1. **Aim: Preparation of permanent slide to demonstrate DNA by Feulgen reaction**

Theory: Feulgen stain is a staining technique discovered by Robert Feulgen and used in histology to identify chromosomal material or DNA in cell specimens. It is darkly stained. It depends on acid hydrolysis of DNA, therefore fixating agents using strong acids should be avoided. DNA (Deoxyribonucleic acids) can be studied in the paraffin sections of salivary glands of Drosophila or chironomous by Feulgen-Schiffs reaction. Feulgen and Rosenbeck (1924) and Pearse (1968) studied above reaction for DNA. In this reaction DNA is denatured by hydrolisation at 60°C. During this process deoxypentose sugar and aldehydes of DNA are separated. After hydrolization tissues are subjected to Schiff's reagent. The chromatin stained and purple colour is obtained.

Materials Required:

(a) 1 M hydrochloric acid
Hydrochloric acid (conc.) 8.5 ml
Distilled water 91.5 ml

(b) Schiff reagent

(c) Bisulfite solution
10% potassium metabisulfite 5 ml
1M hydrochloric acid 5 ml
Distilled water 90 ml

Procedure:

(a) Preparation of Schiffs reagent:

1. Take a 500 ml conical flask and clean it by chromic acid solution. Chromic acid solution is prepared by mixing 10 gm Potassium Dichromate in 100 ml of concentrated Sulfuric Acid.
2. Rinse conical flask by chromic acid solution. Wash the flask 9 times with tap water and 3 times with distilled water.
3. Keep flask in inverted position for drying on a filter paper.
4. Dissolve 1 gm basic fuchsin in 400 ml of boiling distilled water in a conical flask. Borosil or conical flask should be taken.
5. Cool the flask to 50°C and then filter it in another conical flask.
6. To the filtrate add 1ml of thionyl chloride. Let the solution stand for 12 hours in dark.
7. Add 2 gm activated charcoal to the solution. Shake it for 1 to 2 minutes.
8. Filter the solution and store in the dark at 0°_ 4°C.

(b) Feulgen staining method:

1. Bring all sections to water.
2. Rinse sections in 1M HCl at room temperature.
3. Place sections in 1M HCl at 60°C
4. Rinse in 1M HCl at room temperature for 1 minute.
5. Transfer sections to Schiff's reagent for 45 minutes.
6. Rinse sections in bisulfate solution for 2 minutes, repeating twice again.
7. Rinse well in distilled water.
8. Counterstain if required in 1% light green for 2 minutes.
9. Wash in water.
10. Dehydrate through alcohols to xylene and mount.

Result:

In the sections DNA appears red-purplish in colour and the Cytoplasm appears green.

Precautions:

1. Keep Feulgen kit in a tightly closed original package at room temperature.
2. Use only appropriate instruments for collecting and preparing the samples.
3. Valid BioSchiff reagent solution is colourless.
4. Discard after it starts to assume colour because of the SO2 loss.

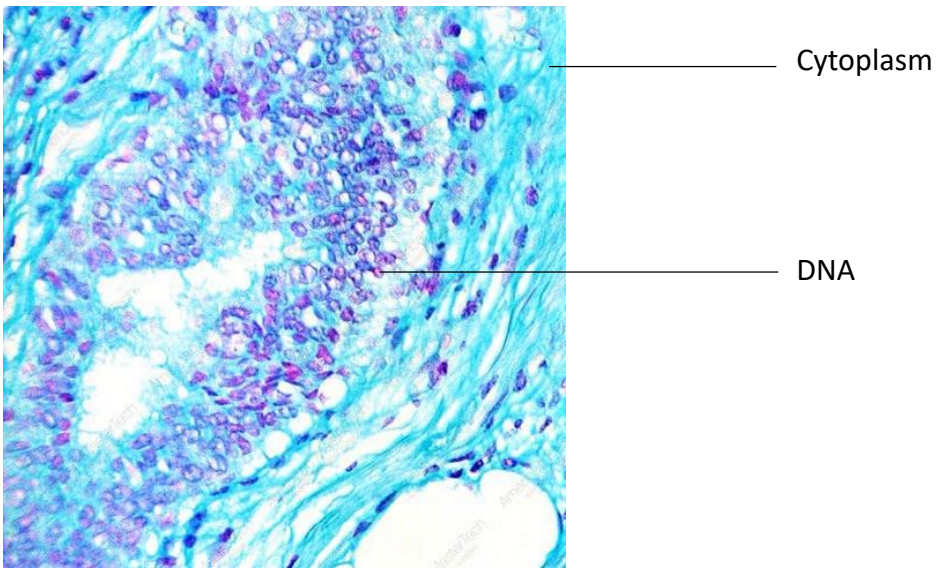


Fig : DNA stained by the Feulgen's Reagent

2. Aim: Preparation of permanent slide to demonstrate RNA by MGP.

Theory: The nuclear chromatin in the nucleus is composed of nucleoproteins. The two types of nucleic acids are deoxyribonucleic acid (DNA), found in the nucleus, and ribonucleic acid (RNA), found in the nucleolus and cytoplasm. DNA functions in cell heredity, and synthesis of RNA. RNA functions principally in protein synthesis.

Methyl green is an impure dye containing methyl violet. When methyl violet has been removed by washing with chloroform, the pure methyl green appears and is specific for DNA. Both dyes are cationic, when used in combination methyl green binds preferentially and specifically to DNA, and pyronin binds RNA.

Materials Required:

Fixed Tissue, Paraffin Wax, Methyl Green Pyronin (MGP) Y is prepared by :

2% methyl green (chloroform washed)	9ml
2% pyronin Y	4ml
Acetate buffer pH 4.8	23ml
Glycerol	14ml

Procedure:

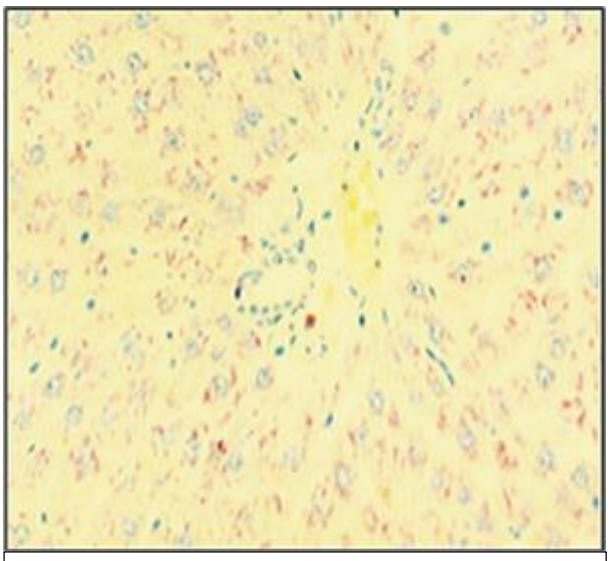
1. Take sections of the tissue down to water.
2. Rinse the tissue in the acetate buffer at pH 4.8.
3. Place the tissue in the methyl green-pyronin Y solution for 25 min.
4. Rinse the tissue in the buffer solution.
5. Blot dry tissue.
6. Rinse in 93% ethanol, then in the absolute alcohol.
7. Rinse in xylene and mount the prepared.

Result :

The cells nuclei are blue to blue-green due to DNA presence. The nucleoli and cytoplasm are pale pink to red due to RNA staining by P.

Precautions:

1. Be careful when handling fixatives and solvents.
2. Do not overstain the sample, as this can make it difficult to distinguish between DNA and RNA.
3. Dehydrate the sample thoroughly before mounting, as this will help to prevent fading.



MGP stained DNA and RNA

3. **Aim: Preparation of permanent slide to demonstrate muco-polysaccharides by PAS reaction.**

Theory: The Periodic Acid Schiff (PAS) reaction is a histochemical staining method used to identify the presence of muco-polysaccharides. It is based on the principle that vicinal diols, which are present in muco-polysaccharides, can be oxidized by periodic acid to form aldehydes. The aldehydes then react with Schiff's reagent to produce a purple colour.

Materials Required:

- Microscope slides
- Cover slips
- Fixative (10% neutral buffered formalin)
- Periodic acid solution (0.5% in distilled water)
- Schiff's reagent
- Counter stain (hematoxylin)
- Dehydrating agents (ethanol and xylene)
- Mounting medium (Permount or DPX)
- Tissue sample containing muco-polysaccharides

Procedure:

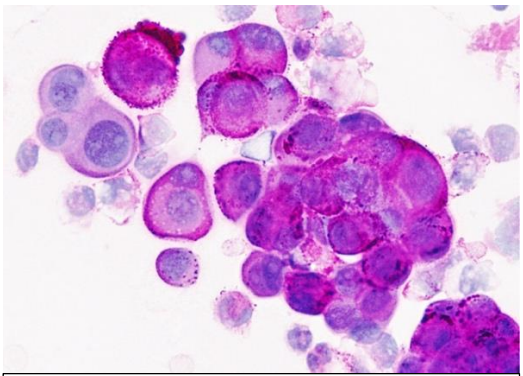
1. Fix the tissue sample in 10% neutral buffered formalin for 24 hours.
2. Wash the sample with water for 5 minutes.
3. Dehydrate the sample with ethanol (70%, 90%, and 100%).
4. Clear the sample with xylene.
5. Place the sample in periodic acid solution for 5 minutes.
6. Rinse the sample with water for 5 minutes.
7. Place the sample in Schiff's reagent for 30 minutes.
8. Wash the sample with water for 5 minutes.
9. Counterstain the sample with hematoxylin for 2 minutes.
10. Wash the sample with water for 5 minutes.
11. Dehydrate the sample with ethanol (70%, 90%, and 100%).
12. Clear the sample with xylene.
13. Mount the sample in Permount or DPX.

Result:

The presence of purple staining in the sample indicates the presence of muco-polysaccharides.

Precautions:

1. Wear gloves and a lab coat while performing the experiment.
2. Be careful when handling fixatives and solvents.
3. Do not overexpose the sample to Schiff's reagent, as this can cause the background to stain.
4. Dehydrate the sample thoroughly before mounting, as this will help to prevent fading.



Mucopolysachharide by PAS Reaction

4. **Aim: Preparation of permanent slide to demonstrate proteins by mercuro-bromophenol blue/Fast green.**

Theory: Mercuro-bromophenol blue (MBB) is a stain that is used to identify the presence of proteins. MBB binds to the basic groups in proteins, such as lysine and arginine. Fast green is a counterstain that is used to stain non-proteinaceous structures, such as carbohydrates and lipids. Proteins are stained blue with MBB, while non-proteinaceous structures are stained green with fast green.
:

Materials Required:

- Microscope slides
- Cover slips
- Fixative (10% neutral buffered formalin)
- Mercuro-bromophenol blue stain
- Fast green stain
- Dehydrating agents (ethanol and xylene)
- Mounting medium (Permount or DPX)
- Tissue sample containing proteins

Procedure:

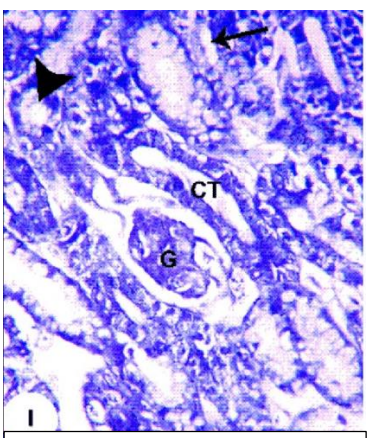
1. Fix the tissue sample in 10% neutral buffered formalin for 24 hours.
2. Wash the sample with water for 5 minutes.
3. Dehydrate the sample with ethanol (70%, 90%, and 100%).
4. Clear the sample with xylene.
5. Place the sample in MBB stain for 30 minutes.
6. Rinse the sample with water for 5 minutes.
7. Counterstain the sample with fast green for 1 minute.
8. Wash the sample with water for 5 minutes.
9. Dehydrate the sample with ethanol (70%, 90%, and 100%).
10. Clear the sample with xylene.
11. Mount the sample in Permount or DPX.

Result:

The presence of blue staining in the sample indicates the presence of proteins.

Precautions:

1. Wear gloves and a lab coat while performing the experiment.
2. Be careful when handling fixatives and solvents.
3. Do not overstain the sample with MBB, as this can make it difficult to distinguish between proteins and non-proteinaceous structures.
4. Dehydrate the sample thoroughly before mounting, as this will help to prevent fading.
5. This experiment can be modified to demonstrate the presence of proteins in specific cell types, such as muscle cells or epithelial cells.



Proteins stained with Mercuro-Bromophenol Blue

5. Aim: Study of mitosis in onion root tip

Theory:

Mitosis is a process of cell division that occurs in somatic (non-reproductive) cells, leading to the formation of two genetically identical daughter cells. It is crucial for growth, development, tissue repair, and maintenance of the organism. Mitosis involves several stages:

1. **Interphase:**
   G1 Phase (Gap 1): The cell grows and carries out its normal functions.
   S Phase (Synthesis): DNA replication occurs, resulting in the duplication of chromosomes.
   G2 Phase (Gap 2): The cell continues to grow and prepares for mitosis.

2. **Prophase:** Chromosomes, which are now composed of two sister chromatids connected by a centromere, condense and become visible. The nuclear envelope begins to break down. The mitotic spindle, a structure made of microtubules, starts to form.

3. **Metaphase:** Chromosomes align at the cell's equator, known as the metaphase plate. Spindle fibers from opposite poles attach to each chromatid.

4. **Anaphase:** Centromeres split, separating the sister chromatids. Spindle fibers contract, pulling chromatids to opposite poles of the cell. Each pole now has a complete set of chromosomes.

5. **Telophase:** Chromosomes reach opposite poles and de-condense back into chromatin. The nuclear envelope reforms around each set of chromosomes, forming two distinct nuclei. Mitotic spindle disassembles.

6. **Cytokinesis:** The division of the cytoplasm occurs, resulting in the formation of two daughter cells. In animal cells, a cleavage furrow forms, pinching the cell membrane. In plant cells, a new cell wall is laid down at the centre, known as the cell plate.

The end result of mitosis is two daughter cells, each with the same number of chromosomes as the parent cell. These cells are genetically identical to each other and to the original cell. Mitosis is responsible for the growth and replacement of cells in multicellular organisms, ensuring that the genetic information is faithfully passed on to the next generation of cells.

Materials Required:

- Onion bulbs
- Microscope slides
- Cover slips
- Microscope
- Hydrochloric acid (HCl)
- 1N NaOH (sodium hydroxide)
- Distilled water
- Acetocarmine or Giemsa stain
- Forceps
- Razor blade or scalpel
- Stopwatch or timer
- Light source

Procedure:

Preparing Onion Root Tips:

Collect Onion Roots: Obtain fresh onion bulbs. Peel off the outer layers until you reach the inner, fleshy part where the root tips are actively dividing.

Cutting Root Tips: Use a razor blade or scalpel to cut off about 1-2 cm of the onion root tips.

Fixation: Place the root tips in a fixative solution, such as a mixture of 3 parts ethanol and 1 part acetic acid, for several hours. This helps preserve the cells.

Softening: Treat the fixed root tips with 1N HCl for about 5 minutes to soften the tissues.

Washing: Rinse the root tips with distilled water to remove excess acid.

Digestion: Treat the root tips with 1N NaOH for 3-5 minutes. This helps to digest the tissues, making it easier to separate individual cells.

Washing: Rinse the root tips with distilled water to stop the digestion process.

Staining and Mounting:

Staining: Use a suitable stain, such as acetocarmine, to colour the chromosomes. Place a small amount of stain on the root tips and let it sit for a few minutes.

Mounting: Place the stained root tips on a microscope slide and add a drop of water. Cover the root tips with a cover slip.

Observing Mitosis:

Microscopy: Place the prepared slide under the microscope. Start with low magnification to locate the root tip, and then switch to higher magnifications to observe individual cells.

Counting Cells: Observe different cells in various stages of mitosis (prophase, metaphase, anaphase, telophase). Count the number of cells in each stage.

Observation and Results:

The following stages of mitosis can be observed in the onion root tip cells:

Prophase: The chromosomes condense and become visible. The nuclear envelope breaks down.

Metaphase: The chromosomes line up in the middle of the cell.

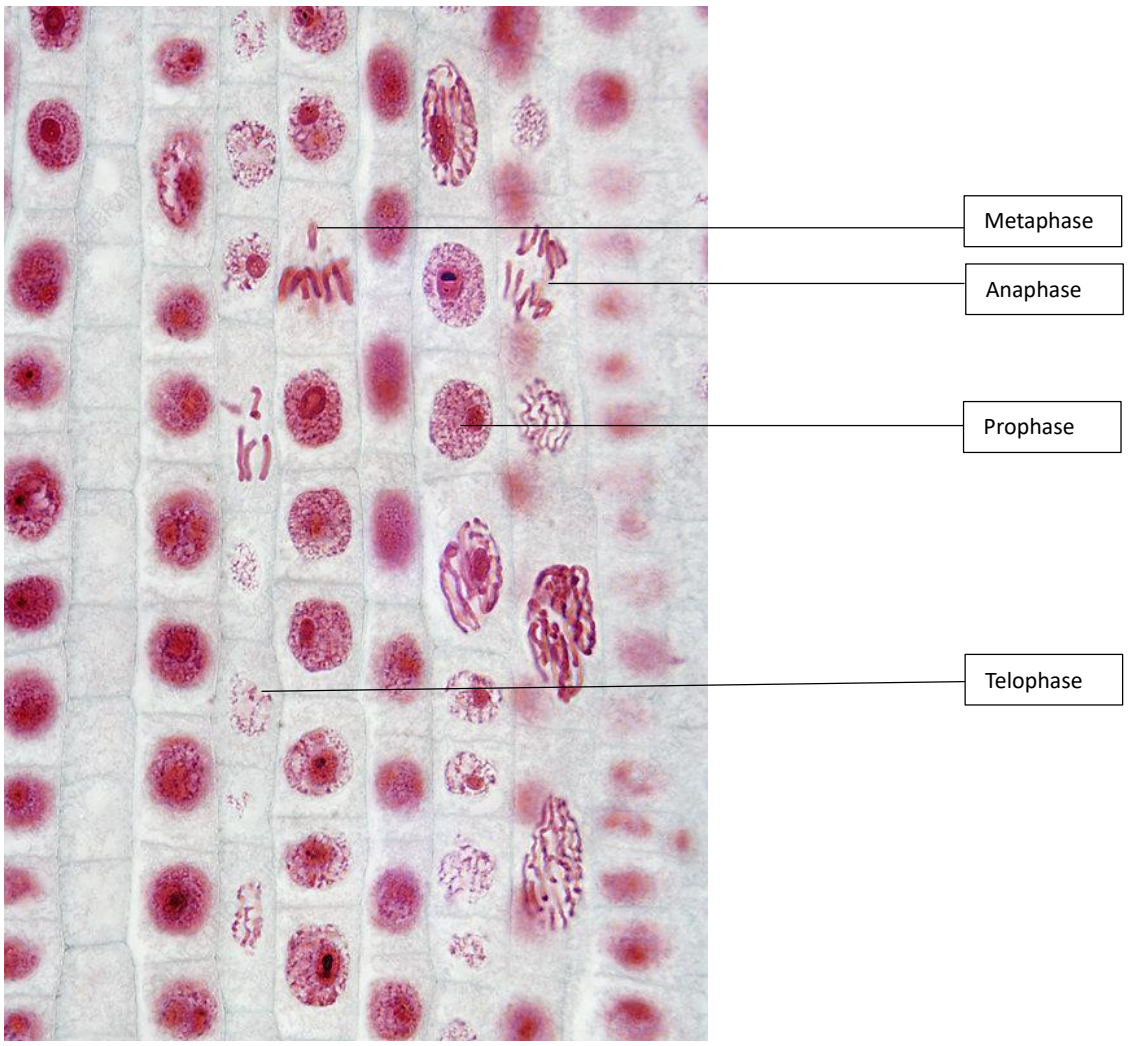
Anaphase: The chromosomes separate and move to opposite poles of the cell.

Telophase: The nuclear envelope reforms around the chromosomes. The cell divides into two daughter cells.

The presence of different stages of mitosis in the onion root tip cells confirms that mitosis is a dynamic process that occurs in all plants and animals.

Precautions:

1. Use a sharp razor blade or scalpel for cutting onion root tips.
2. When using chemicals such as fixatives, acids, and stains, follow proper laboratory safety protocols.
3. Treat root tips gently during the preparation process to avoid damaging cells.
4. Excessive force can distort cell structures and affect mitotic stages.
5. Use fine-tipped forceps to handle root tips and avoid crushing or tearing them.



Mitosis in the Onion Root Tip Cells

6. Aim: Study of meiosis in grasshopper testis

Theory:

Grasshopper testis is an ideal material for studying various stages of meiosis. Grasshopper is of good choice because it is easily available in lawns and fields, males can be easily distinguished from female and testis is easy to dissect. In addition, it has fewer number of chromosomes (locally available species contain 17 or 19 or 21 chromosomes in males; odd number of chromosomes due to XX/XO sex chromosome system) and all chromosomes are of one type, i.e., acrocentric, facilitating unambiguous identification of division stages.

The different stages of meiosis are observed along on the basis of the following features.

Stages of Meiosis I

Prophase I: In this stage, the chromosomes condense and move towards the centre of the cell. It consists of five different sub-phases:

- Leptotene: The homologous chromosomes replicate.
- Zygotene: Synapsis between homologous chromosomes start.
- Pachytene: The sister chromatids separate but the homologous chromosomes remain attached.
- Diplotene: The two homologous chromosomes migrate apart and disintegrate between the chromosomal arms.
- Diakinesis: The condensation of chromosomes stops at this stage and the chiasmata is clearly visible under an electron microscope. The nucleolus and the nuclear envelop disappear at this stage and the centrosome move to the equator.

Metaphase I: The homologous chromosomes that contain two different alleles for each gene, line up on the metaphase plate to be separated.

Anaphase I: The separated chromosomes are pulled towards the centrioles on either side of the cell.

Telophase I: The chromosomes are completely pulled apart and new nuclear envelope forms.

Stages of Meiosis II

Prophase II: In this stage, the nuclear envelope disintegrates and centrioles develop.

Metaphase II: The chromosomes line up on the metaphase plate and the chromatids are on either side of the metaphase plate.

Anaphase II: The sister chromatids separate and are known as sister chromosomes.

Telophase II: The cell divides into two and a new nuclear envelope surrounds the chromosomes.

Materials Required:

- Male grasshopper, Insect saline (0.67% NaCl),
- 1:3 aceto-ethanol fixative, 70% and 90% ethanol,
- 2% acetoorcein stain
- 45% acetic acid,
- Slide, Cover glass, Sealing wax or nail polish

Procedure:

1. Dissect out testes from male grasshopper.
2. Keeping the testes in normal saline, remove the yellow fat.
3. Fix the testis in fixative in a centrifuge tube for 30 min.
4. Remove fixative and add about 0.5 ml of 60% acetic acid, leave for 2-3 min till the testis appears nearly dissolved.
5. Add 5-6 ml of fixative to the tube without removing the acetic acid.
6. Centrifuge at 1,200 rpm for 5 min.
7. Gradually remove the supernatant and add a few drops (~0.2 ml) of fresh fixative and make a suspension.
8. Drop a few drops of cell suspension on a slide and flame dry.
9. Stain the slides with Giemsa stain, rinse in water, dry and observe under a microscope.
10. The slide can be mounted with DPX before observing in oil immersion lens.

Fixation Of Grasshopper Testes:

1. Hold a male grasshopper in hand, give a small incision with scissors at the junction of thorax and abdomen and press the abdomen gently. The testes covered in yellow
2. fat bodies will pop out. Dissect them out and put in insect saline. Remove yellow fat with the help of forceps as much as possible. A pair of testes (each having a bunch of white tubules) will be seen.
3. Transfer the tubules in a tube and fix in aceto-ethanol fixative, close the tube and leave for 20 minutes.
4. Remove fixative and add 90% ethanol, leave for 2hr.
5. Decant 90% ethanol and add 70% ethanol. The testes can be stored in 70% ethanol for a long period of time if the tube is tightly closed. Storing at 4℃ is even better.

Staining And Making Squash Preparation:

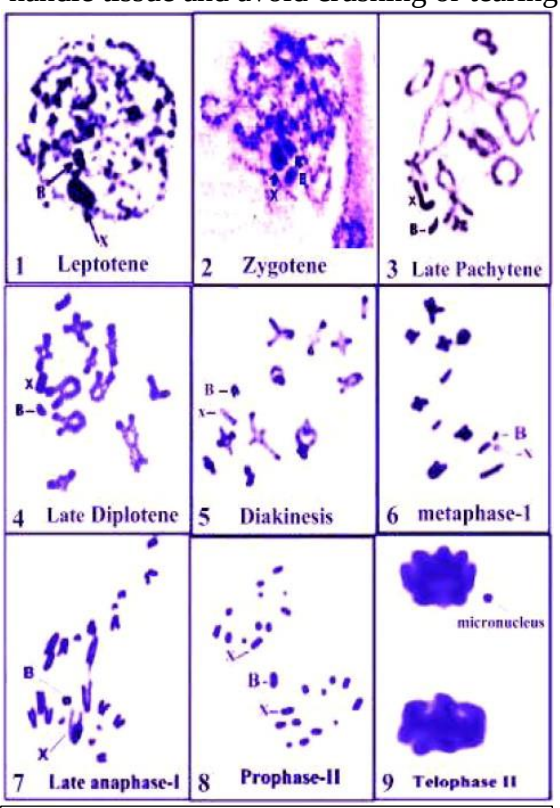
1. Stain the fixed testis in aceto-orceine for 30 min.
2. Take a drop of 45% acetic acid on slide, place a few tubules of testis in the drop, leave for 1-2 min. If acetic acid drop becomes coloured, it can be decanted and a fresh 45% acetic acid drop can be added.
3. Place a cover glass on the tubules and squash using a rubber-end pencil under the folds of a blotting paper.
4. Seal the edges of the cover glass with molten wax or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
5. The slide is ready for observation under a microscope.

Result:

The cells of testis lobes are spread out and became distinct.

Precautions:

1. Use a sharp razor blade or scalpel for cutting Tissue.
2. When using chemicals such as fixatives, acids, and stains, follow proper laboratory safety protocols.
3. Treat Tissue gently during the preparation process to avoid damaging cells.
4. Excessive force can distort cell structures and affect meiotic stages.
5. Use fine-tipped forceps to handle tissue and avoid crushing or tearing them.



Meiosis in grasshopper testes

7. Aim: Study of permanent slides: Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.

Theory: Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules.

Materials Required: Permanent slides of meiosis and compound microscope.

Procedure:

1. Place a slide on the stage of the microscope and focus the objective lens on the cells.
2. If using an immersion objective lens, apply a drop of immersion oil to the slide.
3. Identify the different stages of prophase I by looking for the characteristic arrangement of the chromosomes.
4. Sketch or photograph the cells to document your observations.

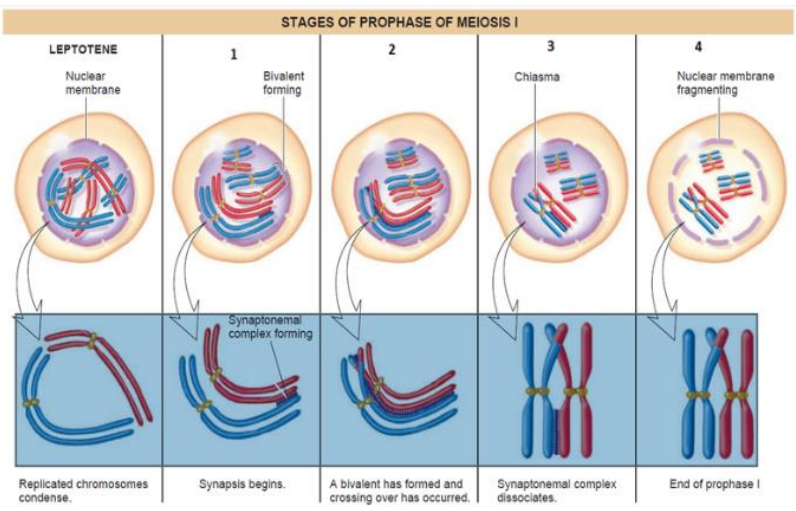
Observations:

1. **Leptotene:** The chromosomes are visible as long, thin threads.
2. **Zygotene:** The homologous chromosomes are paired up and synapsed.
3. **Pachytene:** The homologous chromosomes are still synapsed and the chiasmata, which are the visible sites of crossing over, can be seen.
4. **Diplotene:** The homologous chromosomes are beginning to desynapsed, but remain attached at the chiasmata.
5. **Diakinesis:** The chromosomes are condensed and the chiasmata have terminalized, moving to the ends of the chromosomes. The nuclear envelope has broken down and the spindle is forming.

Result:

The different stages of prophase I can be clearly observed in permanent slides of meiotic cells. The chromosomes undergo a series of morphological changes during prophase I, including condensation, synapsis, crossing over, and desynapsis. These changes are essential for the proper segregation of chromosomes during meiosis.

Prophase I is a critical stage of meiosis, as it is during this time that the chromosomes undergo crossing over, a process that ensures genetic diversity in the offspring. The study of permanent slides of meiotic cells allows scientists to observe the different stages of prophase I in detail and to gain a better understanding of the process of meiosis.



8, Aim: Study of permanent slides: Prophase, Metaphase, Anaphase and Telophase; Barr body.

Theory: Mitosis is the part of the cell cycle when replicated chromosomes are separated into two new nuclei. In general, mitosis (division of the nucleus) is preceded by the S stage of interphase (during which the DNA is replicated) and is often accompanied or followed by cytokinesis, which divides the cytoplasm, organelles and cell membrane into two new cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the mitotic (M) phase of an animal cell cycle (the division of the mother cell into two daughter cells genetically identical to each other). The process of mitosis is divided into stages corresponding to the completion of one set of activities and the start of the next. These stages are prophase, pro-metaphase, metaphase, anaphase, and telophase.

Materials Required: Permanent slides of mitosis, barr body and compound microscope.

Procedure :

1. Place a slide on the stage of the microscope and focus the objective lens on the cells.
2. If using an immersion objective lens, apply a drop of immersion oil to the slide.
3. Identify the different stages of Mitosis by looking for the characteristic arrangement of the chromosomes.
4. Sketch or photograph the cells to document your observations.

Observations:

The process of Mitosis is divided into four stages: Prophase, Metaphase, Anaphase and Telophase:

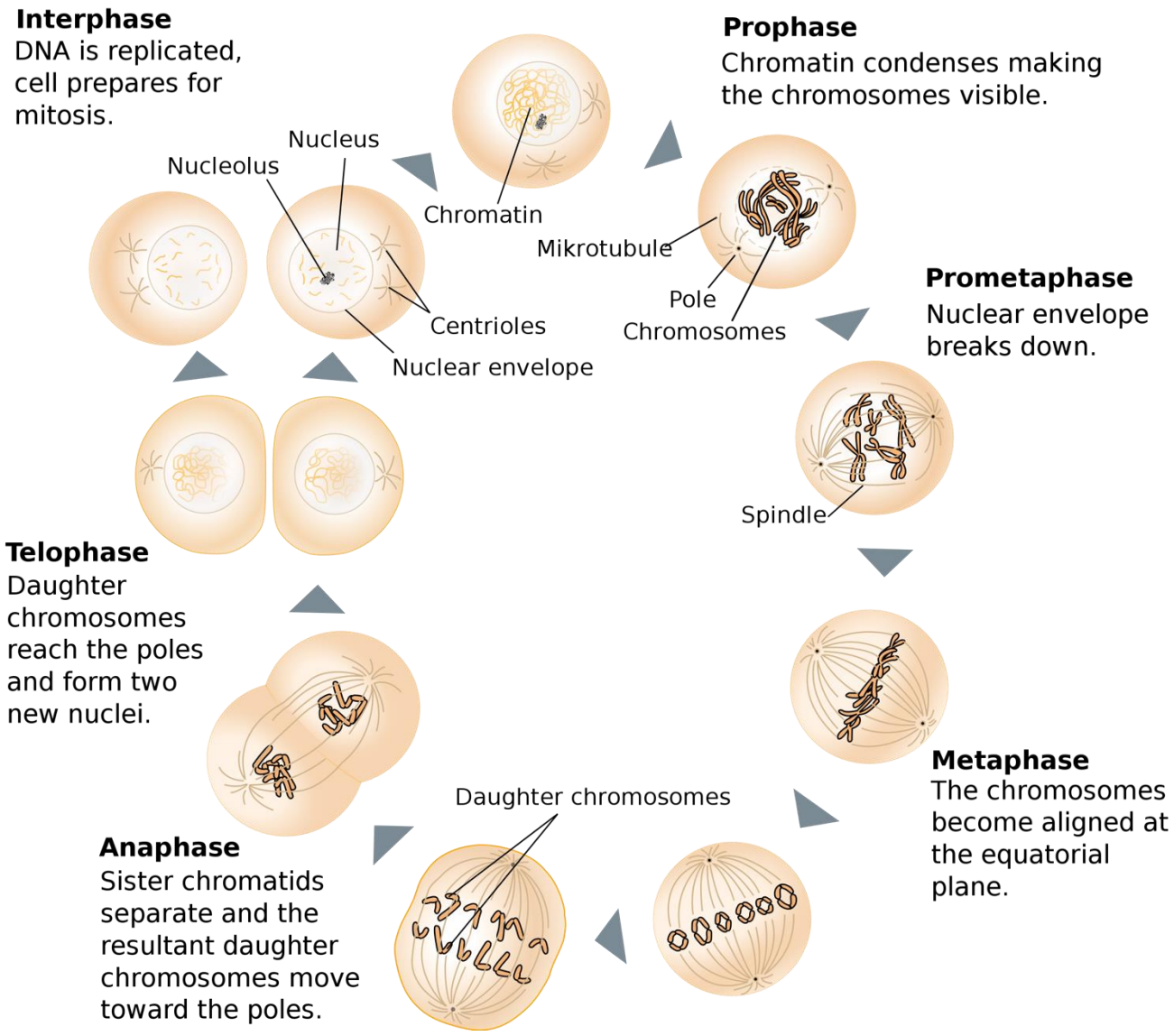
- **Prophase**: During this stage, the chromosomes super coil, condense and become visible for first time during the cell cycle. The spindle fibers start forming. The nuclear membrane starts disintegrating.
- **Metaphase**: During this stage, the spindle fibers reach and attach to centromere of each sister chromatids. The chromosomes align along the center plane of the cell. The nuclear membrane disintegrates completely.
- **Anaphase**: During this stage, the centromeres start splitting and the sister chromatids begin to migrating towards the opposite poles of the cell.
- **Telophase**: During this stage, the chromosomes are clustered on the either end of the cell. The nuclear membrane starts reforming.
- The cell plate (new cell wall) starts to form between the two daughter nuclei. This will be followed by cytokinesis.
- **Barr Body:** Females have two X chromosomes. Since the somatic cells of females are not involved in sexual reproduction. Here one of the two X chromosomes is inactivated by lyonization. This inactive X chromosome is known as a Barr body. The process of X-inactivation was discovered by Mary F. Lyon, a British geneticist. One X-chromosome is inactivated so that unnecessary information is not passed on to the next generation. The amount of expression of X-chromosome genes should be equal in both males and females. The active X-chromosome is enclosed within euchromatin, whereas, the inactive X-chromosome is enclosed within heterochromatin. The inactive X-chromosome is compacted and is not accessible to the molecules involved in transcription. In X-inactivation, the X chromosome is compacted to create a small, dense structure called the Barr body.

Result:

This experiment provides a simple and effective way to study the different stages of mitosis and the Barr body in permanent slides. The results of this experiment can be used to better understand the process of mitosis and its importance in cell division.

Precautions:

1. Be careful when handling microscope slides, as they can be sharp.
2. When using immersion oil, be careful not to get any on the objective lens.
3. When cleaning microscope slides, be careful not to damage the tissue sample.



Mitosis