Practical No#1:

Study of Cell Structure Using Compound Microscope and Elucidation of Ultrastructure from Electron Microphotographs.

★ Cell Theory:

- All living things are made up of cells.
- Cells are the smallest working units of all living things.
- All cells come from preexisting cells through cell division.

★ Cell:

- A cell is the smallest unit that is capable of performing life functions.

Cell Wall:

- Outer membrane of cell that controls movement in and out of the cell
- Double layer

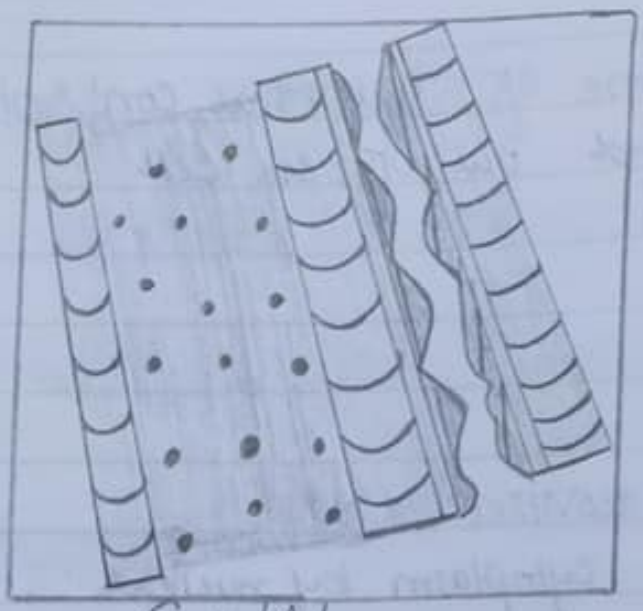
Nucleus:

- Directs cell activities
- Separated from cytoplasm by nuclear membrane.
- Contains genetic material - DNA

Nuclear Membrane:

- Surrounds nucleus
- Mode of two layers
- Openings allow material to enter and leave nucleus

Cell Wall :



Cell Wall

Nucleus :

Nuclear Membrane :

Nuclear Membrane

Nucleolus:

- Inside nucleus
- Contains RNA to build proteins

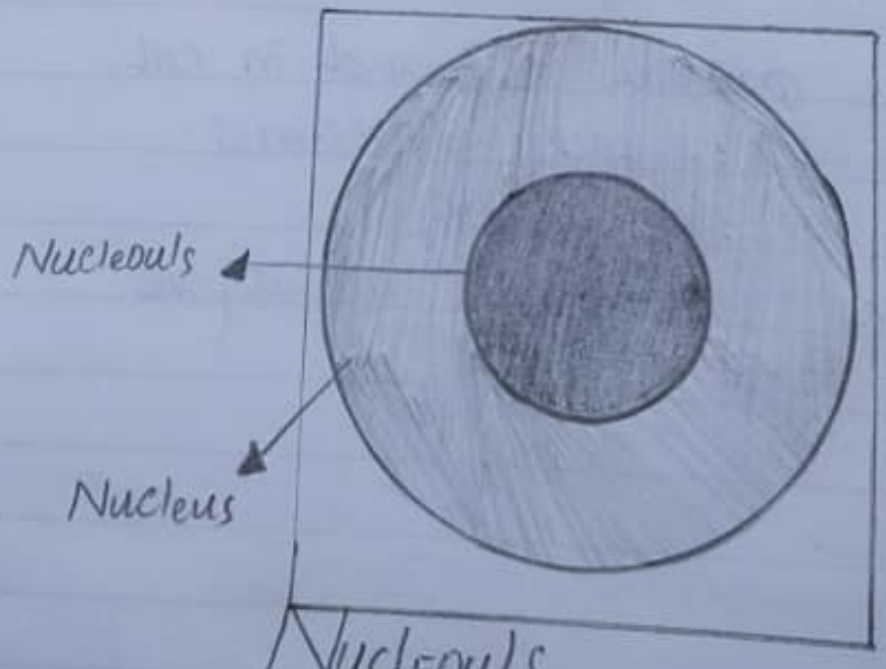
Cytoplasm:

- Gel-like mixture
- Surrounded by cell membrane
- Contains hereditary material

Endoplasmic Reticulum:

- Moves materials around in cell
- Smooth type: lacks ribosomes
- Rough type: (pictured) ribosomes embedded in surface

ENDOplasmic Reticulum



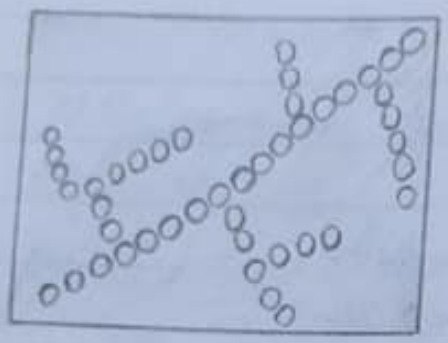
Nucleouls

RIBOSOMES:

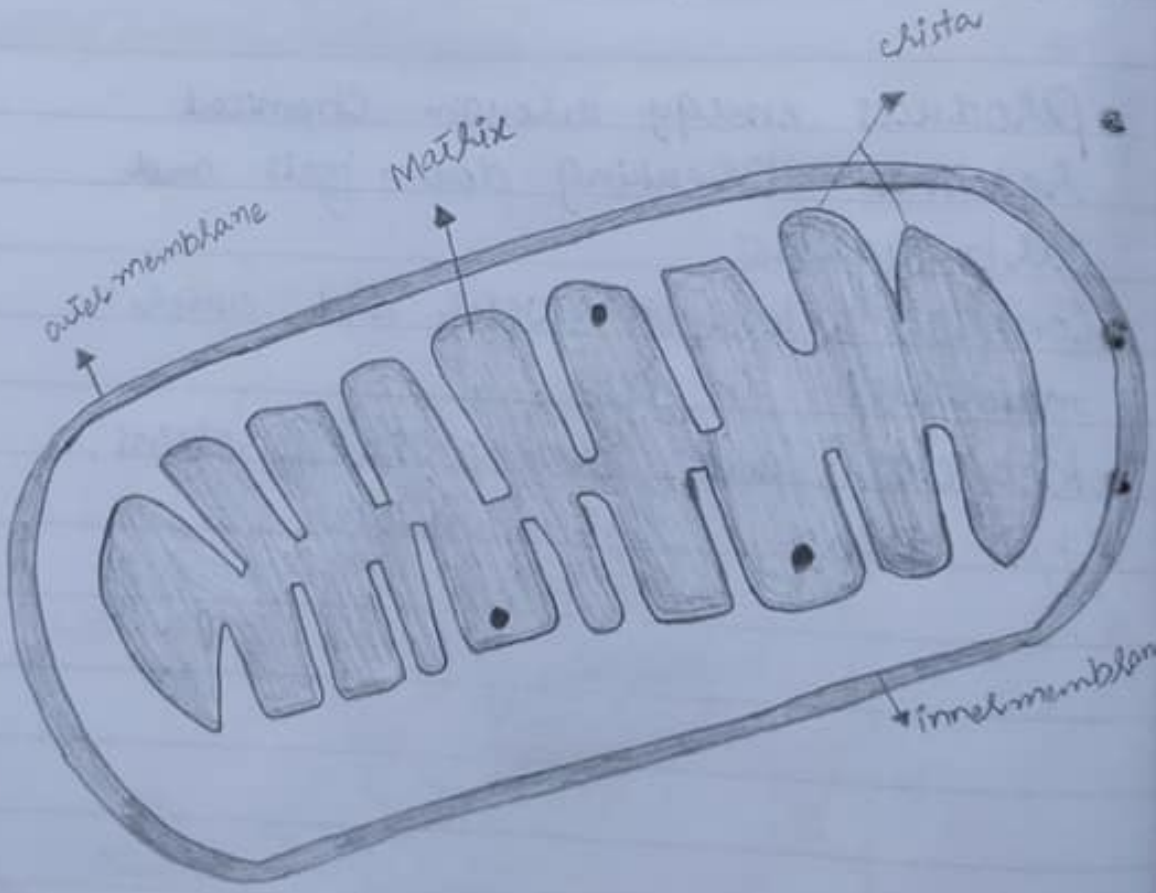
- Each cell contains thousands
- Make proteins
- Found on endoplasmic reticulum and floating throughout the cell.

MITOCHONDRIA:

- Produces energy through chemical reactions- breaking down fats and carbohydrates
- Controls level of water and other materials in cell.
- Recycles and decomposes proteins, fats, and carbohydrates



RIBOSOMES



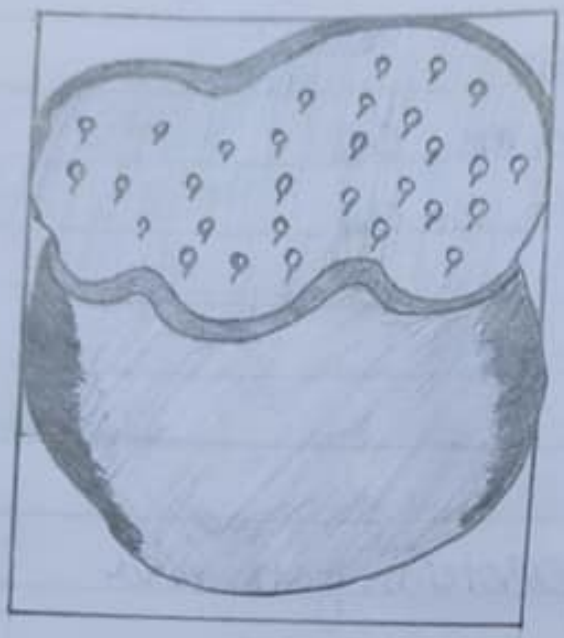
MITOCHONDRIA

Golgi Bodies:

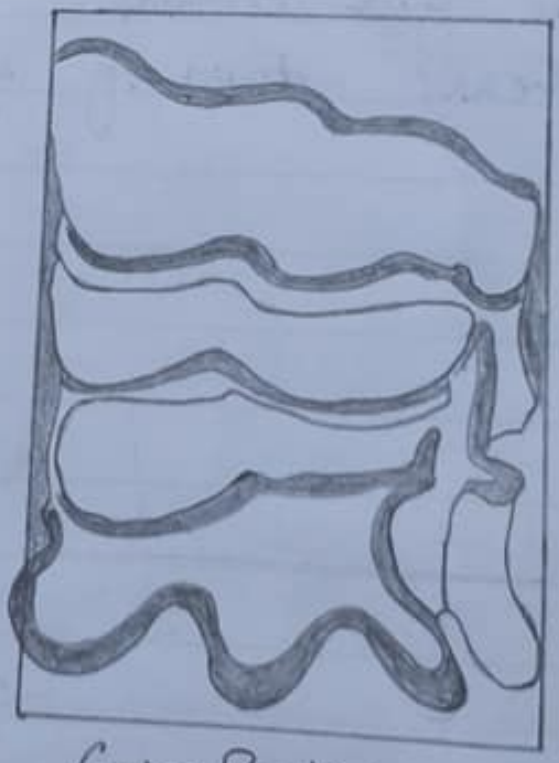
- protein 'packing plant
- Move materials with in the cell
- Move materials out of the cell

Lysosome:

- Digestive 'plant' for proteins, fats, and carbohydrates
- Transports undigested material to the cell membrane for removal
- Cell breaks down if lysosome Explodes.



Lyso Some



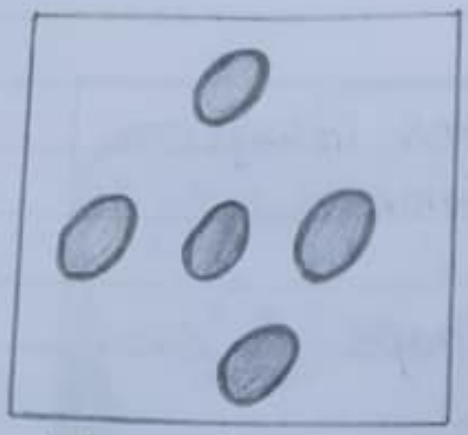
Golgi Bodies

VACUOLES:

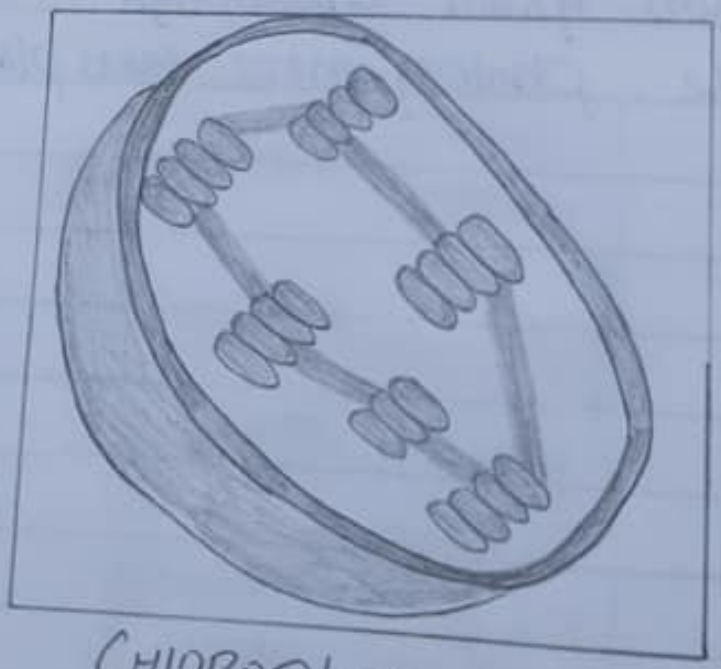
- Membrane-bound sacs for storage, digestion, and waste removal
- Contains water solution
- Help plants maintain shape

CHLOROPLAST:

- usually found in plant cells
- Contains green chlorophyll
- Where photosynthesis takes place.



Vacuoles



CHIOROPLAST

Practical No#2:
Measurement of Cell Size:

Micrometery:

Measurement of small objects by using microscope is known as micrometery.

Types of Micrometer:

- Ocular micrometer
- Stage micrometer

Difference Between Ocular and Stage:

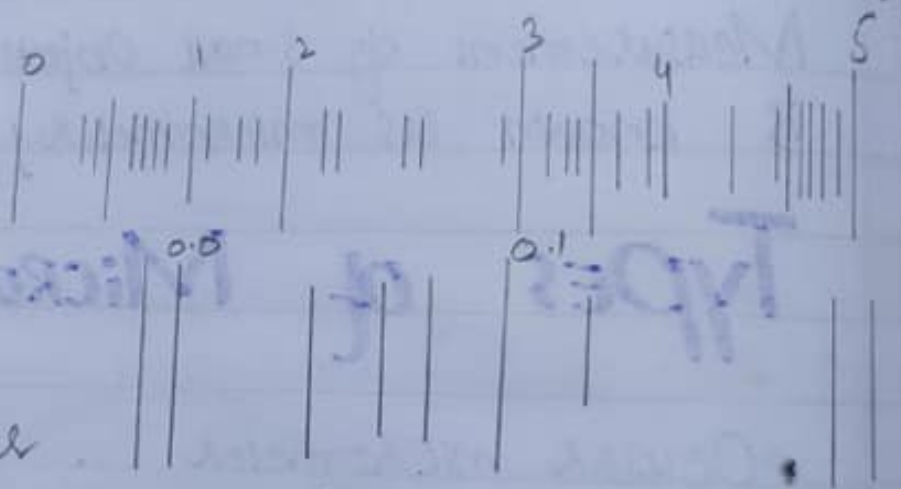
The distance between the lines of an ocular micrometer is an arbitrary measurment that only has meaning if the ocular micrometer is calibrated for the objective being used.

A stage micrometer, also known as an objective micrometer, has scribed lines on it that are exactly **0.01mm** (10 micrometers) apart.

Cell Size Measurements: Ocular And Stage Micrometers:

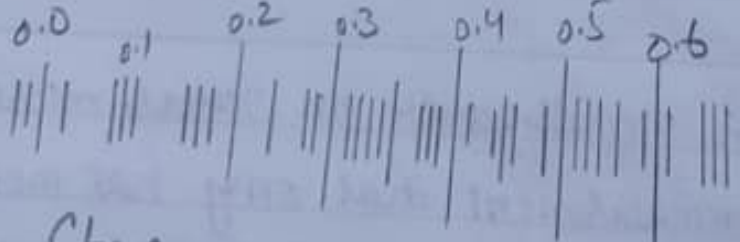
Stage micrometer at 4x

Ocular micrometer



Ocular micrometer

Stage micrometer at 10x



Stage micrometer at 10x

MATERIAL REQUIRED:

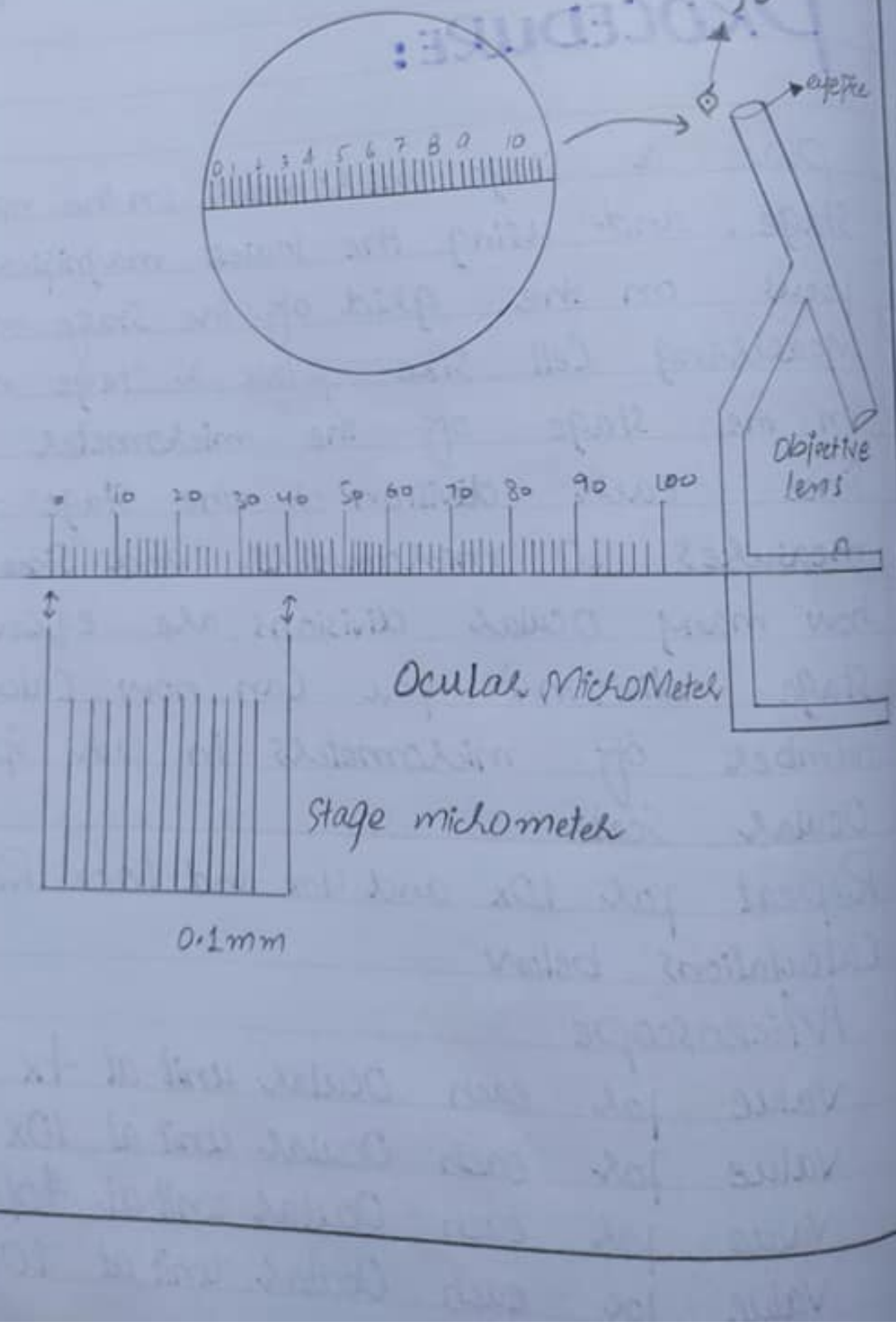
- Microscope
- Ocular micrometer
- Stage Micrometer
- Milimeter ruler
- prepared slids

PROCEDURE:

place a stage micrometer on the microscope stage, and using the lowest magnification (4x) focus on the grid of the stage micrometer. Measuring Cell Size place a stage micrometer on the stage of the micrometer. Since each division of the stage micrometer measures 10 micrometers, and Since you know how many ocular divisions are equievalent to 1 stage division, you can now calculate the number of micrometers in each space of the Ocular Scale.
Repeat for 10x and 40x and 100x Record your Calculations below

→ Microscope

- Value for each Ocular unit at 4x
- Value for each Ocular unit at 10x
- Value for each Ocular unit at 40/45x
- Value for each Ocular unit at 100x


eye
0 1 2 3 4 5 6 7 8 9 10
Objective lens
0 10 20 30 40 50 60 70 80 90 100
Ocular Micrometer
Stage micrometer
0.1mm

Because the individual Cells of any Organisms are too small to be seen with the naked eye. We must use microscopes to magnify them. We can view a Cell at a magnification of up to 1000x under a light microscope. but We can't gauge its actual size just by looking at it.

MAGNIFICATION:

- The size of an image of an object Compared to its actual size.
- Calculated using the formula M=I/A
  I = size of image
  A = actual size of object
  M = Magnification

Magnification:

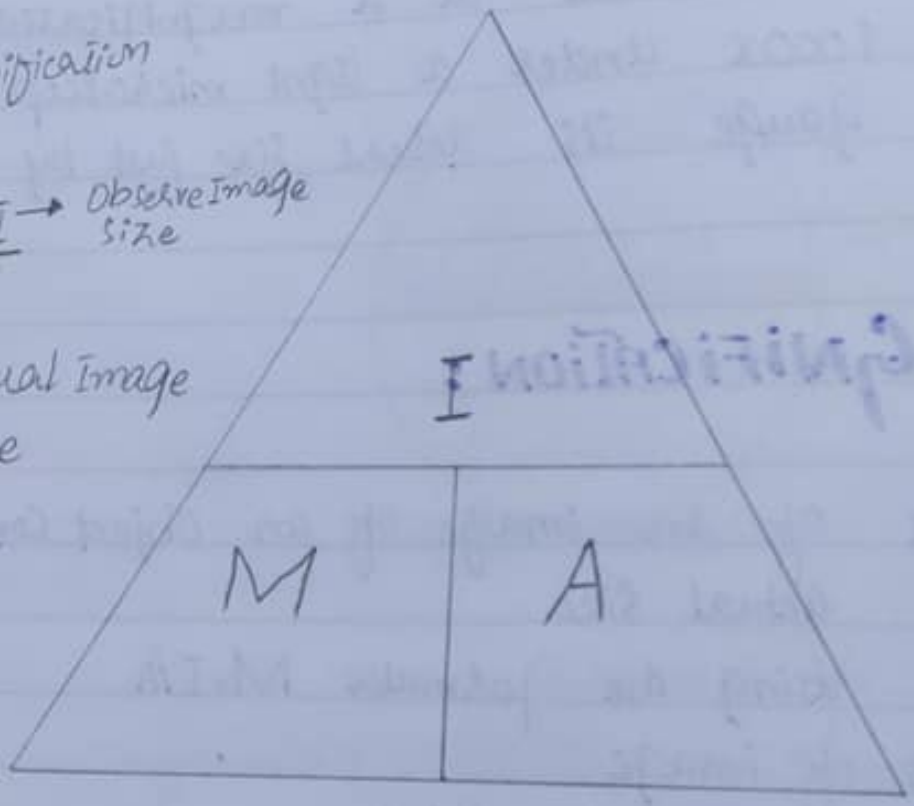
Magnification

$M = \frac{I}{A}$ → Observe Image Size

↓

Actual Image Size

$A = \frac{I}{M}$



Practical No: 3:

Study of Mitosis and Meiosis By Smear/Squash Method AND FROM Prepared Slides

INTRODUCTION:

Squash technique is one of the simple techniques widely used for the study chromosomes. the technique consists of applying a gentle pressure on a small piece of previously stained tissue to flatten the cells and spread the chromosome.

MATERIALS REQUIRED:

- Onion root tips
- Onion flower buds
- Testis of grasshopper
- Acetic Alcohol
- Aceto. carmine or acetoorecin
- Slides
- 2N hydrochloric acid
- Pasteur pipettes

- Cover slips
- Spirit lamp
- Watch glass
- Filter paper, dissection kit
- Nail polish or DPX mountant
- Compound microscope

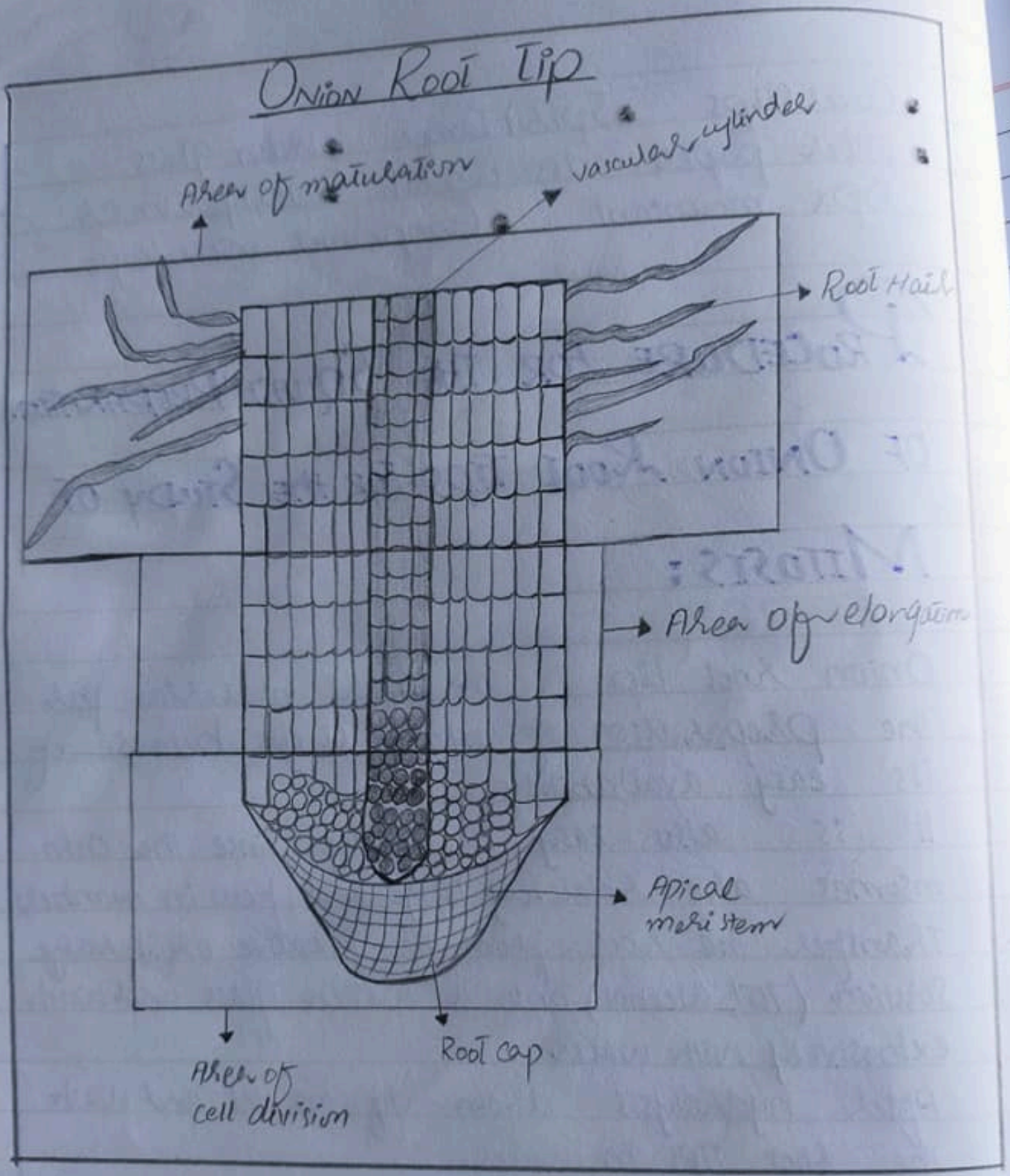
Procedure for the Squash Preparation of Onion Root Tips for the Study of Mitosis:

Onion root tip is an ideal material for the preparation of mitotic stages because of its easy availability.

It is also easy to study since the chromsomes are relatively large and few in number.

1. Transfer the root from the fixative or storage solution (70% alcohol) onto a watch glass and wash extensively with water.
2. After hydrolysis drain off the HCl and wash the root tips in water.
3. Remove the water and add 1% acetocarmine or acctoorcein to the root tips for staining.
4. The staining requires 10 to 15 minutes.

Onion Root Tip



5. Place a drop of 45% acetic acid on the material and carefully place a coverslip over it.
6. Remove the excess acetic acid from the sides of coverslip by using the edge of a filter paper.
7. Seal the edges of the coverslip by applying nail polish so that the fluid evaporation is minimised.
8. Observed the slide under the compound microscope

OBSERVATION AND RESULT:

you are already familiar with the concept of cell cycle and know that cells can be observed in either dividing or non-dividing phase of the cycle.

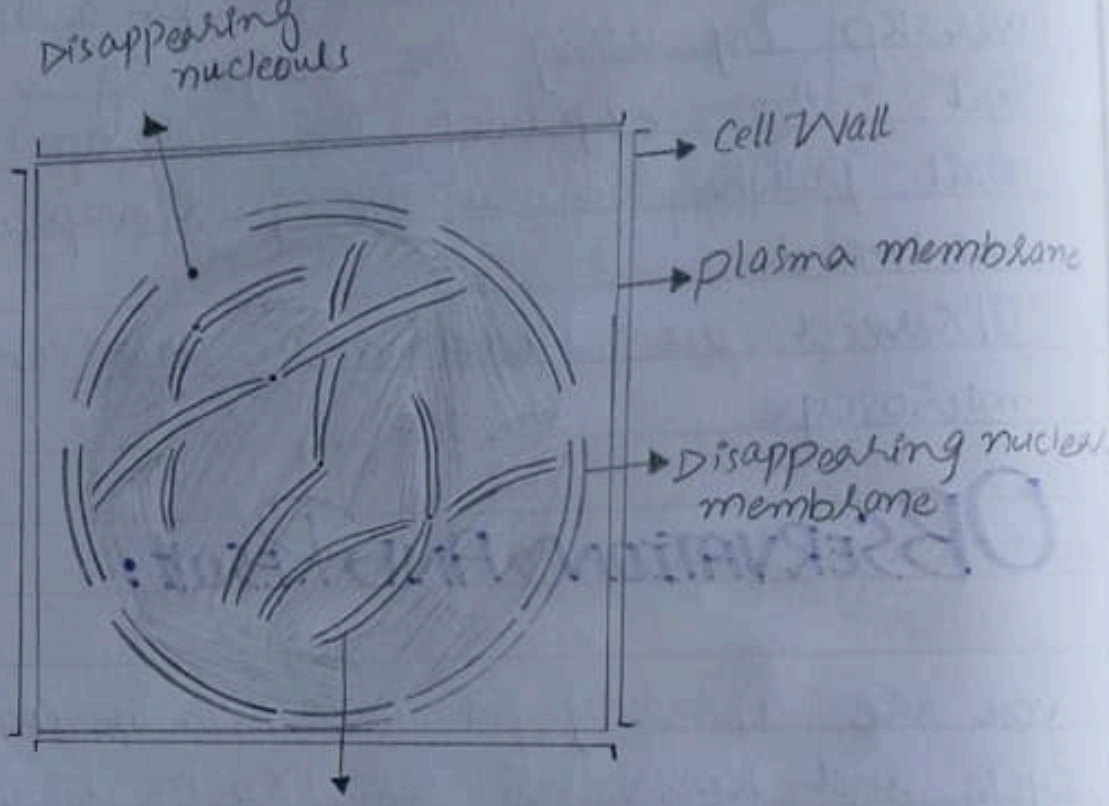
Stages of Mitosis:

1. PROPHASE

prophase, the first stage in the division process is characterised by the unwinding of the chromatin reticulum.
Each chromosome begins to appear as two chromatids with a single centrometer.
The nuclear membrane slowly disappears.

Stage of Mitosis

PROPHASE



2. METAPHASE:

In Metaphase the chromosomes tend to arrange themselves in the middle of the cell called the equatorial plate.
It is possible to count the number of chromosomes at the metaphase stage. When observed carefully, the sister chromatids of the chromosomes can be seen.

3- ANAPHASE:

Anaphase is masked by the movement of the chromosomes the opposite poles of the cell. Essentially the centromere splits and a single chromosome with two chromatids become to independent chromosomes each with ov centromere.

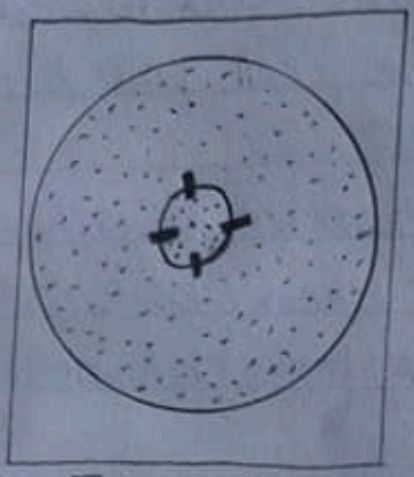
4- TELOPHASE:

In telophase two daughter nuclei each with the number of chromosomes as the parent nucleus formed.

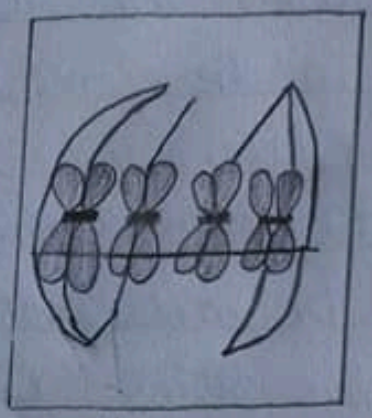
5- Cytokinesis:

Once the nuclear division is complete, cytokinesis that is the division of a cell into two daughter cells occurs.

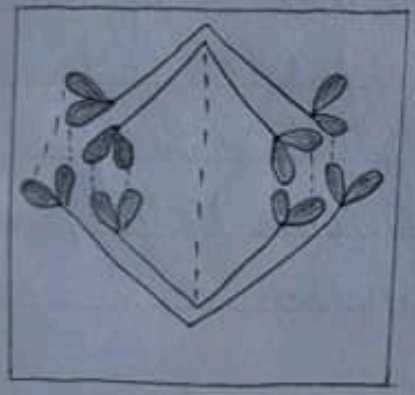
Stages of Mitosis



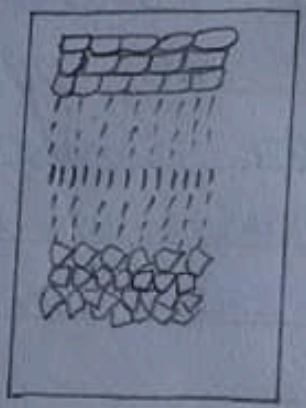
Interphase



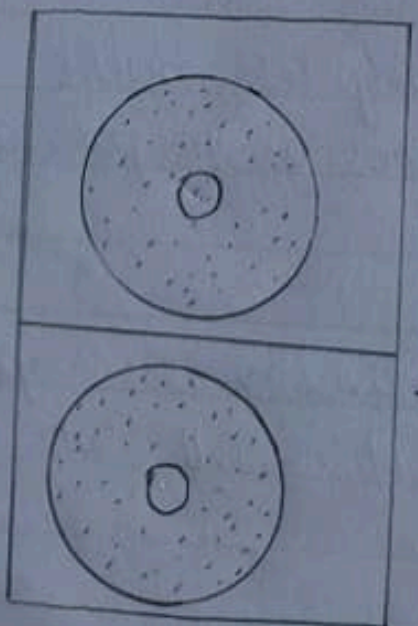
Metaphase



Anaphase



Telophase



Cytokinesis

PRACTICAL NO 14:

STUDY OF CHROMOSOME MORPHOLOGY AND VARIATION in CHROMOSOME NUMBER

CHROMOSOMES:

CHROMOSOMES are the rod-shaped filamentous bodies present in the nucleus, which become visible during cell division.

Chromosomes were first described by Strausberger in 1875.

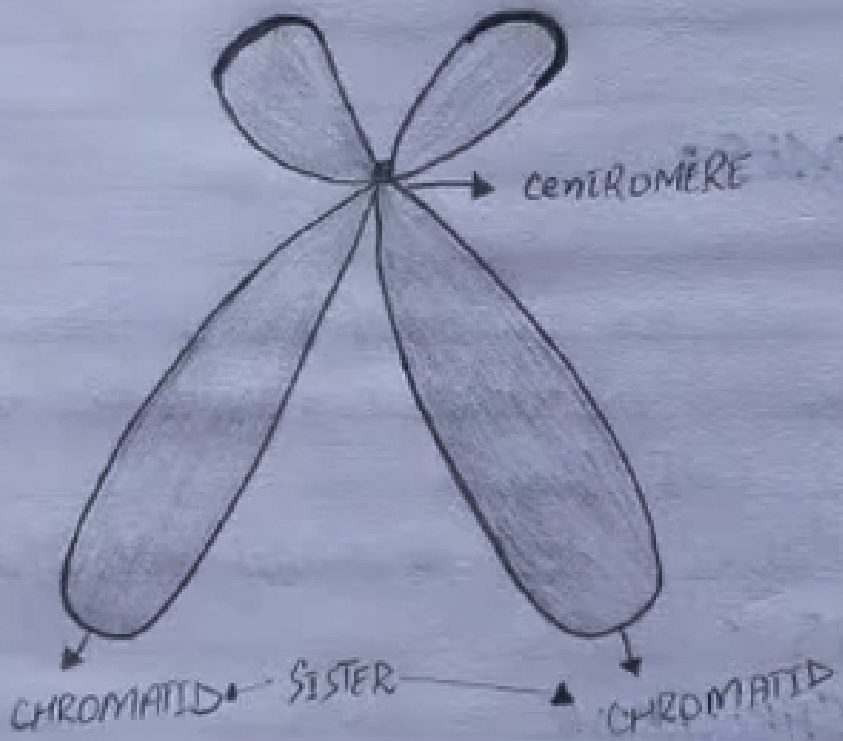
The term **CHROMOSOME** however was first used by waldeyer in 1888

They were given the name Chromosome Chromo = Colour Soma = body due to their marked affinity for basic dyes.

Their number can be counted easily only during mitotic metaphase.

Chromosomes are composed of thin Chromatin threads called Chromatin fibers.

CHROMOSOME



MORPHOLOGY:

Cell division, the following structural features can be seen under light microscope by staining.

i-Chromatid ii-Centromere iii-Telomere
iv-Secondary Constriction and Satellite v-Chromosome

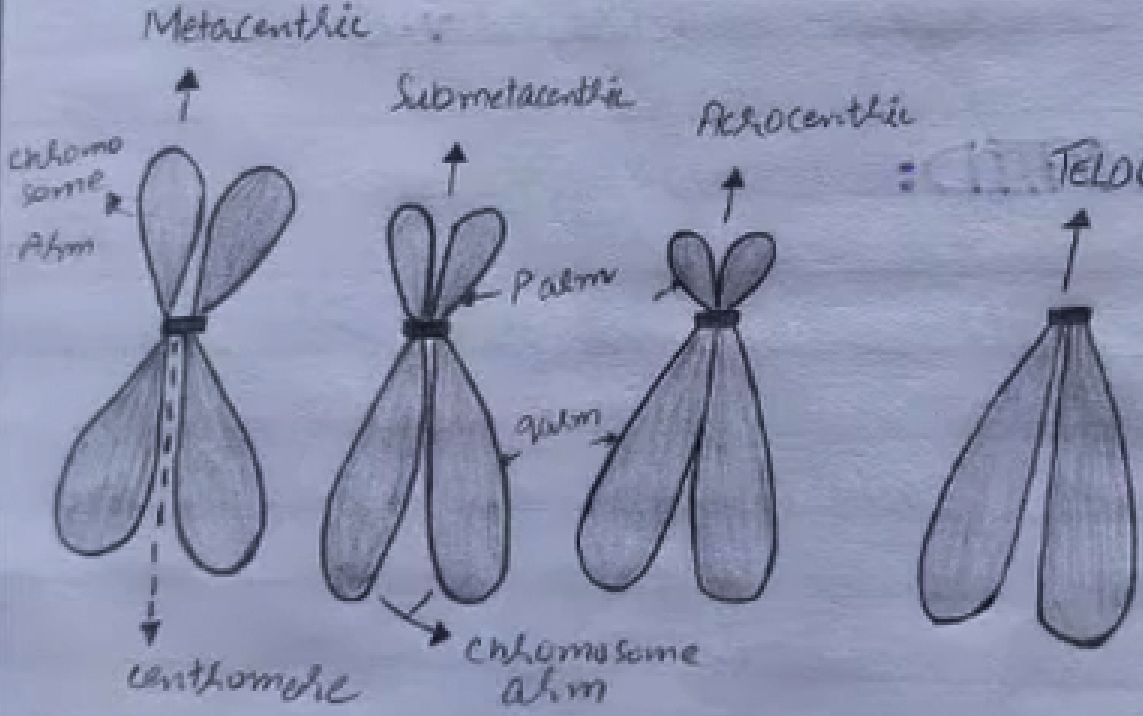
CHROMATID:

It is the structural and functional unit of chromosomes.
At Metaphase, each chromosome appears to the longitudinally divided into two identical parts, each of which is known as chromotid.

CENTROMERE:

The region where the two sister chromatids of a chromosome appeared to held together is known as centromere under light microscope centromere are the first part moving towards the opposite poles during anaphase.

Chromosome Morphology



Types Of Centromere:

On the basis of the position of centromere the chromosome may be divided into four classes

i- Metacentric:

Cnthemere is at the centre of chromosome having equal arms and appeared as V shaped during anaphase.

ii- Submetacentsic:

Centromere is on one side called submedian V or J shaped during anaphase

iii- Acrocentric:

When centromere is located close to one end. they are called as subterminal j or rod shaped

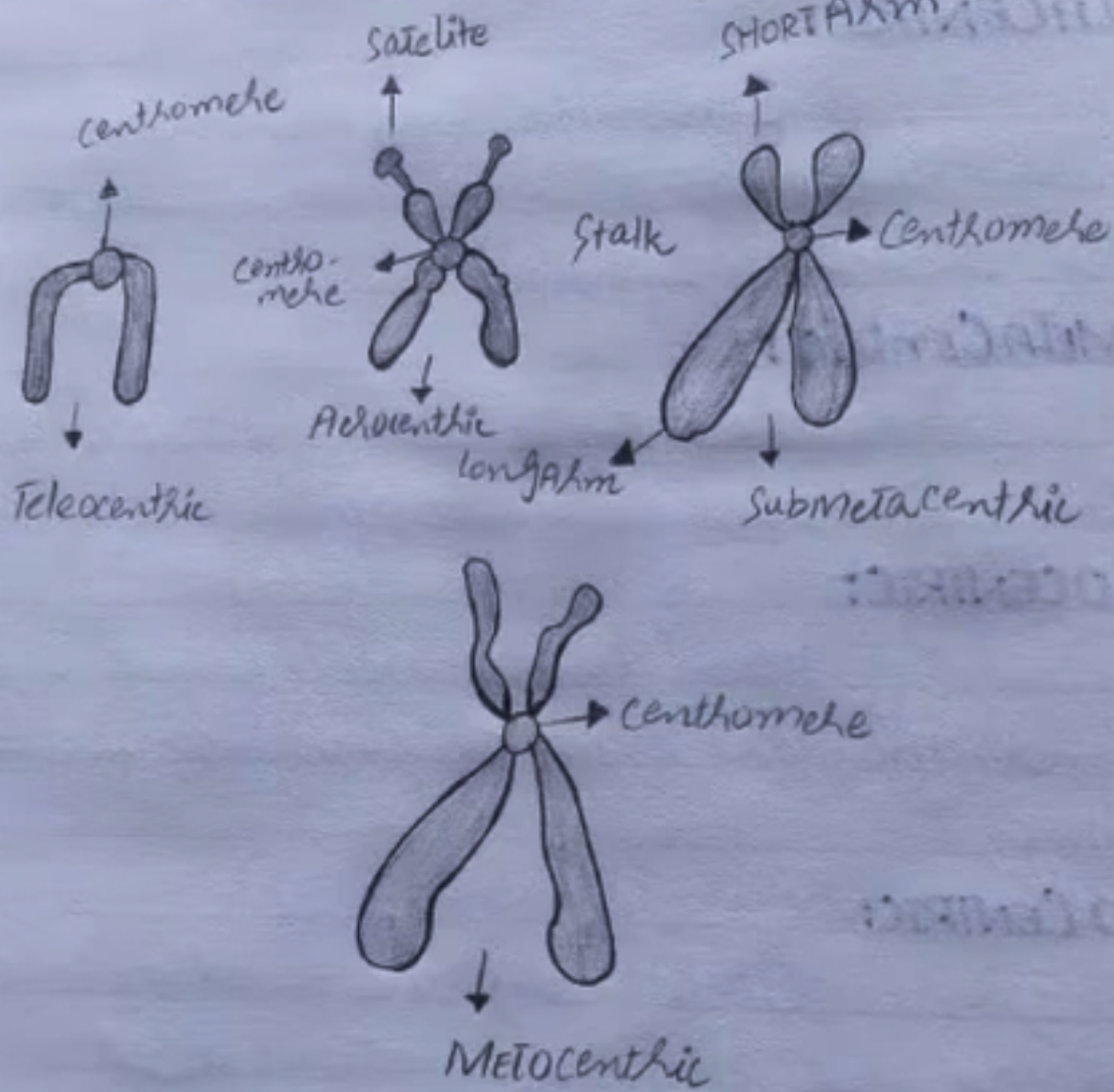
iv- Telocentric:

Occasionally the centromere appears to be at one end of the chromosome, called as Terminal Rod shaped during anaphase they are unstable.

Telomere:

The two ends of a chromosomes are known as Telomeres. they are highly stable and dont fuse with other chromosomes.

Types Of Chromosome



CHROMOSOME MORPHOLOGY:

Chromosomes can be distinguished on the basis of size and the relative location of centromeres

NUMBER OF CHROMOSOMES:

Normally, all the individuals of a species have the same number of chromosomes. Gametes normally contain only one set of chromosome. this number is called Haploid. Somatic cells usually contain two sets of chromosome - 2n: Diploid

3n- triploid 4n- Tetraploid

Aneuploidy:

Monosomics (2n-1)
Trisomics (2n+1)
Nullisomics (2n-2)
Tetrasomics (2n+2)

ORGANISM	NO. CHROMOSOMES
Human	46
Chimpanzee	48
Dog	78
HORSE	64
Chicken	78
Goldfish	94
Fruit Fly	8
Mosquito	6

Number of Chromosome in Organism

Organism	No. Chromosome
Onion	16
Mold	16
Carrot	20
Tomato	24
Tobacco	48
Rice	24
Maize	20
Haploppus gracilies	4
Crepis capillaris	6

PRACTICAL NO#5:

EXTRACTION AND ESTIMATION OF CARBHYDRATES PROTEIN, RNA AND DNA BY PLANT SOURCES

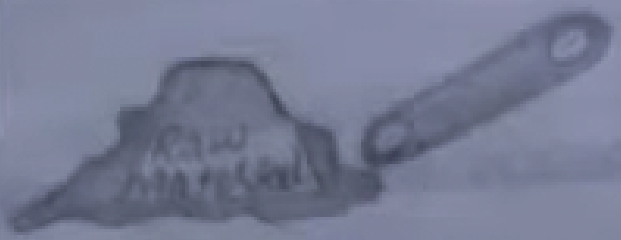
EXTRACTION AND ESTIMATION:

- Extraction are a way to separate a desired substance when it is mixed with others. the mixture is brought in to contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble.
- Estimation is the process of finding an estimate or approximation which is a value that is usable for some purpose even if input data may be incomplete or uncertain.

CARBOHYDRATES:

Carbohydrates are sugars and provide energy when consumed. our bodies break down carbohydrates to extract energy.

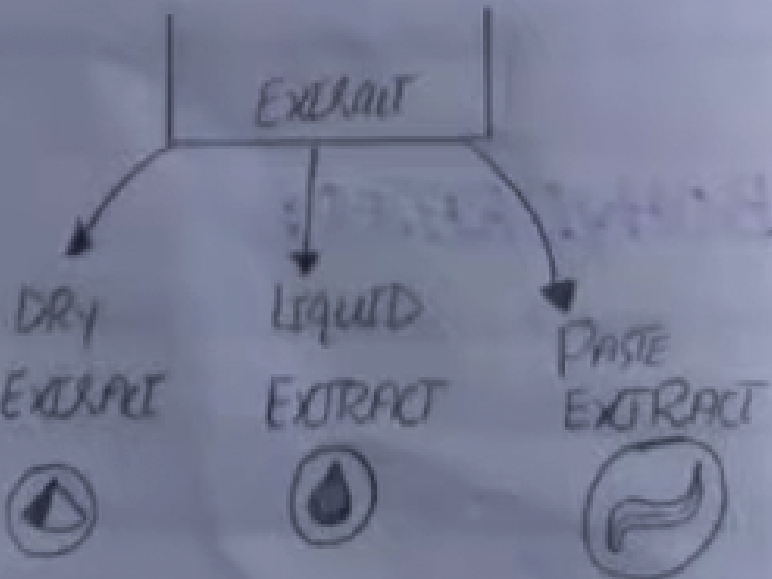
Extraction:



• Maceration

• Filtration

• Decoction and/or Purification



EXTRACTION AND ESTIMATION OF CARBOHYDRATES:

Extract, estimation and to compare the [illegible] and Carbohydrates content from different parts of Costus Speciosus and to test for the phytochemical contents.

MATERIALS AND METHODS:

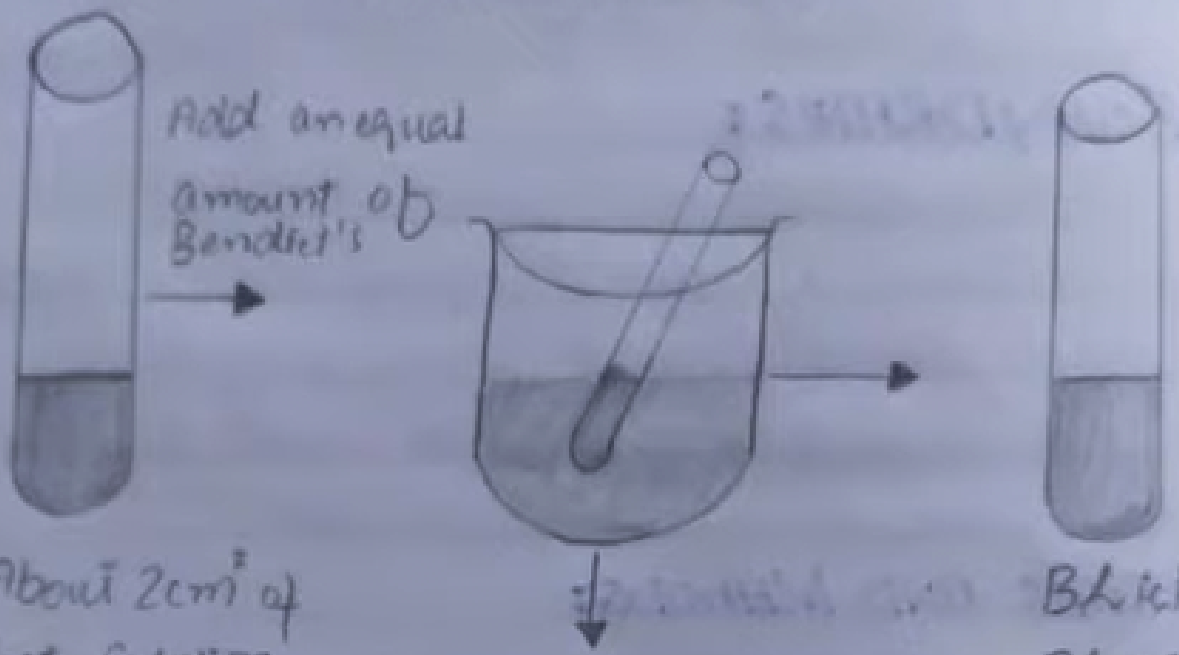
- Extraction was carried out using [illegible] was done for carbohydrates quantification.
- Fehling's Test, Benedict's Test Molisch's Test [Qualitative tests] And Another Method [Quantitative TESTS] Were performed to detect and estimate.
- Photo chemical analysis for components like Saponin, Tannin, Steriods, Glycoside, Phytosterol and Flavanoid.

RESULTS

total Carbohydrates Content were 25.97 ug/ml 20.72 ug/ml and 27.63 ug/ml respectively. Photochemical Such as Saponin, tannins, Steriod, Flavanoid and Glycol were found to be present.

CARBOHYDRATES:

BENEDICT'S TEST:



PROTEINS :

- Proteins are macromolecules formed of amino acids proteins are large size molecules composed of structural units called amino acids
- A total of 20 different amino acids exist in proteins and hundreds to thousands of these amino acids are attached to each other in long chains to form a proteins.

Extraction AND Estimation Of Protein

INTRODUCTION:

The Rhizome, leaves and stem of Cactus Speciosus has been in use for a long time and is believed to have esthogenic, antibacterial, anti inflammatory diuretic, anti diabetic and hepatoprotective property.

MATERIALS AND Method:

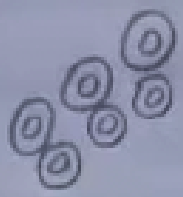
Extraction was carried out using 70% methanol for protein estimation.

Alcanol TEST, Ring TEST (Heller's TEST) [Qualitative TESTS] [Lowel Method] and modified Lowry's Method [Quantitative TESTS] were performed to detect and estimate the total proteins content in different parts of the plant viz, leaves stems and Rhizome.

Phytochemical Analysis for Components like Saponin, Tannin, Steriods, Glycoside, Phytosterol and

Protein Extraction:

Start with cell or tissue



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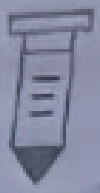
Cell lysis



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Nucleic acid removal and nuclear protein extraction

↓

Nucleic acid free nuclear extract

Flavanoid were present.

Results:

Total protein content of leaf, stem and Rhizome were found to be 99.45ug/ml 86.938ug/ml and 57.94ug/ml respectively.

Conclusion:

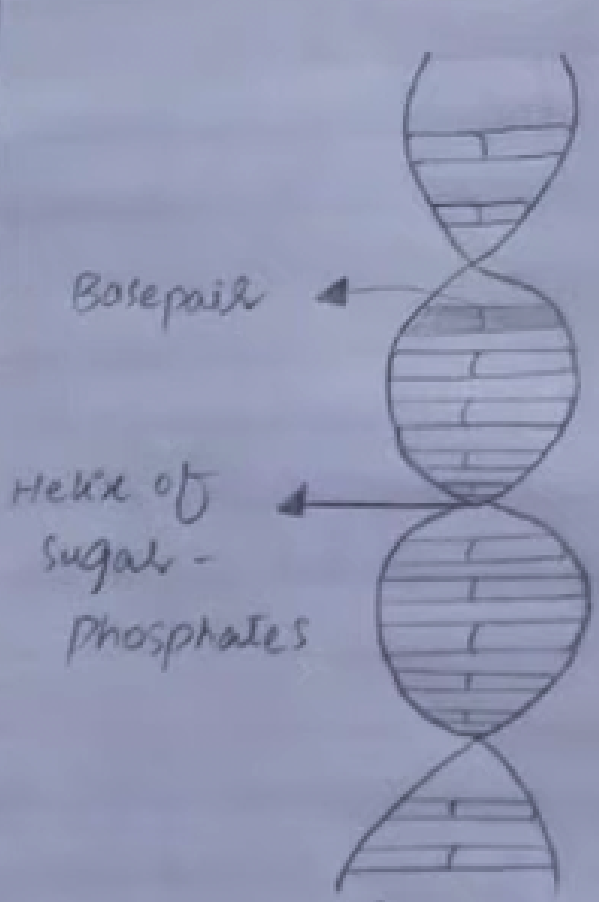
High content of protein along with many important photochemical makes Costus Speciosus an important medicinal plant for clinical research.

Nucleic Acids:

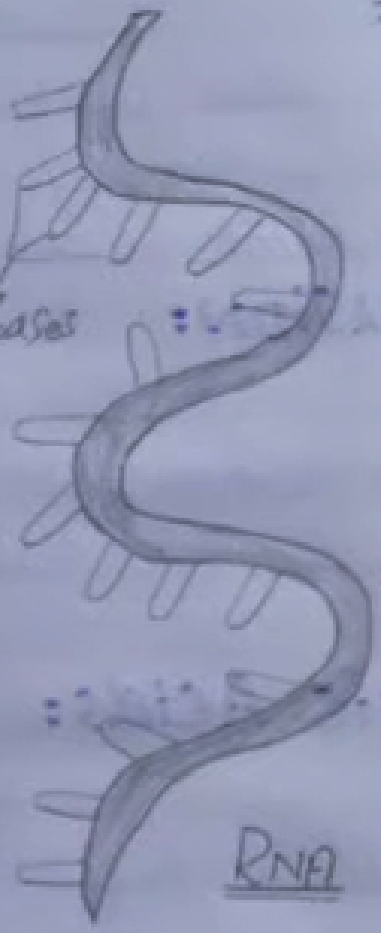
Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis they determine the inherited characteristics they of every living thing.

The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

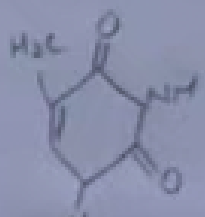
Nucleic Acids:



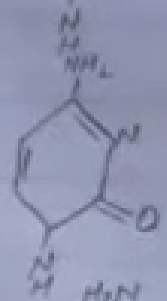
DNA



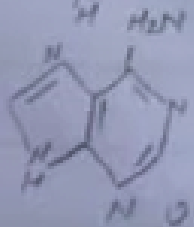
RNA



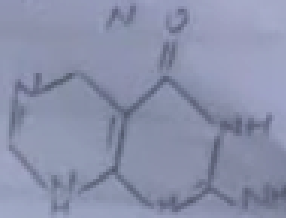
Thymine



Cytosine

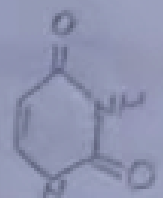


Adenine

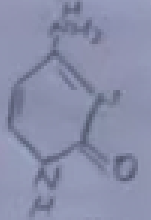


Guanine

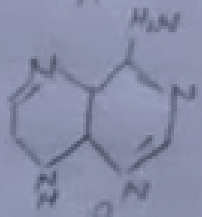
Nucelobases of DNA



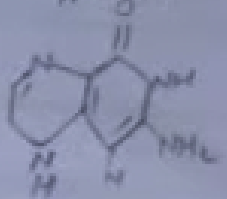
Uracil



Cytosine



Adenine



Guanine

Nucelo bases of RNA

RNA:

RNA abbesviation of ribonucleic acid, Complex compound of high molecular weight that function in cellular protein synthesis and replaces DNA as a carrier of genetic codes in some viruses.

EXTRACTION OF RNA:

MATERIALS:

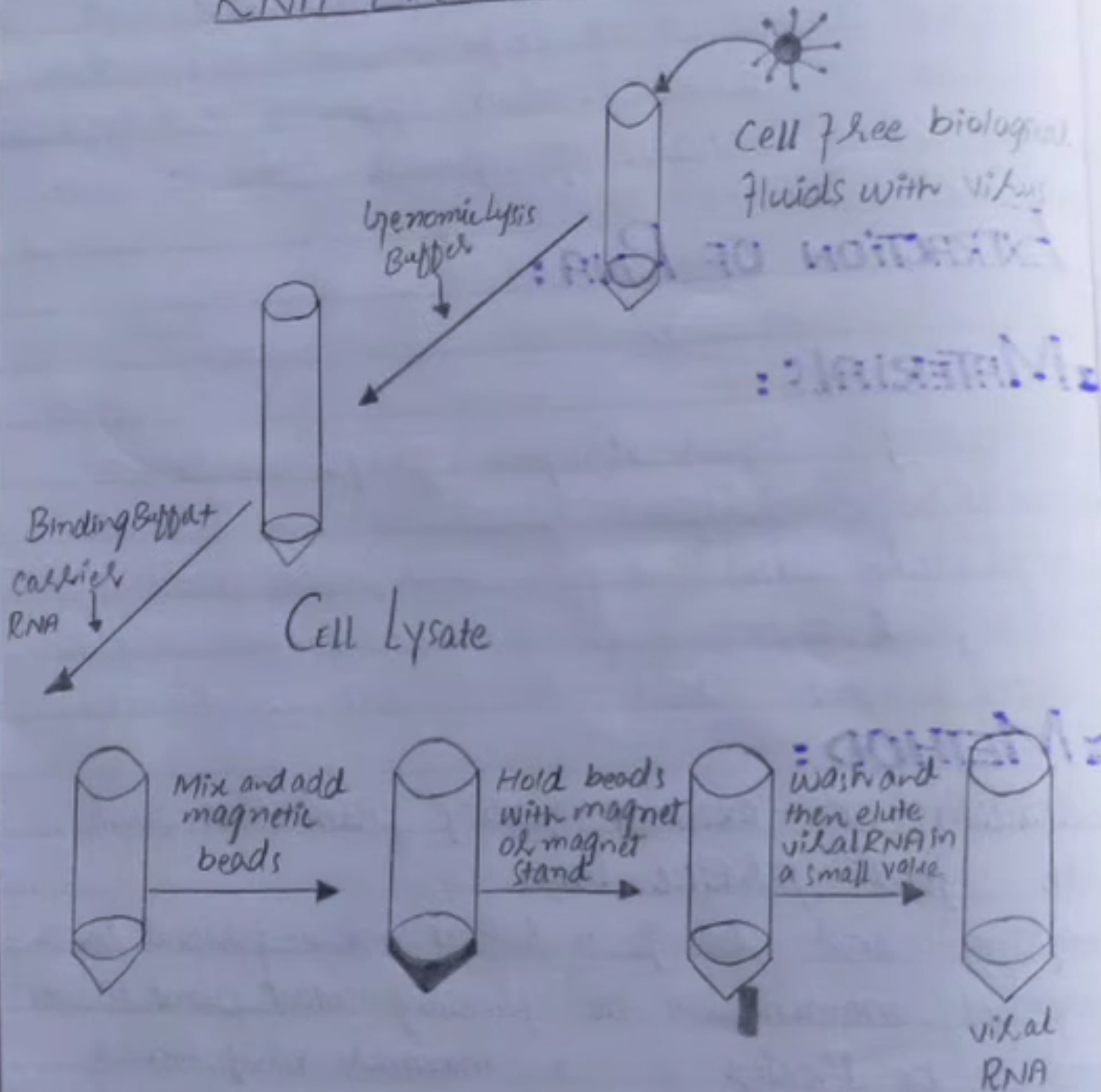
Sampling, liquid nitrogen, polystrene box Sharp knife, scalpel, razor, blade, tweezers. Eppendorf tubes, tinfoil, and plastic bottles. Analytical balance, plant material. Arabidopsis Thizol seagent. Chloroform. Isopropyl alcohol

METHOD:

Extraction of excellent quality plant RNA starts with good practice tissue.

Sampling and storage to reflect mRNA present in a snapshot moment in the growing intact plant tissues need to be treated in a manner very similar to that for Analysis of metabolic intermediates or active enzyme. Partial or complete degradation of mRNA can occur because of tissue sampling and storing techniques. Successful extraction may require alternative techniques.

RNA Extraction:



DNA:

DNA is an organic chemical that contain genetic information and instructions for protein synthesis. It is found in most cell of every organ.

DNA EXTRACTION:

The extraction of DNA from plants is the starting point for genotype analysis.

The approach to preparation of DNA from plants is determind by the species. the type of tissue or sample available and the analysis.

Components of DNA extraction solutions

Buffer. Buffers are used to control the pH of the extraction solution extraction chelating agents. Chelating agents such as EDTA bind metal ions in the extraction. Detergents help to disrupt tissues. Many different detergents

Methods:

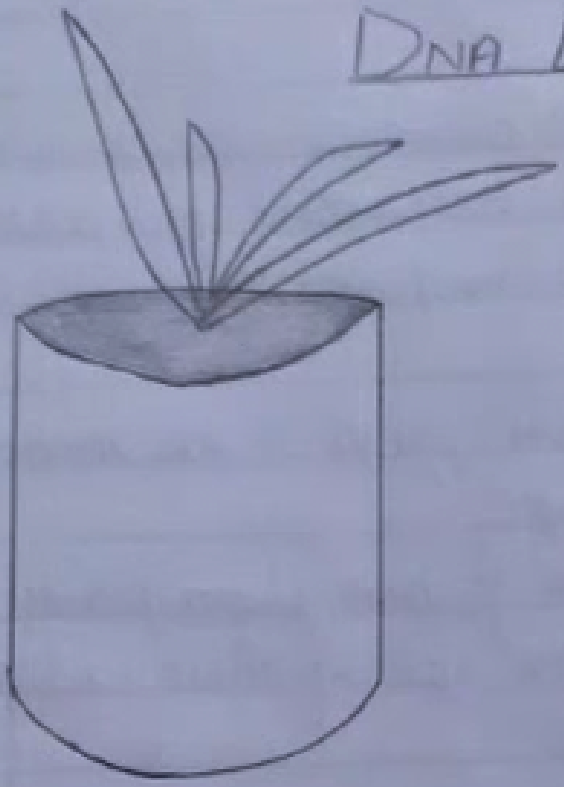
STANDARD Method: Grind sample to a fine powder in liquid nitrogen using mortar and pestle.

Transfer the tissue to a tube of suitable size and add 1 ml of extraction buffer (2% (W/V) hexadecyltrimethylammonium bromide (CTAB), 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA per gram of tissue. Mix by gentle inversion and heat at 55c of 20 min.

Centrifuge at 15,000 g for 5 min.

from isoamyl alcohol (24:1).

DNA Extraction:



DNA can be isolated from a number of plant tissues. young leaf is a good source of DNA.

Leaf tissue is placed in tubes and frozen using liquid nitrogen.

Freezing helps to mechanically break cells.

PRACTICAL NO# 6:

GENETICAL PROBLEMS RELATED TO TRANSMISSION AND DISTRIBUTION OF GENETIC MATERIAL:

GENETICS:-

Genetics is a branch of biology concerned with the study of genes, genetic variation and heredity in organisms. Though heredity had been observed for millenia, Gregor Mendel, a Scientist and Augustinian friar working in the 19th Century, was the first to study genetics scientifically.

Genetic Transmission:-

The transmission of genes to an organism's offspring is the basis of the inheritance of phenotypic traits. These genes makeup different DNA sequences called genotypes. Genotypes along with environmental and developmental factors determine what the phenotypes will be. Most biological traits are under the influence of polygenes.

Genetic disorder:

A genetic disease is any disease caused by an abnormality in the genetic makeup of an individual.

Types of genetic disorders:

⇒ There are a number of different types of genetic
(i) Single gene inheritance (ii) Multifactorial inheritance
(iii) Chromosome abnormalities (iv) Mitochondrial inheritance

Single gene inheritance:

Single gene inheritance is also called Mendelian or monogenetic inheritance. Changes or mutations that occur in the DNA sequence of a single gene cause this type of inheritance. There are thousands of known single-gene disorders.

⇒ Some examples of single-gene disorders include
(i) Cystic fibrosis (ii) alpha- and beta-thalassemias
(iii) Sickle cell anemia (iv) Marfan Syndrome
(v) Fragile x Syndrome (vi) Huntington's disease, and
(vii) Hemochromatosis.

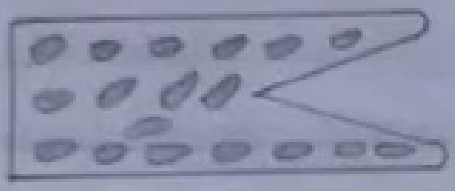
Sickle Cell Anemia:

Sickle cell anemia is an inherited red blood cell disorder in which there aren't enough healthy red blood cells to carry oxygen throughout your body. Normally the flexible, round red blood cells move easily through blood vessels.

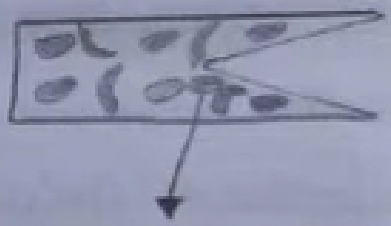
Sickle Cell Anemia:

Healthy Red blood Cell

Sickle Red blood cell



unrestricted blood flow



Blood flow blocked by Sickle Cells

Alpha and beta thalassemia:

The thalassemia are a group of inherited hematologic disorders caused by defects in the synthesis of one or more of the hemoglobin chains. Alpha thalassemia is caused by reduced or absent synthesis of alpha globin chains, and beta thalassemia is caused by reduced or absent synthesis of beta globin chains.

Marfan Syndrome:

Marfan syndrome is an inherited disorder that affect connective tissue - the fibers that support and anchor your organs and other structures in your body. Marfan syndrome most commonly affects the heart, eyes, blood-

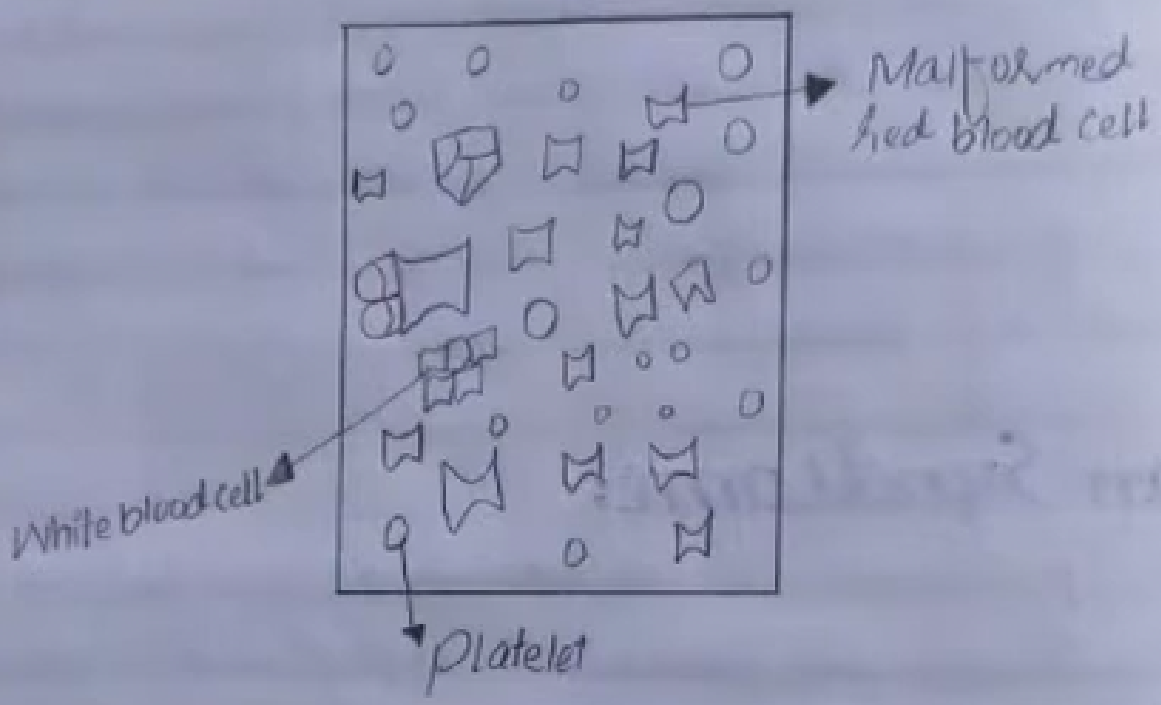
Multifactorial Genetic disorders:

Multifactorial inheritance is also called complex or polygenic inheritance. Multifactorial inheritance disorders are caused by a combination of environmental factors and mutations in multiple genes.

→ Examples of multifactorial inheritance include.

(i) heart disease
(ii) high blood pressure
(iii) Alzheimer's disease
(iv) Arthritis
(v) Diabetes
(vi) Cancer
(vii) Obesity

Thalassemia:



Cancer:

Cancer, also called malignancy, is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Symptoms vary depending on the type. Cancer treatment may include chemotherapy, radiation, and/or surgery.

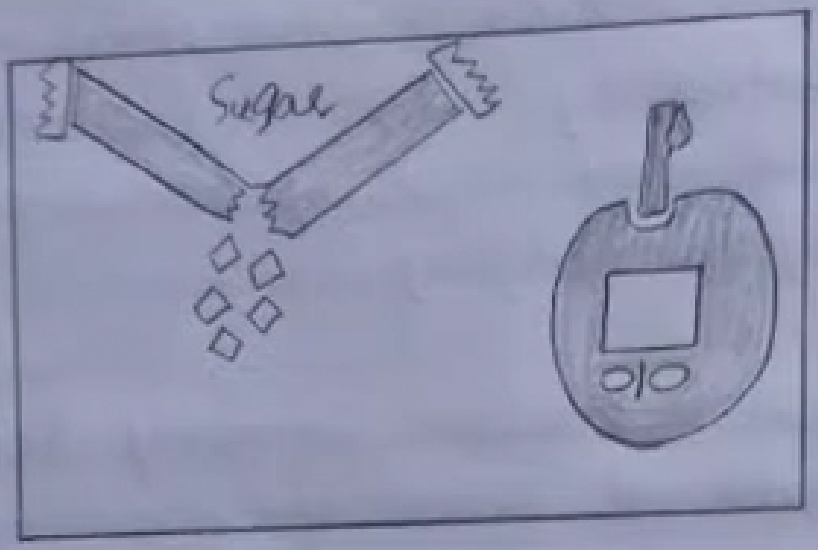
Diabetes:

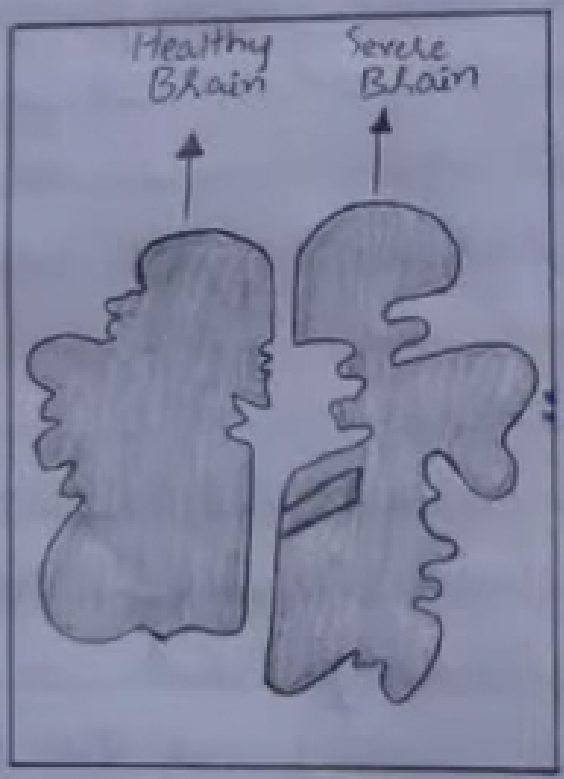
Diabetes mellitus, commonly known as diabetes, is a metabolic disease that causes high blood sugar. The hormone insulin moves sugar from the blood into your cells to be stored or used for energy. With diabetes your body either doesn't make enough insulin or can't effectively use the insulin it does make.

Alzheimer's Disease:

Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks. In most people with the disease - those with the late-onset type - symptoms first appear in their mid-60s.

Diabetes:





Alzheimer's Disease:

Chromosomal abnormalities:

Chromosomes, distind structures made up of DNA and protein are located in the nucleus of each cell. Because Chromosomes are the carriers of the genetic material, abnormalities in chromsome number or structure can result in disease.
Chromosomal abnormalities typically occur due to a problem with cell division.

For Example: Down Syndrome or trisomy 21 is a common genetic disorder that occurs when a person has three copies of chromosome.

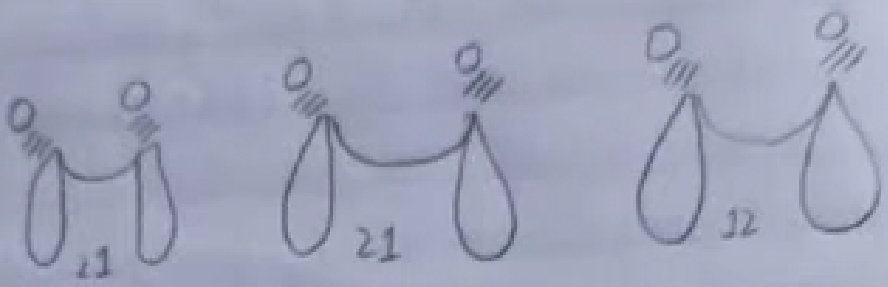
Down's Syndrome:

Down Syndrome or Down's Syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21.
It is usually associated with physical growth delays, mild to moderate.

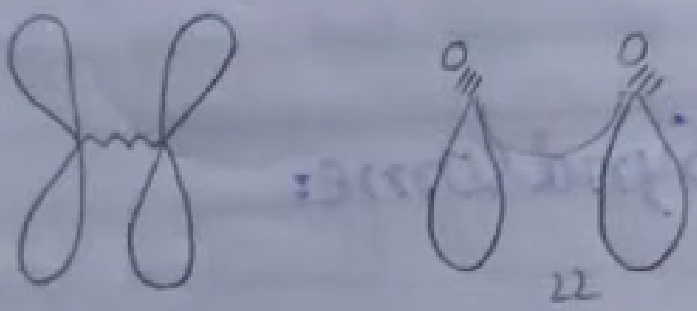
Turner's Syndrome:

Turner Syndrome, a condition that affects only females, results when one of the x chromosomes is missing or partially missing. Turner Syndrome can cause a variety of medical and developmental problems, including short height, failure of the ovaries to develop and heart defects.

Down's Syndrome:

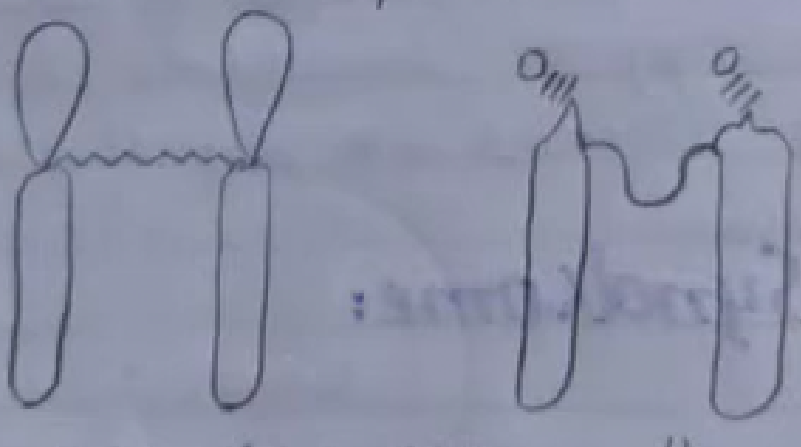


Thisomy of chromosome 21:



21/22 translocation

21/22 translocation



12/21 translocation

12/21 translocation

Distribution of Genetic Material:

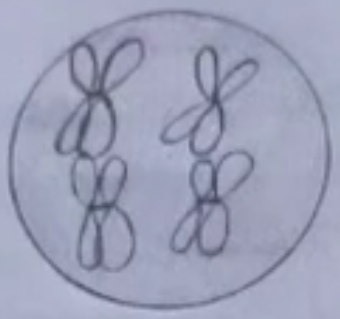
Like mitosis, meiosis is a form of eukaryotic cell division However, these two processes distribute genetic material among the resulting daughter cells in very different ways. Mitosis creates two identical daughter cells that each contain the same number of chromosomes as their parent cell. In contrast, meiosis gives rise to four unique daughter cells, each of which has half the number of chromosomes as the parent cell.

Meiosis I:

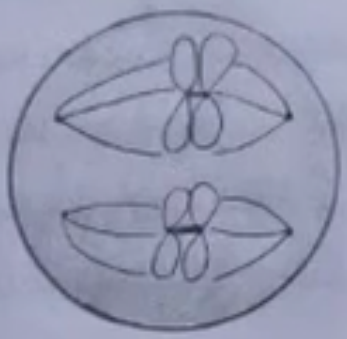
In meiosis I, chromosomes in a diploid cell segregate, producing four haploid daughter cells. It is this step in meiosis that generates genetic diversity.

(i) Prophase I
(ii) Meta phase I
(iii) Ana phase I
(iv) Telo phase I
(v) Cytokinesis

Meiosis I



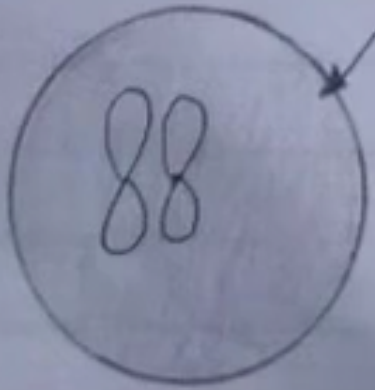
Prophase I
(2n-1)

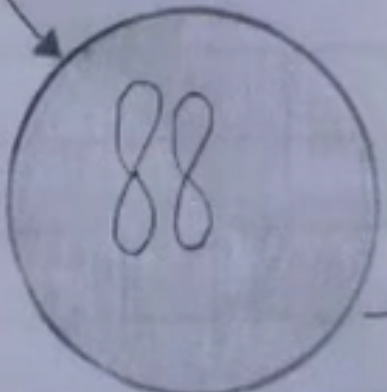


Metaphase I

Anaphase I

Telophase I





Cytokinesis
(n-2)

Meiosis II

Meiosis II

During meiosis II, the sister chromatids within the two daughter cells separate, forming four new haploid gametes.
The mechanics of meiosis II is similar to mitosis, except that each dividing cell has only one set of homologous chromosomes.

(i) Prophase II
(ii) Metaphase II
(iii) Anaphase II
(iv) Telophase II

Significance of Meiosis:

Reproduction in animals takes place through the fusion of gametes i.e. two cells fuse together with their genetic material to develop a Zygote. It germ cells, which give rise to gametes, also maintains their ploidy during devision like the somatic cells, the Zygote will have an accumulation of chromosomes in its nucleus.
The accumulation will keep on increasing with every subsequent generation.

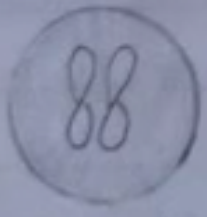
Meiosis II

Prophase II: Nuclear membrane breaks down and chromosomes move towards middle of the cell

Metaphase II:
Chromosomes are aligned at the equatorial plate.

Anaphase II:
Sister chromatids separate and move towards opposite poles an individual

Telephase II:
Chromosomes arrived at the cell poles and nuclear membrane are formed



Cytokinesis
Division of cytoplasm results in four haploid daughter cells.

Practical No#7:
Identification Of Chromosomes in plant material. Carmine/orcein Staining.

Chromosomes:

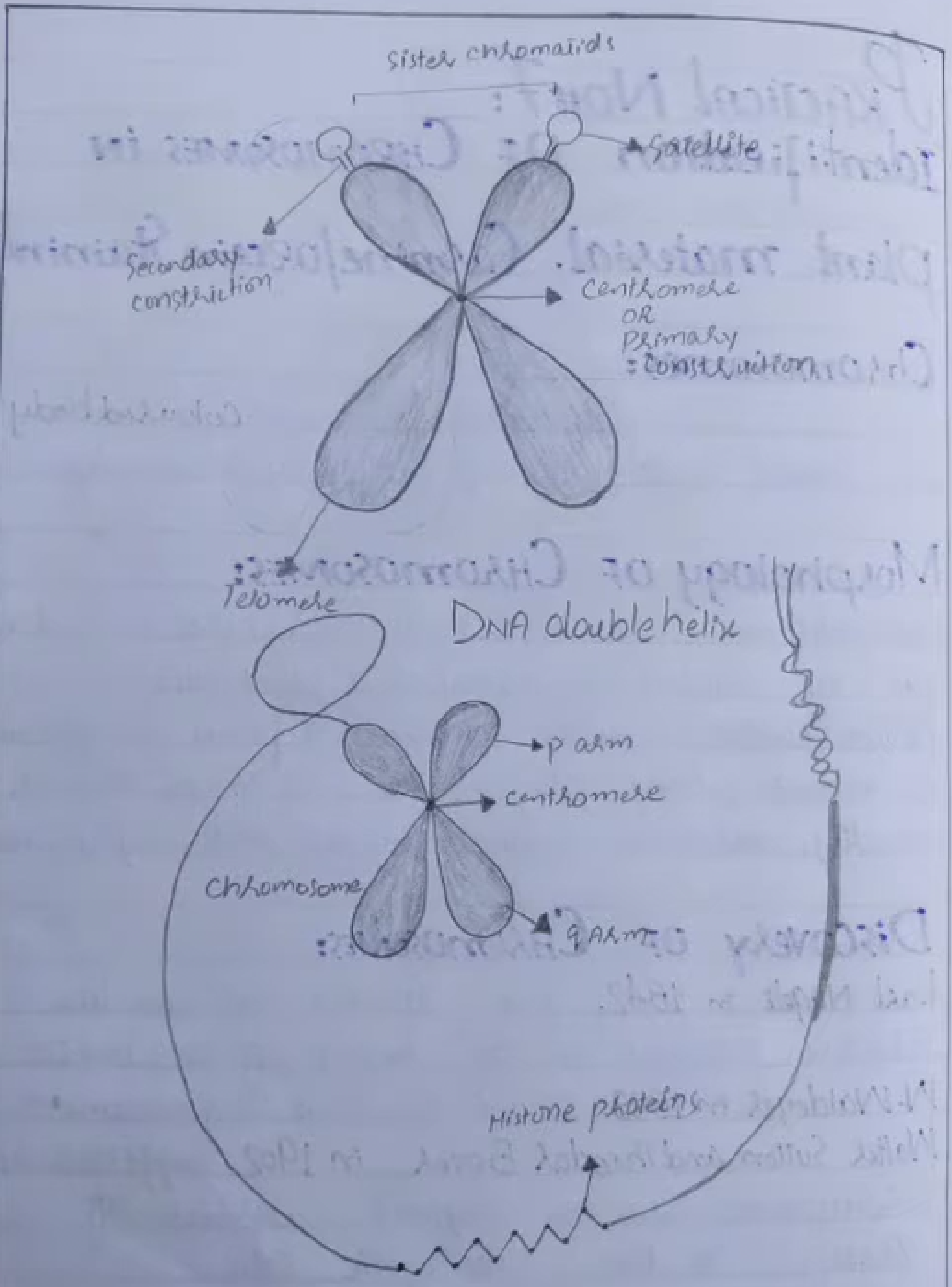
Chromosome means 'Coloured body' that refers to its staining ability by certain dyes.

Morphology of Chromosomes:

Chromosomes are thread-like structures located inside the nucleus of animal and plant cells. Which carries genetic information from one generation to another. they play a vital role in cell division, heredity, variation. mutation. repair and regeneration

Discovery of Chromosomes:

Karl Nageli in 1842, first observed the rod-like structure present in the nucleus of the plant cell. W. Waldeyer in 1888 Coined the term 'chromosome' Walter Sutton and Theodor Boveri in 1902 suggested that Chromosomes are the physical carrier of Genes in the eukaryotic cells.


Sister chromatids
Satellite
Secondary constriction
Centromere OR Primary constriction
Telomere
DNA double helix
p arm
centromere
Chromosome
q Arm
Histone proteins

Chromosome Structure:

Each cell has a pair of each kind of chromosome known as a homologous chromosome.

Chromosomes are made up of chromatin, which contains a single molecule of DNA and associated proteins. Each chromosome contains hundreds and thousands of genes that can precisely code for several proteins in the cell.

Main parts of Chromosomes:

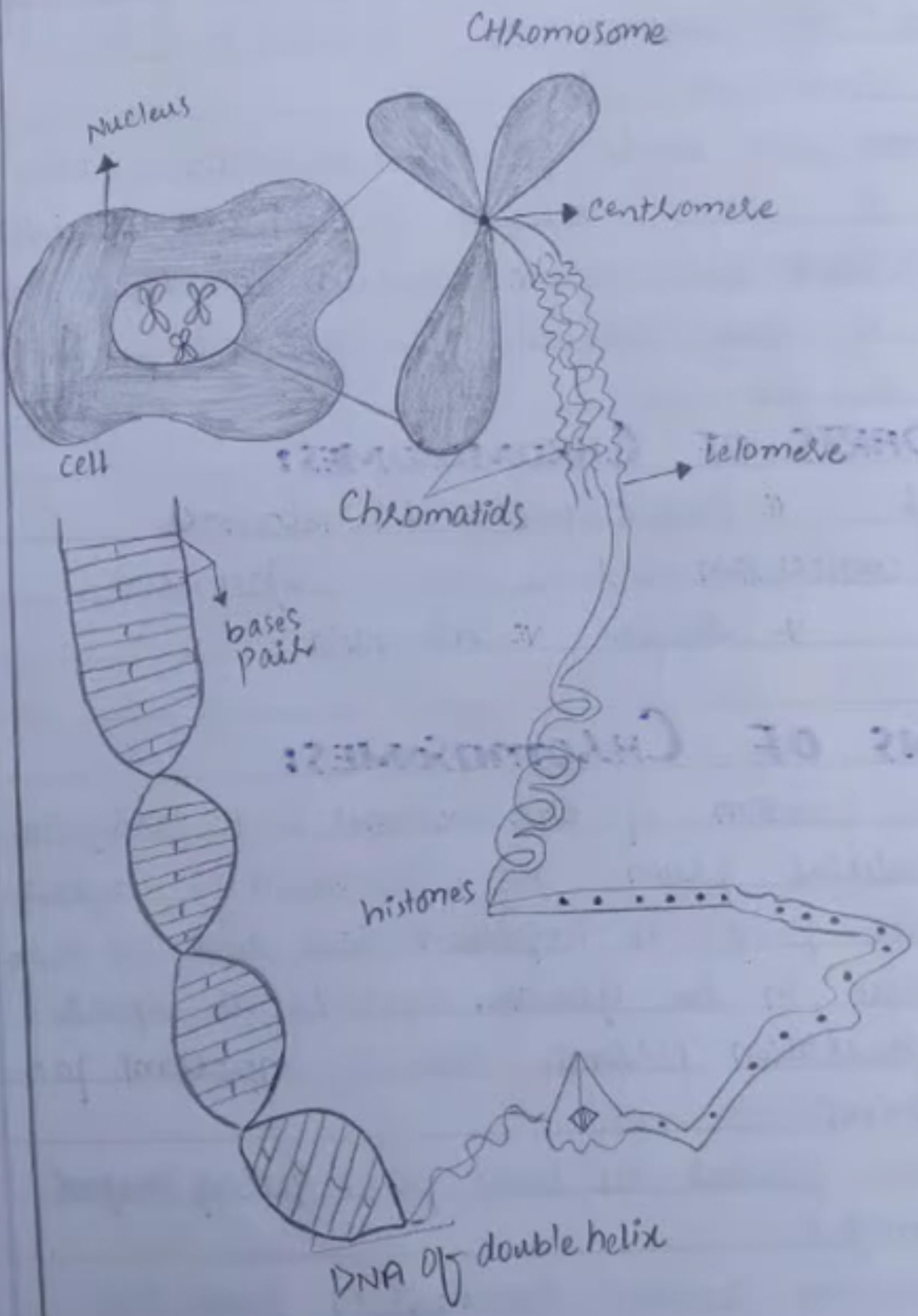
i. Chromatid ii- Centromere and kinetochore
iii- Secondary constriction and nucleolar Organisers
iv- Telomere v- Satellite vi- Chromatin

Functions of Chromosomes:

The main function of chromosomes is to carry the genetic material from one generation to another. Chromosomes play an important role and act as a guiding force in the growth, reproduction, repair and regeneration process, that is important for their survival.

Chromosomes protect the DNA from getting tangled and damaged.

Each chromsome contains thousands of genes that precisely code for multiple protein present in the body.


Chromosome
Nucleus
centromere
cell
Telomere
Chromatids
bases pair
histones
DNA of double helix

METHODS FOR Identification OF CHROMOSOMES:

ACETOCALMINE STAINING:

PREPARTION (1%. Solution)

Carmine is a basic dye that is prepared from the insect Coccus cacti. Dissolve 10 g carmine (Fisher C579-25) in L of 45%. glacial acetic acid. add boileezers, and reflux for 24h.

ACETOCARMINE Staining:

To Stain plant Chromosomes, a 1% Solution of carmine in 45%. acetic acid is used Freshly Fixed material is transferred into 1%. acetocarmine for at least 30 min and then analyzed by the Squash Method.

If the material was fixed for a longer time, it requires a longer staining time to reach good contrast. If the material is to be analyzed immediatly, fix and stain the tissue in one step using the 1%. Acetocarmine Solution.

CHROMOSOMES Squash Technique:

Drain off the fixative and place the roots in 1%. Aceto carmine for 1 to 3h. Heat until the aceto carmine

begins to boil.
cut off the root cap with a razor blade and Squeeze the meristematic tissue out with a lancet needle. Add a drop of acetocarmine or 45% acetic acid.

Staining Procedures For Chromosome Analysis

MATERIAL:

Chromosome Number Callus Culture common Wheat Total 100ml Cereal Species

Abstract:

Many rearrangements of the genetic material of a cell can occur in plant tissue or protoplast culture. These include changes in chromosomes number, deletions of chromosome parts, and rearrangements of the chromosomes such as translocation.
Many of the gross structural changes can be evaluated by convential cytogenetic procedures that involve staining the chromosomes and evaluating the morphology at metaphase of mitosis and their behaviour in meiosis.
The analysis described here should help the experimenter to characterize cultured tissues and regenerated plants.

Differential Staining of plant Chromosomes after Hydrochloric Acid Treatment (Hy Bands):

Abstract:

Differential or preferentially staining heterochromatic segments was achieved in somatic chromosomes of five monocotyledeneous species.

When acetic-ethanol fixed meristems were subjected to 0.1 or 0.2 N HCl at temperatures between 60° and 80°c and stained with aceto-carmine. Suitable incubation time was temperature dependent and afforded 30 to 5 minutes.

Several variations of the procedure were tested. The following plants were investigated. Allium cepa, A. Flavum, A. Carinatum, Scilla Sibirica, Fritillaria meleagris.

PRACTICAL NO 8

Study of salivary gland chromosomes of Drosophila

Introduction

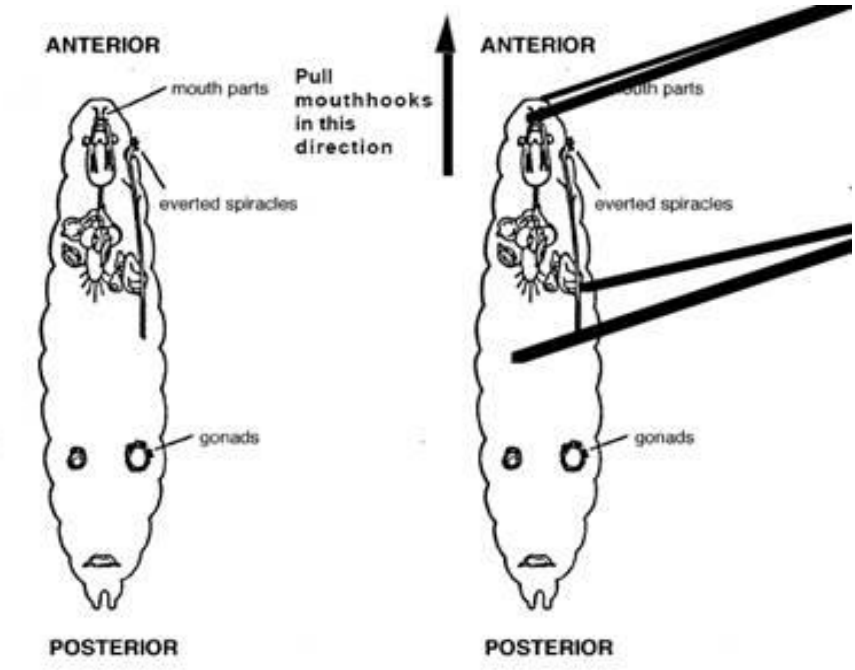
- *Drosophila melanogaster*, or the red-eyed fly, is classified in the family Drosophilidae, and order Diptera (two winged, which also includes flies, mosquitoes and midges.).
- *Drosophila* has been such a model organism for several reasons. They are small, easy to raise in the Lab, have a short life-cycle, have only 4 pair of chromosomes, and contain large polytene (Polytene chromosomes are over-sized chromosomes which have developed from standard chromosomes and are commonly found in the salivary glands of *Drosophila melanogaster*. Specialized cells undergo repeated rounds of DNA replication without cell division(endomitosis), to increase cell volume, forming a giant polytene chromosome. Polytene chromosomes form when multiple rounds of replication produce many sister chromatids that remain synapsed together.) chromosomes.
- Polytene chromosomes, found in the salivary glands of organisms in the order, Diptera, are actually 1,000s of copies of each chromosome lined up in register.
- Areas of dark and light bands contain various concentrations of DNA and protein in the chromatin, and can be seen under a light microscope at a magnification of 450X

Materials

- Drosophila population, males and females
- Culture tubes with food at the bottom
- Breathable cotton material for tube stopper

- Ether (and eye dropper for transferring ether to sleep box)
- Dissecting microscope or hand lens
- Soft, small paint brush for moving etherized flies
- White paper (index card) on which to view the flies
- Compound Microscope (Light)

Procedure

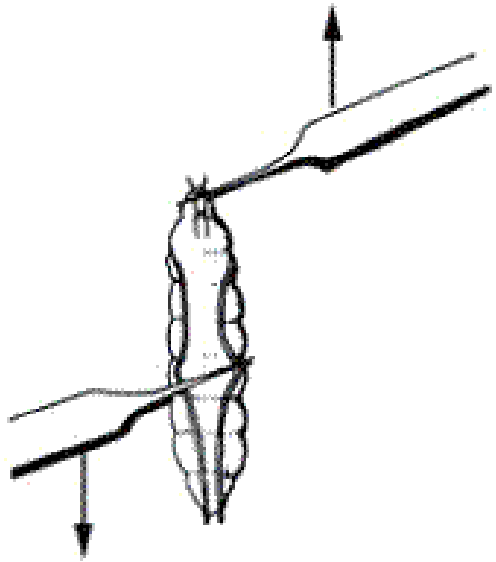


Dissection of the salivary glands and polytene chromosome preparation

1. Place a few drops of insect Ringers (NaCl. 7.5 gm. KCl. 0.35 gm. CaCl2. 0.21 gm) or Saline in a well of a depression slide, watch glass, or small Petri plate.
2. Select a third instar larva from your bottle; they will be found near the bottom of the bottle, as they are just beginning to crawl up the sides. If the one you have chosen is sessile, it has already pupated and is of too late a stage to be used. Remove the larva with a pair of forceps or a dissecting needle and place in the well of your slide.
3. Under intermediate power of the dissecting microscope, place one forceps firmly across the rear half of the larva to prevent movement. Place the second forceps

just behind the mouth hooks at the head region. The head is sharply pointed and the mouth hooks are black and so should be distinguishable from the rear of the larva.

4. Pull the head off the body by firmly pulling the two forceps apart. If you are successful, you should see the head with the two attached salivary glands trailing behind. The salivary glands can be recognized as two long, transparent, sausage-shaped bags with a characteristic translucent fat body along one side and occasionally capping each gland. Always keep the gland moist in Ringers solution.
5. Transfer the glands immediately into a drop of 45% acetic acid placed on a microscope slide. Allow to remain approximately 30-45 seconds. Be sure not to let the glands dry out.
6. Then transfer the glands to a small drop (2-3 mm in diameter) of aceto orcein stain in the center of the same slide. This should all be done under the dissecting scope to insure that the glands don't stick to the forceps. Leave them in the stain for 15-20 minutes (If the chromosomes aren't stained darkly enough, a longer incubation period may be necessary, last year 30 minutes worked well). Again, make sure the stain doesn't dry out during this time period.



7. Gently place a coverslip over this preparation. Put a piece of blotter paper over the coverslip and place a piece of parafilm over the blotter paper.
8. Place your thumb on the parafilm directly over the coverslip and press straight down quite firmly for about 20 seconds. You do not want the coverslip to move relative to the slide.
9. Examine the slide under 10X to locate the squashes. Then, look under 40X to find a good preparation. Ideally, the chromosomes should be well spread and the bands stained darkly. Once you have found such a squash, place a drop of oil on top of the coverslip and examine under 100X. Now, try to identify the chromosomes based on the landmarks familiar to you.

Observation

Drosophila salivary glands chromosomes