Fixation

The foundation of all good histological preparations is adequate and complete fixation. Fixation is required to (1) *prevent* post mortem changes such as putrefaction and autolysis; (2) *preserve* various cell constituents in as life like manner as possible; (3) *protect* by hardening the naturally soft tissue, thereby allowing easy manipulation during subsequent processing; (4) *convert* the normal semi-fluid consistency in cells to an irreversible semi-solid consistency; (5) *aid* in the visual differentiation of structure by application of biological dyes and chemicals. To accomplish these objectives the tissue should be placed in the fixative immediately upon removal from the body or as soon after death as possible.

The choice of fixing agent should be determined by the purpose for which the tissue is to be stained or preserved. Blocks should be cut thin enough so that the fixative will penetrate the tissue within a reasonably short time. To this end the block should not be more than 4 mm in thickness and should be immersed in at least ten times its volume of fixative.

Ten percent buffered neutral formalin is the most widely used fixative because it is compatible with most stains. The length of time for fixation depends upon the size of the block and fixative used. It is well to have a clear understanding of the effects of fixation, the time required for complete fixation of specific tissues, and the post fixation handling of tissue specimens.

That many specimens may be ruined, by poor handling subsequent to proper fixation, has been proven. This generally occurs when one fails to realize that different fixatives require varied times to effect complete fixation; and the specimen may require a particular treatment, immediately following fixation, to insure retention of specific staining properties.

Additional useful knowledge, is the action of a simple fixative on different parts of the tissue specimen. A partial list of the characteristics of certain common simple fixatives and their various effects follows, while more detailed information in this regard can be found in: Baker, J. R.: *Principles of Biological Microtechnique*, New York, John Wiley & Sons, Inc. 1958.

FORMALIN SALINE SOLUTION

37 – 40% formalin	100.0 ml
Sodium Chloride	9.0 gm
Tap water	900.0 ml

A tolerant fixative. Long storage does not create excessive hardening or damage. When not buffered may cause formation of formalin pigment. It fixes nuclear chromatin in a diffused homogenous pattern making it impossible to visualize chromatin distinctly. Therefore, it is not especially useful for routine use. Ideal for the preservation of neuro substances.

BUFFERED NEUTRAL FORMALIN SOLUTION

37 – 40% formalin	100.0 ml
Distilled water	900.0 ml
Sodium phosphate monobasic	4.0 gm
Sodium phosphate dibasic (anhydrous)	6.5 gm

BUFFEERD NEUTRAL FORMALIN MADE WITH TAP WATER

	20 Liters	10 Liters	5 Liters	1 Liter
37-40% Formaldehyde *stock*	2 liters	1 liter	500 mls	100mls
Tap Water	18 liters	9 liters	4.5 liters	900mls
Sodium phosphate monobasic	372 gms	186 gms	93 gms	18.6 gms
Sodium hydroxide	84 gms	42 gms	21 gms	4.2 gms

Dissolve sodium hydroxide in water, make sure the salt is completely dissolved. Add the sodium phosphate monobasic, stir until dissolved. Add formaldehyde, mix well.