ZOOLOGY VIRTUAL LAB 3

(VERTREBRATE MOUNTINGS PART – I)

PRACTICAL 1:

MOUNTING OF BARR BODY FROM BUCCAL EPITHELIUM

AIM: To mount and study the Barr body or sex chromatin in the somatic cell of a female.

BACKGROUND INFORMATION:

Barr and Bertram (1949) observed a condensed body in the nucleus of cells of female cats, which was not nucleolus, whereas male had none. They referred this body as sex chromatin that has been known as Barr body. Lyon suggested that this Barr body is an inactive X – chromosome, which becomes tightly coiled into heterochromatin. A number of evidences support Lyon hypothesis that only one X – chromosome is active in any cell. Some of the evidences can be listed as follows: (1) XXY males show a Barr body, XO females have none whereas XXX female have two Barr bodies. (2) Persons with abnormal number of X – chromosomes have Barr body one less than the number of X – chromosome per cell; (3) Any one X – chromosome becomes inactive at random, and once inactivated all the cells derived from it will maintain the same inactive X – chromosome. This inactivation of X- chromosome occurs when two X – chromosomes are present (inactivation of X – chromosome will not take place in males as they have only one X – chromosome).

It is known in human development that on about the 16^{th} day of embryonic life an X-chromosome is inactivated. Afterward that X- chromosome becomes a Barr body, which lies along the inside of the nuclear envelop in cells of females. Most of the genes of the X –chromosome that forms the Barr body are not expressed. However, small regions of that chromosome remain active.

REQUIREMENTS:

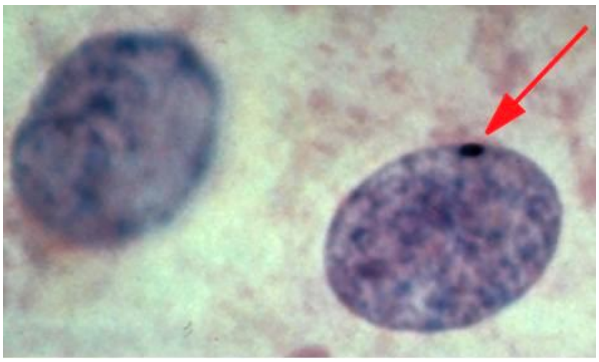
1% Geimsa stain, 70% alcohol, Cotton bud or sterile cotton swab, slides, coverslip, 0.9% saline, microscope, etc.

PROCEDURE:

1. Ask a girl student to rinse and flush the mouth several times with tap water to get rid of food particles sticking to the cheek pouch if any.
2. Take a sterile cotton bud and dip it in 0.9 percent saline to moisten it.
3. Rub the cotton bud several times on the inside wall of the cheek pouch.
4. Smear the cotton bud on a clean dry slide and allow the smear to dry.
5. Fix the smear with 70% alcohol.
6. Stain the smear with Giamsa for 10 min.
7. Wash off the excess of stain with water.
8. Place a coverslip and observe under high power of the microscope.

OBSERVATION AND RESULT:

Barr body stained as bluish-green and is found attached to the nuclear envelope.



PRACTICAL 2:

MOUNTING OF SEX CHROMATIN OR DRUMSTICKS FROM THE WBCS OF A FEMALE

AIM: To mount and study the sex chromatin or drumsticks from the WBCs of a female.

BACKGROUND INFORMATION:

One X chromosome of women is inactivated. In neutrophils this may appear in one of three forms. Drumsticks are nuclear appendages 1.5 in diameter. They are seen in 0.5-2.6% of neutrophils. The inactivated X chromosome may also appear as sessile nodules or as a condensation under the nuclear membrane. The frequency of drumsticks increases with nuclear segmentation. They may be seen in eosinophils but are uncommon as eosinophils have fewer lobes. Racquet forms have a central clearing and should not be confused with drumsticks. They are not inactivations of X chromosomes. The X chromosome is only inactivated in an individual with more than one chromosome. Drumsticks are not seen in individuals having only one X chromosomes [males (XY), Turner's syndrome (XO) and testicular feminization (XY)]. Contrary to expectations individuals who are XXX rarely have cells with two drumsticks. They have an increased incidence of sessile nodule. XXX is also characterized by fewer neutrophil segments. Drumsticks in XXX are less common than normal women. The incidence of drumsticks in patients with Klienfelter's Syndrome (XXY) is lesser than normal women. Shift to left, CML and Down's syndrome is characterized by a decreased drumstick count. It returns to normal in CML following treatment. Drumsticks are more frequent in women with isochromosome of the long are of X. Patients with megaloblastic anaemia and congenital hypersegmentation have a higher frequency of drumsticks in the peripheral smear.

REQUIREMENTS:

1% Geimsa stain, 70% alcohol, Johnson's cotton bud or sterile cotton swab, slides, coverslip, 0.9% saline, microscope, sterile needle, spreader slide and Leishman's stain (readymade - Qualigens), Buffered distilled water ($Na_2HPO_4.2H_2O$ - 3.76 gm., NaH_2PO_4 - 2.1 gm. dissolved in 1000 ml DW), etc.

PROCEDURE:

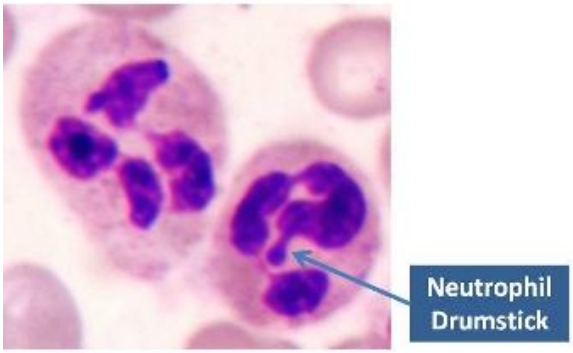
Preparation of sex chromatin or drumsticks from the WBCs:

1. Ask a girl student to clean the fingertip of any finger of left hand with 70 % alcohol.
2. Wait for the alcohol to dry and then prick the area with a sterile needle and squeeze the tip gently. Blood drop appears.
3. Touch the blood drop to a clean slide at one corner.
4. Using the spreader slide make a smear of the blood.
5. Allow the smear to dry and fix it in 70% alcohol.

6. Drain off the excess of alcohol and allow the slide to dry.
7. Stain it with Leishman's stain diluted 1:1 in buffered DW.
8. Wash off the excess of stain in tap water and allow it to dry.
9. Put a drop of oil on thinly spread region and observe under oil immersion lens.

OBSERVATION AND RESULT:

Drum stick Observes as a projection from the nucleus of Neutrophil or Eosinophil which is darkly stained than compared to the other part of the nucleus.



PRACTICAL 3:

MOUNTING OF CYCLOID AND CTENOID SCALES OF BONY FISHES

AIM: To mount and study Cycloid and Ctenoid scales of Bony fishes.

BACKGROUND INFORMATION:

The skins of most fishes is covered with scales. Scales vary enormously in size, shape, structure, and extent, ranging from strong and rigid armour plates in fishes such as shrimp fishes and box fishes, to microscopic or absent in fishes such as eels and anglerfishes. Themorphology of a scale can be used to identify the species of fish it came from. Cartilaginous fishes (sharks and rays) are covered with placoid scales. Most bony fishes are covered with the cycloid scales of salmonand carp, or the ctenoid scales of perch, or the ganoid scales of sturgeons and gars. Some species are covered instead by scutes, and others have no outer covering on the skin.

Fish scales are part of the fish's integumentary system, and are produced from the mesoderm layer of the dermis, which distinguishes them from reptile scales. The same genes involved in tooth and hair development in mammals are also involved in scale development.

REQUIREMENTS:

Bony fishes, Forcepss, Dissecting tray, Slide, Cover slip, Glycerine, Water, Dissecting microscope, Filter papers, etc.

PROCEDURE:

1. Pluck a scale from the body of the fish with forceps and rinse with water.
2. Observe under dissecting and low power of compound microscope.

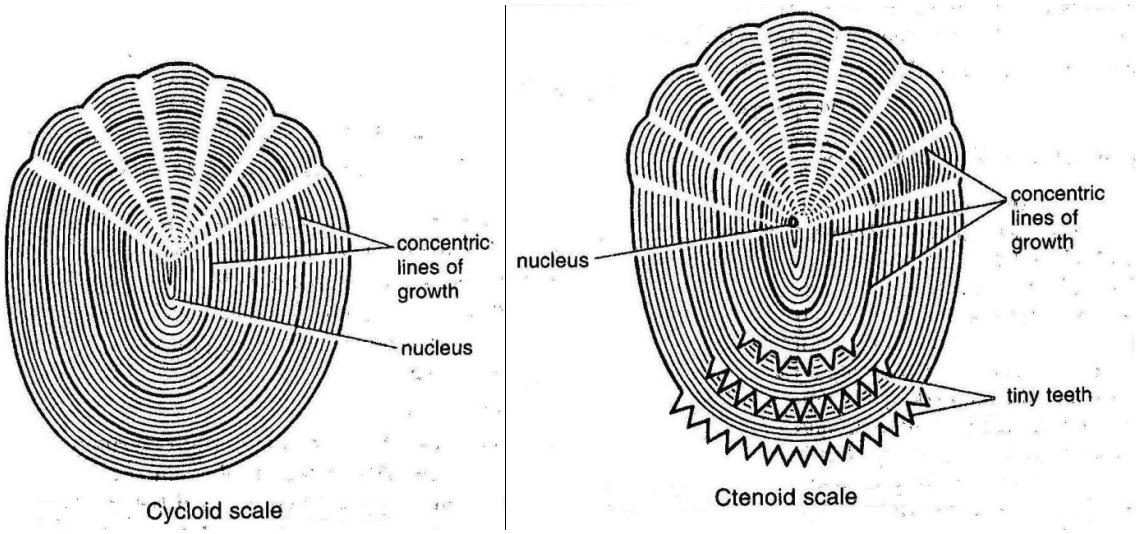
OBSERVATIONS AND RESULT:

Each scale is oval shaped with a spiny posterior margin bearing spines called ctenii. It is made up of inner layer of collagen fibres and an outer organic layer impregnated with calcium salts. These scales partially overlap each other which give them flexibility in the movement. As the fish grows the scale also grows resulting in development of incomplete growth rings. These can be used to estimate the age of the fish. These are found in teleosts i.e. bony fish.

Cycloid (circular) scales have a smooth texture and are uniform with a smooth outer edge or margin. They are most common on fish with soft fin rays, such as salmon and carp.

Ctenoid (toothed) scales are like cycloid scales with small teeth along their outer edges, and are usually found on fish with spiny fin rays, such as the perch-like fishes. They have a rough texture with a toothed outer or posterior edge with tiny teeth called ctenii. These scales contain

almost no bone, being composed of a surface layer containing hydroxyapatite and calcium carbonate, and a deeper layer composed of mostly collagen. The enamel of the other scale types is reduced to superficial ridges and ctenii.



PRACTICAL 4:

MOUNTING OF PLACOID SCALES OF CARTILAGENOUS FISH

AIM: To mount and study the Placoid scales of Cartilagenous fish.

BACKGROUND INFORMATION:

Fish scales are part of the fish's integumentary system, and are produced from the mesoderm layer of the dermis, which distinguishes them from reptile scales. The same genes involved in tooth and hair development in mammals are also involved in scale development. The placoid scales of cartilaginous fishes are also called dermal denticles and are structurally homologous with vertebrate teeth. It has been suggested that the scales of bony fishes are similar in structure to teeth, but they probably originate from different tissue. The skin of sharks is entirely covered by placoid scales.

REQUIREMENTS:

Cartilagenous fishes, Forcepss, Dissecting tray, Slide, Cover slip, Glycerine, Water, Compound microscope, Filter papers, Forcepss, Test tube, Holder, Burner, Watch glass, 10 % KOH, etc.

PROCEDURE:

1. Take a piece of skin of shark in a watch glass and remove attached muscles with the help of Forceps.
2. Transfer the skin into a test tube and add 2-3 ml of 10 % KOH.
3. Gently boil it.
4. Allow the test tube to stand for a few minutes. Decant the supernatant leaving a few drops in the test tube. Pour this residual KOH into a watch glass.
5. Place a drop from it onto a slide.
6. Observe under low power of a compound microscope.

OBSERVATIONS AND RESULT:

Each scale has a flat base plate embedded in the dermis. From the base plate of these primitive scales arise sharp, trident spines. They are found only in cartilaginous fishes (elasmobranchs) i.e. sharks and rays. Structurally they show inner core of pulp, middle layer of dentine and enamel covering and hence modification of the same develops teeth in elasmobranchs. The scales are supported by spines which feel rough when stroked in a backward direction, but when flattened by the forward movement of water creates tiny vortices that reduce hydrodynamic drag, making swimming both more efficient as well as quieter compared to bony fishes.

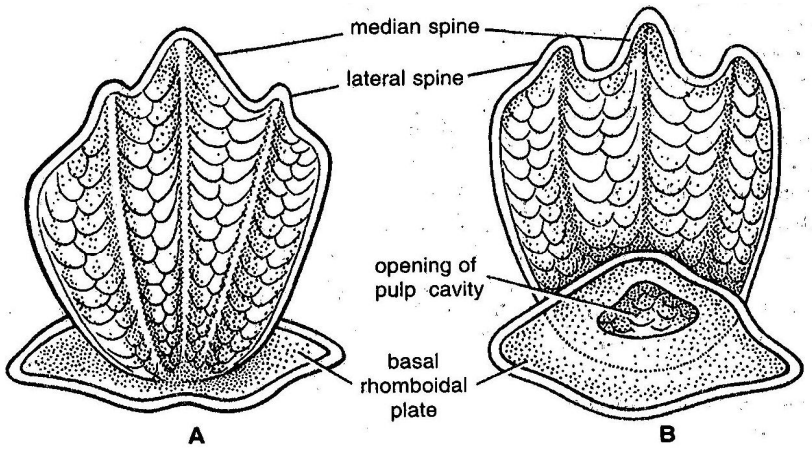


Fig. 6.9. Placoid scales of *Scoliodon*.A–Dorsal view; B–Ventral view.

PRACTICAL 5:

MOUNTING OF SCROLL VALVE IN SHARK

AIM: To mount and study the Scroll valve in Shark.

BACKGROUND INFORMATION:

A spiral valve or scroll valve is the corkscrew shaped lower portion of the intestine of some sharks, rays, skates and lungfishes. A modification of the ileum, the spiral valve is internally twisted or coiled to increase the surface area of the intestine, to increase nutrient absorption.[1]

The intestines of a shark are much shorter than those of mammals. Sharks have compensated for this problem by having a spiral valve, or a scroll valve, inside the intestine to increase the absorbent surface of the intestine. By keeping digestible material in the ileum for an extended period maximum nutrient absorption is ensured. For this reason, many sharks and related fish feed very infrequently.

REQUIREMENTS: Shark, Dissecting tray, Scissor, Forcepss, Water, Watch glass, etc.

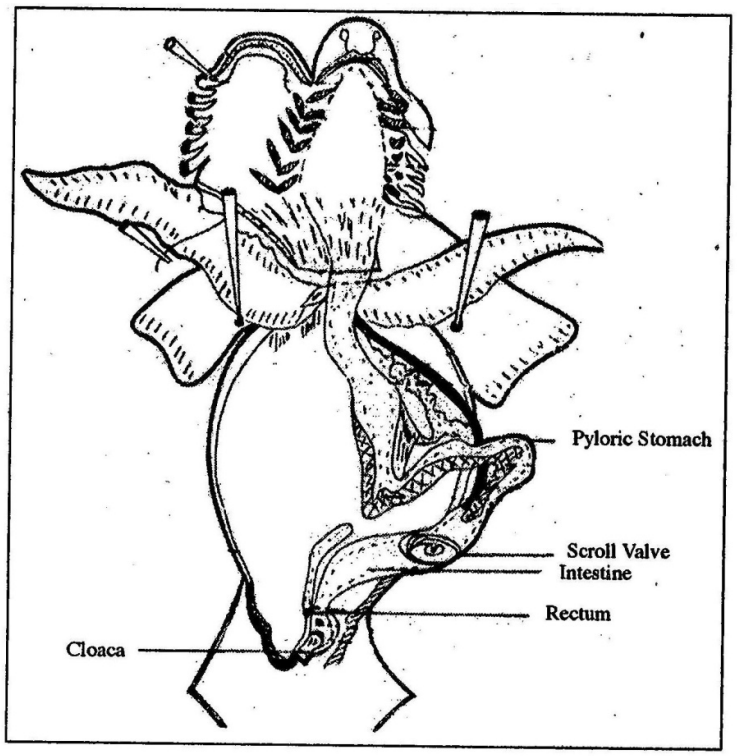
PROCEDURE:

1. With the help of scissor cut open the shark from its ventral side.
2. Trace its alimentary canal so as to locate its intestine.
3. Cut ileum part of intestine and expose its interior part.
4. Observe the scroll valve.

OBSERVATIONS AND RESULT:

The mucous membrane of the intestine is folded to from the scroll valve. The transverse section, it looks like a watch-spring. This valve increases the area of absorption of the intestine. It also does not allow the food to pass through the intestine rapidly.

The food passes into the comparatively short colon of the shark almost fully digested, and then out the cloaca and vent. A consequence of the spiral valve constricting the lumen of the ileum is that sharks cannot pass large hard objects (such as bones) through their lower intestine. Such objects rather remain in the stomach until sufficiently broken down for passing through the valve region, or are regurgitated. Consequently, shark stomachs often contain items of interest that allow to determine what the animals feed on, as well as non-food items ingested during a feeding frenzy.



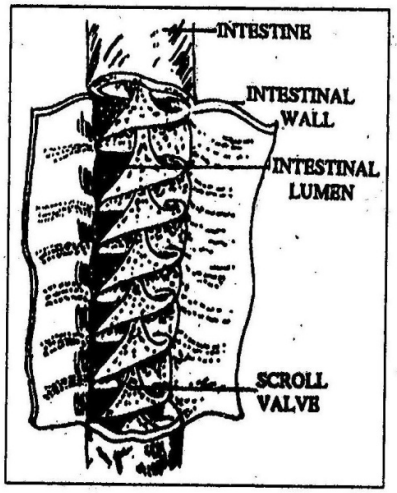


Fig 4.7 A. : Digestive system of shark showing the scroll valve.

PRACTICAL 6:

IDENTIFY THE VARIOUS STAGES OF ESTROUS CYCLE IN THE VAGINAL SMEAR OF RAT

AIM: To determine the phases of estrous cycle in using vaginal smear technique.

BACKGROUND INFORMATION:

In lower mammal menstrual cycle is represented by a homologous condition is called as Oestrous cycle. It occurs during only breeding season. One cycle may occupy whole breeding season, such animals are called Monoestrous (dog). In many oestrous cycles may takes place in same mating season with short resting intervals. Such animals are called as Polyestrous (rats, mice, guinea-pigs etc.)

The Allen-Doisey test be used for the detection of oestrogenic activity, is based on the ability of a compound to produce oestrous in over-ejectomised, sexually mature rats, In the cats and some rodents, the follicle develops, but ovulation does not occurs (ova are not released from follicles) until after copulation and long estrous may occurs, ripening of the follicles and ovulation are initiated by copulation.

REQUIREMENTS:

Cotton swab, 0.9% saline, 70% alcohol, ethylene blue or giemsa, petridish, low power Microscope, female rat

PROCEDURE:

1. Wet the cotton bud in 0.9%saline preferable, so it will select a mature female rat and gently insert the wet cotton bud into the vaginal opening. Slowly rotate bud and also Move it back and tap it. Clear glass slide and make a smear.
2. Allow the alcohol it to dry in air completely. Pore stop of 70% alcohol or Methanol Over. The smear for faring the cell to slide. Allow the alcohol to dry completely and strain the Smear with Methylene blue or Giemsa, only freshly prepared strain are used.
3. Take the slide in a petridish and pour in stain so as to form a thin film or smear. Cover the Petri dish and keep it aside, for 4-10 min, allows it to become completely dry.
4. Rinse, slide with tap water and observe it under low power objectives of the microscope. The types of cell observed seen in smear and determine the phase of cycle as follow in Comparison with figure drawn on opposite page.

OBSERVATIONS AND RESULT:

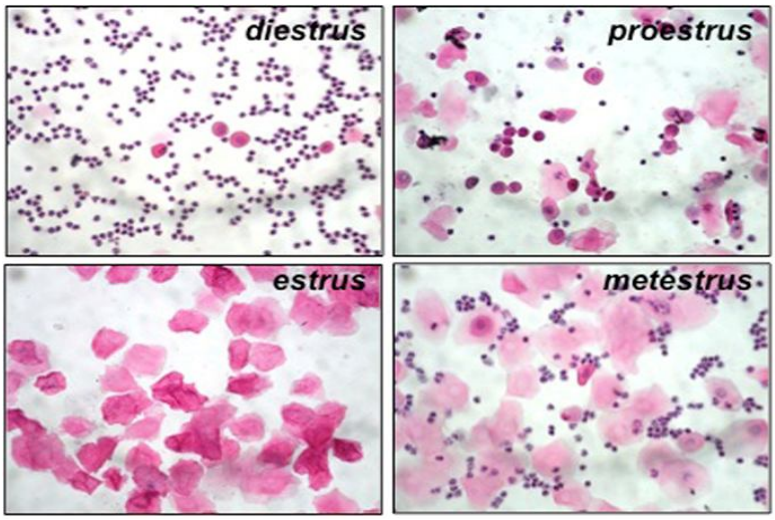
Different phases of oestrous cycle are given below:

1. PROESTRUS (preparatory phase) - Uterus and vagina become congested and secrete a clear sanguinous fluid. Vaginal epithelium proliferates. This is caused by estrogen secreted by

maturing follicles. Vaginal smear show a large number of nucleated cell, broken off from the proliferating vaginal epithelium.

2. ESTRUS (heat period) - This is the period of desire, only when the female will receive the male. This occurs at end of proliferative phase. This time of receptivity immediately prior to ovulation is termed oestrous. Congestion of the uterus become maximum, the vaginal epithelium thicken further and the superficial, layer are fully keratinized, Ovulation takes place at this time so that impregnation is possible. If fertilization takes place, placenta forms and pregnancy begins. If not, it passes on to the next phase. In some animals (eg - cat, rabbits) ovulation does not occurs in absence of copulation. Vaginal smear show a large number of keratinized cells.
3. METAESTRUS (Luteal phase) - Changes initiated in the previous stage proceed still further due to action of progesterone, secreted by the newly formed corpora lutea. In absence of pregnancy, corpora luteal degenerates and the changes of the generative organs subside .In monoestrus animals(bitch)the hypertrophied mucosa break down and is discharged. In certain species of monoestrous animal (rabbit) changes alike pregnancy have been seen to occur even when the female has not been fertilized. These include changes in the uterus and mammary glands and it is named as Pseudo-pregnancy. Activity of corpous luteum is very high and is prolonged in such animals. Vaginal smear show a large number of WBC along with keratinized cells.
4. ANOESTRUS or DIESTRUS-This is the resting asexual period. In monoestrous animals it lasts up to the next mating season, and is known as Anoestrus. In polyestrus animals the resting interval is short up to the next cycle and is called Diesrtous. In rats it last for 4-6days. Vaginal smear show a good number of WBC, a few epithelial cell and mucus.

CELL OBSERVED	**PHASE OF ESTROUS CYCLE**
Nucleated epithelial cells	Proestrus
Cornified epithelial cells	Estrus
Coirnified epithelial cells and leucocytes	Metaetrus
Nucleated epithelial cell and leucocyte very few Cornified cell	Diestrus



PRACTICAL 7:

AIM:

BACKGROUND INFORMATION:

SIGNIFICANCE:

REQUIREMENTS:

PROCEDURE:

OBSERVATIONS AND RESULT:

PRACTICAL 8:

AIM:

BACKGROUND INFORMATION:

SIGNIFICANCE:

REQUIREMENTS:

PROCEDURE:

OBSERVATIONS AND RESULT:

PRACTICAL 9:

AIM:

BACKGROUND INFORMATION:

SIGNIFICANCE:

REQUIREMENTS:

PROCEDURE:

OBSERVATIONS AND RESULT:

PRACTICAL 10:

AIM:

BACKGROUND INFORMATION:

SIGNIFICANCE:

REQUIREMENTS:

PROCEDURE:

OBSERVATIONS AND RESULT: