journal of the

INTERNATIONAL SOCIETY for PLASTINATION

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8th International Conference on Plastination

Fifth Biennial Meeting of the International Society for Plastination

14-19 July, 1996 University of Queensland - Brisbane, Australia

JOURNAL of the

INTERNATIONAL SOCIETY FOR PLASTINATION

Official Publication of the International Society for Plastination

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President's Message



Dear Plastination Colleagues:

It hardly seems possible that almost a year and one half have lapsed since the 7th International congress in Graz. Lots of acetone bubbles have risen in the interim. This note should arrive just in time for you to mark your calendars and begin planning your titles and abstracts for the 8th International Congress which will be convening in "Exciting" Australia, July 14 to 19th. We are looking forward to another great meeting. Thanks to Robbie and Peter for hosting this event. We have several topics which need to be discussed at the 8th Congress: Acetone levels and environmental protection group laws; Financing of future International Congresses and New technology and products. Please send us any items which should be included in the

agenda. Thanks to Ron Wade for keeping the E-mail service up and running smoothly. I trust that all of you have had a great year. As this year comes to an end and we contemplate 1996, I hope you all have a prosperous new year especially in the area of plastination. We look forward to seeing many of you in Queensland.

R. W. Henry, D.V.M., Ph.D. President of ISP

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NERVE PLEXUSES DEMONSTRATED BY THE P-35 TECHNIQUE

Andreas H. Weiglein and Katayoun Bahadori Anatomical Institute, Karl-Franzens University Graz Graz, Austria

INTRODUCTION

Nerve plexuses such as the brachial plexus and the lumbosacral plexus are difficult to display by normal anatomical dissection. They are better displayed by P-35 plastination.

MATERIALS AND METHODS

A cadaver, 43 year old male, was prepared using Thiel's fixation (Thiel, 1992). Dissection began at the distal end of the upper extremity (membrum superius) for the brachial plexus and the distal end of the lower extremity (membrum inferius) for the lumbosacral plexus. Each nerve was marked with a colored thread to avoid any confusion. This was necessary to ensure proper identification of individual nerves after the plexus has been removed from the cadaver. The spinal column was opened from the dorsal aspect and the plexus and distal cord were removed. Plastination followed using the P-35 technique (Weber, 1994).

DEHYDRATION

Dehydration was done by freeze substitution. Each of the specimens were immersed in 15 litres of 99% Acetone at -25° C for 72 hours. There was no need to change the acetone after the initial dehydration took place.

IMMERSION

Immersion of the specimens was carried out in two baths of P-35/A9 mixture for 24 hours each. During both of the immersions the specimens were held below the surface of the mixture using a grid.

FORCED IMPREGNATION

During forced impregnation the specimens were placed in a fresh P-35/A9 mixture for 24 hours. After impregnation they were placed on a glass plate for orientation and trimmed. Proper orientation of the plexuses was very difficult because the nerves were tangled and the specimens became less flexible as time passed. After correct positioning, which was facilitated by the identifying colored threads on the individual nerves, the specimens were embedded.

EMBEDDING

The double glass chamber system of embedding was used.

CURING

Curing was started using UVA-light on both sides of the specimen for 3 hours. Ventilation is very important during light curing because the resin temperature in the embedding chamber must never exceed 35° C. To prevent this, a special cooling system (Fig. 1) was developed for the specimens. The cooling system consisted of two metal tubes with many small holes in them. One tube was placed on each side of the double glass chamber housing the specimen to be cured, and compressed air was forced through them. The air passed through the tubes and out through the holes facing the embedding chamber. As the forced air flowed over the surface of the specimen chamber the heat produced by the exothermic reaction of the curing polymer was dissipated, thus maintaining the curing temperature within safe limits. Using this type of system for curing was much better than using desk-top ventilators because the air flow could be easily adjusted. Curing was completed using a well ventilated oven at 42° C for a period of 3 days.

Subsequently, the glass plates were removed and the plastic trimmed. For demonstration purposes the P-35 plexus plate was placed on a larger plexiglass plate, on which the names of all the nerves shown in the specimen were engraved (Fig. 2). For examination purposes the P35 plexus plate could be easily removed from the plexiglass plate.

CONCLUSION

The P-35 technique for plastination of brain slices has been used as a teaching tool for several years (Weiglein, 1993). By using our method we have developed a very suitable procedure for displaying nerve plexuses and keeping them for long periods of time in perfect condition.

ACKNOWLEDGMENT

We gratefully appreciate the assistance of our laboratory technician, Johann Eder, who assisted us with the plastination procedure and development of the compressed air cooling system.

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Table 1

Timetable for the dissection and plastination of a plexus.

STEP	TIME
Dissection	16 h
Dehydration	72 h
1. Immersion	24 h
2. Immersion	24 h
Forced Impregnation	24 h
Light curing	3h
Heat Curing	96 h
TOTAL	approx. 2 weeks

Fig. 1: Compressed air cooling system.



Fig. 2: Lumbosacral plexus plastinated using the P-35 technique and placed on a plexiglass plate which has the names of the nerves engraved in it.



BRAIN PREPARATION TO SHOW FIBRE TRACTS AND NUCLEI

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INTRODUCTION

The use of brain sections for the teaching of neuroanatomy is well known. Using brain sections along with stained sections to illustrate the differences between white and grey matter is common. In addition many texts contain illustrations outlining the various tracts and nuclei. However, alone or in combination, all of these aids tend to complicate rather than simplify the understanding of neuroanatomy for the undergraduate.

The purpose of this paper is to outline a process of producing 3D models of the brain's fibre tracts and nuclei using Klinger's and von Hagens' procedures.

MATERIALS AND METHODS

This process can be divided into three stages:

STAGE 1: Klinger's method of brain preparation

STAGE 2: Fibre tract and nuclei dissection STAGE

3: Plastination of brains

Stage 1: Klinger's method of brain preparation

Select 3 brains per dissection required Immerse and suspend the brains in 10% formalin overnight

- Add fresh 10% formalin after 24 hours
- Pass a wide bore needle into both ventricles of each brain to assist in the passage of fixative to deeper brain tissues
- Place brains in 10% formalin and refrigerate at -20°C for 8 days

Remove frozen blocks of formalin containing brains from freezer and let thaw under running tap water for 24 hours

When thawed immerse brains in 5% formalin to await dissection

Stage 2 : Fibre tract and nuclei dissection

The above process renders the fibre tracts and nuclei easily distinguishable by blunt dissection. The fibre tracts can be peeled off in strands and the grey matter takes on a granular "brown sugar" like texture. It is delicate and easily removed if meticulously dissected.

- Decide on a tract to dissect. It is best to pick a large tract such as the pyramidal tract (A number of neuroanatomy texts have photographs illustrating this tract)

- Have a clear knowledge of the path of the tract to be dissected and an illustration of it for reference

Roughly define the tract by excising all brain tissue around it

It is useful to begin where the tract is easily definable (i.e. the pyramidal tract in the pyramids of the medulla) If dissecting the pyramidal tract dissect inferiorly to superiorly along the tract towards the internal capsule Define the basal nuclei of the grey matter bordering the internal capsule

Follow the tract through the corona radiata to the precentral gyrus of the cerebral cortex, excising all nonpyramidal fibres as you dissect Instruments required are blunt nosed forceps, to peel off brain tissue and sharp dissecting scissors, to trim the tract fibres neatly

It is helpful to practice on one or two other brains before finally selecting a brain for dissection of the finished product

- A magnifying glass, for dissection of small nuclei and fibre tracts, is required

Stage 3 : Plastination of the brain

The brains were plastinated using the standard S-10 technique (von Hagens, 1987).

SUMMARY

It should be noted that after Klinger's preparation the brains appear more porous under magnification possibly due to the presence of ice-crystals formed when thawing. This also assisted in making the tracts more definable for dissection.

The procedure described above may be supplemented by using a number of other useful techniques such as: opacification of the arterial tree or filling of the ventricles with a transparent resin (Thompsett, 1970).

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von Hagens, G. 1985. Heidelberg Plastination Folder

Fig. 1: Left cerebral hemisphere showing pyramidal tract (P), head of caudate nucleus (C) and corona radiata (R).



Fig. 2: Left cerebral hemisphere showing lentiform nucleus (L), thalamus (T), caudate nucleus (C) and inferior longitudinal fasciculus (I). Arrow indicates location of fibres of the internal capsule.



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.

<u>First Dehydration</u>: 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.

<u>Preparing the Stain</u>: 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.

'Aldrick Chemical Company PO Box 355 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.

<u>Second Dehydration:</u> 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 -IOC) 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.

<u>Plastination</u>: 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.

<u>Use of Impregnation Staining:</u> 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.

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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.

1) 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 (BiodurTM Products Ltd.,

Heidelberg, Germany).

- 1. PlexiglasTM is used when referring to the specific product by its trade name but plexiglass is used when referring to the generic product.
- 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 pushfit 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 where 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 byvonHagens.

 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.

ACKNOWLEDGMENTS

For their patience and help during the preparation of this manuscript we thank Mrs. Marilyn McAuley and Mrs. Brenda McPhail. For his expertise in photography we are indebted to Mr. Henry Verstappen.

We would like to acknowledge the financial support of the Faculty of Medicine and Research Services, at Queen's University, towards travel expenses incurred when this paper was presented at the 7th. International Conference on Plastination in Graz, Austria.

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

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





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



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



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



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. 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.





International

Conference on

Plastination

The fifth biennial meeting of the International Society for Plastination

Brisbane, July 14th - 19th 1996

Venue

The conference will be held at Emmanuel College on the main campus of The University of Queensland in St Lucia, Brisbane.

Accommodation

Accommodation will be available in Emmanuel College and the adjacent Kings College. Rooms are of a higher standard than is usual for student accomodation and they are serviced daily. All have telephones and many have refrigerators. Most have ensuite facilities shared between adjacent rooms. Linen and towels are provided. A small number of 2, 4 and 5 bedroom flats are also available.

Alternatively you can arrange accommodation in a hotel. The St Lucia campus is ten minutes by car from the centre of Brisbane.

Format

There will be plenary sessions for presentation of papers. Facilities for the display of posters and specimens will be available throughout the conference.

Pre-Conference Workshop

A workshop for newcomers to plastination embracing both theory and practice will be held prior to the conference. Numbers will be limited to ensure that all participants can be fully involved. All materials, and lunch, will be provided.

Full information and registration forms will be mailed to members of the International Society for Plastination. If you have not received these documents by January 31st or if you require further information please contact

Carole Lambert University of Queensland Division of Radiology Mater Misericordiae Hospital South Brisbane AUSTRALIA 4101

Tel61 (0)7 3840 8192Fax61 (0)7 3844 1891E-mailp.bore@mailbox.uq.edu.au

The annual conference of the Anatomical Society of Australia and New Zealand will take place in Brisbane between July 10 and 12. For further information contact:

Dr Vaughan Kippers Department of Anatomical Sciences The University of Queensland Brisbane 4072

Tel 61 (0)7 3365 2704 Fax 61 (0)7 33651299 E mail v.kippers@mailbox.uq.oz.au



Kakadu, the rain forest and Fraser Island.	Having travelled to Australia you might include a pre or post conference tour. Suggested places are the Barrier Reef, Ayers Rock,	day and half-day trips to places of local interest.	The accompanying persons program	A workshop for newcomers to plastination will preceed the conference.	specimen displays will take place throughout the conference.	plastinated specimens. Posters and	be devoted to educational uses of	for presentation of papers. One will		to places of interest.	available as well as optional trips	plastination laboratory will be	Visits to the university and the	conference dinner will be included.	within walking distance of the	be available in a range of hotels	Brisbane City. Accommodation will	The conference will take place in
Postal enquiries:	Fax: 617 844 1891 E mail: r.boyes@mailbox.uq.oz.au	Mr Robbie Boyes Dr Peter Bore Tel: 617 840 8192	Conference organisers;				of an anatomy laboratory.	special care and storage facilities	researchers for use anywhere, at	become available to students and	preserved by plastination they	organs or tissue slices are	and free from decay. When whole	dry, odourless, resistant to damage	after outling make the specimen	in which water and lipids in tissue	developed by Dr Gunther von Hagens	Plastination is a technique
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VASCULAR PATTERNS OF PLASTINATED HUMAN HANDS WITH SPECIAL REFERENCE TO ABNORMALITIES OF THE ARTERIAL PALMAR ARCHES

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INTRODUCTION

The vascular anatomy of the human hand is known to be of outstanding importance in medical, surgical and radiological sciences (Coleman and Anson, 1961; Kenesi et al., 1967; Braun et al., 1977), as well as in comparative anatomy and primatology (Manners-Smith, 1910; Sakka, 1972). The constitution, relationship and common abnormalities of both arterial palmar arches make their dissection difficult for students. The superficial palmar arch (Arcus palmaris superficialis) is often damaged during student dissection and subsequently much time is lost. Time is also lost trying to save the deep palmar arch (Arcus palmaris profundus). It was therefore decided to overcome this problem by producing plastinated specimens that would give the students a three dimensional view of the complete arteriovenous vascularization of the hand (Grodin and Olry, 1995). Initial trials of injecting diluted silicone resin were not successful. Part of the red resin, which had been injected first, was pushed from the arterial side via capillaries into the venous system. This led to the production of veins which were partly red, blue and purple. The next trials used pure silicone for the injection. One specimen was dissected to illustrate the superficial arterial palmar arch, a second one for the deep arterial palmar arch, and finally a third one for the venous drainage of the human hand.

SUBJECTS, MATERIALS AND METHODS

Hands, free of any obvious traumatic or surgical history (absence of scars and wrist injuries), were removed from frozen subjects in the midforearm.

Radial arteries from the frozen specimens were cannulated and water maintained at 25 cm of hydrostatic pressure was attached to the cannula. The cannulated specimens were held in cold running tap water for 12 hours while thawing. After thawing, ulnar arteries were also cannulated and any remaining blood was flushed from the vessels using a syringe filled with water via radial and ulnar arteries. A mixture of Biodur S-10/ S3/S6/S2, colored with red or blue Biodur Color Paste (AC50, AC52) was prepared to be injected into appropriate vessels. To prepare the injection mixture, a few drops of color paste were added to 30 ml of S-10/S3 mixture (100:1) and mixed until the color was uniform. After thorough mixing, 0.1 ml of S6 and 0.1 ml of S2 were added to the S-10/S3 color-mix and stirred for 3 minutes. Arterial injection was carried out via the ulnar artery. When the silicone mixture started to ooze out via the radial artery, it was ligated and injection was continued until we noticed red silicone also oozing out from smaller arteries. Then the ulnar artery was also ligated. After arterial injection, venous injection (Specimen 3) was performed via one dorsal metacarpal veins. The proximal ends of the veins were left open for exit of water. When the silicone mixture started to ooze out via many veins, the forearm was mass ligated and injection was continued along with delicate massaging to allow filling of the distal veins.

After vascular injection, hands were placed in cold modified Kaiserling's solution (Kaiserling, 1895) and stored in a cold room. Composition of the fixative solution was: potassium acetate (600 g), potassium nitrate (300g), formaldehyde 37% (400 ml), sugar (2000 g) and water (19.6L). Dissection was started in 24 hours and specimens were kept in the fixative solution for 3 to 5 weeks in the cold room.

After dissection was completed, the specimens were rinsed in cold tap water for 12 hours, dehydrated in 4 successive baths of acetone at -20°C over a 4 week period, and defatted in room temperature in acetone for 5 days before being impregnated at -20°C with Biodur silicone S10, according to the standard technique (von Hagens, 1985; von Hagens et al., 1987).

RESULTS

The superficial palmar arch (Specimen 1) is supplied mainly by the ulnar artery. When the ulnar artery is the main supply to the palmar arch, it is referred to as "ulnar type" (Adachi, 1928). The superficial arch gives rise to three common palmar digital arteries (Aa. digitales palmares communes) and each of these divide into two proper palmar digital arteries (Aa. digitales palmares propriae) (Fig. 1). However, the radial participation to the superficial arch is in this case very particular: it shows what we believe to be a persistent antebrachial dorsal superficial artery (Arteria antebrachialis dorsalis superficialis) which does not traverse the first interosseous space (Georgeiwski, 1905). The relationship of the radial artery and its embryological remnant seem to resemble the type VI described by Adachi (1928) who assigned a frequency of 1.5% to this kind of anomaly (Fig. 2).

The deep palmar arch (Specimen 2), which is known to be less variable than the superficial one (Braun et al., 1977), also showed an anomaly. Its medial origin arises from the palmar digital artery of the little finger (Fig. 3) and not from the ulnar artery, an anomaly which was not described by Adachi (1928).

The superficial venous drainage of the hand (Specimen 3) show's a classical plexiform morphology with some dorsal metacarpal veins and their anastomotic network (Fig. 4). It is noteworthy that the color mixture was stopped here and there by venous valves which even occur in the small veins (Fig. 5). Deep veins were also colored via the numerous inter-connections between the superficial and deep venous networks (Fig. 6).

DISCUSSION

The aim of this study was to provide plastinated human hand specimens which would help students to understand the vascular anatomy of the hand. The complexity of this region, as well as its numerous vascular variations (Adachi, 1928; Coleman and Anson, 1961; Kenesi et al., 1967), dishearten most students who try to dissect the complex venous and arterial patterns of the hand. Plastinated specimens are therefore appreciated as students can regularly refer to models during their anatomical curriculum (Olry and Grondin, 1994; C6te et al, 1995).

We have shown that an appropriate arterial injection is capable of filling and thus coloring the whole arterial network, including very small size subcutaneous vessels (Fig. 6). The size of the smallest colored arterioles were accurately measured using an optical comparator (Mitutoyo). Their size was 0. 1mm. This could provide an opportunity for the analysis of very thin vascular networks in the future.

A complete rinsing of the venous system is very important. A sole injection in a dorsal metacarpal vein allowed the filling of both superficial and deep venous networks provided that the veins were compressed several times when we noticed a venous valve stopping the color mixture. The presence of numerous valves explains that some veins were filled against the current and that water remained trapped in some veins . 5).

ACKNOWLEDGMENTS

The authors wish to thank M. Claude Demers for his help in photography.

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SUMMARY

Specimens were dissected and plastinated in order to demonstrate the arterial and venous vascularization of human hands. Vessels were injected with appropriate colors and plastination was performed according to the standard S-10 technique. We showed that 1) a sole injection via ulnar artery is capable of filling very small size arterioles and 2) injection via only one dorsal metacarpal vein allows to completely fill the superficial and deep venous systems, provided that veins are carefully massaged during the injection to open the valves. **Figure 1.** Palmar aspect of the superficial palmar arch. Ulnar artery (arrow), common palmar digital arteries (arrowheads).



Figure 2. Radial aspect of the radial participation to the superficial palmar arch. Radial artery (arrow), putative antebrachial dorsal superficial artery (arrowheads).



Figure3. Palmar aspect of the deep palmar arch. Deep palmar arch (white arrow), digital artery of the little finger (black arrows).



Figure 4. Dorsal venous network of the hand. Artifact due to the breakage of venous wall at the site of injection (arrow).



Figure 5. Close-up of the dorsal metacarpal veins. Location of small venous valves (arrowheads), remaining water trapped upstream of a venous valve (arrow).



Figure 6. Close-up of the radial aspect of the wrist region. Radial artery (star) flanked by both radial veins, subcutaneous arteriole (arrow).



PREPARING AND USING S-10 AND P-35 BRAIN SLICES

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INTRODUCTION

The S-10 procedure of plastination (von Hagens, 1986; von Hagens et al., 1987) is used for the preparation of half and whole brains as well as brain dissections. With the increasing difficulty in obtaining brains for neuroanatomy courses, the production of S-10 specimens has become important in teaching because these specimens can be kept in good condition for long periods of time before being replaced. S-10 plastination of 1.0-2.0 cm slices of brain tissues can also provide a useful teaching tool.

The P-35 and P-40 methods of plastination are used for the production thin (4,6 or 8 mm), semitransparent slices of brain tissues.

Discussed within this paper will be the methods, for the above mentioned procedures, that are used at the Anatomical Institute in Graz.

METHODS

S-10 Technique

Fixation:

Fresh brains are fixed in 10% Formaldehyde (14 days minimum), rinsed in cold tap water overnight and cooled to 5°C before use. Previously fixed brains (stored for long periods of time in fixative) are washed thoroughly in tap water and cooled to 5°C.

Dehydration:

Brains are submerged in at least two baths of 100% Acetone at -20°C (25L/brain). When the acetone concentration remains at 99%, dehydration is complete (whole brains approx. 3 wks). Dehydration at -20°C is necessary in order to prevent shrinkage of the brains.

Immersion:.

Brains are immersed in a mixture of S-10/S3 (100:1) for 1 week at -20°C. This step helps to prevent shrinkage in the specimens. Longer immersion at this stage will result in shorter impregnation time for the specimens.

Forced Impregnation:

Brains are placed under vacuum for three weeks at -20°C The vacuum is slowly increased until 1-2 mm of Hg is attained. Slow increase of vacuum helps to prevent shrinkage of the specimens.

Gas Curing:

After forced impregnation, the brains are removed from the polymer and excess polymer is drained from their surfaces. The specimens are then placed on a grid and exposed to S-6 (gas cure) vapors for 3 days at room temperature. The vaporization of the S-6 is enhanced by bubbling air through it using a small aquarium pump. This enables fast curing of the brains' surfaces. To assure complete curing of the polymer inside the specimens it is necessary to store them in air-tight plastic bags for an additional two (2) months.

Slicing:

Following curing, the brains can be sliced with a band saw. It is possible to slice very thin (1mm or less) slices from a thicker slice by using a meat slicer. After slicing, if the brain slices do not appear to be fully cured they can be returned to the curing chamber for another 24 hours.

Finishing:

After slicing, the brain slices can be sanded to a smooth finish on a belt sander. A constant supply of water to the surface of the specimens helps in this procedure.

P-35 Technique

Fixation:

Fresh brains were fixed in 10% Formalin for 4-6 weeks. Specimens which have been fixed for longer periods of time or by other methods should be avoided for this procedure. Other fixation methods may alter the P-35 reaction.

Slicing:

After embedding in 20% gelatin (Barnett et al, 1980) brains were cut with a meat slicer into 4mm slices. To prevent degradation of the slices, after cutting and during subsequent handling, they where placed on a piece of wet filter paper before being transferred to stainless steel grids. The grids containing the slices were placed into a stainless steel basket for flushing.

Flushing:

The basket of slices was rinsed with cold tap water overnight and cooled to 5°C before proceeding.

Dehydration:

The basket of brain slices was placed in 100% Acetone at -20°C for 3 days.

Immersion#1:

The basket of brain slices was placed in a bath containing a mixture of P-35/A9 (100:2) for 24 hours at 5° C.

Note: This bath may be the Immersion *2 bath from a previous run of sections.

Immersion #2:

The basket with the brain slices was placed in another bath of P-35/A9 (100-2) mixture for a further 24 hours at 5°C. Note: this bath may be the bath of resin used for forced impregnation during a previous run of specimens.

Forced Impregnation:

The basket of brain slices was transferred to a fresh mixture of P-35/A9 (100:2) and placed under vacuum for 24 hours at room temperature. The vacuum was increased until 10-15mm Hg was attained.

Casting:

The basket of slices was removed from the vacuum chamber, and individual slices were placed between two sheets of glass plates. Each sheet consisted of an outer piece of safety glass and an inner sheet of float glass, the latter sheet facing the specimen. A silicone gasket was placed between the outer edges of the sheets and then clamped in position using fold back clamps. The double glass chambers, containing the specimens, were filled with afresh mixture of P-35/A9 (100:2) mixture.

Light Curing:

After casting the double glass chambers were exposed to UVA light for a period of 3 hours. During this procedure it is necessary to cool the chambers either by ventilators or as described by Weiglein and Bahadori (1995).

Heat Curing:

Following light curing the double glass chambers were placed in a well ventilated oven at 45°C for 5 days.

Finishing:

When curing was completed the glass chambers were dismantled and the sections were trimmed on a band saw. After sawing the edges were smoothed using a belt sander.

P-40 Technique

This technique has advantages over the others. P-40 resin has a lower viscosity than P-35 resin (von Hagens, 1994). The advantages of using this technique are:

- 1) The same polymer can be used for immersion, impregnation, and casting of the specimens
- Only single float glass chambers are necessary when casting specimens as compared with the expensive double glass chambers, containing safety glass, as in the P-35 method
- P-40 is cured by UVA light only, therefore, there is no requirement for an expensive ventilated heat cabinet, as in the P-35 method
- 4) P-40 can be used for production of transparent body slices as well as brain slices.

Experience with the use of this technique is minimal and will be discussed in further publications.

RESULTS AND DISCUSSION

Sliced S-10 plastinated brains provide a good teaching tool. They show good differentiation between white and gray matter in comparison to other types of fixed brains (Fig. 2). This differentiation may be enhanced by staining with such substances as Astra Blue or Aldehyde Fuchsin (Ulfig, 1990; Ulfig & Wuttke, 1990). Slice thickness can be varied from 0.5mm and up. Specimens are flexible and therefore less susceptible to damage.

P-35 plastinated brain slices provide an excellent tool for use in teaching and research. Semitransparent P-35 brain slices maybe observed under direct or transmitted light. They have excellent gray-white matter differentiation and provide gross microscopic detail of some nuclear areas (Fig. 3). P-35 sections provide better macroscopic detail than those sections stained by other methods such as Berlin Blue or Mulligan Staining.

Because of the variability in slice thickness, specimens can be used in a wide variety of ways such as:

- 1) 4-6 mm slices can be utilized when teaching neuroanatomy to medical students, X-ray technicians, physiotherapists and nurses
- 2) 8 mm slices may be employed to give the student a three dimensional concept of neurological structures.

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Fig. 1: S-10 plastinated brain dissection showing internal capsule (1C) and optic radiations (OR).



Fig. 2: S-10 plastinated brain sliced in coronal sections. The first slice shows the pyramidal tract .(P) running between the thalamus (T) and lentiform nucleus (L). Note distinction between gray and white matter.



- Fig. 3: P-35 Plastinated brain slices.
 - a) Horizontal slice: genu (G) and splenium (S) of the corpus callosum, internal capsule (1C), thalamus (T).



b) Coronal slice



Table 1

Steps and Timetable for the S-10 and P-35 techniques for brain slices.

Note that slicing is done before plastination using the P-35 technique and after plastination using the S-10 technique.

Note that the immersion step in the S-10 technique is additional to the standard S-10 technique.

S-10 TECHNIQUE			P-35 TECHNIQUE				
Step	Time	Temperature	Step	Time	Temperature		
FIXATION	3 weeks	room temp.	FIXATION	3 weeks	room temp.		
			Slicing				
Flushing & Cooling	24 h	5°C	Flushing & Cooling	24 h	5°C		
DEHYDRATION	3 weeks	-20°C	DEHYDRATION	3 days	-20°C		
Immersion	1 week	-20°C	Immersion	2x24h	5°C		
FORCED IMPREGNATION	3 weeks	-20°C	FORCED IMPREGNATION	24 h	5°C		
			Double Glass Chamber				
Gas-CURING	3 days	room temp.	Light-CURING	3h	room temp.		
Post-CURING	2 months	room temp.	Heat-CURING	5 days	45°C		
Slicing							
Final Treatment			Final Treatment				

and vacuum infiltrated with S-10 resin at -25°C (von Hagens, 1985; von Hagens et al., 1987).

After infiltration the fetus was cured using S-6 (von Hagens, 1985). Following infiltration, very little curing was necessary because the tissues of the specimen are very thin.

RESULTS

The ossifying bone of the dens was stained red by the Alizarin and the arteries and soft tissues surrounding it were clearly visible. Under the dissecting microscope it was possible to see nutrient arteries entering the bone. The remaining arterial supply of the fetus was also clearly visible and the soft tissues and developing bone and cartilage could be seen without difficulty. The specimen could then be compared to the radiographic images made earlier.

It was found that one of the problems associated with this procedure was that the limb tissues showed marked contraction. We concluded that this was probably due to the fact that our freezer would not go as low as -20°C during the infiltration stage of plastination.

CONCLUSION

It was found that this procedure does produce specimens which are useful for the study of developing bone. As well, the simultaneous staining of cartilage may help to assist researchers in gathering information regarding fetal development.

This procedure also produced specimens that showed the relationship between vasculature, and soft and hard tissues in the fetus. Clarity was lacking in specimens which had been prepared in the past using resin cast modeling (Thompsett, 1970).

Because the plastination procedure was used, the specimens could be handled and used by students, clinicians and researchers without fear of damaging them.

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Fig. 1: Photograph of the left side of the head showing the arteries and bones of the scalp, mandible, face and neck.



Fig. 2: Photograph of the right side of the head showing an intact right globe of the eye and arteries of the palate.



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Fig. 1: Photograph of the left side of the head showing the arteries and bones of the scalp, mandible, face and neck.



Fig. 2: Photograph of the right side of the head showing an intact right globe of the eye and arteries of the palate.



HUMAN TISSUE ACQUISITION AND ITS USE FOR TEACHING IN AUCKLAND, NEW ZEALAND

Peter Cook and Brenda Dawson School of Medicine University of Auckland Auckland, New Zealand

New Zealand is a relatively small country with approximately the same land mass as Britain, but with a population of only 3.5 million. There are only two medical schools, one in Dunedin on the South Island, and one in Auckland on the North Island.

The city of Auckland is the largest in New Zealand with a population base of almost one million people in the greater area. By means of a well-structured donor program, the supply of bequeathed human cadavers is more than adequate to meet the needs of the undergraduate courses in gross anatomy at the School of Medicine.

The Auckland Medical School, Departments of Anatomy and Radiology, currently accept up to 30 cadavers (bequests) per year. Twenty of these requested bodies will be used by second and third year medical students for dissection and the remainder will be saved for prosections, serial sectioning, and plastination purposes.

The medical school enrollment is 120 students per year. In the second year dissection laboratory 5 or 6 students share a cadaver throughout their anatomy course.

The second year students begin their clinically oriented gross anatomy and radiology courses with examination of the lower limb, upper limb, thorax, abdomen and pelvis. In the first semester of the third year, students examine the head and neck region.

The Human Tissue Act

In 1964 an act of parliament was consolidated relating to "The Removal of Human Tissue for Medical Education and Research". This act is known as The Human Tissue Act and sets the criteria under which human tissue may be used (Department of Health, 1965). The Governor General of New Zealand authorizes the establishment of a School of Anatomy and the Minister of Health appoints licensed anatomists. Only licensed anatomists, of which there are only three at the Auckland School of Medicine, are permitted to accept a bequeathed body.

The Anatomy Department, of the School of Medicine, may receive up to 20 formal letters of inquiry in a week from the general public stating in writing their wish to bequeath their body to the school after death. For many of them this decision has been a long-standing wish, of their adult life, to contribute to medicine in some constructive way. The occupations, religions and ages of these bequeathed bodies vary considerably; however, ethnic origin is almost always invariably Caucasian.

The acknowledgment of a written intention to bequeath your body to the School of Medicine, prior to death, is in no way an insurance of final acceptance by the school upon your death. Many factors, at the time of death, enter into the licensed anatomists decision to accept or reject any bequeathed body. Certain infectious diseases and coroner's case autopsies may preclude acceptance of individual bodies. As well, the nextof-kin must be in agreement with the intention to donate.

Under New Zealand law the immediate next of kin is the lawful possessor of the body, of a relative, after death and may have ultimate authority to donate that body and any parts thereof to a medical school or hospital for educational, research or transplant purposes. In all cases the family is contacted by the licensed anatomist, who may at that time address any questions they may have regarding disposal or disposition of remains of the deceased, prior to uplift of the body from the location of death. In instances where the nextof-kin cannot be found the authority to disperse the body to the school may be passed on to an appropriate administrator of the institution at which the individual has died.

Almost all bequests come from the greater Auckland area. There must be no suspicious circumstances surrounding the death of a donor who is being requested for the above mentioned purposes. Upon death, the attending physician must issue a "Medical Certificate of Causes of Death" if an autopsy is not required. Thus, the previously indicated intent of the deceased, the approval of the next-of-kin and the acceptance by the licensed anatomist secures the bequest.

Under the terms of this act all bequeathals of cadavers must be registered under the office of the Detective Superintendent of the New Zealand Police, headed by a special designate the "Inspector of Anatomy". Any receipt of cadavers, intention to dissect them, numbers of cadavers in storage (reported quarterly by the School) or permission to dispose of them, must be reported to the inspector. No dissection may proceed without his/her written approval. The inspector may visit the school and request any documentation (i.e. permission of next of kin) relating to a particular bequest at any time.

Donation of Tissue for Transplantation

The organ tissue and transplantation program is managed separately from the bequeathal program. Generally, no part of a cadaver bequeathed to the School of Medicine is used for therapeutic or transplant purposes. Whether organs or tissues are specifically used for transplantation is the responsibility of a transplant donor co-ordinator. His or her primary area of concern is the post mortem retrieval of suitable donor organs. Permission, to retrieve organs, upon death of a donor, is obtained by the donor co-ordinator from the next-of-kin, if necessary. Following this, heart valves, corneas, skin or bone specimens may be harvested for transplant or research and teaching purposes.

The University of Auckland has recently established a brain bank for the purpose of research into neurological diseases. Brains are acquired from donors, by the tissue co-ordinator, through liaison with the next-of-kin at the time of death.

Disposal of Bequeathed Remains

In all cases the dissected remains are placed with the appropriate cadaver for disposal. Each cadaver is placed in a separate wooden casket, identified by a coded number, prior to removal from the medical school to the crematorium. The cadavers are transported to the crematorium, in a hearse, by a licensed funeral director.

Cadavers are cremated, approximately 3 years after acquisition, and the ashes are disposed of as requested by the next-of-kin. Following cremation and a non-denominational service performed by the university chaplain and attended by the head of the Department of Anatomy and the anatomy mortician, about 75% of the ashes of cadavers used are scattered at a local cemetery. The remaining 25% of the cadaver ashes are returned to the next-of-kin for private internment.

At all times the identity of the donor and the treatment of the remains are maintained and kept confidential; as well the wishes of the next-of-kin are put ahead of those of the School of Medicine.

> PROCEDURE UPDATE RECYCLING USED ACETONE IN PLASTINATION LABORATORIES

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In 1992, we published a technique for recycling acetone inplastinationlaboratories (Grondin andBerube, 1992). Our method includes three steps: step one called <u>"freeze-separation"</u> consists of leaving the used acetone at -20°C for 1-2 days and then filtering it through cheese cloth. This step is very efficient to remove fat from the contaminated acetone. The second step, called <u>"vacuum distillation"</u>, uses the standard equipment (vacuum pump, freezer, manometer) found in a plasination laboratory and produces an acetone that is 97% pure. The last step, called <u>"physical water extraction"</u>, is achieved by adding to the distillated acetone a dessicant that will extract the residual water and bring the purity to 99%. We now use for this step new molecular sieves (Fisher Scientific, catalog number M518-5LB). This new dessicant does not color the distilled acetone like our first one did.

In our original publication (Grondin and Brube, 1992), the second step did not give precise information on the pressure in the system during the distillation process. This was due to the fact that we only had, at that time, a Bennert Manometer which is not suitable to read a pressure higher than 24 cm of Hg. However, as a result of the money we saved on acetone disposal and procurement, we were able to buy a vacuum guage and add it to our system. This permitted us to measure precisely the pressure during distillation. For the distillation process, the acetone is heated to a temperature of 45° C to 50° C. Depending on the degree of contamination of the acetone to be distilled, the pressure will vary from 38 cm (15 inches) to 33 cm (13 inches) of Hg. We will never bring the pressure lower than 33cm (13 inches) of Hg-

When boiling stops by itselt at 50°C and 33 cm of Hg, the residue, which contains around 25% acetone, is put back in the freezer. After 2 days, ice crystals have formed and the solution is filtered to remove this ice. This remaining fluid is now ready to be distilled with our next batch of contaminated acetone.

The Temperature-Pressure equilibrium required to obtain boiling of the contaminated acetone will always depend on the percentage of acetone found in the mixture (Table 1). A mixture of acetone with a high degree of purity requires less energy to boil. It will then be easier to distill. It will boil at lower temperature and higher pressure.

TABLE 1

TEMPERATURE AND PRESSURE REQUIRED TO OBTAIN BOILING OF CONTAMINATED ACETONE						
% Acetone	Temperature	Pressure	Boiling			
70% 70% -	45°C 45°C -	38 cmHg 38 -	Good			
35% 35%	50°C 50°C	35 cmHg 35	Good			
25%	50°C	cmHg 33	Good			
		cmHg	Not			

NOTE: All the pressure measures indicated in this text and table are measures of the <u>real pressure</u> in the system during the process. We are not talking of measures of the vacuum applied to the system.

REFERENCE

Grondin, G.G., Berube, R.: A simple and inexpensive Method for Recycling Used Acetone on Plastination Laboratories. J Int Soc Plastination 6: 17-19, 1992.

Editors Note:

The above article originally appeared in Vol. 8 No. 1, ISP Journal. Due to editorial errors, we are reprinting this article in its entirety.

We regret any problems caused by this mistake and constantly strive to do our best for ISP.

PREPARATION AND UTILIZATION OF BEQUEATHED BODIES IN AUCKLAND NEW ZEALAND

Peter Cook and Brenda Dawson School of Medicine University of Auckland Auckland, New Zealand

The University of Auckland, School of Medicine has recently celebrated its 25th anniversary. Throughout its existence the school has been fortunate to maintain a high quality of facilities for the dissection and examination of human cadavers. It has also been able to maintain an adequate local supply of bequeathed bodies for the body donor program.

Standard Anatomical Embalming Procedure

At the Auckland School of Medicine a bequeathed body is normally received in the Anatomy Department's mortuary within 2 to 24 hours after death. No bequeathed bodies are accepted without communication between the licensed anatomist, the next-of-kin, an official from the medical school, and the donor's doctor.

Regardless of the cause of death, as stated on the Medical Certificate of Death, all bodies are treated as potentially hazardous. After acquisition all bodies are screened for HIV, hepatitis B and hepatitis C viruses. All bequeathed bodies deemed as a risk to staff or students are not accepted for use.

It is standard practice that all cadavers be thoroughly disinfected before use with a high strength virucidal cleanser such as Virkin disinfectant (Antec International, Suffolk, England).

Cadavers are not shaved or de-personalized, as is the case in some other medical schools, thus preserving the individuality of what is virtually the medical students first patient.

• Embalming proceeds with the cutdown and cannulation of either the right common carotid artery or the right femoral artery. Occasional problems are encountered as a result of vascular pathology (i.e. arteriosclerosis) and may necessitate perfusion through other vessels such as the left carotid, left femoral axillary or iliac arteries.

Arterial injection is done using a Portiboy PE10 perfusion pump at a pressure of 10-15 psi. During injection, the jugular or femoral at the sight of injection is isolated and clamped off.

The cadaver is pre-injected with 3-8L of Plasdo-form based embalming fluid (Dodge Chemical Co., Cambridge, Massachusetts, USA) consisting of metaflow and rectifiant. This is advantageous in arterial conditioning. During pre-injection venous pressure is maintained for approximately 30 minutes of arterial perfusion to allow the chemical properties of the metaflow additive to act in dissolving blood clots and expanding the capillaries of the cadaver. After 30 minutes the enlarged jugular or femoral vein is opened carefully to allow drainage of the arterial system resulting in the elimination of any dissolved clotted blood as well as reduction of postmortem discoloring (liver mortis) of the cadaver. Following pre-injection, the cadaver is embalmed using 18-25 litres of embalming solution containing the following:

2704 Formalin	2.5 L
Phenol Glycerine Methylated Spirits Mold-x Iceterine	1.0 L
	6.0 L
	12.0 L
	475ml
	a a a 1

During the initial stages of perfusion 20.0 ml the extremities and the head and neck

regions are massaged with warm soapy water and the superficial venous vessels are "milked out" to assist in the complete and even distribution of the embalming fluid into the smaller capillary systems of the cadaver. Following the arterial perfusion, supplementary local injections of embalming fluid with a large bore hypodermic needle, are administered to the hands, fingers, feet, toes and male genitalia if required. The body is thoroughly examined to ascertain the adequacy of the perfusion and the skin openings are sutured before sealing in a airtight plastic bag and storing at 4°C until required for anatomical dissection. Cadavers are used for dissection, clinical teaching, etc. approximately 12 months later.

DISCUSSION

While this method is excellent for the embalming of cadavers to be used for student dissection it has not proven entirely satisfactory for tissues which will be used for plastination using the S-10 technique (von Hagens, 1985).

Occasional problems of mold growth on some cadavers, exacerbated by high humidity, necessitated considerable experimentation with waterless embalming treatments. These studies have demonstrated that reduction or total elimination of water is an important and effective means of eliminating fungal growth on cadaver tissue (Mitchell et al, 1993).

Originally, the methylated spirit component of our embalming mixture was 6 litres with the remaining 6 litres consisting of boiled water. Initially, we increased the phenol component of the fluid to assist in mold control, however, this practice was abandoned due to the health risks involved in using this chemical. We also found that increasing the volume of glycerine helped to counteract the hardening effects caused from using high volumes of methylated spirits.

The non-water based embalming fluid formulated above is now routinely used, in this department, for embalming cadavers for student dissection.

The relatively warm, humid climate of Auckland predisposes the cadaver tissues to mold growth. To counteract this we have air conditioned our dissection laboratory and maintain it at a constant temperature of 14°C. This measure has proven to be:

- 1) Effective in controlling mold growth on cadaveric material
- 2) A significant factor in reducing the formaldehyde fumes given off by cadavers during dissection.

This air conditioning system works on the laminar air flow principle with 12 complete changes of refrigerated air per hour. Air is expelled from the laboratory through vents situated below the cadaver table levels.

SUMMARY

The methods we have outlined above for embalming and storing bodies in our medical school have proven satisfactory for the past 25 years. Cadavers prepared using these methods have been used on a regular basis by students, staff, clinicians and other health professionals. These techniques are constantly being improved upon by our embalming and prepatory staff.

Our facility is now able to prepare an infection-free, adequately preserved, and cosmetically acceptable, specimen for examination. In addition, the dissection laboratory is maintained as environmentally safe and odor free as possible. This encourages students to use our facility to its fullest extent, thus providing them with a valuable learning resource for the study of human anatomy.

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ANNOUNCEMENT

We are preparing a complete listing of what has been published on plastination since its introduction 20 years ago and wish to present the result of this work at the next International Conference on Plastination in Brisbane, Australia in 1996.

Presently, we have more than 100 references of papers published in 27 different journals, but certainly have missed some.

We would like to include in this work a list of the abstracts, papers or posters about plastination that have been presented at different conferences.

We ask for everyone's assistance. Please send a list of your references, publications (copies if possible) and abstracts to:

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PLASTINATION METHODS USED IN AUCKLAND, NEW ZEALAND

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INTRODUCTION

The acquisition, use and disposal of bequeathed human material in New Zealand is governed by strict regulations specified in the Human Tissue Act of 1964 (Dept. of Health, New Zealand, 1965). The University of Auckland is fortunate to have an adequate supply of body donors and because of this, a valuable teaching resource has been established in its medical school.

The use of teaching specimens prepared using plastination techniques has become an essential component of this resource. The use of plastinated specimens has greatly assisted students in their understanding of anatomy and their being able to correlate these specimens with radiographical images of the human body. Using these plastinated specimens has resulted in the establishment of self directed learning stations which the students may use for their study of anatomy.

PLASTINATION METHODS

In 1983, in the Department of Anatomy at the University of Auckland, School of Medicine, experiments in plastination were carried out on cadaveric specimens using the PEM 27 and PEM 30 methods (von Hagens, 1985), however, these methods gave varying results. Initial trials using the S-10 technique (von Hagens, 1985) and ethanol for dehydration also produced mixed results.

After acquiring an explosion proof deep freezer the S-10, ethanol dehydration procedure was discontinued in favor of the standard S-10, freeze substitution method of plastination (von Hagens, 1985). All subsequent S-10 plastination has been done in this manner.

The majority of specimens acquired for plastination were obtained from embalmed, dissected cadavers. A smaller number of specimens were retrieved from fresh cadavers during autopsies and were used for plastination with good results.

Experiments were carried out using various types of fixatives (i.e. Wentworths' Fixative, Jores' Fixative). We found that an arterial embalming fluid formulated as outlined in a publication entitled "An Improved Method of Embalming Suited to Plastination Techniques" by Cook and Dawson (1995) was the most suitable for our purposes.

Until 1993 all forced impregnation of specimens to be plastinated was carried out at room temperature, at 26°C, in a converted laboratory centrifuge, sealed, vacuum tight, and with a 3cm thick clear perspex lid Plastination at room temperature allowed quicker impregnation of specimens than that carried out in a deep freezer at -25°C. However, because S-10 resin polymerizes more readily at room temperature, it became necessary to find an alternate method to cut down on the expense of buying new polymer.

The acquisition of funding and the design and construction of a new vacuum infiltration chamber gave us the capability of doing all forms of plastination. The chamber was constructed of stainless steel and measured 92.0 cm 42.0 cm. It is covered with a toughened glass plate lid which is hinged to the foam rubber gasketed rim of the chamber. A Javac 60 L/minute double stage vacuum pump is connected to the chamber and the vacuum is controlled by a Whitey needle valve situated between the chamber and the pump. Pressure readings are obtained using a low vacuum gauge during the initial stages of infiltration and nonmeter gauge in the latter stages of infiltration when readings of 1.0 mm of Hg or lower are required. Pump oil is changed after each S-10 impregnation run or after 3-4 runs of E-12 or P-35. Changing of the oil is very important because it removes all traces of acetone or methylene chloride which may have been deposited in it during the infiltration process. These deposits can be harmful to the pump seals. This type of forced impregnation system has proven extremely effective.

We have found that slow seepage, of greasy residues (probably a combination of bone marrow and phenol-glycerine components of the embalming solution used) from some S-10 plastinated specimens has been a problem in the past. To combat this, specimens are immersed in a mixture of 50-90% ethanol containing 5-20% Hydrogen Peroxide. This aids in extracting of embalming fluid ingredients prior to dehydration. Specimens are processed using the standard S-10 technique (von Hagens, 1985).

At present many old and valuable dissected prosections are being processed using the standard S-10 silicone method.

At the Auckland School of Medicine strong emphasis has also been placed on the use of serial sectioned cadavers and organs for teaching. These specimens have been used in correlation with computerized tomography (CT) scans, magnetic resonance imaging (MRI), ultrasound scans and traditional x-rays for teaching and instruction. Serial horizontal sections of head and neck, trunk and pelvis are being presented to the students in large display units for demonstration purposes (Figs. 3&4). Recently, 25 coronal specimens of the head and neck have been added. These specimens are displayed in removable mounts with corresponding annotated photographs and radiographs.

Head specimens, serial sectioned in the coronal plane and plastinated using the E-12 method (Weber, 1993), have been particularly well received for study purposes by both the students and staff. Certain aspects of the intricate anatomical structure of the human head, not clearly identifiable in traditional prosected cadaver material, have been emphasized using these specimens.

Brain sections, plastinated using the P-35 technique (Weber, 1994) have also been produced. These specimens not only show superb white and gray matter differentiation (Fig. 5) but they are free of formaldehyde odor and have a clean, smooth finish which is aesthetically pleasing to the eye.

SUMMARY

The development and perfecting of plastination techniques in the Department of Anatomy, at the Auckland School of Medicine, has provided new avenues for the production of specimens for teaching of modern clinical anatomy. Particularly, the preparation of cross sections of the human trunk and brain, which when accurately correlated with modern diagnostic imaging techniques, provides an invaluable teaching aid.

Plastinated specimens have also proven to be of great value providing a means of producing teaching material which can be kept for longer periods of time, with little or no maintenance, than can traditional prosected specimens. This helps to assure us that we will be able to provide specimens for teaching in the future even though we may experience budget restraints or depleted bequests from donors.

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AN IMPROVED METHOD OF EMBALMING SUITED TO SUBSEQUENT PLASTINATION REOUIREMENTS

Peter Cook and Brenda Dawson School of Medicine University of Auckland Auckland, New Zealand

INTRODUCTION

Plastination of tissues from cadavers embalmed using the standard anatomical embalming solutions has posed problems. The glycerine and phenol components of most embalming fluids are essential for long term tissue preservation but are not ideal for the fixation of tissues to be used for plastination. Because of this, new and improved embalming solutions have been developed specifically for use on cadavers to be used for plastination. Ideally, these solutions must fix the tissues properly and retain natural color within the tissues, while at the same time avoiding the use of long chain alcohols, phenols and glycerines which are detrimental to the infiltration of resins into the tissues during plastination.

In order to assess their fixation ability in respect to plastination some established fixative solutions were used on cadaveric material. It was found that Wentworths and Jores solutions were suitable for preparing individual organs of embalmed cadavers or fixing tissues of unembalmed cadavers obtained at autopsies. A large number of commercially prepared embalming chemicals (Dodge Chemical Company, Cambridge, Massachusetts, USA) were found to be suitable for maintaining color and retaining softness of tissues in embalmed specimens which were to be used for standard prosection techniques.

In order to solve the problem of proper fixation of cadavers for plastination we decided to improve on the performance of past embalming solutions by formulating our own solution and using it for embalming.

METHOD

The cadaver of a thin, 88 year old male, who died of bronchopneumonia, was embalmed through the right common carotid and right femoral arteries. The right jugular vein was used as a drainage point during injection. The total injection consisted of 18 litres of embalming fluid containing chemicals (Dodge Plasdoform based) combined in the proportions shown in Table 1.

Table 1

	700ml
Metaflow	700111
Metasyn Accelerated	700ml
Rectifiant	500ml
Mold-x	500ml
Non-Deionised Water	1600 ml

Perfusion was done using a Porti-Boy embalming pump at a pressure of 10 psi. Following embalming the cadaver was stored at 4°C for 4 months prior to dissection.

DISCUSSION

When the cadaver was dissected for plastination it was noted that there was no degeneration of the skin, fat, muscle, joints or organs. Tissues remained extremely soft with no swelling. Coloring of individual tissues were more realistic than that achieved using other embalming formulas.

Because the tissues within the cadaver remained very moist, there was minimal "firming" effect on either the fatty layers of the body or on the blood within the capillaries and major vessels. The advantage of this was that it allowed for the injection of red and blue latex, into the arterial and venous systems, even though the body had been embalmed some 4 months previous.

Another advantage of using this embalming technique is that muscle bundles and nerve plexuses could be prosected intact instead of piecemeal with no difficulty.

The relatively pleasant aroma of the fluids used for this embalming fluid, in contrast to the pungency of formalin and phenol of other formulas, is another pleasant advantage to using this solution. This is a particularly welcomed advantage to the prosector or student who had previously spent hours hovering over a strong smelly specimen while dissecting.

This solution, though found to be very suitable for the preservation of specimens to be used for plastination and prosection, was not entirely recommended for use on specimens to be used for student dissection.

To date some six cadavers have been prepared using this method. Most recently, cadavers which had been embalmed using the above formula, were used for dissection at a plastic and reconstructive surgery workshop. This workshop is held annually at our facility for groups of international surgeons. It was found that because the cadavers were prepared in this way manipulation of tissues was much easier and that complex surgical techniques, which could not have been performed on cadavers embalmed using other preservation methods, were carried out with ease. Also the tissues of the cadaver more closely resembled that of living tissue.

CONCLUSIONS

The embalming formula outlined in this paper has provided us with a fixative which can be used on specimens for prosections and plastination. It has helped to facilitate the production of high quality specimens for the teaching in our graduate and undergraduate programs.

As well, this technique has allowed us to produce specimens which can be used in the teaching of specialized methods within our curriculum (i.e. reconstructive surgery courses) at the School of Medicine. This has led to international acclaim for the Department of Anatomy and its courses.

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INTERIM MEETING SUMMARY

Tim Barnes Ohio University

This is an UNOFFICIAL account of the interim meeting conducted (Friday, July 14th 1995) at The Ohio State University, Columbus, Ohio. The views put forth here are those of the writer and NOT of the International Society for Plastination.

It was Bastille Day and it was hotter than the hubs of hell, but the only "storming of the fort" that took place was when the interim meeting of the International Society for Plastination took over the auditorium of Goss Hall at the Ohio State University School of Veterinarian Medicine and talked about fixation, dehydration, de-fatting, impregnation, and curing of specimens.

Bill Richeimer, Rita Remy and their assistants had things well in hand organization-wise, and we were greeted by the Dean of the school. Harmon Bickley held forth about the early days of plastination. It's always a pleasure to hear Harmon talk about the wins and losses, trials and troubles, successes and surprizes that he, and those he knows well, have experienced in bringing this technology to its present-day level. One of these days we need to establish a "Distinguished Chair of Plastination" and seat Harmon in it in perpituity.

Bob Henry and I had been tapped to do the standard S-10 dog and pony show. Bob was having some trouble getting a grandchild brought into the world and I had had a premonition that most of those in attendance would be experienced plastinators so I had gathered up several specimens to take along that were other-than-ordinary specimens. One or two had been damaged by hard use. One was an old museum specimen heart that we had had for several years as a wet specimen and was consequently discolored but had a "ball and cage" valve in it along wth the remaining suturing; a longitudinal cut of a Paget's syndrome tibia that showed marrow still in place (there was a discussion of this on the net a month or two ago); a mesenteric artery that illustrated how to deal with difficult shapes when curing a specimen; and an entire infant that illustrated how it is sometimes necessary to do considerable hypodermic on extremities and the interior of the cranium.

Gilles Grondin made two presentations. One concerned his ingenious method of acetone reclamation and the other concerned color injection of the hand to assist anatomy students in understanding both the superficial and the deep vessels. I don't know if Gilles stays up all night thinking up new ways and means to do plastination, but over the years he had come up with some very useful techniques. I am envious of his ability to analyze and overcome hinderances that we all encounter in our work.

I am always impressed by anyone that is willing to share their failures as well as their successes. Bill Martin did just that in relating his experience in plastinating a thorax/ abdomen block from a rabbit. The key to his final success was filling the gut with a gel during the fixation stage.

After having known Alex Lane for several years, I am convinced that Alex only sees people in terms of anatomical planes. He has made outstanding use of plastinated specimens cut in the various planes and compares them to CAT scans of the same area in teaching that dignostic method to students of ancillary medical arts such as med techs, physical therapists, and nurses.

Andreas Weiglein did yeomans duty by making three presentations concerning plastinated specimens and neuroscience. He is now experienced in using some of the newer polymer procedures and the results are outstanding. Graz is lucky to have him.

Michael Wu displayed nicely prepared brain specimens for us to examine.

Bill did a good job of feeding us at noon although he denied staying up all night and doing the cooking. Bill has a well equipped plastination lab and we got to see two students doing some outstanding prosections for the up-coming school year.

As could be expected, we told numerous lies to each other about how much we are being paid, about the tight purse strings at our respective institutions, and about how much we drank at the previous plastination meetings. While this was an abbreviated meeting, much good information was shared amongst the group. I am reminded that I am glad that the plastinator's meetings are a "manageable" size group of people and the individual technician does not get lost in a morass of personalities with a lot of political axes to grind. Those in attendance are there for a learning experience rather than to see and be seen.

An announcement was made about the full international meeting scheduled for next year in Brisbane, Queensland, Australia and the sponsors had forwarded literature for our perusal and pipe-dreaming until we can get there. According to the brochure the dates are 14-19 July 1996. Make your plans early.