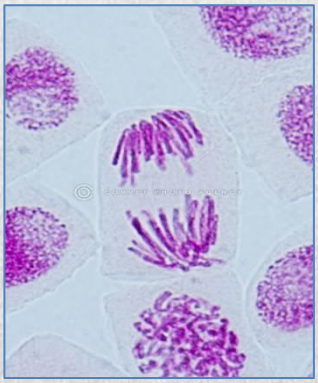
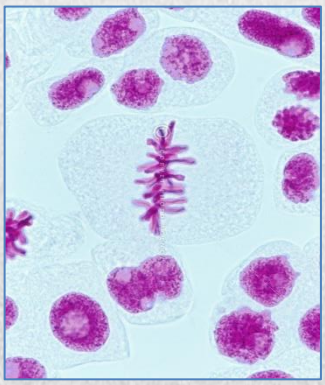
Practical Cell Biology





2nd year students

Prepared by

Dr.\ Hoida Zaki

Botany and Microbiology Department

2022-2023

Index

Laboratory Safety

Safety in the Laboratory Should always is in your mind. Throughout this manual Safety recommendations are given, below are some general consideration that anyone in a laboratory should know.

• General laboratory safety precaution.

1. Follow all instructions carefully. Use special care when you see the word CAUTION in your laboratory instructions. Follow the safety instructions given by your teacher.
2. Determine the location of Fire Extinguishers, Chemical safety showers and Eye washers, Chemical Spill Kits, and alternative exit routes for lab evacuation.
3. Remember that smoking, eating, or drinking in the lab room is totally prohibited.
4. Wear lab aprons when working with chemicals, hot material, or preserved specimens.
5. Wear safety goggles when using dangerous chemicals, hot liquids, or burners.
6. Any chemicals spilled on the hands or other parts of the skin should be washed off immediately with a plenty of running water.
7. If you have an open skin wound, be sure that it is covered with a waterproof bandage.
8. Never work alone in the laboratory.
9. Keep your work area clean & dry.
10. Turn of all electrical equipment, water, and gas when it is not in use, especially at the end of the laboratory period.
11. Tie back long hair.
12. Report all chemicals spills or fluids to your instructor immediately for proper clean up.

• Special precautions for working with heat or fire:

1. Never leave a lighted Bunsen burner of hot object unattended. When an object is removed from the heat & left to cool, it should be place where it is shielded from contact.
2. Inflammable liquid bottles should not be left open, not dispensed near a naked flame, hot electric element or electric motor.
3. Use test tube holders to handle hot laboratory equipments.
4. When you are heating something in a container such as a test tube, always point the open end of the container away from yourself & others.
5. Use only Pyrex glassware's for heating.
6. Allow hot materials to cool before moving them from your lab station.
7. Make sure that Bunsen burner hoses fit tightly.

• Special precautions for working with chemicals

1. Never taste or touch substances in the laboratory without specific instructions.
2. Never smell substances in the laboratory without specific instructions.
3. Use materials only from containers that are properly labeled.
4. Wash your hand after working with chemicals.
5. Do not add water to acid. Instead, dilute the acid by adding it to water.
6. Mix heat generating chemicals slowly.

• Special precautions for working with electrical equipment.

1. Make sure the area under & around the electrical equipment is dry.
2. Never touch electrical equipment with wet hands.
3. Make sure the area surrounding the electrical equipment is free of flammable materials.
4. Turn off all power switches before plugging an appliance into an outlet.

• **Special Precaution for working with Glassware's and other laboratory equipments.**

1. Become familiar with the names and appearance of all the laboratory equipments you will use.
2. Never use broken or chipped glassware.
3. Make sure that all glassware's are clean before you using it.
4. Do not pick up broken glass with your bare hands. Use a pan and a brush.
5. If a Mercury thermometer breaks, do not touch the mercury. Notify your teacher immediately.
6. Do not aim the mirror of your microscope directly at the sun. Direct sun light can damage the eyes.
7. Use care handling all sharp equipments, such as scalpels and dissecting needles.

• Special precautions for working with live or preserved specimens.

1. If live animals are used treat them gently. Follow instructions for their proper care.
2. Always wash your hands after working with live or preserved organisms.
3. Specimens for dissection should be properly mounted and supported. Do not try to cut a specimen while holding it in the air.
4. Do not open Petri dishes containing live cultures unless you are directed to do so.
5. Detergents (detol 5 – 10%) should be used to sterilize and clean benches, glassware and equipment.
6. Safety cabinet should be used while working with microbes.
7. Lab coats should be worn during the work in the lab.
8. Disposable items should be collected and autoclaved.

First Aid

1. Injuries: bleeding should be reduced using bandages; the wound should be cleaned with iodine alcohol mixture and wrapped with sterile bandage.
2. Acid and fire burns: body burns must be washed immediately with tap water. Eye burns must be washed using eye washer, special cream for burns can be used.
3. Poisoning: if any toxic chemical is swallowed, the mouth must be sensed with water, in case of acid, milk is drunk, in case of alkaline, diluted acetic acid (vinegar) can be used.
4. Skin contamination requires washing with water and removal of contaminated clothing, if the contaminant is insoluble in water remove with soap and water.

The Microscope

Highlights:

This Exercise focuses on how to develop a working knowledge of the Microscope and its use. Students should identify the different parts of the Microscope. List and follow recommended procedures in using and caring for the Microscope.

Material:

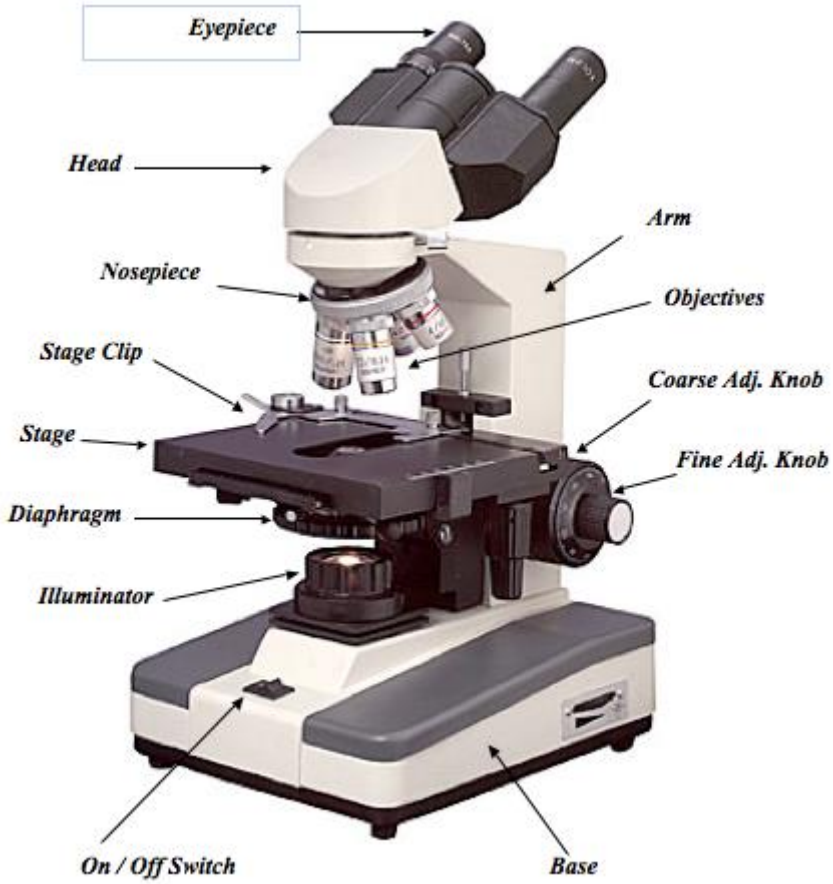
Compound Microscope
Clean Microscope Slides
Cover Slips
Lens papers
Sharp razor blades
Medicine droppers
Scissors
Distilled water
Xylene

Introduction:

Since an unaided eye cannot detect anything smaller than 0.1 mm in diameter, cells, tissues, and many small organisms are beyond our visual capability, so we need equipment to magnified objects which is too small to be seen with unaided eye. There are several types of microscopes but the only one used in this laboratory is the compound light microscope. The compound microscope (sometimes called the student microscope or light microscope); these microscopes are known as compound microscope because there are two magnifying lenses in the microscope. One magnifying lens is in the ocular or eyepiece, which further magnifies the image formed by the objective lens, and one, is in

the objective. Each contributes to the magnification of the object on the stage.

The total magnification of any set of lenses is determined by multiplying the magnification of the objective by the magnification of the ocular. The nose piece rotates the magnification of the microscope. Generally compound microscope magnifies from 40 x to 100 x.



Parts of a microscope:

The compound microscope is a delicate instrument composed of many parts that are accurately filled together in (Figure)

1. Ocular of eyepiece lens.

The ocular lens is the lens you look through, it is inserted at the top of the body tube. If your microscope has one ocular, it is a monocular microscope, if it has two, it is binocular. Its magnification is written on it.

2. Body tube.

Body tube is the optical housing for the objective lenses.

3. Objective lenses.

The objective lenses are a set of three to four lenses mounted on a rotating turret at the bottom of the body tube. The four objective lenses of your microscope and their magnifications are:

Scanning lens	4X magnification
Low power lens	10X magnification
High power lens	40-45X magnification
Oil immersion lens	100X magnification

The magnification of the objective lens is written on the lens.

Note: with the exception of the oil immersion lens all the objective lens is used dry.

The magnification of oil immersion lens requires using the lens with special immersion oil for proper resolution.

4. Stage

The horizontal surface on which the slide is placed is called the stage. It may be equipped with simple clips for holding the slide in place or with a mechanical stage, a geared device for precisely moving the slide. Two knobs, either on top of or under the stage, move the mechanical stage.

5. Condenser lens

Condenser lens system, located immediately under the stage, contains a system of lenses that focuses light on your specimen. The condenser may be raised or lowered using the condenser knob. An older microscope may have a concave mirror instead.

6. Iris diaphragm

Iris diaphragm is located below the condenser or immediately below the stage in microscopes without a condenser. It functions in regulating the light intensity passing through to the stage. More light is required at higher magnification.

7. Light source

The light source has an (ON/Off) switch & may have adjustable lamp intensities & color filters.

8. Base

Base – also called the supporting stand, rests on the bench.

9. Body Arm

The body arm is used when carrying the instrument.

10. Nose piece

Nosepiece is the mounting for the objective lenses which rotates to bring the desired objective into position.

11. Coarse adjustment

Coarse adjustment knob is a large knob located at either side of the microscope which functions in controlling the distance between the objectives and the stage. Use the coarse adjustment only with the scanning (4X) & low- power (10X) objectives. Why? So coarse adjustment is used for rapid focusing of the specimen until the specimen is roughly in focus & then left alone, in which the fine adjustment knob controls precise focusing of the object.

12. Fine adjustment

Fine adjustment is a small knob located at either side of the microscope. This is used for the control of the object, precise focusing you should use just the fine adjustment knob with the higher magnification objective lenses; Because using the coarse adjustment knob with the higher objective lenses may damage the lens &/or the slide you are observing.

Magnification:

Compound microscopes consist of two lens system: the objective lens, which magnifies & projects a “virtual image” into the body tube and the ocular lens, which magnifies the image further and projects the enlarged image into the eye.

The total magnification of a microscope is the product of the magnification of the objective and the ocular. If the objective lens has a magnification of 5X and the ocular 12X, then the image produced by these two lenses is 60 times larger than the specimen.

Microscope safety cautions:

1. Always carry the microscope in an upright position using both hands.
2. Keep the microscope away from the edge of the table.
3. Always examine a slide first with the low-or medium power objective, never use the high – power objective to view thick specimens.
4. Remove slide only after low-power objective has been rotated into viewing position, never when high – power objective is in position.
5. Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned.
6. When returning your microscope to its proper place in the cabinet always:

• Remove the slide from mechanical stage.

• Clean all lens surface and the stage.

• Rotate the nosepiece that the scanning lens is in place.

Steps Used in viewing a slide:

1. Obtain a slide.
2. Check that the ocular and all objective lenses as well as the slide clean.
3. Use the coarse adjustment knob to obtain maximum working distance.
4. Place the slide on the stage, the slide should fit into the slide holder. Use the stage adjustment knob to move the slide over the hole in the stage.
5. Rotate the lower objective in place.
6. Use the coarse adjustment knob to obtain the minimum working distance.
7. Look through the ocular. Adjust the light with the iris diaphragm lever if necessary. Slowly turn the coarse adjustment knob until something comes into focus. Use the fine adjustment knob to sharpen the focus.
8. Using the stage adjustment knob move the slide around until you find an area you wish to examine more closely. Move the slide until the object you wish to examine is in the center of the field.
9. Rotate the high-power objective into place. Use the fine adjustment knob to sharpen the focus. Do not use the coarse adjustment knob. Adjust the light using the iris diaphragm lever if necessary.
10. Rotate the high-power object halfway to the next position, place a drop of immersion oil on the slide, and then rotate the oil immersion objective into place. The objective should be immersed in the oil on the slide. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary.
11. When finished viewing the slide use the coarse adjustment knob to maximize the working distance and remove the slide from the stage. If you are finished with the microscope clean the microscope and return it to storage.

Procedure for cleaning a microscope:

1. Turn off the light.
2. Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.
3. Using lens paper cleans all the lenses starting with the cleanest first ocular, and objectives lens.
4. Clean any oil off of the stage using paper towels.
5. Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.
6. Return the microscope to the appropriate storage area.

Procedure for cleaning a microscope slide:

Before placing a specimen on a slide, it must be clean, as any small foreign body might mislead the observation. If your slide is not clean, do the following:

1. Hold the slide from its ends by fingers of one hand.
2. Using a detergent liquid, rub the slide with one finger of the other hand.
3. Wash the slide under running tap water; rub again, until no trace of the detergent is left.
4. Rinse the slide with distilled water to remove the tap water.
5. Either blot dries the slide by placing it between two towel papers, or place in alcohol solution & keep until used.
6. Never touch the slide from the middle, clean slide always holds it from its ends.

Experiment No: 1

Observation of distinguishing features of prokaryotic and eukaryotic cells

Aim: To observe the characteristics of prokaryotic and eukaryotic cells.

Materials required: Slides, cover slips, stains, microscopes and sample.

Procedure:

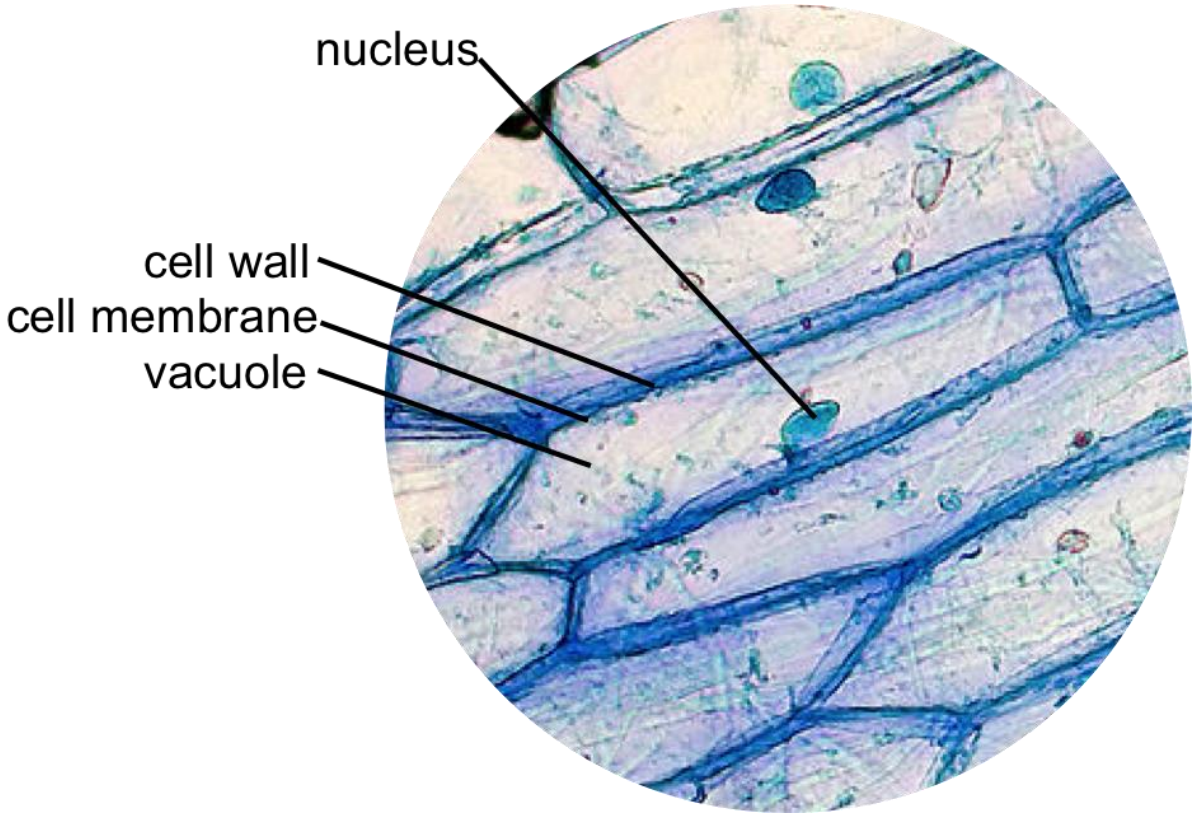
Prokaryotic sample

Prepare a smear of the bacterial suspension on a sterilized clean slide and stain by Gram staining procedure and observe under different magnification**.**

Eukaryotic sample

Peel off the epidermis of onion fleshy leaves and place on a drop of saffranin on a clean slide and observe at different magnification.

Report the differences in cell morphology between them.



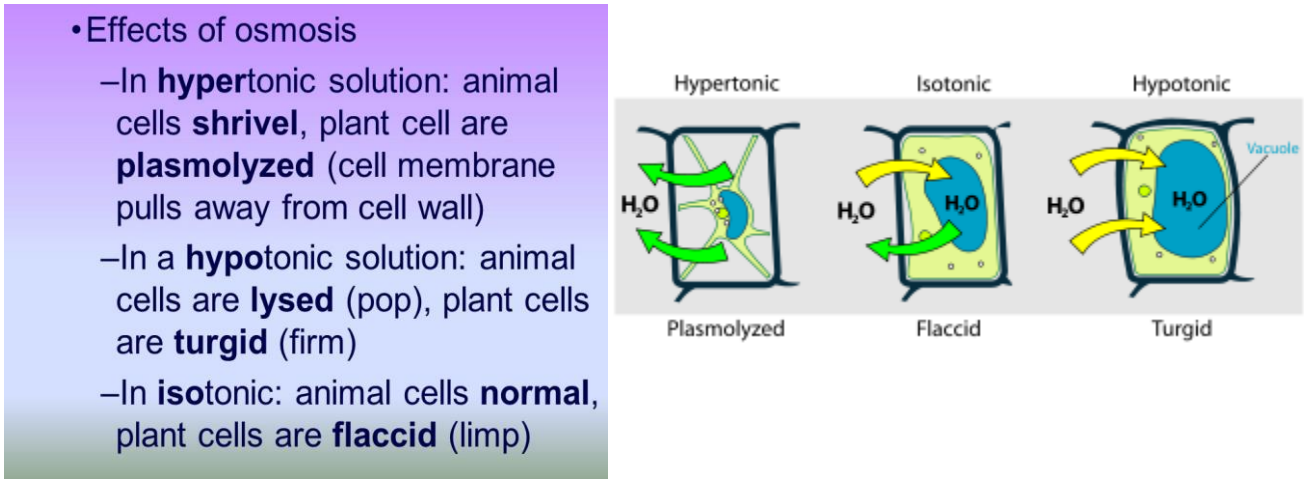
Experiment No: 2

The effect of hypertonic, hypotonic and isotonic solutions on the cell wall.

Aim: To describe experimentally the effects of hypertonic, hypotonic and isotonic solution on the cell wall.

Materials: microscope, NaCl, slides, *Ricinus Communis* petiole**,** Potato

Diagram:



Theory: When a living plant cell is placed in a salt or sugar solution that is more concentrated or stronger than the cell sap (hypertonic solution) water is lost from the cell to the stronger solution. The cytoplasm shrinks and plasmalemma gets detached from the cell wall. A cell placed in a less concentrated solution absorbs water and becomes turgid.

Materials: Distilled water, NaCl solution pond water containing spirogyra cells, microscope slide, and microscope.

Procedure: From the NaCl provided, prepare molar solutions in the following concentrations: 0.2m. 0.4m, 0.6m, 0. 8m and 0.10m. Put a drop of the greenish part of your pond water on each of the five slides provided and examine under the microscope. Once you have observed potato discs or *Ricinus Communis* petiole add each of the molar

solutions one at a time on the five (5) slides respectively. Leave for a few minutes and examine. Note which of the concentrations is hypertonic, hypotonic and isotonic. Make diagrams of the cell from each of the five (5) concentrations you observed under the microscope. Copy and complete the table below.

S/N	NaCl concentrations	Changes observed.
1	(1) 0.2m	
2	(2)0.4m	

In case of *Ricinus communis* petioles

1. In hypotonic soln. curvature of Ricinus communis petioles toward epidermis.
2. In hypertonic soln. curvature of Ricinus communis petioles toward pith.
3. In isotonic soln. there is no change.

Questions:

1. Which of the concentration of NaCl solution is hypertonic, isotonic and hypotonic to the cell plasma?

2. What are the effects of hypertonic and hypotonic solutions on the cell plasma?

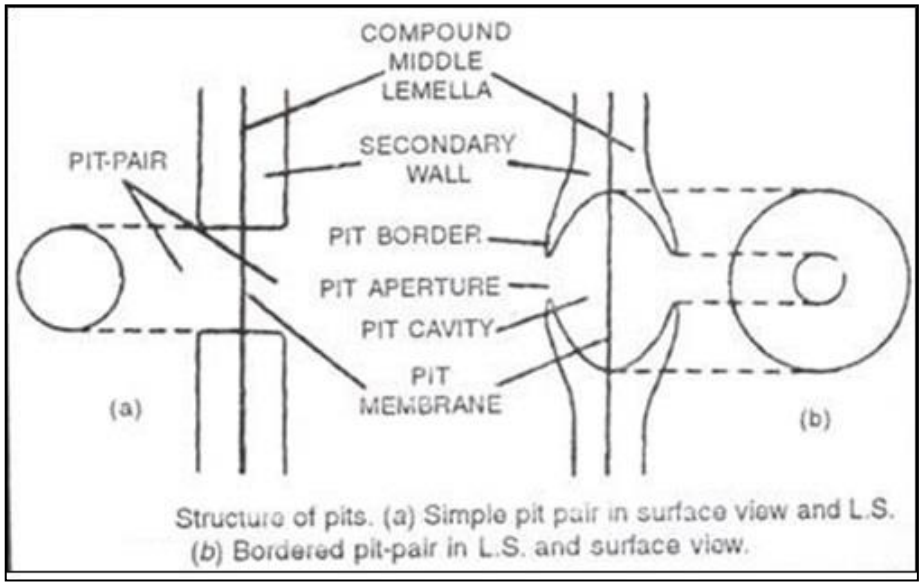
Comments:

Experiment No: 3

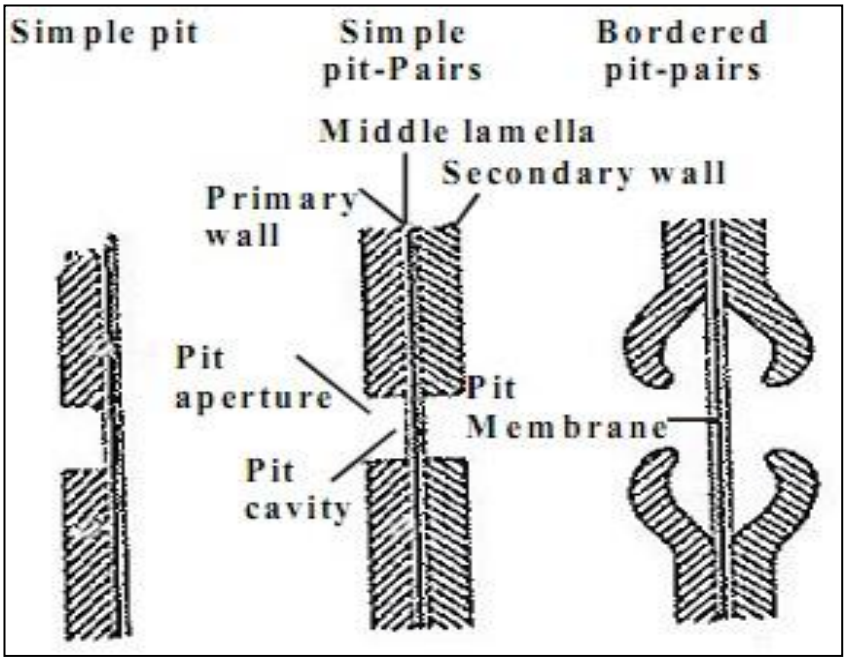
Pits of Cell Wall

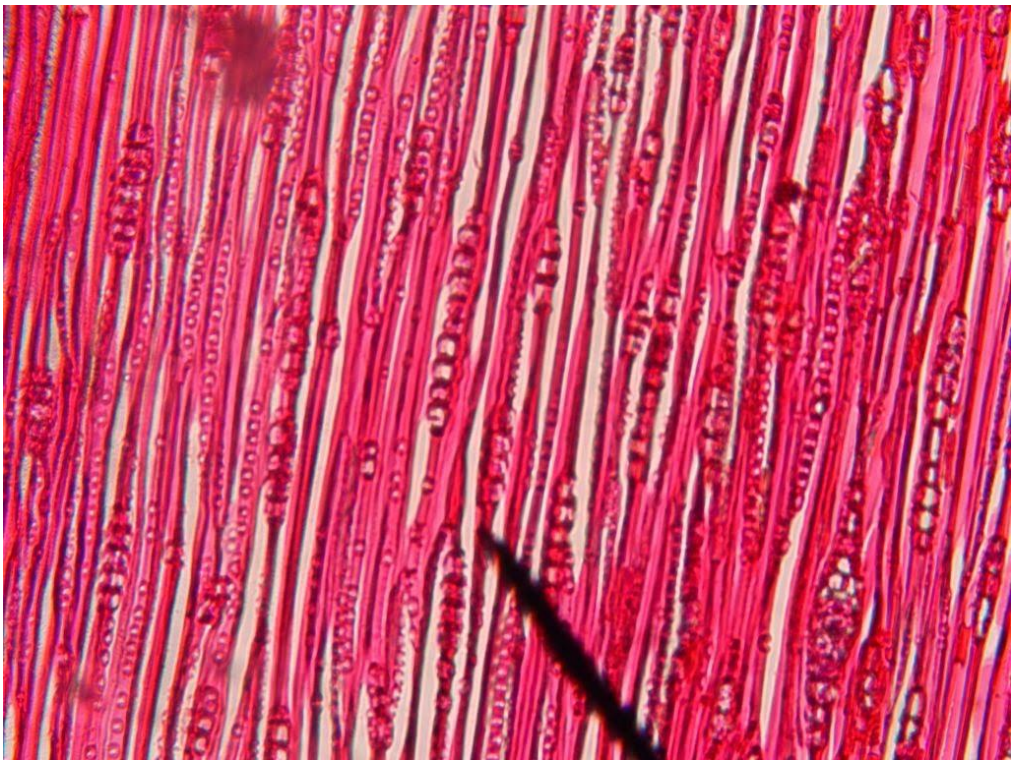
Pits are relatively thinner portions of the cell wall that adjacent cells can communicate or exchange fluid through. Pits are characteristic of cell walls with secondary layers. Generally, each pit has a complementary pit opposite of it in the neighboring cell. These complementary pits are called "pit pairs".

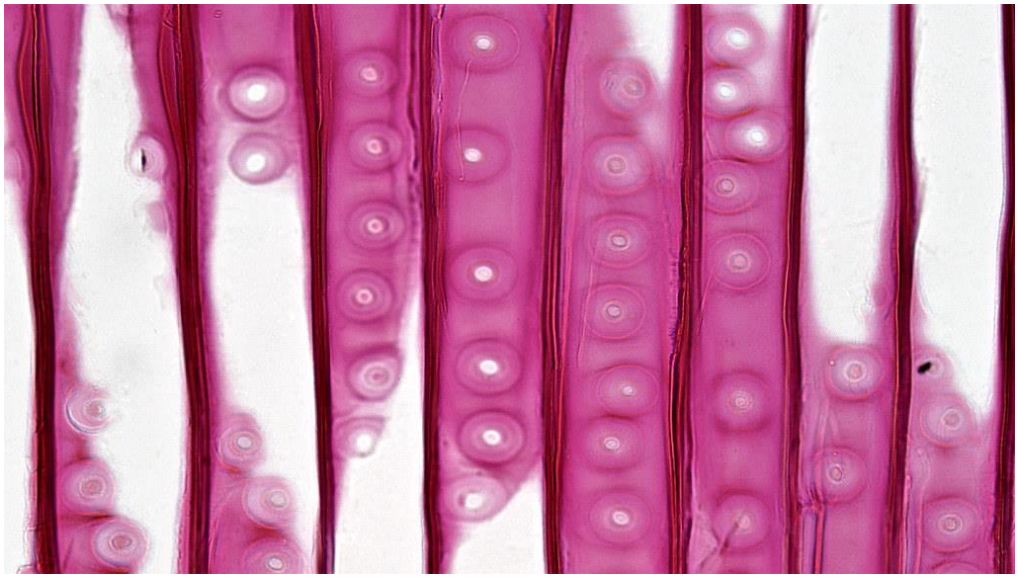
Pits are composed of three parts: the pit chamber, the pit aperture, and the pit membrane.



Structure of pits. (a) Simple pit pair in surface view and L.S. (*b*) Bordered pit-pair in L.S. and surface view.







Experiment No: 4

Living Contents of Cell: Plastid types

The three major types of plastids are:

1. **Chloroplasts** are green and serve as the sites for photosynthesis in the cells.
2. Highly pigmented plastids called **chromoplasts** give plants the colors they use to attract pollinators.
3. Non-pigmented plastids used for storing starches, lipids, and proteins are called **leucoplasts**.

Materials

Leaves of different plants, and algae, slides, coverslips, microscope, water, (leaves of spinach can also be successfully used to observe chloroplasts), etc.

Method

Separate the young leaves from the plants. Mount in water and study under the microscope.

Observations

1. Discoid or oval-flattened chloroplasts can be seen close to the cell wall.

2. Chloroplasts are green in color due to the abundance of photosynthetic pigment-the chlorophyll.

3. Other pigments present in the chloroplast include xanthophylls and carotenes.

4. Chloroplasts are the seats of photosynthesis and therefore, end product in the form of starch grains is also seen.

Study the chromoplasts

Materials

Fruits of tomato, slides, coverslips, microscope, water, etc.

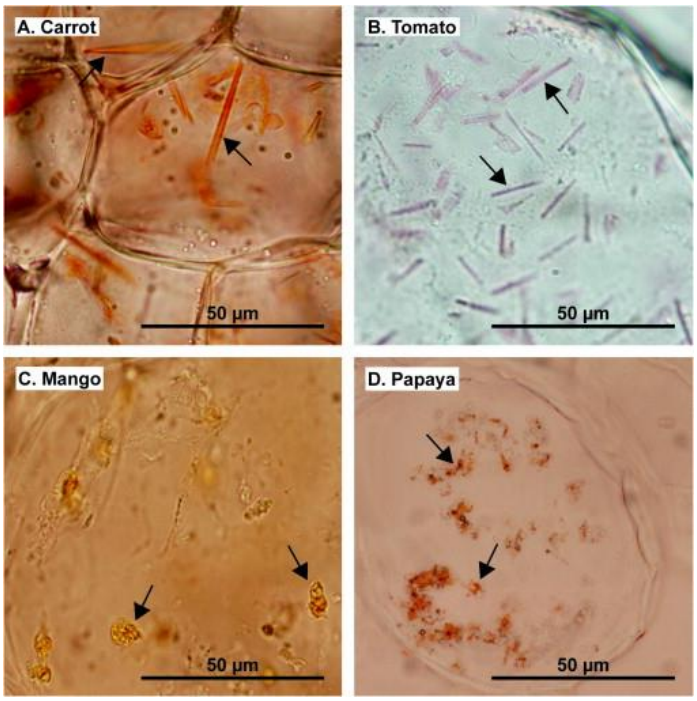
Method

Peel off a part of fruit wall with a small amount of pulp attached to it. Mount in a drop of water and observe under the microscope.

Observations

1. The cells are filled with numerous orange or red coloured chromoplasts.
2. In ripe fruits chromoplasts occur in groups.
3. Chromoplasts may be discoid or flattened. These occur close to the wall.
4. The chromoplasts have abundance of xanthophylls and carotenes and hence their colour. Chlorophyll though present is lesser in amount.

S. The major function of the chromoplasts is to protect the organ from the bright sunlight. It also helps in photosynthesis by absorbing light.



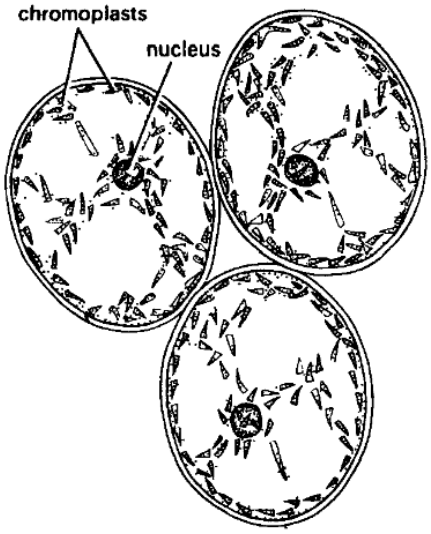


Fig. 9. Chromoplasts in the pericarp of tomato.

Study leucoplasts

Materials

Potato tuber, slides, covers lips, microscope, acid fuchsin, glycerin, water, etc.

Method

1. Cut thin sections of potato tuber.
2. Place them in a watch glass containing 1 % aqueous solution of acid fuchsin. Cover the watch glass with another but larger watch glass. Allow the sections to take stain for at least 3-4 hours.
3. 3. Wash the sections with water.
4. 4. Mount in glycerine.

Observations

1. Leucoplasts are seen as pink-colored structures amongst starch grains.
2. The shape of the leucoplasts is extremely variable.
3. It is filled with numerous starch grains.
4. Leucoplasts are the storage plastids which generally store starch.

Experiment No: 5

Non-living contents in cells

To study starch grains

Materials

Potato tuber, seeds of pea, seeds of wheat, seeds of maize, seeds of rice, slides, coverslip, microscope, Iodine solution, glycerine, etc.

Method

1. Cut a thin section of potato tuber or seed.
2. Place the section on a slide and stain it with a drop of iodine.
3. Wash the section by pouring water and draining it off. Repeat till excess stain is washed off.
4. Mount the section on another clean slide using glycerine as a mounting medium.

Observations

1. Each starch grain has a hilum which is a point of origin of starch deposition.
2. Starch is deposited in layers around hilum.
3. In the starch grains of pea, hilum is located in the centre and the layers of starch are uniformly deposited around it. These starch grains are called concentric and simple. Sometimes two or more starch grains get attached to one another. Such starch grains are called concentric and compound.
4. In the starch grains of potato, hilum is located in one corner and layers of starch are deposited centrically around it. Such starch grains are eccentric and simple. Sometimes two or more starch grains remain attached to one another. Such starch grains are called eccentric and compound.

5. The starch grains are characteristic of a particular plant and can be easily identified. The characters of starch grains of some of the common plants are listed below.

(a) Grains of wheat - simple, concentric, spherical and flattened.

(b) Grains of rice - simple, concentric, with many arms.

(c) Grains of maize - simple, concentric, angular.

(d) Seeds of pea - simple, concentric, spherical or elongated.

(e) Seeds of gram - simple, concentric. spherical or elongated.

(f) Tuber of potato - simple, eccentric, spherical or oval.

(g) Fruit of banana - simple. concentric, spherical.

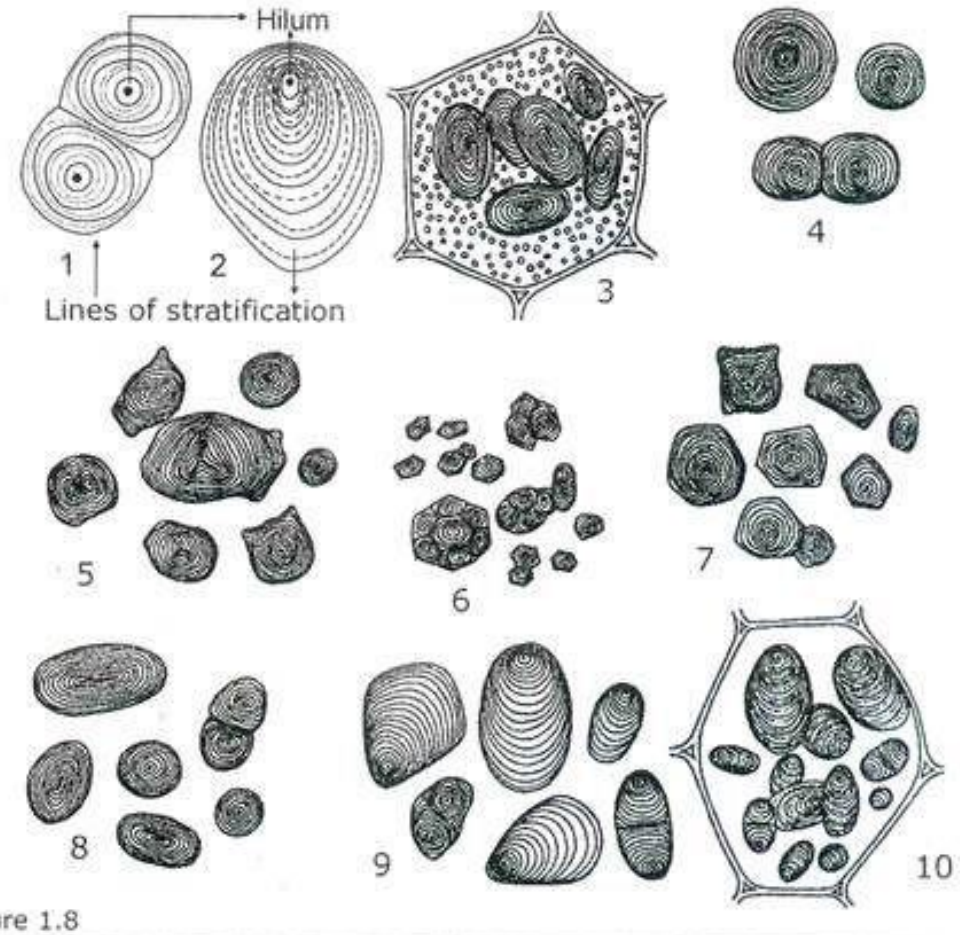
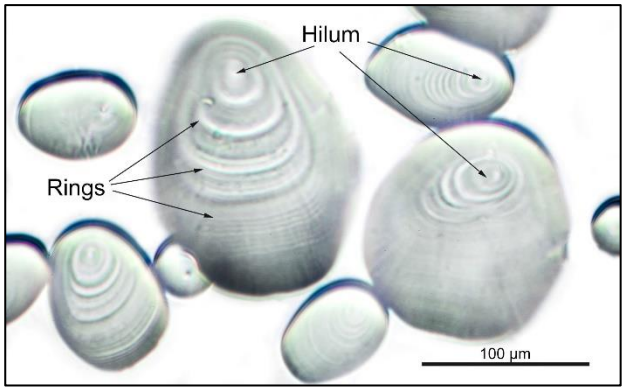
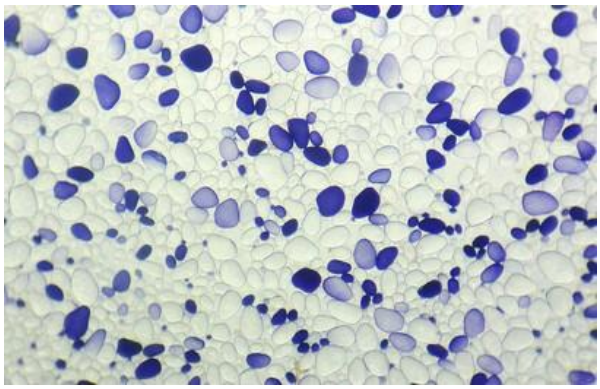


Figure 1.8

Starch grains. 1. Compound starch grain. 2. Simple starch grain. 3. From cotyledon of *Pisum* seed. 4. From flesh of *Musa*. 5. From tuberous root of *Ipomoea batatas*. 6. From endosperm of *Oryza* grain. 7. From endosperm of *Zea* grain. 8. From cotyledon of *Cicer* seed. 9. From tuber of *Solanum tuberosum*. 10. Same in situ.


Hilum
Rings
100 µm



To study Aleurone grains

Materials

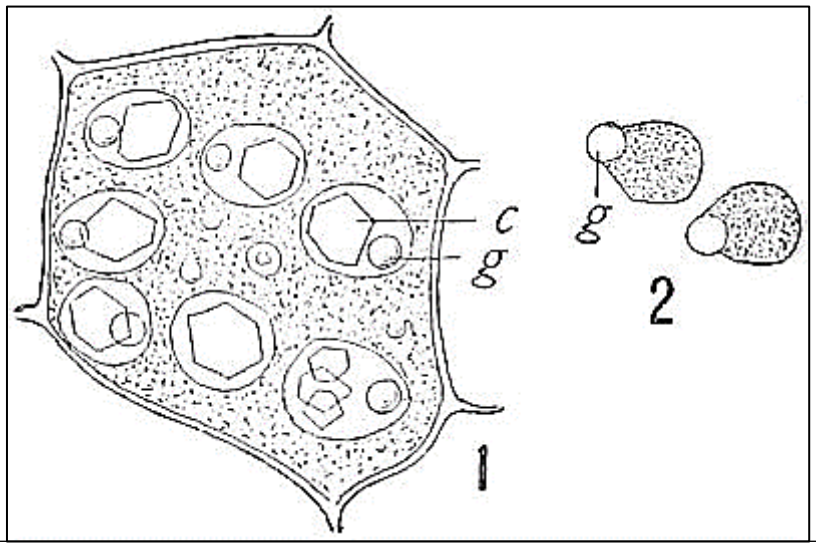
Seeds of castor, safety blade, slides, coverslips, microscope, water, glycerine, etc.

Method

1. Remove the seed coat from castor seeds.
2. Cut a thin section of the endosperm.
3. Mount the section in glycerin and observe under the microscope.

Observations

1. Each cell shows many spherical or oval aleurone grains.
2. Each aleurone grain is made of crystalloid and globoid.
3. Crystalloid is large and has many angles. It is mostly made of proteins.
4. Globoid is a small globular or oval structure. It is made of calcium or magnesium diphosphate.
5. Aleurone grains are abundant in the aleurone layer found in grains of cereals like wheat, maize, rice, etc.



1. A cell from the castor bean, as seen in water, showing roundish aleurone grains imbedded in the protoplasm. In each, one or more crystals, c, and usually a globoid, g.
2. Isolated aleurone grains of the same, as seen in olive oil.

To study Calcium Oxalate

Materials

Onion bulb, safety blade, slides, cover slips, water, glycerine, microscope, etc.

Method

1. Cut a thin section of outer scaly leaf of onion bulb.
2. Mount the section in glycerin and observe under the microscope.

Observations

1. The cells show raphides of different shapes e.g. prism-shaped, rod-like, needle-like, etc.
2. Raphides are the crystals of calcium oxalate.
3. These may be present either singly or in groups. When in groups these become star-shaped (sphaero-raphides) or form bundles.
4. Some of the plants in which raphides are found, are given below

(a) Colocasia (Eng-Taro: Hindi-Arvi, Kachalu)-petiole shows raphides, and sphaeroraphides.

(b) Amorphophallus (Eng.-Elephant-foot yam; Hindi- limikand)-needle shaped raphides in leaves.

(d) Eichhomia (Eng.-Water hyacinth; Hindi- Samundersonkh) - Raphides in the petiole.

(e) Carica papaya (Eng.-Papaya; Hindi-Papita) Raphides in the petiole.

(/) Impatiens (Eng.-Garden balsam; Hindi- Gul-mehndi) - Raphides in the leaves.

(g) Chenopodium (Eng.- Pigweed; Hindi-Bathua) Sphaeroraphides in leaf.

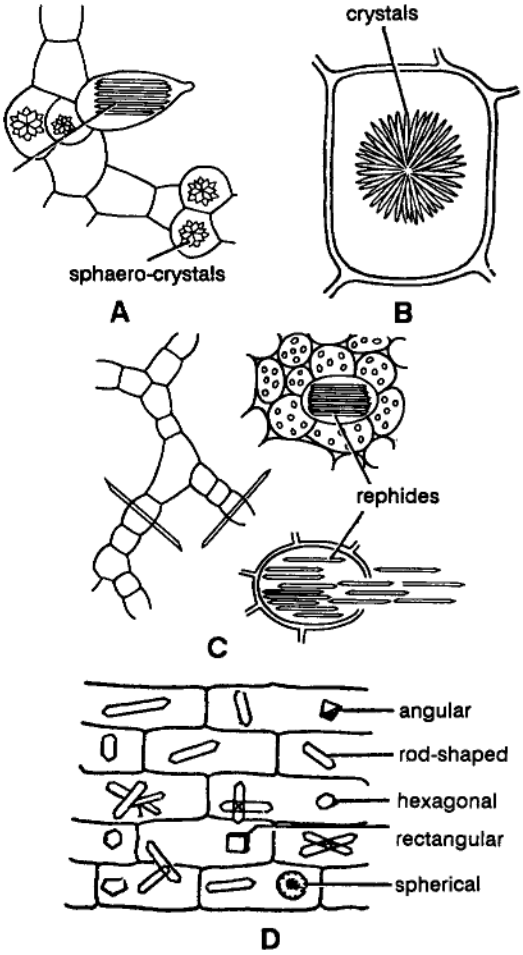
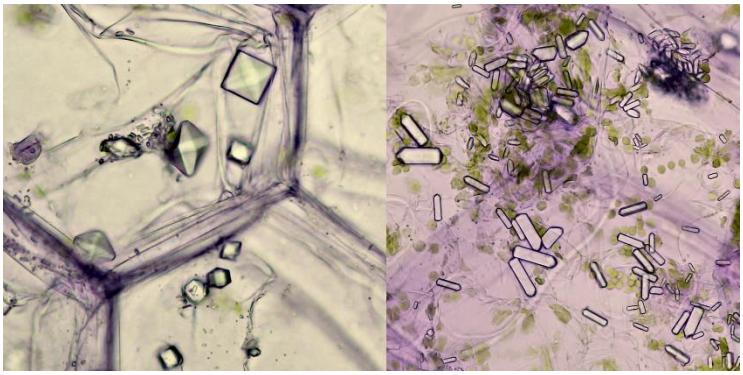
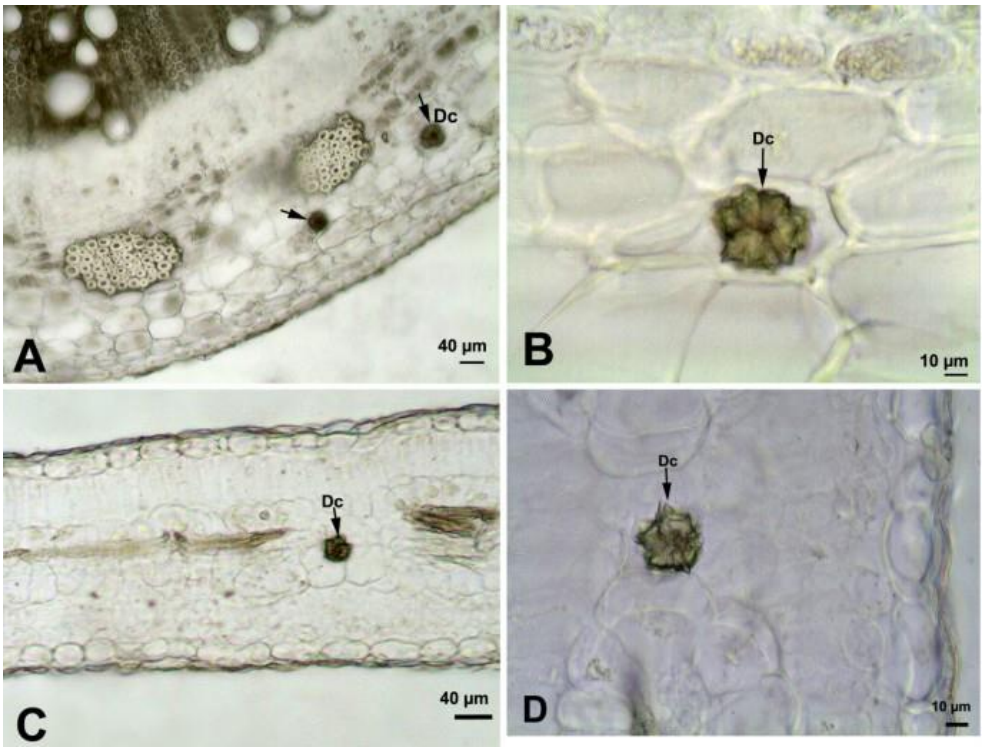


Fig. 14. Diffferent types of raphides. A. Water hyacinth, B. Balsam or Impatiens,C. Sphaeroraphides in Pistia. D. Raphides I the scales of onion.





To study Calcium Carbonate

Materials

Leaves of *Ficus elastica* (India Rubber plant), safety blade, slides, coverslips, water, microscope, safranin, etc.

Method

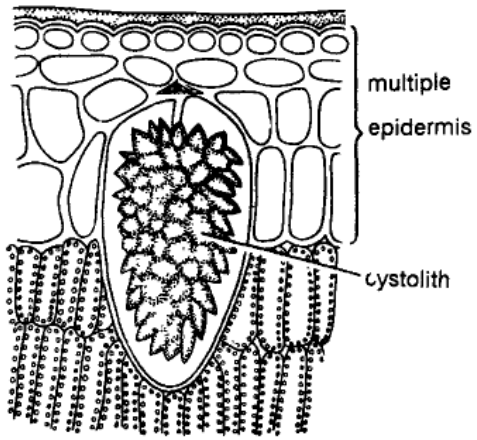
1. Cut a thin section of the leaf.
2. Stain in safranin for about 10 minutes.
3. Wash in water till proper distaining is obtained.
4. Mount in glycerin and study under the microscope.

Observations

1. The epidermis is made of many layers.
2. The cells of the innermost layer of multiple epidermis are elongated.
3. These cells show a peg-like ingrowth produced by the cell wall.
4. Many crystals of calcium carbonate are deposited on this ingrowth (stalk) to form grape -like cluster. This is known as cystolith.
5. Cystoliths are also found in (a) Leaves of Ficus benghalensis (Eng.- Banyan; Hindi-Bargad).

(b) Leaves of Momordica charantia (Eng.- Bitter gourd; Hindi.-Karela).

(c) Leaves of Ruellia tuberosa (Eng.-Meno-wecd).



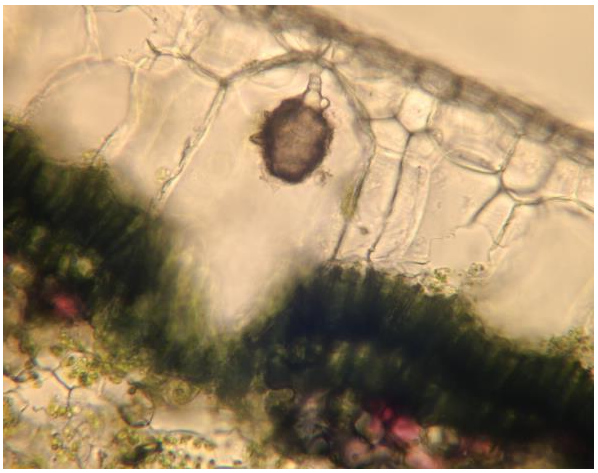


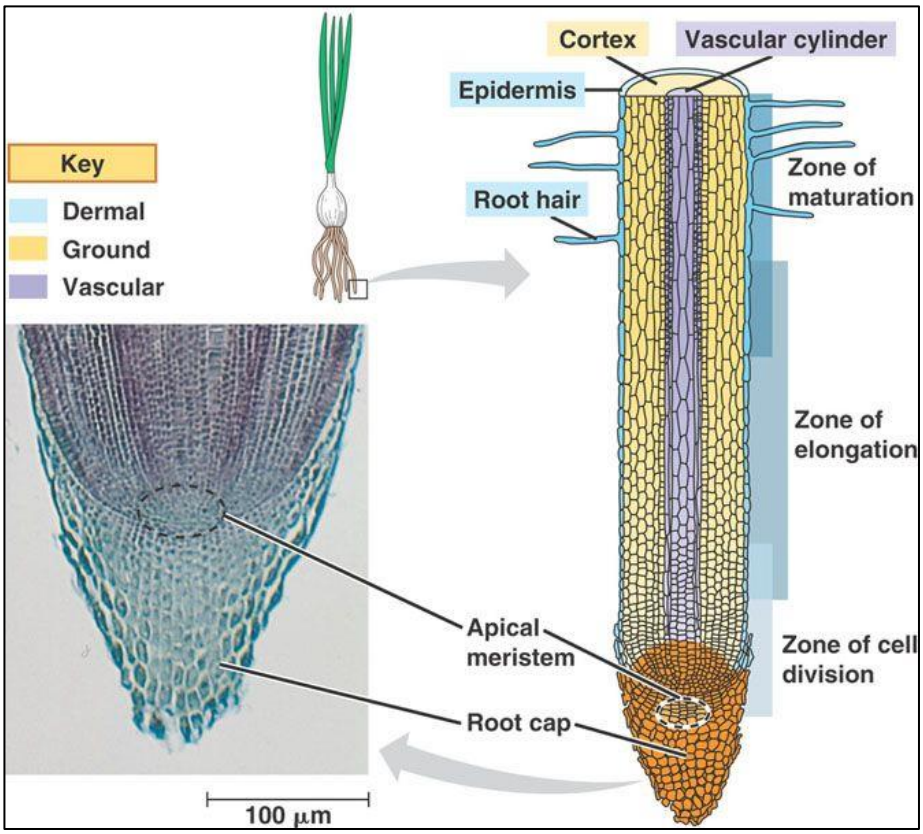
Fig. 15. Cystolith in the leaf of *Ficus elastica.*

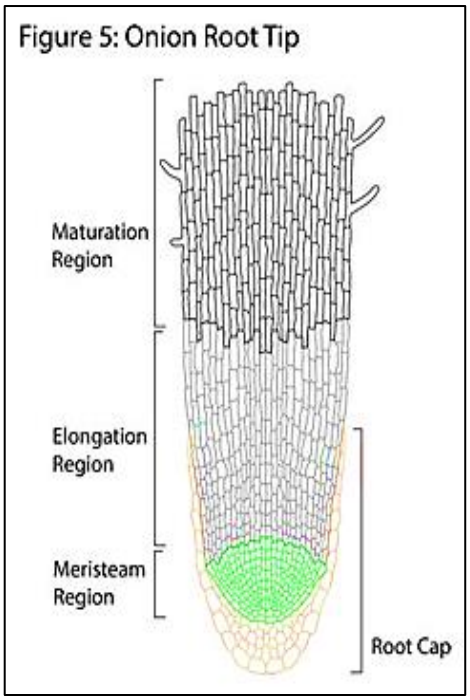
Experiment No: 6

Cell division: Mitosis

Mitosis is a type of cell division which results in the formation of two daughter cells. These cells are identical to the parent cells and have the same number of chromosomes. Mitosis occurs in vegetative cells. It can be best observed in onion root tip.

To study the mitosis by preparing squash of onion root tip





Materials and technique

Onion root tips, acetic acid, aceto-carmine, slides, cover slips, etc. Allow the onion bulbs to grow in bottles filled with water. If the lower root portion of the bulb dips in water, it quickly sends forth large number of

roots. Cut the root tips between 9 a.m. to 12 noon and fix them in Carnoy's fluid.

Procedure

The following procedure is used.

1. Place the fixed root tip in a drop of 45% acetic acid.
2. Place a cover glass over the tip and diffuse aceto-carmine.
3. Tap and apply uniform pressure over the cover glass.
4. The squash preparation is ready.

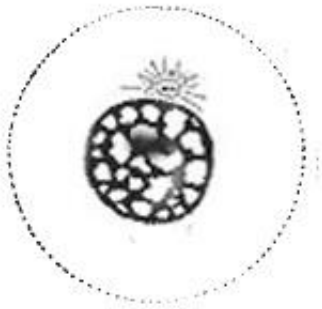
Observations

The slide shows almost all the stages of mitosis.

[I] Interphase

The following characteristics are seen:-

1. This is a stage prior to actual mitotic cycle.
2. The cell appears to be inactive or in resting stage but is metabolically the most active. DNA replication occurs during this period.
3. Nuclear membrane and nucleolus are very distinct.
4. Chromosomes are in the form of chromatin network and individual chromosomes cannot be seen separately.
5. The chromosome appears double stranded i.e., made of two chromatids.



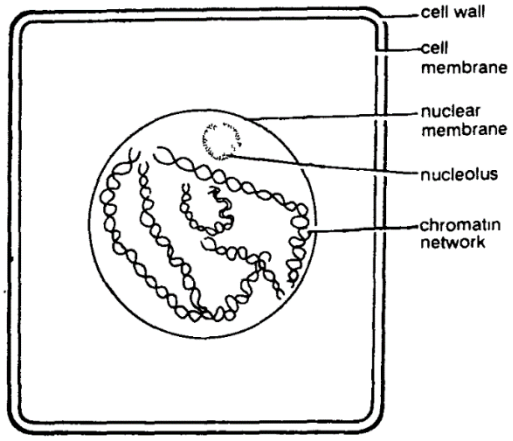


Fig. 9. **Mitosis.** Cell showing interphase.

[II] Early prophase

The following characteristics are seen-

1. This is the first stage of mitosis which is observed under the microscope.
2. Nuclear membrane appears distinct.
3. Nucleolus is also seen clearly.
4. Chromosomes become coiled and shortened and more distinct.

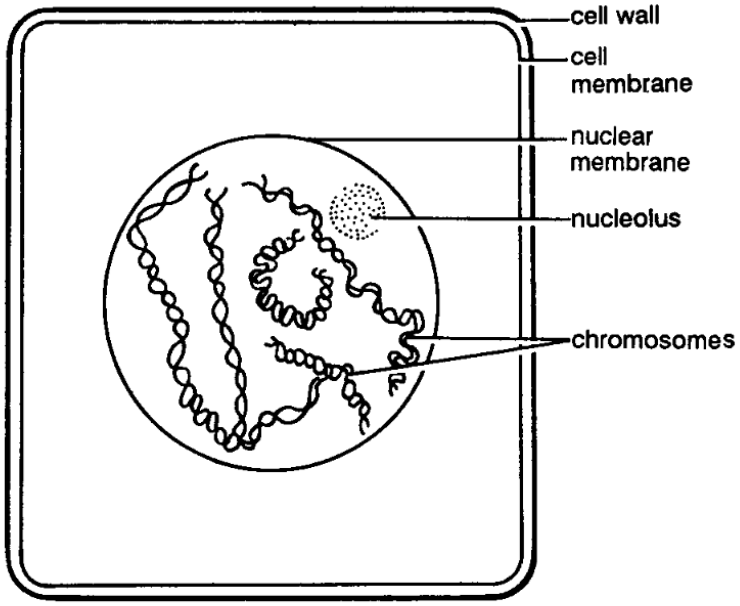


Fig. 10. **Mitosis**. Cell showing early prophase.

[III] Late prophase

The following characteristics are seen-

1. The nuclear membrane and nucleolus have partially or completely disappeared.
2. Each chromosome now begins to show chromatids, primary constriction, secondary constriction and centromeres.
3. The equatorial region appears clearly in the centre of the cell.
4. Chromosomes begin to move and gather near the equatorial plate.
5. Chromosomes are condensed and thus short and thick.
6. Spindle fibers also begin to appear.

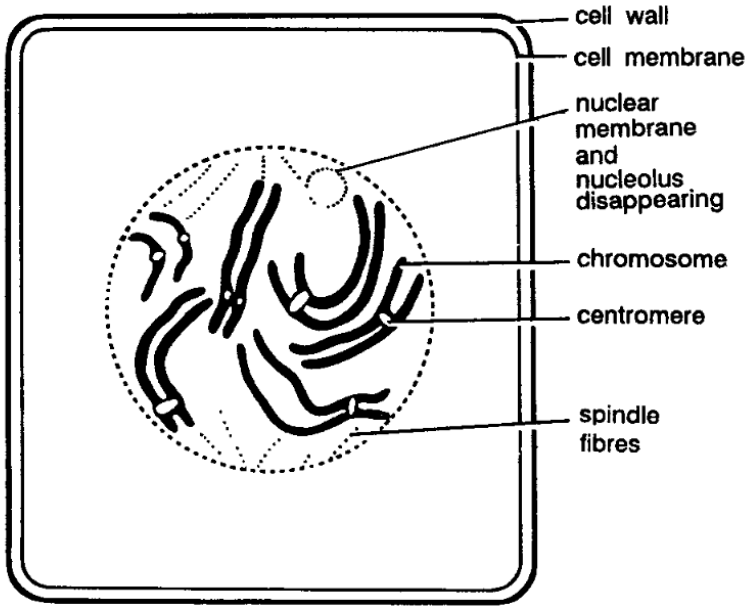


Fig. 11. **Mitosis**. Cell showing late prophase.

[IV] Metaphase

The following characteristics are seen.

1. Nuclear membrane and nucleolus are absent having disappeared.
2. Centromeres of the chromosomes are arranged on the equatorial plate, and each is attached to the spindle fibres.
3. Centrioles are absent and hence aster is not formed in plant cells. This type of mitosis is known as anastral mitosis.
4. The spindle is made of fibres only. The absence of centrioles indicates that it is a plant cell.
5. The chromosomes at metaphase are very distinct. Thus, number and morphology of chromosome is studied at this stage. Each chromosome shows two chromatids, centromere, primary constriction, euchromatic and heterochromatic regions, chromomeres, etc.

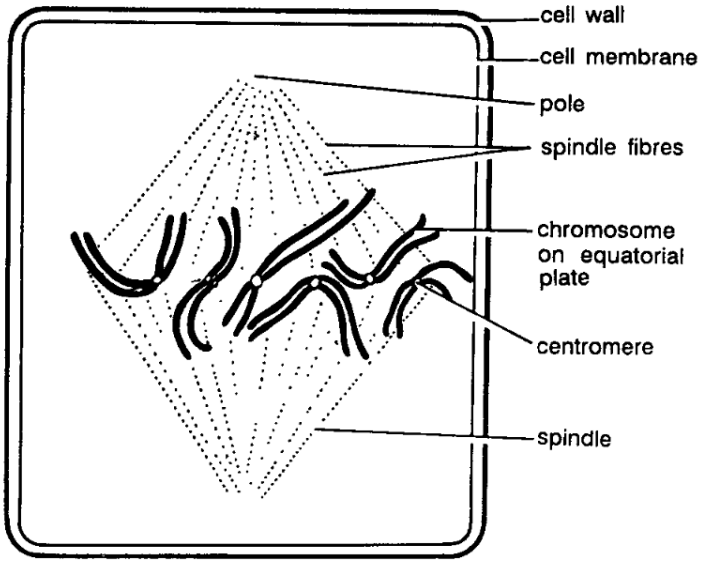


Fig. 12. **Mitosis**. Cell showing metaphase.

[V] Anaphase

The following characteristics are seen:-

1.This stage is completed in a very small period of time.

2. The centromere of each chromosome gets split into two.

3. The chromosome also gets divided into two chromatids. Each chromatid now bears one centromere each.

4. The chromosome becomes shorter and thicker.

5. The separated chromatids are now pulled towards the opposite poles due to contraction of spindle fibres.

6. During movement, each chromosome shows characteristic shape which is dependent on the position of centromere.

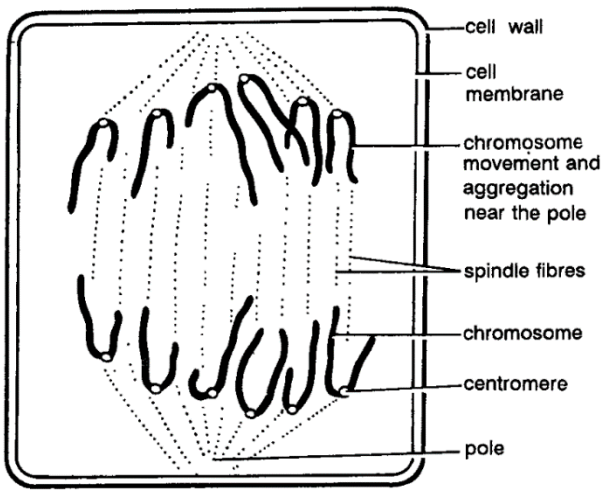


Fig. 13. **Mitosis**. Cell showing anaphase.

[VI] Telophase

The following characteristics are seen~

1. The chromosomes are present at both the poles of a parent cell.
2. The chromosomes increase in length and become thread-like. All the chromosomes together form chromatin network, and their individuality is now lost.
3. The groups of chromatin network at each are surrounded by nuclear membrane Nucleolus is also present.
4. Thus two fully formed nuclei, one at each pole are present in the parent cell.
5. Spindle fibres are absent.

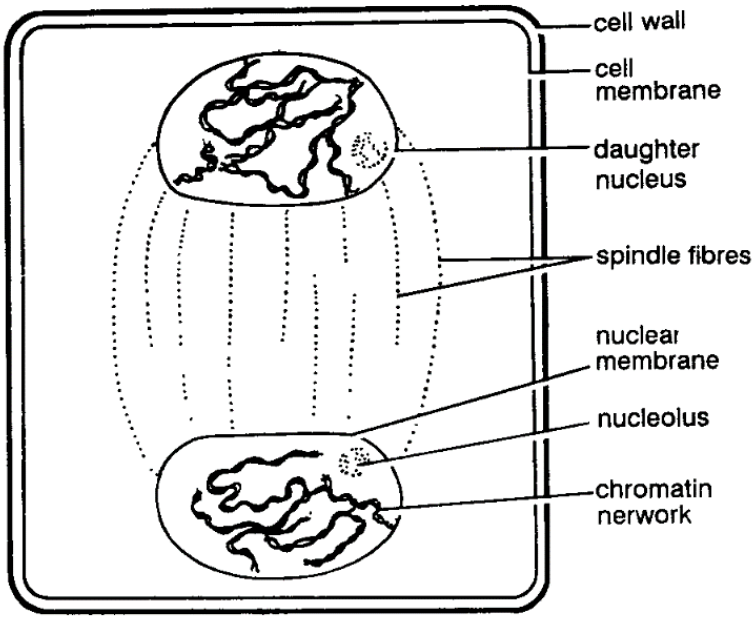


Fig. 14 **Mitosis** Cell showing telophase

[VII] Cytokinesis

The following characteristics are seen: -

1. In this stage, cytoplasm divides into two. It results in the formation of two daughter cells.
2. Division of the cytoplasm is due to formation of a cell plate in the equatorial region.

3. Cell plate formation begins in the centre of the cell and gradually progresses towards the periphery.
4. This results in the formation of two daughter cells. Organelles are also present.
5. The number of chromosomes in each daughter cell is equal to the number present in parent cell.

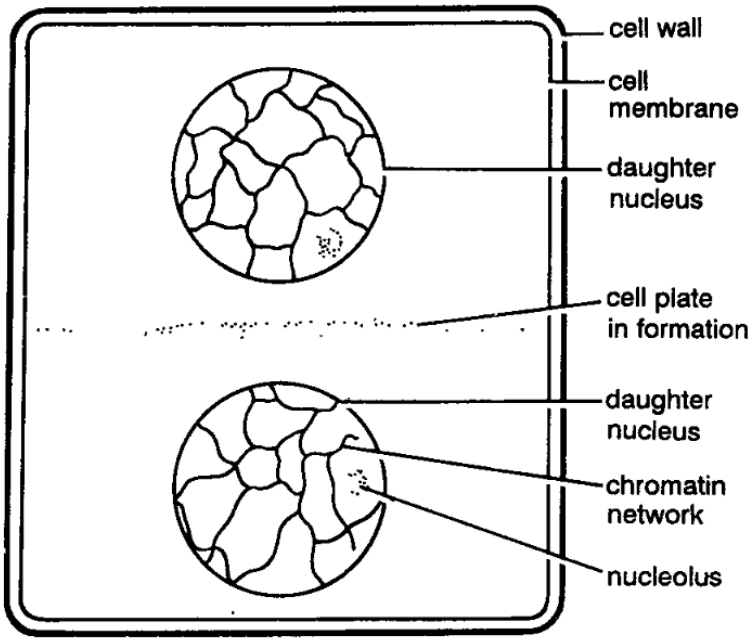
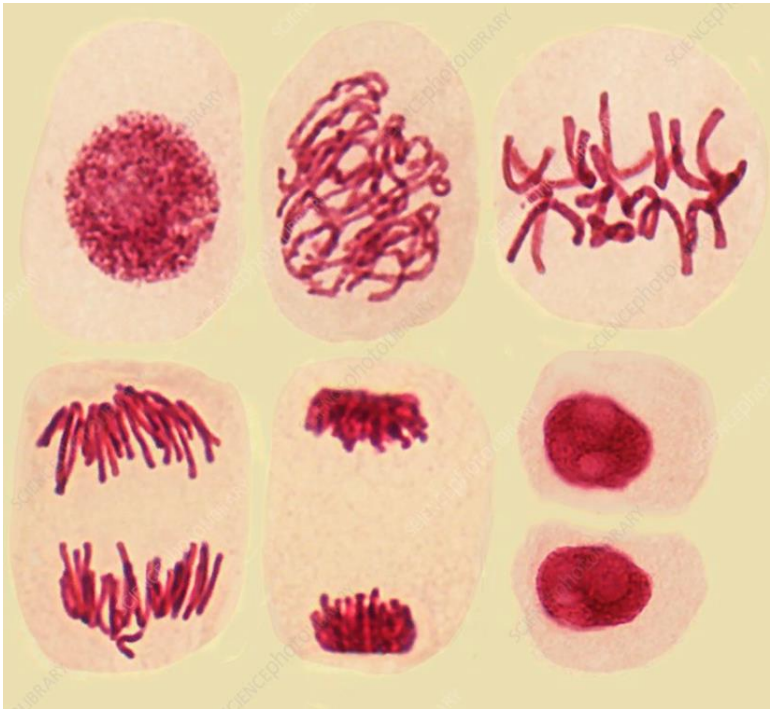
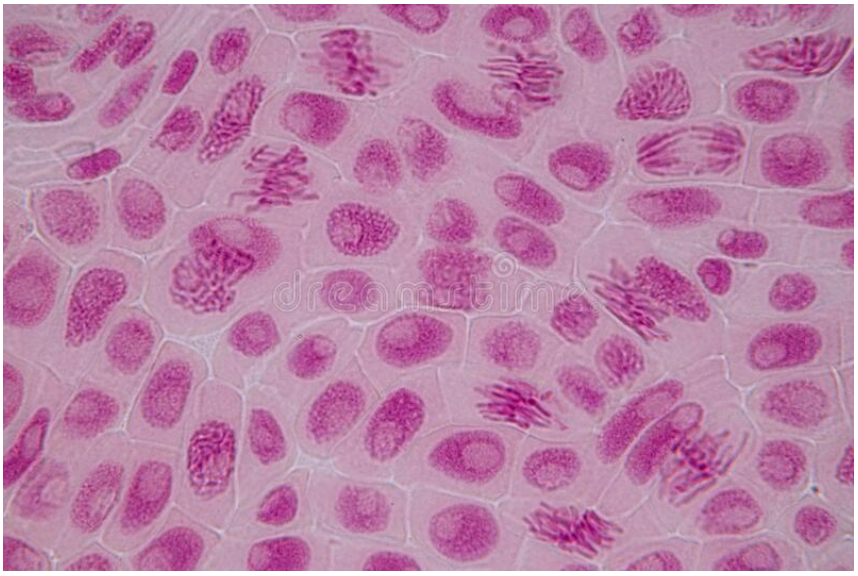
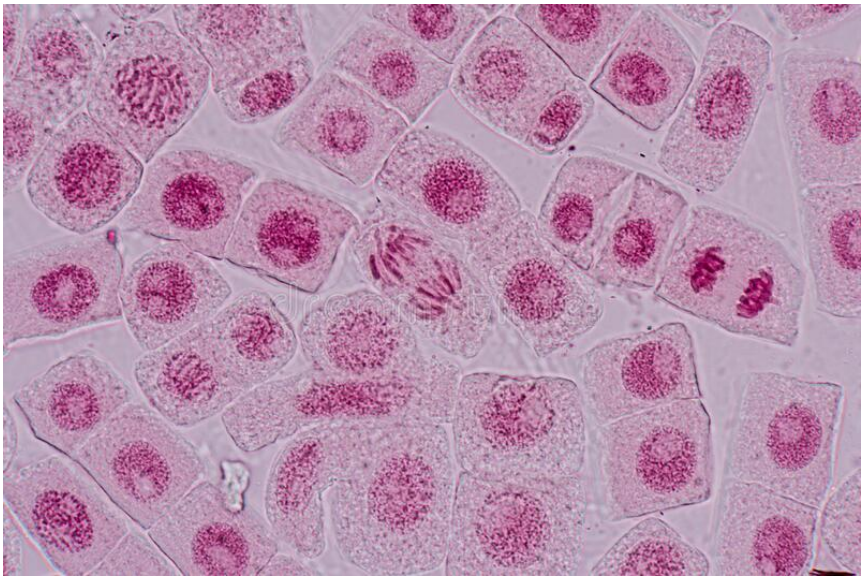
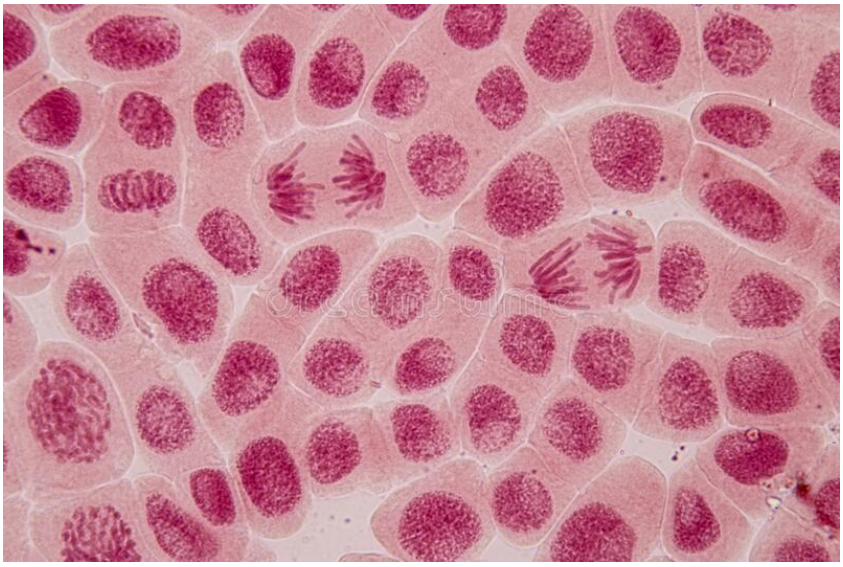


Fig. 15. **Mitosis**. Cell showing cytokinesis.









MEIOSIS

Meiosis is a cell division that is characteristic of organisms which reproduce sexually. During this division, genetic material is duplicated once, and nucleus divides twice. As a result, four daughter cells are formed. These have half the chromosomes as compared to the parent cells. Meiosis also involves crossing over, i.e. exchange of equal parts of non-sister chromatids of the homologous chromosomes. Therefore, the four daughter cells are genetically different from the parent cells. Meiosis consists of (1) Meiosis I and (2) Meiosis II.

Meiosis I involves some very characteristic and important stages such as

(1) Synapsis or pairing of homologous chromosomes,

(2) Recombination due to crossing over and

(3) Segregation of homologous chromosomes.

The stages included in Meiosis I are Prophase I, Metaphase I, Anaphase I and Telophase I. At the end of meiosis, I, two daughter cells are formed. Each cell has half the number of chromosomes compared to parent cell. Meiosis II is similar to mitosis. It results in the formation of four daughter cells, each having the same chromosome number as was present at the end of Meiosis I. Meiosis II is also sub-divided into Prophase II, Metaphase II, Anaphase II and Telophase II.

To study the meiosis by Anther preparation

Materials and Technique

Prepare a smear of young anthers of *Allium cepa* as follow: -

1. Anthers are smeared on the cover glass.

2. It is then inverted on the slide in drop of acetocarmine.

3. Cover glass is sealed with melted wax.

Observations

Following stages can be seen in different slides of Meiosis-

[I] Leptotene (Leptonema) of Prophase I

The following characteristics are seen-

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

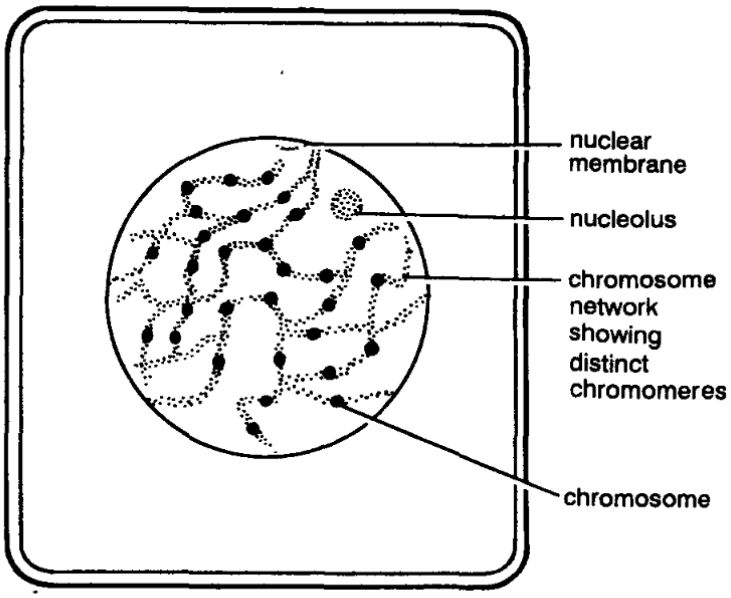


Fig. 16. **Meiosis**. Cell showing Leptotene of Prophase I.

[II] Zygotene (Zygonena) of Prophase I

The following characteristics are seen-

1. Nuclear membrane and nucleolus are still very clear.

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

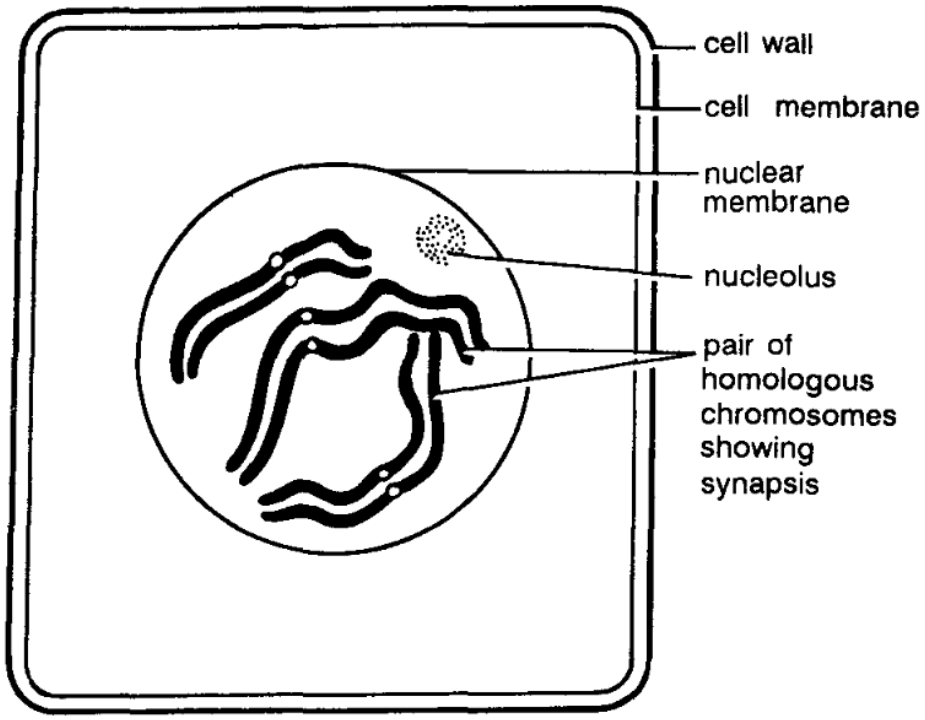


Fig. 17. **Meiosis**. Cell showing Zygotene of Prophase I.

[III] Pachytene (Pachynema) of Prophase I

The following characteristics are seen-

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

3. Chromosomes are very closely coiled. Each chromosome shows its two chromatids. A pair of homologous chromosomes which is intimately coiled upon one other shows four chromatids together.
4. Pair of homologous chromosomes is called bivalent. It is made of four chromatids and hence known as tetrad.
5. The stage is characterized by crossing over. It is the exchange of equal parts of chromatids of two different but homologous chromosomes.
6. Nucleolus is distinctly attached to nucleolar organizing chromosome.
7. The length of the chromosome being more than that found at metaphase, the chromosome at this stage is also used for the study of morphology.

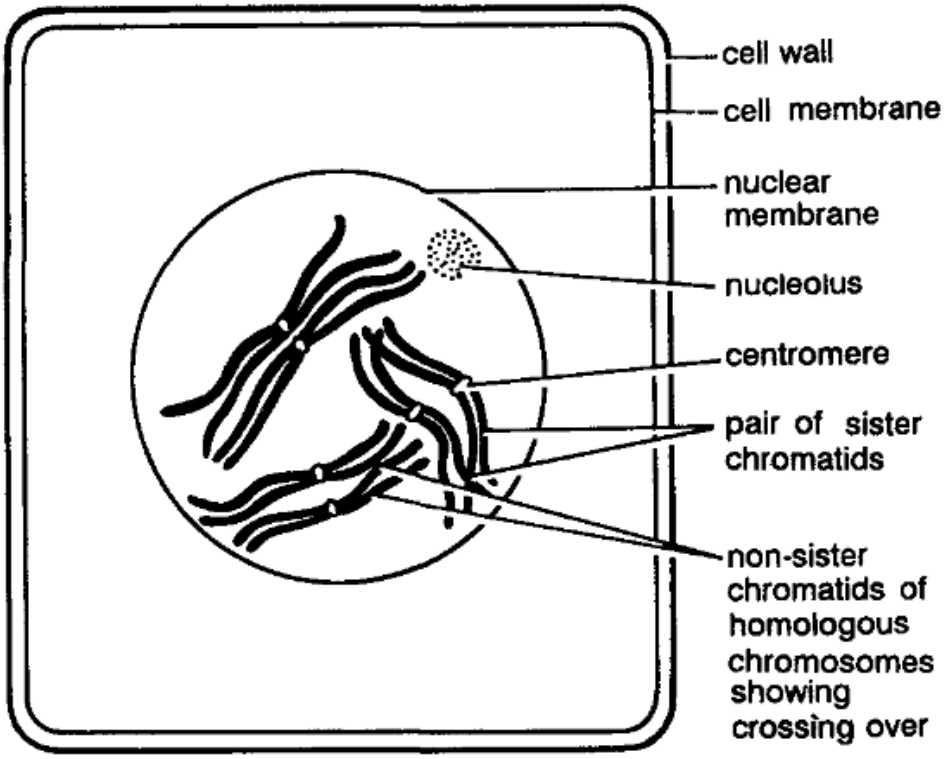


Fig. 18. **Meiosis**. Cell showing Pachytene of Prophase I

[IV] Diplotene (Diplonema) of Prophase I

The following characteristics are seen-

1. The nucleolus is disappearing while nuclear membrane is still intact.
2. The close and tight coiling of chromosomes becomes loose, and chromosomes appear more clear.
3. Homologous chromosomes still remain in contact at some points called chiasmata. These are indicators of crossing over having been completed at these points.
4. Chromosomes shorten and thicken. These become still more distinct by the end of this stage.

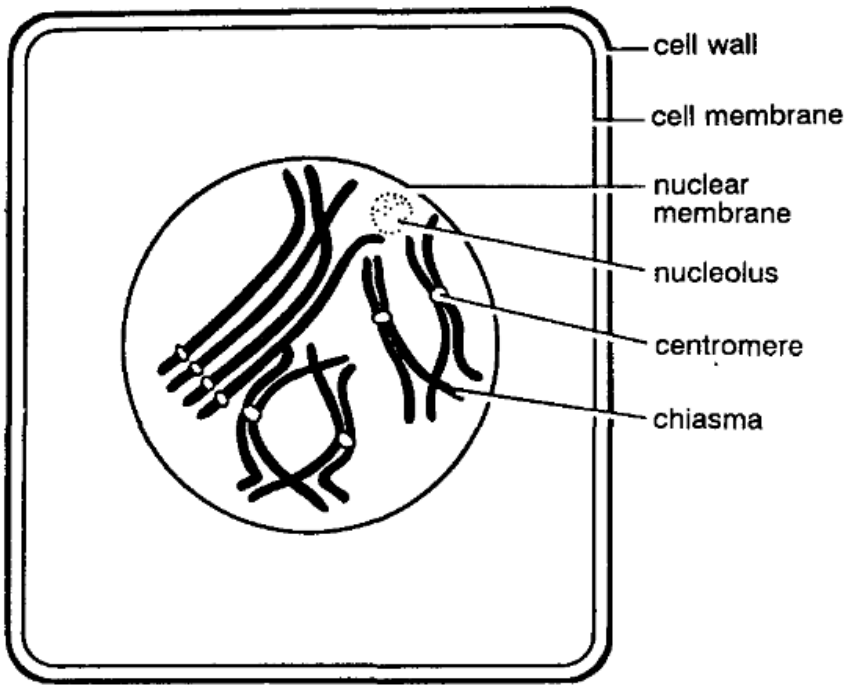


Fig. 19. **Meiosis.** Cell showing Diplotene of Prophase I.

[V] Diakinesis of Prophase I

It shows following characters.

1.Nuclear membrane and nucleolus have completely disappeared.

2. Chromatids start separating, beginning from the centromere towards the end. The chiasmata thus open. This process is known as terminalization.

3. The chromosomes appear almost circular due to continued contraction.
4. Some of the pairs of homologous chromosomes still appear joined with one another.

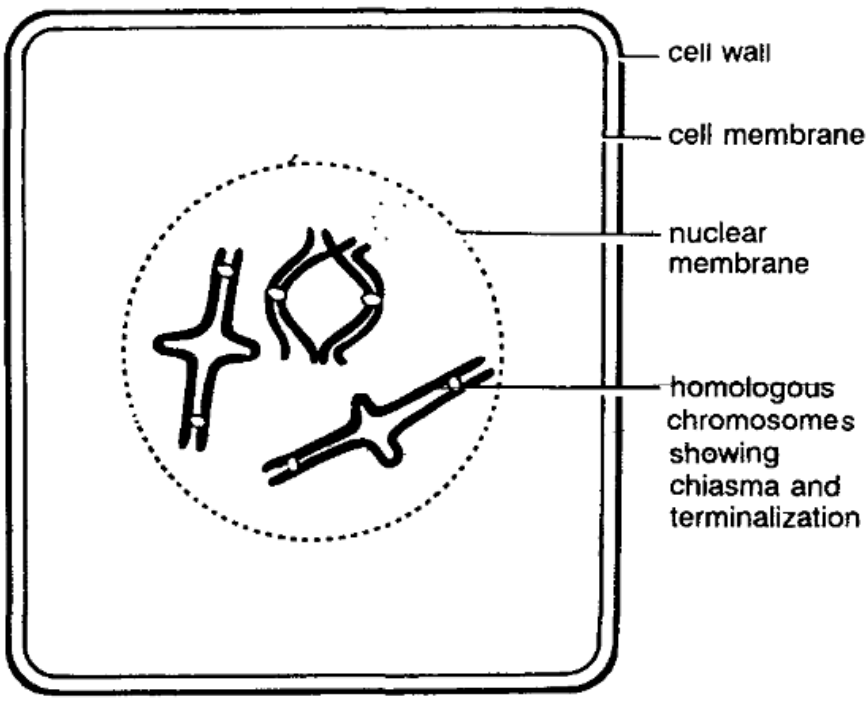


Fig. 20. **Meiosis**. Cell showing Diakinesis of Prophase I.

[VI] Metaphase I

The characters observed during Metaphase I are-

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

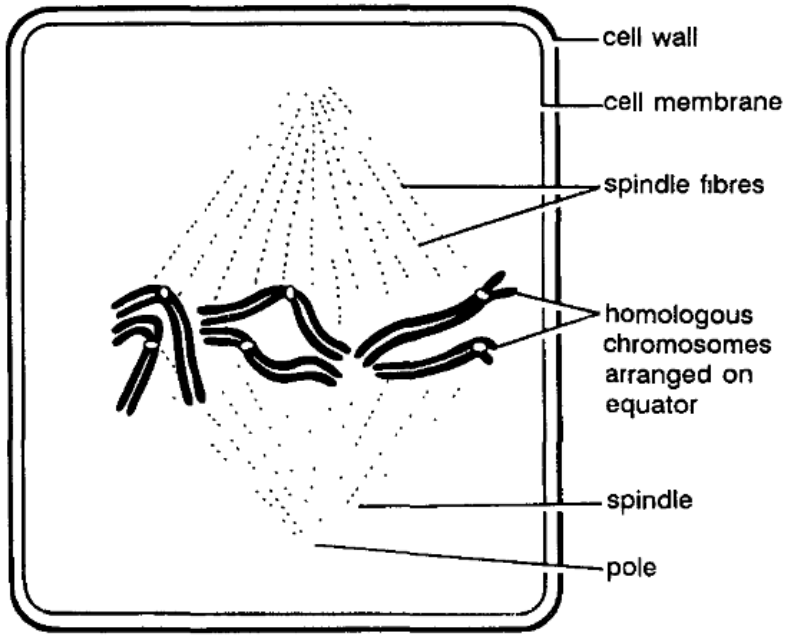


Fig. 21. **Meiosis**. Cell showing Metaphase I.

[VII] Anaphase I

The following are characteristics of this stage-

1. Nuclear membrane and nucleolus are completely absent.
2. The chromosomes separate out of the pair of homologous chromosomes.
3. Spindle fibres contract and pull the centromere along with the chromosome to opposite poles.
4. This results in two haploid sets of chromosomes, one at each pole of the cell.
5. Each chromosome shows characteristic shape during movement.

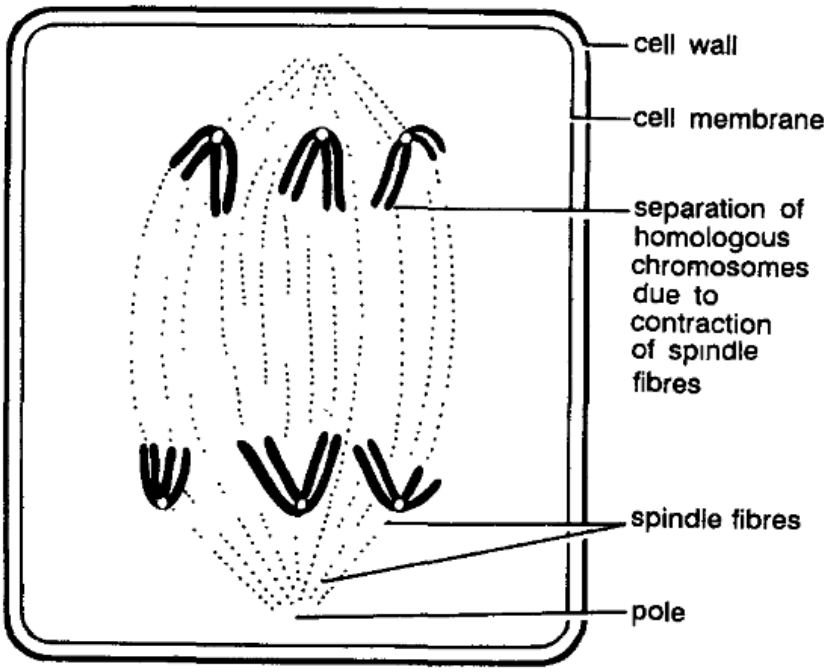


Fig. 22. **Meiosis**. Cell showing Anaphase I.

[VIII] Telophase I

The stage shows following characteristics-

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

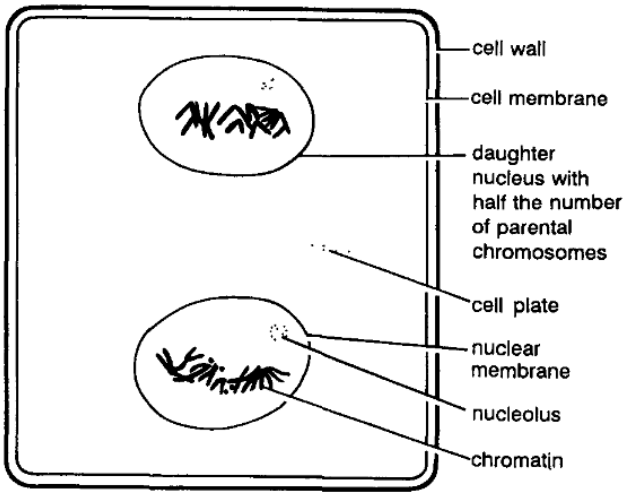


Fig. 23. **Meiosis**. Cell showing Telophase I.

[IX] Prophase II

The following characteristics are seen-

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

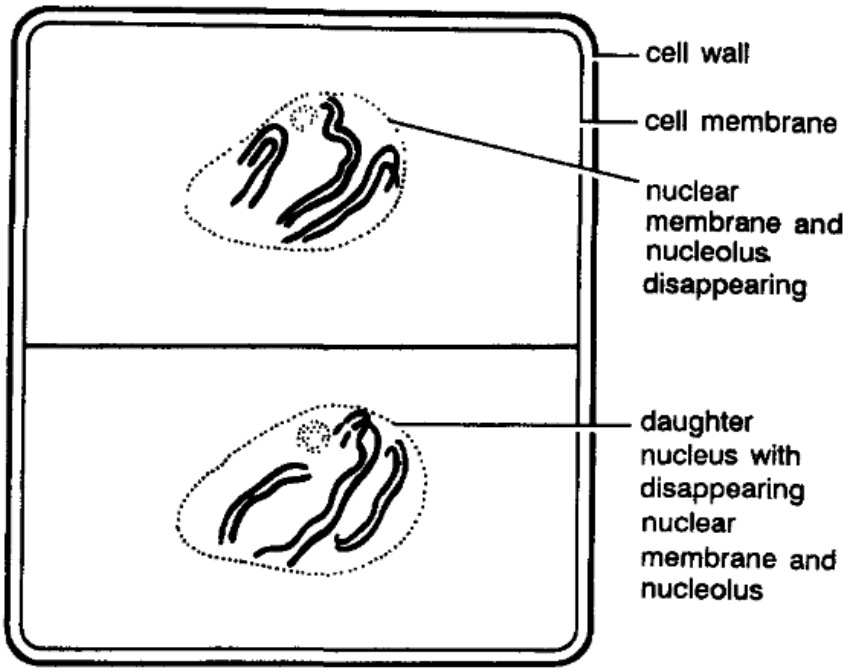


Fig. 24. **Meiosis**. Cell showing Prophase II.

[**XI Metaphase II**

It shows following characteristics-

1. Nuclear membrane and nucleolus both are absent, having disappeared.
2. Spindle fibres are formed. These are organized into a spindle.

3. Spindle fibres are joined with centromeres of the chromosomes.
4. All the chromosomes are aranged on the. equatorial plate.
5. Each chromosome is made of two chromatids held together by a centromere.

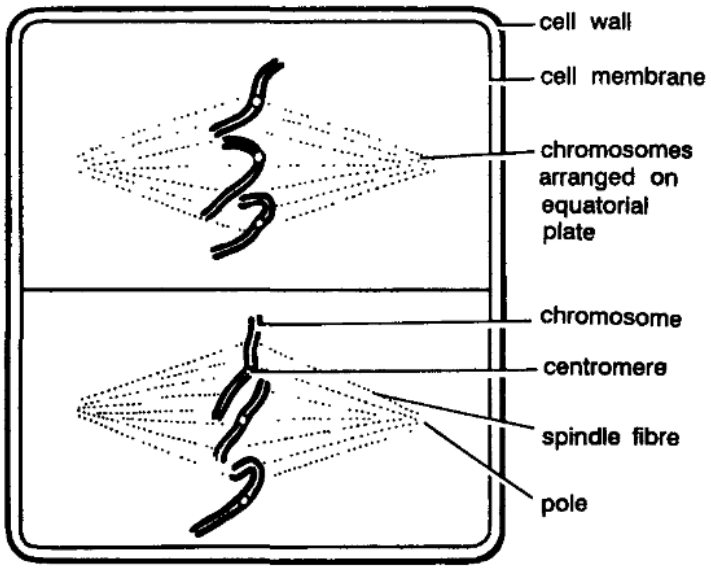


Fig. 25. **Meiosis**. Cell showing Metaphase II.

[XI] Anaphase II

This stage is characterized by the following-

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

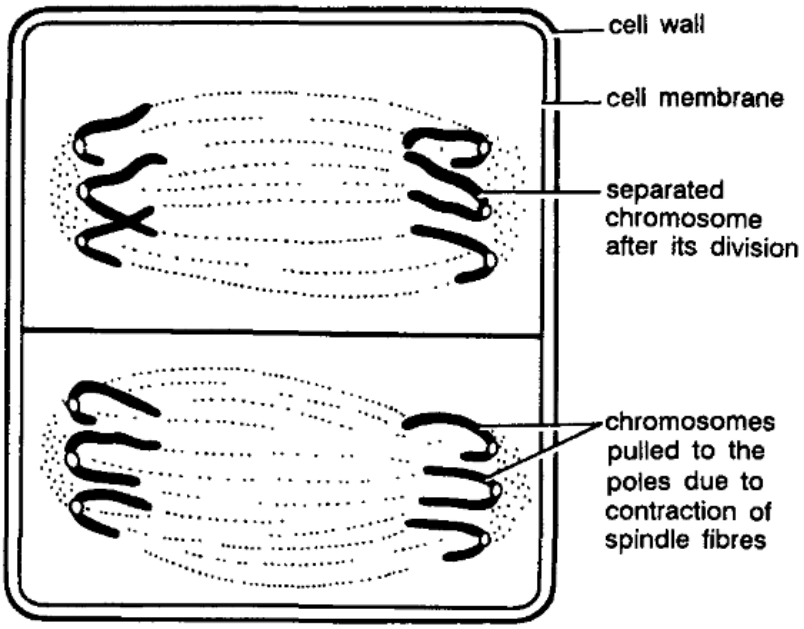


Fig. 26. **Meiosis**. Cell showing Anaphase II.

[XII] Telophase II

The following are characteristic features of this stage-

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

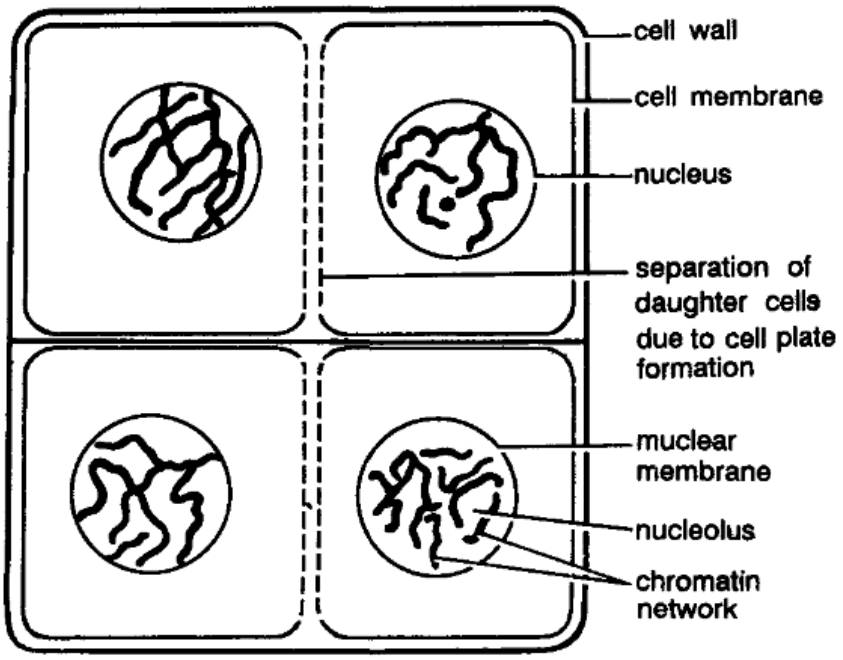
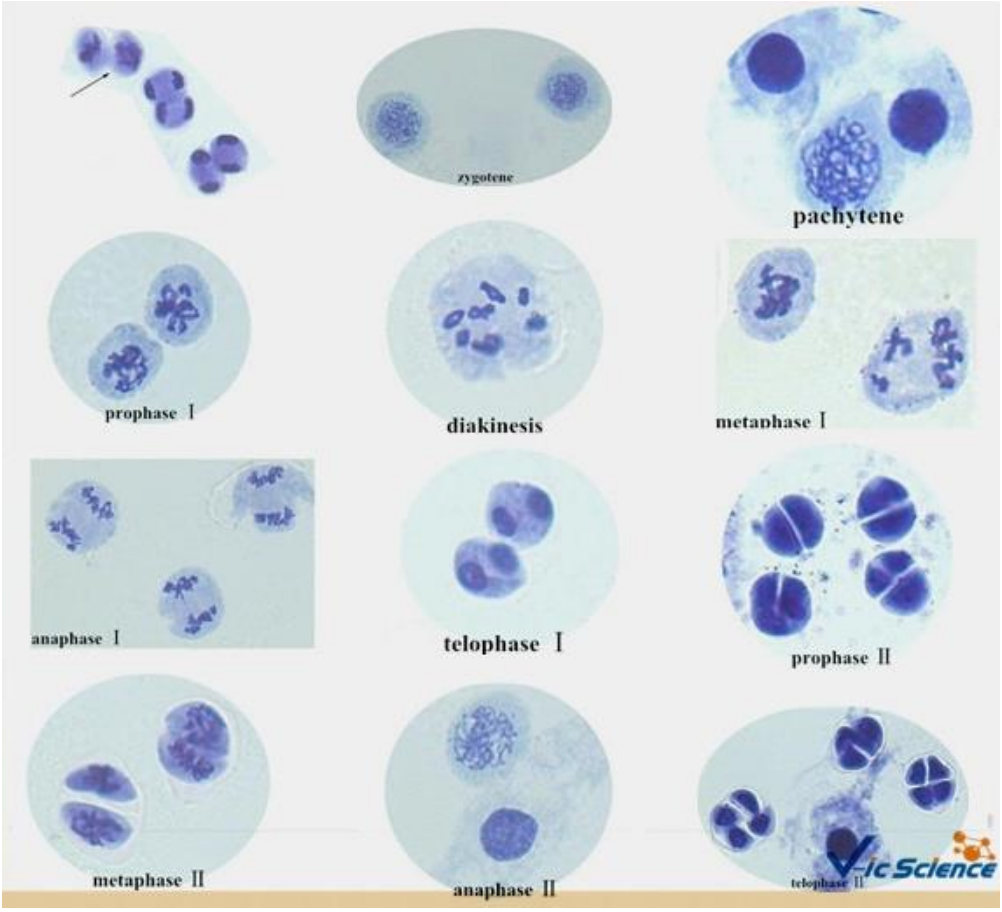
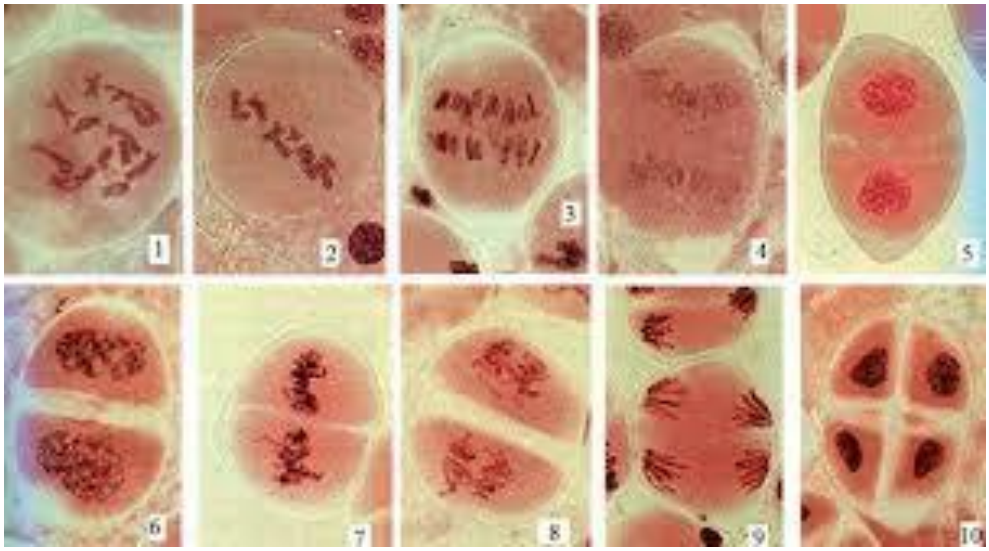
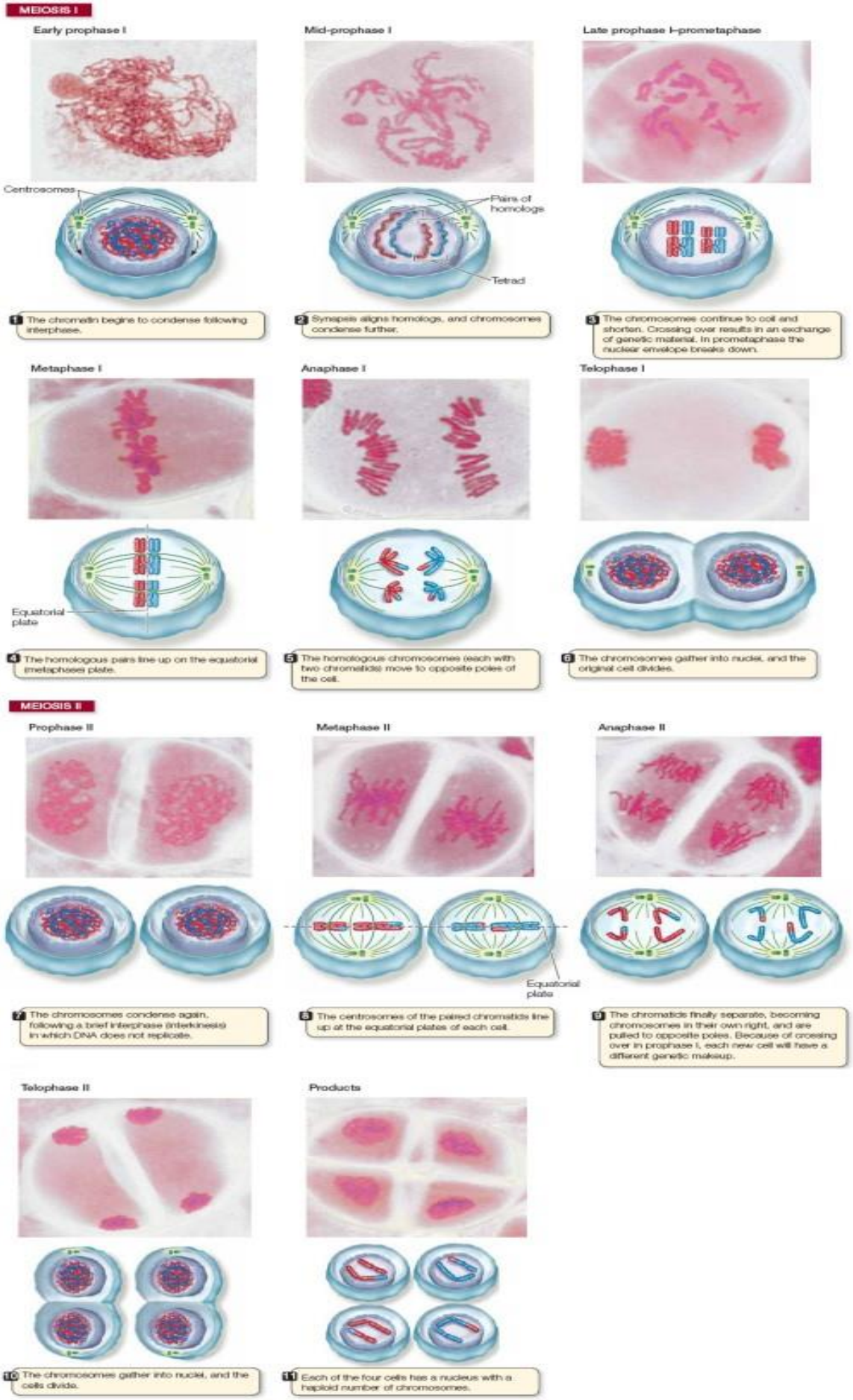


Fig. 27. **Meiosis**. Cell showing Telophase II.


zygotene
pachytene
prophase I
diakinesis
metaphase I
anaphase I
telophase I
prophase II
metaphase II
anaphase II
telophase II
Vic Science


1
2
3
4
5
6
7
8
9
10


MEIOSIS I
Early prophase I
Centrosomes
1 The chromatin begins to condense following interphase.
Mid-prophase I
Pairs of homologs
Tetrad
2 Synapsis aligns homologs, and chromosomes condense further.
Late prophase I–prometaphase
3 The chromosomes continue to coil and shorten. Crossing over results in an exchange of genetic material. In prometaphase the nuclear envelope breaks down.
Metaphase I
Equatorial plate
4 The homologous pairs line up on the equatorial (metaphase) plate.
Anaphase I
5 The homologous chromosomes (each with two chromatids) move to opposite poles of the cell.
Telophase I
6 The chromosomes gather into nuclei, and the original cell divides.
MEIOSIS II
Prophase II
7 The chromosomes condense again, following a brief interphase (interkinesis) in which DNA does not replicate.
Metaphase II
Equatorial plate
8 The centromeres of the paired chromatids line up at the equatorial plates of each cell.
Anaphase II
9 The chromatids finally separate, becoming chromosomes in their own right, and are pulled to opposite poles. Because of crossing over in prophase I, each new cell will have a different genetic makeup.
Telophase II
10 The chromosomes gather into nuclei, and the cells divide.
Products
11 Each of the four cells has a nucleus with a haploid number of chromosomes.

References

- Ashok M. Bendre; Ashok Kumar (2010): A Textbook of Practical Botany Vol. II. Rakesh Kumar Rastogi for Rastogi Publications.

Web Sites:

- http://biology.about.com/od/cellanatomy/
- https://www.wikipedia.org/

"تمت بحمد الله"