Q. What is fixation? Describe the principles of staining with reference to different types of stains used in the study of cell.

The process of colouring the cells, tissues of animal or plant bodies by certain inorganic or organic dyes is known as **staining.** The selection of dye or stain for a particular material usually depends on its chemical nature, the pH value of the fixative used and the chemical reactivity of stain to the material.

Stains. Most cytological stains are solutiions of dyes of aromatic organic compounds which have two kinds of active chemical groups such as, **chromophoric** and **auxochromic** groups. The **chromophoric groups**gives the colour to the dye, *e.g.*, carboxyl (—COOH), azo (—N=N—), nitro ($—NO_2$) quinoid (O =  = O) and indamin (—N=) groups. The **auxochromic group** gives to the dye the ability to attach to the tissue or the material and to dissolve and dissociate, in the water, *e.g.*, *Hydroxy* (—OH) group.

The organic stains are classified into three groups: 1. acidic stains, 2. basic stains, and 3. neutral stains.

1. Acidic stains. The acidic stains are usually used for the cytoplasm and proteins. These have great capacity for the combining with the tissue at low pH than basic dyes. The most common cytological acidic stains are picric acid, acid fuchsin, Congo red, Janus green B, orange G, methyl blue, eosin, aniline blue, Bismark brown and fluorescein.

2. Basic stains. The basic stains are used to stain the nucleus, chromosomes and particularly the nucleic acids. The most common cytological basic stains are basic fuchsin, crystal violet, methyl green, safranine, acridine red, azures, methylene blue, thionine and haematoxylin.

3. Neutral stains. The neutral stains have both the properties of acidic and basic stains.

Acidiphilic and basiphilic tissues. The tissue, cell or cellular component taking acidic stains is known as **acidiphilic** tissue, cell or cellular component, *e.g.*, the cytoplasm. The nucleus, chromosomes and DNA have affinity for the basic stains and known as **basiphilic** organelles.

Metachromasia. Some basic dyes stain certain cell components with a different colour than their original colour. This property of stain is known as **metachromasia** and is very useful for **histochemical** and **physiochemical** tests. The property of metachromasia is displayed by the basic stains such as thionine, azure A and toluidine blue which react with mucopolysaccharides, nucleic acids and acidic lipids.

Mordant and lake. Certain dyes stain the proteins and cytoplasm in the presence of some metal or metallic compound which is known as **mordant.** Usually chemically a mordant is a double salt of potassium or ammonium or ferric sulphate. The mordant and the stain are collectively known as the **lake.** The most important mordant is ferric ammonium sulphate (iron alum), which is used along with the stain haematoxylin and carmine.

Staining for Light Microscopy

For light microscopy both acidic and basic stains are used for the staining of cells or tissue. The stains are specific for different types of cells and also for different organelles of cell. The cytoplasmic

proteins and carbohydrates are stained by acidic stains, while the nucleus, chromosomes, etc., are stained by basic stains.

Staining for Electron Microscopy

Electron microscopy usually requires no stain because beside observing visually, the image is photographed and due to the contrast between cellular components a black and white photograph is received. But certain inorganic stains such as lead acetate, lead citrate, lead hydroxide, urynyl acetate, phosphotungstic acid, osmium tetroxide and potassium permanganate are used to increase contrast between cellular components. Sometimes certain organic stains as azure II, leucofuchsin, orcein, etc., are also used to increase the contrast.

Cytochemical Staining

In addition, there are certain specific stains, called **cytochemical stains** that bind selectively to some specific groups of cellular macromolecules such as proteins, nucleic acids, polysaccharides and lipids. For example, Millon reaction, diazonium reaction and Napthol Yellow 5 stain are used for the proteins; alkaline fast green is used for histone (basic protein); Feulgen reaction (using Schiff's reagent) is used for DNA; methyl green-pyronine stain (Unna-Pappenheim stain) is used in distinguishing between DNA and RNA and it stains DNA green and RNA red; acetocarmine and acetoorcein stains are used to stain chromosomes of dividing cells; periodic acid-Schiff (PAS) reaction is used for the demonstration of polysaccharide materials such as starch, cellulose, hemicellulose, and pectin in the plant cells and mucoproteins (glycoproteins), hyaluronic acid and chitin in animal cells; and fat soluble dyes such as Sudan Red and Sudan Black B are used for the lipids. The Sudan Black B is a specific stain for phospholipids and is used to stain Golgi apparatus.

Vital Staining

Vital stains selectively stain the intracellular structures of living cells without serious alteration of cellular metabolism and function. For example, Janus green B selectively stains mitochondria; neutral red stains plant vacuoles and methylene blue stains Golgi apparatus and also nuclear chromatin of dividing cells.

CENTRIFUGATION

BLOOD VASCULAR SYSTEM IN HERDMANIA

Q. Give an account of the blood vascular system in Herdmania.

Ans→ The circulating system of Herdmania is open type. It consists of :-

(1) Heart and pericardium (2) Dorsal aorta
(3) Ventral aorta (4) Branchio visceral vessels
(5) Cardio-visceral vessels

1. HEART

The heart is situated near stomach. The heart is tubular, thin walled and contractile. It is covered by a membrane called pericardium. The cavity of pericardium, pericardial cavity filled with a pericardial fluid contains corpuscles. The heart remain attached to pericardium on one side through its length by a connective tissue membrane.

The contraction and relaxation of heart rhythmic. The peristaltic wave of contraction arise from one end and move to the other. After a short while these get feeble, stop and start again but in opposite direction. One impulse for contraction originate from two centres called pace makers. The pace makers are situated one at each end of the heart.

There are no valve in the heart. But there is pear-shaped body in the centre of pericardial cavity. It moves end to end of the heart and regulates the blood flow.

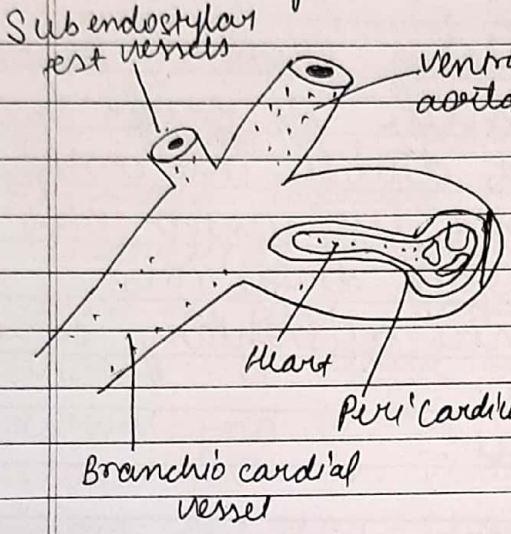

Fig:- Heart & peri'cardium in Herdmania

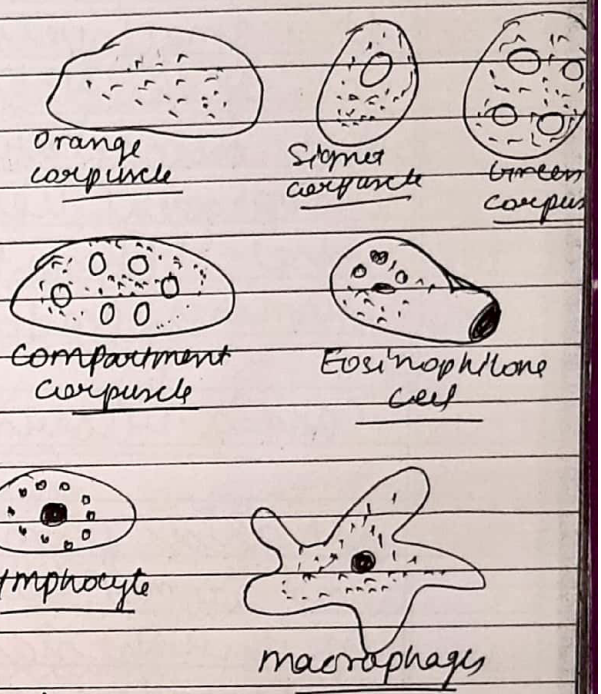

Fig:- Various type of blood corpuscle in Herdmania

BLOOD VESSELS

Ventral aorta :- The ventral aorta arises from the ventral end of the heart. It runs forwards along the ventral side of the pharynx. It gives out paired transverse vessels to the pharynx.

Dorsal aorta :- It lies on the dorsal side of the pharynx. It sends paired transverse vessels to the pharynx. In the pharynx the transverse

vessels of dorsal aorta get connected with the transverse vessels of the ventral aorta.

4. Branchio-visceral vessel:-

It is small vessel arising from the posterior end of the dorsal vessels. Its origin is not demarcated. It divides into a right oesophageal vessel and left ventro intestinal vessel. These two again divide into branches and end into alimentary canal.

Cardio-visceral vessel:-

It arises from the dorsal end of the heart and immediately issue two minor branches on its right side

(a) hepatic branch to the right liver lobe, and
(b) Oesophageo test branch to the oesophagus, stomach, intestine, gonads and test.

BLOOD

The blood is slightly red colour. It composed of colourless plasma and corpuscles. The corpuscles are two types non-pigmented amoeboid leucocytes (macrophages) and pigmented corpuscles. The pigmented corpuscles are with yellow, green, brown or ~~non-nucleated~~ orange pigments. The corpuscles are nucleated or non-nucleated.

Some corpuscles are specialized to extract excretory products from the blood. They are called nephrocytes.

Course of circulation

The course of circulation is ~~pedrocally~~ Periodically reversed because the pace makers work alternately situated at the end of heart. When the pace makers of dorsal end of heart works, a wave of contraction passes from dorsal end to the ventral end. The blood flows into the ventral aorta. At the same time the pear-shaped body present in the pericardial cavity moves towards the dorsal end and passes against the heart wall. This pear shaped body prevents the flow of blood into the dorsal aorta. The blood from the ventral aorta passes into the pharynx through the transverse vessels. Gaseous exchange takes place in the pharynx and the blood is oxygenated.

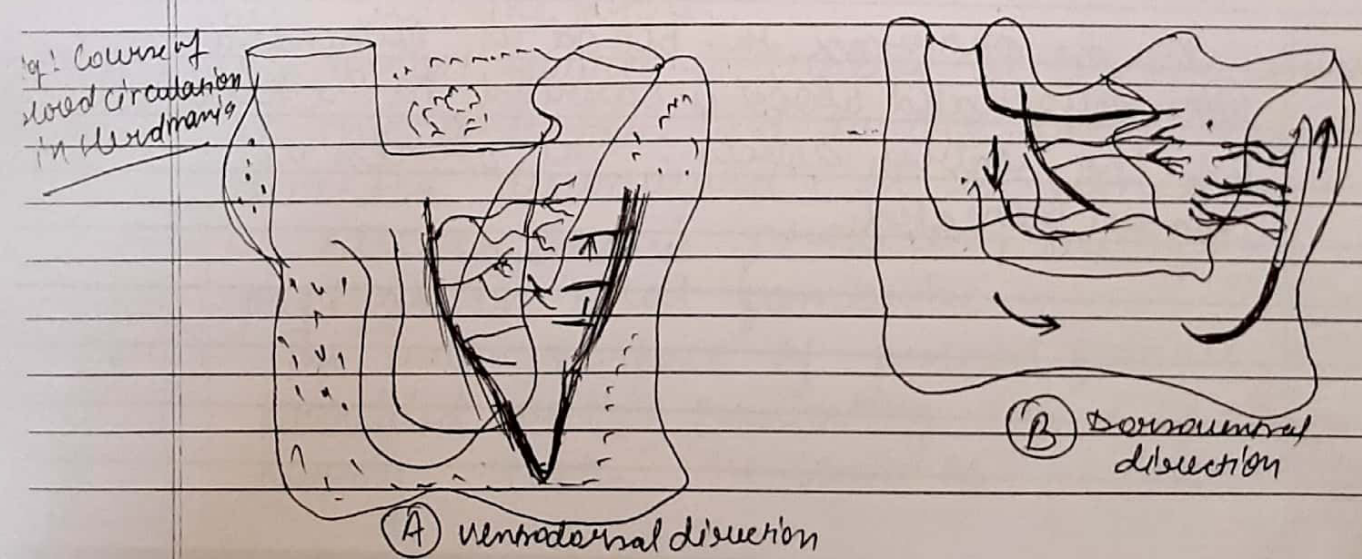

Fig: Course of blood circulation in Hirudinaria

The oxygenated blood passes into the dorsal aorta through the transverse vessels. From the dorsal aorta the oxygenated blood is supplied to the parts of alimentary canal through the branchial-visceral vessels. In these organs the blood is deoxygenated. The deoxygenated blood passes into the heart through the cardio-visceral vessel. Now the pace-maker present in the ventral end of the heart operates & wave of contraction moves from the ventral end of heart towards the dorsal end.

Deoxygenated blood flow into the cardio-visceral vessels. At the same time pear shaped body moves to the ventral end of heart and passes again the heart wall preventing the flow of blood into the ventral aorta. The deoxygenated blood passes into the vesceral organs through the cardiovisceral vessels. From the vesceral organ the deoxygenated blood is carried to the pharynx through the branchio visceral vessels and the dorsal aorta.

In the pharynx the blood is oxygenated. The oxygenated blood is carried to the heart by the ventral aorta. The process is again repeated.