journal of the

INTERNATIONAL SOCIETY for PLASTINATION

JULY 26 - 31, 1992
6th INTERNATIONAL CON.
KINGSTON, ONTARIO, CANADA

August 5 - 7, 1993
3rd Biennial Conference
Mobile, Alabama, USA

Volume 6, No. 1
Summer 1992

JOURNAL of the
INTERNATIONAL SOCIETY for PLASTINATION
Official Publication of the International Society for Plastination

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After months of planning the Second Biennial Conference on Plastination was held at Chaffey College in Rancho Cucamonga, California on August 7, 8, and 9, 1991. More than 70 people attended the three day conference, of which nine were international attendees. Three from Australia, two from Canada, one each from Mexico, Spain, India and Sweden. The attendees covered a wide range of Plastinators, from neophytes to experts alike.

The first day was dedicated to the history, theory and basics of Plastination, as well as, practical information of setting up a lab safely and efficiently, with the emphasis on cost effectiveness. First day speakers were Dr. Harmon Bickley, Mercer University; Mr. Bill Collins, University of California, San Diego; Dr. Robert Henry, University of Tennessee, Knoxville. The afternoon of the first day was originally planned to include closed circuit television demonstrations of the basic techniques, but due to the large number of newcomers to the Plastination field, this was set aside to handle the large amount of questions that were generated.

The second day of the conference loosened up a bit as the attendees and speakers became more accustomed to their surroundings. Dr. Henry presented his technique of "Forced Air Impregnation of Air Dried Lungs". Following a short break, Wolfgang Weber presented a detailed and in-depth discussion on "Sheet Plastination". The afternoon was dedicated to the presentation of abstracts. Presenters were: Dan Whitten and Marc Stamer: "Storage of a Plastinated Cross-Sectioned Cadaver"; Gilles Grondin: "Setting up a Plastination Lab on an Extremely Limited Budget"; Dr. Alex Lane: "Using Sheet Plastinated Sections to Teach Cross Sectional Anatomy"; Dr. S. J. Nagalotimath presented slides of his medical teaching library at the BLDEA's School of Medicine in Bijapur, India. The remainder of the afternoon was devoted to hands on demonstrations of the many aspects of Plastination, Fixation, Dehydration, Color injection, Impregnation, and Curing.

The third day started off with a breakfast sponsored by B/R Instrument Company and was followed by a presentation of an "Acetone Distillation System", manufactured and distributed by the same company. Round Table discussions followed and continued well into lunch. The afternoon was spent with Wolfgang Weber demonstrating "Sheet Plastination Techniques" and allowed all interested a chance to get their hands "gooey".

The attendees all gave a positive feedback and many said that this was the first conference they attended that they had the chance to actually practice the techniques.

For future Conference Planners. Give serious consideration to planning a scientific conference, as "Murphy and his laws" will be sure to participate. Keep a tight reign on your budget, make sure that the people that seem so happy to help you plan in the beginning, will continue when the deadlines start to arrive, and try to think of every thing that will be needed. But first and foremost, don't get into a panic like I did. People associated with Plastination are a fun-loving group of people and most of the schedule changes and other things that don't go as you planned will more than likely be laughed off. Beware of partying with Plastinators, premature fixation of the brain can occur!

For those of you that are wondering where the video of techniques is, don't despair. I am no longer at Chaffey, but will be starting at the University of Southern California, School of Dentistry soon. Hopefully, once I am situated there, I will contact the participants and make arrangements for you to get a copy.

2nd Biennial Interim Conference on Plastination, Chaffey College, 1991.

TO: Jim Johnson
FROM: All Who Attended

THANK YOU!!

It was great. The program, accommodations, and setting were perfect!! All the hard work was appreciated.

Plastination laboratory designs and safety considerations. William R. Collins, UCSD School of Medicine, University of California, La Jolla, CA 92093, USA. PLANNING A PLASTINATION LABORATORY:

A. Budget considerations:
  1. Initial expense:
    a. Basic lab (minimum investment),
    b. Well-equipped lab (standard start-up costs).
  2. Ongoing Expense:
    a. Equipment maintenance and repair,
    b. Personnel,
    c. Supplies, chemicals.

B. Space requirements:
  1. Minimum floor space for complete lab,
  2. Average floor space for well-equipped lab,
  3. Special considerations and requirements:
    a. Adequate ventilation,
    b. Running water,
    c. Adequate lighting,
    d. Emergency power back-up,
    e. Floor plan.

C. Equipment requirements:
  1. A basic lab (minimum needed to get started).
  2. A well-equipped lab.
  3. Dealing with fresh material.

GETTING STARTED:

A. Licenses:
  1. Plastination licensing (Dr. von Hagens):
  a. Educational specimens,
  b. Commercial products.

B. Selecting equipment:
  1. Saving money:
    a. Borrow equipment,
    b. Buy surplus equipment:
      - Campus or State.
    c. Buy used equipment.
  2. Choosing vendors:
    a. Big name vs. small suppliers.
    b. Vendor contracts through your institution?

SAFETY CONSIDERATIONS:

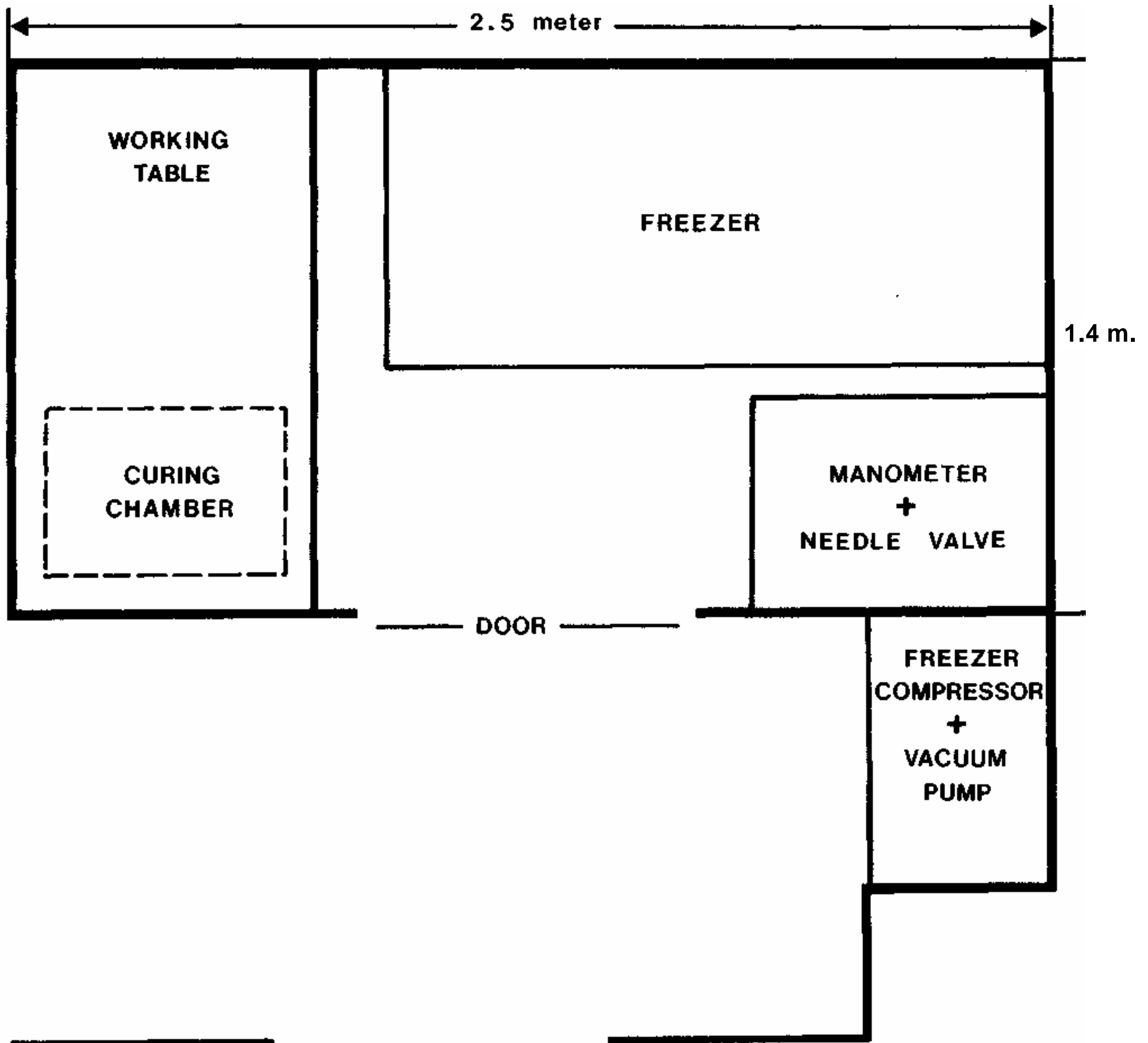
A. VENTILATION! VENTILATION!

B. Hazardous materials:
  1. Special storage requirements:
    a. Flammable storage cabinet.
  2. Special disposal requirements.
  3. Protective gear for personnel:
    - Face shields & Ear plugs.
  4. Testing personnel for chemical exposure:
    a. Regulations vary by state.
  5. Special handling procedures: a. Remote freezer motors. C. Handling fresh material:
    1. Specimen testing:
      - HIV & Hepatitis B.
    2. Vaccinating lab personnel for Hepatitis B.
    3. Standard protective wear.

Starting plastination on a limited budget. Gilles G. Grondin, D6partement of Biologie, Facult6 des Sciences, University de Sherbrooke, Sherbrooke, QUEBEC, CANADA, J1K2R1.

After three and one half years of effort, a plastination laboratory was started for less than $3,000.00 (Canadian $) in Sherbrooke. The pre-plastination years were used to identify and to collect various pieces of surplus equipment which could be used for plastination (old vacuum pump, glass desiccator, tubing and connections, needle valves from a scrapped freeze-dryer) and to harass instructors and administrators concerning finances and laboratory space. The resultant laboratory space was 3.5 square meters (2.5 m x 1.4 m). A glass ceiling was placed below the light fixtures to seal the lights from the room environment thus making the area explosion proof. Ventilation was installed and electrical outlets removed at a cost of $360.00. A household deep freezer was purchased and the thermostat and compressor were removed and installed outside of the plastination room, cost $870.00. A Bennert-type manometer, gas mask and hydrometer were purchased, cost $700.00. Finally, after purchasing 40 liters of acetone and 10 kg of Biodur S 10 silicone resin (cost $900.00), we were ready to commence. Dehydration was carried out in old 4 liter jugs which were covered with glass. A glass desiccator served as a plastination kettle and the curing chamber was a plastic bag inside an old aquarium. We were able to accommodate specimens about the size of a hand, but even with these size restrictions plastination began. After instructors had seen and touched the plastinated specimens, they realized their value and wanted more and larger specimens. At this point it was much easier to get money for a larger vacuum chamber and to discuss the possibility of moving into a larger laboratory. We realized that starting plastination at Sherbrooke might be a long term adventure, but believed that our goal was possible even on a limited budget if we were persistent and were willing to recycle equipment. Once plastination is commenced and specimens are

Starting Plastination on a Limited Budget



produced, you can easily demonstrate its worth and improve your laboratory.

"Forced air" impregnation of air-dried lungs - A recent development in Plastination producing life-like lung specimens. Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.

Air-dried lungs at room temperature were impregnated with a mixture of S10/S3 (polymer-mix) and xylene. Compressed air from the laboratory bench was used to propel the polymer-xylene mix from the air ways into the parenchyma of the lung tissue and on through to the surface of the lung. The lungs were dried using air from the laboratory air supply line. Mixtures of xylene and S10/S3, which varied from 15% to 45%, were forced into lungs, to impregnate the lungs with silicone, via forced air rather than using dehydration and vacuum. The trachea was cannulated and the lungs positioned such that one-half of the polymer-xylene mix was poured into the left portion of the airway and the other half of the mix into the right portion of the airway. Laboratory air (dried medical air), at a high velocity and pressure (30 - 50 Ibs/sq. in.), was

blown into the trachea for four to ten days, in an attempt to force the polymer-xylene mix from the air ways into the lung parenchyma and on to the surface of the lung. To assure that the polymer-xylene mix reached all areas of the lungs, the lungs were continually turned during the first 10-20 minutes of impregnation. For the next 4 to 10 days, the lungs were inverted daily to alleviate pooling of the polymer-xylene mix. The trachea and surface of the lungs were coated daily with excess polymer-xylene mix. When air impregnation was completed, S6 was volatilized by bubbling air through the liquid S6 and directed into the trachea for 15 minutes a day for 3 days. A plastic bag was placed around the lung preparation to serve as curing chamber. Air impregnated lungs, if dried in proper anatomical position, maintain their anatomical relationships. Higher percent xylene lungs were more spongy after curing than lower percent xylene lungs as xylene evaporates at room temperature leaving proportionately less polymer in the lungs. Conversely, lower percent xylene lungs were firmer and more durable because more polymer remained in the lungs.

Plastination - Dehydration of specimens. Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.

Specimens to be plastinated are often moist which necessitates the removal of tissue fluid (dehydration) before forced impregnation or plastination can be carried out. Dehydration removes the specimen fluid (water), as well as, some fat. The fluid is replaced with an organic solvent. To be a dehydrating agent, the solvent must be miscible with water and may be of a variety of chemical structures (ketones or alcohols). Either alcohol or cold acetone may be used as a dehydrant for plastination. Methylene chloride (chlorinated hydrocarbons) is not a dehydrating agent. Shrinkage accompanies dehydration and may be minimized by: 1) using cold acetone (known as freeze substitution) or 2) starting dehydration in a lower % of ethanol. With freeze Substitution, the ice in the specimen is replaced by &e dehydrating liquid (acetone). It is essential to use tn adequate volume of dehydrating liquid (either cold acetone or ethanol). The recommended ratio is: 10 Volumes of dehydrating fluid to 1 volume of tissue. It is necessary to monitor the concentration of the Dehydration fluid at weekly intervals. Once the fluid Content has remained similar for a few days, the specimen is moved to a fresh dehydrating solution, Cold ACETONE (-25°C): Usually has been considered the best method of dehydration, However, dehydration with acetone must be carried out in the cold and not at room temperature; warm acetone will cause excessive shrinkage, Disadvantage: Must be done in a deep freezer, Advantages: Minimal shrinkage; Acetone serves as the intermediary solvent; Superior specimens; and Shorter dehydration time. ETHANOL: Specimens are started in a low % of ethanol (50%) at room temperature, allowed to equilibrate and later placed in ascending concentrations of ethanol, i.e.: 60%, 70%, 80%, 90%, 100%. Advantages: Carried out at room temperature; therefore, less deep freezer space is necessary. Specimens can be stored in 70% ethanol. Specimens from embalmed tissues, containing standard embalming fluids, are cleansed of the polyvalent alcohols (glycerin or ethylene glycol) or phenols. Specimens are defatted. Disadvantages: Shrinkage; Intermediary solvent is necessary; Specific gravity of ethanol and alcohol are similar 0.79 making it difficult to determine when the ethanol has been totally replaced with acetone.

Plastination - Forced impregnation. Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.

Forced impregnation is another important step of plastination. Successful impregnation is possible only after complete dehydration and the availability of an intermediary solvent. As various polymers are available and each require a slightly different process, we will discuss impregnation with silicone polymer, The silicone polymer (S10) is mixed with a curing (hardening) agent (S3) which commences the process of end-to-end linkage of the molecules. At room temperature, this linking is hastened, Therefore, the polymer mix may only be utilized for a few weeks at room temperature before the polymer mixture turns into a various blob and a few more weeks into a solid block. For this reason, the silicone mixture is kept at -25°C. This temperature is sufficiently cold to retard the end-to-end linkage of molecules which enhances the flexibility of the final product. Hence, in the cold, the polymer may be used for many years. Once the mixture is prepared (1 part S3 to 100 parts S10), it is placed into a vacuum chamber which has been placed in a deep freezer. The dehydrated specimens are submerged in the polymer. A mesh placed on top of the specimens

will keep the specimens submerged. The specimens are allowed to stand in the polymer mix over night and hence equilibrate with the polymer. The vacuum is increased gradually over a period of 3 - 5 weeks until nearly total vacuum (one atmosphere) has been reached. This gradual increase in vacuum allows the intermediary solvent which has a lower boiling point (acetone: +56°C and methylene chloride: +40°C), than that of the polymer mix, to be extracted from the specimen and evacuated through the exhaust system of the pump. The loss of the intermediary solvent from the tissue results in a pressure difference between the interstitium of the specimens and the polymer mix allowing the polymer to be drawn into the tissue. The extraction of the volatile intermedium (boiling) must be slow enough to allow sufficient time for the polymer to enter the specimen. Extraction of intermedium is monitored by observing bubble formation on the surface of the polymer mix. When boiling is too fast, the polymer does not have enough time to flow to all parts of the specimen. This may allow the structural framework of the tissue to collapse and the specimen may shrink. Vacuum is controlled by an air inlet valve and pumping speed.

Plastination - Gas curing (hardening). Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.

Curing or hardening of the polymer varies for each polymer used. Curing for the standard silicone procedure will be discussed. After impregnation of the specimen with polymer, the polymer must be hardened. Curing takes place on the surface of the specimens first and then progresses to the interior of the specimen. For the S10 process, Biodur Gas Cure (S6) is the hardening agent. S6 begins the process of side-to-side linkage of the polymer molecules. Side-to-side linkage produces a stronger product. Basically, the S6 vaporizes and reacts with the polymer. Two curing procedures have evolved over the years: Slow cure - in which the specimen is allowed to remain at room temperature for several weeks prior to exposure to S6. This enhances more S3 activity and hence more end-to-end linkage and a more flexible specimen. Exposure to heat may also be used with this methodology. The other procedure is: Fast cure - whereby the impregnated specimen is exposed to a concentration of S6. Volatilization may be enhanced by bubbling air through the S6. The environment for the fast cure should be dehumidified via a desiccant (calcium chloride). An increase in humidity may cause white silicate salt precipitations on the surface of the specimen. A hollow organ may have a concentration of volatilized S6 directed into its lumen to assure that the organ remains dilated and hence in a dilated position after the polymer is hardened. Both the slow and fast cure method utilize Biodur S6 and exposure to S6 is carried out in a closed chamber at room temperature. Once the specimen is exposed to S6, the surface of the specimen is hardened after 12 to 36 hours. However, the interior of the specimen will take longer to harden and is dependent on the S6 penetrating to the depths of the specimen. Yet the specimen may be used before curing is completed to its depths. After curing is complete, the specimen can be stored indefinitely at room temperature.

Plastinated specimens, an aid to understanding standard imaging planes for two-dimensional, real-time echo cardiography m the dog. Robert W. Henry and Janice M. Bright, Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901 USA.

Ultrasonography is a valuable method for evaluating structure and function of the heart. Two-dimensional, real-time echocardiography (2DE) provides images in a single plane, and standard imaging planes have been described for evaluation of the canine heart. Single frame images from these standard imaging planes were obtained from real-time, 2DE examination of healthy dogs. The images are presented with plastinated specimens which were sectioned along corresponding imaging planes in order to illustrate three dimensional anatomy relative to the two dimensional sector. In addition, labelled photographs of the sectioned specimens are displayed adjacent to the echocardiographic images. Standard imaging planes are designated and transducer positions, used to obtain standard imaging planes, are described. To enhance the student's ability to interpret standard imaging planes, a cadaver heart was prepared for plastination by separation of the lungs from the heart and transecting the pulmonary vessels as long as possible. The caudal vena cava and a pulmonary vein were cannulated with an appropriate size of tubing and the heart was flushed to remove blood with warm tap water through both cannulas. The other large vessels were closed by ligating a bleached cork in place (larger end into vessel). The remaining smaller vessels were

closed with suture or hemostatic clips. The heart was hydrostatically dilated with cold water, dilated-fixed with a 5% Kaiserling's solution, dehydrated in cold (-25 °C) acetone, and impregnated with Biodur S10-S3 polymer-mixture. Following impregnation, the heart was drained of the excess polymer-mix and exposed to Biodur S6 (gas curing agent). During curing, the heart was manicured daily to remove excess polymer-mix which accumulated on the surface of the specimens. After curing the heart was sectioned along the planes of standard images.

A new perspective jn teaching human anatomy. Alexander Lane, Triton College and University of Illinois at Chicago, Chicago, IL, USA.

Currently, my research involves a comparison between mathematically computed clinical sections of the human body and sheet plastinated cadaver sections. Computers are making a tremendous impact on the teaching of human anatomy and diagnostic medicine. For instance, as a result of the computer imaging devices, more progress has been made in diagnostic medicine in the past 15 years than in the entire previous history of medicine. There are five major computer body scanning systems. This research is confined to three of these modalities. Comparisons are made between scans from ultrasound (sonography), magnetic resonance imaging (MR) and computed tomography (CT). All of these systems present the body in thin slices without surgery for study and diagnosis.

Sheet plastinated cadaver sections are suitable for a comparison with computer scans because of the similarities in thickness. The purpose of this study is to add a new perspective to the usual methods and approaches in teaching Human Anatomy. This new perspective is needed in order to update teaching methodology and meet the need to properly identify and interpret structures seen on modern computer body scans. Similarities and differences are stressed to demonstrate comparison and correlation between cadaver sections and computed clinical images. The cadaver sections provide a standard and a means of demonstrating the limitations of the imaging modalities. Further, the cadaver section provides confirmation that the slice one gets using an imaging device is a true representation of that area of the body.

In this new perspective, structures of each cadaver or clinical section are classified into four anatomical categories (Lane, 1990). This methodology not only enhances learning but also helps to differentiate the quality of the resolution by imaging devices of various anatomical structures. For example, some imaging devices depict the visceral structures with high resolution but musculoskeletai unit structures are shown poorly by the same device.

LANE, Alexander: Sectional anatomy: Standardized methodology, J Int Soc Plastination 4:16-22, 1990.

High purity solvent recycling of acetone in the Plastination laboratory. Roger R. Roark, Jr., V. P., B/R Instrument Corporation, Easton, MD, 21601, USA.

The recovery of high purity acetone from Plastination techniques has become an interesting topic due to strict regulations promulgated by the U. S. Congress and enforced by the U. S. Environmental Protection Agency. Recycling acetone saves the laboratory money by reducing the purchase of the fresh solvent and the disposal cost of the hazardous waste.

For any solvent recycling operation to be successful, the first priority must be solvent quality that is equal to or better than the original purchased. Acetone with large amounts of water and other contaminants are generated in the plastination process. The recycling system must have a distillation system with sufficient theoretical plates to achieve the separation. B/R Instrument has determined that at least ten plates are necessary for a good acetone-water separation. Twenty to thirty theoretical plate spinning band columns have been shown to recycle a 50:50 acetone-water mix to 99% + pure acetone. The formation of para formaldehyde from formalin contamination is a problem. Further research in this area is needed.

Economic and environmental concerns have made high purity solvent recycling and attractive alternative to solvent purchase and disposal. Like other laboratories generating waste, the plastination laboratory must strongly consider this option.

Sheet plastination of the brain, P 35 technique, filling technique. Wolfgang Weber, Department of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011, USA.

Sheet plastinated specimens have the highest acceptance of preserved body slices. Since the development of the P 35 technique, cross sections of

the brain never were as detailed even when stained, never were as durable, and never were as easy to handle. There are two techniques for sheet plastination. Brain sheets are done with a polyester-compound, BIODUR P 35. While, cross sections of the body are done with an epoxy compound, BIODUR E 12. The procedures are similar, but for the sake of clarity, each procedure will be described in a separate article.

LIST OF NECESSARY EQUIPMENT:

A Vacuum chamber; Manometer; Biodur Polymers: P 35 and A 9, Biodur cobalt accelerator (PB 11), Meat slicer; 2 UVA-lamps, 45°C oven. Stainless steel or aluminum grids; Grid basket (HD 09 or HD 10); Square buckets or appropriate containers for storage and dehydration of specimens. To assemble each flat chamber: 2 Tempered glass plates (5mm) (HS 01), 2 Thin glass plates (1-2mm) (HS 03), 6mm Gasket (HS 06), 4 small (HS 32) and 15 large (HS 30) Fold back clamps or 2 inch binder clamps, Plastic sleeve or hose (to serve as a funnel), and Pressure sensitive tape.

FIXATION OF BRAIN: 10-20% formalin solution.

SLICING THE BRAIN: Flush in tap water. Divide brain into two pieces, return one portion to the water and lay a wet filter paper on the cut surface of the other portion to keep the specimen moist and serve as a support for the slice. Meat slicer at 4mm. Place on a stainless steel grid (filter paper side down).

FLUSH THE BRAIN SLICES: 4 hours via tap water.

COOL IN DEIONIZED WATER: Store overnight at (5°C) in the deionized water.

FREEZE SUBSTITUTION: Submerge in - 20°C cold 100% acetone. One acetone bath of adequate fluid/tissue ratio is sufficient for brain slices.

IMMERSION INTO POLYMER: Polymer mixture of P 35 (resin) arid A 9 (hardener), a ratio of 100:2 and keep in the dark. *Immersion 1:* After 48 to 96 hours of freeze substitution, transfer to cold polymer mixture. *Immersion 2:* After 24 hours, transfer specimens into a new polymer mix (100:2) for 24 hours.

IMPREGNATION: New reaction mixture of P 35 (polymer) and A 9 (hardener) (100:2 ratio) and deaerate. Room temperature, in the dark, and for 21 hours. Increase the vacuum hourly and stabilize at 20mm Hg and leave over night. Do not evacuate below 12mm of Hg.

PREPARATION OF "DOUBLE GLASS PLATES": Tempered glass plates of 5mm and 1-2mm (window glass) thickness are combined to form one side of the flat chamber. Tape the top edge, place fold back clamps on bottom edge.

CASTING: Place 6mm gasket on a "double glass plate" and remove the clamps. Place brain slice on glass. Place second double glass plate, thin glass toward specimen, on specimen. Align gasket and place fold back clamps along edge.

FILLING THE MOLD: Stand mold upright (taped side up), insert plastic sleeve and fill chamber with fresh polymer mix (P 35/A 9). (100:2) (600 cc). Allow trapped air bubbles to rise by spreading the double glass plates and storing for 30 minutes in dark (to prevent early curing of the sheet)

CURING: Initiate with UV-light, complete in a 45°C oven. *Light Curing:* Light source applied to both sides for 45 minutes. *Heat Curing:* Place in 45 "C oven for 5 days.

DISMANTLING: Remove from oven in 5 days. Cool to room temperature.

TRIMMING: Table, radial, or band saw. Smooth edges - belt sander.

CLEANING THE GLASS PLATES: Detergent in a dishwasher at 85°C.

DISPOSAL OF OLD POLYMER: Harden polymer with Cobalt promoter (0.5%).

3rd Biennial Interim Conference on Plastination University of South Alabama Mobile, Alabama, USA

August 5, 6 and 7, 1993

Beautiful white sand beaches on the Gulf of Mexico.

Plan now to attend!!

The emissary veins of the bull's penis. G. H Ardalani, Urmia University, P.O. Box 165, Urmia, IRAN.

To understand the relationship of ccp of mature bulls with csp and csg and the action of erection, injection experiments were carried out on 6 excised organs from Friesian bulls aged *2%* to *5%* years with Microfil and latex. Injection shows that the ccp and csg have very fine connections that act as a safety valve during full erection. Microfil was slowly injected with a syringe into the ccp of specimen 6 at the level of the distal bend of the sigmoid flexure, without previous injection of the dorsal artery. The injection resulted in filling of the dorsal artery and also the dorsal venous system. In the cleared specimen, the csg was also found to be injected, with filling of left and right lateral components of dorsal arterial and dorsal venous systems. Sliced preparations from this specimen showed very clearly the intimate connections established between csp and csg at the apex of the organ. Is it possible that some bulls have fine veins draining the ccp distally? Veins draining the distal ccp that run a straight radial course through the tunica albuginea may limit the pressure that can be attained within the ccp. This has been discussed by Rotter and Schurman (1950) acting as "safety valve" mechanisms to prevent over-distension of the tunica albuginea, yet never becoming large enough to cause leakage that would interfere with erection? On general morphological grounds, it would be expected that such veins, connecting a very high pressure cavernous system with a low pressure venous system would behave in accordance with the three postulates of Thoma (Clark, 1971). These postulates are: 1) Increased rate of blood flow produces an increase in the size of the lumen of the blood vessel. 2) Increased tension in the wall of the blood vessel produces an increase in the thickness of its wall. 3) An increase in blood pressure provides a direct stimulus for the formation of new blood vessels.

Plastination. Harmon Bickley, Department of Pathology, School of Medicine, Mercer University, Macon, Georgia, 31207, USA.

Plastination is a process for impregnating a biological specimen or other porous material with a curable polymer, using the difference in vapor pressure between the infiltrating polymer and a volatile intermediate solvent to propel the impregnation. It is protected by paten;*, however the use of plastination to generate specimens for teaching or museum display may be permitted without royalty if a license to do so is obtained in advance from the holder of the patent. Plastination was invented by Dr. Gunther von Hagens at the University of Heidelberg in the early 1970's. The entire process includes four steps: 1) fixation, 2) dehydration, 3) forced impregnation 4) curing. Each of these will be introduced in this paper and discussed in more detail by speakers that follow. Silicone elastomer is the polymer most widely used in plastination. Epoxy and polyester are employed in special techniques. The qualities of specimens plastinated with each of these will be discussed. Many educational institutions throughout the world are now producing plastinated specimens. They are useful in a number of ways, their most common application being in undergraduate, medical, and veterinary medical education. Plastination promises to be a fruitful technique for research, however more development is needed. A plastination laboratory is not difficult to establish or operate and the equipment may be as simple or elaborate as one's budget permits.

Pitfalls and pleasures of plastinatina pathology specimens. Sally Ford, Kingston General Hospital, Kingston, Ontario, CANADA, K7L 2V7.

The rationale for production of pathology specimens for plastination is different from that in anatomy. In the latter, the objective is simple demonstration of normal anatomic structures and relationships. In pathology, anatomy is distorted by pathological processes, and disordered structure and function require novel approaches in dissection. This paper outlines the problems encountered in setting up a pathology museum of plastinated specimens, and highlights some of the rewards.

Starting plastination on a limited budget. Gilles G. Grondin, D6partement de Biologie, Faculty des Sciences, University de Sherbrooke, Sherbrooke, QUEBEC, CANADA, J1K 2R1.

After 3Jj years of efforts, a plastination laboratory was started in Sherbrooke with less than $3,000.00 (Canadian $). Those years of preplastination were used to collect different pieces of equipment for plastination (old vacuum pump, glass desiccator, tubings and connections, needle-valves from a

scrapped freeze-dryer) and to harass the teachers and the administrators to get money and a room. The laboratory that we obtained was 3.5 square meters (2.5m x 1.4m), covered by a glass ceiling to insulate from the lights and avoid the spark hazard. Bringing in ventilation and getting out wall plug, cost $360.00. Buying a household deep freezer and moving the thermostat and compressor from it, cost $870.00. A Bennert manometer, a hydrometer and a gas mask were bought for $700.00. Finally, 40 liters of acetone and 10 kg of Biodur S 10 Silicone resin cost $900.00, and we were ready to start. The dehydration jars were old 4 liter jugs with the top removed and covered by a glass cover. The plastination kettle was a glass desiccator and the curing chamber a plastic bag in an old aquarium. We were able to treat specimens of about the size of a hand but even with this restriction on the size of the specimens, we started plastination. After teachers had seen and touched the plastinated specimens and realized their value, they wanted more and bigger ones. It was then easier to get money to buy a bigger vacuum chamber and possibly move in a bigger room. We realized that starting plastination may be a long term adventure but it is possible to do it on a very limited budget and that perseverance and recycling may replace money. After it is started, you can easily demonstrate that it is worth while and that you can produce any required specimen. Then it is easier to improve your installation.

A simple and inexpensive method for recycling used acetone in plastination laboratories. Gilles G. Grondin and Serge B6rube^, Departements de Biologie et de Chimie^, Faculty des Sciences, University de Sherbrooke, Sherbrooke, QUEBEC, CANADA, J1K 2R1.

Used for the dehydration and defatting of specimens in the plastination process, acetone represents an important part of the operating budget of the laboratories. We have developed a method that is cheap, efficient, and safe for recycling used acetone. This method consists of 3 steps:

1. Freeze separation: This consists of just storing your used acetone in a freezer at -20°C. Fat, water and other contaminants that may be present will form solid particles big enough to be removed by filtration through cheese cloth.

2. Vacuum distillation: This step may seem complicated, but it is very simple. We just use the same equipment as for the impregnation procedure. The contaminated acetone is poured in a vacuum Erlenmeyer flask and warmed to 45 °C. This flask is connected to a second one that is inside the freezer at -20 °C. The second flask is connected to the vacuum pump and enough vacuum is applied to obtain boiling in the first flask. The acetone will boil from the first flask and condense in the second one.

3. Physical extraction of water: We add to the distilled acetone a desiccant (Molecular Sieve) that will complete the extraction of water from it.

We have used this technique for one year and consider it very efficient. The first step is optional and we use it mainly on the residues of the distillation. The second step is the most interesting. We can distill acetone at a rate of 500 to 600 ml/hour and reach up to 97% purity. The third step is also optional as we can use new acetone for the last dehydration bath. Finally, we needed to invest just $300.00 in the new equipment to achieve our objective, which was to completely recycle our acetone. We remove the cost of disposal of the used acetone, buy just a minimum of new solvent and contribute to the protection of the environment.

Plastination at Queen's: What worked and what did not. Blake Gubbins, Department of Pathology, Queen's University, Kingston, Ontario, CANADA K7L3N6.

When establishing a plastination laboratory, most of the obvious problems, that one might face, have been addressed in the literature or can be solved fairly easily. Our experiences uncovered some areas which deserve further investigation. They include: 1) Selection of a fritted-glass, gas-dispersion tube for the gas cure procedure. 2) How to clean the tube when it gets blocked with precipitate from the S 6. 3) The best place to put your vacuum pressure control valve. 4) Is an explosion-proof freezer really best for plastination? 5) Are we being sufficiently safety conscious in plastination? 6) Canadian Safety Standards as they relate to the latest developments in laboratory design.

The standard *S* 10 technique. Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901, USA.

The standard S 10 technique, although having been improved over the years, is the classical plastination technique. Specimens produced by this method are quite durable. Almost any type of specimen may be produced using this method,

including: whole organs, entire bodies or prosections of portions of the body, whole body slices or brain slices. The steps include: 1) SPECIMEN PREPARATION, 2) MINIMAL FIXATION of the tissue with subsequent removal of the fixative, 3) DEHYDRATION, 4) INTERMEDIARY SOLVENT, 5) IMPREGNATION, 6) CURING or HARDENING. 1) SPECIMEN PREPARATION: An extremely important step as unattractive or inappropriately prosected specimens will remain just as unattractive after plastination. Attractive, useful specimens take time and expertise to prepare. 2) FIXATION: Minimal formaldehyde fixation is the standard. Fixative solutions which are designed to preserve color may be used. The specimen should be fixed in the conformation of the desired final product. Once a specimen is fixed, it is difficult to change its fixed shape or configuration. However, long term fixed specimens may be plastinated. Once fixed, the fixative is rinsed from the specimen using running tap water. 3) DEHYDRATION: is another critical step. Cold acetone (-25 °C) or room temperature ethanol may be used. Cold acetone dehydration, called "Freeze Substitution", has become the accepted method. Dehydration must be complete to avoid shrinkage and provide complete impregnation of the specimen. 4) INTERMEDIARY SOLVENT: Is a volatile solvent whose vapor pressure is such that it can be extracted slowly as pressure is decreased allowing the polymer to be drawn into the tissue as it leaves. Acetone is an intermediary solvent, therefore, this step is accomplished as the specimen is dehydrated. Ethanol's vapor pressure is too low (similar to water) to serve as an intermediary solvent. Therefore, if ethanol was used as the dehydrant, it must be replaced with an intermediary solvent, methylene chloride or acetone. The specific gravity of ethanol and acetone are similar, therefore, the determination of % content of either solution via a hydrometer is not possible. 5) IMPREGNATION: Is the replacement of the intermediary solvent with the curable polymer mixture (Biodur S10/S3). Classically, impregnation is carried out inside a vacuum chamber which is located inside a deep freezer. The cold retards curing (linkage of the polymer molecules). Impregnation may be done at room temperature, but linkage of molecules is accelerated and the pot-life of the polymer reaction mixture is greatly reduced. Impregnation is carried out over a 2 to 4 week period, as absolute pressure is slowly decreased nearly one atmosphere to a final pressure of 1 cm Hg or less. Impregnation is monitored by observing the bubble formation, created as the intermediary solvent is volatilized by the decrease in absolute pressure (increasing vacuum). Impregnation is complete when the bubbling ceases or the absolute pressure is less than 1 cm of Hg. 6) CURING or HARDENING: Is carried out at room temperature after the excess polymer has been drained from the specimen. At room temperature and over a several month period, the S10/S3 mixture will slowly harden via end to end linkage of the polymer molecules. To speed up the curing process, the impregnated specimen is exposed to another hardening agent, Biodur S6, which commences side to side linkage of the polymer and hence seals the surface in 36 to 48 hours. After exposure to S6, the specimen cures from the outer surface inward over a period of several days to weeks.

Vacuum, vacuum gauges and manometers. Robert W. Henry and James R. Thompson, Jr.[1], College of Veterinary Medicine and [1] Department of Physics, The University of Tennessee, Knoxville, TN 37901, USA.

Vacuum is recorded from two perspectives: 1) A decrease in absolute pressure (AP) or 2) An increase in vacuum. These values can be recorded as a fraction of an atmosphere, e.g., 1/3 atmosphere (20 inches or 50.8 cm of Hg, AP), 1/2 atmosphere (15 inches or 38.1 cm Hg), or 2/3 atmosphere (10 inches or 25.4 cm Hg, AP). A column of Hg or vacuum gauge yields a progressively higher reading as absolute pressure is decreased. This is referred to as gauge pressure. However, a manometer yields a progressively lower number and is read as AP. This is because the manometer is reading the difference in two columns of Hg. The vacuum gauge or column of Hg is using atmospheric pressure as point zero, while the manometer is using total vacuum as point zero. Because most manometers used in plastination laboratories utilize two columns of Hg whose difference in height is 20 cm or less, only the last 1/3 or less of the change in the pressure in the vacuum chamber can be monitored. Hence, a vacuum gauge or Hg column is necessary to monitor changes in absolute pressure (vacuum) in the earlier stages (first two thirds) of impregnation. The saturated vapor pressure (similar to boiling point) of dichloromethane is greater than that of acetone, 32.5 mm Hg vs 14.8 mm at -25°C or 78.0 mm vs 35.9 mm at - 10°C. Hence methylene chloride will

vaporize at a higher AP and be extracted before acetone. Pressure is proportional to depth. This results in the pressure being greatest the bottom of the polymer than at the surface of the polymer. At - 25 °C, acetone will remain longer in a specimen or portion of which is submerged 15 to 20 cm below the surface of the polymer. The gauge is reading the surface pressure. If the AP is 2 cm of Hg, then the pressure at the lower level of polymer is the surface pressure (0.38 psi) added to the pressure generated by the 15 inches of polymer (0.51 psi) for a total of 0.89 psi. The force generated when absolute pressure is decreased one atmosphere is 15 pounds per square inch (6.45 cm^). At total vacuum, the one foot cube desiccator, used for plastination, has 2,160 pounds (Ibs) of force on each wall, while an 18 inch by 30 inch surface of a medium size vacuum chamber, has 8,000 Ibs of force, and a 20 x 50 inch surface of a chamber has 15,000 Ibs.

Cadaver preservation methods employed in India: is plastination a viable alternative? Sylvia Kamath. Department of Anatomy, St. John's Medical College, Bangalore, 560034, INDIA.

Cadavers, require to be preserved for various purposes such as study, display, transport, and delaying the last rites. This is achieved by embalming. The body tissues are permeated with an embalming fluid which preserves, hardens and produces asepsis. The main chemicals employed in embalming fluid are formalin, carbolic acid and glycerin. Fluid is run into arteries under pressure, using various techniques. We use a simple machine designed and fabricated in our institution. Embalming techniques are modified under special circumstances i.e., autopsied bodies, post surgery bodies, infected bodies for example AIDS, infective hepatitis, rabies, and in unnatural deaths. Here local injection or local injection combined with evisceration and packing may have to be employed. In the Department of Anatomy at St. John's Medical College, we have devised certain techniques for effective museum display of thin sections (150 /zm) and thick sections (3 mm). Whole organs or parts may serially sectioned and displayed and provide useful models for the understanding and interpretation of CAT scans and MRI pictures.

Freeze-drving and plastination: Useful tools for keeping the bugs out. Wayne Lyons, Department of Anatomy, Queen's University, Kingston, Ontario, CANADA, K7L 3N6.

Over the years, despite the use of standard protocols and procedures, fixation has posed a problem in the preparation of anatomical and anthropological materials. Use of dangerous chemicals, shrinkage and durability of specimens have all plagued previous techniques. Only recently, with the advent of new methods such as freeze-drying and plastination, has the use of harmful chemicals such as formaldehyde and phenol been limited. The ability to preserve fresh, frozen specimens, as well as, pre-treated specimens has not resulted in the production of specimens of inferior quality. Specimens produced in this manner have not, to date, undergone deterioration due to the invasion of "bugs" (microbes or museum pests). Freeze-drying and plastination can be used separately or in combination to help solve additional problems in the preparation of specimens. When using the S 10 method of plastination, shrinkage of whole-brain specimens has caused a great concern among plastinators, however, by combining this method with the freeze-drying technique, this is overcome. The results have produced a superior quality specimen which can be used for teaching. The examination of anthropological materials (bones, fossils) has often proven difficult due to their fragile nature. Using the S 10 technique of plastination, on a fragile North American Indian skull, we have shown that Biodur plastic has functioned as a consolident to eliminate this problem. These approaches (i.e., freeze-drying and plastination) appear to have eliminated the decomposition and destruction of specimens. These techniques have definitely made great strides in debugging the tasks of preserving and maintaining specimens. It is only with continued research and investigation that this will be substantiated.

The use of plastinated transverse body slices for teaching CT anatomy. P. P. C. Nel, Department of Anatomy and Cell Morphology, University of the OFS, Bloemfontein, SOUTH AFRICA.

With the advent of computerized tomography scans (CAT-scans), gross anatomy courses should expose medical students to cross-sectional anatomy. The more recent introduction and wide spread use of MR as a non-invasive, non-ionizing method of acquiring high resolution, multiplanar images of the human body, is making the study of the cross sectional anatomy of the normal human body even more relevant. Clinical department are expecting

their students to have a knowledge of cross-sectional anatomy. Commercial sources for teaching specimens (e.g., transverse body slices) are nonexistent and academic sources available are prohibitively expensive. In order to by-pass the financial burden of purchasing teaching specimens, the department of Anatomy and Cell Morphology at the University of the OFS have, in conjunction with the department of Diagnostic Radiology, at the same university, embarked on a project to determine what the affect (if any) of plastination is on a cross section through a human body. The aim of the study is to determine whether the department could produce its own plastinated body slices for teaching material. A cadaver was CAT-scanned and then embalmed. The location of each scan was carefully marked on the body with a felt-tipped pen. After embalming, the body was sliced into transverse sections. These sections were cut exactly on the markings of the CAT-scans. All sections were plastinated. A comparison between the original CAT-scans and the plastinated body slices show no significant change in the normal anatomy of the specimen. Therefore, the process of plastination, as we apply it in our department, has no effect on the gross anatomy of transverse body slices and plastinated body slices are an excellent answer to the problem of how to teach transverse anatomy on cadaver material.

Plastination and the Mac. Robert H. Parmelee, Department of Anatomy, College of Veterinary Medicine, University of California, Davis, 95616, USA.

The cost and legal difficulties of obtaining animals for classroom dissection exercises, coupled with gradually increasing reluctance by students to participate in a high turnover program in animal use, clearly indicates a need for change in what has been standard operational procedures. At the School of Veterinary Medicine at U.C. Davis, we are using plastination as a preservative technique coupled with interactive computer programs that join text, photography, and plastinated specimens and offer a radically new approach to teaching anatomy. This combination has far exceeded our expectations in both student acceptance and as partial substitute for dissected specimens in anatomy teaching labs. The relative ease in developing these "user friendly" programs with specific plastinated materials makes this system desirable both from an economic standpoint and an instructional one. High quality Kodachromes are taken of the plastinated specimens and loaded into the Macintosh II Si using a slide scanner and Adobe Photoshop software. These images are sized and imported into the Mac Supercard program, which allows several windows to be opened on screen at once so that graphics and instruction can be presented simultaneously. This program allows graphics to be scripted so that students can interact with the program which then becomes more of a tool instead of just a display. A discussion of the problems encountered in the production of S10 plastinated specimens and the development of interactive Supercard programs will be presented.

Monitoring the degree of fixation, dehydration, forced impregnation and cure. M. Ripani, A. Bassi, L. Perracchio, V. Panebianco, M. Perez, M.L. Boccia, State University of Rome "La Sapienza", II Department of Human Anatomy, Roma, ITALY.

The possible technical mistakes in plastination have been examined in eleven parenchymal organs (liver, kidney and spleen) treated with S 10 Standard Technique. All of the four phases were considered: fixation, dehydration, forced impregnation and cure. Possible deformation of the sample was not considered because we routinely fix the specimens on stiff supports. We identified inappropriate dehydration and/or forced impregnation procedures as the most important reason of shrinkage and accidental variations of properties. On the other hand, in our experience during the other phases, damage to the specimens are scanty and nearly always reversible. If standard procedures, found in the literature, are thoroughly followed. Two parameters of dehydration and forced impregnation have been studied: degree and duration. Average values for the eleven organs were calculated and plotted in a diagram. These data make it possible to define the most appropriate treatment in order to reduce the lasting of the procedure, the amount of reagents used and to avoid technical artifacts.

Comparative analysis of a plastination specimen and clinical diagnostic images. M. Ripani, A. Bassi, M. L. Boccia, G. Tomaselli, and G. Marinozzi, State University of Rome "La Sapienza", II Department of Human Anatomy, Roma, ITALY.

This specimen is an invasive apocrine carcinoma of the perineal glands involving the anal canal, the vagina, and the vulva. It was taken from a 62 year-old subject. The patient was studied by Computerized Axial Tomography (CAT) before surgery. The specimen was washed in running tap

water and then in formaldehyde solution. It was dipped into acetone at increasing concentrations and then placed in a -20 °C freezer. Some weeks later, the specimen was dipped into Biodur S 10 plus S 3 and the placed in a vacuum chamber. When forced impregnation was completed, the specimen was exposed to fast cure and then to slow cure to be cleaned. Finally, slices of the specimen were cut following CAT images. By comparing CAT images and photographs of anatomical dissections, it was possible to confirm the accuracy of the *in vivo* instrumental study. Moreover, it was possible to explain in detail, structures which were ambiguous on CAT images. Thus, plastination has proven to be an important means for verifying sophisticated diagnostic images.

Sheet plastination. E 12 technique. Wolfgang Weber, Department of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011, USA.

Sheet plastinated specimens find the highest acceptance of preserved body slices. Cross-sections of the body never were as detailed, never were transparent, never were projectable, never as durable nor as easy to handle. There are two techniques for sheet plastination. Brain sheets are done with a polyester-compound called BIODUR P 35. While, cross sections of the body are done with an epoxy compound called BIODUR E 12. The procedures are similar, but for the sake of clarity, each process will be presented individually.

LIST OF NECESSARY EQUIPMENT: A Vacuum chamber large enough to hold the flat chambers; Ultra-low deep freezer and an additional freezer; Manometer; Biodur Polymers: E 12, E1, AE 10 and AE 30; Meat slicer and/or Band saw with guide stop (refrigerated is preferred); 50*C Oven, Stainless steel (HD 18) or aluminum grids; Polymer mesh (HD 16) or Fly screen; Covered basket (HD 08); Appropriate containers for storage and dehydration of specimens (HD 07). To assemble each flat chamber: 2 Tempered glass plates (5mm) (HS 01 or HS 02), 6mm Gasket (HS 06), 15 large (HS 30) Fold back clamps or 2 inch binder clamps, and Plastic sleeve or hose (to serve as a funnel).

FIXATION OF SPECIMEN: Kaiserling's solution to enhance color of muscle. Fresh specimens with no fixation. Float specimen.

FREEZING THE WHOLE BODY AND PORTIONING: Suspend to minimize flattened areas. Remove and store extremities.

SLICING THE BODY PARTS: Even 2.5 mm slices for best transparency. Band saw with a refrigerated guide stop. Place slices in freezer, scratch saw dust.

ORGANIZATION OF SLICING: Place slice on grid covered with fly screen, in cold acetone (Fly screen-specimen sandwich). Cover with flyscreen and another slice, etc.

DEHYDRATION: Freeze substitution of stacked flyscreen/specimens.

DECREASING: Important for transparent slices. Room temperature acetone or methylene chloride.

IMPREGNATION: Polymer Mixture - E 12 (95 pbw), AE 30 (5 pbw), AE 10 (20 pbw), and E1 (26 pbw). Can not prolong its short life, after mixed. Weight stack of slices to submerge in reaction mixture.

CASTING: 2 tempered glass plates, 6 mm gasket, 5 mm spacer strips. Large fold back clamps.

FILLING THE MOLD: Plastic sleeve (funnel); 600 cc of E 12 (95 pbw), AE 30 (5 pbw), E 1 (26 pbw) mixture per mold.

EVACUATION: in vacuum chamber for 1 hour.

CORRECTING THE POSITION OF SLICES AND REMOVING AIR BUBBLES: Use pieces of stainless steel wire to manipulate air bubbles and the slices.

CLOSING THE MOLDS: The ends of the gasket are used to close the top.

CURING: Molds placed at a 15° angle for 1 to 2 days. 40 °C oven 1 week.

DISMANTLING: Cool to room temperature. Cover with clear plastic wrap.

CUTTING OUT: Mark 1 cm from perimeter of slice and saw on band saw.

SMOOTHING EDGES: Belt sander.

POSTERS

Understanding standard imaging planes for two-dimensional, real-time echocardiography in the dog aided by plastinated specimens. Janice M. Bright and Robert W. Henry, Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901 USA.

A valuable method for evaluating structure and function of the heart is ultrasonography. Two-dimensional, real-time echocardiography (2DE) routinely is used to provide images in a single plane. Standard imaging planes have been described for evaluation of the canine heart. Standard imaging planes were obtained from single frame images of real-time, 2DE examination of healthy dogs. To illustrate three-dimensional anatomy relative to the two-dimensional sector, these images are correlated with plastinated specimens which were sectioned along corresponding imaging planes. Labelled photographs of the sectioned specimens are displayed adjacent to the echocardiographic images. Standard imaging planes are designated and transducer positions, used to obtain standard imaging planes, are described. To enhance the student's ability to interpret standard imaging planes, a cadaver

heart was prepared for plastination. The heart was separated from the lungs by transecting the pulmonary vessels as long as possible. An appropriate size of tubing was used to cannulate a pulmonary vein and the caudal vena cava. The heart was flushed with warm tap water through both cannulas to remove blood. The other large vessels were closed by ligating a bleached cork in place (larger end into vessel). The remaining smaller vessels were closed with hemostatic clips, string, or suture material. The heart was hydrostatically dilated with cold water, dilated-fixed with a 5% Kaiserling's solution, dehydrated in cold (-25 °C) acetone, and impregnated with Biodur using the standard S10-S3 technique. Following impregnation, the heart was drained of the excess polymer-mix and exposed to Biodur S6 (gas curing agent). During curing, the heart was manicured daily to remove excess polymer-mix which accumulated on the surface of the specimen. After curing the heart was sectioned along the planes of standard images and attached to the poster.

The P 35 technique for sheet plastination of the human head. R. T. de Boer-van Huizen, C.J. Cornelissen and H. J. ten Donkelaar, Department of Anatomy and Embryology, Faculty of Medical Sciences, University of Nijmegen, The NETHERLANDS.

Modern imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) require a basic knowledge of cross-sectional anatomy of the human head or central nervous system (CNS). Although excellent volumes on sectional anatomy of the human head and CNS are available as anatomical guides for the analysis of CT and MRI sections, the study of real sections of the human head would be preferable, particularly for teaching purposes. Macrosections of the human head or brain have some disadvantages in that they have to be kept in formalin, fall apart because of handling and the differentiation between white and grey matter of the brain becomes less distinct in time, due to the bleaching effect of the fixative. Plastination, a technique of tissue preservation, introduced by von Hagens offers the possibility to prepare clean, dry and easy-to-handle slices. In the process of plastination, water and lipids are replaced by polymers which are subsequently hardened resulting in dry, odorless and durable specimens. The P 35 plastination technique gives excellent differentiation between grey and white matter of the brain and offers excellent reference material for pre- and postdoctoral training in cross-sectional anatomy. In the present study, the P 35 technique was applied to four mm anatomical slices of a formalin-fixed human head.

A new positioning technique for comparing sectional anatomy of the shoulder with sectional diagnostic modalities: magnetic resonance imaging (MRI). computed tomography (CT) and ultrasound (US). C. A. C. Entius, J. W. KuiperTT w. KoopsJ A. DeGastJ Departments of Anatomy and ^Radiology, ERASMUS University Rotterdam, The NETHERLANDS.

A new positioning technique is presented, based upon MR and CT imaging with surface markers applied to the skin of the specimen. With this technique, the required anatomic section for cryosectioning can be defined accurately. Plastinated slices, of 2 mm thickness, with the correlating MR, CT, and US images of double-oblique coronal imaging of the shoulder are shown. The method is equally applicable to all parts of the body and makes comparison of structures in correlative anatomic studies easier and more accurate.

Special brain preparations to show fiber tracts and vascularization. M. R. Haffajee, G. Mathura, and S. Kassim, University of Durban-Westville, Durban 4000, SOUTH AFRICA.

Although sectioned specimens of brain have reached a high degree of sophistication in plastination for museums, we have found that undergraduates still have difficulty conceptualizing three-dimensional concepts of fiber tracts. We have combined two methods to remedy this difficulty. Fresh brains are injected with a coloured mass to show the arterial tree. The ideal method for showing fiber tracts is by careful dissection following the "freeze-fixation technique". It also enables students to visualize the basal nuclei in three-dimension. Normally, these specimens are kept in formalin but are not amenable to regular use. By plastination using the "S 10" method, although some shrinkage occurs, they are suitable adjuncts to standard sections of the brain for learning.

Plastination of three-dimensional brain sections as a component of a learning module. G. Mathura, M. R. Haffajee, S. Kassim, University of Durban-Westville, Durban 4000, SOUTH AFRICA.

Neuroanatomy is considered to be one of the more difficult areas of study in anatomy.

A special limb plastination using the S 10 technique with roentgenological and angiographic views. G. Mathura, M. R. Haffajee, S. Kassim, University of Durban-Westville, Durban 4000, SOUTH AFRICA.

Using a combination of techniques, a limb preparation method is demonstrated that shows vascularization, and horizontal and longitudinal sections together.

 J Int Soc Plastination, Vol 6:15-16, 1992

USE OF PLASTINATED CRANIA IN NEUROENDOSCOPY

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SUMMARY

Neuroanatomy, studied via the endoscope on plastinated specimens, will play a key role in endoscopial neurosurgery in the future. Plastinated specimens are proving to be an invaluable aid for training in all phases of the neurosciences.

INTRODUCTION

At the neurosurgical clinic, University of Mainz, plastinated specimens have been prepared to help establish endoscopial neuroanatomy and plastinated crania are now used for neuroendoscopial training. Plastinated specimens are dry and available for use in any environment; as opposed to wet, toxic formalin-fixed specimens which are not practical for such training (Resch, 1989). Plastinated specimens reveal precise anatomical detail {Resch and Perneczky, 1990). As the endoscope is manipulated through the prosected regions of the plastinated specimen, new views are possible with the plastinated specimens. Hand-eye coordination is enhanced by using this set up.

MATERIALS AND METHODS

Amputated, formalin-fixed crania were prosected through a dissection microscope. Keyhole approaches up to 15 cm deep were prepared to demonstrate selected areas of neuroanatomy. Four specimens were prepared and were plastinated using freeze substitution and the standard S 10 method for impregnation of the silicone polymer (von Hagens, 1985).

The vessels were injected with colored PEM prior to amputation. The specimens were immersion fixed in a 10% formaldehyde solution with perfusion of the ventricles and subarachnoid space, after which they were dissected. The specimens were dehydrated by freeze substitution in preparation for plastination. The standard S10/S3 technique was used to produce the plastinated specimens (von Hagens, 1985).

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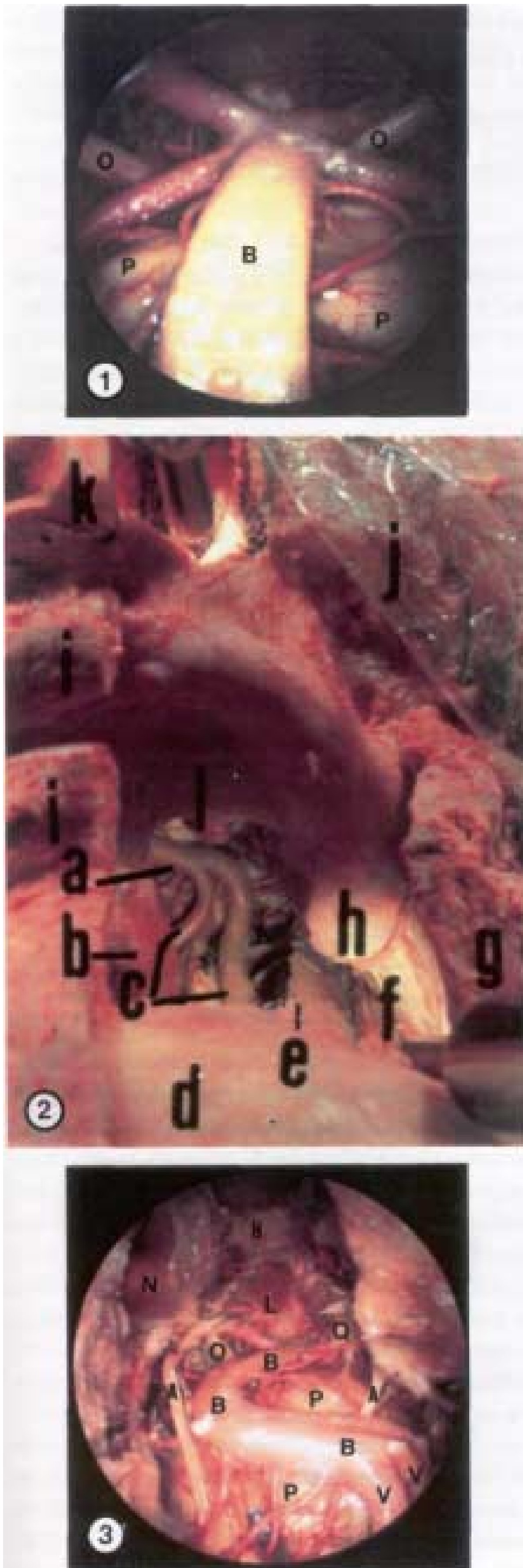
LEGENDS FOR COLOR PLATES <ieft column) ON FACING PAGE:

Figure 1. The entire transoral aspect of the brain stem of the plastinated specimen may observed and magnified using the endoscope. The basilar artery (B) may be followed along the pons (P) to its quatrification. Both oculomotor nerves (0) are in focus.

Figure 2. Transoral view of the brain stem through a large window in a plastinated specimen. Alveolar process (h), Basilar artery (a), Hard palate (I), Mandible (d), Medulla oblongata (origin of hypoglossal nerve) (k), Pontomedullary sulcus (j), Soft tissue of the face (i, g, f), Tongue (b), Vertebral artery (c), Vertebral joint (e).

Figure 3. In the plastinated specimen the endoscope may be placed at different depths and the syntopy of previously hidden structures can be studied. Behind the base of the cranium in the sphenoidal sinus the impression of the carotid artery (N) is seen. Abducens nerve (A), Basilar artery (B), Brain stem structures [Hypophysis (H), Hypothalamus (L), Pons (P)], Oculomotor nerve (0), Vertebral arteries (V).



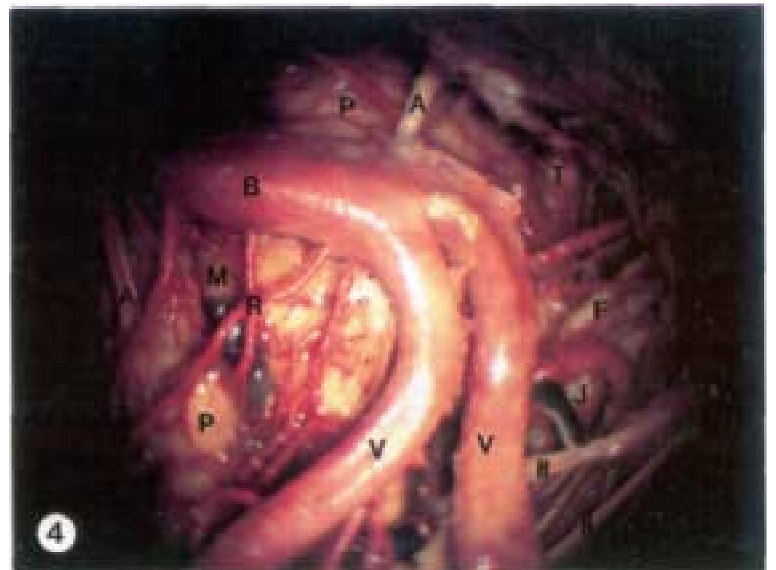


Figure 4. Endoscopic view of Figure 2, the transoral aspect of the brain stem. The optical characteristics are a typical "fish eye" view yielding a much broader field. Many nerves can be followed to their foramina, Abducens (A), Acoustic and Facial (F), Hypoglossal (H), the Jugular foramen group (J) and Trigeminal (T). Basilar artery (B), Basilar vein (M), Pons (P), Pontine perforators (R), Vertebral arteries (V).

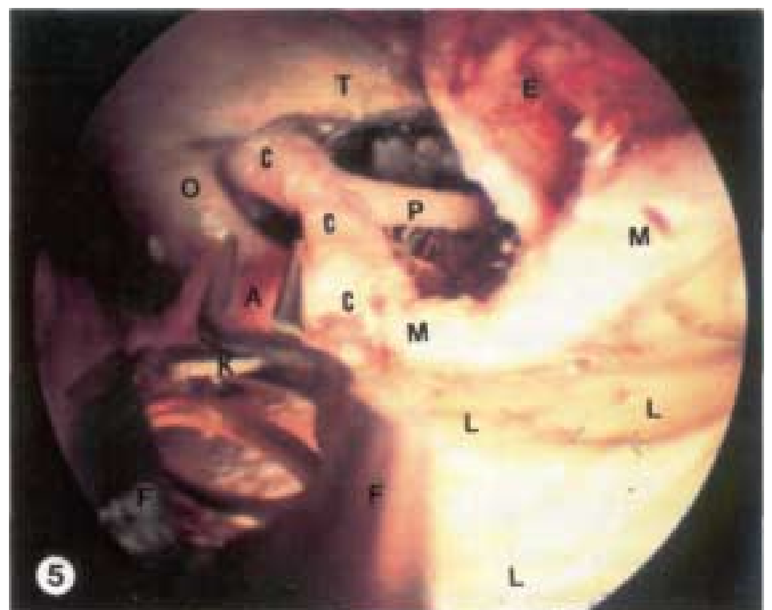


Figure 5. Through a right frontotemporal approach, in the plastinated specimen using the endoscope, an aneurysm clip (K) is introduced with a clip forceps (F) during a training session. Anterior cerebral artery (A), Carotid artery (C), Frontal lobe (L), Middle cerebral artery (M), Optic nerve (0), Posterior communicating artery (P), Temporal lobe (E), Tentorial notch (T).

J Int Soc Plastination, Vol 6:17-19, 1992

A SIMPLE AND INEXPENSIVE METHOD FOR RECYCLING USED ACETONE IN PLASTINATION LABORATORIES

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SUMMARY

This paper describes a technique that is cheap, efficient and safe for recycling acetone. We have developed a method that includes three steps: Freeze-separation, vacuum distillation and physical water extraction. It permitted us to re-use acetone with minimal loss by using equipment that we already had in our plastination laboratory with very few additions or modifications. We were able to bring our used acetone up to 99% purity, so we never had to pay for discarding used acetone and we have purchased only a minimal volume of new acetone.

INTRODUCTION

In most plastination laboratories, acetone is used for dehydration by the freeze-substitution technique and for defatting the specimens, as recommended in the plastination process (von Hagens, 1985). The cost of both purchasing and disposing of acetone represents an important part of the operating budget.

To be able to re-use acetone, plastinators have used their imagination and sometimes, when available, money. Some will leave the used acetone in a freezer until the water and fat congeal and can be separated from the acetone (Henry, 1991). Wealthier laboratories will simply push the button of their sophisticated distillation equipment controlled by a computer. We wanted to find a compromise between these two extremes.

MATERIALS AND METHODS

Procedure 1: which was called "freeze-separation". used three contaminated solutions of acetone: 1. Acetone and water (60% acetone and 40% water); 2. Acetone and animal fat (10 - 20%); 3. Routine contaminated acetone from the dehydration of biological specimens for the plastination process. The contaminated solutions were placed in a deep freezer and at designated intervals, the samples were filtered through cheese cloth to remove the congealed fat or frozen water.

The water contaminated solutions were frozen and then filtered at 1, 2, 3, and 4 week intervals. This step required no special equipment. Space in a deep freezer at -20 °C and buckets and cheese cloth were necessary. The filtered solutions were monitored with a hydrometer (Tralle and Proof Thermo-Hydrometer).

To prepare the fat contaminated solution, animal fat was put into acetone for 1 week at 20 °C and then placed in the freezer. After freezing, the mixture was filtered every 2 days through cheese cloth, 100 g. of the filtered solution was taken, and the remainder of the filtered solution was returned to the freezer. The 100 g. aliquot of filtered acetone solution was evaporated at 30°C on a rotary evaporator (Buchi Rotavapor, model RE 111) and the residual fat was weighed.

Procedure 2: was called "vacuum distillation" and was slightly more sophisticated but not complicated. It required a deep freezer, a vacuum pump, valves, and tubing, available in any plastination lab. Two - 4 liter filtering Erlenmeyer flasks, 2 meters of Tygon tubing, two #13 rubber stoppers, 1 meter of glass tubing, marbles, and a styrofoam box big enough to contain one of the Erlenmeyer flasks were purchased.

Three liters of a contaminated acetone solution were poured into the first Erlenmeyer flask with the marbles acting as anti-bumping granules. This flask was heated in hot water to 45° - 50 °C and transferred into the styrofoam box containing hot water at 50°C. The box and hot water acted as a heating mantle and prevented rapid cooling of the mixture to be distilled due to the extraction of energy generated by the distillation process.

This first flask was connected to the second flask which was placed in the freezer at -20°C. Flask 2 acted as the condenser. It was connected to the vacuum line and enough vacuum was applied to obtain boiling in the first flask. Boiling stopped by itself when the temperature reached 35 "C - 40°C. The cost of the supplementary equipment for this step was less than $300.00.

Procedure 3: called "physical water extraction" was conducted at room temperature by adding granules of molecular sieves (4-8 mesh, beads effective pore size 0.3nm) to the distilled acetone. These granules are utilized to remove residual water from the acetone and completed the purification process. Molecular sieves were bought from Fisher Scientific for $30.00/500 grams.

One liter samples of distilled acetone were put at room temperature. 200 grams of molecular sieves were added and mixed into the 'distilled acetone solution. This mixture was left undisturbed for 1 week. After 1 week, the acetone was filtered using filter paper and purity was monitored with the hydrometer. The molecular sieves were left in a hood to permit evaporation of acetone vapors. The sieves were dried by heating to 120°C for 24 hours and may be reused for many years.

RESULTS

FREEZE SEPARATION:

This procedure was the cheapest way to purify acetone. The efficiency of leaving the samples in the freezer, undisturbed for prolonged periods to aid removal of the two major contaminants found in the dehydration process, water and fat, was much better for fat removal than for water.

1. *Acetone and Water:* Even after 4 weeks at -20"C and filtering through cheese cloth, the purity of the acetone did not improve appreciably.

2. *Acetone and fat:* Table 1 shows the very high efficiency of freeze-separation for removal of fat. Note, the almost complete separation of fat from acetone within 2 days of freezing at -20 °C. Table 2 shows monitoring of the same samples with a hydrometer. It confirms the total inaccuracy of hydrometer monitoring when fat content is high.

3. *Used Acetone From Plastination Lab:* The mixtures of used acetone were kept at -20 °C, filtered through cheese cloth and monitored once a week with a hydrometer for up to 5 weeks. Like the water-contaminated specimens, we did not observe changes in the density of the mixtures even if we had residues left on the filters after each filtration. The only exception happened with one jar that was "forgotten" in the freezer for 4 months. This acetone had been used for freeze-fixation and was 60% pure (hydrometer monitoring). After 4 months at -20°C and one filtration through 4 layers of cheese cloth, the reading was 78%. The filter contained a big residue and gave off a strong formaldehyde smell.

VACUUM DISTILLATION:

The samples that we distilled were mixtures of all the samples used for the freeze separation tests, mainly containing used acetone from plastination. They were monitored with the hydrometer (Table 3).

PHYSICAL EXTRACTION:

When monitored with the hydrometer, the purity of one liter samples of distilled acetone, held at room temperature after addition of 200 g of molecular sieves, increased from 96% to 99%.

DISCUSSION

The described method has been used in our plastination lab for one year and it has proven to be very efficient. This process may seem to be time consuming, but we have not found it so. It just takes a small space in the freezer because our used acetone is always kept at -20°C. After each impregnation, before changing the pump oil, we take a few days to recycle acetone. The most contaminated acetone is taken from the freezer, filtered before it warms up and distilled.

Because of the big difference between the vapor pressure of the acetone (446.7 torrs at 45 "C and 20.16 torrs at -20°C; Weast, 1973), we can condense almost all the vapors. The risks of damaging the pump are negligible because we send less acetone through it during 2 days of distillation than during 3 weeks of impregnation. We have never lost more than 18 ml of solvent during one run which is less than 0.6% of the volume distilled.

We also consider our distillation process as a very efficient method. We typically obtain 97% purity which compare favorably with the maximum purity obtainable by distillation (98.7%; Weast, 1973). Finally, we consider it as a completely safe method because it is all done in a well-ventilated plastination lab with the same equipment that is used for the dehydration and impregnation process.

The first and third steps are optional; however, because they do not require any manipulation there is no reason to by-pass them. The first one helps to reduce the volume of acetone to be distilled and to keep the distillation equipment clean, mainly by removing'the fat. Freeze-separation can also be used to take water from acetone but we believe that the

disadvantages are greater than the advantages because it involves a long term storage of contaminated acetone at -20 °C and a lot of room in your freezers. The third one can also be avoided if you use new acetone for the last dehydration bath. The molecular sieves leave very small particles in suspension in the acetone. It gives a light coloration to the acetone. These particles can be removed by filtration through a very dense filter or by redistillation. We do not remove them and they have not caused us any problem.

Finally, we reached our goals which were to avoid paying for discarding used acetone and to minimize the use of new acetone. We also consider that by reducing the volume of solvent, we make our small contribution for the protection of our planet.

ACKNOWLEDGMENTS

We thank Mr. P. Matton for his support and Mr. D. Thomas for a critical reading of the manuscript. We are also grateful to Mrs G. Girard for the typing of it.

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TABLE 1
FREEZE-SEPARATION OF FAT FROM ACETONE

Sample #	Acetone purity (%) measured by evaporation			
	Day 0	Day 2	Day 4	Day 6
1	82.6	95.9	96.0	95.8
2	87.3	98.1	98.3	98.1
3	91.6	95.1	95.0	94.7

TABLE 2
FREEZE-SEPARATION OF FAT FROM ACETONE

Sample #	Acetone purity (%) measured with hydrometer			
	Day 0	Day 2	Day 4	Day 6
1	94	95	95	95
2	96	98	98	98
3	95	95	95	95

TABLE 3
VACUUM DISTILLATION OF ACETONE AFTER FREEZE-SEPARATION

Sample #	Acetone purity (%) measured with hydrometer			Rate (ml/hr)
	Beginning	Distillate	Residue	
1	75	96	58	565
2	80	97	74	630
3	75	97	66	600
4	62	96	43	
5	76	97	59	
6	83	97	70	
7	56	97	36	

SHEET PLASTINATION OF THE HUMAN HEAD

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SUMMARY

Modern imaging techniques require a basic knowledge of cross-sectional anatomy of the human body. In the present study, the technique of sheet plastination, introduced by von Hagens, was used for the 4 mm anatomical slices of a formalin-fixed human head. In the process of plastination water and lipids are replaced by polymers, which is subsequently hardened, resulting in dry, odourless and durable specimens. The P 35 plastination technique gives excellent differentiation between grey and white matter of the brain and offers excellent reference material for pre- and postdoctoral training in cross-sectional anatomy.

INTRODUCTION

The wide application in the clinical neurosciences of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) has aroused a growing interest in the use of anatomical sections of the human head and central nervous system (CNS). Excellent publications on sectional anatomy of the human head and CNS (Eycleshymer and Schoemaker, 1911; Koritke and Sick, 1983; Schnitzlein et al., 1983; de Groot, 1984; Kretschmann and Weinrich, 1984; Daniels et al., 1987; Gerhardt and Frommhold, 1988; Nieuwenhuys et al., 1988; Talairach and Tournoux, 1988; von Hagens et al., 1990) are available as anatomical guides for the analysis of CT and MRI sections. Study of actual sections of the human head would be preferable, particularly for teaching purposes. Macrosections of the human head or brain have some disadvantages in that they have to be kept in formalin, fall apart because of handling, and the differentiation between white and grey matter of the brain becomes less distinct in time due to the bleaching effect of the fixative. Cryomicrotomy (Rauschning et al., 1983) is a costly alternative requiring expensive equipment. Plastination, a technique of tissue preservation, introduced by von Hagens (1987), offers the possibility to prepare clean, dry, and easy to handle slices. In the process of plastination, water and lipids are replaced by polymers which are subsequently hardened resulting in dry, odourless and durable specimens. The polyester resin P 35 can be used for the production of opaque brain slices, which gives excellent differentiation between grey and white matter (von Hagens et al., 1987, 1990). In the present study, the P 35 plastination technique has been applied to 4 mm anatomical slices of a formalin-fixed human head.

MATERIALS AND METHODS

The standard P 35 technique according to von Hagens (1985, 1989; von Hagens et al., 1987) was used. This technique involves the following steps:

SPECIMEN PREPARATION FOR FIXATION AND SAWING:

Our first "plastination experiments used the head of a human, male, cadaver (73 years of age) which had been preserved in embalming fluid for over three years. The embalming fluid consisted of 500 ml ethanol 96%, 1000 ml formalin 40%, 25 ml phenol liquefied 80%, 300 gm sodium chloride, 300 gm chloral hydrate and 300 ml glycerin in 10 liters of water. The head was removed from the body and rinsed in running tap water for two days. After rinsing, it was placed in a styrofoam box filled with water and frozen at - 20° C with the head oriented so that slices could be sawn parallel to Talairach's (1952) bicommissural (ACPC: anterior commissure - posterior commissure) plane (Bergvall et al., 1988). After three days in the deep freezer, the frozerj styrofoam box, ice, and head were sawn using a handsaw into 4 mm thick slices. The macrosections of the head were placed on glass-plates. After defrosting, the slices were gently rinsed with running tap water to remove the sawdust and pieces of the styrofoam box. After making color photographs of the slices, the slice and plate were submerged into 4% formalin where they were kept until further processing.

DEHYDRATION:

The 4 mm thick head slices were carefully put between stainless steel grids and rinsed for 8 hours in running tap water to remove the fixative. To prevent the brain from becoming too brittle after dehydration in cold acetone, the slices were placed into a solution of 4% sucrose in distilled water at 4"C over night. According to von Hagens[1] protocol (1987), dehydration was performed by freeze substitution using cold acetone of -25 °C. The acetone was changed twice and the specimens were kept in the cold acetone for 2 - 14 days.

IMPREGNATION:

From the cold acetone, the specimens were quickly immersed in a polymer-mix [20 ml of harder (A9) to one liter P 35] at 4°C. After gently removing excessive air-bubbles with the aid of low (minimal) vacuum for 20 - 30 minutes, the specimens were left over night in the first polymer-mix at 4°C. The polymer-mix was changed three times at 24 hour intervals and low vacuum was used for removing air-bubbles each time. Forced impregnation was started after the third change of polymer-mix as the vacuum was increased to 12 mm Hg over a 4 - 5 hour period of time. Vacuum was maintained at 12 mm Hg over night in the dark vacuum chamber at room temperature.

PREPARATION OF THE PLASTINATED SHEETS:

The head slices were carefully removed from the stainless steel grids and placed into the flat chamber consisting of four glass-plates. The two inner plates were separated by a flexible, elastic gasket. A detailed description of this technique is found in the "Heidelberg Plastination Folder" (von Hagens, 1985; von Hagens et al., 1987). To construct the flat chamber for the 4 mm thick slices, a 6 mm thick gasket was used. A small nylon string was connected to the skull to prevent the slice from sinking to the bottom of the flat chamber. After filling the flat chamber with freshly prepared polymer-mix it was again placed into the vacuum chamber and kept under low vacuum for several (3-5) hours to remove the air bubbles from around the specimen, and even more important, most of the small fat globules. After all air bubbles and most of the fat globules had floated to the top of the polymer, the top of the flat chamber was closed utilizing the ends of the gasket. Polymerization was initiated with a 200 Watt UV-A light source for 45 minutes on both sides. After light curing, the flat chamber was kept in an oven at 40 °C for six days to complete the process of polymerization. When this process was completed, the flat chamber was dismantled. The plastinated slice was covered with plastic foil, excessive polymer was trimmed away, and the specimen was polished with waterproof sandpaper and abrasive cloth.

ACETONE VAPOR MONITORING:

Acetone vapor levels were monitored at various stages of the dehydration process. A hexane calibrated TLV-Sniffer was used to measure acetone vapor levels in the deep freezer and at the inhalation level during manipulation of the specimens on the grids and while pouring acetone from one container to another. Vapor levels from pouring both cold (-25 °C) and warm acetone (20 °C) were measured and recorded.

RESULTS

The contrast between grey and white matter of the brain is enhanced during the process of plastination as noted by the 4 mm thick macrosection of the human head (preplastination) and the resulting plastinated slice (Figs. 1, 2). Within the thalamus, the various nuclei can be discerned. Furthermore, large fiber bundles such as the radiatio optica and the forceps major (posterior) of the corpus callosum can easily be identified. The shrinkage of the brain and other soft tissues was minimal. Figure 3 shows a plastinated slice passing through the orbit, the external acoustic meatus, the inner ear and the fossa cranii posterior. The dentate nucleus and pontine nuclei stand out in the surrounding white substance of the cerebellum and pons, respectively. By examining the plastinated slices under a dissecting microscope or with a magnifying-glass small structures such as nerves, blood vessels and meninges can be studied in detail (Fig. 4).

The section in Figure 3 shows the disturbing effects of lipids. The fat globules may accumulate on the surface of the slice.

Acetone concentrations above the open dehydration tank while specimens were being manipulated was 5,000 ppm; while at the inhalation level, the concentration was 850 ppm. When cold acetone (-25 °C) was poured into a dehydration vat, the acetone vapor level at the inhalation level was 45

ppm. When 20°C acetone was similarly poured into the vat, the vapor level at the inhalation zone was 450 ppm.

DISCUSSION

To aid our teaching program, sheet plastination of slices of the human head has been utilized in our laboratory. As already known, the P 35 technique gives excellent differentiation between grey and white matter of the brain (von Hagens et al., 1987, 1990). Macrosections of the head allow the study of the brain and its surrounding structures, i.e., the brain "in situ". Although the process of plastination is time-consuming, the availability of dry, odourless and durable specimens of the human head provides reference material for pre- and postdoctoral training in crosssectional anatomy which is convenient to handle.

Specimens were dehydrated for 2 - 14 days, however, in keeping with von Hagens' data (1989), 4 days appeared to be enough time for adequate dehydration. Longer periods of dehydration can result in extraction of too much lipid from the brain tissue.

The presence of fat globules on the surface of a plastinated slice is most likely due to the extraction of fat from the bone marrow or from an atheromatous cyst in the neck region. It should be noted that during dehydration in acetone and impregnation in the polymer some lipids are extracted from the tissue. For brain tissue, the extraction of lipids should be limited as the differentiation between grey and white matter is achieved by preservation of lipids (von Hagens et al., 1987). Since the skull and skin are still part of the specimen, the lipids from these tissues may cause these disturbing artifacts. They appeared as little white fat globules that sometimes cover the whole plastinated brain slice. These artifacts can be removed using a nylon brush and xylene to dissolve and remove the fat globules. As a result, the surface of the plastinated sheet will be covered with small depressions. These can be eliminated by placing the plastinated slice back into a 1:1 polymer/acetone mixture, for one or two days and then back in the flat chamber where the sheet plastination process is repeated. However, an 8 mm gasket is used to replastinate.

Since dehydration by freeze substitution (cold acetone) is recommended, an inherent problem with the plastination technique is acetone safety. A mixture of acetone and air may be highly explosive. Measurements of the concentration of acetone vapors during our plastination work (Plas, 1991), showed that the highest concentration of acetone was found in the deep freezer while manipulating the specimens on the stainless steel grid in one of the open acetone tanks. This concentration was 5000 ppm just above the open tank. At the inhalation level during this maneuver, the highest concentration of acetone measured was 850 ppm. During the step in which cold acetone of -25 °C was poured out of the tank during the changing of acetone, the highest concentration at the level of inhalation was 45 ppm. When 20 °C acetone was poured, the concentration was 450 ppm. The lowest explosion level for acetone vapors is 2.3% (23,000 ppm). Our highest concentration of acetone in the deep freezer was well below this point. Therefore, the risk for explosion did not exist. The use of an explosion-safe deep freezer, however, has to be emphasized. It should also be stressed that for inhalation these concentrations of acetone are high, so it is advisable to work under a fume hood and if this is not possible, one should wear a mask. As the components of the P 35 polymer are not published, to measure their concentration was not possible. However, the vapor pressure of P 35 is very low. We recommend to work as clean as possible and wear protective clothing, mask and gloves.

Our next step in plastination will be to make other series of plastinated sections in frontal and sagittal planes.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. A.H.M. Lohman of the Department of Anatomy of the Free University of Amsterdam, for offering the opportunity to use their vacuum chamber; Mr. H.J.M. Remers for skillfully preparing the frozen sections of the head; Mr. T.W. Spaan for giving the finishing touch to the plastinated slices; and the collaborators of the Department of Photography of the University of Nijmegen for assistance with photography.

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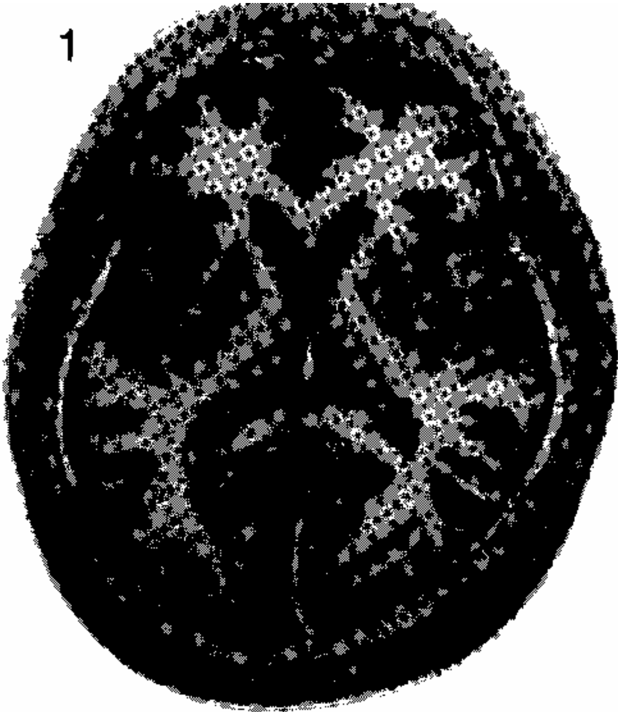
LEGENDS FOR FIGURES ON FACING PAGE:

Figure 1. Section of the human head, parallel to Talairach's (1952) bicommissural plane. 4 mm thick, unstained, X 0.47.

Figure 2. The resulting plastinated slice (same slice as in figure 1), processed by P 35 sheet plastination. 4 mm thick, unstained, X 0.47.

Figure 3. Plastinated slice of the human head. 4 mm thick, unstained, X 0.47.

Figure 4. A close-up (higher magnification) of the sheet shown in figure 3 revealing structures at the cerebellopontine angle and the internal ear. Cerebellum (Ce), Dentate nucleus (De), Cranial nerve eight (n VIII). 4 mm thick, unstained, X 1.25.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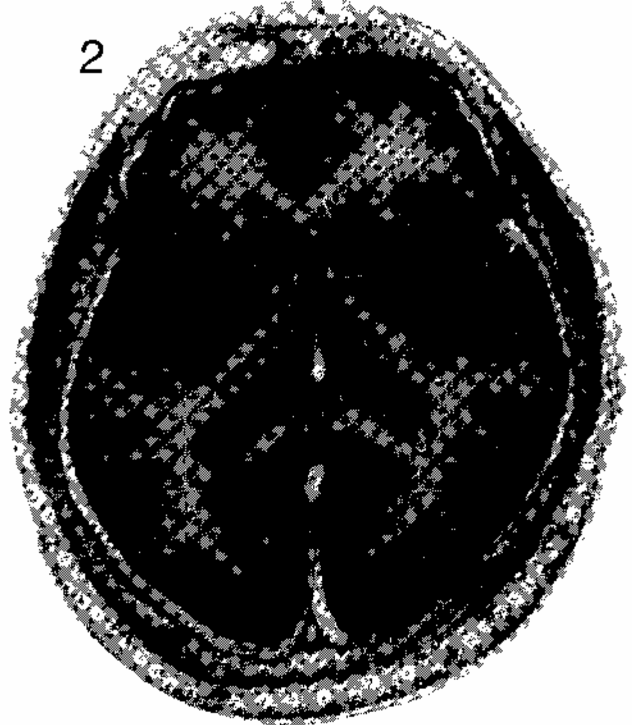
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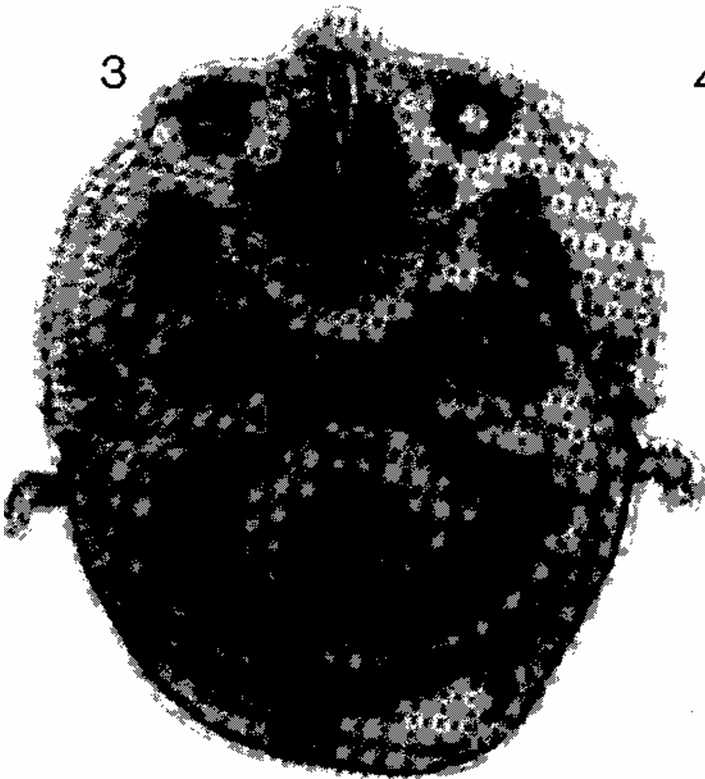
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J Int Soc Plastination, Vol 6:25-28, 1992

EARLY MORPHOLOGICAL CHANGES IN CHONDROMALACIA PATELLAE IN HUMANS - DEMONSTRATED WITH THE PLASTINATION METHOD

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SUMMARY

Twelve human cadaver knee joints with long standing retropatellar knee pain were plastinated and examined for chondromalacia. Early degenerative changes in the hyaline cartilage and in the subchondral area were classified as to stage I or stage II chondromalacia. The plastinated slices were well-suited to visualize the morphology of both chondral and subchondral areas. For the first time, the origin of the idiopathic chondromalacia was detected in the subchondral area and not the cartilage as previously thought. These changes were demonstrated with the sheet plastination method, they were not seen with arthroscopy.

Key words: Chondromalacia patellae, Plastination.

INTRODUCTION

Chondromalacia patellae is a common orthopaedic disease of often unknown origin, which is defined as degenerative changes of the retropatellar hyaline cartilage. In its etiology both endogenous and exogenous factors are discussed in the recent literature (Puhl et al., 1971; Goodfellow et al., 1976; Ficat and Hungerford, 1977; Bjorkstrom et al., 1980; Dick et al., 1980; Zippel and Wei/3, 1981). These factors are summarized in Table 1. The arthroscopic changes of chondromalacia patellae have been classified by Outerbridge (1961) into 4 stages (I - IV). Stage I - Decrease of elasticity and swelling. Stage II - Fissuring and fragmentation of up to 1.3 cm in diameter. Stage III - Fissuring and fragmentation of more than 1.3 cm in diameter. Stage IV - Ulceration and erosion of the cartilage down to the bone. However, despite severe pain and positive clinical signs on examination, both radiographic and arthroscopic examination often fail to detect any abnormality in many cases.

Only a few reports trace the origin of this disease into the subchondral area (Harrison et al., 1953; Brookes and Helal, 1968; Waisbrod and Treimann, 1980). However, they do not demonstrate the changes morphologically as changes are difficult to demonstrate via classic histopathology techniques. Using the sheet plastination technique (von Hagens, 1979 and 1987), the relationship between the structure of cartilage, bone and vascularization are documented. This method allows investigation down into the microscopic range (Graf et al., 1988; Graf et al., 1989; Graf et al., 1991).

MATERIAL AND METHODS

Twelve human cadaveric knee joints with a clinical history of long standing retropatellar knee pain were examined. The mean age was 47 and the ratio of females to males was 1:2. Knee joints were harvested up to 12 hours after death and prepared for sheet plastination. Six specimens had a smooth cartilage surface and were macroscopically free of disease. The other six specimens had smooth cartilage and no true lesions, but with brownish discoloration. Also, one knee joint without macroscopic degenerative changes or a history of retropatellar pain was processed as a control (Fig. 1).

After dissection of the femoral artery and vein, a catheter was inserted into the artery and a 10:1 mixture of epoxy resin (BIODUR E 20/E 2) was infused into the artery using a continuous pressure of 130mmHg. No fixative was used. After freezing at -70 °C, the specimens were cut into 2mm thick slices on a band saw, dehydrated by immersion in cold acetone, and plastinated in sheets using the draining technique as described by von Hagens (1979, 1985). The plastinated sections were placed between polyester foils and glass plates and allowed to cure. After curing, the slices were examined both grossly and with a microscope.

RESULTS

Similar to Outerbridge (1961), our morphological findings were categorized into four stages. Six knee

joints had lesions descriptive of *Stage 1,* but we were able to subdivide stage I into two substages based on morphological changes seen at the transition from cartilage to bone. *Substage la_:* Three knee joints had lesions which were classified in this substage. In this early stage, no chondral defects were observed. The sole abnormality was found in subchondral area where increased vascularization and subchondral sclerosis were observed. The tidemark between calcified and uncalcified tissue became hazed and irregular in outline (Fig. 2). *Substage [b_:* Three knee joints had lesions which were classified in this substage. While retaining its surface relief, the cartilage displayed streaky and spotty discoloration. There were changes at the bone-cartilage border, a zone of disruption in the tidemark area, subchondral sclerosis and increased ingrowth of vascular buds. (Figs. 3 and 4)

Stage //: Two knee joints were identified with lesions in this stage. Destruction of the surface continuity by fibrillation of the articular cartilage was observed. The transition at the bone-cartilage border was ill-defined. The subchondral sclerosis and the increased ingrowth of vascular buds became progressively more pronounced.

Stage III: One joint had lesions of this type. The persistent fissuring of the cartilage lead to the development of ulceration, which extended as far as the tide-mark. Here again, there was subchondral sclerosis and increased ingrowth of vascular buds.

Stage IV: Three knee joints had lesions which were classified in this stage. The morphological findings of this stage were identical with those of retropatellar arthrosis including a decrease of cartilage with defects extending down into the bone.

DISCUSSION:

The etiology in most of cases of chondromalacia is known, i.e. traumatic, dysplasia (Wiberg I-III), and endocrine disorders (gout, diabetes, hypothyroidism).

In cases of unknown origin, we postulate the onset of the degenerative change commences in the subchondral area. In addition to Outerbridge's classification, we subdivided stage I: into a and b substages. Typical findings of *stage la_* were subchondral sclerosis with the absence of cartilaginous changes. The tidemark, which normally is clear cut and well defined, becomes irregular and ill defined. *Stage Ib.* shows, in addition to the findings of stage la, degenerative changes of the cartilage with spotty and streak-like discoloration. These degenerative changes correspond to the fibrils that become unmasked in the histological specimen. The chondral surface in both stage la and stage Ib remains intact and hence, these early changes of chondromalacia can not be visualized by clinical or radiological methods. In neither substage has the early vascular changes in the subchondral bone been documented using conventional histopathology.

The sheet plastination method is presently the only known method to demonstrate these parallel occurring changes and enable to subdivide the lesions into substages la and Ib. Therefore, since sheet plastination of the subchondral area reveals these early changes, this method is suited for detecting early subchondral changes. A study is planned for the future which will use magnetic resonance imaging (MRI) as a mechanism to study knee pain of early chondromalacia patients. The study will attempt to identify and characterize the origin of chondromalacia in the subchondral region via MRI when abnormalities are not seen via arthroscopy.

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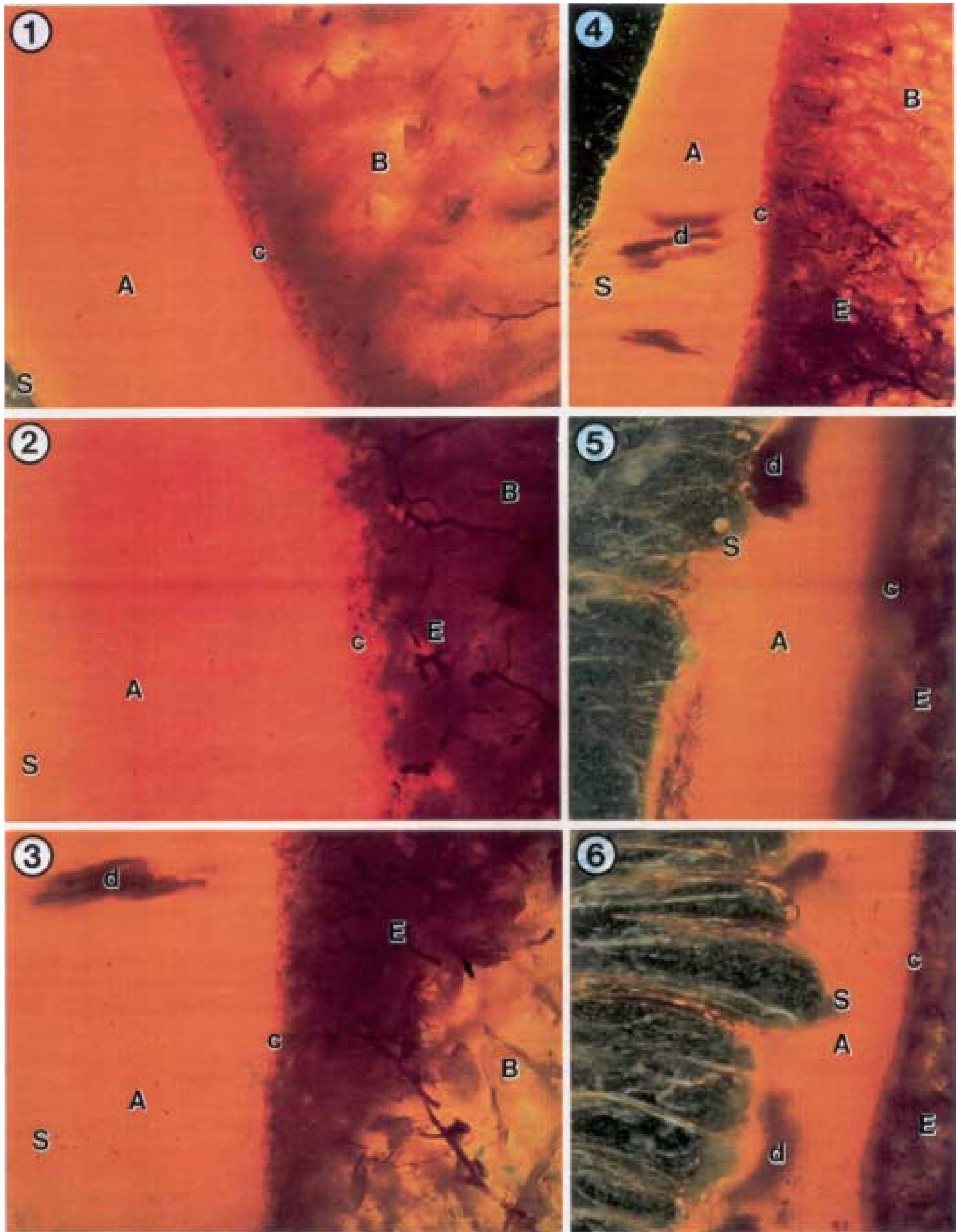
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LEGENDS FOR COLOR PLATES ON FACING PAGE:

Figure 1. Photomicrograph of a sheet plastinated slice of a control knee showing no degenerative changes. A - Cartilage, 6 - 6one, c - Tidemark, S - Surface of hyaline cartilage. 10X.

Figure 2. Photomicrograph of a sheet plastinated slice showing early lesions of Stage la in the subchondral area. A - Cartilage, B - Bone, c - Tidemark, E - Increase of subchondral vascularization and sclerosis, S - Surface of hyaline cartilage. 15X.

Figure 3. Photomicrograph of a sheet plastinated slice showing lesions of Stage Ib in the subchondral area, as well as, at the bone-cartilage border. A - Cartilage, B - Bone, c - Tidemark, d - Degeneration of cartilage, E - Increase of subchondral vascularization and sclerosis, S - Surface of hyaline cartilage. 15X.

Figure 4. Photomicrograph of a sheet plastinated slice showing lesions of Stage Ib in the subchondral area, as well as, at the bone-cartilage border. A - Cartilage, B - Bone, c - Tidemark, d- Degeneration, E - Increase of subchondral vascularization and sclerosis, S - Surface of hyaline cartilage. 6X.

Figure 5. Photomicrograph of a sheet plastinated slice showing Stage II lesions in both the cartilage and subchondral areas. Note the degeneration (d) and destruction of the surface (S) continuity of the hyaline cartilage and disruption of the tidemark (c). A - Cartilage, E - Subchondral sclerosis and Vascular buds of the bone. 15X.

Figure 6. Photomicrograph of a sheet plastinated slice showing Stage III degenerative changes throughout. Note the degeneration (d) and ulceration of the surface (S) of the hyaline cartilage nearly to the bone. A - Cartilage, c - Tidemark, E - Increase of subchondral vascularization and sclerosis of the bone. 10X.

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Table 1. Listing of etiological factors of chondromalacia patella as found in the literature.

ENDOGENOUS FACTORS

Patellar tracking dysfunctions of constitutional origin explicable in terms of aberrant mechanics:

Dysplasias (Wiberg III + IV, patelte parva, patella partita, hypoplasia of the tibial femoral condyle).

Malpositions (lateralization, patella alta).

Dysfunction of the cartilaginous metabolism: Change in the synovia or synovial fluid (infection, primary chronic polyarthritis, gout, diabetes etc.)

Endocrine disregulation (hormonal abnormality, hypothyroidism).

Circulatory disorders.

EXOGENOUS FACTORS

Irregular loading due to disturbed articular mechanism.

Lesion of meniscus or ligament.

Step formation on the posterior aspect of the patella after fractures.

Recurrent luxations of the patella.

Macrotraumas of the patellar cartilage:

Contusion, avulsion.

Structural destruction due to free cartilaginous fragments in the joint, osteochondral fractures or iatrogenic factors.

Microtraumas due to acute or chronic overloading (sport, occupation, weight).

J Int Soc Plastination, Vol 6:29-33, 1992

SHEET PLASTINATION OF THE BRAIN - P 35 TECHNIQUE, FILLING METHOD

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INTRODUCTION

Sheet plastinated specimens have the highest acceptance of preserved tissue slices. Two techniques are available: Biodur P 35, a polyester compound, is used for brain sections, while Biodur E 12, an epoxy compound, is used for whole-body sections. The procedures are similar, but for the sake of clarity, each will be described in a separate article.

This article will describe the P 35 technique. With this technique, sections of the brain are more detailed, more durable, and easier to handle than previous techniques.

MATERIALS AND METHODS LIST

OF NECESSARY EQUIPMENT:

A vacuum chamber large enough to hold the flat chambers (described below); Manometer; Biodur Polymers: P 35 and A 9, Biodur cobalt accelerator (PB 11), Meat slicer; 2 UVA-lamps, Stainless steel or aluminum grids; Grid basket (HD 09 or HD 10); Square buckets or appropriate containers for storage and dehydration of specimens. To assemble each flat chamber: 2 Tempered glass plates (5mm) (HS 01), 2 Thin glass plates (1-2mm) (HS 03), 6mm gasket (HS 06), 4 small (HS 32) and 15 large (HS 30) fold-back clamps or 2 inch binder clamps, Plastic sleeve or hose (to serve as a funnel), and Pressure sensitive tape (von Hagens, 1989).

FIXATION OF BRAIN:

The brain should be fixed as a complete unit in a 10-20% formalin solution. Once the brain is thoroughly fixed it is ready for slicing.

SLICING THE BRAIN:

Flush the brain in tap water, for 4 hours, to remove the fixative. After determining the proposed plane of the sections, the first cut is made with a brain knife. A case with adjustable cutting levels and guide slots for the knife is helpful. After cutting the brain into two pieces, place one portion back into the water and lay a wet filter paper on the cut surface of the other portion. The filter paper keeps the specimen moist, serves as a support to cradle the slice when it is made, and assures that no pieces of the brain slice get lost. With scissors trim the excess filter paper to the outline of each brain slice. To help hold the various portion of the brain slices as a unit, any brain or in particular brains with abnormalities or injuries may be embedded in gelatin prior to slicing.

Once the brain is ready for slicing on the meat slicer, the brain is placed onto the sliding table with the filter paper covered end toward the fence (guide stop). The thickness of the cut is set to produce 4 mm thick slices. Your right forearm may rest on the sliding table while your right hand holds the brain firmly against the guide stop. Keep fingers out of the path of the blade. Using a smooth, even, motion, push the brain all the way through the rotating knife while the left hand supports and holds the resulting brain slice (filter paper side). The slice is placed onto a rigid stainless steel grid (filter paper side onto the grid). Subsequent slices are made in a similar manner, placing a piece of wet filter paper on the new cut surface and trimming to shape to each new slice. Each slice is placed on a grid. It is beneficial for each end of the grids to be bent up IOO". Therefore when stacked, the next tray rests on these bent ends providing a clearance of 4 mm over the brain slice, which assures that acetone and later the polymer have free access to the entire surface of each slice.

The grids with filter papers and slices are placed into a stainless steel basket (capacity of 12 grids is convenient). In order to provide maximum opening size and ease of handling the trays, the basket has no front wall, as well as, no top. A lid designed to cover the top and the front wall is secured to the basket with two long pieces of twine which will

serve as handles to allow the basket to be easily submerged and retrieved from the water, polymer mix, and acetone baths. Square polyethylene pails with lids are convenient in which to submerge the basket with the stacked grids and slices in water for storage until ready to proceed to the next step.

FLUSH THE BRAIN SLICES:

Fill the pail, containing the basket of slices on grids, with cold tap water and allow to flush for 4 hours via a slow flow of tap water from the bottom of the reservoir. Direct the water to the bottom of the pail via a hose.

COOL IN DEIONIZED WATER:

Fill a second pail with deionized water. Transfer the basket containing the slices into this deionized water. Because the brain slices are fragile, the basket should always be moved slowly and carefully. Store the brain slices overnight in the refrigerator (5*C) in the deionized water.

FREEZE SUBSTITUTION:

The next morning momentarily drain the slices and submerge in - 20°C cold 100% acetone. One acetone bath of adequate fluid/tissue ratio is sufficient to dehydrate the brain slices without the need of a second change. Four baskets of brain slices can be properly dehydrated in IOO liters of cold acetone.

IMMERSION INTO POLYMER:

Prepare a polymer mixture (100:2) of P 35 (resin) and A 9 (hardener). An accurate weight of both components is important. *Immersion 1:* After 48 to 96 hours of freeze substitution, transfer the brain slices into a cold (refrigerated) polymer mixture . Refrigerate and store specimens for 24 hours at 5°C. Subsequent first stage immersion may be done with remaining polymer mixture from *immersion* 2 of a previous batch. It is very important to conduct the transfer from acetone to the polymer mix with a quick but careful movement of the basket. The brains must be submerged as quickly as possible, to prevent drying and shrinkage. The exchange of acetone and polymer begins by diffusion.

Immersion 2: After 24 hours, transfer the specimens into a new or good polymer mix (100:2) which may come from the forced impregnation of the preceding batch of brains. The specimens remain in this polymer mix for 24 hours. When this stage is completed, the polymer mix from this stage may be used for *immersion 1* of a following batch.

IMPREGNATION:

Prepare a fresh reaction mixture of P 35 (polymer) and A 9 (hardener) (100:2 ratio) for forced impregnation. Combine and stir the mixture with a stainless steel whip for 5 minutes and then deaerate (degas) via vacuum. After the components are mixed, the mixture must be kept in the dark. After deaeration, transfer the basket with the brain slices into the new polymer mix at room temperature. Loosely cover the container with mylar (clear plastic) wrap and fasten the wrap to the container at a few points with clamps. The clear wrap protects from dust and impregnation can be observed through it. P 35 forced impregnation is conducted at room temperature, in the dark, and takes at least 21 hours. It is convenient to start the vacuum at 11:00 a.m. and evacuate to 55 mm Hg. After this vacuum is achieved, increase the vacuum hourly until the vacuum is stabilized at 20 mm Hg by 5:00 p.m. The most important mechanism for regulation of vacuum is to keep the acetone evaporating steadily with penny-sized bubbles. For example, when impregnating 12 human brain slices, 10 bubbles at a time is not too many. Expect fewer bubbles with less and/or smaller specimens. The next morning the pressure will likely be around 12-15 mm Hg and no acetone bubbles will be observed. Trial and error is the only mechanism for proper regulation of your specific vacuum unit. Do not evacuate below 12 mm of Hg. The polymer contains styrenes which volatilize at 10 mm Hg and will harm the pump oil (von Hagens, et al, 1987).

PREPARATION OF "DOUBLE GLASS PLATES":

Tempered glass plates of 5 mm and 1-2 mm (window glass) thickness are used to make each side of the flat chamber (mold) for the slices. The thin glass plate is flexible so that the plastinated sheet will release from the glass after it has hardened. However, by itself, the 1-2mm glass is too flexible and will bulge out when filled with resin. The 5 mm glass makes the flat chamber more rigid and prevents bowing out. The mold is prepared by combining the two sets of the glasses as follows: 1.) Place a 5 mm plate on styrofoam or a pad to protect from breaking and scratching. 2.) Place a 1-2 mm plate on the 5 mm plate. On the side away from you, secure the

two glass plates together with 2 fold-back clamps (Figure 1). At the front, seal the gap between the glass plates with a piece of removable adhesive tape. Two of these units must be prepared for each slice. In order to prevent scratches on the glass, store the plates with strips of plastic between them and cover to keep dust free. The double glass plates may be assembled before the casting day.

CASTING:

The assembly of the flat chamber (casting mold) is commenced by positioning a "double glass plate" on a styrofoam pad with the thin glass up and the adhesive tape away from you. Remove the clamps, which are on the side toward you. Center the 6 mm gasket, whose length is just shorter than the perimeter of the double glass plate, along the length of the thin glass plate (on the side next to you) 3 cm from and parallel to the edge of the glass plate (Fig. 1). Put disposable polyester gloves on and with a pair of pliers, lift a grid and brain slice out of the P35 bath. Grasp the grid with the free hand, lay the pliers down, and hold the grid with both hands. Let the excess P35 drip into the polymer container. Then quickly bring the grid over the glass plate and turn it over so that the specimen lands on the glass plate. Place the empty grid into a storage pail, along with the lid of the basket and the pliers. With a pair of thumb forceps, remove and dispose the filter paper. Remove the gloves. Place two small pieces of gasket (4 cm) near the rear corners of the glass plate. Check the specimen to make sure that it is all there and arranged properly. Place the second double glass plate, thin glass toward specimen, on the specimen, with the adhesive tape away from you (Fig. 2). Remove the fold-back clamps. Align the gasket and make sure that the "double plates" are exactly one over the other. Place a row of fold-back clamps along the front edge of the glasses which will hold both double glasses against the gasket. Place the gasket, hanging over each end, into the mold, parallel to the ends of the glass plates, remove the two small pieces of gasket at the rear corners, and secure the gasket at each end with a row of clamps. The ends of the gasket are now hanging over the side which is away from you. The mold is ready for casting.

FILLING THE MOLD:

Fold the clamp arms back onto the glass plates. Stand the mold upright (taped side up) in a rack or by using two larger clamps as supports. Insert a plastic sleeve (hose) into the gap between the double glass plates (Fig. 2) and secure the sleeve with small clamps. The sleeve serves as a funnel. Fill the flat chamber with fresh polymer mix (P35/A9) (100:2), about 600 cc. Remove the plastic sleeve and place the filled unit in a rack. To allow trapped air bubbles to rise to the surface, spread the gap between the double glass plates using a stack of 6 or 7 tongue depressors (blades) or a small block of wood. Also, use a depressor to keep the brain slice submerged. Store the unit, for 30 minutes, in a darkened area (to prevent early curing of the sheet) to allow the trapped bubbles to rise. Some bubbles will cling to the specimen and must be removed manually. Modify a few pieces of I mm gauge stainless steel wire, by bending the ends into an eye or hook which will be used to move the bubbles to the surface. A thicker (3-4 mm) rod is useful to position (center) the specimen in the mold for curing. Supporting the flat chamber at a small angle from the vertical, allows the correction work to be done with ease. It is necessary to check for bubbles on both sides of the brain. After the bubbles have been removed and the slice centered, remove the tongue blades and insert the end portions of the gasket into the gap at the top of the unit. Clamp the top of the mold (Fig. 3) and the sheet is ready for curing.

CURING:

Curing of P35 is initiated by UV-light (long-waved UV light, tanning lamps) and completed in a 45 °C oven. *Light Curing:* The UV light source must be applied to both sides of the flat chamber. Make sure that no polymer drains out of the mold and that all clamp levers are folded back. The glass plates must be clean and scratch free to avoid photo effects. This curing process is very exothermic. Therefore, it is necessary to disperse the heat with a fan or blower. It is beneficial to devise a manifold to direct the air stream onto the sheets. After 45 minutes of UV exposure, curing is complete except for the center of the slice. To harden the center of the slice, it is necessary to use heat. *Heat Curing:* The flat chambers are placed in a 45 °C oven for 5 days. Lower temperatures won't activate the peroxide hardener and higher temperatures may cause white spots to appear in the specimen.

DISMANTLING:

After 5 days, the flat chambers are removed from the oven and most of the clamps are removed. A

few clamps are left in place to secure the unit's integrity. Store the sheets upright in a rack for cool down to room temperature. During the cool down, cracking sounds are heard as some of the plastic sheets separate from the glass. However, not all sheets will separate by themselves and it will be necessary to gently spread the glass plates. Sometimes, a scalpel may be useful to initiate the separation. When taking the sheet from the glass, it is important to prevent traces of non-cured polymer from getting on and sticking to the sheet. As the sheet (specimen) is removed from the flat chamber, it should be covered with adhesive plastic wrap for protection.

TRIMMING:

The excess plastic sheet may be cut away from the perimeter of the specimen. It is beneficial to mark the intended saw line prior to sawing. A table, radial, or band saw is adequate. It may be beneficial to make a preliminary cut about 1 cm outside of the intended final circumference. For final cut, use the sharpest blade and use a slow speed. The slower speed will produce neater edges. After sawing, the edges often are sharp and need to be sanded after the protective film has been removed. A belt sander with a water bath is ideal. After the brain sheets have been sanded, they are dusty and need to be washed. Do not use water over 50°C as white spots may be produced. The warm water releases all tension which is produced on the sheet by sawing and sanding. The sheet is now ready for use.

CLEANING THE GLASS PLATES:

The thin glass plates have a polymer residue which is softened and partially removed by washing the plates with detergent in a dishwasher at 85°C. It is beneficial to interrupt the washing cycle after 10 minutes and manually remove the polymer which has been softened by the heat. Otherwise it is necessary to wash the glass several times.

DISPOSAL OF OLD POLYMER:

After a polymer bath has been used for impregnation and two immersion baths, it has to be discarded after it has been hardened. Harden the polymer by stirring in Cobalt promoter (0.5%) and transfer the polymer/cobalt mixture into old cans and keep under the hood. The reaction causes excess heat and fumes. No flammables are to be within the hood and plywood or an insulation pad should be placed under the cans. After the mixture has hardened and cooled, it may be discarded.

Practical hints: The sheet may be labeled by engraving the names of featured structures on the plastic surface. The sheets are easily scratched, therefore, it is advisable to protect them with adhesive plastic wrap prior to student or extended usage. The wrap slightly impairs the visual quality.

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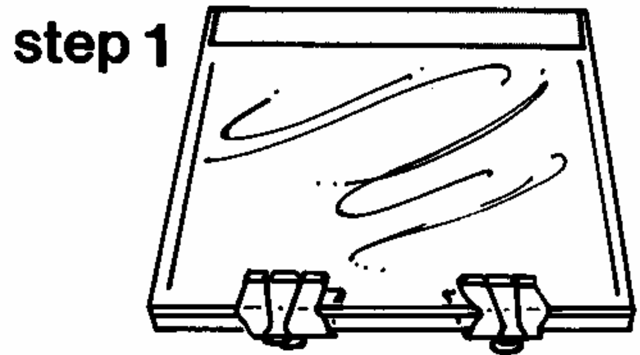
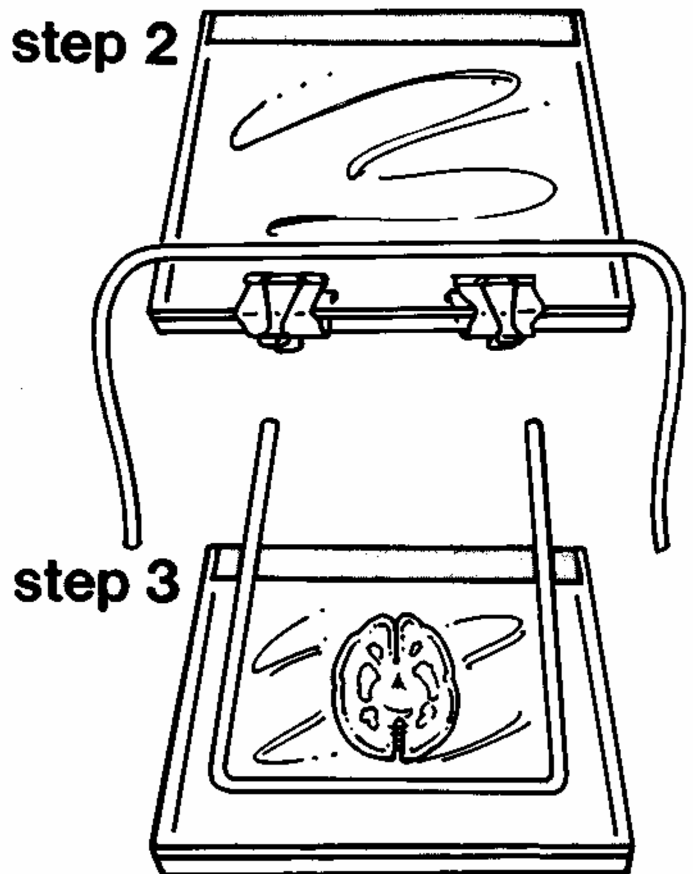
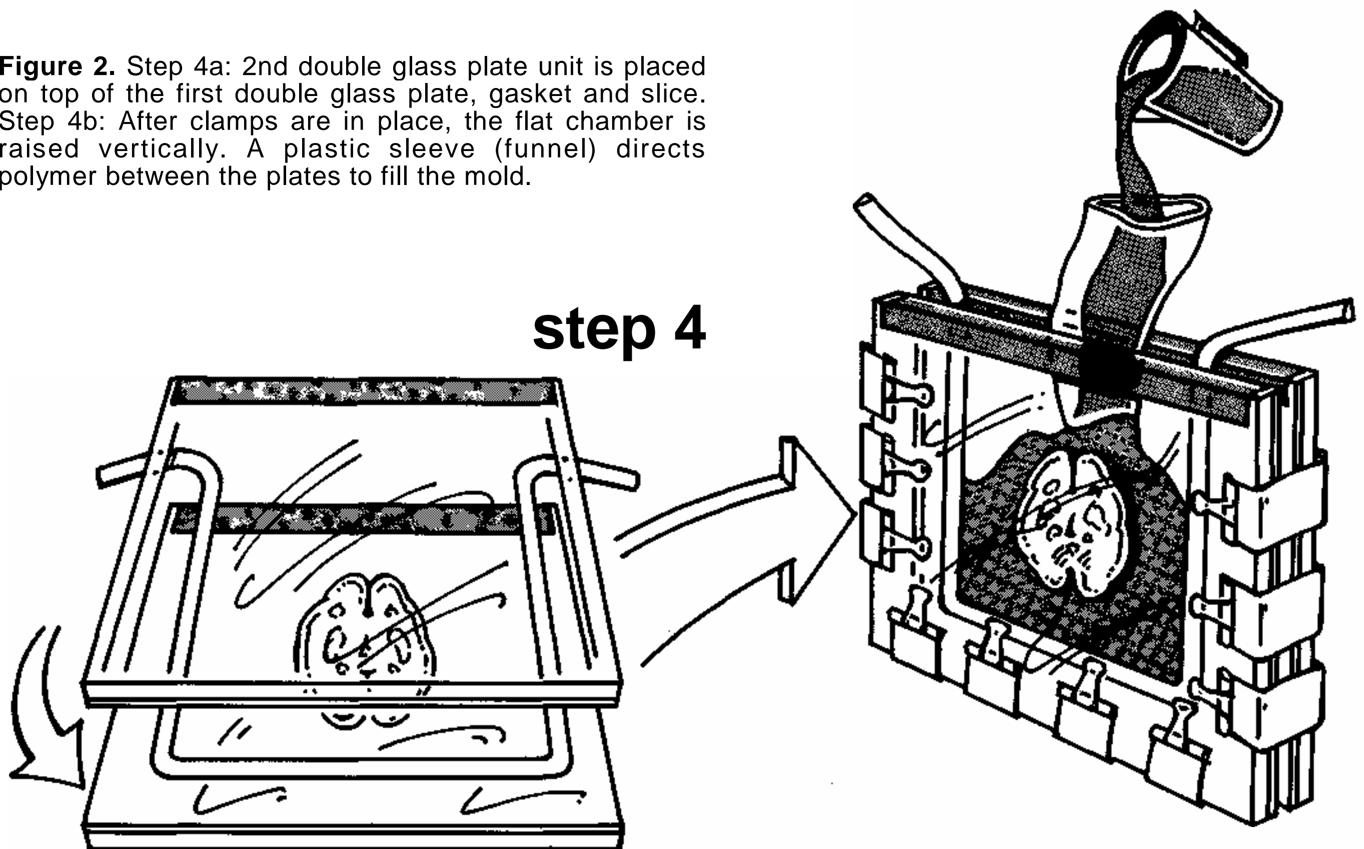


Figure 1. - Step 1: Assembly of double glass plates using



tape and clamps. Note the thin (2 mm) plate is on top. Step 2: Gasket is placed on thin plate. Step 3: Gasket is adjusted and brain slice is placed on thin glass plate side of the double plate.

Figure 2. Step 4a: 2nd double glass plate unit is placed on top of the first double glass plate, gasket and slice. Step 4b: After clamps are in place, the flat chamber is raised vertically. A plastic sleeve (funnel) directs polymer between the plates to fill the mold.



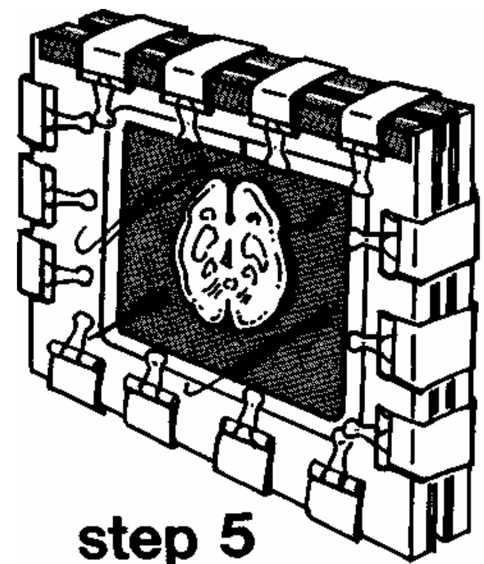


Figure 3. Step 5: The top of the gasket has been tucked in and clamps placed to seal the top of the mold.

ACKNOWLEDGMENTS

Thanks to Carol Haynes of the CVM Art Department, The University of Tennessee for the excellent drawings.

THE USE OF EXPLOSION PROOF FREEZERS IN PLASTINATION: ARE THEY REALLY NECESSARY?

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SUMMARY

At first it was suspected that personnel safety might be a drawback of plastination with increased exposure to toxic chemicals for those involved with preparing and processing the specimens. The data from this study suggests that health and safety are not a major problem as long as proper precautions as described above are observed.

It is recommended, however, that all precautions be taken to minimize the risk of explosion. Although not considered optimum protection it is possible to modify domestic deep freezers, by removal of motors, compressors and lights to achieve some degree of protection.

Despite our results showing the danger of explosion to be minimal, it should be noted that optimal conditions are used throughout our laboratory. Since these optimal conditions are not always possible, it is wise to modify the freezer as suggested by Gubbins (1990).

INTRODUCTION

In the short period of its existence, plastination has proven its superiority over older methods for preservation of biological tissues (von Hagens, 1985). Despite having been adopted by many institutions around the world, the cost for the implementation of a laboratory destined to produce medium and large sized specimens is nearly prohibitive, especially when budget funds are limited.

One primary concern regarding equipment cost is the requirement for explosion proof freezers, that on the average cost $8,000.00. Are these expensive freezers really necessary?

While plastination reduces exposure of students and instructors to formalin, the plastination process itself may add some safety and health concerns to those preparing the plastinated specimens. These concerns are the possible exposure of employees to acetone, various resins and curing agents, as well as, the danger of explosion in the dehydration and curing phases. In explosions, not only are employees at risk but also any resources that might be in the building or adjacent buildings.

For the purposes of this study, an initial hazard review was conducted to determine what hazards might be present during the plastination process. From this prospective, the various steps of the plastination process were evaluated to: 1. determine where exposures to toxic chemicals might occur, 2. quantify possible exposures to hazardous chemicals, and 3. measure flammability of some of the chemicals.

MATERIALS AND METHODS

For the purpose of determining what chemicals would be present during the plastination process, approximately 30 mg of Biodur S10/S3 resin mixture was taken from the vacuum chamber and 10 ml of Biodur S6 (gas cure agent) from storage and both were analyzed by gas chromatography and mass spectrophotometry. The components found were: acetaldehyde or ethylene oxide, ethanol, acetone, xylene, and possible ethylbenzene and tetraethyl silicate.

Various methods were used to determine if ambient concentrations of the various chemicals exceeded levels that are considered safe. These methods included using sorbent media, flammable/toxic/oxygen (triple) gas meter, and an infrared spectrophotometer to collect average ambient concentrations of vapors during different phases of the plastination process. The triple and infrared instruments were of the direct reading type, and the sorbent media required subsequent description and outside laboratory analysis.

The recommended methods of sampling and analysis as set forth by The Occupational Safety and Health Administration and National Institute of Occupational Safety and Health are summarized in Table I.

EXPOSURE MONITORING:

Our vacuum system is setup to exhaust directly into the room air, at approximately 1.5 meters from the work bench. All samples for chemical concentration analysis of the room air were collected just above the work bench. Unless otherwise stated all measurements reflect the concentration of the chemical being manipulated plus those released through the exhaust outlet of the vacuum pump.

Chemicals:

Formaldehyde: The analysis for formalin was done when the specimens were removed from the sealed

bags of fixative and placed in running water for twenty four hours. Sampling was conducted for formaldehyde only and not methanol because the methanol is present in lower concentrations and is of a much lower toxicity.

Acetone: Sampling for acetone was conducted ambiently, at the work bench site, when the specimens were transferred to more concentrated solutions of acetone. To estimate the quantity of acetone vapors that would be released when the freezer is opened, sampling was conducted inside a deep freezer, with the door closed. The freezer contained five containers, each with 10 liters of acetone and two carboys with 20 liters each. Also, the pump exhaust was sampled at the work bench site.

Xvlene: Sampling for xylene was conducted ambiently when the specimens were in the Gas Cure Phase. A cloth saturated with xylene is utilized to wipe the excess polymer from the surface of the curing specimen. This procedure aids in reducing the gloss of the specimen.

Resin components: During forced impregnation, air samples were taken above the work bench to check for the potential release of resin components into the air. During the gas cure stage, samples were taken above the curing chamber.

Equipment:

Sampling was conducted using personal air sampling pumps (SKC Hall 224-43XR) at sampling rates, times and sorbent in media filled collecting tubes as specified by the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. These agencies also recommend methods for analysis (Table I). The sampling media used were two-section sorbent tubes with various media for collection. The first section is designed to collect the contaminants. The second section is used to determine if the first section breaks through to the second section. If so, the results were considered to represent an estimate of the minimum concentration of analyte present in the sampled air. Upon analysis using varied methods (depending on the contaminant), the amount of contaminant detected was divided by the quantity of air to yield the ambient concentration of the contaminant in air during the sampling period. The sample pumps used were calibrated before and after sampling using a primary calibration source (detergent and water solution in a volumetric burette). The burette was inverted and the soapy solution emptied into a beaker. The sample pump was connected by tygon tubing to the top end of the burette and the other open end of the burette was touched to the surface of the beaker of soap water. Bubbles from the soap film move into the burette with the flow of air created by the sample pump. The sampling rate of the sample pump was calculated by the volume of bubbles which passed into the burette divided by the time it took to pass.

The Miran infrared spectrophotometer was used to measure the level of tetraethyl silicate during the curing process.

FLAMMABILITY MONITORING:

Because of the flammable nature of some chemical products used in plastination it seemed prudent to monitor several phases of the process to determine the likelihood of flammable vapors accumulating. A Mine Safety Appliance (MSA) Passport Personal Alarm Monitor with combustible channel calibrated to methane and a Foxboro Miran infrared spectrophotometer were used to estimate vapor concentrations during the dehydration, impregnation, and curing phases. The MSA Passport was used to estimate if potentially explosive levels might be present in the room at the vacuum pump exhaust outlet and inside the deep freezer containing the acetone for dehydration. Air sampling was performed both at room air (above the work bench, at 10 to 30 cm from the vacuum pump exhaust) and also, inside the freezer with the door ajar 1 cm and with door wide open.

RESULTS

EXPOSURE MONITORING:

The concentrations of chemicals in the air measured above the work bench are shown in Table JI. The resultant values for the reported chemicals, correlate with the various steps of the plastination procedure and the constant exhaust of the impregnation pump into the room air. Acetaldehyde was found in an average concentration of 0.04 ppm (Table II).

The room concentrations of acetone vapors, during transfer of specimens, initially demonstrated a concentration of 0.6 ppm over a 78 minute period. However, over a prolonged period of sampling (420 minutes) the acetone vapors increased up to 6.4 ppm. While acetone concentrations inside the deep freezer from two different samples were 1900 ppm and 3800 ppm. Sample break through occurred in the sampling tube.

Ethanol levels were 2 ppm. Ethyl benzene average concentration was 0.046 ppm. Ethyl silicate (found in the gas cure) average concentration was 0.035 ppm. Formaldehyde levels were 0.20 ppm when measured over a 215 minute period. Xylene levels were 0.088 ppm.

FLAMMABILITY MONITORING:

The concentrations, expressed in % lower explosive level (LED, provide by the MSA Passport Monitor are shown in Table III. LEL is the lowest fuel

to air ratio which will support combustion and propagate flame (in the presence of an ignition source). Atmospheres containing up to 10% of the LEL are recognized as safe, within an ample margin of safety.

DISCUSSION

As judged by the data in the Tables, the resultant concentration of most products monitored were very low, in fact most were below the level of analytical detection. The exposure limits given in Table II are thought to be the highest levels that a person can be exposed to on a regular basis without health risk. Generally upper limits are designed to protect more sensitive individuals, as well as, the average person. Given the present exposure limits and the data collected, there appears to be little health risk during the phases of the plastination process which were studied, as long as, the proper precautions and techniques are followed.

Proper precautions include wearing personal protective equipment when there is a possibility for physical contact with any of the chemicals, including the proper respiratory protection when vapor concentrations may near or exceed normal limits. As a good work practice, all containers should be kept tightly sealed. When working with formaldehyde, it is best to use local ventilation or wear a respirator. Ethanol was used prior to dehydration to remove any remaining glycol from the embalming fluid. The specimens were immersed into a solution of ethanol, hydrogen peroxide and water for one week.

As shown in the results, the possibility of flammable concentrations of acetone vapors accumulating during the dehydration phase exists. We must emphasize that the readings of 3,800 ppm and 1,900 ppm should be considered as estimates since sample break through occurred in the 2-chambered sorbent tube. Break through is considered to have occurred when the concentration of analyte in the down stream chamber (chamber 2) is 5% of the upstream concentration (chamber 1). With sample breakthrough the reading is not as accurate because some of the chemical has been diverted into the second chamber (portion of the sorbent tube). The break through may have been due to the high concentration of acetone and the cold temperature. The 3,800 ppm reading occurred when the lids of the plastic containers did not fit tightly. When acetone was transferred to containers with better fitting lids, the concentration in the deep freezer dropped markedly to 1,900 ppm. These concentrations, when converted to percentage by volume of air, were 0.38% and 0.19% respectively. When % LEL were calculated for these two concentrations of acetone, the resultant 0.15% and 0.07%, are below the LEL (2.6%) for acetone, confirming the minimal flammability hazard during the impregnation phase. However, precautions should be taken.

As long as acetone vapors are maintained below zero degrees Fahrenheit, acetone vapors will not likely reach a flammable level. However, if the freezer should malfunction and the low temperature is not maintained, flammable levels of acetone can accumulate and the temperature or sparks from the compressor motor could cause a serious explosion. If vapors are allowed to accumulate in the freezer, it may even be possible for static electricity to ignite the acetone when the freezer is opened. As long as, the containers of acetone have a good seal and are airtight, acetone vapors cannot escape into the freezer and hence a household deep freezer may be considered.

Monitoring of acetone levels during the impregnation stage, suggests that with the pump used, flammable vapors probably do not accumulate around exhaust outlet of the pump. Consecutive readings of the monitor over a period of 25 minutes showed a converted % LEL of 2.3 which is far below the 10% LEL which is considered to be an adequate margin of safety. These results suggest that the flammability hazard in the vacuum pump is probably insignificant.

The vapors of tetraethyl silicate were measured during the gas cure phase. Levels found in the chamber were well below flammable levels, as would be expected, since the flash point of tetraethyl silicate is well above room temperature.

When working with chemicals that are toxic and/or have low flash points, it is prudent to limit the quantity of these chemicals present in the laboratory to only the amount needed for the present time or for a few days. For the obvious reason, larger volume equals larger spills which could result in greater personnel exposure. Greater quantities of flammable material in a given area are just more "rocket fuel" for a fire.

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Table I - Recommended methods of sampling and analysis as set forth by OSHA and NIOSH.

Contaminant of interest	Method Used	Collection Media	Flow Rate	Sample Ml/Min	Analysis Volume (L) Method
Acetaldehyde	OSHA 68	XAD-2	50	3	GC-NPD
Acetone	NIOSH 1300	Charcoal	20	2	IR
Ethanol	NIOSH 1400	Charcoal	50	1	GC-FID
Ethyl benzene	NIOSH 1501	Charcoal	50	10	GC-FID
Ethyl silicate	NIOSH 3 .	XAD-2	20	7	GC
Formaldehyde	OSHA 52	Xad-2	100	24	GC-NPD
Xylene	NIOSH 1501	Charcoal	50	2	GC-FID

GC: Gas chromotography.
GC-FID: Gas chromotography, Flame ionization detector. GC-
NPD: Gas chromotography, Nitrogen-Phosphorus detector. OSHA
- Occupational Safety and Health Administration NIOSH -
National Institute for Occupational Safety and Health.

Table II - Results of room air sampling conducted.

Contaminant of interest	Sample Duration (minutes)	Sample Rate (ml/min)	Results (ppm)	Recommended Exposure Limit (ppm)*
Acetaldehyde	255	101.70	0.04	100
Acetaldehyde	228	82.70	0.06	
Acetaldehyde	209	134.30	0.04	
Acetaldehyde	243	134.50	0.03	
Acetone (room)	78	25.80	0.60	750
Acetone (room)	420	18.90	6.40	
Acetone (freezer)	418	22.80	1900	
Acetone (freezer)	68	25.64	3800	
Ethanol	35	22.60	2.00	1000
Ethanol	48	15.50	2.00	
Ethyl benzene	246	43.00	0.04	
Ethyl benzene	173	50.10	0.05	
Ethyl benzene	201	50.00	0.05	
Ethyl benzene	205	49.60	0.05	
Ethyl benzene	211	55.70	0.04	
Ethyl silicate	355	20.10	0.06	10
Ethyl silicate	207	44.30	0.04	
Ethyl silicate	245	31.90	0.04	
Ethyl silicate	172	58.80	0.00	
Formaldehyde	215	100.0	0.20	1
Xylene	173	50.10	0.10	100
Xylene	201	50.00	0.09	
Xylene	211	55.70	0.08	
Xylene	246	43.00	0.08	
Xylene	205	49.60	0.09	

"Recommended exposure limits are those adopted by the American Conference of Governmental Hygienists, specifying a maximum time weighted average concentration, or Threshold Limit Value, under which it is believed that nearly all workers may be repeatedly exposed, day after day, with no adverse effects.

Table III - Percentage Lower Explosive Level (LED for acetone measured in the room air, inside freezers, and around the vacuum pump exhaust outlet.

Area Sampled	% LEL Direct Reading	Converted
Room Air	0.0	0.0
Vacuum pump exhaust outlet	2.12	2.30
Inside freezer (door ajar)	0.0	0.0
Inside freezer (door wide open)	0.0	0.0

* Time weighted average reading in 25 minutes. The measurement at the vacuum pump exhaust outlet was made between 10 to 30 cm from the outlet. During the air sampling, there were approximately 30 brain slices in the impregnation chamber freezer. Five containers with approximately 10 liters of acetone in each, plus 2 carboys of 20 liters of acetone were inside the dehydration freezer.

SILICONE TRACHEOBRONCHIAL CASTS

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INTRODUCTION

Preparation of beautiful and anatomically precise tracheobronchial casts have an inherent teaching value. To actually see the replica of the airway instead of merely hearing a description of it or only seeing a figure rendered by an artist is an invaluable teaching aid. In addition to education, casts have been used for morphometric studies (Weibel and Gomez, 1962; Horsefield and Gumming, 1968; Pump, 1969). Some previously used resins produce brittle specimens which often shatter and may be totally destroyed if dropped (Tucker and Krementz, 1957). Frank and Yoder (1966) introduced the use of silicone for forming casts. Kilpper and Stidd (1973) devised a complicated method for filling the airways in a wet state to prevent shrinkage artifact, while Phalen and coworkers (1973) introduced making silicone casts of the airways in situ. We have chosen to use air-dried lungs, similar to Wang and Kraman (1988), to produce the casts. There are several manufacturers of polymer and numerous polymers to chose from. The properties of the polymers are just as diverse. Comparisons of the properties of polymers from the various manufacturers are available but not totally accurate. Five polymers of varying properties were used to make tracheobronchial casts.

METHODS

Fresh cat, dog, horse, pig and ox lungs were procured from local abattoirs and from the necropsy laboratory. The heart, esophagus, any other mediastinal tissue and fat were separated from the lungs and tracheobronchial tree similar to McKiernan and Kneller (1983). A cannula of the appropriate diameter was ligated in the trachea. A water source was attached to the cannula and the lungs gently inflated to near capacity. After filling, the water source was removed and the water, mucous and blood allowed to flow out of the trachea. This flushing procedure was repeated 6 to 10 times, until most of the blood and secretions were cleared from the lungs and airways. The surface of the lungs was kept moist during the flushing period. After the majority of the water had drained from the organ, the lungs were inflated and dried using compressed laboratory air directed into the trachea. Smaller lungs were suspended from the trachea and larger lungs were placed on their dorsal (posterior) surface on a tray to contain the extravasated fluid. The air flow was gradually increased until the lungs were inflated and remained inflated to near capacity. Flow and inflation were maintained until the lungs were thoroughly dried (Henry and Butler, 1990).

After drying was complete, the lung was suspended in a vertical position. Aided by an appropriate size funnel, a selected polymer was poured into the trachea and allowed to flow into the lung via gravitational forces. Five polymers were used and two or three casts were made of each polymer. 1. Biodur - S/10/S3/S2 mixture [Biodur Products, Dr. Gunther von Hagens, Rathausstrasse 18, Heidelberg, D-6900 GERMANY]. 2. Dow Corning - Silastic E RTV [Dow Corning Corp., Midland, MI 48640-0994, USA]. 3. General Electric - RTV 11 [General Electric Co., Silicone Products Div., Waterford, NY 12188, USA]. 4. & 5. Rhone-Poulenc - 2 different Rhodorsil Silicones: 4. Rhodorsil RTV II - # 1556 and 5. Rhodorsil RTV II - # 1547 [Rhone-Poulenc, Inc., Specialty Plastics Division, CN 5266, Princeton, NJ 08543-5266, USA]. All product components were combined and mixed using the manufacturer's recommendations. Hardener/polymer ratios were 1:10, except Biodur which was 1:100 (S3:S10) but the S2 was varied from 0.5:100 to 2:100 in 0.5 increments. Approximate quantity of polymer used was .2 - .5 kg/ 20 kg dog, 1-2 kg for adult pig, horse or ox. In larger specimens before the silicone hardened, a heavy wire was placed into the lumen of the trachea to give support to the silicone cast. The polymer mixes were allowed to harden over night. The next morning, the lung unit was placed in a vat of simmering to slowly boiling water for several hours.

The specimens were checked in four to five hours to determine if most of the lung and airway tissue had been macerated away from the cast material. Once most of the tissue had been loosened or removed, the cast was removed from the hot water and sprayed with a high pressure hose to remove much of the remaining tissue. If tissue was not removed with the high pressure water, the cast was submerged either in hot water again or in a 10% sodium hydroxide solution until the remaining tissue was released. The specimens were rinsed with water and allowed to dry before use.

RESULTS

All five polymers produced representative casts of the airways or portions of the airways. The more viscous polymers flowed into the terminal bronchi (Fig. 1) and occasionally into the alveoli. The less viscous polymers flowed freely into the alveoli, thus filling the entire airway (Fig. 2). S2 ratios of 1.5 - 2:100 (S10/S3 mix) caused the polymer to harden too quickly, only allowing filling of the larger bronchioles, while casts of S2 0.5:100 ratios remained tacky. The Biodur mix (0.5-1.0:100) consistently was the least viscous and entered the distal airways. The Rhodorsil products usually entered the distal airways. About 30% of the time, Silastic E RTV reached the terminal airways. The GE polymer seldom reached the alveoli. Biodur, Silastic E, and Rhodorsil 1556 produced specimens which had the most elasticity. The GE, Silastic E and Rhodorsil 1547 casts maintained the shape of the lung best.

DISCUSSION

All six polymers produced durable, representative casts of the airways. The cranial most portion of the cranial (superior) lobes did not fill as uniformly as the caudal (inferior) lobes. This was due to the lungs hanging via the trachea and the least gravitational force was to this area. The type cast which is to be produced must be decided before starting the project. Polymers low in viscosity flow into the alveoli thus filling nearly the entire volume of the lung, whereas more viscous polymers present a more clear view of the branching pattern of the bronchi because less polymer enters the distal airway. Viscosity of the polymer mix may be increased by increasing the time interval between combining the polymer and hardener and actually pouring the mix into the lungs. Therefore, distal airway filling can be decreased or eliminated by waiting 15 minutes to one hour before filling the airway. Thus a cast is produced which allows better visualization of the branching pattern of the airways. All polymer mixes benefited by having a stiff wire inside the tracheal cast for support. The tracheas with the stiff wire insert were better suited to support the airways in a horizontal position, however, steel wire rusted during the maceration process. To prevent rust, a galvanized wire should be used rather than a steel wire. Cost of various polymers is similar, $35.00/kg. Recently, we have used "general household sealant" a 100% silicone rubber product in a caulking tube by General Electric Corporation. The results look promising, especially considering the lower cost, $10.00/kg.

ACKNOWLEDGMENTS

A special thanks to Kreis Weigel and Dr. M. McCracken for help with the photography.

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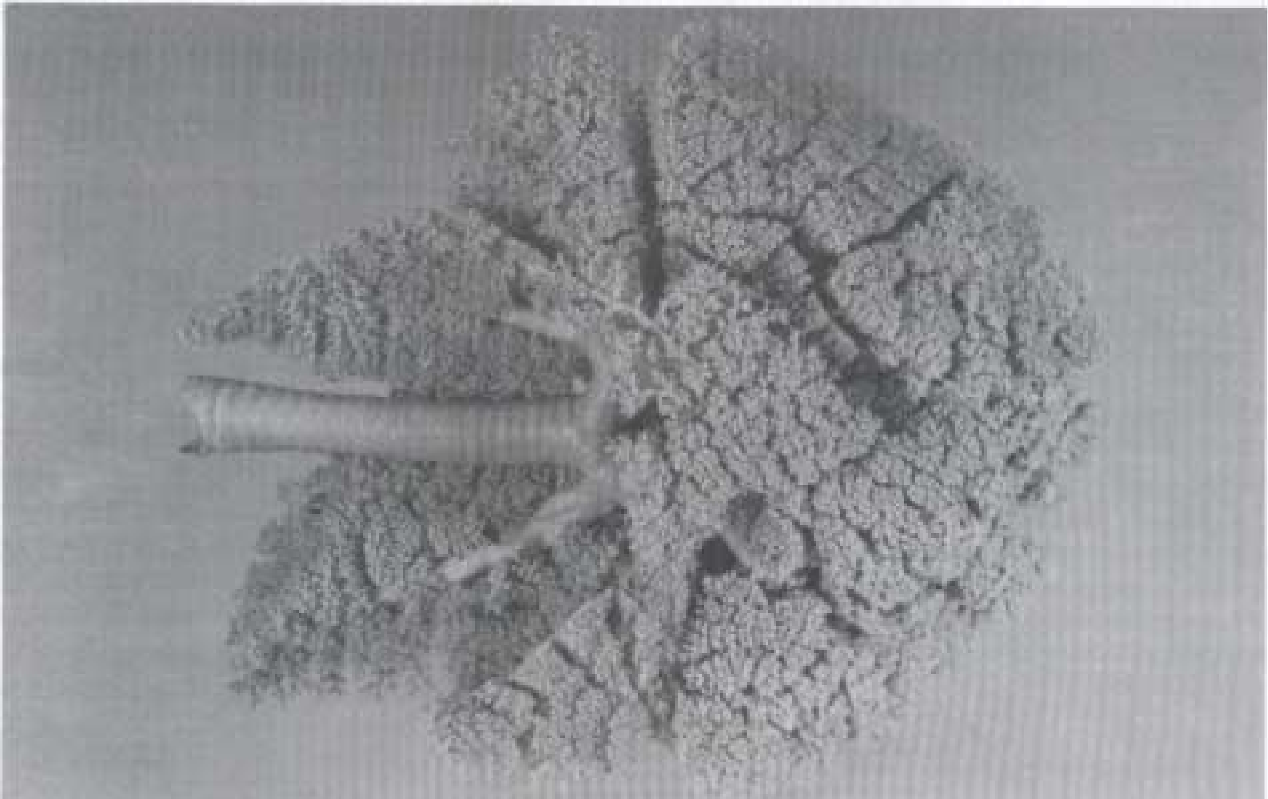

Figure 1. Silicone tracheobronchial cast from a dog. The airways are of # 1547 Rhone-Poulenc polymer mixture (10:1). Note, the airways are filled distally into the alveoli.

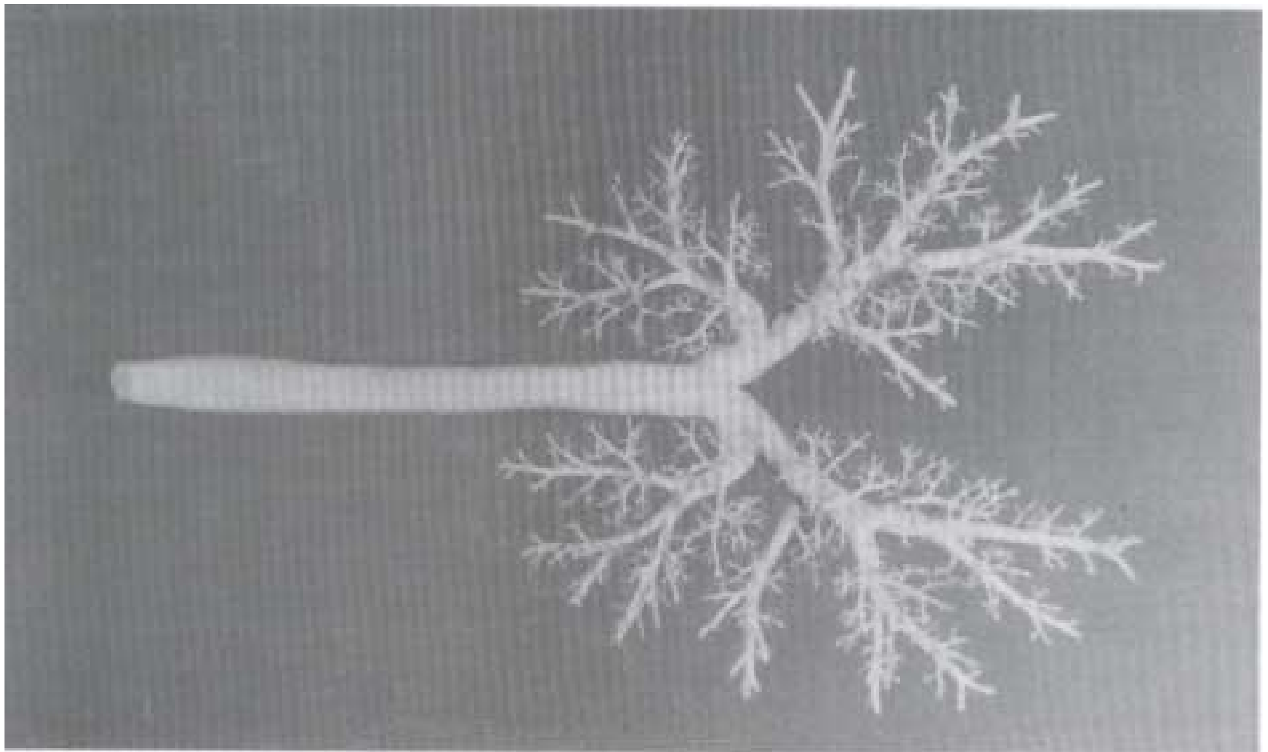

Figure 2. Silicone tracheobronchial from a dog. The airway cast is made of Silastic E RTV polymer mixture (10:1). Note, only the larger airways are filled and the branching pattern is easily observed.

J Int Sac Plastination, Vol 6:41-44, 1992

SILICONE PULMONARY VASCULAR CASTS WITH ATTACHED TRACHEOBRONCHIAL CASTS

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INTRODUCTION

Preparation of anatomically precise tracheobronchial casts with accompanying pulmonary arteries and/or veins are valuable teaching aids. To see the actual replica of the airway and the pulmonary vasculature, clarifies many questions in the student's mind. McLaughlin and co-workers (1961) made similar casts from vinylite and latex which are not as durable as silicone casts. The manufacturers of the various polymers are numerous, while the properties of these polymers are diverse. Charts are available which list the properties of many of the available polymers, thus generic polymers with similar properties can be purchased. Four polymers were used to make tracheobronchial casts with accompanying casts of the pulmonary vasculature.

METHODS

Two silicone polymers were used to inject the pulmonary vasculature of eight lungs (4 each). 1. Biodur - S/10/S3/S2 [Biodur Products, Dr. Gunther von Hagens, Rathausstrasse 18, Heidelberg, D-6900 GERMANY]. 2. Rhone-Poulenc - Rhodorsil RTV II - # 1556 (red or clear) [Rhone-Poulenc, Inc., Specialty Plastics Division, CN 5266, Princeton, NJ 08543-5266, USA]. All components were combined and mixed using the manufacturer's recommendations. Biodur polymer ratio was 1:100 (S3:S10) but S2 was varied from 0.5:100 to 1.5:100 (in 0.5 increments). Since Biodur polymer is transparent, either red or blue color additive [Biodur color paste: AC 50 (red) or AC 52 (blue)] was used to give a color to the vessels. The red Rhone-Poulenc polymer was used as it came from the manufacturer, while the clear was tinted. Rhone-Poulenc hardener/polymer ratio was 1:10. Approximate quantity of polymer used per vascular cast was .2 - .5 kg (20 to 50 cc) / 20 kg dog, 1-2 kg (120 - 200 cc) for adult pig, ox or horse. For the airway casts, either the Rhone-Poulenc polymer # 1556 (clear) or the Dow Corning polymer-Silastic E RTV (white) [Dow Corning Corp., Midland, MI 48640-0994, USA] was used.

Fresh dog, pig and ox lungs were procured from the necropsy area and from local abattoirs. The lungs were cannulated and flushed several time to remove the excess blood and secretions (Henry and Butler, 1990). The pericardium was removed from the heart. For a cast of the airway and pulmonary arteries, the majority of the heart was removed leaving the pulmonary trunk and aorta in situ. A cannula was ligated in place in the pulmonary trunk in preparation for injection of the polymer. The appropriate polymer (Rhone-Poulenc or Biodur) was mixed, tinted, drawn into 60 cc catheter tip syringes, and injected into the pulmonary arteries via the cannula in the pulmonary trunk. Injection proceeded until the color could be seen near the lung surface or until the resistance was notably increased. To aid visualization of filling, a small tear was made in the edge of a lung lobe. When polymer appeared at the tear, injection was ceased. For a cast of the pulmonary arteries and veins, a cannula was inserted through the aorta, past the aortic valves, through the left ventricle and A-V (mitral, bicuspid) valve, into the left atrium and ligated at the aorta. The right ventricle (conus arteriosus portion) was opened and a cannula inserted into and ligated in the pulmonary trunk. The selected polymer (Rhone-Poulenc or Biodur) was mixed, tinted, placed into 60 cc catheter tip syringes, and injected into the pulmonary arteries via the cannula in the pulmonary trunk or the pulmonary veins via the cannula in the aorta. Injection proceeded until the colored polymer could be seen near the lung surface or perimeter or until the resistance was notably increased. After the vessel injection was complete, the esophagus and any other mediastinal tissue and fat were removed from the lungs and tracheobronchial tree similar to McKiernan and Kneller (1983). The lungs were

inflated and dried using compressed laboratory air directed into the trachea. Lungs were placed on their dorsal (posterior) surface on a tray to contain the extravasated fluid. The air flow was gradually increased until the lungs were inflated and remained inflated to near capacity. Air flow and inflation level were maintained until the lungs were thoroughly dried. After drying was complete, a selected polymer (Rhone-Poulenc or Dow Corning) was mixed, using the manufacturer's recommended ratio (1:10), and was poured into the trachea aided by an appropriate sized funnel. The quantity of polymer used per airway was .2 to .5 kg/ 20 kg dog, 1-2 kg for adult pig, horse or ox. In larger specimens before the silicone hardened, a heavy galvanized wire was placed into the lumen of the trachea to give support to the silicone cast. The polymer mixes were allowed to harden over night. The next morning, the lung unit was placed in a vat of simmering to slowly boiling water for several hours. The specimens were checked in four to five hours to determine if most of the lung and airway tissue had been macerated away from the cast material. Occasionally, at this time, a ligature was placed around the right and left primary bronchi and the associated vasculature of the casts. Following maceration, the cast was removed from the hot water and sprayed with a high pressure hose to remove remaining tissue. If tissue still remained, the cast was submerged either in hot water or in a 10% sodium hydroxide solution until the remaining tissue was released. The specimens were rinsed with water and allowed to dry before use.

RESULTS

Both polymers produced representative casts of the airways and pulmonary vasculature. More viscous polymers did not flow into the finest bronchi, alveoli and vessels (Fig. 1). The less viscous polymers flowed freely into the capillary bed and the small arteries (Fig. 2). The Biodur polymer consistently was the least viscous and entered the smallest vessels (Fig. 3). Ratios of 1.5:100 of Biodur S2 usually hardened to quickly to fill the capillary bed while casts of 0.5:100 ratios remained tacky.

DISCUSSION

Representative casts of the airways with their pulmonary vasculature were produced with the selected polymers. The desired cast type must be determined before production. Low viscosity polymers flow freely into the alveoli and fill the entire airway and vessel. Whereas, more viscous polymer mixtures, due to less polymer entering the distal airways and vessels, present a more clear branching pattern. The optimal polymerhardener ratio for the Biodur product was 100:1:1. Viscosity of a polymer mixture may be increased by increasing the time interval between combining the polymer and hardener and actually pouring the mixture into the lungs or by increasing the quantity of hardener. Excess hardener will cause the mixture to set up rapidly and be difficult to flow into the smaller airways and vessels. Therefore, filling of the smaller vessels can be decreased or eliminated by waiting a few minutes before filling the vessels. Thus a cast may be produced which allows better visualization of whatever structures are desired. Securing the components of the cast with a string or wire helped keep the vascular components of the cast in proper orientation to the airways. When filling the pulmonary venous system, if the cannula is in the left ventricle instead of the left atrium, the A-V valve may close and not allow the veins to fill. However, this will produce a nice cast of the left ventricle, aorta and coronary vessels. Polymer cost is similar, $35.00/kg.

ACKNOWLEDGMENTS

A special thanks to Kreis Weigel and Dr. M. McCracken for help with the photography. Thank you to Emily Smathers for assistance with casting.

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Figure 1. Tracheobronchial-vascular cast from an ox. Airways of Silastic E RTV and the arteries (a) of Rhone-Poulenc mixture (10:1). Note only the larger airways and arteries are filled.

Figure 2. Tracheobronchial-vascular cast from a pig. Airways of Silastic E RTV and the arteries (a) and veins (v) of Rhone-Poulenc mixture (10:1). Note both large and small airways (arrowhead) are filled. Also, the polymer reached the smaller venules (arrow) and small arteries.

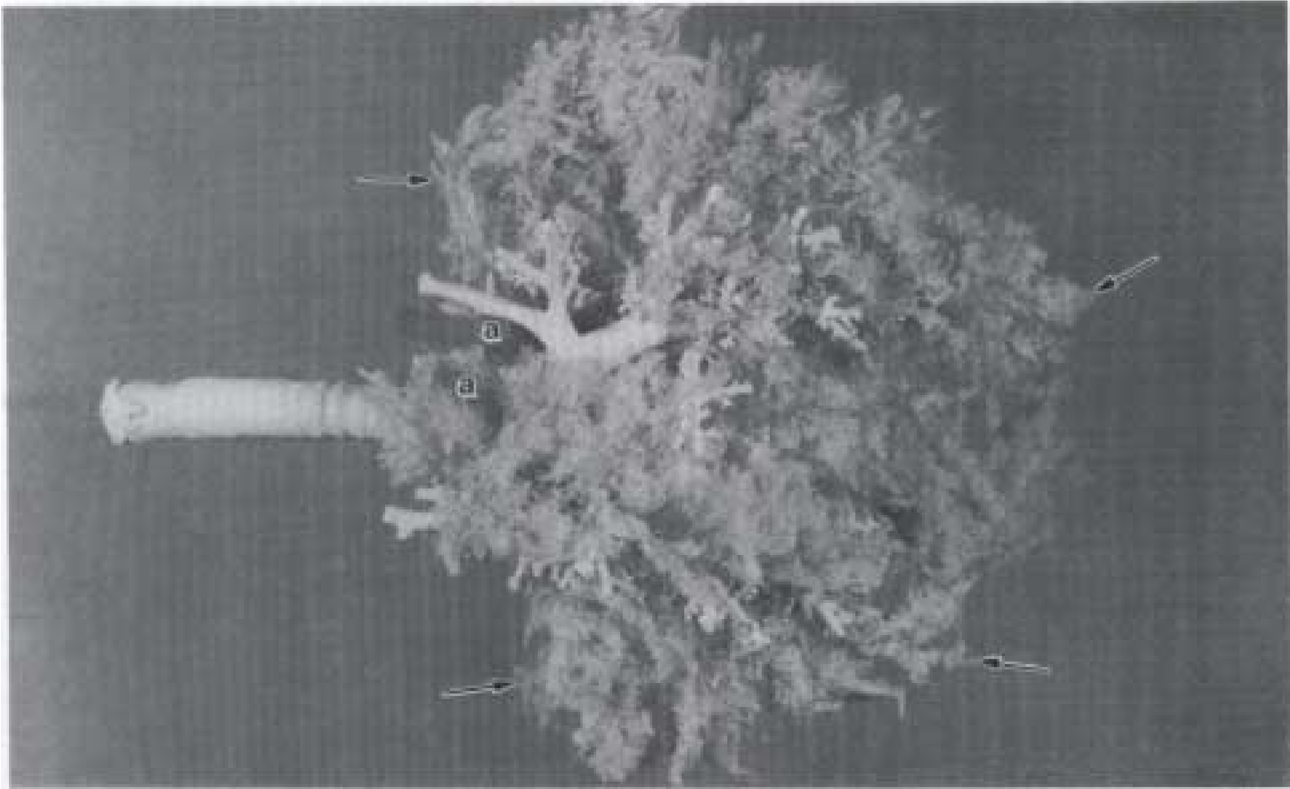

Figure 3. Tracheobronchial-vascular cast from a dog. Airways of Silastic E RTV and the arteries (a) of Biodur S10/S3/S2 mixture (100:1:1). Note only the larger airways are filled, however, the capillary bed (arrow) is complete.

Continued - Abstracts, 6th International Congress

Application of plastination to forensic entomology. Yvette D. LeGrande, William C. Rodriquez, and Paul S. Sledzik, National Museum of Health & Medicine, Armed Forces Institute of Pathology, Washington D. C. 20306-6000, USA.

Forensic entomology is the study of insects and insect larvae to aid medicolegal professionals in determining the time elapsed since death in decomposing human remains. The progression of insects and larvae on a corpse is fairly consistent, allowing the forensic entomologist to estimate the time since death. Currently, forensic entomologists use insect and larval specimens fixed in a 50:50 isopropyl alcohol/water solution to make comparisons with insects and larvae recovered from remains. This paper will discuss the application of plastination in preserving larval specimens for use in teaching and study in forensic entomology. A successful technique of plastinating larval specimens will be presented.

Fixation - The key to good tissue preservation. Dale Ulmer, Department of Pathology, College of Medicine, University of South Alabama, Mobile, AL 36617, USA.

The foundation of all good tissue specimen preparations is complete fixation. The primary function of tissue fixation is to prevent putrefaction and autolysis. Faults in fixation cannot be remedied at any later state, and the finished product can only be as good as its initial fixation. Tissue preservation and plastination is steadfastly becoming a significant increment in the design and implementation of pathology and anatomy museums, as well as, an effective research tool for archaeologists, anthropologists, and general medical historians. Fixation techniques and procedures are expanding and developing at a notable pace, The choice of fixative should be governed by the type of investigation or specimen required, both immediately and in the future. Rarely will one fixative be suitable for a variety of methods. Simple modifications of the basic formalin fixative method, to microwave fixation, to special fixation for color preservation, to holograms from plastinated specimens are being achieved and improved by universities, museums, and other institutions around the world. Special attention and emphasis to the process and development of fixation methods and techniques will ensure the advancement of good tissue specimens and the role of plastination in the future.