B.L.D.E. Association's

S.B. ARTS AND K.C.P. SCIENCE COLLEGE

VIJAYAPUR- 586103

Karnataka State

B. SC I SEMESTER

PRACTICAL LAB MANUAL

DEPARTMENT OF ZOOLOGY

2023-24

LAB COURSE CONTENT

1. Understanding of simple and compound microscopes.
2. To study different cell types such as buccal epithelial cells, neurons, striated muscle cells using, Methylene blue or any suitable stain (Virtual/slaughtered tissue).
3. To study the different stages of Mitosis in root tip of *Allium cepa.*
4. To study the different stages of Meiosis in grasshopper testis (Virtual).
5. Study of parasites in human
   a. Protozoans
   b. Helminthes
6. To learn the procedures of preparation of temporary and permanent stained slides with available mounting materials.
7. Study of mutant phenotypes of *Drosophila* sp. (from Culture or Photographs).
8. Preparation of polytene chromosomes (*Chironomus* larva or *Drosophila* larva).

Experiment - 1

UNDERSTANDING OF SIMPLE AND COMPOUND MICROSCOPES

The optical microscope often referred to as the light microscope, is a type of microscope that uses visible light and a system of lenses to magnify images of small subjects.

There are two basic types of optical microscopes:
1. Simple microscopes
2. Compound microscopes.

1. Simple Microscope:

A simple microscope is one that uses a single convex lens for magnification, such as a magnifying glass.

Principle:

A simple microscope works on the principle that when a tiny object is placed within its focus, a virtual, erect and magnified image of the object is formed at the least distance of distinct vision from the eye held close to the lens.

Instrumentation of Simple Microscope:

(i) Mechanical parts
(ii) Optical parts

(i) Mechanical Parts

These parts support the optical parts and help in their adjustment for focusing the object.

They include the following components:

1. Metal Stand:
   It has a heavy base plate and a vertical rod fitted to it, which provide support and stability to other parts of the microscope.

2. Stage:

- It is a rectangular metal plate fitted to the vertical rod.
- It has a central hole for light to pass from below.
- Slide with the specimen to be observed is kept on the stage, in such a way that, the specimen remains just on the central hole.
- Some microscopes have a pair of slanting wings projecting from both sides of the stage. They provide support to hand for manipulating the object.

(ii) Optical Parts

These parts are involved in passing the light through the object (specimen) and magnifying its size.

The components of the optical parts are as follows:

1. Mirror:
   - A plano-convex mirror is fitted below the stage to the vertical rod by means of a frame.
   - It focuses the surrounding light on the object to be observed.

2. Lens:
   - A biconvex lens is fitted above the stage, to the vertical rod, by means of a frame.
   - It magnifies the size of the object and the enlarged virtual image formed is observed by keeping the eye above it.
   - For proper focusing, the lens can be moved up and down by the frame.

2. Compound Microscope:

The term microscope can be split into two separate words, 'micro' and 'scope', where the term 'micro' means small or tiny, and 'scope' means to view or to observe. Therefore, a microscope can be understood as an instrument to observe tiny elements.

The term "compound" in compound microscopes refers to the microscope having more than one lens.

Parts of Compound Microscope

1. Eyepiece and body tube.
   - The eyepiece is the lens through which the viewer looks to see the specimen.
   - It usually contains a 10X or 15X power lens.
   - The body tube connects the eyepiece to the objective lenses.

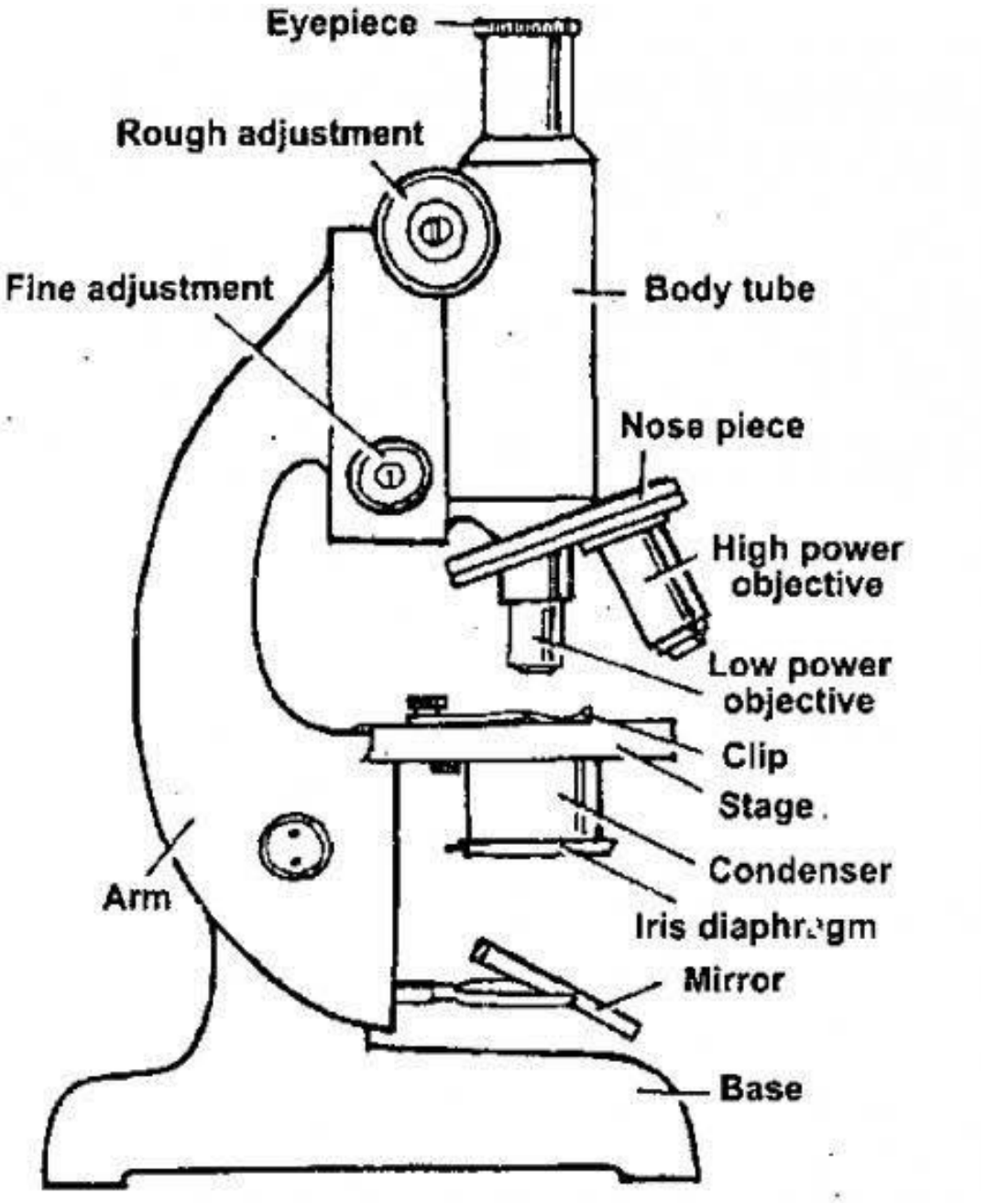
2. Objectives and Stage Clips
   - Objective Lenses are one of the most important parts of a Compound Microscope.
   - They are the closest to the specimen.
   - A standard Microscope has three to four Objective Lenses which range from 4X to 100X.
   - Stage Clips are metal clips that held the slide in a place.

3. Arm and Base
   - The Arm connects the Body Tube to the base of the Microscope.
   - The Base supports the Microscope and its where Illuminator.

4. Illuminator and Stage
   - The illuminator is the light source for a microscope.
   - A compound light microscope mostly uses a low voltage bulb as an illuminator.
   - The stage is the flat platform where the slide is placed.

5. Nosepiece and Aperture
   - Nosepiece is a rotating turret that holds the objective lenses.
   - The viewer spins the nosepiece to select different objective lenses.
   - The aperture is the middle of the stage that allows light from the illuminator to reach the specimen.

6. Condenser, Iris diaphragm, and Diaphragm
   - A condenser gathers and focuses light from the illuminator onto the specimen being viewed.
   - Iris diaphragm adjusts the amount of light that reaches the specimen.
   - The diaphragm is a five holed disk placed under the stage.
   - Each hole is of a different diameter. By turning it, you can vary the amount of light passing through the stage opening.

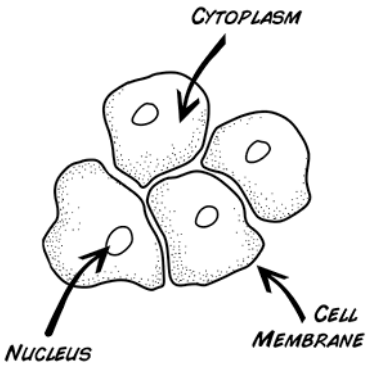


Experiment - 2

TO STUDY DIFFERENT CELL TYPES SUCH AS BUCCAL EPITHELIAL CELLS, NEURONS, STRIATED MUSCLE CELLS USING, METHYLENE BLUE OR ANY SUITABLE STAIN (VIRTUAL/SLAUGHTERED TISSUE)

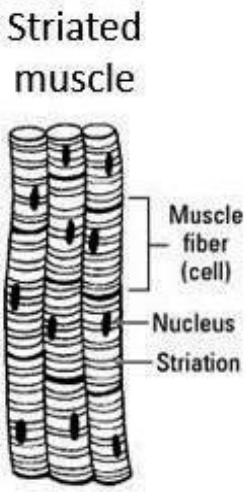
(A) Squamous epithelium :

These are simple epithelial tissue, consist of very thin and flattened, roughly hexagonal Cell. They are closely fitted to give a mosaic
Appearance.



1. The width and depth of the cell is greater than its height.
2. Each cell consists of a distinct, rounded Nucleus in the centre. The cells have faintly stained Granular cytoplasm.
3. The basal surface of the tissue adheres to the basement membrane.
4. It is found in the lining of the lungs, Bowman's capsule and blood vessels.

(B) Striated Muscle :



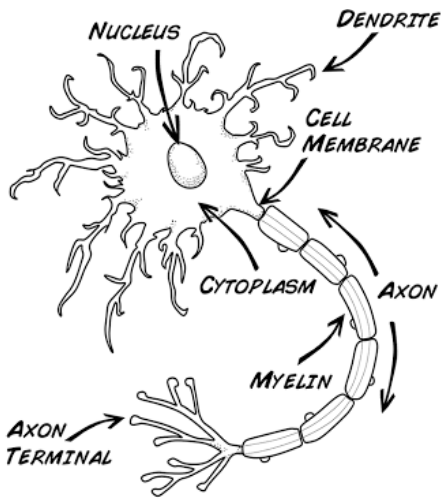
1. Muscle tissue is extremely elongated and Is cylindrical and tapering at both the ends, Which are arranged in bundles, called fasciculi.
2. Each fibre is surrounded by an endomysium And each fasciculous by a perimysium.
3. The muscle fibre consists of sarcoplasm, a Peripherally placed nucleus and covered by a Thin sarcolemma.
4. The centre of the fibre is made up of Longitudinal bundles, the myofibrillae.
5. The myofibrils are striated in a regular Manner and show alternate light and dark Pattern.
6. At cross section myofibril looks like a Series of closely packed dots across the entire
7. These muscles are attached to bones and These includes the biceps, triceps, cuff muscle etc

(C) Parts of a neuron:

1. Neurons vary in size, shape, and structure depending on their role and location. However, nearly all neurons have three essential parts: a cell body, an axon, and dendrites.
2. Cell body- Also known as a soma, the cell body is the neuron's core. The cell body carries genetic information, maintains the neuron's structure, and provides energy to drive activities.

Like other cell bodies, a neuron's soma contains a nucleus and specialized organelles. It's enclosed by a membrane which both protects it and allows it to interact with its immediate surroundings.

3. Axon - An axon is a long, tail-like structure which joins the cell body at a specialized junction called the axon hillock. Many axons are insulated with a fatty substance called myelin. Myelin helps axons to conduct an electrical signal. Neurons generally have one main axon.
4. Dendrites - Dendrites are fibrous roots that branch out from the cell body. Like antennae, dendrites receive and process signals from the axons of other neurons. Neurons can have more than one set of dendrites, known as dendritic trees. How many they have generally depends on their role.



For instance, Purkinje cells are a special type of neuron found in the cerebellum. These cells have highly developed dendritic trees which allow them to receive thousands of signals.

Experiment - 3

TO STUDY THE DIFFERENT STAGES OF MITOSIS IN ROOT TIP OF *Allium cepa.*

Mitosis

Mitosis is the process of cell division in which one cell gives rise to two genetically identical daughter cells, resulting in cell duplication and reproduction.

- The number of chromosomes is preserved in both the daughter cells.
- Mitosis is a short period of chromosome condensation, segregation, and cytoplasmic division.
- The mitosis occurs in the somatic cells, and it is meant for the multiplication of cell numbers during embryogenesis and blastogenesis of plants and animals.
- As a process, mitosis is remarkably similar in all animals and plants.

Aim- To study the different stages of Mitosis in root tip of *Allium cepa*

Objective- Squash preparation of onion root tips to observe stages of mitosis.

Requirements- onion root tips, carnoy's fluid, Microscopic glass slide, cover slip, acetocaramine, Spirit lamp, blotting paper and microscope.

Procedure-

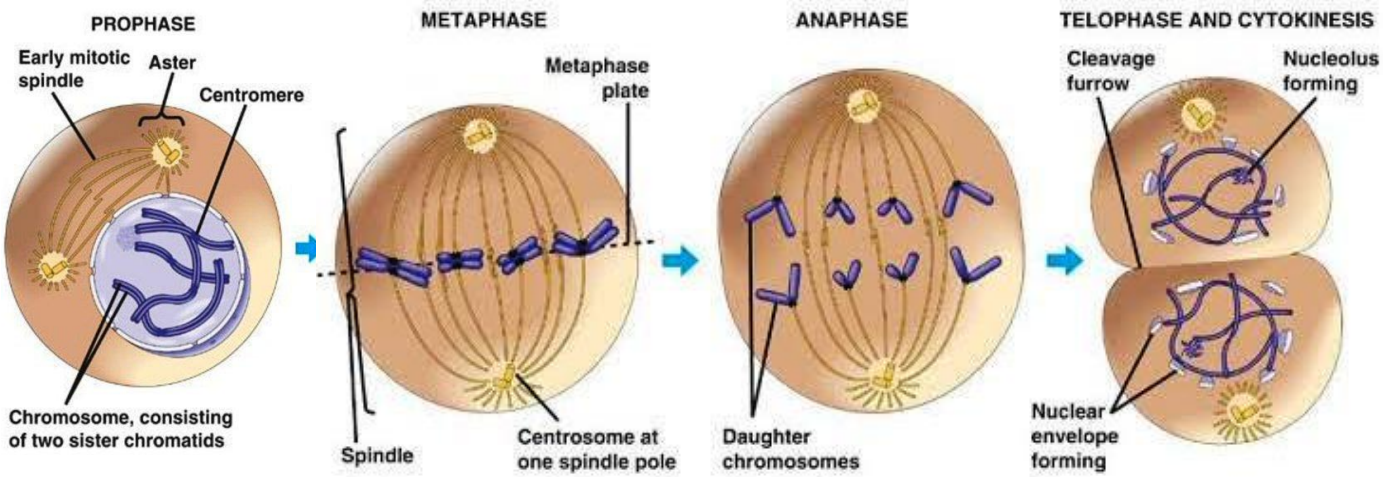
1. Take a drop of acetocaramine on a clean microscopic slide and put on it one or two root tips.
2. Place a coverslip over it and press gently by needle.
3. Warm the slide on the flame of a spirit lamp and then put a blotting paper over it, press it smoothly by your thumb.
4. Examine the slide under the microscope.

Result -

The cells under chromosomes are spread out and become distinct. Carefully observe different stages of mitosis.

<u>Stages of mitosis</u>

Mitosis is a part of the cell cycle and is preceded by the S phase of interphase and usually followed or accompanied by cytokinesis. Replication of chromosomes and synthesis of proteins required for spindle fiber formation are formed prior to the onset of mitosis.



Mitosis is divided into the following phases based on the completion of one set of activities and the onset of the other.

1. **Prophase-**
   - The first phase of mitosis is marked by the early condensation of the chromosomes into visible structures.
   - At first, the chromatins are barely visible but as they continue to Coil, the chromosomes become thicker and shorter.
   - The nuclear envelope is still present during this stage, as are any nucleolar structures.
   - Centrioles are moving to the poles of the cell and spindle fibers are just beginning to form.

2. **Metaphase-**
   - During this phase chromosomes line up in the center of the cell and form a metaphase plate.
   - The spindle apparatus is completely farmed.
   - Chromosomes are attached to spindle fiber.
   - The nuclear envelope has disappeared.

3. **Anaphase-**
   - The moment the anaphase phase begins precisely as the two half off a chromosome, the chromatids, Separate and begin moving to the opposite poles.
   - The centromeres will lead the way in this process, and the chromatids form a V shape.
   - The centromeres pointing towards the respective poles.

4. **Telophase-**
   - The last phase is identified by the aggregation of the chromatids at the respective poles.
   - During this phase, the chromosomes uncoil, the nuclear envelope is synthesized.
   - The spindle apparatus is dismantled and The nucleolus begins to appear

Experiment - 4

TO STUDY THE DIFFERENT STAGES OF MEIOSIS IN GRASSHOPPER TESTIS

Requirements-

Living grasshoppers, chloroform, normal saline, carnoy's fluid, acetocaramine, Slide, coverslip, blotting paper and microscope.

Procedure-

1. Take a chloroformed grasshopper I need dissect 8 in normal saline. Take out its testis and fix them in carnoy's fluid for 2to12 hours.
2. Take a small loop of testis and stain it in acetocaramine.
3. Put the stained lobe on a clean slide and cover it with a cover slip.
4. Adopt squash preparation technique as described in case of onion root tip. Examine the slide under microscope.

Result-

The sense of testis lobes are spread out and become distinct. Carefully observe different stages of meiosis under microscope.

Phases of Meiosis

- Meiosis is composed of two rounds of cell division,
  Meiosis-I and Meiosis-II.
- Each round of division contains a period of karyokinesis (nuclear division) and cytokinesis (cytoplasmic division).

Meiosis I

- In the first meiotic division, the reduction of chromosome number takes place and, thus, two haploid cells are resulted by this division.
- Meiosis I consists of the following steps:

Interphase :

- Just like mitosis, meiosis also consists of a preparatory phase called interphase.
- The interphase is characterized by the following features :
- The nuclear envelope remains intact, and the chromosomes occur in the form of diffused, long, coiled, and indistinctly visible chromatin fibers.
- The DNA amount becomes double. Due to the accumulation of ribosomal RNA (rRNA) and ribosomal proteins in the nucleolus, the size of the nucleolus is significantly increased.
- In animal cells, a daughter pair of centrioles originates near the already existing centriole and, thus, an interphase cell has two pairs of centrioles.
- In the G_2 phase of interphase, there is a decisive change that directs the cell toward meiosis, instead of mitosis.
- At the beginning of the first meiotic division, the nucleus of the dividing cell starts to increase in size by absorbing the water from the cytoplasm, and the nuclear volume increases about three folds.

Prophase I :

Prophase I is the longest stage of the meiotic division. It includes the following substages:

Leptotene

- In the leptotene stage, the chromosomes become even more uncoiled and resemble a long thread-like shape, and they develop bead-like structures called chromomeres.
- The chromosomes at this stage remain directed towards centrioles, so the chromosomes in the nucleus appear like a bouquet in the animal cell. Therefore, this stage is also called the Bouquet Stage.

Zygotene or Synaptotene

- The zygotene stage begins with the pairing of homologous chromosomes, which is called synapsis.
- The paired homologous chromosomes are connected by a protein-containing framework called a synaptonemal complex.
- The synaptonemal complex helps to stabilize the pairing of homologous chromosomes and to facilitate recombination or crossing over.
- The synapsis might begin at one or more points along the length of the homologous chromosomes.
- Synapsis might start from the ends of the chromosomes and continues towards their centromeres (proterminal synapsis), or it might start at the centromere and proceed towards the ends (procentric pairing).
- In some cases, the synapsis occurs at various points of the homologous chromosomes (random pairing).

Pachytene

- In this stage, the pair of chromosomes become twisted spirally around each other and cannot be distinguished separately.
- In the middle of the pachytene stage, each homologous chromosome splits lengthwise to form two chromatids, but they continue to be linked together by their common centromere.
- The chromosomes at this point are termed bivalent because it consists of two visible chromosomes, or as a tetrad because of the four visible chromatids.
- This stage is particularly crucial as a critical genetic phenomenon called “crossing over” takes place in this stage.
- The crossing over involves redistribution and mutual exchange of hereditary material between two homologous chromosomes.
- The enzyme endonuclease breaks the non-sister chromatids at the place of crossing over.
- After the breaking of chromatids, the interchange of chromatid segments takes place between the non-sister chromatids of the homologous chromosomes.
- Another enzyme, ligase, binds the broken chromatid segments with the non-sister chromatid.
- The process of mutual exchange of chromatin material between one non-sister chromatid of each homologous chromosome is known as the crossing over.

Diplotene

- The synaptonemal complex appears to be dissolved, leaving chromatids of the paired homologous chromosome physically joined at one or more localized points.
- In diplotene, chiasmata move towards the end of chromosomes in a zip like a manner.

Diakinesis

- In this stage, the bivalent chromosomes become more condensed and uniformly distributed in the nucleus.
- At this point, the nuclear envelope breaks down, and the nucleolus disappears.
- Further, the chiasmata reach the end of the chromosomes, and the chromatids remain attached until metaphase.

Metaphase I :

- Metaphase I consists of spindle fiber attachment to chromosomes and chromosomal alignment at the equator.
- During metaphase I, the spindle fibers are attached with the centromeres of the homologous chromosomes, which are directed towards the opposite poles.

Anaphase I :

- At anaphase I homologous chromosomes are separated from each other, and due to the shortening of chromosomal fibers or microtubules, each homologous chromosome with its two chromatids and undivided centromere move towards the opposite poles of the cell.
- Because during the chiasma formation, one of the chromatids has changed its counterpart, therefore, the two chromatids of a chromosome are not genetically identical.

Telophase I :

- The onset of telophase I is defined by the movement of a haploid set of chromosomes at each pole.
- The nuclear envelope is formed around the chromosomes, and the chromosomes become uncoiled. The nucleolus reappears and, thus, two daughter nuclei are formed.

Cytokinesis I :

- In animals, cytokinesis occurs by the constriction of the cell membrane while in plants, it occurs through the formation of the cell plate, resulting in the creation of two daughter cells.

Meiosis II

- In the second phase of the meiotic division, the haploid cell divides mitotically and results in four haploid cells. This division is also known as the homotypic division.
- This division does not include the exchange of the genetic material and the reduction of the chromosome number as in the first meiotic division.

Meiosis II division is same as mitotic division

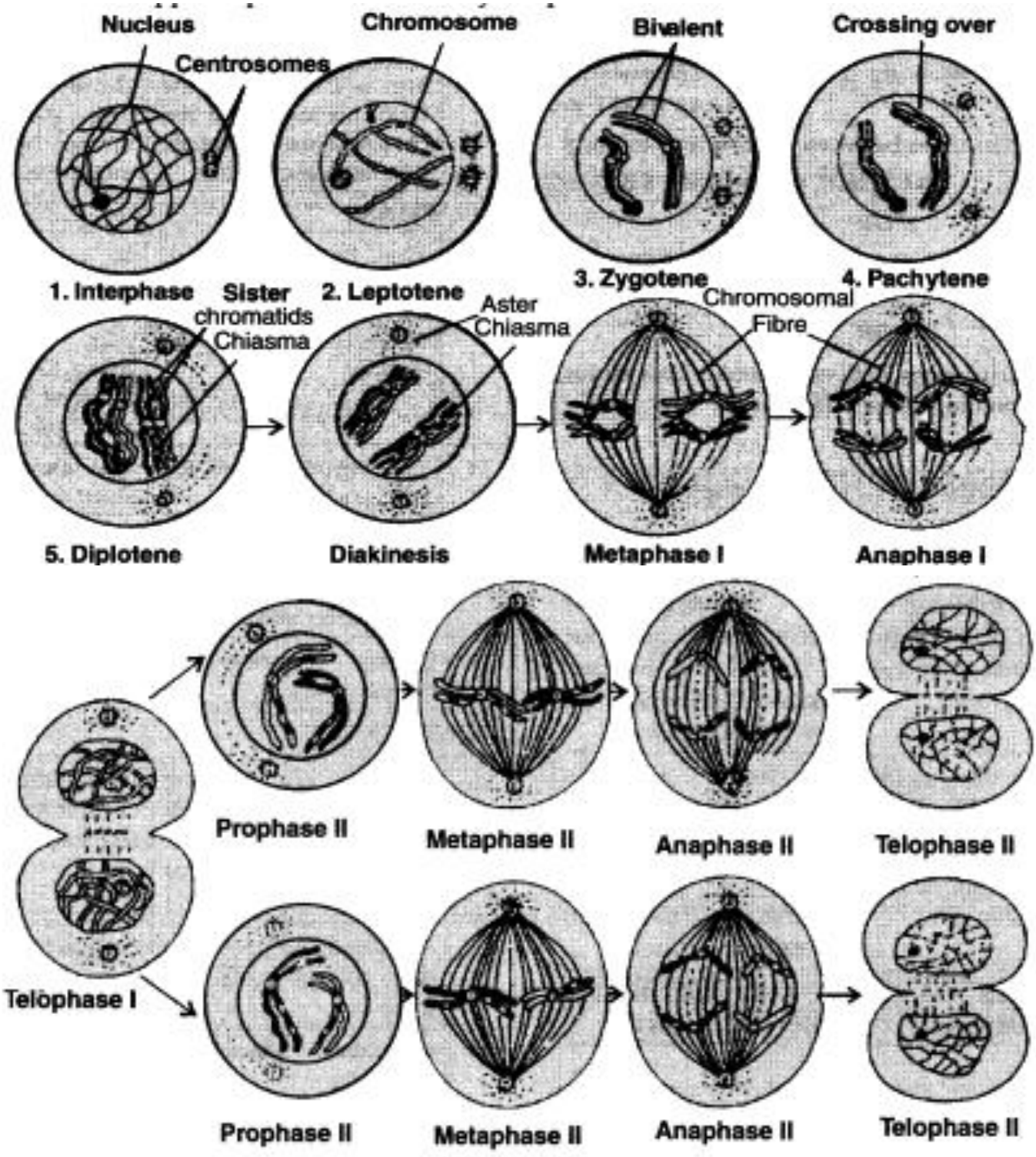


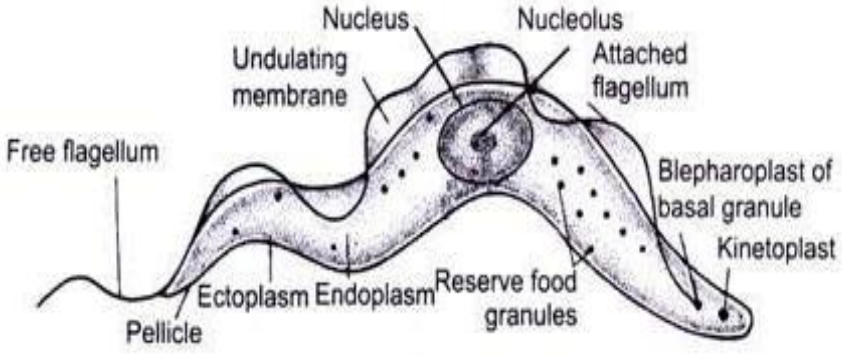
Fig. Stages in meiosis

Experiment – 5

STUDY OF PARASITES IN HUMAN

1) TRYPANOSOMA

- It is a unicellular, parasitic flagellate protozoon.
- Name is derived from Greek word ; Trypano –borer ,soma-body
- It need more than one host to complete its life cycle
- Often transmitted by a vector
- Generally found in intestine, but sometime found in blood stream or in heart.
- TRYPANOSOMA BRUCEI causes African sleeping sickness through the vector Tse Tse fly, TRYPANOSOMA CRUZI causes American sleeping sickness through the vector triatomine bug.



Symptoms:

1) Hemolymphatic: fever, headache, joint pain, itching, weakness, fatigue, weight loss
2) Neurological : parasite invade the CNS by passing through blood brain barrier

- Treatment : Nifurtimox, Posaconazole, Pentamidine

2) WUCHERERIA BANCROFTI

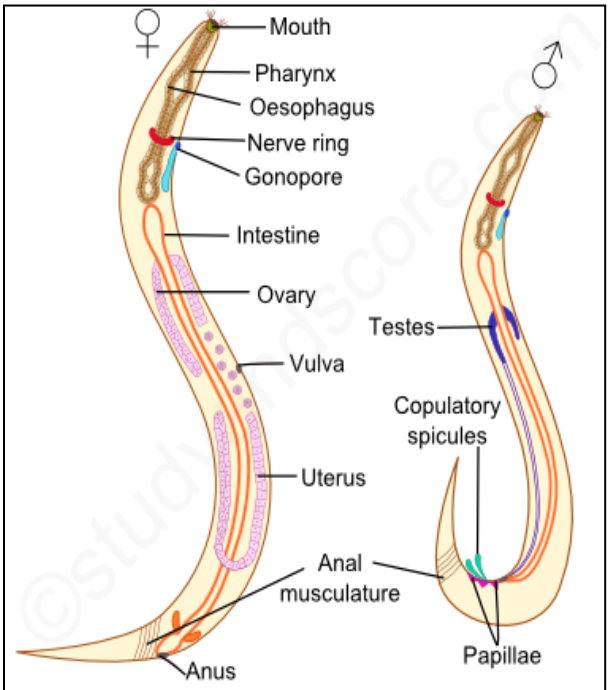
- It is a filarial nematode (roundworm) that is major cause of lymphatic filariasis.
- Lymphatic filariasis is caused by infection with parasites classified as nematodes
- Adult worms nest in the lymphatic vessels and disrupt the normal functions of the lymphatic system

Symptoms:

1) Asymptomatic –showing no external signs of infection while contributing to transmission of the parasitic ,they still cause damage to the lymphatic system and kidneys and alter the body's immune system
2) Chronic-lymphoedema(tissue swelling),elephantiasis (skin or thickening of limbs)
3) Acute – local inflammation involving skin, lymph nodes and lymphatic vessels.

Treatment

- Albendazole
- Ivermectin
- Diethylecarbamazine citrate (DEC) – it is both microfilaricidal and active against the adult worm, is the drug of choice for lymphatic filariasis.



WUCHERERIA - ADULT FEMALE AND MALE

Experiment - 6

TO LEARN THE PROCEDURES OF PREPARATION OF TEMPORARY AND PERMANENT STAINED SLIDES WITH AVAILABLE MOUNTING MATERIALS

Permanent Histological slide preparation

Permanent Histological slide preparation Double staining is applied. Haematoxylin and Eosin stain nucleus and cytoplasm of the cells respectively. Take individual slide and first keep it in xylene to remove wax for 10-15 minutes. Wax is dissolved in xylene and sections are left free. Now pass the slide in descending series of alcohols. 100%, 90%, 70%, 50%, 30%, and water and stain the slide in haematoxylin for 5-10 minutes. Take out slide and again dip in distilled water. Then immerse the slide in a beaker containing tap water. The sections turn blue because of alkalinity of water. If the stain is dark then immerse the slide in acid water and quickly immerse in tap water. Now dehydrate the slide through ascending series of 30%, 50%, 70%and 90% alcohol. After 90%, immerse the slide in alcoholic eosin for 2 to 3 dips. Wash eosin in 90% alcohol. Then keep slide in 100% alcohol for 5 minutes and then in xylene for 15 minutes. Mount the slide in DPX. Keep the grades of alcohol and stains in separate coupling jars. A good staining slide has pinkish colour of cytoplasm and blue of nuclei.

Slide 1 Xylene (10-15 minutes)
Slide 2 Xylene (10-15 minutes)
100% alcohol (5-10 minutes)
90% alcohol (5-10 minutes)
70% alcohol (5-10 minutes)
50% alcohol (5-10 minutes)
30% alcohol (5-10 minutes)
Water (5- 10 minutes)
Haematoxylin (2-5 minutes)
Distilled water & then dip in tap water.

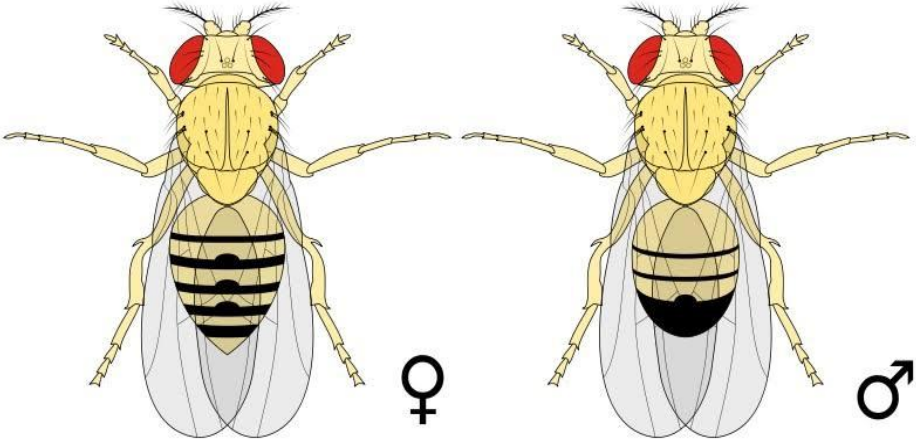
30% alcohol (5 Minutes)
50% alcohol (5 Minutes)
70% alcohol (5 Minutes)
90% alcohol (5 Minutes)
Eosin (2 to 3 dips)
90% alcohol (5 Minutes)
100% alcohol (5 Minute)
Xylene 1 (10-15 minutes)
Xylene 2 (10-15 minutes)
DPX
Cover slip.Then observe under microscope.

Experiment – 7

STUDY OF MUTANT PHENOTYPES OF DROSOPHILA SPECIES (FROM CULTURE OR PHOTOGRAPHS)

Wild type

1. Body can be divided into head, thorax and abdomen.
2. Head bears and Antennae and compound eyes.
3. eyes bear 800 to 1000 facets
4. Color Gray or brown
5. Thorax contains wings, halters and secretellum.



Mutant types:

1. Vestigial wings (vg) :

(a) Wings reduced.
(b) Body can be divided into head, thorax and abdomen. Head bears antennae and compound eyes.
(c) Located in 2nd chromosome; II-67.0.
(d) Recessive mutation.

2. Curly wings (Cy) :

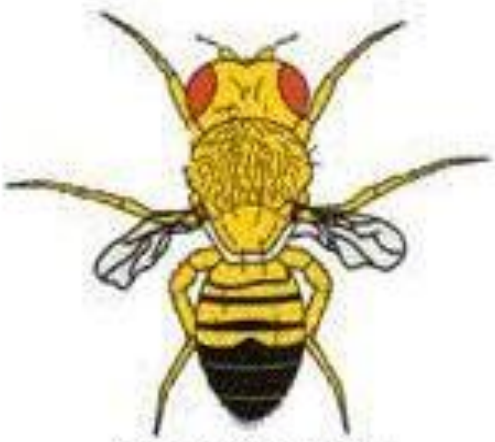
(a) Hind wings are curly they are curled upwards.
(b) Body can be divided into head, thorax and abdomen. Head bears antennae and compound eyes.
(c) Located in 2nd chromosome; II-6.1.
(d) Dominant mutation.

3. Bar eyes (B)

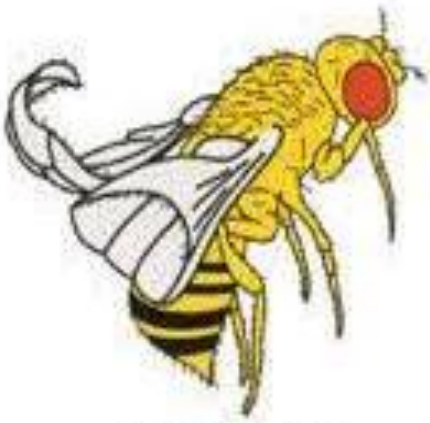
(a) Eye shape oblong or bar like, with facets less than 300. Smaller than normal eyes.
(b) Other characters similar to normal flies.
(c) Located in 1st chromosome; I-57.0.
(d) Dominant mutation.

4. White eyed (w)

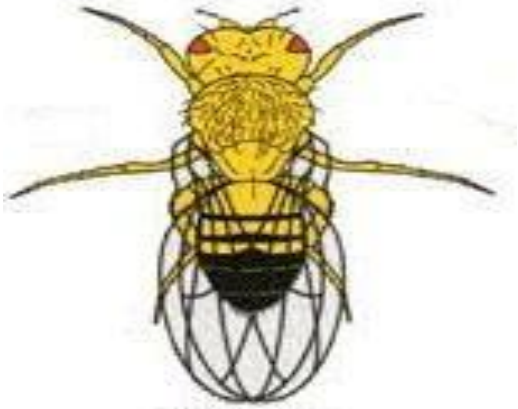
(a) These flies have white eyes. In these flies, the white gene is totally defective: it produces no red pigment at all.
(b) Located in 1st chromosome; I-1.5.
(c) Recessive mutation.



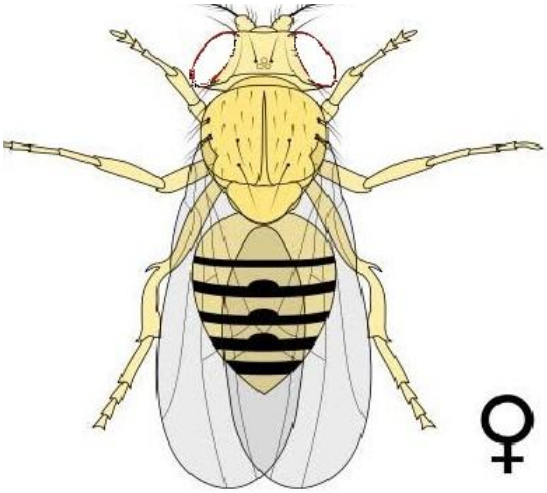
Vestigial wings



Curly wings



Bar eyes

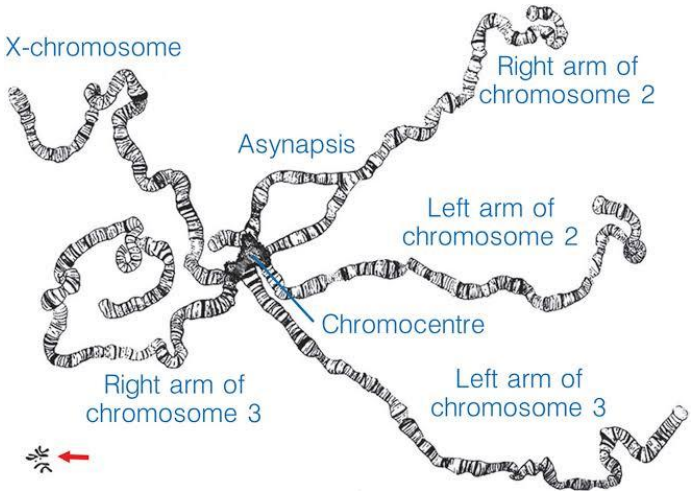


Experiment – 8

PREPARATION OF POLYTENE CHROMOSOMES (CHIRONOMUS LARVA OR DROSOPHILA LARVA)

Procedure

1. A third instar larva is selected, for which the cuticle has not yet hardened, from a wild type culture of *Drosophila* sp. It is placed into a drop of Ringer's insect saline solution on a slide.
2. The slide is placed on the stage of a dissecting microscope (20x-30x) and the larva is viewed against a dark background. The anterior end of the larva is grasped with a fine point forceps and the posterior portion is pinned down with a probe. Gently the head is pulled off and the tail of the larva is discarded.
3. The salivary glands and their attached fat bodies are located. The glands are semitransparent and attached by ducts to the digestive system. The fat bodies are white and opaque. The fat bodies are teased away and discarded.
4. The salivary glands are fixed with a few drops aceto-methanol/ethanol over a slide.
5. The fixative is removed by tilting down the slide. One to three drops of aceto-orcein is placed on fixed tissue over the slide next.
6. A Petri dish is placed over the preparation arid allowed to stand for five to ten minutes. The stained slide is washed with 45% acetic acid for thirty to forty five seconds. Excess acetic acid is tilted off. One drop of aceto-lacto-orcein is put over the slide. The stained salivary gland is then covered with a cover slip.
7. The gland preparation is gently squashed in the following manner:
   - The slide is placed between several layers of paper toweling.
   - The thumb should be placed on the top of the towel immediately over the cover slip and the thumb is gently rolled while exerting a small amount of pressure. One should not move one's thumb back and forth. One gentle roll is sufficient.
   - The slide is removed from the towels, and seal the edges of the cover slip are sealed by using a paintbrush dipped in melted paraffin or nail polish.
8. The slide is examined under the a compound microscope and diagram of the salivary gland chromosome of *Drosophila* sp. is drawn along with the banding patterns that are observed.



Observations:

It is well known that genes responsible for different characters (wild type and mutant) are borne by various chromosomes. Locations of these genes are also precisely pointed out in the different bands of the salivary gland Chromosomes of *Drosophila* sp. Close observation of the salivary gland.

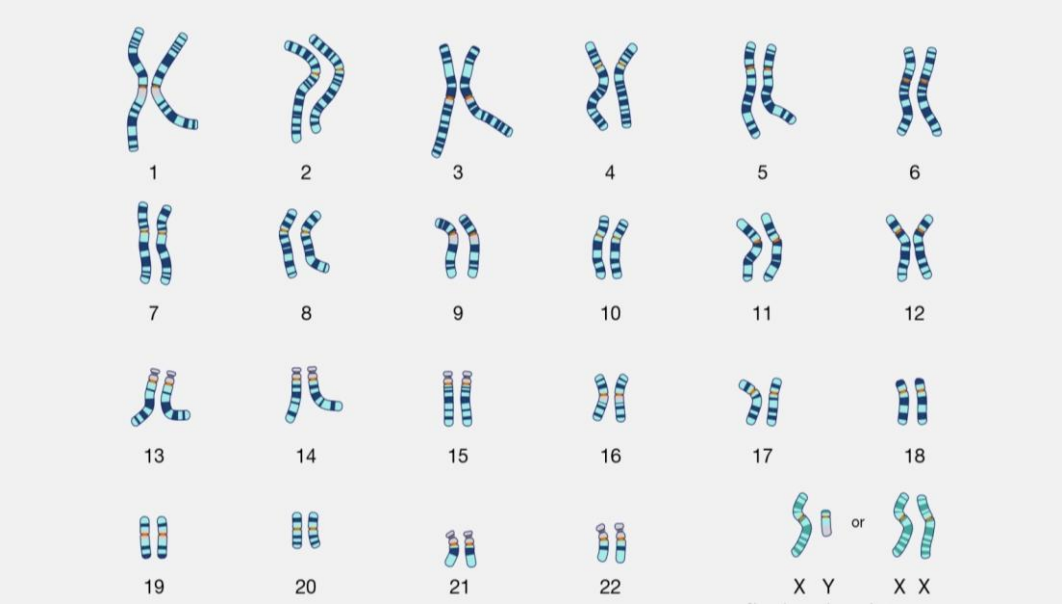
Experiment – 9

PREPARATION OF HUMAN KARYOTYPE AND STUDY THE CHROMOSOMAL, STRUCTURAL AND NUMERICAL ABBERATIONS FROM THE PICTURES PROVIDED.

A karyotype is an individual's complete set of chromosomes. The term also refers to a laboratory-produced image of a person's chromosomes isolated from an individual cell and arranged in numerical order. A karyotype may be used to look for abnormalities in chromosome number or structure.

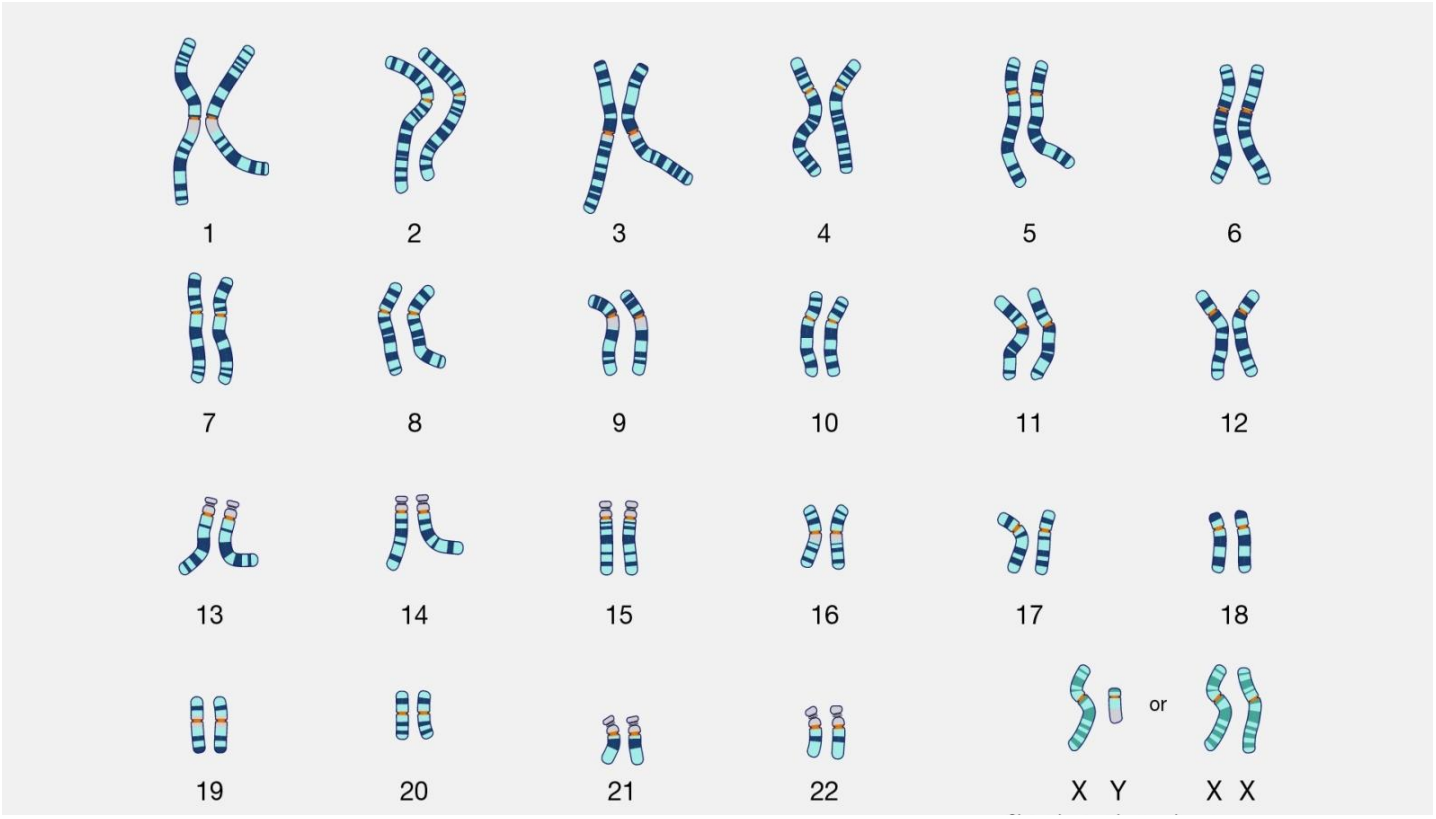
KARYOTYPE OF NORMAL HUMAN FEMALE

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric, equal arms and sat chromosomes.	Normal
Sex chromosomes for female		
Sex chromosomes for male	X chromosomes is of group C type and Y chromosomes group G type.	Karyotype is of human male.



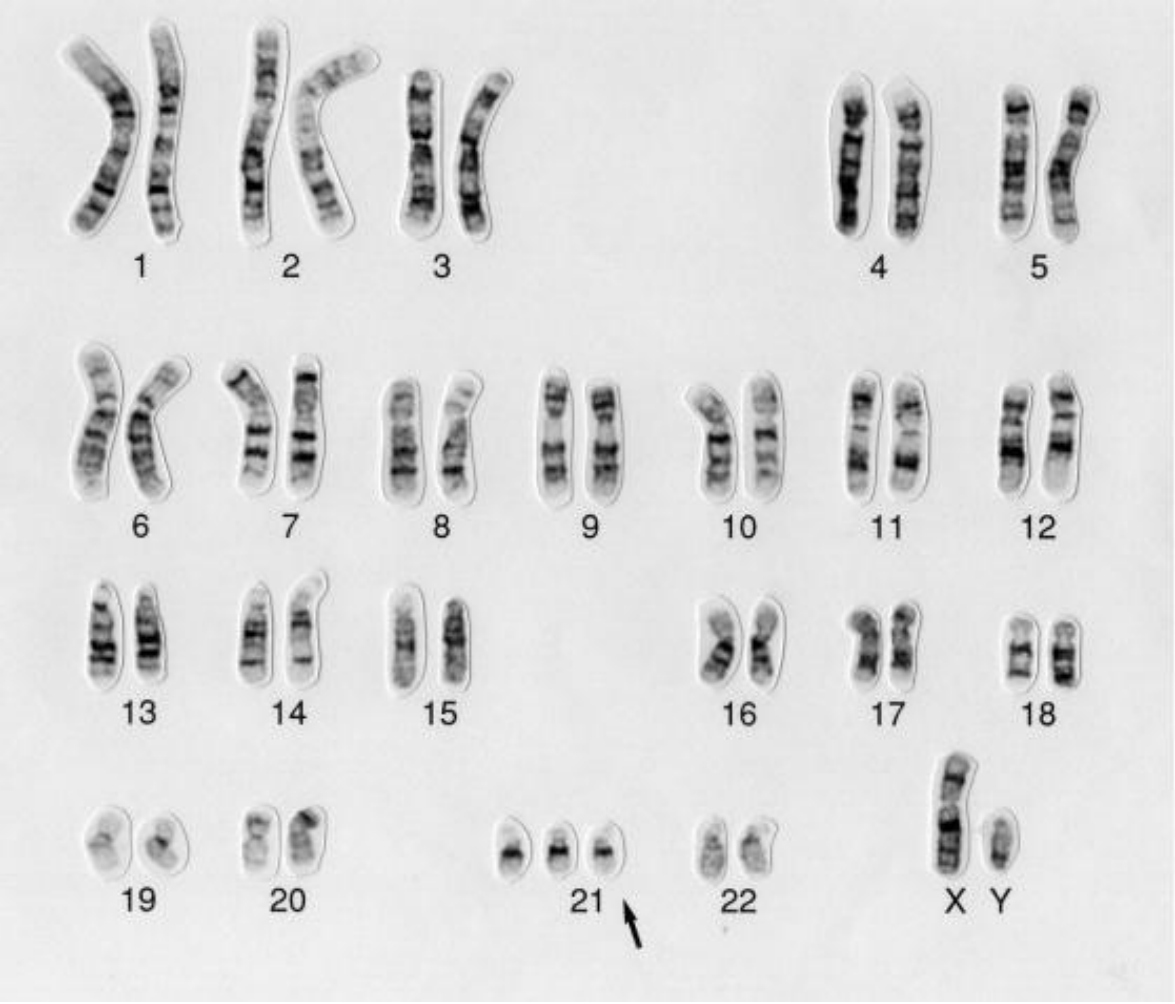
KARYOTYPE OF NORMAL HUMAN MALE

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric, equal arms and sat chromosomes.	Normal
Sex chromosomes	X chromosomes is of group C type and Y chromosomes group G type.	Karyotype is of human male.



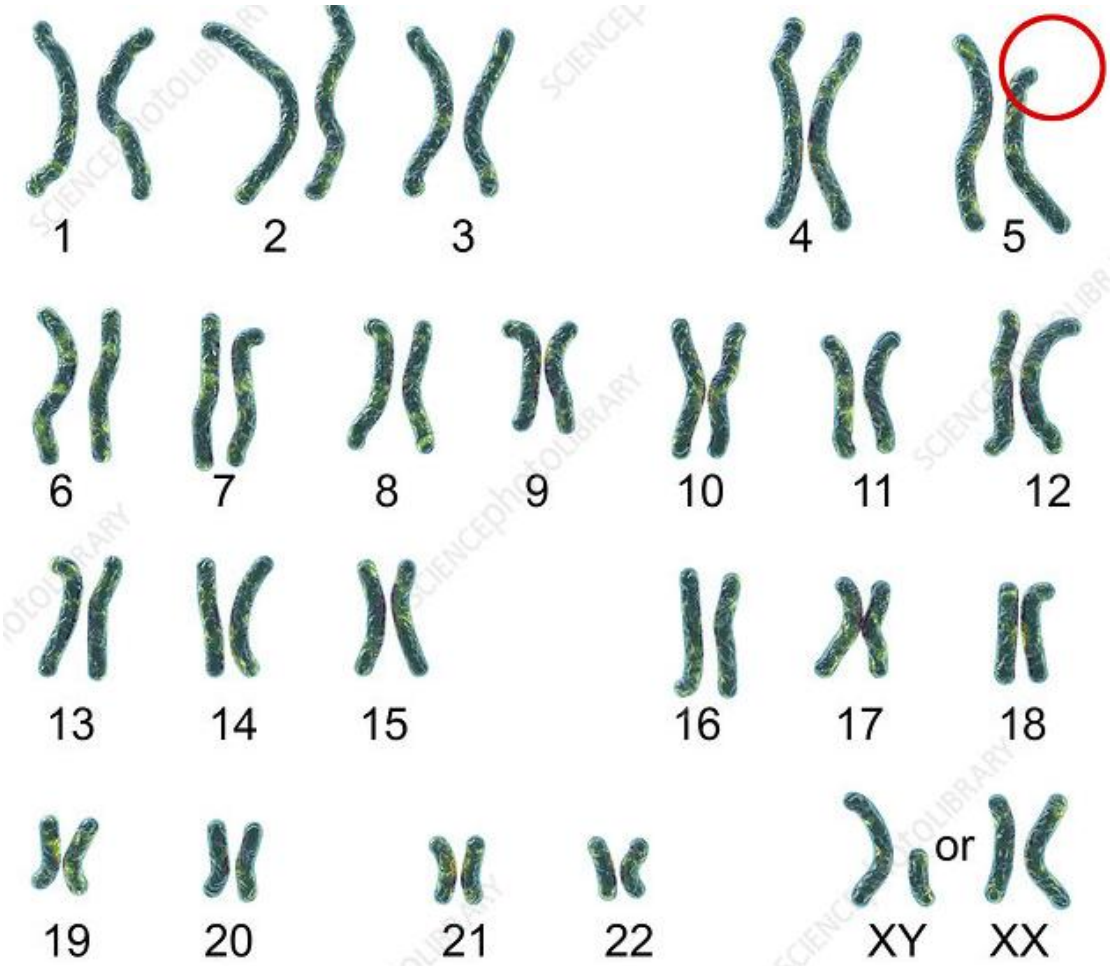
KARYOTYPE OF KLINEFILTER'S SYNDROME

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric, equal arms and sat chromosomes.	Normal
Sex chromosomes	2 X chromosomes and one Y chromosomes.	Karyotype is of Klinefilter's Syndrome



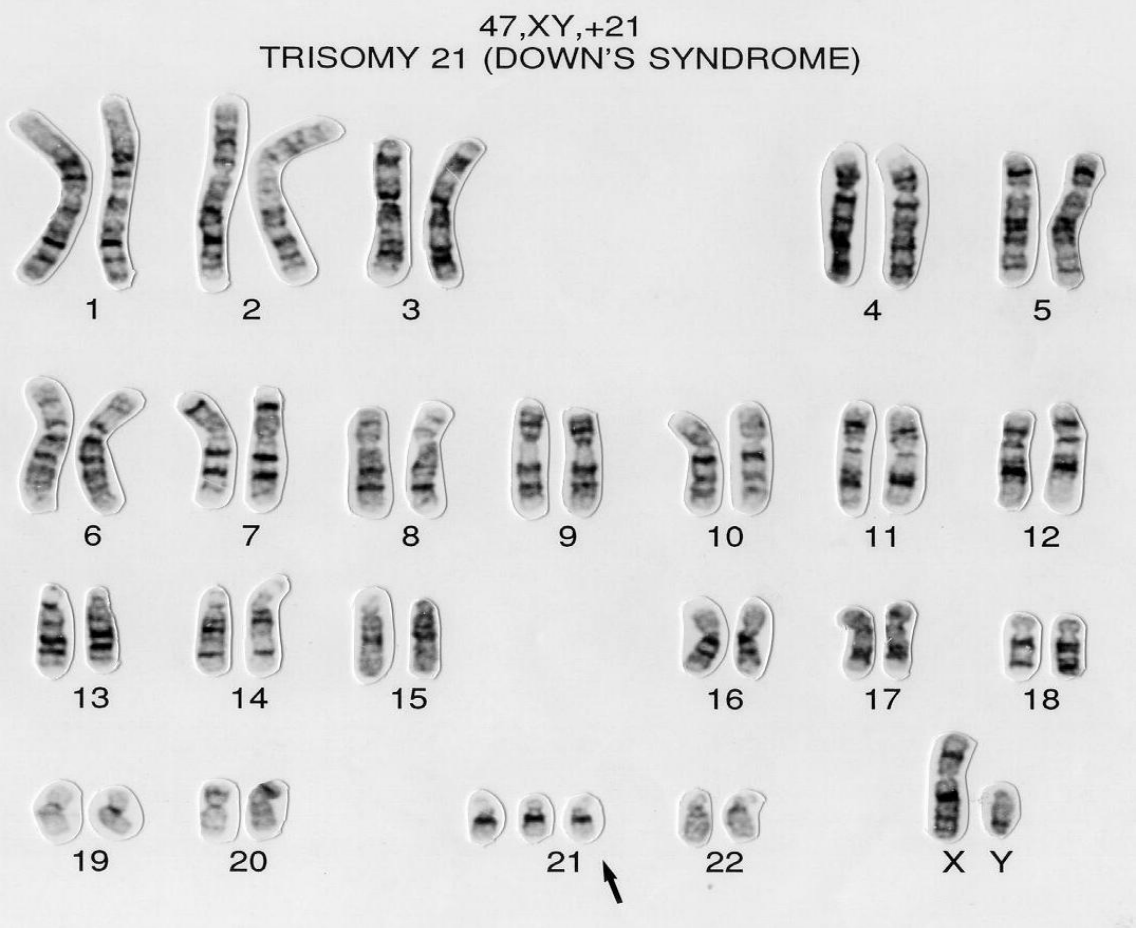
KARYOTYPE OF CATTERY SYNDROME (CRI DU CHAT SYNDROME)

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms and deletion of small part of short arm of 5th chromosomes.	Karyotype of Catery syndrome.
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric, equal arms and sat chromosomes.	Normal
Sex chromosomes	X chromosomes is of group C type and Y chromosomes group G type.	Karyotype is of human male.



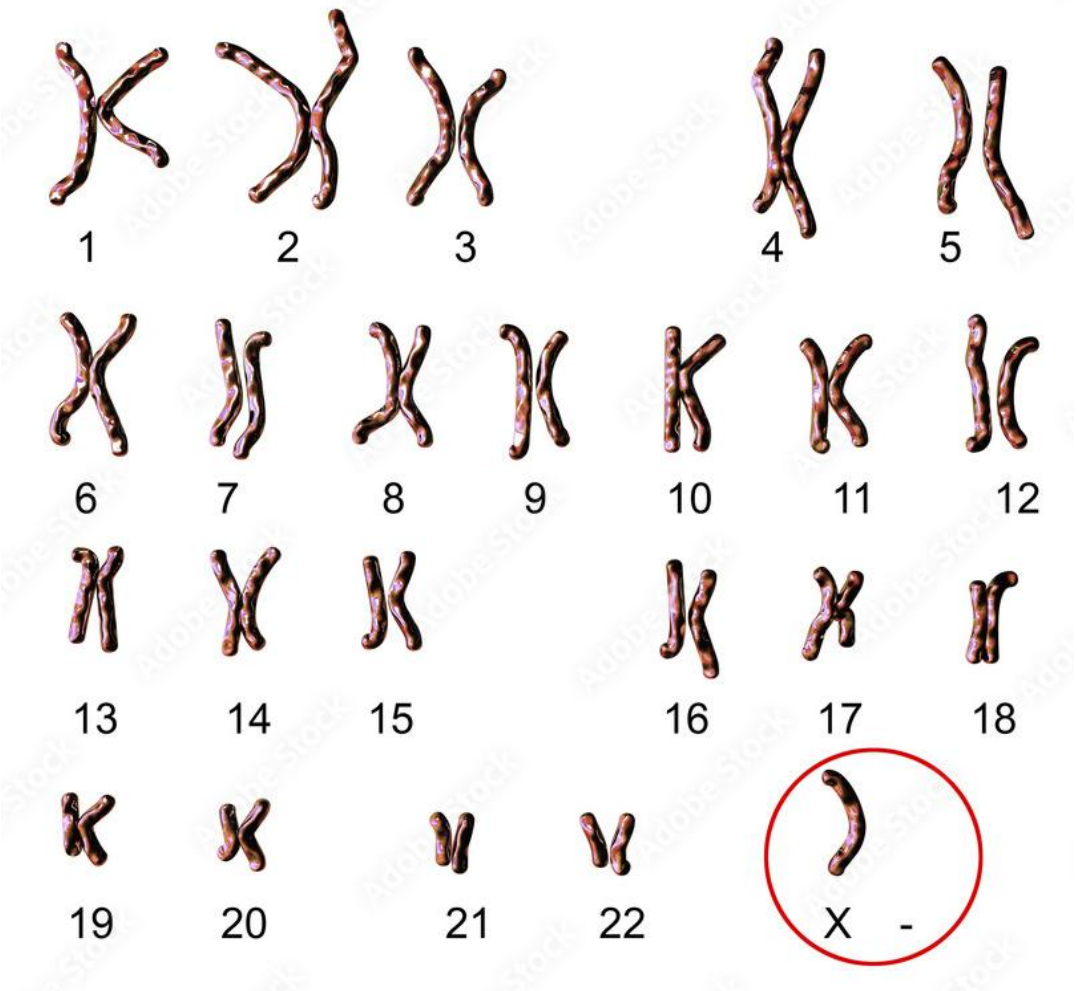
KARYOTYPE OF DOWN'S SYNDROME

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric and Sat chromosomes. 3 copies of 2 chromosomes.	Karyotype of Down's syndrome.
Sex Chromosomes	Two X chromosomes of C type	Karyotype is of human Female with Down's syndrome.



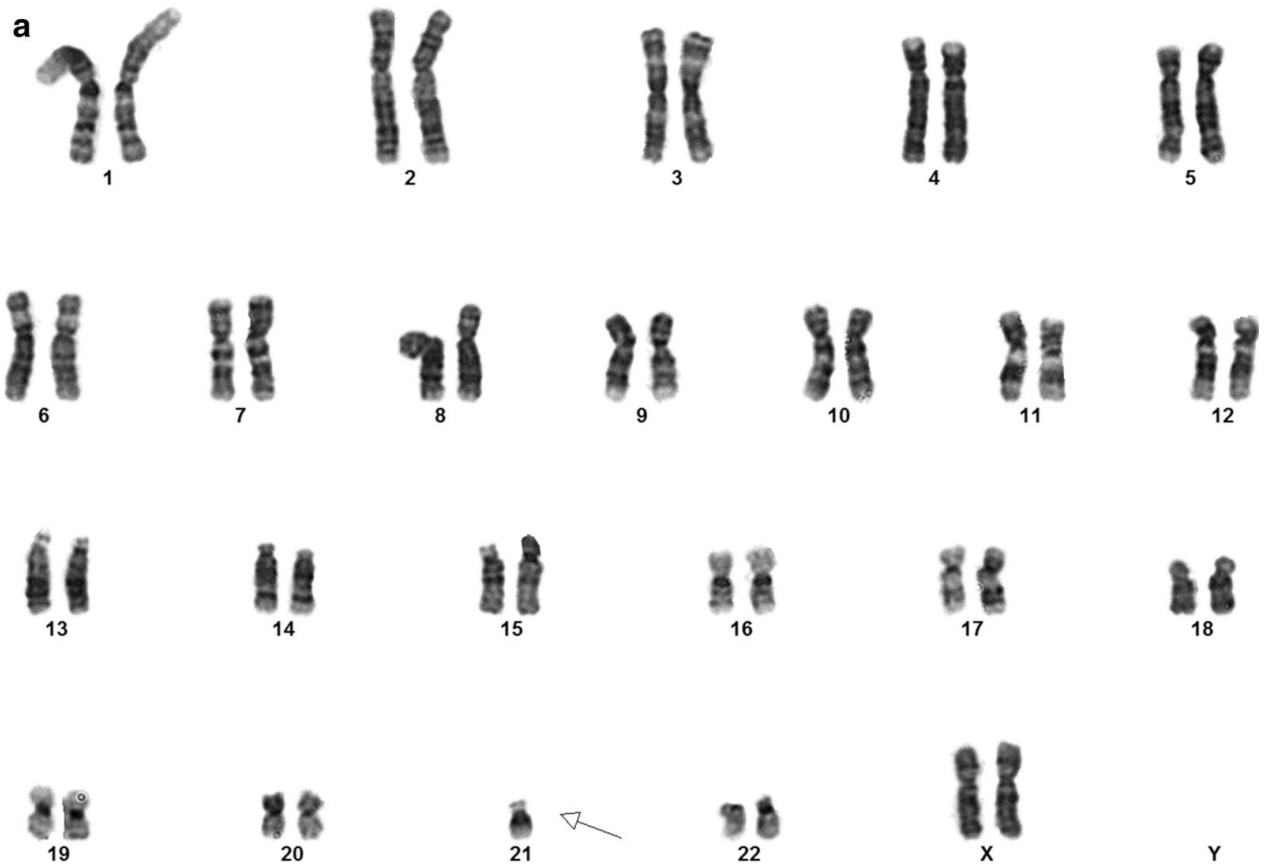
KARYOTYPE OF TURNER'S SYNDROME

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Acrocentric, equal arms and sat chromosomes.	Normal
Sex Chromosomes	X chromosome is of group C type and Y chromosome absent	karyotype is of human female with turner's syndrome. (44+XO)



KARYOTYPE OF 21 MONOSOMY SYNDROMES

Group (homologous pairs)	Observation of chromosomes	Inference
Group A – (1 to 3 Pairs)	Largest chromosomes with approximately median centromere and equal arms.	Normal
Group B – (4 to 5 Pairs)	Next largest chromosomes with submedian centromere and unequal arms.	Normal
Group C – (6 to 12 Pairs)	Medium sized chromosomes, sub median centromere and unequal arms.	Normal
Group D – (13 to 15 Pairs)	Shorter than group C, acrocentric, Sat-chromosomes.	Normal
Group E – (16 to 18 Pairs)	Short, metacentric or submetacentric with equal or unequal arms.	Normal
Group F – (19 to 20 Pairs)	Short metacentric with equal arms.	Normal
Group G – (21 to 22 Pairs)	Smallest, Metacentric, equal arms and sat chromosomes. One chromosome of 21 pair is absent.	Karyotype is of 21 monosomic.
Sex chromosomes	X chromosomes is of group C type and Y chromosomes group G type.	Karyotype is of human Female and male.



B.L.D.E. Association's

S.B. ARTS AND K.C.P. SCIENCE COLLEGE
VIJAYAPUR- 586103
Karnataka State

B. SC III SEMESTER
PRACTICAL LAB MANUAL

DEPARTMENT OF ZOOLOGY
2023-24

B. L. D. E. ASSOCIATION'S
S. B. ARTS AND K. C. P. SCIENCE COLLEGE, VIJAYAPUR.
Department of Zoology

DEPARTMENT OF ZOOLOGY

B.SC III SEMESTER PRACTICAL MANUAL

Si.no	LIST OF EXPERIMENTS
1	To study the principle and applications of simple, compound and binocular microscopes.
2	To study the principle and applications of various lab equipments- pH meter, Electronic balance, Vortex mixer, use of glass and micropipettes, Laminar air flow, Incubator, shaker, Water bath and centrifuge.
3	To prepare Buffer solutions (Phosphate, Citrate, Tris-HCl buffer)
4	To estimate amount of RNA by Orcinol method.
5	Demonstration of differential centrifugation to fractionate components in a given mixture.
6	To estimate amount of protein by Lowry's method.
7	Extraction of DNA from the given animal tissue sample.
8	To estimate amount of DNA by di-phenyl amine (DPA) method.

Experiment No. 1

To study the principle and applications of simple, compound and binocular microscopes.

Simple Microscope Definition

A simple microscope is one that uses a single lens for magnification, such as a magnifying glass while a compound microscope uses several lenses to enhance the magnification of an object. It uses a lens to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image. The use of a single convex lens or groups of lenses is found in simple magnification devices such as magnifying glass, loupes, and eyepieces for telescopes and microscopes. It is actually a convex lens of small focal length, which is used for seeing the magnified images of small objects.

Logan's Simple Microscope (circa 1871)

Principle of Simple Microscope

A simple microscope works on the principle that when a tiny object is placed within its focus, a virtual, erect, and magnified image of the object is formed at the least distance of distinct vision from the eye held close to the lens.

Applications of Simple Microscope

The magnifying power of a simple microscope is given by:

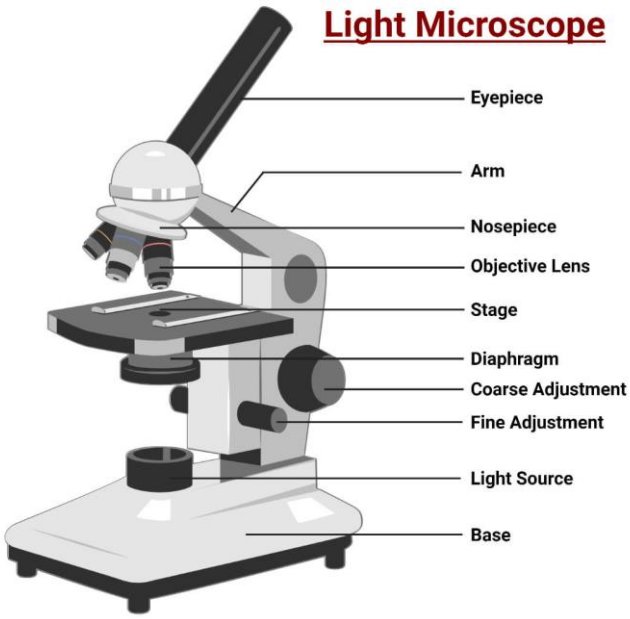
- The focal length of the convex lens should be small because the smaller the focal length of the lens, the greater will be its magnifying power.
- The maximum magnification of a simple microscope is about 10, which means that the object will appear 10 times larger by using the simple microscope of maximum magnification.

Compound Microscope

- The term "compound" in compound microscopes refers to the microscope having more than one lens.
- Devised with a system of combination of lenses, a compound microscope consists of two optical parts, namely the objective lens and the ocular lens.

Working Principle of the Compound Microscope

Compound microscopes have a combination of lenses that enhances both magnifying powers as well as the resolving power.



- The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens.
- A beam of visible light from the base is focused by a condenser lens onto the specimen.
- The objective lens picks up the light transmitted by the specimen and creates a magnified image of the specimen called the primary image inside the body tube. This image is again magnified by the ocular lens or eyepiece.
- When higher magnification is required, the nose piece is rotated after low power focusing to bring the objective of a higher power (generally 45X) in line with the illuminated part of the slide.
- Occasionally very high magnification it required (e.g. for observing bacterial cell). In that case, an oil immersion objective lens (usually 100X) is employed.
-

Applications

- A compound microscope is of great use in pathology labs so as to identify diseases.
- Various crime cases are detected and solved by drawing out human cells and examining them under the microscope in forensic laboratories.
- The presence or absence of minerals and the presence of metals can be identified using compound microscopes.
- Students in schools and colleges are benefited from the use of a microscope for conducting their academic experiments.
- It helps to see and understand the microbial world of bacteria and viruses, which is otherwise invisible to the naked eye.

Experiment No. 2

To study the principle and applications of various lab equipments – pH meter, Electronic balance, Vortex mixer, use of glass and micropipettes, Laminar air flow, Incubator, shaker, Water bath and centrifuge.

pH Meter

A pH meter is a device used in laboratories that measure the H-ion concentration in water-based solutions to determine the acidity or alkalinity of the solution. A pH meter is often termed a "potentiometric pH meter" as it measures the difference in electric potential between the reference and a pH electrode.

Working Principle

- In a potentiometric pH meter, single or multiple glass electrodes, connected to a bulb selective to hydrogen ions, are attached to a metal rod.
- When the bulb with the electrodes is dipped into a solution, hydrogen ions in the solution exchange with positive charges on the electrode generating an electrochemical potential which is displayed in terms of pH units on display.

Applications

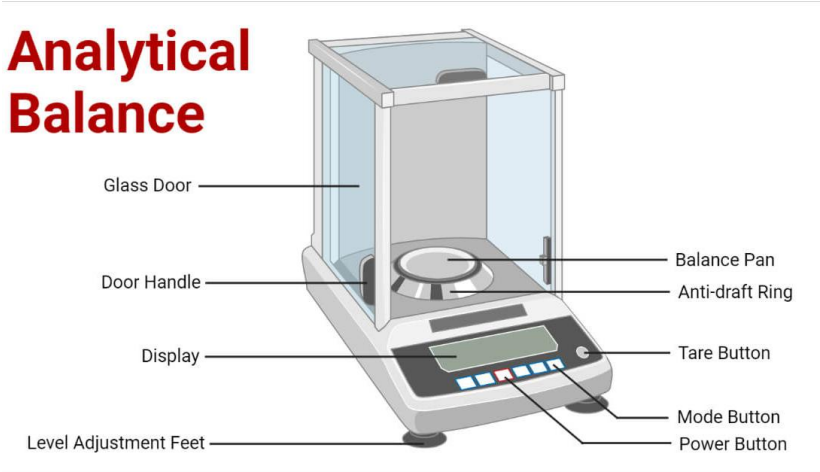
- They are used to analyze the exact pH value of food grade products and chemical products to ensure safety and quality, or they can be used to evaluate acidity/alkalinity of drugs in pharmaceutical and biotechnology industries.

Electronic Balance / Analytical Balance

An Electronic Balance is a type of balance that is commonly used for the measurement of mass in the sub-milligram range.

Working Principle

- These types of balances are made with a measuring pan enclosed in a transparent covering that prevents small particles or air currents from getting collected on the pan.



- An electric Electronic Balance uses the force necessary to counteract the mass rather than measuring the mass itself.
- An electromagnet is used to create a force required to achieve a balance with the mass of the substance, and the resulting force is displayed.

Uses

- As they are highly precise and based on advanced technology, Electronic Balances are explicitly used in laboratories for the effective completion of tasks like weighing test materials and sampling amounts, formulation, density determination, purity analysis, quality control testing, and material and conformance testing.

Principle of Electronic Balance

Electronic Balance calculates weights based on the force required to balance the mass of a sample rather than utilizing actual masses. They produce a force to balance the sample using an electromagnet, then output the result by measuring the force required. A transparent enclosure with doors surrounds the measurement pan of an Electronic Balance (0.1 mg or greater), blocking external influences. As a result, dust does not collect, and air currents in the room do not affect the weighing performance.

Uses/ Applications of Electronic Balance

- Analysis and determination of density
- Preparation of sample
- Differential weighing
- Formulation calculation
- Percent weighing
- Gross-net tare weighing
- Animal tissue weighing

Vortex Mixture/ Vortexer

A vortex mixture is one of the basic technologies used for the mixing of samples in glass tubes or flasks in laboratories.

- **Working Principle of Vortex Mixer**

- The drive shaft of the motor for vortex mixers is positioned vertically, and a cupped rubber piece is mounted somewhat off-center. The rubber component will quickly oscillate in a circular motion when the motor is running. A vortex will form in the sample when it is placed on the rubber piece because the motion is transferred to the liquid. This enables ferocious sample mixing.

Applications of Vortex Mixer

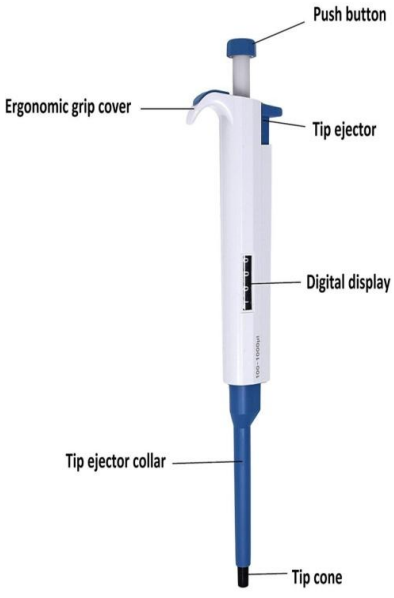
1. It has wide application in the clinical and medical sectors for thawing and mixing samples.
2. The vortexer has been used to suspend cell or tissue samples for use in tissue analysis and cell culture.

3. When investigating proteins and enzymes, a vortex mixer is essential for the homogeneous mixing of samples with reagents and buffer.
4. It is also utilized in heating and mixing samples in pharmaceutical areas.
5. It is employed in schools and universities for practical demonstrations and experiments.
6. Vortexer is used in quality control testing and sample preparation for industrial use.

Micropipette

Working Mechanism of micropipette

Air displacement micropipettes function via the piston driven air displacement. As the piston pulled down, air contained within the sleeve of the micropipette is released because of the force with which the liquid in the micropipettes tip is also eliminated.



As the piston is moved upwards there is a vacuum created in the empty space left due to the movement of the piston. The air at on the tips to expand to fill in the empty space. The tip air is replaced by the liquid that is drawn upwards to the tip.

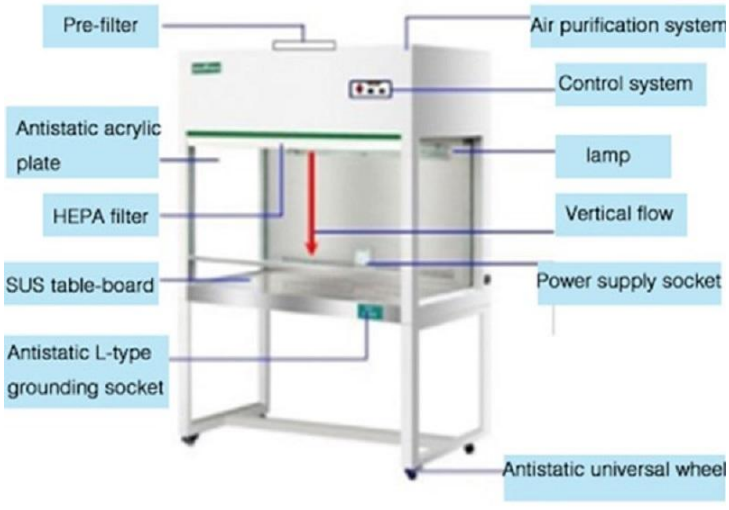
Applications/fields where micropipettes can be used

Micropipettes are typically used in microbiology, chemical and medical labs to ensure the exact and precise transmission of specimens. Micropipettes with a single channel are utilized in laboratories which conduct research in microbiology, molecular biology cell culture, immunology biochemistry, analytical chemistry, and genetics, multichannel micropipettes can be used as a substitute for ELISA (diagnostic tests) molecular screening study of kinetics and DNA amplification.

Principle/ Working of Laminar flow hood

The principle of laminar flow cabinet is based on the laminar flow of air through the cabinet.

The device works by the use of inwards flow of air through one or more HEPA filters to create a particulate-free environment.



The air is taken through a filtration system and then exhausted across the work surface as a part of the laminar flow of the air.

The air first passes through the filter pad or pre-filter that allows a streamline flow of air into the cabinet.

Next, the blower or fan directs the air towards the HEPA filters. The HEPA filters then trap the bacteria, fungi and other particulate materials so that the air moving out of it is particulate-free air.

Some of the effluent air then passes through perforation present at the bottom rear end of the cabinet, but most of it passes over the working bench while coming out of the cabinet towards the face of the operator.

The laminar flow hood is enclosed on the sides, and constant positive air pressure is maintained to prevent the intrusion of contaminated external air into the cabinet.

Uses of Laminar flow hood

The following are some common uses of a laminar flow cabinet in the laboratory:

1. Laminar flow cabinets are used in laboratories for contamination sensitive processes like plant tissue culture.
2. Other laboratories processes like media plate preparation and culture of organisms can be performed inside the cabinet.
3. Operations of particle sensitive electronic devices are performed inside the cabinet.
4. In the pharmaceutical industries, drug preparation techniques are also performed inside the cabinet to ensure a particulate-free environment during the operations.
5. Laminar flow cabinets can be made tailor-made for some specialized works and can also be used for general lab techniques in the microbiological as well as the industrial sectors.

Incubator Definition

An incubator, in microbiology, is an insulated and enclosed device that provides an optimal condition of temperature, humidity, and other environmental conditions required for the growth of organisms.

An incubator is a piece of vital laboratory equipment necessary for cultivating microorganisms under artificial conditions.

An incubator can be used to cultivate both unicellular and multicellular organisms.

Principle/ Working of Incubator

- An incubator is based on the principle that microorganisms require a particular set of parameters for their growth and development.
- All incubators are based on the concept that when organisms are provided with the optimal condition of temperature, humidity, oxygen, and carbon dioxide levels, they grow and divide to form more organisms.
- In an incubator, the thermostat maintains a constant temperature that can be read from the outside via the thermometer.
- The temperature is maintained by utilizing the heating and no-heating cycles.
- During the heating cycle, the thermostat heats the incubator, and during the no-heating period, the heating is stopped, and the incubator is cooled by radiating heat to the surrounding.
- Insulation from the outside creates an isolated condition inside the cabinet, which allows the microbes to grow effectively.
- Similarly, other parameters like humidity and airflow are also maintained through different mechanisms that create an environment similar to the natural environment of the organisms.
- Similarly, they are provided with adjustments for maintaining the concentration of CO2 to balance the pH and humidity required for the growth of the organisms.
- Variation of the incubator like a shaking incubator is also available, which allows for the continuous movement of the culture required for cell aeration and solubility studies.

Uses of Incubator

Incubators have a wide range of applications in various areas including cell culture, pharmaceutical studies, hematological studies, and biochemical studies.

Some of the uses of incubators are given below:

1. Incubators are used to grow microbial culture or cell cultures.
2. Incubators can also be used to maintain the culture of organisms to be used later.
3. Some incubators are used to increase the growth rate of organisms, having a prolonged growth rate in the natural environment.
4. Specific incubators are used for the reproduction of microbial colonies and subsequent determination of biochemical oxygen demand.
5. These are also used for breeding of insects and hatching of eggs in zoology.
6. Incubators also provide a controlled condition for sample storage before they can be processed in the laboratories.

Water Bath

Principle of Water Bath

The sensor converts the temperature of the water into a resistance value, which is then amplified and compared by an integrated amplifier. This produces the control signal, which effectively regulates the average heating power of the electric heating tube and keeps the water at a constant temperature

Advantages

- The alcohol lamp's flame may reach temperatures of up to several hundred degrees Celsius. The melting process of the material cannot be documented or viewed if it is heated directly by the alcohol lamp fire in the experiment. The sample can be heated evenly in the lab water bath, avoiding direct heating's excessive intensity and uncontrollable temperature swings.
- Water baths offer a larger surface area, allowing your samples to be heated more quickly.
- Even if you are simultaneously heating several samples, there is very little chance of temperature variations because water baths can hold a significant amount of heat.

Centrifugation

- Centrifugation is a technique of separating substances which involves the application of centrifugal force.
- The particles are separated from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Principle of Centrifugation

- In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it floats to the top.

- To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge.

- A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a potentially strong force perpendicular to the axis of spin (outward).
- The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances and particles to move outward in the radial direction.
- At the same time, objects that are less dense are displaced and move to the center.
- In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

Applications of Centrifugation

- To separate two miscible substances
- To analyze the hydrodynamic properties of macromolecules
- Purification of mammalian cells
- Fractionation of subcellular organelles (including membranes/membrane fractions) Fractionation of membrane vesicles
- Removing fat from milk to produce skimmed milk
- The clarification and stabilization of wine
- Separation of urine components and blood components in forensic and research laboratories

Shaker

The **laboratory shakers** are useful instruments that can form a homogenous mixture from multiple ingredients. Life sciences uses these shakers in waste water treatments, biotech industries and in life sciences. They are not just used in science labs either. Laboratory shakers can be used in many different industries. Examples of these include cosmetics, electronics, pharmaceuticals and in the food and beverage industries. These machines are an important part in many labs.

Top Uses of a Shaker

There are many different uses that a shaker can do. These include:

- General mixing
- Diagnostic testing
- Cell cultures
- Bacterial suspensions
- Hybridization
- Solubility studies
- Staining
- Destaining
- Extraction procedures

Experiment No.3

To prepare Buffer solutions (Phosphate, Citrate, Tris-HCl buffer)

Potassium phosphate buffer (0.01M - pH 7.4): 500mL of 0.01 M K,HPO, and 500mL of 0.01 Molar KH_2PO_4 are prepared. K_2HPO_4 is placed onto a magnetic stirrer and a pH electrode is inserted. KH_2PO_4 is added slowly to adjust the pH to 7.4.

Sodium saline citrate solution: SSC–85ml of 0.9% sodium chloride solution + 15ml of 0.5% sodium citrate solution usually gives pH 7.4, if not adjust pH.

Tris buffer (1M): There are many variations on the basic Tris-HCl buffer combination, most of which are commercially available. Solutions with EDTA are known as TE buffers, while solutions with EDTA and acetic acid are known as TAE buffers.

The basic buffer is a combination of Tris (tris(hydroxymethyl)aminomethane) and HCI acid. These are sometimes referred to as Tris-base and Tris-HCl solutions. Tris buffers should not be used below a pH of 7.2 or above a pH of 9.0. Tris buffers are also extremely temperature sensitive.

121g of Tris is dissolved in 800mL of distilled water. The pH is adjusted with concentrated HCI. Final volume is diluted of 1 litre.

Experiment No.4

Estimation of RNA by Orcinol Reaction

Aim: To estimate the concentration of RNA by orcinol reaction.

Principle: This is a general reaction for pentoses and depends on the formation of Furfural when the pentose is heated with concentrated hydrochloric acid. Orcinol reacts with the furfural in the presence of ferric chloride as a catalyst to give a green colour, which can be measured at 665 nm.

Requirements:

1. Standard RNA solution- 200µg/ml in 1 N perchloric acid/buffered saline.
2. Orcinol Reagent- Dissolve 0.1g of ferric chloride in 100 ml of concentrated HCl and add 3.5 ml of 6% w/v orcinol in alcohol.
3. Buffered Saline- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure:

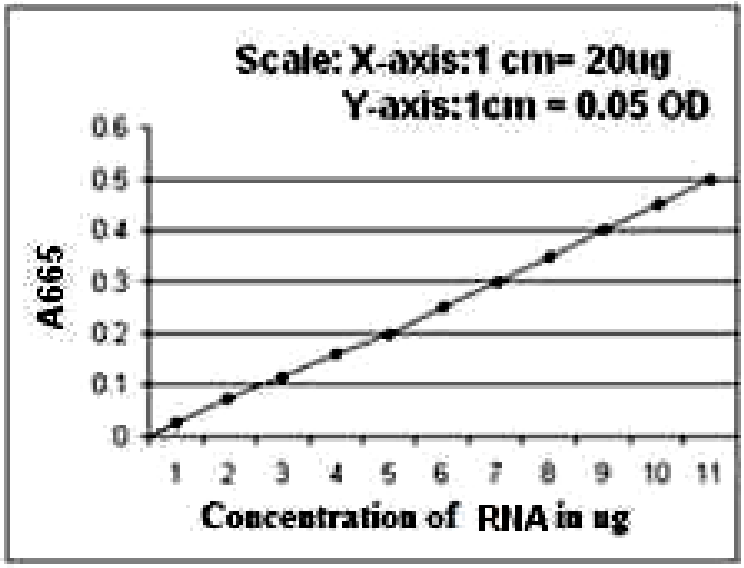
1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled waterserves as the blank.
4. Now add 2 ml of orcinol reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and heat on a boiling water bath for 20 min.
6. Then cool the contents and record the absorbance at 665 nm against blank.
7. Then plot the standard curve by taking concentration of RNA along X-axis and absorbance at665 nm along Y-axis.
8. Then from this standard curve calculate the concentration of RNA in the given sample.

Result: The given unknown sample contains- µg RNA/ml.

Observations and Calculations

Volume	Volume of distilled water (ml)	Concentration ofRNA(µg)	Volume	Incubate in boiling water bath for20 Min & Cool	A665
0.0	1.0	00	2		
0.2	0.8	40	2		0.00
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be estimated	2		

Standard curve for RNA estimation by Orcinol reaction



Experiment No.5

Demonstration of differential centrifugation to fractionate components in a given mixture.

Introduction: Fractionation of samples typically starts with centrifugation. Using a centrifuge, one can remove cell debris, and fractionate organelles, and cytoplasm. For example, nuclei, being relatively large, can be spun down at fairly low speeds. Once nuclei have been sedimented, the remaining solution, or supernatant, can be centrifuged at higher speeds to obtain the smaller organelles, like mitochondria.

Principle: Cells are disrupted in a homogenizer and the resulting mixture, called the homogenate, is centrifuged in a step-by-step fashion of increasing centrifugal force. The denser material will form a pellet at lower centrifugal force than will the less-dense material. The isolated fractions can be used for further purification.

Differential centrifugation Protocol

The organelles of sub-cellular origin (nucleus, mitochondria, lysosomes, Endoplasmic Rerticulum) in the homogenate of a tissue liver can be isolated using these techniques of differential centrifugation. The procedure consists of these steps

⇩

Preparation of homogenate from the chicken liver tissue (ice cold sample, maintaining pH buffer for isotonic condition) in 10 percent sucrose solution.

⇩

A centrifugation of 1000 RPM for 10 mins.

⇩

The pellet is separated from the sedimented that is the nucleus.

⇩

The supernatant that is decanted by the step is then subjected to centrifugation at 3000 RPM for 10 minutes (contains less dense organelles).

⇩

Separation of the sedimented pellet that is containing mitochondria and sediment with Endoplasmic Reticulum and Golgi apparatus.

⇩

The supernatant emitted by the step is then subjected to centrifugation at 16000 RPM for 20 minutes.

⇩

Separation of the pellet that has been sedimented that contains Lysosomes.

⇩

The supernatant that is decanted by the step contains Ribosomes

Experiment No.6

To estimate amount of protein by Lowry's method

Aim: To estimate the protein using Lowry's method.

Principle: The –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a blue colored complex. In addition, tyrosine and tryptophan residues of protein cause reduction of the phosphomolybdate and phosphotungstate components of theFolin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method.

Reagents Required:

1. **Reagent A:** 2% sodium carbonate in 0.1 N sodium hydroxide.
2. **Reagent B:** 0.5% copper sulphate (CuSO4.5H2O) in 1% potassium sodium tartarate. Prepare fresh by mixing stock solutions.
3. **Alkaline copper solution (Reagent C):** Mix 50mL of reagent A and 1 mL of reagent B prior to use.
4. **Diluted Folin's reagent (Reagent D):** Dilute Folin reagent with an equal volume of 0.1 N NaOH
5. **Standard*:*** Dissolve 50mg BSA in 50mL of distilled water in a volumetric flask. Take10mL of this stock standard and dilute to 50 mL in another flask for working standard solution.One mL of this solution contains 200 µg protein.

Apparatus and Glass wares required: Test tubes, Pipettes, Colorimeter, *etc*.,

Procedure:

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
2. Pipette out 1 mL of the sample in another test tube.
3. Make up the volume to 1 mL in all the test tubes. A tube with 1 mL of distilled water serves as the blank.
4. Now add 5 mL of reagent C to all the test tubes including the test tubes labeled 'blank ‘and 'unknown'.

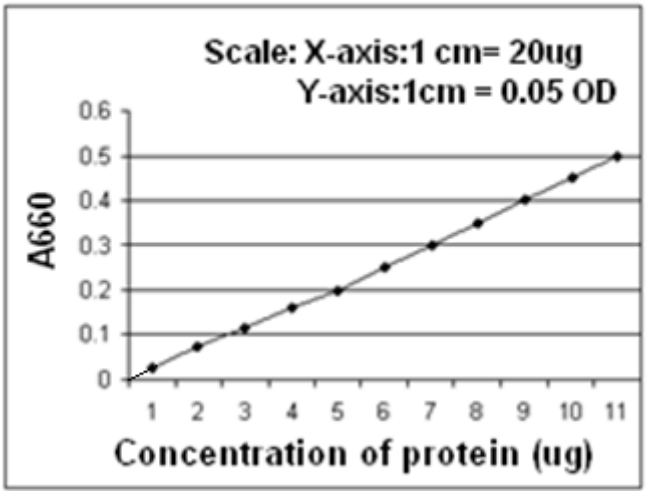
5. Mix the contents of the tubes by vortexing / shaking the tubes and allow to stand for 10min.
6. Then add 0.5 mL of reagent D rapidly with immediate mixing well and incubate at room temperature in the dark for 30 min.
7. Now record the absorbance at 660 nm against blank.
8. Then plot the standard curve by taking concentration of protein along X-axis andabsorbance at 660 nm along Y-axis.
9. Then from this standard curve calculate the concentration of protein in the given sample.

Result: The given unknown sample contains -µg protein/ml.

Observations and Calculations

Volume	Volume	Concentration of protein (µg)	Volume of reagent C	Incubate At Room Temp for 10 min	Volume of reagent D (ml)	Incubate At dark room temp. for 30 min	A660
0.0	1.0	00	5		0.5		0.00
0.2	0.8	40	5		0.5		
0.4	0.6	80	5		0.5		
0.6	0.4	120	5		0.5		
0.8	0.2	160	5		0.5		
1.0	0.0	200	5		0.5		
1.0 UK	0.0	?	5		0.5		

Standard Curve for Protein by Lowry's Method



Experiment No.7

Extraction of DNA from the given animal tissue sample

Introduction

The elucidation of the structure of DNA in 1953 by James Watson and Francis Crick was one of the most exciting discoveries in the history of genetics and molecular biology. After understanding the functional properties of DNA viz, replication, transcription, mutation, recombination and repair, it became possible to manipulate DNA.

Principle

Most methods of DNA isolation involve the breakage or lysis of the cells to release nuclei and further breakage of nuclei to release the chromatin. DNA in cells exists as nucleoprotein complexes and therefore isolation of DNA involves removal of proteins and carbohydrates (if any) associated with it. Finally, the polymeric nature of DNA is utilised to precipitate it and make it free of small molecular contamination.

Reagents, Supplies and Equipment Required

Tissue: spleen/heart/testis/kidney of any vertebrate or coconut endosperm; (2) Mortar and pestles or glass homogeniser;(3) Glass distilled water; (4) Centrifuge (range 3000 to 10,000rpm); (5) pH meter (optional); (6) 10 ml centrifuge tubes; (7) 30 ml test tubes; (8) Test tube rack ; (9) bent glass rod; (10) Sodium saline citrate solution (SSC–85ml of 0.9% sodium chloride solution + 15ml of 0.5% sodium citrate solution usually gives pH 7.4, if not adjust pH.); (11) 12% Sodium chloride solution (Dissolve 12 gms of sodium chloride in 100ml of distilled water); (11) Absolute alcohol (double distilled alcohol).

Laboratory Protocol

(1) Grind about 200mg of the tissues in about 5ml of SSC in a homogeniser or with a mortar and pestle.

(2) Transfer the homogenate into a centrifuge tube and make up the volume to 10ml with SSC.

(3) Centrifuge at 3000rpm for 8 minutes and discard the supernatant.

(4) Rehomogenise the sediment with 5ml of SSC.

(5) Adjust the volume to 10ml, centrifuge at 3000rpm for 8 minutes and discard the supernatant.

(6) Then, suspend the sediment in 10ml of 12% sodium chloride solution and centrifuge at 10,000rpm (at least 7000 rpm) for 15 minutes.

(7) Transfer the supernatant into a 30ml test tube and add 2-3 volumes of absolute alcohol.

(8) Gently mix it by inverting the tube. The white fibrous DNA precipitates.

(9) Spool the fibrous white DNA by winding around a clean sterile bent glass rod.

Result: The fibrous white DNA was observed in the test tube after adding absolute chilled alcohol

Experiment No.8

To estimate amount of DNA by Di-Phenyl Amine (DPA) method

Aim: To estimate the concentration of DNA by diphenylamine reaction.

Principle: This is a general reaction given by deoxy pentoses. The 2-deoxyribose of DNA, in the presence of acid, is converted to ω-hydroxy levulinic aldehyde, which reacts with diphenylamine to form a blue coloured complex, which can be read at 595 nm.

Requirements:

1. Standard DNA solution- Dissolve calf thymus DNA (200µg/ml) in 1N perchloric acid/buffered saline.
2. Diphenylamine solution- Dissolve 1g of diphenylamine in 100 ml of glacial acetic acidand 2.5 ml of concentrated H_2SO_4. This solution must be prepared fresh.

Procedure:

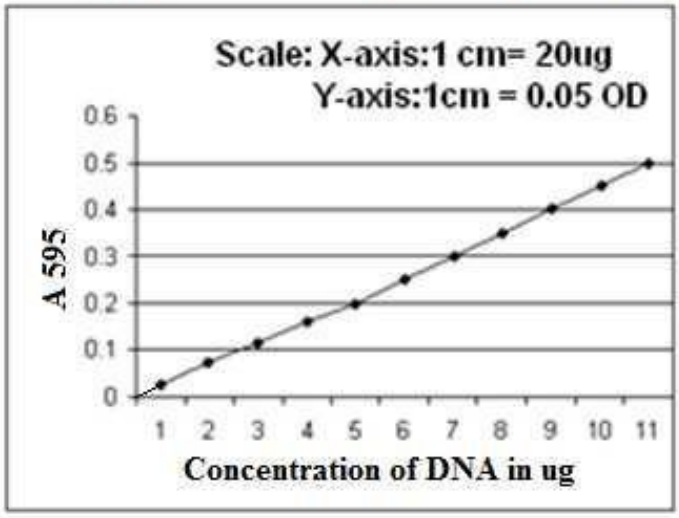
1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled testtubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water servesas the blank.
4. Now add 2 ml of DPA reagent to all the test tubes including the test tubes labeled 'blank'and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate on a boilingwater bath for 10 min.
6. Then cool the contents and record the absorbance at 595 nm against blank.
7. Then plot the standard curve by taking concentration of DNA along X-axis and absorbance at 595 nm along Y-axis.
8. Then from this standard curve calculate the concentration of DNA in the given sample.

Result: The given unknown sample contains…… µg DNA/ml.

Observations and Calculations

Volume of standard (200 µg/ml) DNA (ml)	Volume of distilledwater (ml)	Concentration of DNA (µg)	Volume of DPA reagent (ml)		A595
0.0	1.0	00	2	Incubate in boiling water bath for 10 Min& Cool	0.00
0.2	0.8	40	2		
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be Estimated	2		

Standard Curve for DNA estimation by DPA method



B.L.D.E. Association's

S.B. ARTS AND K.C.P. SCIENCE COLLEGE

VIJAYAPUR- 586103

Karnataka State

B. SC 5th SEMESTER

ZOOLOGY PRACTICAL LAB MANUAL

PAPER-I & II

DEPARTMENT OF ZOOLOGY

2023-24

CONTENTS

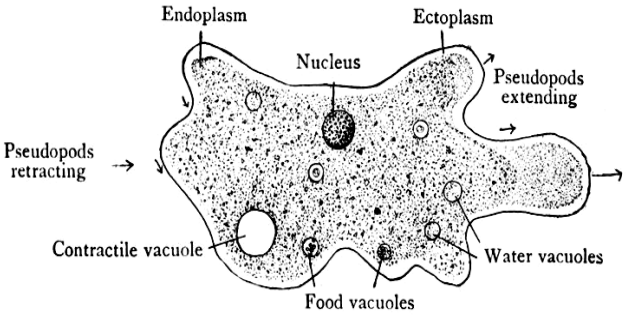
PROTOZOA

Amoeba:

Classification:

Phylum: Protozoa, Class: Rhizopoda, Order: Lobosa

Genus: *Amoeba*



1. Animal is irregular shape, with simple or branched pseudopodia measuring 250 to 600 microns in diameter.
2. Cytoplasm is differentiated into ectoplasm and endoplasm. Ectoplasm contains ectoplasmic ridges.
3. Body of the animal is covered by a thin, delicate and permeable plasma membrane, called as plasma lemma.
4. Endoplasm contains nucleus, food vacuole, contractile vacuoles, water globules and crystals. 5 Permanent posterior end is called as uroid.
5. Withdrawal of pseudopodium and new pseudopodium containing endoplasm is present Feeding may be studied by giving carmine. Nutrition is holozoic.
6. Reproduction by binary fission & Multiple fission
7. Amoeba proteus move by the formation of pseudopodia. Pseudopodia are blunt, finger like extensions of the ectoplasm containing endoplasm (lobo podium).

Euglena:

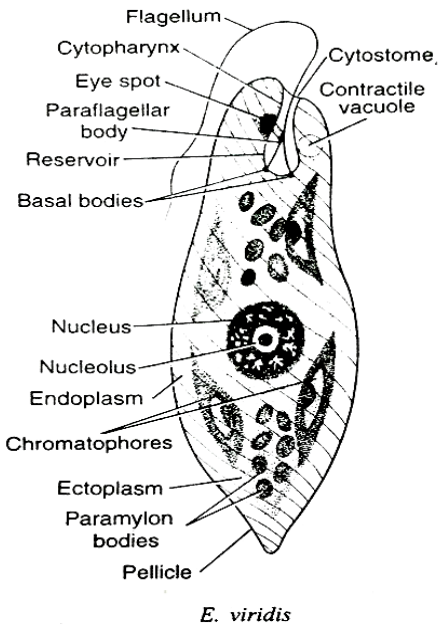
Classification:

Phylum: Protozoa

Class: Mastigophora

Order: Euglenoidina

Genus: *Euglena*



E. viridis

1. Body of animal is simple fuse form, spindle shaped plump like red or green colour.
2. *Euglena* measure 50 to 100 microns in length. Outer covering is called as pellicle which are marked by an spiral striations called asmyonemes.
3. Anterior end has funneled shaped cytosome which leads into a flask –shaped.

4. Cytopharynx on one side is a red mass of hematochrome called stigmata which is photosensitive.
5. Endoplasm contains nucleus ,chloroplast and other ultra structural organelles.
6. Nutrition holophytic or saprophytic and reproduction by longitudinal division or encystment. Euglena does not take solid food but lives entirely by autotrophic and saprozoic nutrition.
7. . It is unique animal with floral mode of nutrition and funnel mode of life and reproduction.
8. *Euglena viridis, E .rubra, E. sanguine* and *E.fusiformis* are common spices they respond to various stimuli such as light heat etc.

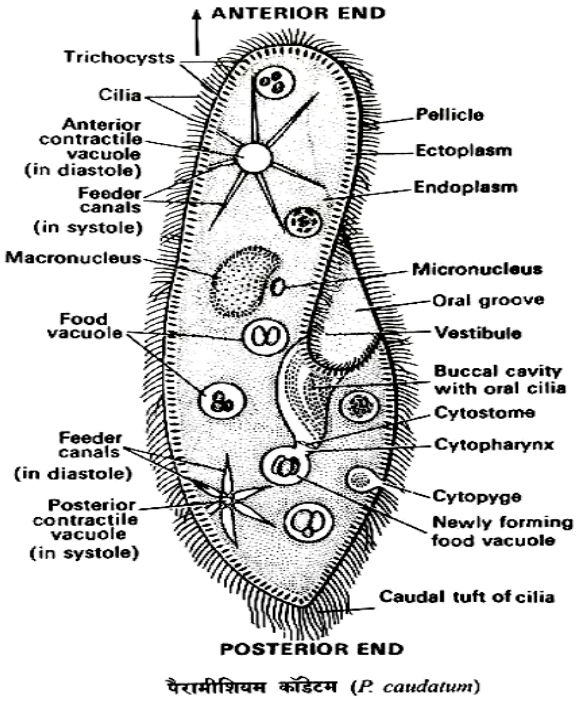
Paramecium:

Classification:

Phylum: Protozoa Class: Ciliata Order: Holotricha

Genus: *Paramecium*

1. Commonly called as slipper animalcule, being microscopic, elongated, slipper- shaped cigar-shaped or spindle shaped.
2. Most familiar and extensively studied protozoans.
3. Pelli Body of animal is simple fuse form, spindle shaped plump like red or green colour.
4. *Paramecia* propel themselves by whiplash movements of their cilia, which are arranged in tightly spaced rows around the outside of their body.



पैरामीशियम कॉडेटम (*P. caudatum*)

5. The beat of each cilium has two phases: a fast "effective stroke," during which the cilium is relatively stiff, followed by a slow "recovery stroke," during which the cilium curls loosely to one side and sweeps forward in a counter-clockwise fashion.

Vorticella:

Classification:

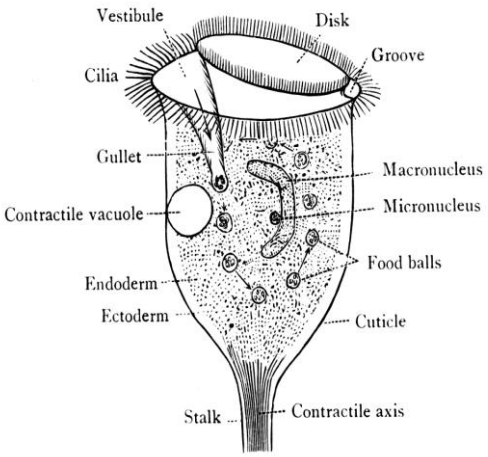
Phylum: Protozoa

Class: Ciliata

Order: Peritricha

Genus: *Vortivella*

1. Vorticella is commonly called bell animalcule.
2. Vorticella has an inverted bell shaped body with a long, simple slender and highly con-tractile stalk for attachment.
3. The bell of Vorticella measures up to 157 microns in length and 99 microns in width and stalk varies from 53 to 4150 microns in length.
4. The free broad end of the body is the oral and the opposite narrow end is the aboral end.
5. The body consists of a thin pellicle, ectoplasm or cortex and endoplasm or medulla.
6. Below the pellicle lie myonemes which are contractile in nature. The rim of the bell is thick and provided with a circle of cilia and the central mass which fills the mouth of the bell is called disc.
7. The peristomial groove is situated between the rim and the disc. On one side the peristomial groove forms a groove which continues into a funnel like structure called vestibule.
8. Mouth is situated at the bottom of vestibule leading into the cytopharynx ending into protoplasm.



PORIFERA

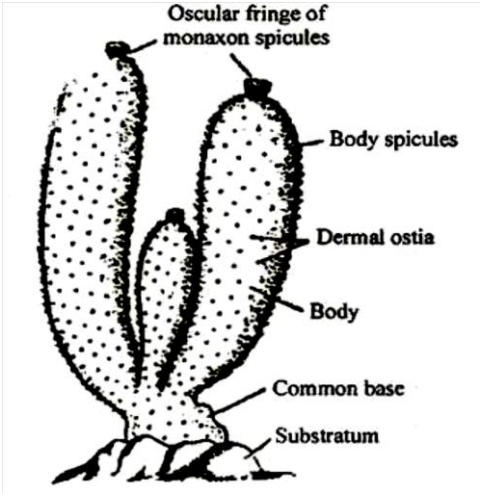
SYCON:

Classification:

Phylum: Porifera Class: Calcaria Order: Heterocoela

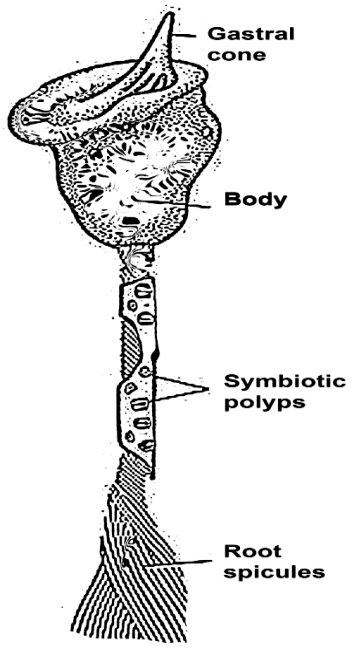
Genus: Sycon

1. Scypha is commonly called Crown sponge.
2. It is a small, solitary or colonial marine sponge found in shallow and well oxygenated water. Commonly distributed in Europe from Rhode Island to Greenland.
3. Body is vase-shaped (measuring 20-25 mm in length) opening to exterior by osculum at distal end.
4. Osculum is encircled by oculars fringe formed by monoaxon spicules. Proximal end or base is attached to the substratum.
5. Body wall is thick having monoaxon, triaxon and tetraxon spicules. Body wall consists of outer dermal



epithelium and inner flattened epithelium which lines the spongocoel separated by a middle layer of mesenchyme.

6. Canal system is syconoid type. Water current passes through Ostia incurrent canal.
7. Prosopyles radial canal apopyles spongocoel osculum exterior. Reproduction by asexual (budding) as well as sexual method.
8. Larval stage is called amphiblastula.



Hyalonema:

Classification:

Phylum: Porifera Class: Hexactinellida Order: Amphidiscophora

Genus: *Hyalonema*

1. *Hyalonema* is commonly called glass rope sponge as it appears like ball of glass wool with projecting tufts of glassy spicules.
2. It is a marine sponge inhabiting 10-15 metres deep sea water. It is found along the New England coast.
3. Body is round or oval and radially symmetrical. The spicules of root tuft are compact, elongated and twisted forming an axis or columella. It helps the organism in anchoring.
4. Several polyps of a Zoanthidea (anemone) grow in symbiotic association with *Hyalonema* at its columella.
5. Osculum contains a sieve plate.
6. Spongocoel can be noticed only when the upper surface of the sponge body is depressed since the excurrent canals open into it, but when the surface is extended into a gastral cone by upward projection of columella, no spongocoel exists.
7. Skeleton consists of small amphidiscs. Whole body is covered by small branching six rayed spicules which resemble to Christmas trees.

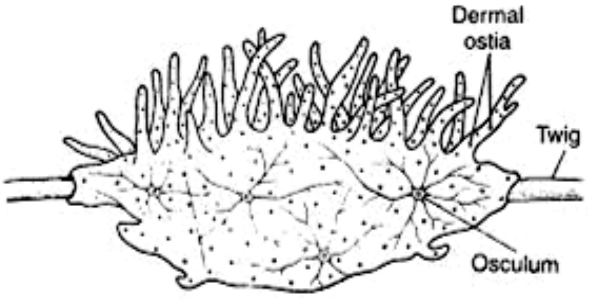
Spongilla:

Classification:

Phylum: Porifera Class: Demospomgiae Order: Haplosclerina

Genus: *Spongilla*

1. *Spongilla* is commonly known as fresh-water sponge as it is found in freshwater ecosystems in Atlantic, Europe and American waters.
2. It is present in the form of profusely branched colony.
3. It exhibits greenish colour due to the presence of symbiotic algae like *Zoochlorellae* within the body.
4. Body wall consists of very thin dermal membrane perforated with dermal pores or ostia and several oscula.



5. Different types of monaxon siliceous spicules are present. They are of large (megascleres) and small (microscleres) size held together by spongin fibre.
6. Canal systen is Rhagon type with choanocytes restricted to small flagellated chambers. Water current passes through dermal pores subdermal cavity incurrent canal several flagellated chambers excurrent canal Osculum.
7. Asexual reproduction occurs by regeneration of fragments or by specialized structures called gemmules.
8. Sexual reproduction by sperm and ova. Devlopment is indirect involving free swimming larva.

CNIDARIA

1. Aurelia:

Classification:

Phylum: Cnidaria class: Scyphozoa order: Semaeostomea

Genus: *Aurelia*

1. *Aurelia* is commonly known as Jelly-fish. It is a solitary, marine, medusoid form inhabiting warm and temperate seas throughout the world.
2. Body is transparent, bluish white in colour. Inner organs are visible from body surface. Circular body measuring about 90 mm in diameter having convex aboral (exumbrellar) and concave oral (sub umbrella) surface.
3. Four red or purple horse-shoe shaped gonads are visible from aboral surface. Mouth is four cornered each corner continues into four long and narrow oral lobes hanging down from oral surface.

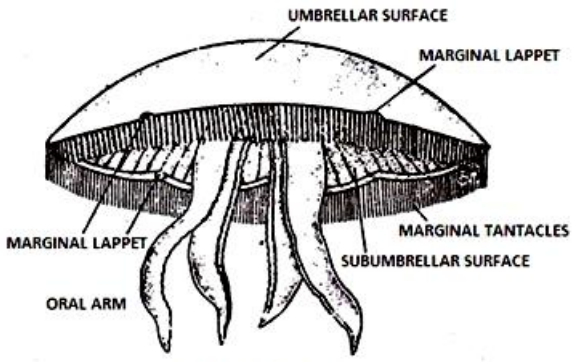


Fig. 169 AURELIA

4. Subumbrellar margin contain marginal tentacles having stinging cells and 8 marginal lappets having sense organs called tentaculocysts (rhopalium).

5. Jelly fish is carnivorous feeding on small animals like molluscs, crustaceans, protozoans, nematodes etc.. It swims by rhythmic contractions of umbrellar surface.
6. *Aurelia* is dioecious i.e. male and female individuals are separate.
7. Life cycle shows alternation of generation.

2. Obelia

Classification:

Phylum: Coelenterata Class: Hydrozoa Order: Hydroidae

Genus: *Obelia*

Obelia is commonly called as sea-fur. It is sedentary marine colonial form attached on the surface of sea weeds, molluscan shells, rocks and wooden piles in shallow waters. It is widely distributed throughout the world.

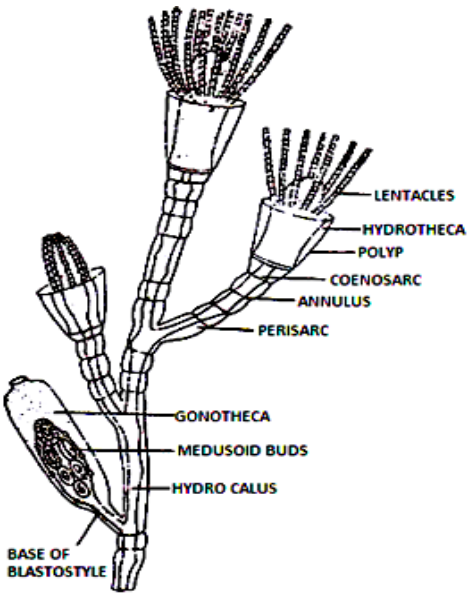


Fig. 101. OBELIA

1. It is a trimorphic colony present in the form of filamentous sea weed measuring several centimeters in height.
2. Hydrorhiza and hydrocaulus are covered by chitinous perisarc which encloses soft inner coenosarc.
3. Coenosarc is the living, hollow cellular tube made up of ectoderm, endoderm and mesoglea. The trimorphic colony of *Obelia* posses three types of zooids polyp (hydranths), gonangium (blastostyle) and medusa (sexual zooid).
4. Polyp is the nutritive zooid. It is a bell shaped cup made up of lower hydrotheca and upper hypostome. Hypostome carries a ciclet of numerous tentacles provided with nematocysts.
5. Blastostyle is the club shaped reproductive zooid enclosed within a covering called gonotheca. It gives rise to buds which develop into medusa.
6. Medusa is the free swimming reproductive zooid consisting of upper exumbrellar (convex) and lowers sub-umbrellar (concave) sides. It is provided with marginal tentacles, four radial canals bearing gonads, a ring canal and a central hanging manubrium on the concave side.
7. It reproduces by asexual as well as sexual methods. Life history of *Obelia* exhibits alternation of generation.

3. Hydra:

Classification:

Phylum: coelenterata class: Hydrozaoa order: Hydroidae

Genus: *Hydra*

1. *Hydra* is a solitary, freshwater and cosmopolitan hydrozoan found attached to some objects in lakes, ponds and streams all over the world but most common in India, Canada and U.S.A.
2. It is a polypoid, tubular and cylindrical coelenterate and measures about 1 cm when fully extended.
3. Proximal end is called basal disc and contains gland cells which secrete adhesive secretion for attachment. The free distal or oral end bears mouth situated on a conical projection called hypostome.
4. Hypostome is encircled by 6-10 hollow, slender tentacles provided with nematocysts. Body wall is diploblastic consisting of an outer ectoderm and inner endoderm separated by mesoglea.
5. Mouth leads to gastrovascular cavity. Lateral buds may be present on the sides of the body which give rise to new individuals by budding.

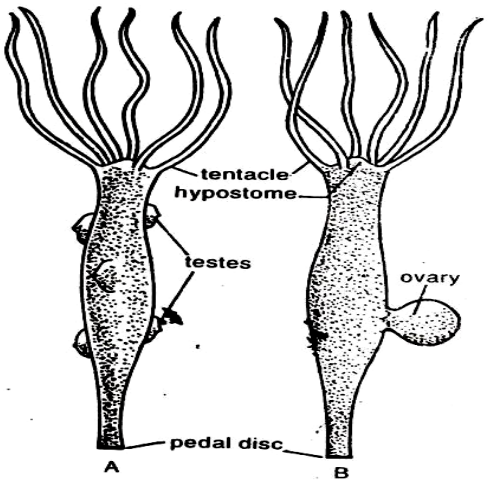


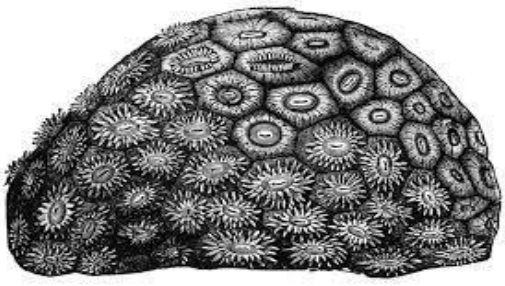
Fig. 20.1. Hydra sp. A. Male, B. Female

6. Gonads are present as buds on the body. Testes are situated near the oral and end while ovaries near the base.
7. Reproduction by both asexual (budding) and sexual methods. *Hydra* posses great power of regeneration. Thus extensively used for experimental studies on regeneration.
8. *Hydra viridis* contains symbiotic green alga Zoochlorellae.

Study of corals:

1. **Astraea:**
   Phylum: Coelentrata Class: Anthozoa Order: Madreporaria
   Genus: *Astraea*

1. Astraea is a massive stony coral.
2. The colony comprises numerous closely fitted polygonal cups or theca.
3. Theca are so closed to each other as to have common walls.
4. Skeleton is very hard made up of calcium carbonate and is secreted by the ectoderm for support of delicate tissues.
5. The colony is formed by buds.



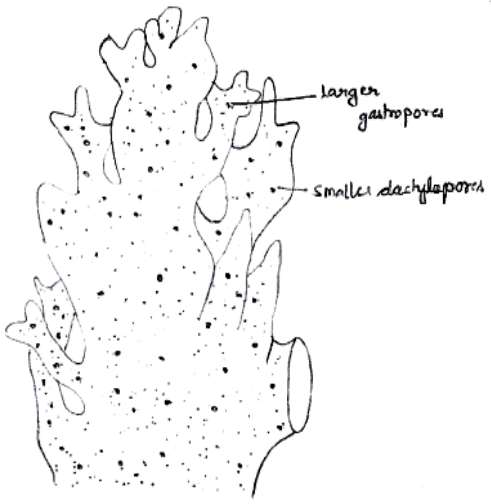
6. The coenenchyme is produced by calcification of coenosarc and also gives rise to corallites which lie in close contact to each other.
7. It is a poreless coral reproducing by gemmation, fissiparous divisions and buddings. Habit and habitat. Astraea is a marine, colonial form. Distribution. Astraea is found on the coasts of warm seas, in U.S.A. Florida and California.

2. Milepora:

Phylum: Coelentrata Class: Hydrozoa Order: Hydrocorallina

Genus: *Millipora*

1. It is commonly known as stinging coral as its powerful nematocysts are painful to man.
2. It is a colonial marine coral distributed throughout the tropical shallow waters of West Indies and U.S.A.
3. Colony consists of upright leaf like calcareous growth, white or yellowish in colours.
4. The surface of colony bears pores of 2 sizes the larger gastropores and smaller dactylopores.
5. Colony has two types of zooids i.e. gastrozooids – shorter having mouth and tentacles and dactylozooids – long, slender, hollow tentacles without mouth.



6. 6. Medusae originate from coenosarcs. They don't have mouth and tentacles and are short lived.
7. 7. Dried colonies form irregular mass.

HELIMINTHES

1. Planeria:

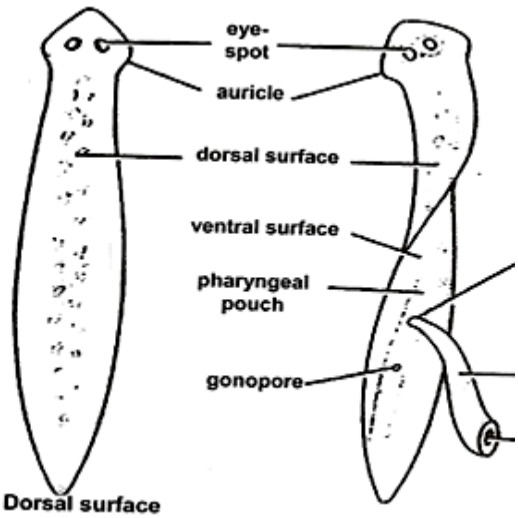
Classification:

Phylum: Platyhelminhes

class: Turbellaria

order: Tricladia

Genus: *Planeria*



1. Planaria (Dugesia) is found in freshwater streams, springs, ponds, lakes and shallow rivers of cold running water (See fig. no. 4.1). They are found in India , Myanmar, U.K ,U.S,A and U.S.S.R.

2. Body elongated leaf like, bilaterally symmetrical, with a broader anterior end and pointed posterior end & dorsoventrally flattened.
3. They are brown or black in color with size vary from 2 to 15 mm. 5Head is broad, blunt, and triangular with laterally on either side auricles or two eyes.
4. Digestive system comprises of mouth (on ventral surface), everted pharynx (proboscis) and branched intestine.
5. Proboscis is covered with the proboscis sheath.
6. Intestine forks into three diverticulated branches, one anterior and two posterior. Genital pore is situated a little posterior to the mouth.
7. Reproduction is sexual and asexual.
8. Planarians are used in experiment for regeneration and grafting.

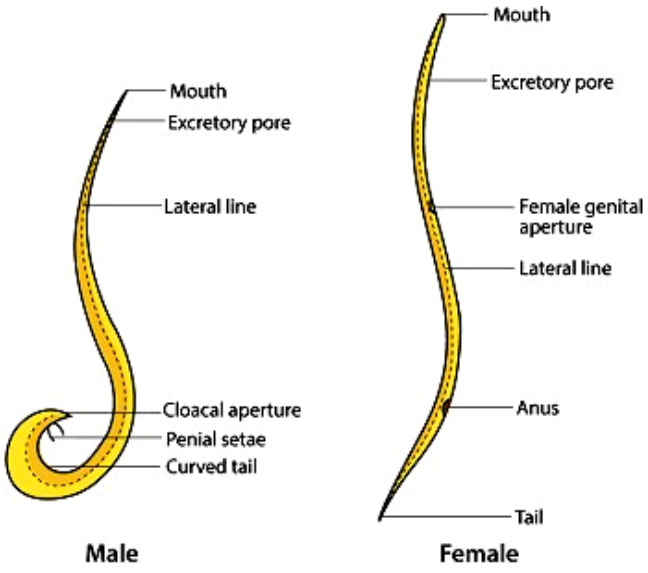
2. Ascaris:

Classification:

Phylum: Aschelimnthes class: Nematoda order: Ascaroidea

Type: *Ascaris lumbricoides*

1. *Ascaris*is a common endoparasite in the small intestine of the man.
2. It causes ascariasis in man especially in children. Infection by eating raw & uncooked vegetables.
3. It shows sexual dimorphism with separate male and female individuals. Male measures 15 to 30 cm and female 20 to 35 cm in length.. Body is elongated, cylindrical, pointed at both ends. Surface of the body is marked with four longitudinal lines.
4. Mouth provided with a median dorsal and a pair of symmetrical sub median ventral lips.



5. Excretory pore is small and lies at the ventral surface with distance of 2 mm away from the anterior end.
6. Tail end of male is ventrally curved containing cloacal aperture, through which two equal isospicules projections.
7. Female genital aperature lies about one-third of the body from the anterior end. Life history is simple and no intermediate host.

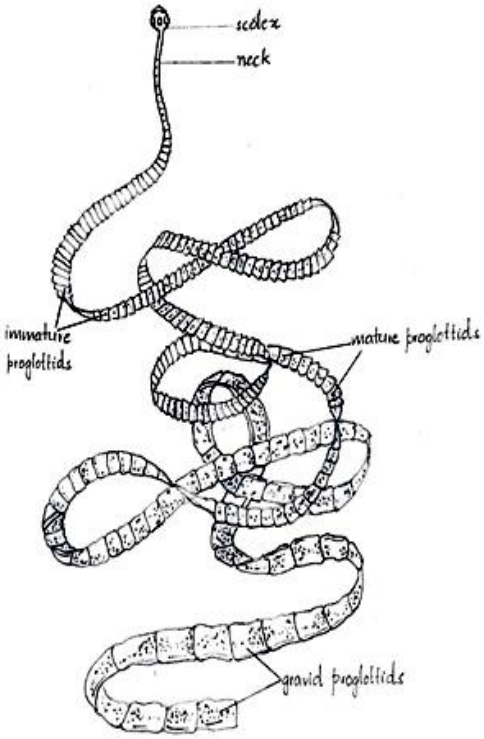
3. Taenia solium:

Phylum: Platyhelminthes,

Class: Cestoda,

Order: Cyclophyllidea

Type: *Taenia solium*



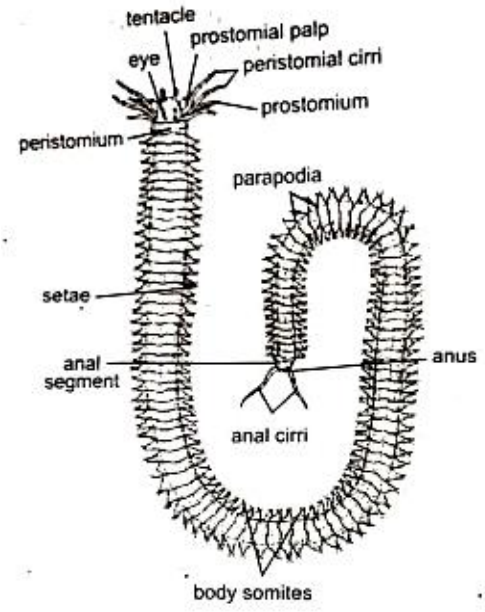
1. It is commonly known as the pork tapeworm. It is an endoparasite inhabiting the intestine of man who eats pork. It feeds on the digested food of the host, and causes nausea, anemia, abdominal pains and nervous disorders.
2. The body is flat, elongated, ribbon like, measures 2-3m in length. It consists of anterior minute pin head like scolex, a narrow short neck and a long strobila.
3. The scolex bears two circlets of hooks and four suckers, meant for attachment.
4. The strobila consists of hundreds of segments called proglottids. They are produced from the neck, by a process called strobilization.
5. Tapeworm does not possess digestive system. Each proglottid has a set of male and female reproductive organs, hence it is hermaphrodite.
6. Life cycle is complicated involves an intermediate host, pig.

ANNELIDA:

1. Neries:

Phylum: Annelida class: Polychaeta order: Errantia

Genus: *Neries*



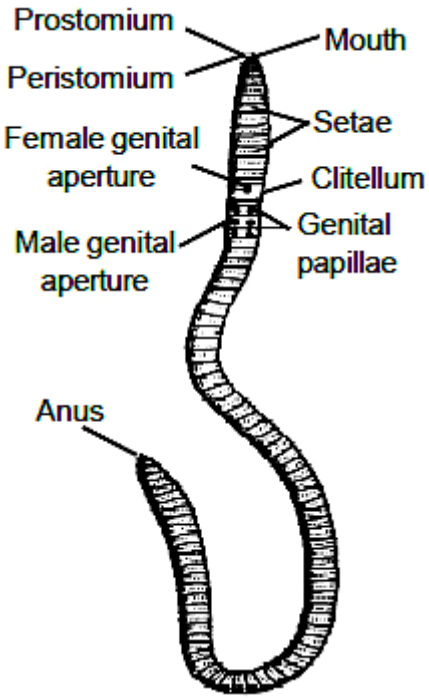
1. Is commonly called rag worm. The body is long, slender, elongated, dorso-ventrally flattened segmented and is divisible into head, trunk and pygidium.
2. Head consists of two parts, the prostomium and peristomium. Prostomium bears a pair of tentacles, two pairs of eyes and a pair of short two jointed palps. Peristomium bears four tentacles and a slit-like mouth.

3. Trunk is made up of several segments, each bearing a pair of lateral parapodia which are Locomotory organs. Setae project beyond the outer margin of each parapodium.
4. Pygidium or anal segment is without parapodia but bears a pair of appendages known as anal cirri and a terminal anus.
5. Respiration happens via blood capillary network of parapodia.
6. Alimentary canal is straight and extends from mouth at the anterior end to the anus at the posterior end.
7. Sexes separate. Fertilization is external.
8. The sexual phase of *Nereis* is known as *Heteronereis*.

2. Earthworm:

Phylum: Annelida class: Oligochaeta order: Neooligochaeta

Genus: *Pheretima*

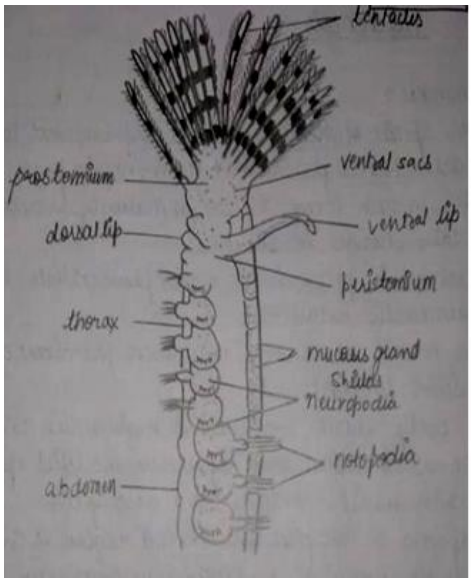


1. Body is bilaterally symmetrical, narrow, long, elongated and cylindrical measuring upto 150 mm in length. Anterior end is tapering and posterior end is blunt.
2. Body is divided into 100-120 ring-like segments by a distinct series of annular grooves.
3. Each segment is provided with setae arranged in a ring with each setae arising from a setigerous sac of the skin. The setae help in locomotion by holding the earth.
4. Mouth is crescentic aperture situated just below the prostomium.
5. The clitellum is a circular band of glandular tissue which completely surrounds the segments from 14th to 16th segment.
6. Hermaphrodite.
7. A pair of male genital pore is situated ventrally in the eighteenth segment while female genital pore are situated at the ventral surface of fourteenth segment.
8. Anus is situated at the terminal end of the last body segment called anal segment.
9. Reproduction is usually sexual. Development takes place in cocoons.
10. Earthworm is used as bait in fishing and as food by many uncivilized people. It also has use in medicines, education, experiments and in agriculture as producer of organic fertilizer.

3. Sabella:

Phylum: Annelida class: sedentaria order: sabelidae

Genus: *Sabella*



1. The body is orange to dull purple in colour. Conspicuous crown of feathery tentacles banded in red, brown and purple, withdrawn into the tube when not feeding.
2. It is commonly known as peacock worm.
3. Chaetae small, slender and unobtrusive.
4. The muddy tube is smooth and flexible and projects up to 10cm above the substrate.

ARTHROPODA

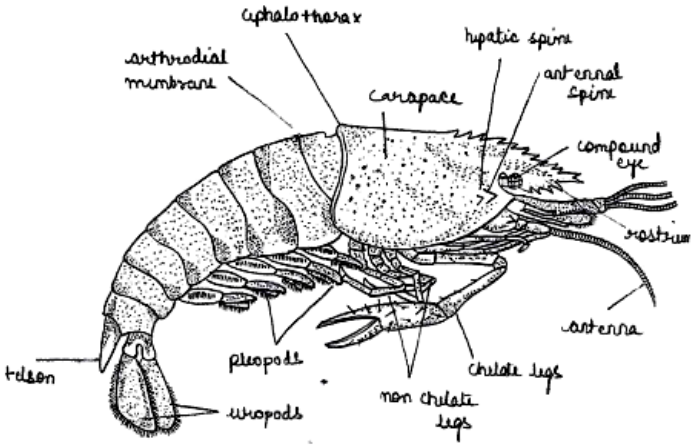
1. Palaemon:

Phylum: Arthropoda

class: Crustacea

order: Decapoda

Genus: *Palaemon*

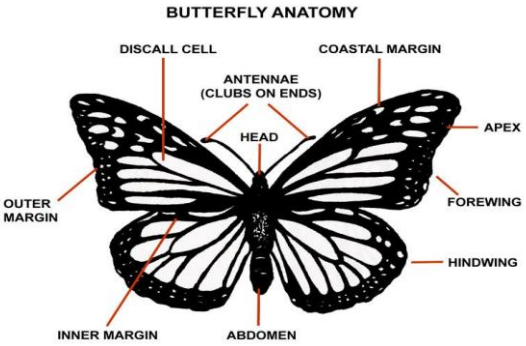


1. Is commonly known as prawn.
2. Body is elongated, spindle shaped and bilaterally symmetrical.
3. *Palaemon* species are of pale-yellow, pale-blue and greenish color with brown tinge or with orange-red patches. Preserved specimens become deep orange-red.
4. The body can be divided into two regions, anterior cephalothorax and posterior abdomen.
5. Cephalothorax is a large, rigid, unjoited, immovable and cylindrical structure. It consists of 13 segments, 5 of the head region, and 8 of the thorax region (Kotpal, 2005).
6. Abdomen is rounded, jointed and compressed laterally. It consists of 6 movable segments and a terminal conical structure, called telson. Each abdominal segment bears a pair of jointed appendages called pleopods or swimmerets.
7. The prawn uses its walking legs for movement at the water-bed. Respiration happens *via* gills, epipodites and lining of branchiostegites.
8. Excretion through a pair of antennary or green glands, a pair of lateral ducts, an unpaired renal or nephroperitoneal sac and the integeument. Sexes are separate. Sexual dimorphism is well developed.

2. Butterflies:

Phylum: Arthropoda class: Insecta order: Lepidoptera

Genus: *Ariadne (*Angled castor butterfly)



1. Butterfly is the most familiar and fascinating brilliantly coloured insect found in dense forests and gardens.
2. The body is slender, delicate, clothed with hairs and scales, and is divisible into head, thorax and abdomen.
3. Head bears a pair of small compound eyes and a pair of clavate antennae. Mouth parts sucking type with a conspicuous long coiled proboscis and are commonly called siphoning type.
4. Thorax bears 3 pairs of legs and 2 pairs of wings covered by scales.
5. Abdomen consists of 10 segments and also covered by scales.
6. The larvae are known as caterpillars.
7. They are diurnal or day fliers and suck the nectar from the flowers. They are useful in pollination of crop plants.

3. Scorpion:

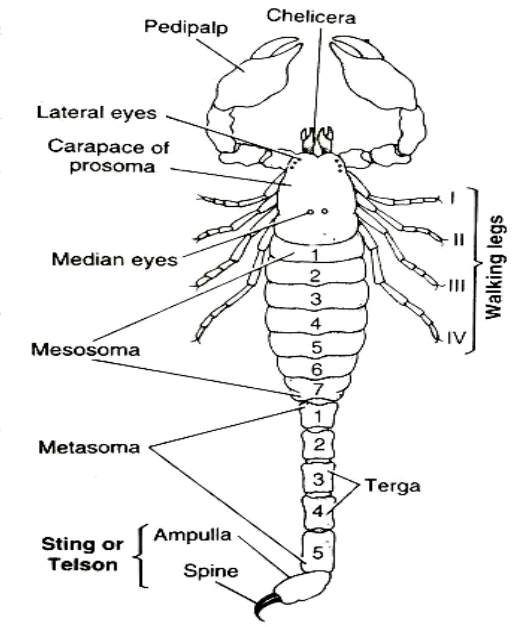
Classification:

Phylum: Arthropoda

class: Arachnida

order: Scorpionidea

Genus: *Buthus tamulus*



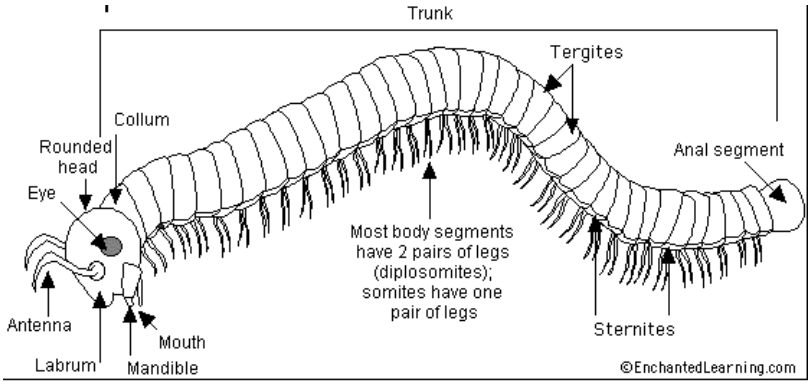
1. Buthus is a common species of scorpion reaching to size of 8cm.
2. The scorpion is a nocturnal animal, hiding during day time under wood, stones, leaves and in crevices and holes. It is carnivorous and feeds on insects and spiders.
3. The body is divisible into prosoma, mesosoma (6segments) and metasoma (6segemnts)
4. The prosoma is not segmented externally. It bears a pair of forceps like chelicerae, a pair of large pedipalp and 4 pairs of walking legs.
5. There are a pair of median eyes and 5 pairs of small lateral eyes. All eyes are simple in structure.

6. The mesosoma bears a genital pore on ventral side, which is clefted in males and entire in females. The second segment bears a pair of comb like structure called pectines. There are 4 pairs of slit like stigmata. These are openings of the respiratory organs called book lungs.
7. The metasoma is 6 segmented more or less cylindrical. The last segment bears a sting with an ampulla and a spine.
8. Sexes are separate. Viviparous.
9. Scorpion is harmful to mankind. Their poison causes extreme pain, fever and sometimes fatal.

4. Millipede:

Phylum: Arthropoda Class: Myriapoda Order: Diplopoda

Genus*: Julus*

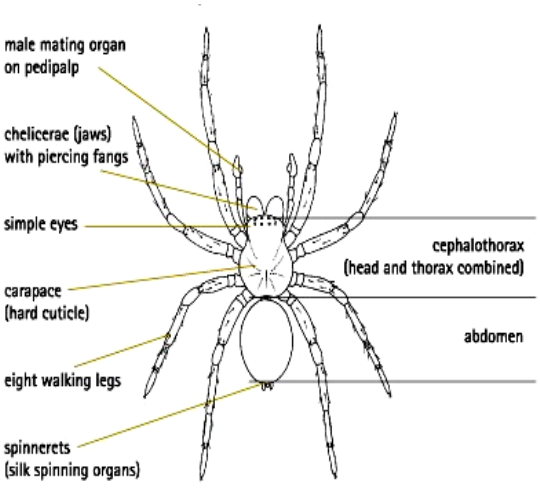


1. Body is elongated and cylindrical consisting of number of segments, and the colour may be yellowish brown or reddish chestnut.
2. Herbivorous, lives in moist soil.
3. Body is divisible into head, thorax and abdomen.
4. Head consists of 5 segments, thorax of 4 segments and abdomen of 20-100 segments.
5. Head bears a pairs of antennae, a pair of mandible and a pair of maxillae.
6. Thorax segments with one pair of legs in each segment while abdomen segments bear two pairs of legs.
7. Stink glands present along the sides of the body secreting noxious substance.
8. Sexes separate and gonopores are situated mid ventrally in 3rd abdominal segment.

5. Spider:

Phylum: Arthropoda Class: Arachnida Order: Araneae

Type: *Argiope anasuja*



1. Argiope, commonly known as Orb-web spider, is found on trees, grasses and in shady places.
2. Body consists of prosoma and opisthosoma. Prosoma is covered by carapace and bears eight eyes dorsally and six pairs of appendages, a pair of subchelate chelicerae with poison glands, a pair of non-chelate pedipalps and 4 pairs of walking legs.
3. Opisthosoma is oval, unsegmented, without appendages and bears spinnerets just anterior to

the anus to produce the threads for making webs.

4. Respiration by book lungs or trachea or by both.
5. Excretion by malpighian tubules and coxal glands.
6. Sexes separate with sexual dimorphism. Pedipalp in male acts as Copulatory organs. Female eats up male after copulation.
7. Spiders are carnivorous and nocturnal feeding on small insects by hunting.

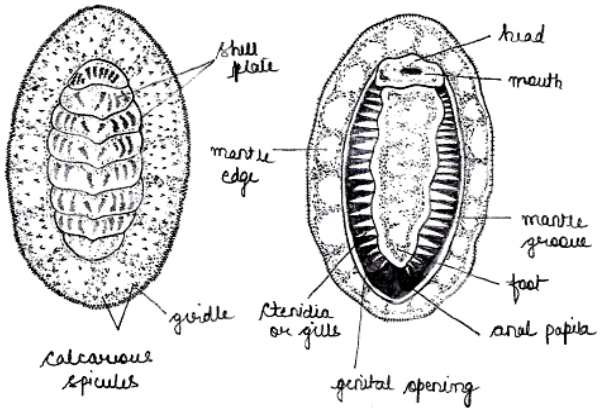
MOLLUSC:

1. Chiton:

Classification:

Phylum: Mollusca class: Polyplacophora order: Chitonina

Genus: *Chiton*



1. Body of Chiton is bilaterally symmetrical, unsegmented and dorso-ventrally compressed.
2. It consists of shell, foot, mantle and the visceral mass.
3. Shell is calcareous and is present on the dorsal side and is composed of eight overlapping plates.
4. Head is not distinct. Eyes and tentacles are absent. Foot is ventral, broad, sole-like and muscular, adapted for creeping and adhering.
5. Mantle covers greater part of body and partly covers the edges of the shell plates.
6. Mouth and anus are at opposite ends.
7. Sexes are separate; gonad is single and is located in the front of the heart. Excretory system consists of two nephridia.
8. Development is indirect through trochophore larva. 12. Chitons are eaten as food and their shells are used for decoration.

Octopus:

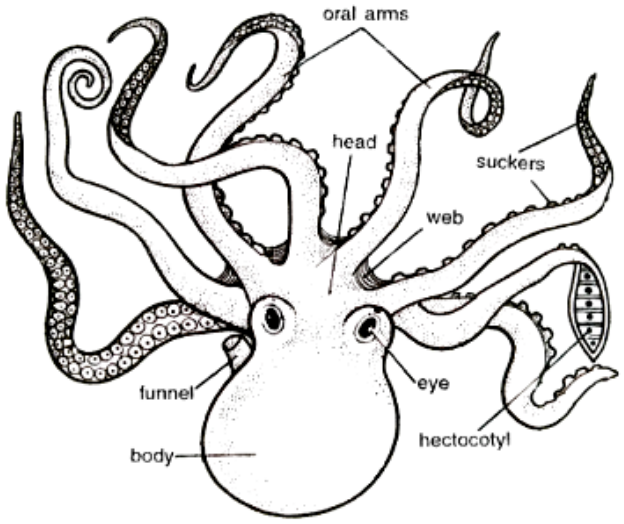
Classification:

Phylum: Mollusca class: Cephalopoda order: Octopoda

Genus: *octopus*

An Octopus is commonly called devil fish. The body is unsegmented, symmetrical and soft bodied animals.

1. The head bears a pair of eyes. The mouth is surrounded by eight elongated equal arms usually bearing suckers.
2. Shell is absent. It moves around by crawling or swimming.
3. For defence it ejects ink from the ink-gland into the surrounding water, producing a smoky cloud.
4. In males one of the arms, called hectocotylized arm, bears a spoon shaped organ at its end. The arm is used to caress the female and deposit spermatophores beneath its mantle.
5. Sexes are separate. 9. Development is direct.
6. It feed upon crabs, bivalves and fishes etc.



Pila

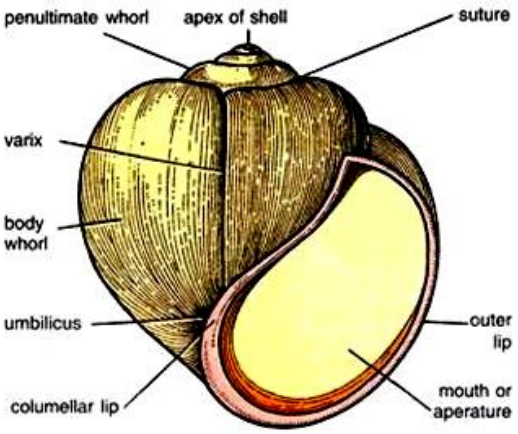
Classification:

Phylum: Mollusca Class : Gastropoda Order: Mesogastropoda

Type: *Pila globosa*

Pila is commonly called as apple snail, found in ponds, pools, streams, tanks etc.

1. The soft body of the animal is divided into head, foot, visceral mass and mantle. And is enclosed within a shell composed of a single valve.
2. The shell is spirally coiled around an axis. The top of the shell is called the apex. And each spiral is called as whorl. Most part of the body, visceral mass is found in last whorl called body whorl. The mouth of the shell is closed by an operculum.
3. Head and foot projects out from the mouth of the shell. But visceral mass remains in body whorl.
4. It feeds on aquatic plants.
5. Respiration by ctenidium, and pulmonary sac.
6. Sexes separate with sexual dimorphism. Development direct.

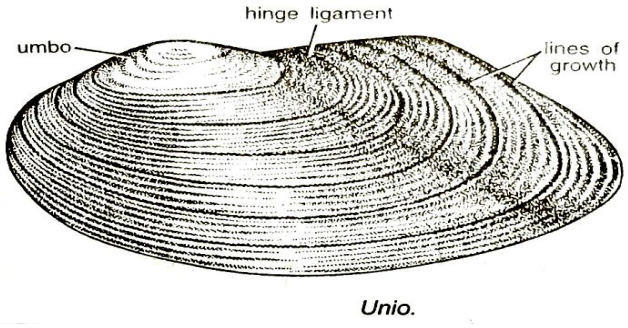


Shell pattern:

1. Unio:

Phylum: Mollusca Class: Pelecypoda Sub class: Palaeocheterodonta

Type: *Lamellidens marginalis*



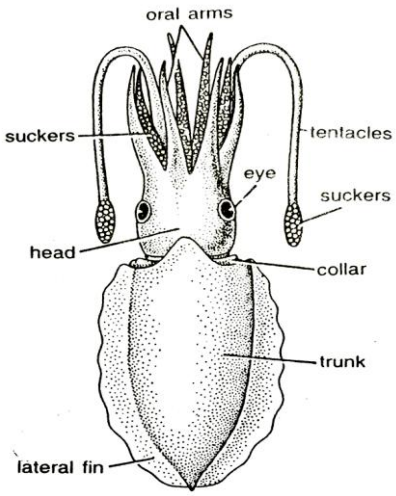
Unio.

1. It is commonly called as fresh water mussel, found in ponds, lakes, streams and rivers, buried in the sand or mud.
2. The soft unsegmented body is enclosed in bivalve shell. The two valves of the shell are joined together by a hinged ligament dorsally. Each shell valve has a raised umbo and numerous lines of growth.
3. A wedge shaped muscular foot projects between the two valves ventrally.
4. The soft body is covered laterally by mantle.
5. Respiration is by leaf like ctenidia. A pair of siphons project from the posterior end. One is the inhalant siphon meant for an entry of water and other exhalent siphon for the exit of water.
6. It is a filter feeder. Mouth and anus are placed at opposite ends.
7. Sexes are separate. Fertilization is external. Development is indirect with Glochidium larval stage.

2. Sepia:

Phylum: Mollusca Class: Cephalapoda, Sub class: Dibranchiata

Type*: Sepia*



Sepia

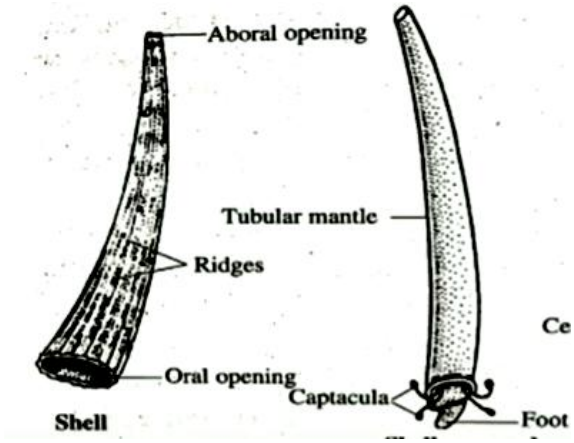
1. Sepia is commonly called as cuttle fish, is a marine mollusc found in shallow coastal waters.
2. Body bilaterally symmetrical, dorso-ventrally flattened, and is divisible into head, neck and trunk.
3. The head bears a pair of large and highly developed eyes and five pairs of arms surrounding the mouth. Four pairs are short and stout with inner surface bearing suckers. The fifth pair of arms is the longest and is known as tentacles. These are provided with suckers only towards their free ends. In male the left fourth arm is hectocotylised to transfer the spermatophores into the female mantle cavity during the reproduction.
4. The neck is inconspicuous connecting the head with the trunk. Trunk is covered by mantle making the flat shell internal. It has two extended thin lateral fins for swimming
5. Mantle is thick and muscular enclosing a large mantle cavity on the ventral side which contains the viscera. Funnel is tubular opening into the mantle cavity.

Ctenidia or gills are the respiratory organs. Single kidney is the excretory organ

3. Dentalium:

Phylum: Mollusca Class: Scaphopoda

Genus : Dentalium entalis



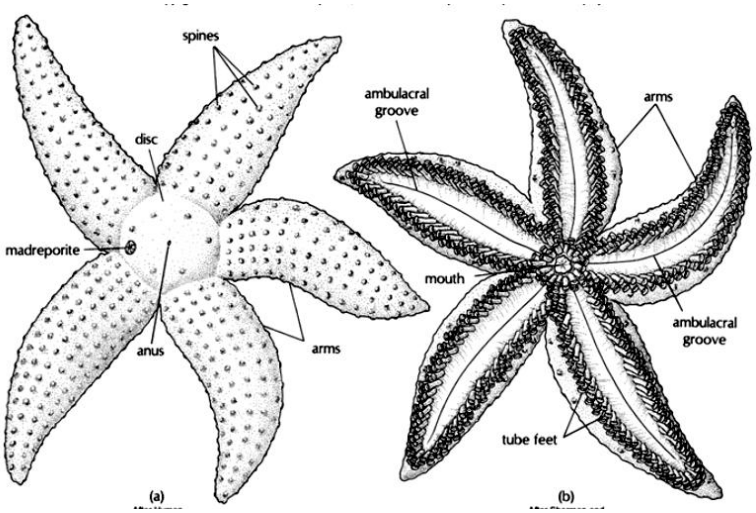
1. Dentalium is commonly known as 'elephant's tusk shell.
2. It is a marine mollusk, living at moderate depths in the sub littoral zone. It is found burrowing in sand or shell gravel.
3. The shell is a slightly curved and tapered tube, opening at both ends and resembling an elephant's tusk.
4. The mantle cavity is long and the gills are entirely wanting.
5. The foot is pointed and the mouth is located at its base in a projection of the pharynx.

ECHINODERMATA

1. Sea star (Asterias):

Phylum: Echinodermata Class: Asteroidea Order: Forcipulata

Genus: *Asterias*

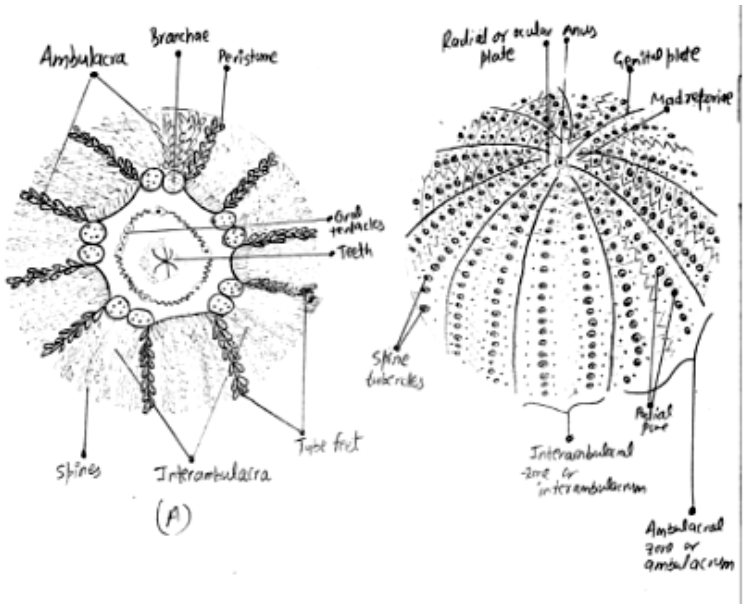


1. It is often called as "sea star" or "starfish" and can be collected on sea shores.
2. The body is flattened with a central disc and five radiating arms.
3. The upper surface is the aboral surface and lower is the oral surface.
4. The anus lies in the centre of aboral surface. A porous plate called madreporite occurs near the anus. The mouth lies in the centre of the oral surface.
5. A groove called ambulacral groove extends from the mouth along each arm.
6. The locomotory organs called tube feet are arranged in grooves in rows.
7. The surface is covered by calcareous plates or Ossicles. They bear very short spines. The spines at the borders of the arms are larger.
8. There are also short forceps like structure called pedicillariae. All these together form the endoskeleton of starfish.
9. The sexes are separate. There is no sexual dimorphism. Development indirect.

2. Sea urchin Echinus:

Phylum: Echinodermata Class: Echinoidea Order: Diamentoidea

Genus: *Echinus*



1. Echinus is commonly known as sea urchin, is widely distributed in the Atlantic, Mediterranean and Pacific waters**.** Echinus is seen in inter tidal zones, attached to the rocks.
2. The body is globular and found enclosed in a rigid globular test or corona.
3. The test is composed of closely fitted calcareous plates or Ossicles. The surface of the test is divided into alternate ambulacral and interambulacral areas. The test bears numerous spines and rows of tube feet in the ambulacral areas.
4. The mouth faces the substratum, and is surrounded by soft membrane known as peristome. Through the mouth project the five teeth of Aristotle's lantern.
5. Anus and madreporite are present on the aboral side.
6. Internally echinus has digestive, water vascular and reproductive system.
7. Sexes separate. Development includes a free swimming echinopluteus larva.

BENIFIFICIAL NON CHORDATES

SERICULTURE:

Types of silk:

- There are five major types of silk of commercial importance, obtained from different species of silkworms which in turn feed on a number of food plants: Except mulberry, other varieties of silks are generally termed as non-mulberry silks. India has the unique distinction of producing all these commercial varieties of silk.

1. Mulberry:

The bulk of the commercial silk produced in the world comes from this variety and often silk generally refers to mulberry silk. Mulberry silk comes from the silkworm, *Bombyx mori* L. which solely feeds on the leaves of mulberry plant. These silkworms are completely domesticated and reared indoors. In India, the major mulberry silk producing states are Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir which together accounts for 92 % of country's total mulberry raw silk production

Non mulberry silk:

1. Eri silk:

Also known as Endi or Errandi, Eri is a multivoltine silk spun from open-ended cocoons, unlike other varieties of silk. Eri silk is the product of the domesticated silkworm, *Philosamia ricini* that feeds mainly on castor leaves. Ericulture is a household activity practiced mainly for protein rich pupae, a delicacy for the tribal. Resultantly, the eri cocoons are open-mouthed and are spun. The silk is used indigenously for preparation of *chaddars* (wraps) for own use by these tribals. In India, this culture is practiced mainly in the north-eastern states and Assam. It is also found in Bihar, West Bengal and Orissa.

2. Oak tasar:

It is a finer variety of tasar generated by the silkworm, *Antheraea proyeli* J. in India which feed on natural food plants of oak, found in abundance in the sub-Himalayan belt of India covering the states of Manipur, Himachal Pradesh, Uttar Pradesh, Assam, Meghalaya and Jammu & Kashmir. China is the major producer of oak tasar in the world and this comes from another silkworm which is known as *Antheraea pernyi*.

3. Tasar:

Tasar (Tussah) is copperish colour, coarse silk mainly used for furnishings and interiors. It is less lustrous than mulberry silk, but has its own feel and appeal. Tasar silk is generated by the silkworm, *Antheraea mylitta* which mainly thrive on the food plants Asan and Arjun. The rearings are conducted in nature on the trees in the open. In India, tasar silk is mainly produced in the states of Jharkhand, Chattisgarh and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh. Tasar culture is the main stay for many a tribal community in India.

4. Muga:

This golden yellow colour silk is prerogative of India and the pride of Assam state. It is obtained from semi-domesticated multivoltine silkworm, *Antheraea assamensis*. These silkworms feed on the aromatic leaves of Som and Soalu plants and are reared on trees similar to that of tasar. Muga culture is specific to the state of Assam and an integral part of the tradition and culture of that state. The muga silk and high value product is used in products like sarees, mekhalas, chaddars, etc.

VERMICULTURE:

Earthworm species used in vermiculture:

1. Eisenia fetida (foetida):

Commonest compost worm used in worm farming and easy to obtain. Usually called Red Wigglers, but also known as Red Worms, Red Wrigglers, Compost Worms, Manure Worms and Brandling Worms. They got their name of red wiggler because as fishing worms as theyare active on the hook and stay alive in water for some time, although they are a bit small for this purpose. They are between 2 to 3 inches long and weigh in at 900 to 1000 worms per pound. They are quick breeders and productive in vermicomposting. They are found throughout the world and as such are no threat to the environment if they escape. Temp range — Extremes: 38°F-88°F / Optimum 70°F -80°F.

2. *Eudrilus eugeniae:*

Common name: African Night crawlers. These worms are much larger than Eisenia Fetida (Red Wigglers) and are commonly. over six inches long. Good compost worms and great for fishing, because of their size and as they are lively on the hook and have a firm skin. They prefer temperatures of around 75°F- 85°F , but can tolerate 45°F- 90°F, cannot tolerate extreme cold and dislike disruption of environment and handling. Weight: 175 to 200 worms per pound.

3. *Lumbricus terrestris:*

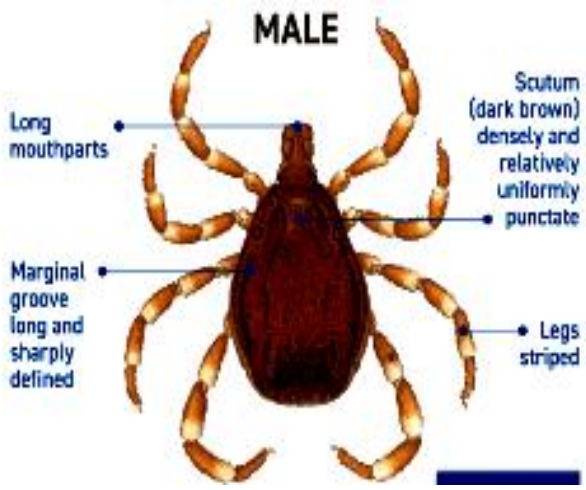
Common earthworm species, sometimes called nightcrawlers. They are not suitable for vermiculture as they are a deep burrowing species (Anecic). Their burrows, are semi permanent and may extend to six feet below the surface — these burrows are lined with mucus and help aerate the soil and improve water retention.

4. *Perionyx excavates:*

Common name. : Indian blue worm. This species has a distinctive iridescent blue sheen to its skin. It is a tropical worm and does not tolerate cold or much handling or environmental disruption. Although small, it is suitable for vermiculture as it is a prolific breeder and matures quickly. It has one major drawback though - it is known for staging mass escapes from the worm farm, for no apparent reason and is somewhat unpopular for this reason. Temperature range — Extremes:
45°F - 90°F / Optimum 70°F - 80°F.

HARMFUL NON CHORDATES:

1. Ticks:



(i) Identification features:
   a. Oval in shape
   b. Cannot distinctly be separated into head, thorax and abdomen.
   c. Contain four pairs of legs but no antennae.
   d. Dorsal surface is covered by a chitinous shield, called scutum.
   e. It has a "head" or capitulum at anterior end.

(ii) Feeds both day and night and cannot stand starvation.

(iii) Important species:

a. Dermacentor sp.
b. Haemophysalis sp.

(iv) Diseases transmitted:

   a. Tick typhus, Kyasanur forest disease (KFD) , Tularaemia, Human babesiosis

DIGESTIVE SYSTEM OF EARTHWORM

Structure of the Alimentary Canal

The alimentary canal is long and straight and runs between the first and last segment of the body.

Parts of the alimentary canal	No. of the segment in the body
Mouth	1
Buccal Cavity	2-3
Pharynx	3-4
Oesophagus	5-7
Gizzard	8-9
Stomach	9-14
Intestine	15-last
Anus	last

- **Mouth:** Mouth is the first segment called **peristomium** and is covered by prostomium, which acts as a wedge to force an open crack in the soil. The prostomium is sensory in nature. The mouth is a crescentic aperture which opens into the buccal cavity. Food ingestion takes place through mouth.
- **Buccal Cavity:** It extends from 2nd to 3rd segment. It is a thin-walled chamber. It can protrude out or retract in with the help of contraction of muscles attached to the body wall. It helps in holding the food during ingestion. Buccal cavity opens into a muscular chamber called the pharynx.
- **Pharynx:** It's a thick-walled, pear-shaped chamber, which extends till 4th segment. It is differentiated from the buccal chamber by a dorsal groove where the brain of an earthworm is present. The dorsal part of the pharynx has a pharyngeal bulb, which is made up of muscle fibre, connective tissues, blood vessels and salivary glands. Salivary glands are unicellular and known as chromophil. Chromophil cells secrete saliva which contains proteolytic enzymes protease which converts protein to amino acids. Mucin is the other enzyme present, which makes the food soft. The pharyngeal wall is connected to the body wall through muscular strands which contract or dilate the pharyngeal lumen. It acts as a suction pump.

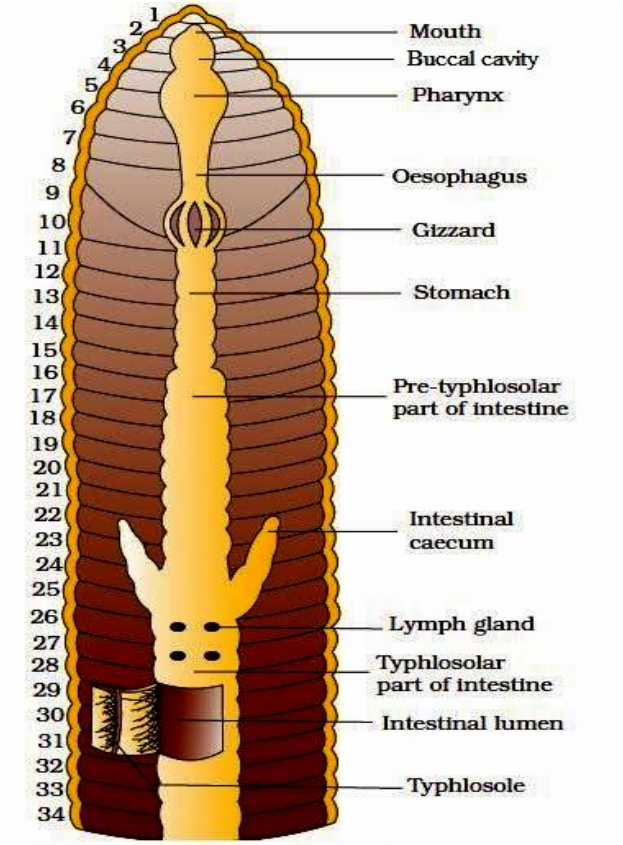


Fig. Alimentary canal of earthworm

- **Oesophagus:** Pharynx continues into the oesophagus. It is a small narrow thin tube-like structure present on 5-7 segment. It passes food to the gizzard. It has no glands.
- **Gizzard:** It is present on 8-9 segment. It is a highly muscular thick-walled organ. It has an inner lining of the cuticle which makes it the hardest part of the alimentary canal. It helps in the grinding of soil particles and decaying leaves.
- **Stomach:** The stomach extends from 9-14 segments. It is a highly vascular and tubular structure. Three pairs of Calciferous glands are present in the segment 10-12, which produces carbonate of lime and helps in neutralising humic acid present in the humus. Glandular cells of the stomach secrete proteolytic enzymes which help in digestion of proteins. Stomach leads to the intestine.
- **Intestine:** It is a long thin-walled tube present from the 15^{th} segment till anus i.e. the last segment. The inner lining of the intestine is ciliated, vascular and glandular. The inner lining is folded to form villi. These villi increase the effective area of absorption in the intestine. The intestine is divided into 3 parts:

- **Pre-typhlosolar region:** present from 15-26 segment. It contains villi. There is a short conical projection on the 26th segment, which is known as intestinal caeca. Intestinal caeca extend upwards till 23rd segment and secrete amylase enzyme. Amylase helps in the digestion of carbohydrates.
- **Typhlosolar region:** Typhlosole is the large internal median fold of the dorsal wall of the intestine forming a longitudinal ridge, which is present after the 26th segment, except the last 23rd-25th segments. Typhlosole increase the area of absorption in the intestine
- **Post-typhlosolar region:** It's the last 25 segments of intestine and also known as rectum. Intestinal villi and typhlosole are absent in this region. It stores faecal pallets and leads to the anus.

Anus: It is a small round aperture present in the last segment. The alimentary canal of an earthworm opens to the exterior through the anus. Undigested food is excreted out through the anal opening in the form of worm casting.

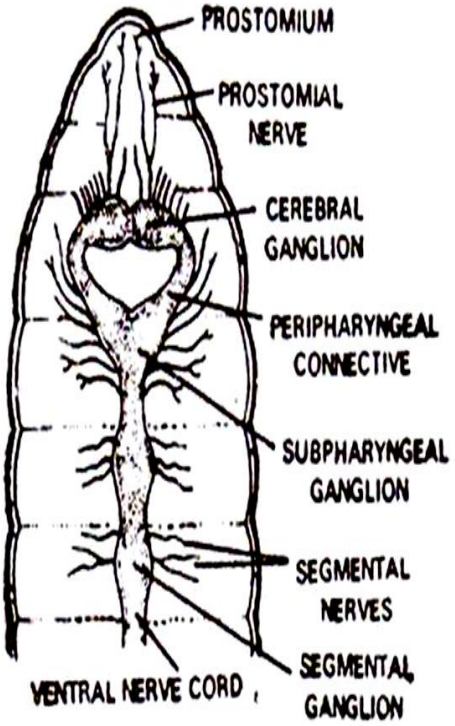
NERVOUS SYSTEM OF EARTHWORM:

The nervous system of earthworm consists of three parts namely,

I. **Central nervous system**
II. **II. Peripheral nervous system**
III. **III. Sympathetic nervous system**

I. Central Nervous System It includes following structures:

i. Nerve ring ii. Ventralnervecord



i. Nerve ring : It is ring like spherical structure which lies around the pharynx in 3rd and 4th segment.
It has 3 parts:

Cerebral ganglia- There are two pear shaped cerebral ganglia fused together at 3rd segment called brain. They are bilobed in structure. A pair of whitish pear-shaped supra pharyngeal ganglia fused to form brain. Lies dorsally in the 3rd segment in the depression between the buccal cavity and the pharynx.

Circum pharyngeal connective- Two circum pharyngeal connectives arise from each cerebral ganglion laterally. They encircle pharynx and fuse at 4th segment. The fused portion is called sub pharyngeal ganglia. It is outer buldging part which extends from 3rd to 4th segment.

Sub-pharyngeal ganglia- Found in lower region of nerve ring which lies in 4th segment.

Ventrally Circum-pharyngeal ganglia meet with a pair of sub pharyngeal ganglia. Thus, a complete nerve ring is formed around the pharynx.

ii. Ventral nerve cord

It is white rod like structure, which starts running from sub- pharyngeal ganglia towards posterior end. In each segment, ventral nerve cord swells which is called segmental ganglia. Actually, there are two cords fuse together to form single ventral nerve cord. Ventral nerve cord is composed of nerve cells and nerve fibers. There are 4 giant fibers on mid dorsal side of nerve cord which conducts impulses rapidly. The outer covering of ventral nerve cord is called peritoneum.

II. Peripheral nervous system

It includes nerve fibers or nerve, which arises from central nervous system.

- From cerebral ganglia, 8-10 nerves arise and supply to prostomium, buccal chamber, and pharynx.
- From circum pharyngeal connectives, two pairs of nerves arise and supply to 1st and 2nd segment.
- From sub pharyngeal ganglia, three pairs of nerve arise and supply to 2nd, 3rd and 4th segment.
- From each segmental ganglion, three pairs of nerves arise and supply to respective segment.

III. Sympathetic nervous system

IV. It consists of nerve plexuses extensively branched and distributed beneath epidermis, alimentary canal that is connected to circum pharyngeal connectives.

Chordates and Comparative Anatomy

PAPER - 2

Fifth Semester B.Sc. (Zoology)

Paper Code: 21BSC5C6ZOO6P (DSCC-12) **Paper Title:** Chordates and Comparative Anatomy (Practical)

Teaching Hours: 4 H / Week **Marks:** 25+25=50

Total hours: 56 hrs **Credits:** 02

Title of the experiments

1) **Protochordata**: Balanoglossus and T. S through proboscis Ascidian/Herdmania and Amphioxus, T.S. of Amphioxus through pharynx and intestine.
   Cyclostomata: Petromyzon, Myxine. Ammocoete larva
2) **Pisces**: Cartilaginous fishes – Narcine, Trygon, Pristis, Myolobaties, Scolidion.
   Bony fishes– Zebrafish, Hippocampus, Muraena, Ostracion, Tetradon, Pleuronectus, Diodon, Echeneis.
3) **Amphibia**: Rana, Bufo, Ambystoma, Axolotl larva, Necturus and Ichthyophis.
4) **Reptilia**: Turtle, Tortoise, Mabuya, Calotes, Chameleon, Varanus.
   Snakes–Dryophis, Ratsnake, Brahmini, Cobra, Krait, Russell's viper and Hydrophis.
5) **Aves**: Beak and feet modifications in the following examples: Duck, Crow, Sparrow, Parrot, Kingfisher, Eagle or Hawk.
   Mammalia: Mongoose, Squirrel, Pangolin, Hedge Hog, Rat and Loris, Platypus, Echidna.
6) **Virtual Dissection/Cultured specimens**: Shark/Bony fish: Afferent and efferent branchial systems, glosso-pharyngeal and vagus nerves.
7) **Comparative account of skeletal system**: Skull, girdles bones of Shark, Frog, Calotes, Pigeon and Rabbit
8) **Comparative account** of heart in Shark, Frog, Calotes, Pigeon and Man.

SCHEME OF PRACTICAL EXAMINATION (Paper-2)

B.Sc. Zoology **V Semester (NEP)**

Paper Title: Chordates and Comparative Anatomy (Practical)

Duration: 3 Hrs **Max. Marks: 25**

Q I. Dissect and display the organ system of available cultured specimens provided and comment.

(Dissection and display-4 marks, comments-2 marks) **(4+2)=06**

OR

Virtual Dissection (Two Specimen) - Identify. Draw labeled diagram and comment on the flagged Systems (Identification of the system – ½ marks: Identification of the flagged part – ½ mark:Labeled diagram of the entire system -1 marks: Description of flagged part -1mark)

(3+3) = 06

QII. Identify with systematics. Draw labeled diagram and comment (**A&B**).

(1 slide/ 1 specimen). (Identification – 1/2 mark; Systematics -1/2 mark; Labelled diagram -1 mark; Comments -1 mark) **(3x2) = 06**

Q III. Identify the endoskeleton **'C'** with neat labelled diagram and comment.

(Identification -1 mark. diagram -1 mark, comment-1 marks) **03**

Q IV. Identify and give the comparative account of skin / heart / brain of two vertebrates **'D'**

(Identification -I mark, diagram -2 mark, comment-2 marks) **05**

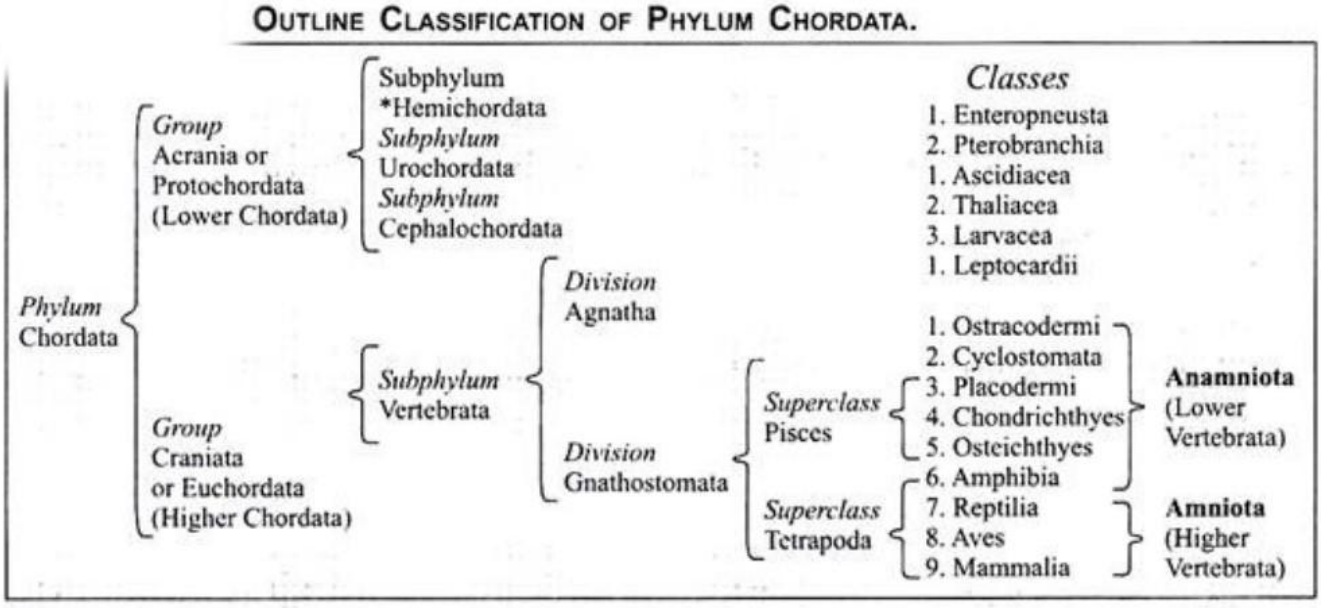
Q V. Record and Viva voce **05**

OBJECTIVE

We will understand the basic characters of chrodates, origin and Ancestrory of chordates.After reading this chapter we will also know about about the general characters and classification of chordates upto order level.

GENERAL CHARACTERISTICS OF CHORDATA

- The Chordata is the animal phylum with which everyone is most intimately familiar, since it includes humans and other vertebrates However, not all chordates are vertebrates.
- All chordates have the following features at some stage in their life (in the case of humans and many other vertebrates, these features may only be present in the embryos).
- Pharyngeal slits – a series of openings that connect the inside of the throat to the outside of the "neck". These are often, but not always, used as gills.
- Dorsal tubular nerve cord – A bundle of nerve fibers which runs down the "back". It connects the brain with the lateral muscles and other organs.



Subphylum Hemichordata is now considered to be an invertebrate group.

- Notochord – cartilaginous rod running underneath, and supporting, the nerve cord. • Post-anal tail – an extension of the body post the anal opening. Animals belonging to phylum Chordata are fundamentally characterised by the presence of a notochord, a dorsal hollow nerve cord and paired pharyngeal [relating to the pharynx] gill slits.
- They are bilaterally symmetrical, triploblastic, coelomate with organ-system level of organisation.

Experiment No: 1 **Date:**

PROTOCHORDATA: BALANOGLOSSUS AND T. S THROUGH PROBOSCIS ASCIDIAN/HERDMANIA AND AMPHIOXUS, T.S. OF AMPHIOXUS THROUGH PHARYNX AND INTESTINE.

Protochordata:

They are lower order members of phylum chordata. The name Protochordate is derived from two words, protos =first; chorda=cord. They are of great phylogenetic significance. It shows first sign of vertebrate formation.

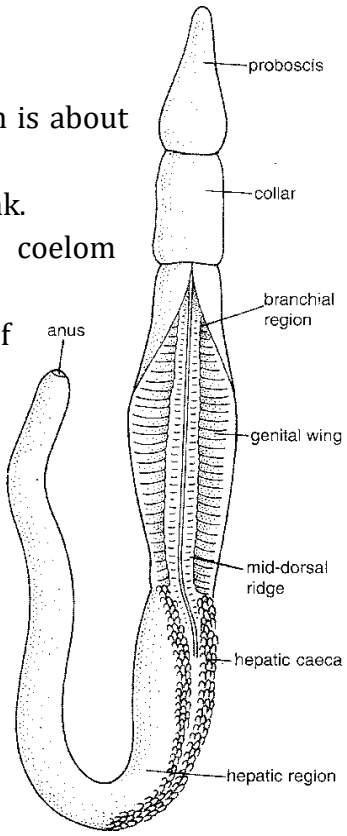
1. BALANOGLOSSUS

Classification:-

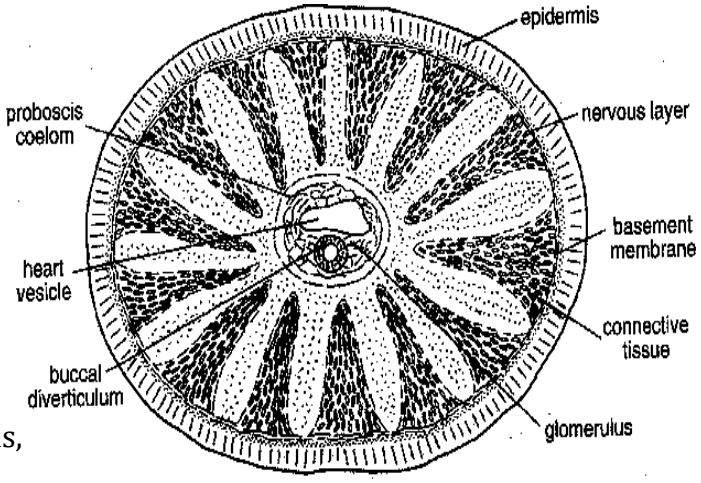
Kingdom:	Animalia
Phylum:	Chordate
Subphylum:	Hemichordata
Class:	Enteropneusta
Family:	ptychoderidae
Genus:	Blanoglossus

General characters:-

1) *Balanoglossus* is found in marine water.
2) They live at the surface of marine water.
3) Their body is soft and cylindrical having ciliated surface. Length is about 10-50 cm.
4) Body is divided into short conical proboscis, collar and a long trunk.
5) Proboscis has thick muscular walls and its cavity -proboscis coelom opens to the outside by a proboscis pore.
6) Collar is short, muscular cylinder like, enclosing a pair of coelomic cavities open to the dorsal surface by a pair of collar pores.
7) Trunk is devoid of segmentation (superficially ringed).
8) Trunk is divisible into anterior branchio-genital region, middle hepatic region and a posterior abdominal region.
9) Alimentary canal is straight and anus is present on the posterior end of the body.
10) Sexes are separate and fertilization is external. Development is indirect.
11) The life cycle comprise of a free - swimming pelagic larva, the tornaria.



T.S of proboscis of *Balanoglossus*



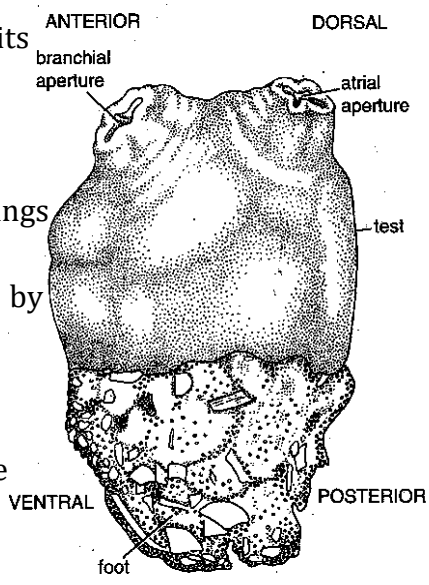
1) Body wall is composed of single layer of epidermis.
2) Nervous layer lies below epidermis.
3) Basement membrane is present just below the nervous layer.
4) Proboscis coelom is very much reduced.
5) Central complex comprises of the buccal diverticulum, glomerulus, central sinus and heart vesicle.

2. *Herdmania* (Sea squirt):-

Classification:-

Phylum:	Chordata
Group:	Protochordata
Subphylum:	Urochordata
Class:	Acidiacea
Order:	Pleurogona
Suborder:	Stolidobranchia
Family:	Pyuridae
Genus:	Herdmania

General characters

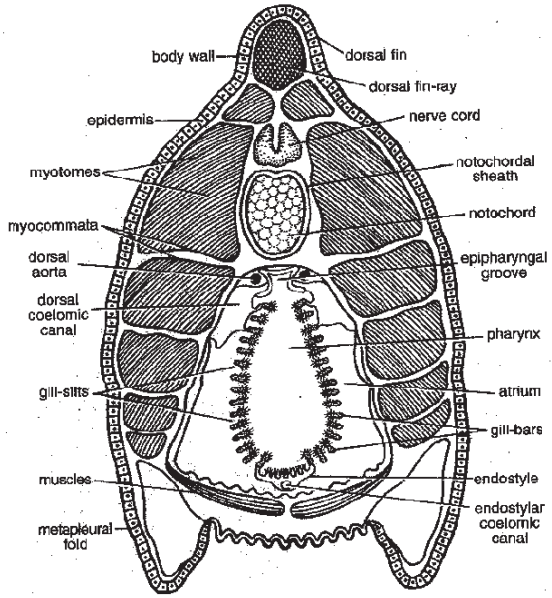


1) *Herdmania* lives a solitary marine life.
2) They attached with the surface of ocean by a foot through its posterior ventral ends.
3) Body is roughly oblong enclosed in a soft leathery test.
4) Color of the body is pink.
5) Free ends of the body are providing with two external openings of atrial aperatures.
6) Mouth opens by branchial aperature or siphon while anus by atrial aperature.
7) Pharynx is sac like perforated by numerous stigmata.
8) Alimentary canal is U shaped.
9) Respiration is through branchial - sac. They also have accessory respiratory organs in the form of test.
10) Blood vascular system is open type.
11) Execratory organ is neural gland situated above the nerve ganglion.
12) Sexes are united or hermaphroditic.
13) Fertilization external & development is indirect.

Amphioxus **(T.S of *Amphioxus* through pharynx)**

General characteristics:-

1) Body wall is formed of epidermis which is composed of a single layer of simple columnar epithelium.
2) Dorsal fin with dorsal fin ray lies at the dorsal surface.
3) Myotomes and myocommata of both the dorso-lateral sides alternate with each other.
4) Dorsal tubular nerve cord is present below the dorsal fin.
5) Notochord composed of vacuolated cells, is surrounded by notochordal sheath and lies below the nerve cord.
6) Pharynx is quite spacious and surrounded by the atrial cavity.
7) Pharynx is perforated by numerous gill slits which on either side separated by primary and secondary gill bars.
8) In the mid-dorsal line of the pharynx is present a ciliated epipharyngeal-groove, while in the mid -ventral line lies a glandular endostyle.
9) The dorsal aortae are present, one on either side of the epipharyngeal groove.



10) The coelom appears as dorsal coelomic canals on either side of the epipharyngeal groove. Parts of coelom are also present in the endostyle and in metapleural folds.
11) The metapleural folds are present on the ventral side.

CYCLOSTOMATA: PETROMYZON, MYXINE,

General characteristics

1) Body is long, rounded and eel like. Found in marine water
2) Skin is soft, smooth and without exoskeleton.
3) Mouth is suctorial devoid of functional jaws.
4) Paired fins or lateral appendages are absent.
5) Skeleton is cartilaginous.
6) Notochord is persistant.
7) Heart is two chambered with many aortic arches.
8) Development is direct or indirect.

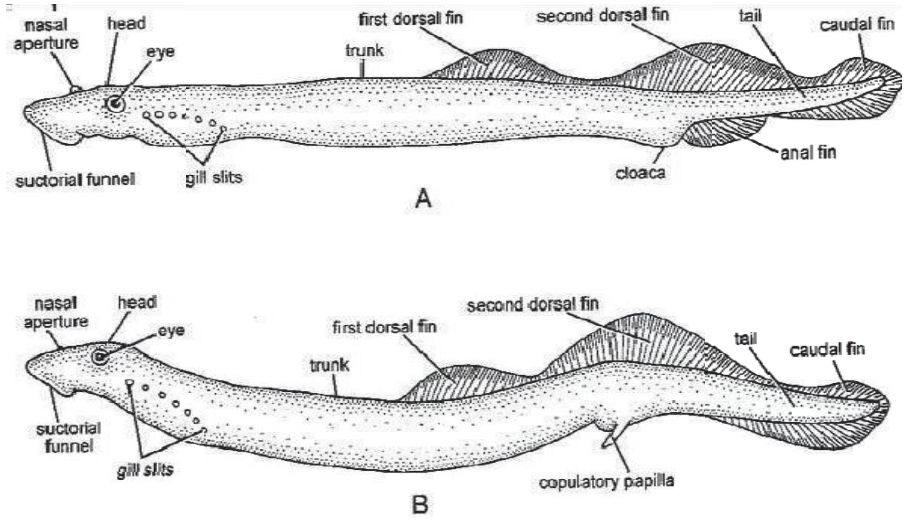
1. PETROMYZON (LAMPREY/ LAMPER EEL):

Classification:-

Phylum:	Chordata
Group:	Vertebrata
Subphylum:	Agnatha
Class:	Cyclostomata
Order:	Petromyzontia
Genus:	*Petromyzon*
Species :	*marinus*

General characters

1) They are found in both fresh water and salty water.
2) They are ecto -parasite on fishes.
3) Body is divided into head, trunk and tail. Length is about 1 meter.
4) Surface of body is smooth and slimy and heavily pigmented.
5) Head region is characterized by great forward development of the upper lip region forming the buccal funnel.
6) Mouth is circular armed with numerous horny teeth.
7) The paired eyes are relatively large and functional.
8) There are two small median eyes-pineal and parietal.
9) Nostril is single and dorsal.
10) Seven pairs of external gill- apertures and well developed cartilaginous rays.
11) Jaws and paired fins are absent.
12) Sexes are separate. Female has large anal fin and male with urinogenital or copulatory papilla.
13) Fertilization is external. Development is indirect includes Ammocoete larva.



Petromyzon (A) Female (B) Male

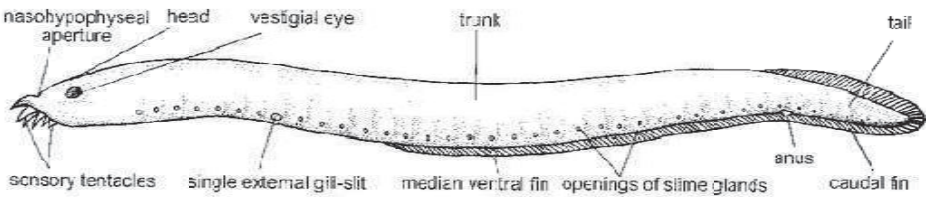
2. MYXINE (HAG FISH OR BORER):

Classification:-

Phylum:	Chordata
Group:	Craniata
Subphylum:	Vertebrata
Division:	Agnatha
Class:	Cyclostomata
Order:	Myxinoidea
Genus:	Myxine

General characteristics

1) Body is eel like divided into head, trunk and tail.
2) Surface of the body is soft and smooth without scales.
3) Mouth is terminal and surrounded by soft lips.
4) Buccal funnel and jaws are absent. Branchial-basket is also reduced.
5) Lateral to the mouth are four pairs of short tentacle supported by skeletal rods.
6) Nostril is single, lies close to mouth and opens terminally.
7) Single pineal eye is visible on top of head.
8) Paired eyes are vestigial or degenerated due to dark & bottom dwelling habbit.
9) 6-14 pairs of gills which do not open separately to the outside but open by a single external gill- opening.
10) Single median fin runs from about the middle of the ventral surface extending around the tail region.
11) Large mucous glands are present opening by mucous pores.
12) They are Hermaphroditic.
13) They are parasitic or quasi - parasitic.
14) They are nocturnal and scavenger.



Myxine

Experiment No: 2 **Date:**

PISCES

Fishes are vertebrates within class Pisces. The word pisces has (come from the Latin word Pisces meaning "fish". They are cold blooded, lacks limbs, having gills for respiration, lay eggs, scales, having fins for locomotion. They are purely aquatic animals found in fresh and marine water on skin. They have jaws and, streamlined body.

General Characteristics

1) They are aquatic vertebrates, always live in water. They have representation both in fresh water and in marine waters and are also represented in brackish water.
2) Their body is invariably stream lined and they swim with the help of tail.
3) They have paired appendages in the form of fins. Unpaired fins are also present. Fins help in balancing during swimming.
4) They have lateral line system that helps them to know the disturbances in the nearby environment.
5) They respire with the help of gills.
6) Have a swim bladder for buoyancy, they show no or little parental care to their young ones
7) Internal skeleton is either cartilaginous or bony.
8) Sexes are separate; fertilization is external or internal.

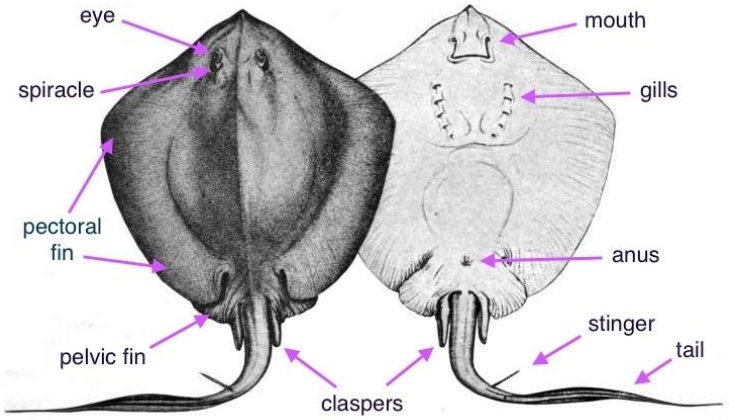
General Characteristics Chondrichthyes (CARTILAGINOUS FISHES)

1) They are marine animals with streamlined body and have cartilaginous endoskeleton. Mouth is located ventrally.
2) Notochord is persistent throughout life.
3) Gill slits are separate and without operculum (gill cover).
4) The skin is tough, containing minute placoid scales.
5) Teeth are modified placoid scales which are backwardly directed.
6) Their jaws are very powerful.
7) These animals are predaceous [shark].
8) Due to the absence of air bladder, they have to swim constantly to avoid sinking.
9) Heart is two-chambered (one auricle and one ventricle).
10) Some of them have electric organs (e.g., Torpedo) and some possess poison sting (e.g., Trygon).
11) They are cold-blooded (poikilothermous) animals, i.e., they lack the capacity to regulate their body temperature.
12) Sexes are separate. In males pelvic fins bear claspers.
13) They have internal fertilisation and many of them are viviparous [give birth to young ones].
14) Examples: Scoliodon (Dog fish), Pristis (Saw fish), Carchaiodon (Great white shark), Trygon (Sting ray).

1. *TRYGON*

Classification:-

Phylum:		Chordata
Group:		Craniata
Subphylum:		Vertebrata
Class:		Chondrichthyes
Order:		Myliobatiformes
Genus	:	*Trygon (Sting- ray)*



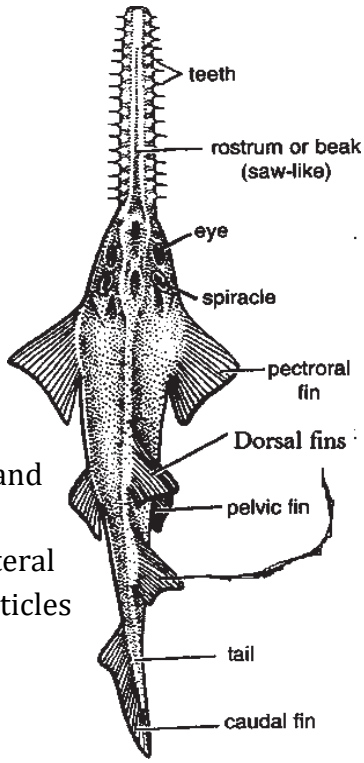
General characters

1) *Trygon* is commonly known as sting -ray because presence of 3 stings or spines in the tail.
2) Body is flat. Disc is sub -rhomboidal or broader than long.
3) Pectoral fins are confluent with the sides of the head.
4) Mouth is ventral in position.
5) A rectangular naso-frontal flap is present in front the mouth.
6) Spiracles are present behind the eyes on the dorsal side.
7) Five pairs of gill slits are present on the ventral side.
8) Tail is long, slender and whip like terminating in a small caudal fin and armed with a sharped serrated poisonous string.
9) Claspers are present near the pelvic fin in the male.
10) Viviparous

2. *PRISTIS* (Saw-fish):

Classification

Phylum	:	Chordata
Sub-Phylum	:	Vertebrata
Division	:	Gnathostomata
Superclass	:	Pisces
Order	:	Chondrichthyes
Sub-class	:	Selachii
Order	:	Hypotremata
Genus	:	*Pristis (Saw-fish)*



General characters:-

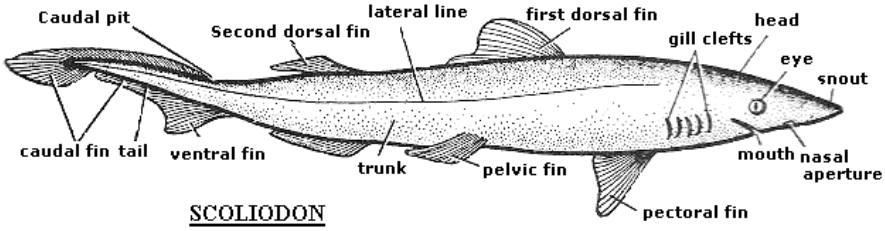
1) Body is elongated (length is about 3-6 metres), depressed and shark like
2) Head and skull prolonged into a long flattened rostrum, lateral margins are provided with a series of strong tooth like denticles giving its appearance of saw.
3) No rostral tentacles.

4) Teeth in jaws are minute and obtuse.
5) Spiracles are present behind the eyes.
6) Dorsal fins are large the first dorsal fin is opposite to the pelvic fin.
7) Tail is well developed and terminating in heterocercal caudal fin.
8) The fish is used for liver oil of high vitamin value and skin for scale boards

3. SCOLIODON

Classification:

Phylum-Chordata
Group-Craniata
Subphylum-Vertebrata
Division-Gnathostomata
Series-Pisces
Class-Elasmobranchii
Order-Lamniformes
Genus-Scoliodon



SCOLIODON

General characters:-

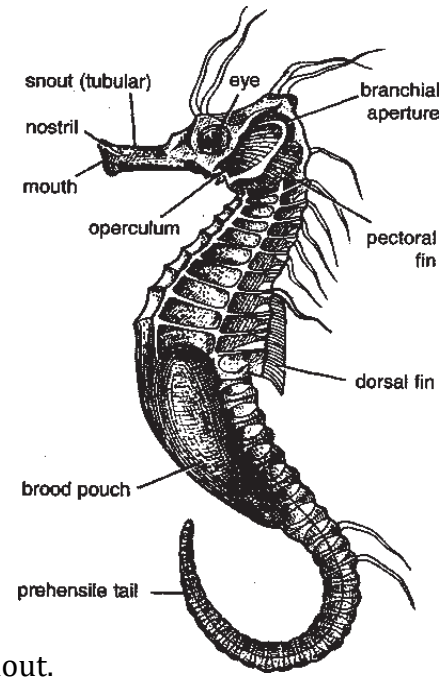
1) It is commonly known as Dog fish.
2) Body is long, laterally compressed and spindle-shaped tapering at both ends.
3) Body is divisible into head, trunk & tail.
4) Head dorsoventrally compressed and flattened into snout.
5) Tail is heterocercal.
6) Five pairs of gill-slits present laterally behind eyes.
7) A pair of pigmented lateral lines extends from head to tail.
8) In male a copulatory organ called clasper is present.

General Characteristics Osteichthyes (Bony Fishes)

1) It includes both marine and fresh water fishes with bony endoskeleton.
2) Their body is streamlined. Mouth is mostly terminal.
3) They have four pairs of gills which are covered by an operculum on each side.
4) Skin is covered with cycloid/ctenoid scales.
5) Air bladder is present which regulates buoyancy.
6) Heart is two- chambered (one auricle and one ventricle).
7) They are cold-blooded
8) Sexes are separate.
9) Fertilisation is usually external.
10) They are mostly oviparous and development is direct.

Examples: Flying fish, Sea horse, Fighting fish, Angel fish etc.

1. HIPPOCAMPUS



Classification:

Phylum:	Chordata
Subphylum:	Vertebrata
Division:	Gnathostomata
Series:	Pisces
Class:	Teleostomi
Subclass:	Actinopterygii
Order:	Syngnathiformes
Genus:	Hippocampus

General characters:-

1) It is commonly known as sea horse.
2) Body is elongated having an exoskeleton of rings.
3) Mouth is at the extremity of an enlongatedtubular snout.
4) Pectoral & dorsal fins are small.
5) Pelvic & caudal fins are absent.
6) Tail is prehensile &used for coiling round the sea weeds.
7) Male possesesa "brood-pouch" on the abdomen, in the brood pouch eggs is retained while they hatch as young ones.
8) It feeds on minute organism.

2. *ECHENEIS* (Sucker fish):

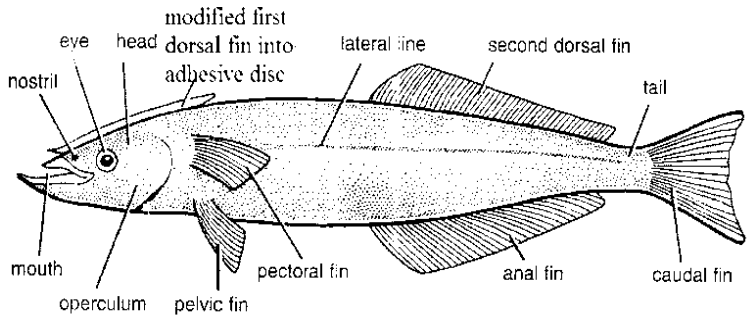
Classification:

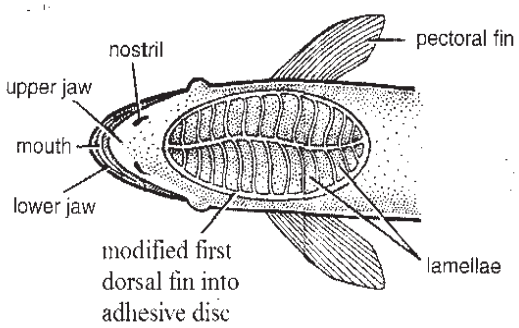
Phylum :	Chordata
Group :	Craniata
Sub-Phylum :	Vertebrata
Division	: Gnathostomata
Superclass	: Pisces
Class :	Osteichthyes
Sub class	: Actinopterygii
Order :	Echeneiformes
Genus :	Echeneis

General characters:-

1) Body is elongated (length is about 1 metre), fusiform and covered with small cycloid scales. Head is depressed and furnished with an adhesive organ.
2) Eye is lateral in position.
3) Mouth cleft is wide and deep.
4) First dorsal fin is modified into an adhesive disc.
5) Second dorsal and anal fins are elongated without spines and opposed to each other.
6) Adhesive disc is flat, oval and transversely furrowed and is used for attachment.

7) Air Bladder is absent.
8) Tail is homocercal & caudal fin bilobed.
9) It is employed for catching the turtles on the east coastal region of Africa.

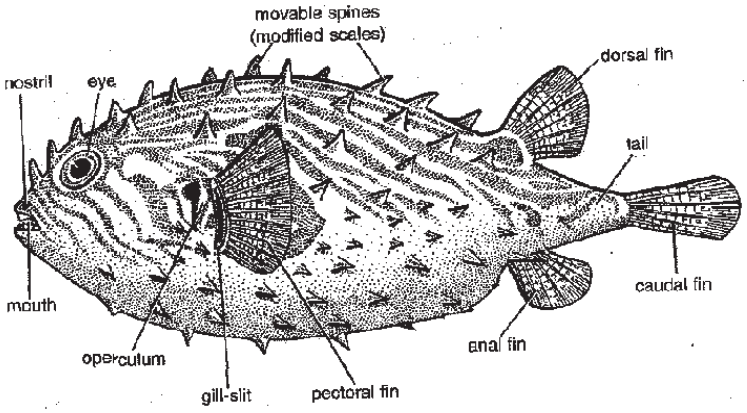




3. ***DIODON*** **(porcupine fish):**

Classification:

Phylum : Chordata
Group : Craniata
Sub-Phylum : Vertebrata
Division : Gnathostomata
Superclass: Pisces
Class : Osteichthyes
Sub class: Actinopterygii
Order: Tetrodontiformes
Genus: *Diodon*



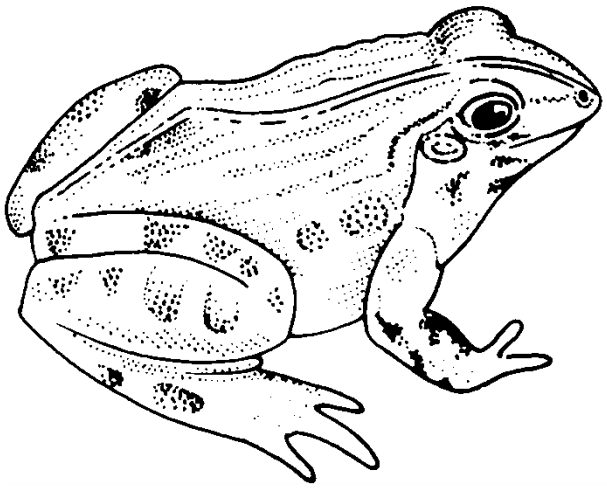
General characters:-

1) Body is globular.
2) Skin is covered with stiff and movable dermal spines, which serve as organs of defence.
3) Mouth opening is small and jaws without median suture.
4) Inter-operculum is rod like and attached to the anterior limb of sub-operculum.
5) Paired pectoral fin near operculum. Dorsal fin near caudal fin. Caudal fin is rounded and tilted upwards anal fin opposite to dorsal fin.
6) Gills are three in number. Gill- slits is situated near the pectoral fin.
7) Air bladder is present.
8) The flesh of this fish is regarded as poisonous.
9) A thin walled inflatable gastric diverticulum is present which allows the whole body to be puffed (leathery) into a globular shape and the spines become defensively erected.
10) This fish is poisonous and non-edible

Experiment No: 3 **Date:**

AMPHIBIA

Amphibians mainly live in water or damp places, none in salt water. They are the commonest in moist temperate regions but some are tropical, one frog ranges into Arctic circle and tree frogs occur above 400 meters in Sierra Nevada of California. Some toads are tree toads and live in deserts, some are nocturnal. The hell bender (Cryptobranchus). Mud puppy (Necturus) and Congo eel (Amphiuma) are strictly aquatic. Some frogs are purely arboreal. Land salamanders hide under stones and logs. Amphibians partly pass their life in water and partly on land (Gr., amphi = dual; bios = life). They are the lowest and earliest tetrapods evolving from Devonian and onwards. Amphibians have educational, experimental and food value.



1. RANA:

Classification:

Phylum:	Chordata,
Subphylum:	Vertebrata,
Super class:	Tetrapoda,
Class:	Amphibia,
Order:	Anura,
Type:	Rana tigrina

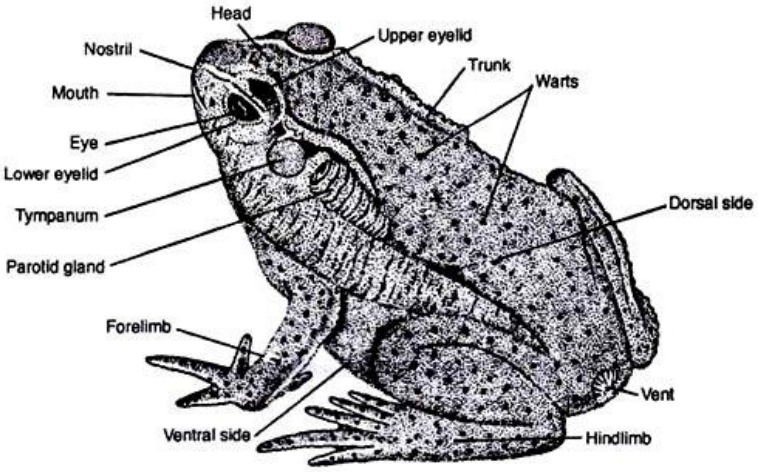
General characters:-

1) Rana tigrina is found near fresh water streams, pond, tanks and paddy fields.
2) Skin is smooth, slippery, and greenish with dark patches dorsally, pale ventrally.
3) Body is divisible into head and trunk. Neck absent.
4) Head carries a pair of large eyes with eyelids, a pair of nostrils, and a wide mouth. A tympanum a little behind the eye.
5) Trunk bears a pair of forelimbs, hind limbs and a cloaca. Forelimb has 4 digits and hind limb 5 webbed digits and an additional digit called prehallux.
6) Feeds on insects, worms, mollusks, caught by it sticky tongue. No teeth.
7) Sexes separate. Male is smaller than female. Male attracts females by croaking sound. Fertilization external. Development indirect with tadpole larva.

2. BUFO

Classification:

Phylum:	Chordata
Group:	Craniata
Subphylum:	Vertebrata
Division:	Gnathostomata
Class:	Amphibia
Order:	Anura
Genus:	Bufo



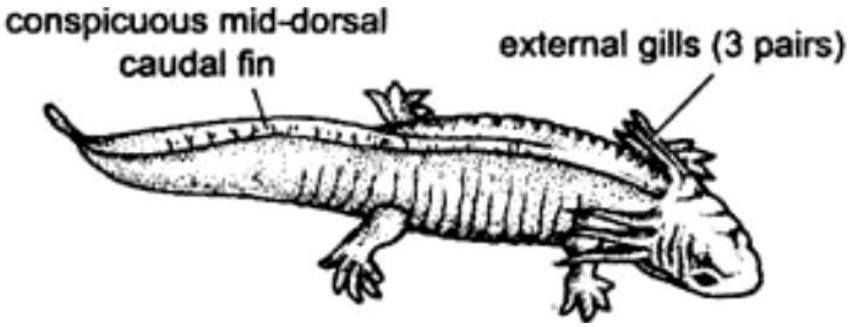
General characters:-

1) It is a common Indian toad, found in damp places all over the country.
2) The skin is rough and warty. Colour depends upon the pigment cells in the dermis.
3) Paired parotid glands are present on the sides of head, secrete milky irritating fluid. The mucous glands are also found.
4) They are nocturnal, feeds on earthworms, snails, and all sorts of insects. Teeth absent. Fingers web less.
5) Males are smaller than females. During breeding season, the toad utters a peculiar shrill sound. The larval life is short.

3. AXOLOTL LARVA

Classification:

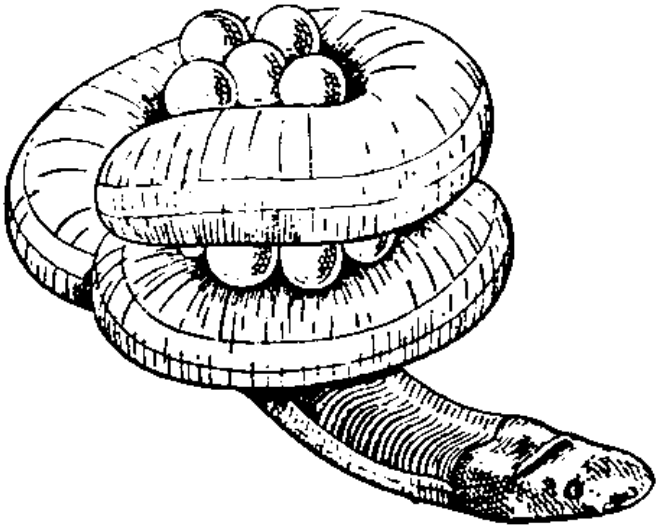
Phylum:	Chordata,
Subphylum:	Vertebrata,
Superclass:	Tetrapoda,
Class:	Amphibia,
Order:	Urodela
Genus:	Axolotl larva



General characters:-

1) Axolotl larva is the larva of Ambystoma tigrinum.
2) Body consists of head, trunk and tail.
3) At the junction of head and trunk, there are 3 pairs of external gills and four pairs of gill slits. And tail having tail fin.
4) Axolotl larva exhibits the phenomenon of neoteny retaining prolonged larval characters due to lack of I_2 content in water which prevents metamorphosis.
5) If sufficient food and water available, it develops gonads, matures and starts reproducing and the phenomenon is called paedogenesis.
6) During metamorphosis gills are lost, tail fin absorbed, gill slits are closed.

4. ICHTHYOPHIS



Classification:

Phylum:	Chordata
Group:	Craniata
Subphylum:	Vertebrata
Division:	Gnathostomata
Class:	Amphibia
Order:	Apoda
Genus:	Ichthyophis

General characters:-

1) It is a burrowing elongated and eel-type animal.
2) The colour of the body is dark brown or bluish with yellow band along the side.
3) Skin is provided with numerous transverse grooves or wrinkles.
4) Minute scales are embedded in the skin.
5) Limbs and limb girdles are entirely absent.
6) Tympanic membrane and columella are absent.
7) Sexes separate.
8) Parental care is very well developed. Female coils herself around the gelatinous egg mass to protect it from other animals.

Experiment No: 4 **Date:**

REPTILIA

1. **TURTLE:**

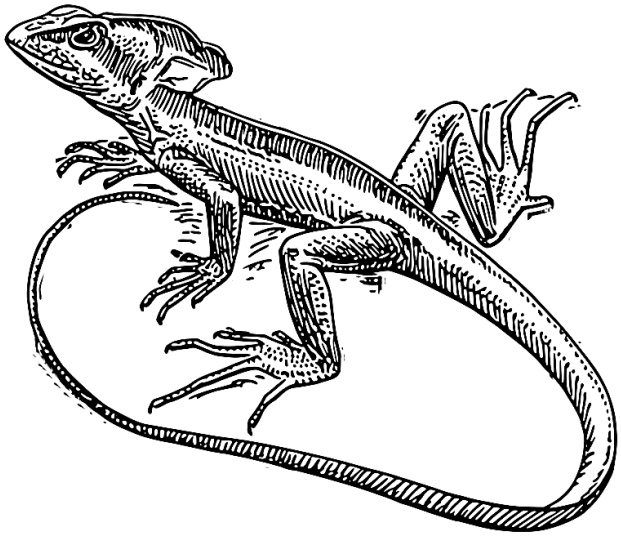
Classification:

Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Reptilia
Subclass:	Anapsida
Order:	Chelonia
Genus:	Chelone mydas

General characters:-

1) It is marine, herbivorous and commonly called the green turtle.
2) Body is divisible into head, neck, trunk and tail. Head bears a pair of nostrils, a pair of eyes and a mouth.
3) Trunk is enclosed in a shell composed of a carapace and a ventral plastron. Head is retractile into the shell.
4) Trunk bears two pairs of limbs. They are modified into paddle like for swimming.
5) Sexes are separate and female is oviparous. Eggs are laid in sand. Young ones hatched from eggs move into the sea.

2. **CALOTES VERSICOLOR**



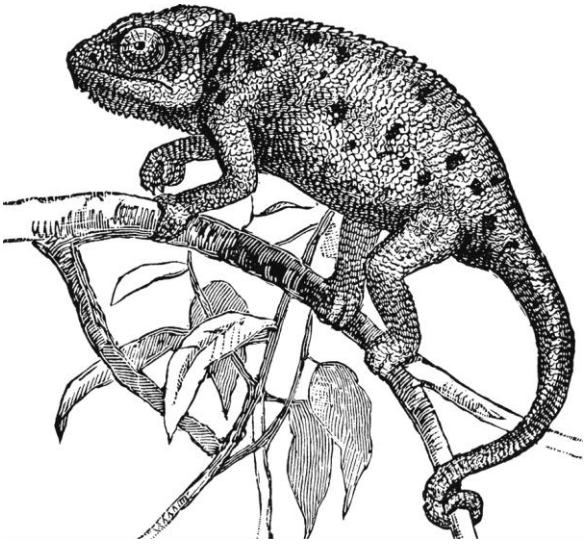
Classification:

Phylum:	Chordata,
Subphylum:	Vertebrata,
Class:	Reptilia
Subclass:	Diapsida,
Order:	Squamata
Suborder:	Lacertillia
Genus:	Calotes versicolor

General characters:-

1) Calotes is commonly called as garden lizard found in open fields.
2) The body is covered with imbricate epidermal horney scales.
3) A crest of sharp spines present on the dorsal surface on the neck and back.
4) Mouth anteriorly placed on the head and cloaca is situated on the ventral surface at the root of tail.
5) It is known for changing its body colour.
6) Feeds on insects.
7) Oviparous.

3. CHAMELEON



Classification:

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Reptilia
Subclass: Diapsida,
Order: Squamata
Suborder: Lacertillia
Genus: Chameleon vulgaris

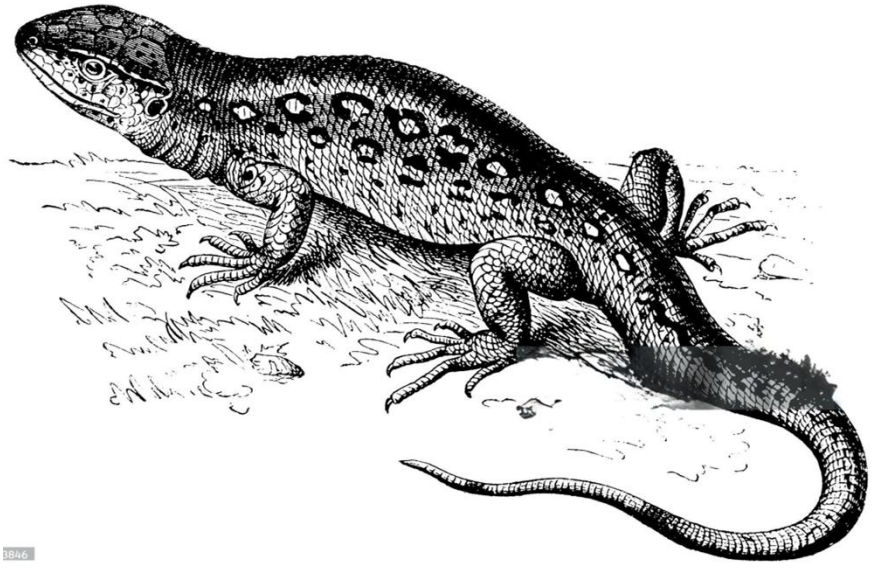
General characters:-

1) Arboreal, lizard like animal with lateral compressed body.
2) Digits fused together to form two opposite sets well suited for grasping the twigs.
3) Tail long and prehensile.
4) Head become helmet.
5) Skin covered with tubercles or granules.
6) Eyes large with thick eyelids and capable of independent movement.
7) Tongue long protrusible, club shaped and sticky at the end to catches insect from distance.
8) It is capable of changing its colour according to its surrounding.

4. MABUYA

Classification:

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Reptilia
Subclass: Diapsida,
Order: Squamata
Suborder: Lacertillia
Genus: Mabuya



General characters:-

1) Commonly called as skink, and is burrowing in habit.
2) Body is covered with imbricate scales. On the dorsal surface red coloured longitudinal stripes are present.
3) Head is small and conical covered with symmetrical shields.
4) Eyes with movable eyelids.
5) Limbs are weak and slender.
6) Tongue with sac like papillae.

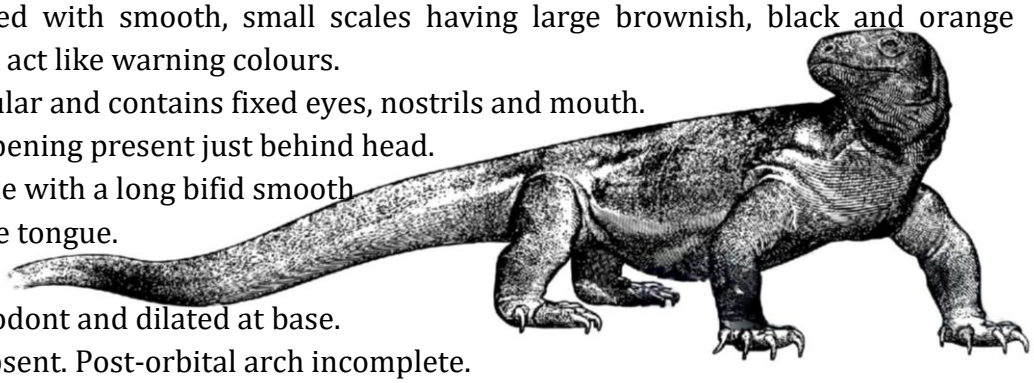
5. VARANUS : MONITOR LIZARD

Classification

Phylum :	Chordata
Class :	Reptilia
Order :	Squamata
Family :	Varanidae
Genus :	Varanus

General characters:-

1) Commonly known as Monitor lizard.
2) Animal measuring 60 to 90 cm in length is divided into head, neck, trunk and tail.
3) Body is covered with smooth, small scales having large brownish, black and orange patches, which act like warning colours.
4) Head is triangular and contains fixed eyes, nostrils and mouth. External ear opening present just behind head.
5) Mouth gap wide with a long bifid smooth and protrusible tongue. Teeth large pointed, pleurodont and dilated at base.
6) Osteoderms absent. Post-orbital arch incomplete.
7) Trunk is large and stout. Tail is long thickened and serves as storehouse for fat.
8) Forelimbs and hind limbs are stout, well developed and adapted for swift movement, but they can hardly lift the body up from the ground. Digits are clawed.



SNAKES

Man has always feared and worshipped snakes in India. Snake bites are often fatal in case of poisonous snakes. A detailed account is given below in order to distinguish the poisonous and non-poisonous snakes.

(1) Non-poisonous snakes - Pythons, trinket snakes and racer snakes.

(2) Poisonous snakes - Cobra, Krait, Vipers, Russell's viper, Saw-scaled vipers.

The poisonous snake bites have two types of toxins present in the venom:

1) Neurotoxins - The neurotoxins act on motor nerve cells and provoke muscular paralysis. In case of cobra snake venom, both convulsions and paralysis may occur.
2) Hemotoxins - The hemotoxins result in haemorrhages, destruction of tissue cells, red cells, blood vessels and specific organs. The victim (patient) in both cases has respiratory difficulties and haemorrhages. As the poison is absorbed into the tissues, the patient feels dimness in vision. Pulse rate becomes rapid and weak. Convulsions are sometimes associated with vomiting. Benadryl acts as antidote to counteract the effect of hemotoxins, local leisons are applied with Antisera. Medicines are injected intravenously.

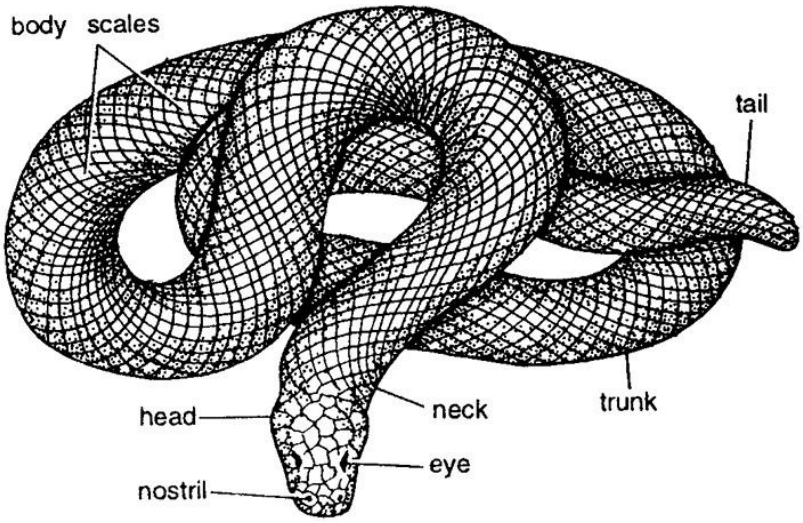
1. ERYX : RAT SNAKE

Classification

Phylum :	Chordata
Class :	Reptilia
Order :	Squamata
Family :	Boidae
Genus :	*Eryx*

General characters:-

1) Commonly called as sand boa, the common (dumuhi). double mouthed snake
2) It is elongated measuring one meter in length. Body divided into head, neck, trunk and tail. Head contains eyes and nostril.
3) Pinkish grey dorsal surface has irregular brown patches while ventral surface is yellowish.
4) Entire body is covered with 40-45 rows of small scales, sometimes keeled in tail region. Ventral scales do not run across the body.
5) Head and neck indistinguishable. Head scales primitive and 3 scales enlarged.
6) Eyes are small with vertical pupil and are reduced due to burrowing life.
7) Nostrils are slit-like. Tympanum absent.
8) Tail region is as thick and blunt as head and hence it is called Dumuhi.



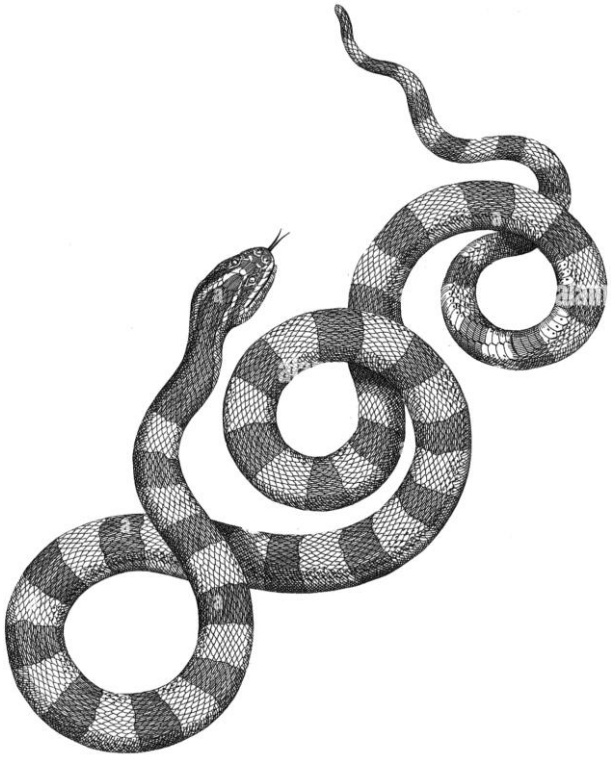
2. BUNGARUS KRAIT

Classification

Phylum :	Chordata
Class :	Reptilia
Order :	Squamata
Family :	Elapidae
Genus :	*Bungarus*

General characters:-

1. Commonly called as Krait.
2. Body is elongated and cylindrical, measuring one meter in length. Body divided into head, neck, trunk and tail.

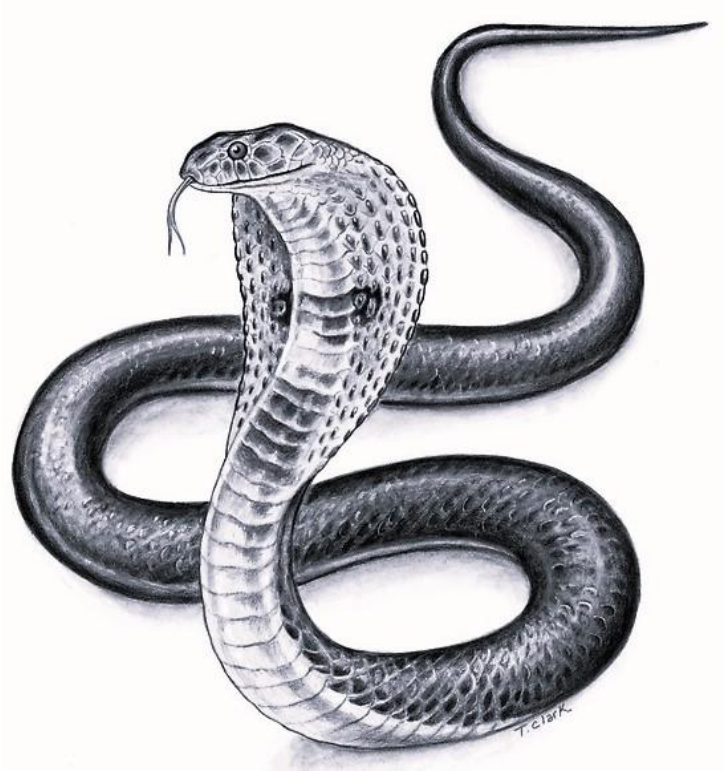


3. Colour of body steel-blue and dark-blue. Dark-blue, patches alternate with white cross bands.
4. Head is not differentiated from the neck. Loreal absent. Post-ocular, preocular and supra-labial 2, 1 and 7 in number respectively. Fangs small. Head contains eyes, nostrils, bifid and protrusible tongue.
5. Eyes are of moderate size with round pupils.
6. Scales are smooth forming 13-17 rows. Ventrals are 194-234 and caudals 42-52.
7. Large mid-dorsal hexagonal scales are present. Ventral scales beyond the anal region are in a single row.
8. Oviparous. Female shows parental care.

3. NAJA NAZA : COBRA

Classification

Phylum:	Chordata,
Subphylum:	Vertebrata,
Class:	Reptilia
Subclass:	Diapsida,
Order:	Squamata,
Suborder:	Ophidia
Genus :	Naja naja



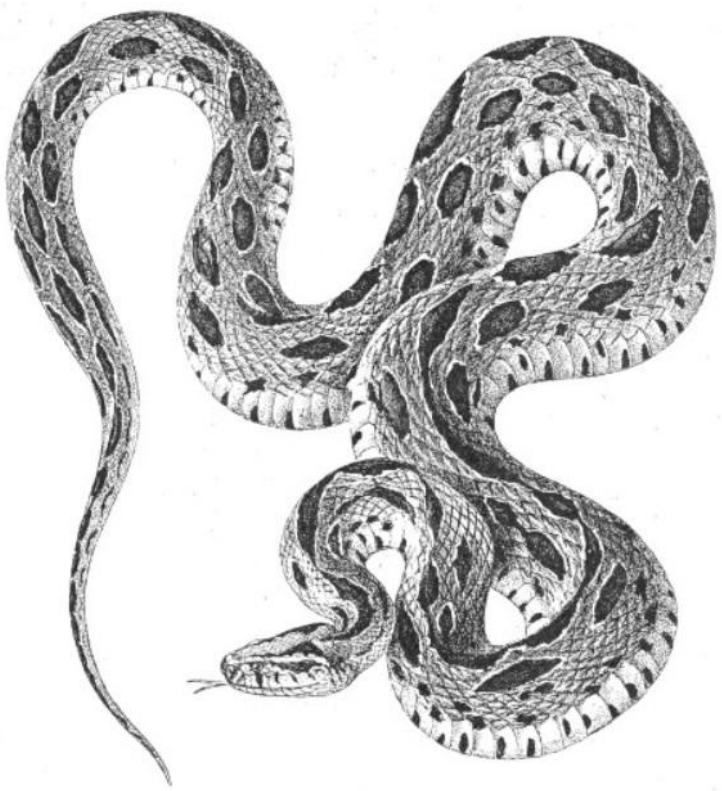
General characters:-

1) It is commonly called as Indian cobra, grows upto 1.75m.
2) It is diurnal, found living in holes and thick vegetation, and is deadly poisonous snake, feeds on frogs, rats and lizards. Its venom is neurotoxic.
3) Body is elongated with pointed tail. Head is differentiated from neck.
4) The expansion of neck and cervical ribs form the hood.
5) Hood bears a spectacle mark on its dorsal side.
6) Head is covered by shield. Ventrales are transversely elongated. The 3rd supra labial touches the eye and nostril.

4. RUSSELL'S VIPER:

Classification

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Reptilia
Subclass: Diapsida,
Order: Squamata
Suborder: ophidian
Genus : Vipera russelli



General characters:-

1) It is commonly called as "Russell's viper". It measures about 5 feet in length.
2) It is common in the plains of Bombay, Madras, and Punjab. It is nocturnal, and
3) Feeds on rats.
4) It has distinct bright oval markings on the body.
5) Nostrils are lateral; eyes are situated far in front.
6) A 'v' shaped mark is placed over the head.
7) It remains coiled with head in the centre of the coil.
8) It makes loud hissing sound when attacked.

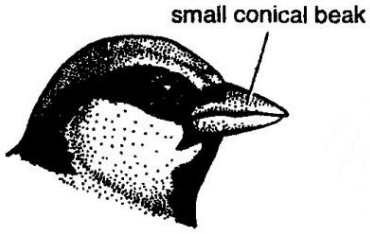
Experiment No: 5 **Date:**

AVES & MAMMALS

Types of Beak Modification

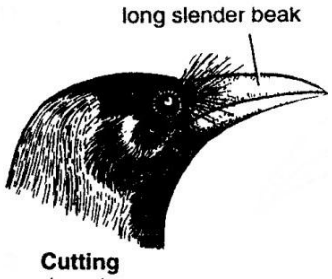
The entire modern avian world is characterized by the absence of teeth. The upper and lower jaw bones become elongated to form a peculiar beak or bill covered by a horny sheath called as rhamphotheca. The modification of forelimbs into wings deprived birds of some of their normal functions. Beak serves both as mouth and hand. The diversity of form of beaks is mainly related to the type of food eaten and to the manner of feeding. Birds exhibit almost indefinite variations in shape, size and structure of beaks, of which only some of the most important and common types are described here.

1. **Seed-eating beak:** Short, stout, peg-like and conical beaks are characteristic of small grainivorous or seed eating birds, such as sparrows, finches and cardinals. The weaker beaks are used for piercing up small seeds, while more powerful beaks are meant for crushing large and hard-shelled seeds and fruit stones.



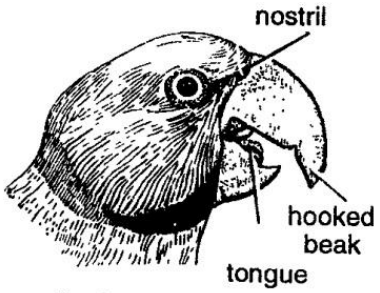
Seed eating
(sparrow)

2. **Cutting beak:** Birds such as jungle crows, possess long and slender beaks with cutting edges which can be used variously for cutting plants.



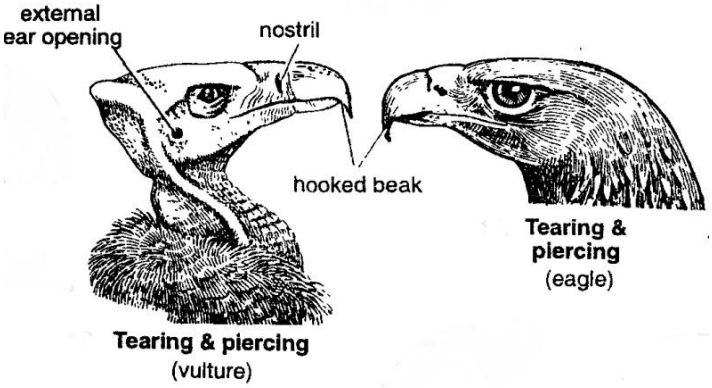
Cutting
(crow)

3. **Fruit-eating beak:** In parrots, the beak is sharp, massive, deeply hooked and extremely strong. It is well adapted for gnawing or breaking open hard seeds and nuts, which form their staple diet. Enormous beak of hornbilis, looking so heavy and cumbersome, is really quite light as its interior is of a cellular structure. It is suggested that these cells act as resonators, thus enabling the bird to produce its exceptionally loud cry.

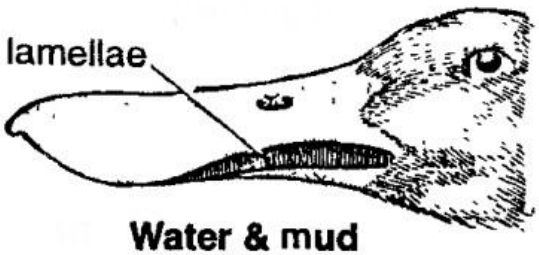


Fruit eating
(parrot)

4. **Tearing and piercing beak :** Carrion-feeding and flesh-eating birds, such as vultures, hawks, eagles, owls and kites etc., have short, pointed, sharp-edged and powerful, hooked beaks for tearing flesh and operated by well-developed mandibular muscles.

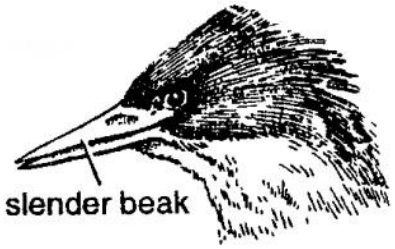


5. **Water and mud straining beak.** In ducks: Teals and geese, the beak is broad and flat. The edges of the jaws are furnished with horny serrations or transverse lamellae, which act as a sieve or strainer, letting the water and mud fall out while retaining the food in the mouth. Such a beak enables the bird to avail itself of the rich store of food in the shape of insects and other organism. In flamingoes, the beak is distally curved downwards and likewise furnished with shifting lamellae. The two halves of lower jaw are considerably enlarged so that the comparatively narrow upper jaw closes upon a wide cavity.



Water & mud straining (duck)

6. **Fish catching beak.** Storks, herons and kingfishers have long, powerful and sharply pointed spearing beaks to capture fish, frogs, tadpoles and similar aquatic animals. Cormorants have long and narrow beaks, the edges of which are armed with sharp backwardly directed, tooth-like processes meant for capture of fish. In snake-birds or Indian darters, these serrations take the form of fine needle-like points.

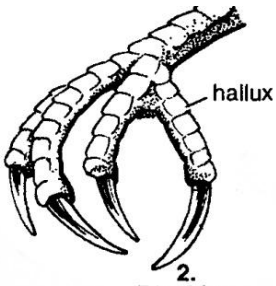


Fish catching (king fisher)

<u>Types of Feet Modification</u>

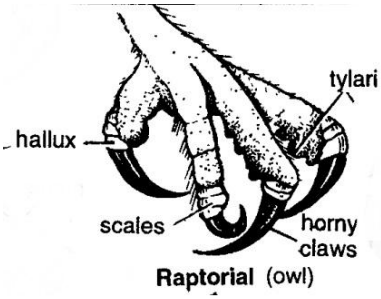
The feet of birds are also modified variously in accordance with the character of the environment and the manner of locomotion. For illustration see figure. 129.

1. **Perching feet :** Majority of birds belong ,to the category of perching birds or such as tinche sparrows, crows, bulbuls, robins, mynahs, etc. Toes are anterior and slender, while one toe or hallux is posterior, strongly built and apposable, so that .they can securely fasten the foot to a branch or a perch.



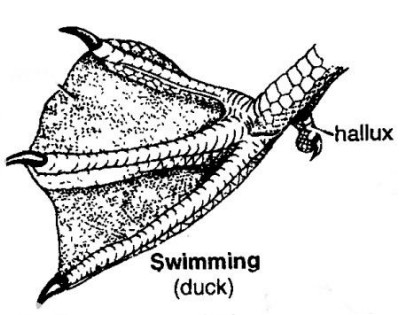
2. Perching (sparrow)

2. **Raptorial feet :** Predatory or carnivorous birds, such as eagles, kites, vultures and owls, etc. have strongly taloned feet for striking and grasping their prey. Toes have strongly developed, sharp and curved claws. Large and fleshy bulbs, called tylari, are found on the undersurface of the toes, especially developed in the sparrow-hawk. In osprey and Ketupa, tylari are absent but horny spines are present, which help in gripping slippery preys such as fish.



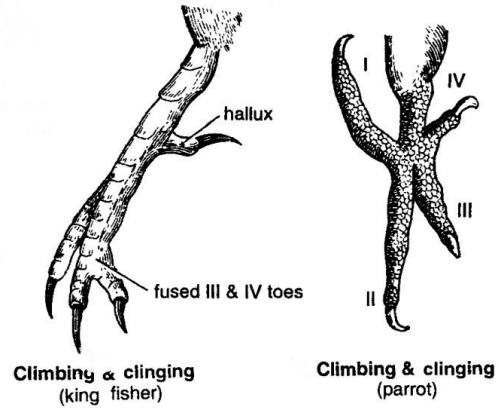
Raptorial (owl)

3. **Swimming feet:** In swimming birds, the toes are webbed, partially or completely. In diving birds, like coots and grebes, the web is lobate and the toes are free. In swimming and paddling birds, such as ducks and teals, avolet only the anterior 3 toes are united in a web. In cormorant all the 4 toes are enclosed in the web.



4. **Climbing feet**: In parrots and woodpeckers the feet are used as grasping organs and especially adapted for climbing vertical surfaces. Second and third toes point in front, while the first and the fourth toes point backwards.

5. **Clinging feet:** In swifts, martinets kingfisher and humming birds, all the 4 toes point forwards and serve to cling to steep faces of cliffs or under caves of houses, etc.

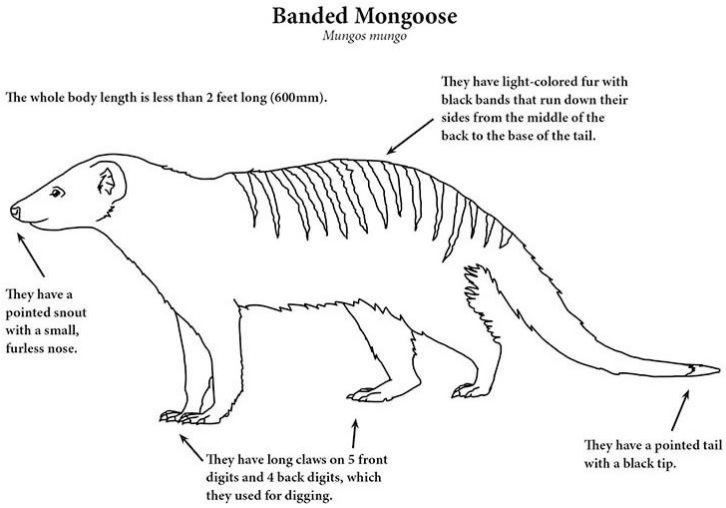


MAMMALIA

1. **MONGOOSE:**

Classification

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Mammalia,
Sub class: Eutheria
Order: Carnivora,
Genus: Herpestes edwardsii



General characters:-

1) It is commonly called as nyola in Hindi.
2) Body is elongated and covered with yellowish grey fur.
3) Head is elongated and possesses pointed snout. Eyes small, ears with small pinnae, which close the ear when animal is buried.
4) Tail is long.
5) Fore and hind limbs are clawed, with five digits.
6) Carnivorous and highly predaceous. It also takes vegetable food.
7) Mongoose is famous for its fight with snakes. It kills the snake and feeds on their blood.

2. SQUIRREL:

Classification

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Mammalia,
Sub class: Eutheria
Order: Rodentia,
Genus: Fanambulus palmarum

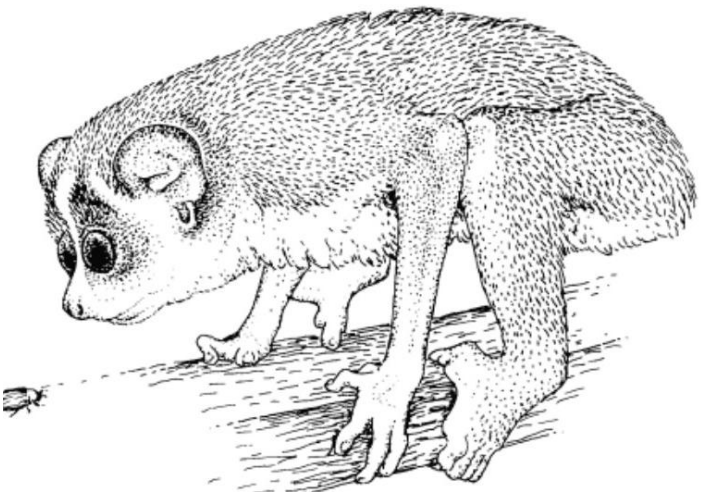
General characters:-

1) These are found all over the world except in Australia and islands of Madagascar.
2) Body is elongated and covered with fur. Five longitudinal stripes of dark colour are present on the back. Tail is long and bushy.
3) The eyes and pinnae are large.
4) The fore limbs have an inconspicuous thumb and hind limbs have four clawed digits.
5) Arboreal and active climber. Feeds on nuts, seeds and fruits.
6) Diurnal and builds nests of twigs and leaves.

3. LORIS

Classification:

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Mammalia,
Sub class: Eutheria
Order: Primata
Type: Loris tardigradus



General characters:-

1) Loris is solitary, nocturnal, and arboreal primate.
2) Body is covered with brownish fur with silver look. Fur is thick and woolly.
3) Body is divided into head, trunk, abdomen and tail.
4) Head contains pointed snout with nostril, large closely placed eyes and small pinna.
5) Teeth thecodont and heterodont.
6) Tail is long but not prehensile.
7) Limbs elongated. Some toes clawed, others with flat nails.
8) Locomotion is remarkably slow. It is often found hanging upside down.

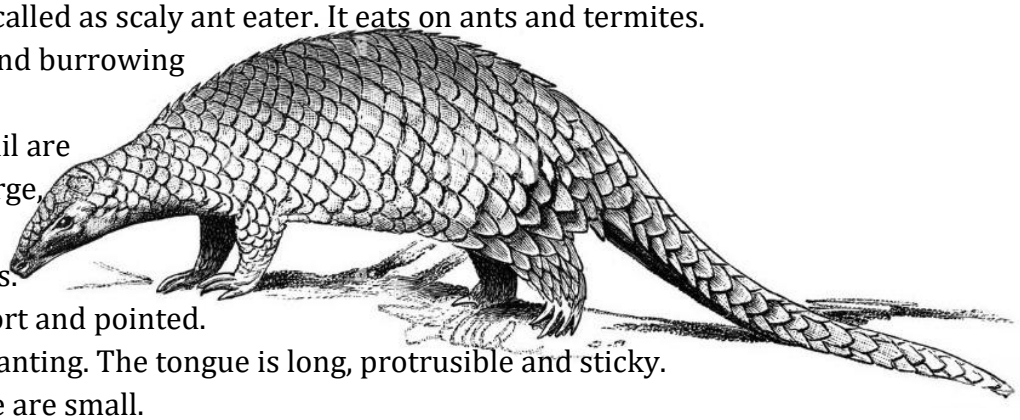
4. PANGOLIN

Classification:

Phylum: Chordata,
Subphylum: Vertebrata,
Class: Mammalia,
Sub class: Eutheria
Order: Pholidota
Genus: Manis crassicaudata

General characters:-

1) It is commonly called as scaly ant eater. It eats on ants and termites.
2) It is nocturnal and burrowing in habit.
3) The body and tail are covered with large, rounded horny epidermal scales.



4) The snout is short and pointed.
5) The teeth are wanting. The tongue is long, protrusible and sticky.
6) Eyes and pinnae are small.
7) The tail is long and broad.
8) The limbs are short and with 5 digits.
9) They roll into ball when alarmed.

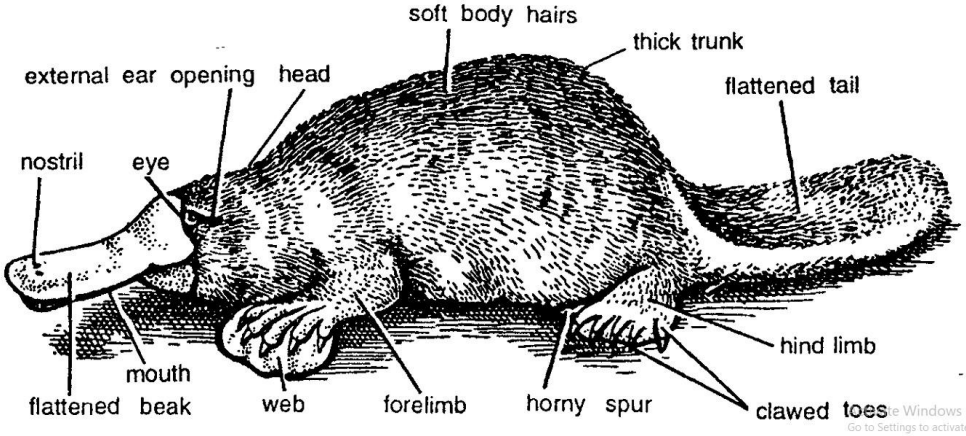
5. DUCK-BILL (ORNITHORHYNCHUS)

Classification:

Phylum - Chordata
Subphylum- Vertebrata
Division - Gnathostomata
Superclass- Tetrapoda
Class- Mammalia
Order- Monotremata
Genus- Ornithorhynchus (Duck·bill)

General characters:-

1) Commonly called as duck-billed-platypus.
2) It measures about 50 cm in length having fine short fur, dark brown colour and combines the characters of a duck



with a mammal.

3) Body is divided into head, thick trunk and tail. Body and tail contain soft hairs.
4) Head distinct. Upper jaw produced to form a flattened beak which is covered with a smooth, hairless skin that forms a free fold at the base of the beak. Head contains nostril mouth and external ear opening.
5) Adult has no teeth. Jaws covered with horny plates. Pinnae absent.
6) Forelimbs and hind limbs have 5 digits, web and clawed toes. Hind limb has horny spur. Tail is flattened and adapted for swimming.
7) Coracoid and precoracoid present. T -shaped interclavicle.
8) Eyes small having nictitating membrane. Mammary glands without nipples.
9) Cloaca present. Ureters open in dorsal wall of urinogenital passage. Testes abdominal, penis conducts only sperms. Oviducts distinct, uterus or vagina absent.
10) Female makes nest of roots and leaves during spring in burrows, lays 1-3 eggs. About 0.5 cm long young one is hatched. It nurses by lapping up milk secreted by scattered mammary glands on the abdomen of female.

Experiment No: 6 Date:

VIRTUAL DISSECTION OF CULTURED SPECIMEN

General Anatomy of *Scoliodon*

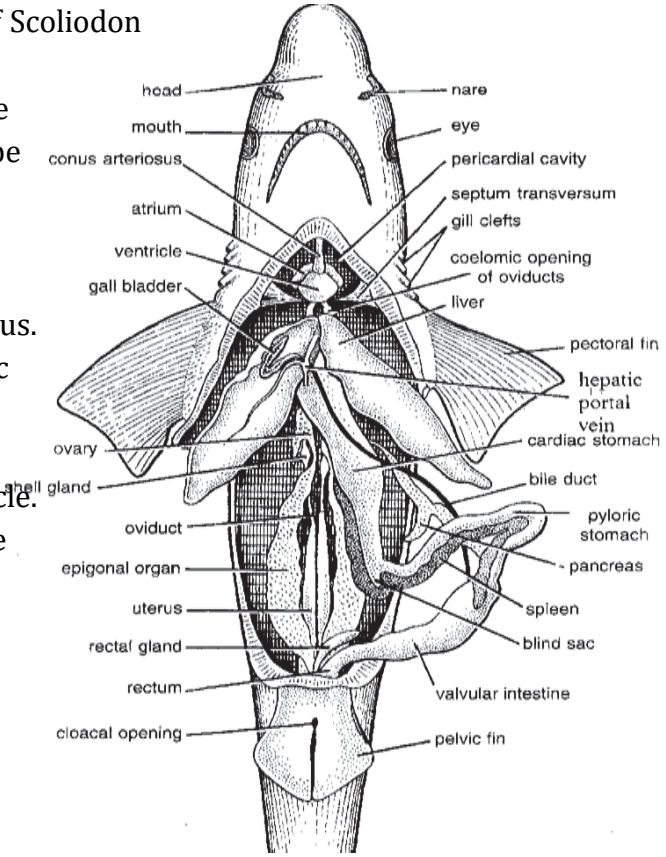
1. Procedure for dissection:

Take preserved *Scoliodon* and wash it with tap water. Lay down the fish in a dissecting tray ventro-dorsally, i.e ventral surface upwards. Fix the specimen by pins. Pins are to be fixed in pectoral fins. The abdominal cavity is opened by a median ventral incision in the body-wall extending from the anterior border of the pelvic fins to the anterior border of the pectoral fins. Cut transversely along the line of pectoral and pelvic fins and pin the flaps of the body-wall.

Study the internal viscera and note the following organs:

Heart, Afferent and Efferent Branchial Arteries of Scoliodon

1. Carefully remove the muscles and expose the pericardium which encloses the heart. Remove the pericardium carefully and thus heart will be exposed.
2. Heart: Heart is dorso-ventrally bent, muscular tube consisting of four chambers, i.e sinus venosus, auricles, ventricle and conus arteriosus.
3. Sinus venosus : It is a triangular thin-walled sac
4. Atrium (auricle) : It is a large triangular sac
5. Ventricle: Ventricle is the most prominent chamber and lies ventral to the atrium or auricle.
6. Conus arteriosus: It is a stout muscular tube extending from the ventricle to the
7. anterior apex of the pericardial cavity. The conus arteriosus through the wall of the pericardium as the ventral aorta.

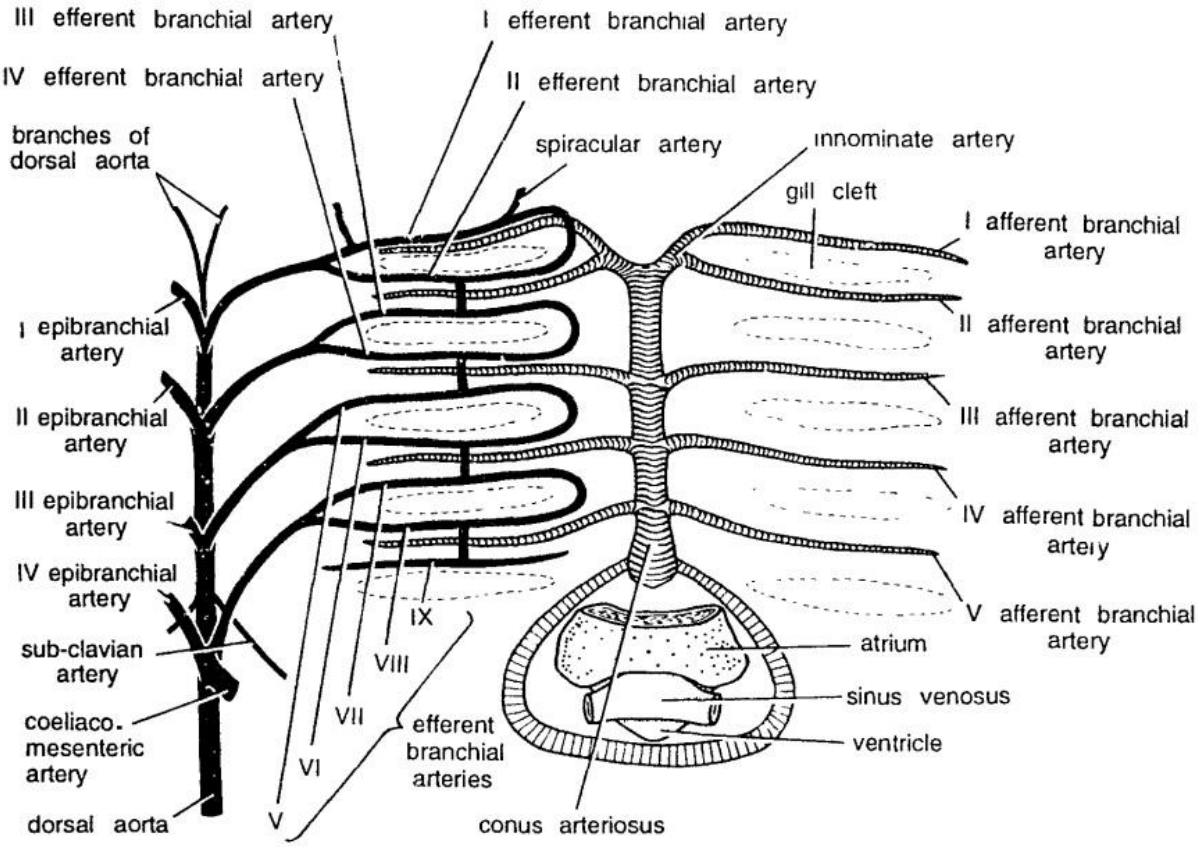


2. Afferent Branchial Arteries

1. Ventral Aorta: it runs up to the posterior border of the pharynx. Distally it divides into two innominate arteries. It bifurcates into two branches the innominate arteries, each of which again divides into first and second afferent branchial arterie
2. First afferent branchial artery-supplies to all the hyoideandemibranch

3. Second afferent branchial artery- supplying branches to both the anterior and posterior gill-lamellae
4. Third, Fourth and Fifth afferent branchial arteries- runs along the outer borders of the second, third and fourth branchial arches

3. Efferent Branchial Arteries:



Dogfish (*Scoliodon*) : Afferent and Efferent branchial arteries.

1. Procedure for dissection: dissect the fish from roof of the pharynx and expose the Efferent Branchial Arteries which are forming loops around each gill-cleft.
2. There are 9 Efferent Branchial Arteries on each side running along the anterior and
3. Posterior border of 5 gill-clefts.
4. I + II , III + IV, V+VI and VII + VIII Efferent Branchial Arteries forms four complete loops round the first four gill-clefts.
5. The IX Efferent Branchial Artery joins with the VIII Efferent Branchial Arteries.
6. The four loops are connected with one another by short longitudinal vessels which connects the posterior efferent of a gill-cleft with the anterior efferent of the next gill-cleft behind.
7. Each of the four Efferent Branchial loops is continued into an epibranchial artery.
8. The four pairs of epibranchial arteries unite to form median dorsal sorts.
9. In the anterior region hyoideanepi branchials and branches of dorsal aorta are seen.

4. Glossopharyngeal nerve

10. IX or Glossopharyngeal nerve is originated from ventro-lateral side of medulla & it
11. has two branches
12. Pre-trematic is sensory in nature & innervated in mucous membrane 1 st gill slit & pharynx.
13. Post-trematic is mixed in nature & innervated in muscles of pharynx.

5. Vagus nerve

14. X or Vagus nerve is mixed in nature, originated from side of medulla and it has 3 branches
15. Branchialisnerve is innervated in gills
16. Viceralis nerve is innervated in visceral organs
17. Lateralis nerve is innervated in lateral line of trunk

Experiment No: 6 **Date:**

COMPARATIVE ACCOUNT OF HEART IN CALOTES, PIGEON AND MAN

	Calotes (Lizard)	Columnba (Pigeon)	Oryctolagus (Rabbit)
1	Heart is situated mid ventrally in the anterior part of the body cavity in the pleuro peritoneal cavity be-hind the sternum.	Same way the heart is located.	Heart is situated in the thoracic cavity, between the lungs of two sides (Mediastinum). It is present slightly towards the left side.
2	Heart is comparatively smaller in size.	Heart is comparatively larger in size.	Heart is comparatively larger in size.
3	It is enclosed by double walled pericardium.	It is also enclosed in the double walled pericardium.	Same.
4	Heart includes a dorsal sinusvenusus aright auricle, a left auricle and a single incom-pletely divided ventricle.	Heart is four chambered, sinus venosus is absent in the adult. Completed divided two auricles and two ventricles by inter auricular septum and inter ventricular septum respectively.	Same as in columba
5	The three vena cavae or two precavals and a post caval vein open into the sinus venosus	The three vena cavae or two precavals and a post caval empty the blood directly into the right auricle.	Same as in pigeon.
6	The left auricle receives two pulmonary veins from the lungs.	The left auricle receives four pulmonary veins from the lungs.	Left auricle receives two pulmonary veins from the lungs.
7	The right auricle possess sinu-auricular ap-erture guarded by valve.	Absent.	Absent.
8	The two auricles are completely separated by inter auricular septum. But the inter ventricular septum in the ventricle is incomplete. Hence an oxygenated and deoxygenated type of blood is mixed to some extent in the ventricle.	Complete inter auricular and inter ventricular septa are present. There is no possibility of mixing the oxygenated blood with deoxygenated blood.	Same as in pigeon.
9	The heart of lizard is in a transitional stage approcarhing the double circuit stage But it has not reached it completely due to incomplete division of the encircle.	The heart is a double circuit heart because of complete division of ventricle into right and left chambers.	Same as in pigeon.
10	The auriculo ventricular aperture is guarded by two flap like semilumar valves.	The right auriculo ventricular aperture is guarded by two large muscular flap like valve and the left by three valves.	The right auriculo-Ventricular aperture is guarded by tricuspid valve and the left by bicuspid valve (mytral valve).

11	There are three aortic arches arising from the ventricle.	Only two aortic arches originate from the ven-tricles.	Only two aortic arches arise from the ventricles.
	i) Pulmonary trunk (ventral most)	i)Pulmonary trunk (from right ventricle)	i) Pulmonary arch (right ventricle)
	ii) Right systemic trunk (arise from left side of ventricle)	ii)Right systemic trunk (from left ventricle) i.e. Right aortic arch is characteristic of birds.	ii) Left systemic aorta (Left aortic arch from the left ventricle) Right aortic arch is absent.
	iii) Left systemic trunk (arise from right side of ventricle) All the trunks are united by connective tissue.		
12	Ductus caroticus is present (connection between carotid & systemic arches)	Absent	Absent.
13	Lizard's heart presents a transitional heart, since it approaches the double circuit heart but has not yet completely attained. So the heart is less efficient.	Avian heart has at tained maximum com olexity and is a doubk circuit heart, i.e. venous blood is com pletely separated frorr oxygenated blood.	Same as in Pigeon.
14	Absent.	Sinu-Auricular Node and Auriculo ventricular node are present.	SA - node and A.V. node are present. In addition bundle of His muscles are also develop.

Experiment No: 7 **Date:**

COMPARATIVE ACCOUNT OF SKELETAL SYSTEM: SKULL, GIRDLES BONES OF SHARK, FROG, CALOTES, PIGEON AND RABBIT

1. AMPHIBIA

Axial Skeleton- Skull of Frog

1. Skull is triangular, dorso-ventrally flattened and broad.
2. Skull is Dicondylic.
3. Cranium is small & narrow.
4. Skull is platybasic

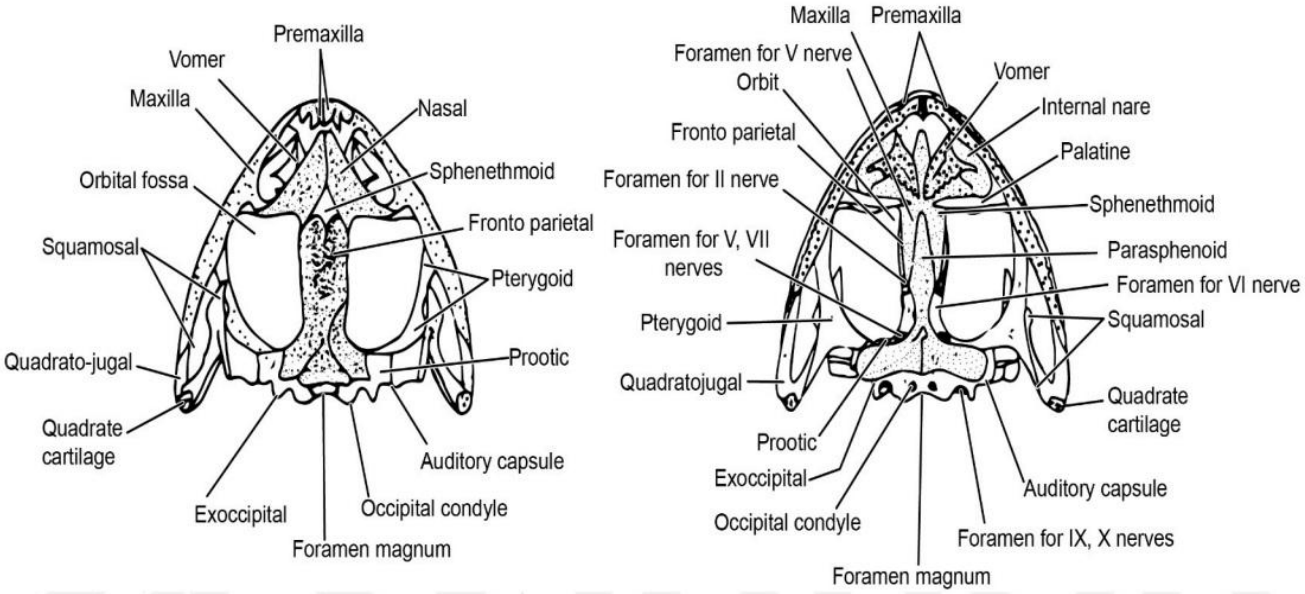
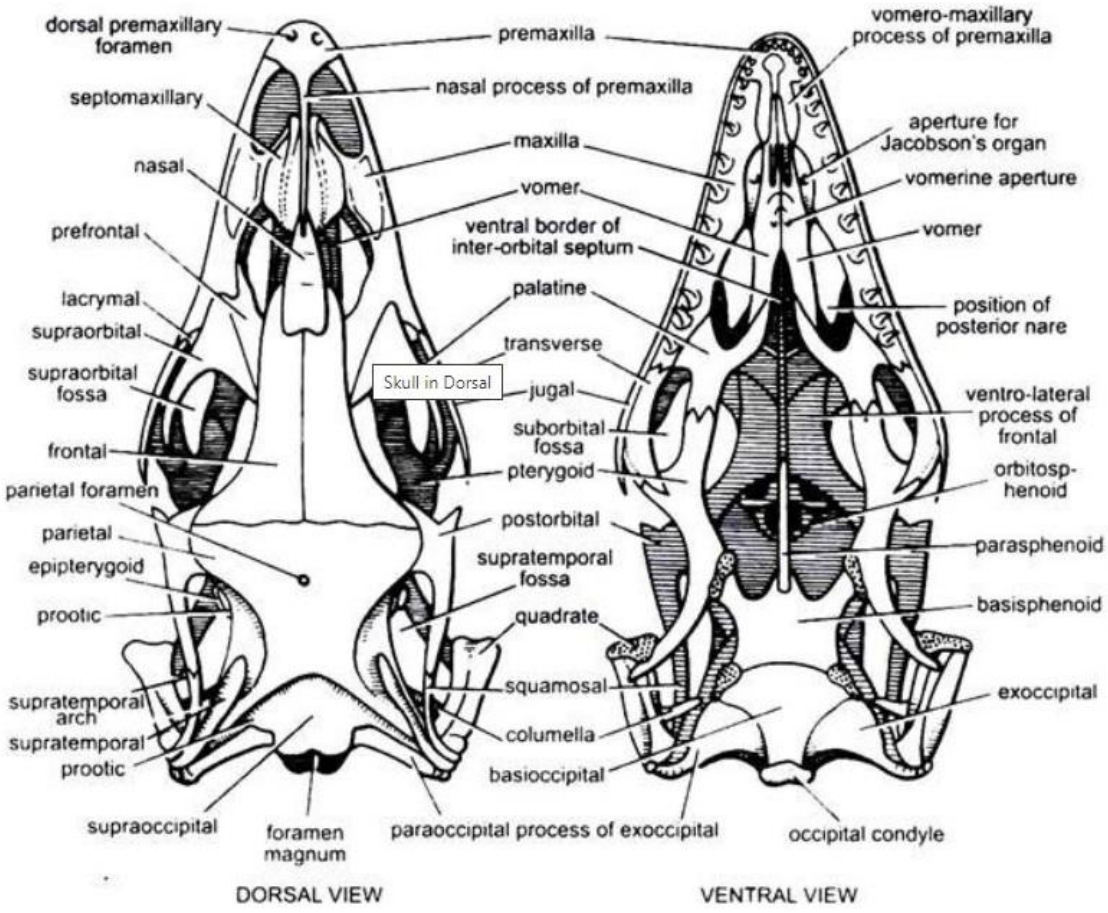


Fig. 2.11: Frog skull. a) Dorsal view; b) Ventral view.

2. REPTILES

Axial Skeleton- Skull

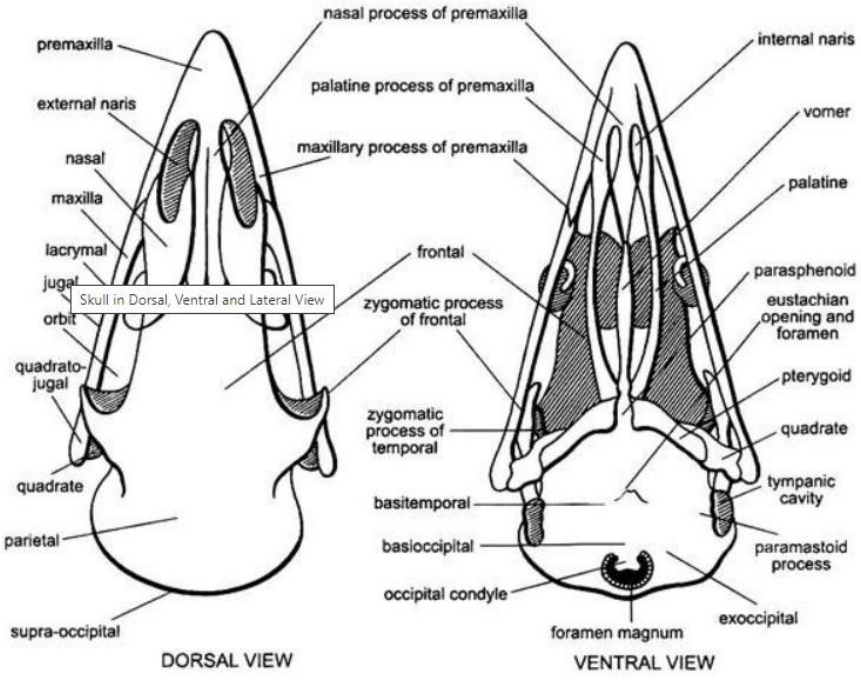
1. The skull is compact and having the narrow anterior end.
2. It is monocondylic.
3. It is tropibasic.
4. Temporal region has t fossae on either side, the supra-temporal fossa, supra-orbital fossaand sub-orbital fossa.
5. Lower jaw attached with quadrate bone of the skull.
6. There are 6 bones in lower jaw.
7. Cranium has three regions: a) Occipital segment b) Parietals c) Frontals



3. BIRDS

Axial Skeleton: **Skull**

(Dorsal View):



1. Skull Compact, devoid of teeth and very light due to spongy bones and presence of air cavities. Lightness is in accordance with flying habit.
2. Distinct feature of skull is the presence of a long pointed beak without teeth. Skull bones very compact, cosely fused, polished and with obliterated sutures.
3. Cranium greatly enlarged to accommodate the larger brain.
4. Monocondylic,single occipital condyle articulates with atlas.

5. Tropibasicskull – the definite inter-orbital septum separates large sized orbitals.

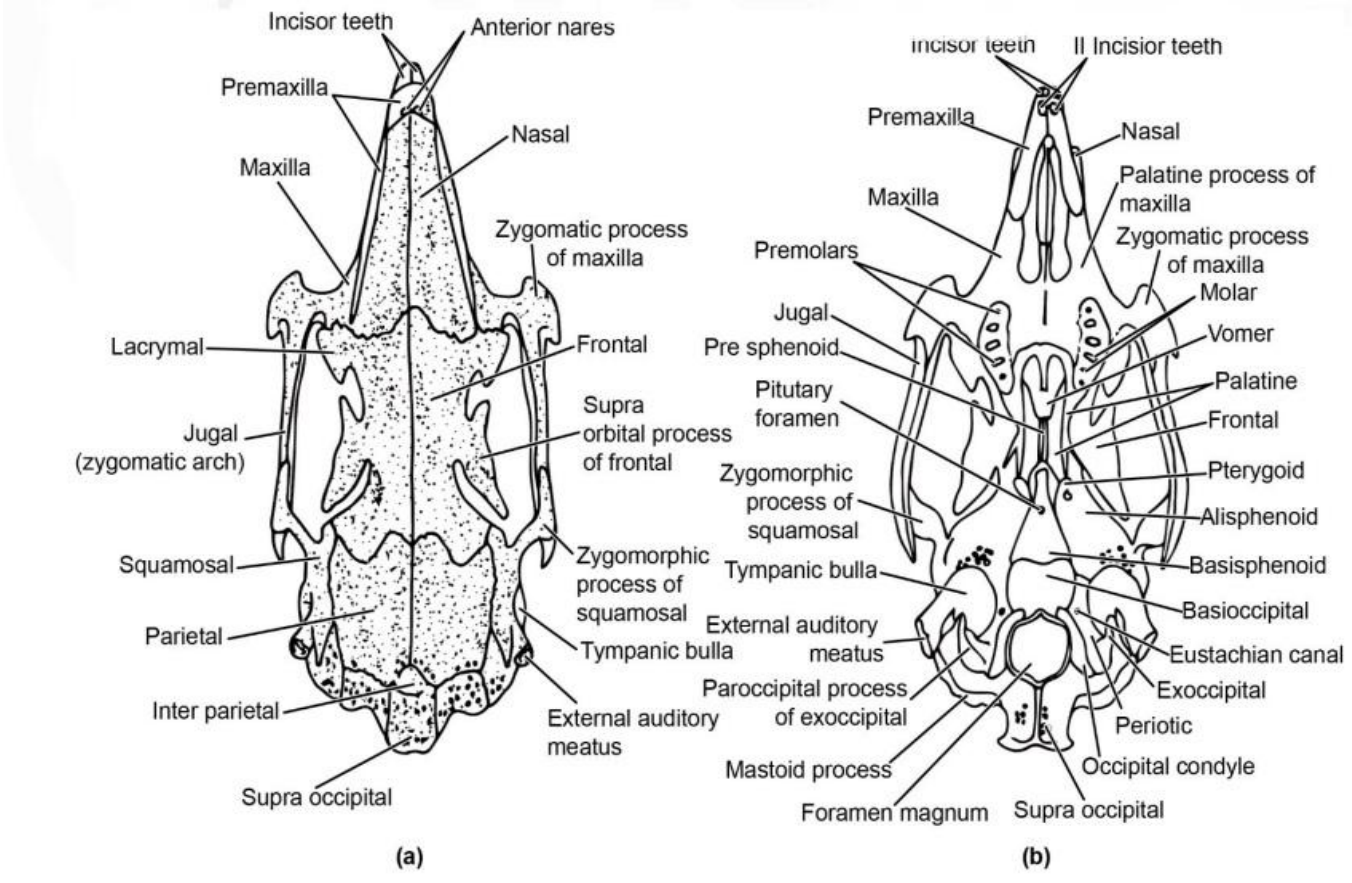
***Axial Skeleton* Skull (Ventral View)**

1. Supra-occipital joins with parietals forming a large prominent ridge known as occipital or lamboidal ridge.
2. Tympanic cavity large, hemispherical and having a single columella.
3. Teeth absent- Palate schizognathous formed by vomers, palatines, pterygoids and palatal prolongations of maxillae.

4. RABBIT

Axial Skeleton- Skull:

1. Completely Ossified with distinct sutures.
2. Dicondyle skull
3. Facial portion is elongated while cranial portion is small deflected at an angle of 60degree.
4. Occipital region composed of four bones
   a) Two Lateral Exoccipital
   b) One Ventral Basioccipital
   c) Dorsal Supraoccipital



a) Dorsal view of rabbit skull; b) ventral view of rabbit skull.

5. Auditory region composed of Periotic bone and tympanic bulla.
6. Each half of the lower jaw consist of a single bone- the dentary.
7. Teeth are heterodont and thecodont.

Experiment No: 8 **Date:**

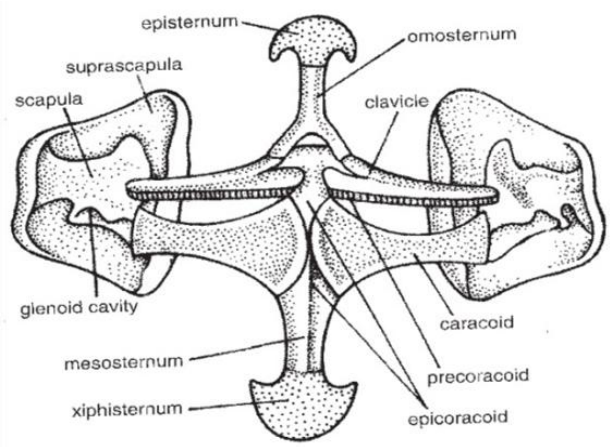
COMPARATIVE ACCOUNT OF SKELETAL SYSTEM: SKULL, GIRDLES BONES OF SHARK, FROG, CALOTES, PIGEON AND RABBIT

GIRDLES BONES

1. FROG

Pectoral Girdle:

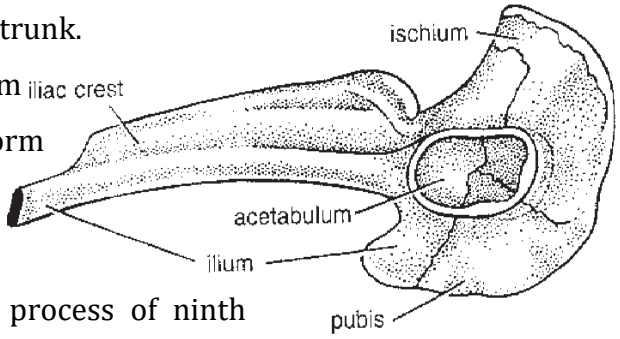
1. Found in thoracic region and give support to the fore-limbs.
2. Each half is consist of scapular and coracoid portions.
3. Two similar halves united mid-ventrallywith sternum and separated dorsally.
4. Scapular portion contains supra-scapula and scapula.
5. Supra-scapula is a thin cartilaginous plate on the dorsal side.



Pectoral Girdle

6. Scapula is flat contains a cup like glenoid cavity into which articulates the head of humerus.
7. Coracoid region having clavicle and coracoid and 2 cartilages- epicoracoid & precoracoid.

Pelvic Girdle:

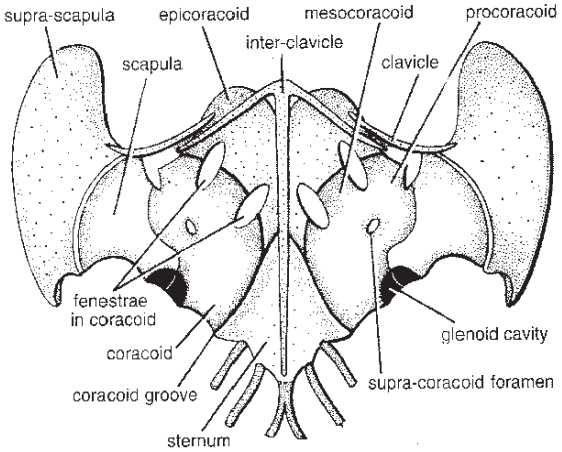


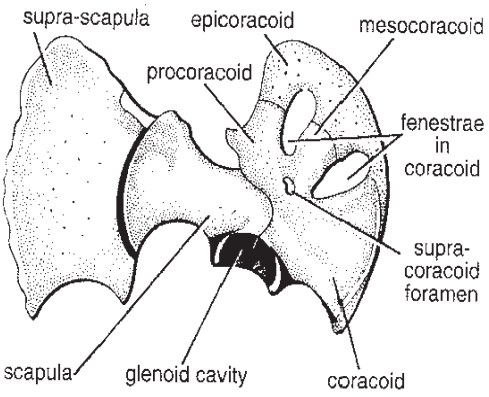
1. Pelvic girdle found in the posterior side of trunk.
2. Each half of girdle is contains ilium, ischium and pubis. Two half join posteriorly to form V- shaped structure.
3. It provide support to the hind-limbs.
4. Ilium is long and joins with transverse process of ninth vertebra.
5. Pubis is a triangular piece of calcified cartilage. Pubic cartilages of both sides arecompletely fused.
6. Ischium is slightly oval bone, form posterior part of disc.

2. CALOTES-

Pectoral Girdle

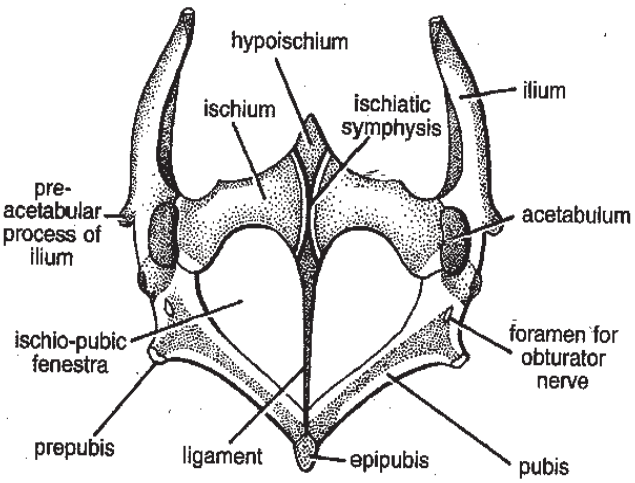
1. Pectoral Girdle is made up of two halves attached with T shaped interclavicle.
2. Each Half is made up of suprascapula, scapula, coracoid, interclavicle and clavicle.
3. Suprascapula is flattened, calcified cartilaginous plate, articulating with scapula.
4. scapula is completely ossified, flattened plate articulating with suprascapula and coracoid.
5. Coracoid is a flat bone partly ossified and partly cartilaginous.
6. Interclavicle are T shaped bone.
7. Clavicle are short, curved dermal bone articulating with suprascapula and interclavicle





Pelvic Girdle:

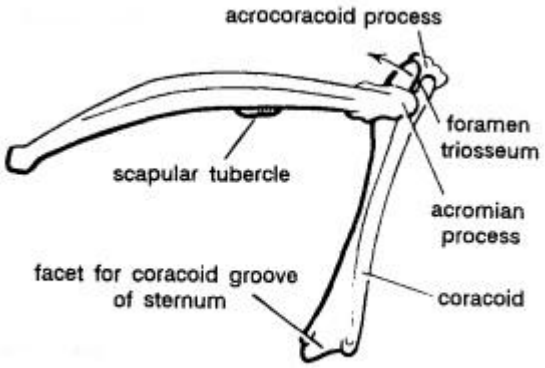
1. It has three bones namely ilium, pubis and ischium.
2. Ilium is a rod shaped bone constituting major part of acetabulum.
3. Pubis is a curved bone. They constitute one-third of acetabulum.
4. Ischium is flat and slightly curved & it articulates with pubis and ilium of its side



3. <u>PIGEON-</u>

Pectoral girdle

1. Pectoral girdle is very stout bony structured connected with sternum on either side tosupport wings.
2. On either side consists of a scapula, coracoids and a clavicle.
3. Scapula is long flattened bone lying above the thoracic ribs and parallel to thevertebral column.

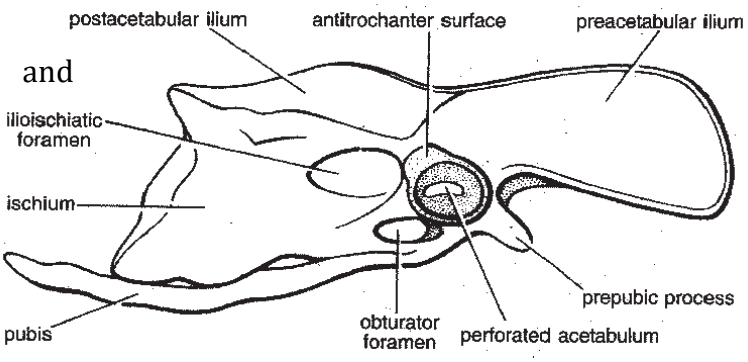


PECTROAL GIRDLE OF FOWL

4. On its anterior outer side it bears shallow depression forming a part of glenoid cavityand its inner surface is produced into an acromian process.
5. Coracoid is stout straight bone directed downwards and articulates with the articular surface of coracoids on the antero-lateral edge of the sternum at the base of manubrium.
6. Clavicles are a pair of slender curved bones connected by their expanded upper ends with the coracoids and scapula.

Pelvic Girdle:

1. Pelvic Girdle consists of two separate halves lying onsynsacrum.Each half is known as os-innominatum.
2. Ilium is an elongated and remarkably expanded bone extending both anterior and posterior to the acetabulum.
3. Ischium is dorso-ventrally flattened bone projecting

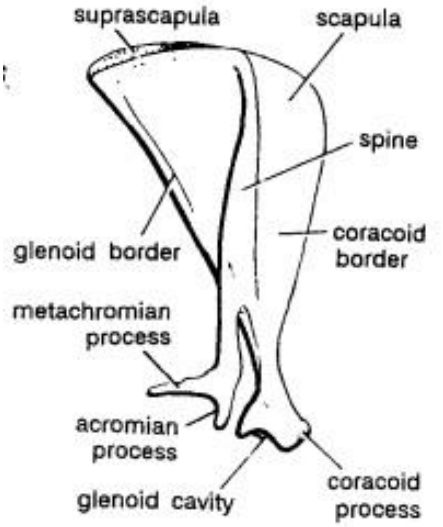


backwards behind theacetabulum and parallel to the posterior part of ilium

4. Pubis is a long slender bone directed backwards parallel to the outer margin of ischium with which it is fused.
5. At the junction of three bones on outer side is present a concavity, the acetabulum for the articulation of head of femur.

4. <u>RABBIT-</u>

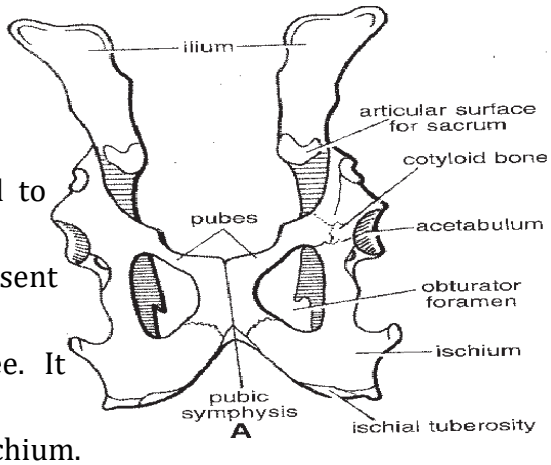
1. Each half of pectoral girdle is made up of clavicle and scapula-coracoid.
2. Scapula-coracoid.
   (i) It is a triangular replacing bone.
   (ii) The apex contains a concavity called glenoid cavity for humerus head.
   (iii) Over glenoid cavity hangs a coracoid process.
   (iv) A distinct vertical spine divides outer surface of scapula and it terminates below into an acromian process, which further gives posteriorly a metacromian process.



PECTORAL GIRDLE OF RABBIT
(Right half in outer view)

<u>Pelvic Girdle</u>

1. Pelvic girdle is composed of 2 equal halves called os-innominata.
2. Os-innominata are united at public symphysis .
3. Each innominatum consists of 3 pieces:
   I. **Ilium:** it is a blade like bone present parallel to vertebral column.
   II. **Ischium:** It is stout and straight bone present opposite to Ilium
   III. **Pubis :** It is smallest bone among the three. It becomes transverse near the acetabulum
4. Acetabulum is present at the union of ilium and ischium.
5. A cavity called obturator foramen is present between pubis and ischium.



A

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS, SHRI. B. M. PATIL ROAD, VIIJAYAPUR-586103

Karnataka

DEPARTMENT OF ZOOLOGY

B. SC II SEMESTER

PRACTICAL LAB MANUAL

2023-24

INDEX

Date	Sl. No.	Title of the experiment.	Signature of supervisor
	1.	Qualitative Analysis Of Carbohydrates, Proteins And Lipids.	
	2.	Qualitative Analysis of Nitrogenous Wastes – Ammonia, Urea and Uric acid.	
	3.	Separation of Amino Acids or Proteins by Paper Chromatography.	
	4.	Estimation of Haemoglobin in Human Blood using Sahli's Haemoglobinometer.	
	5.	Counting of RBC (Erythrocytes) in blood using Hemocytometer.	
	6.	Counting of WBC (Leucocyte) in blood using Hemocytometer.	
	7.	Differential Staining of Human Blood Corpuscles using Leishman stain.	
	8.	Recording of Blood Glucose Level by Using Glucometer.	

GENERAL INSTRUCTIONS TO THE STUDENTS

1. Wear apron before entering the laboratory.
2. Bring the observation book, record book, a set of colour pencils and small napkin for laboratory use separately.
3. Do not eat or drink inside the laboratory.
4. Study the theory background of the practical before coming to the lab.
5. **See that all the instruments are thoroughly cleaned before starting the practical.**
6. Write your observations immediately in the observation book and get it verified by the supervisor at the end of the practical.
7. Clean the instruments and the working place without fail before leaving the lab.
8. After use, bring the microscope to low power, switch it off and cover it.
9. Take all your belongings with you while leaving the lab.

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS, SHRI. B. M. PATIL ROAD

(SOLAPUR ROAD), VIJAYAPUR-586103

DEPARTMENT OF ZOOLOGY

Practical Examination Scheme (NEP), B.Sc., II Semester

COURSE TITLE: **BIOCHEMISTRY AND PHYSIOLOGY**	COURSE CREDIT: **02**
COURSE CODE: **21BSC2C2Z002P**	DURATION OF EXAMS: **4HOURS**
ASSESMENT MARKS:**25**	ASSESMENT MARKS:**25**

QUESTION NO	CONTENT	MARKS
1.	QUALITATIVE ANALYSIS OF CARBOHYDRATE/PROTEIN	07
2.	QUALITATIVE ANALYSIS OF AMMONIA/ URIC ACID	05
3.		05
4.	VIVA	03
5.	JOURNAL	05
	TOTAL	25

Experiment No. **Date:**

QUALITATIVE ANALYSIS OF CARBOHYDRATES, PROTEINS AND LIPIDS

KEY FOR DETECTION OF UNKNOWN SOLUTION

Dip a piece of paper in the given solution

Paper becomes transparent – Lipids present	No transparency of paper – Protein/ carbohydrate may be present

Heat the solution in the test tube

Coagulation – Protein present	No coagulation – Carbohydrate may be present

Soln. + I_2 – Blue coloration- Starch present	O. Sol_n + Benedict's reagent boil and cool under tap water. Green/Yellow/Red ppt – Glucose present	O. Sol_n + Benedict's reagent boil and cool under tap water. No ppt - Sucrose present

C. T for Glucose

Sl. No	Test	Observation	Inference
1.	5cc Fehling's Soln Boil+ 1cc O.S and boil	Green/Yellow/Brick red ppt	Glucose present
2.	3cc O.S + 1cc Picric Acid soln+ 1cc 40% NaoH	Red colour appears	Glucose present
3.	2cc $CuSO_4$+ 2cc O.S + 2cc 40%NaoH Boil	Blue colour changes to brick red on boiling	Glucose present
4.	3cc H_2O + 1 drop Methylene blue + 0.5cc 40% NaoH Boil and add 1cc of O.S	Blue colour disappears	Glucose present
5.	3cc $K_3Fe(CN)_6$ + 1cc 40%NaoH soln Boil + add drop by drop O.S and keep on boiling	Yellow colour of ferricynide disappears	Glucose present and confirmed

Comments: - Glucose is the simple sugar. It is present in natural food. Glucose is white crystalline solid soluble in water. It is source of energy. It is important in cellular respiration

C.T for Sucrose

Sl. No.	Test	Observation	Inference
1.	Benedict's test Heat benedict's reagent 5ml in a test tube ,if colour does not change add 8-10 drops of o.s and heat it again .Allow it to cool and observe.	No change in colour mixture .	Sucrose present
2.	Inversion test Take about 5ml of o.s add 2-3 conc.HCL heat it for few sec,cool it go on add Na2Co3 till get neutralized . a) Hydrolysed o.s +Benedicts reagent heat it. b) Barford's test – 2ml of barfords reagent in test tube and add 2ml of hydrolysed o.s and the mixture for 2min heat the mixture for 2min heat the mixture and allow to cool.	Black red ppt is formed Blue colour of reagent remains as it is.	Sucrose present Sucrose present

Comments: - Sucrose is disaccharide. It is important as source of energy. It is found in fruits.

C.T for Starch

Sl. No	Test	Observation	Inference
1.	2ml O.S + 1-2 drops of I_2 Soln	Blue colour appears	Starch present
	(i)To the above soln add more I_2	Blue colour deepens	Starch present
	(ii)Heat the above soln.	Blue colour disappears	Starch present
	(iii)Cool the above soln.	Blue colour reappears	Starch present
2.	3ml O.S. + 1ml 40%NaoH +few drops of I_2	No colour	Starch present
	(i)To above soln + 1ml Conc. HCl	Blue Colour appears	Starch present
3.	2ml O.S. + 2ml α –Napthol + 1ml Conc.H_2SO_4	Purple ring at the junction of 2 solutions	Starch present & Confirmed

Comments: - Starch is polysaccharide. It is present in plants.

C.T for Proteins

Sl.No.	Test	Observation	Inference
1.	Biuret test: - 3cc O.S + 1cc 40%NaoH+ 1cc $Cuso_4$ and rotate gently.	Violet or pink ring at the junction of the two fluids.	Protein present
2.	Xanthoprotein test: - 3cc O.S + 1cc Conc HNO_3 and boil and add 40% NaOH in excess and boil.	White ppt on boiling changes to yellow	Protein present
3.	2cc O.S + 2 drops Lead acetate	White ppt	Protein present
4.	3cc O.S+ 2 Drops acetic acid + 3Drops of $K_4Fe(CN)_6$	White ppt	Protein present & Confirmed.

Comments: - Proteins are found in animals and plants and are considered as building blocks of body. They have high mol. wt. and contain free amino and carboxyl groups. On hydrolysis they break down into peptones, peptides and proteases.

C.T for lipids

Sl. No	Test	Observation	Inference
1.	Acrolein test: - 1ml O.S + little Potassium hydrogen sulphate mix thoroughly and heat	Irritating odour of Acrolein.	Lipids present
2.	2ml O.S + 2-3 drops Sudan III soln	Red colouration	Lipids present
3.	5ml water + 2 drops O.S shake vigorously	Emulsification	Lipids present
4.	2ml O.S +1 ml KOH(Alcoholic) + Nacl or $cacl_2$	White ppt	Lipids present and confirmed

Comments: - lipids are the food which breakdown to release fatty acids and glycerol. They are energy rich compounds. They are insoluble in water and soluble in organic solvents.

Experiment No. **Date:**

QUALITATIVE ANALYSIS OF NITROGENOUS WASTES – AMMONIA, UREA AND URIC ACID

(I). Ammonia (NH_3)

1.	Urine + 5% NaOH +Nesseler's reagent	Orange yellow colour changes to dirty green	NH_3 present
2.	2ml of Urine in test tube evaporate it to dryness	Fumes of NH_3	NH_3 present

(II) Urea: -

1.	Urine + $HgNo_3$	White ppt	Urea present
2.	5ml urine + 2 drops alcoholic Phenopthalein (1%) +A pinch of soybean flour. Mix thoroughly and heat at 50°C water bath	Pink colour	Urea present
3.	1 ml Urine + Few drops of Urease **Or** Phenol red	Pink colour	Urea present

(III) Uric Acid:-

1.	2ml urine + Na_2CO_3 saturated +Follin's reagent	Blue colour	Uric present
2.	1ml Urine+ 1ml Sodium thiosulphate +1ml Fehling's reagent	Blue colour	Uric present
3.	2ml Urine + 1ml $NaHCO_3$ + 3-5 drops Benedict's reagent	Blue colour	Uric present

QUALITATIVE TESTS FOR ABNORMAL CONSTITUENTS OF URINE

Test	Observation	Inference
Glucose 1) Fehling test 1ml urine + 1ml Fehling A+1ml Fehling B. Mix Boil well	Green/ orange/Red ppt	Glucose present
2) Benedict test 1ml urine + 5ml Benedict reagent. Mix. Boil well.	Green, orange or Red ppt	Glucose present
Albumen 1) 5ml urine.Boil well. Add 1ml 5%Acetic acid.	White ppt	Albumen present
2) Urine + NaoH +2 drops $CuSO_4$	Violet or pink colour	Albumen present
3) Urine + Few drops of Conc.HNO_3	Albumen coagulates	Albumen present
Ketones 1) 5ml urine + 0.5ml Na Nitropruside. Mix well. +2ml conc. Ammonia. Mix.	Red purple colour	Ketones present
2) 5ml urine + 1ml NaOH(1%). Mix.Add excess iodine soln. (about 2 ml)	Yellow ppt	Ketones present
3) 3ml Urine+ Saturated $(NH_4)_2SO_4$ + Crystals of Na Nitropruside and shake well.	Red purple colour	Ketones present
	*Brown colour	*Ketones absent
Bile salts 5ml urine in a small beaker sprinkle sulphur powder.	Sulphur sinks to the bottom.	Bile salts present
	*If sulphur floats on the surface	Bile salts absent

Comments: - If glucose is +ve indicates of diabetes mellitus. +ve Albumen indicates of patients with glomerulonephritis. +ve Ketones indicates of late stages of diabetes mellitus. +ve Bile salts indicates of patients with Jaundice.

Experiment No. **Date:**

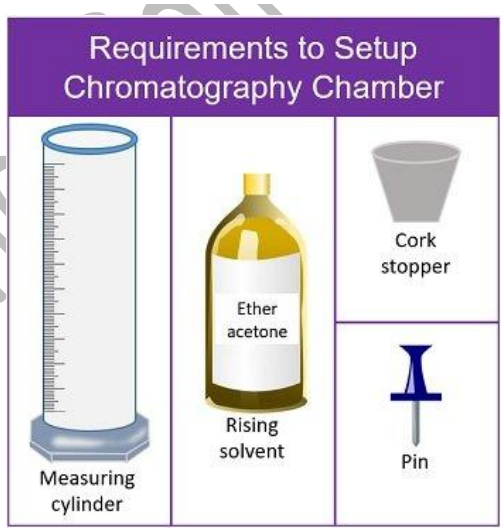
SEPARATION OF AMINO ACIDS OR PROTEINS BY PAPER CHROMATOGRAPHY

Paper Chromatography

The mixtures in compounds have **different solubilities**. For this reason, they get separated distinctly between the stationary and running phase. The **mobile phase** is a combination of non-polar organic solvents. The solvent runs up the stationary phase via capillary movement. The **stationary phase** is polar inorganic solvent, i.e. water. Here, the absorbent paper supports the stationary phase, i.e. water.

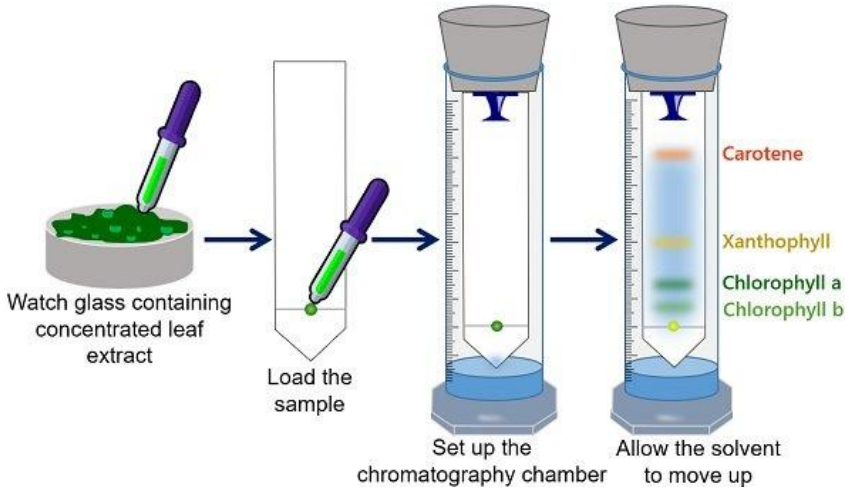
Setup the Chromatography Chamber

This stage requires the materials displayed in the picture below:



- Take a clean measuring cylinder and add rising solvent (ether acetone) up to 4 ml.
- Bend the strip of paper from the top. Then, using a pushpin attach the paper to the bottom of the cork.
- Adjust the length of the paper. The absorbent paper should not touch the surface of the measuring cylinder.
- After that, allow the solvent to move up the absorbent paper.
- When the solvent front has stopped moving, remove the paper.
- Allow it to dry for a while until the colours completely elute from the paper.
- At last, mark the front edge travelled by each pigment.

Steps of Pigment Separation



1. Take Whatman filter paper and draw a line above 2 cm from the bottom margin.
2. You can use a pencil and scale to draw a fainted line. **Note**: A pencil is used because pencil marks are insoluble in the solvent.
3. Then, cut the filter paper to make a conical edge from the line drawn towards the margin end. You can use a scissor to cut the Whatman filter paper.
   Note: The conical end at the bottom of the filter paper results in better separation.
4. After that, load the sample on the chromatography paper.

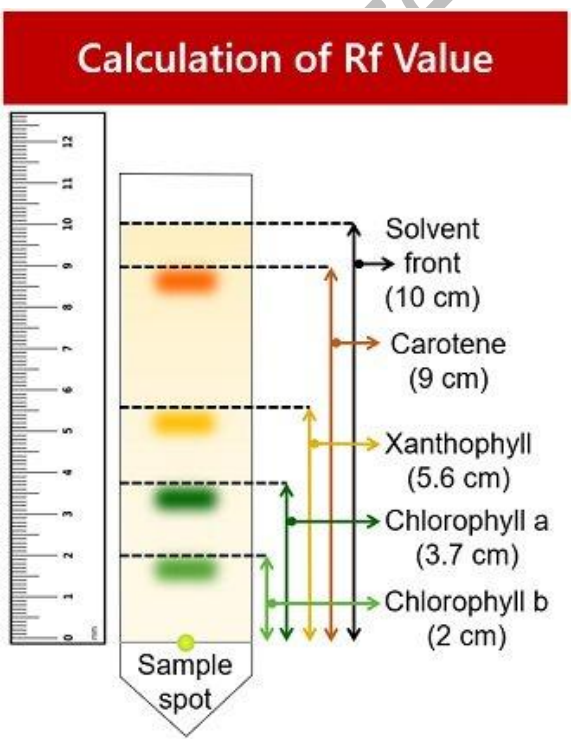
5. Then, keep a paper containing sample spot inside the chromatography chamber containing solvent.
6. At last, allow the solvent to move up to visualize the coloured spots.

Observation

Over the dried paper strip, you will see four different bands. Different colour streaks form because of different affinities with the mobile phase (solvent).

Calculation

Calculate Rf values for each pigment. Rf stands for **retardation** or **retention factor**. You can record the Rf value by knowing the distance analyte travelled by the distance solvent travelled.



1. Light green spot indicates **chlorophyll-b** pigment.
- Rf value= Distance chlorophyll-b travelled / Distance solvent travelled = 2/10 = 0.2

2. Dark green spot represents **chlorophyll-a** pigment.
- Rf value= Distance chlorophyll-a travelled / Distance solvent travelled = 3.7/10 = 0.37

3. The yellow band represents **xanthophyll** pigment.
- Rf value= Distance xanthophyll travelled / Distance solvent travelled = 5.6/10 = 0.56

4. The yellow-orange band indicates **carotene** pigment.
- Rf value= Distance carotene travelled / Distance solvent travelled = 9/10 = 0.9

Factors affecting the Rf values of a particular analyte are:
- Stationary phase
- The concentration of the stationary phase
- Mobile phase
- The concentration of the mobile phase
- Temperature

The Rf value of compounds in the mixture differs by any changes in the concentration of stationary and mobile phases.
Temperature affects the solvent capillary movement and the analyte's solubility in the solvent. Rf value is independent of the sample concentration. Its value is always **positive**.

Experiment No. **Date:**

ESTIMATION OF HAEMOGLOBIN IN HUMAN BLOOD USING SAHLI'S HAEMOGLOBINOMETER

Aim: Estimation of blood haemoglobin by Sahli's method

Principle: Heamoglobin is converted into stable acid haematin by addition of N/10 HCl. This concentrated solution is diluted with water till its colour density matches with that of the standard.

Apparatus: 1. Sahli's comparator 2. Sahli's tube (Heamoglobin tube) 3. Sahli's pipette (Heamoglobin pipette) 4. Thin glass rod stirrer 5. N/10 HCl 6. Distilled water 7. Spirit swabs 8. Blood lancet 9. Filter paper

Sahli Comparator

Procedure:

- Take a clean & dry Sahli's tube and place it in the comparator.
- Using a dropper, add N/10 HCl upto 2 g mark on the percentage scale.
- Under aseptic precautions, prick the finger.
- Wipe the first one or two drops of blood.
- Obtain a good sized drop of blood.
- Draw the blood up to 0.02 ml (or 20 mm^3) mark.

- Avoid any air bubbles. If present, discard and repeat.
- Dip the pipette immediately in Sahli's tube containing HCl and gently blow till the blood is expelled.
- Rinse the pipette twice or thrice with HCl.
- Mix the contents gently by stirring with a glass rod.
- Place the tube in the comparator & wait for 10 minutes (during this period, RBCs rupture and Hb is liberated which reacts with N/10 HCl to form stable acid haematin).
- Go on adding distilled water drop by drop & mix it, till the color matches with that of standard tinted glass against natural source of light.
- When the color of the solution matches, note down the lower meniscus reading.
- Express the haemoglobin content as _____gm/100 ml.

Calculation:

O_2 carrying capacity = Haemoglobin content in gms x 1.34 = _____ml/100 ml of blood.

Note: 1 gm of Hb carries 1.34 ml of O_2

Note: 1 gm of Hb contains 3.34 mg of Fe

Normal range:

- At birth - 23 gm%
- At 1 year - 12 gm%
- In adult male - 14–18 gm% (Average 15.5 gm %)
- Female - 12–15 gm% (Average 14.0 gm %)

Discussion:

Other methods of estimation of Hb are:

Direct methods:

a. Van Slyke's method – Oxygen carrying capacity is estimated
b. Iron estimation
c. Spectophotometry

Indirect methods:

a. Tallquist's method - Hb is converted to Oxy-Hb
b. Haldane's method - Hb is converted to Carboxy-Hb
c. Cyanmethemoglobin method - Hb is converted to Cyanmeth Hb

Experiment No. **Date:**

COUNTING OF RBC (ERYTROCYTES) IN BLOOD USING HEMOCYTOMETER

AIM: Determination of RBC count per unit volume of blood by manual method

PRINCIPLE: The number of RBCs in a known volume of diluted blood is counted and the number of cells in undiluted blood is calculated and reported as the number of red cells per mm^3 of whole blood.

APPARATUS:

1. Microscope 2. Neubauer's counting chamber 3.RBC pipette 4.Cover slip
5. RBC diluting fluid (Hayem's fluid).

Components of Hayem's fluid & their functions:

Sodium chloride: 0.5 gm; maintains osmolarity.

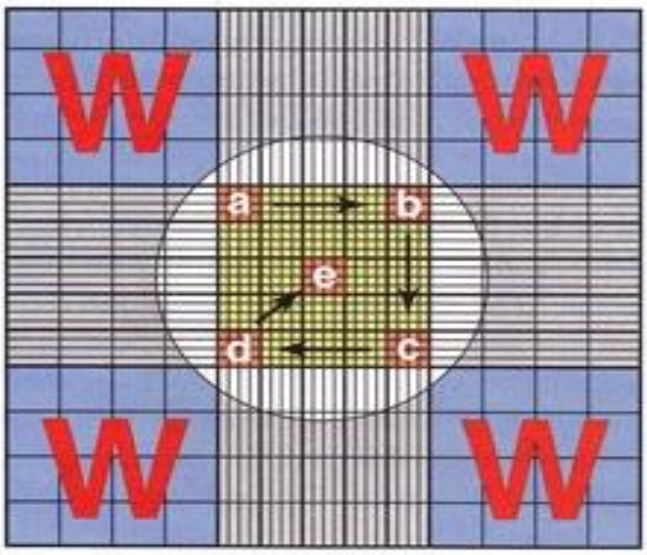
Sodium sulphate: 2.5 gm; prevents aggregation of RBCs.

Mercuric chloride: 0.25 gm; acts as a preservative (antifungal and antibacterial).

Distilled water:100 ml; acts as solvent.

PROCEDURE:

- Clean the pipette, coverslip and Neubauer's chamber thoroughly.
- Place the coverslip on the central platform of Neuauer's chamber.
- Take adequate RBC diluting fluid in a watch glass.
- Under aseptic precautions, prick the finger and suck the blood upto 0.5 mark.
- Suck the RBC diluting fluid upto 101 mark.
- Hold the pipette horizontally and close both ends of pipette, then gently mix the contents of the bulb by rolling between the palms.
- Discard the first two drops of fluid from the pipette.
- Charge Neubauer's chamber by placing the tip of pipette on the surface of the chamber, touching the edge of the coverslip at an angle of 45^0.
- Allow the diluted blood to flow under the coverslip by capillary action.



- Take care to avoid entry of air bubbles and flow of fluid into gutters (recharge, if air bubbles are present or if there is overflow).
- Allow the cells to settle down for two to three minutes.
- Place the chamber on the stage of microscope.
- Observe first under low power and ensure equal distribution
- Focus under high power and start counting the RBCs in 5 medium sized RBC squares.
- To avoid reduplication in the counting process, apply 'L' rule.
- The number of RBCs in each of the 5 medium sized squares should be approximately equal (the difference between the squares should not exceed 20).

Dilution obtained:

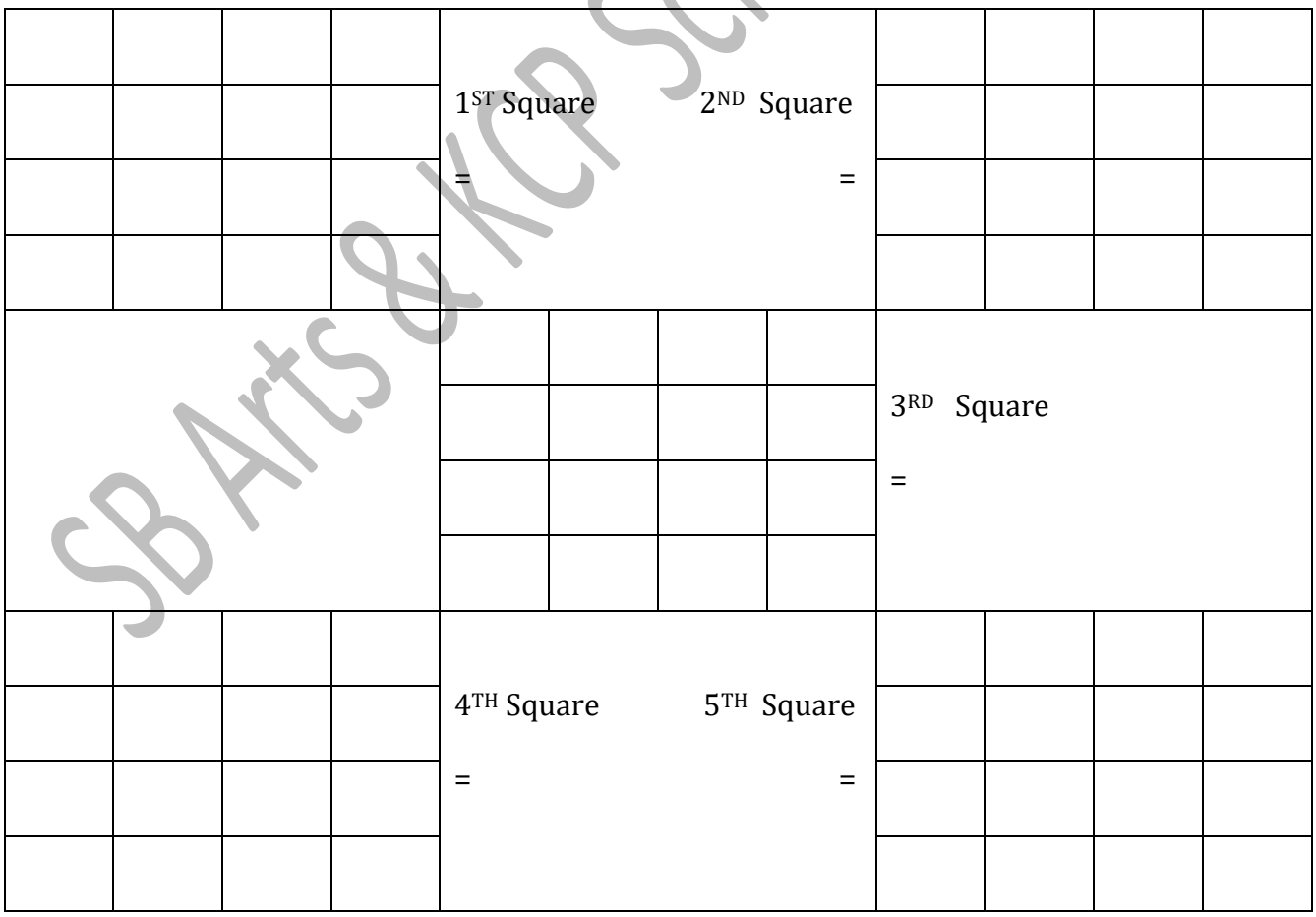
The volume of the bulb is 100.

100 volumes of diluted blood contain 0.5 volume of whole blood.

200 volumes of whole blood contain 1.0 volume of whole blood.

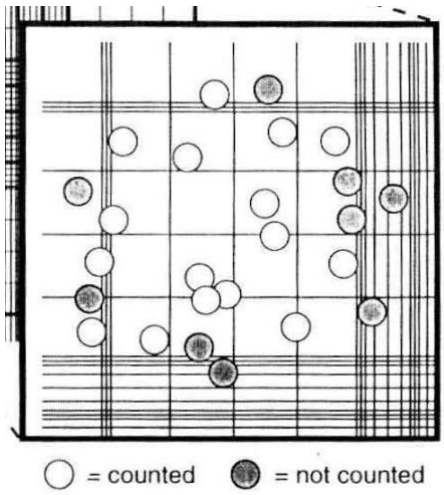
Therefore the dilution is 1 in 200.

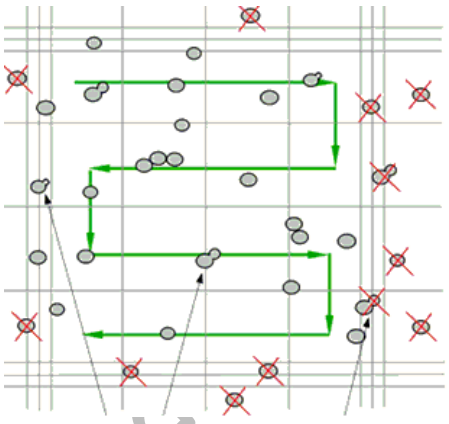
Total count of RBCs



N cells= total cells present in squares 1+2+3+4+5.

Therefore **N cells =**





L – rule with counted & non-counted RBCs

CALCULATIONS:

- Area of 1 medium sized square is 1/5 x 1/5 = 1/25 mm^2.
- Area of 5 medium sized squares is 1/25 x 5 = 1/5 mm^2.
- Volume of 5 medium sized squares is 1/5 x 1/10 = 1/50 mm^3.
- 1/50 mm^3 of diluted blood contains N cells.
- Therefore 1 mm^3 of diluted blood contains 50 N cells.
- Dilution factor in 1 in 200
- 1 mm^3 of undiluted blood contains 50 x 200 x N cells.
- Therefore RBC count =10,000 x N cells

=_____million cells/mm^3

Normal RBC count: Males = 4.5 – 5.5 millions/mm^3 of blood

Females = 4.0 – 4.5 millions/mm^3 of blood

Report:

Name: Age: Sex:

Experiment No. **Date:**

COUNTING OF WBC (LEUCOCYTE) IN BLOOD USING HEMOCYTOMETER

Aim: Determination of total leucocytes per unit volume of blood

Apparatus:

1. Improved Neubauer's chamber 2. Microscope 3. WBC diluting fluid/Turk's fluid
4. WBC pipette 5. Coverslip 6. Blood lancet 7. Spirit swabs

Composition and functions of Turk's fluid:

Glacial acetic acid– 1% (destroys RBCs and makes the WBC nuclei prominent)

Gentian violet– 0.3% (stains nuclei of WBCs)

Distilled water (causes lysis of RBCs osmotically and acts as solvent)

Procedure:

- Clean and dry the pipette, cover slip and Neubauer's chamber thoroughly.
- Under aseptic precautions, prick the finger.
- Wipe the first two drops of blood.
- Obtain a good sized drop of blood.
- Draw the blood up to 0.5 mark. Avoid any air bubbles.
- Suck the diluting fluid upto mark 11.
- Mix thoroughly by holding the pipette horizontally between the palms.
- Discard first two drops of fluid.
- Charge Neubauer's chamber by placing the tip of pipette on the surface of the chamber, touching the edge of the cover slip at an angle of 45^0.
- Allow the diluted fluid to flow under the cover slip by capillary action.
- Take care to avoid the entry of air bubbles and flow of fluid into gutters. (recharge if air bubbles are present or if there is overflow).
- Allow the cells to settle for 2-3 mins.
- Place the chamber on the stage of microscope.
- Observe first under low magnification and ensure equal distribution.

Upper LEFT square _____ cells **Lower LEFT** square _____ cells					**Upper right** square _____ cells **Lower LEFT** square _____ cells			

Total =

Calculation:

Volume of one square= L x B x D

= 1 mm x 1 mm x 1/10 mm= 1/10 mm^3

Volume of four squares= 4 x 1/10 mm^3

= 2/5 mm^3

2/5 mm^3 contains – N no. of WBCs

Therefore, 1mm^3 contains 5 x N/2 no. of WBCs

Dilution factor is 1: 20

1 mm^3 of undiluted blood contains = 5 x N/2 x 20

= 50N cells

= 50 x _____ cells

= _____ cells/mm^3 of blood

Experiment No : **Date :**

DIFFERENTIAL STAINING OF HUMAN BLOOD CORPUSCLES USING LEISHMAN STAIN

Aim: Determination of percentage of various types of WBCs in peripheral blood

Principle: A blood film stained with Leishman's stain is examined for different types of white blood cells and the percentage distribution of these cells is then determined.

Apparatus:

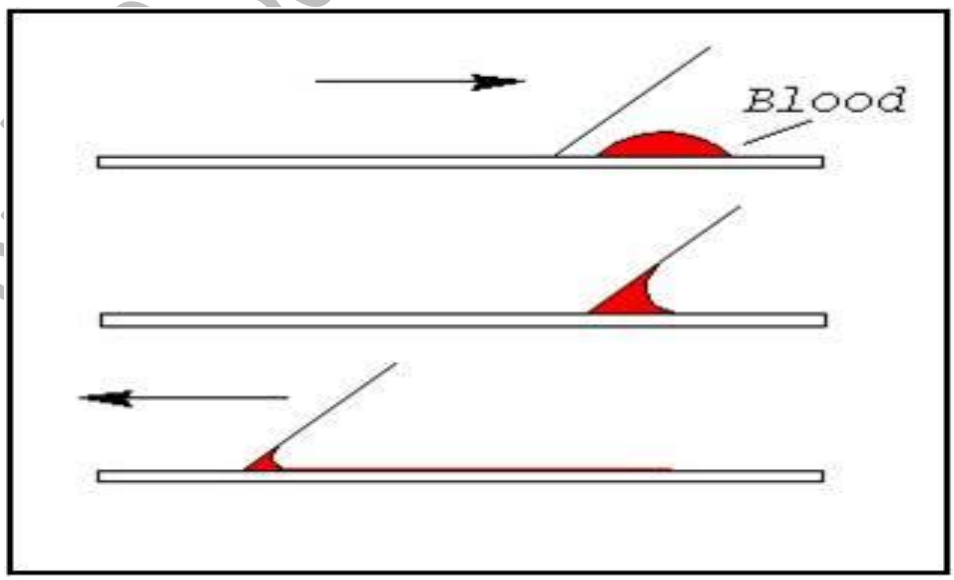
1. Glass slides
2. Leishman's stain
3. Microscope
4. Distilled water
5. Lancet
6. Spirit swabs

Composition of Leishman's stain:

- Contains Eosin & Methylene blue (in acetone free methyl alcohol)
- Methyl alcohol fixes the smear on slide (by precipitating the proteins)
- Eosin stains cytoplasm & basic granules.
- Methylene blue stains the nucleus and acidic granules.

Procedure:

1. **To make a smear**



- Take two grease free slides with smooth edges and select one as a spreader.
- With aseptic precautions prick the finger.
- Place a medium-sized blood drop near one end of slide (do not touch the slide).
- With the left middle finger and thumb, hold the slide.
- Place the edge of spreader just in front of blood drop.
- Draw the spreader back until it touches the drop of blood.
- Let the blood spread over the edges of the spreader.
- Maintain an approximate angle of 30-45^0 between the two slides.
- Push the spreader to other end of the slide by smooth, quick & uniform movement.
- Prepare a second smear using the same smear.
- Dry the smear quickly by waving the slides in the air.

Note: An ideal smear has a thick area of head and gradually thins out at the body and tail end. It is tongue shaped, with no windows or striations (longitudinal or transverse).

2. **Staining the smear:**
   - Counting the drops, pour sufficient of Leishman's stain on each slide (to cover whole of smear & not to overflow).
   - Wait for 90-120 seconds for proper fixation of the smear.

Note: During this period do not allow the smear to dry and add few more drops of stain, if needed.

- Add double the amount of distilled water.
- Mix the stain and water thoroughly by gently blowing air through a Pasteur pipette.
- Let the diluted stain remain on the smear for 8-10 minutes.
- Discard the stain, and wash the smear gently under tap water.

Note: The tap water should not fall directly on the smear as it may get washed off.

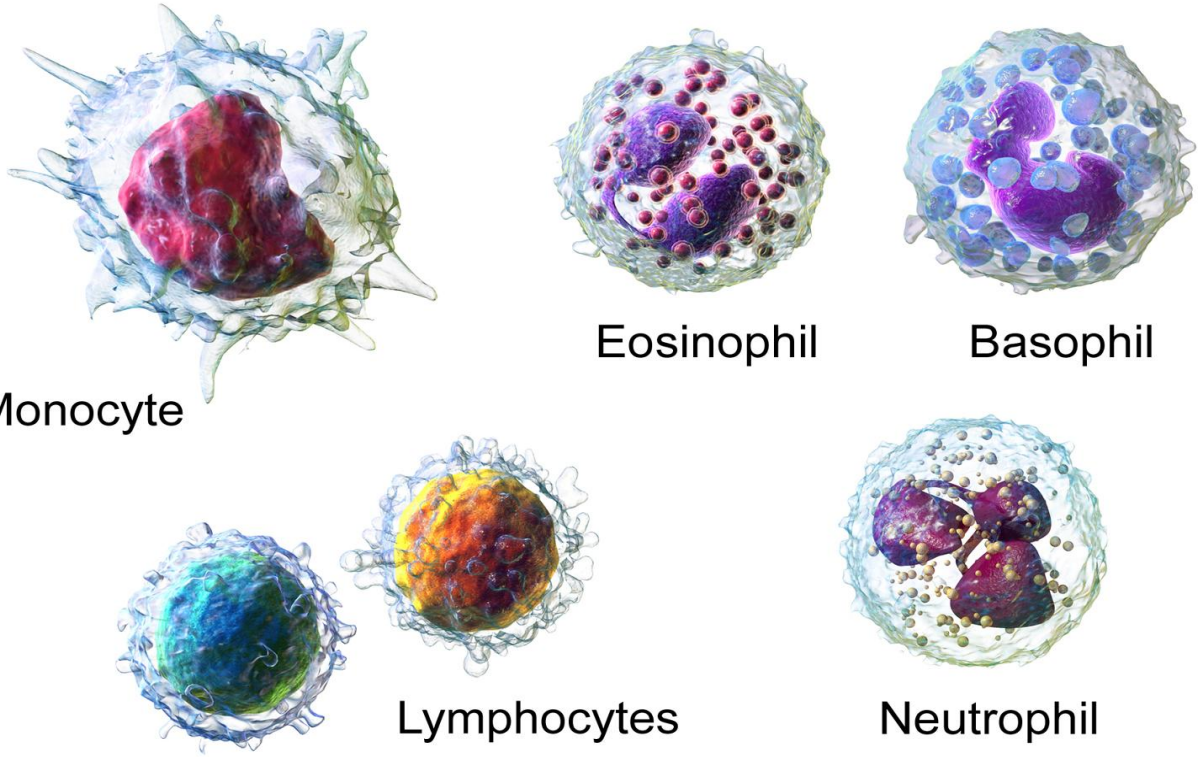
- Let the smear dry in upright position.
- A well stained smear appears faint purple in colour.
- Focus the smear first under low power objective to scan the entire slide and then under the high power for selecting the field.
- Add a drop of cedar wood oil on the selected field.
- Lower the end of oil immersion till it is just immersed in oil drop.
- Then the smear is focused by using fine adjustment screws only.
- Count one hundred WBCs by moving the slide in zig-zag manner.

Points to identify different white blood cells:

1. Cell size
2. Features of the nucleus & no. of lobes
3. Features of cytoplasmic granules
4. Nucleus/cytoplasm ratio

Various types of Leucocytes

CELL TYPE	SIZE	NUCLEUS	CYTOPLASM & GRANULES	NORMAL DIFFERENTIAL COUNT
1. Neutrophil	10-12 µm	3-4 lobes according to maturity	Violet-pink fine granules	50 – 70%
2. Eosinophil	10-12 µm	2 lobes connected by thin strand of chromatin, spectacle-shaped	Coarse red granules	1 – 4%
3. Basophil	12-15 µm	Usually bilobed or trilobed	Cytoplasm laden with coarse bluish granules	0 – 1%
4. Lymphocyte	07-08 µm	Round nucleus practically fills the cell	Thin rim of cytoplasm	20 – 30%
5. Monocyte	14-17 µm	Nucleus is indented & often kidney-shaped	More cytoplasm (ground glass)	2 – 8%



White Blood Cells

Experiment No : **Date :**

RECORDING OF BLOOD GLUCOSE LEVEL BY USING GLUCOMETER

Principle:

Materials Required:

- Alcohol prep pad or soap and water
- A lancing device with a fresh lancet (used to draw blood)
- A test strip
- A way to record results

Procedure:

1. Turn on the glucometer. This is usually done by inserting a test strip.
2. The glucometer screen will tell you when it's time to put blood on the strip.
3. Use the lancing device to pierce the side of your finger, next to the fingernail (or another recommended location). This hurts less than lancing the pads of your fingers.
4. Squeeze your finger until it has produced a sufficient-size drop.
5. Place the drop of blood on the strip.
6. Blot your finger with the alcohol prep pad to stop the bleeding.
7. Wait a few moments for the glucometer to generate a reading.
8. If you often have trouble getting a good blood sample, warm your hands with running water or by rubbing them briskly together.
9. Be sure they are dry again before you stick yourself.

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS, SHRI. B. M. PATIL ROAD, VIIJAYAPUR-586103

Karnataka

DEPARTMENT OF ZOOLOGY

B. SC IV SEMESTER

PRACTICAL LAB MANUAL

2023-24

B.SC IV SEMESTER PRACTICAL MANUAL

Sl. No.	LIST OF EXPERIMENTS
1	Calculate the mean, median, mode and standard deviation (Measurement of pre and post clitellar lengths (with suitable examples)
2	Measure the height and weight of all students in the class and apply statistical measures.
3	Determination of ABO Blood group and Rh factor
4	To study Restriction enzyme digestion using teaching kits (Demonstration only).
5	To detect genetic mutations by Polymerase Chain Reaction (PCR) using teaching kits (Demonstration only)
6	Demonstration of Agarose gel electrophoresis for detection of Nucleic acids
7	Demonstration of Polyacrylamide Gel Electrophoresis (PAGE) for detection of protein
8	To calculate molecular weight of unknown DNA and protein fragments from gel pictures.
9	To learn nucleotide sequence d
10	To learn sequence alignment: Pairwise alignment (Protein/ DNA).

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS,

SHRI. B. M. PATIL ROAD (SOLAPUR ROAD), VIIJAYAPUR-586103

DEPARTMENT OF ZOOLOGY

B.Sc., IV Semester Practical Examination Scheme (NEP)

Course Title/Code: Gene Technology, Immunology and Computational Biology (DSCC5ZOOT4)

Duration: 4 hours **Max. Marks: 25**

I. Identify the ABO and Rh blood group of the given blood sample and comment on the significance of blood typing. 04

(Identification of ABO and Rh blood group- ½+½=1 Mark; Reasons- 1+1= 2Marks; significance-1 Mark)

II. Identify and comment on the spotter A (Immune cells and organs-slides/photographs). 02

(Identification-1 Mark, comments-1)

III. Biostatistics problem on Chapter 7 04

IV. Biostatistics problem on Chapter 8 04

V. Identify and comment on the given spotters B, C and D. 3X2=06

(PCR/PAGE/Restriction enzyme kit/ BLAST, CLUSTALW, FASTA/Database)

(Identification - 1 Mark; comments -1 Mark)

VI. Class record 05

Total - 25

MEASURES OF CENTRAL TENDENCY

Mean: - The average obtained arithmetically is called arithmetic mean or mean. It is obtained by summing up all observations and dividing the total by the no. of observations: -

(a) Ungrouped data: - Suppose each individual observation is $x_1, x_2, x_3, x_4, + - - - x_n$

Then $\Sigma x = x_1, x_2, x_3, x_4, + - - - x_n$

Mean = $\bar{x} = \frac{\Sigma x}{N}$ where $\bar{x}$ = Mean

Σx = summation of observations

N= Total no. of observations.

1. Marks obtained of ten students with Roll numbers is 67, 69, 66, 68, 72,63,76,65, 70, and 74. Find the mean of the data.

Roll No.	Marks
1.	67
2.	69
3.	66
4.	68
5.	72
6.	63
7.	76
8.	65
9.	70
10.	74
N=10	Σx=690

$\therefore \bar{x} = \frac{\Sigma x}{N}$

$= \frac{690}{10} = 69$

2. Haemoglobin percentage of 9 patients of a ward of hospital was obtained as 6mg, 4mg, 8mg, 7mg, 9mg, 6mg, and 8mg. Find the mean from this data.

Solution: -

Σx= 6mg+ 4mg+ 8mg+ 7mg+ 9mg+ 6mg+ 8mg = 60 48

N = 9 7 $\therefore$ Mean percentage $\bar{x} = \frac{\Sigma x}{N}$

$= \frac{60}{9\ 7}$ 48 6.85mg. = 6.66mg

3. Find the mean from the frequency table –

Marks	30	40	50	60	70	80	90
No. of students	15	20	10	15	20	15	5

Solution: -

Marks (x)	No of students (f)	fx
30	15	450
40	20	800
50	10	500
60	15	900
70	20	1400
80	15	1200
90	5	450

$\Sigma f = 100$ $\Sigma fx = 5700$

Here we apply

$$\text{Mean } \bar{x} = \frac{\Sigma fx}{\Sigma f} = \frac{5700}{100}$$

= 57 Ans.

Median: - Median is the middle most point or the central value of the variable in a set of observation, when observations are arranged either in ascending or descending order of their magnitude.

(a) Ungrouped data: - Arrange data in ascending or descending order of magnitude.

(i) If the no. of observations is odd, the value of middle most item is the median. If the no. are even, the arithmetic mean of the two middle most items is taken as median.

When n is odd **Median = M = $\frac{n+1}{2}$ th term**

When n is even **Median =M = $\frac{n/2 + (n/2 + 1)}{2}$**

Examples: -

1. Find the median of the following nos. : -
   21, 12,37,49,88,55,46,63,74

Soln: - In this data the no. of item is n = 9(odd)

$$M = \frac{n+1}{2} = \frac{(9+1)}{2} \text{ th item}$$

$$= \frac{10}{2} = 5^{th}$$

Now the 5^{th} value in the data is 49

M = 49

2. Find the median of the following numbers: -
   72,88,33,29,70,54,86,91,57,61

Soln: - let us arrange the data in order

29,33,54,57,61,70,72,86,88,91

In this data the no. of item is n = 10 (Even)

M = Average of $(n/2)^{th} + (n/2+1)^{th}$ term

= Average of $(10/2)^{th} + (10/2+1)^{th}$ term

= Average of $5^{th} + 6^{th}$ terms

$$M = \frac{61+70}{2} = \frac{131}{2} = 65.2$$

Therefore **M = 65.2**

(b) Grouped data:

Discrete series: Arrange the data in either ascending or descending order of magnitude. Prepare table showing thecorresponding frequencies and cumulative frequencies. Now median is calculated by the following formula : -

$$M = \frac{(n+1)}{2}$$

Example: (3) calculate the median for the following data: -

No.of students	6	16	7	4	2	8
Marks	20	25	50	9	80	40

Soln: -

Let us arrange the data (marks) in ascending order and then form cumulative frequencies.

Marks	No. of students (f)	Cumulative frequency (cf)
9	4	4
20	6	10
25	16	26
40	8	34
50	7	41
80	2	43

Here $\sum f = n = 40$

Median (M) = $(n+1)/2 = (43+1)/2 = 22^{nd}$ Value

The table shows that all items from 11 to 26 have their value 25.

Since 22^{nd} item lies in this interval , therefore its value is 25.

Continuous series: -

(i)Data is given in the form of frequency table with class interval.

(ii) Cumulative frequencies are found out of each value.

(iii)Then median class is calculated

(iv) Now median is calculated by applying the following formula:

$$M = L + \frac{N/2 - C}{fm} \times i$$

L = lower limit of the class in which median lies

N = Total no. of frequencies

C = Cumulative frequency of the class preceding the median class

I = Width of the class interval in which the median lies.

fm = frequency of the class in which median lies.

Example (4): The weekly expenditure of 100 families are given below find the median of weekly expenditure.

Expenditure	0-10	10-20	20-30	30-40	40-50
Families	14	23	27	21	15

Soln: - Let us prepare the table showing the frequency and cumulative frequency.

Weekly expenditure	No. of families	cf
0-10	14	14
10-20	23	37
20-30	27	64
30-40	21	85
40-50	15	100

N/2 =100/2 = 50

It is more than cf 37 but less than 64 hence mediar class is 20-30

L =28 , i=10 c=37 fm= 27

M = 20 +(50-37)/27 x10 = 20+ 13/27 x10 = 24.81

Mode:

Mode is considered as the value in a series which occurs most frequently i.e. , has the maximum frequency.

(a)Ungrouped data:

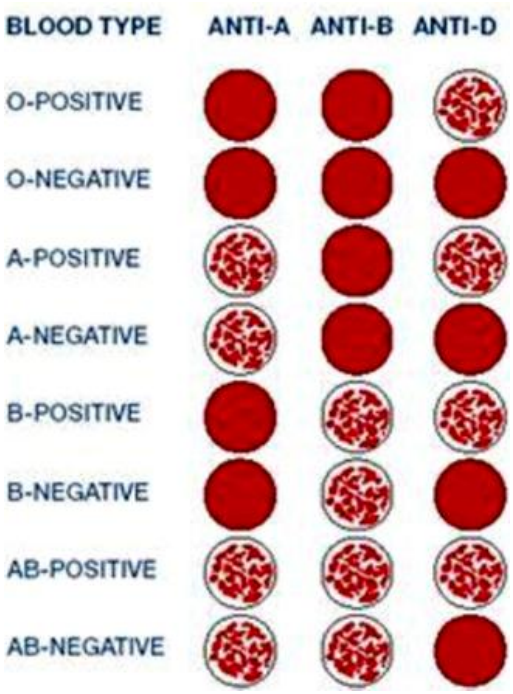
- In case of simple series mode can be determined by inspection only.
- It can be determined by locating that value which occurs maximum no. of times.

Example: (1) : - From the data given below find the mode.

1,3,3,1,5,3,3,5,4,5,2,4,7,6,3,6,2,7,1

Soln: Here the no 3 has occurred maximum no of times than the other so the **Mode is 3.**

Aim: Determination of ABO Blood group and Rh factor



Materials Required

- Toothpicks
- Blood sample
- Alcohol Swabs
- Lancet
- Clean glass slide
- Sterile cotton balls
- Biohazard disposal container
- Monoclonal Antibodies (Anti-A, B, and D)

Procedure

- Take a clean glass slide and draw three circles on it.
- Keep the slide aside safely without disturbing.
- Now wipe the ring finger with the alcohol swabs and rub gently near the fingertip, where the blood sample will be collected.
- Prick the ring fingertip with the lancet and wipe off the first drop of the blood.
- As blood starts oozing out, allow it to fall on the three circles of the glass slide by gently pressing the fingertip.
- Apply pressure on the site where it was pricked and to stop blood flow. Use the cotton ball if required.
- Unpack the Monoclonal Antibodies (MAB) kit. In the first circle add Anti-A, to the second circle add Anti-B and to the third circle add Anti-D with the help of a dropper.
- Mix the blood sample gently with the help of a toothpick and wait for a minute to observe the result.

Conclusion: Here is the chart which predicts the different types of blood groups along with its Rh factor.

To study Restriction enzyme digestion using teaching kits (Demonstration only).

Restriction enzyme is a protein (nuclease) that recognizes a specific, short nucleotide sequence of DNA (known as restriction site or target sequence or recognition sequence) and then cuts the DNA only at that specific site.

They cut the DNA molecule in two ways: (1) Many restriction endonucleases cleave both strands of DNA simply at the same point within the recognition sequence. As a result of this type of cleavage, the DNA fragments with blunt ends are generated. PvuII, Haelll, Alul are the examples of restriction endonucleases producing blunt ends. Blunt ends may also be referred to as protruding ends.

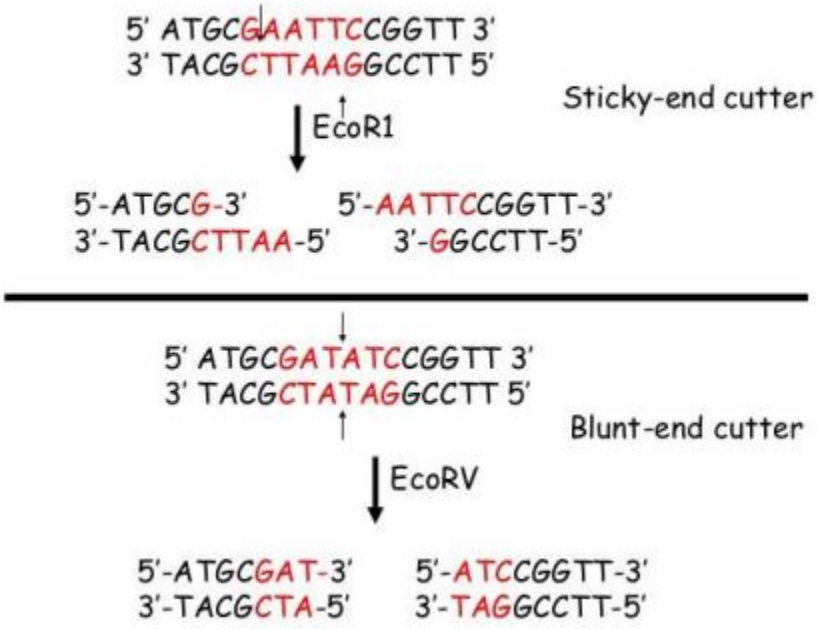
(2) In the other style of cleavage by the restriction endonucleases, the two strands of DNA are cut at two different points. Such cuts are termed as staggered cuts and this results into the generation of protruding ends i.e., one strand of the double helix extends a few bases beyond the other strand. Such ends are, called cohesive or sticky ends.

There are hundreds of commercially available restriction enzymes recognizing many different sequences (many of which are palindromes).

Sticky ends are helpful in cloning and genetic engineering because they hold two pieces of DNA together so they can be linked by DNA ligase enzyme.

Enzyme	Source	Recognition Sequence	Cut type (Sticky or Blunt)
EcoR1	*Escherichia coli* Sticky ends	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
BamH1	*Bacillus amyloliquefaciens* Sticky ends	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
Taq1	*Thermus aquaticus* Sticky ends	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'
Alu1	*Arthrobacter luteus* Blunt ends	5'AGCT 3'TCGA	5'---AG CT---3' 3'---TC GA---5'

Types of DNA cuts generated by restriction enzyme EcoR1



To detect genetic mutations by Polymerase Chain Reaction (PCR) using teaching kits (Demonstration only)

Polymerase chain reaction or PCR is a reaction that is utilised to amplify a gene or fragment of DNA of interest. It is done in vitro using a primer. This technique is used in labs to make billions of copies of the desired gene for research, diagnostic and therapeutic purposes.

PCR was invented by Kary Mullis in 1983. PCR requires a DNA primer designed for the DNA template and DNA polymerase, preferably a thermostable DNA polymerase.

PCR is an integral part of biotechnology, medical biology, diagnostics, forensic analysis, molecular biology research, etc. The amplified DNA can be sequenced, cloned and visualised by gel electrophoresis.

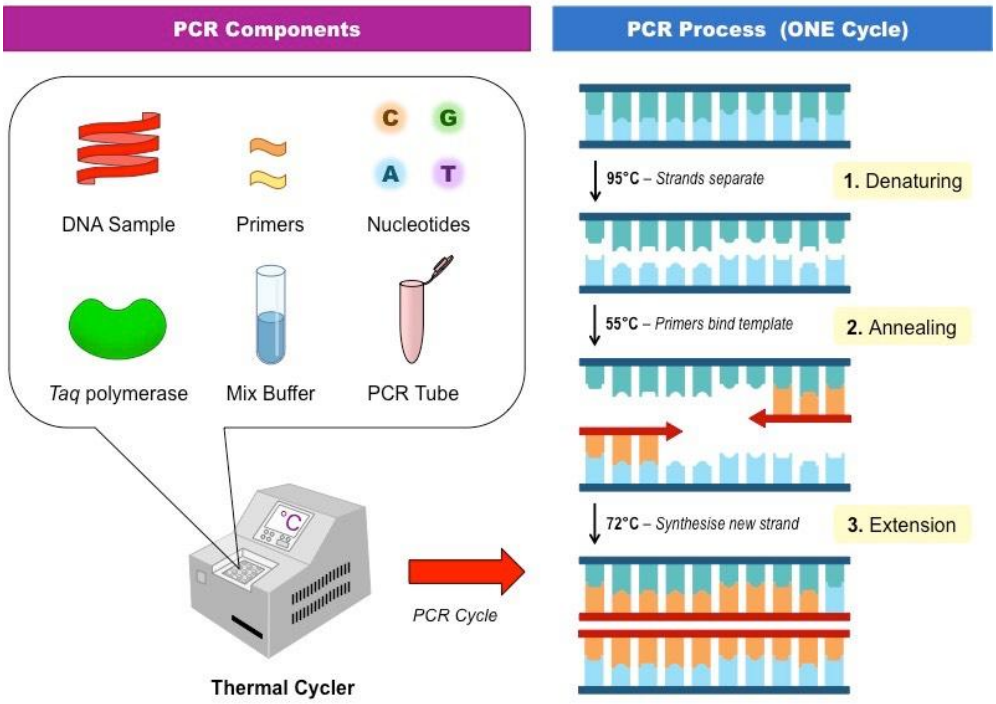
Polymerase Chain Reaction Steps

Each cycle of polymerase chain reaction has three steps. They are:

1. **Denaturation:** The first step in PCR is denaturation. Denaturation is required to separate the double-stranded DNA sample. It is done at 94-98 ℃ for 20-30 seconds. It breaks the hydrogen bonds present between base pairs. Denaturation leads to the formation of single strands of DNA.
2. **Annealing:** The second step is the annealing of the primer. Here the reaction temperature is lowered to allow the complementary base pairing between the primer and the complementary part of the single strands of the DNA template. A proper temperature needs to be maintained in order to allow highly specific and proper primer hybridisation. Then DNA polymerase binds to the template-primer hybrid and starts the DNA synthesis.
3. **Extension:** A thermostable DNA polymerase is used for this purpose. Taq polymerase is commonly used for this purpose. It is done at a temperature of 75-80 ℃ (72℃). The DNA polymerase adds nucleotides in the 5'-3' direction and synthesises the complementary strand of the DNA template.
4. This cycle is repeated 25-30 times and with this, the DNA sample can be amplified a billion times. Gel electrophoresis is used to visualise the result of polymerase chain reaction.

5. PCR reaction requires a primer, thermostable DNA polymerase, template DNA and nucleotides.

Primers for Polymerase Chain Reaction



Two sets of primers are used, which are short stretches of oligonucleotides. They are chemically synthesised and complementary to the part of the DNA template to be amplified. DNA polymerase requires a primer for initiating replication. DNA polymerase cannot initiate the process, it can only add nucleotides, therefore a primer is used for this purpose to initiate the polymerisation process.

Primers are usually 20 nucleotides long. The two primers used, cover the target region of DNA to be amplified and bind to the opposite edges of the template strand. After binding of primers, the region between them gets copied by the DNA polymerase.

Taq Polymerase

The replication or polymerisation of DNA requires a DNA polymerase enzyme. In PCR, Taq polymerase is used. It is thermostable and is isolated from a heat-tolerant bacterium, *Thermus aquaticus*. A thermostable polymerase is required so that it can remain active at higher temperatures. Higher temperatures are used in the PCR process for denaturation of the DNA double helix repeatedly for multiple cycles. Taq polymerase is most active at around 70 ℃.

Demonstration of Agarose gel electrophoresis for detection of Nucleic acids

What is Agarose Gel Electrophoresis?

- Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA , RNA or proteins in a matrix of agarose.
- Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool.

Principle of Agarose Gel Electrophoresis

Gel electrophoresis separates DNA fragments by size in a solid support medium such as an agarose gel. Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current which causes the negatively-charged DNA to migrate (electrophorese) towards the anodal, positive (+ve) end. The rate of migration is proportional to size: smaller fragments move more quickly and wind up at the bottom of the gel.

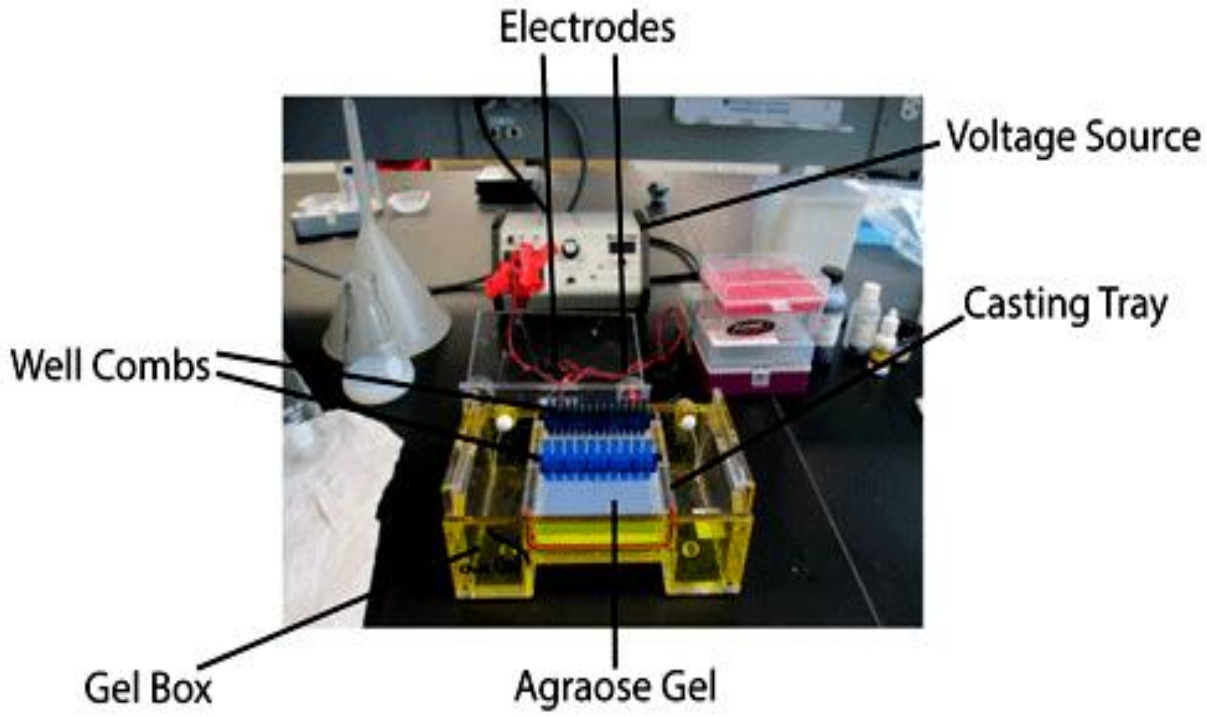
DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce.

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

1. An **electrophoresis chamber** and **power supply**
2. **Gel casting trays**, which are available in a variety of sizes and composed of UVtransparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
3. **Sample combs**, around which molten medium is poured to form sample wells in the gel.
4. **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
5. **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
6. **Staining**: DNA molecules are easily visualized under an ultraviolet lamp when electrphoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When

intercalated into doublestranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate.

7. **Transilluminator** (an ultraviolet light box), which is used to visualize stained DNA in gels.



Applications of Agarose Gel Electrophoresis

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA.

- Estimation of the size of DNA molecules
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
- The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.

Demonstration of Polyacrylamide Gel Electrophoresis (PAGE) for detection of protein

SDS PAGE or Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis is a technique used for the separation of proteins based on their molecular weight. It is a technique widely used in forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility.

Principle of SDS-PAGE

The principle of SDS-PAGE states that a charged molecule migrates to the electrode with the opposite sign when placed in an electric field. The separation of the charged molecules depends upon the relative mobility of charged species.

The smaller molecules migrate faster due to less resistance during electrophoresis. The structure and the charge of the proteins also influence the rate of migration. Sodium dodecyl sulphate and polyacrylamide eliminate the influence of structure and charge of the proteins, and the proteins are separated based on the length of the polypeptide chain.

Role of SDS in SDS-PAGE

SDS is a detergent present in the SDS-PAGE sample buffer. SDS along with some reducing agents function to break the disulphide bonds of proteins disrupting the tertiary structure of proteins.

Protocol of SDS-PAGE

Preparation of the Gel

- All the reagents are combined, except TEMED, for the preparation of gel.
- When the gel is ready to be poured, add TEMED.
- The separating gel is poured in the casting chamber.
- Add butanol before polymerization to remove the unwanted air bubbles present.

- The comb is inserted in the spaces between the glass plate.
- The polymerized gel is known as the "gel cassette".

Sample Preparation

- Boil some water in a beaker.
- Add 2-mercaptoethanol to the sample buffer.
- Place the buffer solution in microcentrifuge tubes and add protein sample to it.
- Take MW markers in separate tubes.
- Boil the samples for less than 5 minutes to completely denature the proteins.

Electrophoresis

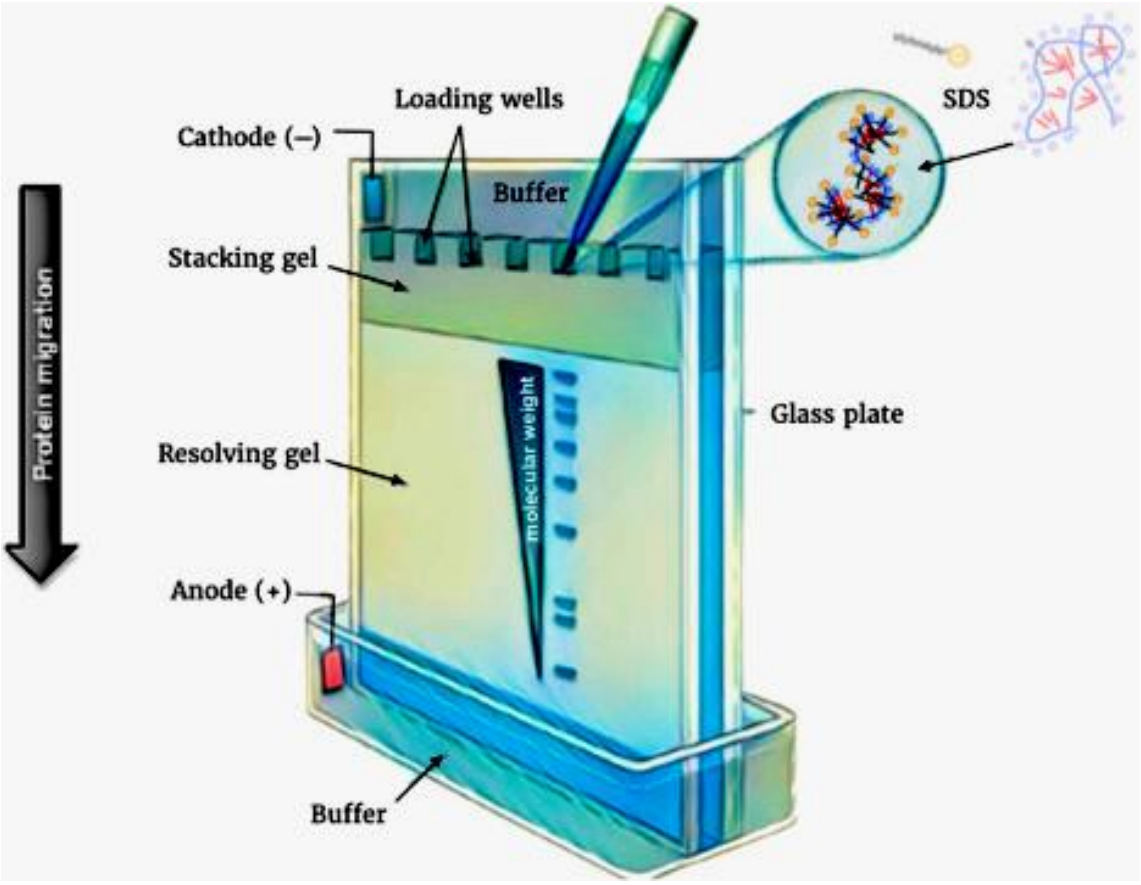
- The gel cassette is removed from the casting stand and placed in the electrode assembly.
- The electrode assembly is fixed in the clamp stand.
- 1x electrophoresis buffer is poured in the opening of the casting frame to fill the wells of the gel.
- Pipette 30ml of the denatured sample in the well.
- The tank is then covered with a lid and the unit is connected to a power supply.
- The sample is allowed to run at 30mA for about 1 hour.
- The bands are then seen under UV light.

Applications of SDS-PAGE

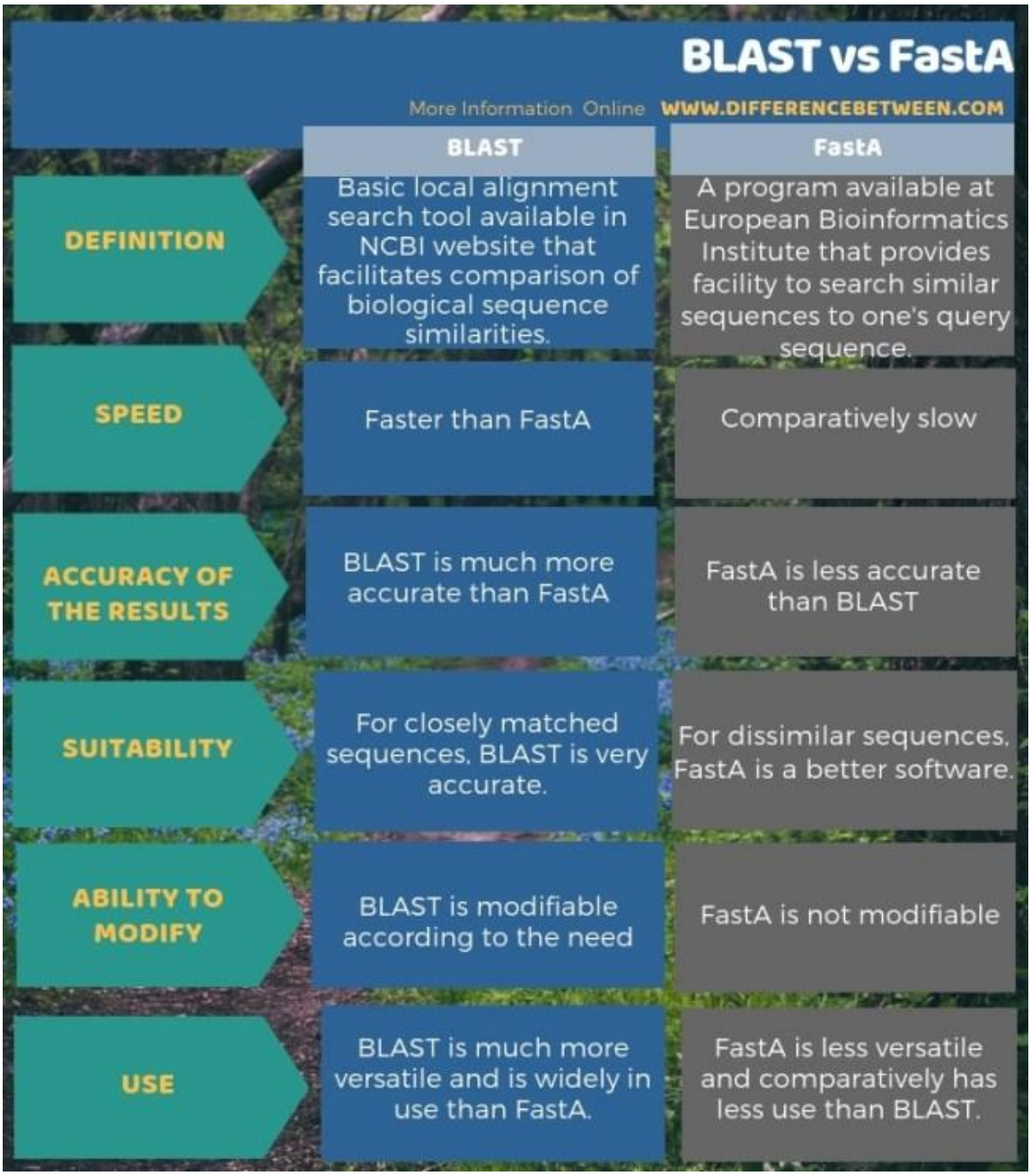
The applications of SDS-PAGE are as follows:

1. It is used to measure the molecular weight of the molecules.
2. It is used to estimate the size of the protein.

3. Used in peptide mapping
4. It is used to compare the polypeptide composition of different structures.
5. It is used to estimate the purity of the proteins.
6. It is used in Western Blotting and protein ubiquitination.
7. It is used in HIV test to separate the HIV proteins.
8. Analyzing the size and number of polypeptide subunits.
9. To analyze post-translational modifications.



BLAST & FASTA



BLAST (Basic Local Alignment Search Tool)

- The BLAST program was developed by Stephen Altschul of NCBI in 1990 and has since become one of the most popular programs for sequence analysis.
- BLAST uses heuristics to align a query sequence with all sequences in a database.

- The objective is to find high-scoring ungapped segments among related sequences. The existence of such segments above a given threshold indicates pairwise similarity beyond random chance, which helps to discriminate related sequences from unrelated sequences in a database.
- BLAST is popular as a bioinformatics tool due to its ability to identify regions of local similarity between two sequences quickly. BLAST calculates an expectation value, which estimates the number of matches between two sequences. It uses the local alignment of sequences.

Variants of BLAST

- **BLAST-N:** compares nucleotide sequence with nucleotide sequences
- **BLAST-P:** compares protein sequences with protein sequences
- **BLAST-X:** Compares nucleotide sequences against the protein sequences
- **tBLAST-N**: compares the protein sequences against the six frame translations of nucleotide sequences
- **tBLAST-X:** Compares the six frame translations of nucleotide sequence against the six frame translations of protein sequences

FASTA

- FASTA stands for fast-all" or "FastA".
- It was the first database similarity search tool developed, preceding the development of BLAST.
- FASTA is another sequence alignment tool which is used to search similarities between sequences of DNA and proteins.
- FASTA uses a "hashing" strategy to find matches for a short stretch of identical residues with a length of k. The string of residues is known as ktuples or ktups, which are equivalent to words in BLAST, but are normally shorter than the words.
- Typically, a ktup is composed of two residues for protein sequences and six residues for DNA sequences.
- The query sequence is thus broken down into sequence patterns or words known as k-tuples and the target sequences are searched for these k-tuples in order to find the similarities between the two.
- FASTA is a fine tool for similarity searches.

White Blood Cell types

Lymphocytes

Lymphocytes play a significant role in the development of antibodies and body protection, the size of which varies from 8 to 10 micrometres and is generally referred to as the natural killer cells. A human body comprises on average 10 to 12 cells of lymphocytes. WBC are colourless cells that develop in lymphoid tissue, hence called lymphocytes. These cells are quite significant in the immune system. The two main lymphocyte types are

- B lymphocytes
- T lymphocytes

Monocytes

Monocyte cells typically have a large bilobed nucleus with a diameter of 12 to 20 micrometres and the nucleus is usually half-moon or kidney-shaped and occupies 3 to 8% of WBCs. Monocytes are Immune System garbage vehicles. The primary functions of monocytes are,

- Migrating into tissues and removing dead cells
- Shielding towards bloodborne pathogens
- They travel very rapidly to tissue infection sites.

Neutrophils

Neutrophils generally found in the bloodstream, they are predominant cells, which are present in pus. About 60 to 70 per cent of WBCs are neutrophils of 10 to 12 micrometres in diameter. The nucleus is lobed with 2 to 5, and cytoplasm has tiny granules. Neutrophil assists with lysosomes in the degradation of bacteria and it serves as a potent oxidant. Neutrophils are only stained with neutral dyes. Neutrophils are often the first immune system cells to react to an invader, like a bacteria or a virus. The WBCs' lifetime lasts up to 8 hours, and are formed in the bone marrow every day.

Eosinophils

Eosinophils are the leukocyte cells that are present in the immune system responsible for combating infections in vertebrate parasites and regulating processes associated with allergy & asthma. Eosinophilic cells are small agranulocytes that are formed in the bone marrow that make 2 to 4% of all WBCs and are present in the digestive tract at high concentrations.

Basophils

The least common of the granulocytes are basophils, ranging from 0.01 to 0.3 percent of WBC. They contain large cytoplasmic granules which play a vital role in mounting a non-specific immune response to pathogens, allergic reactions by releasing histamine and dilating the blood vessels. There are about 20 to 25 percent of basophils in WBCs. Such WBC has the capacity to stain when they are exposed to simple dyes, thus called basophilia, and they are best known for their function in asthma, and results in airway inflammation and bronchoconstriction.

B.L.D.E. Association's

S.B. ARTS AND K.C.P. SCIENCE COLLEGE

VIJAYAPUR- 586103

Karnataka State

B. SC 6th SEMESTER (NEP)

LAB MANUAL

DEPARTMENT OF ZOOLOGY

2023-24

Scheme of Practical Examination

B.Sc. VI Semester (NEP)

Paper Title: Evolution and Developmental Biology

Duration : 3Hrs Marks: 25

I. Identify and comment on the given model **A** 03

(Identification-1mark, Comments-2 marks)

II. Identify and comment on the spotter **B** 03

(Identification-I mark, Comments-2 marks)

III. Problem/Graphical representation of data 04

IV. Identify and comment on the given chart/speciemen with a labelled Diagram **C** 03

(Identification-1 mark, Comments-1 mark,diagram-1 mark)

V. Identify and comment on the given chart/speciemen with a labelled Diagram **D** 03

(Identification-I mark, Comments-1 mark,diagram-I mark)

VI. Identify and comment on the spotter **E**

(Identification-1 mark, Comments-1 mark,diagram-2 mark) 04

VII. Record and Viva -voce 05

EXPERIMENT – 1

VESTIGIAL ORGANS: VERMIFORM APPENDIX, WISDOM TEETH, COCCYX (TAIL BONE), NICTITATING MEMBRANES OF EYE.

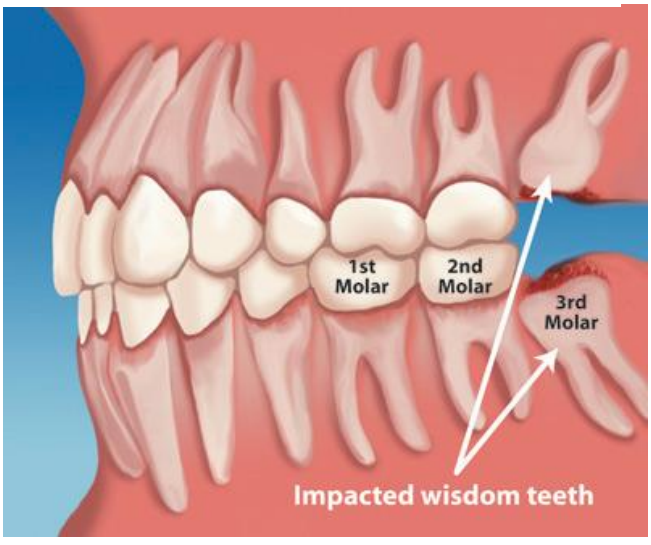
Vestigial organs are organs, tissues or cells in a body which are no more functional the way they were in their ancestral form of the trait. It is authentication of evolution and hence, were helpful in explaining adaptation. Such a structure can arise due to gene mutation which causes a change in the proteins. These mutated proteins result in the formation of vestigial structures. In the population, the occurrence of such structures may, however, increase if it is beneficial enough. For instance, snakes have evolved to slither as they no longer have legs excluding some snakes who still possess rear legs (the Boas). In humans, the appendix is a good example of a vestigial organ. This non-functioning organ eventually degenerates, shrinking in size and disappearing ultimately.

Examining vestigiality should be governed by drawing similarities with their counterparts with respect to their homologous features. The exposure of this occurs through various processes of evolution, one of which is the loss of function of a feature that is not subjected to positive selection pressures in accordance with its surroundings.

Vestigial organs vary from being pointless to favourable based on the selection. Some structures, due to less or no utility, degenerate over a period of time to avoid consequences of genetic drift or selective pressures.

1. Wisdom Teeth

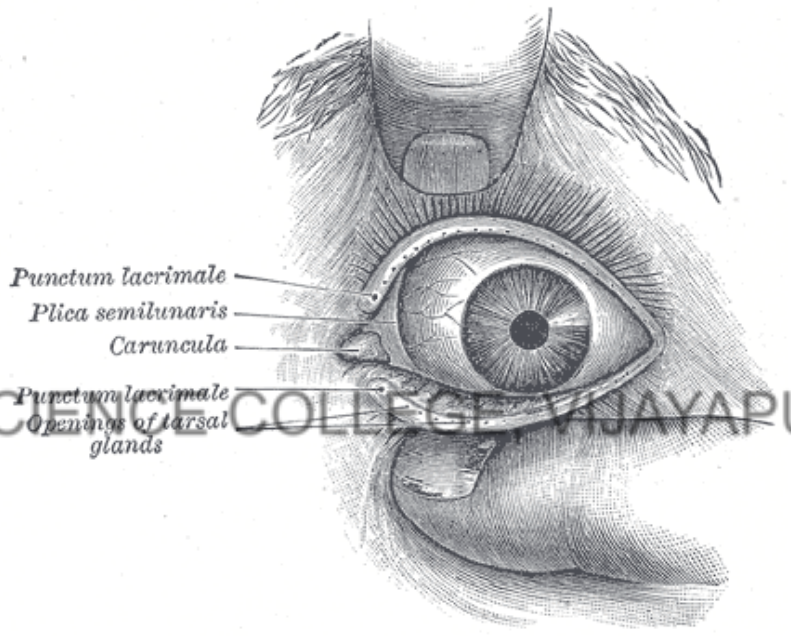
As the human species migrated out of Africa, it came to populate a variety of habitats, and eventually, human civilizations developed. Coincident with those events was a shift in the human diet toward the



consumption of soft and processed foods, which gradually eliminated the need for large, powerful jaws. With a reduction in human jaw size, molars—particularly the third molars, or wisdom teeth—became highly prone to impaction. Increasingly, wisdom teeth are congenitally absent. As a consequence, they are now considered a vestigial feature of the human body.

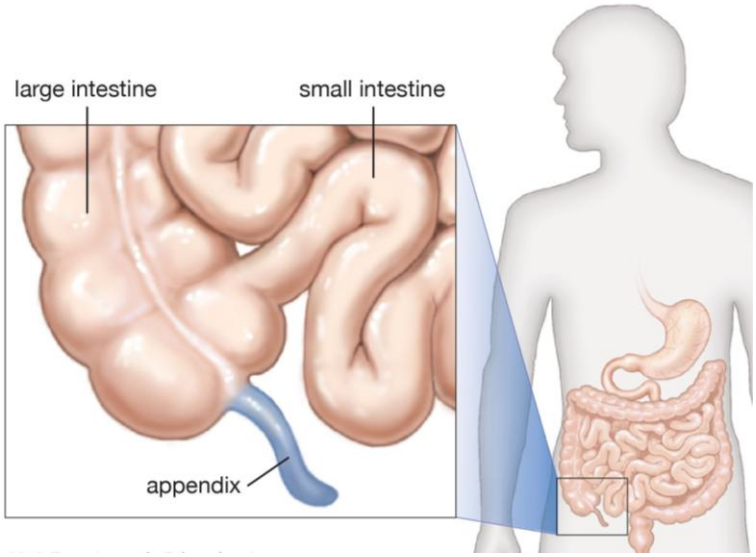
2. Nictitating Membrane

The plica semilunaris is a fold of conjunctiva at the inner corner of the human eye. Its likeness to the nictitating membrane, or third eyelid, of other animals led to the idea that it might be the vestige of such a structure, which is still part of the eye in some primates, including gorillas. In the chimpanzee, however—one of the human species' closest relatives—the plica semilunaris also appears to be vestigial. The function of the nictitating membrane in many animals is protective—for example, keeping the eye clean and moist or concealing the iris from predators. In some species, the membrane is sufficiently transparent so as to enable vision when underground or underwater. Though the reason for the loss of a nictitating membrane in humans in unclear, changes in habitat and eye physiology may have rendered the tissue unnecessary.



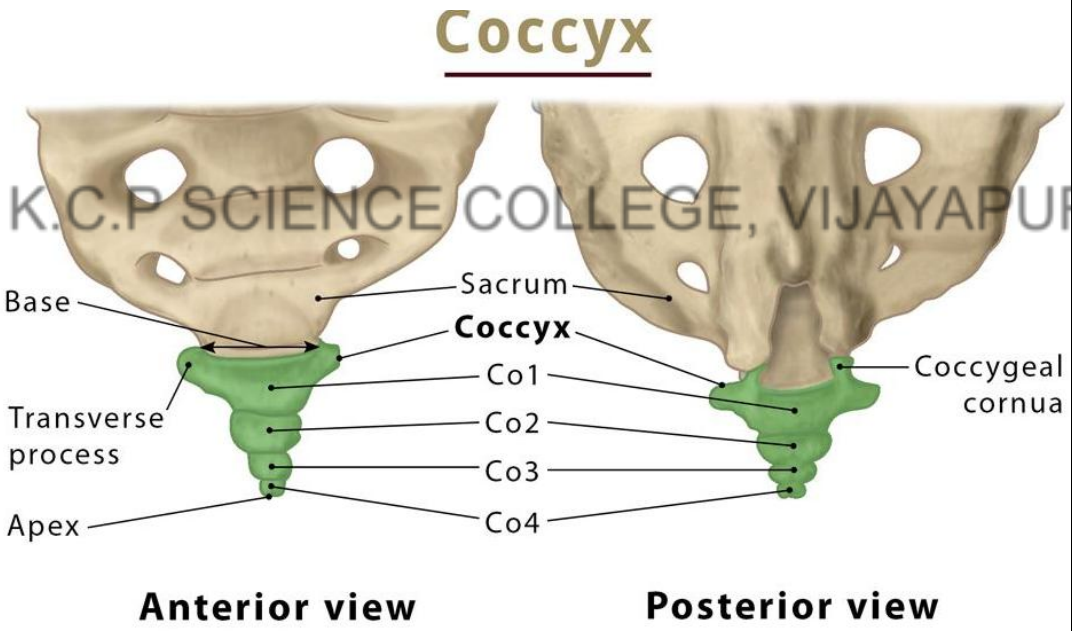
3. Appendix

It is one of the most commonly known vestigial organs. This finger-like tube closed at one end arises from the vermiform process. In prime ancestors, the appendix is believed to have brought about the digestion of cellulose. Today, scientists predict that the appendix may play a role in digestion by bacteria.



4. Coccyx

It forms the last part of the vertebral column, the residue of the lost tail and is often termed as the tailbone. It is observed during human embryogenesis. This formed as the centrepiece of the 'theory of recapitulation'.



EXPERIMENT – 2

STUDY OF HOMOLOGY AND ANALOGY FROM SUITABLE EXAMPLES.

Objective

To study the homology and analogy in animals and plants using charts, models and specimens.

Materials Required

- Specimens required for homology in animals are: Charts or model or preserved specimen of forelimb of bird, bat, man, seal, etc.
- Specimens/charts required for analogy in animal are:
  - Preserved forelimb of bat/bird.
  - Preserved wings of insects.

Theory/Principle

The organs or features which have similar basic structure but performs different functions in different species are called as homologous organs and their study is called homology. These similarities are a result of divergence from a common ancestor, i.e. their origin from same species. Homologous organs are thus structurally similar but due to different habitats and their specific adaptations, they became functionally different.

The organs or features which have common or similar fundamental structures but are involved in different functions in different species are referred to as analogous organs and their study is called as analogy. These similarities arise due to the adaptive convergence of the different organs to be used in similar environment, e.g. wings of bat and wings of an insect are analogous organs.

Homology (similarities in traits of two different organisms) and analogy (similarities in functions of two different organisms) are common in occurrence in animal species. During the course of evolution, the organism adapts/recieves these organs or features from their ancestors.

Procedure

- The specimens of the limbs of both animals and plants are studied closely.
- Note the characteristic shape, structure, similarities, if any.
- Select the charts/figures of different animals and plants and study the details about homologous and analogous organs or features.
- Make a thorough observation of external and internal features and find out the similarity and dissimilarities (in structure and function) between them, then record your observations carefully in your notebook.

Observation

(i) Homology/Homologous Organs

- The structures depict the forelimbs of a man, cheetah, whale and bat (see Fig. 1) in the case of animals.

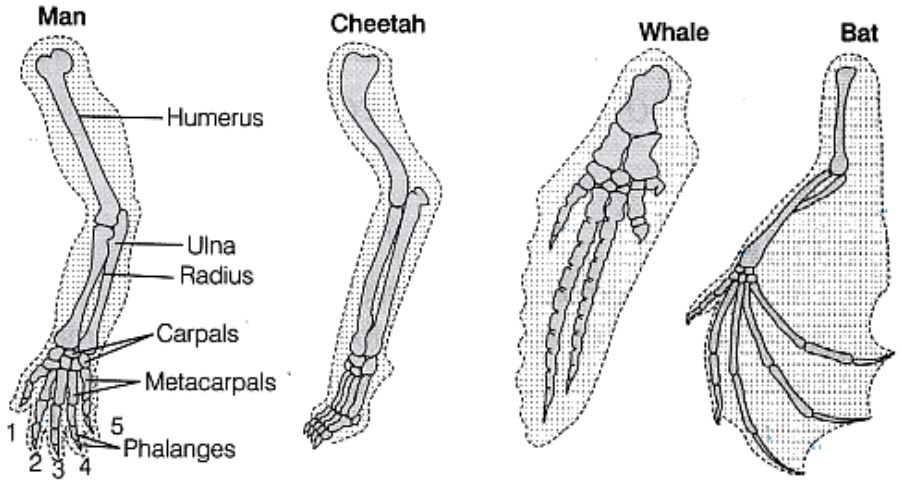


Fig. 1 Homology in animals

- The hand of a man, limb of cheetah, flipper of a whale and the wings of a bat have common set of bones but all of these structures though appears similar but are involved in different functions like grasping, running, swimming and flying respectively.

- These structures of animals under study show homology, i.e. similarity in the fundamental (basic) structures due to shared or common embryonic origin but all of these organs/features performs different functions.

(ii) Analogy/Analogous Organs

- The structures depict the wings of a bat and wings of an insect in case of animals.

- The forelimbs of bats, birds and wings of insects are used for flying by these animals however, they are structurally very different from each other.

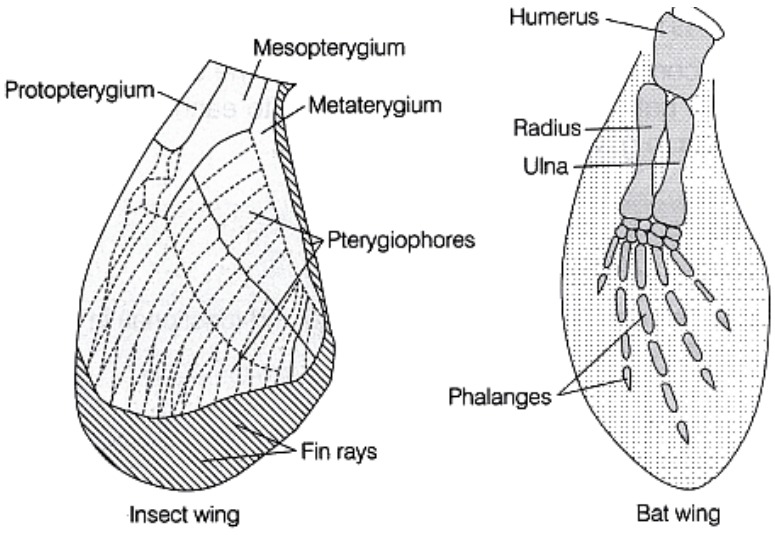


Fig. 3 Analogy in animals

- The above structures of animals under study shows analogy, i.e. they perform similar functions in different organisms but do not share same structure on the basis of origin.

Result

The observations made on the above given structures reveals that during course

of evolution, some organs of same origin in different organisms have taken up different functions, such organs are called as homologous organs, e.g. forelimbs of vertebrates. Some organs, in contrary, have evolved to perform the same function but their origin is not same and they do not resemble or are not similar to each other, such organs are called as analogous organs, e.g. wings of insects and birds.

EXPERIMENT – 3

TYPES OF EGGS BASED ON QUANTITY AND DISTRIBUTION

Introduction:

1. The main form of food reserve present in an egg is the yolk. The yolk appears in the oocytes in the second phase of egg maturation ie vitellogenesis period.
2. As the main components of proteins, Phospholipids and lesser extent neutral fats are present in the yolk of the eggs.
3. According to the predominance of these substances/components, can distinguish Yolk as protein yolk or fatty yolk.
4. As the protein yolk is the main reserve in many invertebrates such as Echinodermates and lower chordates such as protochordates (Amphioxus, Tunicates), in these animals is having a small amount of the yolk.
5. But in Amphibians the protein yolk is formed of large granules which termed as yolk platelets, it contains two main substances such as Phosphovitin and Lipovitin.

6. The amount of yolk present in the egg is related to the nature of embryonic feeding. If the embryo starts feeding at once or takes food from the mother's body, the egg will have less yolk.
   Examples:- Marine Invertebrates and Placental Mammals.
7. In higher invertebrates, unless the embryonic alimentary canal develops, the yolk nourishes /nitrifies the embryo.
8. The amount of yolk is an important determining factor for the further pattern in embryological stages.
9. Eggs are of various types according to the amount and distribution of yolk and by the pattern of their cleavage.

Classification of types of eggs

Types of eggs are divided based on the amount of yolk, distribution of the yolk, on presence and absence of shell, and pattern of development.

1. Amount of yolk:- Eggs are grouped into two types.

A. Alecithal Eggs: - The egg contains little or no yolk, is called Lecithal egg.

Example:-Eutherian Mammals and Human egg.

B. Lecithal Eggs: - The egg with distinct amount of yolk.

Lecithal eggs are categorized as follows.

I. **Microlecithal :-** The eggs with small or little amount of yolk , are also referred to as Oligolecithal.

Examples : Echinoderms, Urochordates, Amphioxus.

II. **Mesolecithal:-** The Eggs with moderate amount of yolk.

Examples:-Molluscs(Aplysia), Amphibians(Frog), Dipnoi and Petromyzon.

III. **Macro/ Megalecithal:-**The eggs with large amount of yolk, is occupied almost the entire part of the eggs and the cytoplasm remains at the top. Examples:- Bony fishes, Reptiles, Birds, Prototherians.

2. Distribution of the yolk:- Eggs are classified into three types

A. **Homolecithal /Isolecithal eggs :-** The yolk uniformly distributed in the cytoplasm (Vegetal pole, Animal pole, Equatorial region) . In such eggs cleavage is deeper. So all microlecithal eggs exhibit Isolecithal.

Examples : Echinoderms, Urochordates, Amphioxus

B. **Heterolecithal eggs:-** Yolk is unevenly distributed in the cytoplasm of the eggs. They are divided on the pattern of yolk distribution such as

a. Telolecithal eggs:- The eggs yolk concentrated highly towards the vegetal pole, the concentration of yolk is smallest at the animal pole.(practically without yolk / with smallest amount of yolk),The amount of yolk is so massive that it occupies almost all the vegetal pole and the active cytoplasm and germinal vesicle (nucleus)remain confined to a small cap at the animal pole. So, Mesolecithal and Macrolecithal eggs exhibit Telolecithal.

Examples:- Fishes, Amphibians, Reptiles and Birds.

b. **Centrolecithal eggs:-** The eggs has it yolk in the centre with the cytoplasm surrounding it.

Examples:-Insects eggs.

3. **Presence or Absence of the shell: -** It is classified into two types, they are

A. **Cleidoic eggs:-** Cleidoic means Sealed Box (in Latin).

- The eggs are laid on dry land must be protected wall. Hence, they are leathery coats or hard shells covered by the calcareous shells.
- To certain accessory cells surrounding the ovum in the ovary secrete a membrane envelope and hard shell (in oviduct).
- To protect them from desiccation, i.e., to the danger of evaporation of water before the development of embryo.
- The shell membrane becomes water tight but has do the gaseous changes. The eggs become a closed system and it is known as Box like egg. Examples:- Reptiles and Birds.

B. **Non- cleidoic eggs:-** In aquatic animals, are fishes and Amphibians lay their eggs in water. There are not having hard covering shell except jelly coat. This type of eggs is also laid by animals in whose the development is internal.

Example: - Mammals.

EXPERIMENT – 4

STUDY OF STAGES OF DEVELOPMENT OF FROG: CLEAVAGE STAGES, BLASTULA, GASTRULA, NEURULA STAGES (WHOLE MOUNT) AND VARIOUS STAGES OF TADPOLE.

Objective: To identify and describe the different developmental stages in frog development from a fertilized egg to gastrula from permanent slides by observing under the microscope.

Introduction: Frogs are amphibians which lay their eggs in water. A single female frog can lay up to 4000 eggs in cluster known as "egg masses". For example: Rana pipiens lay around 2500 eggs, while 20,000 in case of the bullfrog, Rana catesbiana. In most species of amphibians, fertilization is external. In this case, the male frog grabs the female's back and fertilizes the eggs as they are released from a female. The eggs are covered by jelly layers which protect and adheres the eggs to nearby plants or substratum and anchor the eggs. Other species may release their eggs without any support and make them float in the aquatic environment.

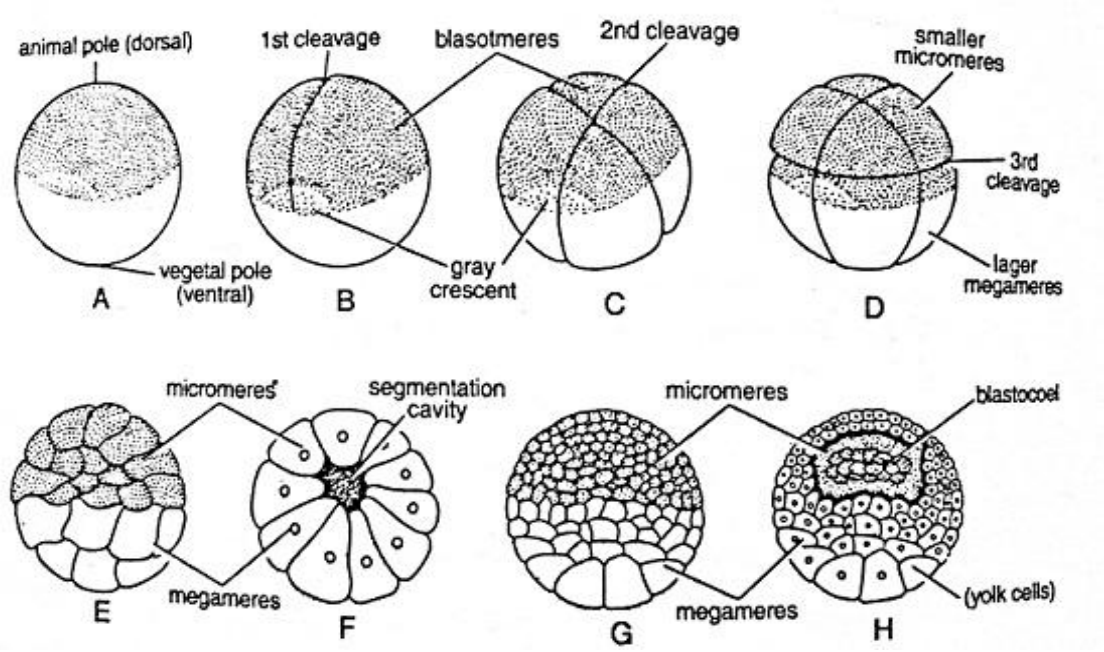
Frogs eggs are mesolecithal, i.e., contains moderate amount of yolk which are concentrated at one end of the egg. Hence, it is telolecithal. All the development stages of frogs occur in the aquatic environment.

Materials Required: Prepared permanent microscopic slides of Frog's Embryo, Compound microscope.

1. Process of Cleavage or segmentation:

- In frog, the division is complete but the cells formed are unequal. So, the cleavage is called **holoblastic** but unequal.
- The first division is vertical and divides the zygote into two blastomeres. The furrow extends from animal pole to the vegetal pole (lower end).
- Second division is also vertical but at right angle to the first resulting in 4 blastomeres.
- The third division is horizontal passing above the equator forming 8 unequal blastomeres. Out of these, 4 upper smaller ones are called **micromeres** and 4 lower larger ones are called **megameres**.

- Two more vertical divisions (fourth cleavage set) take place forming 16 cells (8 megameres and 8 micromeres).



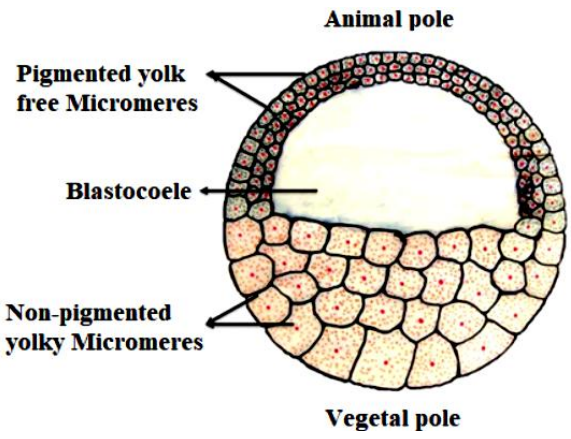
- Two horizontal divisions following this, results in the formation of a 32-celled stage.

2. Morulation (formation of morula):

- After 32-cell stage, cleavages become less regular and difficult to follow.
- Micromeres divide more rapidly than the megameres. This is due to less quantity or lack of yolk in micromeres and megameres having more yolk present on the vegetal end.
- Due to this irregular division of cells, the dividing zygote appears like a mulberry-shaped solid ball of cells called **morula**.

3. Blastulation (formation of blastula):

- As the division continues, the blastomeres arrange at the periphery and a small central fluid filled cavity or space appears within an embryo, called **blastocoel** or **segmentation cavity.**
- Thus, the embryo appears as a hollow ball and is now called a **blastula (coeloblastula).** This process of formation of blastula is called **blastulation**.
- Although, blastula appears to be composed of only micro and megameres, the cells forming future parts of the body can be



identified by special staining methods. The areas are:

1. Animal pole of the blastula represents the **presumptive ectoderm**. This can further be divided into **presumptive epidermis** and **neural plate** (nervous system).
2. A small area near the vegetal pole is the **presumptive notochord**.
3. Close to notochord lies the **presumptive mesoderm**.
4. The remainder of the vegetal pole formed by large yolk laden megameres forms the future **endoderm**.

4. Gastrulation (formation of gastrula):

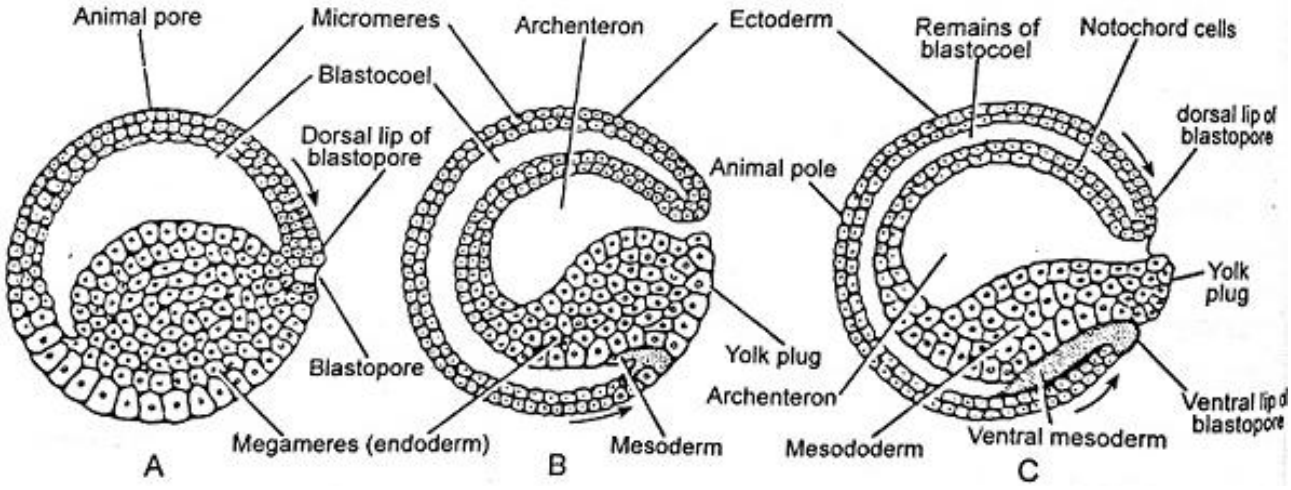
This is formed after blastula stage. The conversion of blastula to gastrula is called **gastrulation** which is completed by four processes:

a. Epiboly (overgrowth of micromeres):

- The micromeres of the animal pole divide repeatedly and spread over the lower megameres.
- Thus, the presumptive notochord, mesoderm and endoderm get enclosed leaving a small area called **yolk plug**.

b. Imboly:

- Behind the presumptive notochord, invagination appears which the beginning of archenterons is.
- The open end of this invagination is called blastopore. Its anterior end is the dorsal lip of the blastopore.
- As archenteron extends inwards, it becomes an extensive cavity called **archenteron cavity** which lies above megameres.



Different stages of Gastrulation

c. Involution or Migration of micromeres:

- Micromeres begin to migrate inwards from dorsal lip and with it the archenteron enlarges. Micromeres form a thick layer or the dorsal surface of archenteron which form the future notochord and mesoderm.
- When the development of archenteron begins, blastopore decreases in size and gradually disappears.
- The migration of micromeres also takes place on the side and on the ventral surface of dorsal lip, forming lateral lips and ventral lip respectively. These lips unite and reduce the size of blastopore. Through this blastopore come out yolk filled megameres called yolk plug.

d. Rotation of gastrula:

- Now the gastrula rotates inside the vitelline membrane. The blastopore comes near the original vegetal pole of the embryo. During this, the yolk plug moves inwards and in the ventral surface of the archenteron.
- The complete disappearance of blastocoel marks the end of gastrulation.
- After all these changes, three layers can be seen in the gastrula;
  - **The outer surface,** which forms the ectoderm (future neural plate and epidermis)
  - The cells on the roof of the archenteron called **chordamesoderm.**
  - Floor and sides of the archenteron forming endoderm.
- Still now, chordamesoderm and endoderm are not clearly distinguishable. At the end of gastrulation, a process called **neurulation** takes place during which notochord is formed from chordamesoderm.

5. Organogenesis:

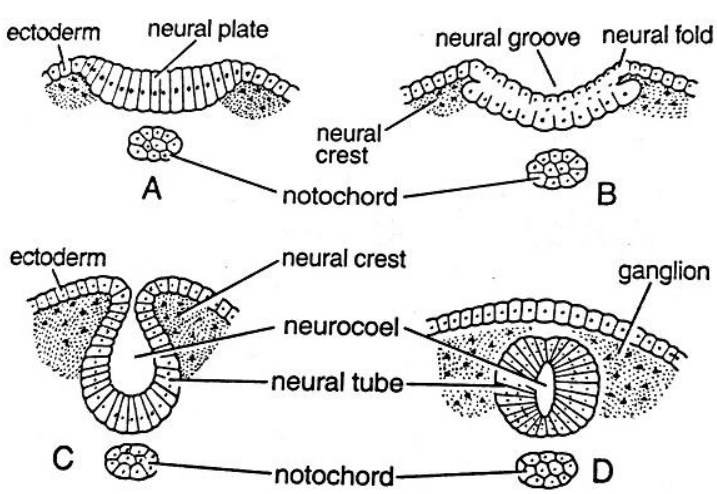
- Gastrulation is completed in about thirty hours after fertilization.
- The three layers of cells; ectoderm, mesoderm and endoderm formed after gastrulation are called three germinal layers (in **triploblastic metazoans**).
- Further development of the embryo is concerned with the formation and differentiation of various embryonic tissues from these three primary germinal layers.
- This phase of development of called organogenesis, which ultimately leads to the formation of an active free swimming larval stage called **tadpole larva**.

Organogenesis involves the following various changes:

a. Neurulation (formation of neural tube):

- On the mid-dorsal region, ectoderm cells thicken to form neural plate. On either side of this are the neural folds.

- Both neural folds increase in size and fuse at the mid-dorsal region forming the neural canal or tube which opens at the anterior end by a small opening called
- Neural tube posteriorly remains connected for some time with archenteron by neurenteric canal.
- At this stage, the embryo is called **neurula**.
- At the end, the neural tube is covered into a closed tubular canal, the anterior part of which is the future **brain** and the posterior portion forms the **spinal cord**.



b. Formation of notochord and mesoderm:

- At the mid-dorsal region, the chordamesoderm forms a cylindrical rod like structure which forms the notochord. The rest of chordamesoderm gives rise to mesoderm.
- On the either side of the notochord lies mesoderm which can be divided into three parts:

1. Dorsally situated **epimere**, which further has three more parts;
   - **Myotomes** forming body musculature
   - **Dermatomes** forming dermis of skin
   - **Sclerotomes** forming axial skeleton
2. Middle mesoderm called **mesomere or nephrotome**, which gives rise to excretory and genital organs
3. Ventral mesoderm called **hypomere or lateral plates**, which on either side divide to form layers with a narrow space in between the layers called coelom. The outer layer of mesoderm forms somatic and inner layer forms visceral layer of coelom.

c. Formation of endoderm:

- The cells forming the floor of archenteron divide, extend dorsally and completely enclose archenteron. This layer is below the mesoderm and forms the endoderm.
- Now the embryo which has elongated to some extent can be said to have three primary germinal layers namely ectoderm on the outer side, endoderm on the inner side and mesoderm in between them.

6. Fate of three germinal layers:

- **Ectoderm** gives rise to:
  - Epidermis and cutaneous glands
  - Lining of the cloaca and mouth cavity
  - Central nervous system, brain and spinal cord
  - Lens, cornea and retina of the eye
  - Olfactory and auditory organs

- **Endoderm** gives rise to:
  - The epithelium lining the digestive canal except mouth and cloaca, and digestive glands like liver and pancreas
  - Larynx, trachea and lungs of the respiratory system
  - Lining of the urinary bladder
  - Thymus and thyroid glands

- **Mesoderm** gives rise to:
  - Dermis of the skin
  - Cartilage and bones of the skeletal system
  - Blood vascular system including blood and blood vessels
  - Excretory and genital organs
  - Spleen, sclerotic and choroid of the eye

7. Morphogenesis:

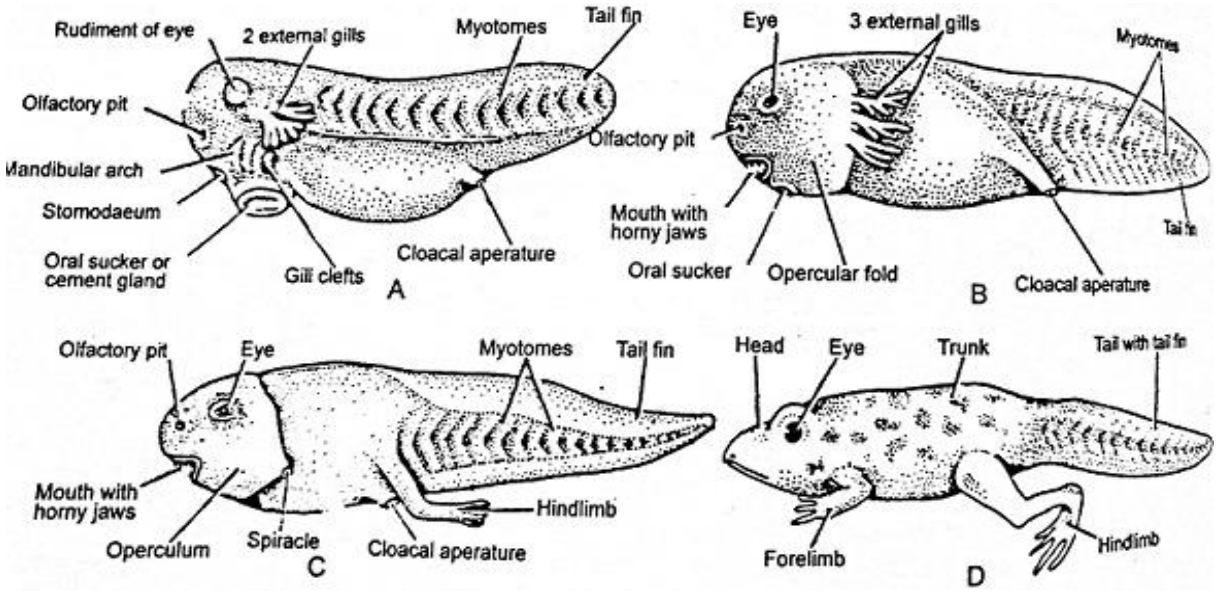
- Further development of elongated embryo occurs leading to the pre-tadpole stage followed by the larval stage.

a. Pre-tadpole stage:

- After about 4 days of fertilization, the embryo becomes about 4mm long and lies within the egg membrane.
- The body can be divided into head, trunk and tail.
- On the head, on either side lie the round elevations showing the position of future **tympanum**.
- On the ventral side of the anterior end is a **U-shaped sucker (cement gland**) formed by mucus gland cells.
- Between sucker and nasal pit is a small depression forming At the posterior end is another depression called **proctodaeum**.
- Posterior to proctodaeum, the body elongates to form the tail. Internally, the embryo contains parts of central nervous system, notochord, closed alimentary canal, liver, heart and rudiment of urinary bladder.
- After development of such organs, it is time for embryo to hatch out.

b. Hatching:

- About 2 weeks after fertilization, the embryo becomes about 6mm in length.
- It breaks out the egg membrane and comes out.
- This is called hatching and the free larval stages of the frog after hatching are called **tadpoles**.



Frog tadpoles

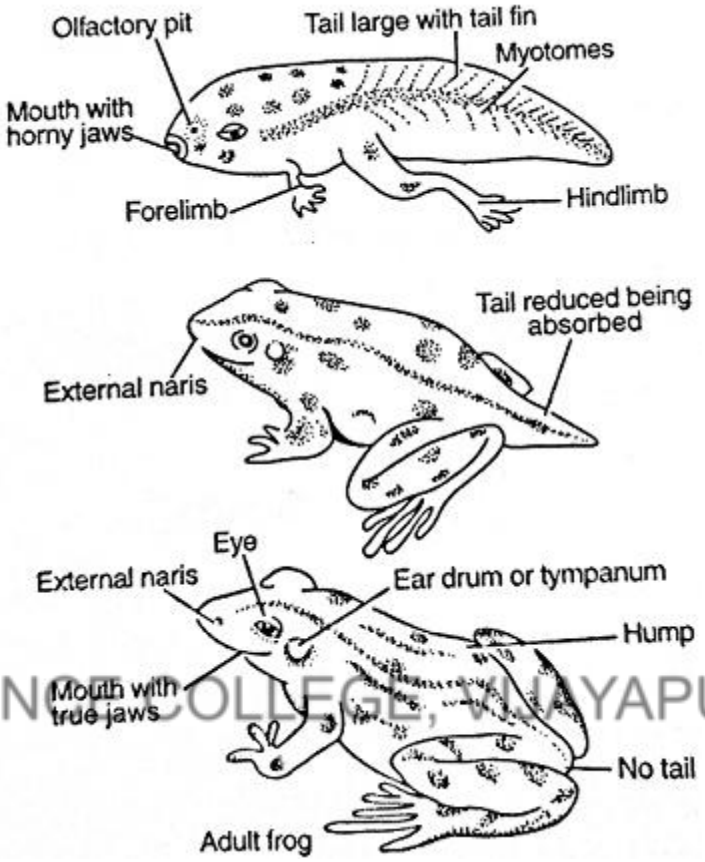
c. Tadpole larva:

- The newly hatched tadpole is a small, blackish, fish-like creature.
- In the beginning, the tadpole gets attached to some aquatic plant by means of its ventral sucker or cement gland.
- First two pairs of external gills develop on either side of the head. Soon they develop into three pairs of branched gills, and help in respiration along with skin.
- The tadpole in its first week of hatching gets nourishment from the yolk still present in the cells of archenteron.
- The mouth develops 7 days after hatching, which is bound by two horny jaws after which it feeds on aquatic plants.
- Stomodaeum and proctodaeum get connected with gut forming a complete alimentary canal. It is at first small and broad but later becomes long and coiled like a spring.
- Slowly three pairs of external gills are replaced by four pairs of internal gills. Internal gills are covered with an **operculum**.
- Mesonephric kidney of adult begins to appear.
- Lateral line system is well-developed.
- Fore limbs and hind limbs start forming. Fore limbs develop slowly than hind limbs as they remain excluded by operculum.

- Lungs begin to appear and when fully formed, a tadpole respires through both lungs and gills.

8. Metamorphosis and growth:

- Tadpole larva completely differs from the adult frog in form and nature.
- Two or three weeks after breathing through lungs, the tadpole undergoes drastic changes to transform into a small young frog.
- The changes that transform a tadpole into an adult frog are called metamorphic changes and the process is called **metamorphosis**.
- It includes morphological, physiological and behavioral changes.
- Before metamorphosis, the tadpole develops a thyroid gland which secretes the hormone, **thyroxine** essential for metamorphosis.



- **Some of the important changes are as follows:**
  - The tadpole stops feeding.
  - The tail tissue forms a nutritive substance which through blood provides nutrition to the larva.
  - The tail begins to disappear slowly.
  - The sectorial mouth becomes wider and a large sticky tongue is developed.
  - The eyes increase in size.
  - The forelimbs come out of the operculum and the hind limbs become longer.
  - Skin becomes vascular, glandular, respiratory and pigmented.
  - The lateral line system disappears.

EXPERIMENT – 5

STUDY OF DEVELOPMENT OF CHICK EMBRYO THROUGH INCUBATED CHICK EGGS UPTO 96HRS

Materials Required

1) Fertilized egg of 24, 48, 72 and 76 hours
2) Forceps and scissors
3) Petri dish tumble
4) Brush, needle
5) 2% Borax carmine solution
6) Binocular microscope/simple microscope
7) Chick saline

Preparation Of Chick Embryo

The fertilized eggs are collected from recognized poultry farm having arrangements for incubation of eggs. A good poultry can supply eggs of definite hour of incubation. Eggs incubated for 24, 48, 72 and 96 hours arc used for the study of development of chick. The eggs are cleaned with 50% alcohol to make germ free. A small pore at the broad end of the egg is made gently with the help of a scalpel. Then the shell is cut around the broad end with the help of scissors till the opening is large enough. The content of the incubated egg is poured in a large Petri dish / tumble filled with chick saline, without damaging the vitelline membrane around the yolk. In the animal pole the embryo appears as a small white body on the surface of the yolk at the centre. The embryo is dissected out and kept it in a wash glass. The embryo is cleaned with a brush in the chick saline. Then the embryo is stained with 2% borax carmine solution. The embryo is thoroughly washed, dehydrated and mounted in DPX. Slide with stained embryo is examined under microscope.

Result

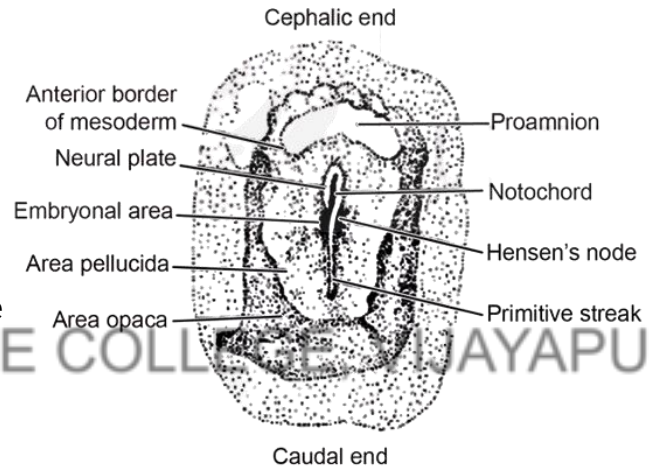
Observation and identification of mounted slides.

EXPERIMENT – 6

STUDY OF PERMANENT SLIDES OF CHICK EMBRYO -18 HRS, 24 HRS, 36 HRS, 48 HRS (WHOLE MOUNT AND T.S OF 18 HRS AND 24 HRS CHICK EMBRYO)

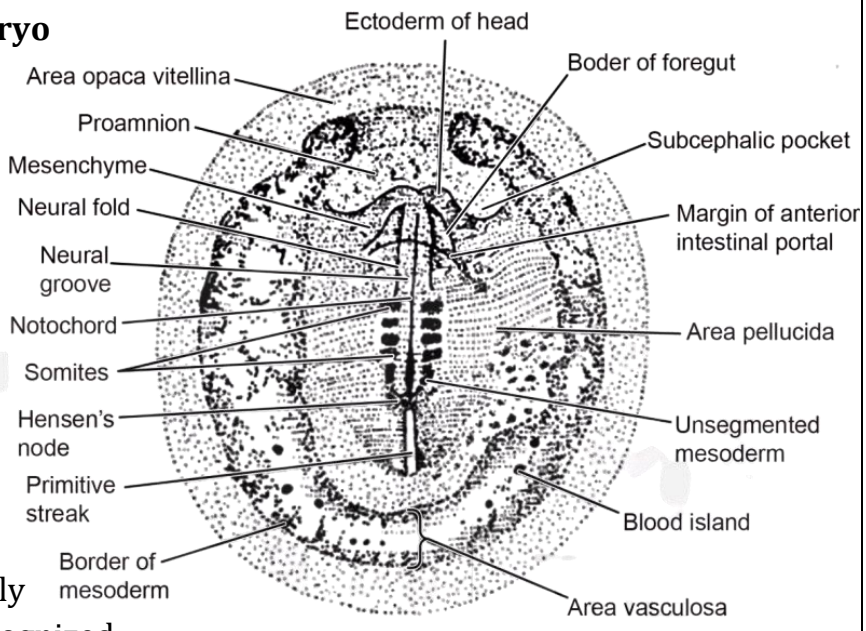
Study of Chick Embryo at 18 Hours

1. Features of whole mount of chick embryo at 18 hours of incubation.
2. In the 18 hour whole embryo you will observe that the notochord has become markedly elongated to form a conspicuous structure.
3. Notochord is seen to extend from the Hensen's node towards the cephalic region present in the middie.
4. Embryo at this stage of development is said to be in the head Process stage.
5. Neural plate develops around the notochord.
6. The dark peripheral area opaca, the inner translucent area Pellucida and the central embryonal area are clearly visible.
7. In the anterior region a small and more translucent portion of area pellucida, known as proamnion can be observed.
8. The primitive streak lies in the middle of the area pellucida in the posterior half.
9. The neural plate and primitive streak can be seen to be separated by Hensen's node.



Characters of 24 hour chick embryo

1. Area vasculosa and area pelucida are distinct.
2. Primitive streak is much reduced at the posterior end.
3. Usually 4 pairs of somites are present in the middle of the body.
4. Neural folds are well developed at the anterior end.
5. Primary optic vesicles in an early stage of development can be recognized.



6. Fore gut development initiates.

Characters of 48 hour chick embryo

1. Primitive streak is almost absent. Both cervical and cranial flexures are well developed.
2. Neural tube differentiated into brain and spinal cord.
3. Usually 26 pairs of somites are visible.
4. Anterior part of the embryo is twisted to the right.
5. Pharyngeal pouches are visible.
6. Amnion development starts.
7. Optic cups are conspicuous.
8. Distinct auditory vesicles are present.
9. Three pairs of arterial arche arise from the ventral aorta.

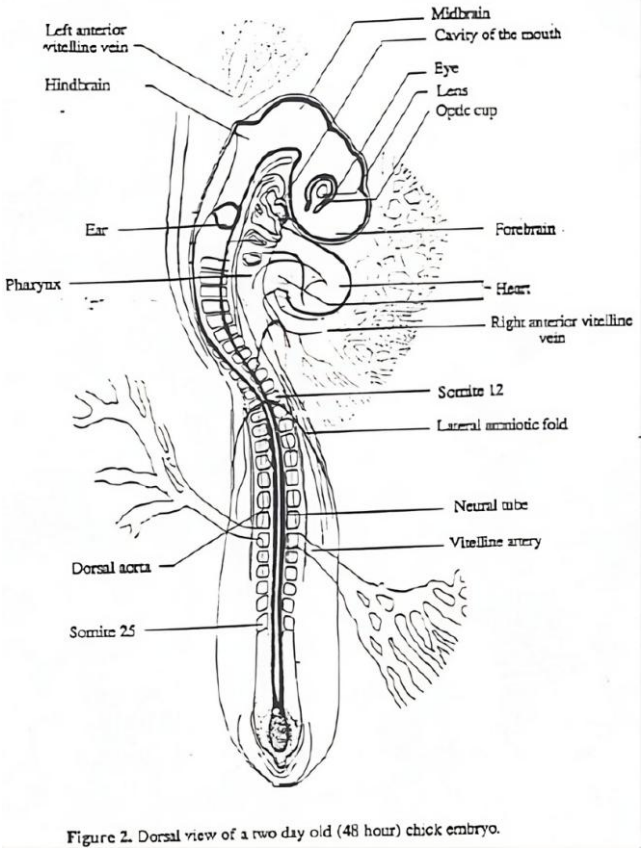
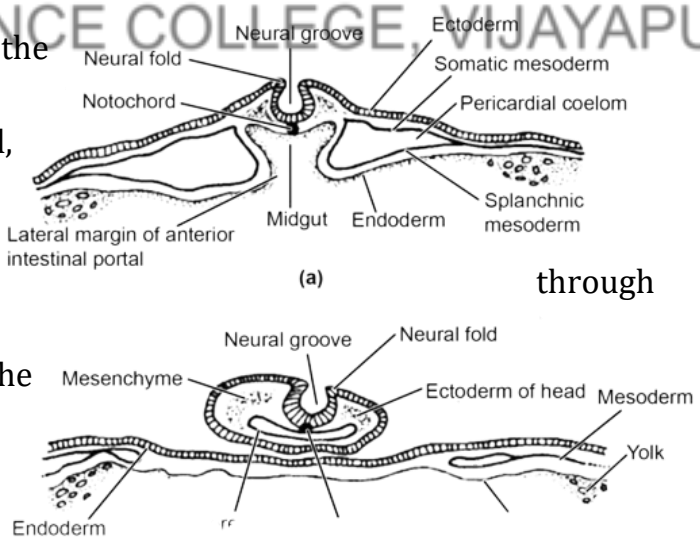


Figure 2. Dorsal view of a two day old (48 hour) chick embryo.

Chick Embryo In Transverse Section (T.S) At 24 Hours

1. The transverse section passing through head region shows the folded neural plate forming a complete tube.

2. The notochord can be seen beneath the neural fold.
3. Mesenchyme, foregut, ectoderm of head, mesoderm and endoderm can be seen in this section.
4. The transverse section passing through mid-body of the chick embryo shows formation of somites and changes in the mesoderm.
5. Mesoderm is seen to be differentiated into:
   i) dorsal mesoderm,
   ii) intermediate mesoderm and
   iii) lateral mesoderm.
6. Other structures seen in the slide are ectododerm, endoderm, lateral margin of anterior intestinal portal, midgut and pericardial coelom.



T.S. passing through a) head region; and b) mid body in chick o

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS, SHRI. B. M. PATIL ROAD, VIIJAYAPUR-586103

Karnataka

DEPARTMENT OF ZOOLOGY

B. SC VI SEMESTER (PAPER-II)

PRACTICAL LAB MANUAL

2023-24

INDEX

Expt. No.	DSCC-16: Course Title: Environmental Biology, Wildlife Management and Conservation-Practical (Code: 036 ZOO 014)	56.hrs / sem
1	Collection of water sample and analysis of physical parameters of water: Temperature, pH, Electrical Conductivity.	4
2	Estimation of chemical parameters of water: Dissolved Oxygen (O_2), Carbon Dioxide (CO_2), Hardness, Chloride, Alkalinity, Total dissolved solids (TDS).	6
3	Analysis of physical parameters of soil: pH, EC, Soil moisture, Soil temperature	2
4	Determination of organic matter in the soil sample	4
5	Study of tropical pond as an ecosystem: Study of flora and fauna and interaction between the various constituents using charts.	4
6	Analysis of air pollution: Air monitoring for particulate matter	4
7	Collection, preservation and estimation of zooplanktons	4
8	Study of threatened animals of India (charts/models/pictures): Tiger, Lion, one horned Rhinoceros, Golden langur, Lion tailed monkey, Musk deer, Kashmir stag, Great Indian horn bill and Indian rock python.	4
9	Location of Tiger reserves, National parks, Biosphere reserves, Wildlife sanctuaries of India on Map.	4
10	Demonstration of field equipments used in Wildlife census: Compass, Binoculars, Spotting scope, Range finders, Global Positioning System, Various types of cameras and lenses.	4
11	Identification of wild animals: Wild animal's pugmarks, hoof marks scats, pellet groups, nest, antlers. Demonstration of field techniques for wild flora and fauna.	4
12	Visit to Zoo/ Sanctuaries/ National parks/ Biosphere reserves	12
13	Any other practical's related to this paper may be added based on the feasibility	

B.L.D.E.ASSOCIATION'S

S. B. ARTS AND K. C. P. SCIENCE COLLEGE, NEW CAMPUS, SHRI. B. M. PATIL ROAD (SOLAPUR ROAD), VIIJAYAPUR-586103

DEPARTMENT OF ZOOLOGY

B.Sc., VI Semester Practical Examination Scheme (NEP), Paper- II

COURSE TITLE & CODE:21BSC6C8ZOO8P: Environmental Biology, Wildlife Management & Conservation

Duration: 3 hours **Max.Marks:25**

QI. Analysis of the quality of given water sample (O_2/,CO_2,/Chloride/Hardness/Salinity] 05

QII. Analysis of Physico-Chemical parameters of soil sample (pH, Temp, Moisture, Organic matter) (Procedure-2Marks,Results-2 Marks 04

QIII. Identify and Comment on the given spotters- A and B (Expt.10 & 11) (2X3)=06

(Identification-1mark,Comments-2 marks)

QIV. Submission of Report (Expt.12) 05

QV.Record Viva -Voce 05

Total - 25

II) Estimation of free carbon dioxide in the water samples

Objective/Aim: To determine the free CO2 from the given samples of water.

Requirements: Burette, Pipette, Conical flask, phenolphthalein indicator, 0.05N of NaOH.

Introduction: The CO2 is one of the most important gases in natural water. The concentration of CO2 in aquatic environment is generally between fraction of mg/liter. The small part of CO2 dissolved in water forms carbonic acid. Which comes mainly from the atmosphere and respiration of plants and animals. Plant photosynthesis sometimes reduces its concentration nearly to zero. Under such conditions plants obtain CO2 directly from bicarbonate ions. Some of the carbonic acid dissociates to form bicarbonate ions with which it is also in equilibrium. Some bicarbonate dissociates to form carbonate ions.

$CO_2 + H_2O \leftrightarrow H_2CO_3$

$H_2CO_3 \leftrightarrow H + HCO_3$

$HCO_3 \leftrightarrow H + CO_3$

Free CO2 reacts with NaOH to form sodium bicarbonate. Completion of the reaction indicated by the development of pink colour, by adding phenolphthalein indicator .

Procedure : Take 50 ml of water sample in a clean dry conical flask. Add 5-10 drops of phenolphthalein indicator.

If the sample turns pale pink colour then free CO2 is absent. If the sample remains colourless, titrate against 0.05NaoH solution until a pale pink colour persists. This colour change is the end point. Record the reading.

Calculation: $CO_2\ mg/L = \frac{M.B.R \times 0.05N \times 44 \times 1000}{50\ ml\ sample\ of\ water}$

Observation.

1) Standard solution taken in the Burret : Sodium Hydroxide
2) Sample taken in conical flask : 50ml of water

Indicator used: Phenolphthalein indicator.

Colour changes: Colo

urless to pale pink colour.

Table :

Reading	**Pilot Reading**	**I**	**II**	**III**	**Mean Burret Reading**
Final Burret Reading					
Intial Reading					
Difference					

BMR =$\frac{\text{I+II+III}}{3}$

III) Estimation of dissolved oxygen in the given water samples

Objective/Aim: To determine dissolved oxygen (DO) from given samples of water.

Requirements: Burette, pipette, conical flask, BOD bottles, mangnoussulphate solution, Alkali-potassium iodide solution. Conc.H2SO4, 0.025N Sodium thiosulphate solution, starch indicator.

Principle: When Mangnous sulphate is added to sample containing alkaline potassium iodide, Mangnous hydroxide is formed which is oxidized by the dissolved oxygen on addition of Sulphuric acid. When sulphuric acid is added the basic manganous oxide liberates iodine which is equivalent to that of dissolved oxygen originally present in the sample. The liberated iodine is titrated aginst standard solution of sodium thiosulphate using starch as an indicator. By calculating the amount of iodine liberated the DO can be determined.

point. The burette reading gives the amount of dissolved oxygen of water sample

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$$

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$ Basic manganic oxide which on acidification gives

$$MnO(OH)_2 + H_2SO_4 \rightarrow MnSO_4 + 2H_2O + [O]$$

$$2KI + H_2SO_4 + [O] \rightarrow K_2SO_4 + H_2O + I_2$$

The liberated iodine (I_2) is titrated against standard sodium thiosulphate ($Na_2S_2O_3$) solution using starch as indicator [**Starch+ I_2 → Blue coloured complex**].

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

Procedure: Carefully fill water sample in a 300ml glass stopper narrow mouthed bottle (BOD bottle). Add 2ml of Manganous sulphate solution in the bottle by pipette, well below the surface of water. Similarly add 2ml of alkali-potassium iodide reagent . Close the bottle with stopper and shake it vigorously. A brown precipitate appeas. Allow the precipitate to settle down. Add 2ml of concenctrated sulphuric acid by the sides of BOD bottle. Mix it thoroughly and allow it to stand for at least 5 to 10 minutes till a clear sample is obtained Take 50ml of above sample in to a clean conical flask and add 1-2 ml of starch indicator.

Titrate against 0.025N sodium thiosulphate solution till blue colour disappears. This is the end Point.The burette reading gives the amount of dissolved oxygen of water sample.

Obsevation:

1) Standard solution taken in the Burret : Sodium thiosulphate0.025N.
2) Sample taken in conical flask : 50ml of water + Manganous sulphate + Alkai potassium iodide +conc. Sulphuric acid

Indicator used: 1 or 2ml Starch indicator.

Colour changes : Blue colour to colourless.

Table :

Reading	**Pilot Reading**	**I**	**II**	**III**	**Mean Burret Reading**
Final Burret Reading					
Intial Reading					
Difference					

BMR =I+II+III

3

Calculation : MBR X N of $Na_2S_2O_3$ X 8 X 1000

V2 (V1 - V)

V1

Note

N of $Na_2S_2O_3$ = 0.025 N
V2 = volume of sample taken
V1= volume of BOD bottle
V= Volume of the reagent

IV) Estimation of Total Hardness in the water samples

Objective/Aim: To determine the total hardness from the given samples of water.

Requirements: Burette, Pipette, Conical flask, Standard EDTA (0.01N) Eriochrome black-T, Buffer Solution.

Principle: The hardness of water is due to presence of various cat ions in the water. Of these Ca and mg ions contribute to a greater extent while others like Fe, Mn etc for lesser extent. When EDTA is added to water containing both calcium and magnesium, it combine first with calcium that is present, and solution becomes wine red. When sufficient EDTA is added the solution turns from wine red to blue. This is the end point of titration.

Procedure: Take 50ml of water sample in conical flask. Add 1-2ml of buffer solution. Add a pinch of indicator (200mg). Titrate against standad EDTA solution solution .The colour change from wine red to blue colour Indication..

The colour of the solution at the end point is blue.The colour change from wine red to blue. Note the ml of EDTA solution consumed.

Calculations: Total hardness as $CaCO_3$ mg/L = $\frac{\text{M.B.R x 0.01Nx 100 x 1000}}{\text{50ml sample of water}}$

Where N is normality of EDTA and 100 is Mol. Wt of $CaCO_3$.

DETERMINATION OF P^H OF SOIL

PRINCIPLE: A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside. This is because the thin glass bulb allows mainly the agile and small hydrogen ions to interact with the glass, the glass electrode measures the electro-chemical potential of hydrogen ions. To complete the electrical circuit, also a reference electrode is needed.

MATERIALS REQUIRED: pH Meter, Buffer Solutions, Soil Samples, Weighing machine, Beakers, Distilled Water, Glass rod.

PROCEDURE:

1. The pH meter was calibrated using pH 7 buffer solution.
2. Then the meter was adjusted with known pH of buffer solution, with 4.0 and 9.2.
3. 20g of soil was weighed and transferred into 100ml beaker.
4. 40ml distilled water was added and stirred with a glass rod.
5. This was allowed to stay for half an hour with intermittent stirring.
6. To the soil-water suspension in the beaker, the electrode was immersed and pH value was determined from the automatic display of pH meter.

OBSERVATION:

1. pH of Garden soil=7.2 ……..
2. pH of Construction soil=7.8 ……..
3. pH of College campus soil=7.6……..

RESULT: The pH of Garden Soil seemed to be more fit for the cultivation because it was close to neutral. On the other hand, Construction Soil is not fit for the growth, as it is close to alkaline nature.

DISCUSSION: The pH factor of soil reflects its acidity level, which is important to consider because all plants require different levels for proper growth. The soil's acidity level also affects the dispersal of other important nutrients in the soil, and an imbalance can block a plant's ability to absorb them. Testing pH levels is important, particularly when planting a garden for the first time in new soil whose acidity is unknown.

pH Requirements: When planning a new garden, it's important to know if your soil is suited to the types of plants you will grow. The soil's pH is rated on a scale of 3.5 to 9.0, and most plants do best in soil that test within the neutral range of 6.0 to 7.0. Growth may still occur if the soil tests higher or lower than this, but plants may exhibit the effects of an improper balance through poor development and fruiting.

I) Study of threatened animals of India

a) Indian Tiger (Panthera tigris) : The most famous animal of Indian wild life is the tiger found throughout India except in the deserts of Rajasthan, Punjab, cutch and sind.

- It is famous for its phantom like grace, beauty, strength and courage.
- It is regarded as "National animal of India "
- It lives in variety of habitats, from thorny jungles to dense terai forests, even up to 3000m altitude in the Himalaya.
- Tiger is rich coloured well stripped animal with a short coat.
- The tiger is almost entirely nocturnal in habit, excellent hunter, and is also good swimming.

- Female give birth to 3 cubs in the wild but only two are raised to maturity.
- Its population is reduction due to killing for its skin, and destruction of its habitats.
- To save the population of tiger "project tiger " was launched by Government of India in 1973 with assistance of World wildlife fund (W.W.F)

b) Indian lion : The Indian or Asiatic lion (Panthera leo persical) which was once widely distributed in many parts of India is now reduced to a population of 205 in small area in Gir forests.

- It has a fairly good mane with the tail tuft bigger and bearing more hair on its elbows.
- It measures 250 to 287cms and weight up to 200 to 250kgs.

- A keen sense of hearing helps it in hunting its food.
- A lioness becomes mother at the age of 2 .1/2 to 3years and gives birth to 2-3 cubs at a time.
- It is endangered species and is being protected under the "Gir Lion " Sanctuary Project " which was started in 1972.
-

c) **Indian Rhinoceros :** The great Indian one horned rhinoceros (Rhinoceros unicornis).

- It is limited in its distribution to the low marshlands on the banks of Brahmaputra in Assam and adjacent areas of West Bengal and Nepal.
- Tall grass of Kaziranga National Park (Assam) is the shelter for rhinos.
- A full grown rhino weight up to 200kgs.
- It has a horn protruding from the top of its snout.
- It is used a weapon of attack. Even elephant is afraid of rhinos horns.
- The poachers kill this animal just for its horn which fetches a high price.
- Horn is alleged to be strong aphrodisiac and an antidote for poison.
- Rhino's urine is considered as antiseptic.
- According to recent census 1,654 rhinoceros are in Kaziranga National Parks.

d) **Golden Langur (Trachypithecus geei) :** Golden langurs are found mainly in the foothills of the Himalayas, along the Assam-Bhutan border.

- The coat of golden Langur is covered with rich golden to bright creamish hair. The face is black and they have a very long tail, which may measure up to 50 cm in lengh.
- The long tail of the golden langur helps it in balancing itself , while leaping across the branches of trees.
- It is mainly found inhabiting the forests of india that have high trees.
- Golden langur is herbivorous feeding on young and mature leaves, ripe and unripe fruits seed buds and flowers.
- Golden langur live mainly in groups of 8 to 50 members. In each group, one male langur attached itself with a number of females.
- Golden langurs are currently considered endangered species in India. Presently, their population is around 10,000.
- One of the major reasons for the dwindling population is the destruction as well as degradation of their natural habitat by humans.

e) **Lion-tailed Macaque (<u>Macaca</u> <u>silensus</u>) :** It is endangered species found only in Nilgiri, Anamalai's cardamom hills and periyar wild life sanctuary (kerala), spending most of their time on trees and is excellent swimmers.

- The coat of lion-tailed macaque is covered with dark-brown or black fu.
- One of its unique features is its silver-white mane, which stretches on from the cheeks to the chin, while surrounding the head.
- The face of Lion-tailed macaque of India is free from any hair and is black in color.
- The tail is of a medium size and reaches a length of approximately 25cm . At the end of the tail is a black tuft, resembling the tail of a lion.
- It is a diurnal. Lion-tailed macaques are social. Seen mostly in groups of 10-20 members (both male and female)
- The leader of the group is an adult male macaque who leads the group and defends its territorial area.
- Its killing for skin and meat purpose, destruction of its habitat has reduced its population to about 800 living in 55 troops in Western Ghaat

f) **Musk deer (Moschus moschiferus) :** Musk deer is found in small population in Jammu and Kashmir, parts of himachal Pradesh, uttar Pradesh.

- A unique features of this deer is the presence of the presence of a musk gland in the male.
- Musk is thick, brown secretion of this gland used in preparing medicines and perfumes.
- Musk costs up to Rs. 40,000 -60,000 per kg, in Internal Markets.
- Its habitat destruction has its population to isolation protected areas in India. In 1974 project Musk deer has launched in Kedarnath sanctuary (U.P) to its population.

VI) Marking of existing Project Tiger Areas and Biosphere Reserves in Indian Map

Project Tiger :

Project Tiger is a wildlife conservation movement initiated in India in 1972.

The project aims at tiger conservation in specially constituted tiger reserve throughout India.

Project Tiger was launched by the Government of India in the year 1973 to save the endangered species of tiger in the country. Starting from nine (9) reserves in 1973-74 the number is grown up to fifty (50). A total area of 71027.10 km is covered by these project tiger areas.

Sl.No.	Tiger Reserve (TR)	State	Total Area (sq Km)
1	Bandipur	Karnataka	914.02
2	Corbett	Uttarakhand	1288.31
3	Kanha	Madhya Pradesh	2,051.79
4	Ranthambore	Rajasthan	1,411.29
5	Simlipal	Orissa	2,750.00
6	Sunderban	West Bengal	2,584.89
7	Periyar	Kerala	925.00
8	Namdapha	Arunachal Pradesh	2,052.82
9	Nagarjunsagar Sagar	Andhra Pradesh	3,296.31

VII)Spotting of the endangered animals conserved in protected areas of Karnataka state (usingKarnataka map)

Asian Elephant :

Indian Elephant is smaller pinnae are broad and fan like but not very long.

Head rounded tip of the trunck has only one sensory projection fore head is concave tasts are large in male but just projection beyond the lips is female.

Generally only the males have large tusks.

Elephants have very poor sight but small and hearing are acute.

Project Elephant started in 1992.

Mass of male 4,000 2.7 meter and 2.2 meter upto shoulder.

Since 1986 the Asian Elephant has been listed as endangered on the IUCN Red list as the population has declined by atleast 50%.

Gharial (Gavialis gangeticus):

The gharial (gavialis gangeticus) also known as the gavial or the fish eating crocodile.

The gharial name they have a distinct boss at the end of the snout which resembles an earthen ware pat known as a ghara hence the name " Gharial".

The gharial is well adapted to catching fish because of its long thin snout and 110 sharp interlocking teeth.

The global gharial populations is estimated at few than 235 indiviuals which are threatened by loss of riverine habitat

The gharial is one of the largest af all living crocodilians measuring upto 625.

Asiatic Cheetah (Acinonyx jubatus venaticus):

The Asiatic cheetah is also known as the Indian cheetah or Persian cheetah it is an extinct species in India and critically endangered cheetah subspecies.

Reduced number is due to the land use change habitat degradation fragmentation by loss of prey as a result of evergreen of granzing from introduced live stock and antelope hunting mining development and road construction near reserves also threats the population.

The Asiantic cheetah is know listed as critically in the IUCN red list of threatened animal.

Indian Peacock (Pavo cristatus):

Indian Peacock is the National Bird of India.

Indian Peacock is known for its beautiful feathers and romatic dance under the black clouded sky male Indian.

The whole of its body measured around 2-3m which includes the length of 1.4-1.6m

The Indian peafowl is not endangered species in the sub continent.

They feed on grains insects small reptiles small mammals and some cultivated crops.

The Indian peafowl is listed by the ICUN.

VIII) Marking of National Parks in Karnataka

Introdution

The state of Karnataka is south. India has a rich diversity of flora and fauna. It has a recorded forest area of the state these forests support 25% of the elephant population and 20% of the tiger population of India many regions of Karnataka are still unexplored and now species of flora and fauna are still found, The western ghats mountains in the western region of Karnataka area biodiversity hotspot two sub-cluster to the western ghats, talaco every and kudremukha in Karnataka are in a list of sites that could be designated or world Heritage sites by UNESCO. The Bandipur and Nagarahole National Parks which foll out side these subclusters were included in the Nilgiri Biospere Reserve in 1986 a UNESCO designation Biligiriranga Hills in Karnataka is a place where Eastern ghats meets western ghats . The state bird and state animal Karnataka are Indian roller and state the Indian Elephant respectively, The state trees and flower are sandal wood and Lotus respectively Karnataka

National Parks of Karnataka

1) **Bandipur National Parks:**

Location : Mysore and Chamarajnagar district of Karnataka,India.

Area covered : 874.2 sq km.

Established : 1974

Speciality of Forest : Tiger Reseve.

Forest type : Tropical Rain forest.

Fauna : Amphibian : Tod frogs etc.

Reptiles : Marsh crocodile, monitor lizard walf snake, flying lizard.

Aves : Grey jungle fowl, Kingfisher, owl, Eagle etc.

Mammals : Tiger,Leopard, Asiatic wild dog, Mangoose, Giant squirrel, ect.

2) **Kudremukha National Park:**

Location : Kudremukha, Mudigere Taluk, Chikkamanglure,District Karanataka, India.

Area covered : 25 km2.

Established : 1984.

Speciality of Forest : Tiger Reserve.

Forest type : Tropical Rain forest, Lion Tailed Macaque.

Fauna : Insects : Butterflies, Aquatic insects Day flying insects etc.

Amphibians : Flying frog, Malabar gliding frog, golden frog etc.

Reptiles : Monitor lizard, Flying lizard, crocodile, Cobra, Rat snake, King Cobra etc.

Aves : Great horn bill, Imperial pigeon, Malabar dragon.

Mammals : Bengal tiger , Indian leopard, Lion tailed Macaque.

3) **Nagarahole National Park:**

Location : Kodagu and Mysore district of Karnataka, India.

Area Covered : 643.39 sq km.

Established : 1983.

Forest type : Moist deciduous forest.

Speciality of Forest : Tiger Reserve and Nilgiri hills lion tailed macaque.

Fauna : Amphibian : Bafo, treefrog, Flying frog, Toads etc.

Reptiles : Crocodile ,Monitor lizards, Python, Common cobra, Lizards etc.

Aves : Common peafowl, grey jungle fowl, Rocket tailed drongo common King fisher etc.

Mammals: Tiger, Leopard, Asiatic wild dog, Mangoose, Flying squirrels, Lion tailed Macaque, etc.

4) **Bannerghatta National Park:**

Location : Banglore Karnataka, India.

Area Covered : 260.15 km2.

Established : 1974.

Speciality of Forest : Biological Reserve.

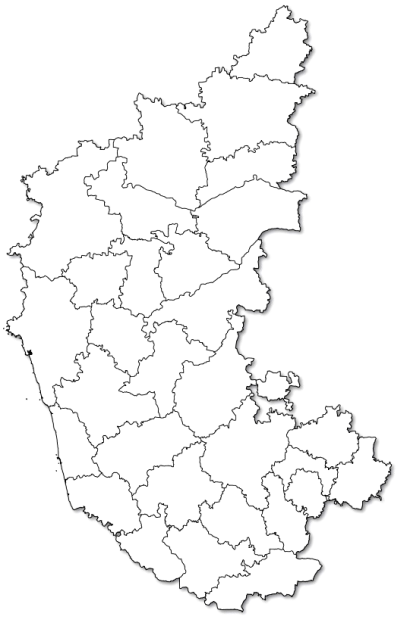
Forest type : Decidous Forest.

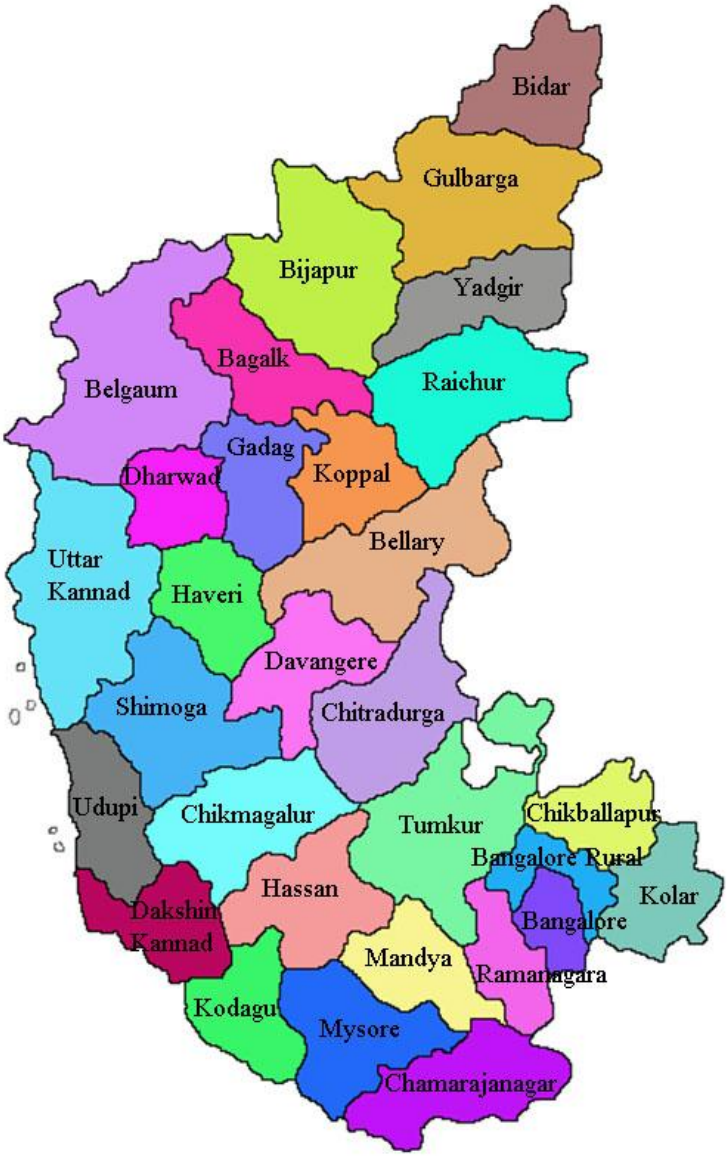
Fauna : Reptiles : Land monitor lizard, Crocodile, Python, Rat snake, etc.

Aves : Peafowl, Grey jungle fowl, wood pecker, sunbird, Eagle etc.

Mammals : Elephants, Leopards, wild dog , mous deer etc.

Insects : Varieties of butterflies, bear ants etc.





IX) Marking of wildlife sactuaries in Karnataka map

1) **Attiveri Bird Sanctuary :**

Location : Attiveri, mundgond taluk, Uttara Kannada district Karnataka, India.

Area Covered : 2.23km2.

Established : 2,000.

Speciality of Forest : Kingfisher, Grey horn bill.

Forest type : Dry Decidous forest.

Avian Fauna : Black headed ibis, Eurarian spoonbill, Pied and kingfisher.

2) **Bankapura Bird Sanctuary :**

Location : Bankapura Adichandranagiri taluk Haveri district Karnataka.

Area Covered : 139. Acres. (0.56km2).

Established : 2006.

Speciality of Forest: Peacock.

Forest type : Moist Decidous forest.

Avian Fauna : Great horned owi, babbler, green bee eater, night jar, spotted myna etc.

3) **Bonal Bird Sanctuary :**

Location : Bonal village, Shorapur taluk yadgir district Karnataka, India.

Area Covered : 700,Acres.

Established : 1998.

Speciality : Purple heron, White necked stork.

Forest type : Wet Land Conservation

Avian Fauna : White ibis, Black ibis, Brahminy duck, Bar headed goose, Indian shag,Snake bird, Purple moorhen etc.

4) **Gudavi Bird Sanctaury :**

Location : Gudavi village, Soraba taluk subdivision Sagar shimoga district, Karanataka, India.

Area Covered : 0.74 sq km.

Established : 1980.

Speciality : Pheasant tailed Jacana ,Crowned night heron.

Forest type : Ever Green forest.

Avian Fauna : Grey heron, Night heron, little carmarant, little grobe, Purple moorhen, Darter,etc.

5) **Kuggaladu Bird Sanctuary :**

Location : Kaggaladu village, sira taluk, Tumkur district ,Karnataka, India.

Area Covered : 0.82 sq km.

Established : 1999.

Speciality : Painted Storks, Black stilts.

Forest type : Moist Ever Green forest.

Avian Fauna : Storks, Grey horons, Pelicans, Black stilts, and Ducks, Grey pelican, etc.

6) **Magadi Bird Sanctuary :**

Location : Magadi village, Shirahatti Taluka. Gadag district Karnataka, India.

Area Covered : 900 Hectares.

Established : 1990.

Speciality : Bar Headed Goose.

Forest Type : Moist Deciduous, Wet land.

Avian Fauna : Headed goose, Grey heron, Purple Heron, Comb duck Oriental Ibis, White Breasted Water Hen, etc.

7) **Mandagadde Bird Sanctuary :**

Location : Mandagadde village, Tirtha halli Taluka Shivamoga,district, Karnataka,Village.

Area Covered : 1.14 Acres

Established : 1990.

Speciality : Median Egrat, Darter, Little Carmorant.

Forest Type : Moist Wet land.

Avian Fauna : Median Egret, Little cotemorant,Darter, White Ibis Black Ibis. , Brahminy duck, Bar headed goose,etc.

8) **Puttenahalli Lake Bird Sanctuary :**

Location: J.P**.** Nagar, Banglore, Karnataka, India.

Area Covered : 13 Acres.

Established : 2007**.**

Speciality : Spot billed duck.

Forest Type : Fresh water Lake.

Avian Fauna : Purple heron, Eurasian coat, India pond heron, Gorganey,common kingfisher, etc.

9) Ranganathittu Bird Sanctuary:

Location : Mandya district ,Karnataka, India.

Area Covered : 40 Acre.

Established : 1940.

Speciality : Painted Stork.

Forest type : Wet land, Banks of Kaveri river.

Avian Fauna : Pied kingfishers, open billed storks, pointed strorts, Snoury egret, great stone,curlews,white ibise,etc.

10) Daroji Bird Sanctuary :

Location : Daroji Ballari district , Karnataka, India.

Area Covered : 82.72km2

Established : 1994.

Speciality : Peacock.

Forest type : Dry deciduous forest.

Avian Fauna : Great hormed owi, babbler,Magipie, Robin, green bee eater, night jar, parakeets, myna,etc.

Identification of wild animals: Wild animal's pugmarks, hoof marks,

Introduction: Pugmarks are the marks which are left by different animal's species while they are walking, running, or moving from one place to another place. Pugmarks refer to the footprints of most animals' species. "PUG" also means foot in Hindi. Pugmarks of some animals are denoted by some different terms. Pugmarks denote "paw print" of most feline animals for e.g. like dog, cat, etc. Herbivore footprints are called as hoofmark. Some of the herbivore animals are like cow, goat, buffalo etc. Mostly the footprints of tigers are termed as pugmarks. Every animal species has different type of pugmark and this factor can be used for their identification purpose.

Procedure for Examination

After the collection of pugmarks, each and every pugmark was observed individually. Both hind foot and fore foot of the different animal species were observed. Different characteristic features were identified through which we can know easily which pugmark belongs to which animals.

Figure 13: Pugmark of buffalo (fore foot)

Figure 14: Pugmark of bull (hind foot)

Figure 7: Pugmark of deer (fore foot)

Figure 8: Pugmark of deer (hind foot)

Figure 21: Pugmark of elephant (fore foot)

Figure 22: Pugmark of elephant (hind foot)

Objectives • Participants will learn to identify types of binocular

A	**SPECIES NAME: ARTIODACTYLA**					
S.No.	**Animal name**	**Characteristic of fore foot pugmark**				
		Shape	**Size**	**Dew mark**	**Claw mark**	**Specific feature**
1	Buffalo	2 bilaterally symmetrical mark shape	Large	Absent	Absent	Uneven sizes of hooves mark are formed i.e one hooves mark is larger than other mark.
B	**SPECIES NAME: PERISSODACTYLA**					
S.No	**Animal name**	**Characteristic of fore foot pugmark**				
		Shape	**Size**	**Dew mark**	**Claw mark**	**Specific feature**
1	Elephant	Round	Large	Absent	Absent	Scales marks are present.

C	**SPECIES NAME: ARTIODACTYLA**					
S.No	**Animal name**	**Characteristic of hind foot pugmark**				
		Shape	**Size**	**Dew claw**	**Claw mark**	**Specific feature**
1	Deer	Oval	Small	Absent	Absent	Tip of the hooves mark are slightly pointed and bottom is circular in shape

Demonstration of field equipments used in Wildlife census: Compass & Binoculars

Binocular Basics

Always use your binocular neck strap so the binoculars are safe around your neck and against your chest, within easy reach of your hands. Some birders prefer to use a binocular harness, which uses straps over the shoulders and across the back to distribute the weight of the binoculars.

The adjustable eyecups keep your eyes the right distance from the lenses. If you wear glasses, keep them on when using your binoculars. The eyecups should be folded or twisted down. If you don't wear glasses, keep the eyecups extended.

Adjust the binoculars at the hinge so that the two circles you see merge into one when looking through both lenses. For children and adults with smaller size heads, some binoculars may not adjust close enough. Try others.

The most useful binoculars for bird watching incorporate a central focus wheel and a diopter focus adjustment. The diopter is often part of the right-hand eyepiece on a binocular. The purpose of the diopter is to compensate for the differences between your two eyes (because no two eyes are the same or have the same ability to focus.) Adjusting both the diopter focus and the central focus is how you get the clearest possible image from your binocular.

Compass

A compass can be defined as a device which is generally used to navigate through paths and to find directions. It is considered the Global positioning system of ancient times, as its use has reduced a little but it is still used at places where GPS signals can not reach like deep oceans and mountains. They generally work on the true magnetic north of the Earth.

Uses of Compass

The uses of a compass are mentioned as follows below:

- It helps in reading maps.
- It is also used to navigate through paths and routes.
- It is also used to conduct surveys.
- It helps in navigating through the water as well as mountains.
- It is also used to detect directional degrees of a certain place.
- It is also used to find the true magnetic north of the Earth.