18BTU112 **CELL BIOLOGY PRACTICAL** **3H-2C**

Total/hour/week: L: 0 T: 0 P: 3 **Marks: Internal: 40 External: 60: Total 100**

Course Objectives

This practical course is designed to impart basic knowledge and practical approach in fundamental concepts of cellular function and be able to critically analyze, the scientific evidence underlying our current understanding of cellular processes.

Outcomes

- Learn and use current techniques and powerful model systems utilized in cell biology research.
- Gain experience in experimental design, data collection, and interpretation of results.

List of Practical's

1. Study of Prokaryotic and Eukaryotic cell, Structure
2. Study the effect of temperature and organic solvents on semi permeable membrane
3. Demonstration of dialysis
4. Study of Plasmolysis and de-plasmolysis
5. Cell division in onion root tip
6. Microtomy: Fixation, block making, section cutting, double staining of animal tissues like liver, pancreas and kidney.
7. Preparation of nuclear, mitochondrial and cytoplasmic cell fractions
8. Determination of enzyme activity in organelles using sprouted seed or any other suitable source

References

1. Karp, G (2013). Cell and Molecular Biology: Concepts and Experiments (7^{th} ed). Hoboken, US: John Wiley & Sons. Inc
2. De Robertis, E.D.P & De Robertis, E.M.F. (2006). Cell and molecular biology (8^{th} ed). Lippincott Williams and Wilkins, Philadelphia
3. Cooper, G.M., & Hausmann, RE (2013). The cell: A molecular Approach (6^{th} ed). Washington, USA: ASM press & Sunderland, D.C., Sinauer Associates.
4. Becker, W.M., Kleinsmith, L.J & Bertoni, G.P (2009). The world of the cell (7^{th} ed). San Francisco: Pearson Benjamin Cummings Publishing.

CELL BIOLOGY PRACTICAL

COMPLETION REPORT

S.No	Name of the Practical	Completion Report
1.	Study of Prokaryotic and Eukaryotic cell, Structure	
2.	Study the effect of temperature and organic solvents on semi permeable membrane	
3.	Demonstration of dialysis	
4.	Study of Plasmolysis and de-plasmolysis	
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1. Study of Prokaryotic and Eukaryotic cell, Structure

Aim: To identify Prokaryotic and Eukaryotic cell types.

Prokaryotic cell

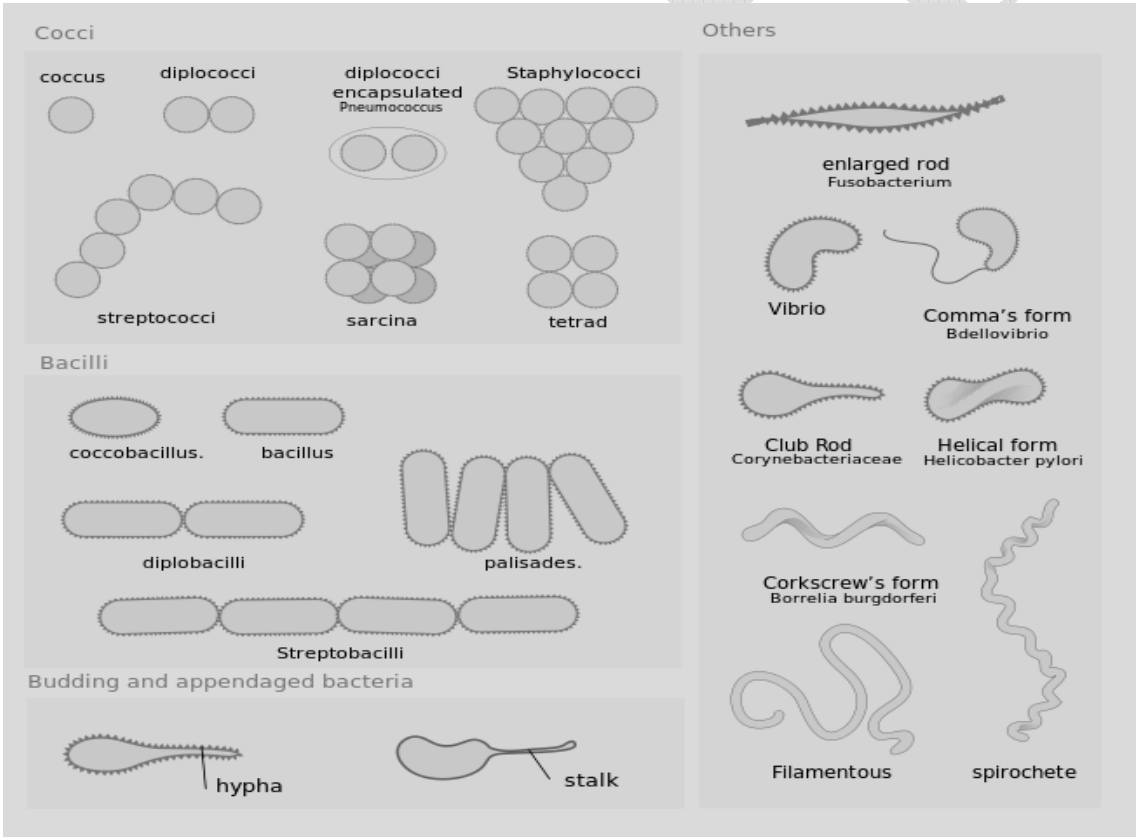
Bacteria

Bacteria have peptidoglycan in their cell walls, and they have no unusual phospholipids. Bacteria have four shapes:

- bacilli (rod shaped)
- vibrios (curved shaped)
- coccus (round shaped)
- spirilli (spiral shaped).

Bacteria can also have prefixes before their names: strepto, indicating chains of the shaped bacteria, and staphylo, indicating clusters of the shaped bacteria.

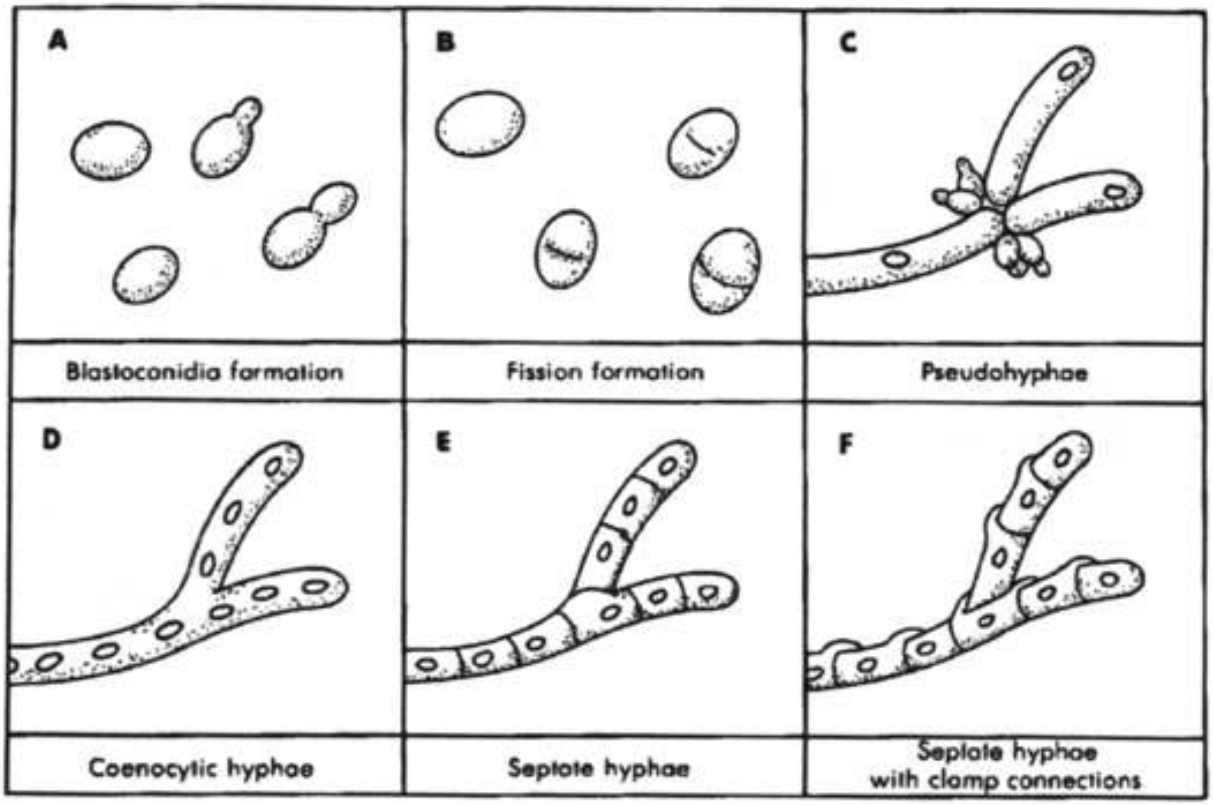
Observations:



Fungi

Fungi represent life that is composed of eukaryotic cells, but the fungal cell type is distinct from both plants and animals. They are distinct in their method of obtaining nutrition, secreting enzymes that digest food outside their bodies. There are fungi that can use essentially any biological material, so they exist essentially anywhere that any other life exists. Their reproduction is quite different from the most common type of cellular reproduction. They produce no embryos, but reproduce by means of tiny reproductive packages called spores. These spores are extraordinarily light and mobile and are propagated widely by air currents and by attachment to any mobile object.

Observations:



Eukaryotic cell and Structure

Plant cell :

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include:

Maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

A cell wall composed of cellulose and hemicellulose, pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

Specialized cell-to-cell communication pathways known as plasmodesmata, pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

Parenchyma cell:

Parenchyma cells are living cells that have functions ranging from storage and support to photosynthesis and phloem loading (transfer cells). Apart from the xylem and phloem in their vascular bundles, leaves are composed mainly of parenchyma cells. Some parenchyma cells, as in the epidermis, are specialized for light penetration and focusing or regulation of gas exchange, but others are among the least specialized cells in plant tissue, and may remain totipotent, capable of dividing to produce new populations of undifferentiated cells, throughout their lives. Parenchyma cells have thin, permeable primary walls enabling the transport of small molecules between them, and their cytoplasm is responsible for a wide range of biochemical functions such as nectar secretion, or the manufacture of secondary products that discourage herbivory. Parenchyma cells that contain many chloroplasts and are concerned primarily with photosynthesis are called chlorenchyma cells. Others, such as the majority of the parenchyma cells in potato tubers and the seed cotyledons of legumes, have a storage function.

Collenchyma cells :

Collenchyma cells are alive at maturity and have only a primary wall. These cells mature from meristem derivatives that initially resemble parenchyma, but differences quickly become apparent. Plastids do not develop, and the secretory apparatus (ER and Golgi) proliferates to secrete additional primary wall. The wall is most commonly thickest at the corners, where three or more cells come in contact, and thinnest where only two cells come in contact, though other arrangements of the wall thickening are possible.

Sclerenchyma cells:

Sclerenchyma cells (Greek *skleros*, hard) are hard and tough cells with a function in mechanical support. They are of two broad types – sclereids or stone cells and fibres. The cells develop an extensive secondary cell wall that is laid down on the inside of the primary cell wall. The secondary wall is impregnated with lignin, making it hard and impermeable to water. Thus, these cells cannot survive for long' as they cannot exchange sufficient material to maintain active metabolism. Sclerenchyma cells are typically dead at functional maturity, and the cytoplasm is missing, leaving an empty central cavity.

Xylem:

Xylem cells are elongated cells with lignified secondary thickening of the cell walls. Xylem cells are specialised for conduction of water, and first appeared in plants during their transition to land in the Silurian period more than 425 million years ago. The possession of xylem defines the vascular plants or Tracheophytes. Xylem tracheids are pointed, elongated xylem cells, the simplest of which have continuous primary cell walls and lignified secondary wall thickenings in the form of rings, hoops, or reticulate networks. More complex tracheids with valve-like perforations called bordered pits characterise the gymnosperms. The ferns and other pteridophytes and the gymnosperms have only xylem tracheids, while the angiosperms also have xylem vessels. Vessel members are hollow xylem cells without end walls that are aligned end-to-end so as to form long continuous tubes. The bryophytes lack true xylem cells, but their sporophytes have a water-conducting tissue known as the hydrome that is composed of elongated cells of simpler construction.

Phloem:

Phloem is a specialised tissue for food transport in higher plants. Phloem cells mainly transport sucrose along pressure gradients generated by osmosis. This phenomenon is called translocation. Phloem consists of two cell types, the sieve tubes and the intimately associated companion cells. The sieve tube elements lack nuclei and ribosomes, and their metabolism and functions are regulated by the adjacent nucleate companion cells. Sieve tubes are joined end-to-end with perforate end-plates between known as sieve plates, which allow transport of photosynthate between the sieve elements. The companion cells, connected to the sieve tubes via plasmodesmata, are responsible for loading the phloem with sugars. The bryophytes lack phloem, but moss sporophytes have a simpler tissue with analogous function known as the leptome.

Animal cell types:

The 4 main types of animal tissues are **epithelial tissue**, **connective tissue**, **muscular tissue**, and **nervous tissue**.

Epithelium

1. can be simple or stratified.
   a. Simple epithelium has only a single cell layer.
   b. Stratified epithelium has more than one layer of cells.
   c. Pseudostratified epithelium is a single layer of cells so shaped that they appear at first glance to form two layers.

Functions of epithelial cells include:

- movement materials in, out, or around the body.
- protection of the internal environment against the external environment.
- Secretion of a product - glands.

2. Study the effect of temperature and organic solvents on semi permeable membrane

Living beetroot cells are suitable materials to demonstrate the effects of high temperature and chemicals on the permeability of cell membranes. Beetroot contains a red pigment called anthocyanin, which is located in the large central vacuoles of the beetroot cells. As long as the cells and their membranes are intact, the anthocyanin will remain inside the vacuoles. However, if the membranes are damaged, anthocyanin will leak out and produce a red colour in the water surrounding the beetroot. The intensity of red colour in the water can be used to assess the degree of damage to living membranes by different factors.

High temperature and organic solvents e.g. alcohols, denature membrane proteins and increase the fluidity of membrane lipids. Organic solvents at high concentrations can also dissolve lipids. Acetone, alcohol and chloroform are organic solvents that severely destroy membranes.

A. By high temperature

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Pipette 5 cm^3 of water into six test tubes and labelled as 30, 40, 50, 60, 70 and 80.
5. Use a water bath to heat a boiling tube containing water up to 80°C.
6. Gently lower 5 pieces of beetroot discs into the hot water and leave them immersed for exactly 1 minute.
7. Carefully remove the discs and place them in the prepared test tube labelled as 80.
8. Leave the tubes for 20 minutes. Shake the tubes occasionally for the pigment to leak out of the cells.
9. Repeat steps (5) to (8) for temperatures at 70°C, 60°C, 50°C, 40°C and 30°C respectively.
10. Remove the discs from each test tube after 20 minutes.
11. Compare the intensity of red colour in each tube. Use a one to ten '+' sign to indicate the relative colour intensity.

B. By chemicals

Procedure

1. Use a cork borer to cut cylinders of tissue from a beetroot.
2. Cut the cylinders of beetroot into thin discs of about 3 mm thick.
3. Rinse the beetroot discs in running water to wash off pigment that leaked out as a result of cutting.
4. Blot dry the discs with paper towels.

Prepare the following test tubes with 5 cm^3 of solutions as below:

Tube	**A**	**B**	**C**	**D**	**E**	**F**
Solution	water	10% alcohol	30% alcohol	50% alcohol	Chloroform	Paraffin oil

5. Put equal number of discs into tubes A to F. Stoppered the tubes with cork.
6. Shake the tubes occasionally.
7. After an hour, take out all discs from tubes A to D.
8. Take out the discs from tube E (chloroform) carefully without damaging them and blot to remove the solution on the surface of the discs.
9. Put the discs from step (9) into another test tube labelled as E1, which contains 5 cm^3 of water. Shake occasionally.
10. Repeat step (9) and (10) for tube F (paraffin oil).
11. After an hour, take out the discs from tubes E1 and F1.
12. Record and compare the colour intensity of the solutions in tubes A, B, C, D, E1 and F1

Note

- Chloroform and alcohol can destroy the structure of cell membrane of beetroot, and the red pigment will diffuse out. The pigment is soluble in alcohol and water, but not soluble in chloroform.
- Paraffin oil and water do not destroy the cell membranes

3. Demonstration of dialysis

Background

The movement of molecules through a cell membrane is termed osmosis or diffusion. Such movement is principally possible because nutritive molecules are smaller than membrane micro pores. If the molecules are too large, no molecular transfer, or diffusion occurs.

Thus, some membranes may transmit selectively and are termed semi-permeable membranes. In the following experiment, cellophane dialysis tubing serves as an excellent representation of the cell membrane. The enclosed tube may, for the purpose of this experiment, be considered a single living cell in greatly enlarged form.

Materials

- Glucose Solution,
- Starch Solution,
- Iodine Solution,
- Glucose Test Strips,
- Dialysis Tubing,
- String,
- 400 ml Beaker,
- 10 mL Graduated Cylinder,
- Scissors,
- Ruler

Procedure

1. Measure and cut 20 cm of dialysis tubing.
2. Place this tubing in a beaker and cover it with water. Allow it to soak for about five minutes.
3. Remove the tubing from the water and rub one of the ends between your thumb and pointer finger to open. Once open, submerge it in water again for about thirty seconds.
4. Tie one end of the tubing in an overhand knot

5. Measure 5 ml of starch solution using the 10 ml graduated cylinder and pour it into the tube.
6. Thoroughly rinse the graduated cylinder, shake dry, and measure 5 ml of glucose solution. Pour this into the tubing as well.
7. Tie the second end of the tubing in an overhand knot and rinse it under the faucet. Set your "cell" on a clean surface.
8. Fill the 400 ml beaker ¾ full with tap water. Add 10 drops of Iodine solution and stir well. (Iodine will turn blue-black in the presence of starch).
9. Test the solution in the beaker for the presence of glucose by dipping a glucose test strip into it. After 30 seconds, compare the color on the strip to the color chart on the side of the bottle. Complete the Initial Status information on your data table.
10. Place the artificial cell (the sealed tubing with solutions) into the beaker of solution and allow it to remain undisturbed for 15-20 minutes.
11. Remove the tubing from the beaker and record your observations in the data table for final status of the solution and the bag.
12. Retest the solution in the beaker and in the bag with a new glucose test strip and record this data in your table.

4. Study of plasmolysis and de-plasmolysis

Aim

To demonstrate plasmolysis and de-plasmolysis in peels of Rhoeo plant in hypotonic and hypertonic solutions using salt solution.

Introduction

Plasmolysis is the process of shrinkage or contraction of the protoplasm of a plant cell as a result of loss of water from the cell. Plasmolysis is one of the results of osmosis and occurs very rarely in nature, but it happens in some extreme conditions. We can induce plasmolysis in the laboratory by immersing living cell in a strong salt solution or sugar solution to lose water from the cell. Normally people use Rheo or Tradescantia plant epidermal cell for experiment because they have coloured cell sap which can be clearly visible.

Plasmolysis and deplasmolysis

When a plant cell is immersed in concentrated salt solution (hypertonic solution), water from the cell sap moves out due to exosmosis. Exosmosis is the passage of water from higher water concentration to lower water concentration through a semipermeable membrane.

When a plant cell is placed in concentrated salt solution, water concentration inside the cell is greater than that which is outside the cell. Therefore, water moves through the cell membrane into the surrounding medium. Ultimately the protoplasm separate from the cell wall and assumes spherical shape. It is called plasmolysis.

When a plasmolysed cell is placed in a hypotonic solution, (i.e., the solution having solute concentration lower than the cell sap), the water moves into the cell because of the higher concentration of water outside the cell than in the cell. The cell then swells to become turgid. It is called deplasmolysis.

If we place living cells in isotonic solution (i.e., both solutions have the same amount of solute concentration), there is no net flow of water towards the inside or outside. Here, the water moves in and out of the cell and is in equilibrium, so the cells are said to be flaccid.

Materials Required

Rhoeo Leaf

Glass Slides

Cover Slips

Sodium chloride 5% solution

Compound Microscope

Sodium chloride 0.1% solution

Forceps

Droppers

Needle

Procedure

- Take two glass slides and place them on the table.
- Take a rhoeo leaf from the Petri dish.
- Fold the leaf and tear it along the lower side of the leaf.
- Using a forceps, pull out two small segments of thin transparent layer from the lower epidermis of the rhoeo leaf.
- Place the epidermal peels on both glass slides.
- Using a dropper, take some sodium chloride 0.1% solution from the beaker.
- Put 1 to 2 drops of solution on one slide.
- Using another dropper, take sodium chloride 5% solution from the beaker.
- Put 1 to 2 drops of solution on the next slide.

- Place a cover slip over the peel of both slides using a needle.
- Place the slides one by one under the compound microscope.
- Observe them under the microscope.

Observation

After half an hour we can observe that cells in sodium chloride 0.1% solution appear turgid, while cells in the sodium chloride 5 % solution show plasmolysis.

Conclusion

When plant cells are immersed in sodium chloride 5 % solution or concentrated salt solution, water moves through the cell membrane into the surrounding medium because the water concentration inside the cell is greater than that which is outside the cell. Ultimately the protoplasm causes shrinkage and assumes spherical shape. This is called plasmolysis. When a plant cell is immersed in sodium chloride 0.1% solution or dilute salt solution, the water moves into the cell because of the higher concentration of water outside the cell than inside the cell. The cell then swells and becomes turgid.

5. Cell division in onion root tip

Aim:

To study the different stages of mitosis in onion root tip cells

Principle:

Onion root tip has meristematic tissue just behind the root cap, hence this serves as a good material for studying various stages of mitosis. The roots can be easily grown if an onion is placed on a water filled conical flask is such a way that the onion disk touches water or else making the onion to sit on wet sand in such a way that the bulb is buried partially in sand. In two days time nearly 1 cm long roots develop, which can then be cut, fixed and stored.

Longer grown roots are not good as it will have more of non-dividing tissue.

Materials required

Onion root tips, 1N HCl, 1:3 acetomethanol fixative, 70% and 90% ethanol, 2% acetocarmine stain (2gms of carmine mixed with 100ml of 45% acetic acid and boiled using a reflux conderser for 1 hr to dissolve carmine), 45% acetic acid, slide, cover glass, sealing wax or nail polish.

Procedure:

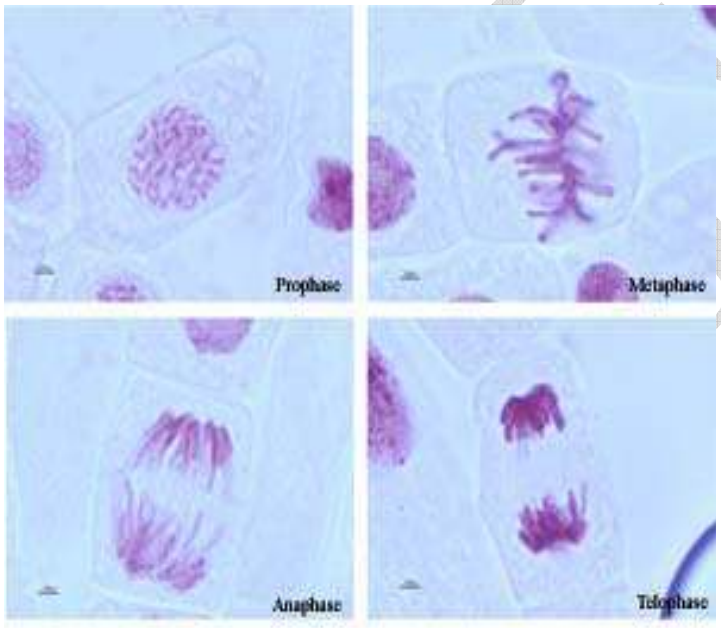
Fixation of root tips:

1. Fix the freshly cut ~1 cm long root tips in acetomethanol fixative for overnight in a specimen tube.
2. Remove fixative and add 90% ethanol, leave for 2hr.
3. Decant 90% ethanol and add 70% ethanol. The root tips can be stored in 70% ethanol for a long period of time if the tube is tightly closed. Storing at 4oC is even better.
4. Staining and making squash preparation:
5. Treat the root tips with 1N HCl for 1 min. This will soften the cell wall.
6. Rinse the tips once in water, transfer to acetocarmine stain and stain for 30 min.
7. Take a drop of 45% acetic acid on slide, place a root tip on the drop, leave for 1-2 min. If acetic acid drop becomes coloured, it can be decanted and a fresh 45% acetic acid drop can be added.
8. Place a cover glass on the root tip and squash it using a rubber-end pencil under the folds of a blotting paper.

9. Seal the edges of the cover glass with molten was or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
10. The slide is ready for observation under a microscope.

Observations:

Different phases of mitosis can be observed as shown in the figures below:



6. Microtomy

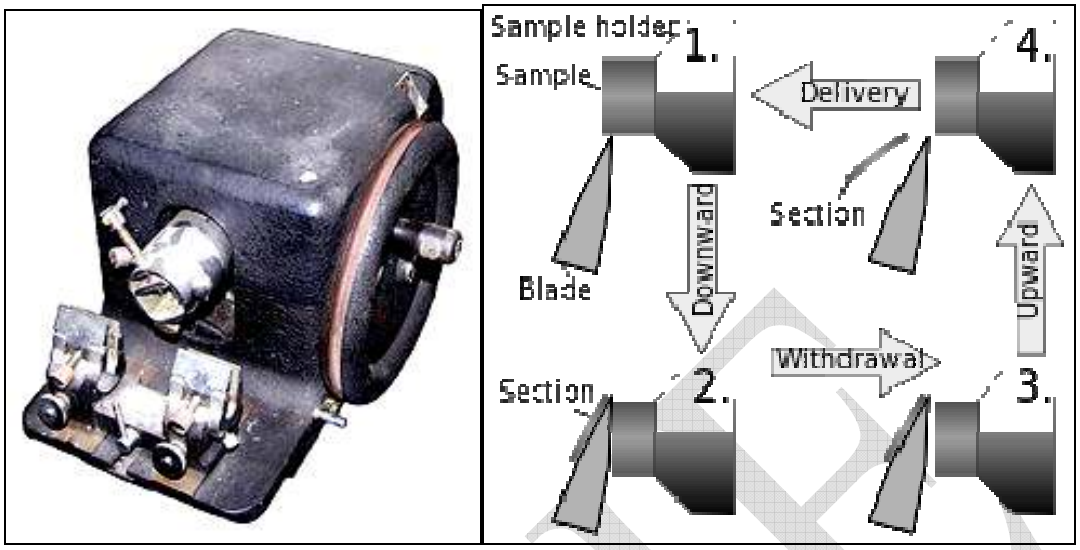
A microtome (from the Greek mikros, meaning "small", and temnein, meaning "to cut") is a tool used to cut extremely thin slices of material, known as sections. Important in science, microtomes are used in microscopy, allowing for the preparation of samples for observation under transmitted light or electron radiation. Microtomes use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. Steel blades are used to prepare sections of animal or plant tissues for light microscopy histology. Glass knives are used to slice sections for light microscopy and to slice very thin sections for electron microscopy. Industrial grade diamond knives are used to slice hard materials such as bone, teeth and plant matter for both light microscopy and for electron microscopy. Gem quality diamond knives are used for slicing thin sections for electron microscopy.

Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to electropolishing and ion milling. Microtome sections can be made thin enough to section a human hair across its breadth, with section thickness between 50 nm and 100 μm.

Application :

Traditional Histology Technique: tissues are hardened by replacing water with paraffin. The tissue is then cut in the microtome at thicknesses varying from 2 to 50 μm. From there the tissue can be mounted on a microscope slide, stained with appropriate aqueous dye(s) after prior removal of the paraffin, and examined using a light microscope.

This instrument is a common microtome design. This device operates with a staged rotary action such that the actual cutting is part of the rotary motion. In a rotary microtome, the knife is typically fixed in a horizontal position.



Out line & Principle of microtome

In the figure to the left, the principle of the cut is explained. Through the motion of the sample holder, the sample is cut by the knife position 1 to position 2), at which point the fresh section remains on the knife. At the highest point of the rotary motion, the sample holder is advanced by the same thickness as the section that is to be made, allowing for the next section to be made.

The flywheel in many microtomes can be operated by hand. This has the advantage that a clean cut can be made, as the relatively large mass of the flywheel prevents the sample from being stopped during the sample cut. The flywheel in newer models is often integrated inside the microtome casing. The typical cut thickness for a rotary microtome is between 1 and 60 µm. For hard materials, such as a sample embedded in a synthetic resin, this design of microtome can allow for good "Semi-thin" sections with a thickness of as low as 0.5 µm.

Sample preparation:

Biological materials are usually placed in a more rigid fixative, in a process known as embedding. This is achieved by the inflow of a liquid substance around the sample, such as paraffin (wax) or epoxy, which is placed in a mould and later hardened to produce a "block" which is readily cut.

Operation of machine:

The majority of microtomes are a knife-block design with a changeable knife, a specimen holder and an advancement mechanism. In most devices the cutting of the sample begins by moving the sample over the knife, where the advancement mechanism automatically moves forward such that the next cut for a chosen thickness can be made. The section thickness is controlled by an adjustment mechanism, allowing for precise control.

7. Preparation of nuclear, mitochondrial and cytoplasmic cell fractions

Aim:

To study the preparation of nuclear, mitochondrial and cytoplasmic cell fractions.

Procedure

All centrifugations should be done at 4°C. Samples should be kept on ice throughout the procedure.

1. Transfer cells from 10 cm plates into 500 µL fractionation buffer, eg by scraping. Incubate 15 min on ice.
2. Using 1 mL syringe pass cell suspension through a 27 gauge needle 10 times (or until all cells are lysed).
3. Leave on ice for 20 min.
4. Centrifuge sample at 720 xg (3,000 rpm) for 5 min. The pellet will contain nuclei and the supernatant will contain cytoplasm, membrane and mitochondria.
5. Transfer supernatant into a fresh tube and keep on ice. This will be dealt with in Steps 8–11.
6. Wash nuclear pellet from Step 4 with 500 µL fractionation buffer. Disperse the pellet with a pipette and pass through a 25 gauge needle 10 times. Centrifuge again at 3,000 rpm for 10 min. Discard the supernatant and keep the pellet that contains nuclei.
7. Resuspend the pellet from Step 6 in TBS with 0.1% SDS. Sonicate the suspension briefly to shear genomic DNA and homogenize the lysate (3 s on ice at a power setting of 2-continuous).
8. Centrifuge the supernatant recovered in Step 5 at 8,000 rpm (10,000 x g) for 5 min. The pellet contains mitochondria. Transfer the supernatant into a fresh tube and keep on ice: this is the cytoplasm and membrane fraction.
9. Process the mitochondrial pellet from Step 8, as described for the nuclear pellet in Step 7, to obtain mitochondrial lysate in TBS/0.1% SDS.
10. For a membrane fraction, centrifuge the supernatant from Step 8 in an ultracentrifuge at 40,000 rpm (100,000 x g) for 1 h. Wash pellet by adding 400 µL of fractionation buffer. Resuspend by pipetting and pass through a 25 gauge needle. Re-centrifuge for 45 min. Resuspend the membrane pellet in the same buffer as used for the nuclei.
11. Optional: concentrate the supernatant by centrifuging through the filter unit. This concentrates the cytosol down to approximately 50–75 µL.

8. Determination of enzyme activity in organelles using sprouted seed or any other suitable source

Aim:

To determine the enzyme activity using sprouted seed.

Materials required:

- 50 mM Tris-maleate
- 10 mM calcium chloride
- 0.5 M sodium hydroxide

Procedure

- Ten whole seeds were ground and extracted for 10 minutes, using a pestle and mortar, in 5 ml 50 mM Tris-maleate buffer, pH 6.2 containing 10 mM calcium chloride.
- The extract was centrifuged at 2000 g for 10 minutes and the supernatant used as an enzyme source.
- One ml of phadebas substrate (10 mg/ml in buffer) was added to 1 ml enzyme extract and 4 ml buffer and the mixture incubated at 37°C for 20 minutes.
- The reaction was terminated by adding 1 ml 0.5 M sodium hydroxide, centrifuged at 2000 g for 10 minutes and the absorbance of supernatant read at 620 nm.
- The test was standardized against pure malt α- amylase using an activity range of 0.02 to 0.2 units, one enzyme unit being defined as the amount of enzyme that will hydrolyze 1.0 mg maltose from starch at pH 6.9 in 3 minutes at 20° C.