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Expt. No. 04 03 FIXATION OF TISSUES Page No. 11

INTRODUCTION

It is a process by which the cells or tissues are fixed in chemical and partly physical state so that they can withstand subsequent treatment with various reagents, with minimal distortion of morphology and no decomposition.

AIMS OF FIXATION

(a) To preserve the tissues as close to their living state as possible.
(b) To prevent autolysis and bacterial attack.
(c) To prevent tissues from changing their shape and size during processing.
(d) To harden the tissues.
(e) To allow clear staining of sections subsequently.
(f) To improve the optical differentiation of cells & tissues.

PRINCIPLE OF FIXATION

Fixation results in denaturation and coagulation of protein in the tissues. The fixatives have a property of forming cross links between proteins, thereby forming a gel, keeping everything in their in vivo relation to each other.

PROPERTIES OF FIXATIVES AND FACTORS AFFECTING FIXATION :-

1. Coagulation and precipitation of proteins in tissues.

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2. Penetration rate differs with different fixatives depending on the molecular weight of the fixative.
3. pH of fixatives - Satisfactory fixation occurs between pH 6 and 8. Outside this range, alteration in structure of cell may take place.
4. Temperature - Room temperature is alright for fixation. At high temperature there may be distortion of tissues.
5. Volume changes - Cell volume changes because of the membrane permeability and inhibition of respiration.
6. An ideal fixative should be cheap, nontoxic and non-inflammable. The tissues may be kept in the fixative for a long time.

Types Of Fixation

- Immersion fixation
- perfusion fixation
- Vapour fixation
- Coating / Spray fixation
- Freeze drying
- Microwave fixation / Stabilization

Simple Fixatives

1. Formaldehyde :- Commercially available solution contains 35% - 40% gas by weight called as formalin. Formaldehyde is commonly used as 4% solution giving 10% formalin

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for tissue fixation. Formalin is most used fixative.
2. Absolute alcohol :- It may be used as a fixative as it coagulates protein. Due to its dehydrating property it removes water too fast from the tissues and produces shrinkage of cells and disortion of morphology. It penetrates slowly.
3. Acetone :- Sometimes it study for enzymes especially phosphatases and lipases.
4. Mercuric chloride :- It is a protein precipitant. It causes great shrinkage of tissues hence seldom used alone.
5. Pottasium dichromate :- It has a binding effect on protein similar to that of formalin fixation with pottasium dichromate tissue must washed.
6. Osmic acid :- It is used for fixation of fatty acid tissues.
7. Chromic acid :- It precipitates all proteins and preserves carbohydrates. Tissue fixed in chromic acid also require through washing with water before dehydration.
8. Osmium tetraoxide :- It gives excellent preservation of cellular details. for electron microscopy.
9. Picric acid :- It precipitates proteins and combines with to form picrates. Tissue can not be kept in picric acid more than 24 hrs.

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COMPOUND FIXATIVES

1. Formal Saline :- It is most widely used fixative. Tissue can be left in this long period without excessive hardening or damage.
2. Formal Calcium :- Useful for demonstration of phospho-lipids fixation time - 24hrs at room temperature.
3. Zenker's fluid :- It contains mercuric chloride, potassium-di-chromate, Sodium sulphate & glacial acetic acid.

Advantages - even penetration, rapid fixation.

Disadvantages - After fixation the tissue must be washed in running water to remove excess dichromate. Mercury pigment must be removed with Lugol's Iodine.

4. Zenker's formal :- In stock zenker's fluid, formalin is added instead of acetic acid.

Advantage :- Excellent micro anatomical fixative especially for bone marrow.

5. Bouins fluid :- It contains picric acid, glacial acetic acid and 40% formaldehyde.

Advantages

Rapid and even penetration without shrinkage.

Points to Remember

* 10% Buffered formalin is commonest fixative
* Tissues may be kept in 10% Buffered formalin.
* The specimen should be completely submerged.
* Special fixatives are used for preserving particular tissues.

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* Formalin vapours cause throat / eye irritation
* Tissues should be well fixed before dehydration.
* Penetration of fixatives takes some time.
* Mercury pigment must be removed with Lugol's iodine
* Biopsies cannot be kept for more than 24 hours.
* Glutaraldehyde and Osmion tetraoxide and used as fixatives for electron microscopy.

Most commonly used fixatives in laboratory are :-

10% formalin

Formaldehyde (40%)	-	10 ml
Distilled water	-	90 ml.

Formal saline

Formaldehyde (40%)	-	100 ml
Sodium Chloride	-	9 gm
Distilled water	-	900 ml.

Bouin's solution

Saturated picric acid	-	750 ml
formaldehyde (40%)	-	250 ml
Glacial acetic acid	-	50 ml

10% Buffered formalin

Formaldehyde	-	10 ml
Sodium dihydrogen phosphate	-	0.4 gm
Disodium hydrogen phosphate	-	0.65 gm
Distilled water	-	90 ml.

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Alcoholic formaldehyde
40% formaldehyde - 100 ml
95% alcohol - 900 ml

Alcohol containing fixatives
Carnoy's fixatives -
Absolute ethanol - 60 ml
Chloroform - 30 ml
Glacial acetic acid - 10 ml

Mercury Salt Containing fixatives
Zenker's fluid
Distilled water - 950 ml
potassium dichromate - 25 gm
Mercuric chloride - 50 gm
Glacial acetic acid - 50 gm

Bs fixative
Stock reagent A -
Mercuric chloride - 60 g
Sodium acetate - 12.5 g
Distilled water - 1000 ml

Stock Reagent B
10% Buffered neutral formalin

Working Solution

Stock reagent A - 90 ml

Stock reagent B - 10 ml

Fixative time - 5-8 hrs.

Adequate time should be given for fixation. Formalin fixation should ideally be given for at least 8 hours before processing.