Processing of material for histological evaluation

Preparation of tissues for study

Histology involves exploring the body's tissues and understanding how they form organs. Tissues consist of two key elements: cells and extracellular matrix (ECM), which comprises various organized molecules like collagen fibrils and basement membranes. The ECM serves multiple functions including providing mechanical support to cells, transporting nutrients, and removing waste products. Cells actively produce ECM and respond to stimuli and inhibitors together.

Each fundamental tissue type comprises various cell types and specific cell-ECM associations, aiding in distinguishing different tissue subtypes. Organs typically consist of a combination of tissues, with the exception of the central nervous system (CNS), which is primarily composed of nervous tissue.

To examine tissues, light microscopes are commonly used, where tissues are illuminated with a beam of light transmitted through them. Since tissues and organs are too thick for light to pass through, they need to be sectioned into thin slices. Proper tissue preparation is crucial to maintain the structure and molecular composition of the tissue as it exists in the body.

Human tissue samples can be obtained through biopsy from living organisms or necropsy after death. The collection process must be swift, safe, and painless for the patient.

It can be obtained by few ways:

1. **Excision** – Cutting Out – samples are cut out by sharp device (Scalpel) – Skin.

2. **Needle biopsy** (puncture) is used for taking samples from solid organs (lymph node, liver, kidney, bone marrow, brain, thyroid, breast, skeletal muscle etc), Fine Needle biopsy – for superficial lumps just under the skin.

3. **Curettage** – endometrial biopsy – mucosa of the uterus

4. **Endoscopic** using an endoscope, taking of samples by means of special tube , the tube gets in to hollow organs and cavities in the body (stomach, intestine, bronchus, urinary bladder, heart)

5. **Exfoliative cytology**– cells are examined in smears – uterus

Every sample requires proper labeling, including details on the container such as the name, year of birth, and birth certificate number, along with a comprehensive dispatch note for histological examination. This note should include patient identification, probable diagnosis, past therapy history, information for insurance purposes, as well as the name and address of the physician.

Important Values:

Sample size < 1cm3 (in em <1mm3)

Sections sliced (LM) – 1-10µm

Tissue processing: The objective of tissue processing is to encase the tissue in a solid medium firm enough to provide mechanical support and adequate rigidity for cutting thin sections measuring 4-10 µm. The duration of the entire procedure, from fixation to observing the tissue under a light microscope, may vary from 12 hours to 2.5 days, depending on factors such as tissue size, the type of fixative used, the embedding medium, and the staining method employed.

Fixation: 24 hours

To achieve permanent tissue sections, it's essential to employ fixation methods to prevent metabolic processes and tissue degradation caused by endogenous enzymes (autolysis) or bacterial activity, thereby preserving the structural integrity and molecular composition. Fixation can be achieved through chemical or, less commonly, physical means, such as freezing with liquid nitrogen. However, freezing methods can affect the cellular water transport function.

Chemical fixation typically involves immersing tissues in solutions containing stabilizing or cross-linking agents known as fixatives. One widely used fixative is formalin, which is a buffered isotonic solution containing 37% formaldehyde. Formalin solutions for fixation purposes typically range from 10-25% formalin (equivalent to 4-10% formaldehyde), often combined with buffers like NaCl or CaCl2.

Various formulations of formalin solutions exist for specific applications, including Formalin Saline, Buffered Formalin (incorporating phosphate buffer for pH stabilization), and Baker's Fluid, which is particularly useful for lipid fixation and comprises a mixture of formalin, distilled water, and CaCl2 (calcium chloride).

The duration of fixation is typically around 24 hours, although formalin fixation does not lead to over-fixation, so fixation times can be extended if necessary.

After fixation– washing in water or 70% ethanol.

The chemistry underlying the fixation process is intricate and not always fully elucidated. Fundamentally, formaldehyde is recognized for its reaction with the amine groups (NH_2) found in tissue proteins.

Other fixatives than formalin:

• Combination of picric acid and formalin (in a ratio of 3 parts picric acid to 1 part formalin), such as Bouin's fluid. This mixture is effective for fixing polysaccharides (evidenced by its yellow color), particularly suitable for preserving glycogen but not recommended for fixing organs with blood or lipids.

• Fixatives containing mercuric chloride, found in solutions like SUSA fluid and Zenker's Fluid. These fixatives are useful for preserving polysaccharides and cell membranes. However, a drawback is the formation of black mercury precipitates, which can be removed through iodination.

• Fixatives containing heavy metal compounds (such as $HgCl_2$, OsO_4, $KMnO_4$).

• Highly concentrated alcohols (e.g., ethanol, methanol): 96% ethanol is used for fixing nerve tissue for Nissl staining, while methanol is preferred for preserving smears. Cooled acetone is used for fixation in histochemistry.

• Organic acids.

Embedding and Sectioning:

Typically, tissues are enclosed within a solid medium to ease the sectioning process. After fixation, tissues are permeated with embedding agents to confer a firm texture. Paraffin is one such embedding material commonly utilized. Paraffin embedding involves two primary stages: dehydration and clearing.

Dehydration: Initially, water is removed from the fragments intended for embedding by immersing them in a sequential series of ethanol-water mixtures, typically ranging from 70% to 100% ethanol.

Clearing: Replacing of ethanol, an intermediary solvent compatible with both alcohol and the embedding medium (paraffin) is used. These intermediary solvents include Benzene, Xylene, Toluene, and Methyl.

Impregnation of the embedded medium: The tissue is immersed in molten paraffin within an oven, typically maintained at a temperature range of 52-60 degrees Celsius. The application of heat prompts the solvent to vaporize, filling the voids within the tissue with paraffin. As a result, the fragments solidify, readying them for sectioning. These hardened blocks containing the tissues are placed into a device known as a microtome, where they are cut into sections ranging from 1 to 10 µm thick using steel or glass blades.

Following sectioning, the sections are affixed to glass supports using albumin as an adhesive.

Alternatively, tissues can be subjected to rapid freezing using liquid nitrogen (a form of physical fixation). Subsequently, a freezing microtome, known as a cryostat, is utilized to section the frozen tissue block. This method is expedient and commonly employed in hospital settings during surgeries. Moreover, freezing does not typically deactivate most enzymes.