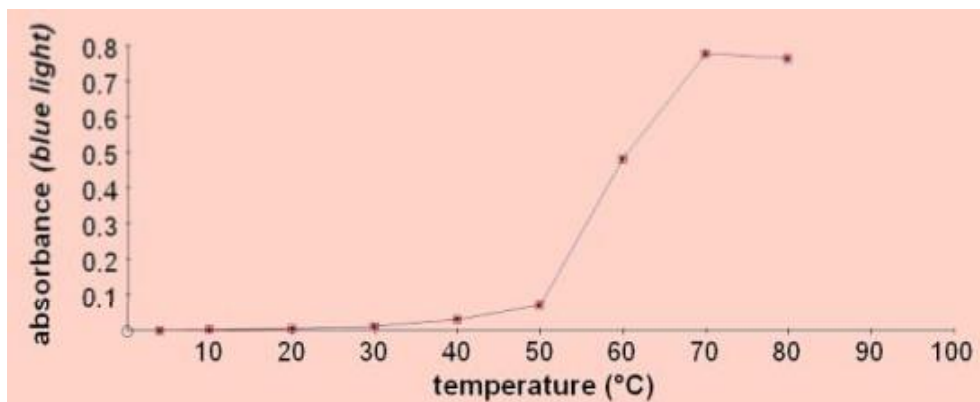


Fig. 10.5: Graph representing the effect of increasing temperature on absorbance.

10.5.3 Results and Discussion

You must have noted that with an increase in temperature, the absorbance of the solution increases. Denaturation of proteins at higher temperatures results in distortion of the membrane and its permeability properties, leading to a release of betacyanin pigment in the external medium. Absorbance measured at 80°C or 100°C may be less because of breakdown of betacyanin pigments. When the tissue is not exposed to high temperature, its pigments, sugars and salt remain retained within the tissue. After heat treatment, the solutes within the cell diffuse into the external solution at a much faster rate because of an increase in kinetic energy of the pigment molecules.

10.6 PRECAUTIONS

1. All beetroot cylinders must be removed from the same tuber as these are at the same osmotic level and tuber should be healthy.
2. Beetroot should be healthy and the cylinders must be of uniform size and colour intensity. Areas with any white patchy specks must be avoided.
3. Beetroot slices must be transferred with the help of blunt forceps to avoid injury to the tissue.
4. Cylinders should be cut underwater and thoroughly washed to remove the pigment sticking to the damaged surface.
5. All tubes should contain an equal amount of solution.
6. Reaction time should be constant for all treatments of varying temperature and organic solvents. However, reaction time can vary depending upon the plant material. You can vary the time, depending on the plant material provided.

10.7 TERMINAL QUESTIONS

1. In which part of the cell anthocyanins/betacyanin pigments are located?
2. Why is beetroot a suitable material for permeability experiments?
3. What is the role of permeability in living systems?
4. What prevents the diffusion of pigments and sugars from the beet root under natural conditions?
5. How does temperature influence the permeability of plasma membrane?
6. Why do you get maximum intensity of red colour at 60% or 80% alcohol concentrations?

10.8 ANSWERS

1. Vacuole
2. Coloured contents in a vacuole

3. Regulation of flow of metabolites from and to the cells
4. Living and functional tonoplast
5. High temperature denatures the membrane proteins thereby making the membrane permeable
6. Anthocyanins/betacyanins are water soluble

Acknowledgements

Fig. 10.1 : <https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fslideplayer.com%2F7873884%2F25%2Fimages%2F4%2FBeetroot%2Bpigments%2BBETACYANIN.jpg&imgrefurl>

Fig. 10.2 : a) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Freader024.staticcloud.net%2Freader024%2Freader%2F2020123115%2F5ae8b4aa7f8b9aee078fd7fb%2Fhtml%2Fbg3.png&imgrefurl>

c) <https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fslideplayer.com%2F7873884%2F25%2Fimages%2F4%2FBeetroot%2Bpigments%2BBETACYANIN.jpg&imgrefurl>

Fig. 10.3 : <http://www.mystrica.com/Pages/Content/Images/membranealc.jpg>

Fig. 10.4 : https://31.media.tumblr.com/b3d625fd4cdde277733716bbe94df7ed/tumblr_inline_mzr9m7ylcA1s0ikvd.jpg

Fig. 10.5 : <http://www.mystrica.com/Pages/Content/Images/membranetem.jpg>

EXPERIMENT 11

TO DEMONSTRATE THE TECHNIQUE OF DIALYSIS – SEPARATION OF STARCH AND SIMPLE SUGARS

Structure

11.1	Introduction	11.5	Observations
	Objectives	11.6	Precautions
11.2	Study Guide	11.7	Terminal Questions
11.3	Materials Required	11.8	Answers
11.4	Procedure		

11.1 INTRODUCTION

Dialysis is a technique which is used to separate macromolecular and micromolecular solutes from a mixture based on their molecular weight difference using a semi-permeable membrane. This technique was first introduced by Thomas Graham in 1861. A dialysis bag (made from cellulose acetate or cellophane) has pores of fixed size. Molecules small enough to pass through these pores can diffuse into or out of this bag along in the direction of decreasing concentration gradient. The molecules larger than the pore size are retained within the bag. In nature, high molecular weight solutes penetrate the membranes through larger pores. In other words, dialysis allows the separation of colloids and crystalloids.

The technique of dialysis finds many applications in medicine where the method of **hemodialysis** removes waste products like urea and creatinine from kidneys in case of renal failure. Dialysis acts as an artificial kidney. Dialysis is used as a routine procedure in biochemistry labs to purify proteins and enzymes as it is one of the most effective technique to separate colloids from crystalloids.

Objectives

After performing this experiment you would be able to:

- ❖ appreciate the principle underlying the separation technique of dialysis; and
- ❖ comprehend the various microchemical tests involved in identifying the components.

11.2 STUDY GUIDE

For satisfactory work you must read theory Units before coming to the laboratory.

11.3 MATERIALS REQUIRED

1. Dialysis bag (cellulose acetate)
2. Thread
3. Clamp stand
4. Beaker
5. Aluminum foil
6. Glass rod
7. Beaker 250 ml
8. Bunsen Burner
9. Fehling's solution A & B (mixture of 5% starch and 5% glucose)
10. I_2KI solution

11.4 PROCEDURE

Cut a piece of dialysis tubing (about 6 cm) from the tubing roll and dip in lukewarm water for 16 minutes. Tightly tie one end of the tubing and pour into the bag containing a mixture of 5% starch (colloid) and 5% glucose (crystalloid). Tightly tie the other end and ensure that there is no leakage. Wash the solution filled bag under running water to eliminate any spill off. Hang the dialysis bag with the help of a thread into a beaker containing water so that it is completely immersed but not touching the walls (see Fig.11.1).

- a) To begin the experiment, pipette out some water from the beaker and test for glucose and starch.
- b) Keep the set up undisturbed for 4 hours. Now pipette out some water from the beaker divide it into two test tubes. Test the contents for glucose (Fehling's test) and starch (I_2KI).

11.5 OBSERVATIONS

- The initial test with Fehling's and I_2KI is negative, and this indicates the absence of any glucose or starch outside the dialysis bag.

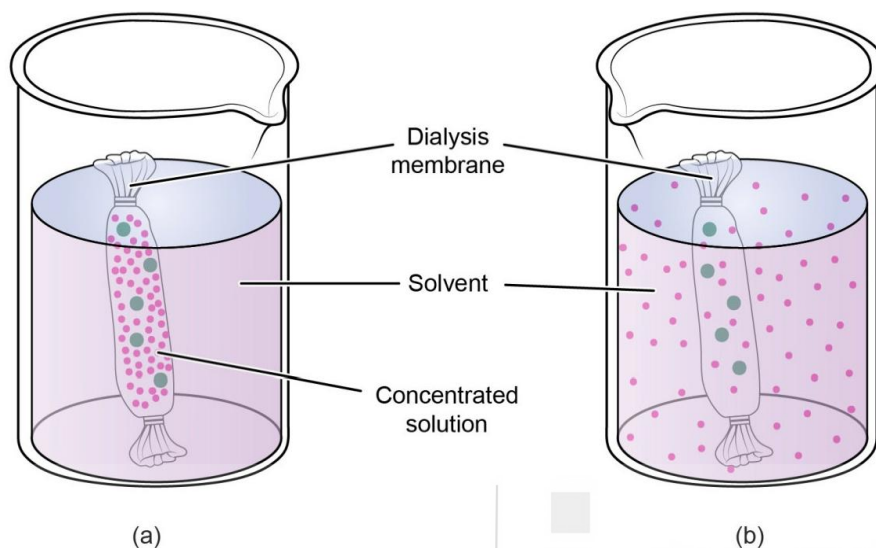


Fig. 11.1: A pictorial representation of the experimental setup. a) start of dialysis; b) at equilibrium.

- You will observe that after four hours, water in one of the tubes turns brick red using Fehling's test thereby indicating that glucose has diffused out of the dialysis bag into the external medium. However, the I_2KI test is negative. This shows that starch molecules have not been able to pass through the dialysis bag (Fig. 11.1).
- To confirm this contention, two drops of I_2KI are added to the water in the beaker. You will observe that after 30 min the contents in the dialysis bag will stain blue or blue black confirming the presence of starch. In fact I_2KI diffuses into the bag and reacts with starch to give blue colour.

11.6 PRECAUTIONS

You should take the following precautions while performing this experiment:

- The dialysis tubing must be dipped in lukewarm water before the experiment.
- Both the ends of the dialysis bag should be tied well to prevent leakage of contents.
- The dialysis bag must be completely immersed and not touch the sides of the beaker.
- Test for sugars must be performed on the water taken out from the beaker. The I_2KI must be added to the beaker only later.

11.7 TERMINAL QUESTIONS

- Why is it desirable to dip the dialysis tubing lukewarm water?

2. Why is it necessary to completely immerse the bag in water and not touch the walls of the beaker?
3. Why is the Fehling's test not performed in the water in the beaker itself?
4. What will happen if the experimental set up is placed in a refrigerator?

11.8 ANSWERS

1. Dipping in lukewarm water opens the pores in the membrane.
2. All the pores will not get a chance to transport the crystalloids.
3. It will interfere with subsequent I_2KI Test.
4. Rate of diffusion will slow down due to drop in temperature.

Acknowledgements

Fig. 11.1 : <https://masteringcollegebiochemistry.files.wordpress.com/2019/01/dialysis-1.jpg?w=1024&h=633&crop=1>



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EXPERIMENT 12

TO STUDY PLASMOLYSIS AND DEPLASMOLYSIS USING *RHOEO* LEAF

Structure

12.1	Introduction	12.4	Procedure and Observations
	Objectives	12.5	Precautions
12.2	Study Guide	12.6	Terminal Questions
12.3	Materials Required	12.7	Answers

12.1 INTRODUCTION

The exchange of gases, water, food material and wastes between cell and its environment involves the processes of diffusion and osmosis. The plasma membrane is selectively permeable and thus allows the diffusion of only certain molecules into and out of the cell. Osmosis is also a special case of diffusion where the molecules move across a selectively permeable membrane from a region of higher concentration to a region of lower concentration. Since in living system water is the most common solvent, osmosis in living cells refers to diffusion of water only.

The fluid content of a cell may be higher, lower, or equal in concentration with respect to its surrounding. A net flow of water out of the cell occurs when it is surrounded by a solution that contains higher concentration of solute particles (hypertonic solution) than the cell (Fig. 12.1a, b). As water moves out of the cell, it shrivels and becomes distorted in hypertonic solution in which the cell has been kept. In a plasmolysed cell, the space between the cell wall and the shrunken protoplasm is occupied by the external solution. With reference to the RBCs, the shriveling and distortion of cell by loss of water to the surrounding is called **crenation**. The phenomenon in plant cells is termed **plasmolysis**.

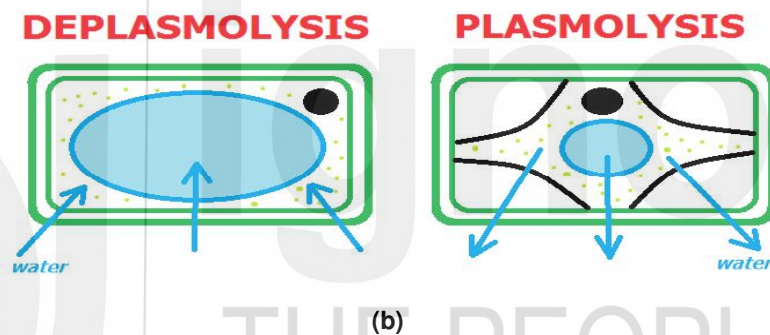
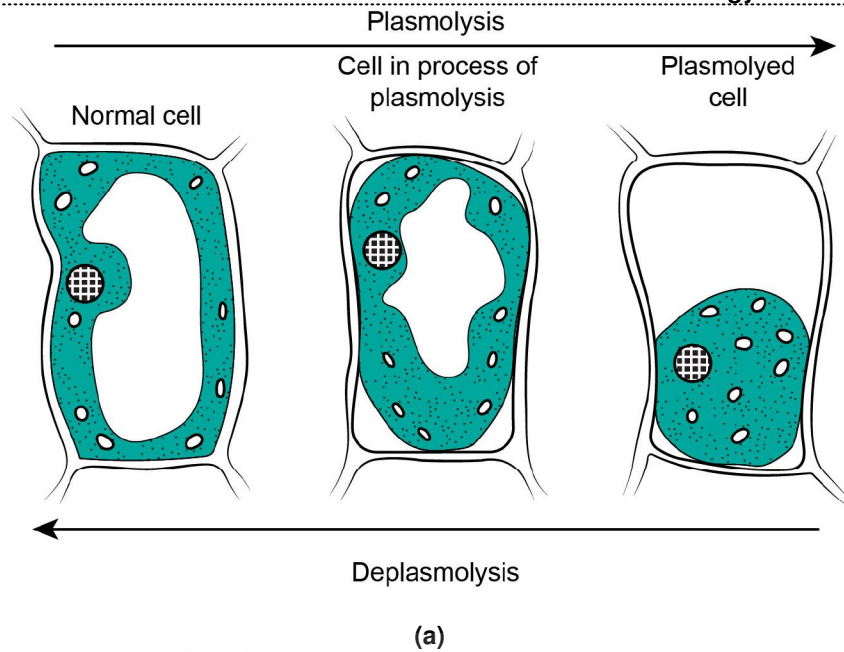


Fig. 12.1: a) Cell showing plasmolysis and deplasmolysis; b) Diagrammatic representation of Plasmolysis and deplasmolysis.

In this lab exercise, you will study plasmolysis and deplasmolysis in the epidermal cells of *Rhoeo discolor*.

Objectives

After performing this experiment you would be able to:

- ❖ distinguish between plasmolysis and deplasmolysis; and
- ❖ appreciate the permeability properties of the plasma membrane.

12.2 STUDY GUIDE

For satisfactory work you must read course BBYCT 137 Plant Physiology.

12.3 MATERIALS REQUIRED

1. Healthy leaves of *Rhoeo discolor* (*Tradescantia spathacea*) (Family Commelinaceae)
2. Microscope

3. Slides
4. Cover slips
5. Beaker
6. Two droppers
7. Razor blade
8. Dissecting needles and a pair of forceps
9. Camel hairbrush
10. Filter paper
11. Tissue paper
12. 12.5 mL each of 10% and 20% sucrose solution.

12.4 PROCEDURE AND OBSERVATIONS

The term plasmolysis was first used by **De Vries**. Plasmolysis is shown by only the living cells and is used to determine the approx. osmotic pressure of a cell.

- Take *Rhoeo* leaf and peel out the layer of purple epidermal cells or cut out a small section nearly parallel to epidermis.
- Place it on a slide and cut about 2 mm square piece with a sharp razor blade.
- Mount this piece on microscopic slide with a few drops of water and place a cover slip over it.
- Observe first under low and then under high power of a microscope. The cells near the edges are generally thinner and thus are better for viewing the details.
- Identify cell wall, plasma membrane, cytoplasm, nucleus, and vacuole in a cell. Draw the cell in your notebook.
- Now, remove water from one side of the coverslip and then add a few drops of 10% sucrose from the other side of the coverslip.
- Alternatively, prepare fresh mount in 10% sucrose instead of water using same specimen. Leave it for 10 minutes and then observe. If you do not observe obvious changes leave it for another 10 or 20 minutes and then observe again.
- Make another slide of fresh specimen with 20% sucrose and observe it after half an hour. Draw the cell showing the changes observed.

Note: You will observe that the protoplast of the cells is not uniformly plasmolysed and at some places cytoplasm seems firmly attached to the cell wall, this could be due to firm holding of cytoplasmic strands particularly at the plasmodesmatal connections. Therefore, the plasmolysed protoplast shows varied shapes (Fig. 12.2).

Plasmolysed cell ←

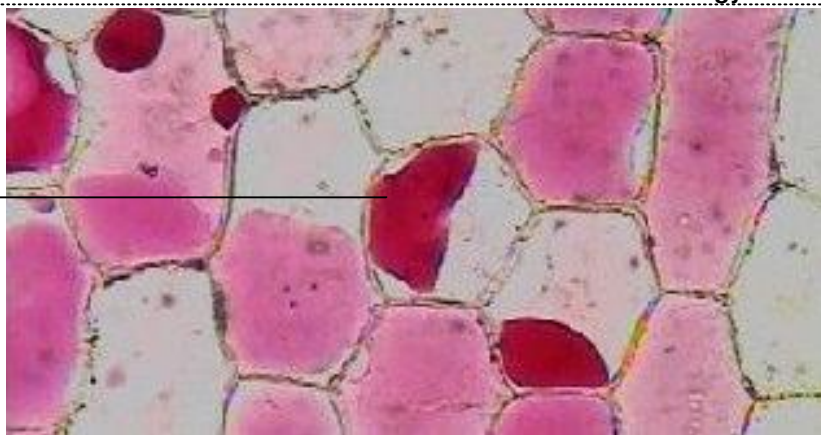


Fig. 12.2: W.M of *Rhoeo* epidermal peel shows normal and plasmolyzed cells.

Deplasmolysis

- Remove the coverslip of the above preparation and take the specimen with forceps, wash it in water, dry with a filter paper and immediately mount it in water. Leave for 20 minutes and observe.

12.5 PRECAUTIONS

1. Fresh leaves of *Rhoeo* must be used for the experiment.
2. The epidermal peels must not be allowed to dry during the experiment

12.6 TERMINAL QUESTIONS

1. List the changes observed in the plant cell when placed in 10% or 20% sucrose solution.
2. Why does a plant cell not rupture when placed in water?
3. List a few day-to-day examples which show plasmolysis and deplasmolysis of plant tissue.

12.6 ANSWERS

1. The concentration of the sugar solution is higher than that of the concentration of sugar in the cell, hence when the plant cell is kept in 20% sugar solution will become plasmolysed (shrink) due to osmosis.
2. When a plant cell is placed in water, the water enters into it. Outer rigid, protective cell wall prevents the bursting of plant cell by inhibiting excessive endosmosis.
3. Some examples of plasmolysis include shrinkage of vegetables in hypertonic conditions, shrinkage of blood cell when they are placed in the hypertonic conditions. Other examples include adding of salt and/or sugar to preserve food and make jams, jellies, and pickles. The cells lose water and become less conducive to the growth of microorganisms such as bacteria, allowing these food items to be preserved.

Acknowledgements

- Fig. 12.1** : a) <https://www.google.co.in/imgres?imgurl=x-raw-image%3A%2F%2F%2Fa1d0a5f9a6ffcb5238e13976975efc1bef294e443ead084ff3c8ff7b73ba4b8a&imgrefurl=https%3A%2F%2Fcdn1.byjus.com%2Fcoredifferences.com%2Fwp-content%2Fuploads%2F2020%2F04%2FDifference-between-Plasmolysis-and-Deplasmolysis.png>
- b) <https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fcoredifferences.com%2Fwp-content%2Fuploads%2F2020%2F04%2FDifference-between-Plasmolysis-and-Deplasmolysis.png>

- Fig. 12.2** : https://slidetodoc.com/presentation_image/74ae27fbf87d96a267f7f161ff37884a/image-35.jpg



EXPERIMENT 13

STUDY OF STRUCTURE OF NUCLEAR PORE COMPLEX BY PHOTOGRAPHS

Structure

13.1	Introduction	13.4	Procedure
	Objectives	13.5	Observation
13.2	Study Guide	13.6	Terminal Questions
13.3	Materials Required	13.7	Answers

13.1 INTRODUCTION

Membranes within a eukaryotic cell regulate the permeability and the traffic of micro and macromolecules. While the plasma membrane acts as a permeability barrier between the cytoplasm and the extracellular spaces, preventing the passage of macromolecules, it is the nuclear membrane which regulates the movement of RNAs and proteins between the nucleus and the cytoplasm. As you have already studied in the Unit 7 of BBYET 141 Cell and Molecular Biology there are many proteins which are transported from the cytoplasm into the nucleus for participating in DNA replication and transcription. At the same time, mRNAs, tRNAs and the ribosomal subunits are transported from the nucleus across the nuclear envelope into the cytoplasm. Thus, it is the nuclear pores present on the nuclear envelope which play a significant role in regulating the nucleocytoplasmic traffic.

In the present exercise, you will study the complex organization of the nuclear pore complex (NPC) with the help of electron micrographs. Since the entire structure of NPC is known to undergo complex conformational changes while transporting the materials, a view of scanning electron micrographs have been supplemented along with diagrammatic representations.

Objectives

After observing and drawing the diagrams from the electron micrographs of the nuclear pore complex (NPC) you will be able to:

- ❖ Study the topography of the nuclear membrane and the location of the nuclear pores, and

- ❖ describe the structural uniqueness of the components of the nuclear pore complex which are supplemented by photographs of diagrammatic representations to relate them to the functions.

13.2 STUDY GUIDE

For doing satisfactory work you must read the Unit 7 Organelles II of course BBYET-141 -Cell and Molecular Biology.

13.3 MATERIALS REQUIRED

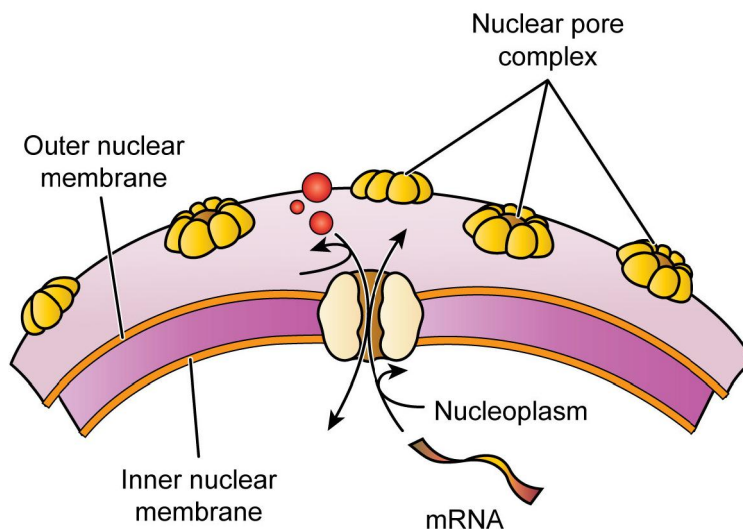
You will be provided with the electron micrographs of the nuclear pore-complex. In addition photographs of the surface view of the nuclear membrane will also be shown to you to understand better the location of the nuclear pores. Photographs of the diagrammatic representations of the nuclear pore complex will also be provided to you to help you to appreciate the components of this complex organization of NPC.

13.4 PROCEDURE

Observe the electron micrographs provided carefully. Make neat, well-labelled diagrams (both outline and a portion enlarged to show the cellular details) in your note book and write comments. It is advised that figures should not be copied from the book but should be drawn after observing the electron micrographs. You can take the help of photographs illustrating the diagrammatic representation of the nuclear pore complex to draw the right structure and proportion of the components.

13.5 OBSERVATIONS

The **nuclear envelope** consists of two cellular membranes arranged parallel to one another and separated by a perinuclear space which ranges from 10-50 nm (Fig. 13.1 a).



(a)

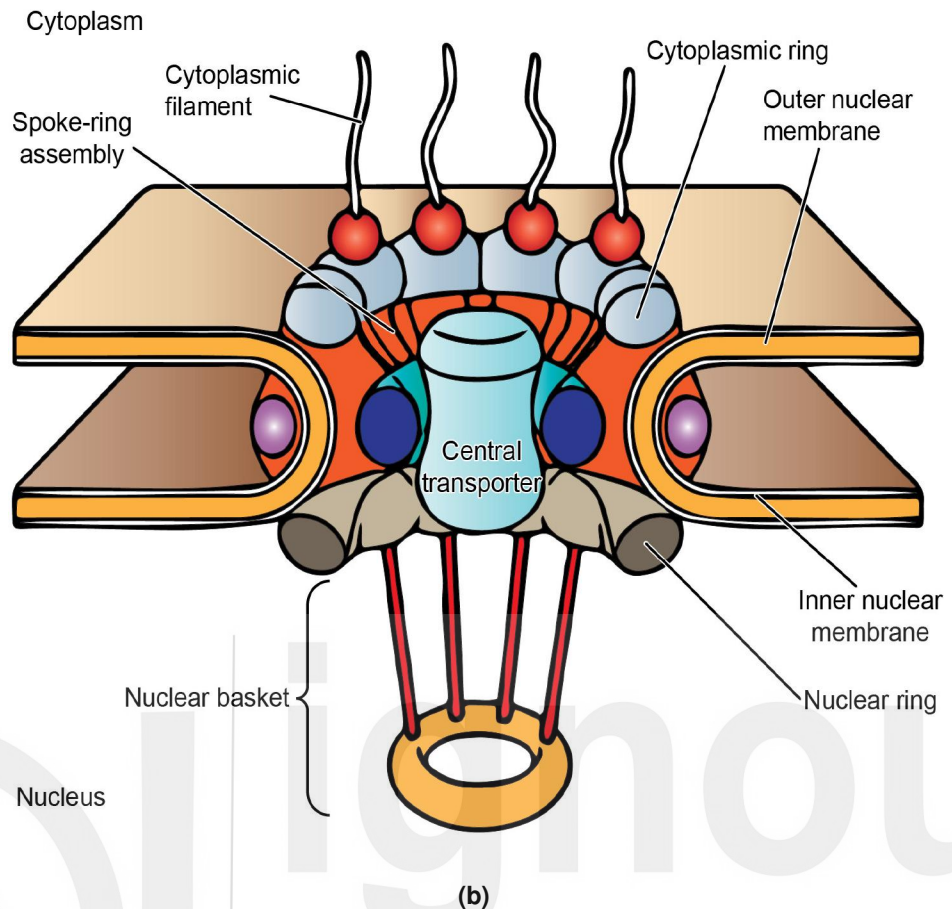
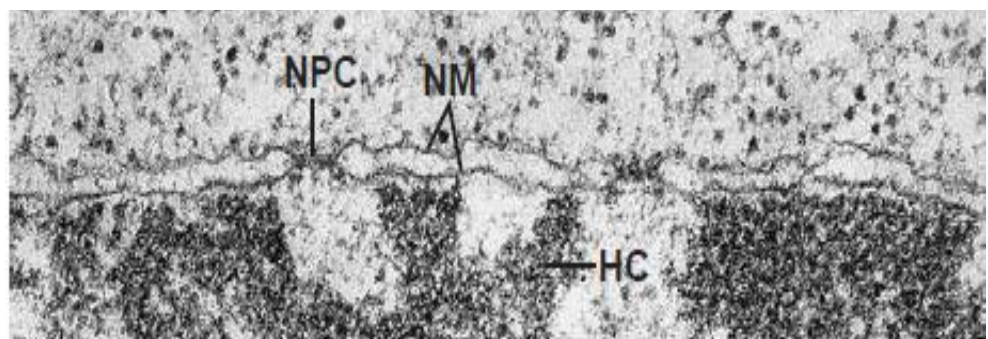


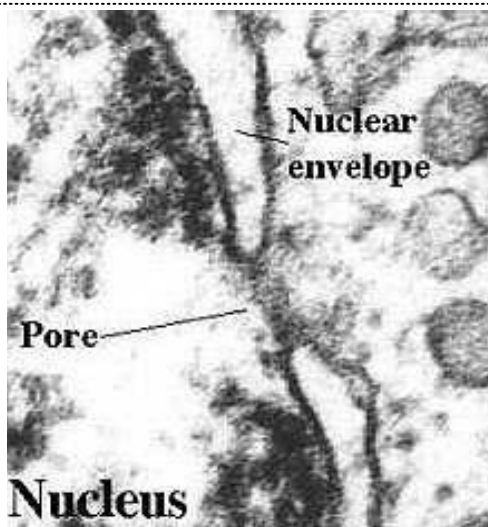
Fig. 13.1: a) Diagram highlighting position of nuclear pore complex; b) A diagrammatic view of the nuclear-pore complex.

There are many transmembrane proteins which link the outer nuclear membrane with the elements of the cytoskeleton (Fig. 13.1 b).

Along the nuclear surface, there are various points where the inner and outer nuclear membranes are fused forming circular pores (Fig. 13.2). These pores contain complex assemblies of proteins. Thousands of such pores are present in the nucleus of a cell. A thin filamentous meshwork, the nuclear lamina is present bound to the integral proteins at the inner face of the nuclear envelope at the inner surface of the nuclear envelope. This meshwork provides mechanical support to the nuclear envelope and serves as a site to which the chromatin fibers get attached.



(a)



(b)

Fig. 13.2: Electron micrographs (a, b) showing the nuclear pore complexes (NPC), nuclear membrane (NM) and heterochromatin (HC).

The nuclear pores are not just open channels but represent a very intricate supramolecular complex system called the nuclear pore complex (NPC). This complex projects from both the sides (nucleoplasm and the cytoplasm) of the nuclear envelope. In magnitude the NPC is 15-30 times the mass of a ribosome. NPC exhibits octagonal symmetry as its several components are repeated eight times or their multiples (Fig. 13.1b).

The NPC consists of a central channel which is surrounded by a ring of eight nucleoporins. The diameter of the central channel can change from 20 to 40nm depending on the rearrangements of the nucleoporins. A subset of proteins possessing phenylalanine-glycine residues (called FG) is present among the nucleoporins. These FG domains show a disordered structure due to their unique amino acid composition and this gives them a flexible organization. The hydrophobic meshwork formed by the FG domains blocks the free diffusion of larger molecules (larger than 40kD) between the nucleus and the cytoplasm.

The NPC gets reassembled after mitosis when the nuclear membranes of the daughter nuclei get reorganized. The NPC is reconstructed step by step from modular components in the nucleus.

13.6 TERMINAL QUESTIONS

1. Enlist the major components of Nuclear Pore Complex.
2. What is the importance of Nuclear Pore Complex?

13.7 ANSWERS

1. Nuclear Pore Complexes are composed of units termed as nucleoporins (Nup) which are the main channels that facilitate transport of small molecules such as small proteins, metabolites, and ions between the nucleoplasm and cytoplasm.

2. Nuclear pore complexes (NPCs) form an important functional entity of nuclear membrane and perform important function of enabling selective transport of materials such as proteins, ribonucleoproteins and RNA across the nuclear membrane in an organized and orderly fashion.

Acknowledgement

- Fig. 13.1** : a) <https://www.google.co.in/imgres?imgurl=x-raw-image%3A%2F%2F4e3d9578648eb7484f4139423bd65356d8e7eff12adfe81d9742638b1640944c&imgrefurl=https%3A%2F%2Fcore.ac.uk%2Fdownload%2Fpdf%2F>
- b) https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fcytochemistry.net%2F_Media%2Fnuclpor13_med.jpeg&imgrefurl=http%3A%2F%2Fcytochemistry.net%2Fcell-biology%2Fnuclear_envelope.html

- Fig. 13.2** : <https://www.google.co.in/imgres?imgurl=http%3A%2F%2Fwww.sivabio.50webs.com%2Fnucl012.jpg&imgrefurl=http%3A%2F%2Fwww.sivabio.50webs.com%2Fnucleus.html>



EXPERIMENT 14

STUDY OF SPECIAL CHROMOSOMES (POLYTENE AND LAMPBRUSH) BY SLIDES/PHOTOGRAPHS

Structure

14.1	Introduction	14.5	Observation
	Objectives		Polytene Chromosomes
14.2	Study Guide		Lampbrush Chromosomes
14.3	Materials Required	14.6	Terminal Questions
14.4	Procedure	14.7	Answers

14.1 INTRODUCTION

Chromosomes are the carriers of genetic information from one cell generation to another. They are composed of nucleic acids and histones. The chromosomes are thread like structures and have different shapes depending upon the position of the centromere. However, in some species the size of these chromosomes become very large, and they are called **giant chromosomes**.

Polytene chromosomes were first recognized as giant bodies by an Italian cytologist E. Balbiani in cells having large nuclei in the salivary glands of the midge *Chironomus* (Diptera). They were named as polytene chromosomes by Kollar. Polytene chromosomes in the larval salivary glands of *Drosophila* are about 100 times longer than the chromosomes of those normally found at the metaphase of mitosis. This happens because cells undergo repeated rounds of DNA replication without cell division. The DNA of the giant chromosome repeatedly replicates but the daughter strands do not separate. Hence the chromosome number remains constant but the DNA content is amplified many fold. The fully developed chromosomes are nearly 0.25 to 0.55 mm long. The chromosomes show alternating dark and light bands, and each band represents a gene. The giant polytene chromosomes display about 5000

bands when stained with dyes. The bands may also show **chromosomal puffs** or **Balbani Rings** which are the localized swellings of the chromosome due to high DNA transcription activity. The amplification of DNA of salivary gland chromosomes in the insect larvae is also known as *polytenisation* and the chromosomes are referred to as polytene chromosomes.

Lampbrush chromosomes are a special type of chromosome found in the growing oocytes (immature eggs) of most animals. They were first observed and described in 1882 by Flemming in amphibian oocytes. A detailed study was made by Ruckert (1892) on the oocytes of Shark. Lampbrush chromosomes are larger than the polytene chromosomes. Chromosomes transform into such form during the diplotene stage of meiotic prophase I due to an active transcription of many genes. The chromosomes have loops projecting in pairs which give lampbrush chromosome its characteristic appearance.

Objectives

After going through these prepared slides and photographs of the giant chromosomes you will be able to:

- ❖ describe the structural uniqueness of each type of giant chromosome, viz., polytene and the lampbrush chromosomes; and
- ❖ appreciate the bands, interbands and the Balbiani rings in the Polytene chromosomes and the somatic pairing and chromatin loops in the lampbrush chromosomes.

14.2 STUDY GUIDE

For doing satisfactory work you must read the course BZYCT-137 Genetics and Evolutionary Biology before coming to the laboratory.

14.3 MATERIALS REQUIRED

You will be provided with a compound microscope and the following permanent slides:

1. Whole mount of the salivary glands of *Drosophila/Chironomus* (Polytene chromosomes), and
2. Whole mount of giant nucleus of *Acetabularia* / primary oocytes of amphibians, reptiles, birds, or mammals.

You will also be provided with the photomicrographs of Polytene and Lampbrush chromosomes.

14.4 PROCEDURE

Observe the permanent slides provided carefully under the compound microscope both under low and high power. Note down the structural details of each type of chromosome. Make neat, well- labelled diagrams (both outline

and a portion enlarged to show the cellular details) in your note book and write comments. It is advised that figures should not be copied from the book but should be drawn after observing the slides under the microscope or the photographs provided to you.

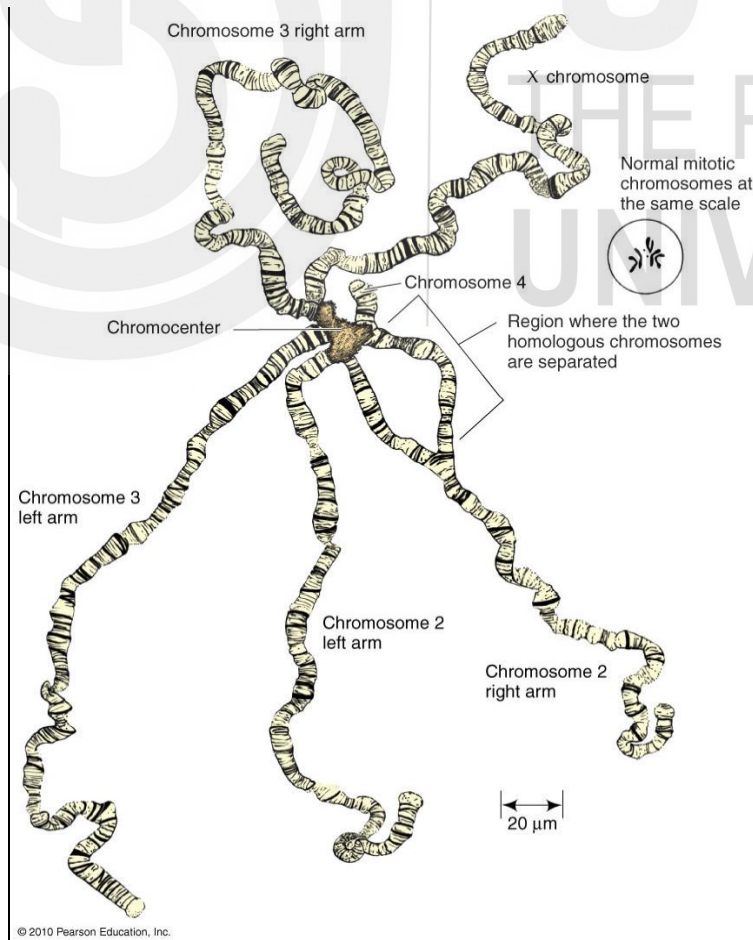
14.5 OBSERVATIONS

You have been provided with permanent slides and the photographs of Polytene and Lampbrush chromosomes. Observe each one of them carefully and note down their characteristic features.

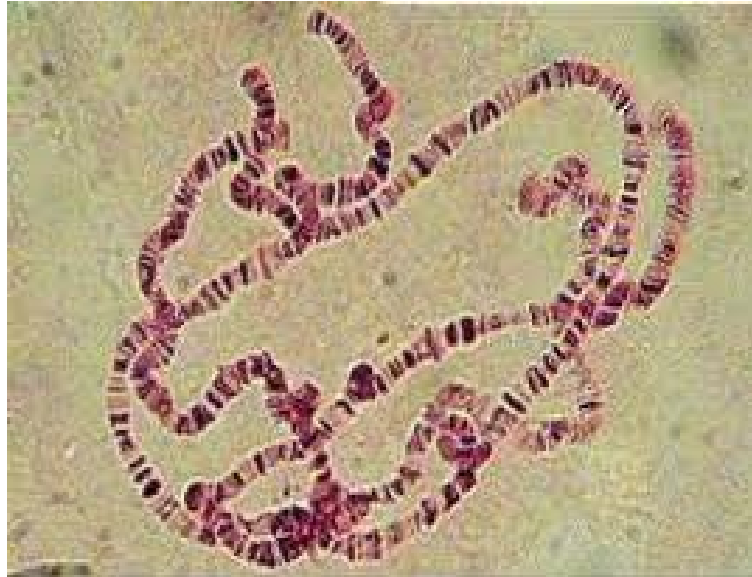
14.5.1 Polytene Chromosomes

A micrograph of the salivary gland cells of *Drosophila* or a dipteran *Chironomus* exhibits the following structural features:

- The salivary gland chromosomes are exceptional as they are clearly visible during interphase.
- They are about 200 times longer than the normal chromosomes. They attain a large size due to **polyteny**.
- Regions with more tightly coiled DNA fibres are represented as darkly stained **bands**. Such bands alternate with relatively clear zones lightly stained zones called **interbands** which are regions of low DNA density (Fig. 14.1).



(a)



(b)

Fig. 14.1: a) A polytene chromosome; b) Polytene chromosome as seen in *Drosophila melanogaster*.

- In certain areas of the bands, the tightly coiled chromosomal fibers open out; the DNA unfolds into loops because of intense mRNA synthesis (gene transcription). This results in a swelling in regions due to increase in area. Such zones are called chromosomal puffs or Balbiani rings (Fig.14.2).
- In *D. melanogaster*, the polytene chromosomes are seen as a complex of five long and one short arm radiating out from a deeply staining, amorphous region called the **chromocenter**. This region is formed to the fusion of the centromeric regions of all the chromosomes (the entire Y chromosome in males). The maternal and paternal homologous chromosomes remain associated side by side. This is called **somatic pairing**.

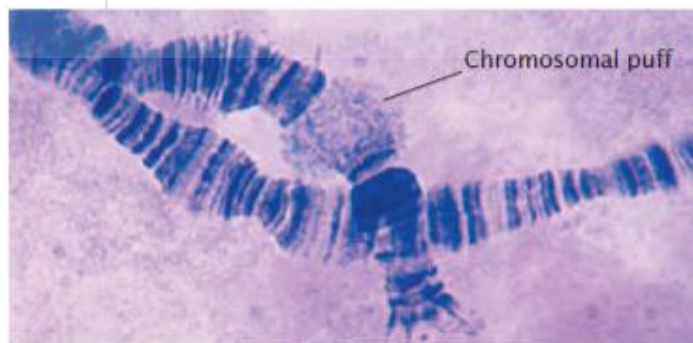


Fig. 14.2: Photomicrograph of giant polytene chromosomes isolated from the salivary glands of larva of *Drosophila*. The chromosomal puffs depict regions of relaxed chromatin with active transcription.

14.5.2 Lampbrush Chromosomes

You have been provided with the electron micrograph and the diagrams the salamander oocytes showing the lampbrush chromosomes. You will observe the following structural characteristics:

- Lampbrush chromosomes are larger than the polytene chromosomes.
- Chromosomes transform into such form during the diplotene stage of meiotic prophase I due to an active transcription of many genes.
- The chromosomes have loops projecting in pairs which give lampbrush chromosomes their characteristic appearance.
- The lampbrush chromosomes are so named because they give the appearance of a brush used for cleaning glass chimneys of old lamps (Fig.14.3).
- They could attain a length of up to 1 mm and a breadth of 20 μm in some amphibian oocytes.

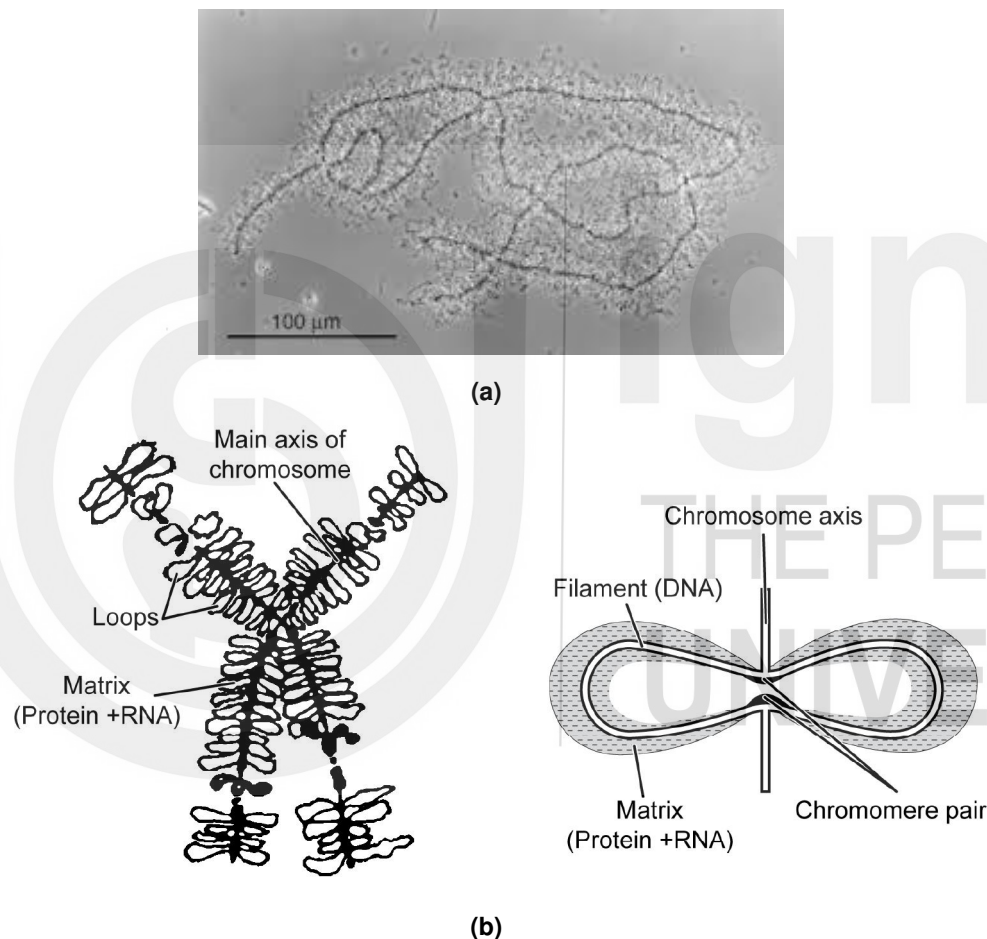


Fig. 14.3: a) Photomicrographs of lampbrush chromosomes as seen in amphibian oocytes. b) Diagrammatic representation of DNA loops.

- These are present in the form of bivalents where a pair of maternal and paternal homologous chromosomes is seen held together by chiasmata (Fig.14.4).
- Each bivalent shows four chromatids and instead of condensing they decondense and stretch out. The axis of each homologue shows a row of granules or **chromomeres** from which arise a pair of **lateral loops** (one from each sister chromatid). Each loop represents extended and unfolded single DNA molecule which is highly active in transcription, i.e., RNA synthesis.

- More than 10000 such loops may be seen per haploid chromosomal set in the oocytes.
- Number of pairs in loops gradually increases during diplotene and this meiotic stage with lampbrush chromosomes may persist for several months.

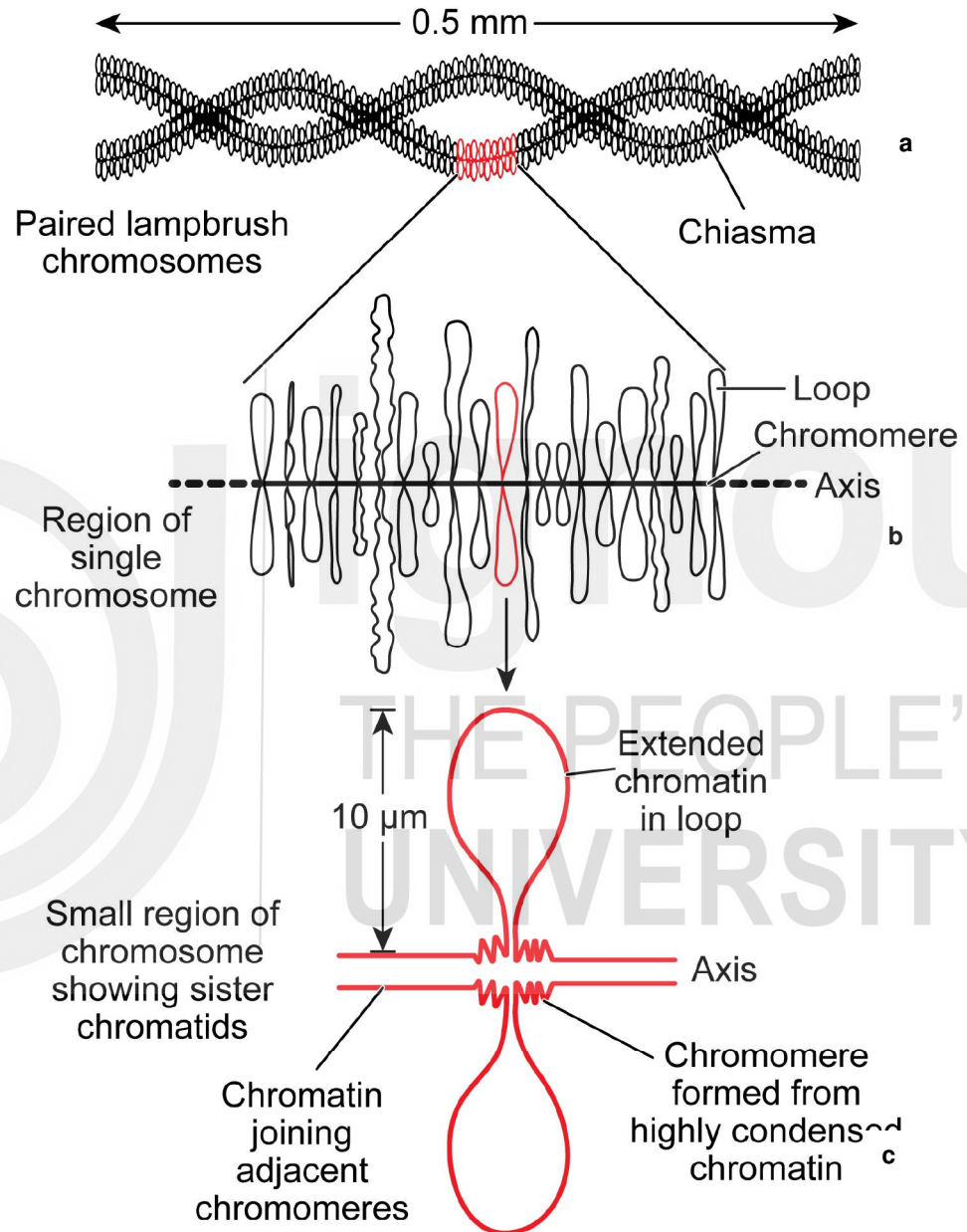


Fig. 14.4: a) A lampbrush chromosome with bivalents showing chiasmata; b) A portion of a bivalent showing loops and chromomeres; c) An enlarged portion of b. with a single pair of loops. (After Alberts *et al* 1989).

14.6 TERMINAL QUESTIONS

1. What is the significance of giant chromosome?
2. What is the role of lampbrush chromosome?

14.7 ANSWERS

1. The interbands found in the giant chromosome are involved in the interaction with the active chromatin proteins, and nucleosome remodeling.
2. Lampbrush chromosome helps in studying gene amplification which is required for growth of oocytes. The loops of lampbrush chromosome are used in studying transcription, chromosome mapping and visualizing gene expression in its natural state

Acknowledgements

Fig. 14.1 : a) Source:
[google.com/imgres?imgurl=https%3A%2F%2Fwww.mun.ca%2Fbiology%2Fscarr%2FiGen3_16_01_Figure-L.jpg&imgrefurl=https%3A%2F%2Fwww.mun.ca%2Fbiology%](https://www.mun.ca/fbiology/fscarr/fiGen3_16_01_Figure-L.jpg)

b) Source:
[https://www.google.com/imgres?imgurl=x-raw-image%3A%2F%2F%2F61cdd42f90e214074c4a0ce70a1bf719d82d4c42305a88f9a63b23e33ea46015&imgrefurl=http%3A%2F%2Fsphinxxai.com%](https://www.google.com/imgres?imgurl=x-raw-image%3A%2F%2F%2F61cdd42f90e214074c4a0ce70a1bf719d82d4c42305a88f9a63b23e33ea46015&imgrefurl=http%3A%2F%2Fsphinxxai.com%2F)

Fig. 14.2 : Source: <https://www.google.com/imgres?imgurl=x-raw-image%3A%2F%2F%2Fbe2ab9e0f0118574c0fcf5d6f3553a692591216bff13a9389bc0d612a97be9c&imgrefurl=https%3A%2F%2Fwww.glocaluniversity.edu.in%2Ffiles%2FeContent%2FeLifeScience%2FPolytene%2520chromosomes>

Fig. 14.3 : <https://www.google.com/imgres?imgurl=https%3A%2F%2Fnateshippo.com%2Fwp-content%2Fuploads%2F2021%2F08%2FLB-1.jpg&imgrefurl=https%3A%2F%2Fnateshippo.com%2Flampbrush-chromosomes-history-definition-structure-and-functions%2F&tbnid>

Fig. 14.4 : <https://image.slidesharecdn.com/lampbrushchromosomeandtheirimportance-180807154130/95/lampbrush-chromosome-and-their-importance-8-638.jpg?cb=1533656660>

EXPERIMENT 15

INVESTIGATION OF HUMAN KARYOTYPES

Structure

15.1	Introduction	15.7	Study of an Unknown Karyotype from Sheet-IV
	Objectives		Questions Sheet IV
15.2	Study Guide	15.8	Study of an Unknown Karyotype from Sheet-V
15.3	Materials Required		Questions Sheet V
15.4	Procedure	15.9	Answers
15.5	Study of karyotypes from Sheet I and II		
	Questions Sheet I and II		
15.6	Study of an Unknown Karyotype from Sheet-III		
	Questions Sheet III		

15.1 INTRODUCTION

Chromosomes are most easily studied during metaphase. At this stage, each chromosome clearly shows the two chromatids connected by a centromere. It is possible to stop the process of mitosis in metaphase by chemical means and get the photograph of chromosomes. The photographs of metaphase smears are arranged in a prescribed manner to obtain the karyotype of that individual.

The chromosomal constitution of a cell is called its karyotype. In other words, a karyotype represents a characteristic fixed size, shape, and number of the chromosomal complement of a species. The actual photographic representation in a karyotype can also be depicted with the help of a schematic diagram called an **idiogram** or karyogram. Here the chromosomes are represented in the form of vertical bars. The haploid set of chromosomes of a species is drawn in a decreasing order of their size.

Objectives

After doing this laboratory exercise you should be able to:

- ❖ prepare karyotypes from the Xerox photographs of chromosomes provided;
- ❖ identify karyotype of normal male and female; and
- ❖ diagnose the chromosomal disorders from the abnormal karyotypes provided.

15.2 STUDY GUIDE

For doing satisfactory work you must read the Unit 8 of BBYET-141 Cell and Molecular Biology before coming to the laboratory:

15.3 MATERIALS REQUIRED

1. Human chromosome photographs
2. Human karyotype forms
3. Pair of scissors
4. Pencil
5. Tape or Glue

15.4 PROCEDURE

Karyotyping

Geneticists have developed a system for identifying each of the 46 human chromosomes. The 22 pairs of autosomes are numbered from 1 to 22 according to their length. The sex chromosomes are grouped according to their shape and size along with the autosomes. The 23 pairs of chromosomes have been arranged into 7 groups according to the size and location of centromere. Table 15.1 gives the information about various chromosomes. This table would be your key guide in the preparation and study of karyotypes in this experiment.

Table 15.1: The Seven Groups of Chromosomes.

Group	Chromosomes	Characteristic
A	1, 2, 3	Very long; centromere in the centre of chromosome
B	4 and 5	long; centromere away from centre of chromosomes
C	6, 7, 8, 9, 10, 11, 12, X	Medium length; centromeres in the centre or slightly away from center of chromosomes

D	13, 14, 15	Medium length; centromeres at or very near the end of chromosomes
E	16, 17, 18	Somewhat short; centromeres in the centre or away from the centre of chromosomes
F	19 and 20	Short; centromeres in the centre of chromosomes
G	21, 22, Y	Very short; centromeres at or very near the end of chromosomes

The chromosomes of the first 22 pairs (Autosomal chromosomes) are similar in all human karyotypes. But the chromosomes of the twenty-third pair, the sex chromosomes are dissimilar. In male there is a larger X chromosome and a smaller Y chromosome, while in females two X chromosomes are present.

In this investigation you would study three abnormal karyotypes.

- i) **Down's syndrome** – an extra chromosome 21 is associated with this disorder.
- ii) In **Turner syndrome** X chromosome in females is missing; and
- iii) In this disorder, extra X chromosome (XXY) is found in males which leads to **Klinefelter syndrome**.

In this laboratory exercise, you will study and identify the different groups of human chromosomes and prepare a karyotype of them from the figures provided. You will also learn that abnormal chromosomal numbers result in specific syndromes commonly found in human males and females.

The procedure to prepare karyotype is as follows:

1. Cut each individual chromosome from sheet I and II. Be careful not to cut any part of the chromosomes. Count how many you have.
Note: The chromosomes cut-outs are very light and are easily lost. Even if a chromosome is lost from the sheet set, the whole set would become incomplete and the exercise will be useless. So be very careful.
2. Arrange the chromosomes in the karyotype forms a and b respectively according to the chromosome characteristics by placing the short arm of each chromosome towards the top.
3. Pair the chromosomes referring to their banding pattern (if provided) and other features mentioned above.
4. After the chromosomes are arranged in order, glue, or tape them in place.
5. Answer the questions based on sheet I (Fig. 15.1) and II (Fig. 15.2) about the karyotypes you have prepared.
6. Similarly study the karyotype sheets III, IV and V and answer the questions based on them.

15.5 STUDY OF KARYOTYPES FROM SHEET-I AND II

A chromosome spread from a normal human and karyotype forms of individuals are given in Fig. 15.1 and 15.2. Observe them and answer the questions given below.

SHEET - I

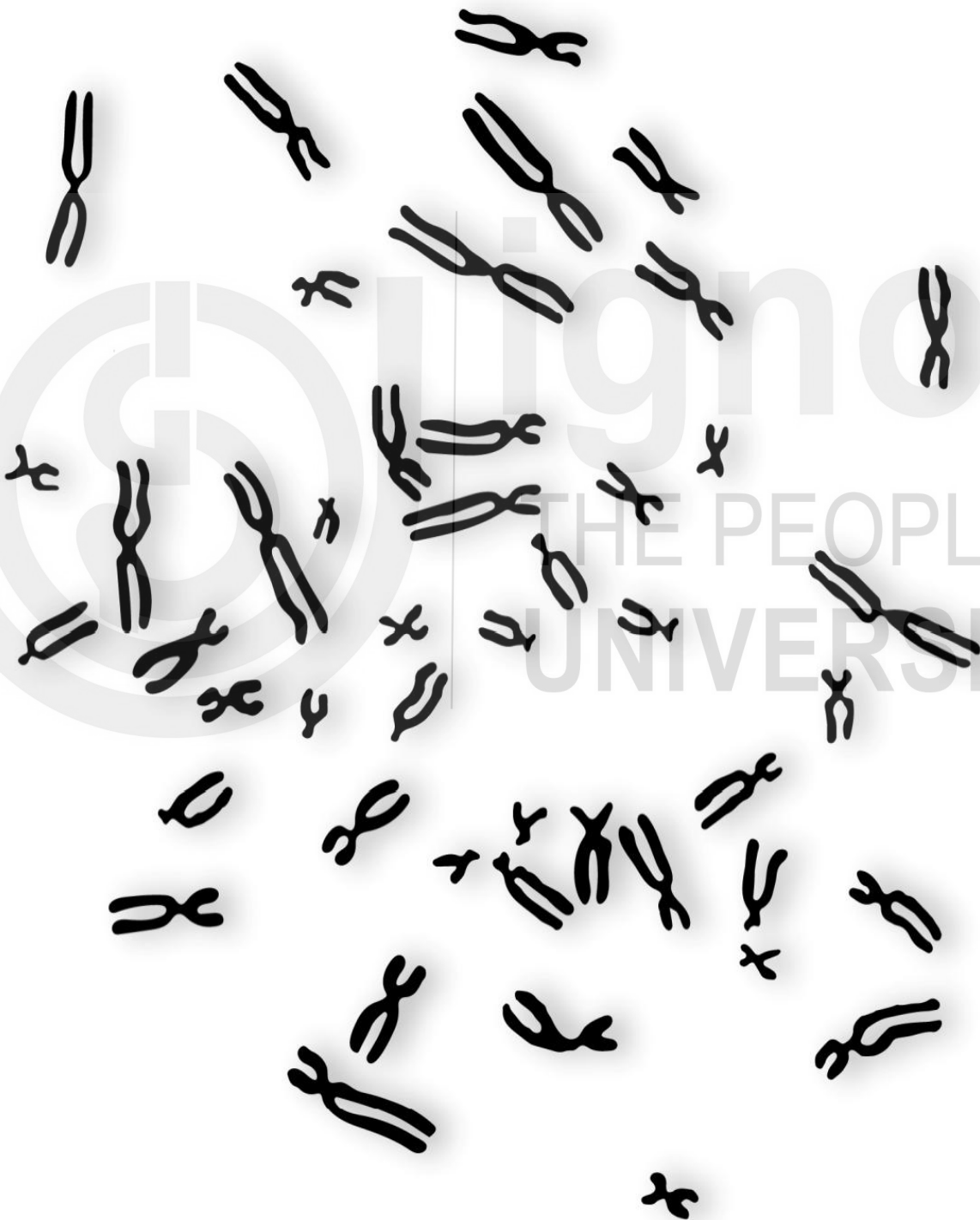


Fig. 15.1: A chromosome spread from a normal human.

Karyotype Form a

1	2	3	4	5		
A			B			
6	7	8	9	10	11	12
C						
13	14	15	16	17	18	
D			E			
19	20	21	22	Sex Chromosomes		
F		G				

SHEET - II



Fig. 15.2: A chromosome spread from a normal human.

Karyotype Form b

1 2 3

A

4 5

B

6 7 8 9 10 11 12

C

13 14 15

D

16 17 18

E

19 20

F

21 22

G

Sex Chromosomes

Questions Sheet I and II

1. Why must the photographs of individual chromosome be cut separately from sheet I and II, and pasted to karyotype it?
2. Why metaphase smears are considered best for karyotype studies?
3. Identify the sex of the individuals whose chromosomes appear in Sheet I and Sheet II respectively.
4. Compare the two karyotypes you have made. What specific difference can you find?
5. How important is this difference? Explain.
6. For what kind of an individual would a genetic counselor, most likely recommend karyotyping done? Why?
7. a) What kind of difficulties did you have in sorting the chromosomes into pairs, b) into groups? c) Would someone preparing a karyotype in a genetics lab have the same difficulties? Comment.

15.6 STUDY OF AN UNKNOWN KARYOTYPE FROM SHEET-III

This is a karyotype of an individual (Fig. 15.3). Observe it and answer the questions given below.

SHEET - III

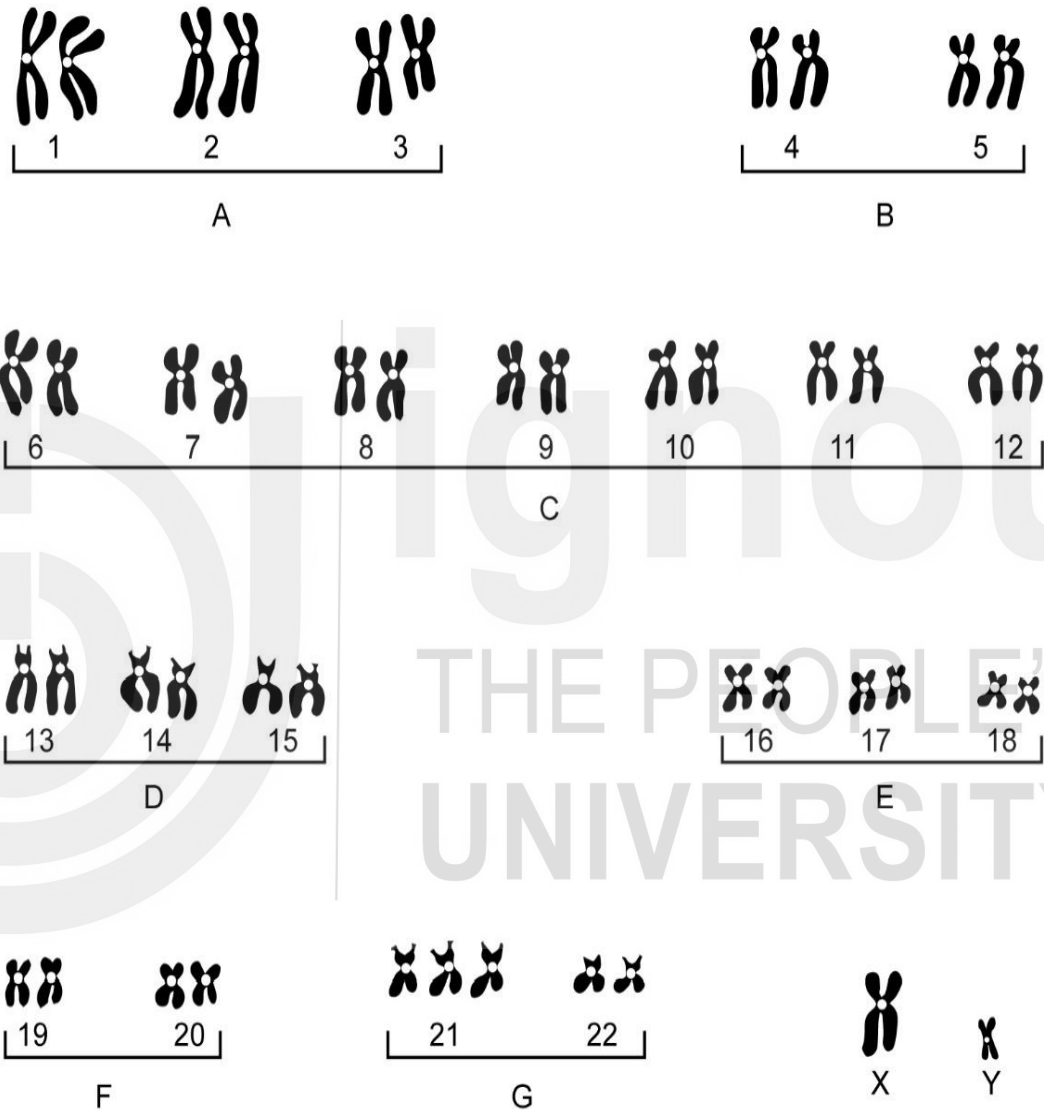


Fig. 15.3: Karyotype of an individual.

Questions Sheet III

1. Is this a karyotype of a normal individual or does it show any abnormality?
2. What is the feature that makes it different from the earlier two karyotypes?
3. Based on (2), name the genetic condition that such person has.
4. What are the prominent physical features of such individuals?

15.7 STUDY OF AN UNKNOWN KARYOTYPE FROM SHEET-IV

This is a karyotype of an individual (Fig. 15.4). Observe it and answer the questions given below.

SHEET - IV

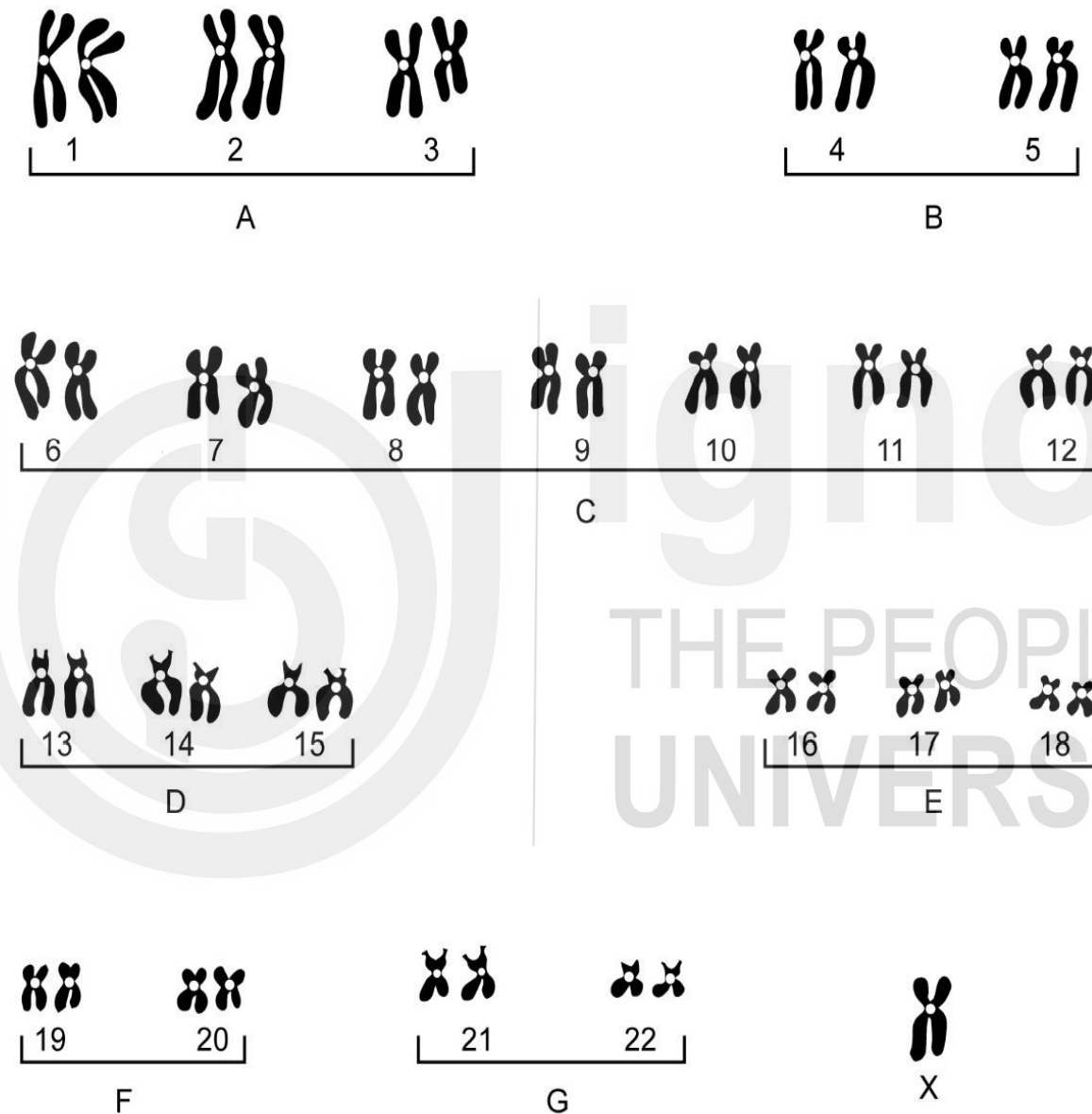


Fig. 15.4: Karyotype of an individual.

Questions Sheet IV

1. Does sheet IV show a normal karyotype or any abnormality?
2. If found abnormal, then what is the difference from the normal one?
3. Such karyotype belongs to person with which kind of genetic aberration?
4. What are the prominent physical features exhibited by such person?

15.8 STUDY OF AN UNKNOWN KARYOTYPE FROM SHEET-V

This is a karyotype of an individual (Fig. 15.5). Observe it and answer the questions given below.

Sheet V

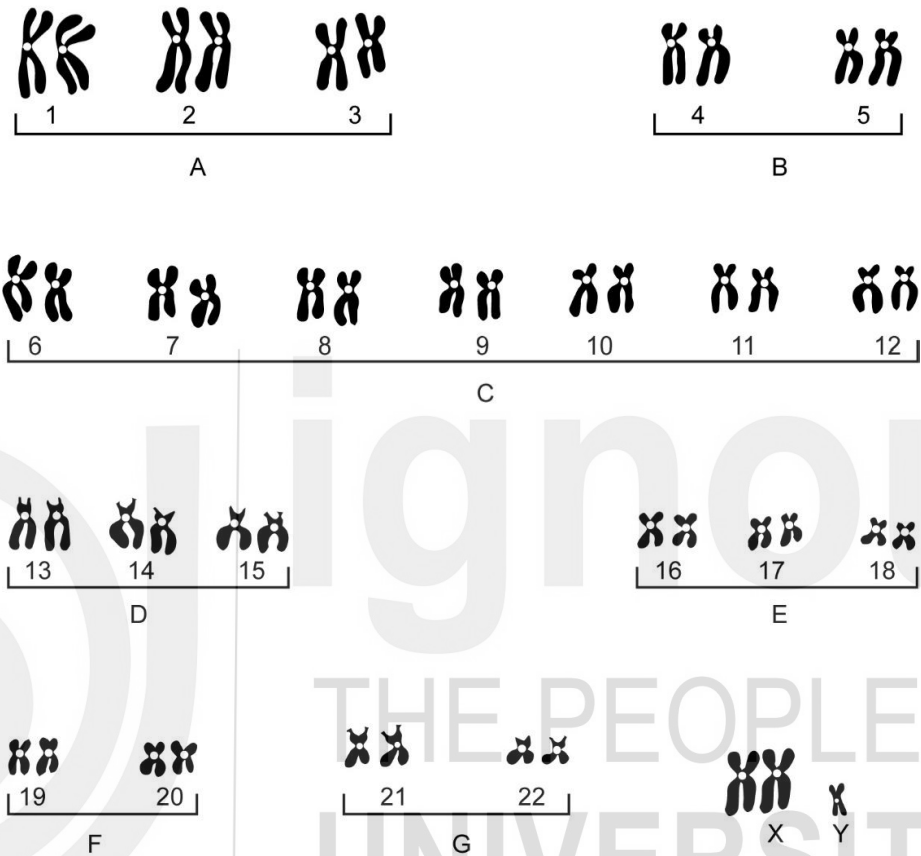


Fig. 15.5: Karyotype of an individual.

Questions Sheet V

- Does the sheet V show a normal karyotype or any abnormality?
- If you have found it abnormal, then what is the difference from the normal one?
- This is a karyotype of an individual having which kind of genetic aberration?
- What are the prominent physical features that help in identifying such individuals?

15.9 ANSWERS

SHEET I and II

- Pasting of the photographs of individual chromosomes help us to make pairs of autosomes, find any odd number of chromosomes and ascertain sex of individual.

2. Since chromosomes are most condensed at the metaphase, their morphology at this stage is the most suited for photography. Their size and shape including the position of centromere is clearly seen.
3. Sheet I - Normal male
Sheet II - Normal female
4. Karyotype in sheet I shows two X chromosomes
Karyotype in sheet II shows one X and one Y chromosome
5. This difference is the basis of sex-determination in human beings.
6. In case there is an abnormality in the form or physical or mental stability.
7. Students are advised to work out the karyotype preparation themselves. The difficulties faced may vary from student to student.

SHEET III

1. This karyotype shows an abnormality. The karyotype records 47 chromosome instead of normal count of 46.
2. This karyotype shows trisomy of the 21st chromosome.
3. Down syndrome
4. Oriental features; short and broad neck, short stubby hands and feet, moon like face with widely separated eyes, flat nose, ears small and malformed.

SHEET IV

1. This is an abnormal karyotype with 45 chromosomes in place of normal count of 46.
2. There is only one sex chromosome –X. So designated as XO (44+ XO)
3. Turner's syndrome.
4. Phenotypic females but sterile, underdeveloped breasts, webbed neck, low set ears, short stature.

SHEET V

1. This is an abnormal karyotype. It possesses 47 chromosomes in place of 46.
2. There are three sex chromosomes (an additional X chromosome) so designated as 44+ XXY.
3. Klinefelter's Syndrome.
4. Sterile males with some degree of breast development, underdeveloped genitalia, sparse body hair.