M.SC. IV SEMESTER
DISCIPLINE SPECIFIC ELECTIVE
Fish and Fisheries
ZOPDLD5: Fish anatomy, physiology and biotechnology
(Lab. Exercises)

Ex.1. General anatomical observations of a bony fish.

Fish identification depends mostly on the external morphological characters of the fish.

Depending upon the habits and habitats of the fish,variations in structure and shape are present in organs.

Snout

The anterior most part of a fish, which in most cases is rounded or obtuse. Variations are

a) Pointed and sharp (Eels).
b) With a groove across on top (*Shismatorhyn chos*nukta).
c) Tubular with jaws at tips (Pipefish).
d) Smooth in most cases covered with thin or thick skin but in some tubercles maybe present (*Gonoprok topterus*, *Barilius* species)
e) Over hanging the mouth (Engraulids)
f) Some have a proboscis developed (*Garranasuta)*

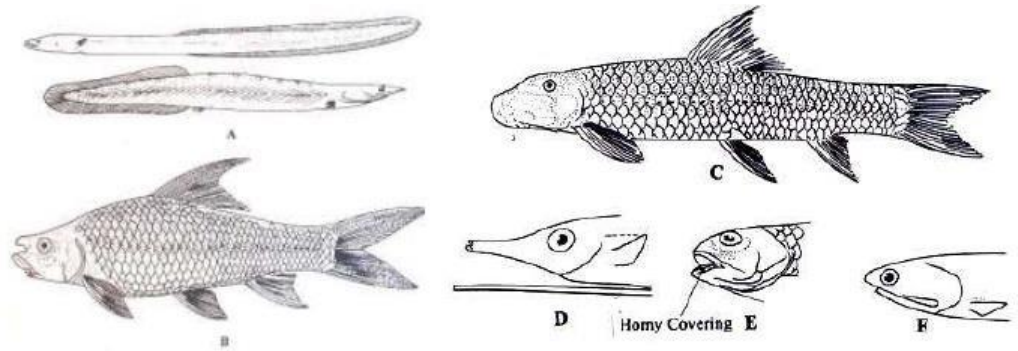


Fig.1. Shape of snout. A. Pointed and sharp. Eel. B. With a groove across on top. *Schimatorhynchos Nukta)nukta*. C.With a well-developed proboscis *Garranasuta*. D. Tubular with jaws at tips. Pipefish.
E. With tubercles. F. Overhanging.

Lips

- The premaxillary and maxillary bones of the upper jaw are covered by the upper lip and the mandible on the lower jaw by the lower lip.
- Mostly these lips are thin smooth membranes but in some they may be with pores, stripes as in *Labeo dero* and *L.dyocheilus* respectively or modified to form a sucker-like disc as in *Garro* species.
- In some as in the Mahseer the lower and upper lips are continuous around the jaws and the labial fold (fold formed by the lips) is uninterrupted by the isthmus or interrupted.
- The lower lip may evenable prolonged as a flap called the mentum.
- In *Torprogenius* the upper lip is modified as a fan shaped structure.

Lip Structure

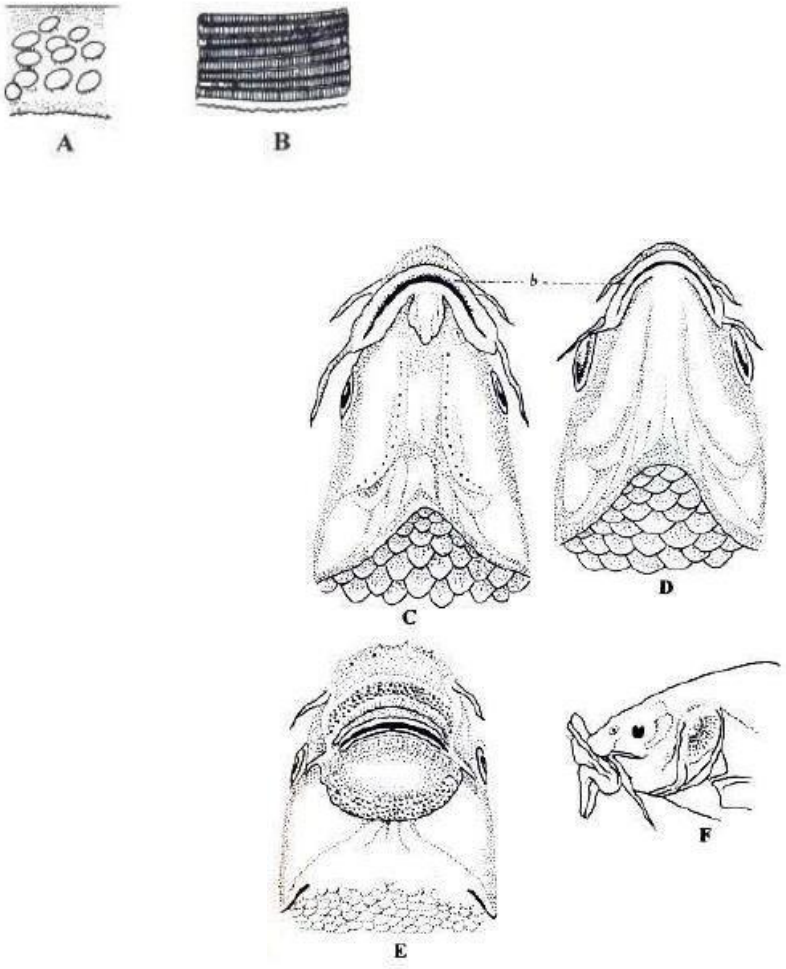


Fig. 2. Lip structure. A. *Labeo dero* with pores. B. *Labeo dyocheilus* with stripes. C. Labial fold continuous with metum. D. Labial fold interrupted.(b=Upper lip) E. With a suctorial disc on lower lip Garra. F. Upper lip with fan-shaped enlargement *Tor progenies*

Mouth

- Mouth is the chief organ for feeding of the fish and based on the type of food it takes, the shape, position, size and form vary.
- In most cases it is terminal or slightly below subterminal. Surface swimmers as *Danio, Puntius*, and *Rasbora* species have a terminal mouth.
- On the other hand hill stream fishes as *Balitora, Bhavania,*
- *Garra* species have their mouth narrow and placed in the ventral side of the snout to suit their scratching of food from the rocks and boulders where they live without being washed away by the surging waters.
- Species of *Glyptothorax* have the terminal mouth placed slightly inferior.
- In Belonidae (freshwater Gars) the mouth is superior, wide and the cleft extends to the border of the eyes (orbit).

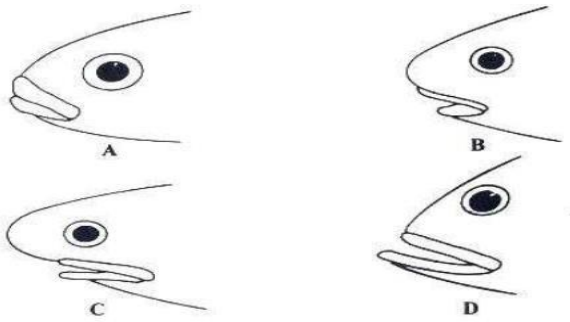


Fig.3.Shape of mouth. A.Terminal (*Danio*, Rasbora, *Putius*). B. Sub-terminal. C. Inferior (*Balitora, Garra*) D. Superior (Belontids).

Teeth

- Teeth are borne on the jaws and palate. All fishes may not have teeth. The teeth are essentially meant for crushing, scraping the food that the fish takes and accordingly they are modified.
- Many as *Chanos chanos* (Milk fish), Cyprinids are without teeth (called edendate).
- Siluroids have sharp teeth.
- The teeth when present are mostly villiform (sharp), conical, molariform (Rita species), canine (*Pseud apocryptes* Goby).
- In Puffer fish (*Tetraodon* species) the teeth are formed like a beak- like dental plate. In most fishes the teeth on the lower jaw are in the form of an arrow or wide band, separated in the middle where as on the upper jaw it is uninterrupted and continuous.
- On the palate they may be in patches, discontinuous or continuous or as a single broad band. The band is nearly curved and may extend deep into the corner of the mouth.

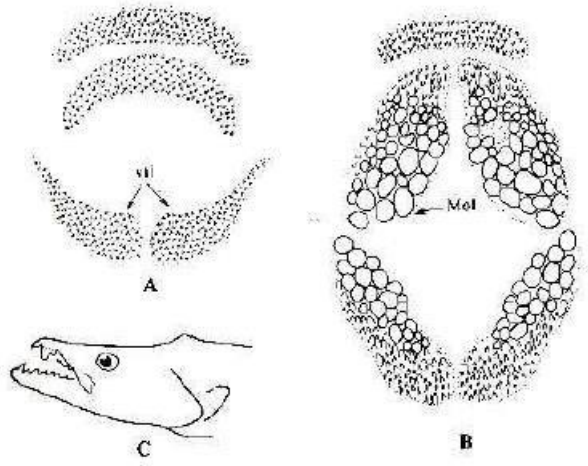


Fig.4 Teeth.A.Villiform.B.Molariform(*Rita*).C.Conical(Gobies).

Jaws

- The pre-maxillaries, maxillaries and mandible bones form the upper and lower jaws.
- The jaws are essentially mean to capture, hold and swallow the prey and the teeth help in munching, grinding and making it fit for passage through the gullet.
- They are united by a symphysis point which enables them to open and close the mouth.
- The jaws bear the teeth and act as the frame for the shape of them outh.
- The palate teeth are borne by the vomer bone, which is not a part of the jaw.
- In most fishes the jaws are more or less of equal length, but in some the upper jaw is longer than the lower.
- In Clupeidae the lower jaw is longer than the upper.
- In Engraulidae the upper jaw is projecting.
- InCtenopsspeciesboththejawsareelongatedtoformasomewhatpipe-shapedmouth.
- In *Hyporhamphus* species (Hemiramphidae) the lower jaw in the adult is elongated as along beak.
- In Pipe fishes (*Ichthyocampus* species) both the jaws are produced as a beak.
- In puffer fishes both jaws are divided by a median suture with a cutting edge and covered by ivory like substance.
- In some the lower jaw may be having a horny covering as in *Labeo fisheri.*

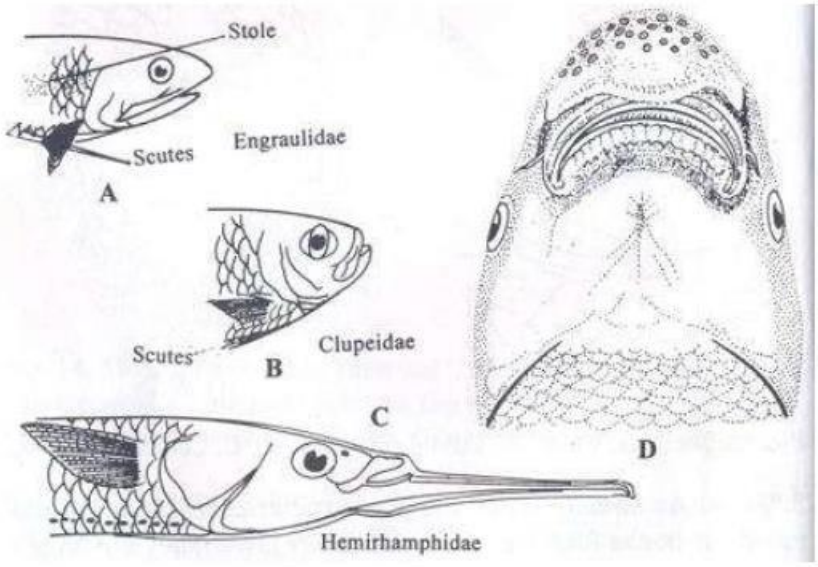


Fig.5 Jaws. A. Upper jaw longer than lower jaw (Engraulidae). B. Lower jaw longer than upper jaw (Clupeidae. C. Lower jaw elongated (Hemiramphidae). D. Jaw ridge horny (*Labeo fisheri*).

Barbels

- Barbels are flexible tactile filaments under the chin surrounding the mouth, on the snout, on the sides, on the ventral side and in between the nostrils.
- In cat fishes they play a very important role in identifying the food objects, locating the extent of the width in crevices and also as a defense organ.
- *Mystus bleekeri*, the fiddler fish of Mysore, erects its barbells in a threatening manner when disturbed.
- In the Ariid genus *Osteogeniosus* the only pair of maxillary barbels are thick and semi-osseous.

- Most siluroids carry four pairs of barbels, but it is not constant; it may be one, two or three.
- The Cyprinids also have barbells but not as long as in the catfishes.
- In *Nemacheilus* the barbells may be well developed and they are used as a sensory organ only.

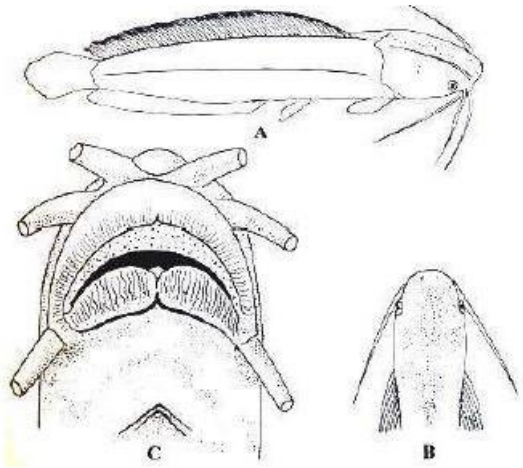


Fig.6 Barbels. A. Soft and muscular Clarias batrachus. B. Stiff and osseous *Osteogeniosus militaris*. C. Simple hollow short tubes. *Noemacheilus labeosus*.

Nostrils

- Nostrils are a pair of apertures or slits on the snout which are the openings for the smell organs leading to the nasal canal on the skull.
- They are mostly small to medium and are sunk in the snout, often covered by mucous especially in catfishes.
- A pair of nasal barbells is often seen,which may be long, short or rudimentary and borne on the posterior one.
- They are generally well separated but in Sisoridae the nasal barbels are closely placed one behind the other, slit-like but separated.
- In Heteropneustidae the anterior nostril is placed on the tip of the snout and produced as short tube.
- In Ariidae they are closely placed and separated by a valve like structure.
- In some Nemacheilines a flap separates them.
- In *Oreonectes*the anterior nostril is prolonged as a long nasal barbel.

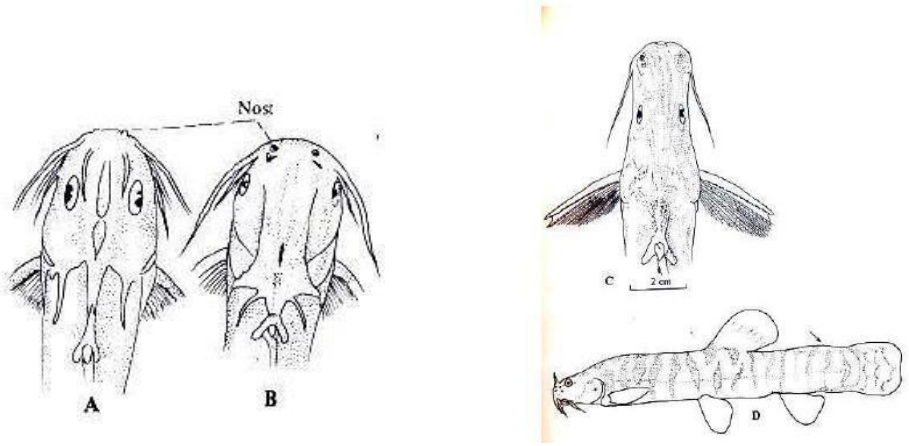


Fig. 7 Nostrils. A. Placed wide apart Bagridae. B. Close together Sisoridae. C. Separated by a valve Ariidae. D. With a barbel in-between *Oreonectes* (*Oreonectes*) *evezardi*

Eyes

- Eyes mainly used for seeing food, enemies and predators are placed in most fishes dorso laterally (at the sides) along a mid-axis line of the body.
- However this position may vary depending upon the habitat of the fish. It may be superior or inferior.
- Many gobioid fishes have the eyes placed on the top of the"head. Species of *Oxyurichthys, Bathygobius, Boleophthalmus* have the eyes placed on top of the head.
- *Mugil corsula* has protruding eyes on the top.
- In some catfishes the eyes are placed low so that they are visible from below the ventral surface.
- Chandramara chandramara, *Horabagrus brachysoma*, Ompok and *Ailia*species show this kind of placement.
- The cat fishes brow seat the bottom and hence the eyes are situated at this level.
- The eyes are generally large in size or moderate, but in the eels and hill-stream fishes they are, small; the latter being denizens of fast powing shallow 'streams, with too much light penetrating, large eyes would be a disadvantage.
- In *Brachyam blyopuiburmanicus* (eel like goby) the eyes are minute and hidden.
- The eyes are subcutaneous and they may be circular, oval in shape. Some cave dwelling fishes are totally blind.

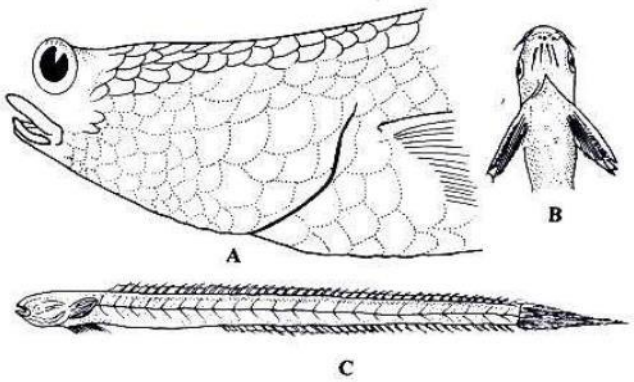


Fig. 8 Eyes. A. SuperiorMugilcorsula. B. Inferior visible from below ventral surface *Chandramara chandramara*. C. Minute reduced, hidden *Brachyamblyopus burmanicus*.

Operculum and Gills

- Operculum and gills form part of the branchial apparatus. On either side of the fish.
- The gill slits are situated which may be wide, narrow or even in the form of a small aperture as in the case of the eels.
- In the snake eels (Ophichthyidae) the gill openings are in the pharynx as wide slits.
- In Moray eels (Muraenidae) they are small, round openings only.
- In hill stream fishes they are greatly restricted to the ventral side (*Bhavania australis*). Where the openings are wide they are covered by a group of flat thin opercular bones joined together by the skin which covers the gills inside.
  The concave pharyngeal margins of the branchial arches are fringed with a double series of

either cartilaginous or bony tubercles or filaments called the gill rakers.
The anterior row of gill rakers on each arch usually interdigitate with those of the posterior row on the preceding arch and in this way the two rows form a sieve like mechanism to prevent any solid particles entering the pharynx with the respiratory current of water and from passing into the gill clefts and clogging it.

- Thegillarchescarrythegilllamellaeandgillrakersorbranchiospines.Thefirstbranchial arch (the anterior- most one) carry rakers on the upper limb and filaments on the lower limb. Five gill arches are placed on either side of the head region.

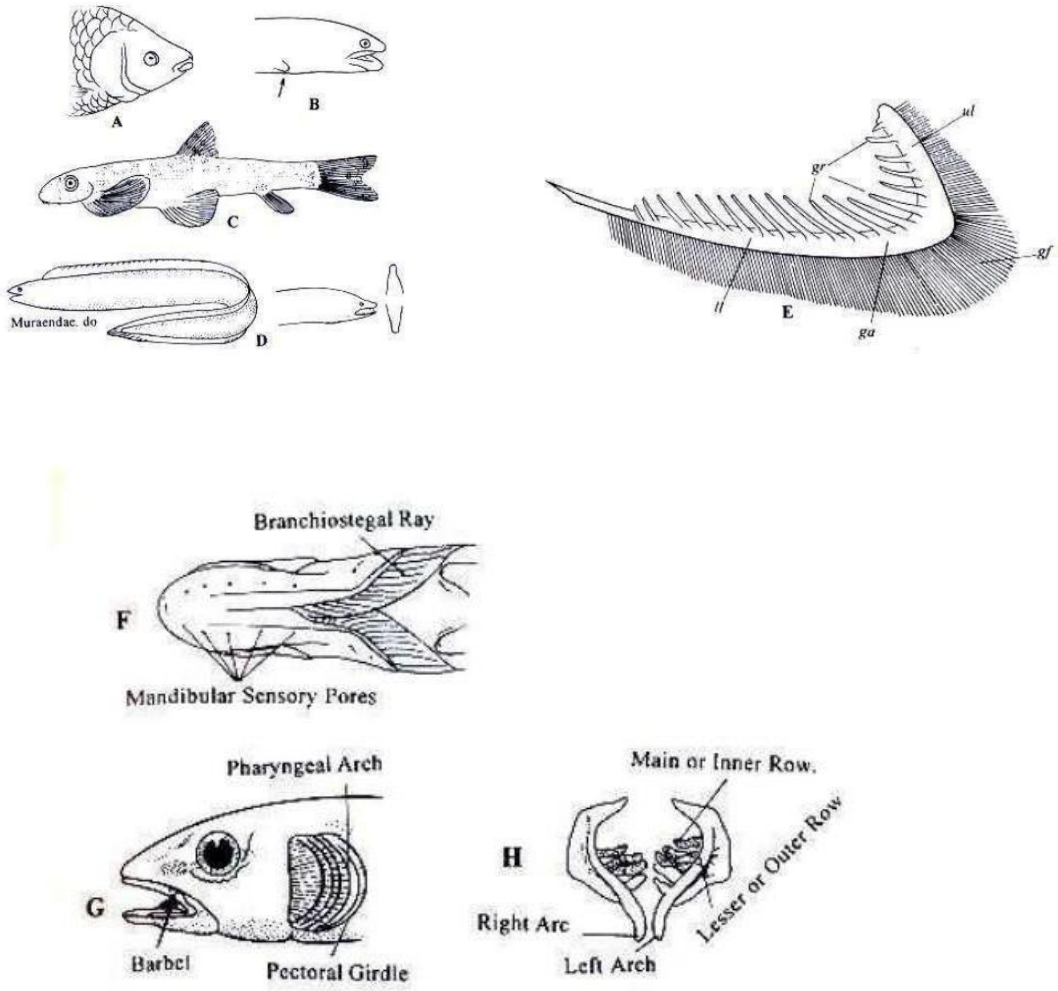


Fig. 9 Operculum and gills. A. Normal. B. Eel as a moderate slit in pharynx near base of pectoral fin. C. Greatly restricted above base of pectoral fins *Bhavania australis*. D. Round andlateralinpharynxMureanidae.E.Structureofagill.(*u.l*)Upperlimb.(*gf*)Gillfilament. (*ga*) Gill arch. (*ll*). Lower limb. (*gr*) Gill rakers.

Median groove

- Median longitudinal groove or fontanel are two longitudinal-externally visible long depressions on the head and covered by skin in catfishes.
- They may be single or double and are in the center of the head extending from near the snout to the base of the occipital process.

- When single it is a continuous depression without a break. When double it is interrupted in the middle by a short bone. These represent the passage for the cranial nerves in the skull. When covered with thick skin its extent can be found by inserting a needle and dragging

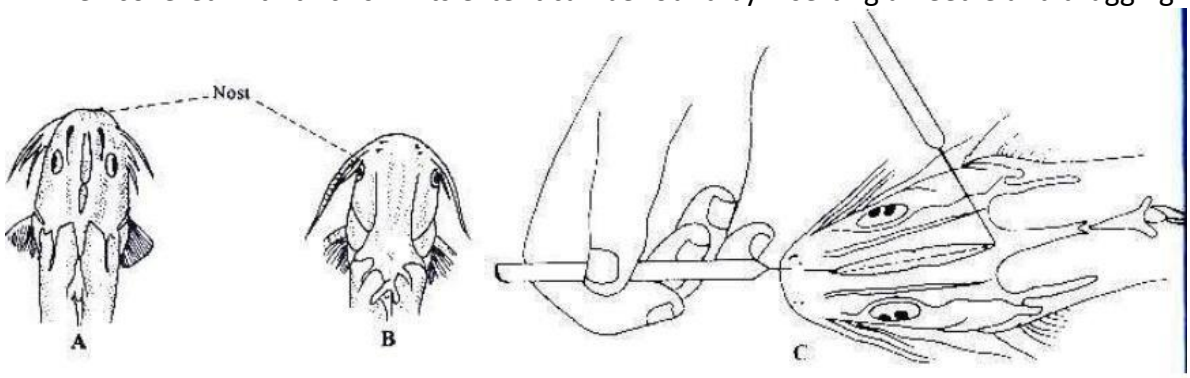


Fig.10 Mediangroove. A. Continuous. B. Interrupted as two fontanels. C. Extentand identification of fontanel.

Body

The Body of the fish carries the paired and unpaired fins, scales, lateral line and internal organs as already started. The main features are

1. Paired fins

The pectoral, pelvic fins are the paired fins since they are two in numbers placed side by side.**Pectoral fins-** are inserted in most cases laterally may be horizontally (Psilorhynchidae, some Homalopterids) or even above the ventral profile (perches, gobies).
They bear the fin rays, simple and branched and in cat fishes the pectoral spine.
In some case the fin rays may be elongated as long filaments (*Ctenops nobilis*).

Pelvic fins(sometimes called ventral fins), are inserted in most cases ventrally and are placed with a distance in between them.
In Gobiidae they are united.
In *Sicyopterus* they are united in the form of a cup shaped disc. The fins bear the simple and branched rays.
In Syngnathids they are much reduced.
The fins are absent in some (eels,Mastacembelidae,Puffer fishes).
In perches the fins when present may be thoracico-jugular in position and bear spines.

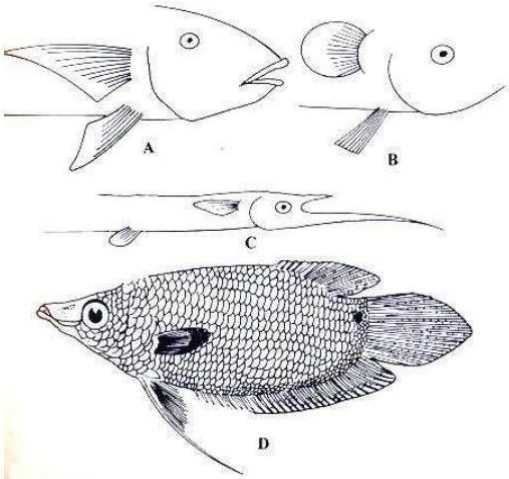


Fig. 11 Pectoral fin insertion. A. Thoracic. B.Jugular. C. Abdominal. D. with filaments *Ctenopsnobilis.*

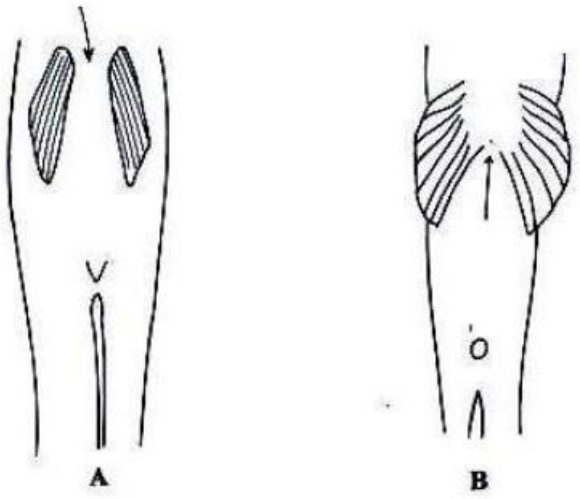


Fig. 12.Pelvicfins:A.Free.B.Unitedasacup (Gobioids).

2. Unpaired fins

The dorsal, anal and caudal fins are unpaired in the sense that they are single and not in pairs.

Dorsal fin-in most fishes is single, concave in shape with smooth or serrated spine with simple and branched rays.

- There may be a procumbent spine in some *Mystacoleucus*.
- In *Megalopscyprinoides* the last ray is prolonged as a Filament.
- In Perches there are two dorsal fins one after the other with the first one separated either by a short or long gap from the second fin or may even be united; both may bear spines and also soft and branched rays.
- In mugils the first fin is with spines only, separated from the second one by a distance.
- In Synbrachids (Swampeels) the dorsal fin is vestigial in the form of ridges only.
  In Mastacembelus the fin is in two parts; the first one with 32 to 40 short depressible spines and 46 to 90 rays.
- In *Sillaginopsis* the second dorsal spine is prolonged as a long filament.
- The fin may be in different positions on the dorsal profile, mostly at the center, but in many may be far posterior above the anal fin. The fin may be free or even confluent with the caudal fin.
- An adipose dorsal fin is present in siluroids and salmons; it is generally smooth, free and not united with the rayed dorsal fin though the inter space between the two may belong or short.
- In *Sisorrhabdophorus* the adipose fin is reduced in the form of a spine.
- In *Chaca chaca* and some other fishes it is confluent with the caudal fin

Anal fin- is inserted on the ventral side and is with simple and branched rays.

- Generally the fin is free, short, but exceptions are there as in the case of *Horabagrus, Clarias*, *Heteropneustes,* Schilbeids, Pangasids., Plotosids.

- In Claridae and Heteropneustidae though long, it is separated from the fin by a short distance. In *Horaichthys* the fin is modified into two parts; the first six rays are separated as an independent gonopodium.
- In *Garnbusiaan* intromittent organ present. In both cases Only the males show this adaptation.

Caudal fin or the tail fin- is the propeller for the fish and acts as a rudder.

- It is the posterior most part of the fish body. It is of varying shapes and is always a single fin, rounded. with or without margins, truncate, furcate or slightly emarginate, forked, lunate or lanceolate, wedge or paddle shaped, notched, rounded or ovate,etc.

Lateral line

- The Lateral line is the sensory line formed along each side consisting of sensory pores to tiny tubes in scales or skin.
- Most fishes have the lateral line, but in some it is absent (Mugilidae).
- It is generallycontinuous, but in some Cyprinids and Perches it may be discontinuous or in two levels.
- Generally it stops at the base of the caudal fin but in *Latescal carifer* it extends beyond into the caudal fin.
- In *Toxotes chatareus* it is interrupted.Scales

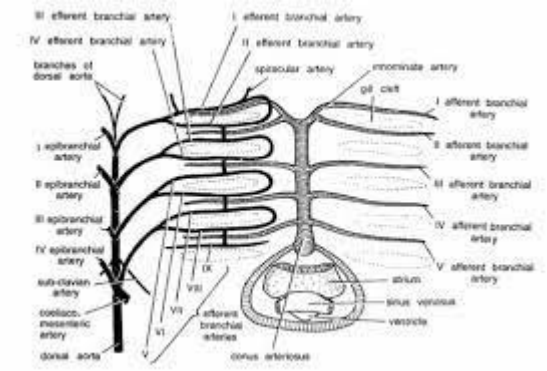
The body of fish is usually covered with scales,which provide protection.There are four basic types of scales:

- Placoid scales (pointed, analogs of vertebrate teeth, e.g.,in Elasmobranchii);
- Cosmoid scales (probably evolved from the fusion of placoid scales, e.g., in Family Ceratodontidae);
- Ganoid scales (rhomboid shaped, modified cosmoid scales;e.g., in Family Lepisosteidae); and
- Elasmoid scales, separated in to cycloid (circular with smooth edges) and ctenoid (circular with combed edges) scales, e.g., in Actinopterygii.

Abdomen

- The **Abdomen** of a fish is mostly rounded except in flat fishes, hill stream fishes and deep sea fishes where they are flat.
- In most Cyprinids the abdomen may be keeled with no barbell surrounded with barbels.
- In Clupeids the ventral profile may be with serrations.
- In the Sisorid fish *Glyptothorax* an adhesive apparatus is developed in which the paired fins, pectoral and pelvics, may be plaited

Ex.2. Display of afferent and efferent branchial vessels.

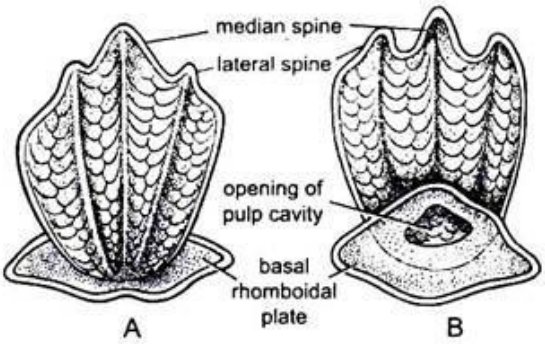


- **Afferent branchial arteries:**
- There are five pairs of afferent vessels.
- -Ventral aorta bifurcates into two.
- Each of which divides again to form the first and second afferent blood vessels.
- The third, fourth and fifth afferent vessels arise from the posterior part of ventral aorta.
- These arteries arise from the ventral aorta which supply deoxygenated blood to the gills for oxygenation.
- **Efferent branchial arteries:**
- These arteries arise from the gills and supply oxygenated blood to all body parts.
- The oxygenated blood from the gills is collected by 09 efferent blood vessels on each side.
- First 08 join in pairs while 9^{th} is connected with 4^{th} pairs of efferent vessels by a longitudinal connective.

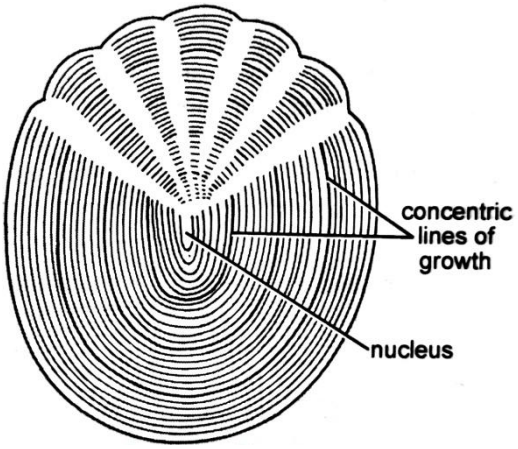
Ex.3. Study of available histological slides of different structures/organs.

Fish Scales

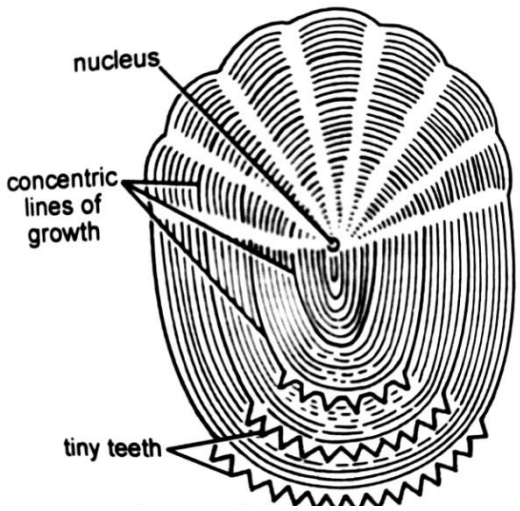
1. Placoid scales: Consists of a basal plate and a spine, giving a rough surface to skin. Basal plate is formed of a substance resembling the cement of teeth, secreted by the dermis. The spine develops from the malpighian layer of the epidermis -The outermost covering of spine is made of vitrodentine while inner layer is dentine which encloses pulp cavity. The basal plate has an aperture through which blood vessels and nerves of the dermis enter into the pulp cavity -The placoid scales do not overlap each other -Ecto-mesodermal in origin, resemble teeth in basic structure.



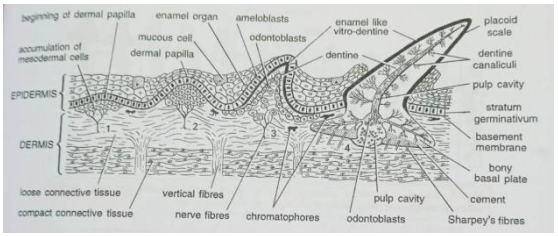
2. Cycloid scales : Thin, transparent, roughly rounded -Shows alternate ridges and grooves -Ridges or circuli are concentric rings –Central part is focus -Oblique grooves or radii running from the focus to the margin -Dermal in origin -First appear on the caudal peduncle of the larva and then on the remaining body -Project diagonally in an imbricating pattern, forming a protective covering over the body -The circuli or ridges are less distinctly seen in the posterior part of the scale to which chromatophores are also attached. Bony material is ichthylepdin. **eg.** Carps (Teleosts).



3. Ctenoid scales: Basically similar to the cycloid scale -Has a serrated margin and spines on posterior part **eg.** Perciform fishes (*Anabas, Nandus, Channa*).



V.S. of skin of *Scoliodon*



1. Skin hard, rough, rigid, leathery and firmly attached to body.

2. Colour of Scoliodon is dark, grey dorsally and pale white ventrally. In fishes, greatest colour patterns amongst chordates.

3. irridophres located in dermis.

4. Body color does not change. Some fishes have protective colouration.

5. No cutaneous respiration. Not permeable to water.

6. Epidermis stratified , thin and without a cornified stratum corneum, thin cuticle present, no moulting.

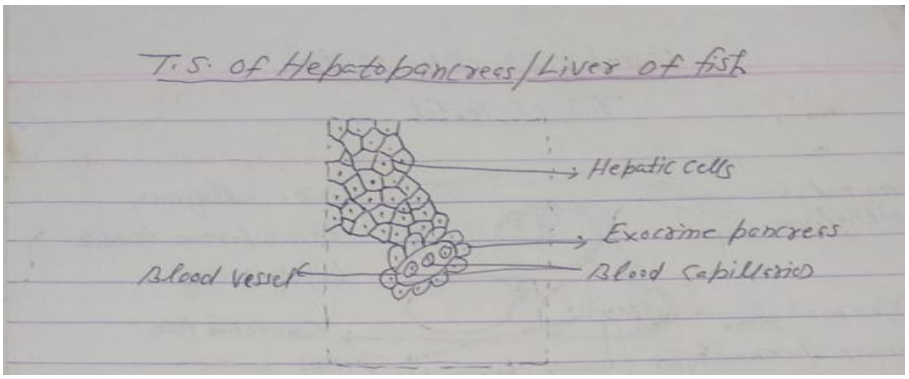
7. Epidermis contains numerous unicellular mucous secreting gobbler gland cells. Multicellular poison and luminescent glands also occur in some fishes.

8. Dermis is typical with connective tissue fibres, blood and lymph vessels and pigment cells. Connective tissue fibres run parallel to surface

9. Dermal scales are present as placoid scales.

10. Exoskeleton present and represented by scales.

T.S. of Hepatopancreas/Liver of fish:



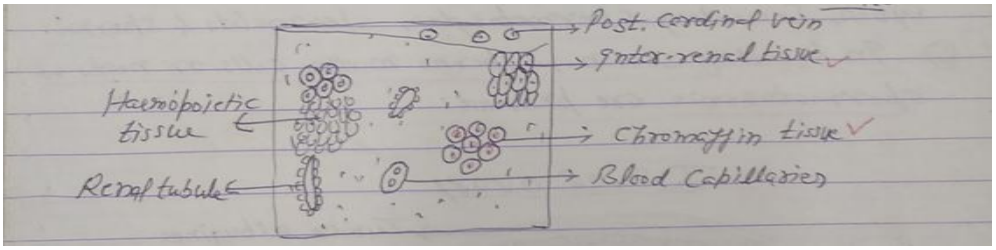
-It is composed of hepatic cells.

-In between hepatocytes, blood vessels and blood capillaries are present.

-In many species, pancreas extends into liver forming hepato-pancreas.

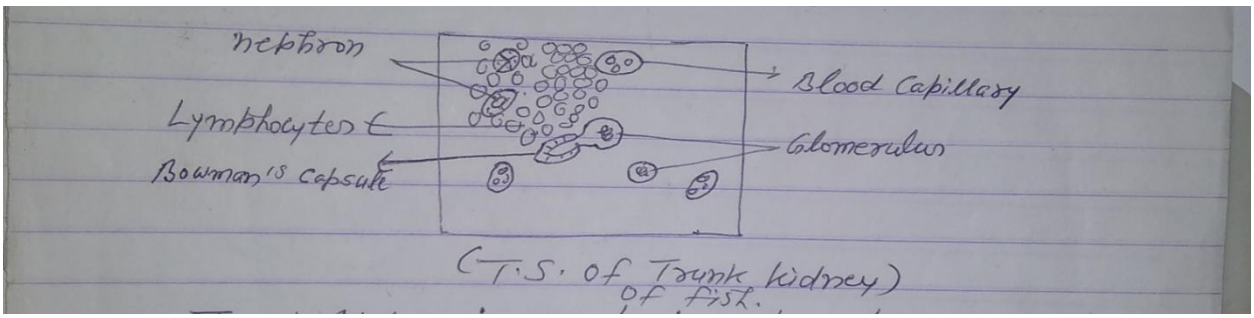
-Exocrine part of pancreas contains zymogen cells.

T.S. of Head kidney through adrenal tissue:



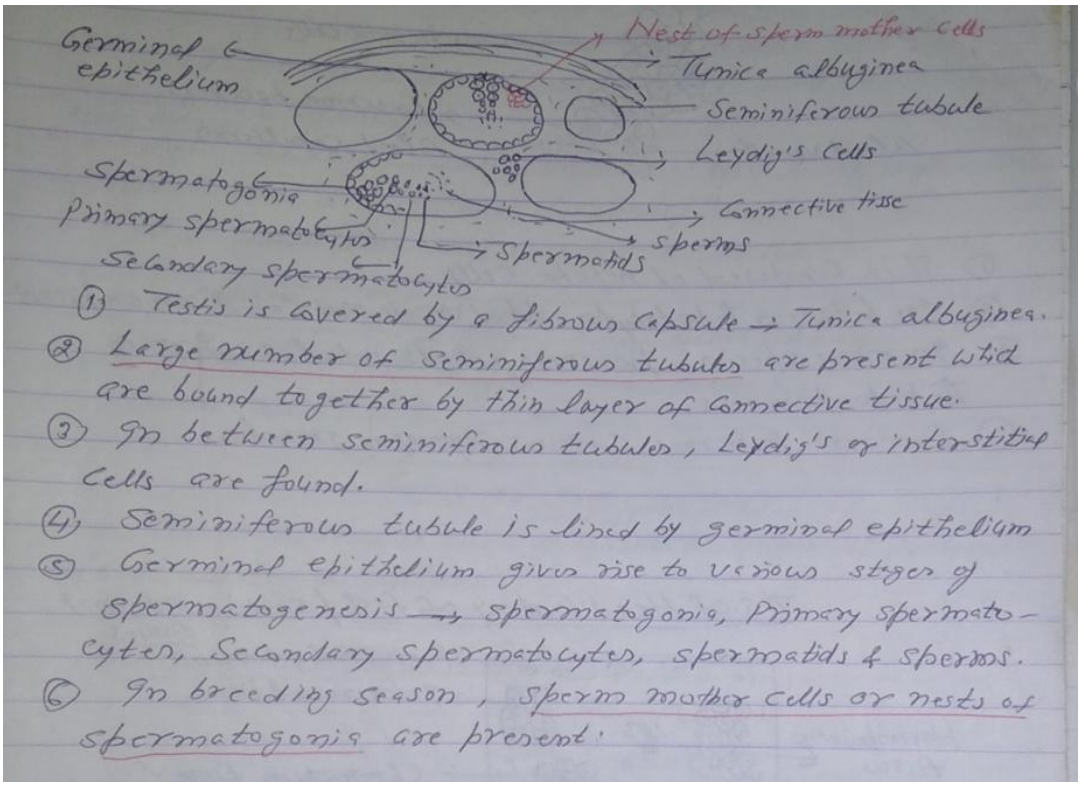
-It contains renal tubules (rare), inter-renal tissue and chromaffin cells.
-Inter-tubular space is full of lymphoidal/haemopoietic tissue.
-Mostly head kidney is not excretory in function.
-Adrenal gland is composed of iter-renal and chromaffin tissue.
- Both are mainly composed of haemopoietic tissue.
-Inter-renal tissue is located along the post cardinal vein and also diffused throughout the kidney and eosinophilic in nature.
-These cells are columnar, cuboidal or polygonal and closely associated with chromaffin tissue.
-Inter-renal cells secrete gluco and mineralocorticoids which affect sugar level and osmoregulation respectively.
-Inter-renals are homologous to adrenal cortex.
-Chromaffin cells are dispersed in head kidney near the post cardinal vein.
-These cells are rounded and basophilic in nature.
-Cells occur singly or in groups.
-These are homologous to adrenal medulla.
-They secrete adrenalin and nor-adrenalin.
-They also contain dihydroxyphenylalanine (DOPA) and 5-hydroxytryptamine (serotonin).

T.S. of Trunk kidney:



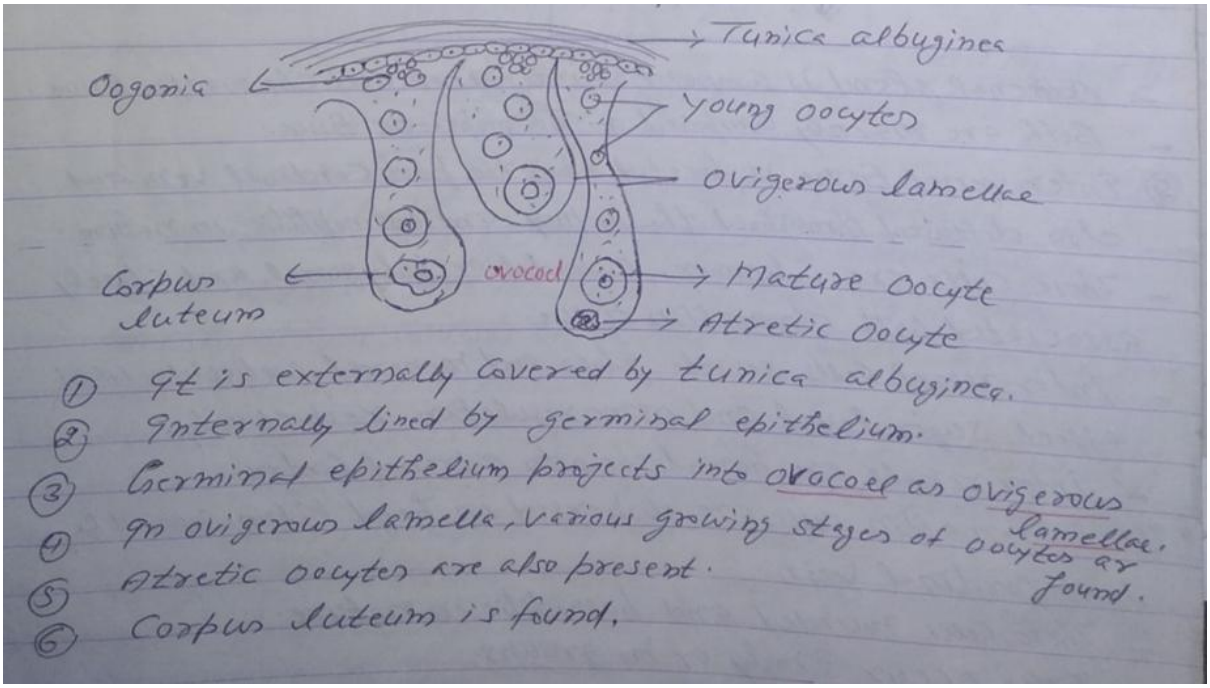
-Trunk kidney is excretory in nature.
-It contains a large number of nephrons.
-Inter-tubular space is filled with lymphoidal/haemopoietic tissue.-In cross section, blood capillaries, Bowman's capsule, glomerulus, etc. are seen.
-Corpuscles of Stannius may be seen.

T.S. of Testis



(1) Testis is covered by a fibrous capsule → Tunica albuginea.
(2) Large number of seminiferous tubules are present which are bound together by thin layer of connective tissue.
(3) In between seminiferous tubules, Leydig's or interstitial cells are found.
(4) Seminiferous tubule is lined by germinal epithelium
(5) Germinal epithelium gives rise to various stages of spermatogenesis → spermatogonia, Primary spermatocytes, Secondary spermatocytes, spermatids & sperms.
(6) In breeding season, sperm mother cells or nests of spermatogonia are present.

T.S. of Ovary



(1) It is externally covered by tunica albuginea.
(2) Internally lined by germinal epithelium.
(3) Germinal epithelium projects into ovocoel as ovigerous lamellae.
(4) In ovigerous lamella, various growing stages of oocytes are found.
(5) Atretic oocytes are also present.
(6) Corpus luteum is found.

Ex.4. Study of haematological parameters- blood corpuscles, T.C, D.C and Hb content

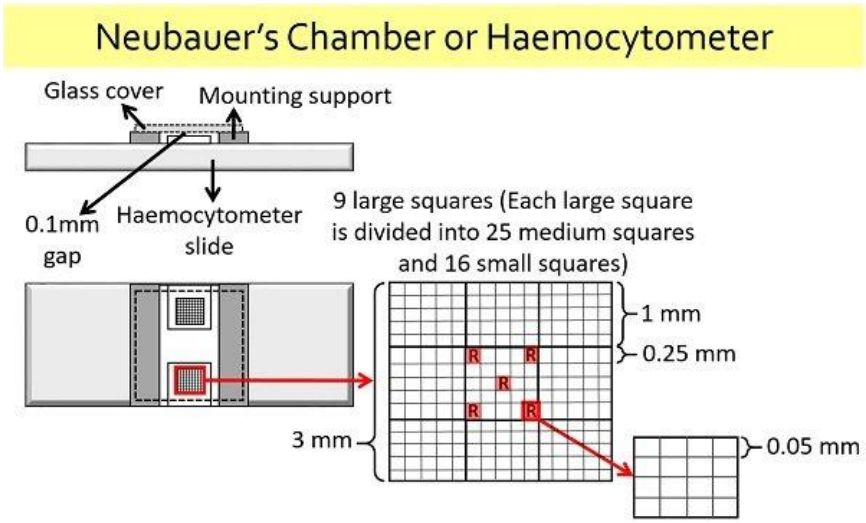
Aim: To estimate the total number of RBCs in human blood.

Principle: The blood specimen is diluted (200 times) with the help of RBC diluting fluid (Hayem's solution) which preserve and fix the Red blood cells. The Hayem's fluid is isotonic to the Red blood cells (salinity of blood- 0.9%) and does not cause any damage to it. RBCs are then counted with the help of haemocyrometer.

Requirements: Haemocytometer, RBC pipette, Hayem's solution (RBCs diluting fluid), microscope, etc.

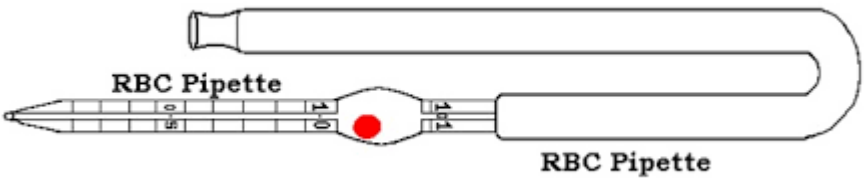
Haemocytometer: It consists of a special glass slide containing two counting chambers (Neubauer's haemocytometer). It is 3 inches long and 1.5 inch wide. It has 4 parallel channels extending across the width of the slide, forming 5 platforms. Central platform consists of 2 counting chambers.

Each counting chamber is 9 sq. mm in size and is divided into 9 small squares, of 1sq.mm each. Central small square is again divided into 25 smaller squares, of 1/25 sq.mm each. Each smaller square is further divided into 16 smallest squares, of 1/25x16 sq. mm each. Thus there are total 400 smallest squares in central small square.



RBC pipette:

1. It consists of capillary tube, central bulb with red bead and 03 graduation marks (0.5 cmm, 1.0 cmm and 101 cmm).
2. For sucking the blood and diluting fluid, a rubber tube is fixed to the broad end.
3. Red bead inside the bulb is for mixing of blood with Hayem's solution.



Hayem's solution: It is composed of the following ingradients:

1. Hg Cl_2 → 0.05 g
2. Na2 SO4 → 2.5 g
3. NaCl → 0.5 g
4. Distilled water → 100 ml.

-Sodium chloride maintains osmolarity and provides isotonicity, so that red cells maintain their shape and size or do not burst. Sodium sulphate prevents aggregation of RBCs; it also acts as an anticoagulant and fixative. Mercuric chloride acts as a preservative.

Procedure: 1. Sterilize tip of ring finger of left hand with sprit.

2. Prick the finger with sterilized needle, so that blood flows freely. Do not squeeze the finger.
3. Then suck the blood in the clean and dry RBC pipette up to 0.5 cmm mark.
4. Wipe out the blood adheres to the tip of pipette.
5. Now immediately suck in the Hayem's solution up to the 101 cmm mark of the RBC pipette. Hayem's solution prevents the haemolysis, rouleaxue formation and cogulation of blood.
6. Held the RBC pipette horizontally and rotate several times. Blood is mixed with Hayem's solution with the help of red bead.
7. Discard 1 to 2 drops of the sample.
8. Now quickly transfer the sample in the counting chambers under the cover slip.
9. When counting chambers are properly flooded, keep the slide for few minutes on a horizontal place, so that RBCs are settled down the bottom of chambers.
10. Transfer the slide gently and place it under the microscope. Start the counting RBCs in counting chambers.

Counting of RBCs: Count the RBCs in 05 different smaller squares (1st, 5th. 13th, 21^{st} and 25^{th}). Avoid the counting of RBCs which are on the triple lines.

Calculation:

$$\text{No. of RBCs/cmm of blood} = \frac{\text{Total no. of RBCs counted x dilution (200) x Total no. of smallest squares (400)}}{\text{No. of smallest squares counted (80) x Height of blood film (0.1 mm)}}$$

Results: No. of RBCs /cmm of fish blood is……

Precations:

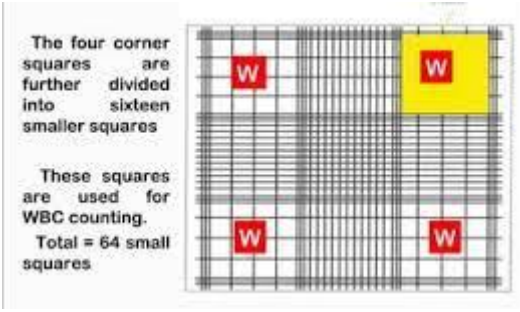
1. Use clean and dry haemocytometer and pipette.
2. Use rectified sprit to sterilize needle and finger tip.
3. Dilution fluid should not exceed the 101 cmm mark.
4. Mix the Hayem's solution and blood properly to get uniformly distributed RBCs.
5. Do not overflow the counting chamber.

Aim: To estimate the total number of WBCs (TLC) in fish blood.

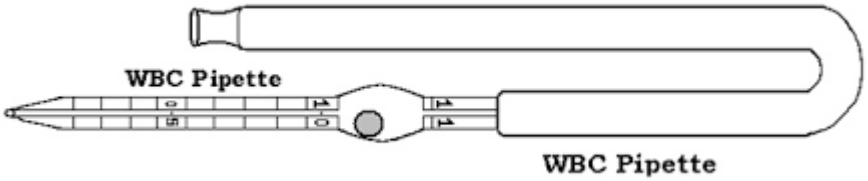
Principle: The blood specimen is diluted (usually in 1:20 ratio) with the help of WBC diluting fluid (Turk'S solution) which stains and fix the White blood cells. The diluting fluid is hypotonic to the blood so RBCs get burst but does not cause any damage to WBCs, A hemocytometer is charged

with the diluted blood, and WBCs are counted in the appropriate chambers using a light microscope.

Requirements: Haemocytometer, WBC pipette, WBCs diluting fluids, microscope, etc.
Haemocytometer: Each counting chamber contains 09 small squares, of 1 sq.mm each. 04 small squares, one on each corner, are used to count the WBCs. Each small square is divided into 16 smallest squares.



WBC pipette:
1. It consists of capillary tube, central bulb with white bead and 03 graduation marks (0.5 cmm, 1.0 cmm and 11 cmm).
2. For sucking the blood and diluting fluid, a rubber tube is fixed to the broad end.
3. White bead inside the bulb is for mixing of blood with diluting and staining fluid.



Turk's solution: It contains 0.2 ml of 1% Gentian Violet + 0.3 ml of Glacial acetic acid (100 %). The mixture is diluted to 1000 ml of ultrapure water.
-It dilutes blood, stains WBCs and destroy RBCs.
Procedure:
1. Suck the blood in the clean and dry WBC pipette up to 0.5 cmm mark.
2. Wipe out the blood adheres to the tip of pipette.
3. Now immediately suck in the diluting fluid up to the 11 cmm mark of the WBC pipette (dilution 20 times) and mix the blood with diluting fluid.
4. Discard 1 to 2 drops of the sample.
5. Now quickly transfer the sample in the counting chambers under the cover slip.
6. After settling the WBCs, start the counting.
Counting of WBCs: Count the WBCs in 04 corner small squares (1st,3rd. 7th and 9th).

$$\text{No. of WBCs/cmm of blood} = \frac{\text{Total no. of WBCs counted x dilution (20)}}{\text{No. of small squares counted x Height of blood film (0.1 mm)}}$$

Results: No. of WBCs /cmm of blood is……

Precations: 1. Use clean and dry haemocytometer and pipette.

2. Use rectified sprit to sterilize needle and finger tips.

3. Dilution fluid should not exceed the 11 cmm mark.

4. Mix thediluting fluid and blood properly to get uniformly distributed WSBCs.

5. Do not overflow the counting chamber.

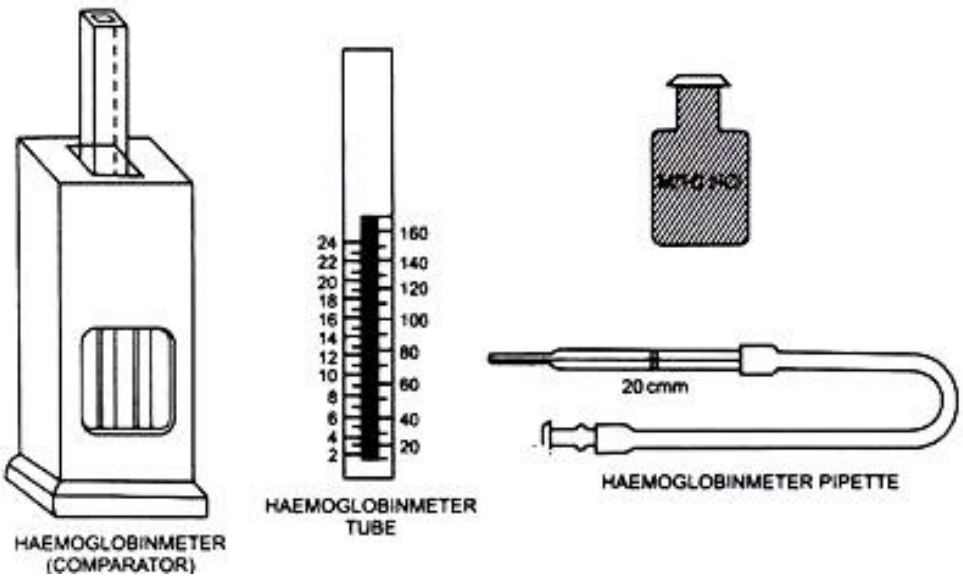
Aim: To estimate the gm percentage of haemoglobin contents in the fish blood.

Principle: When blood is treated with diluted acid/base, a brown coloured haematin compound is produced. Then this brown coloured solution is diluted with distilled water until the colour matches with the colour of standard glass tubes fitted in haemometer.

Requirements: Haemoglobinometer, N/10 HCl, distilled water, fresh fish blood, etc.

Haemoglobinometer/Haemometer: Haemometer consists of two vertical shield tubes containing a standard suspension of the acid haematin. A graduated tube with a glass rod/stirrer of same diameter is also provided which can fit in between vertical tubes. Colour of all the tubes is matched against a white background.

Hb pipette: It is a glass tube, having only one mark of 20cmm, and a rubber tube is fitted with it to suck the blood.



Procedure:

1. Clean the graduated tubes and Hb pipette well.

2. Take out 1ml N/10 HCl in graduated glass tube.

3. Suck the blood in pipette up to 20 cmm mark. Wipe out blood adhered to the tip of pipette.

4. Transfer this blood into graduated tube containing N/10 HCl and rinse 2 to 3 times in the same tube.

5. Mix the solutions with glass stirrer and allow to stand for 2-3 minutes.

6. A brown colour of haematin is developed.

7. Dilute this brown colour with distilled water or N/10 HCl drop wise until the colour matches with that of standard tubes.

8. Note the amount of sample in a graduated tube as it is equal to the gm% Hb per 100 ml of blood.

Result: Blood sample contains…….Hb.

Precautions:

1. Clean the glass tubes and rod carefully before use.

2. Fill the micropipette up to 20 cmm mark avoiding air bubbles.

3. Match the colour of tubes carefully against a white background.

Ex.5. Determination and comparison of hemoglobin content of water-breathing and air breathing fish.

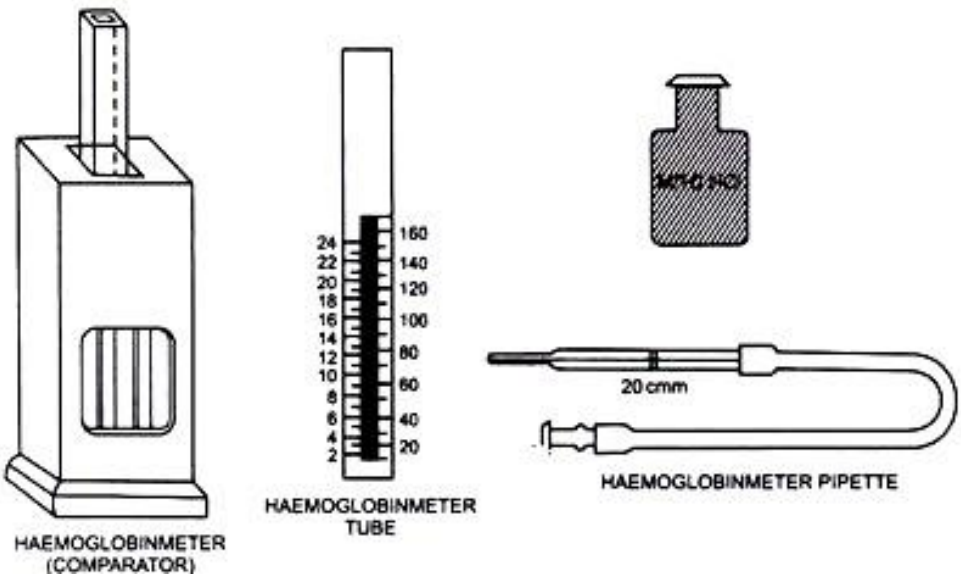
a. Aim: To estimate the gm percentage of haemoglobin contents in the fish blood.

Principle: When blood is treated with diluted acid/base, a brown coloured haematin compound is produced. Then this brown coloured solution is diluted with distilled water until the colour matches with the colour of standard glass tubes fitted in haemometer.

Requirements: Haemoglobinometer, N/10 HCl, distilled water, fresh blood, etc.

Haemoglobinometer/Haemometer: Haemometer consists of two vertical shield tubes containing a standard suspension of the acid haematin. A graduated tube with a glass rod/stirrer of same diameter is also provided which can fit in between vertical tubes. Colour of all the tubes is matched against a white background.

Hb pipette: It is a glass tube, having only one mark of 20 cmm, and a rubber tube is fitted with it to suck the blood.



Procedure:

1. Clean the graduated tubes and Hb pipette well.

2. Take out 1ml N/10 HCl in graduated glass tube.

3. Suck the blood in pipette up to 20 cmm mark. Wipe out blood adhered to the tip of pipette.

4. Transfer this blood into graduated tube containing N/10 HCl and rinse 2 to 3 times in the same tube.

5. Mix the solutions with glass stirrer and allow to stand for 2-3 minutes.

6. A brown colour of haematin is developed.

7. Dilute this brown colour with distilled water or N/10 HCl drop wise until the colour matches with that of standard tubes.

8. Note the amount of sample in a graduated tube as it is equal to the gm% Hb per 100 ml of blood.

Hb contents (gm%)	**Water breathing fish**	**Air breathing fish**
1.		
2.		
3.		
Mean value		

Result: Blood samples contain……Hb in water breathing fish and …….Hb in air breathing fish.

Precautions:

1. Clean the glass tubes and rod carefully before use.

2. Fill the micropipette up to 20 cmm mark avoiding air bubbles.

3. Match the colour of tubes carefully against a white background.

Ex.6. Study of ventilation rate and surfacing activity of fish under different experimental conditions.
Aim: To determine the effect of temperature on the ventilation rate of fish.

Materials required: Medium size fishes in small container, timer, thermometer, crushed ice, normal and hot water, etc.

Background: Counting of opercular movement is a way to calculate respiration rate in fishes. Fish breaths by taking in water through mouth and forcing it over the gills, when the mouth closes. This time oxygen-carbon dioxide exchange occurs between blood of gills and incoming water. Operculum opens to exhale the carbon dioxide rich water. By counting operculum

movement we can get an idea of a fish response to an ecological change. Respiration rate will typically increase as dissolved oxygen concentration in water decreases.

Procedure:

Carefully a fish was taken out from the tank and placed it in the beaker filled with tap water at room temperature.

The temperature of water in beaker is recorded by thermometer. Fish was allowed to calm down for 5-10 minutes.

Counting of respiration was done by looking at the opening and closing of operculum or mouth for 1 minute. The counting was recorded two more times.

The same procedure was followed for cold and hot water.

Observation table:

Water temp. (^{o}C)	Opercula movement per minute			Mean value of opercular movement
27	Observation 1	Observation 2	Observation 3	
30				
40				

*Plot a graph by taking water temperature at x- axis and opercular movement at y-axis.

Inference: The opercular movement of fish is ……

Precautions:

1. Handle the fish carefully
2. Slowly add cold and hot water in the aquarium
3. Record the water temperature acurately
4. Count the opercular movement with utmost care.

Ex.7. Determination of feeding habit of carps and catfishes by analyses of their gut contents

Aim: Determination of gut contents of *Wallago attu.*

Principle: Gut contents analysis throw light on feeding habits of fishes, whether these are herbivores or carnivores or both.

Requirements: Fresh fish specimens, dissection box, petri dishes, microscopes, formaline, cotton, etc.

Procedure:

1. Collect fresh sample of fish from local commercial fishermen in fresh condition.

2. Weigh each specimen nearest 1.0 gm accuracy with the help of single pan balance and the measure the length using scale (± 5.0 mm). After that dissect the fish to collect the gut contents for analysis.

3. Record the weight of the full stomach with the help of weight machine.

4. Open the gut with the help of scissors and then emptied into petri dish.

5. The gut contents of partially or undigested state were removed by using soft brush and collected in separate petri dish and fix in 5% formalin for further observation.

6. The weight of empty stomach was measured again to calculate the difference between the fullness and emptiness of stomach.

7. Short out the collected gut contents animal origin for the analysis of gut contents.

8. Analyse the gut contents qualitatively with the help of microscope.

-Gastro-somatic Index:

GSI helps in determining the feeding condition of fish in different months and conditions. It was calculated by using the following formula.

$$GSI = \frac{\text{Weight of full stomach}}{\text{Weight of fish}} \times 100$$

-Fullness of stomach:

The composition or fullness of the stomach is judged and classified as gorged or distended, full, 3/4 full, 1/2 full, etc. through eye estimation. Methods of gut contents examination are comprehensively distinguishable into two sections, viz., qualitative and quantitative. Quantitative methods for investigation are three types, viz., numerical, gravimetric and volumetric. The qualitative examination comprises of a complete identification of the organisms in the gut contents. In the present study, food items were identified qualitatively and classified them accordingly.

-Dominance method:

Basically the dominance method is a partial advancement of the occurrence method. In an investigation of predominance the bulk of the food material is taken in to record, it can yield only a very rough picture of the dietary of a fish. The items which are less dominant because of a few reasons may escape notice. In the present study, the dominant food items are recognized on the basis of their frequency occurred.

-Point's method.

The point's method is advancement on the numerical method where consideration is given to the majority of the food items. The food items are classified as "very common", "common", frequent", "rare", etc., taking into account harsh tallies and judgments by the eye.

-Length-weight relationship:

Length-weight relationship is shown by a factor (k) known as condition factor or coefficient. Condition factor reflects about the health of fish during a certain period. Condition factor is the ratio of the length to the weight of the fish. It can be calculated as:

$$\text{Condition factor (k)} = \frac{W \times 100}{L^3}$$

Where, L= length (cm), W = weight (gm).

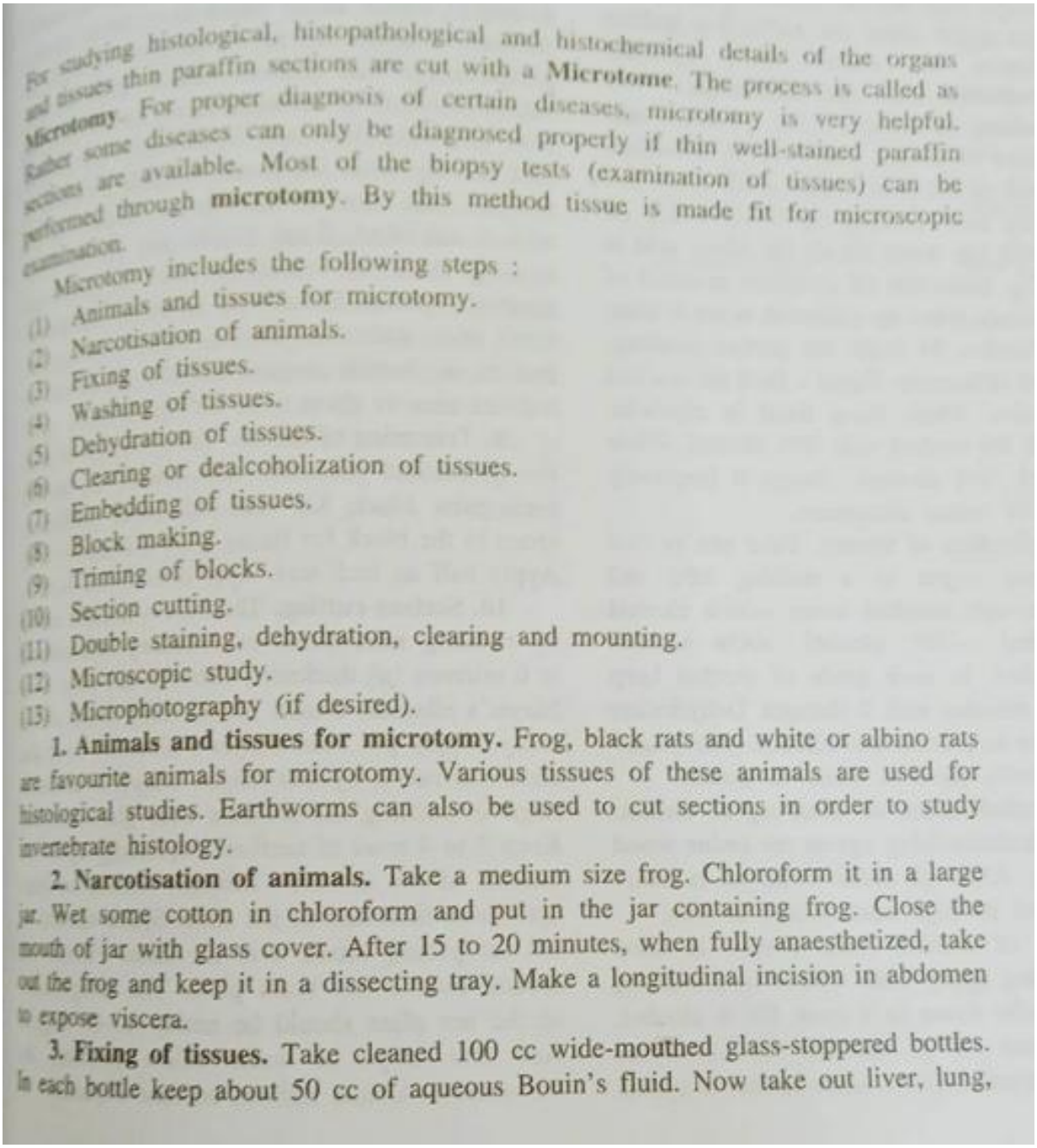
-L^3 is taken as volume because growth in weight is proportionate to the growth in volume.

-Factor 100 is used to bring value of 'k' close to 1.

Results:

Precautions:

Ex.8. Preparation of permanent stained slides of different endocrine glands and kidney of a teleost.



For studying histological, histopathological and histochemical details of the organs and tissues thin paraffin sections are cut with a **Microtome**. The process is called as **Microtomy**. For proper diagnosis of certain diseases, microtomy is very helpful. Rather some diseases can only be diagnosed properly if thin well-stained paraffin sections are available. Most of the biopsy tests (examination of tissues) can be performed through **microtomy**. By this method tissue is made fit for microscopic examination.

Microtomy includes the following steps :

(1) Animals and tissues for microtomy.
(2) Narcotisation of animals.
(3) Fixing of tissues.
(4) Washing of tissues.
(5) Dehydration of tissues.
(6) Clearing or dealcoholization of tissues.
(7) Embedding of tissues.
(8) Block making.
(9) Triming of blocks.
(10) Section cutting.
(11) Double staining, dehydration, clearing and mounting.
(12) Microscopic study.
(13) Microphotography (if desired).

1. Animals and tissues for microtomy. Frog, black rats and white or albino rats are favourite animals for microtomy. Various tissues of these animals are used for histological studies. Earthworms can also be used to cut sections in order to study invertebrate histology.

2. Narcotisation of animals. Take a medium size frog. Chloroform it in a large jar. Wet some cotton in chloroform and put in the jar containing frog. Close the mouth of jar with glass cover. After 15 to 20 minutes, when fully anaesthetized, take out the frog and keep it in a dissecting tray. Make a longitudinal incision in abdomen to expose viscera.

3. Fixing of tissues. Take cleaned 100 cc wide-mouthed glass-stoppered bottles. In each bottle keep about 50 cc of aqueous Bouin's fluid. Now take out liver, lung,

tongue, stomach and intestine and keep them in separate bottles for 30 minutes. From each bottle, after half an hour, make 2 to 3 cm rectangular pieces of each organ, and again keep them in their respective bottles for 24 hours.

The above tissues can be fixed in alcoholic Bouin's fluid also. Mostly for research purposes, tissues are fixed in alcoholic Bouin's fluid.

It is very essential to fix the tissues in fixative agent. Fixation serves 3 functions :

(1) Fixative renders **hardness** to tissues to resist further **postmortem changes**.

(2) Fixative agent **coagulates and renders** the elements of tissues insoluble so that cellular substances may not be washed away.

(3) Fixative agent alters the **refractive indices** of tissues and makes them **optically differentiated** under the microscope.

4. Washing of tissues. After 24 hours take out the pieces of tissues and keep in a beaker. Tie the mouth of the beaker with a thin cloth and keep it under slow running tap water. Keep on washing under tap water till all the picric acid is removed. The indication of complete removal of picric acid comes when no yellowish water is seen. Normally, it takes 24 hours for perfect washing. Tissues fixed in aqueous Bouin's fluid are washed with tap water. While those fixed in alcoholic Bouin's fluid are washed with 70% alcohol. While washing with 70% alcohol, change it frequently till the yellow colour disappears.

5. Dehydration of tissues. Take one or two pieces of any organ in a staining tube and dehydrate through distilled water →30% alcohol →50% alcohol →70% alcohol →90% alcohol →100% alcohol. In each grade of alcohol keep tissues for 5 minutes with 2 changes. Dehydration removes water to prevent putrefaction. The graded alcohol gradually replaces water in tissues.

6. Dealcoholization or clearing of tissues. Clearing or dealcoholizing agents are **cedar wood oil** or **xylene**. Although cedar wood oil is better but because of its high cost, xylene is used.

Removal of alcohol from tissues is done through clearing agent. Take xylene in a staining tube and transfer tissue in it from 100% alcohol. Keep in xylene for 15 minutes till the tissue becomes transparent. Don't leave tissue in xylene for longer period otherwise it would become fragile. Now tissues are ready for embedding.

7. Embedding of tissues. Depending on melting point of wax, adjust the oven at 58°C to 60°C. Take wax with ceresin instead of plain wax. Keep flakes of wax in a beaker of 100 cc in oven 4 to 5 hours before embedding. In another beaker keep some wax plus xylene. Now take the tissue from xylene and first keep it in the beaker containing xylene + wax for 30 minutes. Then transfer the tissue in pure melted wax for embedding for 1 to 2 hours. Normally double time is given for embedding than the time required by tissue to sink at bottom in xylene.

8. Block making. Make blocks either in metal L-shaped angles or in paper boat or in cavity blocks. 'L' pieces are preferred. First apply little glycerine on their internal surface. Pour melted wax at bottom of rectangular cavity formed by two 'L' pieces. Then add tissue and more melted wax to fully cover the tissue. Keep L pieces in a trough. Add water around them. As the melted wax is solidified, flood it with tap water. After cooling, the block comes out from 'L' pieces or remove L pieces. Because of glycerine 'L' pieces don't stick with wax. During block-making, see that no air bubble comes. If there are some air bubbles remove them with hot spatula.

9. Trimming of blocks. Trim the wax around the embedded material and make a perfect rectangular block. On one side keep sufficient space in the block for fixing it on a block holder. Apply half an inch wax layer over block holder.

10. Section cutting. The blocks are cut either by **rocking microtome** or **rotatory microtome** at 6 microns (μ) thickness. Ribbons are kept over Mayer's albumen coated slides. Keep clean slides ready. Apply pin head Mayer's albumen over the slide and rub it by last finger. Mayer's albumen helps in sticking the sections over glass surface. Keep 2 to 4 rows of sections depending upon the breadth of the sections. Ribbons should be kept upto more than half of the slide. Space should be left for putting labels over the slide. Flatten the section over a hot brass plate. The temperature of the hot plate should be nearly 40–45 degree. Add few drops of water below ribbons. As the water is heated ribbons become expanded by

semi-melting of the wax. Sections should not be separated. Never do flattening over spirit lamp as in most of the cases wax melts and sections are burnt. After all the sections and ribbons become flattened, drain off water and leave the slide at room temperature overnight for drying the ribbons. Mark the ribbon side by glass marking pencil.

11. Double staining, dehydration, clearing and mounting. Double staining is applied. Haematoxylin and eosin stain nucleus and cytoplasm of the cells respectively. Take individual slide and first keep it in xylol to remove wax for 5-10 minutes. Wax is dissolved in xylene and sections are left free. Now pass the slide in **descending series** of alcohols 100% →90% →70% →50% →30% →distilled water. After rinsing in distilled water stain the slide in **haematoxylin** for 2-5 minutes. Take out side and again dip in distilled water. Then immerse the slide in a beaker containing tap water. The sections turn blue because of alkalinity of water. Observe the slide under microscope. If stain is dark, then immerse the slide in acid water and quickly immerse in tap water. Now dehydrate the slide through **ascending series** of 30, 50, 70 and 90% alcohols. After 90%, immerse the slide in alcoholic eosin for 30 seconds. Wash eosin in 90% alcohol. Then keep slide in 100% alcohol for 5 minutes and then in xylol for 15 minutes. Mount the slide in D.P.X. Keep ready the following descending and ascending series of stains and grades of alcohols in separate coupling jars.

12. Microscopic study. A good stained slide reveals pinkish colour of cytoplasm and blue colour of nuclei.

13. Microphotography. The slides meant for research may be microphotographed. For microphotography slide must be nicely stained.

Descending series

Slide
↓
Xylene
↓
100% alcohol
↓
90% alcohol
↓
70% alcohol
↓
50% alcohol
↓
30% alcohol
↓
Distilled water
↓
Ascending series

Ascending series

Distilled water
↓
Haematoxylin
↓
Distilled water
↓
Tap water
↓
Acid water
↓
30% alcohol
↓
50% alcohol
↓
70% alcohol
↓
90% alcohol
↓
alcoholic eosin
↓
90% alcohol
↓
100% alcohol
↓
Xylene
↓
Mount in D.P.X.