Histology lab:

1st lab:

Preparation of tissue for study

HISTOLOGY

It is the branch of science which deals with the microscopic study of normal tissue

HISTOPATHOLOGY

It is the branch of science which deals with the microscopic study of tissue affected by disease

:Tissue for study can be obtained from

- Biopsies
- Autopsies

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

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

1-Fixation: Small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure.

2-Dehydration: The tissue is transferred through a series of increasingly concentrated alcohol solutions, ..

fixation

•This is 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 architecture

This is achieved by exposing the tissue to chemical compounds:

fixatives : 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

Processing

Tissue Processing

- In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge .These properties can be imparted by infiltrating and surrounding the tissue with paraffin wax, various types of resins or by freezing .This process is called tissue processing .

Tissue Processing: It can be subdivided into:

a- Dehydration - b- Clearing c- infiltration

Types of tissue processing

•There are two types:

1- Manual Tissue Processing 2. Mechanical Tissue Processing

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

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

2- Dehydration (removal of water)

It is the process in which the water content in the tissue to be processed is completely removed by passing the tissue through increasing concentrations of dehydrating agents

•Tissues are dehydrated by using increasing strength of alcohol; e.g . %70, %90and 100%

•Water is replaced by diffusion

•During dehydration water in tissue has been replaced by alcohol

•The next step alcohol should be replaced by paraffin wax

•As paraffin wax is not alcohol soluble, we replace alcohol with a substance in which wax is soluble. This step is called clearing.

Clearing : Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid (alcohol) and the embedding medium (wax).

•Some clearing agents: - Xylene - Toluene - Chloroform – Benzene

The tissue is kept in a wax bath containing molten paraffin wax

Embedding: is the process by which impregnated tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning Embedding:It is done by transferring the tissue to a mould filled with molten wax & is allowed to cool & solidify. After solidification, a wax block is obtained which is then sectioned to obtain ribbons.