17MBU613A **CELL BIOLOGY - PRACTICAL** **(3H –1C)**

Instruction Hours / week: L: 0 T: 0 P:3

Marks: Internal: **40** External: **60** Total:**100 End Semester Exam:** 6Hours

SCOPE

To describe the basic concept of cell structure, membrane, cellular functions of different types of cell, modes of cellular signaling and signal amplification.

OBJECTIVES

- To explain the cell structure and functions of organelle.
- To determine the transportations through cell membrane.
- To categorize the different receptors and model of signaling.
- To analyze the concept of cell signaling.

EXPERIMENTS

1. Study a representative plant and animal cell by microscopy.
2. Study of the structure of cell organelles through electron micrographs.
3. Cytochemical staining of DNA – Feulgen.
4. Demonstration of the presence of mitochondria in striated muscle cells/ cheek epithelial cell using vital stain Janus Green
5. Study of polyploidy in Onion root tip by colchicine treatment.
6. Identification and study of cancer cells by photomicrographs.
7. Study of different stages of Mitosis.
8. Study of different stages of Meiosis.

SUGGESTED READINGS

1. Hardin J, Bertoni G and Kleinsmith LJ. (2010). Becker's World of the Cell. 8th edition. Pearson.
2. Karp G. (2010) Cell and Molecular Biology: Concepts and Experiments. 6th edition. John Wiley & Sons. Inc.
3. De Robertis, EDP and De Robertis EMF. (2006). Cell and Molecular Biology. 8th edition. Lipincott Williams and Wilkins, Philadelphia.
4. Cooper, G.M. and Hausman, R.E. (2009). The Cell: A Molecular Approach. 5th Edition. ASM Press & Sunderland, Washington, D.C.; Sinauer Associates, MA.

Experiment No: 1

Study of Representative Plant and Animal Cell:

AIM:

To study about the structure of Plant and Animal cells observation by using Light Microscope.

Background Information:

The term "cell" came from a pioneer of microscopic biology, Robert Hooke, while looking at epidermal onion cells under a microscope.The onion's cell walls, like those of other plants, are rigid. Cellulose in the cell walls forms clearly defined polygonal structures. Water within the cell walls gives the walls strength and helps plants resist the force of gravity. The cell's cytoplasm and vacuole contribute to the onion's solidity and its characteristic crisp texture. Below the cell wall is a layer of liquid called the cytosol, primarily composed of water, salts and organic molecules. The cytosol also contains organelles, organic structures that manage all the elements of cell metabolism. The cytosol also carries inclusions, which are starches, proteins and other elements that act as building blocks for a number of functions. The nucleus, also found in this cytoplasmic layer, contains the plant's basic genetic material.

Prepare onion wet mount:

Materials required:

Onion, , Slide, Coverslip Methylene blue solution, Forceps,Microscope.

Procedure:

- Quarter an onion.
- single onion leaves were isolated.
- Then break leaf in half and pull thin layer of epidermis, being careful to not let it fold on itself. If it does fold, attempt to unfold it by using forceps.
- Place the flattened onion epidermis on a microscope slide and Place a small drop of iodine on the epidermis. Methyline blue will stain the nucleus of the cell so it is visible.
- Place cover slip on top of the specimen and, remove any excess stain with a paper towel.
- Place wet mount on microscope.With the scanning objective (4x), focus the specimen and locate a section where the epidermis is a single cell thick. This is usually found on the edges of the specimen.
- Switch to low power objective (10x). Focus in on a section of the epidermis where it is a single cell thick.
- Switch to high power objective (40x) and focus, only with the fine focus knob.

RESULT:

Experiment -1B

Investigating animal cells

Back ground Information:

Cheek cells are epithelial cells that line the interior surface of our mouths. The base layer of cells in an epithelial structure are not actually cells, but a sticky layer on which the cells anchor. The other surface of the epithelial cell touches the outside world (like skin) or an open space (like the mouth). Because of their high rate of division, epithelial cells are found tightly packed together. When you stain your cheek cells, you should be able to distinguish between the nucleus, cytoplasm, cell membrane. If you are very observant (and lucky) you may visualize the nucleolus and other organelles with in the cell.

Materials Required:

Toothpick, slide,coverslip, Metheleneblue stain, Microscope.

Procedure:

- Using a flat toothpick, very gently scrape the inside of your cheek to obtain cheek cells.
- Spread the cells on the end of the toothpick onto the microscope slide.
- Add 1 small drop of methylene blue to the sample. Methylene blue will stain the sample, allowing visualization of the nucleus, cytoplasm, and even some organelles. Note: methylene blue will stain your hands and clothing. Wear goggles, gloves and an apron.
- Place a cover slip on the sample. Press down on the coverslip and remove excess methylene blue with a paper towel.
- Using the scanning objective (4x), focus the specimen and locate cheek cells. Change the objective to low power, refocus on a few cheek cells.
- Finally, visualize your cheek cells at high power. At this point you may need to reduce your light intensity and adjust the condenser aperture.

RESULT:

Experiment No: 2

STUDY OF THE STRUCTURE OF CELL ORGANELLES THROUGH ELECTRON MICROGRAPHS.

AIM:

To study the structure of cell organelles through Electron micrographs.

OBJECTIVES:

To observe the structure and function of following organelles through electron micrograth

- The Electron Micrograph of Mitochondria
- The Electron Micrograph of Golgi Complex
- The Electron Micrograph of Endoplasmic Reticulum
- The Electron Micrograph of Lysosomes
- The Electron Micrograph of Plastids
- The Electron Micrograph of Nucleus

The Electron Micrograph of Mitochondria:

- It is an electron micrograph of cell's largest and most important organelle – the mitochondria and is characterized by the following features
- The name mitochondria was given by Benda (1898) and their ma n function was brought to light by Kingsbury (1912).
- Each mitochondria in section appears as sausage or cup or bowl shaped structure lined by double membranes. Theoretically, the membrane is similar n structure and chemical composition to plasma membrane.
- Two membranes are separated by a 6-8 mm wide fluid filled space called peri-mitochondrial space.
- The inner membrane is projected into the central cavity as finger like outgrowths- the cristae.
- Numerous small, rounded & stalked particles – The oxysomes or F1 or ATPare are attached to the inner surface of inner membrane.
- The central cavity is filled with matrix which theoretically possesses circular DNA 55^{s} ribosomes and respiratory enzymes.
- The main function of mitochondria is to synthesize chemical energy- ATP from glucose as substrate.
- From one molecule of glucose 38 ATP molecules (40%) are synthesized and the rest of the energy (60%) goes as heat.

Fig. 7 ELECTRON MICROGRAPH OF MITOCHONDRIA

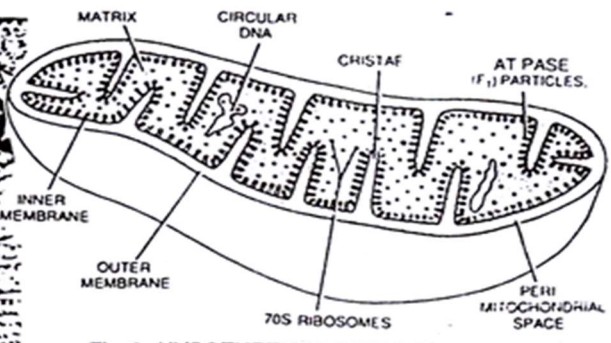


Fig. 8. HYPOTHETICAL DETAILED STRUCTURE OF A MITOCHONDRION.

The Electron Micrograph of Golgi Complex:

- It is the electron micrograph of Golgi complex along with its line drawing and is characterized by the following features:
- It was discovered by Camillio Golgi (1898) and was named after his name.
- The Golgi complex, as is visible in electron microphotograph, is a stack (bundle) of hollow tubules, which in actual form are hollow flattened sacks arranged above each other. On either side certain large globular vesicles and smaller vacuoles are also visible.
- Each tubule or lamella is lined by membrane, which is theoretically similar to plasma membrane in structure and chemical composition.
- The Golgi complex is more prominent and well developed in secretory cells and absent in RBC of mammals and prokaryotic cells.
- Its main function is to glycolise the proteins which are synthesized by ribosomes i.e., It converts these inert proteins into glycoprotein's to act as hormones, enzymes and coenzymes.

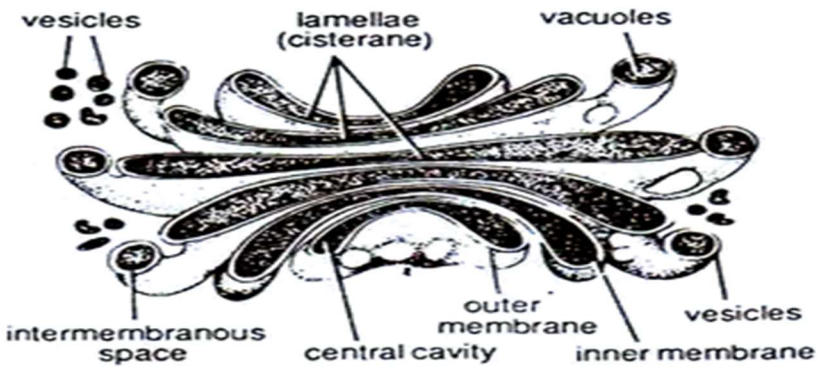


Fig. 10 GOLGI BODY THREE DIMENTIONAL AND DIAGRAMMATIC MODEL

The Electron Micrograph of Endoplasmic Reticulum:

- It is an electron micrograph of endoplasmic reticulum and is characterized by following features
- It was discovered and named by Porter (1948).
- It is made up of large number of interconnected and branched tubules, long, flattened and sac-like cisternae and hollow approximately rounded vesicles present all over in the cytoplasm forming a continuous system.
- Each tubule, cisternae or vesicle is made up of membrane, which is theoretically similar to plasma membrane in structure and chemical composition.
- Some cisternae and tubules bear small, dark, rounded and granular structures, ribosomes, along their surface. This endoplasmic reticulum is called rough or granular E.R. The endoplasmic reticulum without ribosomes is called smooth or agranular ER.
- The main function of rough endoplasmic reticulum is protein synthesis.

The main functions of smooth endoplasmic reticulum are:

a) Detoxification
b) Synthesis of lipids & cholesterol.
c) It is absent in R.B.C. of mammals and prokaryotic cells.
d) Both types of reticulum provide mechanical support, transport with in the cell, conduction of nerve and electric impulses and formation of nuclear membrane at the time of cell division.

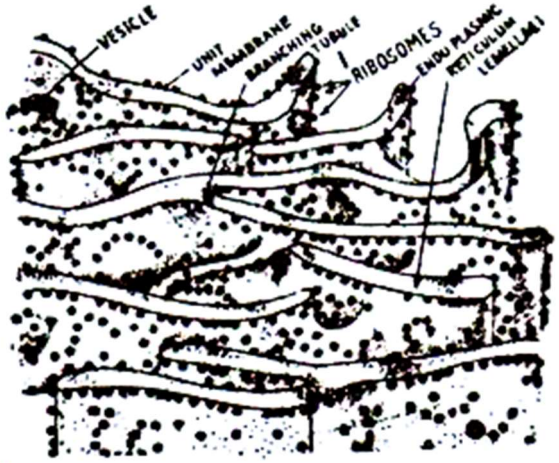


Fig. 12. DIAGRAMMATIC REPRESENTATION OF ENDOPLASMIC RETICULUM IN THREE DIMENTIONAL VIEW

The Electron Micrograph of Lysosomes:

- This is the electron micrograph of Lysosome, and is characterized by following features:
- They were discovered by de Duve (1954).
- They are spherical or irregular membrane bound vesicles filled with digestive enzymes.
- The Lysosomes in a cell occur in three forms viz., primary lysosome, secondary lysosome and residual body.
- The primary lysosomes are nascent lysosomes which are in a dormant stage; the secondary lysosome are those which have fused with phagocytic vesicles and has released their enzyme contents into the vesicle. This is also called phagosome. The residual body is one which has completed its digestive function and is ready to be thrown out of the cell.
- They develop from Golgi complex.
- Besides digestion, their other function is autophagic digestion during extreme starvation or extreme toxicities.

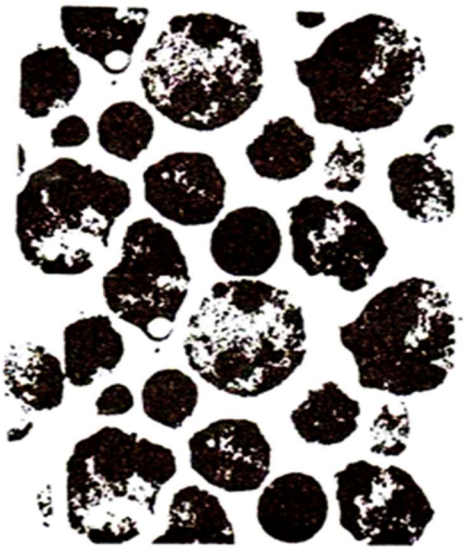


Fig. 13 ELECTRON MICROGRAPH OF LYSOSOMES

The Electron Micrograph of Plastids:

- This is an electron-micrograph of plastid or chloroplast, which is an integral component of all green plant leaves and is characterized by following features.
- They may be spheroidal, ovoid, stellate or collar shaped and differ in size and number in different cells.
- Each chloroplast is a sac-like structure, which is made up of double membranes separated from one another by periplastidial space.

Two types of double membranous lamellae are embedded in the stroma or matrix filled cavity:

a) Smaller flattened disc-shaped lamellae – The thylakoids, placed one above the other in a stack – the grana.
b) Larger tubular lamellae between grana called lamellae or frets which connect adjacent granna.
c) The Inner surface between the two membranes of a thylakoid bear countless granular chlorophyll particles the Ouantasomes.
d) The plastids also have their own circular DNA 55^{s} – Ribosomes and RNA
e) The main function of chloroplast or plastid is to synthesize carbohydrate molecules from $CO_2 + H_2O$ using light energy.

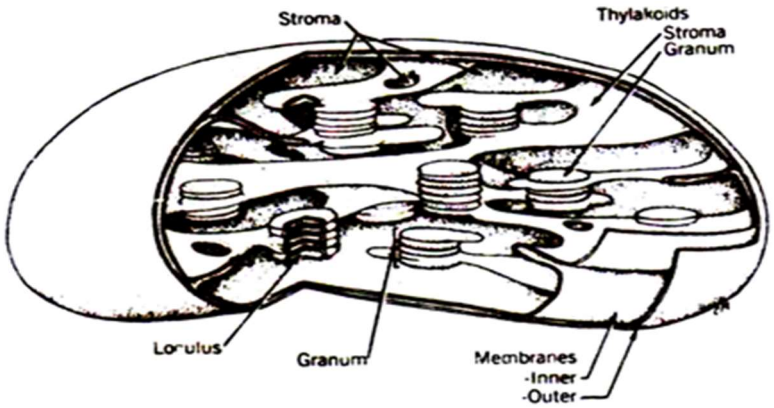


Fig. 16. DIAGRAM SHOWING PLASTID

The Electron Micrograph of Nucleus:

- This is an electron micrograph of nucleus:
- Nucleus was discovered by Brown (1831).
- It is a characteristic entity of almost all eukaryotic cells except mammalian RBCs.
- The nucleus is generally one but may also be two, four or many.
- Each nucleus is surrounded by double nuclear membranes perforated by numerous nuclear pores. Each nuclear membrane is just like unit membrane. Inside, there is present a large darkly stained nucleolus and a network of chromatin threads.
- The nucleolus is responsible for all the ribosomal RNA synthesis and chromatin (DNA) is responsible for controlling all the metabolic activities of cell as well as for all hereditary activities.
- The chromatin threads are made up of double helical DNA molecule which are the carrier of heredity units- the genes.

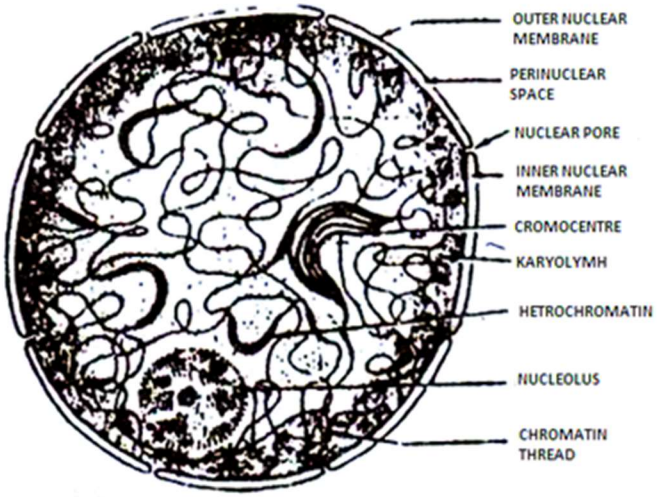


Fig. 18 GROSS STRUCTURE OF NUCLEUS AND NUCLEOPLASM.

Experiment -3

Cytochemical staining of DNA – Feulgen

Aim :

To study about the cytochemical staining of DNA by using feulgen stain.

Background Information:

The Feulgen procedure was invented by German physician and chemist Joachim Wilhelm Robert Feulgen during 1914-1924.. He also discovered animal and plant DNA ("thymonucleic acid") congeniality and estimated the nature of nucleic acids as a polymers of nucleotides with four kinds of nitrogenous bases . It is, probably, the most simple cytochemical method allowing indication and evaluation of DNA levels into cells. Schiff's reagent is the stain used in this technique. It specifically stains the DNA due to reaction of Schiff's reagent with aldehyde groups exposed at C1 as the result of the nitrogen bases cleavage from deoxyribose by 1N HCl hydrolysis at 60°C within about 10 min.

Principle:

Acid hydrolysis, designed to separate selectively 2 purine bases, namely adenine and guanin, from DNA molecule;staining of apurinic acid resulting from hydrolysis with Schiff reagent. This reagent can be used since free deoxyribose changes to aldehyde in acid environment. Feulgen reaction is highly selective for DNA. Moreover, this reaction allows a very precise localisation of DNA .

Materials required:

Schiff's reagent, 1N HCl, sodium or potassium metabisulphite, freshly prepared bleaching solution (5 ml of 10% sodium metabisulphite + 5 ml of 1N HCl + 90 ml of distilled water), 45% acetic acid, mixture glycerol:water (1:1, v/v), hydroponically growing onion.

Preparation of Schiff's reagent :

1. Dissolve 0,5g of basic fuchsin in 90 mL of boiling distilled H2O
2. When cooled to approximately 45 oC, add slowly 10 mL of 1N HCl
3. Cool to room temperature and add 1g of K2S2O5 or Na2S2O5 (metabisulfite can be substituted on sulfite, hydrosulfite or sulfurous acid).
4. Shake for 3 minutes and leave it the dark at room temperature overnight or until a light straw or faint pink color develops.
5. Add 0,5 g of fine activated charcoal and shake for 3 minutes.
6. Filter solution (should be transparent).
7. Store at 4oC in a tightly-stoppered bottle in the dark.

Procedure:

1. Fresh onion root tips are transfer to distilled water then the root tips are treated with 100% ethanol for 5min followed by 90%, 70%, 50% and 30% ethanol 5 min each.
2. After the series of ethanol treatment it has immerged in distilled water for 5min to remove the excess ethanol.
3. Place the root tips in 1N HCL solution for 2min and heat the 1N HCl at 60°c in water bath for 5 mim. Then cool it.

4. Transfer the root tips to distilled water and rinse once.
5. Then the root tips are treated with Schiff's reagent for 5 mins.
6. After that it allows to immerge in bleaching solution for 1min and rinse with distilled water .
7. Place the root tips in 45%acetic acid for 5 min.
8. Finally transfer the root tips in a drop of 45%acetic acid on to glass slide(or in a drop of 50%glycerol (distilled H2O +glycerol 1:1).
9. Cover the sample with coverslip and make a pressured preparation by gently prassing it.
10. Observe the cell at 40x and oil immersion.

RESULT:

Experiment -4

STAINING OF MITOCHONDRIA IN HUMAN CHEEK EPITHELIAL CELLS

Aim:

To study about the staining procedure of mitochondria in human cheek cells.

INTRODUCTION:

Mitochondria are considered as power houses of a cell as it produces ATP by a process called oxidative phosphorylarion. Each cell contains large number mitochondria and they can be observed under a light microscope if stained with Janus green. This stain is bluish green in colour when oxidized and colourless when reduced. When a dilute solution of the stain is applied to stain the cells, it enters in the cytoplasm as well as in mitochondria. Since mitochondrial inner membrane contains cytochrome oxidase enzyme, which can keep the stain in oxidized state, the mitochondria appear stained while in rest of the cytoplasm the stain gets reduced and thus appears colourless.

MATERIALS REQUIRED

Ethanol soaked tooth pick, slide, cover glass, 0.01% Janus green B stain in normal saline (dissolve, leave for 48 hr and then filter)

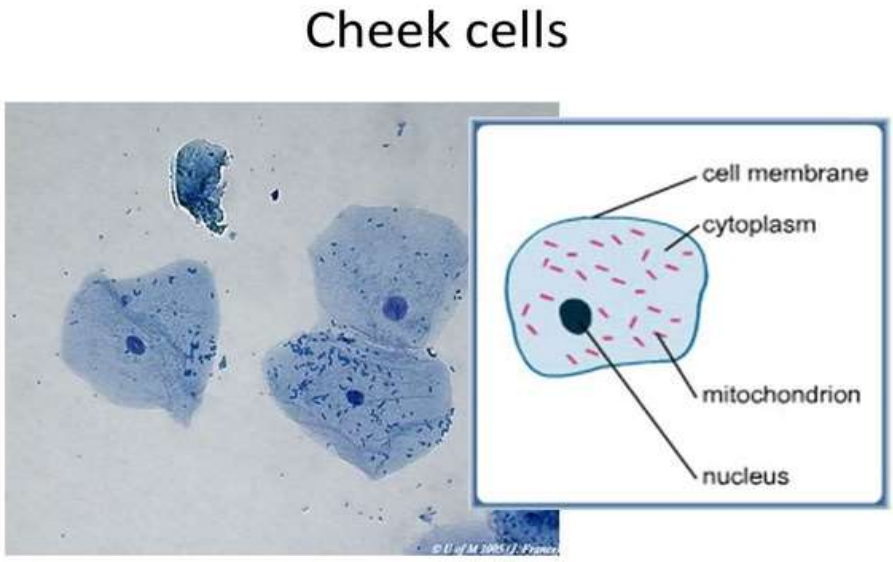
PROCEDURE

1. Dry the ethanol soaked tooth pick in air and scrape gently the inner side of cheek. A large number of cells will come on the tooth pick.
2. Gently rub the tooth pick on slide in one direction to make a spread of cells. Dry the cells on slide so that the cells will not get washed away while staining.
3. Put a few drops of Janus green stain and leave for 5-10 min for staining.
4. After 5 min of staining, rinse cells once with distilled water in such a way that complete stain is not gone and a diluted stain remains. Mount the cells in a drop of distilled water with a cover glass and observe under the bight field students' microscope.

5. The cells can alternatively be mounted in the stain itself. A few air bubbles remaining inside the cover glass give a background stain that makes the viewing easy. The slide can be observed under the high magnification of a student microscope.

OBSERVATION

Each cell is seen to contain a large number of tiny round or elongated bacteria like bodies in the cytoplasm mainly around the nucleus. Generally they are not strongly stained thus appear like pimples on a face. Mitochondrion can be easily distinguished from a bacterium as bacterial cells become more prominently stained and appear sharper than mitochondria. Also as bacteria are on the surface of cells, they will be focused at a slightly different level than mitochondria and can be distinguished.



Experiment -5

Aim:

To study about the Polyploidy aberration using onion root tips by colchicines treatment.

Background information:

A normal cell cycle occurs because of cell cycle and primary infections of cell cycle is duplicating the chrompsomal DNA ,which further divides into two identical daughter cells. In this process there are four main phase G1 phase, S phase ,G2 phase, and M phase. Among these two vital phase are S phase where DNA replication occurs and M phase where mitosis occurs. In mitosis again there are several phase like prophase ,metaphase,anaphase, and telophase. Cell cycle regulation is very important without which detection or repairing of gene is difficult and which can cause uncontrollable division of cell leading to cancer. There are certain alkaloid drugs that can inhibit the uncontrolled growth or division of cell. Colchicines is such drug that has been isolated from the plant called *Colchicum autumunale* and this drug at present is used for treating various diseases like gout,Bechet's disease,

and is under investigation as an anti –cancer drug.it posses the capability of inhibiting the microtubule polymerization by getting binded to protein tubulin therby leading to microtubule degradation.

Requirements:

Sample	-	rooted bulb of Onion
Chemicals	-	1% aqueous solution of colchicines
		Acetocarmine
		45% acetic acid
Others	-	Beaker, water,coverslip, slides,burner,Microscope**.**

Procedure:

- In the small beaker fill with water and keep the rooted bulb of onion in such a way that the roots hung in water.
- Then in another small beaker fill with 1% aqueous solution of colchicines and similarly place the rooted bulb of onion .
- Allow the roots to grow. After that keep the root tips in two separate slides and make the squash by following Acetocarmine technique.
- Observe under the Microscope.

Acetocarmine teachnique:

- Cut the distal end of the root tips and place it in 45% acetic acid and then stain it in acetocarmine.
- Slides cover with a coverslip and squash the root tips by heating and gently pressing.
- After that observe the abnoemalities .

Obsevation:

Separation of daughter chromosome to their pole and doubling of chromosomes there by inducing polyploidy due to colchicines treatment.

Experiment -6

Identification and study of cancer cells by photomicrographs

Aim:

To identify and study about the cancer cells by using photomicrographs.

Backgroud information:

Cancer detection has always been a major issue for the pathologists and medical practitioners for diagnosis and treatment planning. The manual identification of cancer from microscopic biopsy images is subjective in nature and may vary from expert to expert depending on their expertise and other factors which include lack of specific and accurate quantitative measures to classify the biopsy images as normal or cancerous one. The automated identification of cancerous cells from microscopic biopsy images

helps in alleviating the abovementioned issues and provides better results if the biologically interpretable and clinically significant feature based approaches are used for the identification of disease.

Cancer cell detection methods:

For accurate detection of cancer pathologists use histopathology biopsy images, that is, the examination of microscopic tissue structure of the patient. Thus biopsy image analysis is a vital technique for cancer detection. Histopathology is the study of symptoms and indications of the disease using the microscopic biopsy images.

Histopathologists normally look at the specific features in the cells and tissue structures. The various common features used for the detection and diagnosis of cancer from the microscopic biopsy images include shape and size of cells, shape and size of cell nuclei, and distribution of the cells. The brief descriptions of these features are given as follows.

Shape and Size of the Cells:

It has been observed that the overall shape and size of cells in the tissues are mostly normal. The cellular structures of the cancerous cells might be either larger or shorter than normal cells. The normal cells have even shapes and functionality. Cancer cells usually do not function in a useful way and their shapes are often not even.

Size and Shape of the Cell's Nucleus:

The shape and size of the nucleus of a cancer cell are often not normal. The nucleus is decentralized in the cancer cells. The image of the cell looks like an omelet, in which the central yolk is the nucleus and the surrounding white is the cytoplasm. The nuclei of cancer cells are larger than the normal cells and deviated from the centre of the mass. The nucleus of cancer cell is darker. The segmentation step mainly focuses on separation of regions of interests (cells) from background tissues as well as separation of nuclei from cytoplasm.

Distribution of the Cells in Tissue:

The function of each tissue depends on the distribution and arrangements of the normal cells. The numbers of healthy cells per unit area are less in the cancerous tissues. These adjectives of microscopic biopsy images have been included in shape and morphology based features, texture features, color based features, Color Gray Level Co occurrence Matrix (GLCM), Law's Texture Energy (LTE), Tamura's features, and wavelet features which are more biologically interpretable and clinically significant.

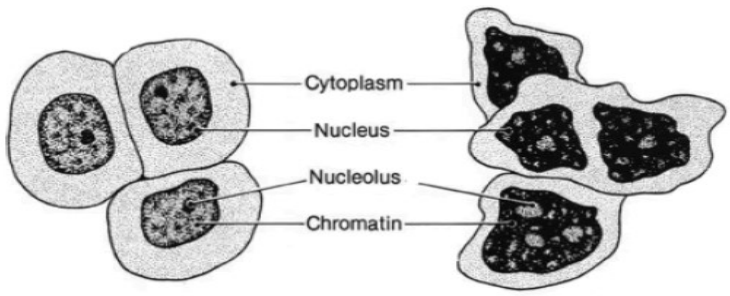


Figure 1. Structure of normal and cancer cells.

Experiment -7

Study of different stages of Mitosis

Aim:

To understand about the different stages of mitotic cell division using Onion root tips.

Principle:

Somatic growth in plant and animal takes place by increase in the number of cells. A cell divides mitotically to form two daughter cells where in the number of chromosomes remains the same as in the mother cell. In plant ,such division rapidly take place in meristematic tissues of root and shoot apices,where the stages of mitosis can be easily observed. In animals, mitotically dividing cells can be easily viewed in the bone marrow tissue of a vertebrate,epithelial cells from gills in fishes .

Requirements:

Onion bulb , wide mouth glass ,glacial acetic acid,ethanol,2-4% Acetocarmine ,Spirit lump, coverslip.blotting paper, light microscope.

Procedure

- Growing of root tips Select a few medium-sized onion bulbs. Carefully remove the dry roots present. Grow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water. Care should be taken so that the stem portion of the bulb (basal part) just touches the water.

- A few drops of water may be added periodically to compensate evaporation losses. New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (1:3:: glacial acetic acid : ethanol). Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future).

- Onion root-tip cells have a cell cycle of approximately 24-hour duration, i.e., they divide once in 24 hours, and this division usually takes place about two hours after sunrise. Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

Preparation of slide

- Take one or two preserved roots, wash them in water on a clean and greasefree slide. Place one drop of N/10 HCl on the root tip followed by 2–3 drops of aceto-carmine or aceto-orcein stain on it. Leave the slide for 5–10 minutes Exercise 6 21/04/2018 21 EXERCISE 6 on a hot plate (or warm it slightly on spirit lamp). Care should be taken that the stain is not dried up.

- Carefully blot the excess stain using blotting paper. Now cut the comparatively more stained (2–3 mm) tip portion of the root and retain it on the slide and discard the remaining portion. After (10–20 seconds) put one or two drops of water and blot them carefully using blotting paper.

- Again put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles. Place the slide in between the folds of blotting paper using the fingers in such a way that the cover slip mounted on the slide is properly held.
- Now slowly tap the cover slip using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells. Carefully seal the margins of the cover slip using molten paraffin wax or nail polish.
- This preparation of onion root tips cells is now ready for the study of mitosis. Study of slide Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells.
- Examine the dividing cells under higher magnification of the microscope to observe the detailed features of mitosis. Observation The stages of mitosis can be broadly categorised into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell).

Interphase :

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus

Stages of Mitosis :

(a) Prophase

- Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible. If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.

(b) Metaphase

- The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell. Each chromosome at this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.

(c) Anaphase

- This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere. Each chromatid now represents a separate chromosome as it has its own centromere.
- The chromosomes are found as if they have moved towards the two poles of the cell. The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them. Different anaphase cells

show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase .

(d) Telophase

- Chromosomes reach the opposite poles, lose their individuality, and look like a mass of chromatin (Fig. 6.1e). Nuclear membrane appears to form the nuclei of the two future daughter cells.

Cytokinesis

- In plants, a cell plate is formed in the middle after telophase. The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells (Fig. 6.2). However, in an animal cell, the two sides of the cell show inpushings or constrictions formed from the peripheral region in the middle of the cell, which grow inward and meet to divide the cell into two daughter cells.

Observation:

Experiment-8

Study of different stages of Meiosis.

Aim:

To understand the different stages of Meiosis cell division by using Lily Anther.

Background information:

In flowering plants such as Lilium, meiosis occurs in the reproductive organs of the flower. Pollen (sperm) is produced in the anthers, and ovules (eggs) are produced in the pistil. Inside the anther, thousands of diploid cells called meiocytes undergo meiosis to produce spherical tetrads, each containing 4 haploid cells. These haploid cells will then mature into pollen grains. The lab staff has already taken lily buds at different stages, removed the anthers and fixed them.

Procedure:

- Place an anther on a clean slide and add a small drop of acetocarmine stain. Using a dissecting needle, smash and chop the anther with the side of the needle in the pool of stain. The meiocytes will leak out into the stain drop on the slide. Using dissecting needles, remove the empty anther sac, leaving the meiocytes in the pool of stain. Do not let the cells dry out.

- Carefully place a coverslip over the sample. Using a paper towel or Kim Wipe, gently blot the excess stain from the sides of the coverslip. Lily microspores are delicate and can be seen without squashing. Too much pressure will lyse the cells and make the stages of meiosis difficult to discern.

- Examine your slide under the microscope, using the instructions on p.28. 6. Each group will need to dissect several anthers from Meiosis I and Meiosis II in order to observe all the stages.

- All the meiocytes within an anther will be at or around the same stage. See if your technique improves with practice. You may also exchange slides with other lab groups.

Results :

Part II – Meiosis:

- From your slides, diagram a cell in each stage of meiosis (prophase I and II, metaphase I and I, anaphase I and II, telophase I and II). Briefly describe what is happening during each stage.

Tips:

- All the daughter cells of a single meiocyte are held together in a sac.
- Document what you see on your slide. Don't try to recreate the tidy cartoons that often appear in textbooks.
- Choose two non-sister cells and count the number of chromosomes in each cell. For each cell, indicate the stage of meiosis, whether you see one or two chromatids per chromosome, and whether you see synapsed homologs. Make additional notes, as appropriate.

Tips:

- A cell is defined by the surrounding plasma membrane. All the daughter cells of a single meiocyte are held together in a sac. Be precise in your language.
- The cells and chromosomes are not flat. Use the fine focus to move up and down through the cell so you can better count the chromosomes.

Observation: