Second Stage\General Histology **د.منار الزهيري**

Monday 10/10/2022

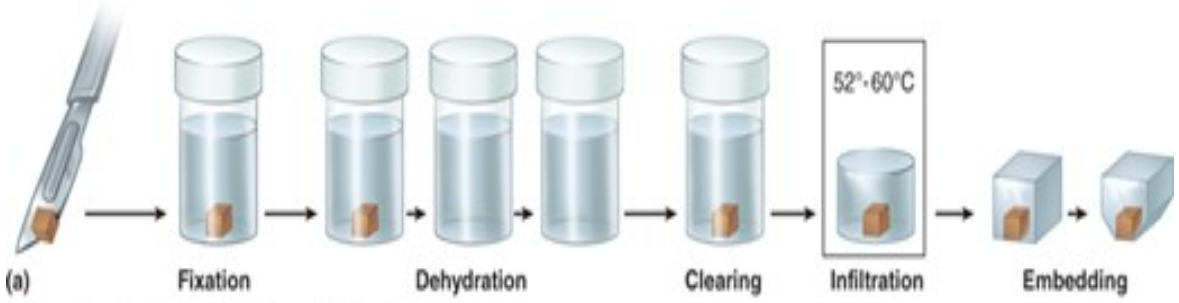
Lec.1

Preparation of tissue for study

Histology:- It is the branch of science which deals with the microscopic study of normal tissue.

The most common procedure used in histologic research is the preparation of tissue slices or "sections" that can be examined visually with transmitted light. Because most tissues and organs are too thick for light to pass through, thin translucent sections are cut from them and placed on glass slides for microscopic examination of the internal structures.

The ideal microscopic preparation is preserved so that the tissue on the slide has the same structural features it had in the body. However, this is often not feasible because the preparation process can remove cellular lipid, with slight distortions of cell structure.



Source: Anthony L. Mescher: Junqueira's Basic Histology, 14th Edition.
www.accessmedicine.com

Most tissues studied histologically are prepared as shown, with this sequence of steps:

1-Fixation: the process by which the constituents of cells and tissue are fixed in a physical and chemical state so that they will withstand subsequent treatment with

various reagents with minimum loss of architecturs. Small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure. Fixatives prevent autolysis and bacterial decomposition and preserves tissue in their natural state and fix all components.

Tissue fixatives

•Buffered formalin (light microscope preparation)

• Buffered gluteraldehyde (electron microscope preparation)

• Osmium tetraoxide (electron microscope preparation, preserve and stain)

• Zenker's formal saline

• Bowen's fluid

-No fixative will penetrate a piece of tissue thicker than 1 cm .Specimen is placed in porous cassettes. Cassettes are collected in fixatives 10% formalin 1mm/hour fixation .

- Generally the specimen should fix for between 6 and 24 hours. Most laboratories will use a fixative step as the first station on their processor.

2- **Processing:-** Tissue have suitable hardness and consistency these properties can be imparted by infiltrating and surrounding the tissue with paraffin wax, various types of resins or by freezing to get tissue block. These block presented to the knife edge of microtome and then cutting the tissue to thin sections of the tissues and placed on slides of microscope.

Types of tissue processing:-

a-Manual Tissue Processing : In this process the tissue is changed from one container of reagent to another by hand.

Note: The processing, whether manually or mechanically, involves the same steps and reagents in same sequence

b-Mechanical Tissue Processing :

• In this the tissue is moved from one jar to another by mechanical device

•Timings are controlled by a timer which can be adjusted in respect to hours and minutes

•Temperature is maintained around 60 ºC

•Automatic tissue processor: Overnight □ 12 Baths □ 16 hours

Tissue Processing: It can be subdivided into:

a- Dehydration - b- Clearing c- Wax infiltration

a-Dehydration: Because melted paraffin wax is hydrophobic (immiscible with water), most of the water in a specimen must be removed before it can be infiltrated with wax. This process is commonly carried out by immersing specimens in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached.

A typical dehydration sequence for specimens not more than 4mm thick would be:

1. 70% ethanol 15 min
2. 90% ethanol 15 min
3. 100% ethanol 15 min
4. 100% ethanol 15 min
5. 100% ethanol 30 min
6. 100% ethanol 45 min

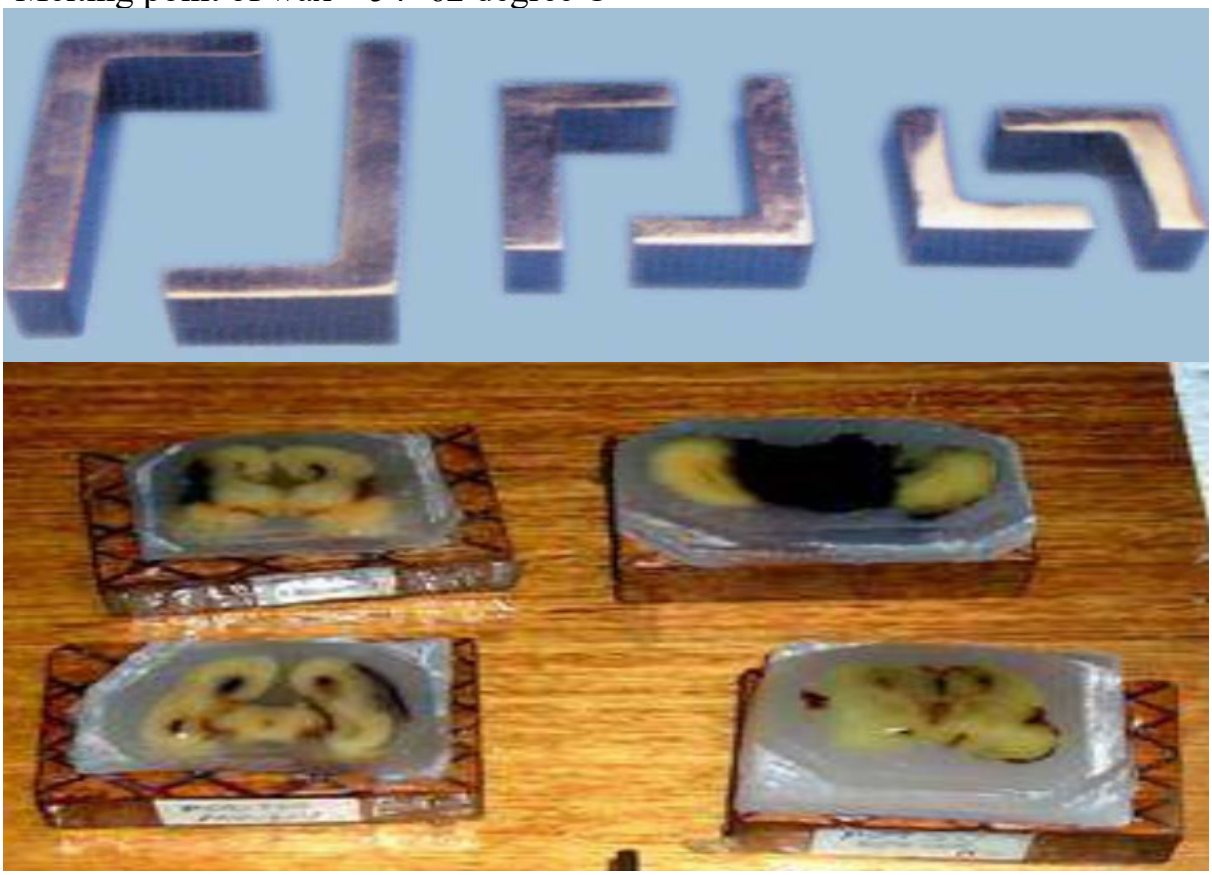
b- Clearing:- removed Alcohol from tissues and cells (dealcoholisation) and replaced by a fluid in which makes tissue transparent.

A typical clearing sequence for specimens not more than 4mm thick would be:

1. xylene 20 min
2. xylene 20 min
3. xylene 45 min

c- Wax infiltration:- after removal of clearing agent the empty spaces in the tissue and cells are poured by molten wax, this process hardens the tissue & helps in section cutting.

-Melting point of wax – 54- 62 degree C



3- Section Cutting:- It is the procedure in which the blocks which have been prepared are cut or sectioned and thin strips of varying thickness are prepared.

- The instrument by which this is done is called as a Microtome.

4-Staining :- Staining of the section is done to bring out the particular details in the tissue under study .The most commonly used stain in routine practice is Haematoxylin & eosin stain.

Procedure :

1. Deparaffinization with xylene.
2. Hydration
3. Wash under water
4. Stain with Haematoxylin for 15 min
5. Wash with water
6. Differentiate with 1 % acid alcohol
7. Wash with water for 10 min
8. Stain with 1% Eosin for 2 min
9. Wash with water.
10. Dehydration
11. Clearing with xylene
12. Dry

5-Mounting:- Adhesives used for fixing the sections on the slides. **Mounting medium is used to attach a coverslip to a microscope slide to protect the tissue during microscopy and storage**. In order to be an effective mounting medium, it is important that DPX (Di-n-butyl phthalate in Xylene). has an optimised viscosity and refractive index (RI).

Good Luck