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The purpose of fixation is to preserve tissues permanently in as life-like a state as possible. Fixation should be carried out as soon as possible after removal of the tissues (in the case of surgical pathology) or soon after death (with autopsy) to prevent autolysis.

The stained cells in a microscope should resemble living cells as closely as possible. Fixation is process by which the internal & external structures of cells & microorganisms are preserved & fixed. It inactivates enzymes that might disrupt cell morphology & toughens cell structures so that they don't change during staining & observation. A microorganism is usually killed & firmly attached to microscopic slide during fixation.

There are two fundamentally different types of fixation. Bacteriologists heat fix bacterial smear by gentle & an air dried film of bacteria. This adequately overall morphology but not structures within cells. Chemicals fixation must be used to protect fine cellular structures & the morphology of larger, more delicate microorganisms. Chemical fixatives penetrate cells & react with cellular components, usually proteins,lipids to render them inactive, insoluble & immobile. Common fixatives mixtures contain such components as ethanol, acetic acid, mercuric chloride, formaldehyde, & glutaraldehyde.

For practical purposes fixation aims to prevent or arrest the degenerative processes which commence as soon as a tissue is deprived of its blood supply. Autolysis, which results in tissue digestion by intracellular enzymes released when organelle membranes rupture, and bacterial decomposition or putrefaction which is brought about by micro organisms which may already present in the specimen, are processes that must be prevented. Loss and diffusion of soluble substances must be avoided as far as possible by precipitation or coagulation of these components or by cross-linking them to other insoluble structural components. The tissues must be largely protected against the deleterious effects of tissue processing including infiltration with hot wax, but, importantly, tissues must retain reactivity to stains and other reagents including antibodies and nucleic acid probes. 1, 2

It is important to realise that a fixative will initially produce a number of changes to the tissues in what is usually an aqueous environment. These will include shrinkage, swelling and hardening of various components. Despite these initial effects tissues will undergo further changes during processing when they are placed in a non-aqueous environment. 2 For example fixation in 10% buffered formalin initially causes slight swelling of tissue specimens. During processing however the specimen may shrink 20% - 30% of its volume. 3 The particular fixative employed will also influence the degree to which individual elements will stain with various histochemical and immuno-histochemical reagents. 4 Thus the total effect on tissues of a particular fixative should be assessed after a tissue has been processed, sectioned and stained to demonstrate the required elements.

2).

Formaldehyde or FormalinFormaldehyde was discovered in 1859 by Butlerov. In 1889 Ttrillat was the first who manufactured formaldehyde com-mercially as industrial reagent. In 1892, Ferdinand Blum recog-nized that formalin could give benefit when used as a fixative.8,9The most routinely used solution for fixation of tis-sue—10% formalin solution v/v—is nothing but an aqueous suspension of formaldehyde In 10% neutral buffered form, formaldehyde is found to be the most commonly used fix-ative in pathology. Reaction between the formaldehyde and macromolecules of tissue seems to be complex. Form-aldehyde reacts with nucleic acids as well as proteins, and it penetrates between nucleic acids and proteins and forms stabilized shell of nucleic acid-protein complex.10-13 As com-pared with other fixatives, formaldehyde causes lesser tissue shrinkage, with exceptions being acetone and ethanol. Form-aldehyde seems to harden tissue more when compared with other fixatives. The lipids are conserved, but carbohydrates are not fixed by formaldehyde.4Formalin comprises 37 to 40% formaldehyde and 60 to 63% water by weight. After continuous storage for long peri-ods, accumulations of white deposits are observed in the solution. These are the precipitates of paraformaldehyde. By storing formalin at low temperature, these white deposits can be avoided. Also, 10% methanol may be added into the formalin to minimize the polymerization reaction that pro-duces paraformaldehyde precipitate. It also contains a slight amount of formate ions. These are obtained from Cannizza-ro reaction. In this reaction, two molecules of formaldehyde react together. One molecule condenses to form methanol and second molecule gets oxidized to form formic acid.14 The solution is acidic in reaction because of formic acid, but acid-ic nature of solution can be counterbalanced with incorpora-tion of magnesium carbonate in little proportion

Picric acid is an example of a coagulant fixative. It forms picrates with basic protein groups, which causes coagulation. For the purpose of demonstration of DNA or RNA, picric acid fixatives are not used as picric acid and can hydrolyze nucleic acids. Also, picric acid is seen to disintegrate calcium depos-its in samples. Although picric acid is not able to fix most car-bohydrates and lipids, picric acid is the most advised fixative to preserve glycogen. Brighter staining is seen by picric acid fixatives.4,18,31Picric acid is an acidic solution. Therefore, sometimes it gets washed out by alcohol. To avoid this, lithium carbonate is added, which acts as a neutralizer. Luna reported that if picric acid is present in the tissue or not completely removed, distortion or obliteration of cellular structures will occur as outcome

For ethanol and methanol, fixation initiates at 50 to 60% concentration and greater than 80% concentration, respec-tively. They are known to be coagulants that cause protein denaturation. They cause interruption in hydrogen and hydrophobic bonding by substituting water in tissue envi-ronment, which results in change in tertiary structure.Ethanol causes mispresentation of cytoplasmic as well as nuclear details, but sometimes it can be used for preservation of glycogen. Methanol is more commonly used for fixation of exfoliative cytology smears and blood films

Acetone is another fixative agent used in histopathology. It acts as an efficacious lipid solvent that results in tissue brit-tleness. Apart from tissue fixation, they are primarily used as an agent for dehydration in tissue processing. Because of extremely volatile as well as flammable nature, they are not recommended for use in automatic tissue processor

Acetic acid is considered as a noncoagulative fixative agent. It acts by causing nuclear proteins coagulation. Incidentally, it stabilizes and assists to prevent nucleic acids loss. Acetic acid, when combined with ethanol, is used as an effective cytolog-ical fixative that helps in conservation of nucleic acids, but if it is used singly, it results in swelling of cells. Time required for fixation by acetic acid is less as penetration of acetic acid is faster into tissues

Potassium DichromatePotassium dichromate is also a noncoagulant fixative, but if used in combination with acid solution, it acts as a coagulant fixative. It is seldom used alone for fixation because chro-mate ions will link with few lipids and makes them insoluble.Chromium seems to react with hydroxyl as well as car-boxyl groups. By increasing the amount of reactive basic groups, the affinity of tissues for eosin staining will boost up. It conserves mitochondria but dissolves DNA. It is suggested that tissues that are fixed with chromate fixatives have to be washed completely in water before processing of tissues any further. This step is important as it avoids establishment of chromate suboxide that is insoluble.

Bouin’s FixativeBouin’s fixative is known as noncoagulant picrate fixative solution and was explained by Pol Andre Bouin in 1897.

Bouin’s fixative is considered as good fixative for conserving delicate as well as soft tissue structures. The major portion of Bouin’s fixative contains picric acid with little quantity of acetic acid as well as formaldehyde. In the samples that have to be undertaken in situ hybridization, Bouin’s solution cannot be used because it decreases the severity of hybridization.

AcroleinAcrolein was introduced by Luft as a primary fixative agent, and it is a three carbon αβ unsaturated monoaldehyde. Acro-lein provides magnificent preservation of structural detail and conserves the virus antigenicity.16,39,40 It is also known as acrylic aldehyde. It reacts with macromolecules that result in formation of cross-links that are reversible.Acrolein is not commonly used because it is unstable at alkaline pH and forms insoluble polymers. Acrolein is high-ly reactive and is found to penetrate tissues rapidly. Acrolein fixatives are chiefly used in enzyme histochemistry.