Miranda House
University of Delhi

INSPIRE INTERNSHIP PROGRAMME 2024

Innovation in Science Pursuits for Inspired Research
An Initiative of DST, Govt of India

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Designing Life:
Small Experiments

Offered by:
Zoology Department

Inspire Internship Programme

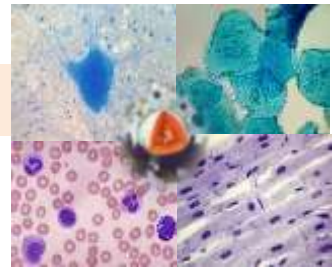
Innovation in Science Pursuit for Inspired Research
An initiative of DST, Government of India

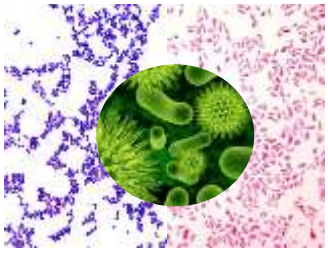
Workshop

Designing Life: Small Experiments

Cells are the building blocks of all living things. They provide structure to the body, take in nutrients from the food, convert these nutrients into energy, and carry out specialized functions. Cells are classified as *'prokaryotic'* and *'eukaryotic'* on the basis of nuclear organization. Bacteria, the most primitive cell type, are unicellular prokaryotes that are present in diverse habitats existing on earth. Evolution of bio-molecules and unicellular organisms resulted in more complex and advance body organization. Understanding these complexities and evolution of different life forms needs study on model organisms. *Drosophila* is one such favorite model organism in the field of biology which in itself is the encyclopedia of life.

***Building blocks of life*: Study different types of cells**





***Unicellular organisms:* Perform gram staining of bacteria**

Multicellular organisms:* Study the life cycle of *Drosophila melanogaster

Dr. Nisha Vashishta, Dr. Jyoti Arora, Dr. Pooja Suman,

Ms. Saba Zulfiquar, Dr. Reetuparna Basak, Dr. Bhawna Chuphal

BUILDING BLOCKS OF LIFE

NISHA VASHISHTA, JYOTI ARORA, POOJA SUMAN,
SABA ZULFIQUAR, REETUPARNA BASAK & BHAWNA CHUPHAL

AIM: To study different types of cells.

OBJECTIVE:

- To learn about the basic units of life.
- To distinguish between the different cells on the basis of their structure, function and location in the body.
- To prepare cells by using different stains to study them under the microscope.

INTRODUCTION:

Cells are the basic building blocks of all living things. The human body is composed of trillions of cells. They provide structure to the body, take in nutrients from food, convert these nutrients into energy, and carry out specialized functions.

Stem cells are mother cells that have the potential to become any type of cell in the body. One of the main characteristics of stem cells is their ability to self-renew or multiply while maintaining the potential to develop into other types of cells.

EQUIPMENT: Compound microscope

CHEMICALS/ SOLUTIONS: Methylene Blue, Giemsa stain, Glycerine, Distilled water

GLASSWARE/ PLASTICWARE: Slides, Cover slip, Forceps, Blotting paper, Dropper, Tooth pick, Needle, Scissors.

PRE ACTIVITY QUESTIONS

1. Define cell.
2. Name different types of cells.
3. Who gave the cell theory? And what is it?
4. Name different types of muscles.
5. What are striated muscle fibres?
6. What are nerve Cells?
7. What are the parts of the structure of the neuron?
8. What is connective tissue?
9. What are the components of connective tissues?
10. What is blood and various types of blood cells?

BACKGROUND

Robert Hooke (1665): looked at a thin slice of cork (oak cork) through a compound microscope observed tiny, hollow, room like structures called these structures 'cells' because they reminded him of the rooms that monks lived in only saw the outer walls (cell walls) because cork cells are not alive.

Anton van Leeuwenhoek (1680): looked at blood, rainwater, scrapings from teeth through a simple microscope (1 lens), observed living cells; called some 'animalcules', some of the small 'animalcules' are now called bacteria.

Matthias Schleiden (1838): discovered that plant parts are made of cells.

Theodor Schwann (1839): discovered that animal parts are made of cells.

Rudolph Virchow (1855): Stated that all living cells come only from other living cells.

EPITHELIAL TISSUE: It is the simplest tissue. It consists of cells arranged in continuous sheets in either single or multiple layers.

Functions:

- Protect the underlying cells from drying, injury and chemical effects.
- Forms lining of mouth and alimentary canal.
- Help in absorption of water and nutrients and elimination of waste products.
- Perform secretary function.

MUSCULAR TISSUE: It forms the contractile tissue and is made of muscle cells. Muscle cells are elongated and large-sized.
The movements of the body or limbs are brought about by contraction and the relaxation of contractile proteins present in muscle cells.

Functions:

- It provides the force for locomotion and all other voluntary movements of the body.
- It undergoes rapid contraction and involved in changing body postures.

NERVE CELL: The basic unit of the nervous system is the nerve cell, or **neuron**. There are approximately 28 billion neurons in the human body penetrating every tissue in every part.
Function: The basic function of the neuron is to transmit information.

CONNECTIVE TISSUE: Connective tissue supports, connects, or separates different types of tissues and organs of the body. Blood is a type of connective tissue which delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets.

Basophils

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine. The nucleus is bi- or tri-lobed. They are characterized by their large blue granules.

Lymphocytes

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes include, **B-Cells and T- Cells**

T-Functions of Blood Cells:

Red blood cells: They help in transport of oxygen to body tissues. They are flexible and oval biconcave discs. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second.

White blood cells: White blood cells or leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist.

Neutrophils defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus.

- They have a multi-lobed nucleus that may appear like multiple nuclei.
- They make up 60-70% of total leukocyte count in human blood.
- The life span of a circulating human neutrophil is about 5.4 days.

1. EPITHELIAL TISSUE (CHEEK CELLS)

- Mouth is rinsed with water.
- The inner side of check is scrapped with a tooth pick
- The scraped material is smeared on to the centre of glass slide, let it air dry.
- The material is then stained with 2-3 drops of the Methylene blue and left for 2 minutes
- The excess stain is washed off from the slide using 2-3 drops of distilled water from a dropper
- Drying of slide is done with blotting paper
- The stained material is mounted in water
- Cover slip is carefully placed on the mount and observed under the microscope.

2. STRIATED MUSCLE FIBRES

- Remove the outer covering of the coxa region of cockroach.
- Take very fine fibres out of the muscles of coxa on the slide.
- Put a 2-3 drops of methylene blue on the fibres.
- After 1-2 minutes, blot excess stain and put a drop of glycerine to it.
- Put the cover slip avoiding the air bubbles.
- Observe it under microscope.

3. NERVE CELLS

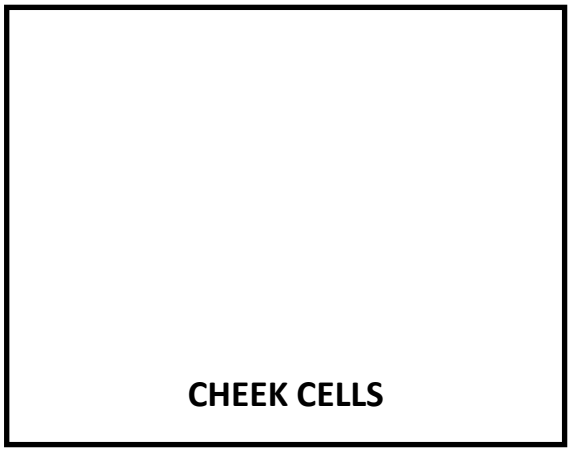
- Cut the nerve cord longitudinally.

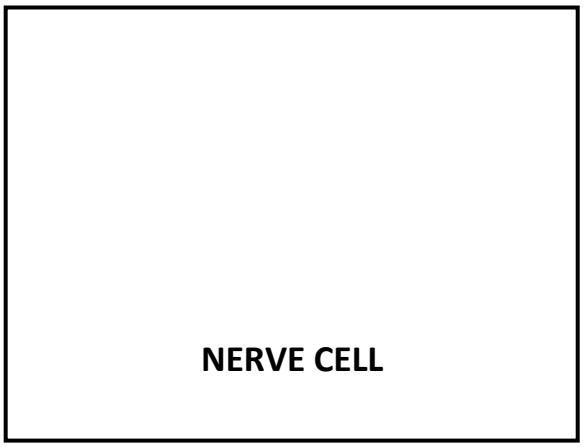
- Use blunt forceps; take out the nerve cell from gray matter.
- Tease it properly with needle.
- After teasing, put it on slide and add 2-3 drops of methylene blue.
- Leave the stain for 2 minutes.
- Blot the excess stain.
- Add a drop of glycerine and gently put the cover slip on the slide.
- After placing cover slip observe under microscope.

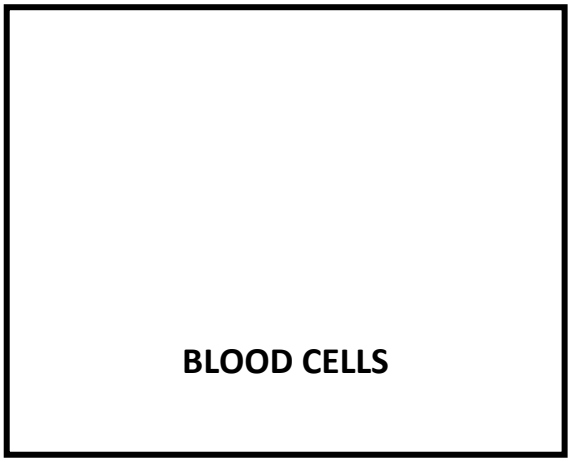
4. CONNECTIVE TISSUE (BLOOD CELLS)

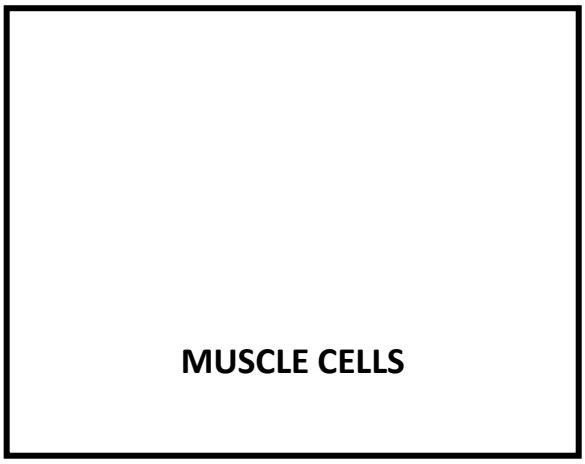
- Sterilize the finger with 90% alcohol.
- Prick the finger with sterilized needle.
- Remove the first drop of blood with cotton.
- Put a drop of blood towards the edge of the slide.
- Take another slide and place it at an angle of 45^{o} to the blood.
- Let the blood to spread on the edge.
- Gently, swap the 2nd slide on the 1st slide making a smooth smear.
- Let it air dry.
- Flood the slide with giemsa stain.
- Keep it for 2-3 minutes.
- Wash the slide with distilled water.
- Let it dry and observe under microscope.

OBSERVATIONS:









EPITHELIAL TISSUE

Structure:-

- The **apical (free) surface** of an epithelial cell faces the body surface, a body cavity, the lumen (interior space) of an internal organ. It may contain cilia or microvilli.
- The **lateral surfaces** of an epithelial cell face the adjacent cells on either side.
- The **basal surface** of an epithelial cell is opposite the apical surface. Hemi desmosomes in the basal surfaces of the deepest layer of epithelial cells anchor the epithelium to the basement extracellular membrane.
- The **basement membrane** is a thin extracellular layer that commonly consists of two layers, the basal lamina and reticular lamina.
- Epithelial tissue has its own nerve supply, but is **avascular** (*a*- without; *vascular* vessel); that is, it lacks its own blood supply. The blood vessels that bring in nutrients and remove wastes are located in the adjacent connective tissue.
  Human cheek cells are squamous epithelium cells.

Nature: - thin, irregular-shaped cells
Occurrence: - forms the delicate lining of mouth, oesophagus, nose and blood vessels.
Function:-protection, forms a selectively permeable surface through which filtration occurs.

MUSCULAR TISSUE

Nature: - The Entire muscle fibres show alternate dark and light striations, so they are called the **striped muscles**. Striated muscle fibres (cells) are long (30-40 cm) or elongated, non-tapering and cylindrical, unbranched. Each muscle cell is enclosed in thin plasma membrane called **sarcolemma**. Its nuclei are peripheral in position. In the sarcoplasm (cytoplasm) of the muscle cells are embedded large number of contractile elements called **myofibrils.**
These muscles work according to our will. (**Voluntary muscle)**
Occurrence:-It occur in muscles of limbs (e.g. biceps and triceps of arms), body wall, face, neck, tongue, pharynx.

NERVE CELL

Structure: Neurons vary greatly in size and shape, with the longest ones—those that extend down the leg as part of the sciatic nerve-measuring over one meter.
All nerve cells have a similar structure. It consists of 3 distinct portions:

(i) Cell body- The cell body of a neuron is called cyton, perikaryon or soma. It contains abundant granules cytoplasm and a large nucleus.
(ii) Dendrites- Dendrites are branched extensions of the cytoplasm of cell body and they conduct impulse toward the cell body.
(iii) Axon-Axon is a longer process and branches distally into many fine filaments called **Telodendrites.** It take the impulse away from the body.

CONNECTIVE TISSUE:

One microliter of blood contains: **4.7 to 6.1 million (male), 4.2 to 5.4 million (female) erythrocytes:** Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles in mammals.

- **4,000–11,000 leukocytes:** White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances.

- **200,000–500,000 thrombocytes:** Also called platelets, thrombocytes are responsible for blood clotting (coagulation).

PRECAUTIONS:

- Slide and coverslip should be dry and clean.
- Overstaining and understaining should be avoided
- Air bubbles should be avoided
- Smearing of the scrapped material from the cheek onto the slide should be done gently.

POST ACTIVITY QUESTIONS

1. Where are Squamous Epithelial Cells located?
2. What are voluntary and involuntary muscle fibres? And to which Striated muscle cell fibres belong?
3. What are the functions of Neuron?
4. Why do we consider gray matter for this experiment?
5. What is the role of RBCs?
6. What is the clinical significance of different WBCs?

GRAM STAINING OF BACTERIA

JYOTI ARORA & SABA ZULFIQUAR

AIM: To perform Gram staining of bacteria.

OBJECTIVES:

- To learn to perform Gram staining.
- To be able to differentiate between different Gram-positive and Gram-negative bacteria.
- To understand the differences between cell wall structure of Gram-positive and Gram-negative bacteria.
- To observe different shapes of bacteria.

INTRODUCTION:

Gram staining of bacteria is a valuable diagnostic tool. The technique is named after its inventor, the Danish scientist, **Hans Christian Gram** (1884). It is the most widely used differential staining procedure, which is used to divide bacteria into two groups based on differences in cell wall structure: Gram positive and Gram negative.

PRE ACTIVITY QUESTIONS

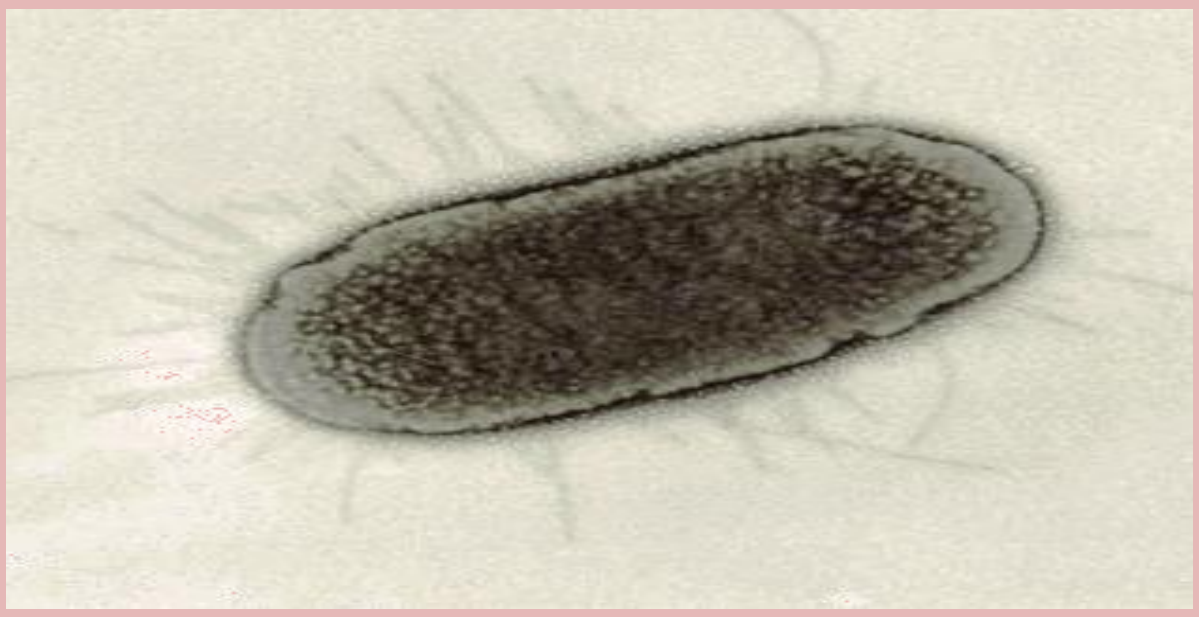
1. What is cell wall?
2. What is the difference between cell wall and cell membrane?
3. What is staining?
4. What types of bacteria are present in curd?
5. Which type of bacteria is *E. coli*?

BACKGROUND

Prokaryotes, the bacteria, are the simplest organisms. Prokaryotic cells are small, consisting of cytoplasm surrounded by a plasma membrane and encased within a rigid cell wall, with no distinct interior compartments. Most bacteria are encased by a strong **cell wall** composed of *peptidoglycan,* which consists of a carbohydrate matrix (polymers of sugars) that is cross-linked by short polypeptide units.

Bacteria can be classified as Gram positive or Gram negative. **Gram-positive** bacteria have a thick, single-layered cell wall that retains a violet dye from the Gram stain procedure, causing the stained cells to appear purple under a microscope. More complex cell walls have evolved in other groups of bacteria. In them, the wall is multilayered and does not retain the purple dye after Gram staining; such bacteria exhibit the background red dye and are characterized as **Gram negative** such as *E.coli.*

A prokaryotic cell is like a one room cabin in which all activities occur in the same room. There are no membrane bound organelles. Thus, the DNA, enzymes, and other cytoplasmic constituents have access to all parts of the cell. Reactions are not compartmentalized as they are in eukaryotic cells, and the whole bacterium operates as a single unit. The entire cytoplasm of a bacterial cell is one unit with no internal support structure. Consequently, the strength of the cell comes primarily from its rigid wall. Bacteria are very important in the economy of living organisms. They harvest light in photosynthesis, break down dead organisms and recycle their components, cause disease, and are involved in many important industrial processes.



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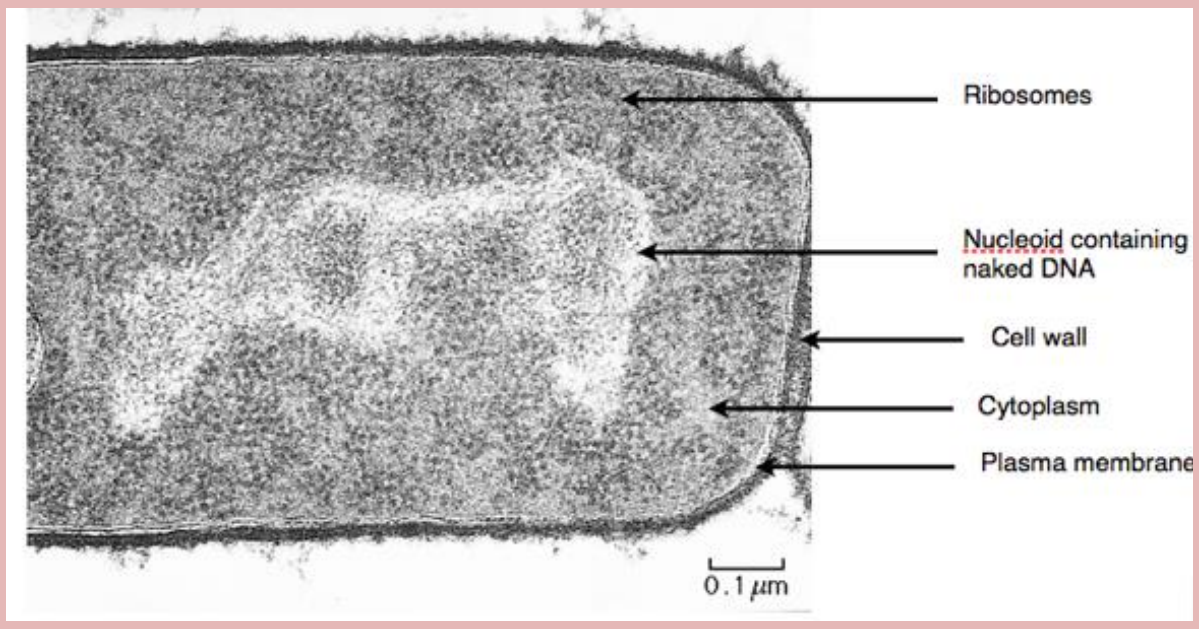
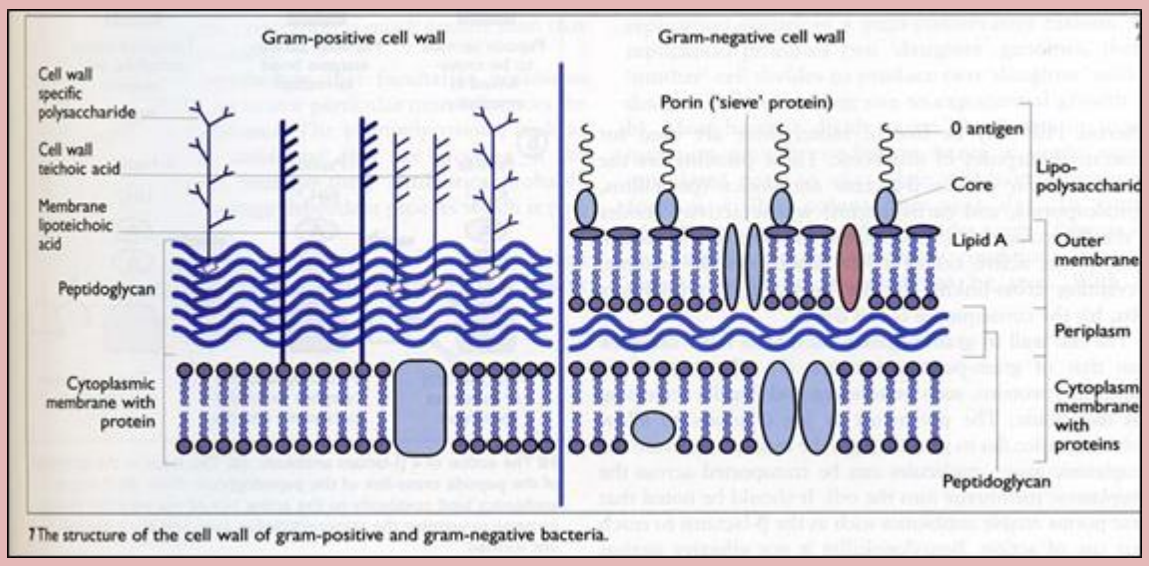


B

Electron Micrographs of a prokaryote, *Escherichia coli* (a bacteria).

A) complete view
B) a portion enlarged

Gram-positive Bacteria	Gram-negativeBacteria
Thick mesh-like cell wall made of peptidoglycan (50-90%)	Have a thinner layer of peptidoglycan (10% of cell wall)
Stained purple by a primary stain - crystal violet	Stained pink by counter-stain – safranin
Resist decolourising action of an alcoholic solution (95% alcohol)	Cannot resist the action of decolourizer.
Sensitive to detergents and antibiotics	More resistant



The structure of the cell wall of gram-positive and gram-negative bacteria.

MATERIALS REQUIRED:

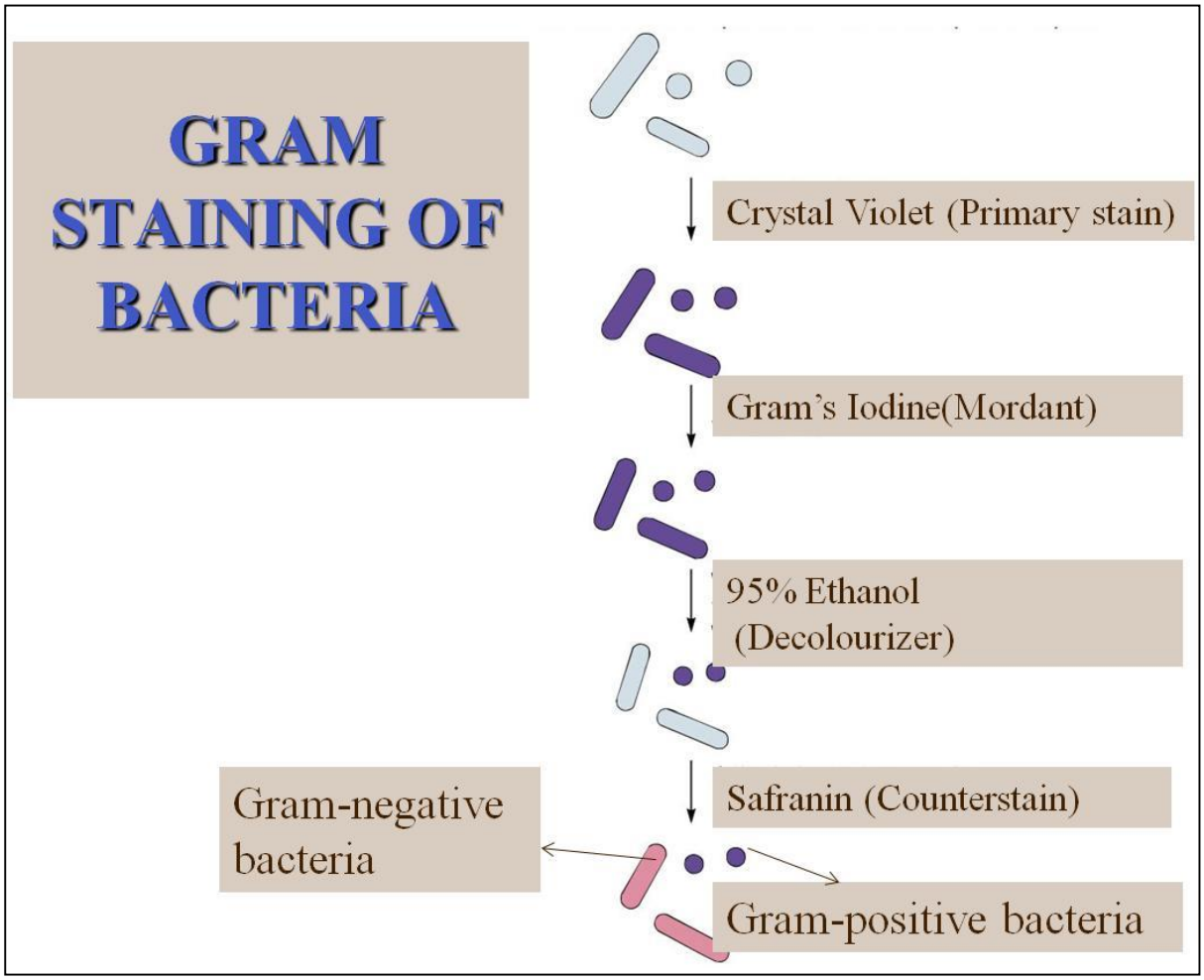
Equipment: Compound microscope

Chemicals: Culture of *Lactobacilli* (Curd) and *Escherichia coli*, Gram stain (crystal violet, 0.1% Iodine solution, safranin, 95% ethanol)

Glassware: slides, cover slip, burner cavity block, filter paper, forceps, dropper, glass rods and distilled water

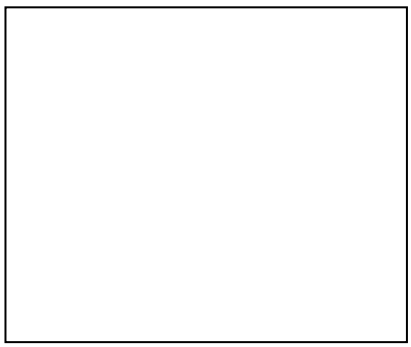
PROCEDURE:

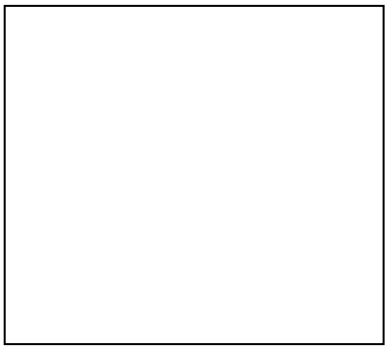
1. Label the slide.
2. Put a drop of bacterial culture on the slide.
3. Spread the bacterial culture on the slide making an even smear by circular motions and air dry it.
4. Heat-fix the bacteria to the slide by gently warming the opposite side of the slide for approximately 30 seconds.
5. Place slide on glass rods over a sink.
6. Flood the surface of the slide with Crystal Violet stain and leave for one minute.
7. Rinse the slide with distilled water.
8. Flood the slide with Gram's Iodine and leave for one minute.
9. Rinse the slide with distilled water.
10. Gently wash the slide with Gram's decolorizer (95% ethanol) keeping slide at an angle till there is no blue tint in the outflow (30secs).
11. Flood the slide with Safranin and wait for one minute.
12. Rinse the slide with distilled water.
13. Blot the slide and scan it under the microscope.



OBSERVATION: Purple, rod-shaped bacteria are seen in culture from curd. Other round bacteria in the culture did not take up the stain. The rod-shaped bacteria which stained purple were *Lactobacilli* and they were Gram-positive. *E. coli* are seen as pink, rod-shaped and identified as Gram-negative.

Microscopic View





DISCUSSION:

The components of Gram stain work as follows:

1. Crystal Violet (Primary Stain): Crystal violet (CV) dissociates in aqueous solution into CV^+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV^+ ion interact with negatively charged components of bacterial cells and stain them purple.

2. Iodine (Mordant): Iodine (I^- or I_3^-) interacts with CV^+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and, therefore, colors the cell.

3. 95% Ethanol (Decolorizer): Decolorizer such as alcohol or acetone interacts with the lipids of the cell membrane. Gram-negative cell will lose its outer lipopolysaccharide membrane, and the inner peptidoglycan layer is left exposed. The CV–I complexes are washed from the Gram-negative cell along with the outer membrane. A Gram-positive cell becomes dehydrated by ethanol. The large CV–I complexes become trapped within the Gram-positive cell due to the multilayered nature of its peptidoglycan. After decolorization, the Gram-positive cells remain purple while the Gram-negative cells lose purple color.

4. Safranin (Counterstain): Safranin (positively charged) is applied last and it imparts a pink or red color to the decolorized Gram-negative bacteria. (The proteinaceous components of the cell cytoplasm) help in the process.

PRECAUTIONS:

- Young cultures (18-24 hrs old) are used as older cultures lose their Gram staining properties due to changes in their cell wall.
- Staining and decolorization is more uniform with thin smears than with thick, uneven smears.
- Fresh reagents of proper strength should be used.
- Decolorizer should not be left for long on the slide as long exposure to decolourizer will remove the crystal violet stain from both Gram-positive and negative cells.

REFERENCES:

Harold J. Benson, 2002. Microbiological Applications: Laboratory Manual in General Microbiology. Mc-Graw Hills, 478pp.

POST ACTIVITY QUESTIONS

1. What is differential staining?
2. What is the significance of Gram staining?
3. Why is it important to fix cells on the slide?
4. What will be the impact of using thick smears on the staining?
5. Why old cultures should not be used?
6. Why is decolorizing time critical in Gram staining technique?
7. Why are Gram-positive bacteria more resistant to antibiotics?

DROSOPHILA -*A VERSATILE MODEL* IN BIOLOGY AND MEDICINE

NISHA VASHISHTA, POOJA SUMAN & BHAWNA CHUPHAL

AIM: To study the life cycle of *Drosophila melanogaster*.

OBJECTIVE:
- To learn about the Holometabolous metamorphosis.
- To observe the different stages of life cycle.
- To know about the multidisciplinary aspects of *Drosophila melanogaster*
- To learn about diet preparation and maintenance of culture.

INTRODUCTION:

Many obvious practical and ethical obstacles severely limit the scope for experiments using humans in biomedical science, thus much of what we know about the underlying biology of cells and tissues comes from studies using model organisms such as the fruit fly *Drosophila melanogaster*. *Drosophila* has been used productively as a model organism for over a century to study a diverse range of biological processes including genetics and inheritance, embryonic development, learning, behavior and ageing.

EQUIPMENTS: Stereo-zoom dissecting microscope

CHEMICAL SOLUTIONS: Ethyl acetate

GLASSWARE/ PLASTICWARE: Petri-dishes, forceps, brush and slides.

PRE ACTIVITY QUESTIONS
1. Who is Cindrella of Genetics?
2. Define an insect.
3. What is metamorphosis?
4. What are the stages of Holometabolous metamorphosis?
5. What is the contribution of T.H. Morgan in genetics?
6. Why *Drosophila* is selected as model organism?

BACKGROUND

***Drosophila* as a Model Organism**

Drosophila has always held a special place in biology and serves as a model organism for a diverse range on experimental studies carried out globally. Numerous scientists have radically shaped the research communities based on their work on *Drosophila.* Most prominent among these, all of whom have been awarded Nobel prizes are - TH Morgan, HJ Muller, Jules A Hoffmann and many others.

Features that make Drosophila an ideal study model include,
- Short life span with a short generation time of about 13-15 days
- Easy maintenance of culture in laboratories
- Distinct sexual dimorphism and general characters
- *Drosophila* genome is 60% homologous to that of humans, less redundant and about 75% of the genes responsible for human diseases have homologs in flies
- Production of a large number of eggs within a short period
- Orthologous homology between *Drosophila* and Chordate *Hox* genes

CLASSIFICATION

PHYLUM: Arthropoda (jointed appendages)
CLASS: Insecta (three pairs of legs)
SUBCLASS: Pterygota (wings present)
DIVISION: Endopterygota (wings are developed inside the body)
ORDER: Diptera (1 pair of wings, hindwings are reduced to form balancing organs; Halteres)
FAMILY: Drosophilidae
GENUS: *Drosophila*
SPECIES: *melanogaster*

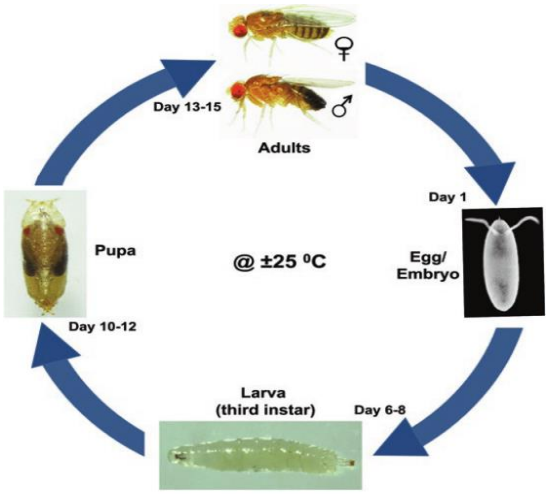
There has been a long history of using Drosophila genetics as a tool for understanding biology dating back to Morgan's experiments over 100 years ago. Thomas Hunt Morgan used the fly to prove the chromosomal theory of inheritance showing that the *white* gene resided on the X chromosome, a finding for which he received a thoroughly deserved Nobel Prize.

GENERAL FEATURES

- Cosmopolitan small fruit flies that are often found near ripe/rotting fruit.
- Body divided into head, thorax and abdomen.
- 2 pairs of wings arise from thorax. Hind wings reduced to balancing organs termed as halteres.
- 3 pairs of thoracic legs. Males have sex comb on foreleg at tarsal segment.
- Sucking sponging mouth parts in adult.
- Pair of Aristate antennae and compound eyes are present

LIFE CYCLE

- Internal fertilisation & holometabolous development.
- Complete metamorphosis with distinct larval and pupal stage.
- The adult and larval stage differ in habit and morphology.
- Courtship behaviour: Mating dance by male accompanied by foot tapping.
- Virgin females become receptive to copulation 8-10 hours after hatching.



METHODOLOGY

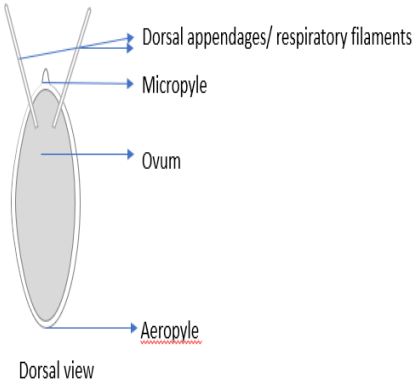
To study the life cycle of *Drosophila melanogaster*, the culture is developed on Corn flour-agar based diet at 25^0C. All the developmental stages are extracted from the diet. Egg, Larvae are present over the diet, pupae are attached to the walls of culture bottle and adults are actively moving on the culture.

DEVELOPMENTAL STAGES:

EGG-

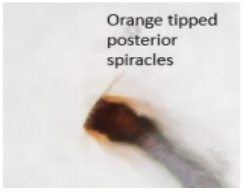
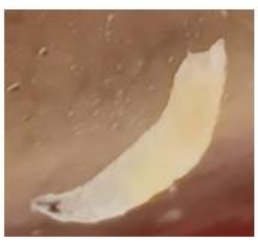
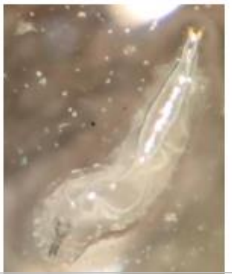
• Ovoid, white, covered by tough and thick chorion envelope.
• 2 anteriorly projecting dorsal appendages that aids in
  - Respiratory exchange
  - Substrate attachment to prevent sinking into semi solid media.

• Micropyle is a small opening present anteriorly. It acts as transport route for sperm.



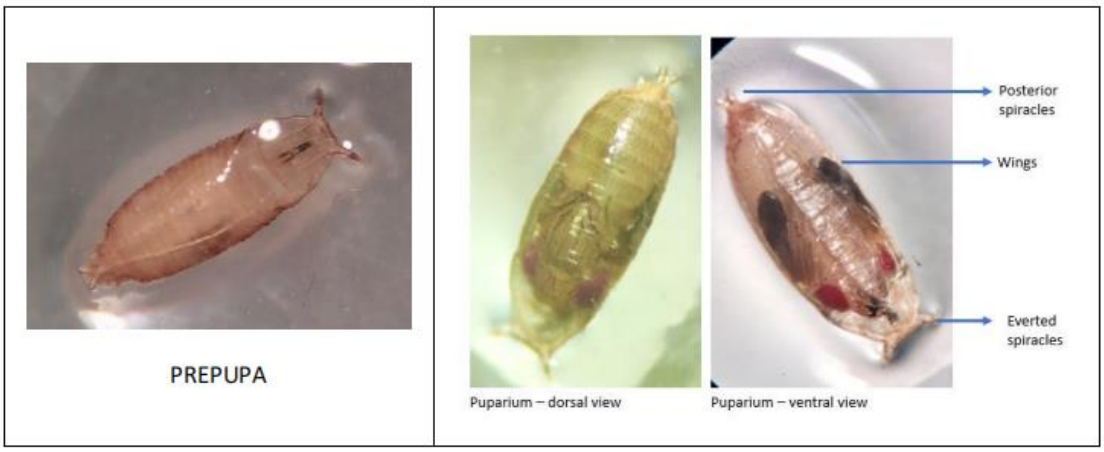
LARVAL STAGES-

• 3 distinct instar larva present that undergo 2 moultings and exhibits sequential progression in 2 features – mouth parts & spiracles.
• All stages have white, segmented, worm like body with a pair of anterior and posterior spiracles for respiratory exchange.
• Larvae have biting chewing mouth parts with darkly pigmented jaws and hooks.

1st instar larvae	*2nd instar larvae*	*3rd instar larvae*
Smallest with indistinct jaw and hooks	Distinct jaw and hooks present Voracious feeders	Well-developed jaw and hooks system
Small spiracles present	Club shaped anterior spiracles	Branched anterior spiracles  Branched anterior spiracles of L3
Unpigmented posterior spiracles	Unpigmented posterior spiracles	Posterior spiracles with an orange pigmented ring 
		

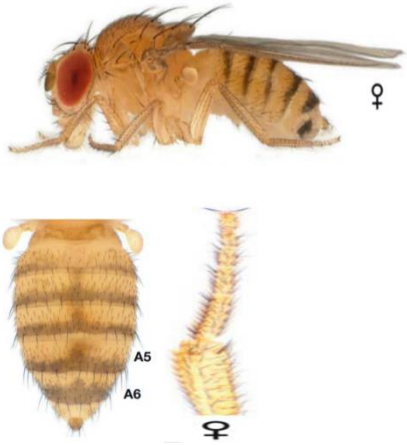
PUPA-

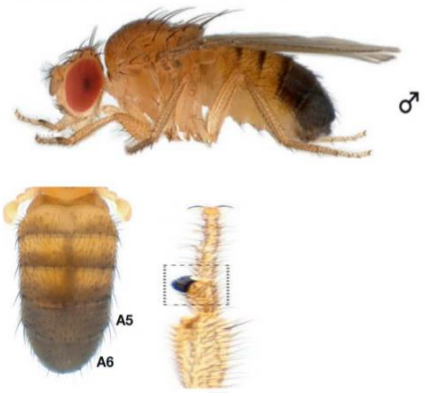
- The 3rd instar eventually stops feeding and finds a pupation site.
- The body shortens, the cuticle shrinks and hardens and the pre-pupa forms.
- Pre-pupa is headless and wingless and everts its anterior spiracles to form pupa.
- Wing and head develop in pupa. The mouth parts are released.
- Eclosion occurs after 4-6 days.



ADULTS-

FEMALE	MALE
• Larger in size. • Tip of the abdomen is elongated. • Abdomen has eight segments with 7 being clearly visible. • Abdominal tip is less darker than male. • Genital appears less darker. • Sex comb is absent.	• Smaller in size • Tip of abdomen is rounded. • Last three abdominal segments are fused, so only 5 segments are clearly visible. • Abdominal tip is much darker than female. • Male genital plate appears rougher and darker. • Possess a sex comb on the 1st tarsal segment of 1st pair of legs.





OBSERVATIONS:

EGG

LARVAE

PUPA

FEMALE

MALE

PRECAUTIONS:

- Culture should be handled carefully.
- Use of needle should be avoided.
- Brush should be used to take the life cycle stages.
- Handle the microscope carefully.

POST ACTIVITY QUESTIONS:

1. What are the various stages in life cycle of *Drosophila melanogaster*?
2. How long adult *Drosophila* takes to develop?
3. What is the function of respiratory filaments in egg?
4. Which larval stage have branched anterior spiracles and orange ringed posterior spiracles?
5. What is pupation?
6. What is difference between molting and metamorphosis?