Ex.No:01

Date:

MICROSCOPIC STUDY OF CELLS

Aim:

To study the shape and structure of various eukaryotic cells.

Materials

Prepared/Permanent Slide:

- Rat pancreas

Animal cells:

- *Adherent cells*
- *Non-adherent cells*

Cell suspension :

- *Chlamydomonas reinharditii*

Fresh dicot plant leaf & stem for cross section

- Microscope
- Clean blade

Preparation of dicot leaf cross section:

Fixation

Since cellular decomposition begins immediately after the slicing, biologists must fix the cells to prevent alterations in their structure through decomposition. Routine fixation involves the chemical cross-linking of proteins (to prevent enzyme action and digestion) and the removal of water to further denature the proteins of the cell. Heavy metals may also be used for their denaturing effect.

Dehydration

If the tissues are to be placed on slide, all traces of water must be removed. The removal of water is called **dehydration**. The dehydration process is accomplished by passing the tissue through alcohol. The leaf sections are then placed in a 100% ethanol solution to ensure that all water is removed. It is important to note that ethanol is hydroscopic and absorbs water vapor from the air.

Difference between dehydration and drying

Tissues should never be allowed to air dry. Dehydration involves slow substitution of the water in the tissue with an organic solvent.

Staining:

Leaf /stem cross sections were stained with acetocarmine

Procedure:

1. Fresh handpicked leaves were brought to the laboratory and washed thoroughly with water.
2. Leaves were wiped with tissue paper and air dried.
3. Small piece of leaf was placed into a cork slit.
4. Using clean sharp blade extreme thin sections were made for leaf and stem.
5. Thin slices were placed on a clean slide.
6. Drop of alcohol was placed on leaf section for dehydration for 30sec.
7. Then few drops of stain were added.
8. Then the stained thin slice was covered with a cover slip. Care should be taken in placing the cross section on the slide rather than placing the leaf surface.
9. Excess stain was blot dried.
10. The slide was placed on a microscope and observed under 10X for fixing the fields and then viewed under 40X.

For cell suspension:

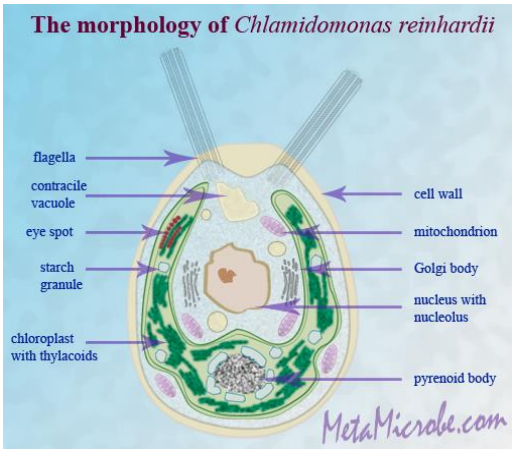
1. One drop of the cell suspension was placed on a clean glass slide.
2. A coverslip was laid on the drop carefully without introducing any air bubbles.
3. The slide was viewed under the microscope to observe the morphology of the cells.

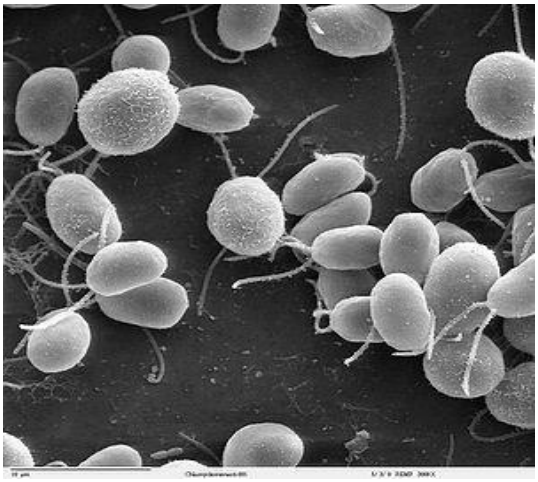
Observation:

RESULT:

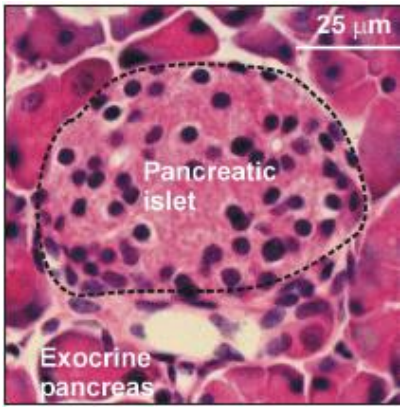
The morphology of various eukaryotic cells was studied.

Chlamydomonas reinhardtii

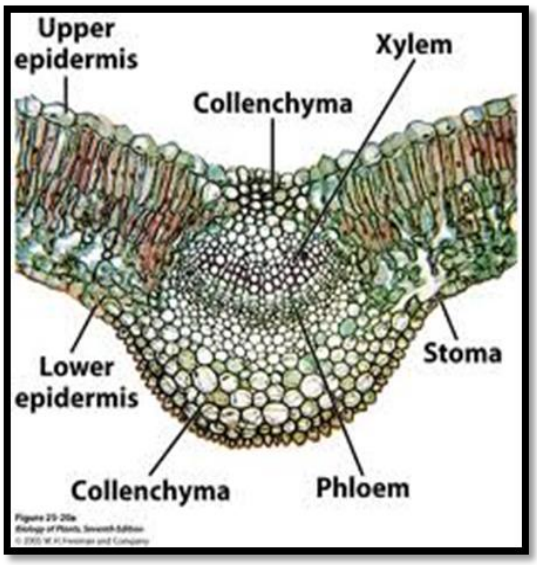


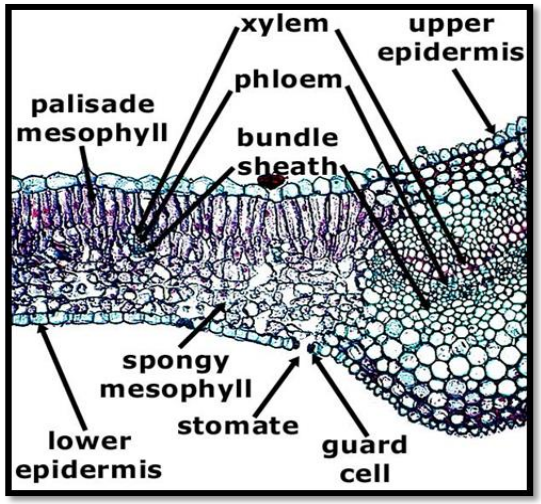


Rat pancreatic cells

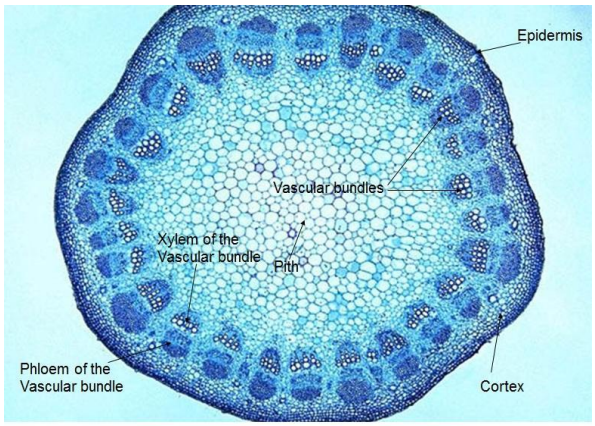


Plant leaf cross section

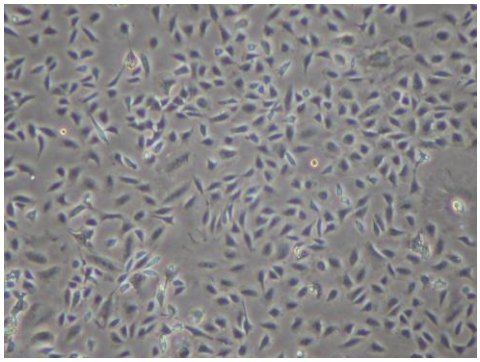




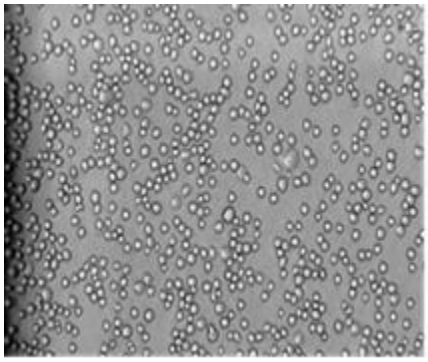
Dicot stem C.S.



Adherent cells



Non-adherent cells



DISCUSSION:

Experiment No.: 02
Date:

Nuclear Staining of Cells

Aim:

To stain the nucleus of adherent cells by Giemsa staining method.

Principle:

It is prepared by the mixture of methylene blue, azure B and eosine. The dye is positively charged. In solution the dye cations are present as dimers. The dimers are attracted to the negatively charged phosphate groups of DNA and adhere to the macromolecule as a result of hydrophobic interactions with the purine and pyrimidine rings of the DNA bases. Attraction of eosin anions by un-neutralized positive charges of bound azure B dimers changes the color of the stained DNA from blue to purple.
Methanol is used as a decolourising agent which removes the excess Giemsa stain. It is also used for fixation of cells.

Requirements:

- Chinese Hamster Ovarian (CHO) Cells
- DMEM
- Petri Plates
- Cover Slips
- Slides
- Giemsa Stain 5%
- Sterile PBS (1X)
- Methanol
- Forceps

Procedure:

- The cells were seeded on the cover slip under aseptic condition and incubated at 5% CO_2 overnight for proliferation.
- The media was decanted using a pipette without disturbing the cover slip.
- The slide was then fixed with methanol for 30-60 seconds (Care was taken to remove the methanol as fast as possible, because the prolonged incubation with methanol may dehydrate the cells).
- Once the methanol is removed, 1 ml of Giemsa stain (5% in PBS) was applied over the cover slip and incubated for 10 min.
- The stain was discarded and the coverslip was washed with 1X PBS.
- Then the cover slip was carefully transferred to a clean glass slide and observed under the microscope.

Result:

Discussion:

EX. No:03
Date:

CELL VIABILITY

Aim:

To determine the viability of the given cell suspension using haemocytometer.

Principle:

The measurement of cell viability plays an important role in cell form of cell culture. It is the main purpose of expression of s/a toxicity assay. Alternatively, it can be used to correlate the behaviour of cell membrane by providing a more accurate picture. Tryphan blue exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cell possess intact cell membrane that include certain dyes, s/a tryphan blue, eosin and propidium; whereas dead cells will have a clear and visible cytoplasm whereas a non-viable cell will have a blue color stain in the cytoplasm.

Requirements:

- Cell suspension
- 70% ethanol
- Trypan blue 0.4%
- Microfuge tubes
- Micropipettes
- Haemocytometer
- Microscope

Procedure:

- The Laminar Air Flow Chamber was sterilized by wiping with 70% ethanol followed by UV irradiation for 15 min.
- Equal volume of trypan blue solution (10µl) was mixed with cell suspension (10µl) in a microfuge tube.
- Haemocytometer was placed on the flat surface of the work bench and the coverslip was placed on the counting chamber.
- 10 µl of cell suspension containing the dye was loaded to a haemocytometer through diffusion by capillary action. It was made sure that there was no air bubbles and over-filling beyond the ruled area.
- The counting chamber was kept idle on the bench for 20 -30 seconds to allow the cells to settle.
- The counting chamber was placed on the stage of the microscope between the clip to hold slide so that the counting chamber can be moved.
- The number of unstained (live) and the stained (dead) cells, seen on the square of any of the 4 corners which were divided into 16 smaller squares, were counted.
- The concentration of live cells in the original suspension in cells/ml was calculated based the formula

No. of Cells/ml = 2 X n X D.F X 10^4

n= total number of unstained cells counted in 1 square corner

D.F= dilution factor

2 = to extrapolate the total number of cells present in 20µl (10 µl of dye and 10 µl of cells)

10^4= to extrapolate the total number of cells present in all the 4 square corners

% cell viability = (Number of live cells/ Total number of cells (live+dead)) X 100

Result:

The number of viable cells was found to be _____ and the % cell viability was determined to be _____.

Discussion:

Exp.No.:04
Date:

ISOLATION OF CHLOROPLAST

AIM:

To isolate chloroplast from the given leaf sample.

PRINCIPLE:

The chloroplasts are important because they are the sites where photosynthesis occurs. Chloroplasts contain chlorophyll. Photosynthesis occurs in two stages, the light reaction stage and the dark reaction stage. The chemical energy produced from this process is stored as sugar. Chloroplasts are plastids that develop in the cells of plant leaves. Chloroplast contains soluble molecules, to match the concentration of the isolation with that in the chloroplast. A concentration too low could result in the chloroplast being rupture due to water rushing into the chloroplasts. If the concentration is too high, water will leave the chloroplast and may disrupt the functioning of the chloroplast.

REQUIREMENTS:

- fresh leaves
- 0.5M Sucrose
- muslin cloth
- mortar &pestle
- scalpel
- centrifuge tube
- glass slide
- cover slip
- microscope

PROCEDURE:

i) The mid rib of few leaves was removed.
ii) The leaves were cut into pieces using a blade.
iii) The pieces were placed on the mortar and were ground finely using a pestle.

iv) 5ml of ice cold sucrose (0.5M) was added and homogenized.
v) To the homogenized mixture, 5ml of sucrose solution was again added and mixed well.
vi) The mixture was then filtered through three layer of muslin cloth, placed on glass funnel.
vii) The collected filtrate was centrifuged at 1000rpm for 5min.
viii) The resultant supernatant was transferred into fresh centrifuge tube.
ix) Centrifugation was done at 2000rpm for 10 min.
x) The resultant pellet was dissolved in 1ml of 0.5M sucrose.
xi) Two drops of this suspension was taken on a slide and layered with cover slip and observed under microscope.

RESULT:

DISCUSSION:

Exp. No.: 5
Date:

Embryogenesis

Aim:

To identify various stages of embryonic development in zebra fish *Danio (Brachydanio) rerio*.

Principle:

Embryogenesis is the process by which the embryo forms and develops. In mammals, the term refers chiefly to early stages of prenatal development, whereas the terms fetus andfetal development describe later stages. Embryogenesis starts with the fertilization of the egg cell (ovum) by a sperm cell, (spermatozoon). Once fertilized, the ovum is referred to as a zygote, a single diploid cell.

Zebrafish is an important vertebrate model organism in scientific research. 75-80 % of the genome of the zebrafish matches that of humans and also because it multiplies very fast and within short time. It is used for endocrine and neurological studies. It has been modified by researchers to produce several transgenic strains.

Different stages of embryogenesis in Zebrafish–

Zygote : 0 - 0.75 hrs

Cleavage : 0.75 – 2.25 hrs

Blastula : 2.25 – 5.25 hrs

Gastrula : 5.25 – 10 hrs

Sedimentation : 10 – 24 hrs

Pharyngula : 24 – 48 hrs

Hatching : 48 – 72 hrs

Staging :

5 broad *periods* of embryogenesis - the zygote, cleavage, blastuIa, gastrula, segmentation, pharyngul, and hatching periods.

The approximate developmental stage of a living embryo can be determined by examining it with a dissecting stereo-microscope illumination and low magnification (about 10x).

Till gastrula stage, the development can be seen as such under the microscope. During the segmentation period the tail elongates, and if the embryo is left within its chorion, the tail eventually curves over the trunk and head so as to obscure the view. When this has happened, one must remove the embryo from its chorion to stage it, either manually with #5 watchmaker's forceps, or by treatment with a proteolytic enzyme.

Late in the pharyngula period the embryo, if removed from the chorion, swims away in response to touch; this can prevented by anesthesia in 0.003% tricaine (3-amino benzoic acid ethyl ester) at pH 7 .Repeated anesthesia and rinsing appears to slightly but significantly retard subsequent development. It is always better to stage embryos while they are alive rather than after killing and fixation.

Table 1: Periods of Early Development

Period - h - Description

Zygote - 0 - The newly fertilized egg through the completion of the first zygotic cell cycle

Cleavage - 3/4 - Cell cycles 2 through 7 occur rapidly and synchronously

Blastula - 2 1/4 - Rapid, metasynchronous cell cycles (8, 9) give way to lengthened, asynchronous ones at the midblastula transition; epiboly then begins

Gastrula - 5 1/4 - Morphogenetic movements of involution, convergence and extension form the epiblast, hypoblast and embryonic axis; through the end of epiboly

Segmentation - 10 - Somites, pharyngeal arch primordia, and neuromeres develop; primary organogenesis; earliest movements; the tail appears

Pharyngula - 24 - Phylotypic-stage embryo; body axis straightens from its the early curvature about the yolk sac; circulation, pigmentation, and fins begin development

Hatching - 48 - Completion of rapid morphogenesis of primary organ systems; cartilage development in head and pectoral fin; hatching occurs asynchronously.

Early larva - 72 - Swim bladder inflates; food-seeking and active avoidance behaviors

Requirements:

- Embryos of zebrafish
- Growth medium
- Dissecting microscope
- Incubator

Procedure:

- The male and female zebra fishes were separated and conditioned for 5-7 days by feeding them with protein rich feed.
- A male and a female pair were put in a tank or in the ratio of male to female 2:1. A mesh was placed at the bottom of the tank so as to prevent the fishes from eating their own embryos.
- The fishes were removed after 1 hour or more after the onset of light cycle.
- The embryos were collected from the bottom of the tank using a pipette.
- The embryos were transferred to the petridishes containing growth medium.
- They were immediately visualized under dissecting microscope for zygote stage.
- The embryos were incubated at 27°C.
- Likewise, staging of embryos at regular intervals were performed to visualize the series of embryonic development.

Observation:

Result:

Discussion:

Exp. No.: 06
Date:

MITOSIS CELL DIVISION IN ONION ROOT-TIP CELLS

AIM:

To observe different stages of mitotic cell division in onion root tip.

PRINCIPLE:

The root of the onion is constantly growing. Right underneath the root tip is an area of dividing cells called the meristem. In order to observe mitosis, actively dividing tissue need to be used.

Acetocarmine is a genetic stain by which chromosomes can be distinctly seen. It is a DNA specific stain like Feulgen stain, so that super coiled chromosome during different stages in onion root tip can be visualized.

MATERIALS:

Acetocarmine

1N HCl

Freshly sprouted onion, about 2-3 cm long root tip

Microscope slides

Razor blades

Cover slips

PROCEDURE:

1. An onion bulb with emergence of root was obtained and its tips were taken (cutting 2- 4 mm) and the rest of the root was discarded.
2. Root tip was covered in 1N Hcl on a watch glass for 2 min to soften it.
3. The root tip was taken with a forceps and blotted on a filter paper to remove Hcl.
4. The root tip was placed on a clean glass slide.
5. 2 – 3 drops of acetocarmine stain was added to it and the slide was gently heated by passing it back and forth. Care should be taken not to boil or evaporate the stain from the slide.
6. The staining procedure was done for 2 minutes.

7. A drop of water was added to the slide and coverslip was laid carefully.
8. Coverslip was gently tapped using blunt end of pencil so that the meristematic tissue of root tip is properly squashed and spread as thin layer of cells.
9. Slide is blotted on filter paper to remove excess stain.
10. Root tip was observed under microscope under low and high power of microscope in the meristematic region for different stages of cell division.

OBSERVATION:

RESULT:

DISCUSSION:

Ex.No:08
Date: **METAPHASE CHROMOSOME SPREAD PREPARATION**

AIM:

To culture leucocytes and to prepare metaphase chromosome spread for karyotyping.

PRINCIPLE:

Leukocytes are used because they have a nucleus that is capable, they are prefferred over erythrocytes. The main use of DNA is to separate out the leukocytes from the entire mixture of the blood.

Colcomid arrests the mitotic cultured cells in metaphase and it should be treated carefully since its mutagenic, tumorgenic and tetragenic. Hypotonic treatment is essential because it moves the chromosomes from a more central position to a more peripheral position where they can be stretched more effectively during mitotic swelling.

The role of fixative is to freeze or stop the cells at the desired stage of cell division without causing distortion, swelling or shrinkage of the chromosomes.

REQUIREMENTS:

For leucocyte culture:

- Fresh venous blood
- Heparin or Trypsin EDTA
- Hikaryo RPMI medium
- Phytohemagglutinin (PHA)
- Fetal bovine serum (FBS)
- Tissue culture flask
- Centrifuge tubes
- CO_2 incubator
- Centrifuge

For metaphase spread:

- 10mg/ml colcemid
- Centrifuge tubes
- 0.075M KCL
- Absolute methanol and glacial acetic acid (3:1 mixture, freshly prepared)

- Ice cubes
- Slides and coverslips

PROCEDURE:

For leucocyte culture:

1. One ml of venous blood was drawn using a sterile syringe and transferred to a centrifuge tube containing 0.5ml of sodium heparin (1000 units/ml) or a pinch of EDTA.
2. 0.5 ml of blood was transferred to a T-25 flask containing 5ml of RPMI medium supplemented with 2µg/ml or 1-1.5% Phytohemagglutinin (PHA) and 5% fetal bovine serum.
3. The culture flask was incubated at 37ºC at 5% CO_2.
4. The culture flask can be gently agitated once or twice daily during the incubation period.
5. An increase in the leucocyte numbers can be noticed at the end of every day.
6. The flask was maintained for 3 days (66-72 hours).

For metaphase spread:

1. 100µl of 10µg/ml of colcemid was added to the leucocyte culture and incubated for additional 2 hours.
2. Entire content of the flask was transferred to a sterile centrifuge tube and centrifuged at 800 – 1000rpm for 10 minutes (at this point it is no longer necessary to be sterile).
3. Supernatant was discarded and the pellet was resuspended in 5ml of hypotonic 0.075M KCl solution and incubated in water bath at 37°C for 15 – 20 minutes.
4. Equal amount of freshly prepared ice cold fixative (Acetic acid: Methanol; 1:3) was added and centrifuged at 800 – 1000 rpm for 10 minutes at 4˚C.
5. The supernatant was discarded and 5ml of freshly prepared ice- cold fixative (Acetic acid: Methanol; 1:3) was added with constant mixing.
6. Cells were left at 4°C for 10-15 min and then centrifuged at 1000rpm for 10 minutes at 4˚C.
7. Step 5 and 6 was repeated for one more time.
8. The supernatant was discarded and the pellet was resuspended in 0.2ml of fresh fixative.

9. One drop of cell suspension was dropped on to a clean cold slide. (Let the drop run down the slide as it spreads)
10. The slide was dried rapidly over a hot plate or hot air oven for 15 minutes (The slide can be air-dried for 1 week).
11. This metaphase spread slide would be taken for karyotype analysis.

PRECAUTIONS:

- Contamination would be the frequent problem and hence maintaining aseptic environment is mandatory.
- Colcemid incubation time of less than 1 hour might result in reduced mitotic index.
- Use only freshly prepared ice cold fixatives.

RESULT:

DISCUSSION:

Ex.No:09

Date: **METAPHASE CHROMOSOME SPREAD PREPARATION (MITOTIC INDEX)**

AIM:

To calculate the mitotic index of the given metaphase spread.

PRINCIPLE:

Mitotic index is a measure for the proliferation status of a cell population. It is defined as the ratio between the number of cells in mitosis and the total number of cells. The mitotic index is an important prognostic factor predicting both overall survival and response to chemotherapy in most types of cancer. It may lose much of its predictive value for elderly populations, for example a low mitotic index loses any prognostic value for women over 70 years old with breast cancer. Additionally, the mitotic index is simply a measurement to determine the percentage of cells undergoing mitosis. Mitosis is the division of somatic cells when genetic information from one single cell is equally dispersed into two daughter cells. Durations of the cell cycle and mitosis vary in different cell types. An elevated mitotic index indicates more cells are dividing, and thus obvious in cancer cells. The mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals,as well as cellular repair the size of injury.

REQUIREMENTS:

- Metaphase spread slide
- Giemsa stain
- Cover slip
- Microscope

PROCEDURE:

1. The prepared metaphase spread slide was stained with Giemsa stain for 15 – 20 mins.
2. After staining, the slide was gently washed with distilled water dropwise to remove excess stain (Do not wash off the smear by excessive washing).
3. The slide was air dried.
4. The slide was then observed under the microscope for counting number of dividing cells and non dividing cells.
5. Mitotic index can be calculated using the calculation below.

Mitotic index = $\frac{\text{Total no. of cells (No.of divided + No. of non-divided)}}{}$

NOTE:
Good metaphase spread slide is needed for finding mitotic index.

RESULT:

DISCUSSION:

EXP.NO:10
DATE: **G-BANDING**

AIM:

To identify the banding patterns in metaphase chromosome using Giemsa stain.

PRINCIPLE:

Types of banding:

G-banding-obtained with giemsa stain following digestion with trypsin. The dark regions tend to be heterochromatic,later replicating AT rich and inactive. The light regions tend to be euchromatic,early replicating and GC rich.

R-banding-reverse of G-banding.

C-banding-stains chromosomes.

Q-banding-fluoresecent pattern obtained using quinacrine for staining. Pattern of band is very similar to that seen in G-banding.

T-banding-visualizes telomeres.

It is used to produce a visible karyotype by staining condensed chromosomes. It is useful for identifying genetic diseases by making the bands appear on the chromosomes. It stains the human cells in purple color and bacterial in pink.

RE**QUIREMENTS:**

For leucocyte culture:

- Trypsin solution (1ml of 0.25% trypsin was mixed with 49ml 0.85% NaCl)
- Salt solution (0.85% NaCl)
- Metaphase spread sheet
- Giemsa stain
- Coplin jar
- Microscope

PROCEDURE:

7. The metaphase spread slides that has been completely dried was taken and dipped in the trypsin solution for 5-30 seconds. (The time of trypsin is dependent on slide preparation conditions, harvesting conditions, material being banded, etc.,).
8. The slide was then dipped salt solution for 10 seconds.
9. The slide was then transferred to a coplin jar containing giemsa stain.
10. Staining was done for 4 minutes.
11. The slide was then gently washed with distilled water dropwise to remove excess stain (Do not wash off the smear by excessive washing)
12. The slide was air dried.

13. The slide was then observed under microscope for G-banding (under 100X magnification with oil).

Note:

1. Prepare the staining solution the day prior to use. Also, slides should be aged at lease 7 -10 days or placed in a 55-65 degrees C oven for 45 minutes before staining, to ensure excellent banding patterns. Aging the slides helps to eliminate fuzzy banding and increase contrast of the bands.
2. Exact timing is important; therefore, a maximum of 5 slides should be stained at one time. Optimum time in the stain appears to be between 2.5-4 minutes. The exact time will vary by several seconds depending on the source of cells; age of slides, the cell concentration on the slide etc.,

PRECAUTIONS:

a) over – trypsinized chromosomes appear fuzzy, somewhat difficult to recognize exact bands.
b) Under-trypsinized chromosomes will have indistinct bands, decreased contrast; very difficult or impossible to determine bands.
c) Adequate trypsinized chromosomes will show telomeres not overly digested and G bands will appear sharp and in contrast.

RESULT:

DISCUSSION:

EXPERIMENT NO: 11
DATE:

POLYTENE CHROMOSOMES

AIM:

To remove the salivary gland chromosome of Chironomide larvae and to observe the polytene chromosomes.

PRINCIPLE:

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. Polytene chromosomes have characteristic light and dark banding patterns that can be used to identify chromosomal rearrangements and deletions. Dark banding frequently corresponds to inactive chromatin, whereas light banding is usually found at areas with higher transcriptional activity. Polytene chromosomes are also used to identify the species of Chironomid larvae that are notoriously difficult to identify. Each morphologically distinct group of larvae consists of a number of morphologically identical (sibling) species that can only be identified by rearing adult males or by cytogenetic analysis of the polytene chromosomes of the larvae. Karyotypes are used to confirm the presence of specific species and to study genetic diversity in species with a wide range. And also to study the insertion and deletion of the chromosomes.

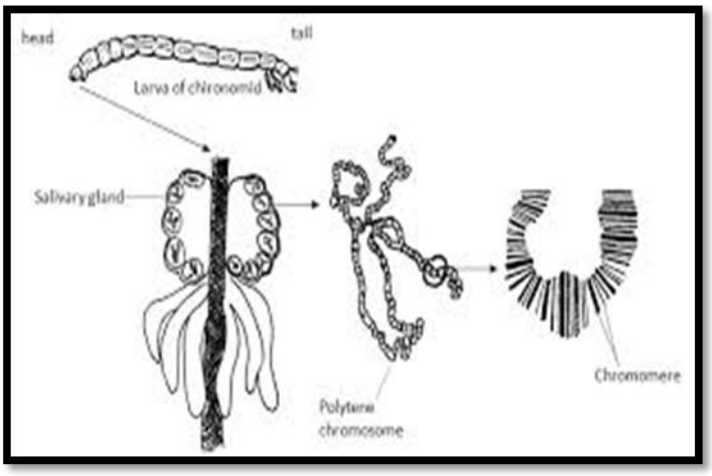
REQUIREMENTS:

- Dissecting microscope
- Third instar larva
- 1N HCL
- Physiological saline
- Acetocarmine stain
- Slides
- Cover slips
- Microscope
- Dissection microscope

PROCEDURE:

1. A drop of 0.7% NaCl was placed on a clean glass slide.
2. An appropriate late 3rd instar larvae (about 5 days old) was transferred to the slide on the stage of a dissection microscope.
3. The posterior end of the larva was finely grasped with forceps and using dissecting needle, the head located just behind the dark pigmentation mouth part was pierced.
4. Using a continuous motion, the needle was pulled away from the body to remove the salivary gland.

5. Salivary glands are recognized by the following feature: They are seen as a pair of whitish translucent elongated structures connected at their anterior ends with a common salivary duct. The fat bodies adhering to glands was removed.



6. The used saline and debris from the slide was removed and the salivary gland alone was place it in 1N HCL for 10 sec.
7. The excess fixative was wiped off with a piece of filter paper (the total duration of fixation should not exceed 20 seconds since longer fixation makes chromosomes brittle and difficult to spread).
8. The slide was then stained with few drops of 2% acetocarmine for 30 minutes.
9. A coverslip was placed on it and gently squashed with blunt end of the pencil (tapping breaks the cell and nuclear membranes and releases chromosomes free in cytoplasm; a very slight movement of cover glass on the slide may be desirable but too strong a tapping would break chromosomes in pieces).
10. Excess stain was removed by gentle blotting with tissue paper and viewed under the microscope for polytene chromosomes.

PRECAUTIONS:

1. Salivary glands from larvae that have not reached the late third instar stage or that are weak due to over-crowding or growth at higher temperature, do not provide

well spread, thick and distinctly banded chromosomes due to their reduced levels of polyteny.

2. Any trace of grease on slides or cover glasses hampers good spreading of chromosomes. The slides and cover glasses must be kept in 95% alcohol for some time and should be wiped dry

3. Just before use with fresh clean and soft cloth - examination of the slide and cover glasses under reflected light (from a lamp) will reveal if traces of oiliness or fibers etc. are present on their surface.

4. Excessive tapping may lead to breaking of individual chromosome arm into pieces.

5. Any lateral movement of the coverglass relative to the slide during squashing results in chromosomes being “rolled” and fragmented: such chromosomes appear as small “rolls” of homogeneously stained material.

6. Imbalanced salt concentrations may cause poor morphology of the chromosomes (the bands do not appear "crisp") or the nuclei may not open up at all (most of the polytene nuclei remain rounded up with their nuclear membrane remaining intact).

RESULT:

DISCUSSION: