Journal of the

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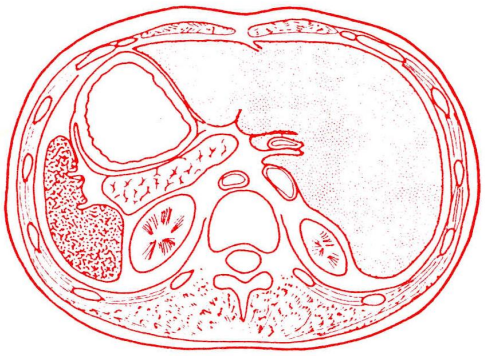

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JOURNAL OF THE INTERNATIONAL SOCIETY FOR PLASTINATION

Volume 1 Number 1 January, 1987

It's always exciting to establish something new. This is the first issue of the first volume of the Journal of the International Society for Plastination, an idea come of time.

Since we have no editorial board (yet), I have elected to adopt a rather flexible set of criteria for our articles. Responses registered on the membership questionnaires indicate that most of you want this journal to emphasize practical information, equipment suggestions and a how-to-do-it approach to technique. In keeping with that principle, the very first article in this issue will cover the very first step in the plastination process, fixation.

Those of you who attended the Third International Conference on Plastination in San Antonio will remember that Karine Oostrom not only told us about fixation but showed us that scientific reporting need not be overly formal to be effective. In preparing her paper for publication I have attempted to retain as much as possible of her charming style.

The next two papers, by Drs Lischka and Prihoda again contain practical, useful information. I was particularly impressed by their description of the institute and its teaching program.

The following paper by Andreas Ghur and collaborators reports a plastination technique that has potential for application in both diagnosis and research. It should attract a wide readership. Unfortunately, our limited budget has precluded publication of the many, excellent half-tone illustrations that accompany this article.

Finally, a paper by the editor and other members of Mercer's pathology department, consisting of a somewhat lengthy discussion of the S 10 Standard Technique. In this article, we have tried to answer the questions most asked us by individuals attempting to start plastination.

We will publish one issue of this journal in January and one in July of 1987. Those of you who are now members will remain in good standing for all of 1987 and will receive both issues.

A simple list of members is also included in this issue. By the time the next one is ready we should have the questionnaire broken down into more detailed information and be able to include more than just names and addresses.

This is a very precarious time in the development of this organization. Like any neonate, we have a higher risk of failure than those with even a little bit of history.

Our needs at the time of this writing are formidable. For example, we need:

1. more members. Dues collected to date will just about cover the printing of stationery and the publication and mailing of two issues of the journal.

2. more manuscripts. Members and others are invited to submit manuscripts of good, practical articles on plastination and related subjects. Diagrams are welcome but halftone illustrations may prove too expensive for our limited budget.

3. sponsorship and advertising copy from industry. If you know of someone who would like to reach many plastinators with little effort, please recommend that they advertise their products or services in the journal.

4. suggestions for enhancing our potential for survival and improving our services.

5. active participation by all members.

Most of you who returned the questionnaires indicated that general meetings should be held every two years. In keeping with this preference, I have scheduled the next (4th) International Conference on Plastination for March 21 through March 25, 1988. Since we have no planning committee (as yet), we will hold this first meeting here in Macon. Succeeding biennial meetings should be held at other locations, preferably coordinated by a host institution at which plastination is being performed. In keeping with our international orientation, the next one ought to be held outside the United States. Anyone like to volunteer?

Please mark your calendars!!!!

4th International Conference on Plastination
Monday, March 21 through Friday, March 25, 1988
Macon, Georgia, USA

This time is unusually good for a meeting in Macon since we will be right in the middle of the Cherry Blossom Festival. I have reserved a block of rooms at the Hilton which is downtown, in the very center of festival activity -- amidst our 70,000 cherry trees, all of which (with nature's cooperation) will be in bloom. Truly spectacular! I am including a brochure on the festival with the mailing of this issue. Other mailings will follow.

Thank you for your cooperation.

Harmon Bickley
Editor (interim) and
Executive Director (pro tem)

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

ESTABLISHING AND OPERATING A PLASTINATION LABORATORY AT THE INSTITUTE OF ANATOMY, UNIVERSITY OF VIENNA

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THE INSTITUTE OF ANATOMY

The Institute of Anatomy, University of Vienna is a renowned European institution. Its history is associated with many important names in the field of Anatomy such as Joseph Hyrtl, who not only elevated the study of human and comparative anatomy to a new standard of excellence but also was very active as a teacher and founder of our specimen museum.

Another outstanding anatomist in the history of the institute was Julius Tandler, author of a widely accepted four-volume textbook. Dr Tandler was also a famous politician. In addition to his contribution as a teacher and scientist, he was responsible for establishing an exemplary system of social welfare in Vienna in the early decades of this century. It is largely through his efforts that the institute became so well-accepted as a part of the community of Vienna.

Because Vienna was the capital of the Habsburg Monarchy, the Medical Faculty of the University of Vienna has always had to contend with large numbers of students. This was a problem in the days of Theodor Billroth and has persisted even to the present, but for other reasons.

Therefore, we are a large institution, consisting of three chairs in the area of research and teaching and a smaller unit that oversees the operation of the museum. This smaller unit also runs the plastination laboratory and is involved in other projects in medical education.

•The staff of the institute comprises three full professors, four positions equivalent to associate professor and approximately 20 • -full-time assistants, all holding medical degrees. In addition, a number of part-time appointments for medical doctors and 45 *I*<such positions for medical student tutors are available. We also < have a technical staff for research and administration, as well ^ as some 12 persons who work in the area of body conservation, | body storage and support of dissection courses.

All this is necessary to cope with classes numbering ^approximately 1200 students per year. Such a large student population requires a yearly intake of about 250 whole bodies, ^obtained through a well-functioning donation system, and ^approximately the same number received as autopsied corpses.

This sizeable effort enables us to provide our students with an intensive learning experience in topographical anatomy. Dissection courses are organized into groups of six students for each body and last for a total of 165 hours. As far as we know this is quite a good standard for anatomy courses offered within German-speaking countries.

Bodies for routine dissection are preserved in the conventional way by infusion via the femoral artery with a mixture of phenol and formaldehyde in deionized water. Final concentrations of both components are less than 1%. We emphasize the importance of using the gravity method and allowing the embalming fluid to find its own way, even when the embalming time is overly long. For routine storage, the bodies are placed on trays and maintained by an automatic system that sprinkles them several times a day with a phenol-free disinfectant fluid. The minimum storage time is six months. On completion of dissection, the remains are buried in a cenotaph, generously provided by the city of Vienna.

As can be seen from these figures, our routine duties have to be quite elaborate to provide an adequate learning experience for such a large student population. Also, the very magnitude of our effort demands considerable expenditure of funds and time. Since much of our instructional material is so short-lived, it would seem that plastination would have been eagerly welcomed when it became available about a decade ago. But, as one might imagine, such a novel and complicated technique did not win easy acceptance, particularly since its establishment required an additional investment of resources (Lischka et al, 1984 ; Lischka et al, 1984)

HISTORY OF PLASTINATION AT THE INSTITUTE OF ANATOMY

We began plastination using nothing more than the equipment available in the workroom of the museum. We had a small vacuum chamber and a pump, formerly used for production of transparent specimens according to Spalteholz. Also on hand were some refrigerators and a dehydration set-up that employed a graded series of ethanol solutions. There was (and still is) one MD-assistant affiliated with the plastination unit, but only for a limited time during a portion of his appointment. In addition, we have one technical staff assistant who attends to such routine duties as dehydration but again, only on a part-time basis.

Essential for starting our plastination efforts was sending our professional assistant to a plastination workshop in Heidelberg. Also extremely helpful was a visit to Vienna by Dr Gunther von Hagens, inventor of the process. It was on this occasion that the term "plastination" was conceived one evening in a Viennese tavern.

Because of the limited dimensions of our first vacuum chamber, we were restricted to small specimens. We still have a number of the products of this chamber, plastinated with polymer formulations no longer in use. Unfortunately some of the best specimens of this period were stolen from a collection exhibited at an anatomical congress.

PRESENT-DAY EFFORTS IN PLASTINATION

From the very beginning it was apparent that we needed a rather strict policy to govern the production and use of plastinated specimens. Not only was such a policy necessary for the most efficient use of our limited resources, moreover it fit well with other administrative procedures at our strongly tradition-minded institution. The major elements of this policy were (and still are) :

1. We will use only a limited number of the wide variety of plastination techniques available. Currently, we work with only S 10 and PEM 27.

2. Plastinated specimens must be complementary to more traditional specimens in their instructional use. Their production and employment will conform to that practiced at other quality medical schools.

3. The cost-effectiveness of plastination must be monitored and compared to that of other means of enhancing our ability to provide teaching specimens, such as enlarging our facilities.

As a result of this policy, we are providing only two categories of specimens, both of which were neglected before the introduction of plastination:

1. Slices of whole body, head, neck and extremities

2. Ligamentous preparations of joints

Production of slices fits very well with recent trends in medical diagnostic imaging, such as computer tomography and nuclear magnetic resonance. Also, it is relatively easy to produce good quality slices without elaborate preparation, whereas colleagues are reluctant to provide intricately dissected topographical specimens of a quality that would warrant plastination.

Specimens of joints with intact ligaments are required in large number for our introductory course which takes place in a room within the museum. Previously, only dried bones could be used because of the inadequacy of air circulation in this area. When compared to the cost of installing ventilating equipment, the use of plastinated specimens has proven impressively cost-effective.

THE S 10 STANDARD TECHNIQUE AS PRACTICED IN OUR LABORATORY

We have adopted the following variation of the S 10 Standard Technique for routine use in our production of silicone-impregnated specimens:

1. Fixation is accomplished in the usual manner using a formalin solution.

2. Dehydration is now done exclusively by freeze substitution.

3. Forced impregnation is performed in a vacuum chamber constructed to fit exactly into an existing deep-freeze cabinet. This chamber measures 1/2 meter in depth and contains approximately 120 liters of polymer reaction mixture, thus allowing impregnation of body slices, but not extremities. The access opening is at the front side, a feature we would not recommend. For the first few days of impregnation, we insert a liquid nitrogen trap between our pump and vacuum chamber.

4. Curing is done by the "fast cure" method, which we find alleviates most of the shrinkage problems associated with slow curing.

5. Unlike other laboratories, we do a lot of surface grinding. We now use an industrial machine which permits wet grinding of silicone and epoxy slices up to 30x70 cm.

Our main difficulties center around the staffing problems that result from temporary workers and lack of skilled technical assistance.

PLANNED MODIFICATIONS OF OUR LABORATORY

Although our present arrangement is safe, our first modification will be to provide explosion-proof equipment for compressed air and lighting. We also will have to provide better ventilation for areas where solvents are handled and stored. An innovation we have in mind is to construct a huge shower compartment in which the wet grinding and cleaning of specimens and equipment can take place.

The staffing problem can only be solved by hiring at least one skilled technician having permanent, full-time affiliation with the plastination unit. Only a totally involved, highly trained person can assure the continued output of quality specimens and the safe handling of solvents and chemicals.

We have attempted to present a brief overview of plastination at Vienna. In summary, our experience has convinced us that plastination is the best means of producing long-lasting specimens of high quality.

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PLASTINATION OF WHOLE-BODY SLICES WITH POLYMERIZING EMULSION

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INTRODUCTION

The availability of plastination has permitted us to introduce sectional anatomy at the Institute of Anatomy of the University of Vienna. Sectional anatomy is a valuable approach to the acquisition of an understanding of body structure. Before our use of plastinated slices, it had been neglected for many years.

Sectional anatomy provides a three-dimensional concept of the relationship among body structures. Up until now, this has been achieved mostly through the use of handbooks and atlases. Also, sectioned specimens can be studied like a puzzle, disassembled and put back together again. This enables the students to trace the course of nerves and bloodvessels through the head, neck, trunk and extremities.

Our experience would also suggest that sectional anatomy is a valuable adjunct to recent advances in diagnostic imaging techniques such as computerized axial tomography, nuclear magnetic resonance and ultrasound. It is very valuable, for example, to have a CAT scan of a plastinated specimen and the specimen itself available for comparison.

SPECIFICATIONS OF AN EFFECTIVE TEACHING SECTION

The full benefit of sectional anatomy can be realized only if the sections conform to certain specifications. We recommend that the following criteria be observed:

1. The specimen should be resistant to the mechanical damage that inevitably will result from passing through many hands.
2. The surface should be dry.
3. Health risk should be minimal.
4. Maintenance and storage should be easy.
5. The specimen should be natural in appearance with vivid surface detail.
6. The specimen should be odorless. ;
7. Production should not be too complicated.
8. The production process should be affordable.

Although methods are available to display cross-sectional specimens in flat chambers, they seldom match all of the requirements listed above. Conventionally preserved specimens are not completely acceptable because they are wet, smell rather bad, emit formaldehyde vapor and require elaborate air circulation and ventilation. We have found plastination with PEM 27 (an epoxy-based emulsion) or S 10 (silicone rubber) to be an elegant and satisfactory means of meeting the desired criteria.

SELECTION OF A BODY FOR SECTIONING

The process of plastination of whole-body slices actually starts with the proper choice of corpses. Our criteria are as follows:

1. The shortest possible postmortem interval is desirable. Normally, a corpse will arrive at our institute 2 to 10 days after death. Because of the autolytic degeneration of delicate tissues, we usually select only those bodies arriving between two and five days postmortem.

2. Although the general state of the circulatory system is more important than age, the deceased should not be too old. Considering the superannuated population we deal with, an age of 50-60 years is looked upon as desirable.

3. The body should be of average stature and weight. This may sound trivial but a trend to obesity can be seen in our population and we must take care to assure that the specimen will fit our equipment.

4. The body should be free of any deformity that would affect the morphology of the sections.

We have found that only 1 to 2 percent (2-5 corpses) per year are suitable for sectioning. Interestingly, among those selected, there prove to be more men than women. This is inconsistent with the composition of the donor population itself which consists of more women than men. In addition to whole bodies selected as above, we also plastinate the skulls of autopsied cadavers with the brain left in situ.

PREPARATION OF THE BODY FOR SECTIONING

Selected bodies or body parts are now embalmed. Generally, whole corpses are injected with 15-20 liters of Kaiserling-I solution, using an irrigator system with approximately 160 cm water column. Kaiserling-I solution consists of 200 ml formalin, 15 gm potassium nitrate, 30 gm potassium acetate and 1000 ml deionized water.

According to our experience, this solution enhances color retention while still providing adequate fixation. After injection, corpses are turned on the ventral surface for overnight storage. Body parts are infused only via their main artery (Lischka et al, 1981)

Embalmed cadavers are now transferred to a tank for long-term storage. In contrast to those stored for dissection, bodies to be sectioned are kept floating in Kaiserling-I solution. Storage time, at present, is usually a minimum of three months. Since we are producing sections 1 to 4 cm thick, prolonged storage does not seem to have an adverse effect. By remaining afloat throughout the entire period of storage, the shape of the corpse does not suffer from deformation. Furthermore, the embalming fluid is not pressed out of the weight-bearing parts, as would happen if they were stored on trays. Corpses are processed in order of their sequence of acquisition.

FREEZING AND SLICING

Before a cadaver is frozen, sectioning lines are drawn. Sections of the head (cross, coronal or saggital) are marked at 1 to 2 cm. Cross-sections of the trunk and limbs are marked at 4 cm. The body is then transferred to a deep-freezer where it remains at-25C for several days.

Sections are cut with an ordinary carpentry band saw, using a blade with 6 teeth per cm. Since we are cutting thick sections, lateral deviation of the blade is minimal. Notches that do occur are ground smooth after curing. Cutting of the skull is complicated by the enormous hardness of the petrous bone and teeth (particularly if dental restorations are present).

As sections are produced, they are immediately submerged (at room temperature) in plastic containers filled with 80% ethanol. Distance is maintained between sections as they thaw by the interspersing of plastic grating sheets. Since ethanol is comparatively cheap, it is changed at least once.

Once thawed, sections are carefully cleaned of any defilement and sawdust. Loose .pieces of intestine are sutured and pinned in place. Using compressed air, vessels and other cavities are cleaned of all detritus.

DEHYDRATION AND DECREASING

The sections are now dehydrated by freeze substitution using acetone of a grade between 96 and 100% purity. This is carried out by precooling the ethanol-saturated sections to 5°C and immersing them in three changes of 10X their volume of acetone at -25"C. Specimens remain in each bath for two weeks. Freeze substitution offers several advantages when compared to other methods of dehydration:

1. The time required is shorter.

2. Specimen shrinkage is reduced.

3. Amount of dehydrating solutions used and discarded is less than that with a graded series of ethanol.

The first and second bath can be reused as long as water concentration does not exceed 5%. Acetone used for the final bath must be 99-100% pure. Because of the differing densities of water, grease and acetone a gradient develops in the dehydrating baths. Water and grease are found at the bottom, in that order, while the top layer consists of fairly pure acetone. Therefore:

1. Specimens should not be placed directly on the bottom.

2. It is important to stir the solution daily.

3. Sections should not be in contact with one another. Adequate interspace should be maintained with plastic grating sheets.

4. Before measuring the water concentration, it is always necessary to stir the bath.

There should always be enough acetone in the dehydrating vessels so that the section closest to the surface does not become dry. Transfer of sections from one bath to the next should be done quickly and gloves should always be worn to prevent skin injury. It is also advisable to wear a gas mask. Dehydration is complete when the water concentration stabilizes at 1% or less.

Since the degreasing capacity of -25°C acetone is not very high, all specimens with osseous structures or considerable fatty tissue are subjected to a separate degreasing procedure. Thorough degreasing enhances the optical quality of finished sections, particularly when plastinated with silicone rubber. Also, storage in the degreasing medium is permissible for a much longer time than in acetone, time that can provide flexibility for one's impregnation schedule.

To accomplish degreasing, we immerse the sections in methylene chloride at room temperature. After two weeks the bath is changed. Sections can remain in this medium for months but must not be left in acetone for this length of time. Long exposure to acetone causes hardening of the surface and interferes with polymer impregnation resulting in poor specimen quality (reduced flexibility).

FORCED IMPREGNATION AND CURING

Both Biodur S 10 and Biodur PEM 27 are used for plastination of body sections. Our procedure for S 10 is described in another paper published in this journal. In this article we will describe only the procedure for PEM 27 (epoxy-based polymerizing emulsion) . We use S 10 (silicone rubber) on specimens of the following types:

1. brain tissue, isolated or in situ
2. specimens to be used by students for self-instruction.

PEM 27 is our choice for all specimens requiring good visual appearance with clear surface detail. PEM 27 specimens should not be used in circumstances where they would be handled by a number of people since they are far more fragile than those impregnated with silicone rubber.

Forced impregnation is carried out according to recommendations in the Heidelberg Plastination Folder - 1985, a publication obtained from Dr Gunther von Hagens of the Institute of Anatomy, University of Heidelberg. A reaction mixture of PEM 27 and E 6 (hardener) is used. Impregnation is started on Monday. Vacuum is applied gradually, maintaining a slow boiling rate. It is then maintained until Thursday when the sections are removed and new sections started.

Impregnated specimens are wiped free of excess resin and placed in an oven at 40C where they remain for 4-5 weeks. Curing is complete when the specimens are thoroughly dry.

GRINDING AND LABELING

Cured specimens are stored until a large enough number has accumulated for grinding. Sections are polished on a wet grinder until cut surfaces are smooth. By grinding the surfaces in this manner it is possible to demonstrate specific structures such as the median atlanto-axial joint, showing the transverse atlantic ligament. Grinding is not *a* popular step because it is accompanied by an impressive amount of noise and dirt. Polymerized resin on the lateral and inner surfaces is removed with the help of drilling devices. So far, six totally sectioned bodies have been prepared in this way.

A permanent, etching type of drawing ink is used for labeling the specimens. Solvent in the ink etches the surface of the plastic and the ink adheres to the etched surface. The area to be marked is prepared with clean ethanol or acetone and wiped dry.

Proper storage and access to specimens is dependent on a complete inventory system. We record the following information on index cards in a file:

1. the index number
2. an anatomical classification
3. the date of forced impregnation
4. the resin used
5. the praeparator
6. location of the storage site and/or the user's name

Specific anatomic structures demonstrable on the specimen should be noted. Sections are usually stacked in cardboard boxes, prominently marked with the index numbers of the specimens it contains.

In the future we hope to acquire a full-time technician and increase the efficiency of our laboratory. When this is accomplished we intend to introduce sheet plastination and plastination of brain tissue with P 35.

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Anat Anz 150:226-234, 1981

COMPLETE EXAMINATION OF MASTECTOMY SPECIMENS USING SHEET PLASTINATION WITH EPOXY RESIN

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INTRODUCTION

In Europe, the malignancy exhibiting highest incidence in the female is breast cancer. When one lesion is found, chances are between 13.4 and 41.6 percent that another growth exists in the same organ (1) (2) . Because of this high rate of multicentric occurrence, it is desirable to have a method that permits examination of each mastectomy specimen in its entirety.

Total-specimen examination has become even more important in recent years, given the emphasis now being placed on conservative surgical treatment such as subcutaneous mastectomy and quadrant-part resection. Specimens obtained from procedures such as these require complete histologic examination so that surgeon and patient alike may be assured of complete tumor resection.

Plastination, a method of impregnating tissue with curable polymer, was developed by one of the authors (GvH) and is now widely used for the preparation of teaching specimens (3) (4) (5). A variation of this technique (sheet plastination with clear epoxy) is ideally suited to the preparation of mastectomy specimens (total, subcutaneous or quadrant-part) for comprehensive macroscopic and microscopic inspection. In fact, it is the only method presently available that permits complete histologic scrutiny of such large specimens on a routine basis.

MATERIALS AND METHODS (EPOXY IMPREGNATION)

The first five steps in the process lead to a sliced specimen completely impregnated with uncured clear epoxy. We will list these in sequence and discuss surface preparation and curing as a separate section.

1. Fixation: The intact specimen is placed in 5% buffered formalin for one or two days until fixed hard enough to be cut by a slicing machine.

2. Slicing: The specimen is now cut into 2.5 mm slices using a commercial meat slicer (see Appendix A) . Each slice should be identified by writing the case and slice numbers on blotting paper which is then stapled to the tissue. Slices may be radiographed at this stage if a comparative image is desired. We recommend a Faxitron table machine, using 45 sec exposure at 20 KeV.

3. Staining: The slices are placed in a hemalum staining bath (Appendix B) until they acquire enough color for macroscopic viewing. This usually takes two or three minutes when the bath consists of one part hemalum and three parts distilled water. If the stain is diluted further with distilled water, the slices may remain in the bath up to several hours.

4. Dehydration: The stained tissue is now dehydrated by progressive exposure to increasing concentrations of ethanol. We use 30%,50%,70%,90% and two or three changes of 100%. It is then immersed in a sequence of three baths of 100% acetone and one of 100% methylene chloride. The slices must remain in each bath for a minimum of two hours. This step accomplishes not only dehydration, but also degreasing and saturation with a volatile intermediary solvent (methylene chloride) that will propel the impregnation occuring in the next step.

5. Impregnation: Tissue slices are now placed in a reaction mixture of the epoxy polymer (Appendix C) contained in a vacuum chamber. Pressure within this chamber is reduced at a very gradual rate, permitting the methylene chloride to vaporize at a gentle boil (indicated by slow bubbling at the surface). It should take about four hours to reach full vacuum, after which the tissue is allowed to remain for 12 additional hours at this low pressure. Air is then readmitted to the chamber over a one-hour period. The slices are now completely impregnated with uncured epoxy.

Dehydration of the tissue slices causes some shrinkage and results in a distorted surface. For proper visual inspection, the surface of each slice must now be rendered smooth.

1. Compression: On a large glass plate (45x45 cm) lay a piece of thick plastic foil the same size. This foil must be heavier than ordinary mylar wrap so that it will not tear on removal. On top of this foil spread a layer of the polymer reaction mixture used in Step 5 (above). Now press the tissue slices firmly into the polymer, so as to remove trapped air bubbles. Pour more of the polymer over the tissue and cover them with a second sheet of plastic foil, similarly coated with a layer of polymer. Again, make sure that no air bubbles are trapped between the tissue and this second foil. A second glass plate is now placed on top of the second sheet of foil and the entire assembly is pressed together with strong clamps.

2. Draining: While still clamped, this multilayered "sandwich" is now placed over a pan to permit draining of excess polymer. Care must be taken to drain only the excess, not material impregnating the slices. We have achieved good results by placing the sandwich at a slight angle (about 10 degrees). At this angle we find that the impregnating resin remains in the tissue and only the excess is removed. Using a greater angle causes polymer to drain out of the tissue, resulting in surface irregularity around the surgical margins. Draining should be continued for about 2 days.

3. Curing: The fully drained sandwich,still firmly clamped, is now placed in an oven, previously heated to 50°C. After 24 to 48 hours at this temperature the epoxy will be fully cured. The sandwich may now be opened and the tissue slices removed.

RESULTS

Without magnification it usually possible to clearly distinguish skin, fat, gland, connective tissue blood vessels, tumor, cysts and lymph nodes. Thus, a preliminary screening can be accomplished using only the naked eye.

Magnification (up to 100X) permits detailed inspection in three dimensions by simply picking a field and adjusting the microscope through a sequence of focal planes. In this manner, a ductal carcinoma can be followed, in continuity, over several slices, from its origin at a terminal duct to its surface manifestation at the nipple.

Hemalum stains only the nucleus of the cell, hence only nuclei can be observed. This permits preliminary evaluation of at least two important diagnostic criteria of malignancy, nuclear pleomorphism and mitosis. If closer inspection is required, thinner sections may be cut from the questionable area of the slice and restained with hematoxylin and eosin for routine histopathological evaluation.

DISCUSSION

Until the development of plastination, two methods for screening breast slices had been reported: radiographic examination and saturation with wintergreen oil. The radiographic technique was described by Egan (6) and further refined by Lagios (7). Marcum (8) and Wellings (9,10) advocated immersion in wintergreen oil.

We have compared radiographic imaging with conventional pathologic examination for the screening of sliced subcutaneous mastectomy specimens (11) and have found that the former is three times more liable to detect residual cancer.

The plastination method herein described is judged to be even more sensitive than radiography, particularly when the specimen contains an abundance of dense fibrous connective tissue. Also, it must be considered that this method permits total, three-dimensional, visual inspection of the surgical margins of a specimen, in continuity. Insofar as we know, such versatility is unique to plastination.

Using the wintergreen oil technique, it is necessary to prepare 100 to 500 thin slices in polyethylene bags (9) . This is much more time-consuming than plastination (which requires only 10-15 slices) and limits the amount of magnification that can be employed. Also, this method involves serious problems regarding storage and the objectionable odor of the clearing medium.

SUMMARY

A method is described for the complete macroscopic and microscopic examination of mastectomy specimens. The entire specimen is cut into 2.5 mm slices, stained and plastinated with clear epoxy. This renders each slice so transparent that it can be examined both grossly and at a magnification of 100X or greater. Thinner sections (4-10 microns) may be cut from a plastinated slice if an area requires even closer inspection. The entire process, including macroscopic and microscopic screening takes approximately seven days. Thus, the method is quite practical for use as a diagnostic regimen.

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APPENDIX

A. SLICING MACHINE

We use and recommend a Bizerba slicing machine. This instrument consistently leaves a smooth surface on each slice, a matter of importance for close inspection. The machine is available from: Bizerba, D-7460 Balingen, West Germany.

B. HEMALUM STAINING BATH (MAYER)

Hematoxylin (Merk #4305) ----------	1 gram
distilled water ----------	1 liter
Sodium lodate (Merk #6525) ----------	0.2 gram
Potassium aluminum sulfate (Merk #1047) — (after 24 hours)	50 grams
Chloral hydrate (Merk #2425) ----------	50 grams
Citric acid (Merk #242) ----------	1 gram

C. POLYMER REACTION MIXTURE*

Biodur E12 Epoxy Resin —	1 kilogram
Biodur El Hardener -----	300 grams

* Biodur products obtainable from:

Biodur
Jahnstrasse 8 D-
6900 Heidelberg
West Germany

PRESERVATION OF TISSUE BY SILICONE RUBBER IMPREGNATION

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INTRODUCTION

In recent years a process called plastination has been introduced that permits excellent preservation of biological material by impregnation with a variety of curable polymers (1). In effect, tissue water (and some lipid) is removed and replaced with plastic. Although this process is protected by several patents (U.S. patents 4205059, 4244992, 4278701 and others), the production of specimens for education or museum display is not restricted, providing written authorization is obtained in advance (2) and such specimens are not sold or traded for profit.

Uses of plastination in anatomic pathology and forensic science include at least the following:

1. Preservation of autopsy or surgical tissue samples in a form useful for teaching
2. Long-term storage of autopsy or surgical tissue samples for later histologic examination
3. Preparation of unusual or historically important material for museum display
4. Preparation of tissue samples for use as evidence
5. Preservation of whole organisms, such as parasites, insects, snakes or plants, for instructional use
6. Preparation of surgically removed facial organs (nose and ear) for use as their own prosthetic replacement (3)
7. Serial sectioning of whole organisms, organs or extremities for detailed examination

PRINCIPLES OF PLASTINATION

Plastination has been performed with a number of different polymers, the most common being epoxy, silicone rubber and polyester. The polymer that has enjoyed widest acceptance in the preparation of specimens for teaching is silicone rubber.

Regardless of the polymer used, the process involves four basic steps: (1) fixation, (2) dehydration (freeze substitution with acetone is recommended), (3) forced impregnation, and (4) curing. Other treatment such as vessel injection is often used for enhancement of specific detail.

Forced impregnation (Step 3) is the key to the entire process and is the one part protected by patent. It is carried out by placing the acetone-saturated specimen (from Step 2) in a reaction mixture of special polymer and slowly reducing the ambient pressure. This causes the acetone to boil out of the specimen, creating a difference in pressure between its interior and the surrounding fluid plastic. Under the influence of this gradient, polymer is drawn into the specimen, filling all intracellular and interstitial space occupied by water in the natural state.

Given the unusual requirements of the process, particularly the need for a polymer of low viscosity that affords a long-enough working time, only the specially formulated plastics developed by the Biodur Company of Heidelberg, West Germany (4) will give uniformly .satisfactory results. In fact, these are the only polymers that can be used in compliance with patent restrictions.

We will first discuss each of the above four steps in some detail, particularly as they apply to the Standard Technique for impregnation with Biodur S 10 silicone rubber. Further information regarding the S 10 Standard Technique is available as a leaflet from Biodur. Information presented in this paper will revise and update that presented in an earlier publication (5).

FIXATION

Biological material must be fixed before plastination to prevent putrefaction and stop the action of other enzymes. Plastination of freeze-dried material has been attempted but such specimens tend to develop an offensive odor. Thus, fixation is presently considered an essential step in the process.

Proper fixation of an organ or tissue sample is very important to the final quality of a plastinated specimen. The usual fixatives such as 5-20% formalin may be used; however exposure must be kept to an absolute minimum if natural color is to be preserved. Care must be taken to avoid fixative fluids containing glycol since this will interfere with the later curing of the S 10 silicone. Color-conserving fixatives, such as Kaiserling's fluid (6) are recommended where appropriate. Color retention is also enhanced by maintaining the fixative bath at +5°C.

Best of all for color preservation, is the practice of freeze fixation. This is defined as the fixation and simultaneous dehydration of specimens in -25°c acetone (7). It is carried out by mixing 5 parts (by volume) of methanol-stabilized formalin (available commercially) with 95 parts of acetone, chilling the mixture to -25°C and using it to fix specimens which have been precooled to +5°C. Fixation time should not exceed two weeks. Maintenance of color, size and shape is excellent and the specimen becomes partially dehydrated as well. In fact, the only drawback to freeze fixation is that the acetone used cannot be easily reclaimed since formaldehyde will precipitate as paraformaldehyde in the condenser of the distillation apparatus. Even so, this can be dissolved by occasional distillation of methylene chloride.

For the most natural-appearing product one should start with fresh tissue; however, if a specimen has been rendered colorless by long exposure to formalin it can still be successfully plastinated and some color restored by staining. In working with fresh or frozen tissue, however, overexposure to fixative fluid must be scrupulously avoided if retention of natural color is important. Assuming reasonable ease of penetration, no more than 48 hours at room temperature should be necessary. If longer exposure is required, the specimen should be sliced to a more easily penetrated thickness or infiltrated with the fixative via a fine needle.

Because fixation imparts some rigidity, the specimen should be fixed in the form that it will exhibit when finished. Lungs, for example, should be fixed by inflation with fixative under hydrostatic pressure introduced through the airway. Smaller solid organs, such as the tongue, should be infiltrated with fixative in addition to being immersed in the same solution. Fixation of the heart should be done by introducing fixative under pressure through one of the great vessels while the organ is suspended in a fixative bath. Bulky organs such as liver should be sliced to an easily penetrated thickness and the slices kept flat by pressing onto a porous surface such as a fine screen or filter paper supported by *a* grid.

DEHYDRATION

Once fixed, a specimen must be thoroughly dehydrated before impregnation. Serial exposure to solutions of gradually increasing ethanol concentration is a familiar and convenient method, however it often results in an intolerable amount of shrinkage.

Far preferable is dehydration by freeze substitution with acetone. In this technique, the specimen is first precooled to +5°C in the fixative bath, removed, blotted and transferred to a container of acetone at -20 to -30°C. Precooling discourages the formation of ice crystals and is particularly important in the dehydration of delicate structures.

The volume of acetone used must be at least 10 times that of the specimen to be dehydrated. Three aliquots of this 10X volume of anhydrous acetone are employed. Specimens the size of a heart or larger should be left in the first for three weeks, in the second for two weeks and in the third for one week. The final aliquot is monitored with a hydrometer to assure that its concentration of water does not exceed 1%. If a higher concentration is detected, a fourth change is used.

Freeze substitution with acetone takes a shorter time than ethanol dehydration and results in a specimen with less dimensional change. In addition, the tissue emerges completely saturated with a solvent that is appropriate for use in the next step. If ethanol dehydration is used, an additional step is required in which the final saturation of absolute alcohol is replaced with a suitable intermediary solvent such as acetone or methylene chloride. Anhydrous acetone will enhance dehydration but methylene chloride is immiscible with water.

FORCED IMPREGNATION

Forced impregnation is carried out by transferring the acetone-saturated specimen to a reaction mixture of polymer (S 10 base material containing 1% S 3 Hardener) and placing the entire assembly under vacuum at freezer temperature. As explained, this causes the acetone to boil out of the specimen, creating a pressure gradient which draws the reaction mixture in.

Vacuum is applied slowly, as determined by the rate at which the mixture bubbles. Rapid boiling must not be permitted because the pressure within the specimen will become too low and it may be compressed or crushed by the force of the inrushing polymer. Also, rapid boiling is liable to result in incomplete impregnation.

Pressure can be be regulated by adjustment of a shutoff valve in the line between the pump and chamber or, better, by a bypass valve that admits air to the chamber at a controlled rate. This permits stabilization of the pressure at any level, a capability that becomes important in a polymer-attenuation technique to be described later.

The use of acetone as a volatile intermediary solvent is highly recommended because it boils readily under vacuum (even at low temperature) and is a good solvent for most uncured resin materials. In some laboratories, dichloromethane (methylene chloride) is used for this purpose because it is more volatile than acetone, non-inflammable and mixes even more readily with uncured resin. Although the use of acetone usually requires a longer impregnation time than dichloromethane (three weeks as compared to two) , it is now used more widely than the latter because it is less toxic in vapor form and, with minimal precaution, constitutes no more of an explosion or fire hazard. Many plastinators prefer dichloromethane, however, and have learned to avoid its irritant properties while taking advantage of its superior qualities as a solvent.

Another feature of acetone that would encourage its use as both a dehydrating agent and intermediary solvent is that it can be relatively easily recycled by distillation, thereby reducing solvent waste, environmental contamination and expense.

An additional point to be made regarding reduction of expense is that in carrying out impregnation at -20 to -30°C, the same polymer reaction mixture can be used indefinitely. Following impregnation, the volume of polymer absorbed is restored by simply adding a freshly mixed aliquot. Temperature this low inhibits the slow polymerization (end-to-end joining) of molecules within the reaction mixture that occurs at room temperature, and prevents the viscosity of the mixture from increasing to a degree that renders it unusable.

CURING

The curing of Biodur S 10 consists of two separate events: polymerization and crosslinking. The process is quite complicated and terminology in general use can be confusing if certain definitions are not established at the outset:

1. PRECURE:
   At room temperature (or more rapidly in a 50 °C oven) S 10 polymer molecules, react with molecules of S 3 Hardener and join end-to-end. This elongation of its constituent molecules renders the reaction mixture first viscous, then sticky. End-to-end joining (polymerization) imparts toughness and flexibility—both desirable qualities of a finished specimen – but will not result in hardness. If it is allowed to happen as the FIRST step after impregnation it is termed the "precure" stage of the whole curing process.

2. GAS CURE:
   The complete curing process consists of both polymerization (as described above) and crosslinking. Crosslinking of its constituent molecules causes S 1 to become firm and hard, also a desirable quality (if not exaggerated). It is accomplished by exposure to a weak acid vapor that acts as a crosslinking agent. This curing vapor is released from a preparation called Biodur Gas Cure 86. Exposure to S6 and the resulting crosslinking is called simply "gas cure."

3. AFTERCURE:
   As S6 gas contacts the specimen it hardens the polymer at the surface and sets up a barrier that slows its own further diffusion. Also, formation of this cured surface "crust" stops continued leakage of uncured polymer from the interior of the specimen and prevents shrinkage.

In effect, it seals the specimen and creates a hardened superficial layer in which the concentration of curing gas is quite high. If the specimen is now placed in a plastic bag, 86 gas within the surface layer will diffuse slowly toward the center and complete the curing. This phase is called "aftercure."

Two variations of the curing process are presently employed, the difference being determined by whether a significant precure phase is used. These two variations are called fast cure and slow cure. In the former, a specimen impregnated with S 10 reaction mixture is wiped free of excess polymer and placed directly into curing vapor in a sealed chamber (kept very dry by the inclusion of open containers of calcium chloride). In the latter, the specimen is allowed to precure on the bench top for a length of time (up to 4 weeks) and then placed in a 50°C oven for an additional interval (again, up to 4 weeks), following which it is placed in curing vapor. Advantages and disadvantages of both methods are summarized in Table 1.

In the fast cure method, the surface is cured first and the interior of the specimen hardens during aftercure. The most conspicuous advantage of this method is that shrinkage is held to an absolute minimum. This is extremely important when one starts with fresh tissue and uses short fixation time to conserve color.

Specimens prepared by the fast cure method have proven somewhat less tough and flexible than those that are slow cured. Also, control of the moisture within the curing chamber is very critical since an excess concentration of water vapor will result in the appearance of white spots on the finished specimen. Despite its need for close attention to detail, however, the fast cure method is recommended when starting with fresh tissue, particularly lipid-rich tissues such as brain and extremity sections because it serves to limit the characteristically severe shrinkage of this kind of material that will result from the oozing out of interstitial polymer during precure.

In the slow cure method, the specimen is allowed a rather long interval for end-to-end polymerization (precure), during which almost all excess polymer drains out of its internal space. Exposure to curing vapor then hardens the remaining polymer thoroughly, resulting in a dry, flexible, resilient specimen. The principal objection is the time required before the specimen becomes useful and its potential for shrinkage. This method is particularly adaptable to bone specimens and those that have been fixed for a long time and will not undergo further contraction.

As experience is gained, most plastinators come to use some variation of the fast cure method because of the shorter time that it takes to produce a useful specimen and because it discourages shrinkage and permits the use of a color-preserving regimen of fixation.

VARIATIONS OF THE STANDARD TECHNIQUE BASED ON POLYMER ATTENUATION

In the S 10 Standard Technique described above, forced impregnation is carried out using a reaction mixture consisting of S 10 silicone base material mixed with 1% S 3 hardener. Undiluted reaction mixture flows into the specimen and fills all available space. For the purpose of distinguishing it from a method to be explained below, this is designated complete polymer impregnation" with S 10 silicone.

If the reaction mixture, rather than used uncut, is diluted with a solvent that remains essentially nonvolatile during impregnation, but is evaporated after curing, the result is somewhat different. Under these circumstances , a sponge-like microstructure is formed in which the polymer permeates the solid components of the tissue but does not completely fill its interstitial spaces. Because the ultimate result is a specimen only partly suffused with polymer this technique is termed "incomplete polymer impregnation" with S 10 silicone.

Incomplete polymer impregnation with S 10 silicone is particularly useful for thin-walled, flexible specimens such as gut or lung because it permits retention of flexibility and simulation of a natural surface. A segment of intestine, for example, when incompletely impregnated with S 10 silicone, will be quite flexible and will exhibit a villous substructure at its mucosal surface that can be appreciated by examination with a hand lens. Lung tissue incompletely impregnated with S 10 silicone remains palpably spongy, while areas of consolidation can be detected by their comparative induration.

Incomplete polymer impregnation is carried out by diluting the standard S 10 reaction mixture with xylene before using it in Step 3 (forced impregnation). The recommended solution consists of the following (in parts by weight):

S 10 Silicone Base Material: 100
S 3 Hardener: 1
xylene (reagent grade): 30

Forced impregnation is carried out in much the same manner as in the standard technique, except that the ambient pressure is not allowed to drop below 15 mm Hg, a pressure at which xylene will boil. The use of xylene as a diluent has the added advantage of lowering the viscosity of the reaction mixture and decreasing the time needed for impregnation to less than one week.

Following impregnation, the specimen is wiped clean and exposed to gas cure in a dry, sealed chamber for one or two days. It is then stored for at least one week in a plastic bag to permit aftercure. To reduce shrinkage to an absolute minimum, the polymer must be allowed to cure rather thoroughly before the xylene is permitted to evaporate.

HISTOLOGIC EXAMINATION OF S 10-IMPREGNATED SPECIMENS

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One of the most interesting and potentially useful qualities of tissue plastinated by the standard S 10 technique is that its microscopic structure remains intact (8). This, of course, means that it can be preserved almost indefinitely in a form that is easily stored while still retaining full potential for histologic examination.

Given the current state of the art, plastination could be used for routine preservation of tissue samples obtained through surgery and autopsy. Indeed, there is no doubt that it would prove more versatile than the paraffin and formalin storage now practiced.

The key to an appreciation of this facility is an understanding of "deplastination". Deplastination takes advantage of the unusual ability of the sodium ion to depolymerize silicone rubber. Tissue samples to be deplastinated are exposed to a saturated solution of sodium methylate in anhydrous methanol until free of poymerized silicone rubber and then subjected to standard histologic techniques. Although some staining procedures are slightly prolonged, the results are comparable to those obtained with more conventional methods.

USES AND ADVANTAGES OF PLASTINATED SPECIMENS IN TEACHING

As previously reported (9) , plastinated specimens have been introduced into the pathology teaching laboratories at the University of Texas Health Science Center at San Antonio, and compared with the same types of specimens preserved in formalin. The educational value of plastinated specimens was judged equal or superior to the latter, and their ease of handling was deemed greatly improved.

Also, plastinated specimens have been in use for some time at Mercer University School of Medicine where they have proven successful as an adjunct to a new, problem-based type of curriculum.

Further experience at both San Antonio and Mercer has shown that, given the availability of a collection of plastinated specimens, they will be employed in circumstances where the use of human tissue samples wound not have been considered. Such specimens are now used as an adjunct to lectures and seminar sessions, both within these institutions and with lay audiences ranging from Rotary Clubs to high school classes.

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Table 1. COMPARISON OF THE FAST AND SLOW CURE METHODS FOR S10

	FAST CURE	SLOW CURE
Duration of Process	Comparatively short. No precure, short gas cure, long aftercure (but specimen may be used during this phase)	Lengthy, long precure, shortgas cure, no aftercure Specimen cannot be used until end of process
Shrinkage of Specimen	Minimal, surface hardens immediately preventing further loss of polymer	may be severe as a result of polymer drainage during precure
Toughness of Finished Specimen	Acceptable but not as tough and flexible as slow-cured specimen	very tough and flexible
Sensitivity of Process to Moisture*	Highly sensitive, curing chamber must be kept very dry with calcium chloride	Relatively insensitive, no need to use calcium chloride
Need for Attention during Process	Polymer is expressed onto surface of specimen and free of polymer must be removed every hour or two during the first day of gas cure	specimen is wiped once per day during precure but only once or twice during gas cure

Newer techniques in which curing gas is bubbled out of solution with an aquarium pump have reduced moisture sensitivity of the fast cure method at this step. Details are available from Dr. von Hagens.

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