

# FIXATION

## The Key to Good Tissue Preservation

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The foundation of all good tissue specimen preparations is complete fixation. The primary function of tissue fixation is to prevent putrefaction and autolysis. Faults in fixation cannot be remedied at any later stage, and the finished product can only be as good as its initial fixation.

With today's modern techniques human tissues from ancient civilizations continue to be scientifically dated and studied.

Mummification is one of man's earliest methods of human tissue preservation. Mummification is a term loosely used to describe different methods used by ancient civilizations to prepare human remains for burial. Various methods of preservation have been described in many articles. Some mummies which have been studied by scientists have become quite famous not only for their part in history as individuals, but also for the wealth of medical information obtained from their mummified bodies. Autopsies performed on mummies have given great insight into medical problems of earlier civilizations. For example, a leading cause of death in Pre-Columbian peoples, going back over 5000 years, was pneumonia. Also noted were lung abscesses, pericarditis, and endocarditis, as well as tuberculosis. These studies have prompted scientists to ponder about which diseases were present before the arrival of Europeans in America.

A major health problem in our population today is neoplasms, particularly malignancies. Medical studies of preserved human tissue from earlier eras have revealed that tumors were scarce in early American man. It is a medical phenomenon that scientists do not fully understand. Many think that this phenomenon is due to longevity, but there are also statistics that indicate 25% of early populations lived past 40 and some lived past 70 years of age. Hopefully, future studies will lead scientists to new and expanded information that eventually will

enable us to have a good overview of daily life and the influence on such factors as: social status, economy, environment, and politics.

Such studies reveal valuable information to archaeologists, anthropologists, and pathologists as well as general medical historians. Thus, plastination and tissue preservation will, in the future, be a significant tool in historical studies.

Pathology and Anatomy museums are again becoming popular. Once confined mainly to teaching hospitals and special institutions, they are now being created in many hospitals and universities. This is due mainly to decentralization of certain aspects of undergraduate and postgraduate medical education, and the increasing involvement of non-teaching hospitals in these activities.

A well organized pathology museum should serve many functions, thus tissue fixation before plastination is of the utmost importance.

Fixation is required to prevent putrefaction and autolysis, and to preserve and harden to a lifelike state.

Fixation agents are often chemical.

### **Functions of Fixing Agents:**

- (1) To set organs or parts of organs so that microanatomical arrangement of tissue elements will not be altered. ( 2 ) To set intracellular inclusion bodies so that the histocytologic and cytologic conditions of cells may be studied.
- (3) To arrest autolysis, putrefaction, and other changes.
- (4) To bring out differences in refractive index of tissues.
- ( 5 ) To render cell constituents insoluble and make them resistant to subsequent processes.

### **Fixation Agents Must:**

- (1) not shrink or swell tissue
- (2) not distort or dissolve tissue parts
- (3) render enzymes inactive
- (4) kill bacteria, molds, and viruses
- (5) modify tissue constituents so that they retain their form when subjected to dehydrants, clearing agents, and embedding media.

Usually, specimens removed at an operation or at necropsy have already been placed in a formalin or saline solution before being sent to the plastination lab. This fixative is most obvious.

Fixatives used today in most museums are based on a formalin fixative technique, and have been derived from Kaiserling (1897). Although there are some modifications to this technique, these tend to be minor and are usually prompted by problems in obtaining certain materials.

The method recommended by Kaiserling was based upon initial fixation in a formalin based fixative which contained salts to give an approximate neutral pH to the solution. This solution contains 10% formalin, potassium acetate, and potassium nitrate. Specimens should be placed in an adequately oversized container with 3-4 times their volume of fixative. Most times one fixation solution will suffice, but larger specimens may require solutions to be changed once or twice. The period for which the specimen should remain in the solution depends on its size, from three days for a small specimen, to 14 days for larger specimens.

Due to the hardening action of formalin, the way in which a specimen will ultimately be presented depends upon maintaining its natural shape during fixation. It is, therefore, important that a specimen does not rest on the bottom of a container thus producing an artificially flat surface or causing unfixed areas.

Cut hollow organs should be padded with cotton, but if uncut, they can be pressure inflated. Fixative can be injected with a hypodermic syringe and the injection pressure required is usually obvious. It is important to avoid over-inflation when dealing with elastic organs such as lungs. A recycling pump with pre-set pressure is recommended.

Solid organs may sometimes be perfused through main arteries. If this is unsatisfactory, the organ should be cut into slices on the proper plane so the organs can fix properly. Spot injection of fixative should be avoided. Specimens should be cut with a sharp, flat blade, knife (at least 30 cm) in one even cutting stroke. This avoids serration and distorted viewing surfaces.

Because of the way in which fixatives affect different types of tissues in the same organ, distortion is a frequent problem. Specimens should be pinned, suspended, inflated, or padded to produce the best results.

Other fixatives encountered before plastination include:

#### **Zenker's fluid**

Mercuric chloride, 5g  
Potassium dichromate, 2.5g  
Sodium sulphate, 1g  
Distilled water, 100 ml  
Glacial acetic acid, 5 ml

This fixative is a good routine fixative giving fairly rapid and even penetration. Following use, tissues must be thoroughly rinsed with water. Small pieces less than 3 mm are fixed in 2-3 hours.

#### **Bouin's fluid**

Picric Acid - saturated Aqueous solution, 75 ml  
Formalin (40% formaldehyde), 25 ml  
Glacial acetic acid, 5 ml

This fixative penetrates rapidly and gives excellent nuclear fixation with preservation of glycogen. It causes considerable shrinkage and destroys most cytoplasmic elements. This shrinkage can be reduced by fixation @ 0°C for 18 hours. Small pieces fix in 15 minutes.

#### **Gough Sections:**

Whole organs may be sectioned on paper by the methods of Gough and Wentworth. These sections provide valuable information on whole organ structure and serve as links between mounted museum specimens and histologic sections.

- 1) Distend organs - fix 2 days - 3/4" slice. Cut and place in water to remove formalin.
- 2) Specimen in 60°C Heated solution to extract air.
- 3) Cast in gelatin solution for microtome cutting, cool sections overnight to harden.
- 4) Specimen placed on Whatman paper and covered with Perspex paper.

### **Oil of Wintergreen/Dawsons Method:**

This staining method is often used for staining sections of embryos, fetuses, and small animals.

- 1) Eviscerate through small midline abdominal incision. Fix in 95% alcohol for 2+ weeks.
- 2) Rinse in tap water and placed in 1% KCO (Potassium Carbonate) for 4+ weeks.
- 3) Clear specimen in 1% KOH (Potassium Hydroxide) for 10 + days.
- 4) Wash in tap water 12 Hours.
- 5) Stain 30-60 minutes in 0-1% Alzirin red to which 6-10 drops of 1% KOH is added.
- 6) Decolorize soft parts in 20 percent glycerin and 1% KOH. This step takes about 2 weeks.

Specimen becomes entirely transparent showing ossified skeleton stained a deep red. This fluid is an excellent defatting agent.

As formalin is the most common fixative, it is likely that much of the tissue material to be used will be so fixed.

Preservation of tissues by freeze drying is often discussed as a method of fixation, but it is, correctly, an alternative to fixation. This method preserves tissue with little alteration in cell structure or chemical composition which permits one to skip stages of dehydration by alcohols and clearing.

The technique of freeze drying consists of two stages:

- 1) Initial rapid freezing (Quenching) 2
- ) Drying of frozen tissue

### **Quenching:**

Cool isopentane to -160°C to -180°C with liquid nitrogen then plunge small pieces of tissue into solution.

It is essential that tissues be absolutely fresh so that rapid freezing inhibits autolysis and prevents diffusion of substances within the cells.

### **Drying:**

Frozen tissue is then placed in a drying apparatus where high vacuum is established and the ice in the tissue is transformed to vapor. Higher temperature is maintained for the faster drying. For example: Raising temp to -60°C to -40°C increases evaporation rate tenfold.

### **Freeze Substitution:**

In 1941, Simpson described a freeze substitution technique as an inexpensive alternative to freeze drying. In general, the results are not always as good, but this method can be employed in a routine laboratory without purchase of expensive equipment.

After cooling in isopentane, these tissues are allowed to reach room temperature slowly and then processed. In 1961, Balfour used freeze substitution as a method of preparing tissue sections for fluorescent antibody staining.

### **Decalcification:**

The presence of calcium salts in tissue prevents good fixation. After decalcification, the natural color of the specimen is lost; it is, therefore, necessary to restore the specimen to as near its natural color as possible. There are many ways this can be done, and the one I recommend is the second stage of Kaiserling's method. This involves removing the specimen from the fixative, washing in running water, and transferring to 95% alcohol. The specimen is placed in alcohol for 1/2 to 12 hours during which time it is watched carefully for color to develop throughout the specimen. If not already done, it is at this stage that the specimen is photographed. When color restoration is satisfactory, the specimen is ready for the plastination process.

Multiple concentrations of formalin fixative can be used. One such method is Klotz solution according to Rodriques (1973) which preserves the natural color.

Briefly, after fixing the specimen in Koltz I for 5-10 days, the specimen is then transferred to Klotz

II where it can remain for an indefinite period of time.

**Kloutz I :**

Sodium Chloride 90g; Sodium Bicarbonate, 50g; Chloral Hydrate, 400g; Formaldehyde, 37%, 300 ml, and Distilled H<sub>2</sub>O, 10,000 ml.

Tissue should be thoroughly (12 hours) washed in tap water before transferal to Klotz II.

**Kioto II Solution:**

Sodium Chloride, 90g; Sodium Bicarbonate 50g; Chloral Hydrate, 200g; Formaldehyde 37%, 100 ml; and Distilled Water, 100,000 ml.

Reduced formaldehyde concentrations are often used with delicate tissues such as subarachnoid spaces. Cerebrospinal fluid should be removed with a syringe before injecting these areas with formalin. Fixation time is 20-24 hours.

Injection of brain tissue via basilar arteries should be accomplished with 100% formalin. After perfusion, the arteries should be ligated. If the brain is only submersed, this should be done with 5% concentration.

Organs to be injected with contrast media should also be worked before fixation. Fixation also includes defatting of tissues. Defatting of specimens can be accomplished with 70% ethanol for three days and then dehydrated in increasing concentrations of ethanol at room temperature. After defatting for 2 days in ethanol, specimens are placed in methylene chloride for three (3) days.

**Microwave Fixation:**

The history of microwave fixation is brief. In my opinion, enterprising and imaginative pathologists will devise new uses for microwave fixation in labs due to the low costs of purchase and short procedure time. These units can be moved close to patients which is good particularly in small hospitals.

New fixation techniques involving microwaves are used in research and have created new possibilities and handling of brain tissue, and

embryos for research.

In short, we are at the beginning and not the end of microwave fixation.

In summary, the choice of fixative will be governed by the type of investigation or specimen required, both immediately and in the future. Large pieces of tissue should be fixed in a tolerant fixative, such as formyl saline, which allows subsequent treatment. Smaller pieces can be removed from the mass and given special treatment if required. It should be remembered that museum specimens to which color is to be restored, should be ideally placed in alcohol or formalin for fixation.

Rarely will one fixative be suitable for a variety of methods.

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