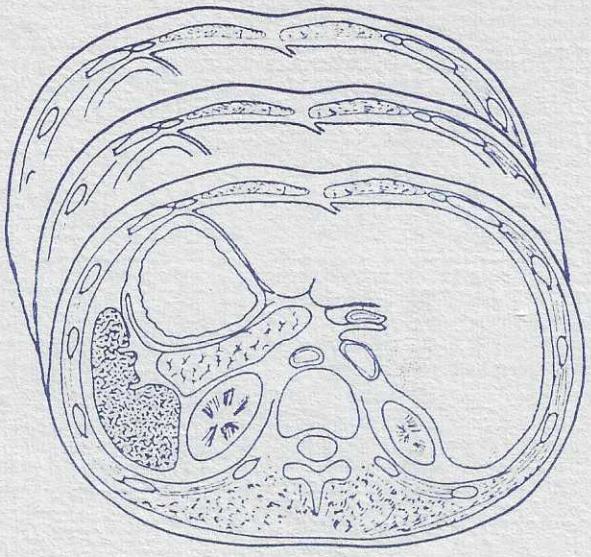
Journal of the

INTERNATIONAL SOCIETY FOR PLASTINATION



Volume 1 ---- Number 2

July, 1987

JOURNAL OF THE INTERNATIONAL SOCIETY FOR PLASTINATION

Volume 1, Number 2
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CONFERENCE COUNTDOWN

Plans are well under way for the 4th International Conference on Plastination and the First Biennial Meeting of the International Society for Plastination. These will be combined into one convention, and be hosted by Mercer University School of Medicine on March 21-25, 1988. If you have not already made plans to attend, let me remind you to do so.

The first three days of the conference will be devoted to scheduled papers and a business meeting. The following two will be occupied with small-group discussions on every aspect of plastination, including the use of plastinated specimens in all kinds of health science instruction. Ample time will be available for informal discussion with the "Heidelberg Mafia," and other practicing plastinators so don't miss this opportunity. The time of the conference coincides with that of the Macon Cherry Blossom Festival, ten days of celebration featuring parades, concerts, dances and 70,000 blooming cherry trees. The Macon Hilton, headquarters for the conference, is located in the downtown area at the very center of festival activity. Even if you bring your whole family (and the dog) there is little chance of their getting bored while you are at the conference.

Once again, we will have a display of equipment and specimens, this time featuring a step-by-step exhibit of the the latest Heidelberg technique for sheet plastination. We encourage you to bring (or send) any equipment, specimens, posters or other exhibit materials that you would like to show off to your fellow plastinators. Or, if you are having trouble with a technique, bring the messed-up specimen and get it discussed by the most experienced plastination scientists in the entire world.

POST-MEETING TRIP

We are arranging a bus excursion for after the meeting that will be both educational and a lot of fun. Those of you who sign up will be picked up at your hotel on the morning of Saturday, March 26th and taken to Orlando, Florida where you will be issued a three-day, unlimited pass to Epcot Center and Disney World. On Tuesday, March 29th, the bus will leave Orlando and return to the Atlanta airport, via Macon. Thus, you will have the option of either returning to Macon or going directly to the Atlanta airport and starting your journey home. Exact times and other information will be available soon. We are trying to arrange it so that the bus will arrive at the Atlanta airport in plenty of time for the departure of overseas flights.

A single fee will cover the cost of round-trip transportation, three nights of hotel accommodation at Orlando (based on double occupancy), buffet breakfasts, transportation to Epcot/Disney World and the three-day unlimited pass. It should amount to a memorable occasion. Family and friends, of course, are welcome.

FORMAT (AND OTHER) CHANGES

You will notice that we coaxed our column device into working for this issue. Also, we are using a new, type-set font, courtesy of the MUSM Department of Pathology. As in the last issue, we are emphasizing a down-to-earth, practical approach. One thing that we did not get around to doing is to develop more information from the membership questionnaire. And so -- this issue will

contain only a simple list of the membership. But, make no mistake; this is a better list! It has been thoroughly updated and revised. If your name remains misspelled or your address distorted, please write us with the corrections so that we can go on bragging.

VACUUM CHAMBERS

R.W. Henry of B&C Enterprises, 925 High Springs Road, Knoxville, Tennessee 37932 USA (615) 966-7380 announces that he will develop, test and service vacuum chambers for plastination. Plastination kettles of various sizes are available and custom-made chambers can be constructed for your freezer.

PLASTINATION SEMINAR

The American Association of Veterinary Anatomists is sponsoring a one-day plastination seminar on Thursday, July 16, 1987 (8:00 AM to 5:00 PM). It will be held at the University of Wisconsin School of Veterinary Medicine, Madison WI. The morning session will feature presentations on all aspects of the basic S 10 Standard Technique. Short formal presentations will be followed by question and answer sessions and a round-table discussion. The afternoon session will cover special topics in plastination, including the processing of pathologic and anatomic specimens and sheet plastination. Faculty will include Harmon Bickley, Phillip Garrett, Robert Henry, Paul Rumph and Wolfgang Weber. Registration fee is $20.00.

For information about the conference or accommodations, please contact Robert W. Henry, PO Box 1071, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901-1071 USA or phone (615) 546-9230 x328.

PLASTINATION WORKSHOPS

Iowa State University College of Veterinary Medicine will conduct a series of two plastination workshops. Workshop No. 1 will be held from September 30 to October 2, 1987 and Workshop No. 2 from October 7, 1987 to October 9, 1987. In addition to a review of fundamentals, these workshops will feature actual, hands-on experience with the S-10 Standard Technique. The number of participants is limited to 15 for each exercise but additional workshops will be scheduled for the spring of 1988 if sufficient interest develops.

For further information, please contact Wolfgang Weber, Iowa State University College of Veterinary Medicine, Ames, Iowa 50011. Phone (515) 294-2832.

ABBREVIATED TITLE

Please note that the "official" contraction for the title of our journal will be J. Int. Soc. Plastination. This designation is consistent with ISI-style abbreviations used in the Science Citation Index.

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Please read the advertisement appearing in this issue. Our thanks to Ahlburg Technical Equipment Service for this vote of confidence.

Remember, the ISP needs your involvement. Recruit a new member, write an article for the journal. Do whatever you want; but do something!

Thank you for your cooperation.

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

A VERSATILE VACUUM CONTROL SYSTEM FOR PLASTINATION

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INTRODUCTION

Piastination is a process for tissue preservation in which tissue fluid (and some lipid) is replaced with a curable polymer such as silicone rubber, epoxy or polyester. This process is undergoing rapid continual development. At present, few equipment items or systems, intended expressly for plastination, are available from commercial sources. For example, most vacuum systems in use today are home-designed and homemade.

The purpose of, this article is to describe the versatile, two-pump vacuum system presently used in our plastination laboratory. Included in this paper are goals of the project, principles of design, construction comments, cost analysis and evaluation after use.

GOALS AND DESIGN

At the outset, several goals were identified. Our plan called for: 1) relative simplicity, 2) minimal cost, 3) commonly available components and, 4) the need for only a moderate level of assembly skills. Because of the almost-universal problem of limited space, size was to be kept to a minimum. Our design was intended to incorporate the following features:

1) for versatility and reliability, vacuum would be generated by two pumps.

2) the system would permit operation of two plastination kettles, individually or concurrently

3) crossover communication between pumps and inlets would allow independent operation of either system with either pump

4) a single battery of manometers would be able to serve either system, as needed

5) traps would be employed to protect the pumps from overflow

6) the entire system would be portable, situated near eyelevel and convenient to use. A schematic diagram of the various configurations is shown in Fig 1

ASSEMBLY

After laying out the vacuum circuits and and determining the minimum area into which they would fit, we selected a wooden table from university salvage on which to assemble them. Wooden supports were attached to the table surface, creating compartments for pumps, controls and manometers. Segments of tubing were then cut to lengths sufficient to communicate between these compartments.

Tools required for assembly included a

tubing cutter, propane torch, solid wire solder (50/50 tin-lead), flux, ruler and pliers. Teflon tape was used to seal all threaded joints. Construction, including table modification, required about six man-hours.

The sediment trap and drains were assembled first, followed by the straight portion of the individual lines. Finally, the lines were connected to each other. Lines to the manometers were attached and reducers were installed for the flexible inlet tubing leading to these instruments.

Alignment during soldering was maintained by temporary braces. After soldering, the system was tested by plugging the inlets, closing all inlet valves, pumping down to 10 mm Hg and closing the pump valves. No change of vacuum was noted on either manometer after six hours.

The final configuration of the system is shown in Figure 2. Its components are listed in Table 1. Our cost for each item is included. Fittings and valves were available from local plumbing supply stores.

EVALUATION

Having used the system for forced impregnation, we offer this evaluation: Its performance has been satisfactory in all respects. Pump-down proceeds - smoothly and vacuum is maintained at less than 1 mm Hg. We find the height and arrangement of valves and manometers convenient. No liquid has Collected in the lines. The sediment traps permit opening the system at any during the pump-down sequence without disturbing the vacuum adjustment Calves. This affords quick return to an | established pressure.

We had intended that each pump be able to work either or both lines. In practice, however, we found that we could not achieve a pressure below 3 mm Hg with one pump working against the other. Placement of shutoff valves at positions A and B in Figure 3 would permit this and would prevent pump oil backflow.

Had sufficient floor space not been available, the entire unit could have been built as a wall unit by mounting the lines on a vertical backboard.

The flexible tubing, manometers and pumps would be common to any system. Thus, the cost analysis presented in this paper applies to our project and is intended to serve only as an example. Since the table was obtained from unversity surplus and the lumber was scrap, they represented no cost. Total cost of tubing, valves and other fittings was $47.23.

SUMMARY AND CONCLUSIONS

We have described a versatile vacuum system for use in plastination. It was constructed at minimal cost, using commonly available components. We are entirely satisfied with its performance in routine use.

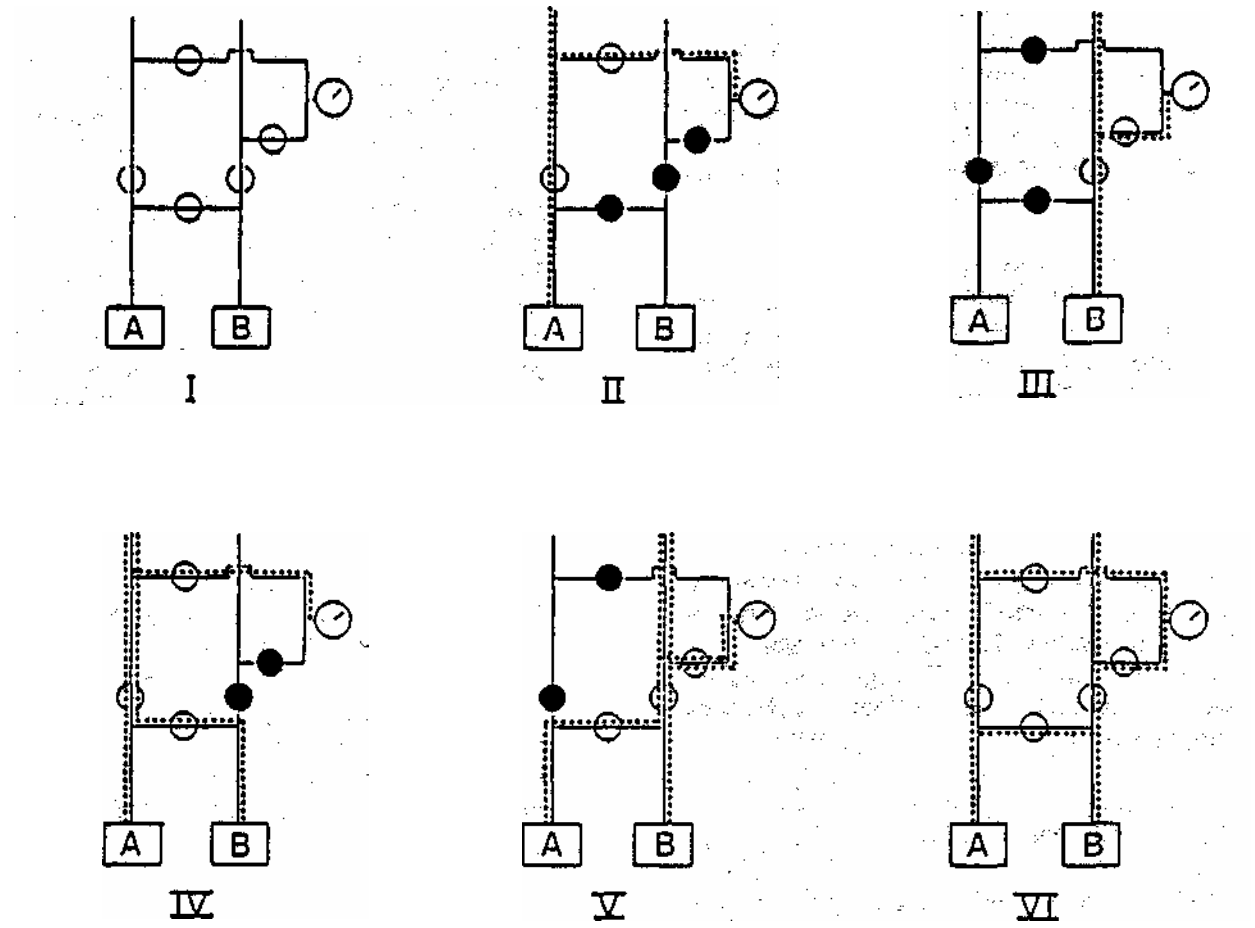

Figure 1. Diagrams of the different configurations of the vacuum apparatus. The squares represent the vacuum pumps; the open circles, the pen valves; and the black circles, the closed valves. A vacuum gauge is represented by a circle at the right of each diagram. The different configurations are: I, basic diagram; II, pump A activating line A; III, pump B activating line B; IV, pumps A and B activating line A; V, pumps A and B activating line B; VI, pumps A and B activating lines A and B.

TABLE 1

ITEM	NUMBER	UNIT PRICE	COST
Pumps, Sargent- Welch Duo-seal, Model #1400	2	$825.00	$1650.00
Bennert manometer	1	$155.00	$155.00
Vacuum gauge, Bourdon type	1	$24.00	$24.00
Parker CPI needle gas valves 1/8"	2	$2.98	$5.96
"Apollo" Bronze gas ball valves, 1/2" sweat Conbraco Industries Inc.	7	$4.00	$28.00
1/2" nominal hard copper 90° elbow	10	$0.11	$1.10
1/2" nominal hard copper tee	9	$0.20	$1.80
1/2" to 3/8" nominal hard copper reducers	6	$0.24	$1.44
3/8" hard copper tubing (price per foot)	12"	$0.31	$0.31
1/2" hard copper tubing	10'	$0.27	$2.70
Female adapter (1/2" copper tubing to 3/8" NPT)	2	$0.67	$1.34
3/8" to 1/8" NPT steel bushing (reducer)	2	$0.45	$0.90
1/8" NPT all thread nipple	2	$0.31	$0.62
Tygon Tubing, R3603 - ID 1/4", OD 5/8" wall thickness 3/16"	5'	$2.50	$12.50
1/2" copper pipe hanger straps	6	$0.15	$0.90
Bell reducer from 1/2" to 3/8" NPT	1	$0.91	$0.91
FTG x MIP copper adapter (1/2" copper tubing TO 1/2" NPT steel pipe)		$1.25	$1.25

1

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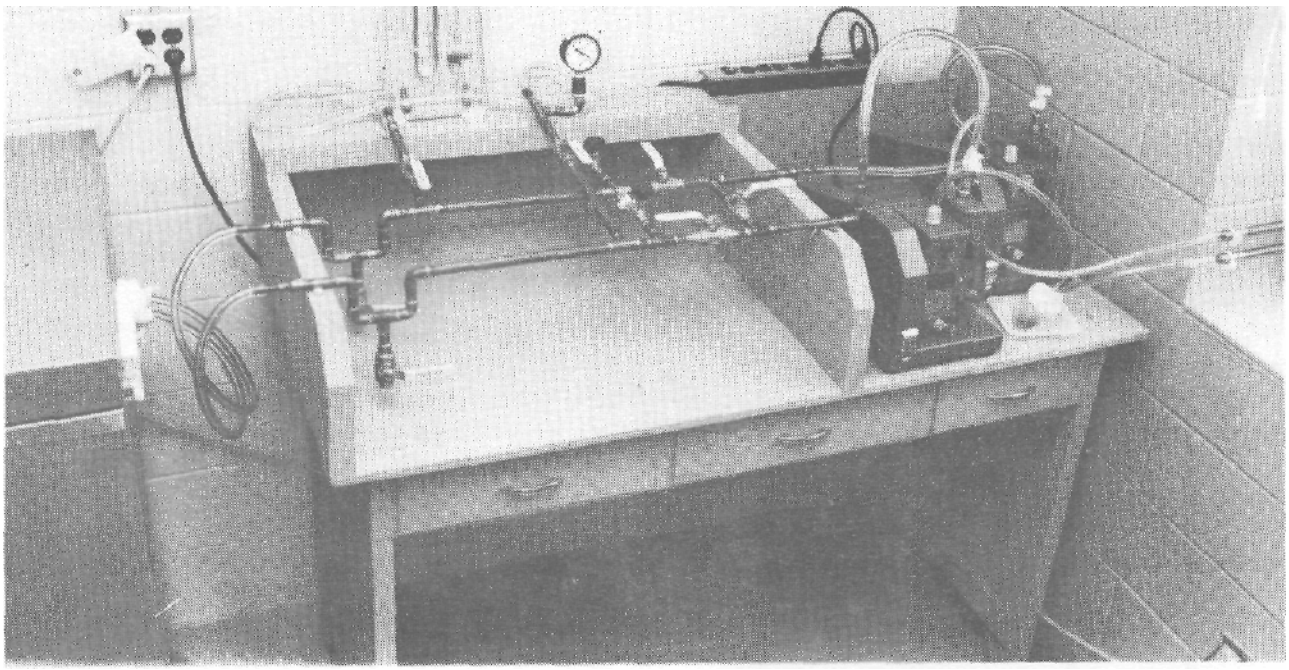

Figure 2. A photograph of the vacuum apparatus. The exhaust lines to the right are connected to copper pipes which pass through the window frame to the outside. A small cup has been rigged to trap oil vapors passing out the exhaust line in use.

DURABLE LABELS FOR PLASTINATED SPECIMENS R.

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INTRODUCTION

Ever-increasing interest has been shown in techniques of plastination and its use, however little has been presented on the rather simple need for low-cost, professional-appearing labels for plastinated specimens. This article will summarize the method used at Mercer University School of Medicine for the production of labels for all specimens, both plastinated and conventional.

We have found this system convenient in that only simple, commonly available equipment is needed. For example, one must have the following on hand:

l)clear or blue transparency sheets of the kind used on photostatic copiers ($0.25 per 8.5 x 11 inch sheet)

2)heat-sealable plastic pouches of the kind used with a laminating press ($0.30 per 3 x 4 inch pouch)

3)metal clasps of the kind used to attach lures to a fishing line ($.02 per clasp)

4)heavy duty utility knife or scissors to trim plastic labels

Additionally, one should have access to the following common office equipment:

l)typewriter or computer with printer

2) photostatic copy machine

3) heat laminating press ($180.00)

4) hobbyist's variable speed drill ($25.00)

5) single-hole paper punch of 1/8 inch diameter ($2.00)

METHOD

Information to be included on the label is typed on a standard sheet of white paper. This should include the identity of the specimen by name of institution, accession number, anatomic site and pathologic process. We have found it desirable to limit such information as much as possible so that the size of the tag can be kept to a minimum. This becomes particularly important when working with small specimens.

Several "rules of thumb" are helpful for the typist. Allow abundant space between labels and the edge of the paper for ease of duplication. Two vertical columns of labels will fit on one 8.5 x 11 inch piece of paper if the width of each column is limited to 30 characters. This leaves six blank columns for margins and the area between labels. Labels should be five lines in height and separated by two lines.

Up to nine labels can occupy a single column (more may be possible on standard

European paper). Thus, at least 18 labels can be typed on one page. Use the smallest type font available so that the largest amount of information can be squeezed into the least area. If the font is small enough, perhaps even three columns of labels could be accommodated on one page. Some photocopiers are able to reduce images up to 1/2 of their original size. This feature should prove attractive for the unusually frugal.

Although small size is desirable, care should be taken to assure that characters are of a size that can be read without magnification. Do not reduce them to microfiche at the copy station. We would suggest that you experiment with various copier settings using white paper to arrive at the best contrast and zoom settings rather than expensive transparency sheets. Duplication is perhaps the most important step in this process since it will determine the quality of the final product.

Once the labels have been imprinted on the transparency sheet, carefully divide it into four equal rectangles and trim away excess plastic so that each of these fits into a 3 x 4 inch plastic laminating pouch. Straighten the rectangle neatly and seal the pouch according to the instructions provided with the laminating press. This usually includes placing the pouch in an aluminum foil sleeve and inserting it between the hot rollers of the laminator.

Perform this step twice to assure the very best seal. Cooling should be accomplished on a hard, flat surface using a book or other flat, heavy object to prevent warping.

Separate the labels in a single laminate using heavy-duty scissors or a utility knife. Even better, use a paper cutter to assure straight edges and square corners. This instrument is usually available in photography laboratories or the copier room. Use caution, however; this step may require a little practice.

Next, a 1/8 inch hole is punched in the upper right area of the label so that a metal clasp can be inserted and used to attach it to the specimen. Care should be taken to place the clasp in a strong area of the specimen. If possible, attach it through a blood vessel or some other location with high tensile resistance. Bony specimens may require creating an artificial hole for the clasp. We have found that a hobbyist's hand-held drill works well for this purpose.

Inevitably, some specimens will defy all efforts to apply the label in a satisfactory manner but, in general, we have found this method accommodates most kinds of tissue and is an excellent adjunct to museum and plastination techniques.

If labels are prepared in significant numbers, our method becomes quite cost-effective. A simple analysis reveals the following:

Supplies (cost per label):

transparency sheet	$0.01
plastic pouch	$0.07
metal clasp	$0.02
white paper	$0.01
Subtotal:	$0.11 per label

Equipment (depreciated for 3 years @ 500 labels/year)

laminating press	$0.12

miscellaneous	$0.01
Subtotal:	$0.13 per label

Labor (3 minutes per label @ $6.00 per hour)

Subtotal: $0.30 per label

Grand Total Cost per Label: $0.54

SUMMARY AND CONCLUSIONS

A method is described for producing durable, professional-appearing specimen labels of high quality. The benefits of these labels far outweigh the cost of their production, even with limited use. If produced in large quantity, their expense can be rendered even lower.

A list of our current vendors is as follows:

1) Art Wire and Doduco
(Attention: Mr Lou Coble)
9 Wing Drive
Cedar Falls, New Jersey 07927
USA

Order "Large Safety Snap #903"
The minimum order is 25 gross and costs $80.00

2) General Binding Corporation
One GBC Plaza Northbrook,
Illinois 60062 USA

Order "Jumbo Laminating Pouches #1210-46"
They are supplied in boxes of 100 at $39.50 per box

4" Mini-Lam Heat Sealing Laminator
Costs $180.00 at time of this writing

3) Local Office Supply Store

Transparency Sheets for Overhead Projector (for use in Canon Copier)
Sparco #95000

Costs approximately $30.00 for 100 sheets

PLASTINATION OF THE HEART

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INTRODUCTION

Processing of a human heart is one of the most demanding techniques in plastination. Therefore, I suggest that it be carried out in the most systematic way possible. As a reflection of this need to be methodical, I will arrange this paper as follows: First, I will present an overview of the principal steps. Second, I will comment on each of these steps. Finally, I will present a short review of these steps in the form of a timetable.

OVERVIEW

1) Removal: A heart that has not been removed properly will be difficult to plastinate.

2) Dilatation: Hearts are dilated with tap water under hydrostatic pressure to relax the muscle. This opens the chambers and provides a clear view of internal structures after plastination.

3) Color Injection: Injection provides better definition of coronary vessels and results in a teaching specimen with more information.

4) Fixation: Hearts are fixed in two stages, first under hydrostatic pressure, then by immersion.

5) Dehydration: This also requires two stages. First, the heart is pre-dehydrated under hydrostatic pressure; then it is completely dehydrated by freeze substitution.

6) Degreasing: This is the latest step to be recommended. Degreasing reduces the likelihood of white spots appearing in the adipose tissue with time.

7) Forced Impregnation: This is the main step. It takes place under vacuum, according to the S- 10 Standard Technique.

8) Cutting: Internal structures are revealed by sawing the heart into two halves with a band saw or by cutting windows in the heart wall.

9) Curing: The heart must be cured in its natural shape since this step will render the specimen rigid.

REMOVAL OF THE HEART

Now we will start the whole procedure over again and take the heart out of the body properly. First of all, it is absolutely necessary that the heart itself remains undamaged during dissection. Also, the great vessels must be cut far enough from the heart so that a workable length remains with the specimen.

The inferior vena cava should be cut out of the diaphragm, leaving a 1 cm margin of muscle still attached if possible. The brachiocephalic trunk, the carotid artery and the left subclavian artery are cut, with at least 2 cm of vessel remaining

attached to the heart. The aorta is cut about 3 cm distal to the decending limb of the arch, again, if possible.

DILATATION

Dilatation is accomplished with tap water under hydrostatic pressure. All vessels must be tied off except those to be used for admitting water and, later, the fixative fluid. In general, vessels should be closed by ligating cork stoppers into the lumen. There are exceptions, however.

1) A polyethylene tube is ligated into lumen of the superior vena cava and one of the pulmonary veins.

2) The inferior vena cava is left open.

3) Aortic branches and small vessels are tied off directly.

Special recommendations resulting from personal experience are:

1) Only stoppers made of real cork should be used. They are resistant to all solvents used in plastination.

2) Stoppers are inserted into vessels upside down, i.e., the very opposite of the way they are used in bottles. This prevents their being forced out by the pressure.

3) Sometimes the stoppers must be pinned to the vessels before thread can be tied around them.

Polyethylene tubing with a diameter of about 1 cm. should be used in the superior vena cava and pulmonary vein. A small detent melted into the wall of the tube with a soldering iron will afford a better hold. Care should be taken because these two plastic tubes remain in place and will be used over and over, even for dehydration.

With all corks and tubing installed, the tube in the pulmonary vein is connected to a water tap equipped with an overflow pressure-regulating device. Hydrostatic pressure of about 40 cm is used. We dilate only the left chamber via, the pulmonary vein. This is because we have experienced rupture of the right ventricular wall when it is subjected to this same pressure. This procedure results in complete rinsing of coronary vessels through the ostia at the root of the aorta and leads to permanent relaxation of the left myocardium. Dilatation pressure is maintained until the heart is noticeably flabby. This usually takes about 24 hours.

COLOR INJECTION

Colored plastic is now injected into the vessels supplying the heart. Red will be injected into the arteries and blue into the veins. (Doing it the opposite way would be silly.) Since we have left the inferior vena cava open, we will have ready access to the coronary sinus and middle cardiac vein.

To be able to inject the coronary arteries, the two longest branches of the aorta must be untied. Using these openings, cannulae with cone-shaped tips are introduced into both coronary ostia. With bent forceps, a path is "dug" through the fat tissue around the stems of the arteries. This is done very carefully. Once the cannulae are inserted, a thread is pulled around the stem of each artery using these same bent forceps. The purpose of this thread is to hold the cannulae in place during injection and to tie off the artery when it is complete.

Next, a thin piece of polyethylene tubing with a shorty somewhat thicker piece of

rubber tubing attached to its end, is pressed into the coronary sinus. The rubber tubing will serve the same purpose as the cone on the end of a cannula; it will assist in holding the tubing in place. This tubing remains in situ after injection. Cone-tipped cannulae are not used here because the wall of this vessel is so delicate that it almost-certainly would be damaged when digging with the bend forceps. Finally, a cone-tipped cannula is introduced into the middle cardiac vein. This canula should not be fixed in place.

Having inserted all instruments needed for injection, the time has now come to mix the colored resin. Meanwhile, the precious heart should not be left unattended. It should be immersed in water until the time of injection.

The resin to be injected into these vessels is BIODUR E 20 RED AND BLUE. Approximately the following amount of each will be needed for a single heart:

ARTERIAL INJECTION MIXTURE

BIODUR E 20 RED -40.0 grams
BIODUR E 2 Hardener - 18.0 "
methylethyl ketone (MEK) 8.0 "
BIODUR AT 10..... 8.0 "

VENOUS INJECTION MIXTURE

BIODUR E 20 BLUE ------- 10.0 grams
BIODUR E 2 Hardener — 4.5 "
methylethyl ketone (MEK) 1.0 "

These components must be weighed with accuracy and the mixtures stirred thoroughly for at least 5 minutes. The tray on which the injection will be performed should be covered with plastic foil to protect it from resin leakage. These materials are very sticky! All of the following tasks should be carried out with alacrity so that injection can be completed before the resins start to become viscous.

We will begin with the arteries. Using a 10 ml disposable syringe, injection is performed slowly and carefully with light, constant pressure. Minimal pressure is used to preclude extravasation of the injected material. First, about 1 ml of red resin is injected into the right coronary artery. This resin can be encouraged to fill the smaller branches by rubbing the vessels with your fingertips.

At this point a quick check should be made to see that the cannula remains in the middle cardiac vein. The small arteries around this vein should now be filled with red resin. If everything is in place, arterial injection is stopped at this point and venous injection started.

Since the small arteries around the middle cardiac vein are now relatively easy to see, a curved needle can be employed to place an atraumatic suture around this vein without puncturing these little vessels. Having placed this suture, the cannula in the middle cardiac vein is fixed in place with finger pressure (do not use the suture yet) and about 1 ml of blue resin is injected into this vessel. The cannula is now removed and the vein tied off with the suture as quickly as possible. About 8 ml of blue resin is now injected into the coronary sinus through the previously installed rubber-tipped plastic tubing, the syringe removed and the tubing bent double and tied to seal it closed.

Returning to the arteries, about 20 ml of red resin is slowly injected into the right artery and 40 ml into the left. This is done in increments, alternating between the two as the injection proceeds. Having developed such skill, this should prove a mere trifle of a challenge. Once this has been completed, step back and admire your work. If these

directions have been followed to the letter, the results will be blindingly beautiful.

Experience has shown me that a few pitfalls lurk in wait for the unwary:

1) If the heart muscle looks too brown-colored, you may be dealing with slight myocardial autolysis. This means that injection will have to be done unusually slowly, Softly and tenderly or resin will be forced out of the weakened vessels and extravasations will appear.

2) If an unexpected extravasation does appear in spite of this gentle handling, it must be vented with a needle to the outside of the heart. Otherwise, it will spread beneath the epicardial surface and cause some very strange-looking ecchymosis, the pathogenesis of which will be difficult to explain to your students.

3) And what if one of the vessels that you are injecting appears to be leaking? You may want to close this sneaky outlet with a small clamp. But, as long as plastic is not accumulating in the tissue, the condition may be tolerable. If it is leaking to the outside, it may smear the tray, the heart, the instruments and your gloves but this can be washed away. Mr. Grant Dahmer (UTHSC at San Antonio, Texas USA), who will not abide such a mess, tells me that he avoids it by injecting the heart under water. The leaking resin simply floats away from the heart and the location of the hole can be easily determined.

4) Our final pitfall, branch-missing, is, alas, quite common. Rather than having to claim later that your heart contains a developmental anomaly, check to make sure that all branches have been injected. If you detect a hold-out, you may have to resort to some rather clever maneuvers to get it. Loosen the thread holding the cannula and keep trying until the little devil capitulates.

Another thing that must be mentioned here is that, following color-injection as described, methylene chloride must never be used in any subsequent step. Should this be done, the resin in the vessels will simply dissolve, even after curing. Acetone is fine but never use methylene chloride on an injected heart.

Let us suppose now that all possible complications have been conquered and the injection finished successfully. The first thing to do is to rinse your cannulae with acetone before the resin cures. Acetone will dissolve the resin so long as it is not cured and makes an excellent solvent for washing instruments.

Next, the heart will need some rinsing with tap water to remove the resin that has leaked into its interior. If the left side is rinsed powerfully via the pulmonary vein, resin remnants will leave via the aortic branches. The right side should be rinsed with 50 ml of acetone via the inferior and superior vena cava to remove any ectopic blue resin. This is followed by a tap water rinse.

This having been completed, the two aortic branches that have been opened are tied off and the inferior vena cava is occluded with a cork stopper. The heart should now be capable of holding pressure. To make sure of this, it is tested with about 40 cm of tap water. If it is nicely water-tight, fixation is begun.

FIXATION

In the first step of the this process the heart will be fixed while dilated by hydrostatic pressure.

The two polyethylene tubes in the pulmonary vein and superior vena cava are connected to a reservoir of 20% formalin placed 80 cm higher than the specimen. This step will take about 1 or 2 hours, therefore each heart will require approximately 10 liters of fixative. It should be performed as soon as possible after injection while the resin is still flexible enough to permit the heart to expand.

The second step in the fixation process takes place by immersing the heart in this same formalin solution for one week.

In earlier publications, the next step recommended was bleaching. This is no longer approved because, on occasion, it appeared to dissolve some of the colored resin.

Having fixed the specimen by immersion for one week, residual fixative is thoroughly removed from the heart chambers. This is best done by connecting the heart to the tap water dilatation device and rinsing for about one hour. Four advantages will result from this:

1) Formalin odor will be reduced.

2) Further work may be done on the heart while it is under water pressure. For example, the Botalli ligament may be dissected out or lymph nodes removed.

3) The heart can be inspected once again for pressure leaks.

4) Dehydrating medium used in the next step will be kept relatively free of formalin.

DEHYDRATION

Just as in fixation, the procedure by which the heart is dehydrated is divided into two subroutines. For the first, 15 liters of acetone, precooled to -20°C will be required.

The acetone is placed in a reservoir 80 cm higher than the specimen which is suspended directly below it in a receiving tank. The outlet of the reservoir is connected to the two polyethylene tubes installed in the heart and the acetone is allowed to flow from the reservoir, through the heart into the receiving tank. As soon as the reservoir is empty, the pre-dehydration step is finished.

This step is used because it affords optimal maintenance of the natural shape of the organ. Thus, the position of all vessels should be checked before beginning. No twisting or compression is allowed.

The following step is done as quickly as possible to prevent drying. Remove the heart from the pressure device and take away all ligatures, stoppers and tubes. Quickly immerse the specimen in -25°C acetone, making sure that the natural shape is maintained. Suspend it carefully for a few minutes until it is frozen in this "natural" shape.

Freeze substitution of a human heart takes about 37 days on the average.
For details about this procedure see other articles in this issue.

DECREASING

This will be a short section. Degreasing is accomplished by simply taking the heart (still in the final change of acetone) out of the freezer and leaving it

at room temperature for one week.

Adipose tissue of the human heart is very difficult to impregnate properly. Without this step, incomplete impregnation of fatty tissue will occur and white spots will appear, marring your otherwise-perfect preparation. Some degreasing will occur during freeze substitution but not to the same extent that is possible at room temperature. Remember, the more fat you get rid of, the fewer impregnation problems you will have later. (Sounds like an advertisement for Weight-Watchers.)

FORCED IMPREGNATION

Impregnation of the heart is almost-exclusively performed with BIODUR S 10 silicone rubber, nowadays.

Detailed information about the S 10 Standard Technique is available in the Heidelberg Plastination Folder so I will not repeat it here. I will include only a few items of data on temperature, time and quantity.

Approximately 0.5 Kg of polymer will be needed for the impregnation of one heart. But a larger quantity is needed for proper immersion. Therefore a total of about 3.0 Kg will be needed for one impregnation procedure. Impregnation is carried out under vacuum at -25°C and takes about three weeks. At the end of this time, the heart is removed from the kettle, excess polymer is poured out of the chambers and it is placed upside down on a grid above the kettle for a few hours to complete draining. All of this is done at -25°C.

CUTTING

The heart must now be opened. This has a number of assorted purposes. For example, it will permit easy viewing of the structures covered by endocardium. Second, it will allow inspection of the endocardial surface during curing so that care may be taken to remove exuded polymer. Third, if cut in a standard way, it will facilitate anteroposterior orientation of the heart during its use in teaching.

The Heidelberg technique is to cut the heart into two halves with a band saw, using a toothless, wavy blade. You will need some experience and skill to do this right. The intention is to cut the heart in such a way that the posterior half contains:

1. intact papillary muscles
2. the inferior vena cava
3. the two inferior pulmonary veins (for orientation)

The anterior half should provide a clear view of:

1. the other vessels
2. the auricles
3. aortic and pulmonary valves

Of course, you are free to cut windows into the heart wall (instead of cutting it in half) if you so desire. This is easier to do in a standard way but, in my opinion, results in a less-useful specimen. I realize that this is a matter of taste, however.

By whatever method it is done, the cut heart is allowed to drain for a few more hours at room temperature. The chambers are then wiped free of excess silicone polymer and injection resin.

CURING

We have now reached the last step in the Plastination process, in preparation for this step, a number of minor chores must first be gotten out of the way.

Heart chambers are stuffed with paper and the great vessels are equipped with stoppers. The two halves of the heart are reassembled and pinned together. These two steps will help maintain the "natural" shape of the organ.

A large plastic pan or tub is "furnished" to serve as the gas cure chamber. The bottom of this chamber will be covered by a grid that will hold the specimen off the flat surface and permit complete circulation of the curing gas.

The heart is placed on a piece of polypropylene foil to prevent sticking to the grid. Several containers of BIODUR GAS CURE S 6 are placed in the chamber, opposite one or two aquarium pumps. These pumps are elevated as much as possible above the level of the containers. Their output is led into the gas cure solution via fine plastic tubing. Circulation provided by these pumps will greatly accelerate the curing process because:

1) bubbles produced in the gas cure fluid speed up the release of curing gas from solution and raises the concentration of this gas in the chamber

2) the pumps draw the gas (which is heavier than air) from the bottom of the chamber up to their level and greatly enhance its circulation

Small trays of calcium chloride (a drying agent of impeccable reputation) are also placed in the chamber to provide for the control of moisture during curing.

Finally, the chamber is closed air-tight with, for example, spray glue and a sheet of plastic foil and the aquarium pumps are started to work.

At the beginning of the curing process, special attention must be paid to proper surface treatment. Curing of a heart involves a great deal of busy work on the first day. A lot can be gained or lost at this stage. For this reason, if time is not available to perform this step properly, the heart should be put in a plastic bag and stored in the freezer. Or, if preferable, the heart could be left in the impregnation kettle until time becomes available.

If curing is to be carried out, place the hearts in the chamber, start the pumps and return every .hour to wipe the surface free of excess polymer and turn the heart over. This must be done with regularity. It is advisable to start the curing process in the morning because, by the end of the day, polymer will have stopped oozing out and the heart can be left in the gas cure overnight without any problem.

After two or three days, separate the heart halves, remove paper and stoppers and put the heart back into the curing chamber for another two or three days to harden the inner surface. Gas cure is complete when the heart is dry and no longer tacky to the touch.

The final step is to store the heart in a closed container to provide aftercure. The high concentration of curing gas at the surface of the specimen diffuses to its interior and hardens the remaining uncured polymer. Aftercure is finished when the specimen no longer smells of curing gas.

REVIEW

And that's all there is to it, Dear Reader. A human heart has been transformed into a sophisticated specimen. Now for the timetable:

removal	
dilatation color injection rinse	24 hours
fixation (pressure)	2 hours
fixation (immersion)	7 days
rinse make final preparations	1 hour
pre-dehydration, open all vessels	1 hour
dehydration by freeze substitution	37 days
degreasing	7 days
impregnation with BIODUR S 10	20 days
drain excess polymer in cold cut heart	1 hour
drain excess polymer at room temperature stuff with paper and stoppers reassemble heart-halves with pins	3 hours
curing with BIODUR GAS CURE S 6 remove paper and stoppers	2 days
cure inner surface	2 days
store in closed container for aftercure (but the heart can be used for teaching purposes)	months

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PLASTINATION OF AN INTEGRAL HEART-LUNG SPECIMEN

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INTRODUCTION

Heart, lung, or heart and lung preparations make impressive teaching specimens, for studying both anatomy and pathology (1,2,3). However, it is frustrating if your specimen does not turn out as beautiful as you desire. All steps of preparation are important but collection, dilation, color injection, and curing may be the crucial steps. Regardless of the outcome, most if not all, plastinated specimens are superior to air or freeze dried, formalin-stored or paraffin-embedded specimens, and artificial models.

Following is an outline of the procedure for preparing plastinated heart, lung, or integral heart-lung specimens:

1) Collection of specimen.
2) Removal of blood from specimen.
3) Dilation using hydrostatic pressure.
4) Separation of heart from lung and ligature of pulmonary vessels.
5) Color injection of coronary vessels.
6) Fixation using hydrostatic pressure.
7) Dehydration (freeze substitution).
8) Impregnation with S 10.
9) Sculpturing heart.
   Inflate lungs with air.
10) Gas cure (S *6).*

COLLECTION

Caution must be used when exposing the heart and lungs so that the serosa or parenchyma of the heart and lungs is not damaged. It is beneficial to start by freeing and reflecting the trachea caudally to the cranial thoracic aperture (inlet). Continue reflection into the thoracic cavity and transect the brachiocephalic trunk, subclavian or carotid arteries, and cranial vena cava near the inlet (as far away from the heart as possible). The trachea serves as a means to grasp the viscera (heart and lungs) without damaging it. As the trachea is pulled caudally, the vena azygos and descending aorta are transected and finally the sternopericardial ligament and caudal vena cava. After removal from the thoracic cavity, the pericardial sac is dissected free, leaving a one centimeter stump attached to the base of the heart. This stump is left to prevent damage to the atria and pulmonary vessels. It may be removed after fixation or curing.

REMOVAL OF BLOOD

The caudal vena cava and a pulmonary vein are cannulated. A ten centimeter piece of tygon (polyethylene) tubing (diameter suitable to fill the lumen of

the vessel) is placed into the caudal vena cava and ligated it in place. An incision is made in a pulmonary vein, and a tube is placed through the incision toward the left atrium and ligated (Fig 1).

Tap water under moderate pressure is used to flush blood from the heart and lungs. One to two hours is usually sufficient. At 15-30 minute intervals, the aorta and cranial vena cava are gently occluded to increase intraorgan pressure and hence achieve better flushing. The organ is flushed until the heart is free of clots, begins to blanche, and the lungs are white. A water tank placed at least 20 centimeters above the organs may be used for a pressure controlled source of water.

DILATION

The heart is dilated to increase chamber size. A systolic or contracted chamber does not allow visualization of the internal anatomy. Therefore, it is desirable to dilate and mimic the diastolic state. To accomplish good dilation, all cut vessels (descending aorta, cranial vena cava, azygos vein, brachiocephalic trunk, left subclavian artery) must either be ligated or preferably closed using a cork stopper (4). The stopper (larger end first) is placed into the vessel and secured by a ligature around the vessel midway on the cork. This prevents it from being pushed out by the increasing pressure in the heart. The stoppers must be prebleached to prevent staining of the vessels by the dark resins in cork. Stoppers are bleached in 5% hydrogen peroxide solution for one week, removed and allowed to dry. This is then repeated for one additional week.

The sealed heart is connected, via the two previously placed cannulas, to a water reservoir. Keeping the reservoir in a walk-in cooler and hence using cold water(5°C) may retard autolysis. Depending on the thickness of the heart chambers and the amount of autolysis present, the height of the reservoir above the heart may be varied from 10-40 centimeters to regulate pressure. Generally the heart is dilated at room temperature (20°C). Occasionally we choose to dilate the heart in a cool environment (5°C). Usually the atria and right ventricle dilate within 12 hours. To get complete dilation including the left ventricle, cat and dog hearts require 1-2 days at room temperature. Horse, cow, and pig hearts may take 2-3 days.

To get maximal dilation it may be necessary to separate the heart from the lungs or place a cork in the trachea. Carefully separate the pulmonary arteries and veins (one to each lobe) from the lung parenchyma and ligate or cork. Continue dilation until the left ventricle expands.

COLOR INJECTION

Once the heart is dilated, red and blue colors (Biodur E 20) may be added via the coronary arteries and sinus respectively. The cork stoppers and cannulas are removed from the aorta and venae cavae. A cannula (4) is directed through the descending aorta or brachiocephalic trunk into the ostium of a coronary artery. A specially designed cannula (Fig 2) makes the injection easier. A ligature may be placed around the artery to hold the cannula in place and to prevent leakage of color around the cannula, or it may be held in place by firm digital pressure. We prefer the later. E 20 (an epoxy resin) plus E 2 (hardener) are thoroughly mixed in a ratio of 6.5 ml to 3 ml in a 12 ml syringe. One ml of acetone may be added to this mix (making it less viscous) to assure greater filling of the capillary

bed. Attach the syringe to the cannula and use constant, firm digital pressure for proper filling of the arteries and capillary bed. 20 ml of mix is needed to fill the average dog heart (12 ml - left coronary and 8 ml - right coronary). After injection is completed, the cannula is removed, and the artery is ligated or clamped with a small curved hemostat.

The coronary sinus is cannulated using an appropriate size tube or cannula, which is introduced through a vena cava. No acetone is added to the blue mix (6.5:3). The venule side of the capillary bed has less resistance. Hence it is easy to overfill and get a blue heart. Usually 10 ml is enough to fill the great cardiac vein. Gentle massaging, rather than increased pressure, is used to work the color the length of the vein. The middle cardiac vein may be cannulated and filled separately. However, blue color may be massaged around the apex through anastomses with the great cardiac vein. The middle cardiac vein will need to be ligated or clamped off with a hemostat after filling, as the coronary sinus or its cannula must be. The epoxy mix hardens in 30 minutes. After injection of the colored polymer mix, the heart chambers are flushed with water and possibly acetone to remove any E 20 leakage.

FIXATION

The corks and cannulas are replaced and the heart submerged in fixative. A reservoir of cool fixative (10-20%), placed a few centimeters above the heart, is used to fill and again hydrostatically redilate the heart before the polymer sets up. Thus the heart is fixed in a dilated state. Small (thinner walled) hearts need to fix only 4-5 days, but may be stored in fixative for longer periods. Larger hearts should be fixed for appropriately longer periods.

The lungs whether attached to or separated from the heart are dilated by filling with 10% fixative via the trachea. The trachea is closed by inserting a cork into the trachea (larger end first) and ligating it in place. The lungs are placed in 10% fixative and covered with a towel or weighted to assure total submersion.

DEHYDRATION

The cork stoppers are removed and the cannulas opened. The fixative is flushed from the specimens using tap water flowing up from the bottom of the container for two to three days. The preferred dehydration method is freeze substitution (5) which minimizes shrinkage. The specimens are submerged in -25°C acetone. At least three changes are used to assure 99% dehydration.

IMPREGNATION

After dehydration, the heart is placed from the acetone into the polymer mix(S 10 + S 3). Complete impregnation is preferred for heart preparations without lungs attached. We prefer to use incomplete impregnation 10 + S 3 + 5-10% xylene) for lung specimens whether separated from or left attached to the heart. Incomplete impregnation of specimens decreases the time required to manicure the specimen during the curing period, and the lung is spongy and more life like.

After impregnation and removal from the cold polymer (S 10 mix), the heart and lungs are allowed to warm to room temperature. The excess polymer is again allowed to drain from the organs.

SCULPTURING OF THE HEART

Cutting windows in the heart to view the

internal anatomy can be done at various times (just prior to or after impregnation or after curing). We prefer to sculpture the heart after impregnation. Make the windows to best demonstrate the desired area or structure you wish to highlight. Windows can be enlarged after curing. Windows made before curing allow for manicuring of the chambers, which is nearly impossible without windows.

GAS CURE

In preparation for the polymerization, the specimens need to be prepared in the following manner: The major vessels of the heart may either have stoppers replaced in them to maintain the contour of the vessels (4) or we prefer to roll lint free paper towels (Teri Wipers, Stock # 34770, Kimberly-Clark Corp, Roswell, GA 30076, USA) into rods and insert the rolled towel into the vessels. This maintains the contour of the vessel and aids in manicuring by absorbing excess polymer. The opened chambers are packed with paper towels to maintain contour and absorb excess polymer. These towels need to be changed every three to six hours.

A gentle flow of air is used to evacuate excess polymer from the pulmonary alveoli and dilate the airways.

Both the lung and heart, after the above preparation, are placed in a closed chamber containing gas curing agent (S 6). It is desirable to continue inflating the lung while in the gas cure. Using an aquarium pump, to increase the amount of volatilized gas cure available in the chamber, will decrease the time a specimen needs to be exposed to gas cure. Removal of excess polymer by wiping and changing toweling is critical 14-24 hours after the onset of exposure to gas cure. It may be beneficial to begin gas cure just before leaving for the day. The next morning is the appropriate and necessary time to manicure specimens. Manicuring should be done three or four times during this day. By evening if the surface of specimens is still seeping, the specimens may be placed in a plastic bag and in a freezer overnight. Gas cure may be continued the next day. Usually the surface ,of specimens is cured and weeping of polymer ceases after 24-36 hours of exposure to gas cure.

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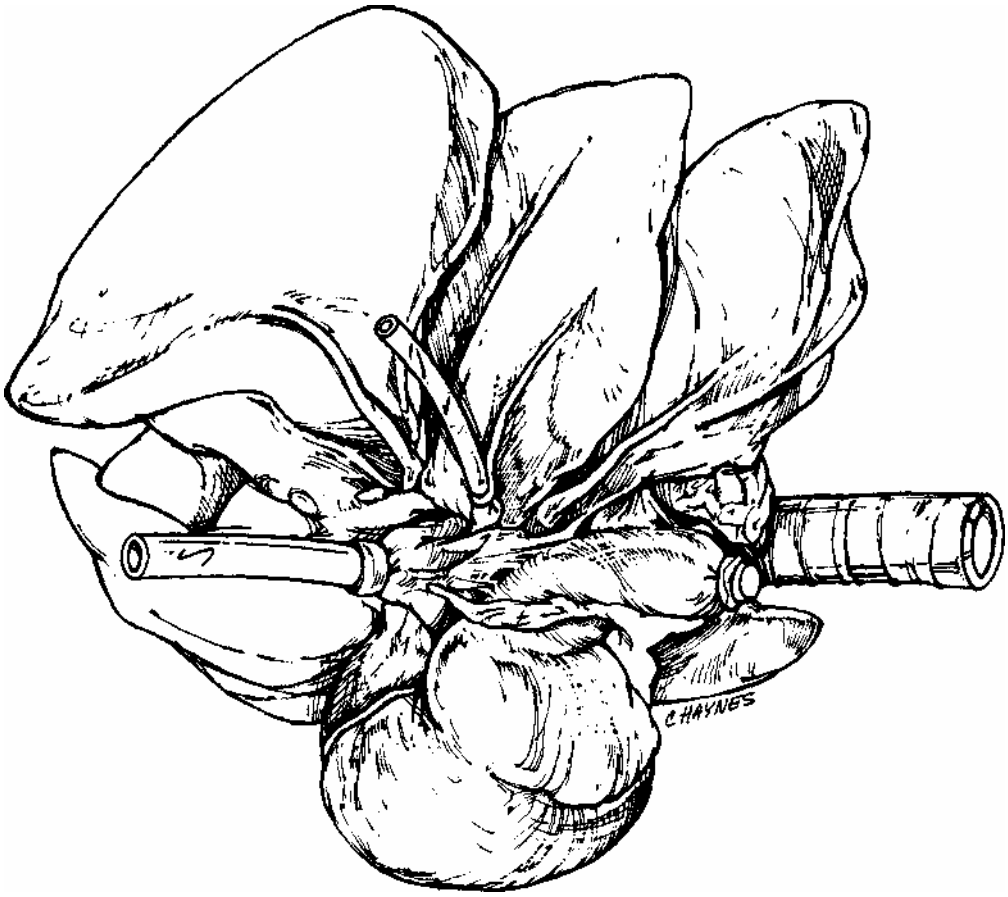

Figure 1. Ventral view of the right canine lung. Pulmonary vein is cannulated with small tubing and caudal vena cava with large tubing. Cranial vena cava is closed with a cork stopper.

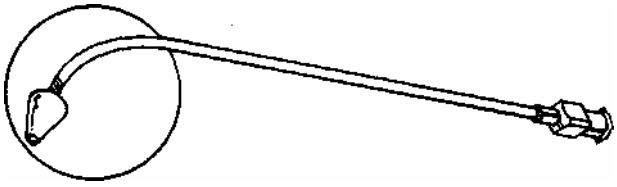

Figure 2. Special cannula for color injection.

TOOLS FOR THE INFILTRATION OF DEHYDRATED SPECIMENS WITH SILICONE RUBBER

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INTRODUCTION

Prior to forced impregnation with Biodur S 10/S 3 mixture, specimens covered with skin (fetus, fish) or capsule (testis, eye bulb), must be infiltrated with polymer. Intermediary solvent exits readily through thick fibrous covering tissue but the more viscous silicone rubber cannot penetrate and exchange fast enough to prevent a vacuum from developing within the specimen. Thus, this step is necessary to prevent compression-induced shrinkage. Also, final infiltration is often needed before curing to restore natural shape.

INFILTRATION OF SILICONE RUBBER

Most attempts to simply inject the S 10/S 3 mixture with a syringe will fail, as pressure on the plunger is far from sufficient to propel such viscous fluid through the usual needle. Table 1 shows some dimensions of disposable syringes. Special notice should be taken of the front surface area of the plungers. Maximum obtainable pressure has been calculated by assuming that the user will exert a thumb force of 20 Kg/cm^2. In practice, most female technicians will use considerably less.

Another reason why these calculations are liable to result in too high an available pressure is because they neglect the detracting influence of friction. Data shown in Table 1 are provided only as relative values and to establish an order of magnitude. The pressure that can be generated with a 1 ml syringe, for example, (more than 100 bar) turns out to be quite remarkable when compared to the low pressures obtainable with larger types.

Pressure is equal to force divided by unit area. The reader is well-aware that a small syringe filled with water and fixed to a thin hypodermic needle is much more easily emptied than a larger one. The needle creates enormous flow resistance which, according to Hagen-Poiseuille's law depends on its length, the viscosity of the fluid (here 700 m Pa's) and the fourth power of its tubular radius.

So, for silicone infiltration, large needles have to be used. The #1 hypodermic needle (20 G x 1 1/2 inch) is the most narrow needle found practical for this step. In fact, for larger specimens, such as fish, all-steel needles, 4 inches in length with a gauge between 19 and 14 are recommended. Needles with internal diameters larger than 2 mm leave big holes and much of the injected polymer may leak out.

The 1 ml size (Fig. 2, A) is the only conventional syringe that will develop enough pressure to be useful for silicone injection. One drawback, however, is that the user's hand hurts after the 10th filling. Despite numerous other limitations (the plunger kinks easily under the heavy pressure; the syringe cannot be filled through the needle; the needle slips

off once it has been lubricated with silicone) it works.
Its minute volume is a real handicap (a testicle has to be injected with 8 ml) — but it does generate a serviceable pressure (Table 1).

The automatic syringe (Fig 2, B) has a leverage grip, the ratchet of which propels a metal plunger rod. Its volume of 10 ml is sufficient but its diameter of about 16 mm limits the pressure that can be obtained. When used with silicone, the automatic syringe becomes heavily stressed. This notwithstanding, it is recommended for stronger users, injecting small volumes. The model that employs a rubber plunger can be improved by replacing its glass barrel with an (unbreakable) aluminum or stainless steel unit. After use, the model using a metal plunger in an interchangeable glass barrel must be carefully cleaned before residual S 10 becomes too viscous. In any case, the needle mount ought to be equipped with a Luer-lock cone. Spare sets of washers and rubber plungers also are necessary.

For infiltration of larger volumes, certain kinds of grease guns are quite useful. They generate pressures of several hundred bar. The cheap cartouche-guns, containing one-component silicone for caulking, turned out to be too weak for our purposes. The author's personal preference is the old-fashioned, bolt-type grease gun (Fig 2, C) with a plunger employing a leather washer. Its screw permits fine adjustment of the outflow. The grease-fitting nozzle at its outlet must be removed and the tube altered for the installation of a Luer-lock cone. Such cones are available commercially, either from a supplier of syringe systems or as tubing adapters (Fig 2, D).

The same change is necessary to adapt the outlet of a larger, lever-action grease pump of the type shown in Figure 2, E. This type will generate sufficient pressure (400 bar), however its rubber washer will suffer somewhat from cleaning with acetone. Both of these types of grease guns must be fixed to the work table and their output tubes are rather stiff.

POLYMER MIXTURE FOR INFILTRATION

The Heidelberg Plastination Folder (1985) recommends the following mixture for infiltration (in parts by volume):

Biodur S 10 Silicone -- 100 S

3 Hardener -- 1 S 6

Gas Cure -- 2

One might inject already-used, hence more viscous, S 10/S 3 mixture, however higher pressures and thicker needles will be necessary.

A tip that will discourage the leakage of injected silicone is as follows:

1) Draw 0.05 ml Biodur Hardener S 2 into a 1 ml disposable syringe.

2) Fill the syringe with S 10/S 3 mixture.

3) Install a large-gauge disposable needle and infiltrate within a few seconds.

Gelation of the polymer will occur within two minutes but the mixture will begin dissolving the syringe. Thus, one must discard both syringe and needle.

SOURCES OF EQUIPMENT

Automatic syringes are sold by companies serving veterinarians, especially large-animal practitioners. Sizes larger than 10 ml are not powerful enough to inject silicone. Models of 5-ml capacity are

quite useful. The price is around $60.00.

Grease guns are cheaper (about $15.00). If the bolt-type is not available in automobile parts stores, try shops specializing in marine equipment.

Attempts by the Heidelberg Plastination Laboratory to construct an infiltration machine resulted in a $1,000.00 prototype (which failed to develop sufficient pressure) and a highly sophisticated $3,000.00 model, employing a reciprocating pump, driven by compressed air. The latter works very well but must be dismantled and thoroughly cleaned after each use.

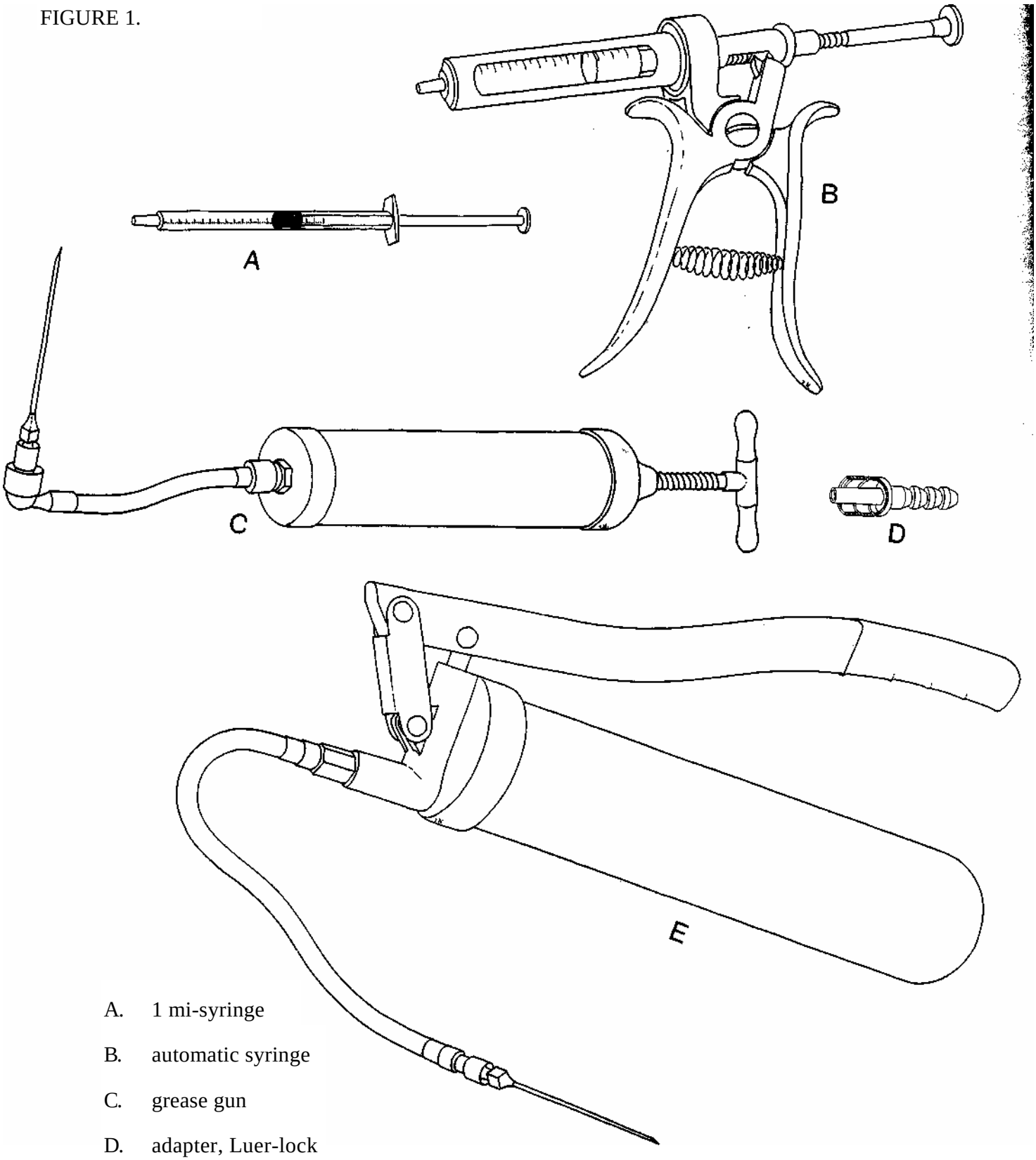
SUMMARY

Silicone-infiltration of specimens covered by skin or capsule requires 50 to 200 bar of pressure. Simple tools that are useful for this purpose include the 1-ml conventional syringe, low-capacity automatic syringes and certain types of grease guns.

TABLE 1. PRESSURES OBTAINABLE WITH CONVENTIONAL (DISPOSABLE) SYRINGES

SYRINGE VOLUME cm) (in ml),	INNER DIAMETER (in mm)	FRONTAL AREA OF PLUNGER (in sq mm)	MAXIMUM OBTAINABLE PRESSURE (at 20Kg/sq (in bar)
1	4.5	15.9	125.0
2	10.0	78.5	25.0
5	13.0	132.0	15.0
10	16.0	200.0	10.0
20	20.0	314.0	6.3
60	27.0	572.0	3.5

FIGURE 1.



A. 1 mi-syringe

B. automatic syringe

C. grease gun

D. adapter, Luer-lock

E. grease pump

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