FIXATION OF TISSUE FOR PLASTINATION: GENERAL PRINCIPLES

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INTRODUCTION

As you examine a plastinated specimen there is nothing to remind you of fixative. It is not wet, it has no odor and it may even have a beautiful color. Nevertheless, fixation is the first of four main steps in the process of plastination. Regardless of specimen or polymer, these steps are the same:

1. fixation
2. dehydration
3. forced impregnation
4. curing

The last three of these are logically a part of the plastination process. But why bother about fixation anyway? Three reasons dictate that we do so:

1. None of us would survive ice-cold acetone baths and being impregnated under vacuum; nor would we like very much being cured. But some enzymes manage to stay intact throughout all of this and remain biologically active. Since this could cause a slight putrefactive odor, we fix tissue to denature these enzymes and avoid this unpleasantness.
2. Chemical incorporation of the fixative into the molecular structure of its tissue makes a specimen firm and better able to resist shrinkage during subsequent steps.
3. Except for fresh joint specimens, very little has been done in plastination without fixation. Therefore, we have almost no experience with omitting or replacing this step.

METHODS AND TECHNIQUES

Now that you know the reasons for fixation you are probably curious about how to do it.

First of all, I would like to make it clear that this paper will report the Heidelberg way of fixation. If you are an experienced fixer and would like to use your own methods then, by all means,
stick to the routines with which you are familiar -- with perhaps one exception. I strongly suggest that you avoid any glycols, glycerides or whatever other glys you may be using because these materials interfere with the curing of silicone rubber.

The fixative most commonly used in plastination is the well-known formalin. As you know, formalin is defined as a saturated solution of the gas, formaldehyde in water, the concentration of formaldehyde amounting to approximately 35 to 38%. We use fixative solutions in the range of 1 to 20% formalin in water, usually 5%. This means that we simply dilute the commercial solution of formalin with tap water. A 5% solution of formalin, for example, would be prepared by mixing 5 parts of formalin with 95 parts of water (by volume).

In order to penetrate all cells of the specimen as fast as possible, five different procedures are in use:

1. IMMERSION: This is the easiest of them all; you just put the specimens into the fixative. A 5% solution of formalin is used most often. Occasionally we use a 1 or 2% solution but a concentration higher than 5% is not advisable. Immersion is especially suitable for thin specimens such as aortas. A lower temperature (+1 to +5 degrees Centigrade) will retard putrefaction and hardly affect the rate of penetration of the fixative. Furthermore,it favors color preservation (to be discussed later).

2. INJECTION: We use this term to denote the introduction of fixative fluid via vessels. In most cases the arteries are used, seldom the veins. A plastic cannula or piece of flexible tubing is inserted into the vessel and serves as its extension. Injection is especially suitable for whole bodies or extremities.

3. PERFUSION: This is defined as the continuous flushing of the vascular system of whole bodies, organs or extremities with fluid. Perfusion liquid is injected into a main artery and flows through the capillaries and veins. Perfusion with tap water is used to rinse blood out of the vessels. Tap water perfusion must always precede fixative perfusion or else the blood will clot in the vessels and block further perfusion. In plastination, tap water perfusion is very commonly used to remove blood from organs such as kidney, heart and liver.

4. INFILTRATION: In plastination, this is defined as "direct injection of fixative into tissue." The vessels are not used. Infiltration is employed for specimens that are too thick for immersion and cannot be injected. It is also handy for specimens whose vascular system has been damaged or injected with colored material.

5. DILATATION: In Heidelberg, this term means the distention of a hollow organ by applying fixative fluid under hydrostatic pressure. The advantage of dilatation is that the natural shape of the organ is maintained. The fixative diffuses through the wall of the organ, thus fixing it in its distended form. Dilatation can be used for fixation of hearts (via the chambers) , kidneys (via the pelvis) and lungs (via the airway).

COLOR PRESERVATION

In the third section of this article I will comment on the measures we can take to achieve color preservation. Color preservation has nothing to do with staining or color injection; it is concerned with the retention of natural tissue differentiation after fixation.

A plastinated specimen should have beautiful color. So, here comes the part where you, the fixer, have to be smarter than the fixative. If you leave your specimen in a formalin solution too long it will do its best to turn the tissue a sort of monotonous brownish-gray -- no preservation of color, no more natural tissue differentiation.

In Heidelberg, three means are used to counteract the fixative's efforts to do this:

1. FIX FOR AS SHORT A TIME AS POSSIBLE: This rule is always applied. An insect is fixed for a matter of hours while a very large specimen might be left for upto three weeks never any longer than is absolutely necessary. Fixation is finished when every cell of a specimen has been in contact with a sufficient concentration of formalin for only one hour. Strive to achieve this as soon as possible. As mentioned, fixation at lower temperatures (+5°C) favors color preservation, retards putrefaction and has very little effect on the penetration of fixative.

2. FREEZE FIXATION: This is the fixation and simultaneous dehydration of specimens (precooled to +5°C) in a mixture of ninety five parts (by volume) of acetone and five parts of formalin, carried out at -25°C. The formalin should be stabilized with 10% methanol, a preparation that is commercially available. Freeze fixation is particularly recommended for fish and fungus. The advantages of freeze fixation are threefold: 1) provided fixation time is not more that two weeks, color preservation is excellent, 2) because the specimen is frozen while being fixed, maintenance of its shape is A-one, 3) water removal starts immediately, therefore dehydration time is shortened. The disadvantage of freeze fixation is that acetone used in this way cannot be easily reclaimed by distillation because the formaldehyde precipitates as paraformaldehyde in the condenser. This can be dissolved, however, by subsequent distillation of methylene chloride.

3. KAISERLING FIXATION: In this method, Kaiserling fluid is used as the fixative. It is particularly recommended for whole bodies which are to be cut into slices and plastinated with Biodur S 10, Biodur PEM 27 or even Biodur E 12. The composition of Kaiserling fluid is: 3 00 gm potassium acetate, 150 gm potassium nitrate, 200 ml formalin and 800 ml demineralized water. For one body, you will need about 20 liters of this mixture.

FIXATION AFTER COLOR INJECTION

Suppose you have a large specimen, such as an extremity or a cow's heart and you want to fix it properly. The best way would be to use the arteries and fix by injection. But what if you have just filled these vessels with a colored polymer to acheive optimum optical results? Don't worry, several other ways are open to you.

With some specimens, like heart and kidney, other paths for the fixative can be found now that you have blocked up the arteries with colored stuff. In heart, the chambers can be used and the organ fixed by dilatation. In kidney, the pelvis could be employed in the same way. If this is not possible, as in the case of an extremity, then you will have to find another way to fix fast enough to prevent putrefaction. Here are three tips:

1) Start the fixation with a very low concentration of formalin, something like a 1 or 2% solution. This low-concentration fixative will penetrate faster because a diffusion barrier of thoroughly fixed tissue is less likely to form in the outer layers of the tissue. Since this low concentration is not sufficient to prevent putrefaction, the formalin content of the fluid will have to be raised in one or two steps to 5%. This can be done either by adding more formalin to the bath or by transfering the specimen to a new solution.

2) Fix at low temperature. Since putrefaction is strongly inhibited by low temperature and the penetration of formalin hardly affected, it is highly recommended that fixation be done at refrigerator temperature (+1 to +5°C).

3) Infiltrate bulky specimens that cannot be injected or sliced. Do not depend upon simple fixative penetration when the thickness to be penetrated is greater than 2 to 3 mm.

PROCEDURES TO BE USED FOR EACH TYPE OF SPECIMEN

Well, dear reader, now that we have struggled our way through the theory, I am glad to be able to direct your attention to something of more practical value. Let us look first at factors that determine the concentration of fixative one should use for any given purpose. Four such factors should be considered:

1. KIND OF FIXATIVE: This factor is easy to control since we almost always use formalin solutions.

2. FIXATION PROCEDURE: It is, for example, not wise to use a 2 0% formalin solution for the immersion procedure because this high concentration would form a "coat" of dense, thoroughly fixed tissue at the outermost layers of the specimen and prevent penetration to its interior. On the other hand, you would be missing an opportunity for very fast fixation if you did not use this concentration for the dilatation procedure.

3. KIND OF SPECIMEN: Fixative concentration should be matched to the physical characteristics of the specimen. Most important, perhaps is the thickness to be penetrated and the availability of injectable blood vessels.

4. DESIRED PROPERTIES OF THE FINISHED SPECIMEN: An example of this is the fact that an aorta fixed using the step wise raising of formalin concentration will be much more flexible than one that is simply immersed in a 5% solution.

As you see, this is pretty complex. If you are just starting, you will need some experience, some intuition, some common sense and maybe even a plastination conference now and again to achieve the best results. At this point I suggest that you peruse Table 1 for a summary of fixation procedures to be used for each type of specimen.

SAFETY

Now we have come to the last section of this paper, a very important one. Let's start with the heaviest stuff.

Formaldehyde, as you know, is a very controversial material. The question of its carcinogenic potential has not yet been answered with authority, however the National Institute of Occupational Safety and Health (NIOSH) recommends that "formaldehyde be handled as a potential occupational carcinogen and that appropriate controls be used to reduce worker exposure."

Other adverse health effects associated with formaldehyde, (depending on concentration) are: burning of the eyes, irritation of the upper respiratory tract, tightening of the chest, palpitation of the heart and even pneumonia. Because of the strong, pungent odor of formaldehyde you probably will never be exposed to concentrations high enough to cause acute damage. Your nose and your eyes will be your best friends here and will warn you long before the concentration gets very high. A very good rule-of-thumb about formaldehyde is that, when you can smell it, the concentration is over the limit established by NIOSH.

The allergic dermatitis caused by formaldehyde is well-known to preparators. So beware of contact with the skin.

Of course, you had better not drink formalin because this will cause necrosis of the gastrointestinal tract, even at concentrations as low as 1%.

From my own experience, I can tell you that it is not pleasant if even a tiny drip is allowed to get in your eye. It hurts!!

Knowing all this, it seems not unwise to avoid intimate contact with formalin. NIOSH recommends in "Guidelines for Minimizing Employee Exposure:"

1. PRODUCT SUBSTITUTION: Relative to this, Dr Becker and Dr von Hagens have developed a new, aldehyde-free fixative for embalming and the fixation of large specimens. Publications will follow soon.

2. CONTAMINATION CONTROL: In the case of formaldehyde, this means that good ventilation is necessary.

3. EMPLOYEE ISOLATION: This does not sound cozy at all. Fortunately, it cannot be achieved unless all the work is done by automatic equipment -- and we are not that far advanced yet.

4. PERSONAL PROTECTIVE EQUIPMENT: In Heidelberg this consists of rubber gloves, plastic aprons and goggles or gas masks. (Those of you who attended the Third International Conference on Plastination in San Antonio will certainly recall the slide in which three young ladies modeled these fashionable accessories, and nothing else. The editor was adamant that we omit this illustration, however it would have served to show that even fixation can be fun.)

TABLE 1: SUMMARY OF FIXATION PROCEDURES BY TISSUE

Tongue:	rinse with tap water overnight; infiltrate with 5-2 0% formalin to restore natural shape (approx 60 ml) ; immerse in 5% formalin until fixed (check periodically to assure preservation of shape)
Lung:	(with blood removed) intubate trachea; rinse with tap water overnight via bronchi; dilate via bronchi with 5% formalin under hydrostatic pressure; immerse in 5% formalin until fixed
Lung:	(with blood) intubate trachea; dilate with embalming fluid under hydrostatic pressure; immerse in embalming fluid until fixed
Heart:	install perfusion tubes; place cork stoppers and secure ligatures; rinse via left atrium with tap water overnight; inject coronary vessels with Biodur E 20 red and blue; dilate under hydrostatic pressure with 20% formalin; immerse in 2 0 % formalin until fixed
Aorta:	rinse (partly cut open) with tap water overnight; fix flat between sheets of filter paper in gradually increasing concentrations of formalin at 5 degrees Centigrade (first day in 1%, second day in 2%, third day in 5%)
Spleen:	if possible, cannulate vein and save capsule; inject via vein or infiltrate via hilus with embalming fluid; immerse until fixed in embalming fluid
Liver:	(to be fixed for slicing) cannulate hepatic artery; inject with embalming fluid; immerse in embalming fluid until fixed
Stomach:	carefully fill with water, then empty (to clean mucosal surface); rinse with tap water overnight; immerse in 2 0% formalin + 5% calcium chloride until fixed (fill stomach with same solution)
Intestine:	flush mucosal surface with water hose; rinse with tap water overnight; flush mucosal surface with 5-10% formalin while immersing in 5% formalin until fixed

Kidney:	cannulate arteries, veins and ureter (save capsule); rinse with tap water (via arteries) overnight; inject major blood vessels with Biodur E 20 red and blue; dilate pelvis and calyceal spaces (via ureter) with 20% formalin under hydrostatic pressure; after dilatation, immerse in 5% formalin until fixed
Uterus:	rinse with tap water overnight; infiltrate with 5-20% formalin (approx 20 ml) to establish natural shape; immerse in 5% formalin until fixed (periodically check for preservation of shape)
Placenta:	cannulate major blood vessels; rinse with tap water overnight (via the vein); inject umbilical vessels with Biodur E 20 red and blue; immerse in 5% formalin (start with shallow depth to keep umbilical cord from being fixed in a floating position)
Testes:	rinse in tap water overnight; infiltrate with 2 0% formalin; immerse in 5% formalin until fixed
Whole Corpse:	(for plastination of thick slices with Biodur S 10) cannulate femoral artery; rinse the body with shower of tap water; inject with Kaiserling fixative under hydrostatic pressure; eventually transfer to bath of Kaiserling fixative while still injecting
Whole Corpse:	(for plastination of thin slices with Biodur E 12) cannulate femoral artery; rinse the body with shower of tap water; inject with Kaiserling fixative under hydrostatic pressure; slice and immerse slices in 5% formalin until fixed