M.Sc. ZOOLOGY LAB MANUAL

1st Semester

Prepared By

Biological Science Dept.

Zoology

MIDNAPORE CITY COLLEGE

This is the first edition of Lab Manual for PG Zoology third Semester, Ecology Special Paper. Hope this edition will help you during practical classes. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topics are not included here; those will be guided by respective teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers and non teaching staff to make this effort complete.

We sincerely thank our Hon'ble Director and Hon'ble Teacher In-Charge for the motivation for making this lab manual.

Non-Chordate Biology, Chordate Biology, Histochemistry and Animal Physiology

(50 Marks, 4 credit)

Syllabus:

1. Non- Chordate Biology:

i) Identification of common Invertebrate and Vertebrate taxa

ii) Minor Dissection:

a. Grasshopper - Reproductive system/ Nervous system

b. Cockroach – Stomatogastric Nervous system

c. Achatina – Reproductive system & Nervous system

2. Chordate Biology:

i) Major Dissection: Vth, VIIth cranial nerves of bony fish

ii) Minor Dissection: Fish scale and pecten of bird

3. Histochemistry

i) Preparation of laboratory fixative mixtures

ii) Microtechnique for routine histological study

iii) Histological study of different vertebrate organs

4. Animal Physiology

i) Demonstration of blood haemoglobin estimation in the animal model

ii) Estimation of pH and its impact on any aquatic animal.

iii) Observation of gut movement in an animal under hypoxia using Dale's apparatus

iv) Estimation of Blood Pressure and Heart Rate

v) Determination of Breath-Holding Time (BHT) in humans

Course No: ZOO 196 (Practical):
Immunology, Methods in Biology, Cell Biology and Cytogenetics

(50 Marks, 4 credit)

Syllabus:

1. Immunology:

a. Study of macrophage.

b. Study of phagocytosis.

c. Determination of human blood group

2. Methods in Biology

a. Characterization of macromolecule through Gel electrophoresis

3. Cell Biology

a. Identification of different stages of cell division and cell organelle.

b. Mitochondrial Staining

c. Cell isolation and cell counting

4. Cytogenetics:

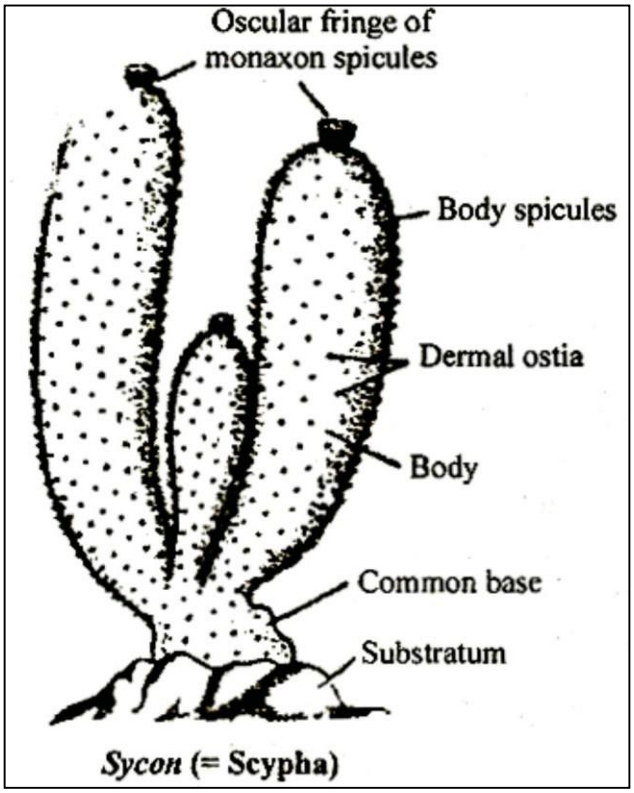
a. The life cycle of *Drosoplila*.

b. Analysis and interpretation of genetic crosses with special reference to *Drosophila*

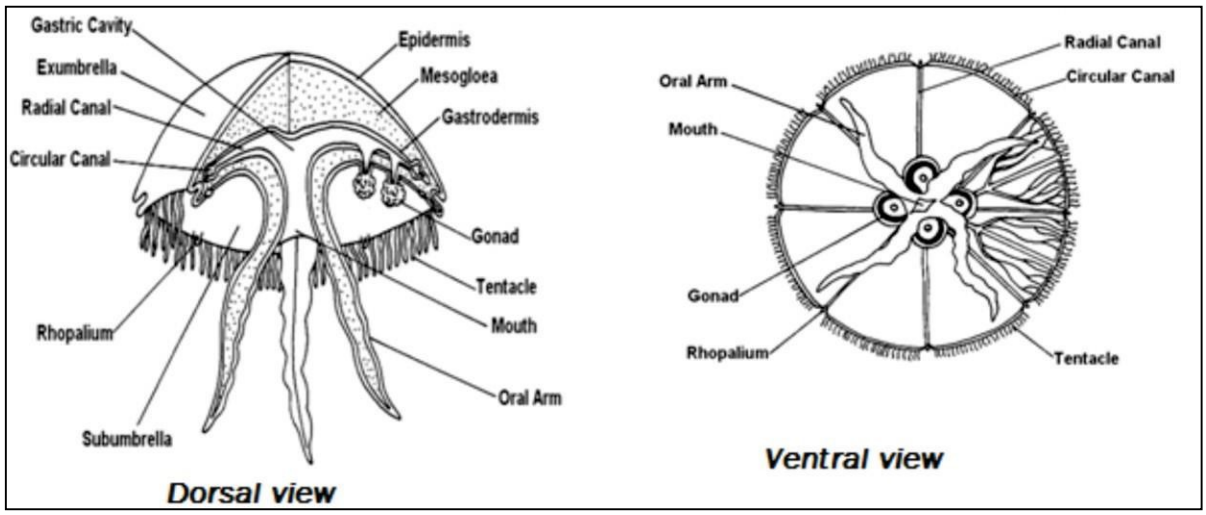
c. Study of the polytene chromosome of *Drosophila*.

ZOO – 195

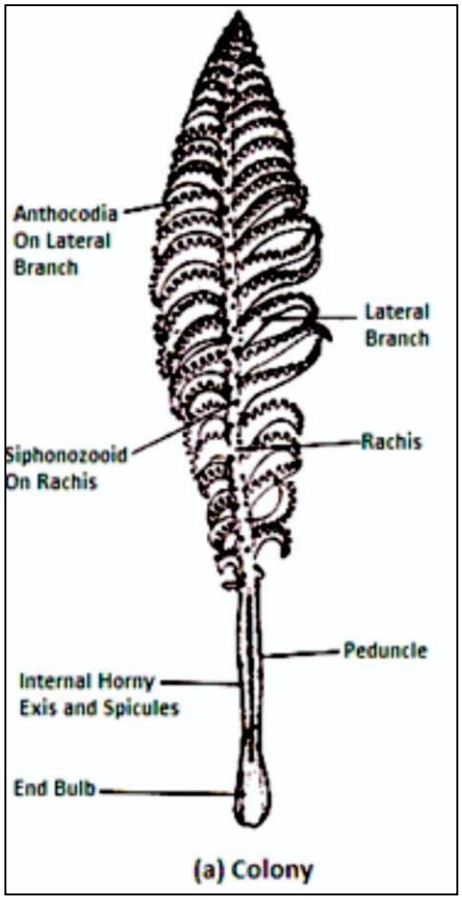
Identification of Common Invertebrate and Vertebrate Taxa:



CHARACTER	INFERENCE
• Body bears a canal system with many ostia and osculum. • Fixed and incapable locomotion. • Non tissue grade multi cellular organism. • Spongocoel is lined by flagellated choanocyte cells. • Diploblastic cylinder shaped body supported by spicules or spongin or both.	Hence, Phylum – Porifera
• Skeleton of calcareous spicules. • Spicules of calcium carbonate. • Spicules are monaxon, triaxon, tetraxon type.	Hence, Class - Calcarea
• Triaxon spicules usually have one long ray.	Hence, Subclass - Calcaronea
• Body shape is vase like. • Body wall is thick form which spicules projected. • Canal system is syconoid type. • Apical ends of each cylinder bears osculum guarded by monaxon spicules. • Group of ostia present at the surface of the body.	Hence, the specimen seems to be *Sycon* sp.



CHARACTER	INFERENCE
• Body soft, biradialy symmetrical, tissue grade. • Diploblastic animal with cnidoblast cell. • Presence of nematocysts. • Presence of gastrovascular cavity. • Ectoderm and endoderm separated by mesoglea. • Polyp and medusoid forms. • Presence of tentacles around the mouth.	Hence, Phylum - Cnidaria
• Medusoid cnidaria. • Presence of endodermal gastric filament. • Mesoglea thick and noncellular. • Presence of oral arms. • Velum absent. • Sense organs are tentacles.	Hence, Class - Scyphozoa
• Highly branched radial canal. • Four horse-shoe shaped and four intra radial gastricpouches present at the surface of the body. • Umbrella shaped body and mouth drawn into 8 long oral arms. • Sub umbrella contains 8 pairs of mesoglea tentacles. • Inner side of oral arms have ciliated groove.	Hence, the specimen seems to *Aurelia* sp.



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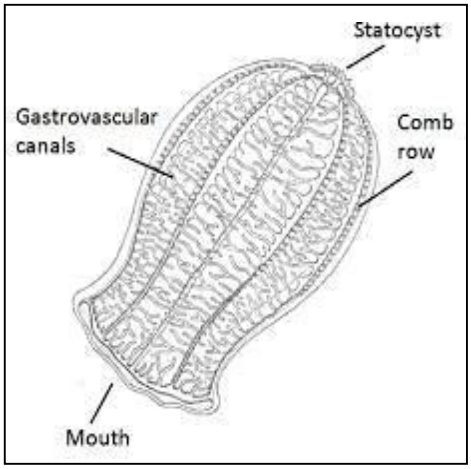
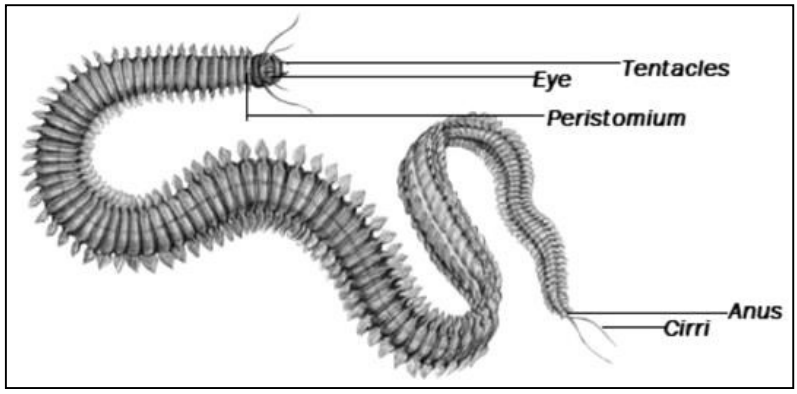
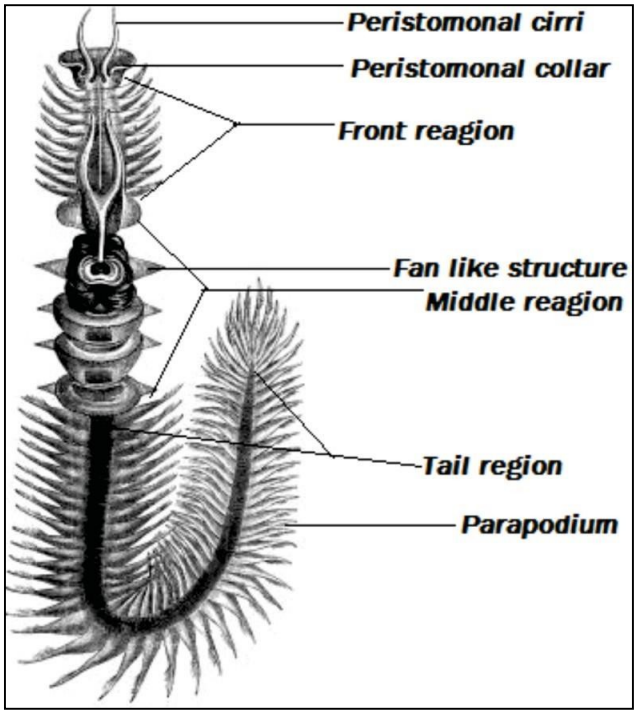
• Only polyp form is present. • Presence of stomodium and mesentries.	Hence, Class - Anthozoa
• Eight number of tentacles and mesentery present. • Tentacles are pinnately branched.	Hence, Subclass -Octocorallia
• Dimorphic feather like colony with single axial polyp and several secondary polyps. • Anterior part of rachis bears lateral pinnules and posterior part bears peduncle with an end bulb. • Siphonozooids are present on the back of the rachis. • Anthocodia are present on the dorsal surface of pinnules. • Pinnules bear anthocodia.	Hence, the specimen seems to be *Pennatula* sp.



CHARACTER	INFERENCE
• Body bilaterally symmetrical, tissue grade and diploblastic. • Presence of 8 comb-plates. • Tentacles when present not more than 2. • An aboral sense organ present.	Hence, Phylum – Ctenophora
• Tentacles absent.	Hence, Class - Nuda
• Body conical shaped and laterally compressed. • Stomodium much wide. • Sense organ rounded at the aboral end. • 8 ribs extended for nearly the whole body length.	Hence, the specimen seems to be *Beroe* sp.



CHARACTER	INFERENCE
• Body bilaterally symmetrical, metamerically segmentedand elongated triploblastic. • Body covered with thin cuticle. • Body coelomate and anus & mouth terminal.	Hence, Phylum - Annelida
• Body segmentation well marked. • Each segment bears one pair of muscular parapodia. • Head distinct and bears pulp, cirri, sometime eyes.	Hence, Class - Polychaeta
• Prostomium distinct and bears appendages.	Hence, Subclass - Errantia
• Body cylindrical and metamerically segmented. • One pair of cirri present. • Head composed of 1. Prostomium, with tentacles, pulp, eyes. 2. Peristomium, with four pairs of tentacles. • Parapodia present in each segment except head and anul segment.	Hence, the specimen seems to be *Neries* sp.



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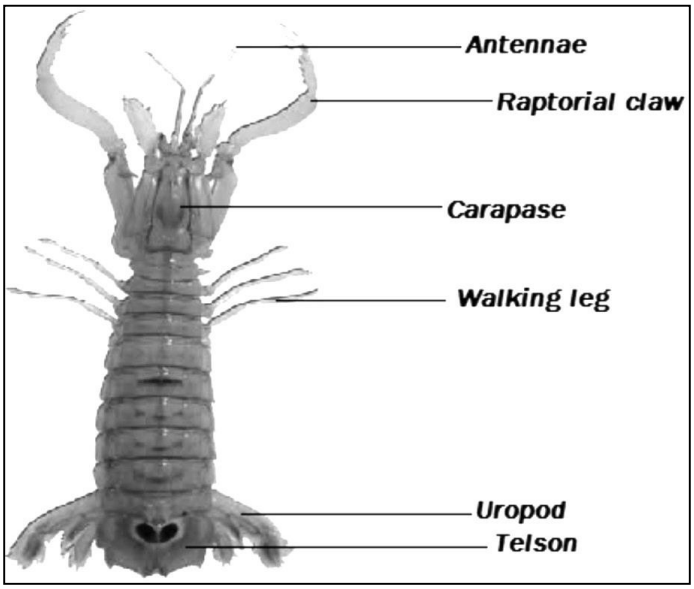
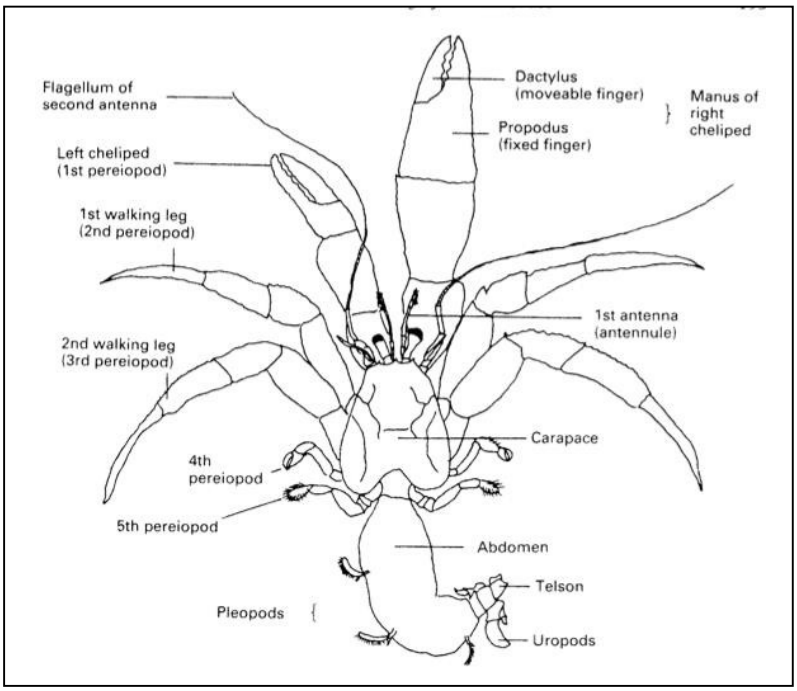
• Prostomium distinct and bears appendages.	Hence, Subclass - Errantia
• Body divided into anterior middle and posterior region with 9,5 & 30 segment respectively. • Middle region bears 3 fan like segment. • Mouth surrounded a collar like peristomium and provided into 2 peristomial cirri. • 10^{th} segment bears one pair of alliform notopodia. • Parapodia present. • Food cup and sucker present.	Hence, the above specimen seems to be *Chaetopterus* sp.



CHARACTER	INFERENCE
• Body segmented and covered by chitinous cuticle. • Each segmented bears paired externally jointed appendages. • Exoskeleton are two types – 1. Chitinous cuticle, thick, tough and covers the body. 2. Arthroidal membrane, soft thin, flexible and connect the segmental exoskeleton.	Hence, Phylum - Arthropoda
• Body divided into three parts – head, thorax and abdomen. • Head bears two or one pair of antennae, two pairs of maxillae and one pairs of mandible.	Hence, Subphylum - Mandibulata
• Dorsal surface covered by carapace. • Thorax and head fused to form cephalothorax. • Head bears two pairs of pre oral and three pairs of postoral appendages.	Hence, Class - Crustacea
• Mandible with pulp. • Presence of with eyes.	Hence, Subclass - Malacostraca
• Body whitish. • Rostrum movable and covers the head region. • Abdomen and telson are large and broad. • Antennules are triramos. • Pleopods are biramose and large. • Last four thoracic are free.	Hence, the specimen seems to be *Squilla* sp.



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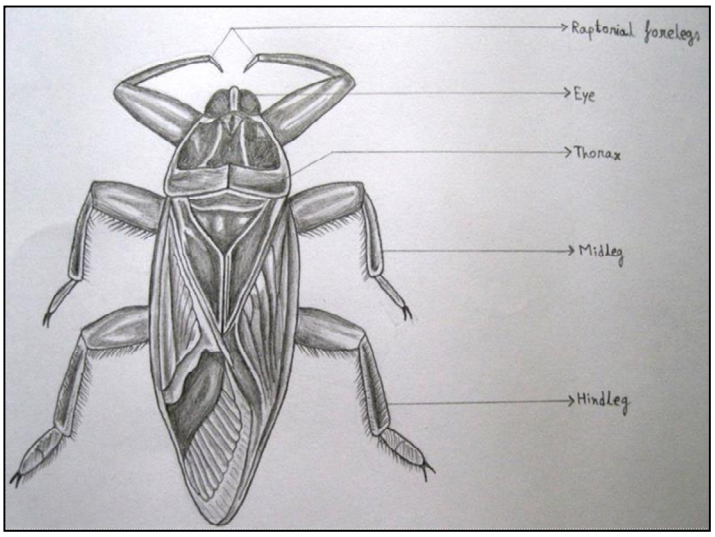
• Dorsal surface covers by carapace. • Thorax and head fused to form cephalothorax. • Head bears two pairs of pre oral and three pairs of post oral appendages.	Hence, Class - Crustacea
• Mandible with pulp. • Presence of with eyes.	Hence, Subclass - Malacostraca
• Asymmetrical body divided into broad cephalothorax and an abdomen. • Abdomen soft fleshy and coiled. • Right chela is larger than other. • Telson reduced. • Uropods hook like.	Hence, the specimen seems to be *Eupagurus* sp.



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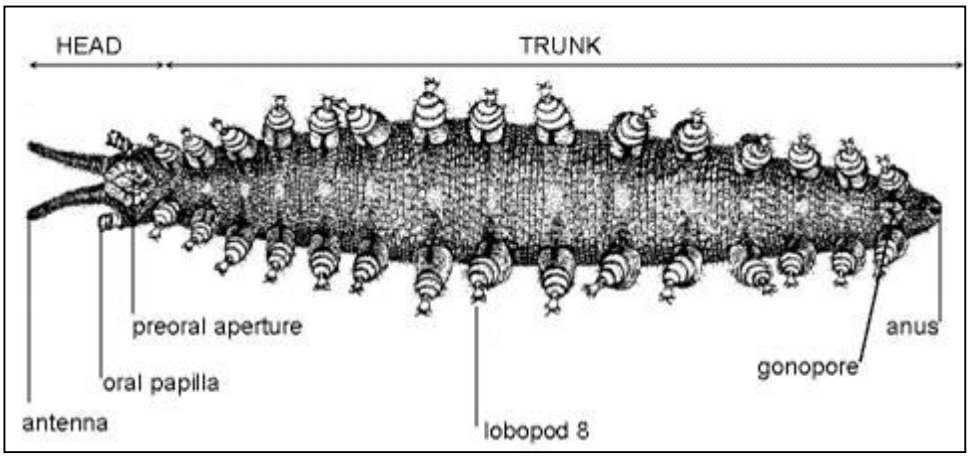
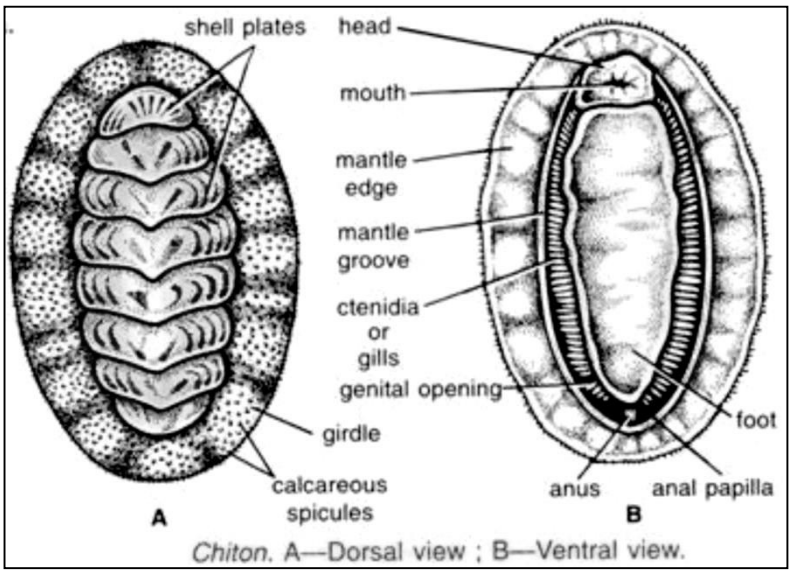
• Body divided into three parts – head, thorax and abdomen. • Head bears two or one paired of antennae two pairs of maxillae and one pair of mandible.	Hence, Subphylum - Mandibulata
• Thorax bears three pairs of jointed walking leg and one or two pairs of wings. • Abdomen bears 7-11 segments.	Hence, Class - Insecta
• Presence of wings in adult.	Hence, Subclass - Pterygota
• Antennae are 4 jointed. • Mouth parts modified for piercing and sucking. • Four legs raptorial type, middle and hind legs modified for swimming.	Hence, the specimen seems to be *Belostoma* sp.

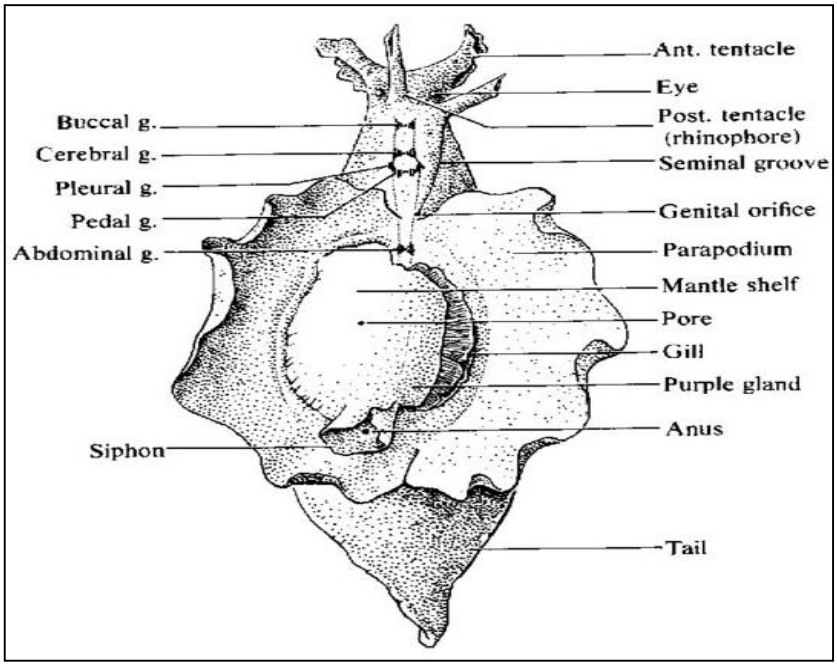


CHARACTER	INFERENCE
• Body is segmented, exoskeleton absent. • Presence of thin cuticle on body surface. • Head consist of 3 segments. One pre oral& two post oral. • Presence of many paired short, unjointed stumpy and clawed appendages.	Hence, Phylum - Onychophora
• Caterpillar like soft body with velvety skin. • Cylindrical body divided into distinct head and elongated trunk. • Mouth and anus at opposite end. • Genital opening between last pair of legs. • Post oral segment bears jaws and oral papillae.	Hence, the specimen seems to be *Peripatus* sp.

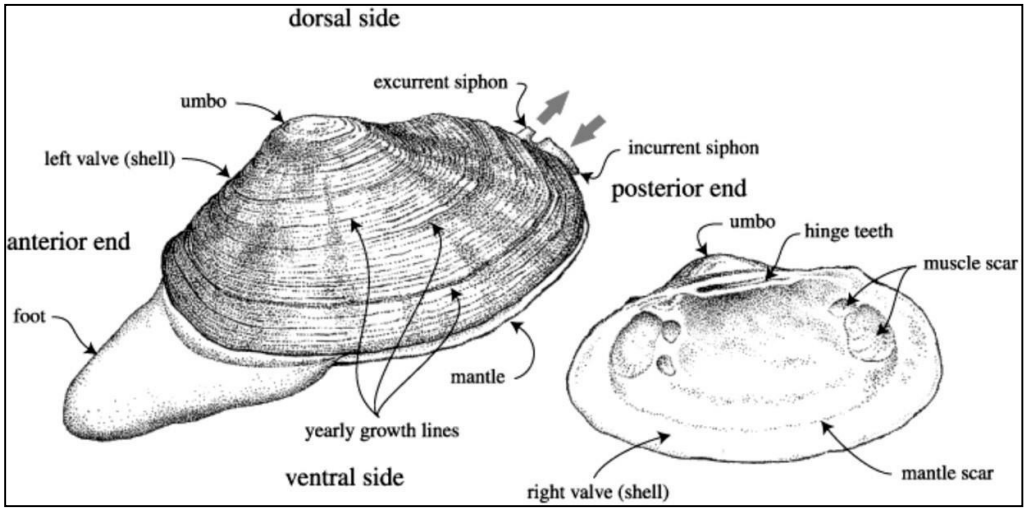


Chiton. A—Dorsal view ; B—Ventral view.

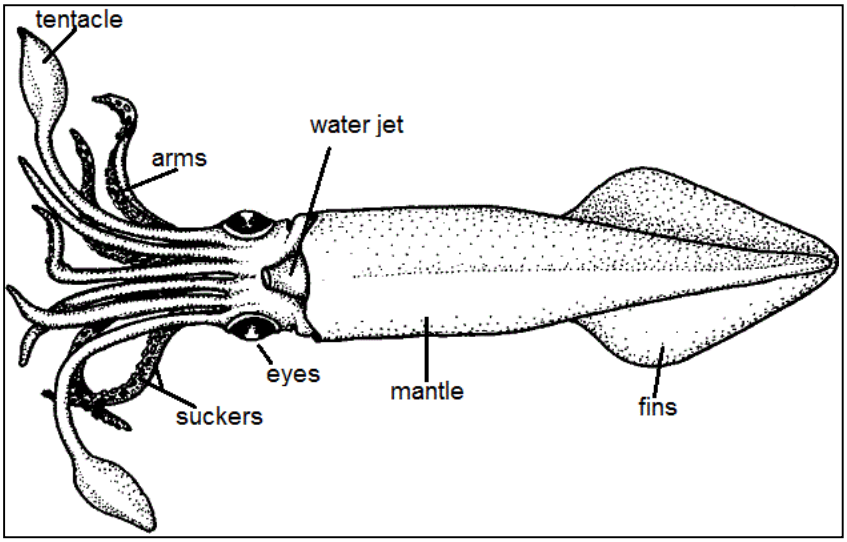
CHARACTER	INFERENCE
• Body soft and unsegmented in adult. • Body divided into head, ventral muscular foot anddorsal visceral mass. • Dorsal visceral mass enclosed by mantle. • External body covered by exoskeleton calcareous shell.	Hence, Phylum – Mollusca
• Body elongated and bilaterally symmetrical. • Pedal and pallial nerve cord present with cross connectives. • Head reduced.	Hence, Class - Amphineura
• Presence of shell valve with insertion plate and teeth. • External gills present on mentle groove. • Dorsal surface is convex and ventral part bears a broad foot.	Hence, Subclass - Polyplacophora
• Dorsal part is convex, ventral part is flat. • Eyes and tentacles absent. • Lines of growth present on valves. • Labial pulp present. • Shall valves overlapping.	Hence, the specimen seems to be *Chiton* sp.



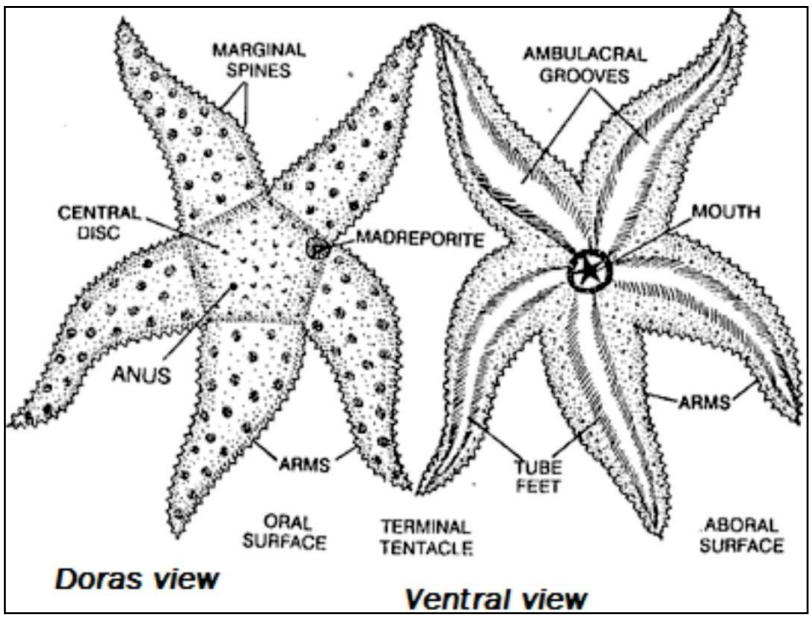
CHARACTER	INFERENCE
• Body soft and unsegmented in adult. • Body divided into head ventral muscular foot and dorsal visceral mass. • Dorsal visceral mass enclosed by mantle. • External body covered by exoskeleton calcareous shell.	Hence, Phylum – Mollusca
• A muscular foot present behind digestive system and visceral mass. • Foot large and flat. • Head bears one or two pairs of tentacles.	Hence, Class – Gastropoda
• Mantle either on posterior end or absent. • Secondary branchia replaces ctenidium. • Shell either reduced or internal or absent. • Anus directed backward.	Hence, Subclass - Opisthobranchia
• Body soft shiny, fleshy and whitish in colour. • Anterior part bears head and neck. • Head bears rhinophores, tentacles and seminal groove. • Eyes present at the base of rhinophores. • Parapodia present.	Hence, the specimen seems to be *Aplysia* sp.



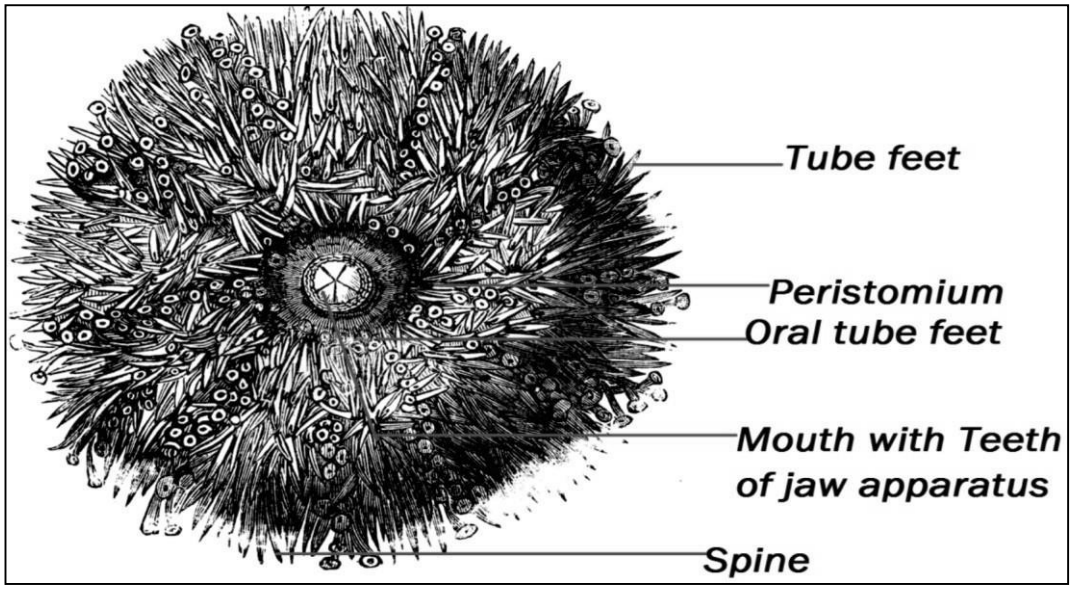
CHARACTER	INFERENCE
• Body soft and unsegmented in adult. • Body divided into head ventral muscular foot and dorsal visceral mass. • Dorsal visceral mass enclosed by mantle. • External body covered by exoskeleton calcareous shell.	Hence, Phylum – Mollusca
• Body is enclosed by two bivalve shell hinged togetherdorsally. • Body bilaterally symmetrical and laterally compressed • Foot ventral and plough shaped • One paired ctenidium present	Hence, Class - Bivalvia
• Mouth present between two labial pulps • Ctenidial filament are folded and inter and inter connected • Siphon present	Hence, Subclass – Lamellibranchi
• Anterior part pointed and posterior part rounded • Foot cylindrical • Presence of byssus threads, projected ventrally • The umbo shifted anteriorly. • Mantle edge lined by mantle tentacles	Hence the specimen seems to be *Mytilus* sp.



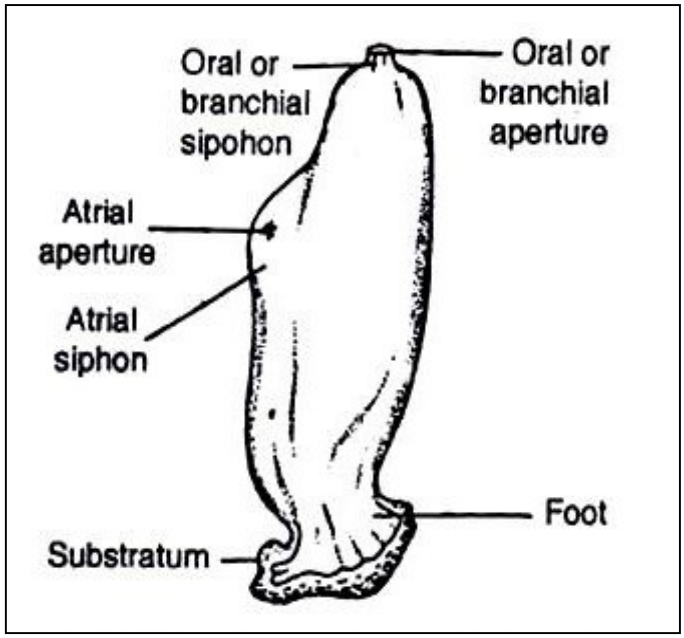
CHARACTER	INFERENCE
• Body soft and unsegmented in adult. • Body divided into head ventral muscular foot and dorsal visceral mass. • Dorsal visceral mass enclosed by mantle. • External body covered by exoskeleton calcareous shell.	Hence, Phylum – Mollusca
• Head well developed and bears circum oral arms and eyes. • Foot modified into oral arms and a siphon. • Mantle naked.	Hence, Class - Cephalopoda
• Eyes prominent with crystalline membrane.	Hence, Subclass - Coleoidea
• Body torpedo like and divided into head trunk & neck. • Lateral abdominal fin triangular and united posteriorly. • Head contain 8 oral arms with suckers. • Presence of two large tentacles with suckers at the tip.	Hence, the specimen seems to be *Loligo* sp.



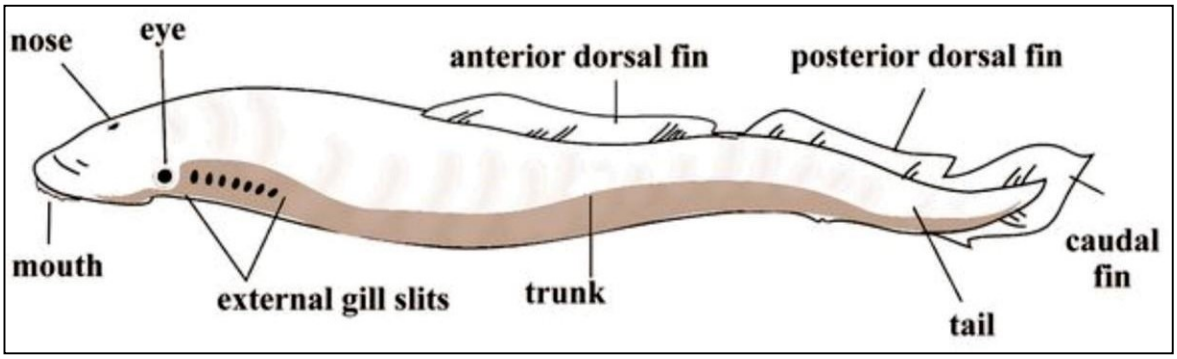
CHARACTER	INFERENCE
• Pentamerically symmetrical body with distinct oral & aboral surface. • Exoskeleton with calcareous spine or ossicles. • Present of five intervening ambulacral grooves on body surface.	Hence, Phylum-Echinodermata
• Body star shaped. • Pentagonal body and ambulacral groove open.	Hence, Subphylum - Asterozoa
• Arms arise from small central disc. • Madreporite on the aboral surface.	Hence, Class - Asteroidea
• Five radiating arms arise from pentagonal central disc. • Aboral surface bears spines all over anus and a madreporite pedicellariae. • Tube feet arranged in two rows, present in the ambulacral groove • Mouth present at the central of oral surface	Hence, the specimen seems to be *Asterias* sp.



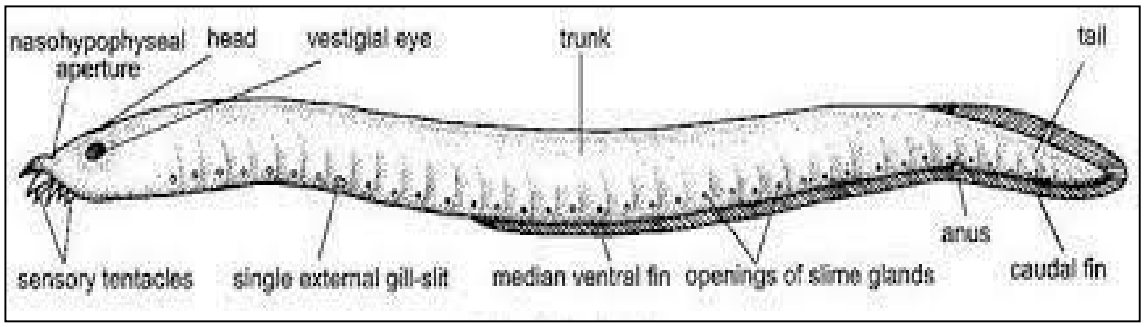
CHARACTER	INFERENCE
• Pentamerically symmetrical body with distinct oral & aboral surface. • Exoskeleton with calcareous spine or ossicles. • Present of five intervening ambulacral on body surface.	Hence, Phylum - Echinodermata
• Mouth on oral and anus on aboral surface. • Ambulacral grooves are covered.	Hence, Subphylum - Echinozoa
• Ambulacral groove meridional in position. • Ambulacral plates bear pores through which tube feet project.	Hence, Class - Echinoidea
• Tube feet with terminal suckers. • Madreporite on aboral surface.	Hence, Subclass - Euechionoidea
• Body nearly spherical with a flat oral and domed aboral surface and covered by spine. • Test rigid, ambulacral and inter-ambulacral zones clearly distinguished. • Presence of Aristotle's lantern, projected from mouth. • Pdeicellariae and sphaeridia present between the spines. • The madreporite and gonopore are present near arms.	Hence, the specimen seems to be Sea-urchin



CHARACTER	INFERENCE
• Bilaterally symmetrical, triploblastic and coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in early stages of life. • Presence of pharyngeal gill silt (at least some stages of life). • Presence of pharyngeal endostyle that may be converted into thyroid gland.	Hence, Phylum – Chordata.
• Notochord present in larva tail. • Body covered by tunic and test.	Hence, Subphylum- Urochordata
• Test permanent and thick. • Mouth terminal. • Adult without notochord and locomotory organ.	Hence, Order - Ascidiacea
• Unpaired gonad lies close to intestine. • Body may or may not be divided into thorax abdomen.	Hence, Order - Enterogona
• Body is wrinkle sac like with a broad base. • Solitary and fixed with one end. • The free end bears two aperture, large, branchial aperture and small atrial aperture borne on siphone. • Terminal branchial siphon is eight lobed. • Sub terminal artial siphon is six lobed.	Hence, the specimen seems to be *Ascidia* sp.



CHARACTER	INFERENCE
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in early stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted into thyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw present.	Hence, Superclass – Agnatha
• Nostril is single and median. • Fins are not supported by fin rays. • Gills slits are 6-14 pairs.	Hence, Order – Cyclostomata
• Body is smooth eel like. • Cylindrical head, trunk and tail laterally compressed. • Upper lip highly developed and formed a buccal funnel. • Mouth at the bottom of buccal funnel with horny teeth. • Skeleton cartilaginous. • One pair of developed eyes covered by transparent skin. • Well developed dorsal fin and a separate caudal fin. • Paired fins absent.	Hence, the specimen seems to be *Petromyzon* sp.



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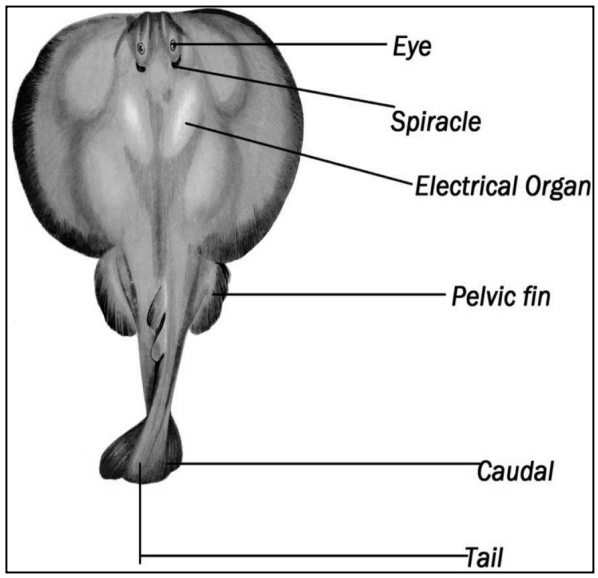
• Functional jaw absent.	Hence, Superclass – Agnatha
• Nostril is single and median. • Fins are not supported by fin rays. • Gills slits are 6-14 pairs.	Hence, Order – Cyclostomata
• Scale less cylindrical body is divided into head, trunk and tail. • Only caudal fin present which contains two ventral and anal fin. • Mouth is surrounding by lips. • Eyes are vestigial. • No buccal funnel. • Single gill slits or branchial aperture present. • Present of a number of mucous pores at the ventrolateral side of the body.	Hence, the specimen seems to be *Myxine* sp.



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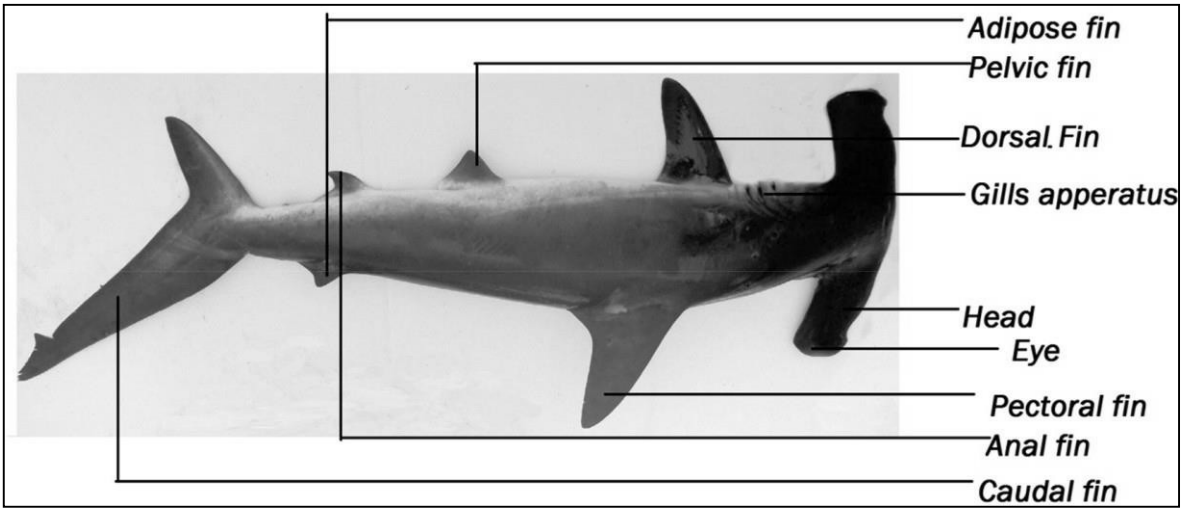
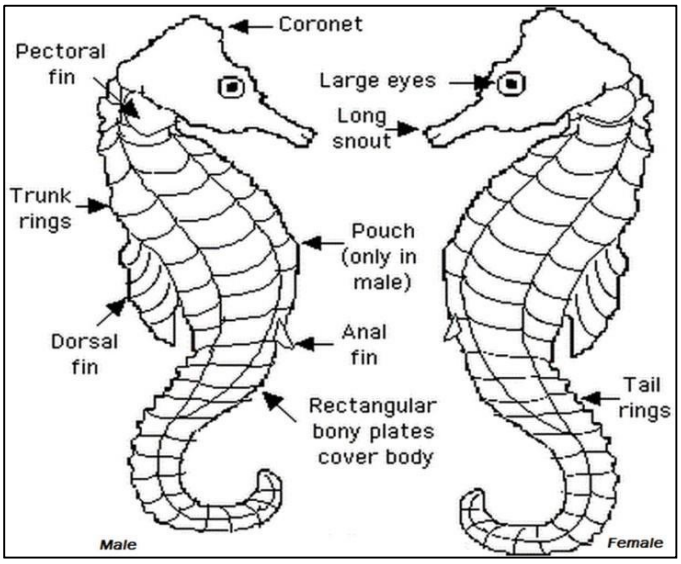
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Gills present. Operculum absent. • Endoskeleton cartilaginous. • Tail heterocercal.	Hence, Class – Chondricthyes
• 5-7 pairs gill slit exterior on each side. • Present of ventrally located subterminal mouth.	Hence, Subclass - Elasmobranchii
• Pectoral disc is rounded. • Eyes on dorsal surface.	Hence,Order – Rajiformes
• Sub circular disc like body. • Skin smooth without scale. • Presence of one pair electric organ between head and pectoral fin. • Tail is short – two dorsal and a caudal fin present. • Spiracle present behind the eyes. • Mouth is slit like aperture.	Hence, the specimen seems to be *Torpedo* sp.

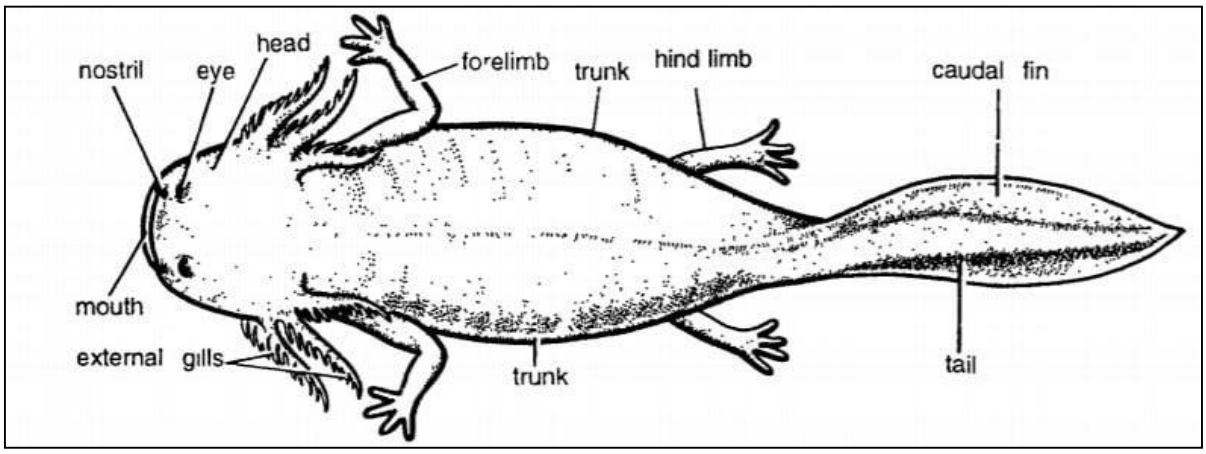


CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in early stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replace by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Gills present. Operculum absent. • Endoskeleton cartilaginous. • Tail heterocercal.	Hence, Class – Chondricthyes
• 5-7 pairs gill slit exterior on each side. • Present of ventrally located subterminal mouth.	Hence, Subclass - Elasmobranchii
• Two dorsal fins without spines present. • Anal fin present. • 5gill slits present. Gill rackets absent. • Present of caudal fin without fin rays.	Hence, Order – Carcharhiniformes
• Head drawn into lateral lobe and looks like hammer. • Eyes located at distal end of the lobe. • Eyes contain nictitating membrane. • Crescent shaped ventrally located mouth. • Dorsal fins are without spine. • First dorsal fin large and second dorsal and anal fin small.	Hence, the specimen seems to be *Sphyrna* sp.

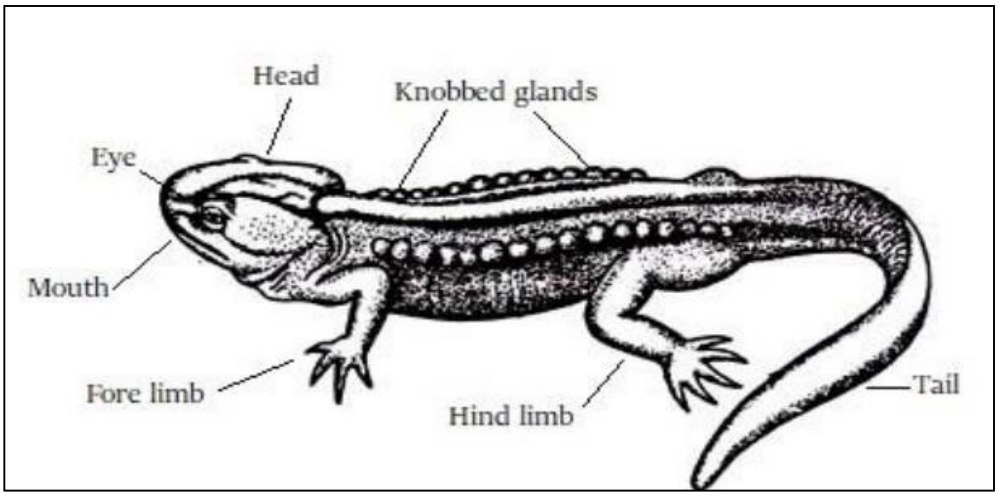


CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in early stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replace by vertebral column.	Hence,
• Cranium present.	Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Operculum present. • Gills are covered by operculum. • Caudal fin is heterocercal. • Bony endoskeleton.	Hence, Class - Osteichthyes
• Fins are supported by fin ray. • Paired fins have broad base.	Hence, Subclass - Actinopterygii
• Scales are covered by epidermis or absent. • Gills are fully filamentous. • Paired fins without central axis.	Hence, Superorder - Teleosti
• Dorsal and anal fins are in opposite position. • Caudal fin absent. • Mouth usually small.	Hence, Order - Gasterosteiformes

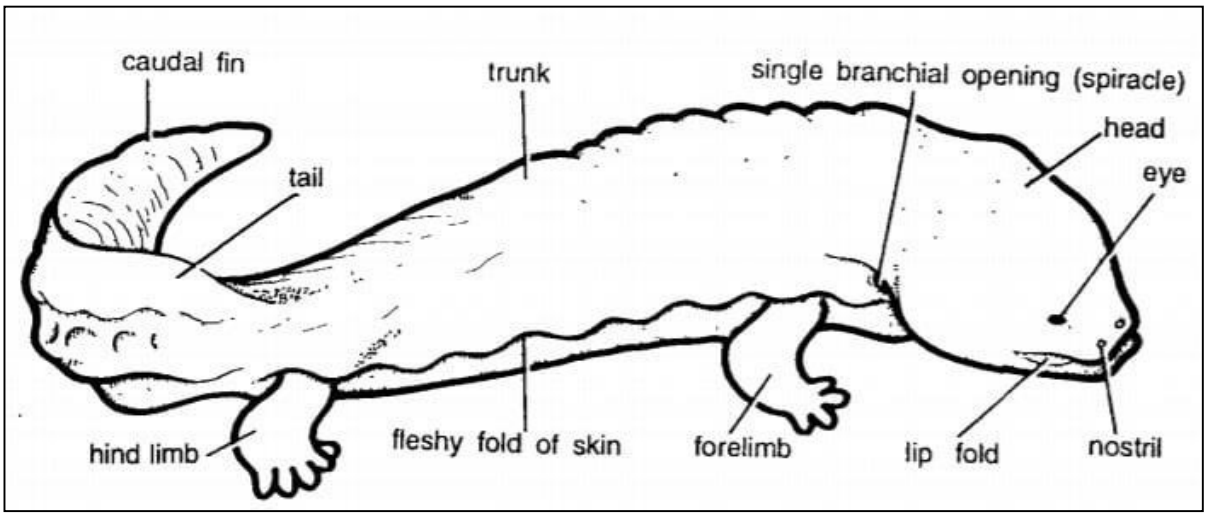
• Trunk with 10-12 exoskeleton rings/ plates. • Tail longer and prehensile. • Mouth is situated at the top of the tubular snout. • Presence of brood pouch on the belly in male. • Presence of anal fin on the belly in female. • Pectoral fin is transparent behind the operculum. • Head bent at the right angle to the trunk.	Hence, the specimen seems to be *Hippocampus* sp.



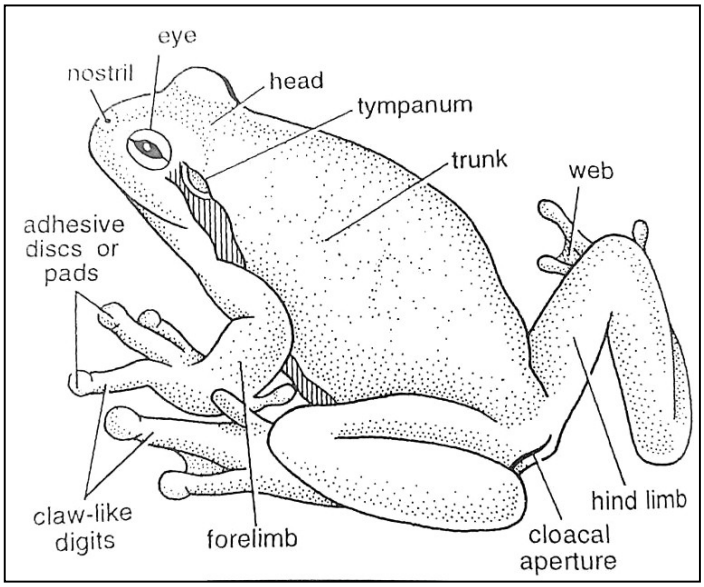
CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages oflife). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Skin naked, smooth or rough and moist and glandular. • Heart three chambered. • Limbs when present are two paired. • Tetrapod animal with 4-5 toes or less.	Hence, Class - Amphibia
• Scaleless smooth skin with numerous glands. • Skin is broad and the orbits are enlarged.	Hence, Subclass – Lissamphibia
• Well developed tail present. • Limbs are two paired, equal and weak.	Hence, Order - Urodela
• Three pairs of gills present at postereolateral side of the head. • Well developed eyes present with lids. • Limbs poorly developed and equal in size. • Tails largely compressed with tail fin. • Nostril present. • Body divided into head trunk tail and caudal fin. • Dorsal fin is continuous with caudal fin. • Jaw with vomarin teeth.	Hence, the specimen seems to be Axolotl larva



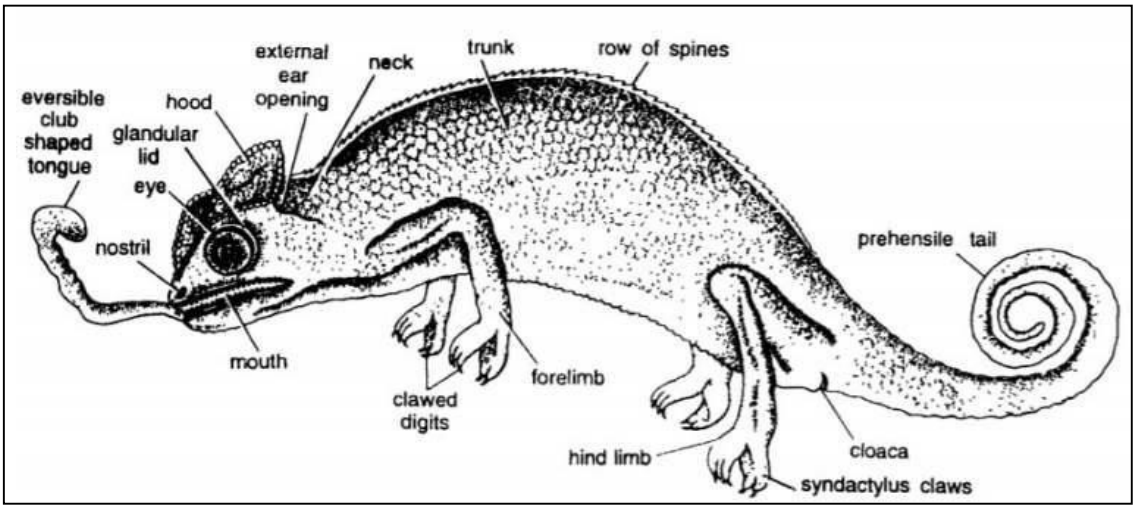
CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Skin naked, smooth or rough and moist and glandular. • Heart three chambered. • Limbs when present are two paired. • Tetrapod animal with 4-5 toes or less.	Hence, Class - Amphibia
• Scaleless smooth skin with numerous glands. • Skin is broad and the orbits are enlarged.	Hence, Subclass – Lissamphibia
• Well developed tail present. • Two pair of limbs, equal and weak.	Hence, Order - Urodela
• Head trunk bears two dorsolateral ridges. • Presence of mid dorsal ventral ridge. • Its colour is above black brown and low is pale. • Tail laterally compressed with a yellowline. • Eyes bulging. • Head broader than length. • Tongue is small sub-circular.	Hence, the specimen seems to be *Tylototriton* sp.



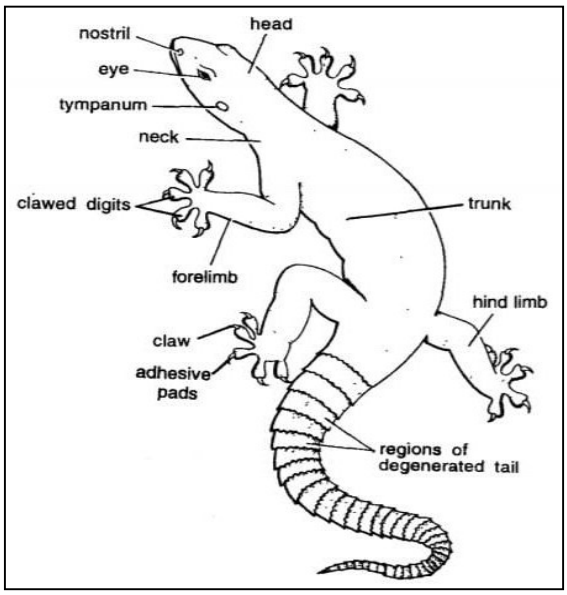
CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Skin naked, smooth or rough and moist and glandular. • Heart three chambered. • Limbs when present are two paired. • Tetrapod animal with 4-5 toes or less.	Hence, Class - Amphibia
• Scaleless smooth skin with numerous glands. • Skin is broad and the orbits are enlarged.	Hence, Subclass – Lissamphibia
• Well developed tail present. • Two pairs of limbs, equal and weak.	Hence, Order - Urodela
• Large sized body. • Tail laterally compressed to form tail fin. • In adult gills are present. • Presence of single branchial aperture in left side. • Eyes are small and lidless. • Limbs are functional with 4 finger and 5 toes. • Body and trunk much flattened.	Hence, the specimen seems to be *Cryptobranchus* sp.



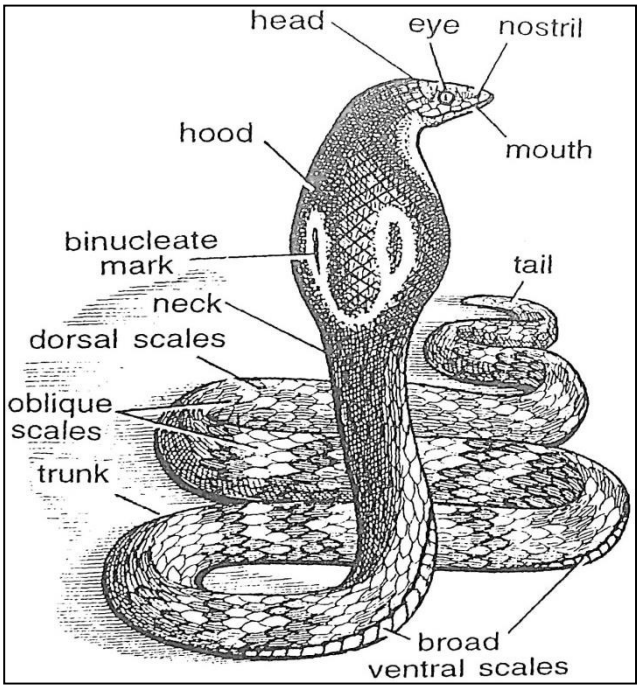
CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Skin naked, smooth or rough and moist and glandular. • Heart three chambered. • Limbs when present are two paired. • Tetrapod animal with 4-5 toes or less.	Hence, Class - Amphibia
• Scaleless smooth skin with numerous glands. • Skin is broad and the orbits are enlarged.	Hence, Subclass – Lissamphibia
• Body is short and tailless. • 4 legs present, posterior legs are longer than anterior. • Well developed eye lids.	Hence, Order - Anura
• Skin smooth and hygroscopic. • Eyes are large and well developed with horizontal pupil. • Teeth present only on the upper jaw. • Digits of the hind limbs are webbed. • Digits and toes have adhesive pads or disc.	Hence, the specimen seems to be *Hyla* sp.



CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass - Gnathostomata.
• Skin dry rough non glandular and usually have epidermal cell. • Tail present. • Pentadactylous limbs present with claw. • Respiratory organ are lungs.	Hence, Class – Reptilia
• Presence of temporal vacuities (diapsid skull).	Hence, Subclass - Lepidosauria
• Exoskeleton with spines, shields or scales. • Quadrate movable. • Head laterally compressed with oral hood.	Hence, Order – Squamata
• Trunk with a row of spine along with mid dorsal line. • Eyes large and bulging. • Very long projectile spoon shaped tongue present. • Tail prehensile.	Hence, the specimen seems to be *Chemaeleo* sp.



CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass - Gnathostomata.
• Skin dry rough non glandular and usually have epidermalcell. • Tail present. • Pentadactylous limbs present with claw. • Respiratory organ are lungs.	Hence, Class – Reptilia
• Presence of temporal vacuities (diapsid skull).	Hence, Subclass - Lepidosauria
• Exoskeleton with spines shields or scales. • Quadrate movable. • Head laterally compressed with oral hood.	Hence, Order – Squamata
• Lizard shaped body. • Skin is granulated, spotted and gray in colour. • Body is covered with minute glandular scale. • Each toe has rigid adhesive pads and sharp claw. • Tail is shorter than the body.	Hence, the specimen seems to be *Gekko* sp.



CHARACTER	*INFERENCE*
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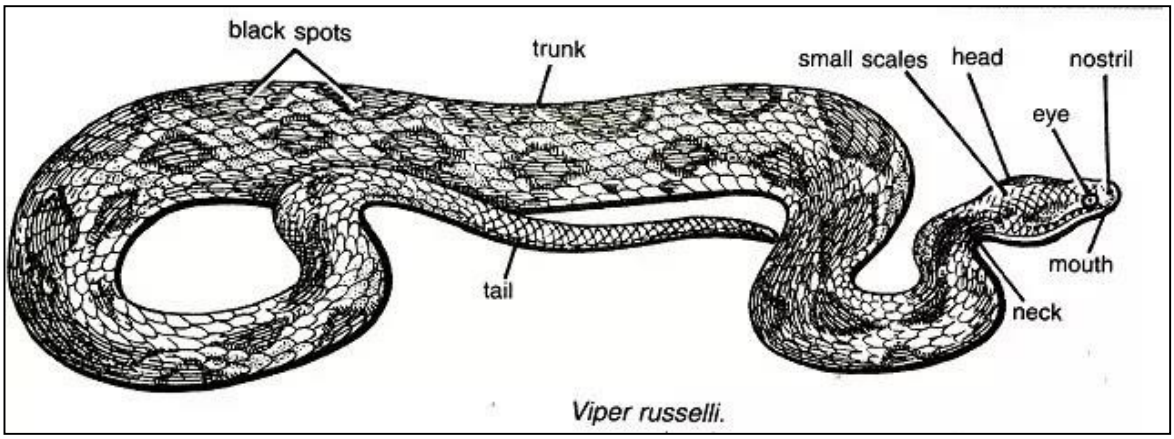
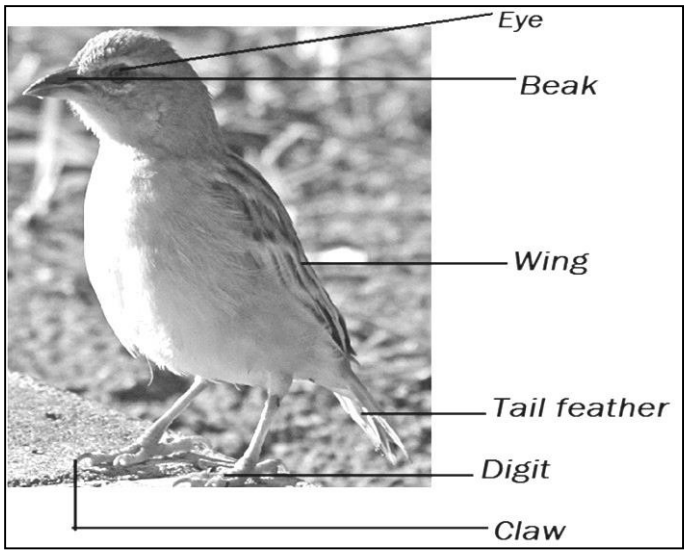
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass - Gnathostomata.
• Skin dry rough non glandular and usually have epidermal cell. • Tail present. • Pentadactylous limbs present with claw. • Respiratory organ are lungs.	Hence, Class – Reptilia
• Presence of temporal vacuities (diapsid skull).	Hence, Subclass - Lepidosauria
• Exoskeleton with spines, shields or scales. • Quadrate movable. • Head laterally compressed with oral hood.	Hence, Order – Squamata

• Body is covered with scale and without limb. • Presence of an eye glass pattern binocellate mark on theback of hood. • Neck is dilated and form hood. • Fang is followed by 1-3 teeth. • Presence of small eyes with immovable eyelids.	Hence, the specimen seems to be *Naja* sp.



Viper russelli.

CHARACTER	*INFERENCE*
• Bilaterally symmetrical, triploblastic coelomate. • A dorsal hollow tubular nerve cord present. • Presence of notochord only in some stages of life. • Presence of pharyngeal gill slit (at least some stages of life). • Presence of pharyngeal endostyle that may be converted intothyroid gland.	Hence, Phylum – Chordata.
• Notochord replaced by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass - Gnathostomata.
• Skin dry rough non glandular and usually have epidermalcell. • Tail present. • Pentadactylous limbs present with claw. • Respiratory organ are lungs.	Hence, Class – Reptilia
• Presence of temporal vacuities (diapsid skull).	Hence, Subclass - Lepidosauria
• Exoskeleton with spines, shields or scales. • Quadrate movable. • Head laterally compressed with oral hood.	Hence, Order – Squamata
• Head larger triangular and flat covered with small scalewith 'V' marked. • Body is elongated and cylindrical, no limbs. • Presence of large black patch on the back. • Body covered by keeled scale. • A pair of fang present in front of upper jaw. • Nostrils large. • Ventral plate large completely covered the body.	Hence, the specimen seems to be *Vipera* sp.



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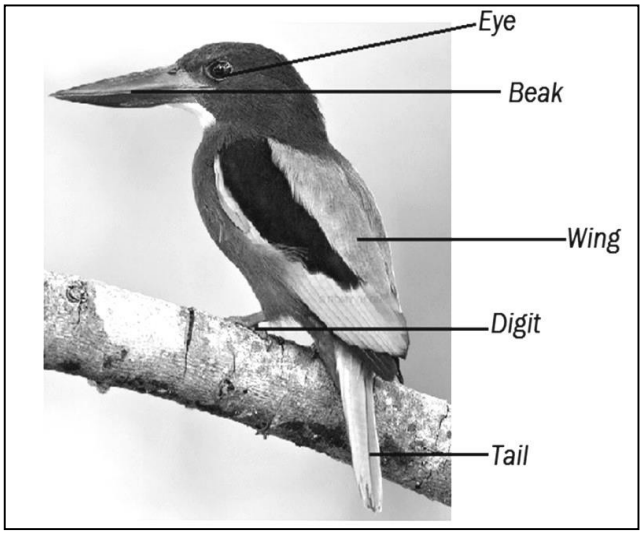
• Notochord replace by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Beak present. • Feather present. • Forelimbs covered into wings and hind limbs bear clawed digits and scales. • Air sac present. • Bone spongy and light.	Hence, Class – Aves
• Tail short with a terminal pygostyle.	
• Tail feather are semi circularly around on pygostyle. • Beak toothless.	Hence, Subclass - Neornithes
• Skull neognathus. • Feather with barbs, barbules and hooklets. • Well developed sternum with keel.	Hence, Superorder - Neognathae
• Plumage variously coloured. • 4 toes present 3 toes in front and toes behinds. • Foot adapted for perching.	Hence, Order - Passeriformes
• Dark streak fulvous brawn above and plain whitish fulvous below. • Beak stout, small and pointed. • Plumage dull – gray and white.	Hence, the specimen seems to be *Ploceus* sp.



CHARACTER	*INFERENCE*
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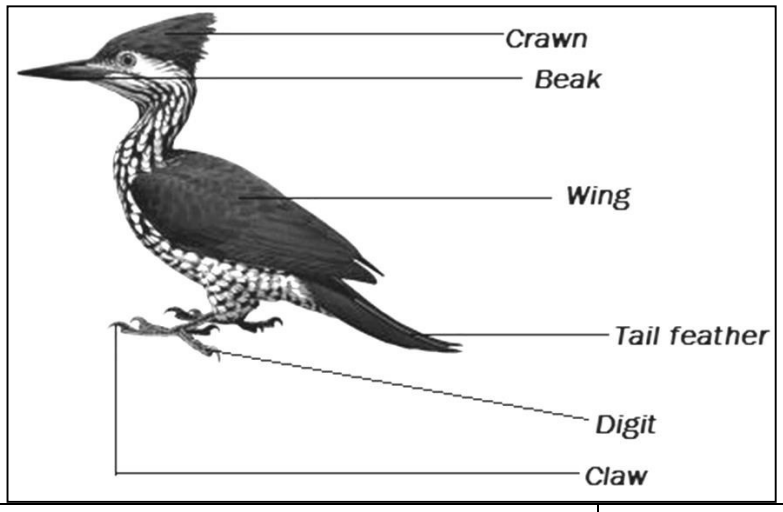
• Notochord replace by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Beak present. • Feather present. • Forelimbs covered into wings and hind limbs bear clawed digits and scales. • Air sac present. • Bone spongy and light.	Hence, Class – Aves
• Tail short with a terminal pygostyle. • Tail feather are semicircularly around on pygostyle. • Beak toothless.	Hence, Subclass - Neornithes
• Skull neognathus. • Feather with barbs, barbuls and hooklets. • Well developed sternum with keel.	Hence, Superorder - Neognathae
• Long strong and pointed beak present. • 3^{rd} and 4^{th} toes fused at the base.	Hence, Order - Coraciiformes
• Brilliant carburise blue with deep chocolate brown head to neck and breast. • Conspicuous white breast. • Long, heavy, pointed red beak.	Hence, the specimen seems to be *Halcyon* sp.



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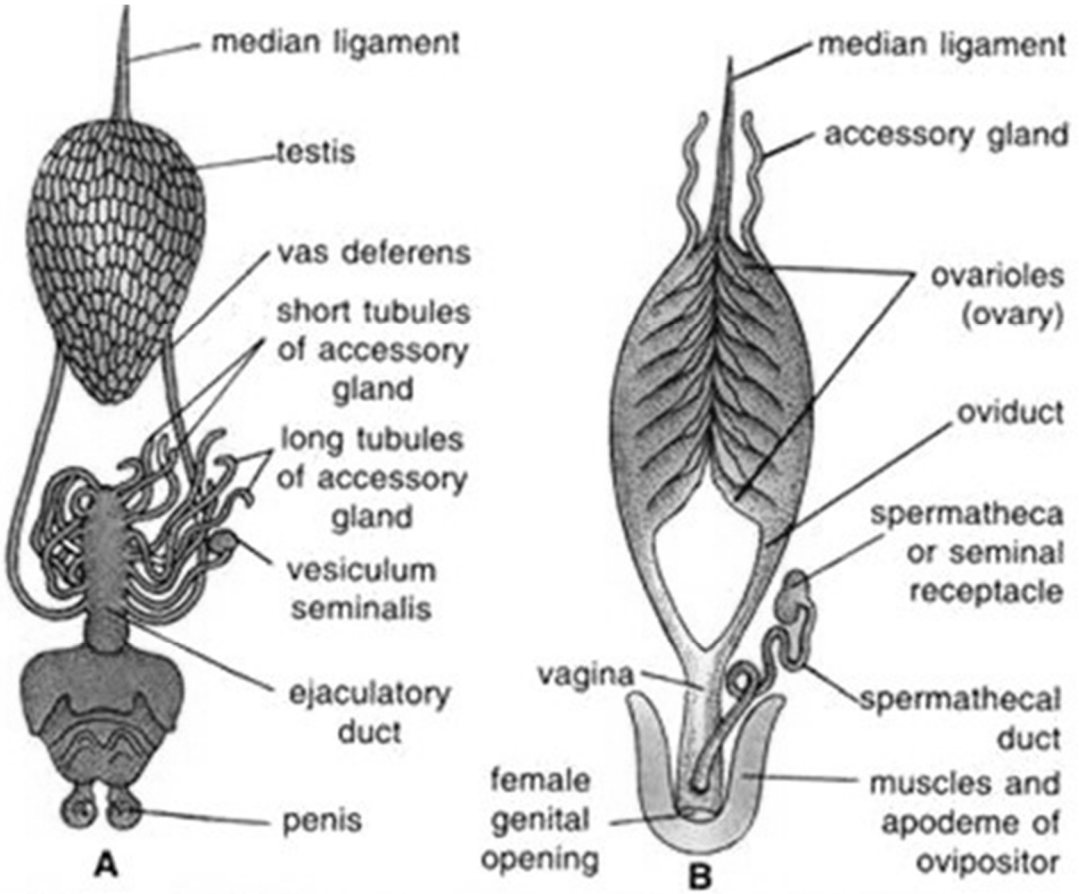
• Notochord replace by vertebral column. • Cranium present.	Hence, Subclass – Vertebrata
• Functional jaw absent. • Paired appendages present. • Teeth may or may not be present.	Hence, Superclass – Gnathostomata.
• Beak present. • Feather present. • Forelimbs covered into wings and hind limbs bearclawed digits and scales. • Air sac present. • Bone spongy and light.	Hence, Class – Aves
• Tail short with a terminal pygostyle. • Tail feather are semicircularly around on pygostyle.	Hence,
• Beak toothless.	Subclass - Neornithes
• Skull neognathus. • Feather with barbs, barbuls and hooklets. • Well developed sternum with keel.	Hence, Superorder - Neognathae
• Hard and powerful beak present. • 2nd and 3rd digits anteriorly place and 1st and 4th digitback warding directed.	Hence, Order- Piciformes
• Presence of crown on head. • Large eyes. • Upper plumage contains black and white spot.	Hence, the specimen seemsto be *Dinopium* sp.

ii) Minor Dissection:



Grasshopper. A—Male reproductive system; B—Female reproductive system.

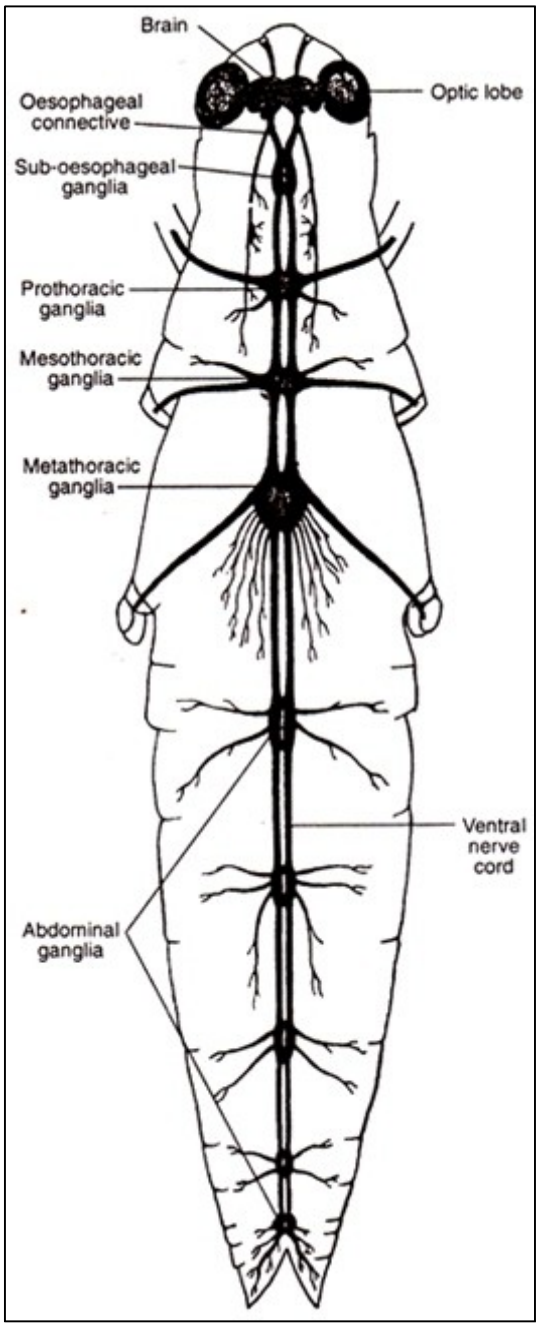


Fig: Nervous System of Grasshopper

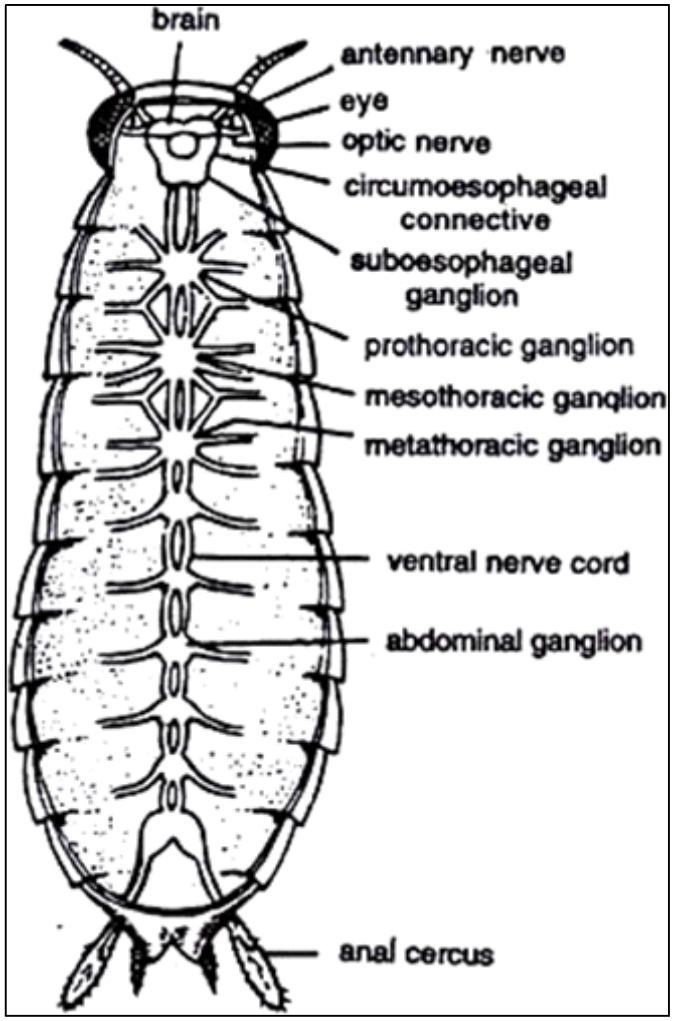


Fig: Stomatogastric Nervous System of *Periplaneta* sp.

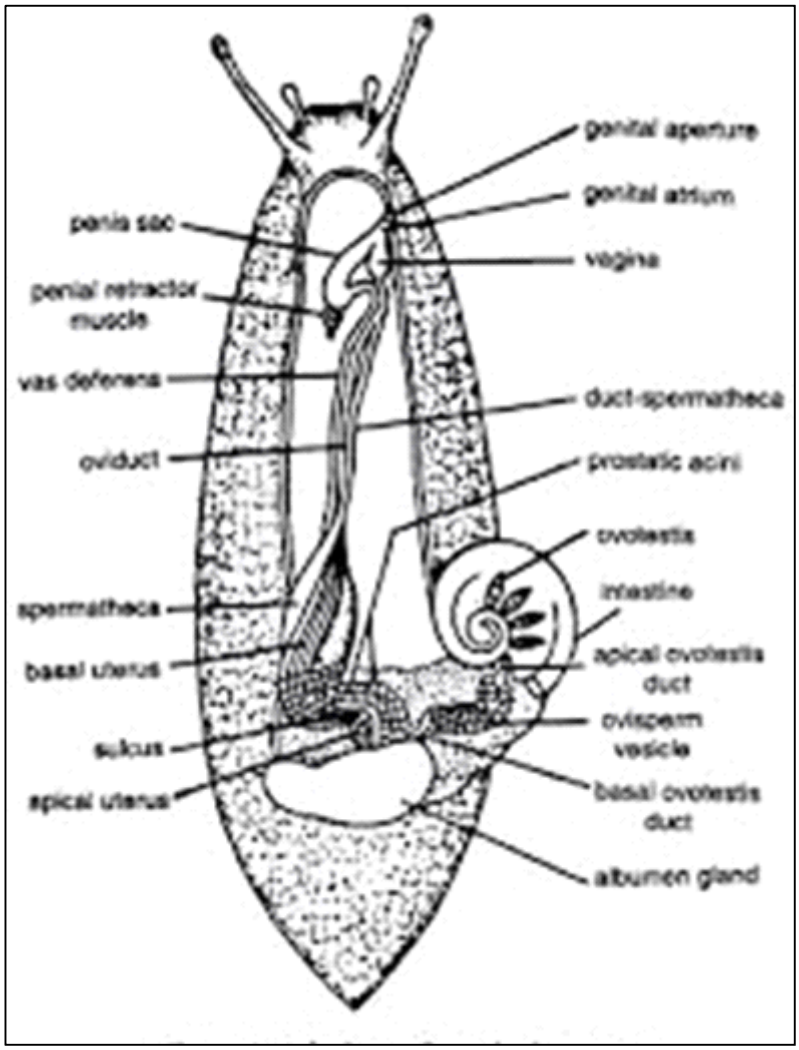


Fig: Reproductive System of *Achatina sp*.

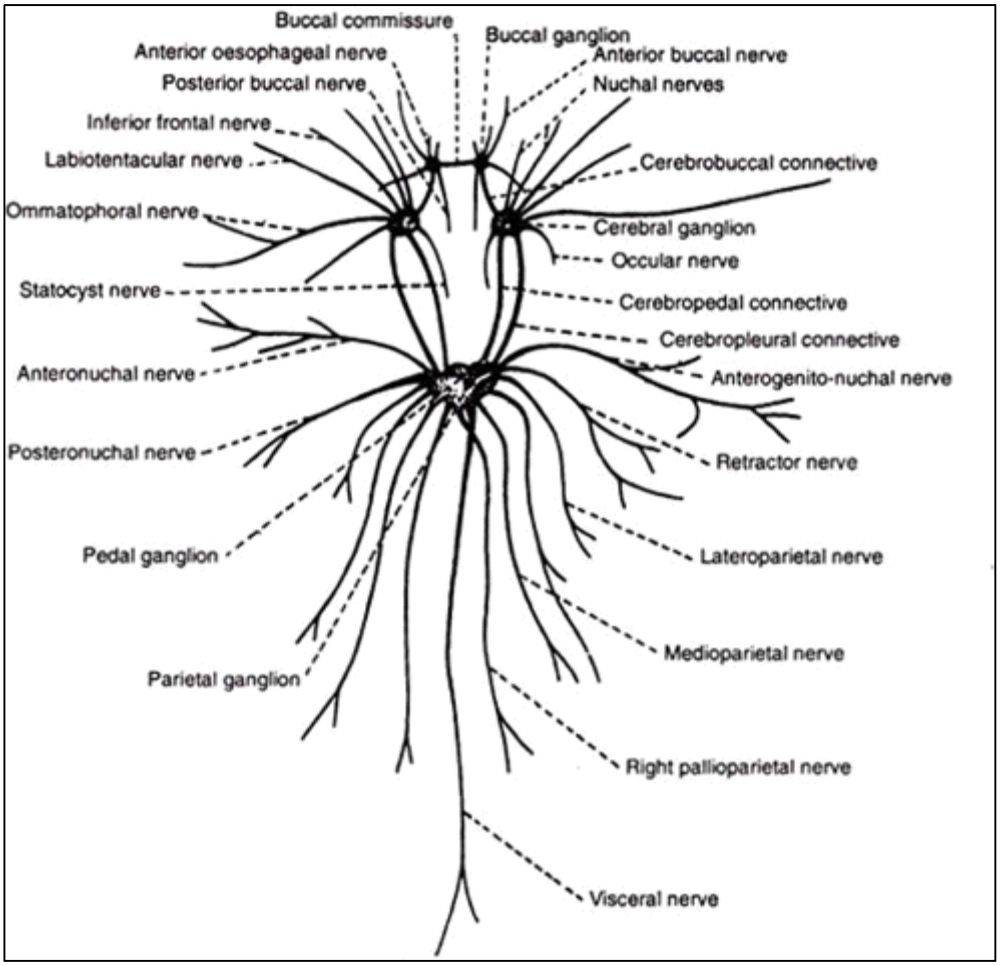


Fig: Nervous System of *Achatina sp*.

2. Chordate Biology:
i) Major Dissection:

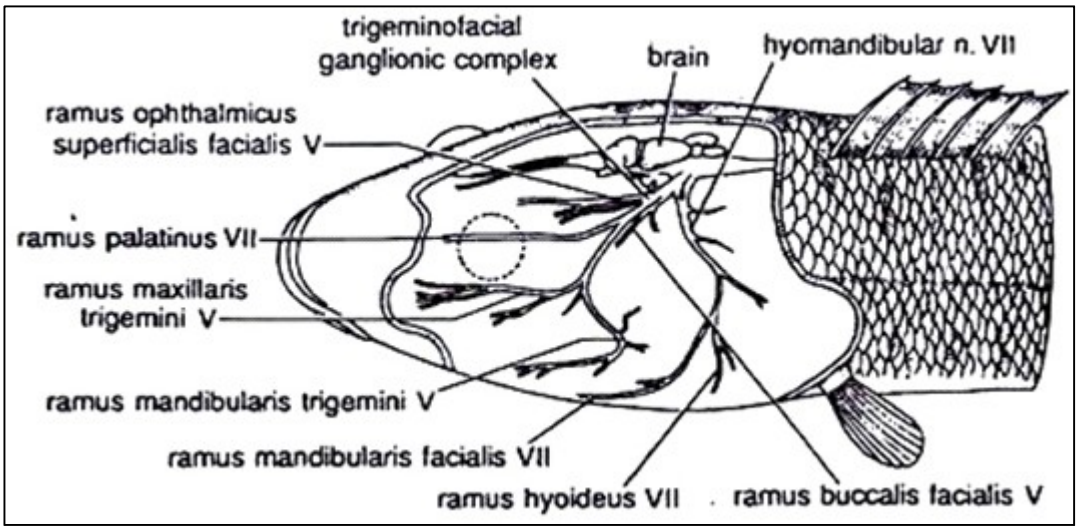
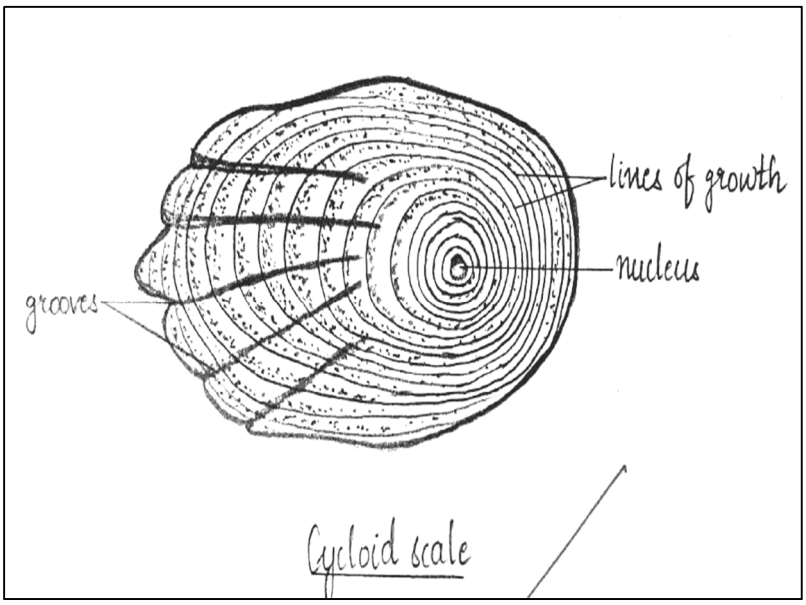


Fig: Brain, V, VII Cranial Nerves of *Chana sp.*

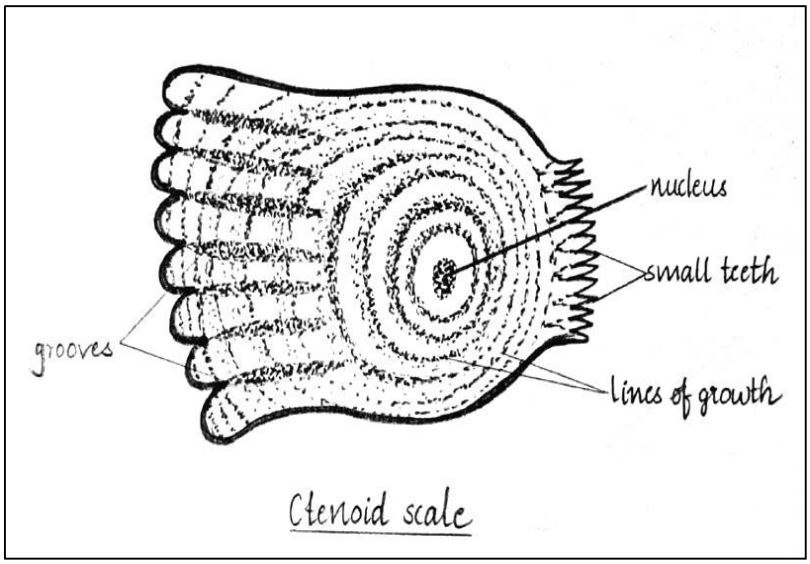
Minor Dissection: Fish scale and pecten of bird

A. Fish scales
Cycloid Scales



Identifying Characters
1. Thin, flexible, translucent plates.
2. Circular in outline, thicker in the centre and marked with several concentric lines of growth which can be used for determining the age of the fish.
3. They are found in a large number of teleost fishes having soft rayed fins, such as ***Labeo, Catla, Barbus, Cirrhina***, etc.
4. The central part of the scale is called the ***focus*** and is the first part to develop. In many species, oblique grooves or radii run from the focus towards the margin of the scale.

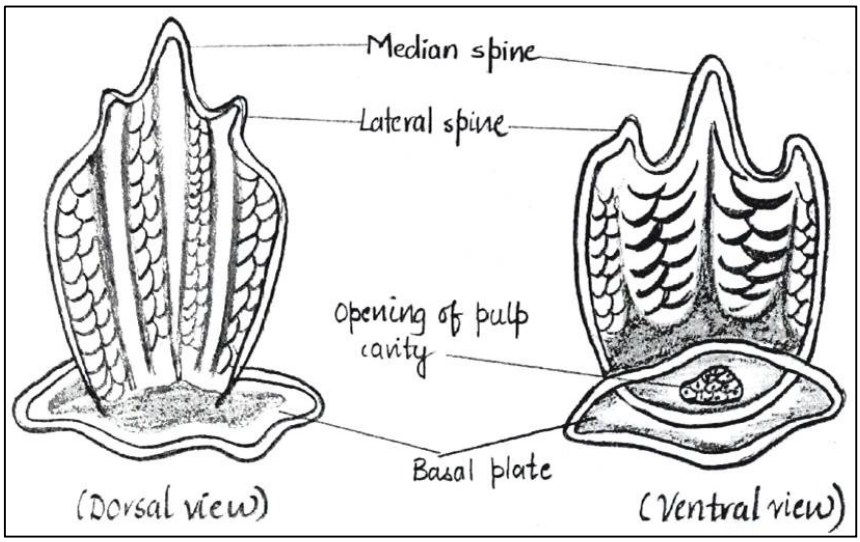
Ctenoid Scales



Identifying Characters

1. The ctenoid scales are also circular and can be distinguished from the cycloid scales by having a more or less serrated free edge.
2. Several spines are present on the surface of the posterior area of the scale. These scales are characteristic of modem higher teleosts such as **perch, sunfish**, etc.
3. They are more firmly attached and their exposed free hind parts which are not overlapped bear numerous small comb-like teeth or spines.

Placoid Scales



Identifying Characters

1. Characteristic of elasmobranchs only. E.g Sharks and rays.
2. Disc-like basal plate embedded in the dermis and a spine projecting out through the epidermis.
3. Resembles a tooth.
4. External covering of enamel-like, hard, transparent material called ***vitrodentine***, followed by a layer of ***dentine*** enclosing a pulp cavity from which several branching dentine tubules radiate in different directions.

B. Pecten of bird

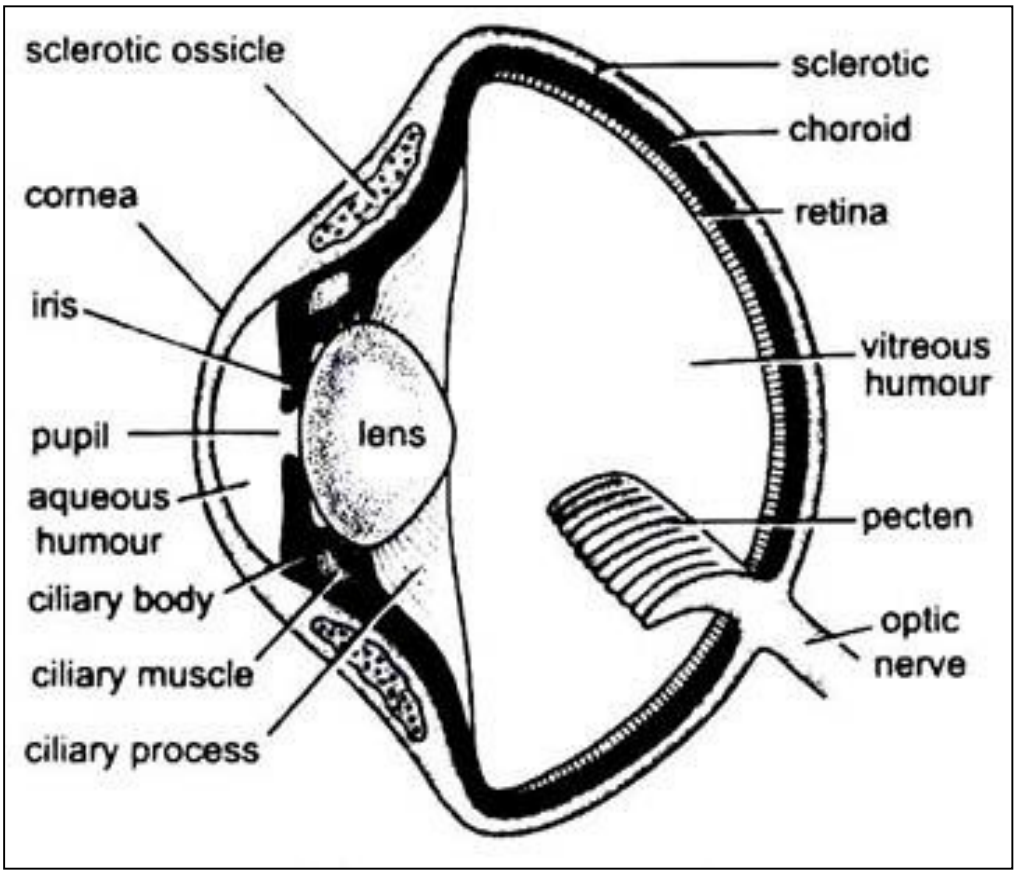


Fig: Bird eye in sagittal section

HISTOCHEMISTRY

MICROTECHNIQUES:

The microtechniques for tissue preparation consist from many steps to study the cellular structure which forming the body of the living human or animals, which can't be seen by naked eyes, but can be seen by using Light microscope or Electron microscope.

There are three types of methods using for microscopic preparations are:

1- The paraffin Technique.
2- The celloidin technique.
3- The freezing technique.

THE PARAFFIN TECHNIQUE:

In this technique using paraffin wax, following are the steps used to prepare histological sections and are summarized as follows:

1. Obtaining of the specimen: First the specimen (animal) is collected and the tissue of interestis taken out as rapidly as possible.

2. Fixation: The first step in the preparation of tissue using 10% formalin solution. The purpose for using formalin is to maintain the tissue and its contents in the state it was in the living body or near from that and the fixation process through chemical reactions and interactions physical between the active groups of the fixative substance and effective groups of chemicals in tissue (Carbohydrate - protein - fat - enzymes - metal salts-dyes) and the fixation process made upon stop fragmentation and disintegration process for bacteria and fungi activity, as well as stop the autolysis process of the tissue by lysosomal enzymes.

3. Washing: The sample must be washed before and after fixation by using tap water currentfor 24 hours, to remove the remainder of the formalin from the specimen.

4. Dehydration: Dehydration by using series spiraling of ethyl alcohol beginning with (50%, 60%,70%, 80%, 90%, 90%, 100% and 100%) for 30 mins for each concentration, to gradual removal of water from the tissue to prevent tissue shrinking.

5. Clearing: Replacement of alcohol in tissue by clearing fluid like (Xylene, benzene, or acetone) for twice and ½ hour for each solution.

6. Impregnation or Infiltration: The tissue specimen infiltration by putting the tissue specimens in paraffin wax on (58 - 60°C) for two times and 2 hours for each step. It provides a strong support to prepare them for cutting with microtome, and helps to save them in normal conditions for a long time without any harm.

7. Embedding: The specimens are embedded within paraffin wax for 24 hours, to made a template so that the specimen is surrounded by paraffin waxed.

8. Trimming: After preparation of wax templates the blocks are preferably trimmed with a sharp blade so that the specimen be in a position suitable for cutting edges so that they become parallel and can be applied to the edge of the knife microtome.

9. Sectioning: Paraffin block are cut by microtome using metal knife, into thin sections ~ 6µ.

10. Mounting: The sections are spread on the hot plate and mounted on glass slides using a thin smear from the Mayer's egg albumin to fix the sections strip on glass slides.

11. Rehydration: Rehydration by alcohol of descending series beginning by (100%, 90%, 80%, 70%, 60% and 50%) for 30 mins for each concentration.

12. Staining: Finally staining the specimens by different stains, like H&E, PAS, Van Gieson's and Verhoff's stains to differentiate their different components.

13. Cover slipping: The histological slides are dried by a hot plate at (40 °C) for 2 hours. Mounting is the process of adding the amount of (Canada balsam) and then putting the cover slide on the sections strip on glass slides and cover it.

Haematoxylin and Eosin Staining

These are two stains used in the examination of thin slices of biological tissue. Contrast is created by the stains where Haematoxylin turns the nuclei blue while Eosin turns the cytoplasm as well as other parts pink or red.

Procedure

1- A rehydrated section is stained in a solution of haematoxylin for 20 to 40 minutes

2- The section is then washed in tap water for about 3 minutes until it turns blue,

3- The section is the differentiated in 70percent ethanol that contains 1 percent of HCL for about 5 seconds to remove excess dye and allow the nucleus to emerge,

This is then washed in tap water,

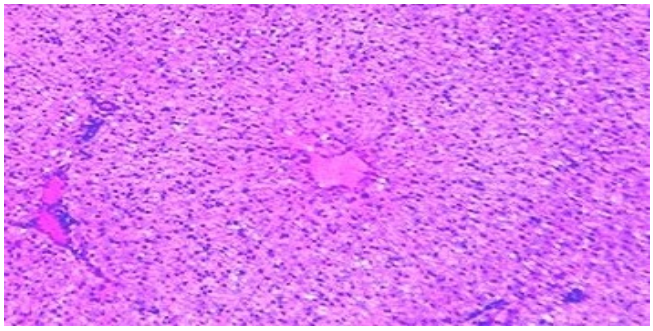
5- Stain with eosin for 10 minutes,

6- Then wash for about 1 to 5 minutes in tap water,

7- Dehydration, clear and mount on a rack

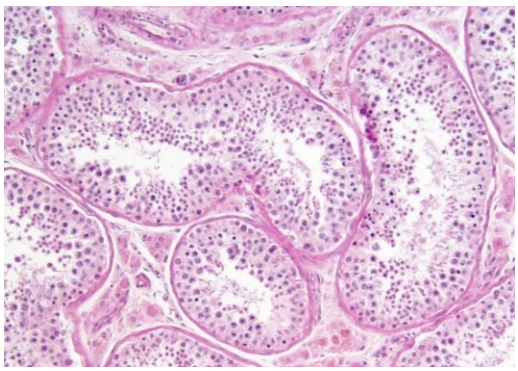
TISSUE IDENTIFICATION FROM CHORDATES:

Specimen 1



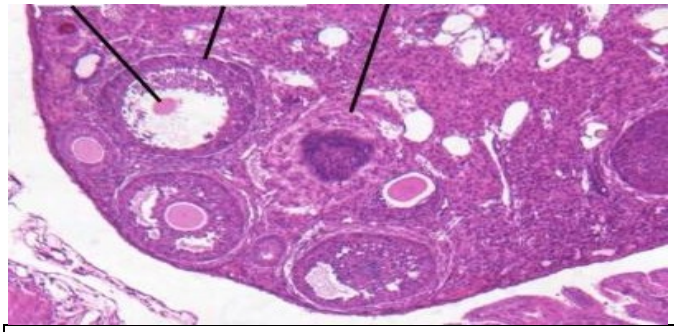
IDENTIFYING CHARACTERS	INFERENCES
1. Presence of large number of polyhedral lobules. 2. Presence of nucleated hepatic cells with hepatic cords. 3. Presence ofcentral vein. 4. Presence of hepatic sinusoids.	Hence the specimen seems to be the T.S of mammalian Liver.

Specimen 2



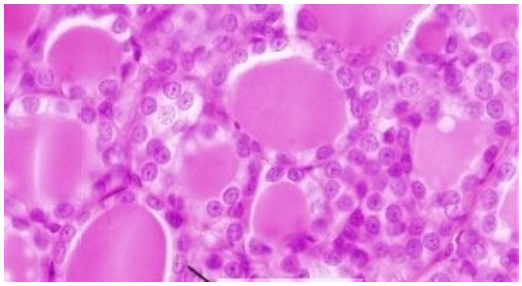
IDENTIFYING CHARACTERS	INFERENCES
1. Presence of outer thick capsules made up of fibrous connective tissue – tunica albuginea. 2. Presence of large number of rounded seminiferous tubules, Leydig cells and blood cells. 3. Presence of spermatogonia, primary and secondary spermatocytes.	Hence the specimen seems to be the T.S of mammalian Testes.

Specimen-3



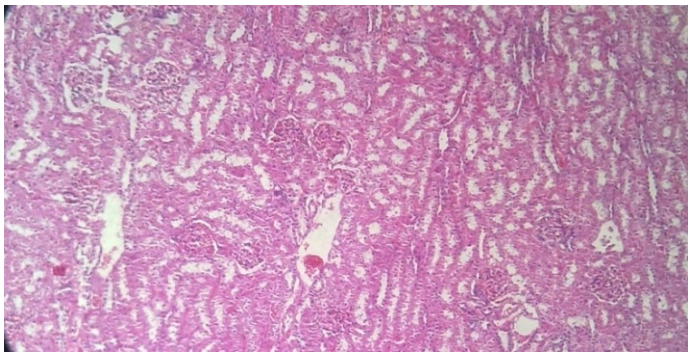
IDENTIFYING CHARACTERS	INFERENCES
1. Presence of superficial layer of cubical cells- germinal epithelium. 2. Presence of Graafian follicle. 3. Presence of fibrous and vascular connective tissue forming stroma.	Hence the specimen seems to be the T.S of mammalian Ovary.

SPECIMEN-4



IDENTIFYING CHARACTERS	INFERENCES
1. Presence of two layered capsules (outer and inner) made up of fibrous elastic connective tissue. 2. Each lobule is made up of large no. of follicles supported by thin basement membrane. 3. The cavity of follicle is filled with viscous fluid calledcolloid.	Hence the specimen seems to be the T.S of mammalian Thyroid gland.

SPECIMEN-5



IDENTIFYING CHARACTERS	INFERENCES
1. Presence of granular outer cortex and striated medulla. 2. Presenece of closely packed uriniferous tubules. 3. Presence of Bowman's capsule and Glomerulus	Hence the specimen seems to be the T.S of mammalian Kidney.

Animal Physiology:

Estimation of haemoglobin using Sahli's haemoglobinometer

Introduction

Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe^{2+} and Globin protein. It is Hemoglobin in RBCs that carries the oxygen from the lungs to the tissues and CO2 from body tissues to the lungs for excretion. Hemoglobin (Hb or Hgb) is responsible for the appearance of Red color RBCs and blood.

Hemoglobin is a chromoprotein consisting of Globin molecule attached to 4 red colored Heme molecules. Hemoglobin synthesis requires the coordinated production of Heme and Globin. Heme is a prosthetic group that medicates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the Heme molecule. The Estimation of hemoglobin in the blood is commonly prescribed in various physiological and pathological conditions and as both diagnostic and prognostic test especially in case of suspected Anemia which can be caused by various factors. Nowadays in many laboratories, the Hemoglobin estimation is done by using Automatic Hematology Analyzers but still in many other labs the following method is Commonly used to determine the Hemoglobin concentration in patient's blood.

- Sahli's Method a.k.a. Acid Hematin Method
- Cyanmethemoglobin Method (CMG) a.k.a Drabkin's Method

Principle

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to N/10 Hydrochloric acid (HCl), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound. The color of the formed acid hematin complex corresponds to the Hemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with N/10 hydrochloric acid or distilled water until the color of acid hematin complex match with the color of the standard.

Reagents required

- N/10 hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid 0.98 ml
- in distilled water and volume is made up 100 ml).
- Distilled water
- Apparatus & equipments required
- Sahli's Apparatus
- Hemoglobin pipette (0.02 ml or 20 µl capacity)
- Sahli's graduated Hemoglobin tube
- Thin glass rod Stirrer for Hemoglobin Tube
- Sahli's Comparator box with brown glass standard
- Spirit swab
- Blood Lancet
- Dry cotton swab
- Pasteur pipette

Procedure

⇒ N/10 Hydrochloric acid is taken in Hemoglobin tube (has two graduations – one side gm/dl and other side shows the Hb %age) up to the mark 20 – the lowest marking (yellow marking).

⇒ For capillary blood draw, boldly prick the tip of the middle or ring finger with the help of Blood lancet or pricking needle. Wipe out the first drop of blood and suck the blood from the second drop in Hb pipette up to the mark of 20 µl. Fill the Hb pipette by capillary action.

⇒ Wipe out the surface of the pipette with the help of tissue paper/ cotton so that excess blood may not be added to the Hb tube.

⇒ Dispense the blood into N/10 hydrochloric acid taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer.

⇒ Place the tube at room temperature for 10 minutes for complete conversion of hemoglobin into acid hematin.

⇒ After the reaction completes, place the Hb tube in the column in Sahli's Comparator box and start diluting the dark brown coloured compound (Acid Hematin) formed in the Hb tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mix with the help of stirrer after each addition.

⇒ This process is done until the endpoint comes matching the color of standard with the color of the test.

⇒ Once the color is matched with the standard brown glass, lift the stirrer up and note down the reading in Sahli's Hb tube by taking the lower meniscus in consideration.

⇒ Now add one more drop of distilled water and mix it properly with the help of stirrer. If color is still matching with the standard add another drop till it matches with the standard and note down the reading and, if it gets lighter after adding the first extra drop, it shows reading taken before dilution was correct. Note down that reading as the final result.

⇒ Reading of this method is expressed in Hemoglobin gm/dl (gram/100 ml) of blood.

Precautions

⇒ Sahli's apparatus especially the Hemoglobin pipette and Sahli's Hemoglobin tube should be clean and dry before use.

⇒ Suck the blood exactly up to the mark of 20 µl (0.02 ml) and air bubbles should not be present in the pipette with blood.

⇒ Mix well the acid and blood and wait for at least 10 minutes after adding the blood in acid.

⇒ Add distilled water drop by drop and mix well after each dilution. Avoid over dilution of the content.

⇒ The matching of color should be done against the natural source of light or electrical tube light (white light) to avoid any visual errors.

⇒ Blood sample and N/10 HCl acid should be taken in an accurate and precise amount in the Hb tube.
⇒ The Hb pipette should be wiped off properly in order to avoid the excess addition of

Advantages
⇒ It is the simple and easy method and may be done at any place because apparatus can be picked up anywhere.

Disadvantages
⇒ Visual intensity may be different for different individuals by this method, we are not able to measure the inactive hemoglobin.
⇒ This method estimates only oxy Hemoglobin. Carboxyhemoglobin and methemoglobin cannot be estimated.
⇒ The endpoint disappears soon so it is difficult to know the actual endpoint and also the Proper stable standard is not available
⇒ The resulting solution is not a clear solution but a suspension due to the action of hydrochloric acid on the proteins and lipids.

Normal values of hemoglobin
- Adult Male: 14-16 gm/dl
- Adult Female: 13-15 gm/dl
- Newborn: 16-18 gm/dl

Clinical significance of hemoglobin estimation
Hemoglobin estimation gives a brief idea of the pathological conditions to the physician so that your physician can easily understand the cause of pathology and prescribe an effective treatment for it.

Raised Hemoglobin Content
- Polycythemia Vera
- Associated with Hypoxia
- Cyanotic Congenital Heart disease
- High Altitudes
- Heavy smoking
- Methemoglobinemia
- Elevated erythropoietin levels
  - Tumors of Kidney, Liver, CNS, Ovary etc.
  - Renal Diseases (Hydronephrosis & Vascular impairment)
- Adrenal hypercorticism
- Therapeutic androgens
- Relative causes of high hemoglobin content
  - Dehydration – Water deprivation, Vomiting, Diarrhea
  - Plasma loss – Burns, Enteropathy

Reduced Hemoglobin Content
Low Hemoglobin value means anemia caused by the following conditions
- Leukemia
- Tuberculosis
- Iron deficiency anemia
- Parasitic infections severely in hookworm infection

- Sickle cell anemia
- Thalassemia
- Aplastic anemia
- Hemolytic anemia
- Loss of blood

i) Estimation of pH and its impact on plankton/ insects /fishes

PRINCIPLE:

pH is the most convenient method of expressing Hydrogen ion concentration. It's a figure not the stable of the solution, that is neutral acid or alkaline. pH is negative logarithm of the hydrogen ion concentration.

$$pH = -\log [H+]$$

MATERIALS:

1. pH meter
2. Different buffer solution (pH=7,4,9)

PROCEDURE:

1. Buffer solution (pH=7) was taken in a beaker.
2. Electrode was washed with distilled water and then electrode was blotted by tissue paper.
3. Electrode was dipped into the beaker containing buffer,
4. Adjustments of pH medium as per pH value of sample solution by calibration in pHmeter.
5. Again electrode was washed with distilled water and then electrode was blotted by tissuepaper.
6. Electrode was dipped into the unknown sample solution.
7. pH value shown in pH meter, that was recorded.
8. This process is repeated for 3 times and the mean is determined.

- RESULTS:

Number of observation	Sample Type	pH value of unknown sample	Mean
1			
2			
3			

- REMARKS:

pH value of unknown sample=4.3. So, the unknown sample is highly *ACIDIC/ALKALINE/NEUTRAL.*

Impact on Fish

Fish perform all their bodily functions in water. Because fish are totally dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce, understanding the physical and chemical qualities of water is critical to successful aquaculture. To a great extent water determines the success or failure of an aquaculture operation.

Very high (greater than 9.5) or very low (less than 4.5) pH values are unsuitable for most aquatic organisms. Young fish and immature stages of aquatic insects are extremely sensitive to pH levels below 5 and may die at these low pH values.

High pH levels (9-14) can harm fish by denaturing cellular membranes. Changes in pH can also affect aquatic life indirectly by altering other aspects of water chemistry. Low pH levels accelerate the release of metals from rocks or sediments in the stream. These metals can affect a fish's metabolism and the fish's abi lity to take water in.

High pH Levels Effect

At high pH (>9) most ammonium in water is converted to toxic ammonia (NH_3) which can kill fish. Moreover, cyanobacterial toxins can also significantly influence fish populations.

NOTE: One critical parameter is pH: Not only for the health of the fish, but for the bacteria have that cleaned up the water as well as nitrifiers that remove excess nutrients.

pH is important in aquaculture as a measure of the acidity of the water or soil. Fish cannot survive in waters below pH 4 and above pH 11 for long periods. The optimum pH for fish is between 6.5 and 9. Fish will grow poorly and reproduction will be affected at consistently higher or lower pH levels

The Effects of pH on Warm-Water Pond Fish

pH	Effects on fish
4	Acid death point
4 to 5	No reproduction
4 to 6.5	Slow growth
	to 9Desirable ranges for fish
	reproduction9 to 10 Slow growth
≥11	Alkaline death point

Recording of gut movement in frog/rat/fish under hypoxia using Dale's apparatus or model

DALE'S APPARATUS:

The movement of studding behaviour of plain muscle under different condition was 1st devised by Dale and according his name it is known as *DALE'S APPARATUS AND EXPERIMENT*. The apparatus consist of a central vessel of 100ml capacity with an outlet its lower and end for draining off the fluid. The central vessel is enclosed in a water jacket which is kept at 37°C by means of an adjustable electric heater. A hollow glass tube filled with a hook at itslower end is clamped to a pillar by a bass head so that it cannot carry balanced lever with afrontal writing point given a magnification of about 3-4 times.

The central vessel is filled with Dale's solution and outer vessel with tap water. The temp of the outer vessel is maintained by between 37°C-40°C and inner vessel at 37°C by varying the position of heater.

An air supply pipe with a screw clamp remains attached with a hollow pipe through which air may be bubbled from an aerator.

COMPOSITION OF DALE'S FLUID:

The Dale's fluid has a composition similar with that mammalian tissue fluid. Dale's fluid is prepared from stock solutions on of NaCl, KCl, $CaCl_2$, $MgHCo_3$ and glucose.

The composition of fluid is as follows: -

1. 18gm% NaCl stock -> 50ml (0.9 gm %)
2. 4.2gm% KCl stock -> 5ml (0.42 gm %)
3. 2.4gm% $CaCl_2$ stock -> 5ml (0.24 gm %)
4. 0.5gm% $MgCl_2$ stock -> 50ml (0.02 gm %).

EFFECT OF HYPOXIA ON NORMAL MOVEMENT OF RAT'S ISOLATED INTESTINE

PRINCIPLE:

Segments of small intestine continue to contact and respond to various stimuli if kept in a suitable medium at optimum temp with provision for oxygen supply.

REQUIREMENTS:

1. DALE'S APPARATUS:

   This consists of a large rectangular box keeping water at appropriate temp with help of aheating element and thermostat. There is a central vessel both of 20ml capacity which isprovided with an outlet. There is a hollow beat glass tube curved at the lower end far fixing in intestine and other tissue. This tube can be invested in the centre of the oxygenboth and is also used for supplying O2 through the solution. A formal lever can be attached to the assembly for recording movement on a moving kymograph.

2. DALE'S FLUID:

   The composition of Dale's fluid –
   [FOR PREPAIRING 1LT OF STOCK SOLUTION]

   18gm% NaCl = 50ml
   4.2gm% KCl = 5ml
   2.4 gm % CaCL2 = 5ml
   0.5 gm % mgCL2 = 1ml

   - ❖ Petridish
   - ❖ Thread and needle
   - ❖ Oxygen
   - ❖ Lever
   - ❖ Kymograph and smoked drum
   - ❖ Pipe and a rat

PROCEDURE:

1. The rat was kept fasting overnight as the intestine of fasting shows good contraction.
2. The oxygen bath was filled with Dale's fluid, outer bath filled with water, thelamp kept at 37°C-40°C.
3. The rat was stunned and the abdomen was opened.
4. A small part of jejunum close to duodenum and took out the intestine was placed in a Petridish containing Dale's fluid.
5. The threaded needle was passed through wall of one of cut end of segment and loop was made from thread.

6. At the other end of intestine segment, a long thread for attaching to the lever was tied.
7. The segment in organ both filled with Dale's fluid was moved securing thethreshold loop to curved end of glass tube.
8. Oxygen was allowed to enter through solution at a rate of 2-3 bubble 1 second.
9. The temp of water bath was kept of 37°C and cheeked.
10. The intestinal wall was attached to frontal lever.
11. Normal movement of intestine was recorded on a slow moving drum.
12. After recording normal movement the O_2 supply was stopped and effect of hypoxia was recorded. After a few seconds, the O_2 supply was again started and normal movement was recorded.

OBSERVATION:

When O_2 supply is stopped, the frequency and amplitude of contraction was decreased. After sometime a straight line-like, movement observed.
When O_2 supply was again started, slowly intestinal muscle regained its normal movement.

Recording of Blood Pressure Using a Sphygmomanometer
Blood pressure is the force of blood against the walls of the arteries. Blood pressure is recorded as two numbers, the systolic pressure (the pressure when the heart beats) over the diastolic pressure (the pressure when the heart relaxes between beats). Normal systolic pressure is 120 mmHg (millimetres of mercury) and the diastolic pressure is 80 mm Hg that would describe the blood pressure as '120 over 80', written 120/80.

Figure 1. Sphygmomanometer

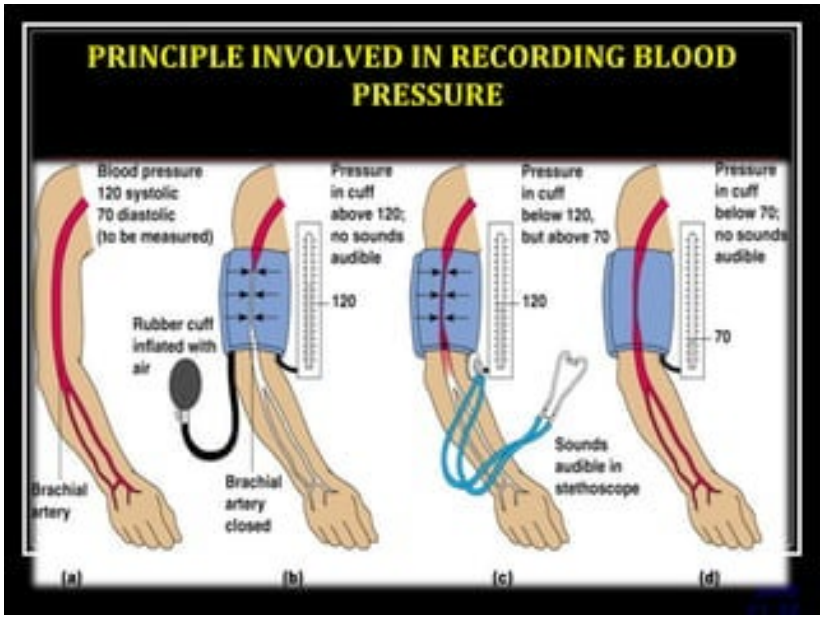
Blood pressure may vary according to whether the patient is lying down, sitting or standing. It is normally recorded with the patient sitting.

Required apparatus

- Sphygmomanometer
- blood pressure cuffs: small, medium, large
- stethoscope
- chair
- patient's care notes or observation chart
- alcohol wipe

Procedure

- Ask the patient to loosen any tight clothing or remove long-sleeved garments so that it is possible to access the upper arm. Do not use an arm that may have a medical problem.
- Place the cuff around the upper arm and secure.
- Connect the cuff tubing to the sphygmo-manometer tubing and secure.
- Rest the patient's arm on a surface that is level with their arm.
- Place the stethoscope over the brachial artery (in the bend of the elbow) and listen to the pulse
- Pump up the cuff slowly and listen for when the pulse disappears. This is an indication to stop inflating the cuff.
- Start to deflate the cuff very slowly whilst watching the mercury level in the sphygmomanometer.
- Note the sphygmomanometer reading (the number the mercury has reached) when the pulse reappears: record this as the systolic pressure.
- Deflate the cuff further until the pulse disappears: record this reading as the diastolic pressure.
- Record these two measurements, first the systolic and then the diastolic (e.g., 120/80), in the patient's notes or chart.
- Tell the patient the blood pressure reading.
- Disinfect the stethoscope drum and ear pieces with the alcohol wipe.
- Wash and dry your hands.
- Report an extremely low or high reading to the clinically qualified person in charge of the patient's care.



ZOO - 196

Immunology

STUDY OF MACROPHAGE FROM PERITONEAL FLUID OF WHITE RAT (*Rattus nervegicum*)

- INTRODUCTION:

Different kind of cells activity involved for smooth running of our body's immune system in contrast of our immunogenicity. The main event is to from a specific different mechanism against foreign particles. In the regard macrophage plays an important role. Macrophage can migrate from one place to another place in our body. As we know Kupffer's cell when it reaches in liver, mesangial cell in kidney microgeal in brain etc.

In this phage of study our object is to study the morphological features of macrophage which has been collected from peritoneal fluid of white rat.

- MATERIAL REQUIRED:
  - Specimen (white rat)
  - 0.85% NaCl solution
  - Slide
  - Watch glass
  - Disposable syringe
  - Leishman's stain
  - Microscope
  - Distilled water
- PROCEDURE:
  1. At first specimen (white rat) was lied on a tray by stretching its tail and holding itsneck. In this way anesthesia was done by using chloroform.
  2. Abdomen of the rat was opened by dissecting out the dermis and then about 3.5ml of 0.85% NaCl solution (hypotonic) was injected into the peritoneal region of the rat with the help of disposable syringe.
  3. The waiting for minimum 20-30 minutes is necessary.
  4. The peritoneal fluid of the white rat was drawn by using some syringe.
  5. One drop of that peritoneal fluid was taken onto a clean slide and then air dried.
  6. Then few drops of filtered.
  7. Leishman stain was added to the sample and keep for 5-10mints.
  8. The stain was washed with distilled water.
  9. Lastly the slide was observed under compound microscope (40X objective).
- OBSERVARION:

  After completion of single staining procedure we observe stained slide under microscope noticed some cell approaches towards foreign substance. We also observed that some foreign substances are already in engulfed state with the help of pseudopodia present in macrophage.

ENGULFMENT OF FOREIGN SUBSTANCE BY MACROPHAGE COLLECTED FROM PERITONEAL FLUID OF WHITE RAT (*Rattus nervegicum*)

- INTRODUCTION:

Different kind of cells activity involved for smooth running of our body's immune system in contrast of our immunogenicity. The main event is to from a specific different mechanism against foreign particles. In this regard macrophage plays an important role. Macrophage can migrate from one place to another place in our body. As we know Kuffer's cell when it reaches in liver, mesangial cell in kidney microgeal in brain etc.

In this phage of study our object is observed phagocytic nature of macrophage and also point out proper incubation period by which macrophage can engulf foreign substance used.

- MATERIAL REQUIRED:
  - Specimen (white rat)
  - 0.85% Nacl solution
  - Slide
  - Watch glass
  - Disposable syringe
  - Leishman's stain
  - Foreign substance (human blood)
  - Sterile needle
  - Rectified spirit
  - Cotton
  - Distilled water

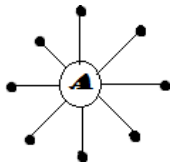
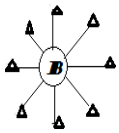
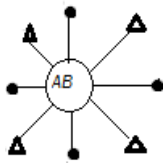
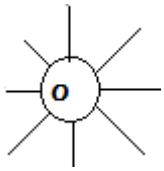
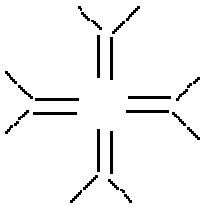
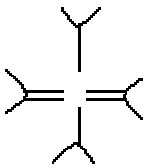
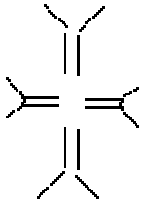
- PROCEDURE:
  1. At first specimen (white rat) was laid on a tray by stretching its tail and holding its neck. In this way anesthesia was done by using chloroform.
  2. Abdomen of the rat was opened by dissecting out the dermis and then about 3.5ml of 0.85% NaCl solution (hypotonic) was inject into the peritoneal region of the rat with the help of disposable syringe.
  3. The waiting for minimum 20-30 minutes is necessary.
  4. The peritoneal fluid of the white rat was drawn by using some syringe.
  5. Now 2-3 ml of peritoneal fluid is put is put on a watch glass and few drops of human blood is added to it.
  6. It is mixed well and incubated for 15-20 minutes.
  7. One drop of incubated fluid is taken onto a clean slide and spread it properly.
  8. Then it will be air dried.
  9. The few drops of filtered Leishman's stain was added on the sample and kept it for 5 mints.
  10. The stain was washed with distilled water.
  11. Lastly the slide was observed under compound microscope (40X objective).

- OBSERVATION:
  After completion of staining procedure (following single stain by Leishman) we observed the prepared slide under microscope (40X objective).
  There are various types of irregular cells, some cells possess pseudopodia like structure and we also observe a mass of cell with prominent nucleus. Hence, the observed slide contains macrophage collected from peritoneal fluid of white rat.

DETERMINATION OF HUMAN BLOOD GROUP

PRINCIPLE:

In 1900 Karl Landsteiner grouped human blood into 4 group based on presence of two antigens on the surface of RBCs. This groups are designated as A,B, AB & O. This are commonly called ABO Blood group. This blood group system is determined on the basis of presence or is absence of antigen on RBC on antibody in blood group is designated as a blood group. B antigen is present on outer membrane of RBC and anti-a antibody present blood plasma. This blood group is designated by B+ blood group. A, B both antigen are present on outer membrane of RBC, this blood group is called AB+ blood group.
A, B both antigens are absent on outer membrane of RBC, this blood is called O+.
In addition to antigen on ABO system the red cells of 80-85% also have an addition antigen called RH+ antigen or Rh factor.

RBC TYPE	GROUP-A	GROUP-B	GROUP-AB	GROUP-O
				
ANTIBODY IN PLASMA	Anti-B 	Anti-A 	None	Anti-A Anti-B 
ANTIBODY IN RBC	A	B	AB	None

In 1940 Karl Land Steiner and Weiner reported that Rabbit sera contain antibodies against of Rhesus monkey that agglutinates RBC of some human being. This antigen was latter named as Rhesus factor (Rh Factor).

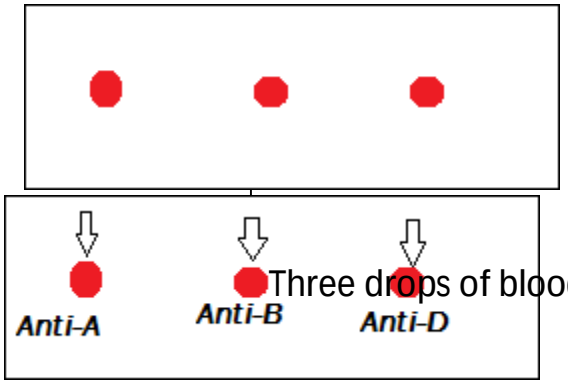
During RH determination, Anti-D serum is mixed with blood and if there is agglutination then the blood is (Rh^+) and if the blood does not agglutinate the blood is (Rh^-).

MATERIAL REQUIRED:

1. Rectified spirit
2. Cotton
3. Sterile needle
4. Human blood
5. Glass slide
6. Anti-A(monoclonal)
7. Anti0B(monoclonal)
8. Anti-C(monoclonal)
9. Glass rod

** ***Monoclonal Antibody***

Anti-a
Anti-b
Anti-c



Three drops of blood taken in a slide

One of the monoclonal antibodies Anti-A

PROCEDURE:

1. Three area on a slide are marked and labeled them A, B and D.
2. The finger tip is punctured by a sterile needle and three drops of blood is taken in aclean glass slide.
3. One drop of Anti-A is added to area 'A' are drop of Anti-B is added to area 'B' and one drop of Anti Rh is added to area D.
4. With separate application glass rod blood is mixed reagent well.
5. After a few seconds the drop are examined.

OBSERVATION:

1. Agglutination occurs when reaction is made with Anti-A serum.
2. Agglutination does not occur when reaction is made with Anti-B serum.
3. Agglutination occurs when tested with Anti-D serum.

CONCLUSION

On the basis of the above observation it can conclude that my blood group is A+.

PRECAUTION:

1. The blood should be mixed with antigen quickly, otherwise it will gel coagulated quickly.
2. The slide used should be dust free.
3. The drop of the blood and the drop of anti-sera should be equal or else we cannot confirm if agglutination has occurred or not.

Methods in Biology

Characterization of macromolecule through Gel electrophoresis

PRINCIPLE:

Agarose gel electrophoresis used to analyze and quantitate nuclic acid. The agarose for agarose gel electrophoresis is purified from agarose. Agarose is a linear polymer made up of repeating units of 1.3-linked D galactopyranase and 1.4-linked 3.6 anhydro a 1 galactopyranose. Agarose has an average MW of 12000 and contains about 35-40 agarobise units. Agarose in solution exists as left handed double helices. About 7-11 such helices from

bundles which extend as long as rods and appear to intertwine with one another, further strengthening the frame work of the gel. The cross links are held together by hydrogen and hydrophobic bonds. By changing the gel conc. the pre size can be altered. Higher the concentration of agarose smaller the pre size and vice versa because of large pre size even at low concentration. Agarose gels are widely used for separation of DNA and RNA.

MATERIALS REQUIRED:

1. Agarose solution.
2. Ethidium bromide
3. Electrophoresis buffer
4. 6×gel buffer
5. DNA sample
6. DNA standard

EFFECTS OF AGAROSE CONCENTRATION ON SEPARATION RANGE:

The following table describes the relationship between agarose concentration and separation range of nucleic acid.

AGAROSE CONCENTRATION (%)	SEPARATION RANGE(kb)
0.3	5 to 60
0.6	1 to 20
0.8	0.8 to 10
1.0	0.4 to 8
1.2	0.3 to 6
1.5	0.2 to 4

FACTORS WHICH AFFECT THE RATE OF MIGRATION OF NUCLEIC ACIDS IN AGAROSE GEL:

Rate of migration of nucleic acids in agarose gel depends mainly on five important parameters.

1. AGAROSE CONCENTRATION:

   Higher conc. of gel is used for the separation of lower weight DNA & RNA fragments and vice-versa.

2. MOLECULAR WEIGHT:

   A duplex DNA fragment migrates at rates inversely proportional to the log molecular weight. A plot of log MW mobility gives a straight line.

3. CONFORMATION:

   Super coiled DNA moves faster followed by linear forms & relaxed open circular forms.

4. APPLIED VALTAGES:

   At low voltages (<5V/cm) the rate of migration is directly proportional to the applied voltage.

5. BASE COMPOSITION & TEMPERATURE:

   Base composition & running the gel between 4 & 30°C don't change the mobilities.

PREPARATION OF STOCK SOLUTION FOR DNA GEL:

Two different buffer systems are used for separation of nucleic acid by agarose gel electrophoresis. There compositions are given below:

TBE buffer:

Tris borate (1XTBE)	10X buffer/ltr.
89 ml tris base	108.0g
89 ml boric acid	55.0 g
25 ml EDTA	9.3 g

TAE buffer:

Tris acetate (1X TBE)	10X buffer/ltr
50ml tris base	302.5 g
25 ml acetic acid	71.4 g
1 ml EDTA	18.6 g

Sterilize the stock solution by autoclaving.

- PREPARATION OF ETHIDIUM BROMIDE(Stock solution):
  10mg ethidium bromide into sterile tube & dissolve in 10ml. Sterile distilled water. The stock is stored in 4°c.

- PREPARATION OF AGAROSE SOLUTION FOR GEL CASTING:
  Dissolved the agarose by placing the flashes in boiling water bath cool to Luke warm. Cover the sides of a tray using cello tape & place the comb about 1cm from the top of the tray.
  Pour the agarose without making any bubbles, cool it for 20mints and take off the combs and uncovered the tapes.
- PREPARATION OF SAMPLE LOADING DYE GLYCEROLLS BROMOPHENOL BLUE(6X):
  3ml glycerol(30%), 25mg bromophenol blue(0.25%), dH_2O to 10ml.

PROCEDURE:

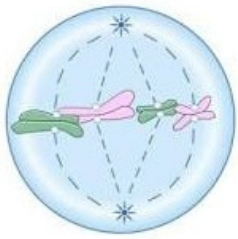
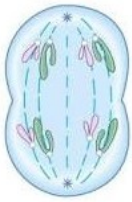
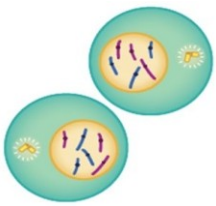
1. The DNA sample is mixed with the loading dye(for 5µl of DNA sample 1µl of 6X dye is used) and loaded in the well carefully using pipetman & capillary tube.
2. One the sample is loaded in to the well the cathode is connected towards the top end of the gel and anode(Red positive) terminal is connected towerds the bottom end of the gel. The maximum volume that can be loaded onto a well formed from a 105mm thickness tooth of the comb is 30µl. The electrophoresis is started by switching on the D.C. power pack.
3. The gel is run at 5V/cm. As the bromophenol blue has moved 1cm above the bottom end. The current is switched off the power supply is disconnected and the gel along with the platform is stain is plastic tray containing 0.5µg/ml ethidium bromide in the sterile distilled water.
4. After about 30-40 min the platform & gel is rinsed with distilled water & by keeping the platform in a standing position, the gel is gently pushed onto the uv transluminator. npw UV light is switched on and the DNA bands are seen and photographed at 5.6 for 10 seconds with an orange filter.

RESULT: After electrophoresis DNA bands can be visualized under VU light and they appear as orange fluorescence.

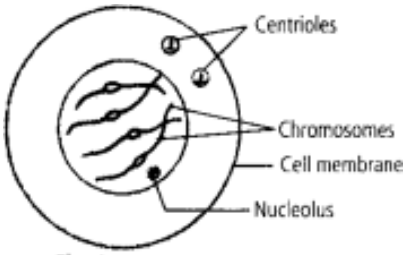
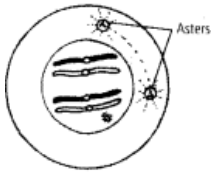
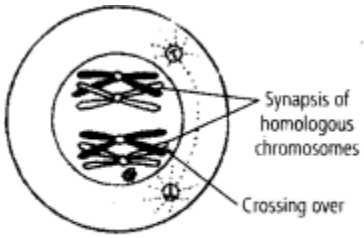
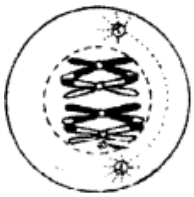
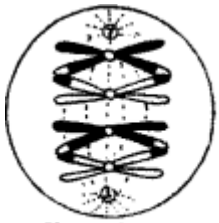
Cell Biology-

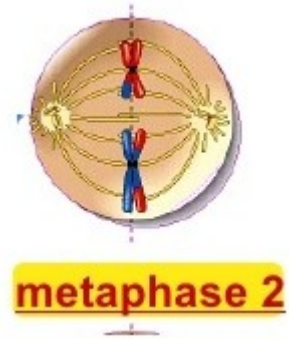
Identification of different stages of cell division and cell organelle:

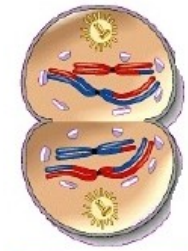
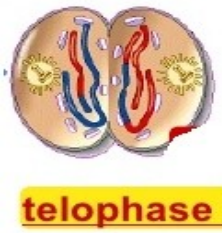
MITOSIS PHASE

	INTERPHASE: • Nuclear membrane is present. • Coiled chromosomes are dense. • Nucleolus can be found.
	PROPHASEE: • Nuclear membrane almost disappeared. • Chromosome shape is not vivid. • Chromatids are not distinct. • Nucleolus disappears.
	METAPHASE: • Spindle fibre has been formed. • Chromosomes are attached with the spindle by their centromeres. • Chromatids are extended on either side. • Axis that is equatorial in position.
	ANAPHASE: • Chromosome centromere divides and daughter chromosomes are formed. • Spindle fibre extended more. • Daughter chromosomes move to the pole of the spindle.
	TELOPHASE: • Spindle disappears. • Daughter chromosomes are assembledto the opposite pole. • Nuclear membrane formation again starts.
	CYTOKINES: • Chromosomes become indistinct. • Cell plate is formed. • Nuclear membrane reappears.

MEIOSIS PHASE

 Fig.: Leptotene	LEPTOTENE: • The reticulum of meiotic nucleus is opened out. • Chromosome is more distinct. • Chromosome appears as long slender and threads. • Chromosome numbering is not possible. • Chromosome looks beaded like.
 Fig.: Zygotene	ZYGOTENE: • Paired chromosomes intimately associated. • Forms bivalent. • Chromosomes are much shorted andthicker.
 Fig.: Pachytene	PACHYTENE: • Chromosomes are thicker than theearlier stage. • Chromosomes are associated in pairthroughout their length. • Presence of nuclear membrane. • Formation of chiasma.
 Fig.: Diplotene	DIPLOTENE: • Nuclear membrane present. • Separation of paired chromosomes noted • Bivalents with four chomatids visible. • Formation of chiasma also seen. • Single unpaired X-chromosome is shortand thicker.
 Fig.: Diakinesis	DIAKINESIS: • Chromosome shorter and thicker. • Loops few in number. • Terminalisation in the way of completion. • Some of the bivalents completely terminalized showing end to end attachment with the figure like circle. • Terminalized and incompletely terminalized chromosomes are scatteredthroughout the cytoplasm. • Single rod shaped X-chromosome is present.

	PROPHASE II: • Chromosomes become visible due todehydration of cell. • Each arm of chromosome passes two chromatids. • Nuclear membrane and gradually disappears. • Centrioles move to the opposite site.
	METAPHASE I: • Each chromosome pair occupies a position onthe equatorial plates. • Polar view gives an arrangement just as a line of the circle. • Almost all chromosomes are terminalized • Chromosomes are exceptionally shortand thicker.
	METAPHASE II: • Nuclear membrane has broken down again. • Short rod shaped chromosomes are arranged in the equatorial plate in pairsand parallely. • Absence of chiasma. • Polar view shows as circular arrangement at the periphery.
	ANAPHASE I: • Chromosomes form a diad. • Movement of chromosomes towards the pole by separation of chromatids. • Each pole contains a set ofchromosomes. • Sex chromosome at one pole.
	ANAPHASE II: • Chromosomes move towards pole by separation of chromatids. • Chromosome rod shaped. • Chromosomes are shorter and thicker. • At each side of the equatorial plate equal numbers of chromosome are present.

	TELOPHASE I: • Chromosome reaches at pole. • Chromosome appears elongated and thin. • Cytokinesis may or may not occur. • Nuclear membrane appears around each group of chromosome(not visible).
	TELOPHASE II: • Presence of shorter cluster of chromosome at each pole. • Cytokinesis is visible. • Nucleolus and nuclear membrane appear.

STAINING OF MITOCHONDRIA IN HUMAN CHEEK EPITHELIALCELLS

INTRODUCTION:

Mitochondria are considered as power houses of a cell as it produces ATP by a process called oxidative phosphorylation. Each cell contains large no of mitochondria and they can be observed under a light microscope if stained with Janus green. This stain is bluish green in colour when oxidized and colour less when reduced. A a dilute solution of the stain is applied to stain the mitochondria in a cell. Since mitochondrial inner membrane contains cytochrome oxidase enzyme which can keep the stain in oxidized state. The mitochondria appeared stained while in rest of cytoplasm the stain gets reduced and thus appears colourless.

MATERIAL REQUIRES:

1. Ethanol soaked tooth pick
2. Slide
3. Cover glass
4. 0.01% Janus green B stain in normal saline

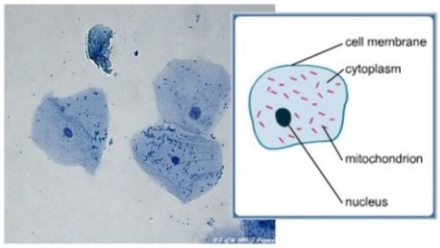
PROCEDURE:

1. Dry the ethanol soaked tooth pick in air and scrape gently the inner side of cheek. A larger no of cells will come on the tooth pick.
2. Gently rub the tooth pick on the slide in one direction to make a spread of cells. Dry the cells on slide. So that the class will not get washed away while staining.
3. Put a few drops of Janus green stain and leave for 5-10 min for staining.
4. After 5 mints of staining, rinse cells once with distilled water. So that complete stain is not gone and a diluted stain remains. Mounts the cells into drop of distilled water with cover glass and observe under the students light microscope. The cells can alternatively be mounted in the stain itself. A few air bubbles remaining inside the cover glass give a background. Stain that makes the viewing easy. The slide can be observed under the high magnification of a student microscope.

OBSERVATION:

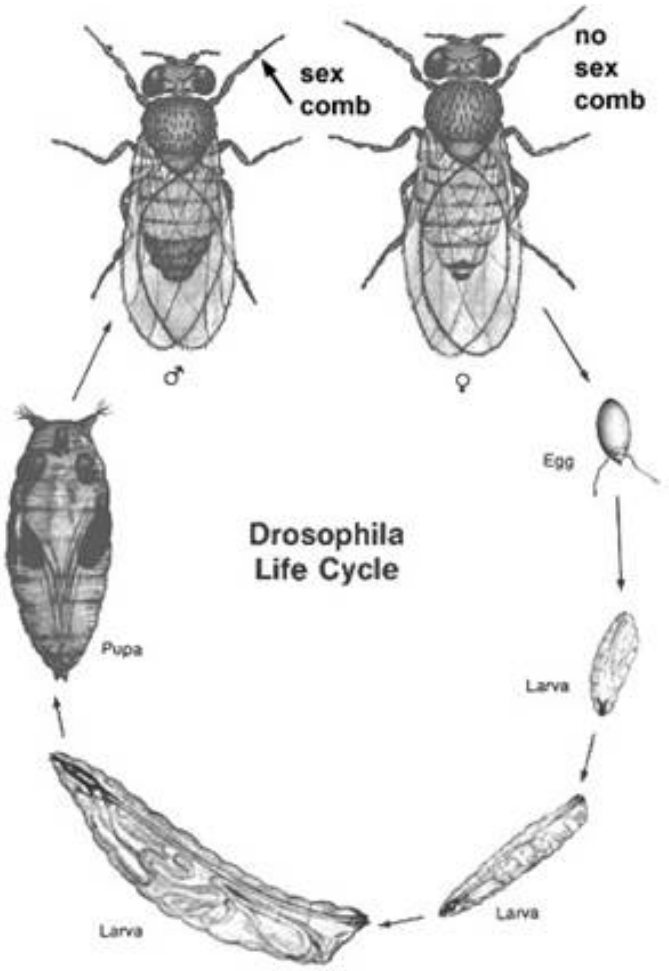
Each cell is seen to contain a large number of tiny rounds on elongated bacteria like bodies in the cytoplasm mainly around the nucleus. Generally they are not strongly stained and thus appear like pimples on a face. Mitochondria can be easily distinguished from a bacterium as bacterial cells become more prominently stained and appear sharper than mitochondria also as bacteria are on the surface of cells. They will be focused at a slightly different level than mitochondria can be distinguished.

Cheek cells



CYTOGENETICS

Life cycle of Drosophila



Fruit flies are holometabolous insects; that is, they undergo complete metamorphosis during their life cycle. The life cycle consists of four distinct stages: egg, larva, pupa, and adult. The rate of development is dependent on temperature, being more rapid at higher temperatures. For instance, at 20°C, the life cycle is completed in 14 or 15 days, but at 25°C, the cycle lasts about 10 days.

Mating and Eggs: Mated females store sperm to fertilize eggs that are subsequently laid. Therefore, to ensure that the desired cross is achieved, it is necessary to place females that are virgins with their intended male mates. Female flies are unable to mate for several hours

after they have eclosed as adults from their pupal cases. Therefore, virgin females can be obtained by clearing all of the flies from a vial and collecting all newly-eclosed females several hours later. These virgin females can be kept separated from males for several days until needed for crosses.

Oviposition by the female starts as early as the second day after its emergence from its pupal case. It increases for about a week until a female adult may be laying 50-75 eggs per day for a total of approximately 400-500 eggs in 10 days. The egg is ovoid, covered outside with a thin but strong envelope (chorion) from which project anteriorly two thin stalks whose terminal portions are each flattened into a spoon-like float. The latter serve as "water-wings" to prevent the egg from sinking and drowning in a semiliquid medium. At the anterior end of the egg is a minute pore (micropile) through which the spermatozoa enter the egg as it passes down the oviduct into the uterus. Although many sperm may enter the egg as it passes down the oviduct, only one fertilizes the female pronucleus and the others are soon absorbed in the developing embryonic tissue.

Larva: The larva is a white, segmented, worm-shaped burrower with black mouth parts (jaw hooks) in the narrower head region. For tracheal breathing it has a pair of spiracles (air intakes) at both the anterior and posterior ends. Since insect skin will not stretch, the young small larvae must periodically shed their skins (cuticle) in order to reach adult size. There are two such molts in *Drosophila* larval development that are accompanied by shedding of the mouth parts as well as the skins. During each period between molts, the larva is called an instar, i.e. the first instar is between hatching and the first molt. Both the size of the larva and the number of teeth on the dark colored jaw hooks are an indication of which instar the larva has reached. After the second molt, the larva (now third instar) feed until ready to pupate. At this stage, the larva crawls out of the food medium onto a relatively dry place, ceases moving, and everts its anterior breathing spiracles.

Pupa: Soon after everting its anterior spiracles, the larval body shortens and the cuticle becomes hardened and pigmented. A headless and wingless prepupa forms. This stage is followed by the formation of the pupa with everted head, wing pads, and legs. The puparium (outer case of the pupa) thus utilizes the cuticle of the third larval instar. The adult structures that seem to appear first during the pupal period have actually been present as small areas of dormant tissues as far back as the embryonic stage. These localized preadult tissues are

called anlagen (or imaginal discs) and because of the ease in which they can be isolated have often been used in studies of developmental genetics. The main function of the pupa is to permit development of the anlagen to adult proportions. The breakdown of larval tissuesto furnish material and energy for this development is therefore a prime feature of pupal metabolism.

Adult: Adults exhibit a typical insect anatomy, including compound eyes, three-part bodies (head, thorax, and abdomen), wings, and six jointed legs. The various types of bristles and hairs found on the body are characters that we will use to identify different phenotypes of flies.

ANALYSIS AND INTERPRITATION OF GENETIC CROSS WITH SPECIAL REFERENCE TO DROSOPHILA

INTRODUCTION:

Mendelian genetics refer to the inheritance of chromosomal genes following the laws governing the transmission of the chromosome to subsequent generations. Gregor Johan Mendel was the first person to discover the fundamental principle of heredity. In modern terms the principle of segregation simple references to the fact that two homologous chromosomes separate from each other during the formation of gametes. Based of experiment involving more than one gene Mendel proposed that principle of independent assortment. It simply means that the orientation of one pair of homologous chromosome at the equatorial plate during cell division is independent of the orientation of other such pair character of phenotype ratio called Mendelian ratio follow both the principle that is principle of segregation and principle of independent assortment.

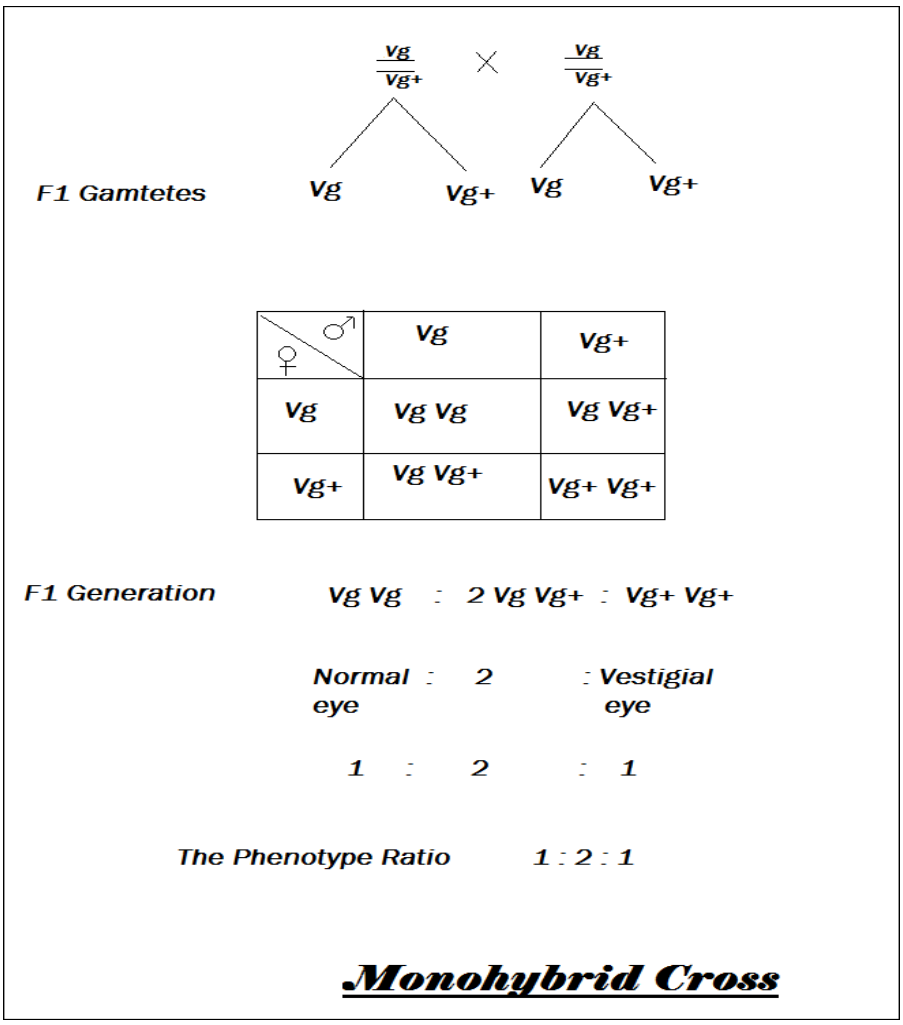
MATERIAL REQUIREMENT:

1. Drosophila culture bottle containing culture media.
2. Different stock of *Drosophila melanogaster*.
3. Dissecting binocular microscope.
4. Etherizer and ether.
5. Brush

PROCEDURE OF MONOHYBRID CROSS:

It is a cross involved only one trait then it's called monohybrid cross.

1. Take 10-15 virgin females from homozygous stock for vestigial wings and autosomal recessive gene are collected.
2. 10-15 males from wild type stock (Vg+) are collected. These flies are put inside a culture bottles.
3. The males are allowed to male for 2 days.
4. The cultures bottles are kept in constant temperature BOD incubator (25^{o}C) for 10days.
5. The flies are collected and examined.
6. The observations are recorded.
7. Cross between F1 generation flies are also set up.
8. F2 flies are collected, anaesthetized, examined.
9. The observations are recorded.

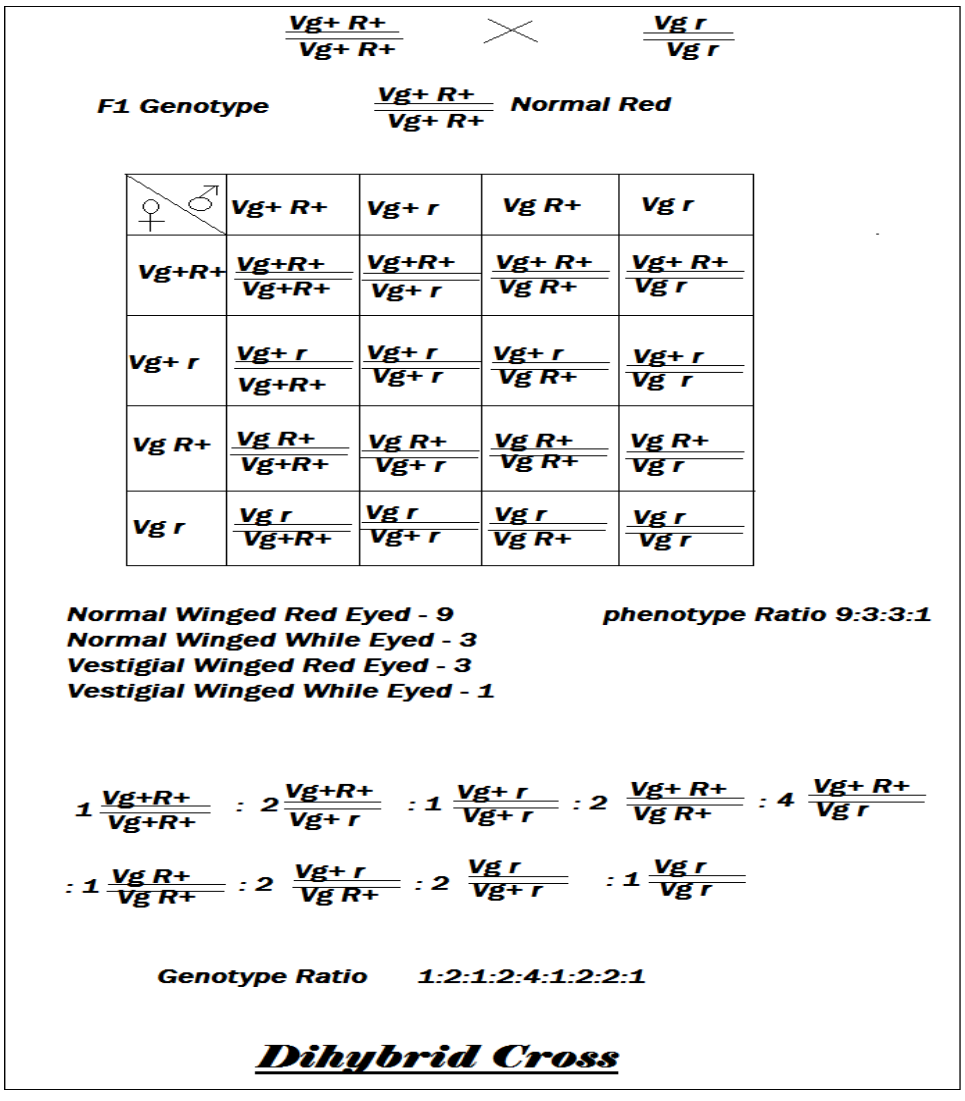


DIHYBRID CROSS

If a cross involves two traits that it's called dihybrid cross.

PROCEDURE:

1. Take 10-15 virgin females from homozygous stock for vestigial wings and autosomal recessive gene are collected.
2. 10-15 males from wild type stock (Vg+) are collected. These flies are put inside a culture bottles.
3. The males are allowed to male for 2 days.
4. The cultures bottles are kept in constant temperature BOD incubator (25^0C) for 10days.
5. The flies are collected and examined.
6. The observations are recorded.
7. Cross between F1 generation flies are also set up.
8. F2 flies are collected, anaesthetized, examined.
9. The observations are recorded.



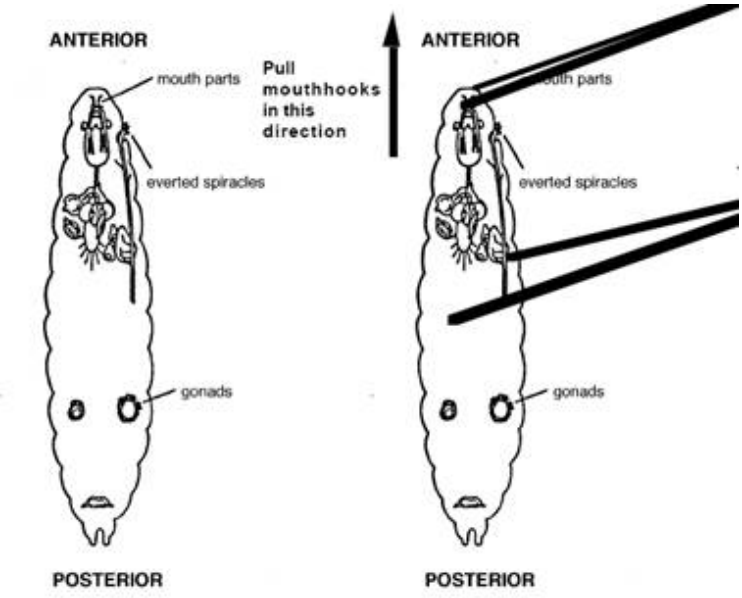
Study of polytene chromosome of Drosophila

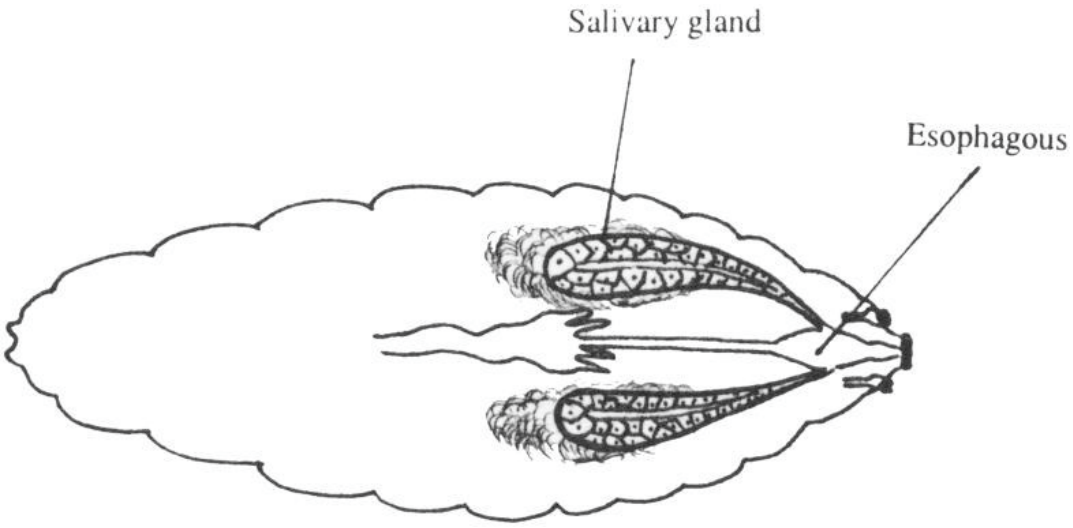
Introduction:

Thomas Hunt Morgan and students demonstrated the relationship between chromosomes and the hereditary information. Morgan chose chromosomes from the Drosophila larval salivary gland for his studies, because of their unusually large size and clearly visible banding. The large size of these chromosomes results from an atypical cell cycle in which they undergo ten rounds of DNA replication (S phase) without intervening mitoses. The polytene cell cycle of the salivary gland produces chromosomes consisting of1000 (210) sister chromatids each. This amplification of the genetic material allows the larvae to produce the large quantities of gene products needed for it to undergo a rapid growth in size as it progresses from the first to third instar stages of development. The genetic experiments of Morgan showed that the Drosophila genome is subdivided into four linkage groups, which correspond to the four pairs of chromosome homologues observed in squashes of chromosomes from a tissue that undergoes a typical cell cycle ofa single S phase and Mphase. The four chromosomes of the Drosophila genome are held together at their centromeres that donot undergo polytenization and, therefore remain tightly adhered to one another to form what is known as the chromocenter. In a well spread polytene chromosome squash such as the one in, it is possible to identify five chromosome arms (the left and right arms of chromosomes 2 and 3 and the single long arm of the X chromosome). The very small 4^{th} chromosome is also sometimes visible. The genes along the sister chromatids of each visible arm are held in register along the arms to form a characteristic and reproducible banding pattern on each arm. Also note that the polytene nucleus of the salivary gland is diploid, and each arm contains a pair of homologous chromosomes aligned in register along their lengths.

Required materials:

1) Dissecting binocular
2) Bottle of wild type Drosophila stock
3) 10 ml Saline (0.7% NaCl)
4) 1 ml 45% Acetic Acid
5) Aceto- Orcein stain
6) H_2O wash bottle
8) Slides
9) Coverslips
10) Forceps





Procedure

1. Use your forceps to remove 8-10 actively crawling larvae from the sides of thecultivation bottle.
2. Place them on a dry microscope slide (slightly to one side).
3. Use a Pasteur pipette to place a drop of saline solution in the middle of the same microscope slide.
4. Use your forceps to transfer one of the larvae to the saline drop, with its trachealtubes facing up .
5. Use one pair of forceps to hold the anterior end of the larva in place at the spot just above the neural ganglion, while using a second pair of forceps to grab the top layerof cuticle from a position on the larva at about one third body length from the anterior end.

6. The salivary glands and ventral ganglion (brain) will usually remain attached to thehead region, separate from the rest of the body, after a clean dissection.
7. After you have accumulated 5-6 salivary glands, prepare a slide for a squash of 1-2 glands. Use a tissue paper to wipe its surface clean, then blow any dust from its surface before placing a 10µl drop of Lacto/Aceto Orcein stain on the slide.
8. Then place a drop of 45% Acetic Acid fixative on the slide next to the drop of saline holding your salivary glands and use forceps to transfer the salivary glands from the saline to it and hold in place for ~30 seconds.
9. Then immediately transfer the glands to the drop of Lacto/Aceto-orcein stain on the slide.
10. Place a clean coverslip onto the surface of the glands and use your forceps to gently tap the surface of the coverslip in a circular pattern.

    Place the slide on the microscope. Using the lowest power objective and standard light source, scan around the slide until squashed material has been found.

Observations:

1. It is well known that genes responsible for different characters (wild type and mutant) are borne by various chromosomes. Locations of these genes are precisely pointed out in the different bands of the salivary gland hromosomes of Drosophila sp. Close observation of thesalivary gland chromosome morphology reveals the following regions:
    a. The individual chromosome appears as six long arms that radiate out in both male and females. Five long arms radiate from a common chromocentre which is heterochromaticin nature.
    b. Each arm is basically composed of two homologous chromosomes as revealed by splitappearance of each arm.
    c. The chromosomes exhibit alternating dark bands and interbands.Bands that are deeplystained with aceto-orcein . These may be single or double. interband regions are very little stained. In Drosophila sp. there are about 5000 bands and 5000 interbands.
    d. Puffs on the different chromosomes are very lightly stained.