Expt. No.

# Microtechnique

Page No.
Date 29/09/08

## Introduction :→

The objective of a histology course is to lead the student to understand the microanatomy of cells, tissues and organs and to corelated the structure with function.

The study of the histology is purely based on a practical work of microtechnique. This technique given rise to micro of very thin section of tissue.

The first step in preparation of a tissue or organ sample is fixation to preserve structure.

The tissue processing methods for the different embedding and sectioning methods. In paraffin wax embedding methods the tissue processing includes-

i) Fixation of the tissue.
ii) Dehydration of the tissue.
iii) Clearing of the tissue.
iv) The tissue is prepared for embedding in paraffin to permit sectioning.
v) The tissue is stained to permit examination.

## I. Fixation of the Tissue

### a) Aim of fixation

The purpose and objectives of fixation are as follows –



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i) Fixation arrests postmortem decay, prevents or stops autolysis and bacterial decomposition.

ii) To coagulate and harden the tissue for the preservation of easily diffusable substances and for sectioning the tissue into thin slices.

iii) Fixatives have the property of forming crosslinks between proteins thereby forming a chain.

iv) Soluble proteins are fixed to structural proteins and thus rendered insoluble and the whole structure given some mechanical strength which permits the protection of tissues during subsequent stages.

v) To increase the affinities of tissue constituents to certain dyes so that the cellular part become easily stainable with differentiation.

vi) To protect the cell and the tissue constituents from subsequent process such as dehydration, embedding, infiltration etc.

vii) To change the refractive index of various constituents of the cell and make them clearly visible through the lens.

viii) To represent the tissues as close to their living condition as possible.



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## Coagulant Fixative

Fixative that causes coagulation of cytoplasmic proteins destroy or distort organelles as such mitochondria and secretory granules but they do not seriously disturb the supporting extracellular materials which are already partly solid before fixed. It is thought that coagulant fixative produced a sponge-like proteinaceous reticulum that is easily penetrated by the large molecules of melted paraffin. Sectioning of wax embedded material is facilitated by coagulant fixative.

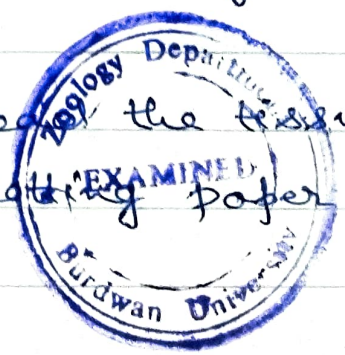
## Non-Coagulant Fixative

Non-coagulant fixatives cross-link protein molecules. They convert the cytoplasm into an insoluble gel in which the organelles are well preserved but which is thought to be easily penetrated by paraffin.

## Method of Fixation

For collection of living tissue from an animal, it is to be sacrifised by killing the animal by using chloroform or anasthetic ether.

As soon as the animal is dead the tissues are taken out and blot in a blotting paper and fixed in fixative.

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Date.

In case of larger tissue initial subdivision should be made in the same plane by which it will later be sectioned. After the suggested period of fixation, the tissues are transferred into the subsequent other chemical necessary for the purpose. Proper labelling is usually done during this work when the tissue of the different organ of the animal are fixed at a time.

## Types of Fixative

Some fixatives used in histological studies are

a) Aqueous Fixative :-

1. Bouin's Fixative

Composition:

i) Saturated aqueous solution of picric acid - 75 ml.

ii) 40% formaldehyde - 25 ml.

iii) Glacial acetic acid - 5 ml.

Fixation time - 16-18 hours.

2. Gendre's Fixative

Composition:

i) Saturated alcoholic (90% ethanol) solution of Picric acid - 80 ml.

ii) 40% formaldehyde - 15 ml.

iii) Glacial acetic acid - 5 ml.

Fixation time - 4 hours.



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## b) Alcoholic Fixative :-

### 1. Carnoy's Fixative

Composition :

i) Absolute alcohol - 60 ml.

ii) Chloroform - 30 ml.

iii) Glacial acetic acid - 10 ml

iv) Fixation time - $1\frac{1}{2}$ - 2 hours.

### 2. Alcoholic Formal-Acetic Acid (AFA)

Composition :

i) Ethanol - 85 ml.

ii) Formalin - 10 ml.

iii) Glacial acetic acid - 5 ml

Fixation time - 4-25 hours.

## c) Aqueous Aldehyde Solution :-

### 1. Formal Saline

Composition :

i) Sodium chloride - 0.9 gm.

ii) Water - 90 ml.

iii) Formalin - 10 ml.

### 2. Formol Sublimate

Composition :

i) Saturated aqueous solution of mercuric chloride - 90 ml.

ii) Formalin - 10 ml.



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d) Mercuric chloride containing Fixative :-

1) Zenker's Fluid

Composition :

i) Mercuric chloride - 5 gm.

ii) Potassium dichromate - 2.5 gm.

iii) Distilled water - 95 ml.

5 ml of glacial acetic acid to be added immediately before use.

2) Helly's Fixative

Composition :

i) Mercuric chloride - 5 gm.

ii) Potassium dichromate - 2.5 gm.

iii) Sodium sulfate - 1 gm.

iv) Distilled Water - 100 ml.

Immediately before use 5 ml of 40% formaldehyde is added to this fluid.

## II. Dehydration

The removal of water from the intercellular spaces of tissue is called Dehydration. It is the most essential state and must be done in a very perfect manner. The dehydration of the tissue is usually done by the treatment with series of alcoholic solutions of ascending concentration of upto 100% alcohol with 2 to 3 changes to complete the removal of water from the tissue.



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