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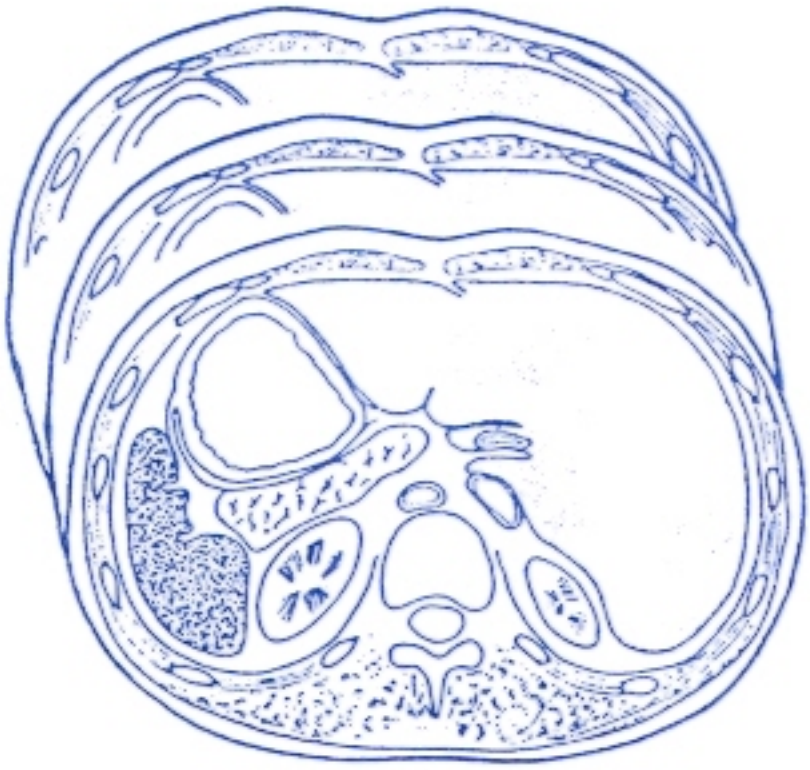

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A TECHNICAL NOTE FOR IMPROVEMENT OF THE E12 TECHNIQUE J.

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INTRODUCTION

The E12 plastination technique is used for impregnation of tissue slices with transparent epoxy resin (1). To obtain the most useful specimens, one must start with sections no thicker than 2-3 millimeters. Several methods have been suggested for the production of acceptable sections. These include the conventional sawing of specimens frozen at -20°C (2), the sawing of frozen specimens with additional ice laid against the stop (3) and the sawing of frozen specimens using specially constructed stops in which cooling is accomplished with a counter-current device (4).

The first two of these methods are not suitable for producing slices with a large surface area such as whole-body transverse sections. Neither are they satisfactory for producing multiple serial sections that must be cut in one session. The third may be used with large specimens and in serial sectioning but breakdown or other operational faults are likely to occur. The purpose of the present investigation was to develop a reliable and simple means of producing tissue sections appropriate for use in the E12 technique.

MATERIALS AND METHODS

A sectioning apparatus was developed by altering a conventional band-saw (Berkel 444, manufactured by Maschinenfabrik K.M. Reich, Niirtingen, Federal Republic of Germany). Modification of the sawing table and the stop were required.

SAWING TABLE: The table supplied with the saw was removed and replaced with one constructed from a plate of 4mm sheet aluminum. The replacement table measured 600 x 360 mm. Backward and forward motion was provided by four wheels running on appropriately aligned rails. Special care was taken to obtain a smooth, gliding action with the table running exactly parallel to the stop.

THE STOP: The stop was cut from 20 mm sheet aluminum and measures 800 x 200 mm. An oblong aluminum container (840 x 150 x 35 mm with a wall thickness of 2 mm) served as a receptacle for liquid nitrogen. Its seams were sealed with a durable, highly elastic, leak-proof glue. A 14 mm hole was bored at each end of the upper surface, one to allow the nitrogen to be introduced and the other acting as a vent. This container was fastened to the back of the stop with screws. The clamping mechanism which holds the stop in place was arranged in such a way as to allow expansion and contraction due to temperature change.

RESULTS AND PROCEDURE FOR USE

A picture of the modified saw is shown in Figure 1. A view of part of the stop is shown in Figure 2. Corresponding construction diagrams are provided in Figure 3.

The procedure recommended for using this saw is as follows:

1. The specimen from which slices are to be cut is frozen thoroughly at -70°C. The time required for this will, of course, vary with its size. A torso, for example, should be kept at -70° for one week.

2. The saw is fitted with an appropriate blade (e.g., a blade of type B or G from Fischmeister, Pansdorf, Federal Republic of Germany).

3. A millimeter scale, calipers and a small brush are placed close at hand. These will be used in setting the stop, monitoring the thickness of the slices and cleaning the slices.

4. Two acetone baths, prepared in advance, are made available. This acetone must have been kept in a freezing cabinet at -25°C for at least two days before the saw is used.

5. The stop (including funnel and supports) is cooled to -70°C for two hours before the saw is used.

6. Liquid nitrogen (20 liters) is made available.

The saw is ready for use when the stop has been aligned 2 mm from the blade and the container filled with liquid nitrogen (approx. 3 liters). The specimen is then cut into 2 mm slices. If there is any sign of thawing, more liquid nitrogen should be added.

After quickly checking its thickness with the calipers, each slice is transfered immediately to an intermediate -25°C acetone bath where it is carefully freed of ice fragments and sawdust with the brush. The cleaned sections are then stacked in a second acetone bath at -25°C, where dehydration can begin.

DISCUSSION

The difficulties involved in preparing sections to be plastinated with the El2 technique arise from the fact that heat generated by the friction of the saw blade tends to soften the tissue. And it is impossible to obtain clean, smooth sections unless the tissue being sawed is kept frozen. The only way to eliminate this difficulty is to employ some means of maintaining the specimen at a temperature below freezing while it is being sawed. The method described here is based on cooling the stop with liquid nitrogen. In addition, both the stop and the specimen are kept at a low temperature before sawing is begun. We have found that the combination of these two measures prevents tissue from thawing while being sawed.

Equipment needed for this procedure includes a deep-freeze capable of reaching -70°C and a modified band saw. Construction is uncomplicated and the cost of the materials modest (approx. US $200.00). The device, therefore, can be rated simple, reliable and inexpensive.

SUMMARY AND CONCLUSIONS

Tissue slices of 2-3 mm uniform thickness are needed for the E12 technique. When large specimens are sawed to these dimensions, friction generated by the saw blade tends to soften the tissue and render the sections unacceptable. This paper describes a means of modifying a commercially available band saw so that the tissue being sectioned is cooled with liquid nitrogen. Construction is relatively easy and inexpensive (US $200.00). We have found that using this instrument according to the procedure recommended results in completely acceptable sections.

ACKNOWLEDGEMENTS

Our grateful thanks are due to Mr H. Abt (†) for his excellent technical assistance and to Mrs J. Steel, MA, for translating the manuscript.

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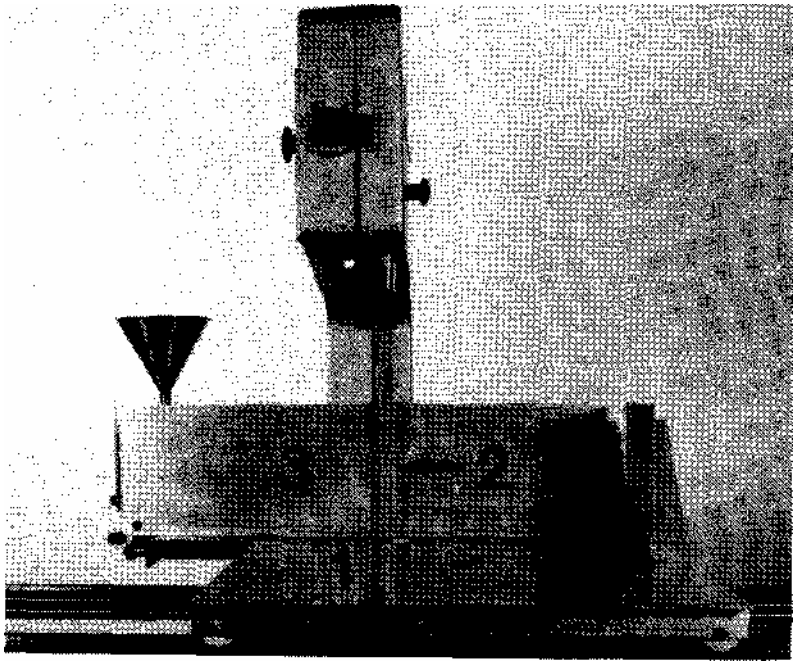

Fig. 1 A view of the complete saw. 1 table, 2 blade, 3 stop.

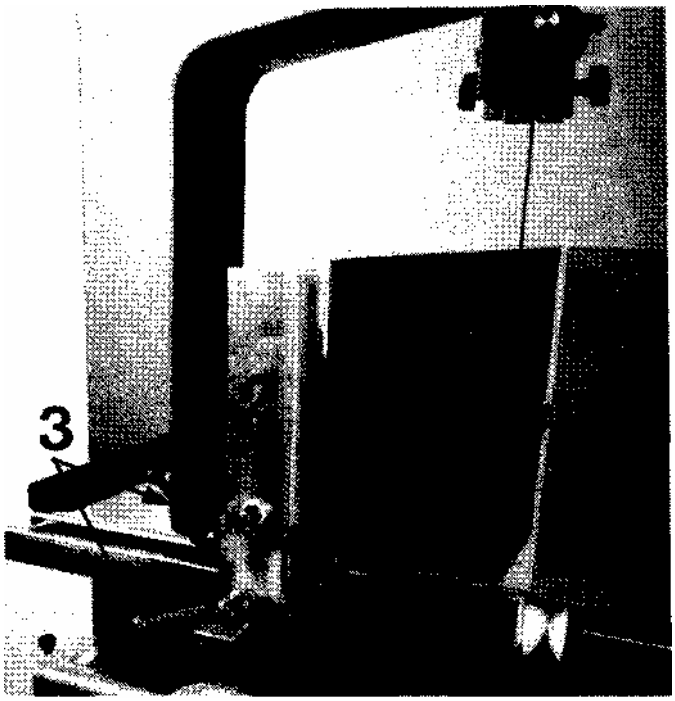

Figure 2.

The stop, as seen from above and to one side. 1 stop, 2 receptacle for liquid nitrogen, 3 setscrews.

Figure 3.

Side elevation of the stop. 1 blade, 2 stop, 3 receptacle for liquid nitrogen.

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FIXATION OF THE HUMAN BRAIN FOR PLASTINATION: SPECIAL CONSIDERATIONS

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INTRODUCTION

Two materials are used for the impregnation of brain tissue in plastination:

1. Silicone Rubber: for plastination of whole brains in the S-10 Standard Technique.

2. Epoxid Resin: for plastination of brain slices in the P-35 Technique.

To produce an acceptable specimen by either technique one must begin with proper fixation. In this paper, an improved method for removing and fixing the human brain will be presented. Also included is a review of the literature dealing with brain fixation.

Fixation methods described to date have not proven suitable for preparing the human brain for plastination. Fixing a brain in a manner satisfactory for plastination requires that the specimen exhibit a natural form after fixation and that all anatomic components be completely and uniformly fixed. There should be no distortion of natural shape and no border of fixative penetration observable at the surface of a section.

Beginning with methods described in the literature, the author has developed a procedure to fulfill these demands. This procedure has been used for four years and has yielded consistently acceptable results.

Although it was developed for plastination, it is completely applicable to the routine fixation of autopsy specimens. The procedure begins with fresh tissue. No fixation is performed in situ. The untreated brain is removed and the body turned over to the undertaker.

A human brain in the fresh state is unable to retain its shape without support, hence, once removed from the cranium, it must be transferred to a special, cranium-shaped vessel which will sustain it properly and determine its form during the steps to follow. We refer to this container as a "hedgehog mold." Use of a hedgehog mold permits immediate release of the body and allows subsequent steps to be performed slowly, thereby reducing the potential for specimen damage.

REMOVAL OF THE BRAIN

In removing the brain, the calvarium is exposed and the cap of the skull is cut away with the usual, circumferential incision. This incision is made with a circular saw, having a blade diameter of 5 cm. The blade is equipped with a plate that limits the depth of the cut to 0.4 cm. Since the thickness of the skull varies from one area to another, caution must be exercised. The depth-limiting plate cannot be relied on completely to protect

underlying structures. The depth of the cut must be regulated, by hand, to prevent damage to the brain.

Final removal of the cranial cap is accomplished with a cross-pledget. Again, great care must be exercised. This instrument is gripped lightly and used with minimum pressure to avoid penetration of the brain. At intervals of approximately 2 cm the bony cap is chiseled from its attachment and removed. The exposed dural surface may now be inspected for damage. With surgical forceps, the dura mater is then carefully lifted from the cerebral hemispheres and, using a pointed scissors, it is cut along the line of incision. The falx cerebri at the front of the brain is severed with slightly opened scissors and the dura is reflected, as far as possible, toward the middle of the brain. This is necessary to avoid rupturing a vessel.

The next step is to remove the brain. During this entire process, the occipital lobes must be supported with one hand while the other is used to free the brain from the cranial fossae. First, the olfactory nerves are gently detached by elevating them from the frontal base of the skull, using light pressure on the underside of the anterior brain. Next, the optic nerves, which are now under tension, can be cut. This is followed by cutting both carotid arteries, the tuberal hypophysis and the oculomotor nerves.

Next, both temporal lobes are freed from their cavities using blunt dissection with the fingers. The tentorium cerebelli should now be visible and both sides may be cut along the petrous portions of the temporal bone, using a long, pointed knife. The cut should be performed in a mediolateral direction. Once the tentorium has been cut, the cerebellum can be gently pushed back, away from the temporal lobes.

A long, thin knife is now used to cut around the spinal cord as it passes through the foramen magnum. The spinal cord is then transected as far as possible into the spinal canal. Supporting the cerebellum, the brain and remaining spinal cord are now gently lifted out of the cranium and placed in the hedgehog mold.

FIXATION OF THE HUMAN BRAIN: A BRIEF REVIEW OF THE LITERATURE

Table 1. provides a summary of the most important historical steps in the evolution of brain fixation techniques. Before the advent of formaldehyde in 1893, ethanol or an aqueous solution of zinc chloride or potassium chloride was used. Blum introduced formalin as a preservative of biological specimens. In his very first publication (1893) he recommended fixation of brain tissue by immersion in 10% formalin.

Two categories of opinion seem to prevail in current literature. Some authors recommend immersion alone as an adequate means of fixation; others favor injection in situ, followed by immersion. With regard to injection, some prefer to introduce fixative into the subarachnoid space; others favor using the carotid arteries. Subarachnoid injection has been performed via a lumbar, nasal or orbital approach or by drilling a hole into the cranial cavity. Carotid injection may be done within the skull, at the site where the internal carotid arteries enter the brain (Hasenjaeger and Spatz) or at the neck as they branch from the common carotid arteries (Boettger). All three authors emphasize the necessity of saline injection before formalin injection and of immersion of the brain in a formalin solution following injection.

Since 1931, it has been suggested by many authors that an isotonic solution of potassium chloride should be used in the preparation of formalin fixative. Schroeder claims that, if 0.9% potassium chloride is used, brain tissue will remain

dimensionally stable during fixation. Without potassium chloride, he found that swelling of about 15% is inevitable. Several other authors have recommend the use of isotonic sodium chloride for this same reason, in both immersion and injection.

Since its recommendation by Klinger in 1935, most laboratories immerse the human brain in 5% formalin for fixation. As advised in 1918 by Reichardt, many laboratories also suspend the brain by a basilar artery during immersion.

Brain fixation techniques in common practice today have three basic disadvantages:

1. None incorporate a means for support of the fresh brain. Fixation must be done outside the body, starting with fresh tissue. Although in-situ fixation is highly desirable, it is usually not possible in the routine autopsy because of time limitation.

2. The natural shape of the brain is often deformed during extracranial fixation.

3. No currently practiced method permits complete fixation of a fresh brain, once it has been removed from the skull.

In the remaining sections of this paper, specific recommendations for overcoming these problems will be presented.

RECOMMENDATIONS FOR BRAIN FIXATION

The first step after removal of a fresh brain is to transfer it to a hedgehog mold for establishment of a near-natural form. (Incidentally, a hedgehog is a small, brain-shaped animal, found in great numbers in Europe. The hedgehog mold is so named because its general outline bears a reasonable resemblance to this animal.) The hedgehog mold has a capacity of 1300 ml and weighs 290 grams. It is possible, therefore, to weigh the brain while it is contained in this vessel. At our institute, a brain remains within this mold during the clinical conference which is held 2 or 3 hours after autopsy. Immediately after the conference, fixation is begun.

A catheter is inserted into one vertebral artery until its tip has entered the basal artery. The second vertebral artery and both internal carotid arteries are clamped off and 150 ml of 100% formalin is gently injected. After a few hours, the brain is immersed, suspended by a basilar artery, in about 4 liters of 5% formalin, where it remains for one week.

USE OF 100% FORMALIN AS A PRE-IMMERSION INJECTION

The use of 100% formalin for pre-immersion injection was determined by an experiment, performed by the author. In summary, the following comparisons were made:

1. 12 brains were fixed by simple suspension in 5% formalin and 12 brains were injected with 100% formalin and then immersed in 5% formalin. Four brains from each group were cut after three hours and four after one week.

2. Sections were compared for adequacy of fixation and both groups were further compared to others that had previously been prepared by injection of various dilutions of formalin, followed by immersion.

Injection with 100% formalin prior to immersion resulted in a far superior specimen than either simple immersion or injection with diluted formalin followed by immersion. The use of isotonic saline or plasma expander solutions as a pre-

injection rinse did not offer any advantage nor did it improve the results.

Injection of 100% formalin led to complete fixation in one week, about half the time needed when the brain was fixed by immersion alone. The contrast between white and gray matter was improved and large histologic sections adhered much better to a slide.

PREPARATION OF THE FIXED BRAIN FOR DEHYDRATION

After an immersion time of one week, the dura is removed and the brain rinsed with tap water for about 30 minutes. At this stage, it is advisable to check for symmetry and for damage incurred while opening the skull. Acceptable specimens are then immersed in a freshly-prepared, 5% solution of formalin and kept at refrigerator temperature (+5°C) for 24 hours. This step is required to prevent ice crystal formation during freeze-substitution (dehydration). Basilar artery suspension is not necessary during this immersion.

SUMMARY OF THE PROCEDURE RECOMMENDED FOR FIXATION OF THE BRAIN

1. Open the skull, remove the brain and transfer it immediately to the hedgehog mold.
2. Prepare injection instruments and 100% formalin for injection.
3. Insert a cannula into the vertebral artery and clamp off the remaining vertebral artery and both internal carotid arteries.
4. Inject about 150 ml of 100% formalin into the brain.
5. Prepare about 3.5 to 5.0 liters of 5% formalin in a suitable container.
6. Suspend the injected brain, imme'fsed in the 5% formalin, by a basilar artery for one week.

TABLE 1. Evolution of

Brain Fixation Technique

YEAR	AUTHOR	CHEMICAL USED	TECHNIQUE
1887	Lenhossek	alcohol or aqueous sol Zinc Chloride	immersion
1890	Obersteiner	aqueous sol potassium bichromate	immersion
	Blum	formalin 10%	immersion
	Parker & Floyd	60 ppv 95% ethanol 40 ppv formalin	immersion on cotton
1898	Sainton and Kattwinkel	formalin 6%	orbital injection
	Reichardt	formalin 10%	suspended by artery
	Bergl	formalin 10%	lumbar injection
	Schroeder	formalin 10% in 0.7% NaCl	immersion
1934	Lagerloef & Torgersrud	buffered formalin in 1.75% aqueous NaCl	immersion
	Klinger	5% formalin then bleaching at +5°C (hydrogen peroxide)	immersion
1935		formalin 20%	nasal inject plus immersion
1937	Ostertag	warm NaCl sol then ?% formalin	carotid injection in situ
1937	Hasenjager & Spatz	formalin	skull hole injection
1938	Fahr	formalin 20% in 0.7% Carlsbad salt	nasal injection
1949	Ostertag	0.9% NaCl then formalin 20%	carotid injection
1951	Boettger		

A SILICONS-IMPREGNATED KNEE JOINT AS A NATURAL MODEL FOR ARTHROSCOPY

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INTRODUCTION

During conventional dissections of the knee joint, the boundaries and attachments of the synovial capsule remain indistinct. Specimens exhibiting a distended joint capsule (induced by either synovial effusion or injection) are particularly valuable for orthopedic or anatomic instruction because they enable the limits of this capsule to be more clearly demonstrated.

In the last decade, operative arthroscopy has become increasingly important (1)(2). The teaching of arthroscopy and arthroscopic surgery would benefit from the availability of specimens that would permit simulation of actual clinical procedures, particularly if such specimens were to incorporate a distended capsule. Knee joint specimens, plastinated with Biodur S10/S3 (3), make ideal, natural models of this kind. Used intact they permit rehearsal of arthroscopic procedures. Cut into halves, they reveal most anatomic details of the joint and suprapatellar bursa.

PLASTINATION OF A KNEE JOINT WITH DISTENDED CAPSULE

SPECIMEN SELECTION: Only knee joint specimens from fresh bodies should be used. A joint capsule from an embalmed cadaver will not expand sufficiently. The entire specimen should be approximately 25 cm in length. Saw cuts should be made 10 cm below and 15 cm above the femorotibial joint.

FIXATION: Fixation is begun with the injection of 20% formalin into the capsule, using a 12-15 gauge needle. When working without an arthroscope, the usual clinical approaches (anterolateral or anteromedial) are not recommended. The injection may over-infiltrate the infrapatellar fat pads (4) and, two months later, the joint may become filled with a meringue-like mass. Superomedial or superolateral injection (into the suprapatellar bursa) is less precarious. The joint capsule is filled, close to bursting, with 120-250 ml of fixative. During injection, the patella must lift off from the trochlear groove and the knee will assume approximately 20 degrees of flexion. When the injection is complete, the joint is immersed in 5% formalin for about one week. The needle and syringe are left in place to maintain pressure within the capsule. Application of a gauze binding will help preserve the natural shape of the severed muscles.

DEHYDRATION: Before dehydration, the specimen is rinsed with running water to remove most of the formalin. The dilated joint capsule is flushed several times, using the injection needle as a means of access. This same puncture site is used for all treatment of the joint capsule, including the final curing. Dehydration is

accomplished by freeze-substitution with acetone. The joint is blotted, the capsule filled with acetone (precooled to -25°C) and the joint immersed in a 5x volume bath of this same agent. Dehydration will require three weeks. The acetone is maintained at -25°C in a freezer and the joint capsule is rinsed occasionally. Two changes of the acetone bath are mandatory. The joint is then transferred to a room-temperature solvent bath for defatting. This will require at least three days in each of two baths of either acetone or methylene chloride.

FORCED IMPREGNATION: The specimen must now be impregnated with Biodur S10/S3 polymer mixture at -25°C. The first step is to remove the defatting solvent from the joint capsule and refill it with polymer. The entire specimen is then immersed in this same mixture. Vacuum impregnation is started at a negative pressure of 60 mm Hg during the first day and adjusted down to zero over a period of 12 days, at which time, impregnation should be complete.

CURING: Before curing, the joint capsule must be emptied of all excess polymer. This is accomplished by introducing compressed air via the injection needle. Within two hours, the fluid silicone polymer should be completely forced out through the puncture site and the porous walls of the capsule. The joint capsule must now be cured, starting from the inside. This is done by blowing gaseous Biodur S6 curing agent into the joint space. A gas washing bottle is assembled in such a way that compressed air is led into a chamber containing 100 ml of S6 solution. Incoming air percolates through this solution, vaporizing the S6 and carrying it into the capsule (Figure 1). Gas flow is permitted to continue for one day under a fume hood. The needle is then withdrawn and the the whole specimen cured for 4 days according to the usual S6 procedure.

The specimen is now ready. After drilling a hole for the anterolateral or anteromedial approach, it can be used for teaching. At this stage, we prefer to cut the specimen into sagittal halves with a band saw. This permits direct inspection of the joint cavity. Also, the specimen can be reassembled and held intact by a gauze stocking' for simulated arthroscopy. The optimal plane of section runs through the middle of the femur, patella and tibia, parallel to the ligamentum mucosum (Figure 2).

We have found that fat remaining in the marrow cavity will become rancid after two months. Therefore, we recommend that the cured specimen halves be defatted once again for six days in two baths of pure, room-temeprature acetone, reimpregnated with S10/S3 and re-cured in the usual manner.

DISCUSSION

The technique outlined in this paper for pressure distention of the capsule of the knee joint can be used for all joints of relevance to arthroscopy. Today, even small joints are inspected with the arthroscope (5). The elbow joint, for example, may be examined, almost in its entirety, by using a posterior radial approach. Even the posterolateral pouch of the ankle joint can be seen with a posterolateral approach. Similarly, bronchial trees, that can serve as natural models for bronchoscopy, can be obtained, by preparing lung/airway specimens in a comparable manner.

The actual operative effort required for completing a knee joint specimen as described here is not excessive, however, because of the sometimes-lengthy segments of time needed for soaking and impregnation, at least 10 weeks are required before the specimen can be used. This includes the second defatting and impregnation which is recommended because it precludes offensive odor and

renders the specimen less greasy to the touch.

The two most common pitfalls in the procedure are insufficient expansion of the capsule or, at the other extreme, rupture of the capsule (usually into the gastrocnemius or suprapatellar bursa). According to clinicians, it takes between 60 and 100 ml to fill the cavity (4). Some authors advocate the use of an infusion bottle, hung just below the ceiling, rather than syringe pressure, for distention (2).

During subcutaneous dissection, piercing of the fasciae must be avoided. All-attempts to cut away muscles also will endanger the integrity of the capsule and may permit its communication with numerous synovial bursae about the knee. With radiography, especially CAT scans, both expansion of the capsule and the plane of the final sagittal section can be monitored.

Although a knee specimen, as prepared here, is very close to natural, it cannot provide all of the desirable characteristics of a fresh joint. Normal flexion and extension is not possible because the fully cured silicone rubber is not sufficiently supple. Application of varus and valgus stress (to expose the posterior meniscus) also is not feasible. Despite these limitations however, actual comparison has shown that a well-expanded, plaStinated knee exhibits more than 80% of the details that can be studied arthroscopically in_the patient. Also, plastinated structures look enough like their fresh counterparts to generate familiarity with their natural appearance. Many objections can be overcome by having a second joint, plastinated in a more flexed position, to complement the first one.

On the other hand, a specimen of this type provides certain unique advantages. For example, when the patella stands 3mm away from its articular surface on the femur (as it does in these preparations), the suprapatellar bursa and the cartilagenous face of the patella both can be inspected through an insertion hole aimed primarily at the cruciate ligaments and the anterior horns of the menisci (anterolateral approach).

For training of the beginner in arthroscopy, the ability to study the cut surface of the joint is an important asset. In fact, it has been our experience that every orthopedist who was given the opportunity to examine a distended, plastinated knee joint wanted to obtain one for his own use.

SUMMARY

After distention of the joint capsule by formalin injection, a knee joint can be plastinated and used as a natural model for arthroscopy. Maintaining a distended capsule during the curing of the impregnation polymer provides a well-defined and permanently expanded joint cavity. The whole process, including a second defatting and impregnation (required for a completely acceptable specimen), takes 10 weeks. While not an exact model of natural structure, the plastinated knee joint specimen provides many important advantages in arthroscopy training.

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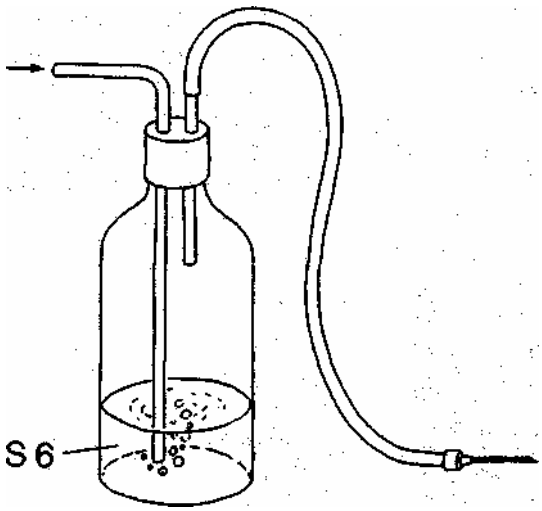

Fig. 1 Application of the Biodur Gascure S6 under sustained pressure.

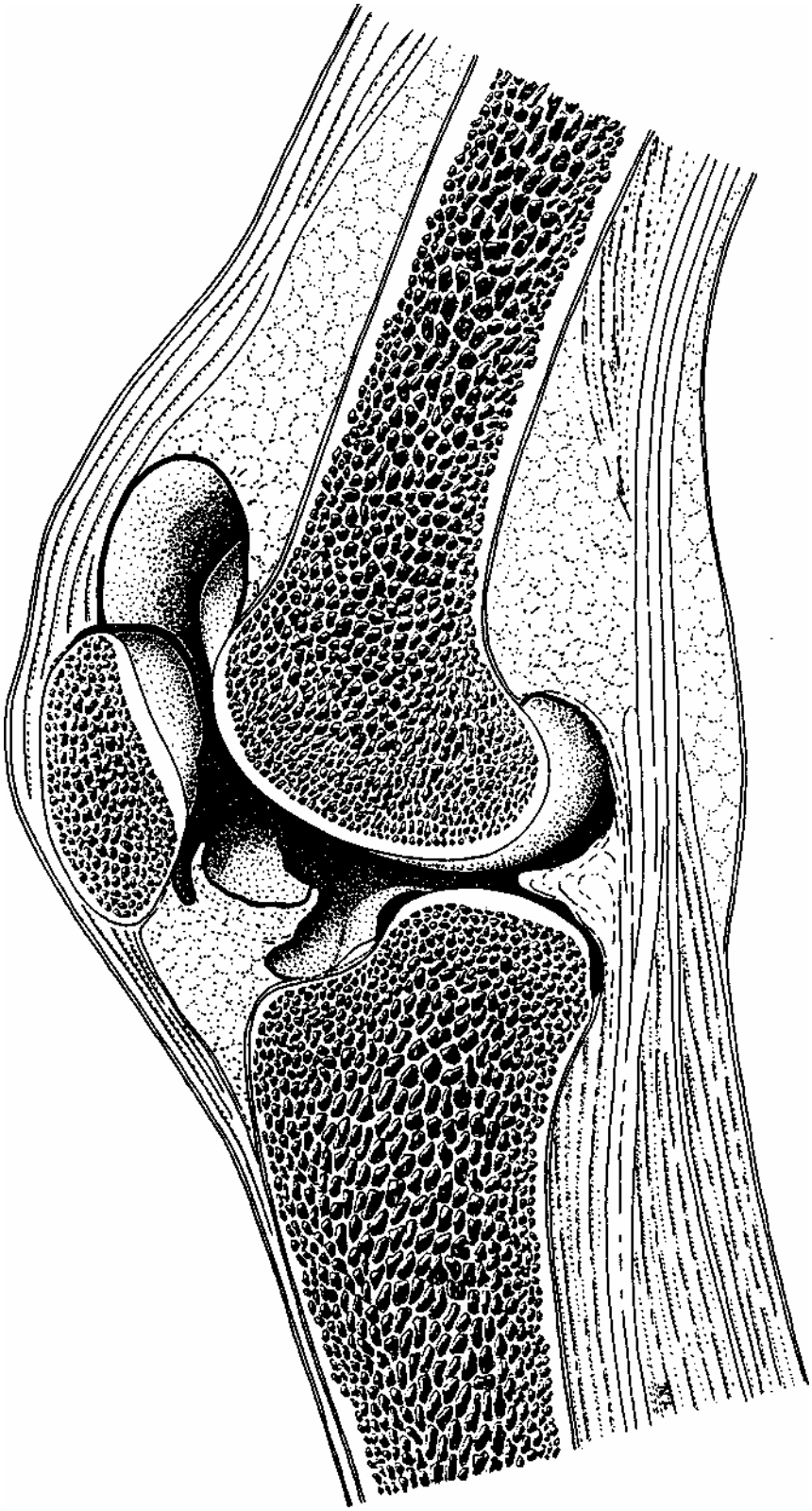

Fig. 2 Plastinated knee joint with expanded joint cavity, lateral compartment and gutter. This half of a natural model for arthroscopy shows the most instructive cutting plane. The cruciate ligaments are preserved in the other half.

PLASTINATION OF THE HUMAN PLACENTA

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INTRODUCTION

The placenta is one of our favorite specimens. This is not because it is particularly attractive. Most would agree that other organs, such as the heart, would win a beauty contest from a placenta. Nor is the placenta one of the most difficult specimens to plastinate. If you're looking for challenge, we would not hesitate to recommend the kidney. The reason for our appreciation of the placenta is because it is one of the few plastination projects in which the person who provided you with the specimen will be able to share your enjoyment of the results.

We will review the process of plastination of the placenta in the sequence in which the actual procedure would be carried out. In doing so, we hope that even the novice plastinator will then be able to follow the recommended steps and arrive at an acceptable teaching specimen.

SELECTION OF SPECIMENS

If you have a relationship with an obstetrician, in which he or she is willing to provide you with a constant supply of placentas, you are in an ideal position. You may then become very picky and select only the most charming examples.

In Heidelberg, where a relationship such as this does exist, we have developed standards to which placentas must conform before they will be accepted for processing. First and foremost, they must be intact and free of injury. Punctured vessels or damaged placental lobes will cause you nothing but misery. You'll be plagued by leaking fluids and experience an awful mess during color injection. Lamentably, most placentas are just not worth the trouble.

Second, we appreciate specimens that are extraordinary in some way. For example, we greatly esteem those which

- have elongated umbilical cords,
- exhibit deviant features, like a velamentous insertion of the umbilical cord,
- are of large size,
- represent unusual (but otherwise normal) conditions, like twin-placentas.

There is one, conspicuous exeption to these rules, however. No matter how ordinary, damaged or even unattractive a placenta may be, we'll go to the very frontier of our ability to transform it into an exquisite specimen if we're doing it for personal reasons. Nobody can be objective about the placenta of his or her own

offspring! This may seem like a weird preoccupation, but sometimes we wonder why people who bronze their children's first shoes, record their first words and take dozens of pictures of every stage of their development, will simply discard their infant's placenta as if it was an empty cigar wrapper! Of course, not everyone is aware of the personal/sentimental possibilities of plastination. But we would suggest to you, the insider, that you never tolerate this kind of callous negligence.

REMOVAL OF BLOOD

Once we have decided which are the favored specimens, we can "go for it." Let's start by getting rid of the blood. The best way to do this is by simply rinsing the vascular system. This can be done very easily if you cannulate the umbilical vein with plastic tubing, and connect it to a water tap. This "cannulation" is not meant to be a sophisticated procedure. Just insert a piece of tubing into the vein and tie a thread around the whole umbilical cord to hold it in place. Be sure to use an overflow device to keep the hydrostatic pressure below 2 meters. Rinsing will take about 12 hours, so it's wise to do it overnight.

Incidentally, rinsing provides you with a fine opportunity to check the vessels for damage. Veins or arteries that spray water will make you wet and attract your attention immediately.

COLOR INJECTION

This brings us to the most complicated step in the whole procedure, color injection. The goal of color injection is to arrive at a specimen that demonstrates the course of the umbilical and placental vessels and exhibits something close to a natural hue.

With the removal of blood, the placenta turns into a very dejected-looking, pale thing. Here is where we will use our cosmetic skills to restore its natural beauty. In order to inject the vessels of the placenta with colored resin we will have to cannulate them. Cannulation of the vein is a mere trifle, as we pointed out before. Unfortunately we cannot say the same about the arteries. These little devils are so tiny that cannulation will take considerable effort and skill. We have developed two methods to get it done.

1. FREE-HANDED CANNULATION: We will call the first method, "free-handed cannulation," because it does not require complex preparation. Instruments and materials needed for this step are as follows:

   - scalpel
   - scissors
   - elastic string
   - a tiny plastic cannula

   Take the end of the umbilical cord between your fingers, look for an artery and cut across this artery with the scalpel. The best place to do this is just below the thread you used to hold the venous cannula in place for rinsing. This same venous cannula and vein will be used for color injection so don't cut them.

   Once the selected artery is bisected, you will be able to see its lumen in cross-section. Position the cannula in the lumen, hold it with your fingers and tie the elastic string around the whole umbilical cord to keep it in place.

2. DISSECTION CANNULATION: We call the second method "dissection-cannulation". This is intended to mean that one must dissect the artery out of the umbilical cord before inserting a cannula. It may sound easy but, alas, it isn't! Sure, you'll get the artery

separated from the cord without damage but, once you have inserted the cannula, any ill-considered move could ruin its delicate wall. In contrast to the artery used in the first method, this one has been removed from its adventitia and is very fragile. As you will notice, more tools will be needed to get the job done. We would suggest the following, in addition to those needed for the first method:

- a frame to hold the cannula in place during injection. This must be locally constructed.
- clamps and hemostats

Once the artery is dissected free of the rest of the umbilical cord, the cannula can be inserted and ligated into this vessel, using the elastic string. The string should be tied around the artery, leaving a generous length of loose ends. After injection, these ends can be used to stabilize the vessel and keep it from moving and becoming damaged. This, of course, would result in a very undesirable leakage of the resin you have just injected. The frame and clamps are used to hold the cord and the cannulated artery while injection is under way.

Now that you are familiar with both methods, the time has come to discuss when to apply each. The rule of thumb is that one uses free-handed cannulation whenever possible. There are circumstances, however, in which this method simply will not work. For example, what if the vessels in the umbilical cord are so spiraled, that you can't find a piece of artery long enough to put the full length of the cannula in? The only possibility is dissection.

And what if the umbilical cord happens to have "false knot" along its course? You would have to put so much pressure on the syringe that you would loose your hold on the slippery umbilical cord. The cannula would come flying out of the artery, and there you are, a colored resin mess. To avoid this misery you had better employ dissection cannulation in this case as well.

Now let's move on to something simple. What quantity of which mixture should be injected into which vessel in what sequence? Well, we start by injecting the artery -- but we inject it with BLUE resin. Remember that the oxygenation of blood in this organ is opposite that in most others. Arteries are blue and veins are red. Once the artery is adequately injected and tied off, we may turn our attention to the vein. Two kinds of resin material are available for use in this step, epoxy and silicone rubber. We will briefly outline the composition the of injection mixtures associated with each (in parts per weight).

1. EPOXY INJECTION MIXTURE:

BIODUR E 20 red/blue	:	100 ppw
methyl ethyl ketone	:	40 ppw
BIODUR E 2	:	45 ppw

These components should be added in the order given and thoroughly stirred for at least 5 minutes. Because BIODUR E 2 is a hardener, these mixtures have a limited pot life. Don't worry, you should have enough time to get your specimens injected. But we would advise you to mix and inject the blue resin before starting on the red.

2. SILICONE INJECTION MIXTURE:

BIODUR S 10 red/blue	:	100 ppw
methyl ethyl ketone	:	40 ppw
BIODUR S 3	:	3 ppw
BIODUR S 6	:	1 ppw
BIODUR S 2	:	1 ppw

Again, the components should be added in the order shown. Precautions regarding pot life and injection sequence apply here as well.

Whether you select epoxy or silicone mixture is a matter of personal preference. Epoxy resins have been used successfully for a long time in this role. But they are more rigid and more allergenic than the silicone. The silicone injection mixture is a rather new development. It seems to be very promising, but you might say that it's "just beyond the experimental stage." We hope that a lot of brave researchers will elect to try it. About 10-20 ml of blue injection mixture and 100-200 ml of red injection mixture is required per placenta.

One more thing about arterial injection. We have been talking of cannulation and injection of just one artery. But, as we know, the standard umbilical cord contains two arteries. You needn't bother about the second one. The two communicate in such an extensive collateral network that the second artery will be filled by retrograde flow in almost 100% of the specimens. If not, you can always cannulate it separately.

When you have completed arterial (blue) injection in the free-handed method all you have to do is remove the cannula from the injected artery and the elastic string will pull itself tight around the umbilical cord constricting the injected artery. This serves very nicely to prevent resin leakage without injury to the vessel. In the dissection method, leave the cannula in place and put a stopper on it to get the same result.

Now that we have cannulation under control, we must look at the instruments and material needed to get the venous injection process started.

- injection resin (epoxy or silicone)
- acetone, to be in use as a cleaning agent
- scissors
- cotton thread
- syringe
- adapters to connect the syringe to the plastic tubing
- an in-line shut-off valve or faucet
- a rather strong hemostat
- a rubber band

The in-line valve will enable us to remove the syringe from the adapter without spilling any resin. We need that because we'll have to refill the syringe several times.

Load the syringe, connect it to the adaptor on the plastic tubing and off you go. When the venous injection is finished, pull the plastic tubing out, clamp the whole cord off with the hemostat, and wrap the rubber band around the cord several times to serve as a final stopper. Now the hemostat can be removed and no leakage will occur.

HARDENING

It's hard to believe but we finally made it through that section on color injection! Now we must talk about hardening. There is really nothing special about hardening. All we have to do is to give the injection resin some time to solidify before we proceed with the plastination of the placenta. What turned out to be the best method in Heidelberg is the following:

- put the placenta in a container, making sure that the uterine surface is on top
- fill this container with just enough water to cover it
- put a cloth over the placenta to keep it from drying
- leave it standing like that overnight at refrigerator temperature

The reason for putting the uterine surface on top and filling the container with just enough water to cover it is that this will prevent the umbilical cord from floating away from the placenta. It looks very unnatural to have the umbilical cord sticking out of the placenta at an angle of

90 degrees. On the following day you can remove the resin remnants and we are ready for the next step.

FIXATION

Fixation is about as uncomplicated as hardening. All you do is immerse the placenta in a 5% solution of formalin for about 3 weeks, making sure that you keep the umbilical cord submerged.

DEHYDRATION

Dehydration is covered in great detail elsewhere, particularly in Prof. Klaus Tiedemann's article that will appear later in this volume. We will do no more than mention a few details that might come in handy whilst plastinating a placenta.

Dehydration of the placenta is performed by freeze substitution in acetone. Three baths are usually required and the acetone must be maintained at -25°C. Each bath should consist of approximately 5 times the volume of the specimen. Defatting is not necessary. The whole process will take approximately 2 weeks. How's that for brief?

FORCED IMPREGNATION

Impregnation of the placenta is performed almost excusively with BIODUR S 10 nowadays. This whole method has been extensively described and is known as the S 10 Standard Technique. Again, I will mention only a few details.

Our placenta, now soaked with acetone, is put into the polymer solution used for impregnation. BIODUR S 10 is a liquid silicone rubber. It has to be mixed with 1% BIODUR S 3, which is a suitable hardener. By means of a vacuum, the acetone is then extracted from the tissue. (You might say that we encourage it to evaporate.)

A pressure gradient develops between the inside and the outside of the specimen and this causes the polymer to be drawn into the tissue. For further details, please refer to the bible of plastination, the "Heidelberg Plastination Folder."

Impregnation of one placenta will actually use up about 500 grams of polymer. But, in order to immerse it properly duing impregnation, more will be required. Impregnation is carried out very gradually and takes about 3 weeks. It is wise to do the impregnation at about -20°C, for this will grant you an almost unlimited pot life for the BIODUR S 10/ S 3 mixture.

CURING

Curing is a process whereby the fluid polymer, which now saturates our specimen, can be rendered solid. It is carried out by exposing the placenta to a preparation called, BIODUR GASCURE S 6. Before we start curing, however, we might want to give the fetal side of the placenta some extra gloss. This looks quite nice on the finished specimen. It makes the placenta seem all wet and natural. To accomplish this very chic "wet look", we remove the amnion from the fetal side of the placenta, and apply a layer of BIODUR S 49, mixed with 1 % BIODUR S 3.

The specimen is then put to rest in the arms of BIODUR GASCURE S 6 in a closed container for about 1 week. Don't forget to use calcium chloride as a drying medium and, of course, contrive to speed up the evaporation and circulation of the curing agent by means of aquarium pumps. And that's it!

ACKNOWLEDGEMENTS

There is nothing left now except for the first author to acknowledge those people

who offered her the opportunity to show her enthusiasm about the wonderful world of plastination. Thanks are due to

- Prof. Pim van Doorenmaalen, my anatomy professor, who introduced me into this technique.
- Dr. Gunther von Hagens, who taught me all I know about it
- The "Stichting De Drie Lichten," a Netherlands foundation that supported my research in Heidelberg.
- Prof. Harmon Bickley, who enabled me to communicate with you by re-editing this paper and polishing my English.

and last but not least

- my husband Bas, who stuck to my side, serving as both thesaurus and therapist while I prepared this paper.

THE DIAGNOSTIC IMAGING CHARACTERISTICS OF PLASTINATED ANATOMICAL SPECIMENS

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INTRODUCTION

Since 1981, whole-body radiography has been performed on cadavers used in the gross anatomy course at the Uniformed Services University (1). This technique offers a unique opportunity for radiographic/anatomic/pathologic correlation and facilitates understanding of complex anatomical relationships by first-year medical students. Also, it has revealed numerous morphological variants and pathological conditions (Fig la).

Heretofore, it has not been possible to retain examples of lesions or anomalous development discovered by this procedure and they were routinely cremated, along with less-interesting material. A relatively new method, called plastination (2) (3), now provides the potential for indefinite preservation of 65-70 of these specimens per year.

Principles of plastination and details of its procedures have been presented elsewhere (4). In this paper, we will demonstrate the change in imaging properties that result from plastination and discuss the teaching of radiographic correlations using plastinated specimens.

MATERIALS AND METHODS

SKELETAL IMAGING (RADIOGRAPHIC): Routine, plain-film radiography of teaching cadavers before dissection resulted in the detection of many examples of skeletal disease. Once dissection was complete, these specimens were collected. They were then subjected to specimen radiography (Hewlett-Packard Faxitron Unit) before and after plastination with silicone rubber (SR). The trabecular pattern of pre-plastination specimens was compared with that of post-plastination specimens. Also, the occurrence of artifacts (e.g. bone dust) due to specimen preparation and plastination was recorded.

SKELETAL AND SOFT TISSUE IMAGING (MAGNETIC RESONANCE): MR imaging of a silicone-impregnated, plastinated knee specimen was performed, utilizing the routine head coil on a 1.5T super-conducting magnet (Technicare Corp, Solon, Ohio). Five (5) mm sagittal sections with a 1 mm intervening gap were obtained, using a 256 x 256 matrix. A 700 msec TR (repetition time) and a 33 msec TE (echo delay time) were used. A multislice technique was used and, in all cases, the number of signal averages was

two. MR images of SR-impregnated plastinated heart and brain were similarly obtained. All images were subsequently evaluated for anatomic definition.

SOFT TISSUE IMAGING (ULTRASOUND): Tomographic ultrasound (US) images of SR-impregnated plastinated heart, brain and placenta were obtained on a real-time, dedicated breast unit with a 2.5MHz transducer. The images were evaluated for echogenicity and through-transmission.

COMPUTER-ASSISTED TOMOGRAPHIC IMAGING: CT scans were performed on several epoxy resin (ER)- and SR-impregnated specimens, using a 4th-generation CT scanner. Axial images were compared with normal scans for anatomic detail, artifact production and tissue attenuation characteristics.

RESULTS

All seven SR-impregnated bone specimens showed decreased trabecular definition after plastination (Fig. 1C). Irregular medullary fat replacement was responsible for silhouetting of the trabeculae by the dense polymer. Before plastination (Fig Ib), the only artifact detected on two of these specimens was bone dust. After plastination, linear medullary lucencies, globular metaphyseal densities and soft-tissue shrinkage were noted.

MR imaging of three SR-impregnated specimens (knee, brain, heart) yielded uniformly poor anatomic definition (Fig 2). Presumably due to a dearth of mobile hydrogen ions, signal acquisition was markedly decreased, despite adequate acquisition time.

US imaging of three SR-impregnated specimens (placenta, brain, heart) was equally disappointing (Fig 3). Echogenicity could not be assessed because of the lack of through-transmission. The ultrasonic beam was strongly reflected at the incident surface of all three organs.

CT images of soft tissue and bone were degraded by shrinkage and air. Anatomic definition was variable (Fig 4a). Cortical and endosteal surfaces remained well-differentiated from adjacent soft tissue and air interfaces. Soft tissues, however, were homogeneously dense, unless permeated by air. Tissue attenuation numbers were similar for medullary space (o=578H) and soft tissue (o=574H), regardless of the polymer used (SR, ER, SR-ER copolymer) (Figs 4b and c). Nevertheless, the loss of anatomic definition noted on CT was considerably less than that seen with US and MR.

DISCUSSION

Most teachers of anatomy and pathology would agree that the use of gross tissue specimens in the laboratory is invaluable. They provide a very desirable correlate to the microscopic and radiographic study of normal and diseased structures. However useful, the preparation and handling of such material is difficult.

Plastination preserves perishable biological specimens indefinitely while greatly improving their handling qualities and durability. At present, the half-life of a conventionally stored gross pathology specimen collection at this institution is less than two years. Continuous replacement of formalin preservative is time-consuming, expensive and a health hazard. Yet, even despite such effort, specimen deterioration is inevitable.

Direct handling of formalin-stored specimens by faculty or students is impractical. Plastic display bags lose clarity very rapidly after handling. Furthermore, student manipulation quickly destroys delicate tissue or disrupts special dissections. With improved modes of therapy, exemplary cases of many classic

diseases such as disseminated infection or neoplasm, have become more difficult to obtain. A more reliable means of preservation was clearly needed.

Plastinated specimens resolve many of these problems. Their potential for enhancement of the teaching of anatomy, physiology and pathology is vast (5). They can be taken into lecture and seminar sessions and simply passed around, without wetting the students' fingers or their textbooks. Also, they may be dissected, laminated or sectioned to improve information content.

For several years, the Department of Radiology/Nuclear Medicine has contributed instructional time and staff to the gross anatomy and pathology laboratory courses. Our main effort has been in the area of radiographic-anatomic and radiographic-pathologic correlation, concepts that physicians use extensively in their daily clinical activities. Plastinated specimens would be an ideal replacement for formalin-preserved material in this exercise because they are much easier to handle. However, they have not proven a practical substitute.

EFFECTS OF PLASTINATION ON IMAGE GENERATION

In the process of plastination, all tissue water and some fat is removed and replaced with plastic. The inevitable result is alteration of imaging properties. This is because radiologic resolution is ultimately dependent on differential air, fat, water and mineral content of tissues and their interfaces. Since all of the water and a significant amount of the fat is removed and replaced by an evenly distributed, dense, high-molecular-weight polymer, radiologic resolution is rendered less effective. This problem is most severe with imaging methods (US and MR) that are most dependent on tissue water for resolution.

Silicone rubbers (siloxanes) are materials consisting of high-molecular-weight molecules, each of which is a linear polymer of SiO_2 with sidechains that determine special characteristics. The general siloxane formula is

$$\begin{array}{c} R \\ | \\ -Si-O \\ | \\ R \end{array}_{n}$$

where the sidechain (R) can be methyl, ethyl or oxygen. More viscous silicones are made up of longer polymeric molecules. End-to-end joining of these molecules results in the viscosity of the material. The connecting of two adjacent linear molecules by a condensation of their sidechains is called crosslinking and forms a unified matrix of the hardened polymer (6). Both of these reactions require different agents. The combination of hardening and cross-linking is called curing.

The density of cured silicone rubber effectively blocks through-transmission of an ultrasound beam (Fig 3) while its crosslinking, by markedly decreasing the number of mobile hydrogen ions, diminishes signal acquisition to a level impractical for MR (Fig 2).

CT demonstrates polymer replacement of soft tissue water and fat. It also shows the replacement of medullary fat by polymer which is of comparable density but has Hounsfield (H) numbers in the range of bone (400-600 H) (Figs 4b and c). It is therefore not surprising that silhouetting of trabecular bone by polymer was observed in plastinated bone specimens (Fig Ic). Nor is it surprising that all soft tissue structures appear uniformly dense and lose mutual differentiation.

USE OF PLASTINATED SPECIMENS IN THE TEACHING OF RADIOGRAPHIC CORRELATIONS

Despite its degrading effect on imaging, plastinated specimens retain excellent gross detail and remain histologically intact. In fact, with sheet plastination, a technique for preparing thin slices, it is possible to screen whole organs for histologic changes at considerably less expense than would be incurred with conventional methods. Areas suspected of harboring disease can then be removed from the larger screening sections and processed for conventional light or electron microscopy (7).

The altered imaging properties of plastinated specimens do not preclude their use in teaching radiographic correlations. Imaging can be performed before plastination and the plastinated specimen kept on hand for comparison. Perhaps, with further development, a polymer may be discovered that even enhances imaging.

ACKNOWLEDGEMENTS

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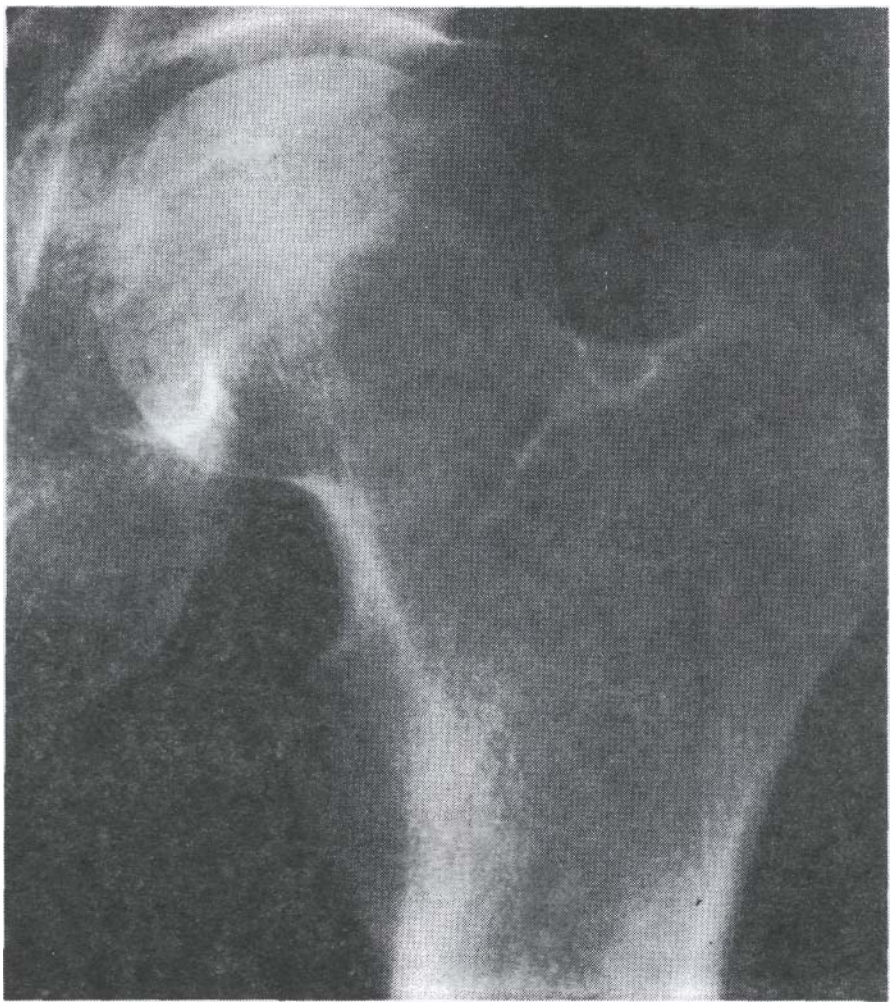

Figure la: Metastatic carcinoma of the stomach. Cadaveric AP hip shows a Grade IB lytic geographic lesion in the left femoral neck.

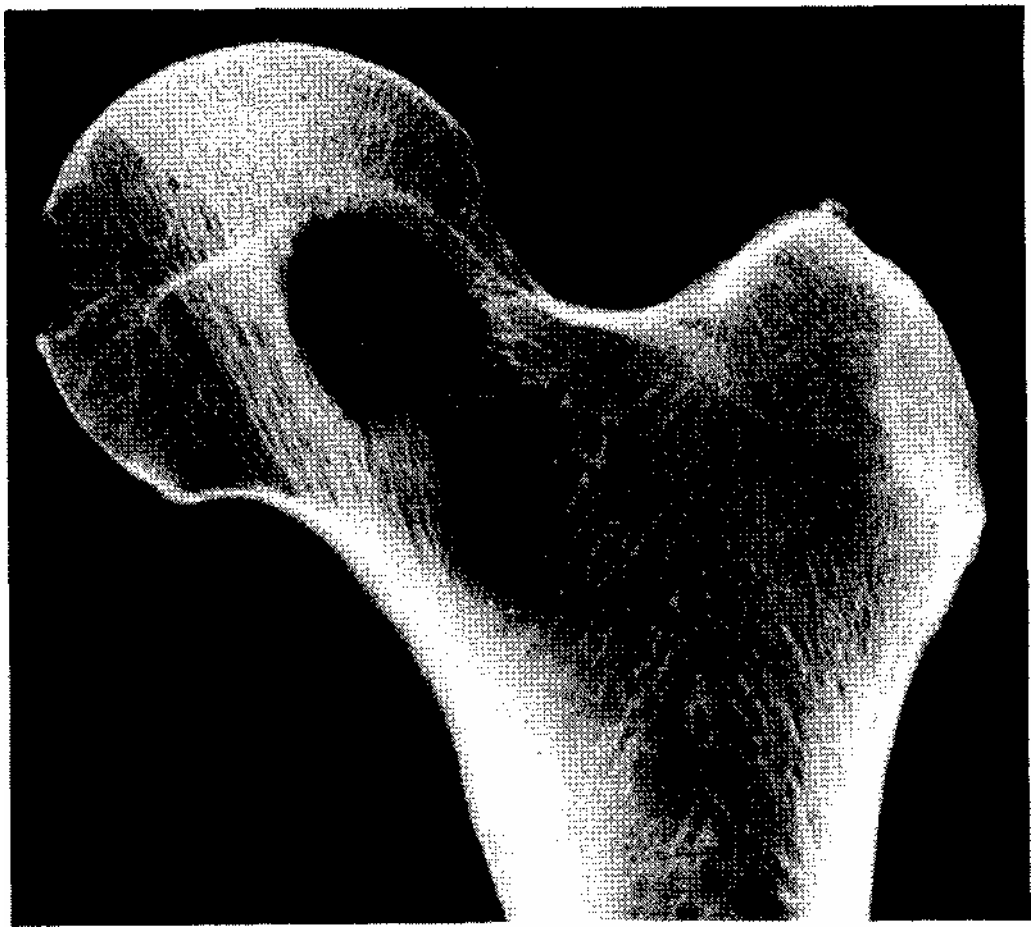

Figure Ib: Metastatic carcinoma of the stomach. Specimen radiography before plastination reveals the abrupt transition between normal trabecular bone and the metastasis, characteristic of a IB lesion. Another metastasis (arrow) causing less trabecular destruction is not seen in the previous radiograph.

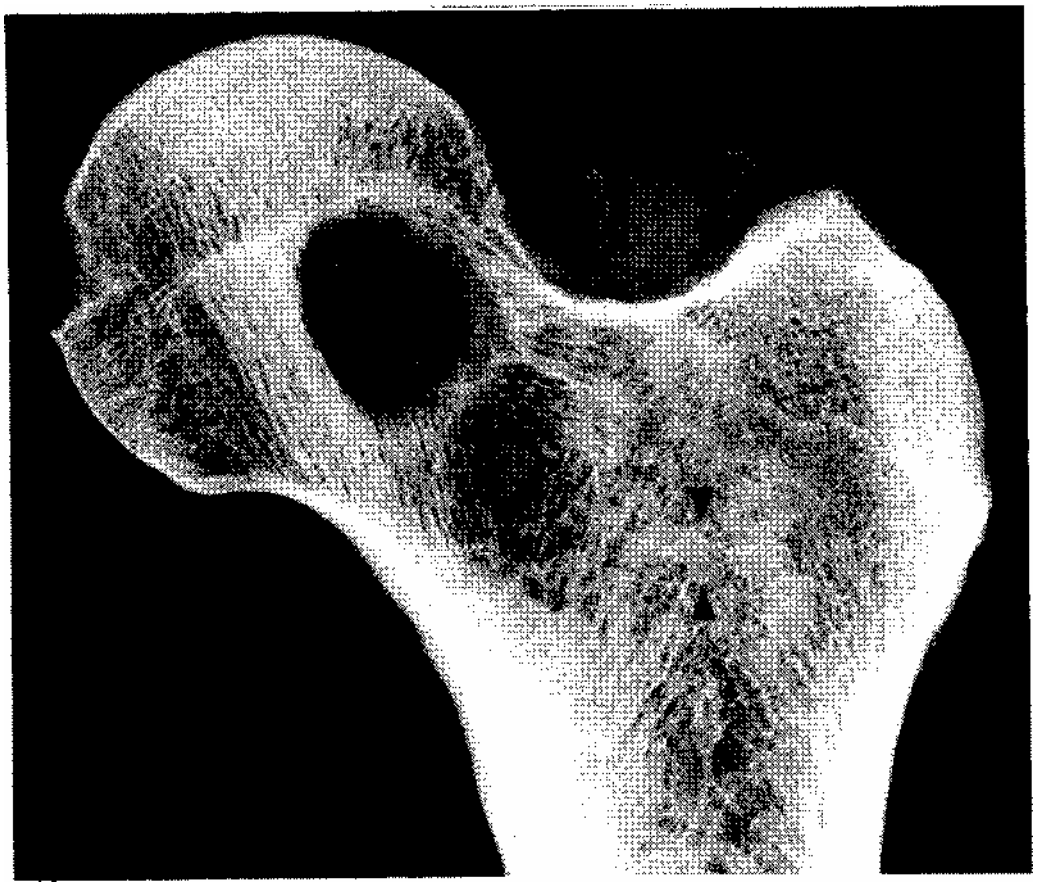

Figure Ic: Metastatic carcinoma of the stomach. Specimen radiography after plastination shows globular metaphyseal densities (the SR polymer) silhouetting the femoral trabeculae and decreasing their definition (arrowheads). Soft tissue shrinkage of the femoral neck metastasis is also noted.

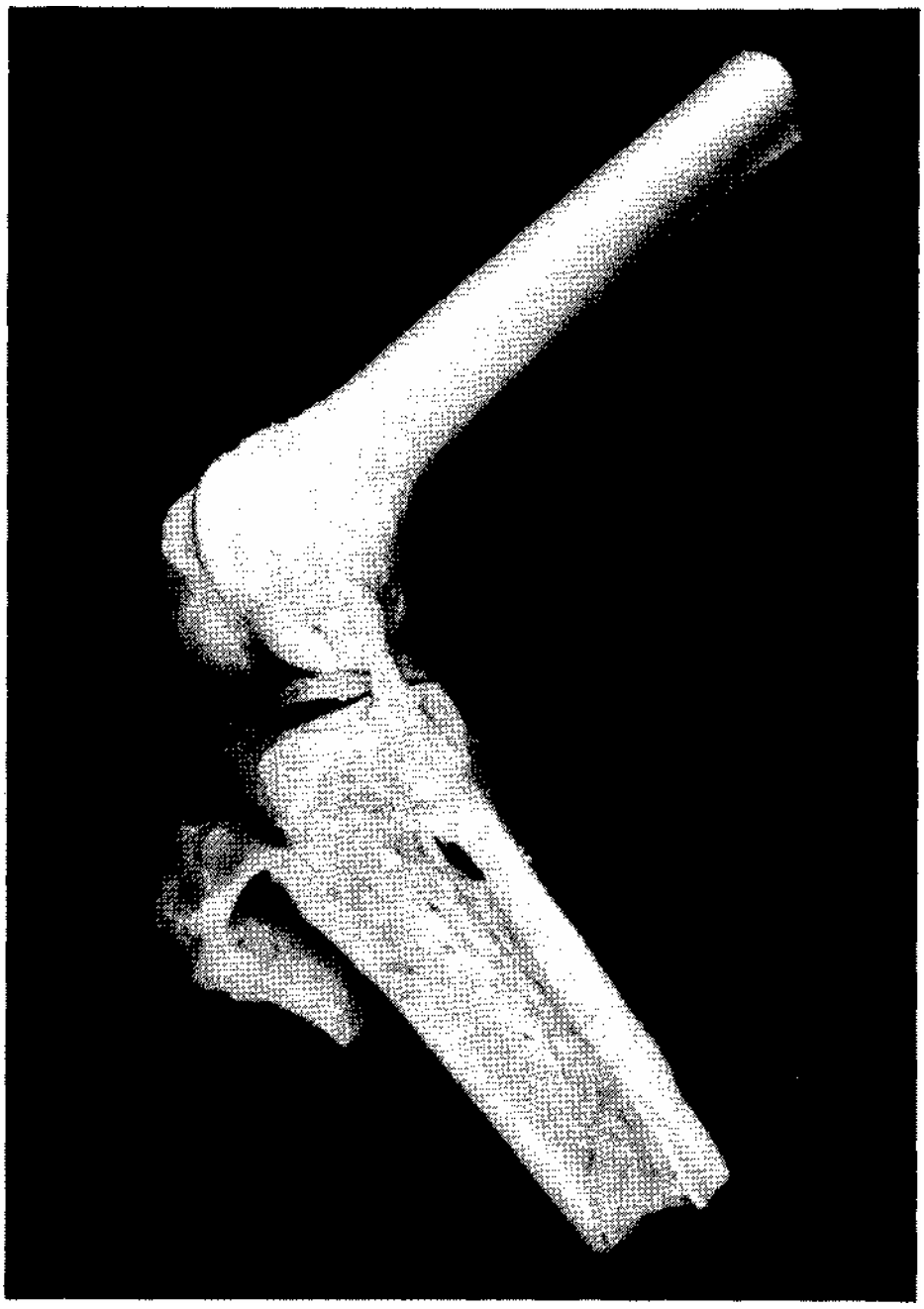

Figure 2a: Plastinated knee specimen from which MR image was made.

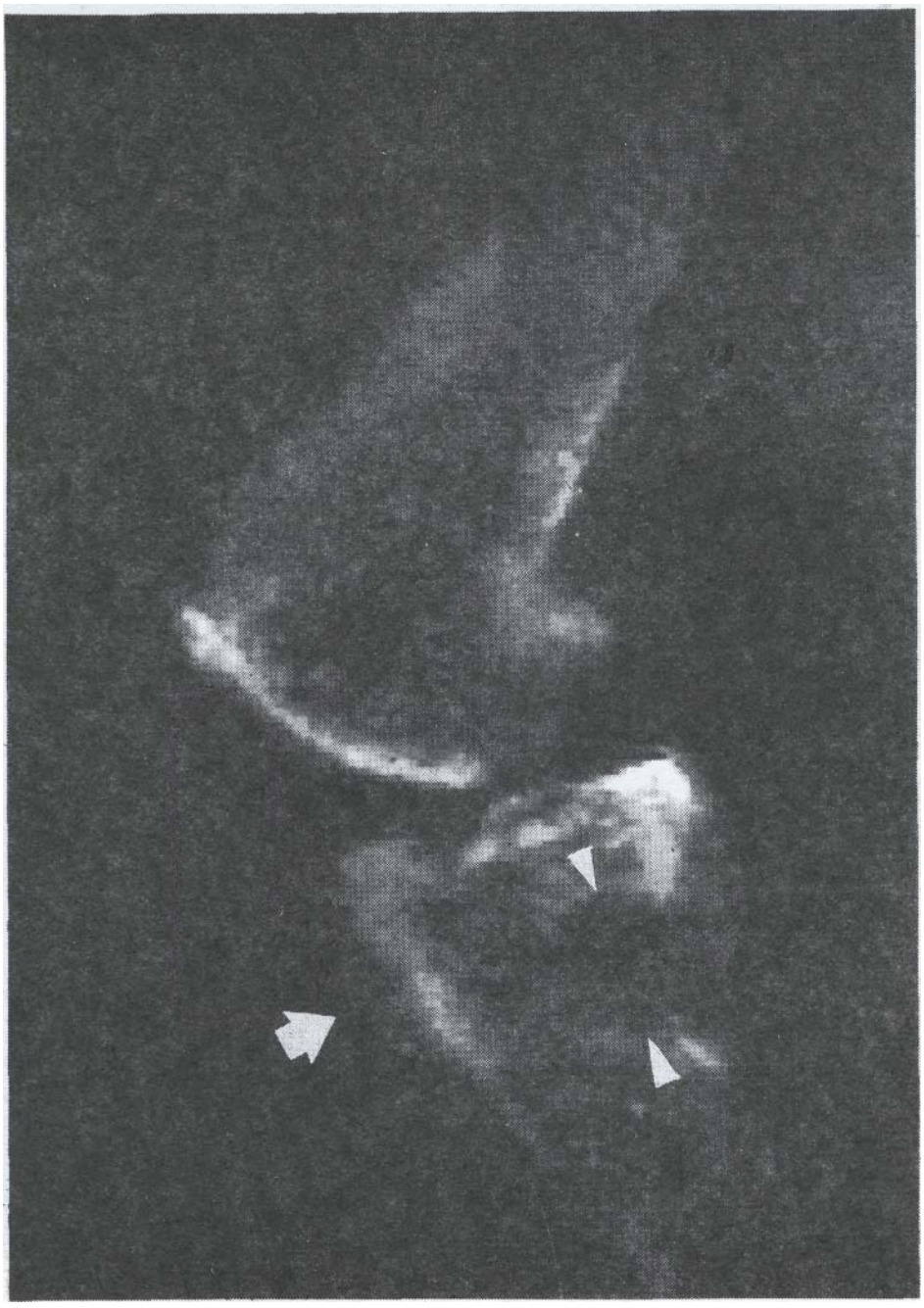

Figure 2b: MR image of a plastinated knee showing marginal imaging of the patella (arrow). Replacement of medullary fat by globular SR is manifested by a low signal area (arrowheads) on the image.

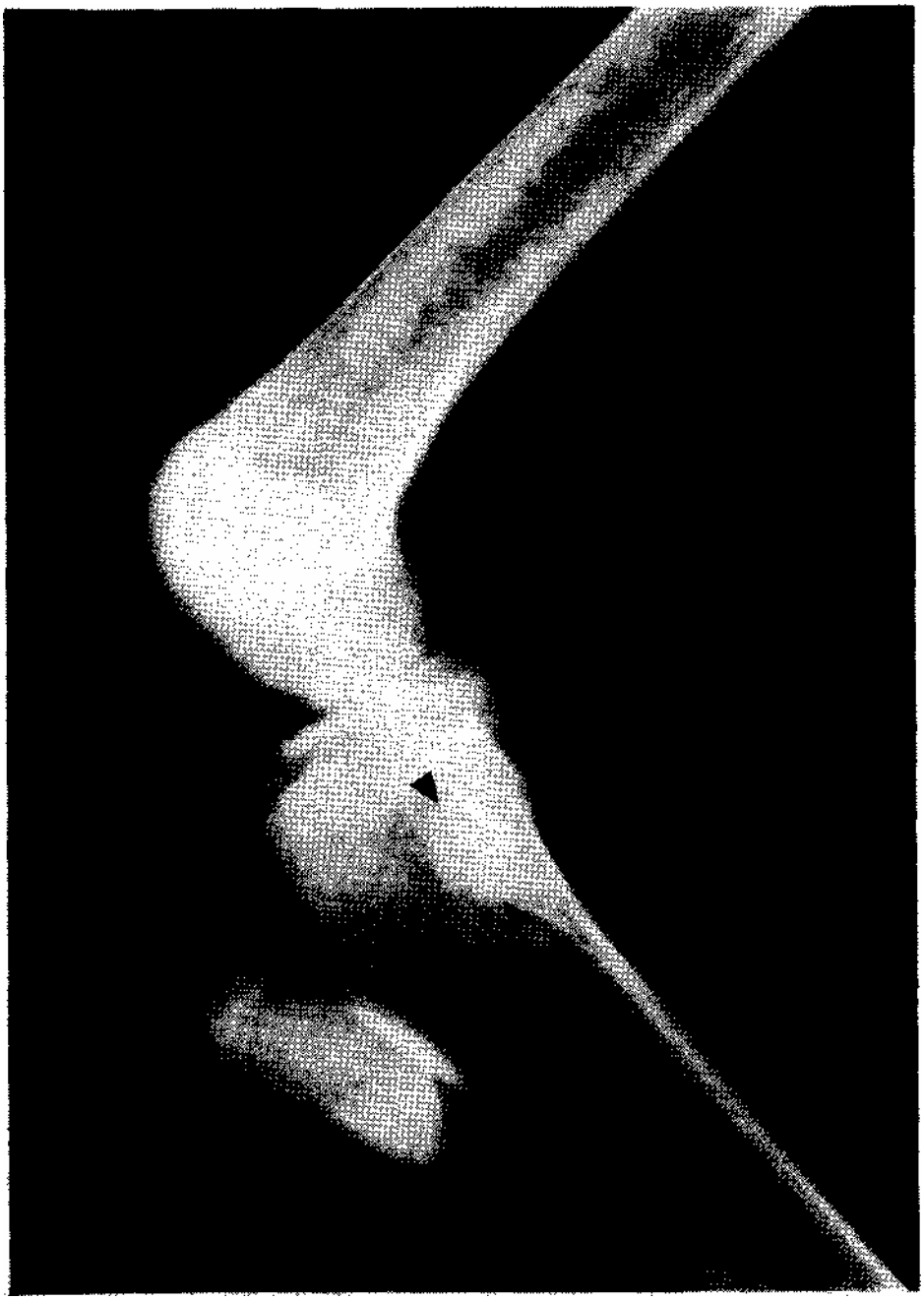

Figure 2c: Radiographic image of plastinated knee showing that low signal area represents a medullary globule of radiographically dense SR polymer (arrowheads).

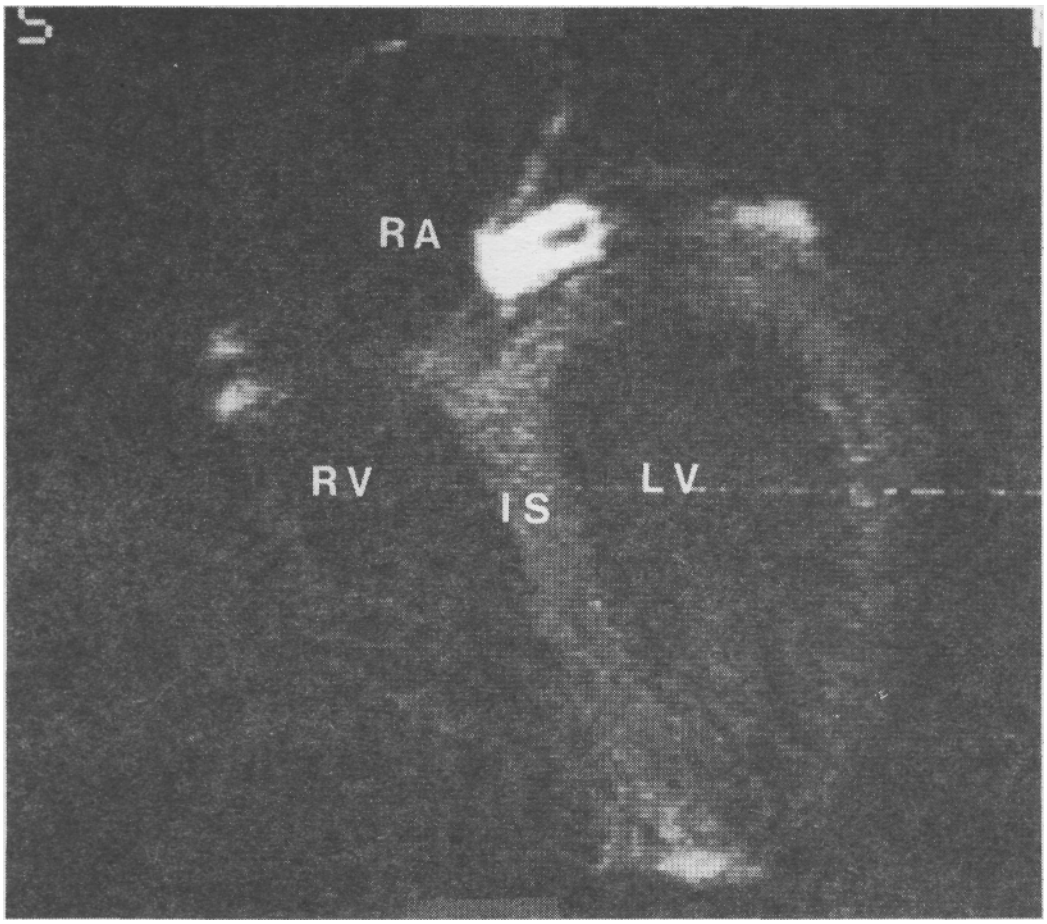

Figure 2d: MR image of a plastinated heart. Except for the intramuscular septum, (IS), left (LV) and right (RV) ventricles, and the right atrium (RA), anatomic detail is lost, the high signal noted between the atria is a plug in the atrial septum.

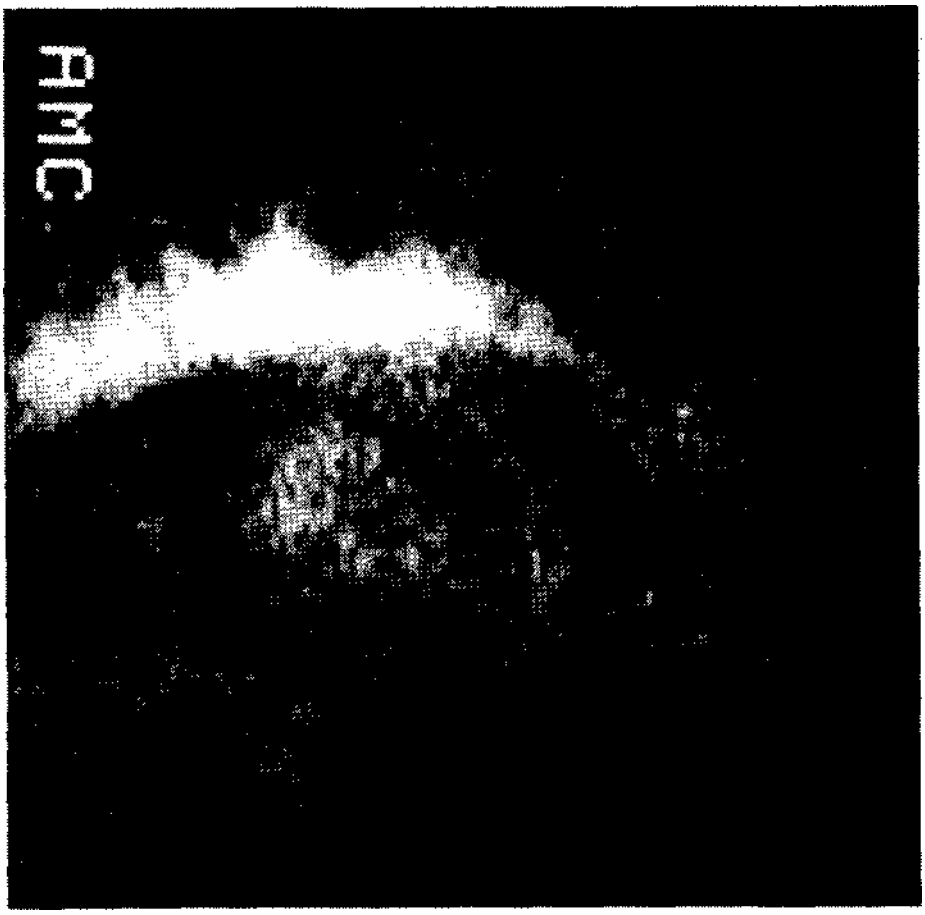

Figure 2e: MR image of brain. Diminished anatomic definition is due to less mobile hydrogen ions within the silicone polymer.

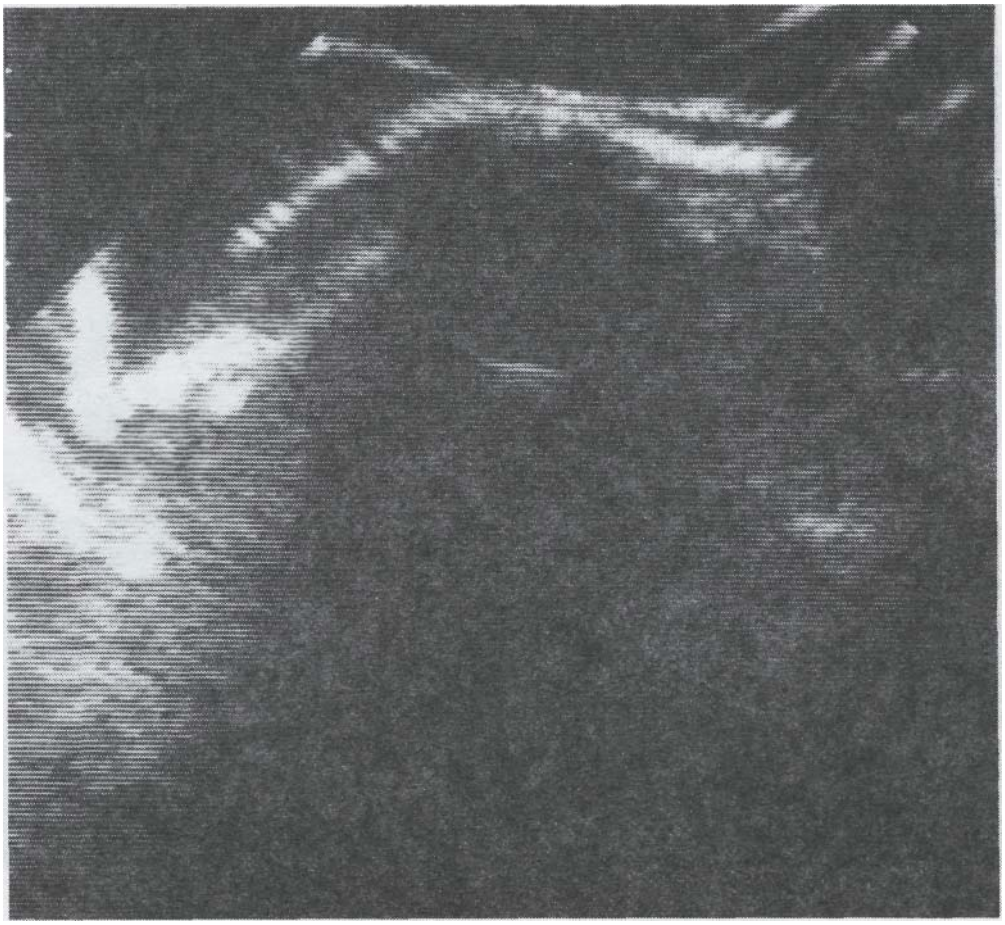

Figure 3: US image of plastinated heart. There is reflection of the beam at the incident surface resulting in distal acoustical shadowing. The linear sonodensity located centrally within the acoustical shadow is the atrial plug.

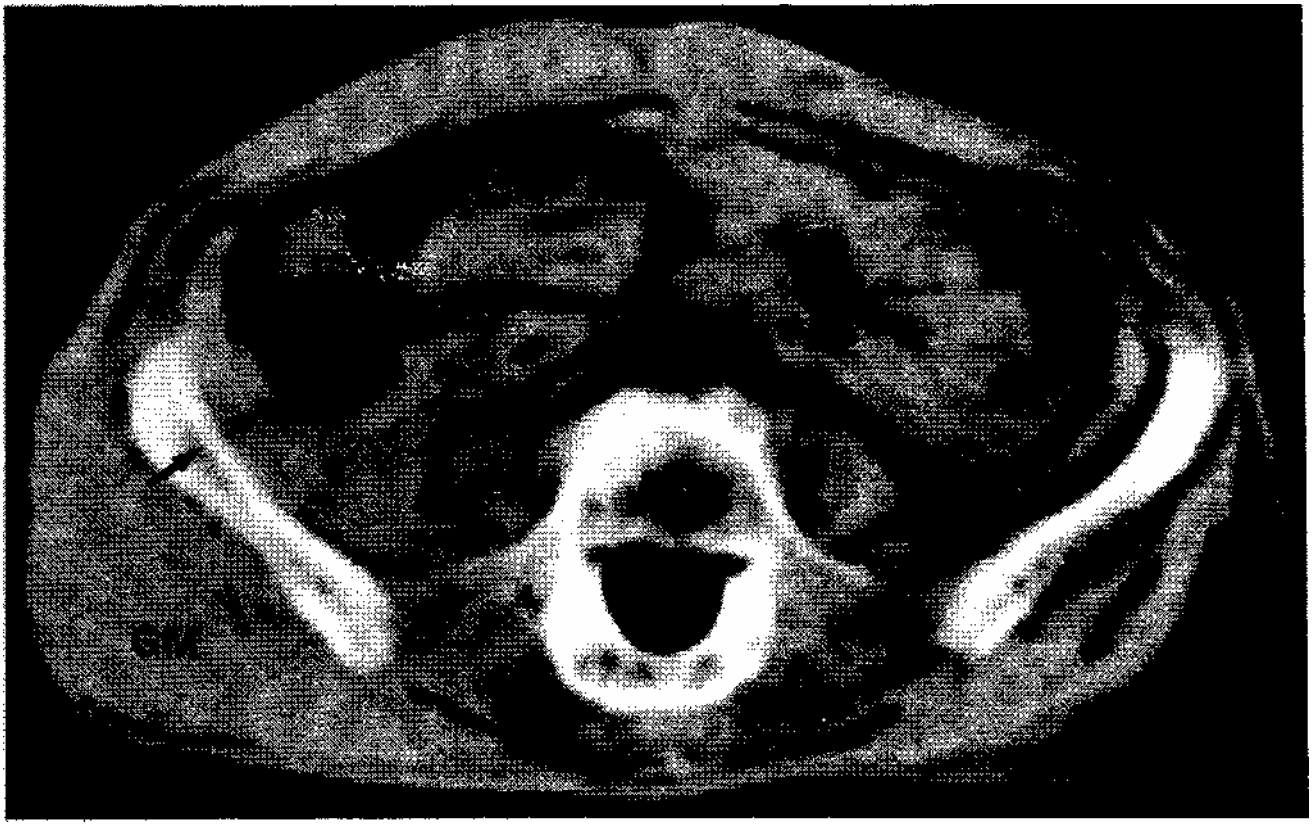

Figure 4a: CT image of plastinated pelvis, showing diffuse intra-and extrapelvic air artifact. The right gluteus maximus (GM) is much better defined than the left. Cortical and endosteal surfaces retain their definition (arrows) since the compact bone of the cortex is not significantly replaced by polymer.

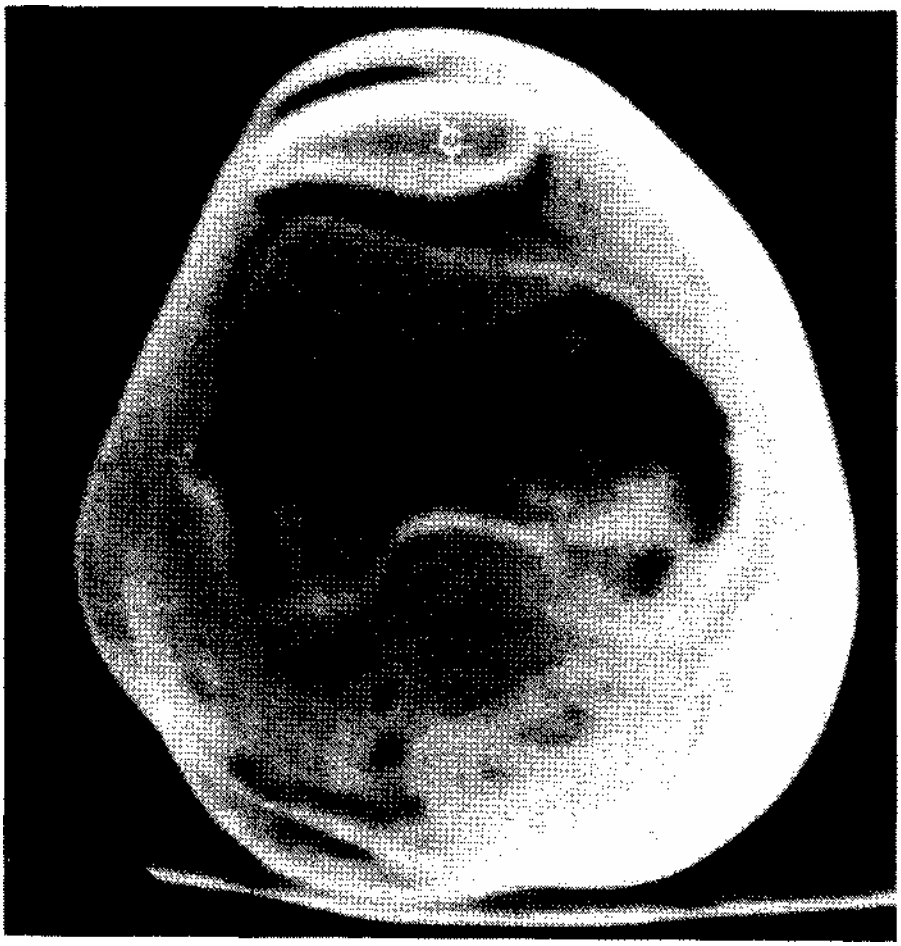

Figure 4b: CT image of plastinated femur (distal metaphysis). SR-ER impregnated specimen shows air artifact in medullary space.

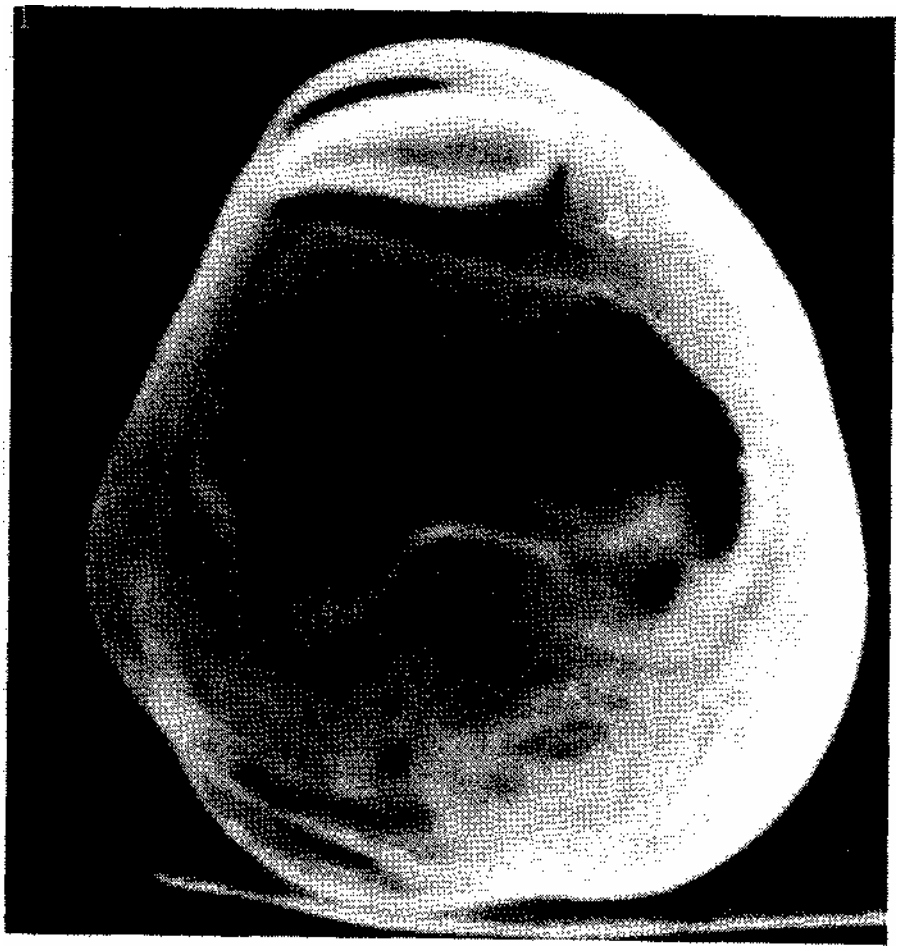

Figure 4c: CT of plastinated femur (distal metaphysis). Medullary space of the patella and adjacent soft tissue have similar attenuation characteristics.

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TECHNICAL COMMUNICATION: ROUTINE MICROSCOPY OF DEPLASTINATED TISSUE

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INTRODUCTION

During presentation of our (silicone rubber) plastinated gross specimens at national meetings, we have often been questioned about the fate of the specimens' histology. Concern, some of which stems from today's medicolegal climate, has been expressed that the plastination process might destroy or at least alter a specimen's microscopic detail and thus its diagnostic value. Although we had read that plastinated tissue was still suitable for histologic study (1), we elected to undertake the following study with our own specimens.

MATERIALS AND METHODS

Representative blocks were taken from 14 silicone rubber impregnated specimens (eight carcinomas and 6 benign lesions). The sections were immersed in 5% sodium methoxide in methanol for 48 hours to depolymerize the silicone. They were subsequently washed in fresh methanol, processed on an automated tissue processor, embedded in paraffin, and sectioned. Hematoxylin and eosin (H&E) stained slides were prepared simultaneously with routinely processed companion sections of each specimen for comparison.

RESULTS AND CONCLUSIONS

In each instance, the histologic and cytologic qualities were equal to that of its nonplastinated counterpart. The microscopic appearances were not always identical but diagnostic pathologic features were readily discernable in the deplastinated tissue. In some cases the microscopic appearance of the deplastinated material was judged superior to that of the nonplastinated; this may have been due to better initial fixation of the former. Figure 1 is a high power photomicrograph of a deplastinated, H&E stained section of an esophageal squamous carcinoma. Figure 2 is its nonplastinated counterpart. In both preparations it is possible to see the pleomorphic neoplastic cells, their high nuclear/cytoplasmic ratios, prominent nucleoli and intercellular bridges. The nuclear detail, however, is better preserved in the deplastinated section.

One technical aspect to underscore is the absolute necessity of removing all the silicone polymer from the sections prior to processing. Residual silicone will interfere with proper paraffin infiltration and subsequent microscopic slides will be unsatisfactory. We also found initially that deplastinated tissue seemed somewhat resistant to paraffin infiltration and that an enclosed tissue processor with alternating pressure/ vacuum produced better paraffin permeation of the sections.

Under most circumstances there is little call for microscopic examination of plastinated tissue. Our results support those of earlier reports that plastination

of a gross specimen does not preclude later histologic study.

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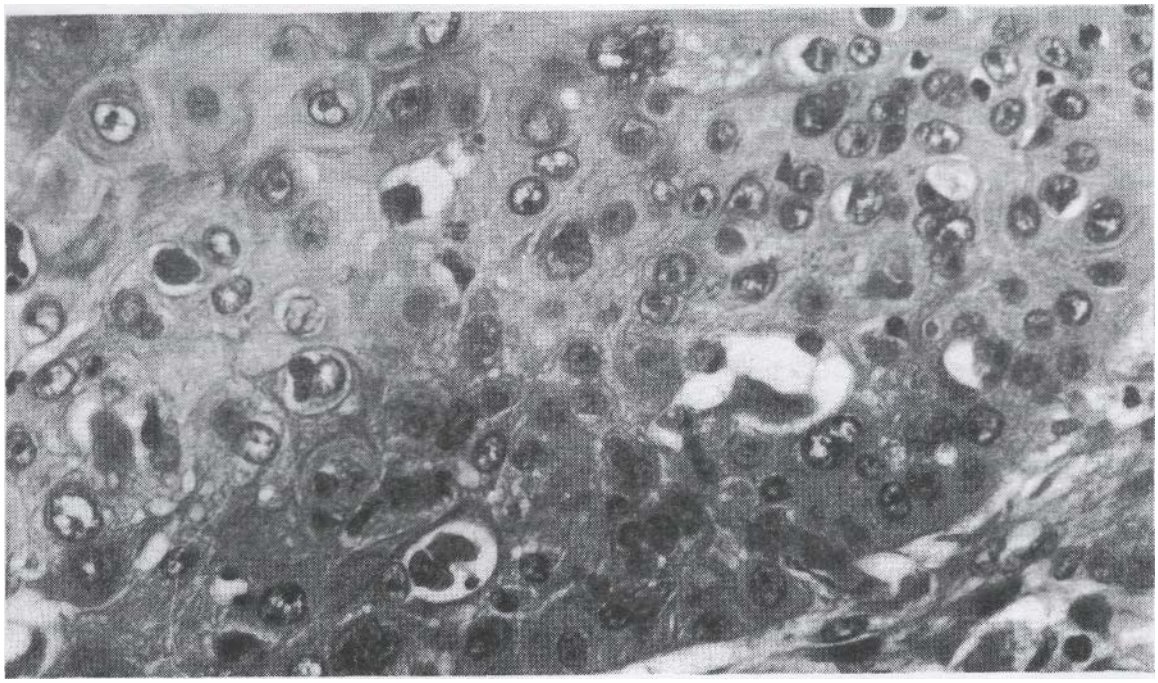

Figure 1: Deplastinated section of a squamous carcinoma of the esophagus (hematoxylin and eosin x 400)

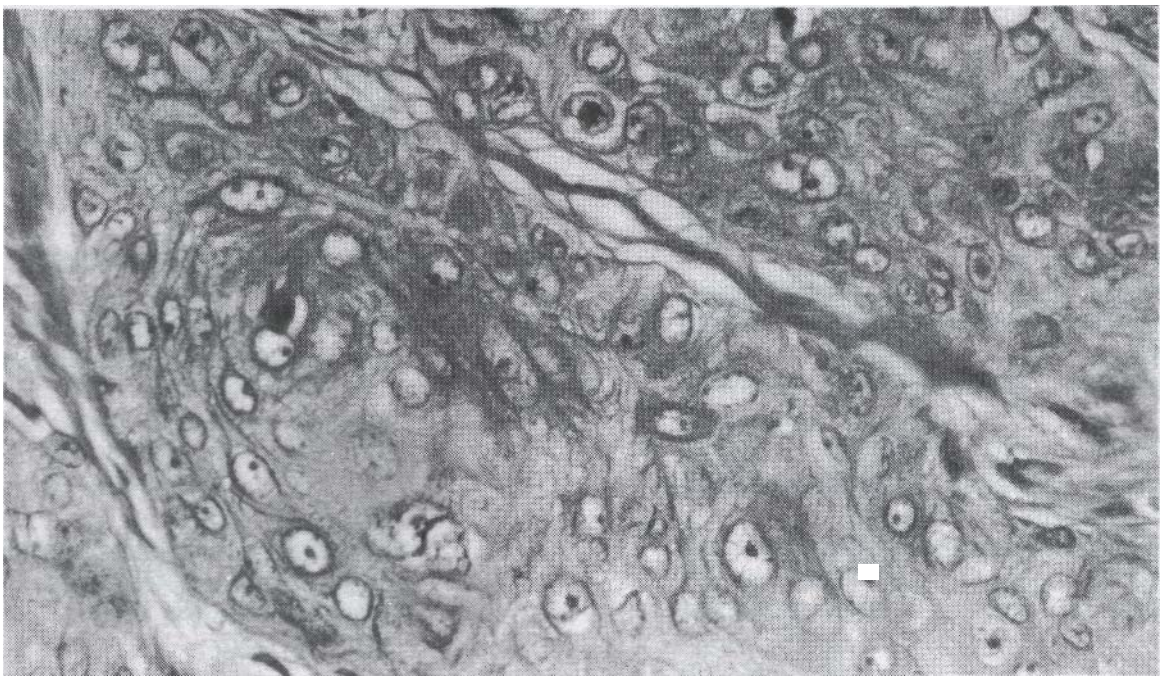

Figure 2: Nonplastinated section of the same tumor, sampled prior to plastination (hematoxylin and eosin x 400)

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