

THE STAINING OF BRAIN SLICES BY IMPREGNATION

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INTRODUCTION

Sheet plastination of brain tissue, using the standard P-35 technique with 4mm sections, results in outstanding differentiation between gray and white matter. Gray matter is rendered brown, while white matter remains close to its natural color. On occasion, however, modification of this effect maybe desirable to permit a different perspective. In this paper a method will be outlined for the quick, convenient staining of brain tissue slices for sheet plastination.

MATERIALS AND METHODS

This procedure was developed using 2 mm coronal sections of formalin-fixed human brain. It has not been used on 4 mm sections and may require some modification of stain concentration for this application.

Sectioning and Rinsing: Sections of fixed human brain, 2 mm thick, are prepared using a commercial meat slicer, modified in such a way that the blade is irrigated with a jet of water. These slices are then separated by filter paper discs and stored in 10% formalin until used. Sections to be processed are removed from storage, blotted dry and placed in a container that will serve for all subsequent steps. They are then rinsed in running tap water overnight.

First Dehydration: The next morning, the rinsed sections are blotted dry and placed in a generous aliquot (at least 10X the combined volume of the specimens) of 100% acetone which has been precooled to -20C. These sections, in acetone, are then placed in the freezer at -20C and allowed to dehydrate for approximately 24 hours.

Preparing the Stain: A stock solution of astra blue is prepared in the manner described by Ulfig, 1990. This consists of dissolving 0.1 gm astra blue¹ in 1 litre of distilled water and adding 1 ml of concentrated hydrochloric acid. This stock solution is then diluted to prepare a working solution of sufficient volume to cover the specimens in their impregnation chamber. The dilution determines the intensity of tissue coloration. Brain tissue exhibits unusual avidity for this stain in a slightly acid medium and individual specimens differ in uptake.

It is therefore recommended that several dilutions be tried. This should start with at least 1 unit of stock solution to ten or twenty of 0.1% Hcl diluent. Higher dilutions are quite feasible. Delicately stained sections are most useful.

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Milwaukee WI 53201 USA

Impregnation Staining

Dehydrated specimens are drained of acetone and covered with the working solution of the stain. They are then placed in an appropriate chamber at room temperature and subjected to strong vacuum. This evaporates the acetone and draws the stain solution into the tissue. Bubbling caused by vaporizing acetone will usually stop within an hour or so (with 2 mm sections) and impregnation can be terminated.

Second Dehydration: At this point, overstained sections can be destained by rinsing in dilute 0.1% Hcl. Ideally, the gray matter will be green and the white matter a translucent blue. This metachromatic effect may be due to the nucleic acid content of cell bodies of the cortex and nuclei. When the color is satisfactory the sections are rinsed briefly, first in distilled water, then in room-temperature acetone. They are then covered with a 10X aliquot of acetone (which has been precooled to -20C) and placed in the freezer (at -10C) overnight. The following day, the acetone is poured off and replaced with the same amount of fresh, precooled acetone. The specimens are returned to the freezer for another 24 hours.

Plastination: After dehydration the sections can be impregnated with polyester resin in the usual manner and mounted in sheets (Weber, 1994). Neither the second dehydration nor the plastination should affect the color.

Use of Impregnation Staining: As mentioned above, the procedure outlined here was developed for 2 mm sections of fixed human brain. There is no reason however, why this method could not be adapted to thicker tissue samples, or even to whole brains (or other organs). An entire organ could be stained and then sectioned or even stained, plastinated and sectioned.

REFERENCES

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AN ECONOMICAL APPROACH TO SHEET PLASTINATION

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In the past many plastinators have shown little interest in setting up a facility for sheet plastination because of the initial costs involved. Unless there is a great demand for sectional anatomy specimens, it is not economically feasible to buy the

equipment required for the process. Therefore it is important to develop methods to reduce the costs of this technique. Described here are several ideas and practical solutions to help the plastinator achieve this goal.

INTRODUCTION

Due to ever increasing costs, it has become more important to build your own equipment or to re-use old equipment. It is with this thought in mind that we have put together a series of hints and suggestions to simplify the procedures of sheet plastination. The methods of equipment construction and time saving procedures described herein allow the plastinator to produce top quality sections at a much reduced cost.

MATERIALS

The following materials and supplies were used to build pieces of equipment used in the sheet plastination technique:

- a) Styrofoam Boxes (various sizes can be saved from equipment packaging discards).
 - b) Gelatin crystals (can be purchased from most scientific chemical supply companies but much less expensive when purchased from a restaurant supply facility).
 - c) Cooling fence manufactured by C.M.G. workshop.
 - d) Feeding mechanism manufactured by C.M.G. workshop.
 - e) Liquid nitrogen (-170°C) available from Canox Canada Ltd.
 - f) Stainless steel plastination container (manufactured by Tackaberry Heating and Refrigeration Supplies, Kingston, Ontario).
 - g) 2.0 mm perforated steel sheets (scavenged from old ventilation grillwork).
 - h) 5.0 mm mesh hardware cloth (purchased from local hardware store), i) Wire coat hangers, j) 30.0 cm. lifting hooks (manufactured from wire clothes hangers), k) Various lengths and sizes of square tubular steel (can be purchased at a local hardware store or metal fabricating shop) For our purposes we scavenged the material from discarded bookshelving units.
 - l) Push-fit corner inserts (available from local hardware outlet),
 - m) 10 cc hypodermic syringes and 22 gauge spinal needles (available from Becton Dickinson & Company, Rutherford, N.J., 07070 U.S.A.).
 - n) 6.0 ml sheet plastic for manufacturing filling funnel, o) Tubing clamp, flatjaw pincock 16 mm. p) Polyethylene Tubing, various diameters (Fisher Scientific Co. Ltd., Ottawa, Ontario), q) Silicone Gasket, 6 mm (Biodur™ Products Ltd., Heidelberg, Germany).
1. Plexiglas™ is used when referring to the specific product by its trade name but plexiglass is used when referring to the generic product.
 2. C.M.G. refers to the Clinical Mechanics Group, Faculty of Medicine, Queen's University, Kingston, Ontario, K7L 3N6 Canada.

- r) Laboratory retort stand with ring support.
- s) Various thicknesses and sizes of Plexiglas™G (transparent methyl methacrylate, manufactured by Rohm & Haas Company, Philadelphia PA, available in Canada from Rohm & Haas Canada, Inc., West Hill, Ontario), t) A solid core heater bar of variable length manufactured from low resistance steel alloy (available from Nedco Canada Ltd., Kingston, Ontario) and mounted in a bending jig. u) Curing Oven, composed of an undercounter heat insulated cupboard fitted with a thermostatically controlled strip heater, v) Ancillary equipment: Welding apparatus, brass welding rods, heat sealing device, hacksaw, handsaw, disc sander, sandpaper (various grits), metal bending jig, table saw, plexiglass bending jig.

METHODS

The following outlines the construction of several pieces of equipment necessary for handling sections for both the P-35 and E-12 sheet plastination techniques. All tissue processing techniques followed general procedures as outlined in "Heidelberg Plastination Folder 1985" (vonHagens, 1985).

- a) Styrofoam Embedding Boxes (Fig. 1):
To stabilize specimens during cutting, a Styrofoam box was used as a mold into which the fixed specimen was placed. 20% gelatin was then added to the mold and allowed to set. When slicing brain tissue, using a Hobart meat slicer (Hobart, Model 1100) it was advantageous to wet the blade, using moistened paper towelling, during slicing. This facilitated cutting of both the gelatin and the tissue. Small body sections (e.g. knee joint, etc.) were also easily cut on a band saw when embedded in gelatin prior to cutting. The gelatin helped in orienting the specimens for cutting and small portions of tissue remained intact throughout the procedure (Barnett, 1980).
- b) Cooling Fence (Fig. 2):
A cooling fence, used to maintain the frozen state of the tissue during cutting, was constructed of 5.0 mm aluminium sheet metal and measured 6.5cm x 38.0cm x 30.0cm. The fence was equipped with a vented lid and stopcock emptying port. The fence was attached to the thickness guide of the band saw and filled with liquid nitrogen prior to cutting (Weber, 1993).
- c) Feeding Mechanism (Fig. 2):
A Feeding mechanism, used to control the pressure applied to the tissue during slicing, was manufactured of 5mm aluminum sheet metal and measured 14.5cm x 21.5cm. It had a 18mm diameter, 40cm adjustment arm which could be secured by a clamping mechanism attached to it. The clamping mechanism was attached to the moveable feed bed of the band saw.
- d) Stainless Steel Plastination Chamber (Fig. 3):
A 20 gauge stainless steel plastination chamber 19.0 cm x 21.0 cm x 30.0 cm (with lid) was purchased from a local heating supply firm. This was used to process the specimens.

- e) **Plastination Chamber Insert (Fig. 3):**
An insert 18.0cm x 20.0cm x 29.0cm. was made for the plastination chamber and used for holding processing racks during the dehydration and infiltration of the tissues. It was constructed from 2.0mm perforated steel plates, scavenged from discarded ventilation grids. The plates were cleaned of dirt and old paint using Acetone and then measured, cut to size and all joints were welded.
- f) **Hardware Cloth Racks (Fig. 4a, 4b):**
Racks for transferring sections during processing were constructed from 5.0mm grid hardware cloth (galvanized steel mesh). For our purposes the racks had overall dimensions of 17.0cm x 19.0mm with 5.0mm folded edges, bent in a triangular shape, to facilitate stacking of the sections. These racks, containing the sections, could be placed in the plastination chamber insert for easy movement between processing solutions.
- g) **Rack Handles (Fig. 4a):**
30.0 cm pieces of coat hanger wire were used to construct handles for lifting the hardware cloth racks during processing. The wire was bent in an upside down "U" shape, with the terminal 5.0mm of the U-arms bent to 90°.
- h) **Infiltration Chamber (Fig. 5):**
A vacuum oven, large enough to accommodate the plastination chamber, was used to infiltrate the specimens. The oven was placed on its end and a monometer was attached in line between the intake of the oven and the vacuum pump. The exhaust of the pump was vented to a fume hood.
- i) **Curing Rack (Fig. 6a, 6b):**
To facilitate the curing of several specimens at a time, a curing rack was constructed from tubular steel and push-fit corner inserts. The following pieces of tubular steel were cut:
- i) 4 - 84.0cm x 2.5cm x 2.5cm
 - ii) 4 - 15.0cm x 2.5cm x 2.5cm
 - iii) 4 - 22.0cm x 2.5cm x 2.5cm
 - iv) 68 - 10.5cm x 1.0cm x 1.0cm
- The 10.5cm pieces were welded along the length of the 84.0cm pieces, at an angle of 105°, at 5.0cm intervals. The rack was assembled using push-fit corner inserts.
- j) **Filling Funnel (Fig. 7a, 7b):**
A 10.0mm diameter funnel for filling the tissue molds during the casting procedure was constructed from 6.0mm clear plastic. Its edge was reinforced with coat hanger wire. The funnel was supported with a circular laboratory retort stand. A flatjawpincock 16.0mm tubing clamp was used to control the flow of resin from the funnel into the tissue molds.
- k) **Air Bubble Remover (Fig. 8):**
Bubbles were removed from the tissue molds, using a BD

Spinal Needle, Type *5148, 12.7cm long, attached to a BD IO.Occ syringe. The needle was rinsed with Acetone between uses or when clogged.

- 1) **Gaskets (Fig. 9):**
Gaskets used in the construction of casting molds can be made of several different materials. Either solid silicone gaskets or construct gaskets using a combination of a solid gasket and polyethylene tubing may be used. This type of gasket is easily assembled by feeding or blowing (using an airhose) a smaller diameter (i.e. 6mm OD) solid silicone gasket into a larger diameter (i.e. 6.4mm ID, 9.5mm OD) polyethylene tubing.
- m) **Gasket Holder (Fig. 10):**
A gasket holder with a 28.0cm x 22.0cm x 12.0mm plywood base and 8.0cm diameter roller, 28.0cm in length, was constructed using 3.0mm Plexiglass G.
- n) **Curing Oven (Fig. 11):**
An under counter cupboard, heat insulated and fitted with a thermostatically controlled strip heater, was used as a curing chamber. The curing process was done as described by vonHagens.
- o) **Plexiglass Mounts and Legend Holders (Fig. 12):**
Several specimen mounts were constructed from 2.5cm Plexiglass G. The mounts varied in size depending on the type of specimens to be displayed. Legend holders, large enough to accommodate a 7.6cm x 12.7cm filecard were fabricated on a bending jig using 3.0mm Plexiglass G (Lyons, 1987).

DISCUSSION

Using gelatin molds to stabilize specimens during slicing (Barnett, 1980), resulted in considerably less damage to them. The gelatin caused no apparent problem during any of the remaining steps of the procedure.

As described by Weber (1993), slicing of anatomical material with a band saw, is much easier when carried out using a cooling fence. It is also essential in maintaining the integrity of the sections. The cost of this device can vary from a few dollars to several hundred. Therefore, it is important to take care when selecting a manufacturer for your cooling apparatus. We have found that developing a good liaison with your physical plant facilities, engineering departments, etc. can be a money-saving proposition. Use of a feeding mechanism attached to the moveable table of a band saw during the cutting procedure was helpful in equalizing the pressure on the specimens as they were guided through the saw blade. The feeding mechanism maintained a constant pressure on the specimen and helped to minimize the distortion of the sections.

The use of stainless steel processing chambers was governed by the cost involved. We found that it was more cost effective to have containers made locally rather than purchase them from a scientific supplier. It is advisable to include an insert for your vessel(s). By using the insert system of handling, damage to the specimens is minimized.

Using hardware cloth racks for support of the sections during handling also prevented damage. The racks, because of their gridded structure, provided freedom for exchange of processing fluids to all areas of the tissues. They also assured that tissues were separated adequately during the dehydration, defatting and infiltration processes.

Using an existing vacuum oven as an infiltration chamber resulted in considerable savings. It is advantageous if the vacuum oven has a glass insert in the door which allows for careful monitoring of polymer bubbles during the infiltration process. Another advantage of using the vacuum oven was that it provided a chamber for bubble removal in the specimens prior to curing.

Constructing your own curing rack enables you to customize it to your own needs. The push-fit corners allow for easy dismantling. Varying lengths of holders can then be inserted and the unit can be reassembled. This type of construction allows single or double tier curing capacity and provides ideal support for the specimens during curing.

Ease of bubble removal in the specimens, prior to curing, was facilitated by using the BD Spinal Needle. The needle was placed near the bubble to be removed. The inner stylet of the needle was removed and a 10 cc syringe was attached to the needle. The syringe was then retracted and the bubble was drawn into the needle. The advantage of using this technique was that the bubbles could be easily accessed without doing harm to the specimen. Use of a filling funnel during the casting of the specimens prevented over filling the molds and reduced the entrapment of air in the specimen.

Use of a solid silicone gasket material in combination with an outer wrapping of relatively inexpensive polyethylene tubing allows creation of gaskets of different diameters. This enables the plastinator to accommodate specimens of varying thicknesses. Another advantage to having this two-part gasket system is that the inner core, solid silicone gasket material can be retrieved after curing of the specimens is complete and subsequently reused at a later date.

Using a curing oven of the type shown (fig. 11) facilitated curing of many specimens at once. In most cases it was possible to cure all sections of a specimen at the same time; in our case thirty-four, P-35 specimens were processed together. Because color and cellular detail, in the P-35 and E-12 techniques, are enhanced during the curing process, constant and even distribution of heat to the specimens is essential. With an undercounter oven of this type we found we were able to achieve this. An oven of this type can also be constructed by a local contractor or an in-house physical plant facility. In our particular case we found this to be considerably less expensive than purchasing one of its commercially built counterparts.

Once processed it was essential to present the specimens in the best possible way, to either students for teaching, or for display. Therefore constructing your own mounts or legend holders allows the flexibility of adapting them to fit the specimen. Mounts fabricated in your own workshop and legend holders, constructed on a homemade bending jig (Lyons, 1987),

are considerably less expensive than those commercially produced.

The hints for sheet plastination outlined here have been used by us in the production of a wide variety of teaching specimens for our Anatomy Learning Centre. Applying these techniques has saved us considerable time and money without jeopardizing the quality of the specimens.

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Fig. 1: Recycled styrofoam box with human knee specimen embedded in gelatin.

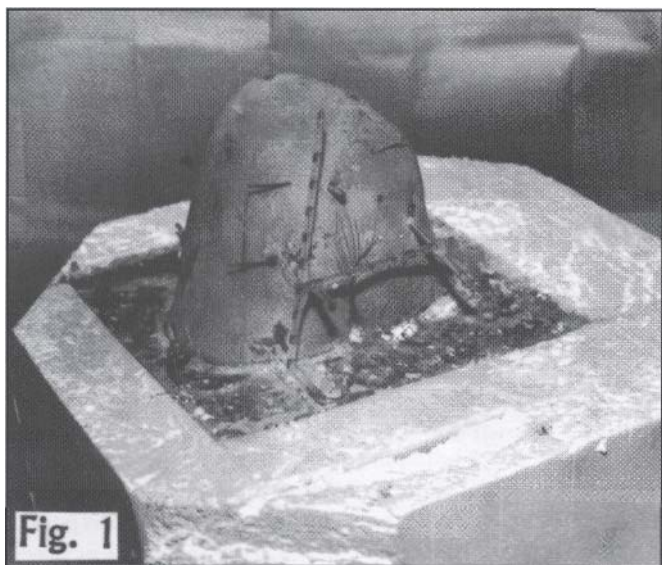


Fig. 3: Stainless steel plastination chamber (a) with insert (b).

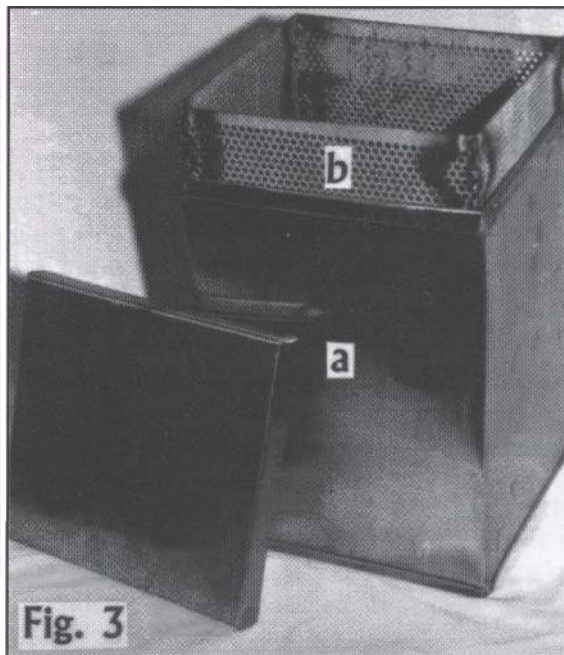


Fig. 2: Modified band saw with cooling fence (a) and feeding mechanism (b) attached.

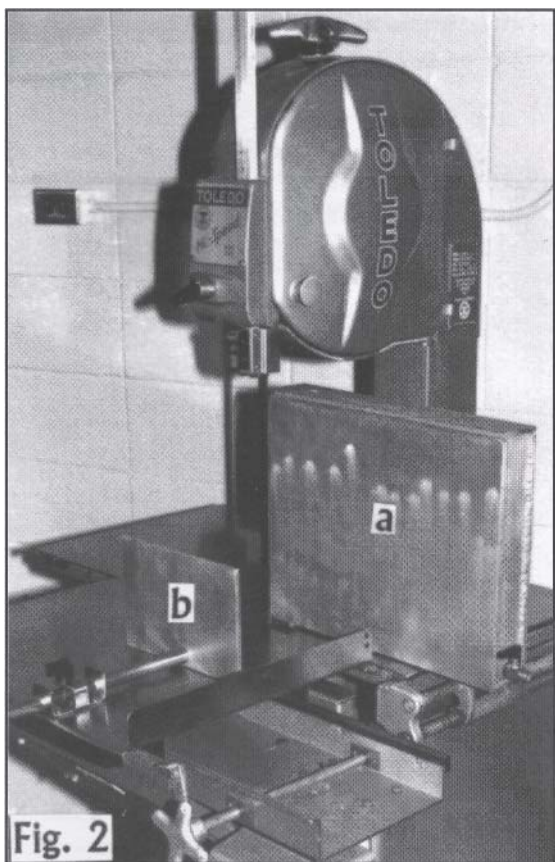


Fig. 4a: Hardware cloth rack with coat hanger wire handle.

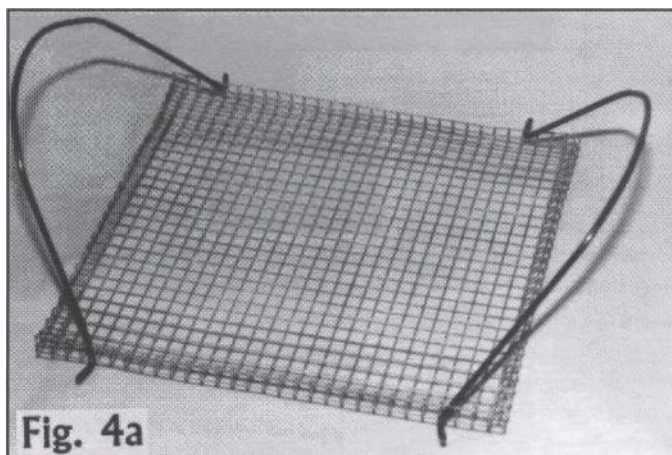


Fig. 4b: Hardware cloth racks stacked for processing of specimens. Note- folded edges of racks serve as spacers between specimens.

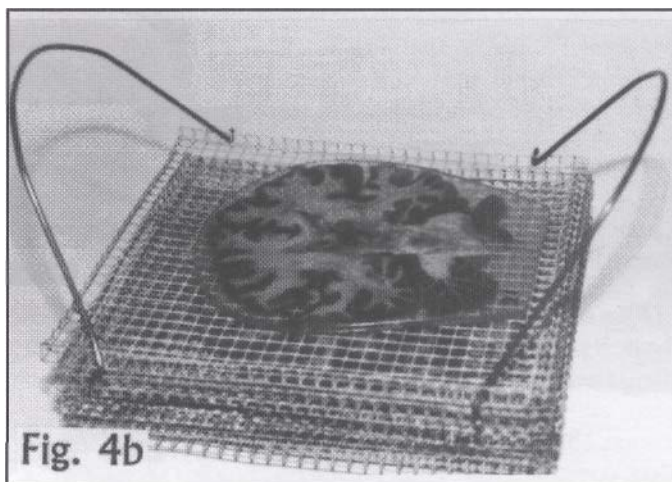


Fig. 5: Large vacuum oven placed on end being used as infiltration chamber. Size required is dependent upon size of plastination chamber used.



Fig. 6b: Curing rack, single layered, with casted specimens in place. *Note- Uprights of curing rack are welded at an angle of 105°.

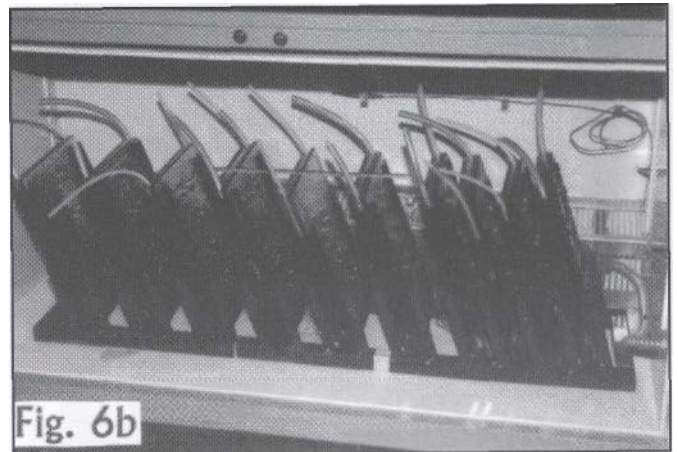


Fig. 7a: Filling funnel constructed of 6.0mm clear plastic. *Note- upper edge of funnel is reinforced with coat hanger

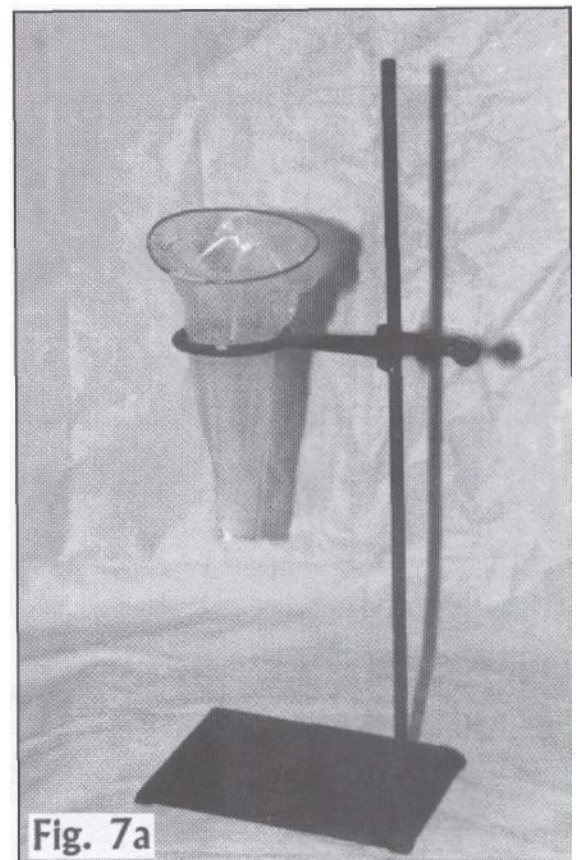


Fig. 6a: Curing rack. May be used single layered or double layered as shown.

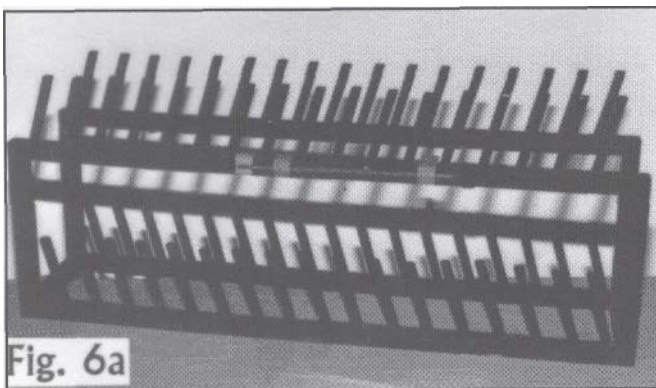


Fig. 7b: Filling funnel being used to fill specimen cast during embedding.



Fig. 9: 6mm OD silicon gasket placed inside of 9.5mm OD polyethylene tubing.

*Note - This type of gasket was used when making mold as seen in Fig. 7b and Fig. 8.

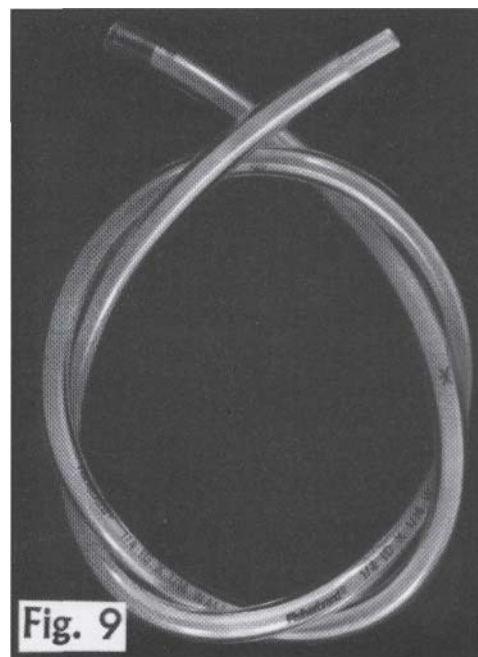


Fig. 8: BD Spinal needle being used to extract bubbles from cast specimen prior to curing.



Fig. 10: Gasket holder with silicone gasket.

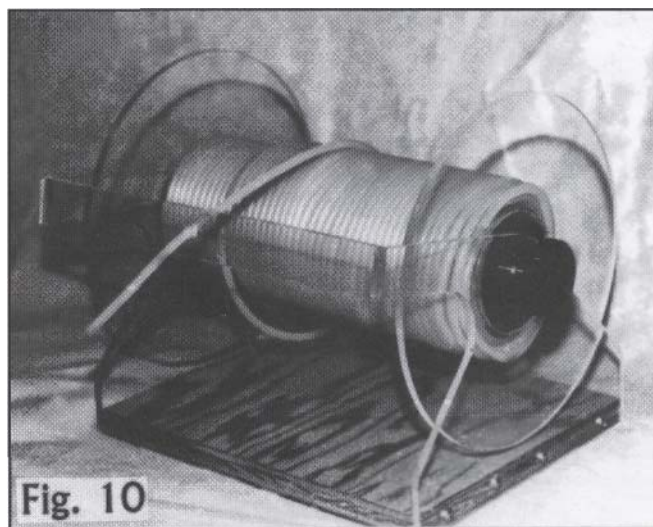


Fig. 11: Undercounter curing oven with specimens in place on curing rack.

'Note - External thermostatic control mechanism can be seen attached to upper right corner of oven on side panel.



Fig. 12: Plexiglass mounts and legend holders used to display specimens.

