MICROTOMY

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The objective of histology is to study the microanatomy of cells, tissues & organs, & to correlate the structures with their functions. The method employed by histologists is extremely diverse. Histological methods for Light Microscopic studies are generally used in laboratory. However, for more detailed interpretation of microanatomy the techniques for Electron Microscopic studies are also used because of its greater resolution & useful magnification. In general, since tissues & organs are usually too thick for light penetration & transillumination, they must be sectioned to obtain thin transparent sections. The term microtechnique is used for the technique of thin sectioning of tissues, for the study of histology. Microtome is the machine used to carry out the work of thin sections.

This technique of microtomy basically has 2 parts:-

1) Microtomy or thin sectioning,
2) Making contrast of the tissue by several staining techniques.

A) MICROTOMY:-

a) **Tissue processing:** Prior to sectioning, the tissues must undergo preparatory treatment & this preparatory treatment is called tissue processing. There are different tissue processing methods for tissue processing. In paraffin wax embedding method, the tissue processing includes-fixation, dehydration, clearing, infiltration of paraffin wax inside the tissue, embedding or casting the tissue within paraffin wax.

i) ***Fixation:-*** It is the most important step in histology. The primary objective of fixation is to preserve protoplasm with least alteration from the living state. The chemicals or fluids used to preserve the tissue are called fixative.

The fixing fluids(fixatives) penetrate the tissue rapidly & acts as preservative inhibiting autolytic changes & bacterial growth. Generally they coagulate protoplasm thus rendering it insoluble & harden the tissue, so that sectioning is facilitated. Besides, many fixatives also increase the affinity of protoplasm, for certain stains.

Materials required for fixation: a large jar with lid; cotton, chloroform; dissecting instruments; some labeled containers having the fixative (Bouin's fixative); normal saline water & the animal (rat).

Some common fixatives:-

- Bouin's fixative: it is of two types- viz; aqueous & alcoholic.
  - Aqueous- saturated picric acid in distilled water(75ml); formalin,i.e.40% formaldehyde(25ml); glacial acetic acid(5ml).
  - Alcoholic—saturated picric acid in 70% alcohol(75ml); formalin(25ml); glacial acetic acid(5ml).

N.B. everything should be kept ready prior to killing the animal, so that no delay occurs once the animal has been killed. Fixation time varies from tissue to tissue,

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generally 12 hours to several days. After fixation, tissue is washed in 50% & 70% alcohol repeatedly to remove excess picric acid from the tissue.

- Carnoy's fluid: absolute alcohol (60ml); chloroform(40ml); acetic acid(10 ml).
- Zenker's fluid: mercuric chloride(5gm); potassium dichromate(2.5gm); sodium sulphate(1gm); distilled water(100ml).

Process of fixation: - i) an animal is sacrificed after anaesthesizing deeply by using chloroform or anaesthetic ether. ii) Different tissues are dissected out from the animal very rapidly & rinsed in normal saline. Iii) The organs are then cut into small pieces & placed in freshly prepared fixative (aqueous Bouin's fixative). iv) The tissue pieces are then fixed for 12-14 hours for proper penetration of fixative.

ii) ***Dehydration:*** - The fixed tissues are taken out from the Bouin's fixative & are thoroughly washed with by 50% & upper graded alcohols as long as yellow colour fades out.

The main purpose of dehydration is the removal of water from the tissue in order to allow paraffin to infiltrate; water must be totally removed from the cell because water & paraffin are miscible.

iii) ***Clearing:*** - Ethanol does not mix with paraffin wax, hence after dehydration tissues are treated with a reagent that mixes with both alcohol & paraffin wax. It may remove easily in the process of infiltration. Most of the reagents used in this purpose make the tissue more or less transparent; hence this step is called clearing. The popular clearing reagents are-xylene, toluene, benzene, chloroform & cedar wood oil.

iv) ***Infiltration:*** - Infiltration involves replacing of dealcoholizing agent from the tissue. Paraffin can be obtained with various melting points from 40°C- 70°C. In winter season 52° C to 54° C & in summer 58° C to 60° C melting points of paraffin wax are usually suitable. The molten paraffin should be filtered before use.

Procedure of infiltration:- The tissue after dealcoholization is transferred to a porcelain cup containing xylene & paraffin mixture(1:1 by volume) & the cup is placed on the oven for 15-20 min. After that period the tissue is transferred to another cup containing pure molten paraffin & placed in the same oven. The tissue material is kept in molten paraffin for about 60-90 minutes with 2-3 changes to replace the clearing agent completely.

v) ***Embedding:-*** After the block of tissue has been completely infiltrated with wax(paraffin), the tissue is placed in a mould containing hot paraffin. The paraffin is now allowed to solidify. This process is called embedding or block preparation.

Procedure of embedding:- The suitable sized mould is first filled with molten paraffin. The paraffin in the mould is allowed to cool except the upper surface. Application of a warm scalpel prevents the cooling of the upper surface. The infiltrated tissue from the porcelain cup is then quickly transferred to the molten paraffin of the mould & properly oriented with the help of warm scalpel & forceps. When the block has been cooled sufficiently, it should be emerged in cold water for complete solidification.

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b) **Tissue sectioning:-** Embedded tissue can be cut into thin sections with the help of an instrument called Microtome. The process is known as Microtomy. There are several types of microtome machines; the rotary microtome, freezing microtome (for frozen sections), vibrating knife microtome, ultra-microtome, etc. Among these the Rotary microtome is widely used for sectioning the paraffin tissue block.

For sectioning the prepared block is first trimmed & mounted on a black holder which is fixed to microtome. Several tissue sections can be cut in the form of a ribbon by rotating the microtome wheel. The entire process is undertaken by the following 3 steps:-

1) **Trimming of the block: -** The paraffin containing tissue material, to be sectioned, is first trimmed to correct shape. For this process, the excess wax is scrapped with the help of a scalpel. Scrapping should be done in a way that the block becomes rectangular in shape & the material is slightly visible.

   Atleast, 1-2 mm paraffin should be left infront & all sides of the material; again atleast 6-7 mm paraffin should be left behind the material to be cut.

2) **Mounting of the block: -** The trimmed block is attached to the mound layer of wax on the disc of microtome block holder with the help of a warm scalpel. The block holder with the attached block should now be allowed to cool at room temperature.

3) **Sectioning of the tissue block by microtome:-** At last sectioning is done with the help of microtome machine.

   Procedure of sectioning:- 1)The block holder along with the block is to be fitted into the object holder of the microtome by adjusting the screw. 2) A sharp razor is to be fixed in the knife carriage in an angle of 45° by adjusting the screws. 3) The knife including its carriage is then moved towards the tissue block so that it might touch the block during the downward movement. 4) All screws are checked & tightened properly before moving the drive wheel. 5) A number of sections are cut at 6-7 mm. They are cut in the form of ribbon by rotating the microtome wheel. 6) The ribbons are to be removed carefully with the help of a forecep. The ribbons are to be kept away from dust particles & air current.

c) **Affixation:-** The tissue sections are stretched & fixed on glass slides by this process. In this process, the slide is first albuminized with Mayer's Albumin solution.

   Composition of Mayer's Albumin Solution:- i)Egg albumin- 50 ml; ii)Glycerine- 50 ml; iii)Sodium salicylate-1gm..

   The solution is shaked & filtered before use. The filtering process is very slow & takes about 1-2 days.

   Procedure of affixation:- 1) a small drop of Mayer's albumen is taken on the upper surface of a clean slide & rubbed on it by a finger. 2) A few drops of water is poured on the albuminised surface of the slide. 3) A small piece of paraffin ribbon is then placed on the water of the slide with the help of a forcep. 4) The glass slide with a section on it, is now warmed on a hotspot to make the sections perfectly flat, called stretching. 5) The stretched sections are then arranged in 2-3 parallel rows on the slide called orientation. 6) The slide with its stretched & oriented section is dried at room temperature & stored in dust free place until it is processed.

B) STAINING:-

Stains are coloured substances used to dye tissue in order to give a detail colour contrast in microscopic examination. Staining substances are combined with tissue in different ways.

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Chemically, a stain has two structural parts:- 1) Chromogen-which is the colour imparting part. & 2) Auxochrome- which is part of the molecule that attaches with the substrate.

Examples of classes of stain:-

1) Basic stain:-these stains are used to stain nuclei & major cationic compounds of cytoplasm. Examples include- basic fuchsin, methyl violet & hematoxylin,etc.
2) Acid stains:- these stains demonstrate cytoplasmic & extracellular proteinaceous material. Examples include- eosin, acid fuchsin, light green orangy,etc.

Stains used in histology- Eosine (acid stain) & Delafield's Hematoxylin(basic stain) are the most common stains used in histology.

a) Composition of Hematoxylin- 1)Hematoxylin-4gm; 2) 95% ethyl alcohol- 25 ml; 3)saturated solution of ammonium alum in water-400ml; 4) 95% methyl alcohol- 100 ml; 5) Glycerine- 100 ml.
b) Composition of Eosine- 1) Eosine powder- 1gm; 2) 90% alcohol- 100ml.

Procedure of staining:- Since, both hematoxylin & eosine are used for staining; the procedure is called double staining. The steps are- 1)the paraffin ribbon oriented slide is deparaffinized in xylene for 10 minutes. 2) after the reomoval of paraffin, the slide is kept in absolute alcohol for 8-10 mins. 3) the slide is then passed through downgraded alcohols(90%-70%-50%-30%). 4) the hydrated slide is stained in Delafield's hematoxylin for 1-5 mins. 5) The stain is washed in running tap water until the nuclei becomes blue. 6)Over stained slide is differentiated in acid water & washed well in tap water until the nuclei turns blue. 7) the properly differentiated slide brought to 90% alcohol through upgraded alcohol(50%-70%-90%). 8) the slide is then transferred to alcoholic eosin for 10-20 mins. 9)the slide is then rinsed in 90% alcohol & kept in absolute alcohol for 30-45mins or more. 10) the completely dehydrated slide is closed in xylene for 5-10 mins & mounted in DPX.

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