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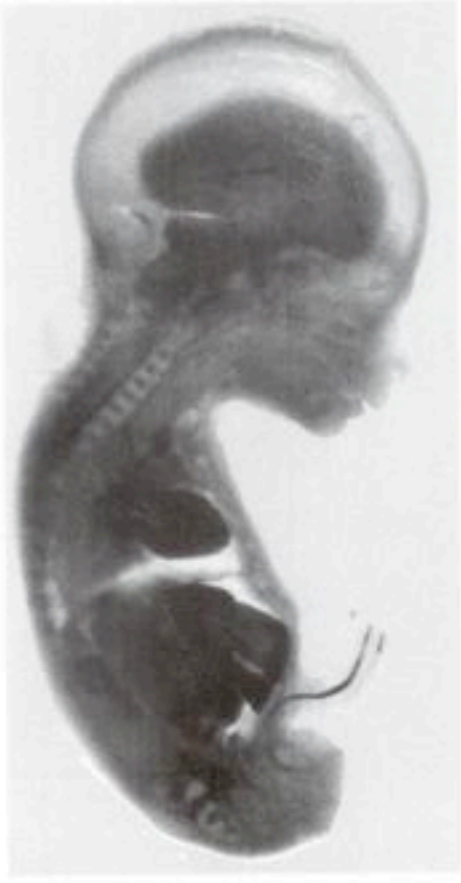
# INTERNATIONAL SOCIETY for PLASTINATION



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## Letter from the President

Dear fellow plastinators,

The 11th International Conference on Plastination held in San Juan, Puerto Rico was a new experience. We had very low attendance, obviously due to both organizational and financial problems. However, the exchange of ideas and experience during the workshop and the conference was excellent among the small group of plastinators. Those who undertook the long and expensive trip to San Juan, definitely were compensated by the beauty of the Caribbean island and the hearty hospitality of our hosts.

In order to assure an attractive and affordable environment for future conferences, the newly appointed conference planning committee has started to investigate what are the desirable and tolerable conference fees and the ideal length of the conference for the membership (see minutes of the 8th biennial meeting, page 37 of this issue).

Utilizing the results of this investigation and help of the committee, our host for 2004, Prof. Dr. Rafael Manuel Latorre Reviriego, promises a well organized conference with a moderate cost and thus, hopefully a well attended conference in Murcia, Spain.

After all the transitions of the journal and the treasury within the last years, all areas of the ISP are functioning very well now. The newly elected executive board and the new journal editors have accepted the challenge to dedicate their time and effort to the ISP-members for the next two years. The first results of this devotion can already be observed in this new edition of our journal - inclusion of the minutes of the meetings from St. Etienne and San Juan as well as the updated bylaws.

We have inserted a proposal for further updating of the bylaws which will include clarification and improvement of our nomination and election process that caused some discomfort within the last year.

I personally want to express my gratitude to you for your confidence in our work and to the newly elected and reelected officers for devoting their time for the ISP.

With the kindest regards from Graz, Austria

Andreas H. Weiglein
ISP-President

# Plastinators in Paradise

It's hard to pay attention to "fixation, dehydration, impregnation and curing" when the palm trees are swaying, the waves are lapping on the shore, and the salsa beat is throbbing in the background. But that sums up what it is like to attend an ISP conference in Puerto Rico.

At the Pre-Conference Workshop conducted by Bob Henry, my first thought was about the excess airfreight charges he must have paid to bring along all of the equipment that we unpacked for him. He brought EVERYTHING (except a freezer) to show the new kids on the block how to do the standard silicone procedure. The man is simply amazing! It's no wonder that the ISP awarded him a Distinguished Member Award.

When the general conference got started with a reception at the host hotel, we enjoyed Latin music and munchies and lots of tall tales about what we had done since departing St. Etienne two years ago. The usual horror stories about travel woes and new regulations about airport security were many. Beat Riederer claims that he never received his luggage while in San Juan - - - - but I am fully convinced that he had "7 identical shirts and 7 identical pairs of trousers". Considering the heat and humidity, if it had been the same shirts and trousers, we would have made him sit by himself on the other side of the room. There were also some good stories about low-budget hotels and their door locking habits and lack of hot water. These are all of the things that make for good memories and can be greatly enlarged for the entertainment of the folks back home.

Good food was a real plus at this conference. The waters of the Atlantic produced lots of smiles and lip-smacking at the lunches and especially the beachside banquet that was produced for us. Who can forget the "pyramids" of rice? The evening meal on the boardwalk was memorable because we were ideally located to look across the bay at the lights of downtown San Juan after the beautiful sunset. It was a clear night and the temperature was exactly right.

Since many of the plastinators are experienced in the use of alcohol - - - both as a dehydrating agent and as a cure-all for travel ailments - - what could be better than a trip to a place that provides you with free alcohol? The Bacardi people were very generous to us on an outing to their distillery. I like the smell of molasses but I never dreamed that you could be overwhelmed by it.

..... and then we went to jail. A grand reception and dinner was on the program but we had no idea that it would be in the most famous lock-up in the Caribbean. The old jail of the city of San Juan was the site. The mayor's office and other officials were on hand to extend the hospitality of the city and island. Good food, good music and good libations. What more can you ask? The Bacardi bar was open again to us and was staffed by two of the island's most comely ladies that one could hope for. It is amazing to see young men (and old ones too) get a glazed look in their eyes when they see a sight such as these ladies were. But the conference ladies had an equal opportunity for ogling when the fine musicians appeared. Latin men have a worldwide reputation for their appeal to the ladies. But the real question is: How much energy did a certain young man from Chili expend dancing to the Latin rhythms with our conference hostess?

Cruise ships in the harbor, white beaches, picturesque forts and buildings from the distant history of the island, the fine people of Puerto Rico - - it goes on and on. It was a good conference and I hope that I have been able to give those of you that were not able to attend some of the flavor and ambience of our "plastinators in paradise" conference. *By: Tim Barnes, Our resident plastinator correspondent.*

**Restoration of Air-dried Specimen Using Parts of the Standard Plastination Equipment and Technique.** ***De Jong KH.*** *Department of Anatomie en Embryologie, AMC Universiteit van Amsterdam, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.*

Part of the Department of Anatomy in Amsterdam is the Vrolik Anatomical Museum where anatomical specimens are on exhibition which have been collected from the 18th century until today. Amongst them are approximately 50 air-dried specimen of muscles and tendons of arm and leg, placenta and several inner organs. Most of these specimens were collected in the early 20th century. Their method of drying is uncertain. On inspection a few years ago, most of these specimens proved to be infected with an unknown insect that acted as a termite: the specimen was destroyed from the inside out by eating it. A proven way to kill these insects was putting the specimens in a gas-chamber for a long period of time. However, as it was uncertain where the infestation came from, the specimens were not protected against a new infestation until an alternate way of sterilizing and protection was found. As an experiment, the dried specimen was placed in acetone in vacuum in order to de-aerate the specimen thoroughly and impregnate the specimen with acetone. After one week in acetone, the specimen was placed under a fume hood to let the acetone evaporate. This procedure was performed 3 times. The main goal was to kill the insects by dehydrating them, but as a side effect, it also proved to clean the specimen thoroughly. After the third acetone bath, the specimen was allowed to dry thoroughly under the fume hood. After drying, the specimen was first submerged and then brushed repeatedly with a mixture of fully pre-cured Biodure S10/S3 silicone dissolved in methylene chloride (3 ppv. S10/S3 in 100 ppv methylene chloride). The large amount of methylene chloride acts as a vehicle for the large slO molecules to penetrate deeply in the specimen thus covering the entire surface (inside and outside) of all structures in the specimen with a thin layer of silicone. After each brushing, the methylene chloride was allowed to evaporate under a fume hood. Finally, the specimen was normally cured using Biodur s6 gas hardener. No re-infestation was reported after this procedure. A nice side effect proved to be the possibility to clean dusty specimens under running tap water without ruining them.

**Starting a plastination facility as a beginner: required equipment and approximate costs.** ***De Jong KH.*** *Department of Anatomie en Embryologie, AMC Universiteit van Amsterdam, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.*

Starting a plastination facility as a beginner requires an important decision before starting that is which technique is going to be performed. As the silicone technique is the most basic, and requires the simplest equipment, it is recommended to take this technique as the starting one. When more experienced, one is always able to expand to other techniques. This paper assumes one will use the Biodur® silicone S10 method. Plastination requires the following steps: 1) making the specimen, 2) preparing the specimen for plastination, 3) dehydrating the specimen, 4) impregnating the specimen with polymer and 5) curing the specimen. Each step may require specific equipment. Making a specimen for plastination doesn't differ from making a specimen for normal anatomy teaching and doesn't ask for special equipment. Preparing the specimen for plastination means (if necessary) the removal of glycerine and phenol from embalming fluid out of the specimen. This can be achieved by submerging the specimen in 50% ethanol and requires adequate containers. Dehydration, mostly performed in acetone at -20°C, requires a normal household deep freezer and at least 2 containers with a volume of approximately ten times the volume of the specimen. Stainless steel containers with a lid are preferred. A temperature calibrated acetonometer is used to monitor the progress of the dehydration. Impregnation under vacuum requires a vacuum kettle that can be placed in the deep freezer and has a see through lid in order to allow one to follow the progress of the process of impregnation. As a pressure of 5mm Hg has to be reached, a vacuum pump adequate for the plastination kettle is required. Several valves are used in order to adjust the pressure. Pressure is measured with a mercury Bennert manometer. The specimen has to be placed in an adequate container that fits in the vacuum kettle and has a volume big enough to contain the specimen and an amount of silicone rubber to keep the specimen submerged until the end of the impregnation process. The silicone impregnation bath has to be mixed according to the subscription of the manufacturer. Curing the specimen requires a container that can be closed airtight in which the impregnated specimen is placed and a hardener vaporized. Working with silicone rubber requires protective clothing, disposable gloves and paper towels to protect the worker and the working environment from smearing with silicone rubber. Initial costs of the equipment needed may differ with the amount of equipment already present in the laboratory. In Amsterdam, we were able to start with a budget of $2000.00.

**Design of a Web-Based Cataloguing System for a Large Plastinated Pathology Museum Using High-Resolution Digital Imagery.** ***Feener T, Gubbins B, Kell K, Kennedy L.*** *Department of Pathology, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada.*

In the late eighties the Department of Pathology at Queen's University implemented one of the first plastination programs in Canada. The objective of the program was to create durable, and non-toxic, plastic impregnated specimens to replace the existing formalin mounted specimens used in teaching medical students and residents. The program used the standard S10 technique to plastinate fixed tissue specimens collected during routine autopsy procedures. Throughout the 1990's, the collection grew by approximately one hundred specimens a year and presently numbers over one thousand. While such a large and diverse collection is an invaluable teaching resource, the sheer size has become prohibitive to many faculty and professional staff who cannot afford the time required to search through the specimens. To overcome this, a novel project was undertaken to construct and implement a web-based cataloguing system using digitized images of the specimens that could be user-friendly, easily searched and fully automated for low maintenance requirements. To begin, all specimens in the collection were photographed with a high-resolution digital camera and file names were written to include index codes as well as keywords. The images were then transferred to a previously established Lab Information System (LIS) Image web server designed to function as a secure on-line catalogue and index for storing routine autopsy and surgical images. The LIS Image server provides professional staff with immediate access to autopsy, and now plastinated museum images, from their office computers. We will detail the design of the on-line catalogue system giving special consideration to image file nomenclature, hardware selection, use of freeware and open source software, web-design, data back-up, security, and searchability as well as camera hardware and image quality. Finally, we will highlight the many benefits that have resulted from the on-line system.

**Naked Silicone Impregnation.** ***Henry RW, Reed RB.*** *Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA.*

The standard S10 technique (Biodur™) has been used for impregnation and production of beautiful, high-quality specimens for 25 years. The Biodur™ process is most often used at cold temperatures. However, it may be used at room temperature. Its down side is that it commences to thicken in a few months. Four years ago, room temperature products were introduced that were stable at room temperature. This new room temperature process (Dow/Corcoran) yields good quality specimens and cuts production time by at least 50%. However, a distracting film occurs on the surface of the specimen. In order to speed up the process and still have the highest quality specimens, impregnation of silicone without catalyst/chain extender or cross-linker was carried out on a variety of specimens. Impregnation time ranged from one day to eight days. Because the viscosity of the polymer used was very fluid (40 centistokes), draining and manicuring time for the specimens was negligible. In fact, some specimens were wiped with silicone to rehydrate the surface which we believed was too dry in appearance. Two methods were used to cure the specimens: 1. Specimens were first exposed to the gaseous cross linker (S6) for 2 days then catalyst/chain extender (S3) were wiped onto the surface twice at 24 hour intervals. 2. Catalyst/chain extender were wiped onto the surface and allowed to sit for 24 hours, at which time, S3 was applied again. After another 24 hours the specimen was exposed to cross linker. Both methods resulted in polymerization of the outer layer of the specimen within three or four days with a sealed, dry surface. When specimens were sectioned two and four weeks later, the interior of the specimen contained uncured polymer. Time will tell if and how long it will take for the polymer to cure throughout the depths. The quality of the specimens appears to be similar to that of specimens produced via the classic Biodur™ method. The most noticeable plus for this method is that polymer runs freely off of hair-covered specimens which virtually eliminates the tedious manicuring task associated with the classic method. We believe that this is another useful method for producing specimens more rapidly and with out the need of refrigeration.

**Polymer Chemistry in Silicone Plastination.** ***Henry RW, Seamans G[1], Ashburn RJ[1].*** *Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA, 'Silicones Inc., 205 Woodbine Street, High Point, North Carolina, 27261, USA.*

Understanding basic polymer chemistry may help understand the plastination process. In any industry, various terms are used which may not be the standard but are entrenched in every day conversation. The silicone type used for plastination is silanol (in industry terms) and has repeating silicone molecules with terminal hydroxy groups. In general, polymers are

composed of very high molecular weight molecules. Silanol can be blended in any number of viscosities. Biodur™ S10 [500 - 700 centistokes (cstk)] is more viscous than some of the more recent entries (50 - 100 cstk) (North Carolina, Dow/Corcoran) into the plastination arena. The longer the silicone molecule, the more viscous the polymer. The more fluid the polymer, the easier to enter the specimen. In silicone plastination and in the silicone industry, a basic formula is a three-part system: polymer (S10), catalyst (S3) and cross linker (S6, hardener). The similar three components are used in the Dow/Corcoran method. The Chinese process appears to utilize a two-part system. The Biodur™ method is actually a four-part system. S3 is more than the catalyst. It also contains a chain extender. These components obviously are named in part for what the components do. The catalyst reacts with the cross linker and readies the silicone polymer for curing (cross linking). For silicone plastination, the recommended ratio of silicone polymer to catalyst (100:1) is quite forgiving. This may not be the case with epoxy polymer.

**Vacuum Monitoring in Plastination.** ***Henry RW.*** *Department of Comparative Medicine, College* ***of*** *Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA.*

Vacuum and decrease in pressure are often used synonymously. However, when the reading is recorded, zero for both are at opposite poles. When there is a decrease in absolute pressure (AP), vacuum is increased. Atmospheric pressure is around 76cm Hg (760mm) = 30 inches Hg at sea level. Values of decreasing pressure or increasing vacuum may 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 38.1cm Hg), or 2/3 atmosphere (10 inches or 25.4cm Hg, AP). Torr is a unit of measure also used with pressure. One torr equals 1/760 of an atmosphere. The reading obtained when measuring the change associated with pressure changes varies depending on the type of instrumentation. A column of Hg or vacuum gauge yields a progressively higher reading as absolute pressure is decreased. This is referred to as gauge pressure or units of vacuum. However, a manometer is read by the difference in height of two columns of mercury. Therefore, 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 22cm or less, approximately the last 1/3 or less of the change in the pressure/vacuum 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. Therefore, understanding the variation of gauging methods and vapor pressures of solvents being used is necessary. The saturated vapor pressure (similar to boiling point) of methylene chloride is greater than that of acetone, 78.0mm Hg vs. 35.9mm Hg at -10°C or 32.5mm Hg vs. 14.8mm at -25°C, respectively. Hence, methylene chloride will vaporize at a higher AP and be extracted before acetone. An important item when using deep vats of silicone for impregnation is: pressure is proportional to depth. This results in pressure being greater at the bottom of the polymer than at the surface of the polymer. Therefore, acetone in the specimen will remain in a specimen that is submerged 15 to 20cm below the surface of the polymer longer than in a specimen near the surface. The gauge hooked to your apparatus is likely reading surface pressure.

**Dehydration with Alcohol at Room Temperature and Use of Locally Available Polymers to Plastinate Human Tissue.** *Jimenez R, Isaza O. Morphology Department, Medicine Faculty, Antioquia University, Medellin, Columbia.*

Biodur® polymers are used in plastination around the world using a variety of techniques described by different authors. These polymers are produced in Europe and are relatively expensive. The plastination technique using Biodur® polymers usually includes dehydration with acetone. Acetone is not readily available in Columbia due to legal restrictions. The difficulties involving cost of polymer and lack of acetone prompted us to work locally available polymers and solvents at room temperature to vary the technique of tissue impregnation. This will allow us to produce permanent human specimens for teaching anatomy in pregrade or postgraduate studies and for anatomical museum pieces. Our work modified the standard techniques described by von Hagens and other authors. Our procedure involved tissue fixation, dehydration in a graded series of isopropyl alcohol, immersion in xylene, impregnation with Cristalan 818® polymer and curing of the specimen. All of these products are readily available in Columbia. The results are good and permit us to continue in the search for the best results using this modified plastination technique (see front cover).

**Anatomy of the Equine Tarsus: A study by MRI and Macroscopic Plastinated Sections (S10 and P40).** *Latorre R, Vazquez JM,* ***Gil*** *F, Ramirez G, Lopez-*

*Albors O, Ayala M,* ***Arencibia*** *A. Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30071 Murcia, Spain.*

The objective of this work was to define the normal gross anatomic appearance of the adult equine tarsus using plastinated sections and magnetic resonance imaging (MRI). Six equine pelvic limbs from adult horses were utilized. The arteries, veins and synovial structures were injected with red, blue and green latex respectively, using colored pigment paste (2% ppv): AC50 (red), AC52 (blue) and AC54 (green) (Biodur™, Heidelberg). Arterial injection was carried out via the femoral artery and injection was continued until red latex oozed from smaller arteries. After arterial injection, venous injection was performed via one of the two plantar digital veins, as distal as possible to avoid valve interference. The proximal ends of the veins were left open until blue latex started to ooze from them. The tarsocrural joint (Articulations tarsocruralis) was injected via its plantar pouches. The frozen limbs were sectioned transversely and sagitally from the tibia to the metatarsus with a high-speed band saw at the desired thickness (0.3 to 1 cm.). After slicing, fixation and dehydration, the slices were plastinated according to either the standard cold S10 technique (Biodur™) or a room temperature process, P40 technique (Biodur™). Using a scanner with a 1.5 Tesla magnet, image sequences were acquired in transverse and sagittal planes. Comparison between anatomical sections and MRI of this joint enabled us to establish the normal reference in MR images of different structures. Optimal image planes were identified for the evaluation of articular cartilage, subchondral bone, flexor and extensor tendons, tarsal ligaments and synovial structures. P40 plastinated sections and MR images provide a thorough evaluation of the anatomic relationships of the structures of the equine tarsus and essential information for diagnosis. The tarsocrural joint is an anatomically complex area and an understanding of this is a prerequisite for accurate diagnosis of injuries in this joint.

Epoxy Impregnation with no Hardener. *Latorre RM, Reed RB[1], Henry RW[1]. Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30071 Murcia, Spain, department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, USA.*

A modification to the classic Biodur™ epoxy impregnation method was evaluated for attributes which might lead to increased casting time, increased ease of bubble removal and decreased yellowing of cured polymer. The impregnation bath was also evaluated as a reusable source of impregnation medium. Tissue slices were impregnated, in the absence of hardener, with epoxy polymer. Impregnated tissue was cast using modified reaction mixtures composed of varying amounts of epoxy polymer, hardener and glass separator. All cast tissue slices cured using the various reaction mixture combinations. Casting time was increased from 12 hours to 2 years. Bubble removal was easier due to the decreased viscosity of the experimental reaction mixtures. Blemishes did occur when tissues touched the glass of the casting chamber. These blemishes were easily repaired by recasting with a thicker gasket or by direct application of reaction mixture. Yellowing of the cured epoxy did occur but was not as severe as that which is seen with the classic epoxy impregnation method. Previously used impregnation mixture was reused two limes to successfully impregnate tissue slices.

**Animating Dural Hematomas Using Plastinated Human Brain Sections.** ***Lozanoff S[1], Lozanoff BK[2], Sora MC[3], Rosenheimer J[1], Keep M[1], Tregear J[4], Jacobs J[5], Saiki S[5,6], Alverson D[7].*** *'Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, HI; [2]SURFdriver Software Company, Kailua, HI; [3]Department of Anatomy, Anatomical Institute, Vienna University, Vienna, Austria; [4]Department of Media Services, University of New Mexico, Albuquerque, NM; [5]Department of Medicine, University of Hawaii School of Medicine, Honolulu, HI; [6]Tripler Army Medical Hospital, Honolulu, HI, [7]Department of Pediatrics and Obstetrics and Gynecology, University of New Mexico School of Medicine, Albuquerque, New Mexico, USA.*

Computerized animation is becoming an increasingly popular method to provide realistic and dynamic presentation of anatomical concepts. However, most animations use artistic renderings as the base illustrations that are subsequently altered to depict movement. In most cases, the artistic rendering is a schematic that lacks realism. Plastinated sections may provide a useful alternative to artistic renderings to serve as a base image for animation. The purpose of this paper is to describe a method for developing animations using plastinated sections. This application is used in a problem-based learning environment involving traumatic head injury that results in an epidural hematoma with transtentorial uncal herniation. In addition, a subdural hematoma is animated permitting the student to contrast the two processes for a better understanding of dural hematomas in general. The method outlined utilizes P40 plastinated coronal brain

sections that are digitized and to which contiguous anatomical structures are rendered in Adobe Photoshop. Once the base illustration is rendered, it is loaded into Kai's SuperGoo and morphed. Finally, the animations are viewed with QuickTime to which audio narration is added and uploaded for viewing on the web. This method demonstrates how realistic anatomical animations can be generated quickly and inexpensively using plastinated brain sections. Supported by OAT, HHS, 2 DIB TM 00003-02.

Plastination of Pathologic Specimens via **Room** Temperature S10. *Miklosova M. Department of Anatomy, Medical Faculty, University of P. J. Safarik, Kosice, Slovak Republic.*

The standard silicone technique (S10) is the classic plastination method. Plastination preserves the normal anatomy, whether human or veterinary, and the relationships among structures. Plastinated pathologic specimens give a different view of anatomy. The normal form of these specimens is altered by the pathologic process and may require a different approach. In addition, the post mortem examination often distorts or even destroys the anatomical relationship. Such specimens require individual thought following the necropsy to enable a useful specimen to be prepared and preserved. Detailed knowledge of typical macroscopic changes of the organ is the basic premise for correct interpretation of the pathologic process. Plastination is a unique method for preserving these altered biological tissues. Specimens (fibroma molle, fibroma durum, uterine myomas) were prepared for the Department of Veterinary Pathology using room temperature impregnation. To enhance specimen quality and preserve color differences, a special fixative composed of: formalin, pyridine, nicotine acid, Dithionit (natrium hydrosulfurosum) and distilled water was formulated. This fixative preserves color and furry coats and enhances flexibility. Specimens were fixed for 4 weeks and then flushed with water. All specimens were dehydrated in cold (-25°C) acetone using three weekly changes. The dehydrated specimens were impregnated in a room-temperature polymer reaction-mixture of S10 and S3 (100:1) for impregnation. After impregnation of eight weeks was completed, the specimens were drained and the polymer was stored in the deep freezer and used for another group of specimens at a later time. The specimens were drained of excess polymer and hardened by exposure to S6 for two days. These specimens highlight the mentioned pathology and are being used as a teaching supplement in our veterinary anatomic pathology teaching laboratories.

**Plastination of Stained Biological Specimens: Their Use in a Teaching Environment.** *Mizer L, Schneck P. College of Veterinary Medicine, Cornell University, Mica, New York, USA.*

Teaching resources for the study of bone and cartilage in gross anatomy, microscopic anatomy, comparative anatomy and embryology are frequently restricted to formaldehyde or glycerin immersed specimens. In many of these disciplines, we use fetal and early neonatal specimens that have been cleared and subsequently stained with alizarin red and/or alcian blue to demonstrate the relative amounts of cartilage and bone in the developing skeleton. Although such specimens are useful as originally prepared and stored in glycerin, they can be messy to handle and examine under a dissecting microscope. Our laboratory has undertaken the plastination of alizarin red and/or alcian blue stained late gestation fetuses and early neonates to provide a relatively durable specimen that is easy and clean to manipulate and study. Using pre-existing alizarin or alizarin/alcian blue stained glycerin-immersed specimens or formaldehyde or alcohol fixed specimens that we subsequently stained, we have plastinated fetuses and whole body or sectioned neonates from various species. Although not ideal in their positioning they do provide examples of the quality of specimen that may be attained for use in multiple settings in the veterinary curriculum at the College of Veterinary Medicine at Cornell University.

Epoxy Under Vacuum. *Reed RB, Henry RW. Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA.*

Epoxy reaction mixture is commonly placed under vacuum to remove air bubbles from the casting chamber. This often appears to create more air bubbles within the chamber than were originally present. In order to determine the source of these newly created gas bubbles, we subjected components of the epoxy reaction mixture (El, El2, AE30), singly and in various combinations, to full vacuum. Bubbles first generated at moderate low pressure were most likely air introduced into the mixtures at an earlier point in time. At low pressure, bubbles were generated in all beakers in which polymer (El2) and hardener (El) were present. Bubble production was greatest in mixtures containing El2. Bubble production also occurred to a lesser extent in mixtures containing El in which no E12 was present. Glass separator alone generated no gas bubbles. These findings suggest that exposing epoxy reaction mixture to low pressure causes the release of a component of both El and El2 in a gaseous form which adds more

bubbles to the casting chamber. Loss of these components does not affect the curing of the epoxy reaction mixture.

**Plastination Dehydration Mediums: Time and Temperature.** ***Reed RB, Henry RW.*** *Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA.*

To determine the minimal length of time required for tissue dehydration using acetone, various animal organs were exposed to room temperature and cold acetone which was changed every 24 or 48 hours according to the experimental protocol. Dehydration of specimens in cold acetone which was changed every 24 hours ranged from 5 to 7 days while those in room temperature acetone ranged from 5 to 6 days. Dehydration of small animal organs with room temperature and cold acetone that was changed every 48 hours ranged from 8 to 10 days while tissues from large animals required 10 to 12 days. Our findings also demonstrate that dehydration is most rapid early in the process making more frequent acetone changes desirable for the most expedient dehydration of tissues.

**Shrinkage Assessment with Classic Plastination Dehydrants.** ***Reed RB, Henry RW.*** *Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, USA.*

To assess the shrinkage effects of current plastination dehydrating agents upon tissues, a variety of animal organs were dehydrated with room temperature acetone, cold acetone and room temperature methanol. The average shrinkage to tissues dehydrated with cold acetone was 14.5% of the original size of the tissue. The average shrinkage caused by room temperature acetone was 20.2% of the original tissue size. The largest average tissue shrinkage, 22.6%, was attributed to room temperature methanol. To minimize tissue shrinkage during dehydration, cold acetone should be the preferred method of the three examined in this study. The shrinkage to tissue caused by room temperature acetone and methanol is most likely great enough to alter results in quantitative studies using such organs. However, their general appearance is not distorted which renders them useful for general anatomical studies in the classroom. Room temperature dehydration is effective for plastination purposes; however, the increased shrinkage factor must be weighed against the benefits of room temperature plastination.

**Use of** Plastinated **Specimens in Medical Teaching Modules.** ***Riederer BM.*** *Instiut de Biologie Cellulaire et de Morphologie, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland.*

The necessity to reduce the number of courses and dissection hours in teaching human gross anatomy in the second year of medical studies required a profound reorganization. In consequence, among the modules to restructure were the dissection of upper and lower extremities. One module usually consisted of 27 hours of dissection distributed over 9 afternoons, but had to be reduced to three blocks of two hours of self-directed learning on prosected specimens. Assistants prosected specimens and so got familiarized with the teaching material. They were also in charge to supervise and guide the students and furthermore gave them a good opportunity to learn topographical anatomy in more detail. During the two first hours of the self-directed teaching module, cutaneous innervation and articulations were studied. This was followed by the topography of the thigh and popliteal region, and finally lower leg and foot were studied. The prepared wet specimens were usually kept in 50% alcohol solution between courses and preserved well for the exception of more fragile structures. Delicate nerve structures of different cutaneous regions resisted only for a short time the inquisitive approach of our students. Even nerves sutured to the tissue were soon torn apart. In order to reduce the number of dissection specimens, we now started to prepare specimens for plastination, this mainly to have more resistant pieces. Specimens were plastinated by the standard S10 method. An approach by dissection permits one to slowly progress in defining the topographical anatomy and prepare for the important structures to locate, while dissected tissues are ready for teaching without tedious search and allowing an immediate approach and therefore considerably reduce the number of teaching hours. Although more care was given to have ideal specimens, some critique rose from students that felt the pressure to learn more in a shorter time and the lack of experiencing exploratory learning. Advantages of introducing dissected and plastinated tissues are manifold. Fewer bodies are used because of a reduced necessity to prepare new specimens every year. Furthermore, delicate structures become more resistant and well dissected tissues preserves for a long time. In addition, plastinated samples can be used also outside the dissection hall. The introduction of plastinated samples added another tool to teaching gross anatomy. However, it does not change the capacity of students to learn more in lesser time.

**Inferior Alveolar Nerve Anatomy Revisited: A Study Based on Dissection and Plastination.** *Weiglein* ***AH[1]*****,** *Kqiku* ***L[2]*****, Pertl C[2].** *'institute of Anatomy, [2]Department of Dental Surgery and Radiology, Karl-Franzens-University, Graz, Austria.*

Dental implants sometimes cause pain due **to** pressure on the inferior alveolar nerve. If there are multiple implants, it is impossible to select and subsequently remove or change the implant that causes the pain. In order to enable the accurate selection of the cause of pain, we dissected 10 mandibular canals of cadavers embalmed with the Graz embalming technique and proved the findings by both histological and plastinated serial slices. Whole mandibles were plastinated with polyester resin and finally enclosed in a block of polyester. After complete curing, the polyester block was sectioned by a diamond band saw (Exakt 310 CP) with laser-oriented section control into 100µm slices. The mandibular canal contains a bundle of nerves that comprise two larger nerves that are separately wrapped in perineural sheaths, one of which is the dental nerve supplying branches to the dental alveoli. The other is the mental nerve that emerges from the canal through the mental foramena to supply the skin and mucosa of lower lip, cheeks and chin. The mental nerve lies anterior to the dental nerve in the posterior molar region and passes it inferiorly in the anterior molar region to finally emerge from the canal laterally in the premolar region. Based on these anatomical findings, the implant causing the pain can be selected accurately and the source of pain can be removed to relieve the patient.

**The International Society for Plastination: Mission and History.** *Weiglein AH. Institute of Anatomy, Karl-Franzens-University, Graz, Austria.*

Twenty-four years ago, the first publication on plastination entitled "Impregnation of large specimens with polymers" was issued in the German journal Verhandlungen der Anatomischen Gesellschaft. A year later, in 1979, it was published in Anatomical Record and later on in 1979 the term "plastination" appeared in The Preparator. The first conference on Plastination was held in San Antonio, Texas in 1982. Since then, plastinators from all over the world meet every even year for an international conference. Interim meetings, which are primarily workshop based and usually take place in the USA, were started in 1989 in Knoxville, Tennessee. In 1987, the first issue of "The Journal of the International Society for Plastination" was published. In 1996, the first issue of the Current Plastination Index, an index listing all publications dealing with plastination, was issued. However, it was not before 1996 that the International Society for Plastination (ISP) was officially founded during the meeting in Graz. Since then, the ISP serves as a forum for the exchange of information about plastination that is accomplished by the publication of the journal, by holding conferences, meetings and workshops on a regular basis and by our web page and list server.

**Plastination: A Tool for Teaching and Research.** ***Weiglein*** *AH. Institute of Anatomy, Karl-Franzens-University, Graz, Austria.*

Plastination is a unique technique of tissue preservation developed by Dr. Gunther von Hagens in Heidelberg, Germany in 1978. In this process, water and lipids in biological tissues are replaced by curable polymers (silicone, epoxy, polyester) which are subsequently hardened, resulting in dry, odorless and durable specimens. Silicone is used for whole specimens as well as for body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices. Polyester is mainly used for brain slices to achieve excellent distinction of gray and white matter. The standard technique consists of four main steps: 1) Fixation, 2) Dehydration, 3) Forced Impregnation, 4) Hardening (Curing). Fixation can be done by almost all conventional fixatives. Dehydration is achieved mainly by acetone because acetone also serves as the intermediary solvent during impregnation. Because this step is originally done at -25°C, it is also known as freeze substitution. Forced impregnation is the central step in plastination: vacuum exchanges acetone by the polymer. Finally, the impregnated specimen is hardened by exposing it to a, usually gaseous, hardener (silicone) or by UVA-light and heat (polyester, epoxy). Although they cannot replace dissection, because they are not flexible, plastinated specimens are perfect museum models and thus, teaching aids. Sheet plastination is also used in research, particularly in comparison with CT- and MRI-images. New devices for sectioning (diamond wire and diamond band saws) provide a new tool for the production of thin slices (approximately 100µm - and in combination with a diamond grinding device down to 10*µm)* of large specimens (up to the size of a human head).

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# The Use of Confocal Microscopy for the Examination of E12 Sheet Plastinated Human Tissue

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*Abstract:* E12 sheet plastination has been used as a teaching aid for several years. More recently, El2 sheet plastinated tissues have been used as a research tool in a variety of areas. This paper describes a new procedure of viewing E12 sheet plastinated material. Skin and subcutaneous tissue from four formalin-fixed cadavers was used in this investigation. The blood vessels of the tissues were perfusion stained with diluted Gill's hematoxylin #1. The tissues were then processed for E12 sheet plastination. Light microscopy and confocal laser scanning microscopy were used to view the E12 plastinated specimens. It was found that autofluorescence was dominant within this tissue at 488nm excitation. Due to the emission spectrum and the spatial distribution of the autofluorescence, this autofluorescence is likely to be due to the connective tissue - in particular, the collagen. The results of this study indicate that, using serial optical sections, the confocal laser scanning microscope provides a much higher resolution image, and reveals structures that are virtually invisible by light microscopy.

*Key words:* **E12 sheet plastination; autofluorescence; collagen; connective tissue**

## Introduction

Over two decades ago the technique of plastination was developed by Gunther von Hagens, in Heidelberg, Germany (von Hagens, 1979). Plastination involves substituting the tissue water in a specimen with a polymer. The polymer is subsequently cured to form a dry, firm, flexible, and durable specimen. One of the more recent forms of plastination is E12 sheet plastination (Weber and Henry, 1993). Since its development in the mid-1980's El2 sheet plastination has generally been used only for teaching because of its strong correlation with both CT and MR-images. More recently, El2 sheet plastination has been used as a research tool, and is now becoming an integral part of a wide range of anatomical research, especially at the macro-micro level (An and Zhang, 1999; Johnson et al., 2000a; Johnson et al., 2000b; Phillips et al., 2000; Zhang and An, 2000).

E12 sheet plastination has been used extensively by our group for a variety of research projects (An and Zhang, 1999; Johnson et al., 2000a; Johnson, et al., 2000b; Phillips et al., 2000; Zhang and An, 2000). E12 plastinated slices are conventionally viewed with either no magnification or with low magnification under a dissection microscope. However, some structures are not able to be effectively viewed by this method, especially when the structures of interest are relatively small and the natural colors and contrasts of the surrounding tissue obscures the region of interest,

Conventionally confocal laser scanning microscopy (CLSM) relies on a fluorochrome to produce the

fluorescent image of a specific structure, which is detected electronically. However, some tissue types (e.g. connective tissue) and chemicals (e.g. aldehydes) are autofluorescent, and thus, do not require a fluorochrome. Autofluorescence may be from a number of sources. Several elements of normal tissue, such as collagen, are known to be auto-fluorescent at specific wavelengths (Koenig and Schneckburger, 1994). Tissue autofluorescence has been used for a wide range of applications - from basic science research (Swatland, 1988; Kollias et al., 1998), to clinical diagnostics (Koenig and Schneckburger, 1994; Glassman et al., 1995; Banerjee et al., 1998). Auto-fluorescence may also be due to chemicals used in the fixation during the tissue preparation. This has been shown to be true in the case of fixatives such as formalin, and its derivative, formaldehyde (Clancy and Cauller, 1998; Aisa et al., 2001).

This paper describes the procedure of viewing E12 sheet plastinated material with CLSM and outlines the possible benefits and limitations of such a technique.

## Materials and methods

Four lower limbs from four cadavers were prepared for this investigation (two female, two males; 65-87 years). The arterial and venous systems of the cadavers were thoroughly flushed via the right femoral artery with normal saline via a gravity feed system, at 1.5m elevation, until the efflux from the femoral vein was clear. This was followed by 20 liters of 10% neutral

buffered formalin to fix the tissue. The limbs were then removed from the cadavers and the vascular system of the lower limbs was then stained using dilute Gill's hematoxylin #1 (diluted 1:1 in distilled water) via the femoral artery for five minutes, and then rinsed with 10 liters of tap water. This protocol of staining the blood vessels with Gill's hematoxylin #1 has allowed identification of blood vessels from the large arteries, through the capillaries, to the venous system. Being able to detect these blood vessels has been very useful in identifying the spatial distribution of the autofluorescence.

Skin flaps (70mm x 120mm), including skin, subcutaneous tissue and deep fascia, were removed and embedded in 20% gelatin. The gelatin blocks were deep frozen to -85°C for 24 hours. The gelatin block was oriented so that the tissue was is the sagittal plane and frozen 2.5mm sections were then cut using a band saw. The gelatin was not removed from the tissue as it assisted with tissue orientation. The plastination protocol was used as previously described by An and Zhang (1999). Briefly, the tissue sections were then put through an ascending series of acetone (94-100%) at -20°C until the tissue was completely dehydrated (approximately four weeks). Following dehydration, the acetone and tissue sections were removed from the freezer and warmed to 25 °C for approximately two weeks to aid with the degreasing process. The acetone was not changed during the degreasing process. The tissue slices were then impregnated with an epoxy reaction-mixture (E12/E1/AE10/AE30 at a ratio of 100:28:20:5 (parts by weight); Biodur, Rathausstrasse 18, 69126, Heidelberg, Germany) in a vacuum chamber at 0°C. Resin impregnated slices were laid flat, covered with epoxy reaction-mixture and cured between 250µm amorphous plastic sheets in a 37°C oven.

The El2 plastinated slices were viewed using light microscopy (LM) and CLSM (BioRad). The slices were systematically viewed in entirety by 0.5-1.5µm thick optical sections at both 488nm and 568nm excitation. Digital overlay images were collected from exactly the same point in both the LM and CLSM images by the BioRad CLSM system (including 2-dimension images and 3-dimensional reconstructions). This allowed judgment to be made on the spatial localization of the fluorescence within the tissue.

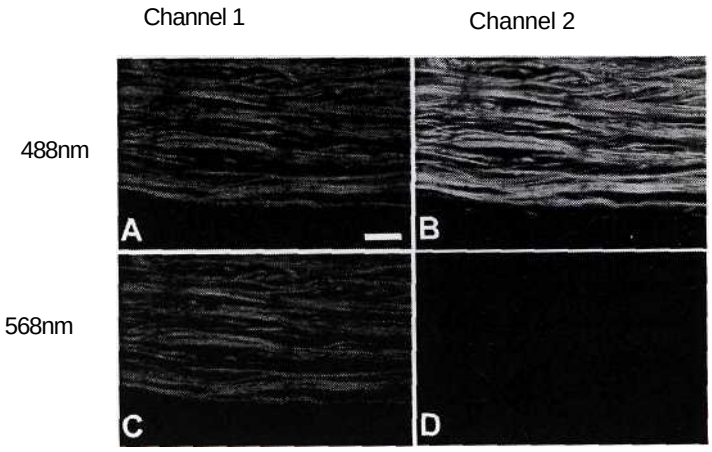



Figure 1. Shown are the emission images from the E12 sheet plastinated tissue when viewed by either 488nm or 568nm excitation wavelengths. 'Channel *V* is the channel at which the image from the 568nm emission is detected, while 'Channel 2' is the channel at which the image from the 488nm emission is detected. It would normally be expected that there should only be fluorescence detected at 488nm excitation/Channel 2 (B), or, 568nm excitation/Channel 1 (C). Here we can see that there is also fluorescence present at the 488nm excitation/Channel 1 (A). This is due to the long excitation 'tail' on the 488nm peak, which is partially extending into the 568nm wavelengths. For this purposes of this investigation the 488nm excitation was used, and detected on channel 2, due to the fact that this arrangement gives the strongest autofluorescence signal from this tissue. Scale bar = 200microm.

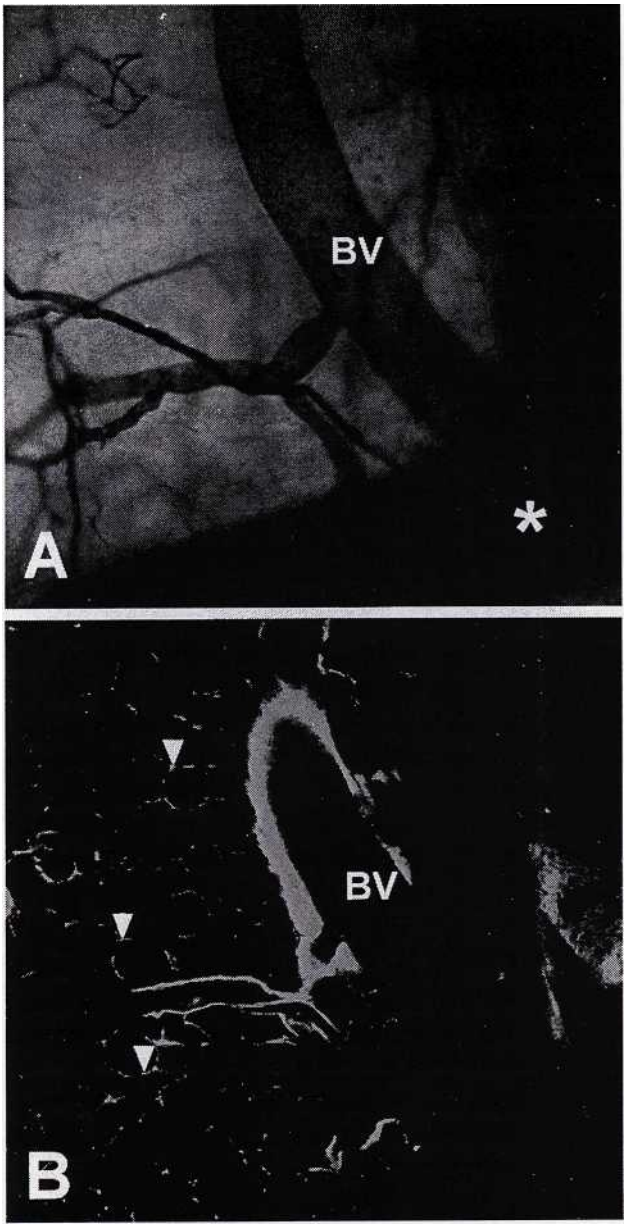
# Results

## *E12 sheet plastinated tissue is fluorescent*

The E12 sheet plastinated material we examined was formalin fixed, with the vascular structures stained by hematoxylin perfusion. After these pre-treatments autofluorescence was found to be present within the tissue at both 488nm and 568nm excitation wavelengths (Fig. 1A-D). The 488nm excitation gave a much stronger emission signal from the tissue.

## *Visualization of previously unseen structures by CLSM ofE12 specimens*

CLSM of the E12 tissue highlighted the collagen distribution and arrangement in loose connective tissue surrounding adipocytes (Fig. 2), epineurium surrounding small cutaneous nerves (Fig. 2) and the structure of the deep fascia.



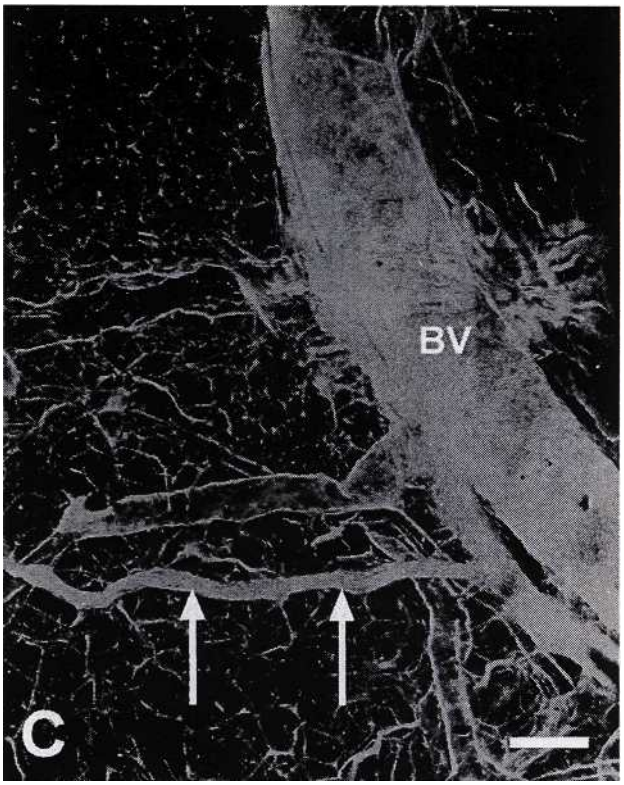




**Figure 2.** A. Light microscopy image of El2 plastinated tissue. The blood vessels (BV) are stained with dilute Gill's hematoxylin #1. Note the obscuring shadow left by a large out-of-focus vessel (asterisk). B. This is a O.5-1.5microm thick image from the confocal laser scanning microscopy. The connective tissues surrounding the adipocytes are clearly visible (arrowheads), along with the blood vessel walls. C. This is a three-dimensional reconstruction of the confocal laser scanning microscopy images from 0.5microm thick optical sections through a total of 210microm of tissue. Note the complex three-dimensional arrangement of the loose connective tissues surrounding the adipocytes. Arrows point to a small coetaneous nerve that was invisible in the LM image (A). Scale bar =

# Discussion

Both the 0.5-1.5µm thick optical sections, and the three-dimensional reconstructed images, from the CLSM allow research to be undertaken on structures within the El2 sheet plastinated material which would otherwise have not been visualized. The best example of such a structure is the arrangement of the collagen within connective tissue structures associated with various organs such as epineurium and loose connective tissue. These structures are virtually invisible in LM images of the corresponding E12 sheet plastinated specimens. Thus, CLSM provides a new perspective of viewing the E12 sheet plastinated tissues.

### *Tissue differ entiation/definition*

CLSM uses a physical pinhole to serially optically section tissue. Due to the fact that this is an optical section, no tissue is lost to cutting or preparation damage. The resolution of the CLSM is such that each optical section through the tissue is 0.5-1.5µm in thickness, thus giving a very high-resolution image that is virtually two-dimensional.

The serial optical sections obtained by CLSM are very well suited to be reconstructed into a three-dimensional image (Fig. 2). This reconstructed image allows direct comparison to be made between the LM image, which is an image of a three-dimensional structure, and the corresponding reconstructed CLSM image (Fig. 2).

### *Source of autofluorescence from the E12 sheet plastinated tissue*

Due to this excitation wavelength, and the spatial distribution of the emitted fluorescence, it was indicated that the detected autofluorescence was likely to be derived from the connective tissue, in particular the endogenous fluorescence from collagen (Kollias et al., 1998; Banerjee et al., 1999). It has been shown that collagen from skin, cervical tissue, bone, ligament and colon is autofluorescent (Prentice, 1967; Leffell et al., 1988; Swatland, 1988; Glassman et al., 1995; Banerjee et al., 1998; Kollias et al., 1998; Banerjee et al., 1999). Many of these studies report that collagen is fluorescent at a much shorter wavelength. Kollias et al. (1998) demonstrated that a pepsin-digestible collagen was present at 340nm, and a collagenase-digestible collagen was present at 360nm. Following this, Banerjee et al. (1999) showed that collagen IV is identified by auto-fluorescence at the 365nm wavelength. This difference may be explained. It has been shown that ultraviolet (UV) light changes the auto-fluorescence peaks (Swatland, 1988; Kollias et al., 1998). Swatland (1988) found that exposing various bovine tissues to UV light for one minute altered the auto-fluorescent emission from 410-420nm to 450-470nm. Similarly, after 30min exposure to UV light, the emission peak at 450-470nm was not present, instead a peak was most intense at approximately 530nm. If we acknowledge that these E12 sheet plastinated specimens of human skin and subcutaneous tissue have been subjected to very low, but chronic levels of UV light for 65-87years then it seems possible that the auto-fluorescence peak is of a longer wavelength than those found in experiments on laboratory based animal models with relatively short life-spans.

### *Fixative derived fluorescence*

It should also be acknowledged that CLSM does carry with it some inherent limitations. In this investigation all of the tissue studied had been fixed in 10% neutral buffered formalin. Literature indicates that formalin, and the similar chemical formaldehyde, have auto-fluorescent properties (Clancy and Cauller, 1998; Aisa et al., 2001). For this reason the type of fixative used in tissue preparation for E12 sheet plastination may be very important. It would be of great interest to investigate the effect that different fixation protocols, or no fixation, have on the auto-fluorescence of plastinated tissue. However, it is our policy that all human tissue for plastination must be fully fixed prior to plastination.

### *Limitations of CLSM and E12 sheet plastination*

A limitation of CLSM of E12 sheet plastinated material is that the physical size of the specimen. In order to be able to view specimens under the BioRad confocal microscope the specimens must be no larger than approximately 100mm x 40mm. This means that large specimens used either for other research projects or teaching are not able to be investigated by CLSM. Generally specimens are required to be produced specifically for CLSM examination, although larger specimens may be 'cut-down' to a size which is able to be used for CLSM.

Another limitation of CLSM of E12 specimens is that the penetration of the light into the E12 specimens from the CLSM laser is relatively limited. Under optimal conditions the laser light may only penetrate 300µm into the specimen. This means that in a 2.5mm thick E12 sheet plastination specimen, a total of only 600µm (300µm from each side of the tissue), the remaining 1900µm of tissue thickness is not detected by the CLSM and may only be viewed by conventional LM. There is, however, a major benefit to the lack of penetration of the CLSM laser light into the tissue, the 600µm of tissue thickness that are visualized by CLSM have incredibly high definition and differentiation. Due to the fact that this very high definition image is only

0.5-1.0 microm in thickness, and with no 'missing sections' between adjacent optical sections, the series of optical sections can be reconstructed to form a high resolution image. This reconstructed image has one major benefit over either LM images (which are images of a three dimensional structure) or large 'whole mount' fluorescent images, due to the fact that the reconstructed CLSM images have no out-of-focus images or 'background noise'.

In conclusion, CLSM has been shown to be a highly effective tool for viewing the connective tissue, in particular collagen, within E12 sheet plastinated specimens. This method is likely to prove to be very useful as a research tool for the investigation of connective tissue architecture.

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# Epoxy Impregnation without Hardener: To Decrease Yellowing, to Delay Casting, and to Aid Bubble Removal

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*Abstract:* Epoxy slices often yellow shortly after casting and the few hours following impregnation can be overwhelming as all slices need to be cast in a short period of time. A modified technique for producing epoxy slices was developed as a measure to address these problems. Tissue slices were impregnated using only epoxy polymer; no hardener was used during impregnation. The impregnated tissue slices were cast using modified casting-mixtures of epoxy polymer, hardener and glass separator. This modification of the classic E12 method (Biodur™) was done to determine: 1. If it is possible to indefinitely extend the casting time after impregnation of epoxy slices and produce quality slices; 2. If the impregnation bath could be reused for casting; 3. If transparency, bubble removal and aesthetics of the final sheet could be enhanced; and 4. If yellowing of the cast could be reduced. The unreacted epoxy impregnated slices were stored in the impregnation mixture for up to one year prior to casting. Hardener was painted on random slices prior to casting. All slices were cast with a polymer reaction-mixture containing 20 to 27% El (hardener) and 1 or 4% AE30 (glass separator). All cast manufactured slices cured. Tissue slices, which rested on the glass, had small areas that did not cure properly. These blemishes were corrected by recasting using a thicker gasket, placing polymer reaction-mixture on the blemished surface and covering with a glass, or placing polymer reaction-mixture on the blemished surface with no glass cover. All recast slices cured and were useful. After a few days, over 50% of the slices turned yellow. However, the intensity of the yellow was much less than that of slices produced by the classic E12 method.

*Key words:* epoxy; slices; **plastination;** yellowing

## Introduction

Today, because utilization of sectional imaging modalities has become the norm in medicine, increased interest as well as necessity has heightened the need to understand sectional anatomy. Therefore, the production of appropriate, high quality anatomical aids in a timely manner needs to be addressed. Various polymers (silicone, polymerizing emulsion, epoxy, polyester) have been used for producing anatomical

sections (von Hagens, 1979; Bickley et al., 1981; von Hagens, 1982; Guhr et al., 1987; Lischka and Prohoda, 1987) and each has their uniqueness. Sheet plastination has been an integral part of the plastination community for over two decades and epoxy slices have been routinely produced and used for study of sectional anatomy (von Hagens et al., 1987; McNiesch and von Hagens, 1988; Cook and Al-Ali, 1997; von Hagens and Whalley, 2000; Latorre et al., 2001; Windisch and Weiglein, 2001). The classic epoxy (El2, Biodur™) method (von Hagens, 1985; von Hagens, 1989; Weber and Henry, 1993) can be extremely challenging to an under-staffed, an emerging or even a well-established laboratory. Certainly, the day on which impregnation is completed is extremely challenging. The pot-life of the polymer reaction-mixture is about forty-eight hours and impregnation takes thirty-six hours. Therefore, when impregnation is complete, there is only a short window of time in which to cast the slices. As well, the reaction-mixture has become extremely viscous which increases the likelihood for bubbles to remain trapped in or around the specimen. Removal of trapped bubbles presents another time issue. Additionally, epoxy yellows with time thus decreasing the optics and the aesthetics of the slice. These challenges prompted us to seek a remedy beyond recruiting more hands.

## Materials and methods

Fresh tissues of various regions of the body (limb, head, trunk and body cavities) and from various species (bovine, canine, equine) were collected from cadavers, prepared for sectioning and frozen (-70°C). Two to six millimeter slices were sliced on standard carpenter or butcher saws and placed on grids. Liquid nitrogen or ice was used to cool the saw table for production of the 2mm slices. Saw dust was removed by submerging tissue slices in cold acetone and scraping or flushing with a small stream of cool tap water with minimal scraping. Cleaned slices on their grids were submerged in 90$^{+}$%, -20°C acetone. Two changes of cold 100% acetone were carried out (day 3 and day 6). Fifteen slices were submerged in methylene chloride for seven days. Dehydrated slices on their grids were submerged in one of three epoxy polymer mixtures (Biodur™): #1. 79% E12 polymer, 17% AE10 thinner and 4% AE30 glass separator, #2. 95% E12 and 5% AE30, or #3. 100% El2. The slices were allowed to equilibrate overnight in the polymer. As well, used polymer mixture was reused to impregnate slices six and twelve months after its initial use for impregnation. The submerged specimens, in their container, were placed in the vacuum chamber and vacuum was applied. Pressure was slowly decreased by incrementally closing the air by-pass valve over a 3 to 6 day period. Pressure was monitored with vacuum measuring devices [gauge (initially) and Bennert-type manometer] and by observation of bubble formation and bursting. As a control, some slices were impregnated using the classic E12 method and impregnation polymer-mix (65% El2, 18% El, 14% AE10, 3% AE30) (von Hagens, 1985; von Hagens, 1989; Weber and Henry, 1993).

Impregnation was judged complete when a pressure of 2-3 mm Hg was reached and maintained and bubble formation was greatly reduced. After impregnation was complete (6-10) days, pressure was returned to ambience. The impregnated slices were held in the impregnation polymer at room temperature. At intervals of 1 day, 1 week, 1 month, 6 months and 12 months, slices were removed from the impregnation polymer and cast in flat-chambers. Flat-chambers were made from 2-3mm window glass, appropriate diameter gasket and fold back clamps. The top of the flat-chambers remained open for insertion of the slices and filling with epoxy reaction-mixture. Four casting formulas were used: #1. 76% E12 polymer, 20% El Hardener, 4% AE30 glass separator; #2. 73% E12, 23% El, 4% AE30; #3. 69% E12, 27% El, 4% AE30; #4. 75% E12, 24% El, l%AE30.

Before casting, each slice was briefly drained of excess impregnation polymer. Two-thirds of the slices were inserted into flat-chambers at the prescribed intervals. The other one-third of the slices had either: 1. Surface polymer blotted for 24 hours and then cast with one of the casting formulas, 2. Surface polymer blotted for 24 hours, then hardener painted on the surface and cast, or 3. Surface coated with hardener.

Each flat-chamber containing a slice was filled with a polymer reaction-mixture. The chambers were allowed to sit upright for 1 to 2 hours to encourage bubbles to rise to the surface. As needed, flat-chambers were tilted 20-30° off the vertical plane to aid the rise of bubbles from each side of the flat-chamber. Remaining trapped bubbles were freed using a teasing wire. Some specimens were placed upright in a vacuum chamber for 1 to 2 hours and pressure was lowered to 15cm Hg to aid in bubble removal. After bubbles were removed, the filled flat-chambers were laid 15° off the horizontal plane and allowed to cure overnight. After 24 hours, the flat-chamber was inspected for drifting of the tissue slice. If the slice had drifted, it was realigned in the flat-chamber. As well, blanks were made by filling flat-chambers with the used classic impregnation-mixture or each casting-mixture to evaluate yellowing.

After 3 to 5 days of room temperature curing, most of the flat-chambers and contents were placed upright in an oven of 50-60°C for 3 to 5 days for final curing.

Three flat-chambers filled with the classic El2 impregnation-mix and casting formula #1 were allowed to cure totally at room temperature over a two-week period. After curing, the oven was turned off and allowed to come to ambience prior to removal of the flat-chambers. The flat-chambers were disassembled and slices were removed, inspected for complete curing, wrapped in foil, and their perimeters squared using a band saw. Any slices with damp areas were either: 1. Recast using a thicker gasket to allow polymer reaction-mixture to cover the surface of the slice, 2. Covered with epoxy reaction-mixture and a glass placed on top of the polymer-mix, 3. Damp spots were covered with epoxy reaction-mixture, or 4. Damp spots were painted with hardener. All types of repairs were room temperature and heat cured as in the original casting protocol. After curing, random slices were transected at one week and one and one-half years to inspect the core of the tissue slice.

### *Impregnation and casting*

*#1:* Dehydrated slices were submerged in methylene chloride for seven days prior to impregnation. Impregnation was via the classic method and polymer-mix (E12, El, AE10, AE30). Slices were cast in formula #1.

#2: Dehydrated slices were submerged in methylene chloride for seven days prior to impregnation. Then into the impregnation polymer-mix (E12, AE10, AE30) without hardener. Slices were cast in formula #1.

*#3:* Dehydrated slices were submerged in El2, AE10, AE30 for impregnation. Slices were cast in formula #1.

*#4:* Dehydrated slices were submerged in El2, AE30 for impregnation. Slices were cast in either formula #1 or #2.

#5: Dehydrated slices were submerged in El2, AE30 for impregnation. Slices were cast in formula #3.

*#6:* Dehydrated slices were submerged in El2 for impregnation. Slices were cast in formula #4.

#7: Dehydrated slices were submerged in previously used El2, AE30 for impregnation. Slices were cast in formula #3.

## Results

All manufactured casts cured and were useful (Fig. 1). However, tissue slices which rested on the glass had small areas that remained damp and excoriated if not repaired (Fig. 2). These often were the thicker (6mm) slices. Some slices that had not been **blotted but** coated with hardener before casting frequently had similar damp areas on the surface of the slices, which excoriated if not repaired. Of the defective manufactured slices: the ones which were recast using a thicker gasket and casting reaction-mixture or whose surface was coated with casting formula whether or not topped with a glass plate cured routinely. An occasional bubble was trapped in glass-topped repaired slices and slight raised areas remained on the surface when polymer without glass was placed on the defect (Fig. 3). All slices whose damp areas were coated with hardener alone did not cure and excoriated if not repaired. However, even these slices, when coated with reaction-mixture and covered with glass, were repairable. Occasional refractive areas (Fig. 4) are seen especially with some of the excoriated areas. The slices that have been completed for 18 months retain a quality similar to that when they were first completed.

Cast tissue slices from all protocols, including those bathed in methylene chloride, began to yellow within the first few days following casting (Fig. 5). However, 2 mm tissue slices that remained in the polymer for six and twelve months before casting showed only a very light yellowing after casting, as well as, six months later. The new polymer reaction-mixture in the perimeter around the recast-manufactured slices did not yellow (Fig. 5). Blank casts of the classic and experimental impregnation-mixtures demonstrated the greatest yellowing. Blank casts of the casting mixtures showed no to only slight yellowing. The blank casts cured only at room temperature showed the least tendency to yellow. By one year post-casting, epoxy had yellowed more.

Bubbles rose easily in the relatively thin polymer-mix. Remaining bubbles were easily teased out using a wire. Flat-chambers placed under vacuum to aid the rise of bubbles developed numerous pinpoint size bubbles (Fig. 5). Of these vacuum induced tiny bubbles, many did not rise to the top but remained distributed throughout the polymer.

Random slices that were transected to inspect the interior of the slice revealed that the epoxy polymer in the core of the tissue slices was not cured. The core of specimens, both one week and one and one-half years post-impregnation that were checked, had uncured polymer.

## Discussion

Epoxy impregnation without hardener extended the casting time for one and one-half years and yielded both high quality and lesser quality manufactured slices.

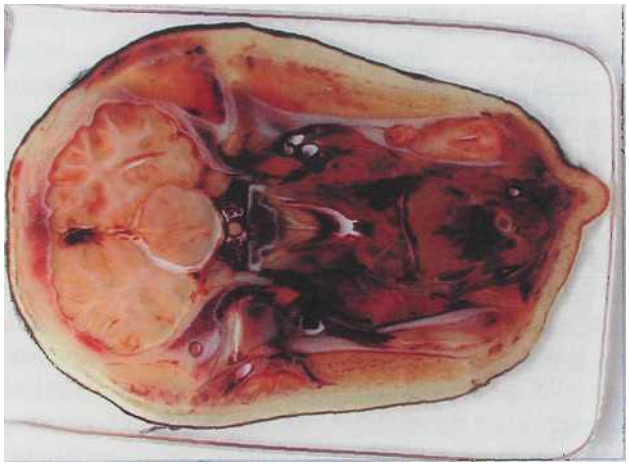

**Figure 1.** Tissue slice plastinated using a modified E12 method lacking hardener in the impregnation step.

**Figure** 2. Modified E12 plastinated tissue slice showing surface defect (arrow).

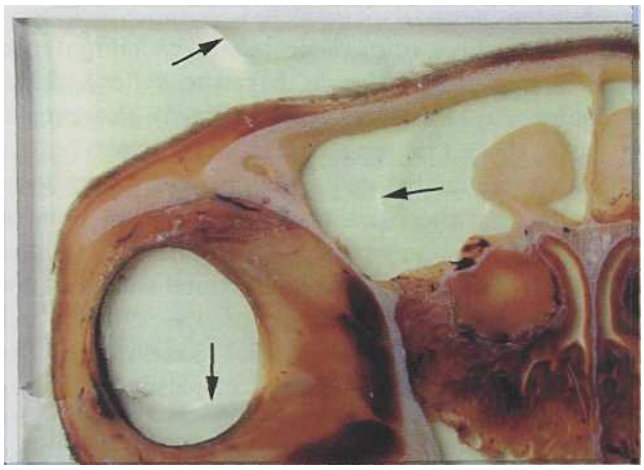

**Figure 3.** Modified E12 plastinated tissue slice showing raised edges of repaired defect (arrows).

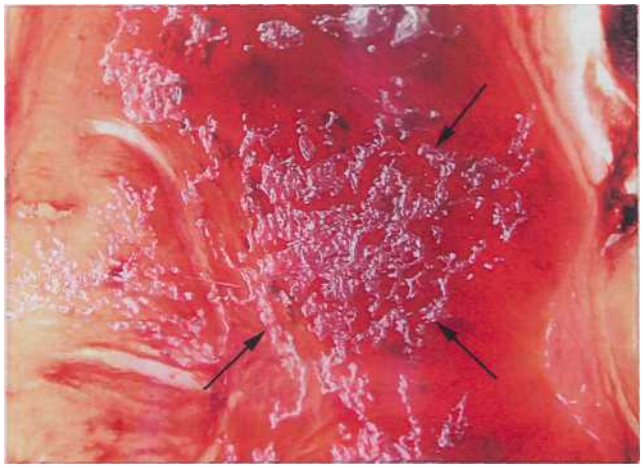

**Figure 4.** Modified E12 plastinated tissue slice showing reflective areas caused by trapped air (arrows).

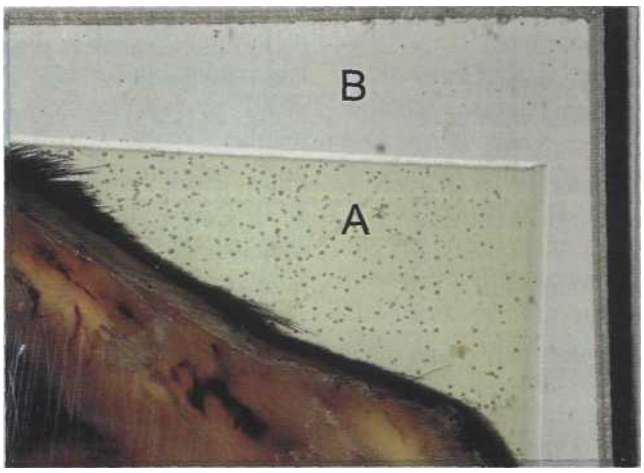


**Figure 5.** E12 plastinated tissue slice recast after yellowing of original cast material. Original cast material (A), second cast (B).

Lesser quality slices were less likely to occur when using very thin tissue slices or a thicker than usual gasket (9-10mm). This is likely due to the fact that thinner specimens float more uniformly and hence do not settle and remain on the bottom glass. When the specimen settles onto the glass, the lack of polymer reaction-mixture between the tissue slice and the glass yielded a manufactured slice with damp (uncured) areas on its surface. Damp areas were evidenced in all slices, which were not blotted but coated with hardener before casting. Flat-chambers may be turned from side to side every few hours to prevent settling of specimens against the glass.

With very thin tissue slices (2mm), there may be a relationship to residual acetone and the yellowing process. The manufactured slices containing two millimeter tissue slices that remained in the polymer for six months prior to casting yellowed only slightly. However, all thicker slices (3-5mm), including those remaining in the impregnation bath long term, yielded manufactured slices that yellowed in a few days of curing. However, the degree of yellowing was less than with the classic method. To best compare the degree of yellowing, experimental and classic slices should be prepared at the same time. We intend to do this project in the future. Unless epoxy polymer in some way ties up acetone, all acetone should have evaporated from all the long-term impregnated slices. If indeed all the free acetone has evaporated, this suggests that certain tissue components or heat curing may cause the yellowing of the cured slices. Even the methylene chloride specimens yellowed. Some casting formulas that were cast with out tissue slices yellowed slightly, however, some did not yellow. The perimeter of recast-manufactured slices did not yellow and they were both room-temperature and heat cured. This would add credence to a tissue reaction causing the yellowing since the tissue slice is sealed from the recasting polymer-mix. More testing in this area is needed to determine if there is an inherent cause for the yellowing in this first generation polymer or if it is reacting with some tissue component, fixative or heat.

All defective areas were repairable. The repaired slices were not perfect but quite useful. The method of repair that produced the most aesthetic slice was to recast. A second repair method, which also worked almost as well, was to coat with casting-mixture and place a glass on top. The most distracting feature of a defective slice was the small refractive areas found randomly in the cast tissue slices. These refractive areas are trapped air. Air is likely introduced as the thin impregnation-polymer either drains or is blotted from the slices. We believe that it is necessary to have minimal unreacted impregnation epoxy with the tissue slice as it is cast. The raised areas, which resulted from covering defects with polymer and no glass, detract from the aesthetics of the slice. However, the ease of this type repair seems to out weigh the work of recasting or placing a glass cover on top of the coated slice.

AE10 (thinner) is not needed when impregnating without hardener since the impregnation-mix does not thicken due to the fact that no hardener is present. AE10 was used in one impregnation formula to mimic the classic impregnation-mixture as close as possible. Because the impregnated slices did not have the thickened impregnation-mixture surrounding them, the thinner polymer-mix in the flat chambers allowed bubbles to rise freely to the surface. By tilting the filled cast from side to side for a few minutes most remaining bubbles surfaced. Those that did not surface were freed with a teasing wire. Vacuum application greatly increased the number of bubbles. It was not possible to get all of these numerous tiny bubbles to rise. They remained in the manufactured slice. This may indicate some breakdown or evacuation of a component of the casting mixture. The use of vacuum for evacuation of bubbles from epoxy flat-chambers should be used cautiously.

Uncured polymer-mix in the center of the tissue slice was predictable. When preparing epoxy polymer as per von Hagens (1985), it is important to thoroughly mix hardener and polymer to assure that the hardening reaction will proceed. Even one and one-half years after curing, the core of the tissue slices remains uncured. In spite of this, the embedded tissue retains its normal anatomical qualities. These slices produced by a modification of the classic epoxy method have similar optical properties as the slices made via the classic method. Thus these specimens should be useful for anatomical aids to be used in conjunction with medical imaging. This technique is a valuable alternative to the classic method for an understaffed lab. Since the tissue slices can be cast at any time, one person can cast the slices at their leisure. The thinner polymer surrounding the tissue slice allows bubbles to rise freely. The manufactured casts seem to be less yellowed. However, this modified method yields a number of blemished casts that need to be repaired, whereas the classic method produces nearly perfect slices every time which will yellow.

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# Shrinkage During E12 Plastination

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*Abstract:* The goal of this project was to determine the amount of shrinkage that occurs during E12 plastination. A human pelvis was transversely sliced into 3.5mm sections and processed using the standard El2 plastination process. After initial slicing and after three of the processing steps (both acetone baths and curing), the area of each slice was traced and recorded using IMAGE TOOL v.2.0 software. The total shrinkage percentage was calculated for the entire process, as was percent shrinkage between each recorded measurement. Total shrinkage (decrease in area) was 6.65%. The greatest shrinkage (4.52%) occurred between the final acetone bath and curing.

*Key words:* **human pelvis slices; polymer E12; shrinkage**

## Introduction

The El2 plastination process is a well-established preservation technique used for demonstration in teaching and also in research (von Hagens et al., 1987). In spite of that, we found no data concerning the shrinkage of plastinated slices. Knowing that shrinkage may lead to incorrect conclusions in morphometric or topographic questions, this study was designed to determine the shrinkage of slices using the El2 plastination technique. These data may partially fill the lack of information about shrinkage in plastination. This is part of a greater project on shrinkage in plastination that started with a study on P40 shrinkage (Sora et al., 1999). A previous publication using plastination in 3D reconstruction (Sha et al., 2001), did not address shrinkage. Slice shrinkage after E12 plastination should be taken into account when reconstruction of structures is intended.

## Materials and methods

### *Material and slicing:*

A male human pelvis was removed from an unfixed cadaver and then frozen at -80°C for one week. Transverse slices (3.5mm) were cut, starting at the level of the first sacral vertebrae and finishing just distal to the minor trochanter. Thirteen slices were used for this study. Numbering markers were placed on the superior surface. The slices were stored at -25°C overnight prior to processing for El2 plastination.

### *Scanning:*

The original size of the frozen slices (Fig. 1) was recorded by scanning their superior aspect via an EPSON GT-10000+ Color Image Scanner. A cm scale served as a calibration marker. The slices were scanned three more times: Twice on cold acetone saturated slices (after the first and last acetone bathes) and Once on the dry final cast slice (after El2 plastination) (Table 1). The slices, including the fresh-frozen, were covered by a transparent foil on both sides to protect the scanner and to decrease acetone vaporization. Using the UTHSCSA MAGE TOOL v.2.0 for Windows software (The University of Texas Health Science Center in San Antonio), the area of the slices was calculated.

### *Dehydration and degreasing:*

Twenty-five liters of cold (-25°C) technical quality

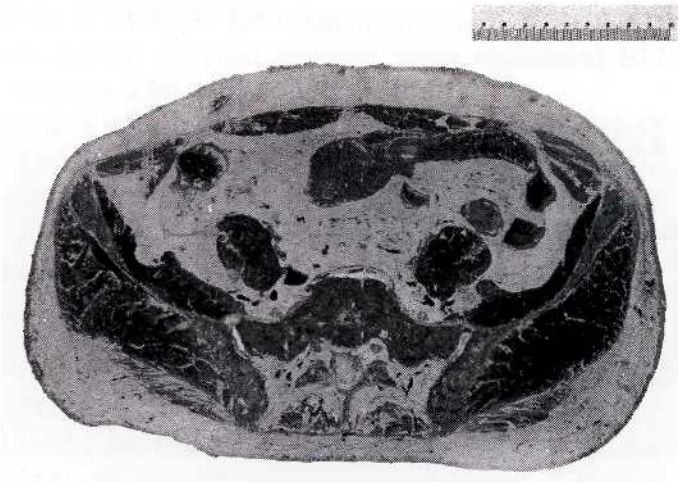

**Figure 1.** Frozen cross-section of human pelvis.

| | Temperature | Measurements | Days |
|---|---|---|---|
| Fresh | -80°C | yes | 0 |
| Acetone 1 | -25°C (96%) | yes | 4 |
| Acetone 2 | -25°C (99%) | yes | 3 |
| MeCl | 15°C | no | 7 |
| Impregnation | 5°C | no | 2 |
| After E12 | 15/45°C | yes | 6 |

**Table 1.** Plastination conditions.

acetone was used for each dehydration bath for the 13slices. Each slice was placed between plastic grids to allow better circulation of the dehydration fluid. Dehydration time was 7 days. Acetone was changed on day 4 and day 7 using cold technical quality acetone. When dehydration was complete, the freezer was disconnected to warm to ambience (15°C) overnight. On day 8, acetone was replaced with room temperature methylene-chloride (MeCl) for 7 days of degreasing (Table 1).

*Impregnation:*

Impregnation was performed at 5°C using the classic epoxy (E12) reaction-mixture: E12/E1/AE10 (95:26:10 pbw) (von Hagens, 1985). The slices were removed from the methylene chloride bath, submerged in the El2 reaction-mixture and placed in a vacuum chamber. Pressure was continuously reduced over the next two days to 2mm Hg. Temperature was kept under surveillance in order to avoid El2 crystal formation which will take place if the temperature decreases below 0°C.

*Casting and curing:*

The slices were cast between two sheets of tempered glass and a flexible gasket was used as a spacer (4mm). The following E12 reaction-mixture was used for casting: E12/E1/AE30 (95:26:5). The slices were placed between glass plates and sealed. The flat-chambers were filled with casting-mixture and placed in a vacuum chamber at 3mm Hg for one hour to remove small air bubbles present in the resin. Large bubbles were removed afterwards manually. After bubble removal, the flat-chambers were placed horizontally inclined at 15° and left until the next day. The polymer became more viscous and sticky. The next day the flat-chambers containing the slices were placed in a 45 °C oven for 4 days. After removal of the flat-chambers from the oven and cooling to room temperature, the glass plates were removed carefully and the sheets were cut as desired.

## Results

The plastinated E12 slices obtained were of high quality (Figs. 2, 3). Their transparency and color were perfect and shrinkage was not evident. Average tissue loss between sections was lmm due to the saw blade. The finished E12 slices were semi-transparent, easy to orient and offered a lot of anatomical detail down to the submacroscopic level. The transparent loose areolar and adipose tissue contrasted perfectly with the muscle and epithelial tissue.

The average total percent shrinkage after El2 plastination per slice was 6.65% (+/- 1.123 standard deviation) (Table 2). Average shrinkage after the first acetone bath was 1.33%. Average shrinkage during the second acetone bath (prior to warming to room temperature) was 0.8%. Average shrinkage per slice during MeCl and the epoxy process was 4.52%. The acetone percentage at the end of acetone bath one was 96%. The acetone percentage at the end of acetone bath two was 99%.

## Discussion

Since the beginning of plastination, the El2 technique is the elected method for producing transparent body slices. Transparent body or organ slices are used for teaching and research purposes, because they allow the study of the topography of all body structures in a non-collapsed and non-dislocated state. In addition, the specimens are useful in advanced training programs in sectional topography (resident training in computed tomography and magnetic resonance imaging). Many research studies deal with the topography of anatomical structures. However, if distances between structures or calibers of vessels are to

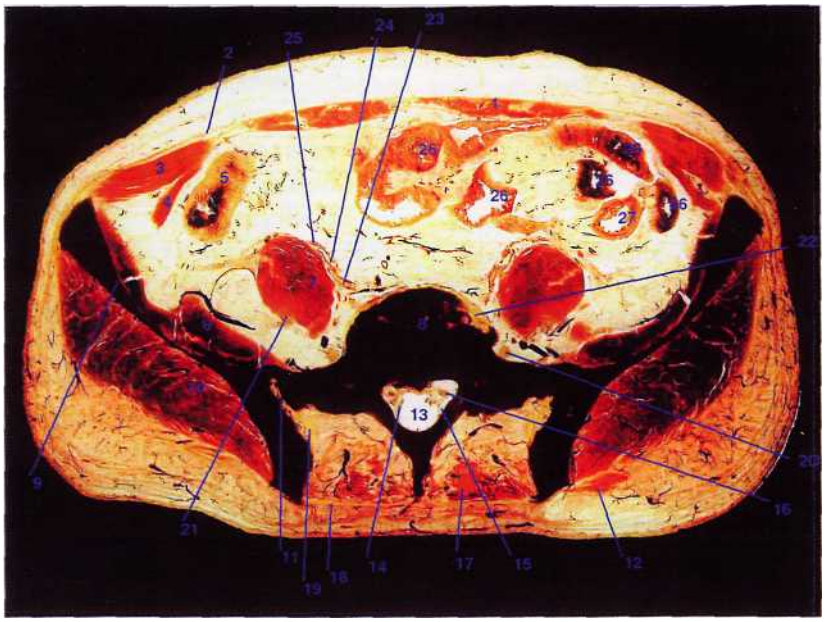

Figure 2. E12 Plastinated human pelvis section. 1. M. rectus abdominis, 2. Externusaponeurose, 3. M. obliquus internus abdominis, 4. M. transversus abdominis, 5. Caecum, 6. M. iliacus, 7. M. psoas major, 8. Promontorium/ Os sacrum, 9. Os ilium/Fossa iliaca, 10. M. gluteus medius, 11. Articulatio sacroiliaca, 12. M. gluteus maximus, 13. Canalis sacralis, 14. Cauda equina, 15. Dura mater spinalis, 16. N. spinalis(sacralis 1), 17. M. erector spinae, 18. Fascia thoracolumbalis, 19. Ligg. sacroiliaca posteriora, 20. Truncus lumbosacralis, 21. N. femoralis, 22. Lig. longitudinale anterius, 23. A. iliaca interna, 24. A. iliaca externa, 25. Ureter dexter, 26. Ileum, 27. Colon descendens.

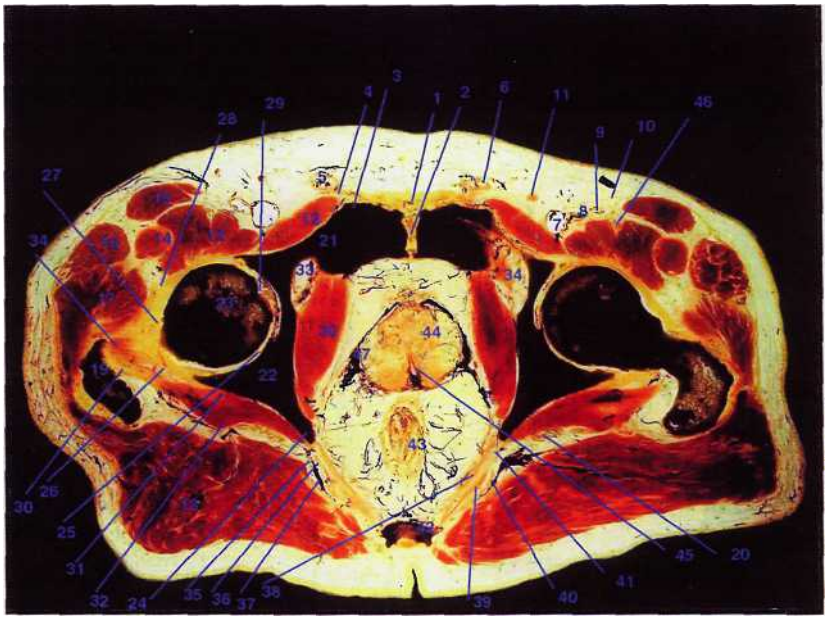

Figure 3. E12 Plastinated human pelvis section. 1. Externusaponeurosefasern, 2. Symphysis pubica, 3. Tuberculum pubicum, 4. Lig. Inguinale, 5. Funiculus spermaticus, 6. Ductus deferens, 7. V.femoralis, 8. A. femoralis, 9. A. circumflexa femoris lateralis, 10. Fascia lata, 11. Nodus lymphaticus inguinalis superficialis, 12. M. pectineus, 13. M. iliopsoas, 14. M. rectus femoris, 15. M.sartorius, 16. M. tensor fasciae latae, 17. M. gluteus medius, 18. M. gluteus maximus, 19. Trochanter major, 20. N. ischiadicus, 21. Ramus superior ossis pubis, 22. Corpus ossis ischii, 23. Caput femoris, 24. Spina ischiadica, 25. Fossa acetabuli, 26. Lig. ischiofemorale, 27. Capsula aricularis, 28. Lig. pubofemorale, 29. Fovea capitis femoris, 30. M. obturator internus, 31. M. gemellus superior, 32. M. gemellus inferior, 33. Canalis obturatorius, 34. M. obturator externus, 35. A. pudenda interna, 36. N. pudendus, 37. V. pudenda interna, 38. M. levator ani, 39. M. coccygeus, 40. Lig. sacrotuberale, 41. Lig. sacrospinale, 42. Os coccyges, 43. Rectum, 44. Prostata, 45. Urethra(Pars prostatica), 46. N. femoralis, 47. Plexus venosus prostaticus.

| NR. | Fresh | 96% Acetone | 99% Acetone | After E12 |
|---|---|---|---|---|
| 35a | 61612.36 | 61235.11 | 60546.46 | 57946.42 |
| 36a | 61741.68 | 61167.09 | 60778.50 | 56627.71 |
| 37a | 63263.41 | 62241.22 | 62055.07 | 57639.29 |
| 38a | 63483.53 | 62017.89 | 61286.99 | 60182.38 |
| 39a | 63669.02 | 62567.11 | 62249.21 | 59282.22 |
| 40a | 64292.74 | 63455.28 | 63058.32 | 59663.66 |
| 41a | 63746.73 | 63112.97 | 62337.92 | 60253.40 |
| 42a | 63837.84 | 63075.22 | 61948.24 | 59701.14 |
| 43a | 64801.36 | 64521.07 | 64347.75 | 60861.43 |
| 44a | 64767.05 | 63687.45 | 63322.74 | 60233.35 |
| 45a | 65120.47 | 64034.88 | 63551.06 | 61252.31 |
| 46a | 64929.94 | 64012.37 | 63319.67 | 60527.69 |
| 47a | 64667.38 | 63787.56 | 63438.67 | 60916.67 |
| Average **Shrinkage** = | | **1.33 +/- 0.502%** | **2.13** +/- **0.668%** | **6.65+7-1.123%** |

**Table 2.** Surface area of tissue slices measured in mm at different stages of El 2 plastination.

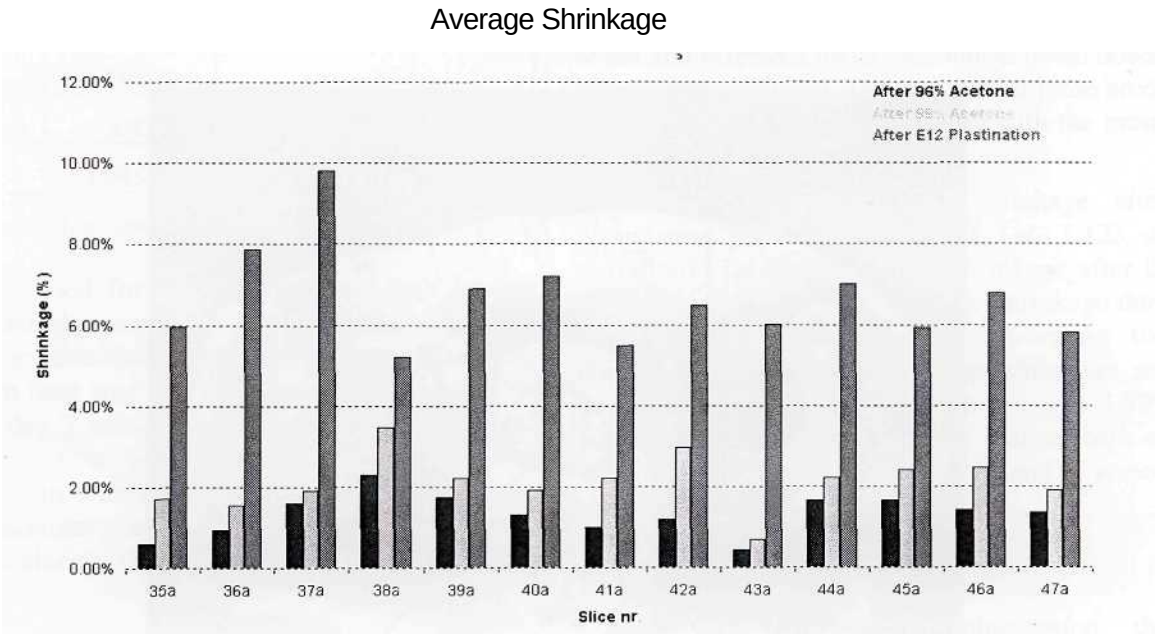


**Figure** 4. Percent shrinkage of tissue slices at different stages of E12 plastination.

be measured on plastinated slices, correct results can only be obtained when the shrinkage rate is considered. However, we did not find any data regarding the shrinkage of E12 slices in the literature.

Two factors contribute to the amount of global shrinkage: Shrinkage of the epoxy polymer itself and Shrinkage of the body slices during the entire plastination process. Because no data was found on the shrinkage rate of BIODUR E12, we designed and carried out experiments with E12 polymer by casting 4mm flat-chambers without inserting body slices. The observed shrinkage of E12 polymer was less than 0.2%. These results are comparable to data obtained from the CIBA Company (Ciba Spezialtatenchemie GmbH,

Breitenfurterstrasse 251, A-1231, Vienna, Austria) who use similar epoxy resins.

The shrinkage values in this study represent only two-dimensional shrinkage. Determination of shrinkage in the third (vertical) direction would have been very difficult, and almost impossible to make after each plastination step. The thickness of the plastinated slices was defined by the gasket (4mm) used for construction of the flat-chambers. Only by transecting the slices and scanning the edge, would it be possible to obtain information on vertical shrinkage. We did not perform this step.

The superior surface of each slice was used to measure the area. By evaluating the obtained data we were able to determine the total shrinkage of the area of each slice. As well data was obtained from three stages throughout the process. Although the slices were removed from acetone for scanning, it is improbable that this led to shrinkage, because the slices were covered with foil to decrease acetone vaporization. Also, scanning took only one minute.

It is essential to be aware of the global shrinkage percent, due to plastination, when dealing with measurements. When considering the plastination process, an average shrinkage value of 6.65% seems reasonable. The percent of shrinkage during the first cold acetone bath was very low (1.33%). Shrinkage in the second cold acetone bath was only 0.8%. Hence the total shrinkage during cold acetone dehydration was 2.13%. This low value could be expected when one considers the principals of freeze substitution (von Hagens, 1985). Shrinkage during the period of room temperature acetone and methylene chloride and during the E12 process was 4.52% and might also be considered satisfactory. This value represents the shrinkage that occurs during the transition from -25°C acetone to room temperature acetone, during degreasing in methylene chloride at room temperature, during epoxy impregnation and finally during curing. It would have been valuable to have measured the shrinkage after the methylene chloride bath. However, methylene chloride is very aggressive and the slices were not measured at this stage in order not to risk ruining the scanner. By comparing the data for each slice (Table 2, Fig. 4) it is evident that some slices shrunk more than others. A possible explanation could be that these slices contained more lipid tissue than others. The shrinkage bars given in figure 4 reveal that shrinkage curves for Acetone 1 and Acetone 2 are similar, whereas the curve after E12 plastination shows some deviations.

By comparing the shrinkage rates occurring after different steps of the plastination process, it may allow us to make suggestions about the processing temperature. It is known that dehydrating tissue at lower temperatures (-25° to +5°C) will keep shrinkage lower (von Hagens, 1985). Shrinkage may have been reduced if the temperature of the final acetone and methylene-chloride bath would have been 5°C or lower. However, lower temperature may increase the risk of getting less transparent slices because of insufficient removal of the tissue lipids (Cook and Al-Ali, 1997) unless the length of degreasing time is substantially increased.

When plastinated slices are used for morphometric studies, the shrinkage of each slice should be assessed in order to obtain accurate results. The total shrinkage determined in the present study should be useful to help estimate the shrinkage during E12 plastination, but other body regions (such as the thigh - essentially only bone and muscle or the distal antebrachium - more bone than muscle as opposed to the thigh) should also be studied to see if tissue type alters shrinkage. Our goal is to extend our study to determine specific tissue shrinkage: i.e. connective tissue, muscle tissue and nervous tissue.

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# Effects of Dehydration Mediums and Temperature on Total Dehydration Time and Tissue Shrinkage

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*Abstract:* To assess the efficacy of current popular plastination dehydration techniques, a variety of organs were dehydrated to: 1. Determine the minimal length of time necessary to thoroughly dehydrate specimens for impregnation and 2. Measure tissue shrinkage during dehydration. Dehydrating agents commonly used for plastination (both room and cold temperature acetone and room temperature methanol) were evaluated. Cold acetone dehydration produced the least amount of tissue shrinkage. Shrinkage was greatest in the graded methanol series. Minimal length of time necessary for acetone dehydration was five days for both cold and room-temperature acetone dehydration.

*Key **words:*** **acetone; dehydration; plastination; shrinkage**

## Introduction

Dehydration has been an integral part of the plastination procedure since its inception in 1977 (von Hagens and Whalley, 2000). Cold acetone has been the standard dehydration agent used in plastination since then. However, dehydration for preservation of biological material and its principals predate plastination 8,000 years. At least a partial dehydration was carried out in the mummification process by the ancient Chinese (Qing, 1996) and by the ancient Egyptians (Strub and Frederick, 1967). During the period of Egyptian embalming, 6,000 BC to 600 AD, an estimated 400,000,000 human mummies were prepared. Egyptian embalming reached its pinnacle during the 21st Egyptian dynasty, 1090-945 B.C. One of the steps in the most expensive methods of embalming utilized alcohol preservation and dehydration (Strub and Frederick, 1967). After other treatment and prior to bandaging the body, the body was exposed to the sun to complete the dehydration (Gale, 1961; Strub and Frederick, 1967).

The 20th century witnessed the rise and continued use of alcohol as an effective dehydrant for biological tissues (Deegener and Berndt, 1915; Berg, 1920; Hochstetter and Schmeidel, 1924; Ball, 1928; Guyer, 1930; Fessenden 1938; Bensley and Hensley, 1947; Jones, 1950; Boyde and Wood, 1969; Jones and Hamer, 1975; Gusnard and Kirshner, 1977; Fredenburgh, 1990). Alcohol may also be used as an efficient dehydrant for plastination (von Hagens, 1979a; von Hagens, 1979b; Bickley et al., 1981; Schwab and von Hagens, 1981; O'Sullivan and Mitchell, 1995). Variations of alcohol dehydration for plastination have been suggested and found to work (von Hagens et al., 1987; Kularbkaew et al., 1996). An inherent problem with alcohol dehydration and to some degree with other dehydrants is the reported and experienced high percent of tissue shrinkage (Schwab and von Hagens, 1981; von Hagens et al., 1987; Henry, 1990, 1992, 1995; Kularbkaew et al., 1996).

Acetone is another effective dehydrant that was not reported as such until the late 19th century (Pfitzer, 1895; Gatenby and Cowdry, 1928). Since then,

numerous accounts of acetone's dehydrating prowess have been reported (Mahon, 1938; Weidemann, 1938; Armed Forces, 1957; Ashworth et al., 1966; Knudsen, 1966; Lutz, 1969; Thijssen, 1972; Glauert, 1974; von Hagens 1980, 1981a, 1981b, 1982). However, acetone is also capable of causing tissue shrinkage of varying degrees. Cold acetone is supposed to cause the least amount of shrinkage.

Cold acetone dehydration (freeze substitution), was first used to obtain enhanced preservation in the field of electron microscopy (Fernandez-Moran and Dahl, 1952; Fernandez-Moran, 1959; Bullivant, 1965; Schwab and von Hagens, 1981; Tiedemann and von Hagens, 1982; Hangay and Dingley 1985). For many years, the classic protocol for acetone dehydration has been: 1, Pre-cooled specimens are submerged into cold (-25°C) 100% acetone and let equilibrate for three weeks. 2. The specimens are placed in the second cold, 100% acetone bath for two more weeks. 3. Finally, the specimens are placed in a final cold, 100% acetone bath for 1 week. In all three acetone changes, the recommended tissue to acetone ratio is 1:10 v/v (Schwab and von Hagens, 1981; Tiedemann and von Hagens, 1982; von Hagens, 1985; Bickley et al., 1987; Lischka and Prohoda, 1987; von Hagens et al. 1987; Nicaise et al., 1990). Other protocols call for 2-4 changes of cold acetone at weekly intervals (Tiedemann and Ivic-Matijas, 1988; Ripani et al., 1994). In recent years, both 100% room temperature acetone and graded series of cold or room temperature acetone have been shown to be effective means of dehydration even at lower tissue/fluid ratios (1:5 v/v) (Tiedemann and Ivic-Matijas, 1988; Zheng et al., 1996, 1998; Henry et al., 1998). Any of the above protocols have been shown to produce thoroughly dehydrated specimens for plastination. The method which produces the least amount of tissue shrinkage has not been determined. The recommended length of time that specimens are to be submerged in acetone has decreased over the years (Tiedemann and Ivic-Matijas, 1988; Henry, 1995, 1998) yet no one has standardized a shortened procedure.

The purpose of this project was: 1. To determine the minimum length of time necessary for specimen tissue fluid to be replaced with acetone in preparation for impregnation with a silicone polymer reaction-mixture and 2. To measure tissue shrinkage caused by various dehydrating agents that are routinely used in the plastination process.

## Materials and methods

Two experiments were carried out on a variety of organs including kidney, heart, liver and testicle. After collection, organs were cleaned and submerged in 5% formalin for minimal fixation overnight. The next day, specimens were flushed in running tap water for six hours to remove formalin. The specimens were then submerged in different dehydrating solutions: Room temperature acetone (ra), Cold (-15°C) acetone (ca) or Room temperature methanol (rm), according to trial protocol.

***Experiment** one:* To determine minimal length of time for proper dehydration for impregnation with a silicone reaction-mixture, the fixed organs were submerged into either room-temperature or cold (-15°C) acetone. Seven groups (1, la, 2, 2a, 3, 4, 5,) of smaller specimens each containing: 2 dog hearts, 2 dog kidneys and 1 cat kidney and four groups of larger specimens containing either: 2 porcine kidneys and a heart (6L, 8L), 2 bovine hearts (7L) or 2 bovine livers (9L) were used. The tissue to dehydrant ratio was approximately 1:10 v/v and one of the following protocols was followed for each group of tissues. As specimens completed dehydration, they were stored in pure acetone for up to 7 days. This was necessary so that all of the dehydrated specimens (whether dehydration time was 5 or 12 days) could be impregnated at the same time.

*Protocol A:* Daily, small animal specimens (groups 1, la, 2, 2a) were placed in new 99.9% pure acetone either at room temperature (1, la) or cold temperature (-15°C) (2, 2a) until 99.9% purity was maintained. Dehydrant purity was measured using an appropriate acetonometer [1-100% (Fisher) or 90-100% (Biodur™ # HD 02)] calibrated at 15°C and 20 °C respectively.

*Protocol B:* Small animal (groups 3,4, 5) and large animal (groups 6L, 7L, 8L, 9L) specimens were placed in new pure room temperature (groups 4, 5, 6L, 7L, 8L, 9L) or cold temperature (group 3) acetone every other day until 99.9% purity was maintained. Dehydrant purity was measured every other day using a 90-100% acetonometer calibrated at 20°C (Biodur™ # HD 02). After dehydration, all specimens were plastinated using the standard cold-silicone technique (von Hagens, 1985). *Experiment two:* To quantify the amount of shrinkage encountered during each of the three dehydration regimes classically used for dehydration in preparation for impregnation with a silicone reaction-mixture, specimen volume was accessed by fluid displacement pre and post dehydration. A tissue fluid ratio of 1:10 v/v was used to dehydrate four small animal specimen types (cat hearts, cat & dog kidneys, cat livers, and dog testicles) in room-temperature methanol or acetone, or cold (-15°C) acetone. Three changes of 100% acetone were carried out at weekly

intervals. The methanol series (70, 80, 90, 95, 100, 100%) was updated weekly. To determine the initial volume of the specimen by fluid displacement, the fixed, water-flushed organs were submerged in water in an appropriate vessel (graduated cylinder or beaker). Specimens were rotated to free any trapped air. Specimen volume was measured via fluid displacement and recorded. Each specimen type was divided into three groups (ca, ra, rm) and submerged in one of the three dehydrants (ca, ra, rm). After the three acetone changes or six methanol changes, the volume of the specimen was determined again by fluid displacement using the appropriate vessel and dehydrating fluid and recorded.

After dehydration, ports were cut into the right and left ventricles and atria prior to impregnation to facilitate submersion of the hearts in the viscous silicone polymer. The specimens were impregnated using the cold-silicone reaction-mixture technique Biodur™ (von Hagens, 1985). Prior to curing, one half of the kidney specimens were sliced either longitudinally or transversely and ports in the hearts were enlarged to determine if the silicone reaction-mixture had penetrated the depths of the tissue.

# Results

***Experiment one (minimal dehydration time):***

For protocol A, specimen dehydration time in cold acetone and room-temperature acetone was 5 to 7 days and 5 to 6 days respectively. The purity of the used acetone dehydration bath is recorded in Table 1. Specimens that completed dehydration first were held in pure acetone for up to 7 days. For protocol B, specimen dehydration time in cold acetone and room-temperature acetone ranged from 8 to 10 days for small animal organs and 10 to 12 days for large animal organs. These results are presented in Table 2 as percent of acetone purity after 2 days of immersion and prior to immersion into pure acetone. After impregnation, sliced organs revealed the silicone reaction-mixture oozing from the entire plane of the cut surfaces.

| | 24hrs | 48hrs | 72hrs | 96hrs | 120 hrs | 144 hrs | 168 hrs |
|---|---|---|---|---|---|---|---|
| #l,ra | 95.5 | 98.2 | 99.0 | 99.6 | 100 | - | - |
| #la, ra | 94.2 | 98.0 | 99.1 | 99.5 | 99.8 | 100 | - |
| #2, ca | 97.0 | 90.5 | 93.5 | 99.1 | 100 | - | - |
| #2a, ca | 94.0 | 96.2 | 98.2 | 99.2 | 99.6 | 99.7 | 100 |

Table 1. Cold (ca) and room-temperature (ra) acetone dehydration by using daily changes of pure acetone. Percent used acetone purity is recorded for each specimen group (1, la, 2, 2a) at daily intervals.

| | 48 hrs | 96 hrs | 144 hrs | 192 hrs | 240 hrs | 288 hrs |
|---|---|---|---|---|---|---|
| #3, ca | 87.0 | 94.3 | 98.7 | 99.5 | 100 | - |
| #4, ra | 96.6 | 98.3 | 99.0 | 100 | - | - |
| #5,ra | 86.0 | 98.7 | 99.5 | 100 | - | - |
| #6L, ra | 96.3 | 99.2 | 99.8 | 99.8 | 100 | - |
| #7L, ra | 95.3 | 98.2 | 9.5 | 99.8 | 100 | - |
| #8L, ra | 95.4 | 97.4 | 99.4 | 99.8 | 100 | - |
| #9L, ra | 95.3 | 98.2 | 99.8 | 100 | 99.8 | 100 |

Table 2. Cold (ca) and room (ra) temperature acetone dehydration by using every other day changes of pure acetone. Percent used acetone purity is recorded for each group (3, 4, 5, 6L, 7L, 8L, 9L) at 48 hour intervals.

| | cold acetone | room temp, acetone | room temp, methanol |
|---|---|---|---|
| Cat Heart | 10.7 +/- 2.41 | 19.6 +/- 7.01 | 23.0 +/- 8.20 |
| Cat & Dog Kidney | 12.8+/-3.29 | 18.2+/-3.95 | 21.4+/-7.32 |
| Cat Liver | 24.6 +/- 6.03 | 29.7 +/- 7.77 | 29.2 +/- 6.66 |
| Dog Testis | 8.8+/- 1.13 | 13.2+/-0.71 | 14.9 +/- 0.85 |
| Average | 14.5 +/- 7.01 | 20.2 +/- 7.26 | 22.6 +/- 7.68 |

Table 3. Average percent shrinkage of specimens as determined volumetrically.

*Experiment two (shrinkage):*

The average shrinkage of cold temperature acetone dehydrated specimens was 14.5% with a range of 8.2% to 29%. The average shrinkage of room temperature acetone dehydrated specimens was 20.2% with a range of 11.1% to 34.3%. The average shrinkage of room temperature methanol dehydrated specimens was 22.6% with a range of 14.3% to 34.5%. The results for shrinkage for all three dehydration methods are displayed in Table 3. The average percent shrinkage for specimens with the liver data removed was 11.2% (ca), 17.9% (ra) and 20.8% (rm).

## Discussion

Minimal length of time to dehydrate carnivore hearts and kidneys was five days, which was achieved by daily changes of cold and room temperature acetone. These findings seem to correlate with the findings of Tiedemann and Ivic-Matijas (1988) who reported cold acetone penetration of tissue blocks to be 4-5 mm the first day with a maximum of 10mm in 4 days. Their experiments used tissue blocks while these carnivore specimens were entire organs whose maximum tissue thickness was about 2.5cm. Ripani et al. (1994) suggested a markedly decreased dehydration time from the classic six weeks for parenchymatus organs (liver, spleen, kidney). They changed the cold acetone every other day and recommended a minimum of 3 to 4 baths for the best results. Their minimal length of dehydration time of six to ten days seems to correlate with our times. As well, Ripani et al. (1996) dehydrated rat organs, which are smaller than dog organs, in 48 hours.

After dehydration was complete, the carnivore organs were held in 100% acetone for 3 to 5 days to allow all specimens to be completely dehydrated. This allowed all specimens to be impregnated together as a group. Therefore, it is possible that if residual fluid was present in tissues after the 5 day dehydration period, it would have been removed during this holding period. All organs were impregnated and appeared normal twelve months post impregnation. All depths of incised tissue were impregnated with the polymer reaction-mixture.

The largest organs, cow heart and liver, took the longest time for dehydration, 12 and 14 days respectively. It was interesting that dehydration time for room temperature acetone dehydrated specimens was one or two days quicker than cold acetone dehydrated specimens. This could be a slight advantage for the room temperature dehydration procedure.

Daily changes of small specimens resulted in dehydration times of five to seven days, while every other day changes had dehydration times of eight to ten days. These findings indicate that dehydration is most rapid the first day in the dehydrant and dehydration rates slow slightly thereafter. This would suggest that optimum tissue penetration by acetone is via pure acetone with slowing of penetration upon dilution by tissue fluid. Tiedemann and Ivic-Matijas (1988) proposed that after 3 three days was the most efficient time to change the first acetone bath. Our findings, as well as the above, certainly seem to antiquate the old dehydration regime of three weeks, two weeks and then one week (Schwab and von Hagens 1981; Tiedemann and von Hagens, 1982; von Hagens 1985). Recall, this dehydration regime has produced beautiful specimens for 25 years. For economy and quality assurance, three or four days minimum in the dehydrating solution would seem to be prudent.

Shrinkage was only 14.5% in cold temperature acetone while room temperature acetone was 20.2% and room temperature methanol was 22.6%. These room temperature shrinkages were lower than the 35% reported by Holladay (1988) and the 44% and 53% reported by Schwab and von Hagens (1981). Part of the difference likely lies in the fact that their tissue included nervous tissue. Nerve tissue is known to have a higher percent shrinkage at room temperature than other tissues (von Hagens, 1985). Small animal livers had the highest percent shrinkage of the four organs tested. If liver tissue data is removed from all dehydrated specimen groups, the shrinkage percent is lowered across groups by 1.7 to 3.3%. This represents a similar decrease among groups. This finding was somewhat surprising since the liver is a parenchymatous organ. One might surmise that a densely cellular organ would shrink less than one with a lot of intercellular space because of the compactness of the cells resisting collapse. These results may indicate that less silicone enters the cells and more is extracellular, since liver tissue has minimal intercellular tissue. It would be interesting to look at this plastinated tissue histologically and see if there was a significant decrease in cell size.

Another factor that could affect results is the method and utensils used to measure shrinkage. Volume displacement seems to be an appropriate method to determine shrinkage. However, it is difficult to obtain precise measurements with conventional lab ware especially with larger specimens. This is because of the courser calibration of lab ware as the container gets larger.

The shrinkage observed using room temperature dehydration is likely large enough to alter data in quantitative studies. However, for routine gross anatomy demonstrations, it probably would not be

perceived. Certainly shrinkage can be reduced by using freeze substitution. However, the ease of a room temperature dehydration operation (no lifting of 50L or large containers of acetone in and out of the freezer), as well as, the need of fewer deep freezers may be worth the added 10% decrease in the size of the specimen. This decreased size may be a benefit when considering the storage of large specimens. Our findings confirm past speculation and reports about the shrinkage associated with dehydration using acetone and alcohol.

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