Tissue fixation and Processing

Fixation

1. **Definition-** it is a complex series of chemical events which brings about changes in the various chemical constituents of cell to preserve morphology and structural detail.

2. **Principle of fixation-** The fixative brings about crosslinking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state for easy manipulation of tissue.

Aims and Effects of Fixation

1. To preserve the tissue as if like life as possible.
2. To prevent postmortem changes like autolysis and putrefaction.
3. Preservation of chemical compounds and microanatomic constituents so that further histochemistry is possible.
4. *Hardening* : the hardening effect of fixatives allows easy manipulation of soft tissue like brain, intestines etc.
5. *Solidification*: Converts the normal semifluid consistency of cells (gel) to an irreversible semisolid consistency (solid).
6. *Optical differentiation* - it alters to varying degrees the refractive indices of the various components of cells and tissues so that unstained components are more easily visualized than when unfixed.
7. *Effects of staining* - certain fixatives like formaldehyde intensifies the staining character of tissue especially with haematoxylin.

Type of Fixatives

1. **Aldehydes:** include formaldehyde (formalin) and glutaraldehyde. Tissue is fixed by cross-linkages formed in the proteins, particularly between lysine residues.
2. **Mercurials:** fix tissue by an unknown mechanism. They contain mercuric chloride and include such well-known fixatives as B-5 and Zenker's.
3. **Alcohols:** including methyl alcohol (methanol) and ethyl alcohol (ethanol), are protein denaturants and are not used routinely for tissues because they cause too much brittleness and hardness. However, they are very good for cytologic smears because they act quickly and give good nuclear detail

Type of Fixative con.

4. **Oxidizing agents:** include permanganate fixatives (potassium permanganate), dichromate fixatives (potassium dichromate), and osmium tetroxide. They cross-link proteins, but cause extensive denaturation.
5. **Picrates:** include fixatives with picric acid. Foremost among these is Bouin's solution. It has an unknown mechanism of action.
6. **Microwaves:** MW irradiation may serve as the primary method of fixation, MWs can also accelerate the fixing action (cross-linking) of aldehydes or alcohols.
7. **Vapour fixation:** various chemicals which act as vapour fixatives include aldehydes (formaldehyde, glutaraldehyde and acrolein), osmium tetroxide, chromyl chloride, ethanol, diethylpyrocarbonate, benzoquinone, and diacetyl; the most common being formaldehyde, osmium tetroxide, and perhaps alcohol.

Factors Affecting Fixation

There are a number of factors that will affect the fixation process:

1. **Buffering:** there must be buffering capacity in the fixative to prevent excessive acidity. Acidity favors formation of formalin-heme pigment that appears as black, polarizable deposits in tissue
2. **Penetration:** Penetration of tissues depends upon the diffusability of each individual fixative, which is a constant.
3. **Volume:** There should be a 10:1 ratio of fixative to tissue.

Factors Affecting Fixation con

4. **Temperature:** increasing the temperature, as with all chemical reactions, will increase the speed of fixation, as long as you don't cook the tissue.
5. **Concentration:** Concentration of fixative should be adjusted down to the lowest level possible, Too high a concentration may adversely affect the tissues and produce artifacts similar to excessive heat.
6. **Time interval:** very important is the time interval from of removal of tissues from the body to fixation. The faster you can get the tissue and fix it, the better.

Clearing Agents

Choice of a clearing agent depends upon the following:

- the type of tissues to be processed, and the type of processing to be undertaken the processor system to be used
- intended processing conditions such as temperature, vacuum and pressure
- safety factors
- cost and convenience.

- **Xylene:** Commonly used, tends to harden tissue if left in too long, neurotoxic
- **Benzene or toluene:** Commonly used; clears overnight.
- **Cedarwood oil:** Slightly slower in penetrating than benzene is; does not cause hardening; does not interfere too seriously with paraffin penetration if it is not completely removed.
- **Methyl benzoate** Very good for clearing; does not harden tissues. It penetrates almost as fast as does cedarwood oil (12 to 24 hours) and is most valuable with the Peterfi celloidin-impregnation technique.
- **Dioxane:** which is miscible both with water and paraffin. It is used primarily when time is important because the tissues may be embedded with paraffin within 4 hours after fixation. The tissues are transferred to dioxane straight from Bouin's fluid or a formalin fixative.

Tissue Processing

This process includes **dehydration**, **clearing** and paraffin wax **infiltration** using the Automated tissue processor.

Fixed specimens are **dehydrated** as follows:

- 70% ethanol 1 ½ hr.
- 95% ethanol 1 ½ hr.
- 95% ethanol 1 ½ hr.
- 100% ethanol 1 ½ hr.
- 100% ethanol 1 ½ hr.
- 100% ethanol 1 ½ hr.

Clearing

- 50:50 (100% ethanol: xylene) 1hr.
- xylene 1hr.
- xylene 1hr.

Infiltration (embedding) media

- Paraffin wax (Tissue Prep, Fisher Sci., melting point 56-57°C
- Paraffin wax 1hr.