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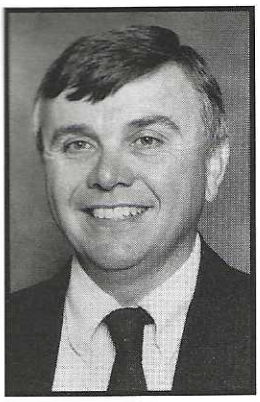

Dear Colleagues,

I hope that each one of you have had a wonderful year. I am hoping to see many of you at the 9th International Conference and our 5th Biennial meeting of IPS. Dr. Peter Bore and Robbie Boyes have put together a tremendous program. Peter and Robbie, thanks for your great effort!

Several things are happening in the Society. A reference index has been prepared by Mr. Gilles Grondin and Dr. Regis Olry. Thanks for sending in your references and supporting this project. The committees on "certification of plastination expertise" and on "Finances" have prepared a report for the ISP meeting, we will also select the "host site" for the 10th Conference and elect a slate of officers.

As a new Society, we are experiencing some "Growing Pains". Please, let us know your concerns and suggestions. I want to thank the executive committee for their help, and would encourage each of you to join our e-mail "list serve". Thanks to Ron Wade for putting that together. I wish each of you a success the remainder of 1996.

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

Editors Note

Due to late arrival of Conference Abstracts, they are printed as submitted without edification.

Wishing everyone the best who attended this conference and thinking of those who were unable.

I'm sure that Robbie and Dr. Bore have done a super job and wished I could have attended. Thank you for past assistance with journals and articles submitted.

I would like to sincerely thank the Editorial Staff for many helpful suggestions.

See you at next meeting!

Dale Ulmer
ISP Editor

BRISBANE INTERNATIONAL MEETING
ABSTRACTS / POSTER PRESENTATIONS

PLASTINATION - A NEW TECHNIQUE FOR THE PRESERVATION OF VISCERA & TISSUE FOR TEACHING AND RESEARCH PURPOSES

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Plastination is a process for impregnating a biological specimen with a curable polymer, using the difference in vapor pressure between the infiltrating polymer and a volatile intermediate solvent, to propel the impregnation.

Objectives

1. To do long term, preservation of bodies, and body tissues and organs for teaching, demonstration and research pur poses without repetitive use of fresh tissue or sacrificing live animals.
2. To minimize the shortage of cadavers/organs/hu man tissues (in the presence of and increasing the number of teaching institutes) for teaching and research purposes.
3. To develop this speciality area of professional activity in order to encourage other institutions to adopt plastination preservation methods and to invite individuals to learn and practice plastination as a carer in science.
4. To demonstrate that it is safer, more economical and better method of preservation of body/organs/tissues for medical education. With the development of the technique, the repetitive use of gloves in handling the specimens can be avoided. Museum glass jars are not required. Funds required for buying formaldehyde and other preservative chemicals can be reduced. It also ensures the safety of the workers.
5. To build the library of clean and odorless teach ing and research specimens.
6. To use plastinated sections as a guide for comput erised axial tomography (CAT) scans, MRI and ultrasound tech niques and will provide a new three dimensional perspective of normal anatomy and its variations.

In the fall of 1992 I was asked to initiate the development of a Plastination Laboratory to prepare anatomic teaching specimens for undergraduate and postgraduate medical students. After three and one half years of effort, funds have been allocated to start the Plastination Laboratory at all India Institute of Medical Sciences, New Delhi, India.

A SHARK HANDSAW BLADE SIGNIFICANTLY ENHANCES THE QUALITY OF CUTS MADE FOR PLASTINATED SPECIMENS

Alston, Markl, Janick, Larry i, Wade, S. Ronald 2,
Weber, Wolfgang 3, Henry, R.W. l

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University of Tennessee, Knoxville, TN
2 State Anatomy Board,
University of Maryland, Baltimore, MD.
3 College of Veterinary Medicine
Iowa State University, Ames, Iowa, USA

The popularity for use of band saws in preparing plastinated specimens has increased significantly over the past few years. This use has brought forth many modifications that have facilitated the production of even higher quality specimens.

In this study a Shark Band* blade was evaluated and found to be superior over more commonly used (10 to 12 teeth per inch) blades. Trials were performed upon frozen tissues in preparation for S10 plastination, E12 sheet plastination, as well as for slicing cured S10 plastinated whole brains. The advantage of a Shark Band blade is that by a reduction to only 3 teeth per inch the creation of, and subsequently contamination of specimens by, sawdust is significantly reduced. The minimal set and thickness (14 guage/.014 inches) of the teeth and blade similarly promotes a smooth and very fine cut. The effects of this blade can be even further enhanced through an increase in the blades cutting speed.

Unfortunately the same design that facilitates the quality of the cut also greatly reduces the blade life. When cutting dense bone or enamel the blade teeth are quickly dulled and taken out of set. This necessitates the replacement of the band after only a few cuts.

PREPARATION AND PLASTINATION OF THE CEREBRAL DURA MATER WITH THE SKULL BASE

Grondin, Gilles and
Olry, Regis
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a Trois-Rivieres, Quebec, CANADA

The aim of this work was to provide a plastinated specimen of the cerebral dura mater and its main partitions (falx cerebri, tentorium and falx cerebelli). The skull was sawed one centimeter above the junction of its base and its vault, the latter being broken up and then removed in order to expose the convexity of the dura mater without damaging it. The eye balls and the accessory organs of the eye were also removed and the sheath of the optic nerve was carefully dissected. One side of the dura mater was opened, the brain was broken up and removed and the specimen was dehydrated and impregnated according to the standard S10 technique. For the gas curing the whole dural cavity was filled with absorbent paper in order to restore the normal shape of the cerebral dura mater. Finally the opening was enlarged to show the falx cerebri with its inner free margin and the incisura superior sagittal sinus to be seen. This plastinated specimen will help students to understand the topography of the dura mater and the transtentorial herniations.

E 12 TECHNIQUE: AN AID TO STUDY SINUSES OF PSITTACINE BIRDS

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Janick, Larry l, Orosz, Susan l

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The University of Tennessee, Knoxville, TN:
2 Animal Medical Center,
New York, NY, USA.

Infraorbital sinus infections are commonly encountered in clinical practice in companion birds. They form the bulk of upper respiratory tract infections (URI) and require medical and/or surgical management for the avian patient. The infraorbital sinus has extensive connections to structures of the head and its dorsal drainage into the nasal cavity makes if difficult to treat these infections. A more complete understanding of the anatomy than is described in the fowl would enhance the clinical diagnosis and management of URI in companion avian species, particularly psittacine birds. For comparison and as an aid in determining the location and extent of the sinuses of the parrot and

macaw, computed tomography (CT) images of cadaver heads were completed at 2mm scans. The scan lines were marked of the skin to allow sawing in a similar plane. Subsequently, the sinuses of the imaged heads were injected via a 20 gauge needle inserted into the infraorbital sinus with a mixture of colored epoxy (E20 and El mixture) in a ration of 2:1. The epoxy was allowed to harden over night and then the specimens were frozen (-25C) for sawing. A shark band blade was used to saw 2mm thick sections which corresponded to the CT scans. The sections were placed on grids (covered with screen) and submerged in cold acetone for saw dust removal and dehydration *(freeze* substitution). Two changes of 100% acetone were used. After 24 hours at room temperature, the filled flat chambers were placed in a 45C oven overnight. The next day the chambers were removed from the oven, allowed to cool, and dismantled. Individual slices were sawed apart. The slices were photographed and compared to the CT images and the sinuses delimited.

ENDOANAL MAGNETIC RESONANCE IMAGING OF THE ANAL SPHINCTER COMPLEX: CORRELATION WITH SHEET PLASTINATED SLICES AND HISTOLOGY

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vanRijn R.R. 3, Zwamborn A.W. 3,
denHollander J.C. 4, Lameris J.S.4

Departments of Radiology 1, Anatomy 2,
Experimental Radiology 3, and Pathology 4
University Hospital Dijkzigt Rotterdam and
Erasmus University Medical School, The
Netherlands

Introduction: The purpose of this presentation is to correlate in vivo endoanal MRI findings of the anal sphincter to the cross-sectional anatomy and histology.

Materials and Methods: Fourteen patients with rectal tumors were examined with a ridged endoanal MR coil before undergoing abdomino-perineal resection. In addition, twelve cadavers were used to obtain sheet plastinated slices. The imaging findings were correlated with histology and anatomy of the twelve cadavers.

Results: The data of the eight patients, eleven rectal preparations and ten cadavers, could be compared. In these cases, there was an excellent correlation between endoanal MRI and the sheet plastinated slices and histology. With endoanal MRI, all muscle layers of the anal canal wall, comprised of the sphincter ani internus, conjoint longitudinal muscle, the sphincter ani externus and the puborectalis muscle were clearly visible. Also the levator ani muscle and ligamentous attachments were well depicted, the perianal anatomical spaces, containing multiple septae, were clearly visible.

Discussion: Endoanal MRI is excellent in depicting the anal sphincter complex and the findings show a good correlation with the sheet plastinated slices and histology.

PLASTINATED CANNE GASTROINTESTINAL TRACTS USED TO FACILITATE TEACHING OF ENDOSCOPIC TECHNIQUE AND ANATOMY

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College of Tennessee, Knoxville, TN, USA

Plastinated specimens, when prepared with a design for endoscopic use, can serve as a practical model for teaching. Intact alimentary canals were excised from fresh canine cadavers. Cannulas in excess of the intended endoscope size, were placed in restrictive openings (pyloric/cardiac sphincters and ileocolic orifice). These cannulas allow ingesta to be removed and maintain adequate diameters for scoping. After flushing out the gastrointestinal contents, specimens were formaldehyde fixed overnight in dilated anatomical conformation. Prior to S10/S3 impregnation, fixative was flushed from the specimens and they were dehydrated in acetone. After impregnation, a slow cure (elongation of S3 molecules at room temperature) was initiated for approximately 1 week with the GI tracts maintained in a dilated conformation by a positive pressure air flow. When polymer seepage was minimal, small quantities of S6 (second curing agent for crosslinking of polymer molecules) were volatilized into the air flow providing a final cure of the specimen. Vapors form the S6 were further contained around the specimen by enclosing them in plastic bags. The completed specimens retain their dilated anatomical conformation, and may be used to teach both endoscopic technique and gastrointestinal anatomy.

COMPARISON OF FOUR CLASSES OF ANATOMICAL LAYERS OF PLASTINATION SECTIONS WITH FOUR ANATOMICAL LAYERS OF COMPUTER IMAGES

Lane Alex
Triton College, River Grove, IL, USA

Several demonstrations of each class of body tissue layers have been photographed from E12, S10 and P35 plastinated human sections and computer images (MRI and Ultrasonography). These photographs are labelled to reflect four classes of tissue layers which include somatic, extra visceral (visceral), intravisceral luminal, and intravisceral nonluminal classes of layers. The four classes of layers are based upon embryonic layers and location.

The somatic layers correspond with layers derived form somatopleure in the developing embryo. The intravisceral layers include the internal organs positioned in deliberate sequence and derived from splanchnopleure of the embryo. Intravisceral luminal layers are organs with a prominent lumen or canal which courses through the organ. Intra visceral nonluminal layers include organs without a prominent lumen.

OUR OWN PLASTINATION LABORATORY

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With the acute problem of getting new cadavers in most countries, there is the need to plastinate available cadavers. However, the cost in setting up such a laboratory may not be within reach of many departments.

Our lab was set up in 1991 using a room 1400 sq. feet in size, adjoining the campus workshop. The laboratory is divided into various areas without partitions. The plastination tank, curing chamber and separator were made locally at a cost of US$1,700. Three items of equipment were ordered form Germany, namely vacuumometer, vacuum pump and acetonometer. The total cost was about US$2,000.

Personnel from two neighboring universities have visited our laboratory to enable them to set up their own laboratories. The department's next project is to undertake sheet plastination. In the meantime, the laboratory is the pride of the medical school.

PREPARATION OF THE CORONARY ARTERIES FOR PLASTINATION

Riepertinger Alfred l, Heuckendorf Evelyn 2

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Muchen-Schwabing, Munich, Germany
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Berlin, Germany

The technique of preservation of anatomical specimens using the method of Dr. Gunther vonHagens provides excellent demonstrations for teaching and further education. Of particular interest in the heart is its vascular supply, which we can display in plastinated specimens injected with red Biodur E20.

The heart should be removed from the thoracic cavity taking care to divide the vessels some distance from the heart. Then the heart is rinsed with cold tap water and all vessels are blocked with cork stoppers. The stoppers for the aorta and aortic valve and the pulmonary valve are in as almost completely closed position.

Injection of the coronary arteries with 49.5 gram Biodur E20 red requires only slight pressure to fill the coronary vessels. For the dilatation-fixation 15L of 20% formalin solution are infused into the ventricle form a height of 1.5 meter. A week of immersion-fixation in 5% formalin solution at a temperature of +5C follows.

The sub indocardial fatty tissue and all facia are carefully dissected away with scalpel, scissors and forceps. Subsequently the specimen is bleached in a 2% hydrogen peroxide solution with 5% fonnalin added to achieve the desired color. After washing for about 1 hour the auricles are tamponaded with polyethylene-foils and the specimens are frozen overnight in a plastic bag. Dehydration lasts for 3 weeks at -25C in the deep-freezer. During this time the acetone is changed twice.

Forced impregnation with Biodur S10 plus S3 hardener takes place for 3 weeks at 25C in the deep-freezer. After removal from the vacuum kettle and draining overnight in the freezer, the specimen drains for further day at room temperature. Then the specimen is placed into a plastic container for gas hardening with Biodur S6. This takes 2 weeks. Finally, a comprehensible injection specimen of the heart is available in a most life-like condition for instruction and research in medicine.

COMPUTERISED LABELLING: A NEW METHOD FOR S10 PLASTINATED SPECIMENS

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The correct labelling of anatomical specimens not only allows the specimen to be readily identified, but also makes salient information regarding the deceased easily accessible. Well prosected specimens, with emphasis on the relevant morphological structures, are aesthetically pleasing. In teaching and research it is essential that the structure being studied is clearly visible. In order to avoid large surface labelling, and appropriate method which wouldn't obscure important parts of the specimen was sought.

Initially, several factors were of concern; cost, accessibility (within the specimen), resistance to chemicals (in particular formaldehyde), size and strength. The Trovan Transponder System meets all these requirements. The transponder chip is approximately 10X3mm in diameter and can be easily inserted into an appropriate area of the specimen. A suitable applicator can be purchased or made at minimal cost. This ensures deep penetration into the hardened silicone matrix (S10), as well as the security of the chip. In cases of minimal skin coverage, a hole of approximately 3.5mm diameter is drilled into bone. The chip is placed within the hole which is then sealed using a suitable adhesive. The Trovan Minireader unit is battery powered. It has digital readout capabilities as well as a serial port for direct input to a computer. At the University of Melbourne data on the chip corresponds to details of the specimen and cadaver from which the tissue was taken, and therefore includes information such as age and cause of death. Of the forty S10 plastinated specimens labelled in this fashion, only a few of the original injection sites are visible. All of the specimens have their own electronic serial number which can be sensed by the minireader at varying ranges. Significantly, the high presentability of the plastinated specimens remains unobscured by this labelling technique.

AN IDEAL CURING CABINET FOR S10 PLASTINATION

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Curing of specimens is an integral part of the S10 process. On completion of this final stage the specimens are touch dry and resilient for everyday use. The fast curing variation of the technique requires a chamber of sufficient size to house the specimens. It needs to be relatively air tight, easily accessible, adjustable and conducive to the curing environment. This presentation details the development of a custom made curing cabinet at the University of Melbourne which has significantly enhanced our ability to produce specimens of a consistently high quality.

Because of the high turnover in prosected "wet" specimens the Departmental anatomical technologies laboratory is involved in the production of as many S10 plastinated specimens as possible. This necessitated the design of a curing cabinet capable of accommodating a large number and variety of specimen sizes. Because specimens at the curing stage need to be constantly monitored the chamber also needs to be transparent. Perspex satisfies all of these requirements, it is relatively inexpensive and is easy to work with. It may be glued, moulded, bent or drilled making it very suitable to our needs. A variety of additional requirements can be accommodated, such as variation in size of the cabinet to suit fume cupboard, or the connection of an extraction system. By assembling the cabinet with a removable front panel the specimens can be readily manicured and the concentration of volatilized S6 varied. Further modifications may be made, such as adapting the shelving to suit the specimens, by gluing several small pieces of perspex to the inside walls of the unit.

PLASTINATION IN CLINICAL MEDICINE

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The changing trends in medical education warrants the availability of clinical material for training of doctors at venues beyond the confines of the medical school. The present training of medical doctors uses patients in the clinical context where patients often endure physical indignities and stress when being subjected to examination by students, registrars and even consultants when gaining experience in various clinical procedures. Futhermore, with newer clinical procedures evolving recently, and with tremendous advents in minimally invasive surgery the

need has arisen for patients not to be used repeatedly in the training of medical students and registrars in clinical procedures. Futhermore, ethical issues which were previously largely ignored by clinicians in third world settings are now enforced in the transforming South Africa. For these reasons, extended applications of plastinations are now more needed than ever.

In the Faculty of Medicine at the University of Natal, Plastination Laboratory has been specifically focused to provide this need. The combination of plastinated specimens in the skills laboratory for the training of undergraduate and postgraduate studented is been fully exploited with this potential in mind.

Plastinated specimens are used at undergraduate levels to teach medical students not only basic human anatomy and embryology as well as neuroanatomy but also clinical examination techniques such as nasal, aural, oral, rectal and vaginal examinations obstetrics as well as gynecological cases. In the teaching of dermatology, plastinated biopsies of skin segments could be used for demonstration rather than using slides or video tapes. This obviates the use of patients in the training of students.

On a different level, medical and surgical procedures such as inserting intercostal drains, pericardiocentesis, cutdowns, biopsies of various organs and bone marrow aspirations could be attempted and perfected by medical personnel in training. While initially, normal procedures could be performed using plastinated specimens, the technique could be expanded to include pathological specimens such as those used in the study of surgical pathology. In this regard, plastinated specimens of benign prostatic hypertrophy, malingnancies of the breast and congenital deformities could be prepared.

On the medical front, procedures such as sigmoidoscopy, colonoscopy and gastroscopy could be accomplished on plastinated torsos. Complicated procedures such as endoscopic retrograde pancreatico-cholangiogram could be performed in a plastinated specimen. On the highly specialized side, angioplasty of the coronary arteries could be practised of plastinated specimens. In reconstructive surgery, injected arteries in plastinated specimens could be used by surgeons to devise myocutaneous flaps in complicated reconstruction of traumatized cases which are prevalent in South Africa where violence is endemic in major cities and townships.

With the emergence of minimally invasive surgery, expertly prepared plastinated specimens could be used in laparoscopic surgery to acquaint the operator with basic anatomy as visualized through the laparoscope, albeit the aspect of hemorrhage would be nonexistent in these "test patients", but the techniques of laparoscopic surgery could be mastered without any inconvenience and morbidity to patients.

In conclusion the use of silicone impregnated plastinated specimens has great potential and could be of tremendous value in the training of medical and Paramedical personnel in the 21st Century.

A NEW BUILDING IS PROPOSED TO RESOLVE FIRE CODE INFRACTIONS AT ISU

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The production of plastinated and skeletal teaching specimens for veterinary anatomy requires large volumes of acetone to accommodate the parts of the carcasses of large animals. However, the uniform building code limits the use or dispensing of flammable liquids to 10 gal per 100 square feet of floor space. Using quantities in excess of this limit requires space classified as H-2 occupance. In this paper, the requirements of an H-2 occupant room are explained, and a strategy of obtaining such a facility is explored.

According to the Uniform Building Code (UBC) of the USA, and H-2 occupance room needs to have the following features :

1. Electric wiring, class 1 division II NEC (National Electric Code)
2. Ventilation to maintain air/fuel mixture below OSHA TLV levels
3. Back up power
4. Two exits, one of which leads to the outside
5. Two hour fire separation with 11/2 hour fire door
6. Sprinkler system
7. Spill containment
8. Blow out panel

Electric wiring: All electric wiring in as H-2 occupancy room must conform with class 1 division II NEC. This means that motors of standard freezers as well as motors of "explosion proof" freezers are not allowed in this room. The so-called explosion proof freezers provide an explosion proof interior while still allowing air and fumes to enter the motor from the exterior. A solution to this problem is to relocate the motor into a adjacent room and connect the cooling coils to the freezer through the wall. The expense of an explosion proof freezer is unnecessary. Vacuum pumps need to be relocated as well. Obviously, it would be a code violation to operate any non explosion proof power equipment such as band saws in this area. Ventilation: The ventilation system will be of the exhaust type exchanging the air at a 1:1 ratio 6 times per hour. In addition, the plans call for local exhaust systems at the perimeters of the freezers and degreasing tank. We will evaluate whether an exhaust duct at the back of the freezers is sufficient to remove fumes form the freezer box.

Back up power: Back up power, provided by an emergency generator, will ensure that even during power failures the air exchange will continue and the acetone in the freezers will remain cold.

Second exit: The Uniform Building Code requires a second exit if the distance of the remotest corner of the room to the door exceeds a specifies distance. Both exits need to be equipped with panic hardware.

Fire separation: The Uniform Building Code requires that the walls be able to withstand 2 hours of fire and the doors 1 1/2 hours to ensure that a fire would not spread to adjacent rooms before the building has been evacuated.

Sprinkler System: The sprinkler system for the planned plastination room will be able to deliver 4000 gal in 20 min. Some of the sprinkler heads will be pointed directly at the freezers and the degreasing tank.

Spill containment: The spill containment system must accommodate the volume of liquids that are present in the room (200 gal of acetone) as well as the volume of water that is discharged by the sprinkler system in the event of a fire. That means 4000 gal of water in 20 min. plus approximately 200 gal of acetone which must be initially contained and drained to a secondary containment. The initial containment of 4200 gal could be accomplished by providing a 4" liquid tight curb along the perimeter for the room. This will necessitate a ramped sill at the entrance of the room. A potential spill must then be drained to a "safe location". The preliminary design of the facility will place the floor drains for the secondary spill containment in front of the emergency exit eliminating the need of a second ramp in the room. A safe location for the spilled liquids could be an underground tank. However, this would require a double wall construction with leak detection equipment making this a very expensive solution. The alternative is an open pit, which not only accommodates the design flow of the sprinkler water, but also the volume of the largest container in this room, plus the 24 hour rainfall as recorded from the worst storm of the last 25 years. This would be accomplished by constructing a pond with a plastic liner using a feeder line of 12" diameter and providing a drainage valve to empty the pond after regular rainfalls. A fence surrounding the pond has been suggested to limit access.

Blow out panel: One outside wall or the roof of an H-2 occupancy room must be designed to vent the intial blow of an

explosion while the other walls must withstand the remaining pressure. This is required to limit the damage to the building in case of an explosion. The new building would be constructed out of precast concrete panels and the roof would be equipped with a blowout panel similar to a skylight.

Obviously, it cannot be cheap to construct such a facility, the preliminary estimate is based of $200.00 per square foot or 2 million dollars for the facility. Several Departments at ISU are under pressure to obtain an H occupancy room for their operation. Sharing a facility is perceived much more cost efficient than attempting to achieve compliance with the code at each location. The Department of Veterinary Anatomy needs a facility to handle large quantities of acetone. The Veterinary Diagnostic laboratory needs a facility to handle ethanol, methanol and agricultural herbicides and pesticides. The laboratory for greyhound racing chemistry needs room for ethanol. And finally the Department of Environmental Health and Safety needs a facility to handle chemical waste created throughout the university. E, H&S currently is using a facility outside the contiguous campus grounds which necessitates a tremendous amount of paperwork with the Iowa Department of Transportation to permit the tracking of chemical wastes from the various campus locations to the existing facility. It would be in their interest if the money would be spent in an on-campus facility, rather than upgrading their existing-off campus steel building.

It is our hope that this alliance of interest will help find funding for a new facility.

1. Paul Rietz, Rietz Engineering Consultants, Ames, Iowa: College of Veterinary Medicine Rooms 1304, 1304 A & 1304B Fire Safety Improvements Feasibility Study, September 1994, Page 1.

2. Gerhard Penz, University of Toronto, Banting Institute, Department of Pathology, Telephone conversation.

CORROSION CASTS OF SPLENIC ARTERY AND VEIN AS A MODEL FOR THE STUDY OF VASCULAR ANATOMY OF SPLEEN

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Morphological segmentation of the human spleen was described on the basis of presence of fibrous septae (Kyber 1870). Vascular segmentations of the spleen on the basis of the splenic vein was reported by Dreyer et al 1952 in their studies on diagnostic splenic venography. Vascular segments to the spleen were also demonstrated by corrosion casts of the splenic artery and its branches. (Gupto et al 1976). By radio opaque techniques, the human splenic segment was found to have an independent hilar branch of splenic artery and a tributary of the splenic vein (Braithwaite et al 1956). This technique cannot be used routinely for the study of vascular anatomy to spleen. The present study was therefore conducted on human spleens to demonstrate the various vascular segments and their interconnections on the basis of the distribution of both the splenic artery and vein using corrosion casts.

Forty fresh adult human spleens were obtained from AIIMS mortuary within 24 hours of death. The splenic artery and vein were dissected out and cannulae were passed into artery and vein. The spleen was washed with tap water to remove blood. With the help of syringe, red solution of cellulose acetate butyrate (CAB) dissolved in acetone was injected in the artery and blue CAB was injected in the splenic vein. Injected specimens were transferred to 10% formal saline for 24 hours for butyrate to harden. The specimen were then transferred to a jar of concentrated hydrochloric acid until corrosion of soft tissues was complete. The resulting cast was then cleaned and examined for the splenic artery and its branches and the splenic vein and its tributaries.

The vascular segmentation of spleen was determined on the basis of the distribution of both splenicd artery and vein. Variations in the vascular segments of the spleen and their interconnections were seen when both artery and vein were taken into consideration.

SHEET PLASTINATION OF THE VASCULARITY OF THE LUNATE BONE

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Introduction: The aetiology of lunate necrosis (Kienbocks disease) remains unclear. Pressure measurements show that the preferred working position of the wrist-dorsiflexion-leads to significantly increased intraosseous pressure in the lunate. The phenomenon can be explained by an impairment of the venous drainage of the lunate in dorsiflexion.

Material and Method: .12 fresh cadaver forearms were injected on the arterial and venous side by El2 technique followed by sheet plastination. 6 arms were fixed in neutral position and 6 were fixed in dorsiflexed position.

Results: the lunates showed an abundant arterial blood supply in wrist neutral position, the arterial supply of the lunates was accentuated on the palmar aspect. In dorsiflexion the arterial supply of the lunate was accentuated on the dorsal aspect. The stretched palmar wrist capsule compressed the palmar vessels, but there was no difference of the rich intraosseous blood supply in the lunates between neutral and dorsiflexed position. The venous drainage of lunates was maintained by dorsal and palmar plexas. In wrist dorsiflexion the venous drainage of the lunates was impaired by the dorsal capsule wedged between the distal radius and the capitate. The palmar capsule was stretched in dorsiflexion and compressed the palmar vessels. Intraosseous veins were congested.

Discussion: Venous congestion in dorsiflexion of the wrist was demonstrated by sheet plastination. Pressure characteristics and sheet plastination showed concordant results. We conclude that there is increased risk for lunates to be damaged by venous congestion when the wrist is dorsiflexed.

LOW COST PLASTINATION TECHNIQUES FOR DEVELOPING COUNTRIES

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The use of plastination is increasing, due to its many advantages. Developing and the underdeveloped countries are yet to catch up because of the high cost.

Some modifications have been made to the procedure to reduce the cost and save time. The specimens so plastinated are better for museum display, demonstrations and discussion, compared to the specimens in jars.

Fresh specimens were selected and cleaned in running water; preserved in 10% formalin for 48 hours; dehydrated using 70-80-90-Absolute alcohol changes spending 3-4 days in each; cleared with Benzene for 3-4 days; transferred to the resin; after 48 hours, exposed to hardener for 2 hours and then air dried. The dissection was done to display the part of interest and then mounted on wood/acrylic base using wires. Colors were added to enhance contrast.

Advantages are a minimal use of materials and the procedure is done at room temperature and pressure. This results in greatly reduced cost.

PREPARING AND USING S10 AND P35 CLEARED RABBIT FETUSES TO DETERMINE SOME OSSIFICATION CENTERS

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Introduction: The determination of some ossification centers of rabbit fetus is part of a study performed by the author using various animals. It is of great significance for academic and medico-legal studies. Similar work on the goat was done in 1992, by the use of clearing, Alizarin staining, and normal radiological procedures. The present study on the rabbit is a trial of plastination in our research.

Material and Methods: Pregnant rabbits were killed and their fetuses were cleared and stained with Alizarin red. Some specimens were plastinated, using either S40 or P35 (S10 P35).

Results: The presence of ossification centers in different parts of the skeleton in relation to different fetal age was recorded.

Conclusion: The results obtained are of great interest and importance as a means of aging the embryos through determining the state of the ossification centers.

THE USE OF CORROSION CAST TECHNIQUE AS MODEL TO STUDY THE PATTERNS OF SEGMENTAL ANATOMY OF LIVER

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Introduction: Advances in hepatic surgery and the trend to conservative hepatic surgery prompted the study the segmental anatomy of the liver in greater detail. Compared with gross dissection, histological and radiological techniques, the corrosion cast preparation appears to give a better display of intrahepatic anatomical relationship. The corrosion cast may be of immense help in localozating and understanding various pathological processes and in planning the consequent surgical approach. Segmental anatomy of the liver has been studied by Glisson (1954), Goldsmith and Woodburne (1957), Gupta et al (1977) and Ralph (1989). Because of the conflicting accounts in the literature it has been decided to make an exhaustive study of the anatomical segmentation of the liver.

Aims: This study aimed to formulate a practical classification of the various segments of the liver particularly on the basis of intrahepatic course of the portal vein, hepatic artery and bile duct by using corrosion cast preparation.

Material and Methods: Liver specimens in block (all males) were randomly selected from post mortem examinations. Cadaver showing intraabdominal pathology, previous surgical exploration of the abdomen or abdominal trauma were excluded from the study. All the samples were injected with different colored cellulose acetate butyrate (CAB) granules dissolved in acetone to exhibit the intrahepatic patterns of hepatic artery (red) portal vein (blue) and bile duct ramification (green). Casts were prepared according to the method described by Tompsett (1970).

Results: Each cast was studied separately for its segmental pattern. Hepatic segmentation was done on the basis of intrahepatic distribution of the portal vein, hepatic duct and the hepatic artery. The study showed the marked variation in the sizes of the segmentation.

LOW COST PRESSURE INJECTOR

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There are a number of situations when plastinated specimens lose some of their natural contours during processing. This may be due to inadequate fixation, loss of fat during the dehydration and defatting phases or failure of polymer to penetrate intact skin and dense fascial layers. Some of these problems can be rectified by injection of polymer into the specimen before or after impregnation. To inject viscid polymer requires substantial pressures (up to 10,000 p.s.i.) and the equipment is correspondingly costly.

Sometimes quite sophisticated equipment is relatively cheap if it is mass produced. Our laboratory does not use custom made stainless steel vacuum chambers costing $2,000 but buys instead similar sized vacuum chambers which are parts of milking machines and thus made in significant numbers. They cost $500.

We therefore sought a source of mass produced pressure injection equipment capable of producing 10,000 p.s.i. and soon located it in the form of a hand powered grease fun used to inject lubricating grease into bearings.

It has been modified. Instead of a cartridge of grease, a cut down 50 ml disposable syringe filled with polymer can be loaded into the grease chamber. An adaptor screws into the existing grease outlay and has a male Lure taper fitting as the new outlet. It incorporates a screwed collar to retain the needle and prevent it form becoming a projectile.

While it has limited capacity and would be tiresome to use of large volumes needed to be injected it has the outstanding advantage of having cost $35. There are other forms of greasing equipment some of which is fed form 25 liter drums and which may be powered by compressed air. This equipment may also prove suitable for modification to enable it to inject polymer.

THE DESIGN OF LIFTING GEAR FOR A PLASTINATION LABORATORY

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With increasing suspicion about the harmful effects of formaldehyde one of the advantages of plastinated specimens is the improved safety to staff and students which accrues form not using formalin preserved Specimens. However, plastination suffers from a considerable safety handicap in that it usually utilizes acetone (di-methylkentne) which is colatils flammable and can form explosive mixtures with air. This property dominates the design of a plastination laboratory with particular emphasis being paid to the elimination of sources of ignition. Spark-proof light fittings, explosion proof ventilation fans and the removal of freezer compressors to an adjacent room are common precautions.

Many plastination laboratories have had to utilize existing buildings and the Brisbane laboratory is no exception. It has a standard single doorway. In order to accommodate the largest possible freezers these were designed to just pass through the doorway without their lids which are fully detachable. In order to accommodate the largest possible freezers were designed to just second design feature was to incorporate extra insulation to ensure that -25 Celsius could be readily maintained during a Queensland summer when ambient temperatures sometimes approach 40 degrees Celsius. In consequence the freezer lids are bulky and heave and not easily manhandled by a single operator and it was not before a back injury was sustained.

Also the movement of drums of acetone or polymer into or out of the freezer is a task that can only be accomplished if they are almost empty.

There was an obvious solution; some form of lifting gear. However this had to operate safely in the presence of flammable vapor. To meet this requirement it proved necessary to consider a number of factors which included considerations of the nature of acetone, the circumstances in which explosive vapors were used and finally the design feature of the lifting gear and operational regulations for its use.

DEVELOPMENT OF AN ANATOMICAL TECHNOLOGIES LABORATORY

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This presentation documents the procedures followed in establishing an anatomical technologies laboratory for the preparation of plastinated specimens in the Department of Anatomy and Cell Biology at the University of Melbourne. Funding for the laboratory was obtained through a Faculty of Medicine teaching development and innovation grant, supplemented by the Department. A space was found in the basement to the building, next to the embalming room and consisted of a "tank room" which was formerly used for the storage of cadavers. This was converted into two suitable work areas, one a preparation room and the other a spark-proof area housing the freezer with an isolated vacuum pump and refrigeration unit.

Part of the funds obtained form the Faculty have been used to employ a technical officer who has dedicated responsibility to the facility and its day-to-day operation.

The laboratory has been fully operational for over a year and a large number of plastinated specimens have been prepared during that time. We are in the process of developing 'sheet plastination' and have a project under-way with radiologists from the Royal Melbourne Hospital correlating cross-sectional plastinated specimens with CT-scans and magnetic resonance images.

It is intended to direct most of our work towards the Department's teaching requirements, however we also intend using the laboratory to train technicians in anatomical techniques and encourage honors and higher degree students in the area.

PLASTINATION AS A CLINICALLY BASED TEACHING AID AT THE UNIVERSITY OF AUCKLAND

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As a concerted move toward closer integration of the clinical and pre-clinical aspects of the undergraduate medical curriculum at the University of Auckland, the Department of Anatomy has established a formal link with the Department of Radiology resulting in a structured program of clinically based teaching of gross anatomy to second and third year medical students.

As sophisticated diagnostic techniques and methods of treatment have become common place, our teaching program has been tailored to accommodate a greater degree of case based learning within the undergraduate course.

Dissecting room demonstration is provided by Radiology, Pathology, Ophthalmology and Surgical registrars with a number of clinical procedures, pathological observations and diagnostic methods employed during the routine dissection of the cadaver.

Through use of a number of plastination techniques, the learning process if enhanced and aided on a number of levels. The E12 epoxy method for producing MRI and CT based serial sectioned cadaver specimens has allowed an accurate and highly detailed orientation of the planes of the body and provides the student with a clearer understanding of anatomical structure and pathological anomalies as seen with modern imaging techniques.

ULTRASTRUCTURE OF THE REACHEOBRONCHIAL EPITHELIUM OF THE NORMAL AND FORMALDEHYDE-EXPOSED GUINEA PIG

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Formaldehyde has long been used as a preservative of cadavers in most of anatomy departments all over the world. This work was designed to study the ultrastructural changes of the tracheobronchial epithelium of guinea pig after formaldehyde exposure.

Forty adult male guinea pigs divided into four groups were exposed to 0.5 part per thousand formaldehyde for 4 hours per day. The period of exposure was 3 days, one week, two weeks and four weeks for the first, second, third and fourth group respectively. A fifth group was examined without exposure as a control.

Many changes were observed in the tracheobronchial epithelium according to the period of exposure. These changes included increased mucous secretion, epithelial desquamation, erosion, ulceration thickening of the basement membrane and proliferative changes in the form of basal cell hyperplasia, squamous metaplasia, dysplasia and micropapillomatosis. The cilliated cells showed numerous changes including vesicuolization of cytoplasm, dilation of rough endoplasmic reticulum, chromatin margination and many ciliary changes. The ciliary changes were in the form of loss of cilia, swollen cilia, truncated cilia, intracellular axonemes, disoriented basal bodies, disorganized cilia in the form of *8+2) and (9+0), and vesiculation and rupture of the ciliary membrane. Three forms of ciliated cell loss were unidentified, the first was desquamation of intact cells, the second was rupture of the apical cell membrane and release of its organelles into the lumen and the third was ciliocytophthoria.

The goblet cells underwent massive secretion of their contents and the mode of secretion was changed from merocrine to apocrine. Later on, goblet cell hyperplasia occurred but many cells contained only few abnormal secretory granules, new cells were observed after formaldehyde exposure and also were the tunnel cells which were considered to be degenerating cells. In the subucosa, many undifferentiated cells were observed after two and four weeks formaldehyde exposure. The presence of these cells raises the possibility of fibrosis and permanent airway obstruction as sequel of long term exposure.

The similarity between the lesions produced by formaldehyde and the early lesions occurring during carcinogenesis raises the possibility of carcinogenic effect of formaldehyde. Assessment of formaldehyde carcinogenicity, teratogenicity and its effect on different parts of the body deserves further studies which are planned to be done in our department in the future. From our results, we recommend stopping, or at least minimizing the use of formaldehyde and employing other safer methods e.g. plastination.

A REPORT ON PRODUCTION OF THE FIRST TRANSPARENT HUMAN BODIES IN IRAN

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In 1991, after taking part in the workshop held in Heidelberg, we started our activities in the field of plastination. In 1994, during the 7th International Conference of Plastination, we announced our success in starting plastination using existing equipment in our country. Now this year we are honoured to declare the production of the first transparent human bodies in Iran.

Additionally, we are working on plastination of the dissected whole body. A more detailed description will be given during the lecture.

A SIMPLE AND ECONOMICAL METHOD IN DISTILLATION OF ACETONE FOR A PLASTINATION LAB

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Because large amount of acetone are used in a plastination laboratory, it is necessary to reuse it by distillation. However, a distillation unit is very expensive so we designed a distillation unit by existing equipment in our laboratory. A detailed description will be given in the paper.

CORRELATING SHEET PLASTINATED SLICES, COMPUTED TOMOGRAPHY IMAGES AND MAGNETIC RESONANCE IMAGES OF THE PELVIC GIRDLE: TEACHING TOOL

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Introduction: In the Dutch medical curriculum there is only a limited amount of time reserved for practical anatomy lessons. As a result the time spent in the dissection rooms has decreased steadily. In order to compensate for this problem we use pre-dissected specimen for educational purposes.

Most of our pre-dissected specimens are conserved using formaldehyde fixation or silicone impregnation. Recently the epoxy resin impregnation technique was introduced in our departments. With this technique we have produced anatomical slices of many different body structures which allowed us to study their topography in an anatomical correct state. These slices can also be used for teaching medical students.

Materials & Methods: The pelvis of a female cadaver was used, the bowels were removed before scanning and preparing the pelvis. Using a Computer Tomograph (CT) (somaton plus, Siemens, Germany) 2mm thick coronal coupes were obtained (120 kV, 210 mAs). For this purpose we mounted the pelvis on a wooden board as described by Entius et all. With manual reposition we obtained good coronal positioning. Using the reference crosses, as projected by the light beams of the CT, we marked this coronal position with a permanent marker. The CT data were used to obtain a 3-D reconstruction of the pelvis. Thereafter the pelvis was stored in a freezer (-20C) for about 7 days. The deep-frozen pelvis was sawed into 36 slices of approximately 2mm, using a handsaw (AEW 600, 5 teeth inch-1). The slices obtained were kept between plastic netting to keep them separated and allow more easy penetration of the acetone. Plastination was performed using an epoxy resin (E12, BiodurTM) as described by von Hagem.

Magnetic Resonance Imaging (MRI) (Gyroscan T5, Phillips Medical Systems, Best, the Netherlands) of a preserved pelvis, using formaldehyde, is impossible due to soft-tissue changes, especially in the musculature. Therefore we had to compare the coronal CT images and plastinated slices with coronal MRI of the pelvis of a healthy female volunteer.

Results: We obtained 36 anatomical slices of the female pelvis. The most ventral part of the pelvis could not be sectioned because of technical problems (the risk of hand trauma to the handsaw operator was too high). We found a good overall correlation between the CT images and the anatomical plastinated slices. Correlation with selected MRI slices was also high.

The plastinated slices in cimbiation with CT and MRI, were used or teaching and examination purposes. It was found that the use of 3-D images strongly enhanced the comprehension of 3-D anatomical structures.

Discussion: The combined use of plastination and radiological techniques as we have described will allow students, radiology residents, and anatomists to gain a better insight in 3-D aspects and the mutual relation between the various anatomical structures of the pelves region. In order to gain insight in normal variations of the anatomy of the female pelvis, plastinated slices of different bodies could be useful.

The major problems with this technique is deviation from the coronal plane during sawing. This is due to the inhomogeneity of the pelvis and is very difficult to prevent.

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THE CURRENT PLASTINATION INDEX

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Since the introduction of plastination some seventeen years ago, the developments of this process and its applications led to an increasing number of publications and communications in several languages all around the world. The aim of the Current Plastination Index (CPI) is to provide a data bank to those who decide to learn plastination, as well as to experience plastinators who want to search the literature about plastination. The CPI will be revised at regular intervals as a result to the information that you pass on to us. The participation of every plastinator is therefore necessary in order to compile and classify (according to language, journal, key-words) the world literature on plastination.

PLASTINATION IN ORTHOPEDIC SCIENCE FIRST RESULTS OF AN ORTHOPEDIC MANAGED PLASTINATION LABORATORY

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We started to use the plastination method in our own laboratory one year ago, in the Department of Orthopedic Surgery at the University of Heidelberg. We have been interested in the following questions:

1. Is there a relationship between the intra-osseous vascularization of the patell and degenerative changes in the hyalive cartilage?
2. Rupture of the Achilles Tendon. Is it a problem of the vascularization?
3. The vascularization of the Anterior cruciate liga ment. Where does its blood supply come from?
4. Is it possible to demonstrate arterial and venous vessels in bone with different colored plastic material?
5. The vascularization of the lunate bone in osteochondritis. Studies during extension and flexion of the carpus of man.
6. An experimental study in sheep. Treatment of osteonecrosis of the hip joint by drilling. Demonstrated by plastination.
7. Case report. Degenerative changes in a human meniscus demonstrated by plastination

As a result, we think that plastination is a very good method to investigate problems in orthopedic surgery. In all the above mentioned cases it has not been possible to use other methods in our experiments.

RECLAMATION OF ACETONE IN PLASTINATION LABORATORIES: A SIMPLE AND INEXPENSIVE METHOD

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The cost of acetone used for the dehydration step in the plastination process has always been considered an important factor in the cost of plastination. Discarding used acetone also presents a problem because it must be treated as a toxic waste.

we have developed a method in three steps that permits us to re-use our acetone. The first step simply consists of storing the contaminated acetone in the freezer and separating by filtration the frozen water and fat that has congealed. The second step is vacuum distillation of the acetone and can be carried out with the freezer vacuum pump that is already found in any plastination

laboratory, with just a few additions. It produces 95% -97% pure acetone. The last step uses a desiccant to remove the residual water from the distilled acetone and brings the purity to 99.5%.

We used the above process for some time and now have modified it by adding two distillation coils and two collection reservoirs in series. Each coil was made from 2.5mm to 6mm copper tubing. Acetone is warmed to a temperature of 40-5-C and vacuum is applied and adjusted to maintain a moderate boil. The vaporized acetone passes into the distillation coils which are inside a deep freezer.

With these methods, we have reduced to a minimum the amount of acetone to be purchased and completely eliminated the cost of discarding used acetone.

As a additional reevaporation process, we direct the exhaust line form our vacuum pump during impregnation into the freezer and collect the vaporized acetone by condensing it into a jug inside the deep freezer.

THE USE OF PLASTINATED EQUINE HEAD SECTIONS OR THE GODFATHER PART V

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As part of a study investigating ethmoid haematomas, a rare disease in equines, the ethmoid region was examined using conventional radiology (CR), computed tomography (CR) and magnetic resonance imaging (MRI). An atlas of correlative imaging of the normal equine head was produces. Photographs of transverse sections of 6 normal heads were matched with the corresponding CT and MRI images. Selected sections were useful for referencing when identifying anatomical structures during the completion of the atlas. In addition, sections form the head of a horse diagnosed with an ethmoid haematoma were plastinated, providing a permanent record of the destructive nature of this disease.

SPECIMEN PREPARATION FOR SILICONE PLASTINATION

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Specimen preparation is a very important step of the silicone plastination process, especially when using fresh tissue. If you plastinate specimens that have been poorly or inadequately prepared, the final product will be inferior no matter how well the specimen is impregnated with silicone.

Minimal fixation may help assure a more natural looking specimen. Care must be taken to keep the specimen in its normal anatomical position. Loose portions of the specimen may be held in position with suture. Intravascular injection of colored silicone, gelatin, latex or epoxy may be used to highlight vessels. Hollow organs need to be flushed, cleaned, dilated and then fixed in a dilated position. Dilation of hollow organs will increase the flexibility of that organ by overcoming rigor and hence a thinner wall. Intestinal specimens may be opened to remove ingested matter, sutured closed and then dilated. Ostia with strong sphincters must be held open. Appropriate sized cannulas or tubing may be used. All cut vessels of heart preparations must be closed by ligatures or inverted corks ligated in place, except for one vena cava and one pulmonary vein. These will have tubing ligated in place and used for dilating each side of the heart. The atrioventricular valves of the heart can be accentuated by holding them away from the chamber wall and semilunar valves aided by packing with cotton or other materials. Joint capsules, distended with 20% formalin, aid in studying the internal anatomy of the joint.

Plan the theme of the specimen and limit the focus of the specimen to fewer items especially musculoskeletal preparations. Holes drilled into the marrow cavity of long bones in less noticeable areas enhance defatting and prevent sticky/greasy specimens years later. Old faded long-term fixed brains may be rejuvenated and made more useful by sectioning or prosecting and staining with astra blue, aldehydefuchsin or Darrow red to highlight the gray matter. Specimens may be plastinated following histochemical studies for long term review or study. A pink color may be added to the surface of the specimen using Biodur stain in the last acetone bath.

Beautiful specimens do not just happen.

CONSTRUCTION AND SERVICE OF A DATABASED OF PLASTINATED SPECIMENS

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About 200 plastinated specimens were made at the Medical Museum, University of Tokyo between 1991 and 1995. All of the specimens were registered in a databased system on a personal computer (Machintosh, Apple Co.) with commercial software FileMaker (Clarin Co.) and HyperCard. This database is used not only to catalogue the specimens but also to show the specimens to those who cannot come to the museum. Besides the database, information about the characteristics of specimens and methods of making specimens are edited with HyperCard for beginners. A part of the database was modified and demonstrated at two public exhibitions on plastinated specimens in 1995 at Tokyo. The database is also distributed on the Internet (http:/ 7202.230.170.207).

MAGNETIC RESONANCE IMAGING (MRI) VERSUS THIN PLASTINATED CADAVERIC SECTIONS AS AN **ANATOMIC LEARNING TOOL**

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The advent of new medical imaging technologies, such as magnetic resonance imaging (MRI), computed tomography (CT) and ultrasonography demand that cross-sectional anatomy be studied. Hence, anatomy students of all disciplines, as well as physicians and medical technologists, must understand the three-dimensional structural relationships of the body from a perspective different to that observed during dissection of a cadaver or conventional radiography.

It is believed that the relatively new technique of plastination, developed by Gunther von Hagens (1979, Anat. Rec., 194:247) can serve as a highly effective anatomical teaching tool. Thin body slices used for educational purposes are well suited to plastination using various epoxy resins.

One half and two whole human cadaveric heads were scanned with an MR imager. Coronal, sagittal and transverse planes of imaging were recorded. After the heads had been frozen using dry ice they were sliced, with sections being cut to a thickness of approximately 6mm in the desired plane. These were then subjected to sheet plastination using either the PEM 11 or E12 technique. Thus, the intrinsic three-dimenstioal anatomical detail is preserved in specimens that are dry, odorless, durable and are pleasant to handle.

Measurements of certain anatomical structures will be taken on both the plastinated specimen and its corresponding MRI to determine the degree of correlation between the two modalities. The efficacy of plastinated sections as teaching tools will be evaluated by testing two groups of students studying head and neck anatomy. The existing anatomical knowledge of each group will be pre-tested using a correspondingly labelled MRI and plastinated specimen. Individual scores will be recorded, however student anaonymity will be maintained.

Each group will then be given 30 minutes to revise the topographical arrangement of approximately 20 labelled features. One group will use MRI's and the other plastinated specimens. After this "study" period, students will again be presented with correlated plastinated sections and MRI's and be required to

identify the same labelled structures. Students will also be asked to complete a questionnaire asking for their personal preference on methods of learning anatomy. The pre and post test scores will be statistically analyzed to determine the educational value of the two distinct anatomical media.

PROBLEM ENCOUNTERED IN E12 SHEET PLASTINATION TECHNIQUE

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Introduction: With the standard technique of E12 Sheet Plastination I have found separation of glass plate difficult to impossible.

Method: Fresh aborted fetus was played on a cork board and left in deep freeze at -25C for 3-4 days. On the 4th day using a fine-toothed saw, the fetus was sliced sagittally through the center. Thin slices (34mm) were made of each sagittal section, cleaned of sawdust and placed on a gauze for dehydration. Dehydration commenced with cold acetone in deep freeze. After 2-3 weeks dehydration was complete.

The specimen was now immersed in E12 solution and placed in a vacuum chamber for impregnation. Impregnation solution was made as follows: E12 lOOmls/El Hardener 28 p.b.w./AE1020 mis.

After 24 hours these slices were placed in fresh E12 solution. The next day these slices were cast between glass plates (flat chamber method) using E12 casting solution. The specimen looked excellent. Casting Solution El2 lOOmls/El Hardner 28 p.b.w./AelO 20mls/AE30 5 p.b.w.
They were allowed to dry for 3-4 days and then placed in an incubator at 50C.

Results: The problem arose when attempts to separate the plates became difficult to impossible because the solution remained "sticky". Any forced separation resulted in ruining the specimen. This became worrisome because having gone through the whole plastination process, the specimen was destroyed at this late stage.

Consideration: Is it the shelf life that has no effect? The chemicals were 18 months old. However, E12 + El Hardener were dept at +18C and AE10 and AE30 were kept at 4C. Previously, I have had no problems with this technique. If this is a problem of chemical storage:

1. What is the shelf-life of the chemicals?
2. Where should one store these chemicals - In the deep freezer?

APPLICATION OF PLASTINATION TO PRIMATE LOCOMOTOR ANALYSES

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We have attempted to apply the double method for plastination to analyse the structural-functional relationships of macaque extensor digitorum et hallucis brevis muscle)EDB and EHB), the single intrinsic muscle of the forsum of the foot.

The muscle samples were dissected, fixed in 10% formalin and embedded in 10% gelatin. Using a Sartorious microtome, the specimens were sliced with section thicknesses ranging for several millimeters to 30mm perpendicularly (5X20mm sheets) or horizontally (15X25mm Sheets) to the longitudinal axis of the muscle bellies. Some 30mm thick sections were stained with Sudan Black B. The other sliced specimens were dehydrated in increasing grades of ethanol and plastinated with epoxy resin (Epon/DDSA+MNA=0.35/0..65, MNA/ DDSA=1/1). The thick specimens (3-5mm) were re-sliced at 3-20mm sheets after being incubated at 60C and were then stained with toluidine blue (40C).

These specimens showed several characteristics of good preservation and cutting quality which enabled the depiction of structural and functional relationships. Muscle fiber types (type 1 and typell) were differentiated in sections stained with toluidine blue or Sudan Black B. There was sharp contrast between muscle fibers and myelinated nerves.

Using this method in the serial sections, muscle spindles canalos to reconstructed in three dimensions. Therefore, it is possible to calculate precise innervation ratios of EDB and EHB muscles by using the number of muscle spindles, muscle fiber population and myelinated thick nerves in given areas. In addition, the three dimensional distribution pattern of the muscle fibers arising form the lateral and dorsal side of the calcaneus can be traced until they divide into four bellies which extend to the four medial toes. We have found that the plastinated specimens obtained in the present study are superior to those of previous methods.

PREPARATION OF THE CORONARY ARTERIES FOR PLASTINATION

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The technique of preservation of anatomical specimens using the method of Dr. Gunther von Hagens provides excellent demonstrations for teaching and further education. Of particular interest in the heart is its vascular supply, which we can display in plastinated specimens injected with red Biodur E20.

The heart should be removed from the thoracic cavity taking care to divide the vessels some distance from the heart. Then the heart is rinsed with cold tap water and all vessels are blocked with cork stoppers. The stoppers for the aorta and the pulmonary trunk contain tubing, so that during the dilatationfixation the aortic valve and the pulmonary valve are in an almost completely closed position.

Injection of the coronary arteries with 49.5 gram Biodur E20 red requires only slight pressure to fill the coronary vessels.

For the dialtation-fixation 15 L of 20% formalin solution are infused into the ventricle form a height of 1.5 meter. A week of immersion-fixation in 5% formalin solution at a temperature of +5C follows.

The sub endocardial fatty tissue and all facia are carefully dissected away with scalped, scissors and forceps. Subsequently, the specimen is bleached in a 2% hydrogen peroxide solution with 5% formalin added to achieve the desired color. After washing for about 1 hour, theauricles are tamponaded with polyethylene-foils and the specimens are frozen overnight in a plastic bag. Dehydration lasts for 3 weeks at -25C in the deep freezer. During this time the acetone is changed twice.

Forced impregnation with Biodur S10 plus S3 hardener takes place for 3 weeks at -25C in the deep freezer. After removal from the vacuum kettle and draining overnight in the freezer, the specimen drains for one further day at room temperature. Then the specimen is placed into a plastic container for gas hardening with Biodur S6. This takes 2 weeks. Finally, a comprehensible injection and research in medicine.

LOW COST PLASTINATION TECHNIQUES FOR DEVELOPING COUNTRIES

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J.S.S. Medical College Mysore, India

The use of plastination is increasing, due to its many advantages. Developing and the underdeveloped countries are yet to catch up because of the high cost.

Some modifications have been made to the procedure to reduce the cost and save time. The specimens so plastinated are better for museum display, demonstrations and discussion, compared to the specimens in jars.

Fresh specimens were selected and cleaned in running

water; preserved in 10% formalin for 48 hours; dehydrated using 70-80-90-Absolute alcohol changes spending 3-4 days in each; cleared with Benzene for 3-4 days; transferred to the resin; after 48 hours, exposed to hardener for 2 hours and then air dried. The dissection was done to display the part of interest and then mounted on wood/acrylic base using wires. Colors were added to enhance contrast.

Advantages are a minimal use of materials and the procedure is done at room temperature and pressure. This results in greatly reduced cost.

STUDENT PARTICIPATION IN PLASTINATION AS **A LEARNING EXERCISE IN A SCIENCE DEGREE EMBRYOLOGY** UNIT

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Students enrolled in their third year of a Bachelor of Science course at Curtin University used plastination techniques to preserve their dissolved specimens as part of the practical component of the embryology module of the Human Structer and Development unit coordinated by GMF at Curtin University. The experience and expertise of GW in S10 plastination technique was utilized. Students attended and information session on the methodology of plastination, which related well to their experience and knowledge of histological techniques using resin embedding. They then examined fetal piglet specimens, measured and weighed them to determine their various ages, and subsequently decided which aspects of development they wished to portray in their plastination specimens.

Dissection work was carried by each student, and the resulting specimens were dehydrated and plastinated over several weeks. Progress was monitored by the students with respect to dehydration, shrinkage and color retention. After 8 weeks, the plastinated specimens were examined and their component parts identified. The specimens were photographed with a digital camera and the resulting images imported into a hypercard stack representing the work of the class. The images were labelled and linked to information about their age and sectioning, and the plastinated specimens retained for use in the next years class.

As a result of participation in the plastination process, students were engaged in the technical aspects of their work and this increased their feelings of responsibility to the resulting dissection. Students gained insight into fixation and embedding techniques combined with image digitisation and multimedia presentation of graphical and text information.

Results of student evaluation of the plastination componet of the unit, and examples of their work will be presented with this paper.

INVESTIGATIONS OF LYMPHATIC VESSELS WITH PLASTINATION TECHNIQUE: FIRST EXPERIENCE

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The lymphatic system remains one of the less well investigated systems. At the same time a number of pathologic processes are associated with the changes of lymph outflow. Demonstrations of the lymphatic vessels for teaching are also rather limited because of the difficulties in the preparation of permanent specimens. Plastination technique can solve these problems.

Lymphatic vessels were studied in rats and dogs. Intraorgan and surface vessels were detected by injection of either a mixture of indian ink with 10% gelatin or latex. This was followed by formalin tixation, dehydration and impregnation with Alizain stain. Epoxy resins were used for plastination. Currently attempts are being made to visualize lymphatic and blood vessels using plastination.

A POTENTIAL BEST STEREOGRAPHIC PRESENTATION OF THE PLASTINATED ANATOMICAL SPECIMEN PART 1 DEMONSTRATION OF REAL AND STEREOGRAPHIC IMAGES OF THE PANCREAS AND DUODENUM COMPLEX

Sadao Shimaguchli; Meiko Taguchli; Fumihiro Koikea; Shouzou Takais; Hajume Tamurat; Masao Akahanres; Soutarou Iwamotoe; Eisaku Kanazawa?; Takasi Satake?; Kunihilo Kimuras; Yutaka Yoshida9; Makoto Iwaharaio
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In the 7th Congress at Graz, Dr. Yoshida,one of our authors, presented a Poster on the brain cortex and medulla to distinguish cortex in the process of making the plastinated brain slices. Hereafter, he made many sort of plastinated specimens and felt the volume of the plastinated specimen was decreased compared to the classical alcohol-solution immersed specimen mainly due to no use of the glass-ware. However, the dimensionality was not changed, although the silicon plastinated specimen hold such fabulous qualities as dryness, odourlessness, durability and tangibility, potentiality of literally being palpated and grasped. Space occupying characteristics is not improved through the plastination. In general, space occupying lesion is well known as a cancer. Only one of the disadvantages of the plastination is this space occupying characteristics. There is a cancer in the plastination technique.

Just at the very time, Mr. Iwahara of JVC proposed a classical stereographical technique of photography, the volumegraph the principally of which will be explained later in the second part by Dr. Koike. I think the method can be suffice for the deficit of the plastination, that is to say, the volumegraph cure the cancer of plastination. Surely, the plastination tech nique has marvellous qualities as a preserving method, com pared to the alcohol immersion system but no dimensionality is lost. The reduction of the dimensionality is attained by the sheet film stereographic presentation; The 3-dimension of the specimen in the glassware is converted into 2-dimension of stereographic sheet film of the volumegraph by Iwahara (JVC) without loss of stereographic images of the specimens. Futhermore, the volumegraph shows the same level of the color representation as that of the plastinated specimens as far as the naked eyesight is concerned. The volumegraph can be copied easily and less expensively and imported without completing a Quarantine Declaration.

EDITORIAL REVIEW
"A PHAROAHS FAREWELL: THE MAKING OF A MUMMY"

Ulmer, D.: Titford, M.
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This article appeared in The National Funeral Directors Association Journal. In it the author describes an experiment conducted by Robert Brier of Long Island University and Ronald Wade of The University of Marymand to copy exactly the mummification process perfected by the Egyptians over 2,000 years ago. Bronze and stone tools were prepared, exact replicas Egyptians used for embalming. Oils, spices, linen, and hundreds of pounds of natron, the salt used to preserve the body was purchased in Egypt.

The body was eviserated by removing the brain through the nose and the internal organs through a four inch abdominal incision. The body was washed with Palm wine and myrr and packed with natron for 35 days. The organs were packed separately. Linen was then wrapped around the body using tree resin as an adhesive. The experiment was a success, although the mummy appears different to those of antiquity from Egypt. Tissue samples will be taken periodically to evaluate the mummification process.

While this process is not true plastination, it is however a forerunner to the now popular process that we as Plastinators now use. From time to time, I believe it is good to examine and see the yester years.

"A PHARAOH'S FAREWELL: THE MAKING OF A MUMMY"

Korbeck, Sharon

As early as 2500 B.C., Egyptians used mummification to preserve kings, commoners and even animals.

Today, two East Coast scholars have recreated the ancient craft in hopes of capturing the mystery and magic of mummies.

Robert Brier, chairman of the philosophy department at Long Island University, and Ronald Wade, director of the Anatomical Service Division at The University of Maryland Medical School, began making a mummy last spring - a process they believe hasn't been replicated in more than 2,000 years.

"Egyptians were the first to use artificial means of preservation, and we wanted to gain some understanding of an ancient process by recreating its steps exactly," says Wade.

Brier, who initiated the project, likens it to a murder mystery in which clues must be recreated to solve the puzzle. His school funded much of the project, and Wade's laboratory donated the space, manpower and the ultimate necessity-a body.

Wade had not known Brier previously, and the need for a body was what brought Wade on board. "In Maryland, people really believe in body donation," he says. Their subject was a 76 year-old Baltimore man who had died of a heart attack.

The facilities at the University of Maryland School of Medicine, were Wade is located, were ideal for the project. Wade is a licensed funeral director, and his anatomical expertise was also required.

Brier had previously traveled to Egypt gathering oils, spices, linen and hundreds of pounds of natron, the salt used to preserve the body. Using handmade bronze or stone replicas of ancient Egyptian tools, the team began its work in May.

The body was first eviscerated, which Wade calls the most challenging portion of the project. In keeping with the Egyptian process, they removed the brain through the nose. Then, through a five-inch incision in the abdomen, internal organs were removed with each organ intact, treated and placed in canopic jars. As per tradition, only the heart remained in the body.

Brier and Wade swabbed the internal cavities with palm wine and myrrh and filled them with linen-wrapped natron packets. Then the body was ready for the long drying process.

The body was covered head to foot with natron and left to cure in a 105-110ϒ F room. "We tried to get the environment most like Egypt," Wade says.

After 35 days, the body was ready to be uncovered. "I was amazed that the natron removed about 100 pounds of water from the body", Wade recalls. The body was then wrapped in linen, using tree resin as adhesive, and kept in the heated room.

Wade took tissue samples from the body prior to final wrapping. The samples allow him to study the degree of preservation and dessication to gauge the project's success. The mummy will be stored in a Ziegler case, and Wade will study tissue samples every 6-12 months.

National Geographic filmed the project's progress for its "Explorer" television program, which aired August 28.

With the mummy only several months old, Wade calls the project a success. "The mummy looks a bit different than those in Egypt, but it is very well preserved."

How long will the mummy stay preserved? Wade believes it could be for several thousand years. But learning about the ancient craft will continue.

"If one or two years from now there is decay, we will know that we missed something that was key to the process."

"The Egyptians were involved in magic. There were secrets, things that were never written down. If this doesn't work, we may never know how they really did it."

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USE OF PLASTINATED SPECIMEN IN A MEDICAL SCHOOL WITH A FULLY INTEGRATED CURRICULUM

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ABSTRACT

The Science University of Malaysia is the youngest of three medical schools in Malaysia, and the only one practicing the fully integrated system. Students are exposed to many disciplines at the same time. The Anatomy Department gives input to courses for students in Medicine (Yr. 1-3), Medical Technology (Yr.l), the Postgraduate Nursing and and the Masters Program in Surgery and Medicine. Students do not do cadaver dissections, but are exposed to four learning materials. These are prosected wet specimens, plastinated specimens, pots and models. In order to assist the department plan, the availability of teaching materials, a survey was conducted among medical students in various years. They were asked to grade each material in terms of handleability, realism, information, suitability for examination and condition of the specimens. The results showed that students generally preferred plastinated specimens. However, with respect to being most informative and most realistic, wet specimens scored the highest. In conclusion, plastinated specimens have a definite use and preference in teaching anatomy where detailed knowledge is not essential, but prosected wet specimens still have a place in our medical school.

INTRODUCTION

The School of Medical Sciences, University Sains Malaysia, was established in 1979. It had its first intake of medical students in 1981. The school adopted an innovative curriculum with specific objectives of producing competent medical practitioners who would be able to identify themselves as part of the health-care team and people.(Roslani, A.M.M.1980)

The teaching-learning methods have been adopted to reflect the curriculum design which is summarized as SPICES, which is Student-centered, Problem-based, Integrated, Community-oriented, Electives and Spiral/Self-learning. The curriculum itself spans over 5 years, divided into 3 phases: Phase I being Year I; Phase II being yr. II and III, and Phase III being year IV and V. Phase I consists mainly of basic medical sciences; Phase II organ-system based, and Phase III clinical postings.

Phase I curriculum is divided into 13 blocks:

Cell & Tissue
Growth & Development
Blood
Musculoskeletal
Cardiovascular
Endocrine
Environment
Reproductive
Nervous
Gastrointestinal
Respiratory
Urinary
Host & Nutrition

Students in Anatomy do not do cadaver dissections. Deep and detailed knowledge of anatomy is not really necessary. They are exposed to prosected specimens and other learning materials.

The teaching-learning processes in Phase II are designed as multi-disciplinary packages centering around clinical problems. The organ-systems are divided into 12 blocks:

Gastrointestinal
Cardiovascular
Renal
Respiratory
Communicable Disease
Psychiatry & Skin
Musculoskeletal
Reproductive
Hemaopoietic
CNS
Head & Neck
Endocrine

Each block lasts for 5 weeks and the students are given a new problem each week. An example of an integration in the Gastrointestinal block is as follows:

ANATOMY INPUT

Anatomy gives input to all blocks. Fixed learning module or FLM, forms an integral part of the problem based approach to learning. Such modules are prepared by staff members, and are displayed in the multi-disciplinary laboratory (MDL) so that the students can study on their own. Each module consists of posters, charts, diagrams, specimens, models, histology slides and X-rays. Each week, a different FLM is displayed. Students are encouraged to study the FLM in groups. It is in the FLM that plastinated specimens play their most important role. They are left in the MDL for students to study at their leisure as these specimens do not need to be kept in formalin as wet specimens.

The department of anatomy at University Science started to produce plastinated specimens in 1990. Due to lack of experience, funds and raw materials, only the silicone-impregnated specimens are produced. To date, there are over 100 specimens in the department, ranging from single organ to the whole thorax or limbs.

The practical examination in Phase I and II are in the form of objective structured practical examination (OSPE) or objective structured clinical examination (OSCE). There are usually at least 20 stations in each examination; each lasting for 3-5 minutes. A student will have to go through all stations, answering the questions based on the specimen or material placed at each station. Plastinated specimens are most suitable for such an examination as there is no formalin smell and being dry, there is no need for hand washing or wiping. Plastinated specimens are also used in postgraduate surgical examination.

THE SURVEY

In order to help plan the availability of learning materials in the department, a survey was conducted among 189 Yr.II and Yr.III medical undergraduates who were exposed to all

types of specimens or learning materials in the teaching of anatomy for over two years. The specimens are pots, which are prosected specimens in sealed glass jars; wet specimens, which are specimens soaked in formalin; plastinated specimens; and plastic models. They are asked to give scores on five properties of the specimens namely: handleability, realistic quality, information, suitablility for practical examination and the condition of the specimens. The responses are recorded as favorable or unfavorable.

RESULTS

Results revealed that in terms of handleability, plastinated specimens scored the highest, being the most favorable. Wet specimens are considered to be more realistic and more informative than plastinated ones. For examination, both plastinated and wet specimens are preferred. The wet specimens are considered not to be in good condition unlike the other specimens. This means that both plastinated and wet specimens have their own roles in anatomy. Models will continue to be used to complement the biological materials as these models demonstrate certain structures more clearly.

DISCUSSION

There is no doubt that students prefer to handle plastinated specimens, rather than wet specimens which are unpleasant to the nose and fingers. Pots and models are also dry, but one cannot touch the potted specimens, while models are not realistic. In terms of being informative, wet specimens score the highest, the reason being such specimens are softer than plastinated specimens, and thus one can study the deeper tissue with more ease. Wet specimens are also considered to be more realistic as structures can easily be differentiated between each other as each one has different texture, feeling, and color. Plastinated specimens are preferred for practical examination possibly because students do not want to get their hands dirty during such stressful exercise. However, wet specimens are also favored for examinations. This is possibly because the students can easily manipulate the specimens in order to identify the structures more systematically, for example to follow the course of a nerve deep to the muscles and around the bones. For obvious reasons, plastinated specimens remain in good condition, almost like models and pots. In our department, only the good specimens are plastinated while the old, partly damaged ones are kept as wet specimens. The latter are still used in teaching as there is an acute shortage of such specimens. From the survey, it is obvious that students do realize that our wet specimens are in poor conditions.

CONCLUSION

In conclusion, we have seen that in the integrated system of a medical curriculum, plastinated specimens have significant roles in the teaching of gross anatomy. They are favored because of their handleability in the class as well as in examination. As such, specimens remain in good condition for a long time, are material-saving, and cost-effective. Wet specimens still have their place in certain aspects.

ACKNOWLEDGEMENT

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PLASTINATED PATHOLOGY SPECIMENS AT ROOM TEMPERATURE IN THAILAND

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INTRODUCTION

Plastination is a relatively new process (1982) that is now widely used to preserve perishable biological specimens with high water content. In this technique, tissue water and lipid are replaced with curable polymers. The completely impregnated specimen is cured by a gaseous vapor.

Dr. Gunther von Hagens of the University of Heidelberg, Germany, developed the suitable polymers and four variations of plastination techniques based on the same fundamental process providing for difference of specimens, (von Hagens, 1979a, b, 1985/1986, 1987). Silicone impregnated specimens (S-10) are resilient and flexible and are mainly used in teaching purposes. Whole organs, limbs, student prosections and even whole bodies may be plastinated with the silicone impregnation method. Specimens produced with polymerized emulsions (P.E.M.) are as opaque as the silicone specimens but are rigid and to some extent breakable. The use of this technique is in production of thick body slices exhibiting a sharp contrast between fat tissue, which shows up white, and all other more intensively stained parenchymas. Transparent body or organ slices

are produced with epoxy resins. This process is known as E-12. For research purposes, these 2.5 mm. to 4.5 mm. thin slices which allow macroscopic study of the topography of all body structures with the naked eye in an uncollapsed and non-dislocated state. In addition, the specimens are useful in advanced training programs in sectional topography (resident training in CT and NMR). As with specimens produced with polymerized emulsions, the eopxy embeded specimens are cured with heat. Opaque brain slices (P-35 process) of 4 mm. thickness, are impregnated with polyester resin, and allow a unique discrimination between fiber and nuclear areas as a direct result of contrast properties within the resin activated by curing the specimen by U.V.A. light sources.

The general procedure of Standard silicone technique S-10 (von Hagens, 1985/1986) has 4 steps.

1. Fixation by using formalin in concentrations between 5 to 20%. It takes about one week in this step. The lower the percentage of formalin the better the color retention within the tissue specimen.

2. Dehydration by freeze-substitution in acetone (-25ϒC) until water content is below 1%. It takes from 1-5 weeks.

3. Impregnation with Biodur* S10/S3 in vacuum at -25ϒC for 10 days to 5 weeks.

4. Remove from silicone polymer bath, drain, wipe off and harden by gas vapour curing process. It takes about 3 weeks. The total time required is about 12 weeks.

In Thailand, because of the limited availability of some equipment, especially the large deep freezer (-25ϒC), we try to modify the standard silicone technique to allow all steps to be carried out at room temperature (average room temperature is about +25 to +30ϒC).

MATERIALS

We use surgical specimens that are sent to the department of pathology for pathological diagnosis. The specimens are fixed in 10% buffered formalin at room temperature.

Polymer (S-10), hardener (S-3) and gas cure (S-6) are required. They are only available from Biodur (Heidelberg, F.R.G.) Ethanol and acetone are also required.

METHODS

FIXATION

The pathology specimens have been collected and fixed in 10% buffered formalin at room temperature for at least 1 week. In this step, care is taken that the specimens maintain their natural shape, or the shape they will exhibit when finished.

DEHYDRATION AND DEGREASING

We use stepwise dehydration in increasing graded ethanol (50%, 60%, 70%, 80%, 90% and 100%, one week for each concentration), and then 2 baths of acetone for complete dehydration and degreasing. The specimens remain in each acetone bath for about 2 weeks at room temperature or until water content is below 1%.

FORCED IMPREGNATION

This is the most important step in plastination, in which the intermediate solvent (acetone) is replaced by curable polymers by means of a vacuum during forced impregnation. The curable polymer is composed of a silicone base material (S-10) and hardener (S-3). We use 10Kg. of S-10 mixed with 0.1 L. of S-3. Many kinds of tissue with varing thickness from 0.5-10 cm. can be done at the same time. It takes about 2 weeks at room terperature.

GAS CURING

The specimens are removed from the mixture of curable polymers and then wiped of excess polymers. The specimens remain in pre-cure at room temperature for about 1 week to allow the excess polymer to drain off. After that, the specimens are cured by a silicate containing gas, evaporating from a fluid (S-6) for about 2 weeks. This step ends when the specimens become solid and dry to the touch.

The total time required is approximately 16 weeks.

RESULT

This technique provides satisfactory results with minimum equipment, because all steps can be done at room temperature. This technique can be used in all laboratories without a large deep freezer (-25ϒC).

The resulting plastinated specimens are dry to touch, odorless, durable, lifelike and non-toxic. They do not deteriorate with time, are maintainance free and resistant to damage caused by handling. They maintain their original shape and are close in color and consistency (figure 1 and 2). They can be stored at room temperature indefinitely. They are useful in the teaching of pathology, especially when used in small groups and are suitable for exhibition purposes as museum specimens.

DISCUSSION

The specimens preserved by plastination techniques are superior to those preserved in formalin. In this method we modify the standard silicone technique so that all steps can be done at room temperature. In the step of fixation we use surgical specimens that are sent to the department of pathology for pathological diagnosis. As the specimens can be used in the plastination process only after complete diagnosis, fixation in 10% buffered formalin at room temperature for at least 1 week is neccessary. This makes the tissue firm, and reduces susceptibility to shrinkage in the subsequent plastination process. However, little change of color can be observed. This problem can be solved by using the Kayserling-Fixation method or staining with BIODUR STAIN C (von Hagens 1985/1986).

Dehydration in graded ethanol causes considerable shrinkage and is time consuming (Schwab and von Hagens, 1981;

von Hagens, 1987) This problem is reduced by using the specimens fixed in 10% buffered formalin for a long time and not over dehydrated. The specimens which have high fat content, such as breast tissue, still shows shrinkage.

Following dehydration and degreasing, the cellular spaces within the specimens originally filled with water and lipids are now occupied by a volatile intermediary solvent (acetone). In forced impregnation the intermediary solvent is replaced by curable polymers by means of a vacuum. The intermediary solvent has a high vapor pressure and a low boiling point (acetone: +56ϒC), while the polymer solution has a low vapor pressure and a high boiling point. Therefore, when a vacuum is applied, only the intermediary solvent is continuously extracted gradually out of the specimens and through the surrounding polymer solution in the form of gaseous bubbles. (von Hagens, 1987). It takes about 2 weeks to complete forced impregnation. Various types of tissue with varying thicknesses (0.5-10 cm) can be done at the same time. The residual polymer in the force impregnation bath can be reused. In this experiment the step of forced impregnation is carried out at room temperature so the viscosity of the polymer increases. This problem is solved by mixing the curable polymers with acetone before reuse for a new batch of specimens.

Following forced impregnation, the specimens are cured by a silicate containing gas, evaproated from a fluid S-6. The hardener S-3 contained in the impregnation bath initiates the curing of the silicone molecules by end to end polymerization. Due to crosslinking during the final gas curing, the silicone rubber within the specimen will become solid and dry (von Hagens, 1987). This step ends when the specimens become dry to the touch. The degree of flexibility of the finished specimen is dependent on the ratio of S-3 hardner to the number of days of the S-6 gas curing.

This technique provides both a satisfactory result with minimum equipment and can be done in all laboratories without a large deep freezer (-25ϒC).

SUMMARY

The modified standard silicone technique (S-10) has been used to plastinate our pathology specimens for teaching and museum exhibition pruposes. All steps have been carried out at room temperature in Thailand where the average temperature is about +25ϒC to +30ϒC. The specimens have been collected, fixed in 10% buffered formalin for at least 1 week and dehydrated in increasing grades of ethanol (50%, 60%. 70%. 80%, 90% and 100%), with one week in each concentration, and then 2 baths of acetone for complete dehydration and degreasing. The specimens remain in each acetone bath for about 2 weeks until water content is below 1%. Forced impregnation and gas curing have also been done at room temperature. It takes about 2 weeks for forced impregnations, 1 week for pre-cure and 2 weeks for gas curing. The total time required is about 16 weeks.

The resulting plastinated specimens are dry to the touch, odorless, durable, life-like and non-toxic. They do not deteriorate with time, are maintainance free, and resistant to damage from mechanical strain caused by handling. The finished specimens maintain their original shape and are close in color and consistency. They can be stored at room temperature indefinitely so they are suitable for exhibition pruposes. They are useful in the teaching of pathology especially when used in small groups and self directed learning. This technique provides satisfactory results with a minimum of equipment.

KEY WORDS: Plastination, standard silicone technique, room temperature, preservation of biological specimens.

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LEGENDS

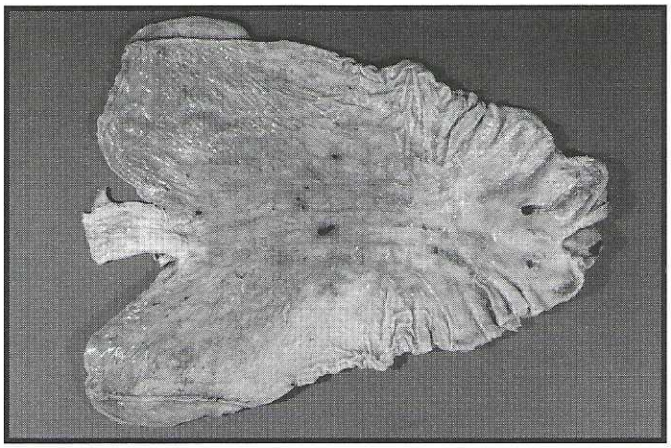

Figure 1 Plastinated specimen of stomach showing stress ulcer.

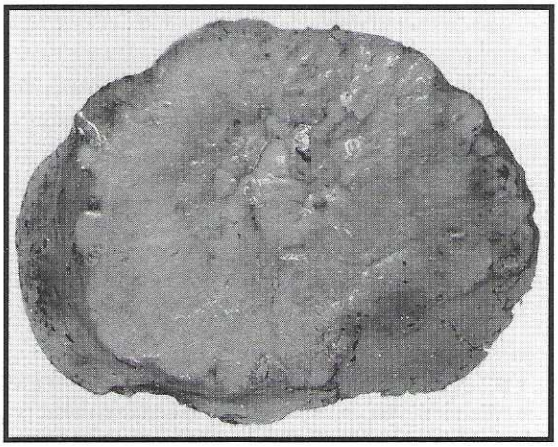

Figure 2 Plastinated specimen of liver showing cholangiocarcinoma mass.

THE USE OF NATRON IN HUMAN MUMMIFICATION: A MODERN EXPERIMENT

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Ever since Herodotus first described the Egyptian use of natron in human mummification, questions have been raised about the exact method of use. To our knowledge, no researcher in modern times has attempted to answer these questions by replicating an Egyptian mummification using natron and a human cadaver. On May 21, 1994 we began such a procedure and concluded it 35 days later on June 25, 1994. We were hoping to gain knowledge in three areas relevant to mummification: (1) Tools used by ancient Egyptian embalmers. Replicas of copper, bronze, and obsidian tools were fabricated and used in the procedure. In addition, copies of ancient storage jars, canopic jars, mummification board, etc., were made and used throughout the experiment. Details of these findings will be discussed in a subsequent paper. (2) Removal of the brain and internal organs. The brain was removed via the nose and internal organs through an incision in the left abdomen. Details of this procedure will be presented in a separate paper. (3) The use of natron in human tissue preservation. This paper will deal with this aspect.

INTRODUCTION

Natron is a naturally occurring compound of sodium carbonate and sodium bicarbonate that exists in several regions of the world.[3,4,5] As it occurs in Egypt, it always contains salt (sodium chloride) and sodium sulphate as impurities. Because the salt content is often high, there has been some confusion, even in ancient times between salt and natron. The ancient Egyptian name for the Wadi Natron, one of the two primary sources of natron for Egyptian embalmers, indicates this confusion. In the ancient Egyptian literary work "The Eloquent Peasant" dating from approximately 2000 B.C., the chief character is described as, "A peasant from the Wadi Natron." The term for Wadi Natron literally means "salt fields", so at least in the toponym, salt and natron are associated. This salt/natron confusion is compounded by the fact that several researchers at the beginning of this century concluded that the ancient embalmers used salt and not natron for mummification. Sir Armand Ruffer suggested that the main desiccant in mummification was salt[6] and in their landmark work, Egyptian Mummies, Smith and Dawson state that, "—at most periods common salt (mixed with certain natural impurities) was the essential preservation agent employed by the Egyptians for embalming."[7] The conclusion that it was salt and not natron that was used for embalming was based on early chemical analysis of mummies on which traces of salt were found. Lucas has conclusively demonstrated that the amount of salt is not sufficient to establish that pure salt was used in embalming, and that the traces could have derived from salt impurities in natron. He concluded, "There is no evidence that salt, either solid or in solution was used in embalming until early Christian times...."[8]

In support of the position that it was natron and not salt that was used by the embalmers, it should be noted that embalmers' refuse inevitably contains natron and not salt. Unfortunately one of the discoveres of two such caches, H.E. Winlock, uses the words "salt" and "natron" interchangeably, both when referring to his finds",[10] and to those of Theodore Davis.[11] Other excavators such as Quibell are more precise and made clear that it is natron that they have discovered.[12]

The ancient Egyptians certainly distinguished between salt and natron. In "The Eloquent Peasant", when the goods the peasant is taking to sell are enumerated we have hm3t (salt) and hsmn (natron). Thus, they were distinct commodities to the Egyptians.

Also in favor of the theory that it was natron and not salt that was used in embalming is the fact that natron had a religious and ritualistic use of natron, but not salt. This is indicated by another of its written forms: hsmn. Here the pouch shows how the natron was kept and the banner, indicating a god's emblem, its divine nature. This aspect is clearest in natron's simplest written form, from which derives the Greek and the English nitre. Similarly, from Arabic comes our "natron". In all these uses and occurrences, the word

denotes something other than salt.

The purely religious use of natron is well attested, both from texts and excavations. In Tutankhamen's tomb, natron was placed at locations where it had no mundane purpose. For example, in two dishes resting on alabaster stands in front of the king's canopic chest.13

Aside from its religious function, natron probably had medical uses as well. The Edwin Smith Surgical Papyrus suggests that ntryt be bound to an abscess of the breast14, and this may well be natron. Ntryt is also mentioned in Papyrus Ebers where it is listed as part of the physicians' pharmacopeia. (48,16; 48,18; 79,1) Among its better established uses is its role in the manufacture of glassis but by far its most discussed use is in mummification.

The earliest mention of natron in connection with mummification is Herodotus' famous description of Egyptian embalmingie. Here he discusses the three types of mummification and mentions natron twice. In one place he says, making it clear that it is natron that removes the flesh. In the other place where "natron" appears, Herodotus uses it to modify the verb which means to preserve like fish. Here he says, "with natron", both Egyptian and Greeks of the period salted their fish so Herodotus had to make clear that it was not salt that was used. The use of the verb has caused considerable confusion and discussion as to how the natron was used. It has been translated as "soaked, bathed, and steeped" all of which seem imprecise in that they require a solution and this is not necessarily how fish was preserved. Unfortunately, in one of the seminal works on mummies, Thomas Pettigrew quotes Laurnet's translation of Herodotus, where "steep" and "lay in brine" are used.17 Thus throughout his work, Pettigrew assumes a bath of natron was used. A century later Dawson continued this error and stated that the procedure was to wash the body "....and then to immerse the corpse in the salt-bath."15

There are several compelling arguments against natron having been used in solution. First, if the purpose is to dehydrate the body, as surely the egyptians realized, then it is both counter-intuitive and counterproductive to immerse it in a bath. Second, Herodotus states that the body cavity was packed with crushed spices and then placed in natron. The spices would have washed out since the incisions were generally not sewn closed. Third, to submerge the mummies of Egypt in a solution of natron would have taken hundreds if not thousands of large vats. If this were the case, surely some, or at least fragments of some with traces of natron would have been found. We must note that Dawson gives as evidence a pottery figure of a person in a jarw and says that it is a mummy in natron solution. We do not find this convincing.

Given all of the above, we find it reasonable to conclude that ancient Egyptian embalmers used natron not salt and that they used it in its natural form rather than in solution. With this premise, we proceeded with our experiment.

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ABSTRACT
"PLASTINATION AS A CLINICALLY BASED TEACHING AID AT THE UNIVERSITY OF AUCKLAND"

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As a concerted move toward closer integration of the clinical and pre-clinical aspects of the undergraduate medical curriculum at the University of Auckland, the Department of Anatomy has established a formal link with the Department of Radiology resulting in a structured program of clinically based teaching of gross anatomy to second and third year medical students.

As sophisticated diagnostic techniques and methods of treatment have become common place, our teaching program has been tailored to accommodate a greater degree of case based learning within the undergraduate course.

Dissecting room demonstration is provided by Radiology, Pathology, Ophthalmology and Surgical registrars, with a number of clinical procedures, pathological observations, and diagnostic methods employed during the routine dissection of the cadaver.

Through use of a number of plastination techniques, the learning process is enhanced and aided on a number of levels. The E-12 epoxy method for producing M.R.I, and C.T. based serial sectioned cadaver specimens has allowed an accurate and highly detailed orientation of the planes of the body and provides the student with a clearer understanding of anatomical structure and pathological anomalies as seen with modern imaging techniques.

COMPARATIVE STUDY OF NMR-CAT IMAGES WITHIN SERIES PLASTINATED SECTIONS

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The complexity of the results from CAT and NMR examinations very often require the aid of normal morphology support to get to certain and undoubted diagnosis. For this purpose, the study and preservation of "slices" from the head of a corpse, after a precise radiography diagram was made.

MATERIAL AND METHODS

In this study, fixed corpses were used. The corpse was frozen at -25°C in order to allow head resection along a transverse-cervic-thoracic plane. After carefully studying the radiography the following points were found: repere, glabella and opisthocranium, necessary to carry out the first transverse section. Other sections, parallel to the first one were then cut. Each "slice", to either cranial or caudal direction, and was 1 cm thick. After cutting the frozen specimens by means of a circular band saw, the sections were treated to remove any residual organic substance on the cut surface. The sections were then set on blotting-paper to absorb defrosted liquid. A soaked pad with hydrogen peroxide (H202) was used to liquify the coagulant on the structures. A solution of hydrogen peroxide was also injected into the blood vessels to remove further obstructions. After this treatment, perfectly cleaned sections, from an anatomical (no residual organic substance) and chromatic viewpoint, were obtained, and the surfaces were photographed with a professional camera (LINHOF). At the end of this phase, each section was set on a suitable metallic support to maintain its morphology.

A cylindrical lattice, 50 cm. high was built. Some transverse planes, made up of metal grids, were attached to a steel wire. The planes were 2 cm. apart from each other and were covered by blotting-paper. The sections were at a distance of 4-5 cm. from one another in order to be thoroughly immersed into the substances used in the next phases. Dehydration was by means of acetone at -25YC. The sections were immersed in that solution for 24 hours. The control of the percentage of acetone in the solution follows. If the value of acetone is less than 98%, it will be replaced with new absolute acetone (99%).

The controls were done every 24 hours until acetone remains unchanged (i.e.,not absolute acetone) to avoid section shrinking. After this dehydration, forced impregnation with S-10 resin and S-3 hardener start. The lattice was immersed in the resin in the vacuum room and the pump was brought into action. After 5-10 minutes, the resin gradually begins to replace acetone. As this happens, bubbles moving toward the surface of the substance can be observed through the glass sealing the room.

The pump operator should ensure that a constant amount of bubbles be present without pressure changes. In fact, a large quantity of bubbles means that forced impregnation of the sections is too quick. This causes a quantitative change between the amount of acetone going out and the resin replacing it, which causes a shrinking of the organs. Impregnation at room temperature lasts seven days and finishes whenever pressure at 25 mbar doesn't produce any more bubbles. The sections displayed on the lattice grids are taken out of the vacuum to be drained of resin excess present on the surface. Then, the last phase, the cure, begins. For this purpose a slow cure was used. The sections were arranged in two layers in a vessel at a distance of 10 cm. from each other. A small vessel, filled with KOH (Potassium hydroxide), was set on each layer. The potassium hydroxide absorbs humidity therefore catalyzing the hardening reaction of resin. Each day the sections dried because of continuous secretion of small amounts of resin. After one week, when the sections were dry, this phase was complete.

SUMMARY

The interpretation of CAT and NMR images is sometimes very difficult and requires the support of normal morphology to reach the formulation of certain unequivocal diagnosis. Following a careful radiological indication we performed a study of the conservation of cadaver's serial sections of some human body regions. The cadaver, frozen in a refrigerator at -25YC was cut in serial cross sections, by a circular belt disk saw, after indicating points on the anatomic specimen. The sections obtained, with a thickness of 0,5-1 cm., after cleaning were mounted on a metal grid. Next, they were subjected to the standard plastination (BIODUR S-10) The careful study of obtained sections and the correspondent NMR-CAT images, provided the direction of the cross-sectional images and the anatomical cuts coincide, and the anatomical support can be clearly useful for the diagnosis.

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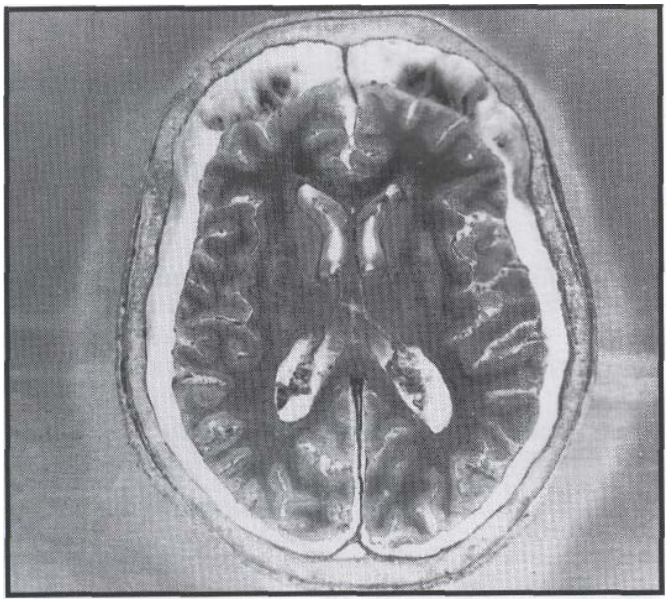


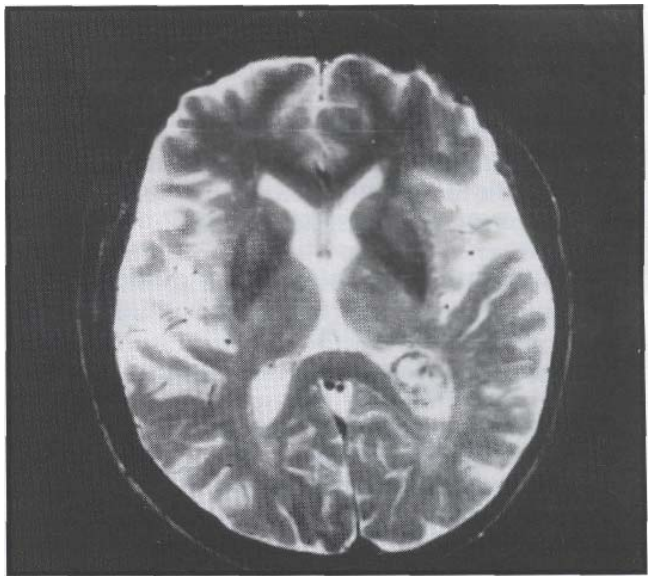
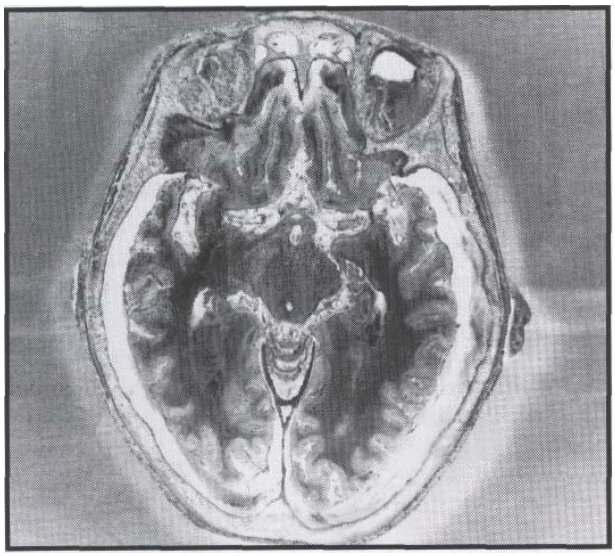


Fig. 1 - Meningioma of chorioid plexus ventriculi lateralis



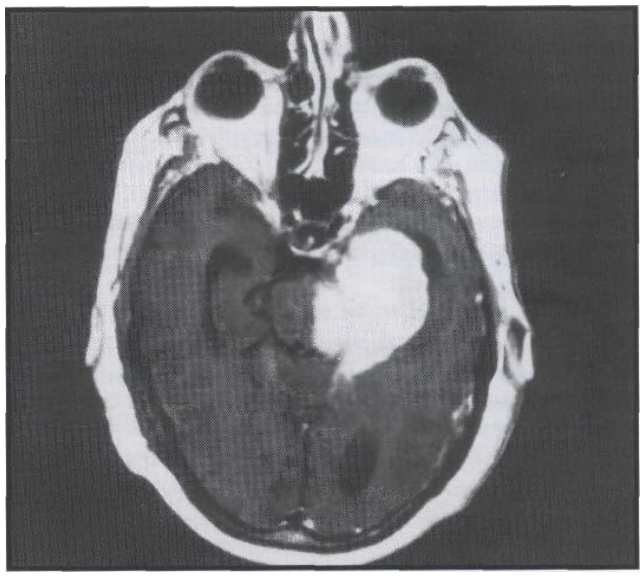
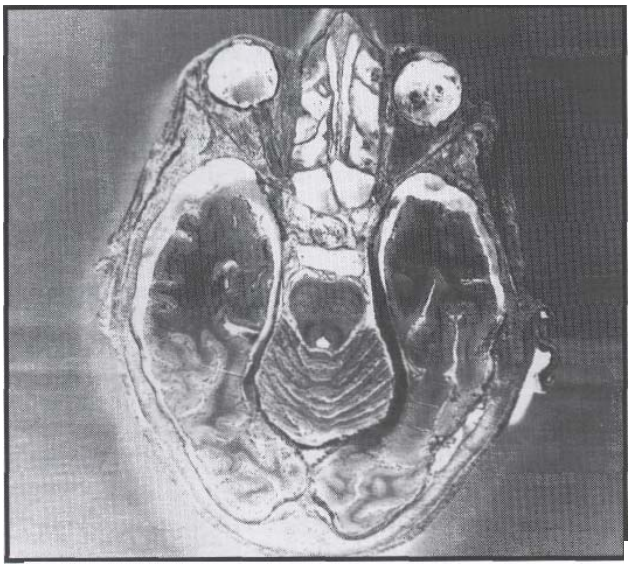


Fig. 2 - AVM (arteriovenosus malformation) on the mesial surface of temporal lobe



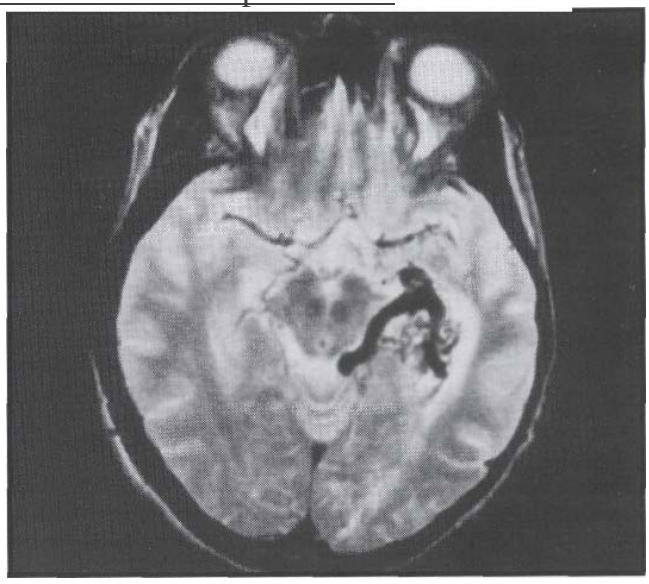


Fig. 3 - Sphenotentorial meningioma: compression of temporal lobe and cerebral peduncle

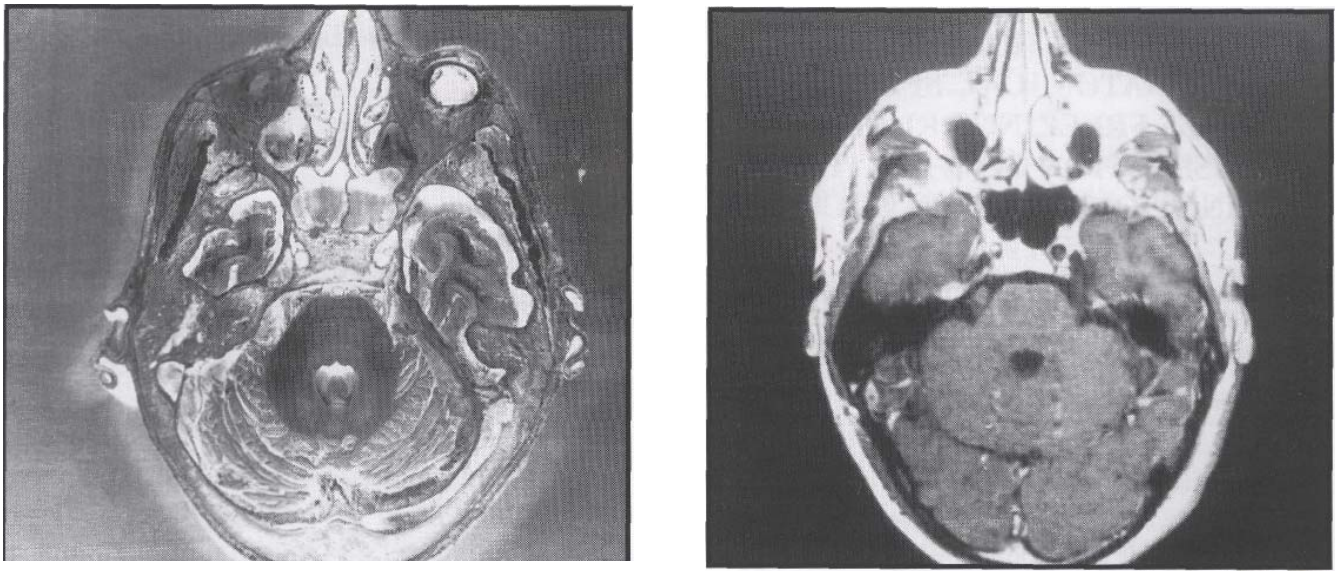


Fig. 4 - Schwannoma in relation to the fifth cranial nerve

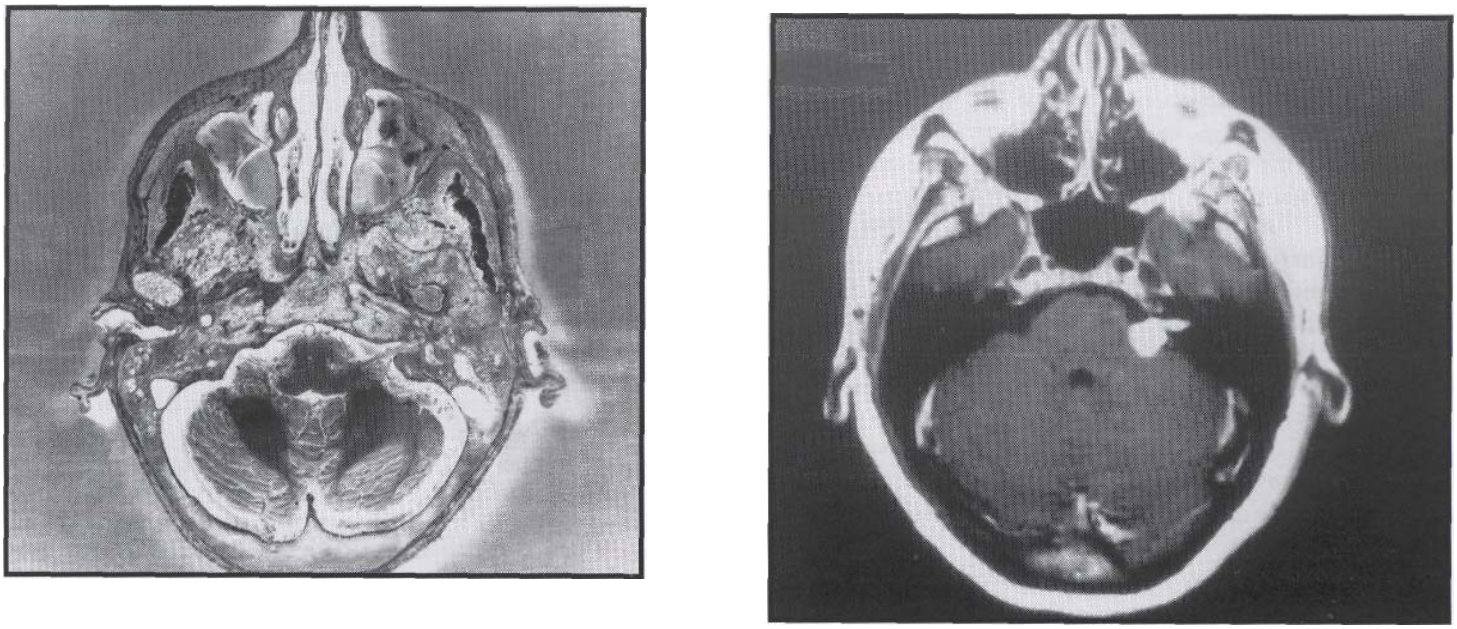


Fig. 5 - Schwannoma

PLASTINATION OF THE NEUROANATOMICAL SPECIMENS: DOES FREEZING PRIOR TO DISSECTION GIVE BETTER DISTINCTION BETWEEN NUCLEI & FIBRE TRACTS?

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ABSTRACT

With the recent decline in the number of brains available for dissection, the use of pre-dissected, plastinated specimens has become increasingly important in the teaching of neuroanatomy. For the specimens to be of the most value, however, it must be possible to distinguish nuclei from fibre tracts. Since freezing of brain tissue prior to dissection generally increases the distinction between gray and white matter in unplastinated specimens,~the following study was carried out to see if the freezing method resulted in better differentiation between nuclei and fibre tracts in plastinated specimens.

Brains previously fixed in 10% formaldehyde were washed overnight in cold, running water, then placed in a plastic bucket containing fresh 10% formaldehyde, and stored in a deep freeze, at -25YC, for 8 days. The brains were then thawed under cold, running water, for 24 hours. The freezing and thawing procedure was repeated twice more before dissection was performed. These dissected specimens, along with those of fixed brains that had not been previously frozen, were then plastinated together, using the standard S-10 method.

It was found that freezing the brains made the dissection of both nuclei and fibre tracts easier. However, the cortex of the "frozen" brains was more fragile and , therefore, more prone to damage during dissection. Nuclei and fibre tracts were equally distinctive in both "frozen" and "unfrozen" brains, suggesting that, for the purposes of plastination, freezing the brains does not enhance the distinction between nuclei and fibre tracts.

INTRODUCTION

The number of donated brains available for the teaching of neuroanatomy has decreased significantly in recent years. Consequently, it has become increasingly important to produce dissected specimens, that are long-lasting and durable. This goal can be achieved by plastination. To be of optimal value, however, the specimens must be of the highest quality. In dissected specimens, this means that there should be good definition of gray and white matter, particularly fibre tracts running through the white matter. In this respect, it has been found that the distinction between gray and white matter in "wet" specimens can be enhanced by freezing the brain prior to dissection (Gluhbegovic & Williams, 1980). The present study was carried out to see if the freezing method produced similar, advantageous results in plastinated specimens.

METHODS

Control Specimens

Brains which had been well fixed in 10% formalin were washed in cold, running water for 24 hours, then dissected, using straight or curved fine forceps and micro-dissecting knives, to show features such as subcortical fibre tracts and basal ganglia.

Experimental Specimens

The methods used in preparing the experimental specimens were adapted from Gluhbegovic & Williams (1980). Following washing in cold running water, as above, fixed brains were:

1. Placed in a plastic bucket containing 10% formalin and stored in a deep freeze at -25YC for 8 days.
2. Thawed under running cold water for 24 hours.

Steps 1 & 2 were repeated three more times before dissections, similar to those performed in control specimens, were carried out.

Control and experimental specimens were plastinated together, using the standard S-10 method described by von Hagens (1986).

RESULTS AND DISCUSSION

Freezing and thawing was found to facilitate dissection in the experimental specimens, and distinction between grey and white matter was just as good in control specimens as in experimental specimens. Two specimens from the experimental group are shown in Figure 1A and Figure IB. Features that can be identified include the caudate nucleus, internal capsule and parts of the pyramidal system. Two specimens from the control group are shown in Figure 1C and Figure ID. They also show features of the caudate nucleus, internal capsule and parts of the pyramidal system. The fibre tracts running through the white matter are equally visible in control and experimental specimens. The distinction between grey and white matter is also similar in the two groups. Note that the cerebral cortex in some areas of the experimental brains is damaged. This may well be due to the rapid temperature change during the specimen preparation. The composition of the grey matter (cell bodies) and white matter (myelinated axons) are quite different. Protein is an essential part of the cell body while the myelinated axons are enclosed in the myelin sheath of which lipid is the main component. The temperature change resulting from freezing and thawing may cause the breakdown of the protein, with little or no effect on lipids, resulting in damage to cortical areas (grey matter), during the handling of the experimental specimens, as shown in Figure IB.

The results of this study suggest that the extra time and effort involved in freezing and thawing specimens prior to dissection is not warranted, since the same quality of fibre and

grey/white matter distinction can be achieved using brain specimens which have been fixed and washed at room temperature.

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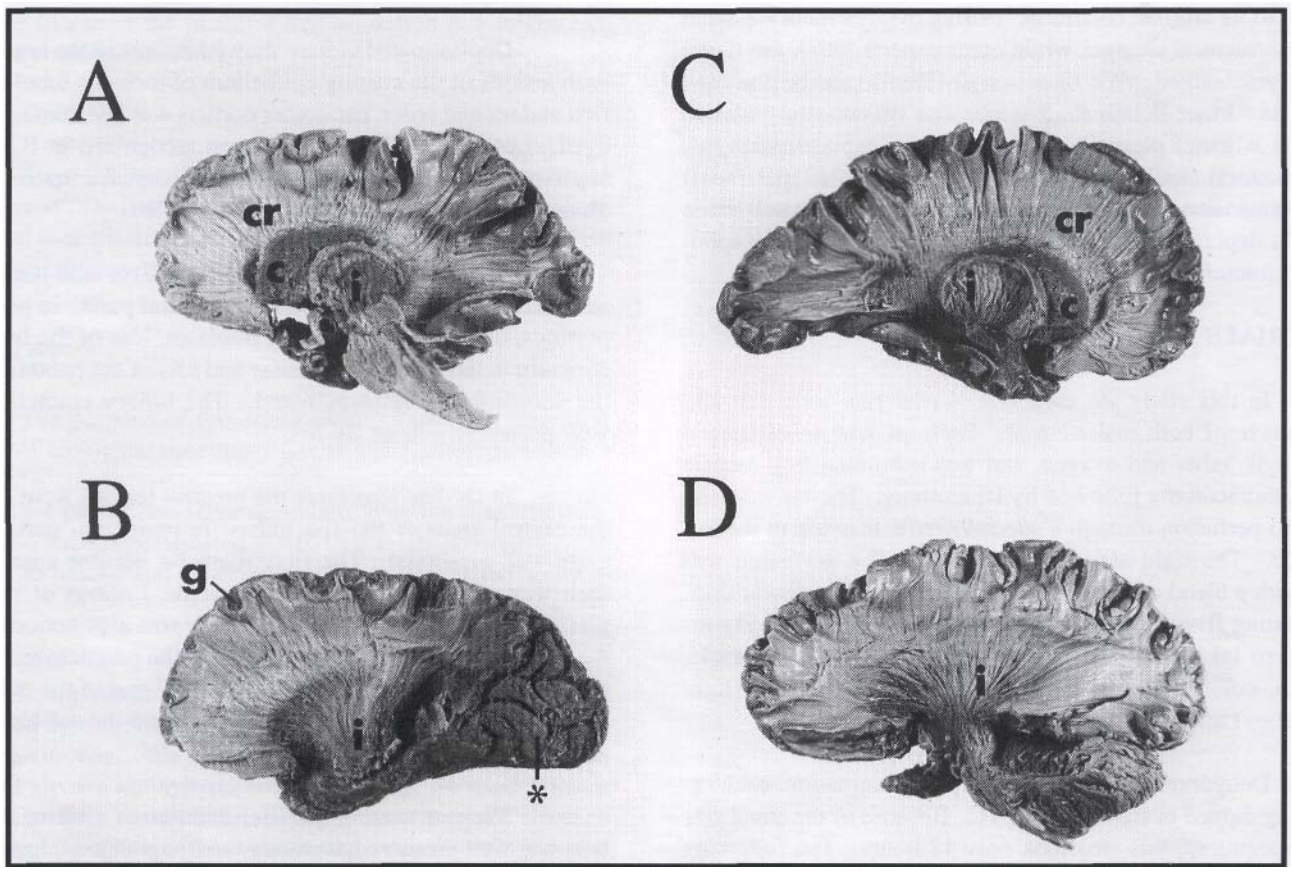

Fig.l

Examples of dissected specimens from the experimental (frozen) group (A and B) and control group (C and D). Features that can be identified include the caudate nucleus, internal capsule and pyramidal system. The fibre tracts running through the white matter are equally visible in control and experimental specimens. The distinction between grey and white matter is also similar in the two groups. Damaged cerebral cortex in one of the experimental brains is marked with an asterisk. Abbreviations: c: caudate nucleus; cr: corona radiata; g: cortical grey matter; i: internal capsule.

LIGHT MICROSCOPY OF PLASTINATED TISSUE. CAN PLASTINATED ORGANS BE CONSIDERED VIABLE FOR STRUCTURAL OBSERVATION?

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INTRODUCTION

Plastination is a technique that allows preservation of anatomical and surgical specimens for a long time without surface morphological modification. It can be effectively used for teaching Macroscopic Anatomy, and is also useful for the radiologist's understanding of the TAC-RMN images. After experience with this technique over several years, we often wondered what the results would be of histological studies of plastinated samples. We found different options in the literature. Some experts think that tissue, once plastinated, cannot be restored to its original condition. During the treatment it passed through structural changes while other experts, think the tissue remains unchanged. We therefore decided to study plastined organs as further detailed. The purpose of our study was to ascertain whether plastination, followed by deplastination, affects structural modifications on biologically treated specimens. At the same time, we tried to locate the most suitable substance to use in deplastination, granting the alteration of the morphological structures of these organs.

MATERIALS AND METHODS

In this study we used five Wistar rats weighing 250 grams each, of both male/female. Each rat was anaesthetized with ethylic ether and oxygen, and was submitted to a median sagittal thoracotomy followed by laparotomy. The rat was submitted to perfusion through a special needle inserted in the left ventricle. The right atrium was opened. The perfusion was made with a blend of heparin and 10% of formalin slowly dripping (lasting five minutes). Then, samples of the following organs were taken: lung, heart, liver, spleen, kidney, muscle, stomach, colon. They were precessed both for L. M. (light microscopy) and plastination.

Dehydration was the first step of plastination: each organ being dipped in acetone at -25YC. Because of the small size of the specimens, this step took only 48 hours. The following step was the so-called forced impregnation in a solution made up of resin and hardener (Biodur S-10 + Hardener S-3). In this phase, the escape of a small amount of bubbles from the surface of the specimens was noticed, probably due to the small dimension of the specimens. Forced impregnation at room temperature lasted 72 hours. Finally, the cure was done slowly using KOH over one week. The specimens were then submitted to the process of deplastination.

For this purpose, it was necessary to to identify the most effective substance to be used as a resin solvent. The following substances are more commonly used: alcohol, methylbenzene, methylene, bichloride acetone. Eight plastinated specimens of human ureter were used in order to carry out our research; they were submitted to transverse dissection; each specimen was 0.5 cm thick. Each sample was immersed into the various substances for the deplastination. The immersions lasted from 24 to 168 hours. From the tests, we found that the best method to obtain good results in the phase of deplastination, was first to immerse the specimens in 99% alcohol for 24 hours, and afterwards in methylbenzene for 48 hours. It is necessary to remove all the resin from the specimens before proceeding to the next phase.

The deplastinated specimens were processed for L.M. through the standard technique, i.e. using hematoxyilin and eosin stain. Then the specimens were observed with the light microscope.

RESULTS

Through L.M. exam of both deplastinated and control specimens, different findings were made for each organ.

Deplastinated kidney showed lesions in the renal tubule with lesions of the coating epithelium of tortuous tubules of the first and second order.The cortex corticis was less seriously damaged. Lesions of the gomeruli were recognized in Bowman's capsule, as well as enlargement of the capsular space. Other glomerli were well preserved (Photo la-lb).

Microscopic examinations of the liver and hepatocytes showed extensive vacuolation in the central parts. In peripheral portions, disappearance of the cytoplasm, loss of the trabecular structure in the Kieman's laminae and loss of the endothelium of the centrilobular veinwas noted. The biliary epithelium was well preserved (Photo 2a-2b).

In cardiac structures the greatest lesions were found in the central areas of the specimens; in peripheral parts, it was quite well preserved. The fibre diameter became smaller, and their threadlike structure was damaged. Lesions of the cytoplasm of the common myocardial cells was also noticed.

In the spleen, a vacuolation of the parenchyma cells of the white and red pulp, (only of the central part of the pulp), was observed. The stroma connective tissue and the red blood cells were well preserved.

Skeletal muscle, parallel bundles of multinucleate fibers was well preserved; however the fibers showed loss of their original volume as well as segmentation and fragmentation, in the central portion. The cytoplasm was hyperchromatic, and the nucleus showed an accumulation of chromatin.

The lung was less severely altered than other organs. A cell coarctation and a loss of volume of the epithelium of the bronchi was observed. In addition, the chondrocytes in the cartilage of the coating plates of some bronchial branches showed vaculoation. (Photo 3-a-3B).

The structure of the entire stomach wall was well preserved. Volume and stain changed, in the underlying layers of the lamina propria and in the inner connective tissue of the

mucous membrane. The structure of the colon was well preserved (Photo 4a-4b).

Changes of volume (coarctation) in the cells of the loose connective tissue, of lymphoid tissue and of the cells of the epithelium infiltration were found.

From the comparisons among deplastinated and control specimens studied at the light microscope, we found out that plastinated organs maintain their seperate histological and morphological characteristics. However, we have found some changes, in the structure of the fragments central zones, mainly in the epithelial and lymphoid tissues. In the specimens of deplastinated organs, these tissues presented some vacuolation and regressive changes. In other words, all the cells with changes of their small living matter during the process of plastination are those mostly damaged. We also noticed that deplastinated specimens were resistant to paraffin infiltration and we believe this is due to the induced changes on the organic structure by dehydration and by forced impregnation of biological samples, as stated in international literature. Finally, we concluded that the best substance to use in the phase of deplastination is a mixture of alcohol and methylbenzene.

CONCLUSIONS

From the results, we can confirm that plastination and successive deplastination modify the structure of the treated organs; and that plastination of a sample doesn't prevent other histological studies, within the above mentioned limits and interpretative caution.

SUMMARY

The purpose of this study was to verify the structural changes of biological specimens due to plastination technique.

We plastinated some specimens with the standard technique, and then deplastinated diem. Then we processed them for L.M. technique and observed them to assess the tissue integrity.

First of all, it was necessary to isolate the best deplastinating substance. For this study we immersed eight plastinated specimens into the most frequently used substances for deplastination. We chose the best solution to remove the resin and alcohol methylbenzene. For study, we used Winstar rat organs: lung, heart, liver spleen, kidney, muscle, stomach and colon. Each organ was associated with its own control. Rat control organs were immediately processed for L.M. The other specimens, were plastinated with the standard technique of plastination, (S-10).

Finally, the plastinated specimens were immersed in the deplastination solution to remove the resin. The deplastinated organs were processed for L.M. microscopic study and showed the substantial preservation of organ structures. Nevertheless, changes in the structure of lymphatic and epithelial tissues were visible. From the results we can answer the basic question of our research: does plastination and its successive deplastination slightly modify the structure of the treated tissues.

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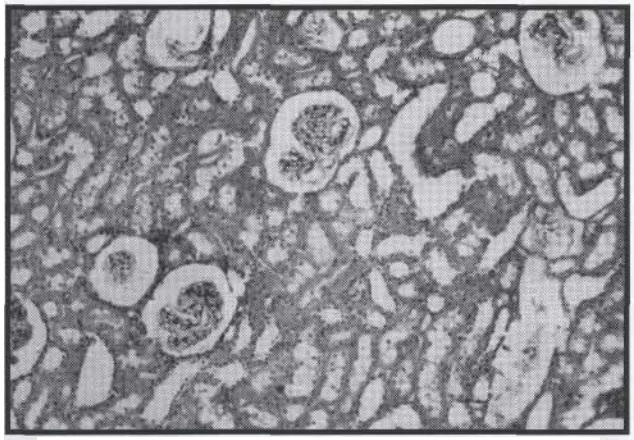

Photo la - Kidney: Control Specimen (L.M. 200X)

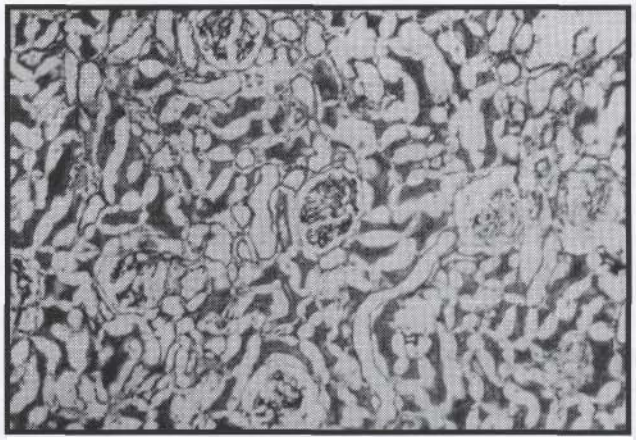

Photo Ib - Kidney: Deplastinated Specimen (L.M. 200X)

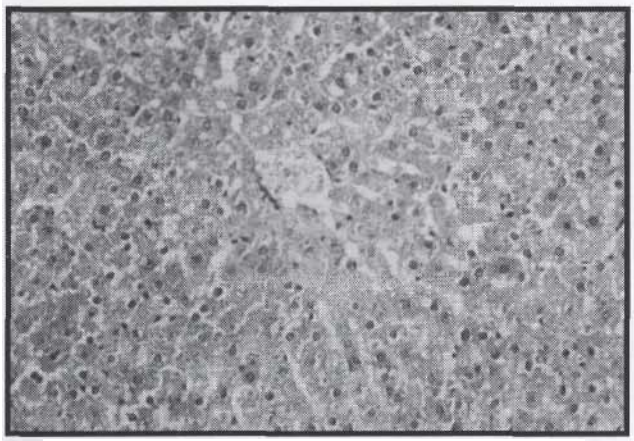

Photo 2a - Liver: Control Specimen (L.M. 200X)

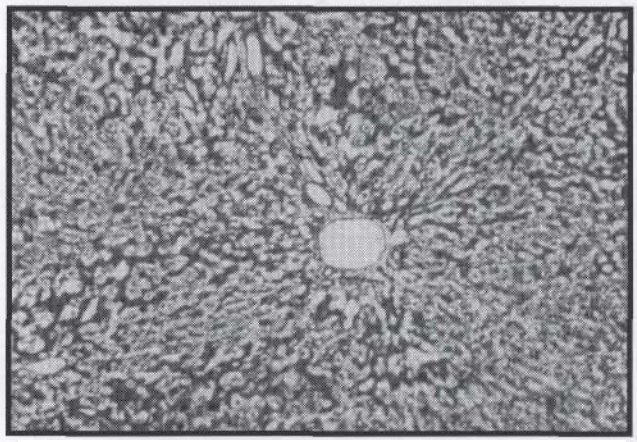

Photo 2a - Liver: Deplastinated Specimen (L.M. 200X)

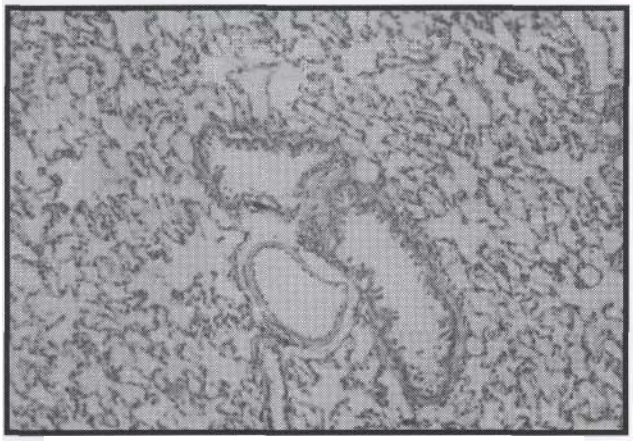

Photo 3a - Lung: Control Specimen (L.M. 200X)

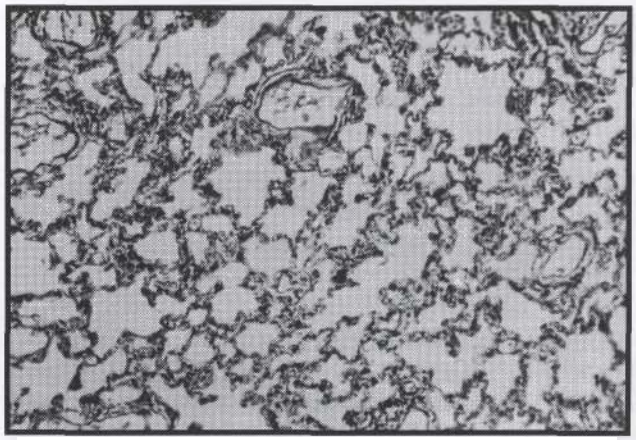

Photo 3a - Lung: Deplastinated Specimen (L.M. 200X)

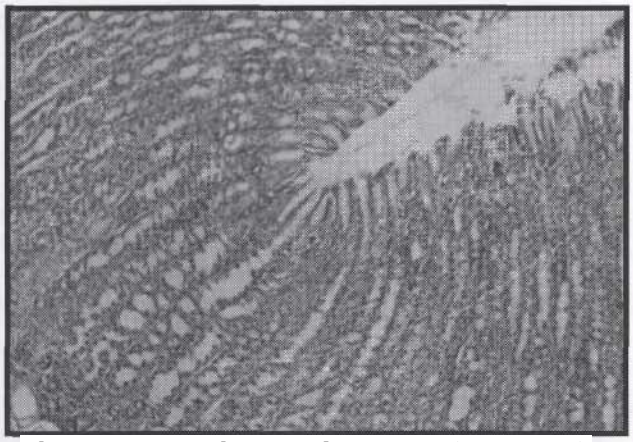

Photo 4a - Stomach: Control Specimen (L.M. 200X)

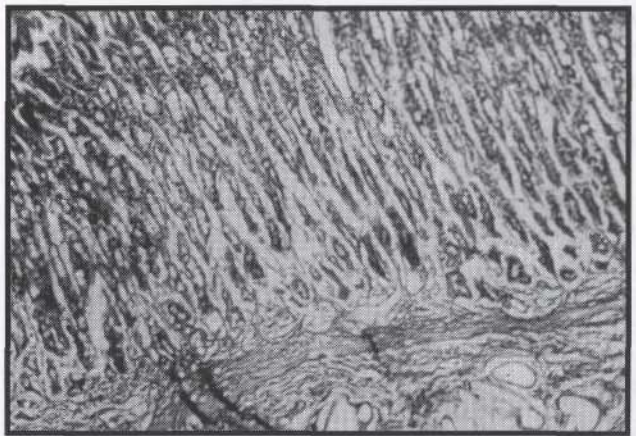

Photo 4a - Stomach: Deplastinated Specimen (L.M. 200X)

PLASTINATION: TECHNICAL ADVICE IN THE PHASE OF FORCED IMPREGNATION

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INTRODUCTION

A special technique of preservation of biologic specimens called plastination has been used over several years at the Institute of Human Anatomy of the University "La Sapienza" of Rome.

This technique, discovered by Prof. Gunther von Hagens, consists of four phases: fixation, dehydration, forced impregnation and curing.

The stages of fixation and cure are always done at room temperature, while dehydration is at either room temperature using ethyl alcohol, or by freezing specimens (at -20TC), using acetone (dehydration freeze substitution with acetone). Forced impregnation, a technique at room temperature was used. Although subjected to the following technical problem: when acetone obtained from the anatomical specimens set in a vacuum-room (fig.l) reaches the lift pump and mixes with lubricating oil, changes its physical characteristics, causing structural damages to the mechanical elements of the pump. We eliminated the problem by means of a simple technical device called LIQUIFIED NITROGEN TRAP described as follows.

MATERIALS AND METHODS

Materials used: Thermically isolated cylindrical pierced lid (h=20cm; d=15cm). Thermical isolated cylindrical cover, abridgment (h=3cm; d=15cm). Graduated beuta (h=18cm; ability=500ml;base diameter=10cm).Double exit glass link. Rubber, elastic muff, Liquified nitrogen.

GENERAL SYSTEM

PREPARATION OF TRAP:

We interrupted the hydraulic circuit between the vacuum and the lift pump by interposing a double exit glass link, connected to a graduated beuta and a plastic muff, ensuring a perfect seal between the two faces.

Liquified nitrogen was poured into the thermically isolated cylindrical container filling two thirds of the container. The beuta, now inserted in the circuit, was placed in the thermic container. The latter was closed by a pierced lid then thermically isolated (the hole allows the linkage between the beuta and the hydraulic circuit).

This system permits keeping only the graduated beuta in the small refrigerated cell. After about twenty-four hours the liquified nitrogen must be changed.

HOW THE TRAP WORKS:

The device, made up of the glass link and the graduated beuta, and connected in series to the circuit, was stored in the refrigerated cell; the acetone, drawn out of the vacuum chamber by the low (below the earth's atmosphere) pressure created by the pump, is brought into the beuta, then into an isolated system, whose inside temperature is noticeably lower than the surrounding temperature.

This process doesn't prevent the pump from continuing to create the negative pressure in the vacuum chamber, due to the presence of the double exit link.

CONCLUSION

The main advantage from using this technique lies in the possibility to that the process of forced impregnation at room temperature does not damage the pump. A further advantage is the possibility of withdrawing and measuring the acetone obtained from the plastinated specimens whenever the liquified nitrogen is replaced. The forced impregnation procedure can be completely controlled.

SUMMARY

The Plastination laboratory at the Institute Human Anatomy of State University of Rome "La Sapienza" has developed a technical device which can be used during the phase of forced impregnation at room temperature. The device is very useful to the technique of plastination at room temperature. Thanks to this device, the mechanical structures involved in the phase of forced impregnation do not show morphological alteration of specimens.

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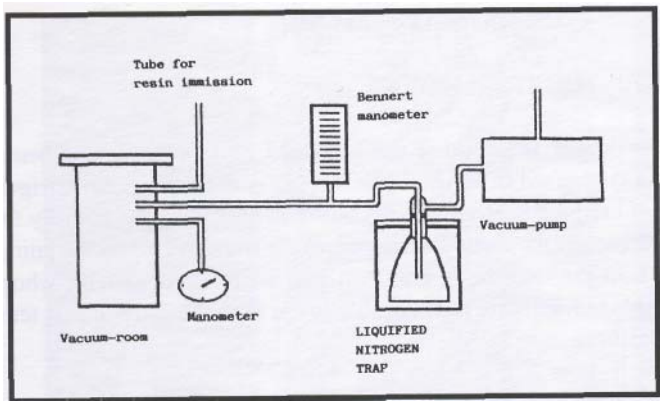
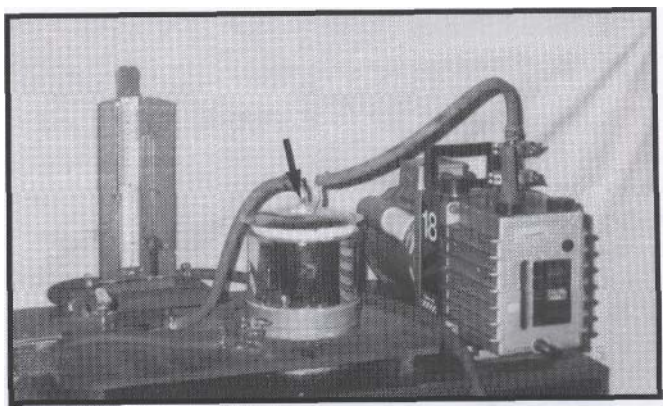


Fig. 1- Scheme of system in the phase of forced impregnation.

Photo 1- Vacuum-pump and Liquified Nitrogen Trap. The outside of duble exit glass link is visible.



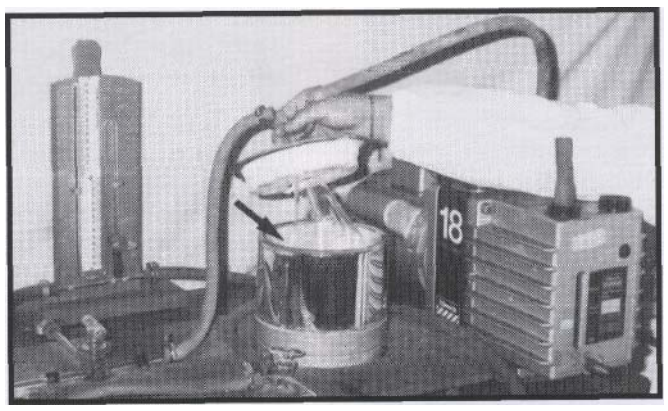


Photo 2- Inside the Liquified Nitrogen Trap. Note the gratuaded beuta and the double exit glass link

PLASTINATION AT ROOM TEMPERATURE

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INTRODUCTION

In the standard plastination procedures, originally developed and described by von Hagens (see von Hagens et al., 1987), the dehydration of specimens is normally achieved by freeze substitution in acetone at -25°C; forced impregnation is also carried out at this same, low temperature usually in a deep freezer. Since 1993 in Iceland and 1995 in Hong Kong we have been able to develop procedures which successfully allow these steps to be carried out at room temperature (15-20°C); the results of which are high quality gross anatomical specimens prepared in this way.

MATERIALS AND METHODS

We perform stepwise dehydration at room temperature in a graded series of acetone solutions of increasing concentration from 70% to 100%. After fixation in 10% formalin or a formalin/phenol/alcohol-based embalming fluid the removal of formalin and other compounds is effected by immersion in running tap water for 2-3 days. The specimens are then transferred to a 70% solution of acetone. Once the acetone level is stable, the specimens are removed to the next higher concentration of acetone solution. In this manner, the specimens are gradually brought to the 100% acetone bath when they are ready for polymer impregnation. The volume ratio between the specimens and the acetone solutions ratio is maintained at about 1:10. We have found that at room temperature, dehydration is complete for most specimens in about 3-5 weeks, which is long enough for the specimens to be completely dehydrated and degreased. This is markedly shorter than the time needed for dehydration at low temperature and there is no danger of ice-crystal formation within cells and tissues.

After the completion of dehydration and degreasing, specimens are submerged in a polymer mix of resin S10/S3 at room temperature for 3-5 days which allows the acetone to escape and the specimens to equilibrate with the polymer mix. After this initial period of equilibration, the specimens are transferred to a vacuum chamber (designed and built locally to our own specifications) and the pressure decreased slowly over a period of 3-4 weeks. The vacuum is monitored by a manometer, vacuum gauge, and the progress of impregnation is checked by observing the evolution of acetone gas bubbles from the surface of specimens. The acetone gas bubbles should rise slowly to the surface of the polymer mix.

Each working day, the vacuum is re-established and slowly decreased. At the end of the working day the vacuum is released and the chamber opened to allow the specimens to be moved around to relax them and facilitate further equilibration with the polymer mix. Over time, the pressure is gradually lowered to about 1-3 mm Hg (0.13-0.40 kPa) and the vacuum maintained for 3-4 more days until no more acetone gas bubbles appear, which indicates that the polymer mix retains a much lower viscosity than at -25YC, permitting faster penetration, and acetone gas bubbles can escape more easily.

DISCUSSION

Omitting the requirement for substitution and impregnation at low temperature reduces capital costs and reduces the likelihood of explosion should acetone vapor be generated in an enclosed space in a freezer which is not designed for complete spark-proof operation. More than 300 large and small anatomical specimens have been plastinated in this way and they have remained in good condition and with stable color for up to three years. In our experience the shrinkage of specimens has been less than 5%.

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SUMMARY

Our current practice in plastination techniques at room temperature is described. The practice is inherently safer than the traditional method and produces durable specimens with stable color, the shrinkage of which has been less than 5%.

ACKNOWLEDGMENTS

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PLASTINATION - A TEACHING AND RESEARCH TOOL

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Plastination is a unique technique of tissue preservation developed by Dr. Gunther von Hagens in Heidelberg, Germany in 1978. In this process, water and lipids in biological tissues are replaced by curable polymers (silicone, epoxy, polyester) which are subsequently hardened, resulting in dry, odorless and durable specimens. Silicone is used for whole specimens and thick body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices. Polyester is used for brain slices to gain an excellent distinction of gray and white matter.

The technique consists of four main steps: 1. Fixation 2. Dehydration 3. Forced Impregnation and 4. Curing/Hardening. Fixation can be done by almost all conventional fixatives. Dehydration is achieved mainly by acetone because acetone also serves as the intermediary solvent during impregnation. Forced impregnation is the central step in plastination: vacuum forces the acetone out of and the polymer into the specimen. Finally the impregnated specimen is hardened by exposing it to a gaseous hardener (silicone), or by UVA-light and heat (polyester, epoxy).

Plastinated specimens are perfect for teaching, particularly for neuroanatomy. Silicone plastinated brains are useful because they can be grasped literally and they are almost everlasting. Polyester plastination of brain slices provides an excellent distinction of gray and white matter and thus better orientation.

The plastination techniques for brain (polyester) and body slices (epoxy) are also used in research, particularly in comparison with CT-and MRI-images.

PLASTINATION OF BRAIN SLICES

Brain slices may be produced by both the S-10 standard plastination technique and the P-35 technique.

The S-10 standard technique is mainly used for plastinating whole brains. These plastinated brains can be sliced after final curing. This results in smooth surfaces of the slices and thus slices are exactly adjacent to each other. Moreover, these slices show good differentiation between gray and white matter, due to freezing and thawing during the S-10 standard procedure. This technique allows the production of brain slices from 0.5 mm up to several centimeters. Therefore slicing S-10 plastinated brains is much better than plastinating pre-sliced brain slices.

For the S-10 technique I recommend adding the following steps to the standard procedure. Before starting the forced impregnation start with an immersion period. During this immersion-step the brains are immersed in the S10/S3 mixture for several days at -20YC. The longer the immersion time, the shorter the impregnation time will be. Moreover, this will also minimize the shrinkage of the brains. After curing is completed, the brains are cut into slices of desired thickness.

P-35 plastinated brain slices provide an excellent tool for teaching and research, because the differentiation between gray and white matter is superior to all other techniques. The thickness of the P-35 slices can vary between 4-8 mm.

The P-35 technique consists of the following steps: Fixation - Slicing - Flushing - Dehydration - 1. Immersion - 2. Immersion - Forced impregnation - Casting - Light Curing - Heat Curing. Light curing may be omitted if light-curing equipment is lacking.

PLASTINATION OF THE BRAIN: DOES FREEZING PRIOR TO DISSECTION PROVIDE BETTER DISTINCTION BETWEEN NUCLEI AND FIBRE TRACTS?

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With the recent decline in the number of brains available for dissection, the use of pre-dissected, plastinated specimens has become increasingly important in the teaching of neuroanatomy. For the specimens to be of the most value, however, it must be possible to distinguish nuclei from fibre tracts. Since freeing of brain tissue prior to dissection generally increases the distinction between gray and white matter in unplastinated specimens, the following study was carried out to see if the freezing method resulted in better differentiation between nuclei and fibre tracts in plastinated specimens.

Brains previously fixed in 10% formaldehyde were washed overnight in cold, running water, then placed in a plastic bucket containing fresh 10% formaldehyde, and stored in a deep freeze, at -25YC, for 8 days. The brains were then thawed under cold, running water, for 24 hours. The freezing and thawing procedure was repeated twice more before dissection was performed. These dissected specimens, along with those of fixed ,brains that had not been previously frozen, were then plastinated together, using the standard S-10 method.

It was found that freezing the brains made the dissection of both nuclei and fibre tracts easier. However, the cortex of the "frozen" brains was more fragile and , therefore, more prone to damage during dissection. Nuclei and fibre tracts were equally distinctive in both "frozen" and "unfrozen" brains, suggesting that, for the purposes of plastination, freezing the brains does not enhance the distinction between nuclei and fibre tracts.

FIXATION RECIPES FOR THE PLASTINATOR

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The technique of plastination has proven to be a very forgiving process. Variances in technique can be instituted to fit one's equipment capabilities, time restraints, and even the occasional oversight. However, one thing plastination does not do is to miraculously improve the appearance of a fixed specimen. The time spent in preparation of a specimen, in both the detail of the prosection and the type of prefixation, greatly determines its esthetics value. With embalmed specimens one is limited to solutions that are efficacious enough to retard autolysis and mold throughout the study. Although Biodur FX-10, FX-20, and ethylene glycol have demonstrated very good color and tissue preservation as an embalming solution, the cost for these solutions is high. A greater degree of flexibility in preservative is available however with fresh specimens. Although the mechanics of the plastination process does not definitively demand that a specimen be fixed, time is often needed to provide the perfect anatomical prosection. Fixation qualities of 10 solutions were evaluated for short term preservation of fresh specimens. Considerations used in their evaluations were: hazardous material designation, cost, color preservation, texture maintenance, and overall fixation quality. The highest quality specimens were produced when fixation time was limited to 48 hours, followed by submersion in water until ready for dehydration. Shorter exposures times did not provide adequate fixation while extended times invoked severe color loss. The acetone and alcohol solutions failed to merit the classification of a preservative due to tissue degradation within 2 days after a 48 hour fixation. The environmentally safe fixatives, Streck Tissue Fixative (STF) and Histochoice, although suitable for fixation duration and texture maintenance, rated low in color preservation. The modified lore's solution provided the best color preservation while maintaining a reasonable degree of texture. However, for those specimens which require a more rigid fixation (Hollow organs) a 2 percent formalin solution would be preferred over the Jore's.

BI-PHASIC ACETONE RECLAMATION

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Through a bi-phasic program of reclamation, the collection of large volumes of acetone can be both simple and inexpensive. Phase I is a process of freeze vacuum distillation which reclaims 96-98% acetone from the waste acetone (contaminated to < 65% by water and fats) produced during specimen dehydration. Volatization is induced by the application of vacuum to acetone which has been heated by a warm water bath. Condensation is similarly enhanced by the cooling of the acetone vapor in the freezer and hence the liquid collection. Phase II is the reclamation of acetone that is being extracted from specimens during the impregnation stage of plastination. This volatized acetone is typically discharged into the laboratory atmosphere with the vacuum pump's exhaust. By directing the vacuum line to a collecting canister (at room temperature during lower levels of vacuum and in a freezer at higher vacuum levels), 1 1/2-4 liters of 99% acetone are reclaimed per 25 kilograms of tissue being plastinated. Operating expenses are reduced by both minimizing the production of hazardous wastes and the recycling of such an integral component of the plastination process.

COMPARISON OF BODY TISSUE LAYERS OF PLASTINATED SECTIONS WITH BODY TISSUE LAYERS OF MAGNETIC RESONANCE SCANS

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A steady growth and development in computer imaging modalities require a methodology for teaching, research and interpretation of cadaver sections and computer imaging scans. This paper adds to the guidelines already established for the

teaching of anatomy to correlate with the sectional scans depicted by computer imaging modalities. Enlarged (close up) photos were taken of sheet plastinated and in some cases S-10 sections of human cadavers to represent each class of layers.

The purpose of this paper is to explain and demonstrate four classes of body tissue layers as one guideline used for the teaching, research and interpretation of sectional anatomy. In order to do this the layers of plastinated sections are compared to similar layers of magnetic resonance images. Each of the four classes of tissue layers (Lane '95) are matched. That is, the somatic tissue layers of the plastinated section is compared to the somatic tissue layers of a similar MRI scan. Likewise, the extravisceral, intravisceral luminar and intravisceral nonluminar classes of tissue layers of plastinated sections are correlated with similar tissue layers of the MRI scans.

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