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

ACKNOWLEDGEMENTS

I would like to thank Prof. Klaus Tiedemann, Dr. Gunther von Hagens and Prof. Harmon Bickley for their indispensable help and advice in producing this paper.