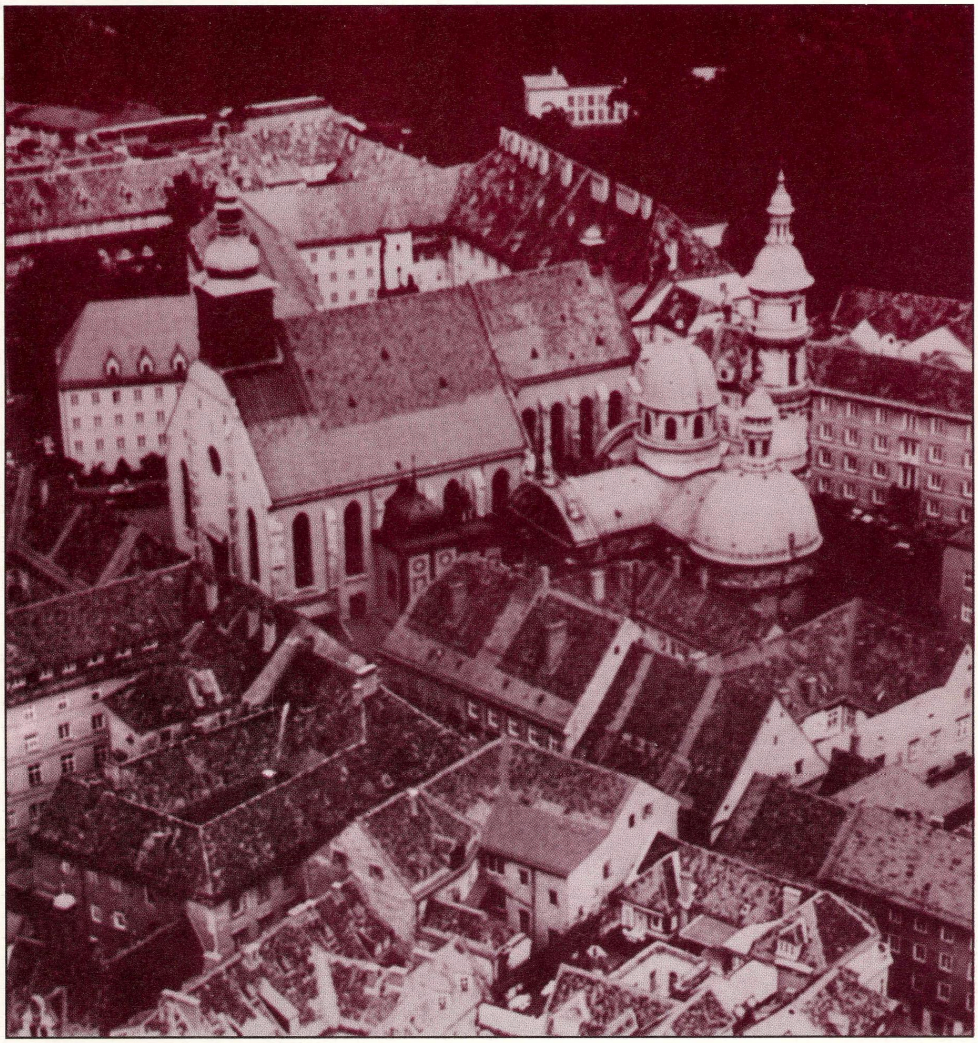
VOL 8 # 1

journal of the INTERNATIONAL SOCIETY for PLASTINATION



7th INTERNATIONAL CONFERENCE ON PLASTINATION

GRAZ Our way of being a town...

Fourth Biennial Meeting of the International Society for Plastination

24 - 29 JULY, 1994 KARL - FRANZENS - UNIVERSITY GRAZ, AUSTRIA, EUROPE

JOURNAL of the

INTERNATIONAL SOCIETY FOR PLASTINATION

Official Publication of the International Society for Plastination

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93 Interim Conference A Success

August 3-7, 1993, Mobile, AL., was the place to be. This year's conference was well attended with 83 registrants. Everyone in attendance was well pleased with the program and the nighttime forays. Wayne and Bill especially enjoyed themselves.

Wolfgang Weber talked about and provided hands on demonstrations of sheet plastination. These specimens are the first actual brain slices to be done in the U.S. Most of them turned out to be fine teaching specimens.

Other speakers included: Dr. Harmon Bickley; Dr. Bob Henry; Dr. Vincent DiFabio; Ron Wade; Dale Ulmer; Tim Barnes; and the meeting host, USA Pathology Chairman, Dr. William A. Gardner, Jr.

Many good ideas were exchanged as speakers lectured and answered questions. Experienced plastinators mixed with first time conference attendees and shared many valuable tips and hints. Approximately one-half attending this conference were newcomers and were extremely interested in becoming part of and participating in our society.

An informal meeting of members was held with many of the opinion that it is probably time to elect society officers in order to be more organized. This is to be on the agenda for '94 GRAZ meeting. Bill Wise has offered for the '95 Interim meeting to be held in Raleigh, NC, and most seemed highly receptive to this invitation. Good luck, Bill! You'll do super.

As the '93 Interim is history, I would be remiss if I didn't especially thank everyone for all the help and effort in putting this meeting together: Ms. Rosemary Farmer; Mrs. Betty Clark; Mrs. Jan Erdelen; Mrs. Debra Reynolds; Mrs. Rebecca Moring; and Dr. William A. Gardner, Jr., as well as the USA College of Medicine.

Looking forward to seeing everyone in GRAZ, Austria, July, 1994.

Dale Ulmer, PA

MONITORING AND ENHANCEMENT OF FIXATION, DEHYDRATION, FORCED IMPREGNATION and CURE in the STANDARD S-10 TECHNIQUE

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Valeria Panebianco, Monica Perez, Maria Letizia Boccia,
and Giulio Marinozzi University of Rome "La
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SUMMARY

The goals of this study are: 1) to monitor and improve some phases of plastination in order to reduce any waste of reagents and time of processing of parenchyma! organs; 2) to avoid distortion of morphology; and 3) to enhance chromatic yield. This study was carried out using the standard S-10 technique.

INTRODUCTION

Using the protocol according to Von Hagen (1985) we have carried out the plastination technique using various Biodur polymers, (S-10, E-12, P-35). Technical mistakes can give rise to specimen shrinkage of over 20%, changes in chromatic yield, changes in mechanical properties, and/or distortion of the whole sample. We closely examined the various steps of our plastination process in order to find an ideal protocol for processing a parenchymal organ, as well as, identify and rectify any specific problem areas of the procedures that could possibly endanger the best result of the S-10 process. In the different sections of this paper, we will outline the procedure which we found to produce the best results and will underline the changes made in the standard technique. Finally, we will suggest a new method of monitoring dehydration and forced impregnation.

MATERIALS AND METHODS

Eleven parenchymal organs (liver, kidney and spleen) were used for this project. Fixation was performed in 3% and 6% formalin solutions (1,2) Dehydration was performed in cold acetone (-20°C) by freeze substitution (1-3). An alcoholometer (acetonometer) and an immersion thermometer were used to determine acetone purity.

Forced impregnation was performed in a vacuum chamber at room temperature with Biodur S-10 polymer mixed with the S-3 hardener. The basic protocol was followed according to current literature (1), however, the protocol was adapted to the different physical properties of the organs. Moreover, by observing our technical artifacts, mechanical features, and weight of the specimen, an enhanced procedure was developed.

ENHANCE PROCEDURE:

To prepare the specimen for plastination, the organ was washed in running tap water for about 24 hours. The first phase is an accurate surgical preparation of the features of the organ which are most interesting for the teaching of anatomy (e.g. hilum). Special care is taken in shape preservation. Unfortunately, most of the material we plastinate is isolated and deformable organs. To avoid any alteration of their morphological structures we cling the specimen onto a metallic grid linked to a metallic basket (1). It is necessary to make certain the specimen does not lie on the bottom of the receptacle. This enables us to move the basket into various tanks containing fixative, dehydrating, or impregnating solutions. This technique makes it easier and is harmless to the specimen. The specimen was suspended for the first three days in 3% formalin, followed by *6%* formalin for 10-15 days for organs weighing <500 g. and for 17-22 days for organs weighing > 1000 g. Specimen weight and fixative fluid ratio was 1:5 (kg/L). The organs were fixed at +5°C to give rise to a better chromatic preservation than fixation at room temperature.

Dehydration

Dehydration was performed by freeze substitution at -20°C with specimen/acetone ratio of 1:5.

The specimen was first placed into a 97.5-98% acetone bath. From the beginning of the dehydration, between the 24th and the 48th hours the first change with pure acetone was performed. We agitated the acetone solution and controlled the acetone concentration every 2 days from the 3rd day to the 10th day for organs weighing <500 g and from the 3rd to 14th day for organs weighing >1000g. If the solution concentration was not lower than 97.75% the organs achieved the best result. Two baths are adequate only if acetone concentration in the second bath remains above 97.75%. If during the 2nd bath, the concentration falls lower than 97.75%, additional baths of 100% acetone must be used in order to keep concentration of acetone above 98%. From the 10th day to the 14th day, according to the specimen weight, should the concentration be 98% or less a new dehydration with 100% acetone should be performed for two consecutive days. When acetone concentration remains stable for a minimum of three days at least (99% - 100%), dehydration is complete. We received the best results with organs which passed through 3 or 4 baths on average. Bath duration was 11-14 days.

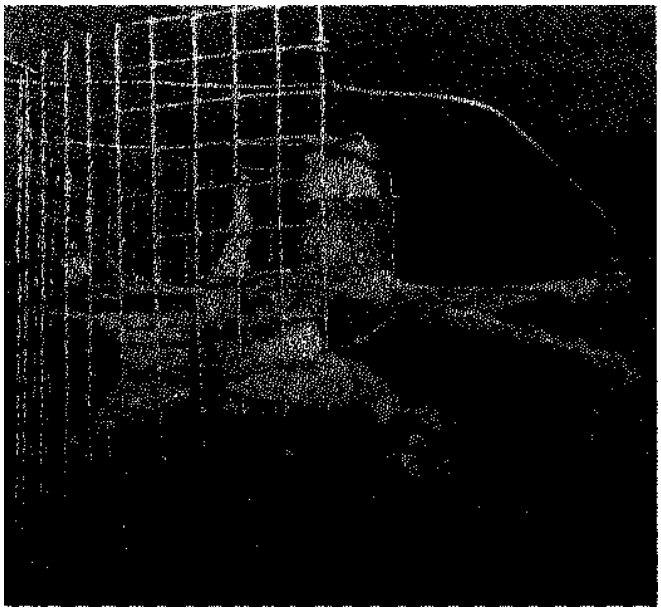
Forced Impregnation

Forced impregnation commenced after dehydration was complete by submerging the specimen in a mixture of Biodur S-10 and Biodur S-3 in a vacuum chamber at room temperature. At this temperature the resin is fluid and flows in to the organ quickly. Table 1 shows the vacuum values we tried to achieve. Not increasing the vacuum for one day could be convenient to the operator and profitable for the result of plastination. From our experience it is better than interrupting the vacuum increase in an intermediate impregnating time. When absolute pressure was stabilized at 15 mm Hg for 24 hours, the specimen was considered completely impregnated. The plastinated specimen was placed in a receptacle for 28 days to let the excess polymer drip from the specimen, it was then wiped of any remaining excess polymer. Afterwards, the specimen was exposed to the action of the rapid polymerizing Biodur S-6.

Monitoring the dehydration and forced impregnation

We carefully monitored these two phases. Because of our experience technical mistakes during dehydration and forced impregnation can give rise to artifacts which are not possible to correct, while some fixation and curing mistakes may be remedied. Dehydration time must be kept as short as possible to prevent brittleness and a decrease in the flexibility of specimens. In order to know the degree of dehydration of the specimen it is important to measure the water content of the acetone solution. However, the percentage reading of acetone purity increases with temperature. Therefore, the proper use of the alcoholometer, including temperature adjustment, is important for monitoring the acetone purity. Alcoholometers are generally calibrated to calculate the acetone concentration at a given temperature. We use the Tralle's alcoholometer which is calibrated within a range between (+10° and +30-40°C). Von Hagen (1) described the measuring of the water content of dehydrating baths by warming the acetone to the temperature for which their alcoholometer is calibrated. Warming acetone to room temperature can be time consuming. Using the following method one can quickly calculate the water content of the dehydrant while the acetone is still cold. In order to achieve this we devised a warming trough and a diagram of an average of three pure acetone solutions. Therefore, for 3 pure acetone solutions we calculated the concentration values at a temperature between -25°C and +25°C. Following this diagram the value of the pure acetone concentration (value obtained by alcoholometer) at +15°C is 100% (fig. 2). At different temperatures the calculated values of acetone concentration will be different. The difference between the real concentration and the "pure solution line" permits us to know the level of the water percentage in the dehydration solution. The impregnation can be monitored with a manometer when the pressure valve into the vacuum chamber is steady. We consider steady vacuum when release of the bubbles is very few or none. In order to allow described measurement, we calculated the pressure in the vacuum chamber, at the beginning and at end of the working day (from 8 AM to 4 PM for safety).

We suggest not exceeding the pressure valves of table N-l. Finally we can monitor the grade of an extraction from the specimen by the observation of the amount of bubbles on the impregnation solution surface. This control is easy to perform at room temperature.



DISCUSSION

According to our goals the organs we plastinated by means of this protocol achieved the good quality that we desired. The monitoring of the dehydration permitted us to know the essential minimum amount of acetone to use and reduced the impregnation time (about 10 days) (4). The use of an expensive freezer for impregnation was eliminated.

This paper may be useful for laboratories that are going to begin basic plastination or do not do a large amount of processing. Although, performing impregnation at room temperature limits the Biodur time life, on the other hand, it reduces the impregnation time. This is important if the laboratory plastinates small organs and stores the resin in a freezer at *-25°C* among the working intervals.

FORCED IMPREGNATION

Days	1	2	3	4	5	6	7	8	9
Specimens weight <500g	100	90	SO	pause	40	25	15		
Specimens weight >1000g	125	100	80	60	pause	50	30	20	15

(Pressure expressed in mbar)

Tab. 1

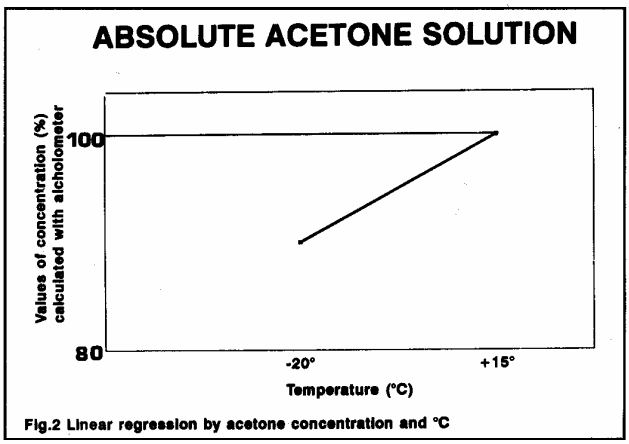


Fig.2 Linear regression by acetone concentration and °C

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PLASTINATION MODEL IN ORAL AND MAXILLOFACIAL SURGERY

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Drs. DiFabio, Williams, and Staropoulos, Amber Meadows Prof. Bldg., Frederick, MD; UMAB Medical School Anatomical Facility MD, Anatomy Board, Baltimore, MD

Plastinated cadaveric material has been used for medical teaching purposes for over fourteen years. The literary search in oral and maxillofacial surgery and head and neck surgery has shown little work in a plastinated model. The process of plastination was developed by Dr. Gunther von Hagens in Germany in 1978 and has been modified many times since. The basic process involves the extraction of lipids and water from fresh cadaveric specimens and replacing the lipids and water with a silicon-type material.

The use of plastinated specimens in teaching head and neck anatomy has several advantages over embalmed specimens. The plastinated specimens are non-toxic, non-lipidic, and odorless. Multiple sections of these specimens can reveal the three dimensional aspects of anatomy and whole specimens can be used for demonstration of surgical techniques such as arthroscopic surgery.

The process of plastination begins with fresh, frozen, whole or 5mm sections of the head which are placed in a 20% formalin solution for a period of one week. The 5mm sections were obtained in the three anatomical planes using separate specimens. A "freeze substitution" dehydration method is utilized that impedes rapid dehydration and produces less tissue shrinkage and distortion. The water in the tissues was replaced by an acetone solvent. After fixation the specimens were transferred into several cold acetone baths, which were pre-cooled at -25 degrees centigrade over the course of three weeks. By the end of the third week, the acetone was measured at 99%. The container with specimens was left under the hood for three days to allow for the removal of some lipid content of the specimens and returned to the dehydration freezer for pre-cboling in preparation for silicon impregnation.

The impregnation is performed using a dedicated ultra-cold, chest-like, explosion safe freezer which is maintained at a temperature of -28 degrees centigrade. This freezer has been specifically designed with built-in ports for vacuum lines and has a full length frost free viewing window in the lid. Inside the freezer is a large stainless vacuum chamber which contains Biodur S-10 (silicon) of sufficient quantity to allow for immersion of the specimens being impregnated. After forced impregnation of the silicon material, the specimens are cured for several days to allow for hardening under a fume hood.

The sectioned material can give a three dimensional view of facial anatomy which is now preserved for future study and examination. The whole material can be used for demonstration of surgical procedures or for teaching Temporo-mandibular Joint arthroscopy.

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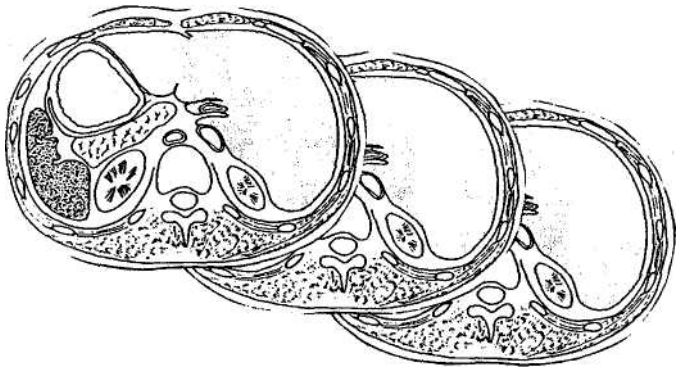

FIXATION
The Key to Good Tissue Preservation

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The foundation of all good tissue specimen preparations is complete fixation. The primary function of tissue fixation is to prevent putrefaction and autolysis. Faults in fixation cannot be remedied at any later stage, and the finished product can only be as good as its initial fixation.

With today's modern techniques human tissues from ancient civilizations continue to be scientifically dated and studied.

Mummification is one of man's earliest methods of human tissue preservation. Mummification is a term loosely used to describe different methods used by ancient civilizations to prepare human remains for burial. Various methods of preservation have been described in many articles. Some mummies which have been studied by scientists have become quite famous not only for their part in history as individuals, but also for the wealth of medical information obtained from their mummified bodies. Autopsies performed on mummies have given great insight into medical problems of earlier civilizations. For example, a leading cause of death in Pre-Columbian peoples, going back over 5000 years, was pneumonia. Also noted were lung abscesses, pericarditis, and endocarditis, as well as tuberculosis. These studies have prompted scientists to ponder about which diseases were present before the arrival of Europeans in America.

A major health problem in our population today is neoplasms, particularly malignancies. Medical studies of preserved human tissue from earlier eras have revealed that tumors were scarce in early American man. It is a medical phenomenon that scientists do not fully understand. Many think that this phenomenon is due to longevity, but there are also statistics that indicate 25% of early populations lived past 40 and some lived past 70 years of age. Hopefully, future studies will lead scientists to new and expanded information that eventually will enable us to have a good overview of daily life and the influence on such factors as: social status, economy, environment, and politics.

Such studies reveal valuable information to archaeologists, anthropologists, and pathologists as well as general medical historians. Thus, plastination and tissue preservation will, in the future, be a significant tool in historical studies.

Pathology and Anatomy museums are again becoming popular. Once confined mainly to teaching hospitals and special institutions, they are now being created in many hospitals and universities. This is due mainly to decentralization of certain aspects of undergraduate and postgraduate medical education, and the increasing involvement of non-teaching hospitals in these activities.

A well organized pathology museum should serve many functions, thus tissue fixation before plastination is of the utmost importance.

Fixation is required to prevent putrefaction and autolysis, and to preserve and harden to a lifelike state.

Fixation agents are often chemical.

Functions of Fixing Agents:

(1) To set organs or parts of organs so that microanatomical arrangement of tissue elements will not be altered. (2) To set intracellular inclusion bodies so that the histocytologic and cytologic conditions of cells may be studied.
(3) To arrest autolysis, putrefaction, and other changes.
(4) To bring out differences in refractive index of tissues.
(5) To render cell constituents insoluble and make them resistant to subsequent processes.

Fixation Agents Must:

(1) not shrink or swell tissue
(2) not distort or dissolve tissue parts
(3) render enzymes inactive
(4) kill bacteria, molds, and viruses
(5) modify tissue constituents so that they retain their form when subjected to dehydrants, clearing agents, and embedding media.

Usually, specimens removed at an operation or at necropsy have already been placed in a formalins or saline solution before being sent to the plastination lab. This fixative is most obvious.

Fixatives used today in most museums are based on a formalin fixative technique, and have been derived from Kaiserling (1897). Although there are some modifications to this technique, these tend to be minor and are usually prompted by problems in obtaining certain materials.

The method recommended by Kaiserling was based upon initial fixation in a formalin based fixative which contained salts to give an approximate neutral pH to the solution. This solution contains 10% formalin, potassium acetate, and potassium nitrate. Specimens should be placed in an adequately oversized container with 3-4 times their volume of fixative. Most times one fixation solution will suffice, but larger specimens may require solutions to be changed once or twice. The period for which the specimen should remain in the solution depends on its size, from three days for a small specimen, to 14 days for larger specimens.

Due to the hardening action of formalin, the way in which a specimen will ultimately be presented depends upon maintaining its natural shape during fixation. It is, therefore, important that a specimen does not rest on the bottom of a container thus producing an artificially flat surface or causing unfixed areas.

Cut hollow organs should be padded with cotton, but if uncut, they can be pressure inflated. Fixative can be injected with a hypodermic syringe and the injection pressure required is usually obvious. It is important to avoid over-inflation when dealing with elastic organs such as lungs. A recycling pump with pre-set pressure is recommended.

Solid organs may sometimes be perfused through main arteries. If this is unsatisfactory, the organ should be cut into slices on the proper plane so the organs can fix properly. Spot injection of fixative should be avoided. Specimens should be cut with a sharp, flat blade, knife (at least 30 cm) in one even cutting stroke. This avoids serration and distorted viewing surfaces.

Because of the way in which fixatives affect different types of tissues in the same organ, distortion is a frequent problem. Specimens should be pinned, suspended, inflated, or padded to produce the best results.

Other fixatives encountered before plastination include:

Zenker's fluid
Mercuric chloride, 5g
Potassium dichromate, 2.5g
Sodium sulphate, lg Distilled
water, 100 ml Glacial acetic
acid, 5 ml

This fixative is a good routine fixative giving fairly rapid and even penetration. Following use, tissues must be thoroughly rinsed with water. Small pieces less than 3 mm are fixed in 2-3 hours.

Bouin's fluid
Picric Acid - saturated Aqueous solution, 75 ml
Formalin (40% formaldehyde), 25 ml Glacial
acetic acid, 5 ml

This fixative penetrates rapidly and gives excellent nuclear fixation with preservation of glycogen. It causes considerable shrinkage and destroys most cytoplasmic elements. This shrinkage can be reduced by fixation @ 0°C for 18 hours. Small pieces fix in 15 minutes.

Gough Sections:
Whole organs may be sectioned on paper by the methods of Gough and Wentworth. These sections provide valuable information on whole organ structure and serve as links between mounted museum specimens and histologic sections.

1) Distend organs - fix 2 days - 3/4" slice. Cut and place in water to remove formalin.
2) Specimen in 60°C Heated solution to extract air.
3) Cast in gelatin solution for microtome cutting, cool sections overnight to harden.
4) Specimen placed on Whatman paper and covered with Perspex paper.

Oil of Wintergreen/Dawsons Method;

This staining method is often used for staining sections of embryos, fetuses, and small animals.

1) Eviscerate through small midline abdominal incision. Fix in 95% alcohol for 2+ weeks.
2) Rinse in tap water and placed in 1% KCO (Potassium Carbonate) for 4+ weeks.
3) Clear specimen in 1% KOH (Potassium Hydroxide) for 10 + days.
4) Wash in tap water 12 Hours.
5) Stain 30-60 minutes in 0-1% Alzirin red to which 6-10 drops of 1% KOH is added.
6) Decolorize soft parts in 20 percent glycerin and 1% KOH. This step takes about 2 weeks.

Specimen becomes entirely transparent showing ossified skeleton stained a deep red. This fluid is an excellent defatting agent.

As formalin is the most common fixative, it is likely that much of the tissue material to be used will be so fixed.

Preservation of tissues by freeze drying is often discussed as a method of fixation, but it is, correctly, an alternative to fixation. This method preserves tissue with little alteration in cell structure or chemical composition which permits one to skip stages of dehydration by alcohols and clearing.

The technique of freeze drying consists of two stages:

1) Initial rapid freezing (Quenching) 2
) Drying of frozen tissue

Quenching:

Cool isopentane to -16O°C to -180°C with liquid nitrogen then plunge small pieces of tissue into solution. It is essential that tissues be absolutely fresh so that rapid freezing inhibits autolysis and prevents diffusion of substances within the cells.

Drying:

Frozen tissue is then placed in a drying apparatus where high vacuum is established and the ice in the tissue is transformed to vapor. Higher temperature is maintained for the faster drying. For example: Raising temp to -60°C to -40°C increases evaporation rate tenfold.

Freeze Substitution:

In 1941, Simpson described a freeze substitution technique as an inexpensive alternative to freeze drying. In general, the results are not always as good, but this method can be employed in a routine laboratory without purchase of expensive equipment.

After cooling in isopentane, these tissues are allowed to reach room temperature slowly and then processed. In 1961, Balfour used freeze substitution as a method of preparing tissue sections for fluorescent antibody staining.

Decalcification:

The presence of calcium salts in tissue prevents good fixation. After decalcification, the natural color of the specimen is lost; it is, therefore, necessary to restore the specimen to as near its natural color as possible. There are many ways this can be done, and the one I recommend is the second stage of Kaiserling's method. This involves removing the specimen from the fixative, washing in running water, and transferring to 95% alcohol. The specimen is placed in alcohol for 1/2 to 12 hours during which time it is watched carefully for color to develop throughout the specimen. If not already done, it is at this stage that the specimen is photographed. When color restoration is satisfactory, the specimen is ready for the plastination process.

Multiple concentrations of formalin fixative can be used. One such method is Klotz solution according to Rodriques (1973) which preserves the natural color.

Briefly, after fixing the specimen in Koltz I for 5-10 days, the specimen is then transferred to Klotz

II where it can remain for an indefinite period of time.

Kloutz I :
Sodium Chloride 90g; Sodium Bicarbonate, 50g; Chloral Hydrate, 400g; Formaldehyde, 37%, 300 ml, and Distilled H_2O, 10,000 ml.

Tissue should be thoroughly (12 hours) washed in tap water before transferal to Klotz II.

Kioto II Solution;
Sodium Chloride, 90g; Sodium Bicarbonate 50g; Chloral Hydrate, 200g; Formaldehyde 37%, 100 ml; and Distilled Water, 100,000 ml.

Reduced formaldehyde concentrations are often used with delicate tissues such as subarachnoid spaces. Cerebrospinal fluid should be removed with a syringe before injecting these areas with formalin. Fixation time is 20-24 hours.

Injection of brain tissue via basilar arteries should be accomplished with 100% formalin. After perfusion, the arteries should be ligated. If the brain is only submersed, this should be done with 5% concentration.

Organs to be injected with contrast media should also be worked before fixation. Fixation also includes defatting of tissues. Defatting of specimens can be accomplished with 70% ethanol for three days and then dehydrated in increasing concentrations of ethanol at room temperature. After defatting for 2 days in ethanol, specimens are placed in methylene chloride for three (3) days.

Microwave Fixation;
The history of microwave fixation is brief. In my opinion, enterprising and imaginative pathologists will devise new uses for microwave fixation in labs due to the low costs of purchase and short procedure time. These units can be moved close to patients which is good particularly in small hospitals.

New fixation techniques involving microwaves are used in research and have created new possibilities and handling of brain tissue, and embryos for research.

In short, we are at the beginning and not the end of microwave fixation.

In summary, the choice of fixative will be governed by the type of investigation or specimen required, both immediately and in the future. Large pieces of tissue should be fixed in a tolerant fixative, such as formyl saline, which allows subsequent treatment. Smaller pieces can be removed from the mass and given special treatment if required. It should be remembered that museum specimens to which color is to be restored, should be ideally placed in alcohol or formalin for fixation.

Rarely will one fixative be suitable for a variety of methods.

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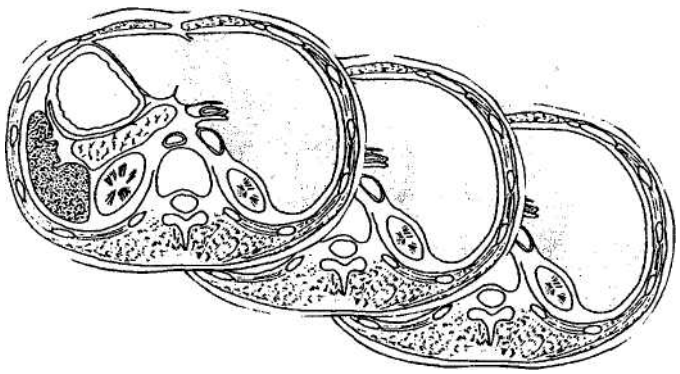

PROCEDURE UPDATE

RECYCLING USED ACETONE IN PLASTINATION LABORATORIES

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Last year, we published a technique for recycling acetone in plastination laboratories (Grondin and Berube, 1992). Our method includes three steps: The first step is called "freeze-separation" which consists of leaving the used acetone at -20°C overnight and then filtering it through cheese cloth. This step is very efficient in removing fat from the contaminated acetone. The second step, called "vacuum distillation", uses the standard equipment (vacuum pump, freezer, manometer) found in a plastination laboratory and produces an acetone that is 97% pure. The third step, called "physical water extraction", is achieved by adding to the distilled acetone a desiccant that will extract the residual water and bring the purity to 99%. The molecular sieves (Fisher Scientific, Cat. No. M518-5LB) are used for filtration which eliminates the light yellow coloration of the acetone.

In our original publication (Grondin and Berube, 1992), the second step did not provide precise information on the pressure requirements in the vacuum system during the distillation process. This was due to the fact that we did not have an adequate pressure gauge to monitor the pressure. The manometer used was a Bennert Manometer which does not register a pressure reading high enough to meet the requirement for the procedure. But, as a result of the money saved on acetone disposal we were able to purchase a vacuum gauge which precisely measures the pressure during the distillation. In beginning any exacting procedure, good reliable equipment must be used, and not simply trying to save money just to start up.

REFERENCE

Grondin, Giles G, S. Berube: A simple and Inexpensive Method for Recycling Used Acetone; Vol. 6, J. Intl. Society Plastination, p 17-19.

See Details on Page 31

COMPARATIVE ANALYSIS of a PLASTINATION SPECIMEN and CLINICAL DIAGNOSTIC IMAGES

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Giampaolo Tomaselli, Laura Giacomelli, Silvio Messinett and Giulio Marinozzi.
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SUMMARY

An invasive apocrine carcinoma of the perineal glands, involving the anal canal, vagina and vulva was surgically removed from a 62 year-old woman. The specimen was plastinated and macroscopically sectioned serially. The sections were photographed and compared to CT images made prior to surgery to verify and support the accuracy of the clinical diagnostic images.

MATERIALS AND METHODS

A CT study of a 62 year-old woman revealed a neoplasm involving the left ischiorectal fossa, with neoplastic encasement surrounding the left heme-circumference of the anal canal, invading through the subepithelial structures of the vulva and posterior wall of the vaginal canal. The tumor was classified clinically as a perianal mucous-secreting adenocarcinoma. The tumor was surgically removed along with the bilateral inguinal lymph nodes, and colostomy was performed. Plastic surgery of the perineal area was performed using a myocutaneous flap of the left gracilis muscle and a slip of the anterior abdominal wall.

After a biopsy was taken for grading the tumor, the specimen was immediately processed for plastination. Specimen was cleansed of blood and fecal material and immersed in running tap water for 6 hours. To avoid alteration of shapes or anatomic relationships, the specimen was suspended in a metallic net. Dehydration was performed by freeze substitution with acetone at -25°C. The acetone concentration was checked daily and upgraded as necessary with 100% acetone. We agitated the acetone solution before checking its concentration. Dehydration was complete in 8 days when the acetone concentration remained steady at 100%. Forced impregnation was begun by emerging the specimen in a mixture of Biodur polymer (S-10) and the hardener (S-3). Vacuum was applied and increased at one hour intervals during the day. We increased the vacuum throughout the day but we did not further increase it during the night hours (vacuum pump on from 8 AM to 4 PM for safety). Impregnation was ended when pressure was steady near 15 mm Hg. Pressure was steady when bubbles on the polymer surface practically disappeared. The specimen was removed from the vacuum chamber and excess polymer mix was allowed to drain from the specimen. The surface of the specimen was manicured and the specimen cured initially by the gas cure (S-6). The specimen was then sectioned serially (thickness was from 5 mm to 15 mm) and both sides of the section were photographed using a Linof professional camera.

RESULTS

The neoplasm was a perianal mucinous adenocarcinoma. The plastinated specimen demonstrated the neoplasm invading the colon. The tumor distorted the normal morphology and rearranged the normal anatomic relationships (Fig. 1). Both the CT image and plastinated specimen placed the neoplasm in the left perineal area. The most caudal section of the plastinated specimen and CT image demonstrated the jagged outline of the tumor almost completely obstructing the rectal canal (Fig. 2,3).

In the cranial (9th section) sections, the neo-plasin was located perirectally with minor evidence of invasion of the rectal wall (Fig. 4) and CT images of (Fig. 5) the prerectal and perirectal vessels were disorganized and displaced on the left side.

In the most superior section, the neoplasm was not found around the rectal wall (Fig. 6, 7).

CONCLUSIONS

By comparing CT images and photographs of the macroscopic sections, it was possible to verify the accuracy of the in-vivo instrumental study. It was also possible to explain details ambiguously shown by CT. Thus, plastination can be a useful tool for verifying sophisticated clinical and instrumental images.

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A: Rectum
B: Vagina C: Neoplasm D: Annulus
E: Pubic Symphysis
F: Peritoneum G: Bladder H: Uterus
I: Sacrum
L: Femur
M: Rectum Muscle
N: Coccygeus
O: os coxae
P: Perirectal vessels

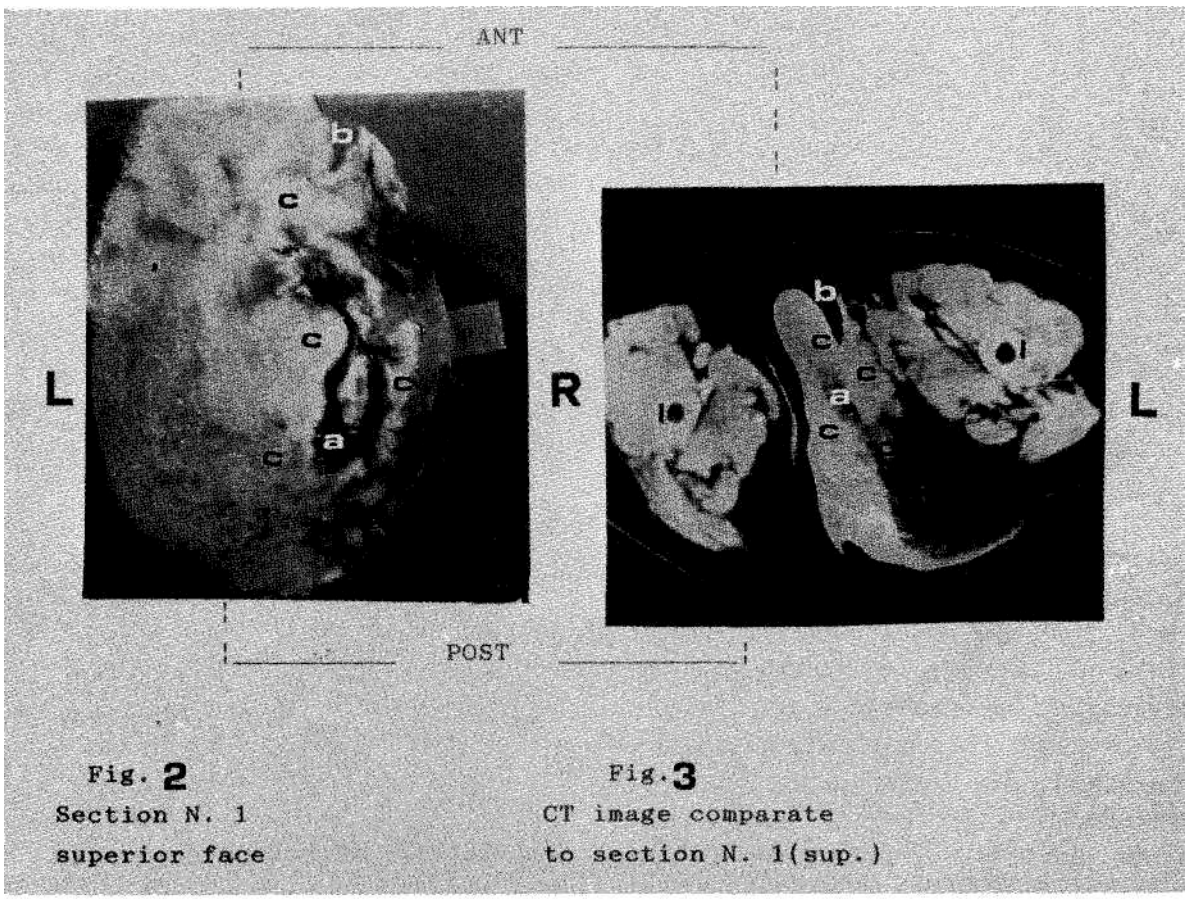

Fig. 2
Section N. 1
superior face

Fig. 3
CT image comparate
to section N. 1(sup.)

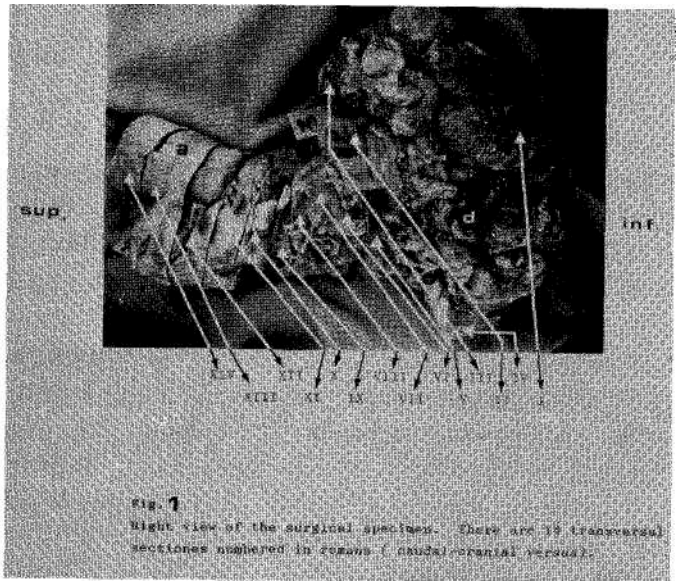

Fig. 1
Right view of the surgical specimen. There are 14 transversal sections numbered in romans (caudal-cranial versus).

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Right view of the surgical specimen. There are 14 transversal sections numbered in romans (caudal-cranial versus).

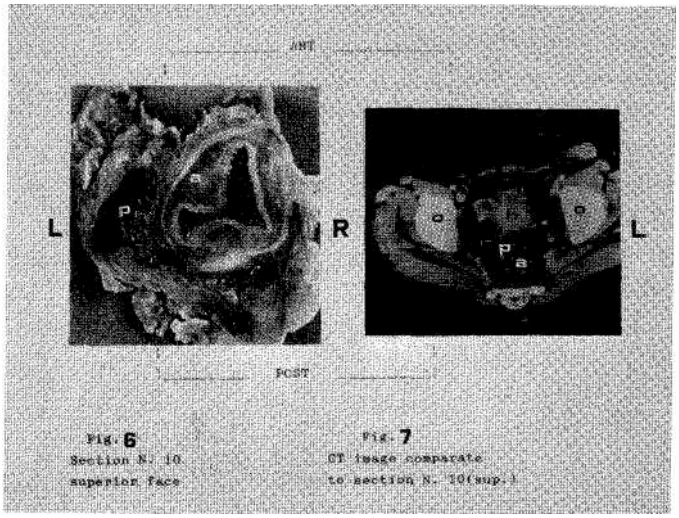

Fig. 6
Section N. 10 superior face

Fig. 7
CT image comparate to section N. 10(sup.)

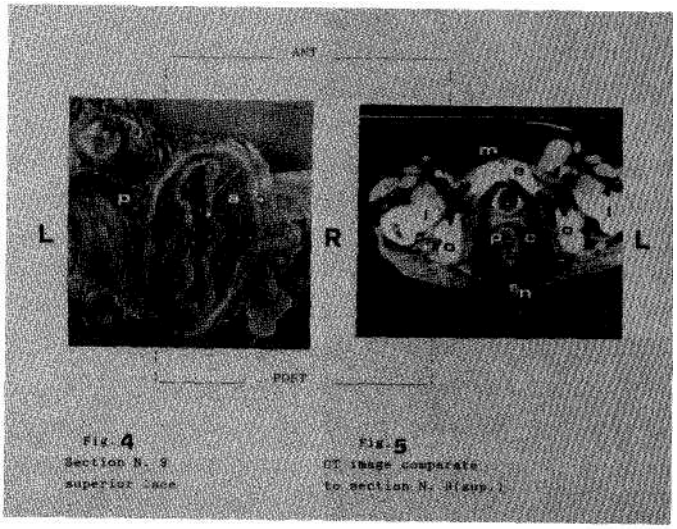

Fig. 4
Section N. 9 superior face

Fig. 5
CT image comparate to section N. 9(sup.)

USE OF PLASTINATED ANATOMICAL PREPARATIONS IN TEACHING REGIONAL ANESTHETIC TECHNIQUES

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INTRODUCTION

Human (cadaveric) anatomical preparations have always been an excellent medium for trainees to learn the correct anatomic landmarks and relationships for performing various nerve blocks. The difficulties in handling and the problems of preservation of human anatomical preparations are eliminated with the process of plastination which has received worldwide acceptance for its value in preparing durable material for teaching and museum display.

At The Uniformed Services University of the Health Sciences School of Medicine, The National Naval Medical Center, Bethesda MD., and The Hartford Hospital, Hartford CT, plastinated anatomical sections have been prepared to help establish training aids for teaching regional anesthetic techniques such as brachial plexus block via the interscalene and axillary approaches, subarachnoid, epidural and lumbar plexus blockade, as well as blockade of numerous peripheral nerves of the upper and lower extremity.

The plastinated preparations are non-toxic, dry, available for use in any environment, and maintain and reveal precise anatomical detail. In addition, variations in impregnating and curing result in preparations that are resilient and hold up to placement of needles as one would do in performing regional anesthesia. As the needles are advanced through the tissue, a direct visualization of the "the minds eye at the tip of the needle" is appreciated and hand-eye coordination is enhanced.

In these training institutions, the anatomical preparations have received an enthusiastic reception by both instructors and students, with requests and ideas for different regional preparations an applications.

MATERIALS AND METHODS

The standard S-10 plastination technique, with its four fundamental steps (fixation, dehydration, forced impregnation and gas curing) was used to prepare human tissue transverse sections which will aid in the teaching of regional anesthetic techniques. Plastination methods described by von Hagens (1985) was used as a general guide. Two cadavers relatively young in age, of average body stature, with no apparent trauma and no metastatic processes were selected for the plastination process (Lischka and Prohoda, 1987).

Cadaver 1; The body was kept frozen for several weeks in a deep-freezer, and upon its removal, was sectioned transversely into 1.5 cm sections on a laboratory band saw. To facilitate processing and prevent sections from laying on top of one another, (which impedes the flow of acetone and silicone into the tissue); the sections were layered between aluminum grids lightly fastened with string. The grids were cut to fit into the dehydration and impregnation containers. The strings allowed easy immersion of the whole preparation into the cold acetone and removal from it. A total of twelve sections were fixed by immersion in a 5% formalin solution for several days. After the period of initial fixation, the sections were removed and placed under cold running water to remove excess fixative. This step ensured the removal of any fixative odor and prevented fixative from diluting the dehydration bath later on. The sections were cooled overnight in a refrigerator at +5°c to minimize the chance of ice crystals forming when placed into cold acetone.

Figure 1. Plastinated cross section of the lumbar subarachnoid space at the level of L4 identifying the subarachnoid space, epidural space, cauda equina, spinous processes and possible approaches to the subarachnoid space with a spinal needle.

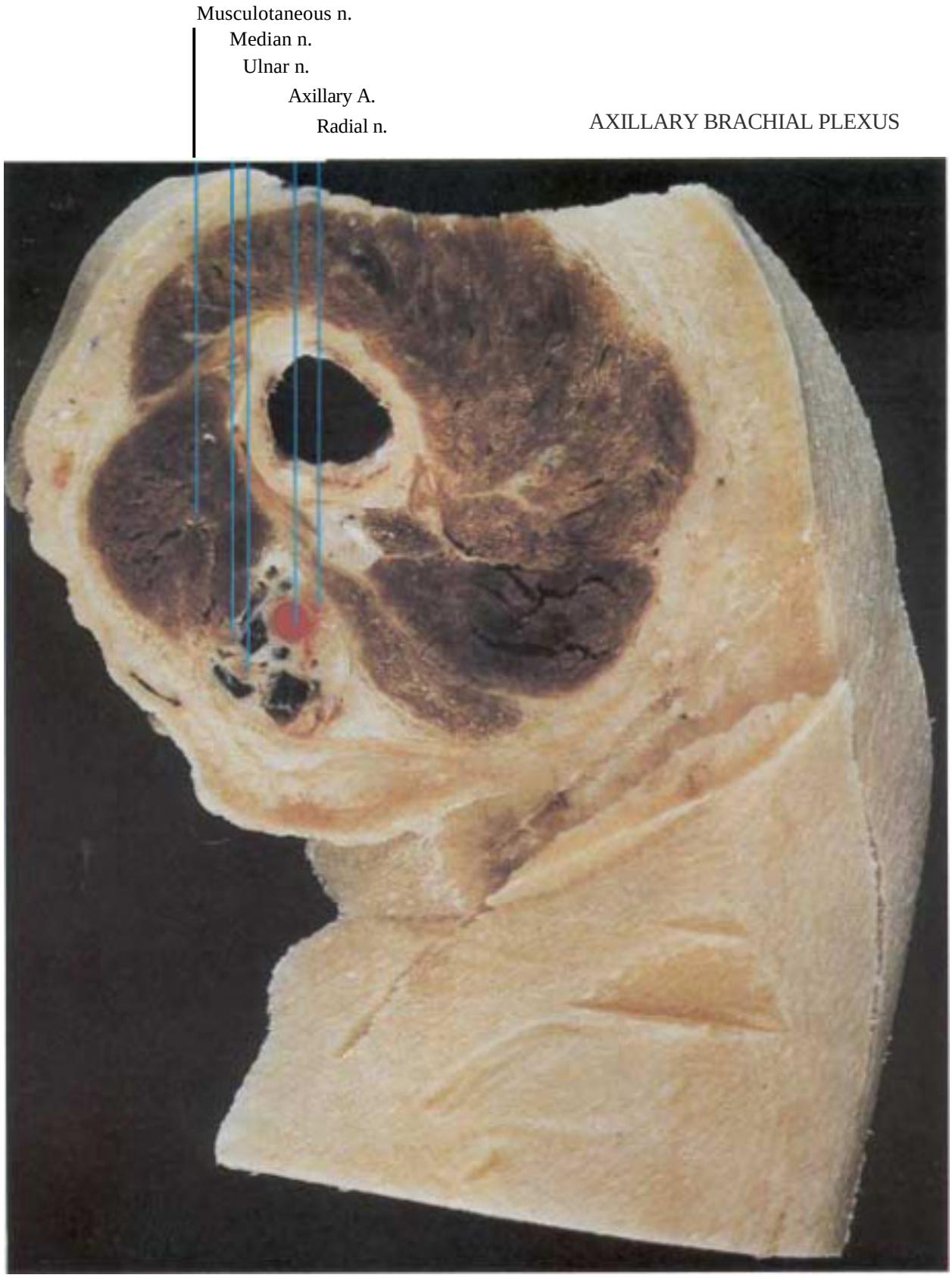
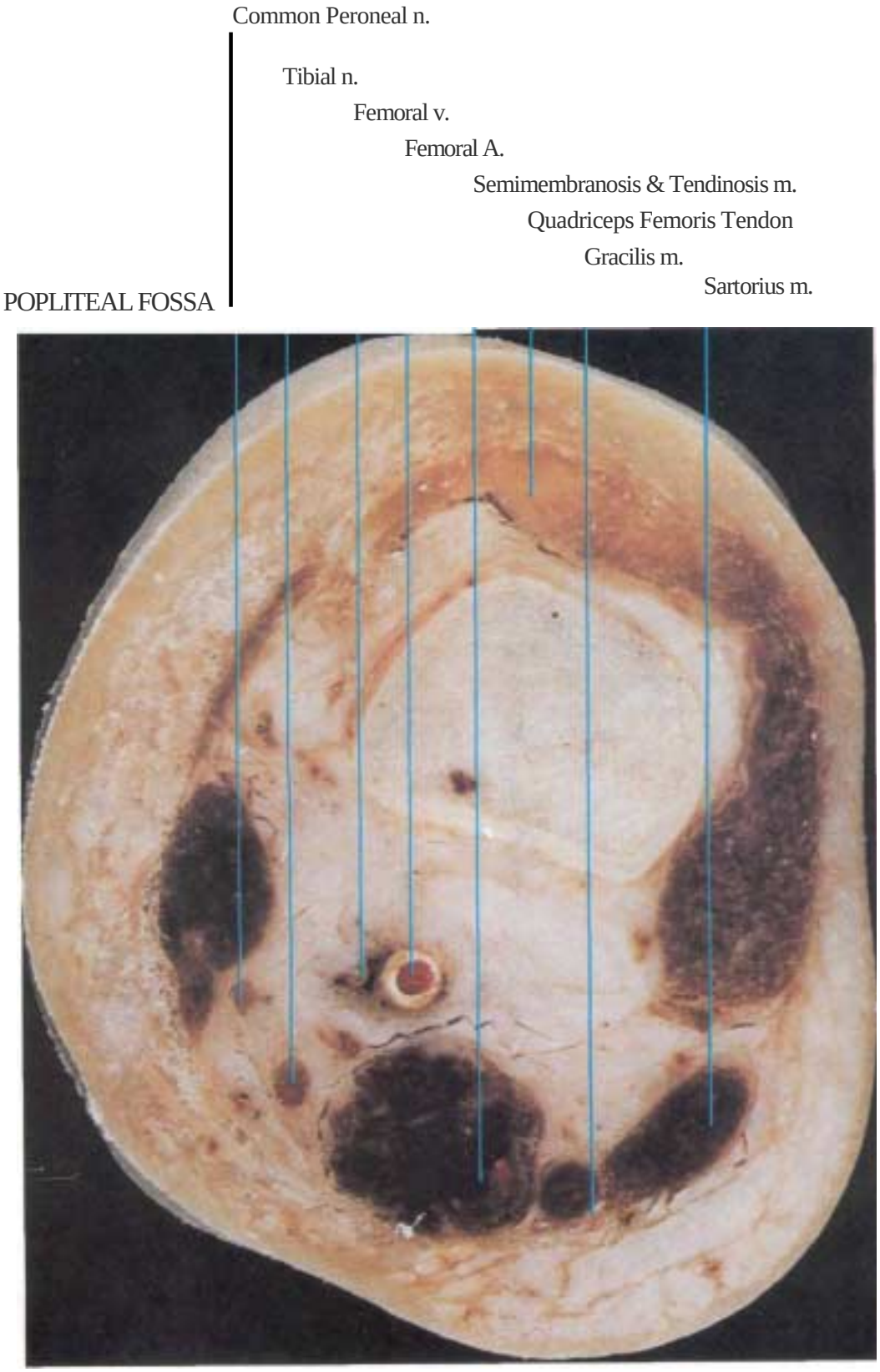


Figure 2. Specimen of a cross section of the upper arm at the axilla identifying the brachial plexus and the relationship of each of its components to the bone, muscles, vascular structures, and regional block needle.



Dehydration by freeze-substitution in acetone at -25°c was used to remove tissue water and some lipids. Dehydration was complete, when the water concentration measured less than *1%*. Prior to impregnation, the sections were kept in a bath of warm acetone for one week for additional degreasing. Using Biodur S-1O/S-3 resin, the sections were plastinated by forced impregnation in a vacuum chamber at -25°c, followed by final hardening with the standard gas curing procedure (Baptista et al., 1989; von Hagens, 1985).

Cadaver 2: This body was "lightly" embalmed using a 10% formalin solution. After several days, injection with a mixture of colored (red & blue) S-10 silicone (1%) + S-3 hardener (1%) and S-6 gas cure (2%) via the femoral and axillary vessels was performed. To allow for the silicone to completely harden within the vessels, polymerization time was approximately 48-72 hours. During this time the body was frozen solid and prepared for sectioning. Transverse sections of 1.5 cm were cut on a newly designed band saw. Modifications were made to the standard Hollymatic HiYield 16 meat cutting saw to permit thin frozen slicing of whole body sections with greater precision, and minimal damage, debris and thawing while cutting. The sections were rinsed and cleaned in water and placed directly into cold (-25°c) acetone for dehydration by the freeze-substitution method. With less than one percent residual water in the tissue, the sections were immediately transferred to a vacuum chamber containing a mixture of Biodur S-10/S-3 at -25°c. Vacuum was slowly increased over a four week period, until bubbling ceased on the surface of the silicone. The sections were then removed, drained of excess silicone and cured with S-6 gas cure until the surface was no longer tacky. Additional curing took place in sealed bags for one to two weeks, after which the sections were removed and ready for immediate use.

RESULTS

All of the normal anatomic relations within the sections were maintained following the standard S-10 plastination process. The colorizing material used for injection highlights the vasculature of each section while enhancing its teaching potential. We did, however, observe noticeable blue extravasation in the corpus cavernosum and at other various points where the vessels weakened. This is due most likely to the application of excessive pressure during the injection procedure. These sections, though not aesthetically pleasing like the others, are still useful for teaching. The anatomical positioning of the cadaver should also be considered and appropriately positioned during fixation, otherwise the final product will be distorted (Henry, 1990). The sections from the first cadaver have been shown and demonstrated in a scientific exhibit at the annual meeting of The American Society of Anesthesiologists, and sections produced from the second cadaver will be used to produce an atlas on regional anesthetic techniques.

DISCUSSION

Understanding anatomical relationships and correlative anatomy is the cornerstone of a competent practice of regional anesthesia. Plastinated human anatomical preparations for use in teaching regional anesthetic techniques are presently playing a key role in anesthesia education and will have increasing application in the future.

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PLASTINATION DOWN UNDER

Peter Bore, Robbie Boyes; University of Queensland
South Brisbane, Australia

It has been well known to generations of clinical teachers that medical students do not bring from the dissecting room to the hospital, sufficient anatomic knowledge for their clinical studies. The reasons for this are multifactorial and not relevant to this article but two of the less controversial are:

1. There has been a large reduction in the time students spend dissecting, with a consequent reduc tion in the quality of the dissections, such that many students are not left with a clear visual image of anatomy but depend for their anatomic knowledge on remembering the words in text books.

2. Few medical schools have facilities for students to revise anatomic knowledge during the later parts of the curriculum. It is particularly difficult in The University of Queensland since the Department of Anatomical Sciences with its dissecting room facilities is a cross city trip of ten kilometers from the teaching hospitals where clinical studies are conducted. Moreover, within the last year a fifth teaching hospital has been established in Townsville, a thousand kilometers to the north.

One of us (PB) first heard of plastination in 1990. It sounded vaguely interesting, and enquiries were made as to whether any specimens were available locally. A local company, Scientific Educational Supplies had some specimens which had been produced by Gunther von Hagens and it required only a few minutes of inspection of these for one to realize the potential of this material to assist in the teaching of medical students. It was relatively easy to pass on the enthusiasm to the second member of the team (RB).

With good educational reasons for using this material for teaching in a geographic environment where the portability of plastinated material offers enormous advantages one might have expected its introduction to be relatively easy. Alas, plastination costs money. It took some two years before a modest amount of money was promised from interested departments. Surgery, Anatomical Sciences and Radiology. Over a year was spent discussing whether such a thing as plastination was legal in Queensland (some aspects of it are still under discussion) and then several months more ascertaining the requirements of the Department of Occupational Health and Safety with regard to spark-proof and concentrations of acetone vapor.

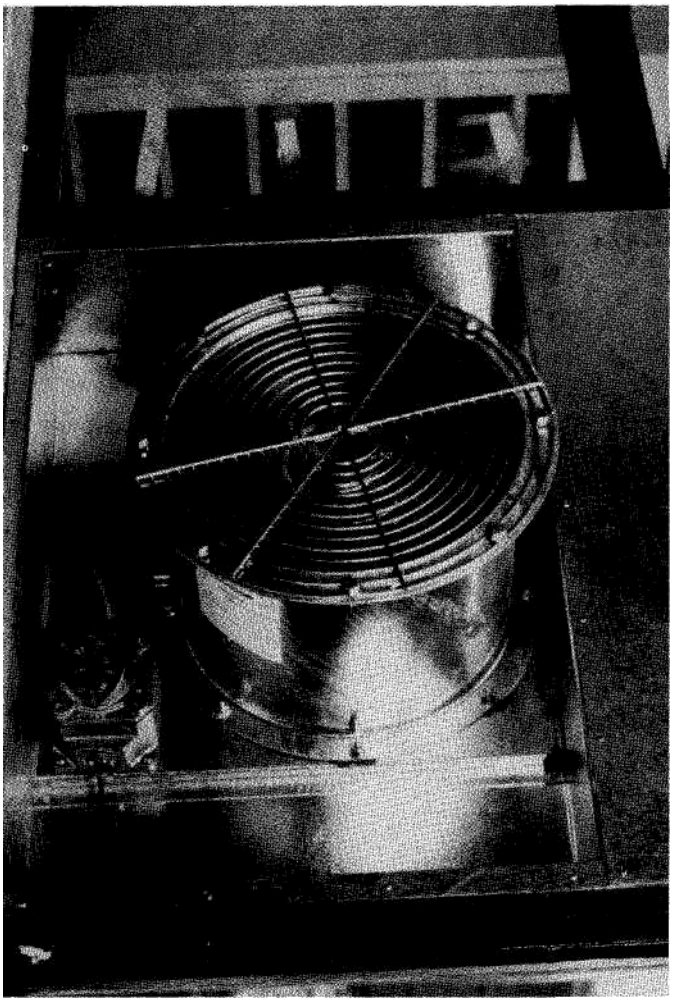
Eventually we initiated work at the end of 1992 with what was clearly, in retrospect, an inadequate budget. However, the University's Building and Grounds Department were extremely helpful in minimizing the costs in creating a spark-proofroom, and our freezers, two enormous custom-built items which can easily maintain -30°c in a Queensland summer were financed by Scientific Educational Supplies and built by a local refrigeration firm.

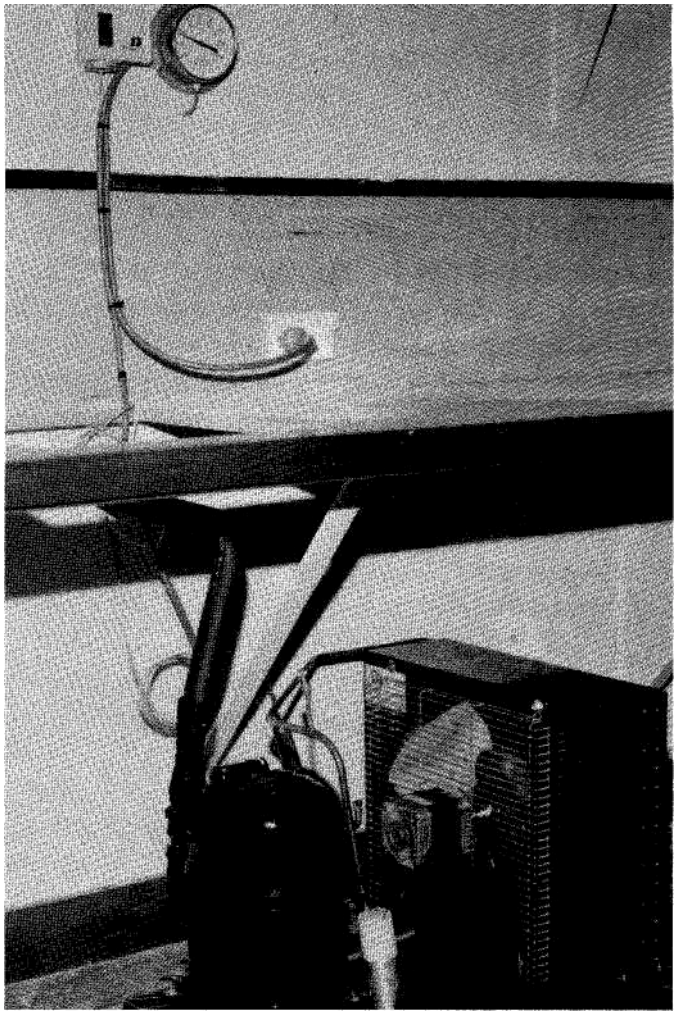
A special Australian problem is that paying for items priced in Deutschmarks with Australian dollars is particularly painful. One must then add either the significant costs of air freight or the significant delays of sea-freight. We have therefore endeavored to buy items locally and occasionally have been spectacularly successful, as, for example, with vacuum chambers. We discovered that stainless steel vacuum chambers with perplex lids are a standard part of Alfa-Laval milking machines. Alfa-Laval were helpful in producing the slight modifications we required at minimal cost and we are now able to buy stainless steel vacuum chambers for around BOO US dollars.

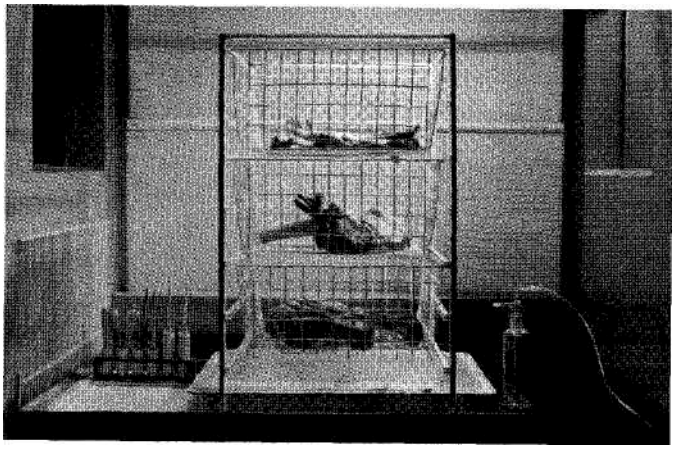
A conscious decision was made that we would make every effort to get things right from the outset and thus a large proportion of our precious budget was spent sending Robbie Boyes to the workshop in Heidelberg and to visit Ron Wade's Plastination Laboratory in Baltimore. This has paid dividends. By the middle of 1993 we had acquired the necessary facilities and equipment to commence S-10 plastination. We were both delighted, and one of us somewhat surprised that our very first batch of specimens turned out to be highly satisfactory.

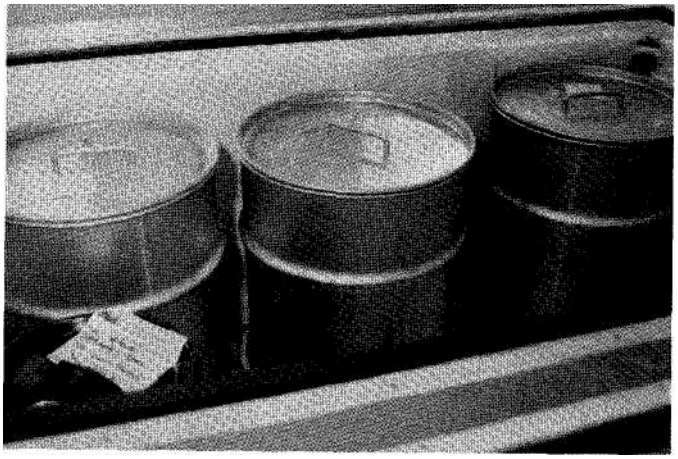
Our current production is entirely of S-10. We are hopeful of progressing to sheet plastination in the not too distant future though funding and time remain precarious. However, now that we are in production we are succeeding in attracting considerable interest from various departments within the University and from other institutions in Australia. We are even considering such commercial activities as plastinating coral or cane toads for sale to tourists.

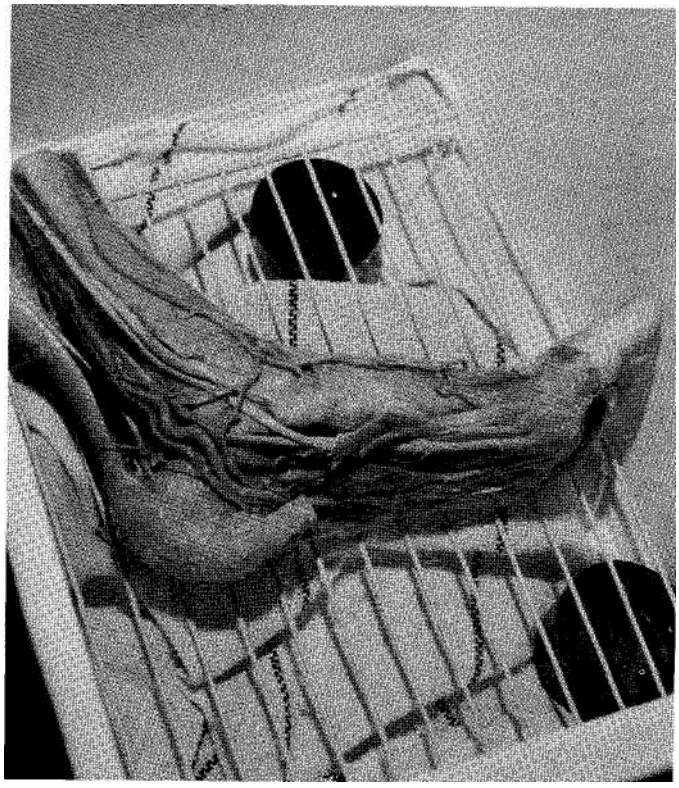
The University of Queensland is not the only or the first place to be involved in plastination in Australia. Plastination, is being or has been, carried out by Russell Barnett, University of Otage, New Zealand, Peter Cook at the University of Auckland, Richard Borg, University of Sydney, Carolos Kordjan at Flinders University, South Australia and Ken Parsons also from the University of Sydney and possibly others.











Photos of lab
Brisbane, Australia

ABSTRACT: PLASTINATION OF LARGE SPECIMENS.

Robert W. Henry College of Veterinary Medicine,
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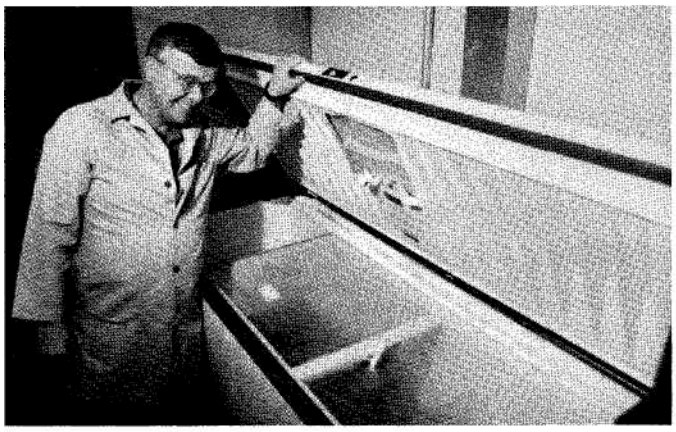
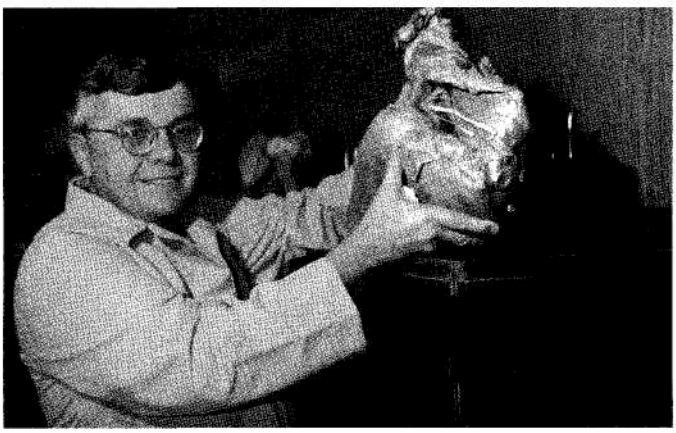
Large specimens which are to be plastinated present some special problems. Obviously, containers for fixation, dehydration and impregnation must be of adequate size. Specimen preparation is important and care must be taken to preserve the normal anatomical detail and position. Large hollow organ specimens may be full of ingesta, time consuming, and tedious. Select a less obvious site and make an incision large enough to adequately remove the ingesta. After removal of the ingesta and adequate flushing, suture the incision closed and dilate fix the organ. Removal of the sutures prior to dehydration makes that step easier because the specimen will submerge easier in the dehydrating fluid. Large specimens tend to flatten on the resting surface. Thus allowing the specimen to float in storage or fixative solutions prevents flattened disfiguration. Fixation of hollow organs via dilation helps to assure proper anatomical detail of the final product. Specimen baskets for dehydration and impregnation aid in transferring and draining specimens. A plastination chamber of adequate size is essential. During curing of the large specimens, large volumes of air may be used to inflate hollow organs. Incisions in hollow organs should be sutured closed to allow for inflation with air. Part of the organ may be suspended by a string or tubing to prevent flattened areas on dependent areas of the specimen during the curing process. The specimen should be turned frequently to also prevent flattening of the dependent surfaces of the organ. The organ should be turned daily until the organ has cured throughout its depths.





ABSTRACT:
SHEET PLASTINATION OF BRAIN SLICES.

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Plastinated sheet specimens are highly desirable. Biodur polyester P-35 impregnated slices of the brain yield specimens with high anatomic detail. The brain must be well-fixed in a 10-20% formaldehyde solution. The brain is flushed in running tap water, bisected, and then sliced into 4 mm sections on a meat slicer. Moist filter paper is placed on the cut surface of the brain to support each resulting slice. Each slide with filter paper is placed onto a grid and the grids are stacked. The stack is flushed in running tap water then stored in distilled water in the refrigerator over night. In the morning they are submerged in cold acetone (-20°C) for dehydration via the freeze substitution method. One acetone bath is sufficient for the slices if an adequate fluid/tissue ratio is used. After 2 to 4 days in the acetone, the slices (on the grids) are submerged into the cold (5°C) polymer mixture (P-35/A-9, 100/2 parts) for 24 hours and then placed in a new polymer mixture for 24 hours. Next the specimens are impregnated at room temperature in a new polymer mix with the impregnation chamber darkened. Vacuum is increased hourly and finally stabilized at 20mm Hg and left at this vacuum overnight. Two sets of double glass plate units are composed by mating a 1/4" tempered glass plate with a single strength regular glass plate before they are used to make a chamber for casting the plastinated brain slice. A brain slice is placed on the thinner glass of one of the double glass plate units. A 6mm gasket is placed around the perimeter except for the top of the double gasket with the thin glass facing the specimen. Fold-back clamps are used to hold the mold together and the gasket in place. The mold is stood upright and filled with polymer mix. The unit is allowed to stand for 30 minutes in the dark to allow any bubbles to rise. Curing is initiated with a UV-light and then completed in a 45°C oven for 5 days. After cooling to room temperature, the mold is dismantled. The slides are then sawed to the desired size and the glass plates cleaned.

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Editors Note:

The above list has not been updated or corrected for some time now. Please forward changes and phone numbers to editor if you desire.

Additionally, please forward names of any members not listed.

7TH INTERNATIONAL CONFERENCE ON PLASTINATION

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Fourth Biennial Meeting of the International Society for Plastination

24-29 July, 1994
Karl-Franzens-University

Program Outline

Sunday	Welcome reception, Anatomical Institute Graz
Monday	Principles of Plastination (fixation-dehydration-forced impregnation-curing)
	Reception by the Mayor of Graz, City Hall
Tuesday	Plastination techniques (S 10, E 12, P 35)
	Reception by the Governor of Styria, Eggenberg Castle
Wednesday	Small group discussions, tours to anatomy and pathology
	Old town sight-seeing tour and armory
Thursday	Developments in plastination, teaching applications,
	Styrian Evening, Landhauskeller
Friday	Applications in research

REGISTRATION FEES

Members of the International Society of Plastination:

Registration Fees received:

Postmarked before May 31, 1994	3,000 - ATS
Postmarked before June 15, 1994	3,250 - ATS
After June 15, 1994	3,500 - ATS

All others:

Registration Fees received:

Postmarked before May 31, 1994	3,200 - ATS
Postmarked before June 15, 1994	3,500 - ATS
After June 15, 1994	4,000 - ATS

ATS...Austrian Shillings

Registration fee includes:

Conference Kit, Luncheons, Coffee Breaks, Reception on Sunday evening, Reception by the Mayor of Graz, Reception by the Governor of Styria, Graz - Old town sight-seeing walk, reduction on price of the Styrian Evening.

<u>For Additional Conference Information:</u>

Coordinator: Dr. Andreas Weiglein
Karl-Franzens - University Graz
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