P. G. 2nd semester Zoology (1)

By Dr. V. Kumar.

Topic : FIXATION

[Cytological study of Preserved cells]

The Fixation is the process that brings about sudden ~~death~~ death of the cells or tissues in such a manner that their morphological and ~~chemical~~ chemical composition is retained in a condition almost identical to that as existed during life. This can be achieved either by use of chemicals or by freezing.

Aims & effects of Fixation

- Fixation hardens the tissues and gives them a constant form
- It prevents autolysis and bacterial decomposition
- It coagulates the tissue, renders the contents insoluble and prevents loss of easily diffusible substances.
- It avoids cell shrinkages and distortion in form due to post mortem changes.
- Improves the optical differentiation of cell components by changing refractive indices and thus increases their visibility.
- Prepares the tissue for staining
- It fortifies tissue against the harmful effects of various stages in the preparation of sections.

[A] chemical fixation.

The tissue is fixed by some chemical compounds such as Formaldehyde, mercuric chloride, Picric acid, chromic acid, osmium tetraoxide, acetic acid and Ethyl alcohol. These are called fixatives.

(a) Simple fixatives

<i> Formaldehyde → 4 to 10%. Formalin Solution.
→ used for fixing Golgi apparatus, Mitochondria and enzymes.
→ It fixes & hardens the tissue but causes little or no shrinkage.

<ii> Mercuric chloride
- It is an intolerable fixative and used only in combination with some other fixatives.
- It hardens and causes shrinkage in tissue but does not distort it.
- It precipitates the protein and fixed lipids.

<iii> Picric acid
- It precipitates proteins and nucleoproteins
- It produces shrinkage, thus not used for cytological studies.

<iv> Chromic acid
- 0.5 to 1% chromic acid is used to fix those tissues which are studied for Golgi complex and Mitochondria.
- It precipitates all proteins & fixes carbohydrates

<v> Osmium tetroxide
- 0.5 to 2% solution of osmium tetroxide is used for fixing cytoplasm, Golgi complex, mitochondria & fact.
- It is extensively used for electron microscopy.

vi) Potassium dichromate.
- 2.5 to 5% Potassium dichromate solution is used in conjuncture with some other chemical substance.
- It renders Protein insoluble in water and fixes lipids.
- It is used for fixation of chromosomes.

vii) Acetic acid
- Glacial acetic acid is used along with other fixative.
- It precipitates nucleoproteins, but not cytoplasmic proteins. It destroys Golgi complex & mitochondria. Thus, uses for fixation of nucleus & chromosomes.

viii) Ethanol:
70% absolute alcohol is used as fixative.

(b) Compound fixative

The most essential feature of a compound fixative is its quick penetration power. some of them are-

(i) 10% Formal Solution - Mixture of Formalin & normal Saline Solution.

(ii) Formal alcohol

- Mixture of 10 ml Formalin, 90 ml of 90% alcohol, and 5 ml glacial acetic acid.
- It is used as a fixative for polysaccharides and nucleoproteins.

(iii) Carnoy's solution

→ Mixture of ethanol 60 ml & glacial acetic acid 10 ml and chloroform 30 ml.
- It fixes nucleoproteins and chromosomes.

(iv) Bouin's fluid

- It is mixture of 75 parts Picric acid, 25 parts formalin & 5 parts glacial acetic acid.
- It precipitates all proteins, penetrates rapidly and produces little shrinkages.
- It fixes chromosomes. & used for histological studies.

Procedure of Fixation

- When a piece of tissue is immered in the fixative. Cellular death does not occur instantaneously and "post-mortem" changes due to anoxia, changes in the concentration of hydrogen ions and enzymatic action (autolysis) may occur.
- The fixative penetrates the tissue by diffusion in such a way that the most external cells are fixed more rapidly and better than the central ones.
- Thus, every fixed tissue has a gradient of fixation, which depends upon the permeability or quickness of diffusion of the fixative and its progressive dilution with the liquid of the cells.
- The rate of Penetration of the fixative depends upon the type of protein barrier of precipitation produced at the periphery of the tissue.

Chemical Basis of Fixation

A/c to recent view most useful fixatives produce polymerisation of tissue compounds. Since proteins are the main components of living tissue, the fixative forms cross-linkages between protein molecules which render them insoluble. Therefore, by fixation proteins are precipitated.

For Example, formaldehyde reacts with amino Carboxyl and indol groups of a protein forming methylene bridges with other protein molecules.

$$-\underset{\underset{H}{|}}{N}-H + HCHO \longrightarrow -NHCH_2OH$$

amino group. methylol.

$$-NHCH_2OH + -\underset{\underset{H}{|}}{N}-H \longrightarrow -NH-CH_2-NH + H_2O$$

methylene bridge.

The most widely used osmium tetroxide ~~tetroxide~~ tetraoxide forms double bonds with lipids and forms unstable osmium esters. This decomposes to deposit osmium oxides or hydroxides causing gelation of protein.

In case of fats and lipids double bond cross linkages are formed.

[B] Fixation by Freezing

For histochemical and Electron microscopic studies, the tissues may be killed and fixed by rapid cooling. This is brought about in special frozen chambers where temperature is maintained at $-100^{\circ}C$ to $-190^{\circ}C$.

The following methods of freezing-fixation techniqne are employed.

<1> Freeze drying

- Very small pieces of fresh tissue are freized by immersing them in isopentane or Propane Cooled by liquid nitrogen to about $-160^{\circ}C$ or $-190^{\circ}C$ (quenching).
- The material is then transfer to the drying Chamber, which has Vaccum (0.01 mm Hg) and

temperature of −30°c to −60°c. In drying (5) chamber, the water is removed from the tissue by sublimation.

— By freeze drying method, the tissue does not shrink, fixation is homogenous and the soluble substances are not removed or displaced. It means the chemical composition of the cell remains the same.

(ii) Freeze Substitution

— In this technique, the living tissue is frozen rapidly, and kept at low temperature (−60°c to −70°c) in a reagent that dissolves the ice crystals.

— The reagent used may be absolute alcohol or acetone.

— This method is also used in electron microscopy but its result is not always good.

(iii) Freeze Etching

The tissue is placed in 20% glycerol and is frozen at −100°c.

— It is mounted on a chilled holder and is splintered with a knife to get its cross section

— The splintered preparation is freeze dried and is covered with a platinum and carbon coating in the high vaccuum of a freezed ultramicrotome, and dried material is taken out and placed in water, so that replica starts floating on the water surface.

— The replica is washed in some basic solution to remove the cellular material and is mounted on a grid and dried for electron microscopic study.

— The replica gives the outline of various cell structure.

Vijay W.
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