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Letter from the President

Dear fellow plastinators,

First, on behalf of the International Society for Plastination, I would like to thank you, who accepted our invitation to participate in the 12th International Conference of Plastination in Murcia, Spain, 2004 and in the 8th International Interim Conference on Plastination in Ohrid, Macedonia, July, 2005. Especially I would like to thank all of you, who will contribute actively to the success of the 13th conference by giving oral or poster presentations.

Our particular thanks go to Dr. Rafael Latorre and Dr. Vlatko Ilieski and their staff who, besides their already tough schedule, offered to host these conferences and to bear all the load, all the concerns, all the troubles, all responsibility and to do all the work, much of which is not obvious or even visible to those who never were involved with the organization of such an event, to make our conference as successful as possible.

It would be a great honor to receive you at The 13th Conference on Plastination to be organized in Austria, 2006 in the city of Vienna. The Center of Anatomy and Cell Biology of the Medical University of Vienna is proud to host this scientific meeting of international prestige. We are looking forward to bringing together so many researchers in the field of morphological sciences. On behalf of the Anatomical Institute, we would like to invite you to take part in a scientific event that hopefully will fulfill your expectations. We wish you a pleasant stay in Vienna.

Moreover, you will shortly be asked for nominations of officers, i.e. president, vice-president, secretary and treasurer. Please begin to think about nominees and also about your own willingness to serve as an officer for a two-year term.

Also, I would like to welcome all new members of the ISP. You did an important step by applying for membership in the ISP. You will not only get price reductions on conference, meeting and workshop fees, but you will also get information via our ISP-homepage which is linked to many other sites of interest for plastinators. You can obtain information from members all over the world via our List-server (isp-l@kfunigraz.ac.at) within a few hours. Moreover, Gilles Grondin has set up a wonderful online version of the Current Plastination Index (http://www.uqtr.ca/plastination/) where you can find any reference concerning plastination.

Finally, I want to thank the ISP members for their input into the society, especially via email, and the Executive Committee members for their support for pushing the quality of our journal.

With the kindest regards from Vienna, Austria
Yours sincerely,
Mircea-Constantin Sora

Letter from the Editor

Dear readers,

This volume of The Journal of the International Society for Plastination marks twenty years of the journal. In order for us to continue producing this vehicle for delivering information concerning plastination to the scientific community, we rely heavily upon your submissions. There are many abstracts that are presented at International Society for Plastination meetings that are never submitted as full manuscripts. I encourage you to do so as much of this information is extremely important to plastination and abstracts simply do not contain the full measure of data. When submitting manuscripts to the JISP, please include as many figures as possible of results as much of what is to be gained from reading the manuscript resides in viewing the photographs of finished products. In the future, I may prompt individuals submitting manuscripts to include more figures.

On the subject of journal reviewers, the editorial board membership is a little low. If you would like to be a member of the editorial board, please contact us at rbreed@utk.edu. If you know of someone you believe would make a good reviewer, please nominate them and we will be glad to contact them.

Yours sincerely,
Dr. Robert Brackin Reed, Jr.

Uses and abuses of the word "Plastination"

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Dear Editor,

The term "plastination" appeared for the first time in scientific literature in two manuscripts published in the German journal Der Praparator in 1979 (von Hagens, 1979a; Knebel, 1979). A manuscript published in The Anatomical Record did not use the word plastination but gave a precise description of the impregnation process that is the key to the plastination technique (von Hagens, 1979b). In these papers, plastination is described as, "a new method of impregnating perishable specimens with resins...by utilizing the difference between the last intermedium's high vapor tension...and the polymer's low vapor tension". This description was confirmed later as, "...a method for the impregnation of biological specimens with curable polymers...This method is called "plastination.", (Bickley et al.,1981). The plastination technique has since been widely recognized and used around the world for describing various preparations for teaching and research purposes (Grondin, 2004).

It is generally accepted that the plastination technique involves four steps: fixation, dehydration, impregnation and curing. Even if fixation can be avoided in some specific cases, generally when one uses epoxy polymer, the remaining three steps are the constituent parts of the technique with the impregnation step being the most important of all. My research to produce and update the plastination index brought me to at least three papers in which the word "plastination" is used in an improper if not abusive manner.

The first instance occurs in a paper published in 2002 by Fickert et al.. The abstract states, "The biliary tract was studied by plastination,". The materials and methods section states, "Plastination of the Bile Duct System:...After hepatectomy, the bile duct system was filled with a colored methacrylate polymer mixture...the liver was submerged in 30% potassium nitrate solution and macerated". This description is nothing more than a description of the casting of bile ducts. These casts are falsely described as plastinated specimens.

The second misuse of the word plastination occurs in the summary of a paper by Eckstein et. al. (2003). It states, "The degree of ICA diameter stenosis was determined by ex-vivo plastination of the surgically removed...". The patients and methods section states, "The eversion specimens...filled with a liquid acrylat,...After the acrylat had hardened, the specimens were incisioned and removed....We measured the minimal diameter of the surface print of the acrylic specimen." These are the acrylic casts that were measured and used for the study. We have here another example where the production of an acrylic cast is presented as plastination.

For many years, casts have been presented along with plastinated specimens as these two types of preparations represent a valuable complement to one another. Over the years, plastination polymers have been presented as good material to prepare excellent casts (Henry, 1992a; Henry 1992b; Pretorius and Geyer, 1995). Vascular casts (Graf et al., 1991, Durand 1998) or casts of cavities within plastinated specimens (Grondin et al., 2000) or in association with these (Henry et al., 1997) were also presented many times. However, in each of these articles, the casts were never presented as plastinated specimens. Additionally, the production of these anatomical casts was never called plastination of specimens.

The third incorrect use of the word plastination occurs in paper published by Ronel et al. in 2004. The abstract states, "...performing detailed anatomical dissections on 10 embalmed and plastinated cadavers." Again, when referring to the methods section we read, "Two of the arms were fresh specimens: These were injected with red silicone and then mildly plastinated". After verification with the authors to gain further insight into the so called "mild plastination", I was told that they, "arterially flushed out the two extremities, injected first with disinfecting

solution (no formalin) in the brachial artery, then some of the Corcoran Silicone (very thin viscosity/watery) with a small amount of catalyst to saturate tissue and finally casted the artery with red silicone". In this case, the arms were simply perfused with silicone until saturation. They were not dehydrated nor impregnated. Should this type of procedure be called plastination? I do not believe it should.

These are three examples of improper uses of the term plastination. Why would someone call a technique that is not plastination, plastination? Could it be that plastination has reached a certain degree of notoriety that using it's name is being used merely to impress others? Is it possible that presenting plastination as a casting method or a perfusion method could eventually lead to the perception that the plastination technique is nothing but a banal and extremely simplistic technique? What could and should the ISP do to prevent such improper use of the word plastination?

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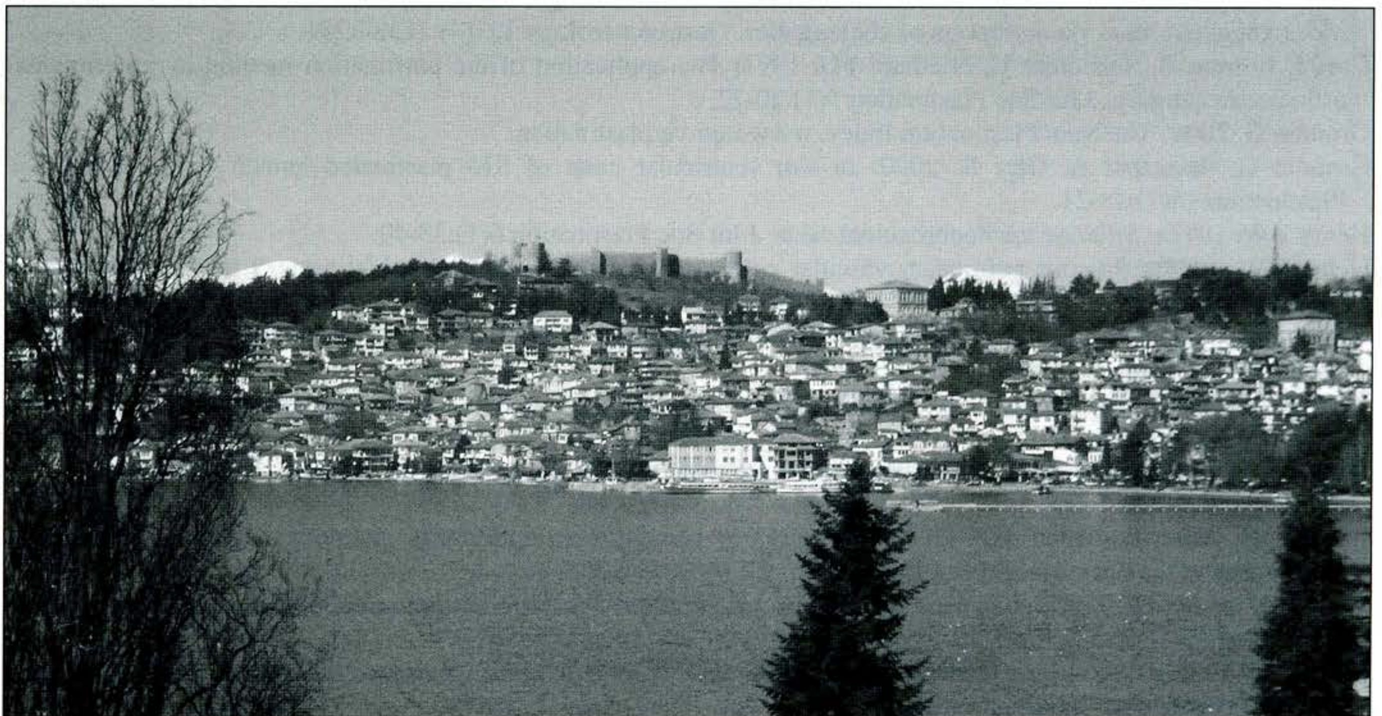
The 8th Interim Conference on Plastination

Marvelous Macedonia, Lake Ohrid, was the venue for the 8th Interim ISP congress. Hosted by the Faculty of Veterinary Medicine at the University of St. Cyril and Methodius - Skopje, each day was filled with perfect weather, the splashing of brilliant blue Lake Ohrid onto the shore by the hotel, new ideas and faces, aroma of fantastic cuisine, and most of all lasting memories and friendships. Traveling to the exquisite old cities of Ohrid, Struga and St. Naum on the boat was mesmerizing while staring into the deep blue water of one of the deepest lakes in Europe. A great line up of scientific presentations and posters filled every mind. Sixty participants from literally around the world enjoyed a wonderful unique conference and workshop as well as every need being catered to by Vlatko and Lazo and their amiable colleagues. Thank you to the Department of Veterinary Anatomy, Faculty of Veterinary Medicine in Skopje, Macedonia.



The 13th International Conference on Plastination, 2-7 July 2006, Vienna, Austria

Dear Colleagues,

On behalf of the International Society for Plastination and the Organizing Committees, we warmly invite you to take part in the 13th International Conference on Plastination, to be held in Vienna, July, 2006. There is no doubt that in the near future, plastination with its multidisciplinary facets will continue to play a key role in everyday clinical practice while contributing to pushing back the frontiers of basic understanding.

We would be very pleased to welcome many participants from all over the world. We desire to promote a very successful congress with high quality contributions. We sincerely hope for a lively exchange of experiences and we especially look forward to meeting old friends and making new ones amongst the participants from all over the world.

Welcome to Vienna
O. Univ. Prof. Dr. Wilhelm Firbas
President of the Congress

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Fax: 43 1 4277 61170
Email: plastination2006@meduniwien.ac.at
http://www.meduniwien.ac.at/plastination2006/start.htm

Accompanying Persons Program
Historical city tour with Schönbrunn Palace
Panoramic city tour with boat ride
Viennese serenade and Grinzing
Spanish riding school - morning exercises with music and guided tour
Mayor's Reception in the City hall

Please find all the information concerning the 13th ISP International Conference, including the call for papers, registration, preliminary program, social events and accommodation details on the conference web site: www.meduniwien.ac.at/plastination2006/start.htm.

We welcome you all to Vienna and hope that it will prove a memorable experience both in terms of the conference itself and the social events.

Conference Organizer: Ass. Prof. Dr. Mircea-Constantin Sora

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Plastination of Pathological Specimens - A Continuing Challenge

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Abstract: Numerous recent studies have acknowledged the merits of plastination in the anatomical sciences. Specimens conserved in this way can be handled easily and hygienically which broadens the possibilities in the presentation of anatomical preparations. The present study was aimed to introduce plastination in another morphological discipline, pathology. Conserving pathological organs or tissues with polymer impregnation and curing offer completely different challenges than when plastinating healthy tissues. Diseased tissues often have color and/or consistency changes which may be washed out or altered during preservation. However, preservation of these tissue changes in disease is of primary diagnostic importance. It can be demonstrated that color differences can be preserved in many cases as well as fixation of loosely anchored tissues without alteration of consistency is possible. Subtle, but typical alterations on the surface of diseased organs are demonstrable as well. At the same time, differences between neighboring healthy and pathological tissues can completely disappear which means a loss of important diagnostic cues. Regarding the large number and diverse kinds of plastinated specimens that have been processed, plastination serves as a useful tool in preserving pathological tissues.

Key words: **plastination; pathology; tissue**

Introduction

Plastinated specimens were introduced to morphological disciplines more than twenty-five years ago and have proven to be of great use (von Hagens et al., 1987). They are dry and hygienic which enables them to be used in an everyday environment (von Hagens et al., 1985). Our previous study along with numerous others reported that both instructors and students have found plastinated specimens as useful supplements in the anatomy curriculum (Mansor, 1996; Alpár et al., 2001; Latorre et al., 2004; Lozanoff, 2004; Riederer et al., 2004; Seródio et al., 2004; Latorre et al., 2006).

Recently we have broadened our activity in plastination to include pathological specimens as have many institutions (Kularbkaew and Cook, 1996; Martin-Alguacil and Martin-Orti, 2004). More than one hundred specimens have been plastinated in the Department of Anatomy, Histology and Embryology for the Department of Pathology, Semmelweis University of Medicine. Preservation of pathologic specimens has produced new challenges. Instead of the fixation of healthy organs, pathologically transformed tissues have to be preserved showing the characteristics which differ from those of normal tissues. Tumorous, inflamed, or degenerated tissues need to be plastinated with their typical surroundings preserved as well. In other cases, subtle color differences which were of primary importance in pathological diagnoses need to be

retained.

Although plastinated specimens cannot replace the experience of dissection and the survey of fresh, unfixed organs, they can be used outside the dissection room. For this reason, they are useful supplements to student studies and in the preservation of precious rarities.

Materials and methods

In order to investigate the conservation of different types of diseaseded tissues, specimens destined for plastination were selected from a broad range of diseases. Specimens were fixed in 4% formalin for a minimum of three weeks. Subsquently, they were dehydrated by freeze substitution with -25°C acetone. Four changes of acetone were carried out over an eight-week period. After dehydration, specimens were impregnated in a reaction-mixture of SR 10 polymer (Biodur™) with 1% Catalyst SH 03 (Biodur™). Vacuum was adjusted to a slow rising of bubbles in the silicone. Bubbles lasted four weeks. The specimens were returned to atmospheric pressure, removed from the silicone bath, warmed to room temperature and prepared for curing with Gas Cure SH 06 (Biodur™). In order to preserve the often rather vulnerable mutations, specimens were handled with care. Specimens were placed in a closed atmosphere, which was saturated by gas cure using an aquarium air pump working continuously for seven hours per day the first two days. Calcium chloride was used to control moisture in the curing chamber. Specimens were wiped every two hours the first day. Thereafter they were kept in the container for seven days and wiped as needed.

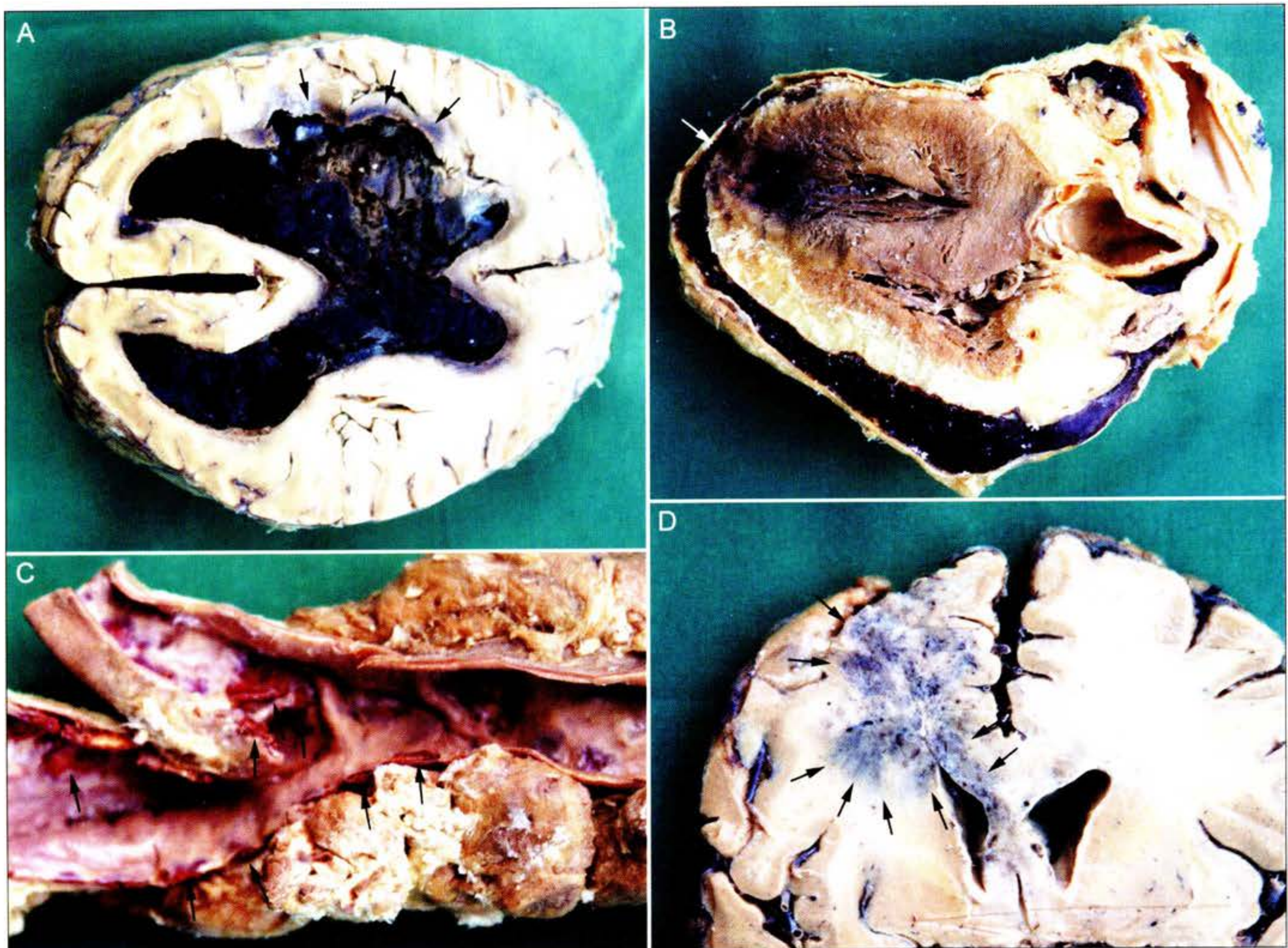

Figure 1. Coronal section of human brain with blood filled lateral ventricles (A), longitudinal section through human heart and pericardial sac demonstrating cardiac tamponade with the causative lesion at arrow (B), longitudinal section of human descending aorta with ulcerated, hemorrhagic atherosclerotic plaques at arrows (C), transverse section through human brain with intraparenchymal hemorrhage at arrows (D).

Results

Hemorrhage

Although significantly different in consistency, extensive brain hemorrhage which fully invaded the ventricles was plastinated preserving the typical location of the hemorrhage (Fig. 1a). Similarly, pericardial tamponade was also demonstrable along with the exact locus of the ventricular wall rupture (Fig. 1b). The striking color of ulcerated, hemorrhagic atherosclerotic plaques was preserved after plastination in the wall of the descending aorta (Fig. 1c). Preservation of color differences aided identifcation of intraparenchymal bleedings in the brain caused by multiform glioblastoma (Fig. 1d).

Tumors

The typical fine structure of the tumor was recognized on most specimens. The vortical, fibrous construction of an ovarian fibroma was visible (Fig. 2a) as was the spreading and osteolytic character of a bone metastasis (Fig. 2b).

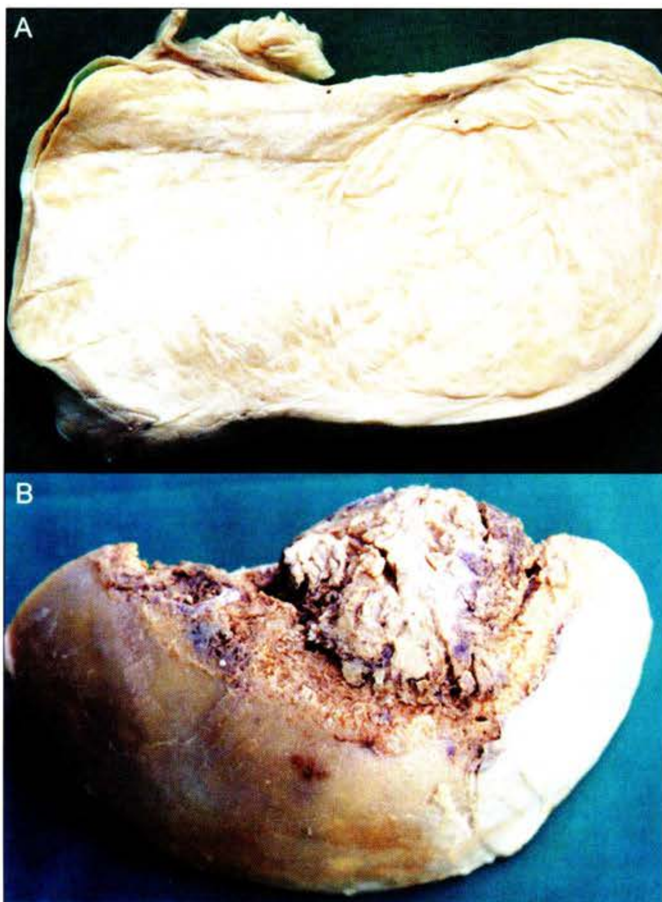

Figure 2. Longitudinally sectioned human ovary with ovarian fibroma (A), osteolytic metastasis in the epiphyseal region of a bone (B).

Inflammation

Characteristic features, i.e. color or surface differences of inflamed tissues were preserved. The typical multiple pin shaped and sized microabscesses of purulent pyelonephritis were recognizable on the surface of the kidney (Fig. 3a). The yellow-green color of a purulent meningitis could also be demonstrated after plastination (Fig. 3b).

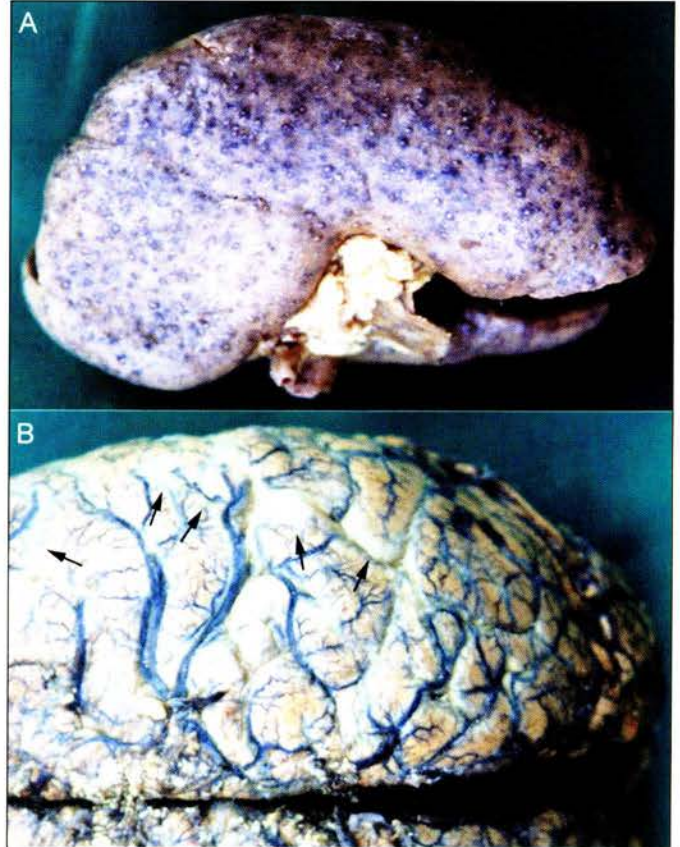

Figure 3. Plastinated kidney demonstrating purulent pyelonephritis (A). Plastinated brain (B) demonstrating purulent menengitis at arrows.

Surface preservation

After plastination the characteristic fibrous, uneven surface of fibrinous pericarditis remained demonstrable (Fig. 4a). As well, erosions could also be obsereved on the mucosa of a metastatic infiltrated stomach (Fig. 4b).

Discussion

During the last decade, the Department of Pathology at Semmelweis University of Medicine has collected a hundred specimens which represent outstanding examples of different pathological diseases or rarities in medicine. Tumors and other pathologies are fixed to healthy tissues which presents a challenge in collecting, as well as, preparing tissue for plastintion. In spite of these problems many pathological tissues have now been plastinated.

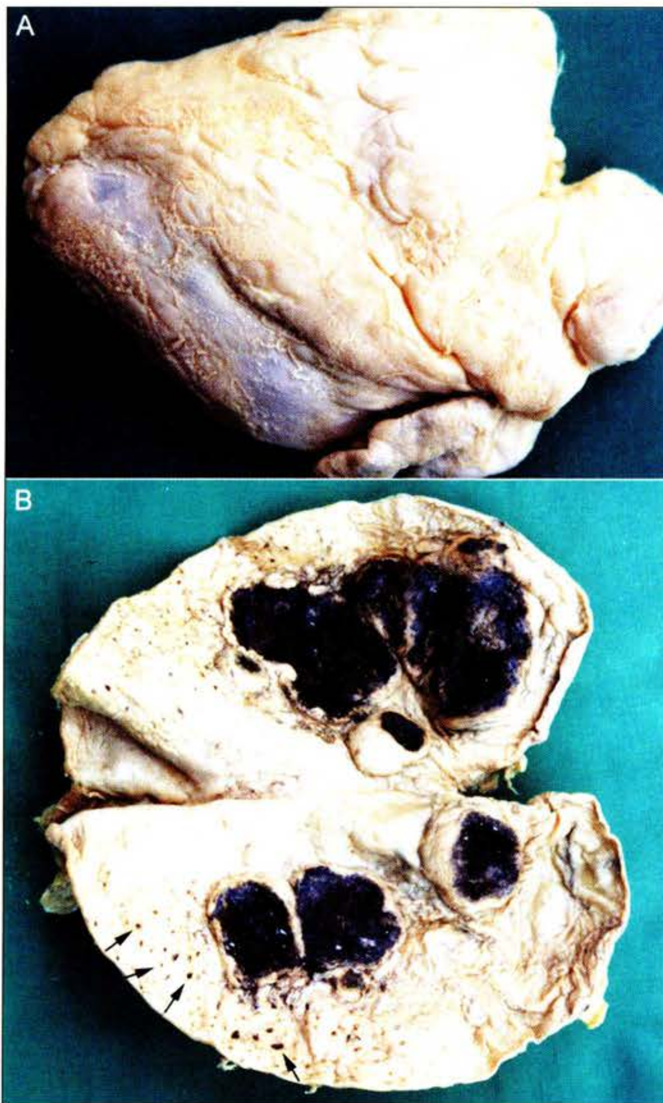

Figure 4. Subsinuosal surface of plastinated human heart demonstrating fibrinous pericarditis (A), mural erosions (at arrows) in a metastatic infiltrated human heart (B).

Numerous preparations of various diseases and organs were successfully plastinated which suggests that diverse types of pathological tissues can be properly conserved by this method. The present study offers insight into the possibilities in the plastination of pathological specimens. A great challange in preserving these specimens by plastination is the preservation of small, but diagnostically important colors and textures of the tissues.

Subtle changes on the surface of organs remained demonstrable irrespective of the kind of the mutation; degenerative (Fig. 1c), tumorous (Fig. 2b) and inflammatory (Fig. 3a) changes were equally recognizable. The inner structure of various diseased tissues could also be studied on the cut surface of the organ, typically in tumors which enabled the observer to identify both the type and the extension or demarcation of the tumor (Fig. 2).

Color differences are of primary importance in pathological diagnostics which can be properly studied only on fresh and unfixed specimens. On most plastinated preparates, the true colors were faded. This was due to formalin fixation and dehydraton but not to the silicone plastination process. Nevetherless, typical color differences could be properly demonstrated on numerous specimens (Figs. 1a, 1c, 3b).

Diagnostics also depend upon recognition of alterations in the consistency of the diseased tissue. This helpful feature is completely lost when the organ is plastinated which must be acknowledged as an inevitable shortcoming of the method. It has to be mentioned, however, that the consisteny of both healthy and tumorous or degenerative tissues is changed during formalin fixation as well.

Fixation of non-stable tissues, e.g. bleeding (or hemhorrage) is another problem to be solved. In addition to the risk that the hemorrhage could be easily washed out, its shrinkage is different from that of the surrounding tissues. Still, hemorrhages could be plastinated *in loco* on several specimens (Fig. 1A-C).

Considering the large number and variety of plastinated specimens, we suggest that plastination can be usefully applied in the conservation of pathological tissues.

Acknowledgements

We would like to thank M. Tóth and L. Patonay for the quality photos.

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Improved Method for Dried Anatomical Specimen Preparation

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Abstract: Dessicated organs used for teaching purposes are susceptible to insect damage as well as damage due to improper handling. Injection of expanding foam products into the lumen of dessicated organs followed by varnishing produces anatomical specimens resistant to insect damage and more resilient to the strains of handling. This study will describe the production of such specimens.

Key words: **plastination; desiccation; preservation; anatomy; organ**

Introduction

For thousands of years, desiccation of biological tissue has been a useful and inexpensive means of specimen preservation (Kitchel et al., 1961; Strub and Frederick, 1967; Church, 1968). Plastination, though well known for its unique preservation qualities of anatomical specimens (von Hagens et al., 1987; Nicaise, 1990; Weiglein, 1996; Latorre et al, 2001, 2002) is still more costly than desiccation in many regions today. However, insects have a predilection for consuming organs dried in such a manner. A simple mechanism to prevent infestation of desiccated organs with insects thus preventing organ destruction would serve to greatly increase the longevity and usefulness of desiccated specimens. This study will describe a mechanism of organ preservation and protection.

Materials and methods

Hollow organs were collected from animal cadavers for use in this study. The stomach, large colon and descending colon were harvested from a horse. Stomachs, lungs and female reproductive tracts were removed from a cow, sheep and dog. The organs were flushed with tap water until free of ingesta. The adipose tissue, omenta and mesentery were removed close to the organ using caution not to damage the outer muscle layer. Once clean, the organ was prepared for classic air drying by cannulation of both ports with appropriate sized tubing and hooked to a laboratory air source. The organ was first dilated to the desired degree of inflation. Air flow and hence organ size was either controlled by adjusting the inflow and/or the exhaust port by either occlusion or throttling by partial closure. Depending on the size of the organ, drying takes three to four days. The stomach (monogastric and ruminant), small and large intestines, lungs and uterus and vagina have been preserved by this method. The second phase commences after drying is completed and consists of gradual injection of the plastic expanding foam, [Tekapur (Bosnia-Hercegovina) or Great Stuff (USA)]. It was beneficial to have an exhaust in addition to the inflow. If too much loss of foam occurs via the exhaust, it can be decreased in size or closed. The next day, more

foam may be injected through one of the ports to fill areas that are devoid of foam to assure complete lining and filling of the organ. The hardening time is eight hours to one or two days depending on the volume of the organ. Varnish was sprayed and brushed onto the external surface of the organs. As well, regions of the organ or the entire organ may be painted.

Results

Cleaning and air drying the organs resulted in a desiccated specimen representative of those occurring *in situ*. (Fig. 1) The surfaces of the desiccated organs were dry to the touch and free of any greasy residue.

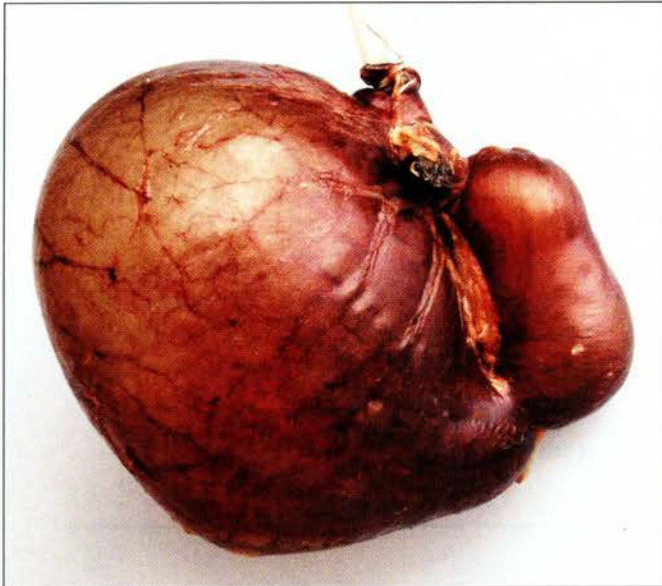

Figure 1. Visceral view of canine air dried stomach. Cannula is in the esophagus.

The injected plastic expanded 2 to 4 times in volume in all directions and hardened gradually. Areas of the organs which partially collapsed following disconnection from the air source were reinflated with the expanding foam. The hardening time for the injected foam is eight hours to two days depending on the volume of the organ. The resulting dried, foam filled organs were light weight and anatomically precise (Fig. 2). Application of the varnish to the outer surface of the organs was accomplished without the production of a runny, streaked appearance. Paint was applied with a brush to highlight anatomical information (Fig. 3).

Discussion

Air-drying of organs has been used for anatomical applications for many years (McKiernan and Kneller, 1983; Henry, 1992). However, filling air dried organs with expandable foam protects the otherwise vulnerable inside of the organ from insect damage while the application of varnish does the same for the external

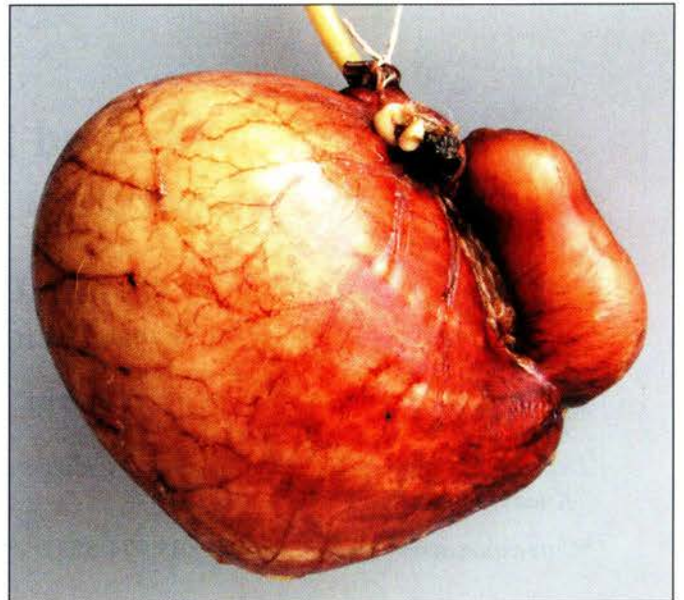

Figure 2. Visceral view of foam filled canine air dried stomach. Note fat on minor curvature that must be trimmed off.

Figure 3. Parietal view of foam filled, varnished and regionally painted equine air dried stomach.

surface. The foam also protects the organ from collapsing under normal handling conditions. Various methods and products have been used to make air dried organs resistant to insect damage including fiberglass (Kitchel et al., 1961), flexible plastic resin (Updike and Holladay, 1986) and silicone (Henry and Butler, 1990). During filling it is beneficial to allow air to exhaust during inflation of organs. Continual flow of air through the specimen allows the organ to dry quicker. If the

muscular wall of the organ is cut, a herniation or blow out of the mucosa may result from inflation without an exhaust port. If too much foam is lost through the exhaust port during injection, the portal may simply be closed.

It is possible this expanding foam could also be used to dilate silicone impregnated hollow organs prior to polymerization in the plastination process. It is imperative to remove all adipose tissue from the specimen prior to desiccation. The failure to do so results in greasy specimens to which the application of varnish is troublesome. The foam products tested are unstable when exposed to acetone. Acetone dissolves the foam and reduces it to a sticky substance. This would preclude the use of the foam prior to impregnation of specimens during plastination during which step the acetone is removed from the specimen. Alcohol saturates the foam but does not dissolve it which opens the possibility of its use in plastination when alcohol is the intermediary solvent. The external surface is protected from insect damage by varnishing. Once varnished, the external surface of the organ may also be painted or labeled for demonstration of information. This method produces specimens that maintain normal anatomical form, are durable and inexpensive to produce.

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Plastination of Coronal Slices of Brains from Cadavers using the P35 Technique

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Abstract: Stringent requirements for obtaining consent for acquisition of human tissues in New Zealand have led to difficulty in obtaining fresh brain tissue from post-mortem. In contrast, the Medical School's body donor program is well supported, and body donation often includes permission to retain organs, including the brain. However, it has been difficult to obtain brains from bodies before embalming due to higher priorities necessitating that most cadavers to be embalmed. Therefore, we have recently trialled plastination with the P35 technique on thin slices from two cadaveric brains, one embalmed with an alcohol-based mix and the other with a 10% formalin formulation. The brains were sliced into 3mm slices and processed separately. Each basket of slices was flushed in running water, immersed in distilled water at 5°C, dehydrated in -25°C acetone, immersed with P35 at 5°C for 48 hours then placed under vacuum at room temperature for 24 hours. Impregnated slices were cast individually in float glass chambers and initially cured under UVA lights for 3 hours followed by heat-curing in an oven at 45°C for 5 days. Slices were finally removed from the glass chambers and the edges trimmed, sanded and polished. The brains from the two cadavers have yielded P35 slices of similar quality to those obtained from formalin fixed, post-mortem brains.

Key words: **plastination; P35; polymer; embalmed; brain; neuroanatomy; education**

Introduction

P40 plastinated brain slices have been used extensively in our neuroanatomy courses (Jones and Barnett, 1998). These P40 slices were produced from fresh brains removed from post mortem cadavers for the intent of slice production. Then the slices were fixed in 5% neutral buffered formalin (NBF) at 5°C with four changes of fixative over a period of 9 weeks. This procedure yielded high quality P40 slices with high contrast between white and grey matter (von Hagens, 1994; Barnett, 1997). Brains fixed in 10% formalin have also been processed successfully with the P35 technique. (Weber, 1994; Weiglein, 1996).

The New Zealand human tissue Act of 1964 allowed use of human tissue, from post mortems at government controlled facilities, for the purpose of teaching and research. This act does not require consent for human tissue to be used in this manner. Until 1996 it was common practice for the department to receive brains from government controlled post-mortem facilities (public hospitals) without consent. Since 1996 due to the change of the ethical climate, the practice of using tissue without consent is considered to be inappropriate. (Jones and Galvin, 2002). Thus, due to this conflict concerning acquisition of fresh tissue from post-mortem sources, fresh brain material for plastination is rarely available. For this reason, we have turned to cadaveric material obtained through the University of Otago's body donor program in order to obtain brain specimens for dissection and plastination. Our University program can provide the possibility for fresh tissue use.

However, due to the demand for cadaveric material in various areas of teaching and research in our department, fresh cadaveric specimens including brains are rare, as all of the cadavers are embalmed. Therefore, brains are only available from embalmed cadavers and not in the fresh state. Most polyester protocols preferably use fresh formalin fixed brains. Results of slice-plastination trials with the P35 technique on brains from two cadavers embalmed by two conventional means are reported. Slice plastination of embalmed cadaveric brains with the P40 technique was also tried, but was unsuccessful when compared with the P35 technique, and so is not further reported.

Materials and methods

Specimen preparation

Fixation was carried out by embalming with a Portaboy 95 and a closed circuit perfusion system. Each body was perfused with 20L of one of the two embalming fluids. The first cadaver was embalmed with Crosado mix which consisted of 60% alcohol, 15% glycerine, 15% water, 7.5% phenoxytol and 2% formalin. The second solution used was Dodge anatomical arterial mix (supplied by Regal Manufacturers, Wellington, New Zealand). For commercial reasons, we have been unable to find out the chemical composition of this fixative other than it contains 4% paraformaldehyde (10% formalin solution). The brains were removed from the cadavers after a minimum time of six months post-embalming.

The cadaveric brains were removed from the bodies, sliced into 3mm coronal slices on a conventional bacon slicer and stacked in a grid basket. The brain slices fixed with the Crosado mix were well-preserved so they were washed for 24 hours in running tap water. The centre of the brain slices fixed with the Dodge mix were pink in colour and soft, indicating they were not thoroughly fixed. Therefore, they were immersed in 10% NBF for three days until the pink colouration was gone. They were washed for 24hours. Each basket of brain slices was immersed in distilled water at 5°C overnight.

Plastination

Each basket of slices was dehydrated by the freeze substitution method using 100% acetone at -25°C for 24 hours and changed into fresh acetone for another 24 hours.

Immersion and impregnation was carried out according to the standard procedure for the P35 technique (von Hagens, 1990; Weber and Henry, 1992; Weiglein, 1996). This consisted of two immersion baths of P35/A9 (100:2) for 24 hours each at 5°C. Vacuum was applied for 24 hours at room temperature to the slices. The second immersion bath was used for the impregnation mixture. Vacuum was increased until a pressure of 10mm Hg was attained.

The basket of slices was removed from the vacuum chamber. Each slice was cast in an individual glass chamber consisting of two float glass plates 250 x 220 x 2mm. Silicone tubing (8mm) was placed between the two glass plates to provide a gasket and the chamber was clamped on three sides. The glass chambers were placed upright and the slices slipped into the top of the chamber with a spatula. Each chamber was filled with a fresh mixture of P35/A9 (100:2). Air bubbles were removed with the aid of a wire.

The chambers were placed between UVA lights at an angle of approximately 15 degrees with the open side of each chamber raised so the resin would not leak out. The brain slices were then positioned in the centre of the chamber with a wire before the lights were switched on. Light curing was undertaken for three hours and fans were used to cool the surfaces of the chambers.

Following light curing the glass chambers were placed in an oven at 45°C for five days. The oven was then switched off and allowed to cool before the chambers were removed.

Finishing

After curing was completed the glass chambers were dismantled and the edges of sections were trimmed on an electric planer, sanded and polished.

Results

The Crosado fixed brain was smaller when removed from the cranium than the Dodge arterial mix brain. Both brains from the embalmed cadavers (Crosado mix and Dodge anatomical arterial mix) were successfully sliced and plastinated using the P35 technique and yielded good results. Dodge arterial mix (10% formalin base) demonstrated excellent definition between the grey and white matter, down to the finest details (Fig. 1). The individual nuclei of the thalamus are demarcated, as is the white matter of the cerebellar folia. Within the white matter, even the dense optic radiation is easily distinguished from the general cortical white matter. Crosado mix (60% alcohol base) yielded good gray/white definition but the finer details within both grey and white matter are less apparent (Fig. 1). The Crosado fixed brain slices were smaller that the Dodge brain slices.

Discussion

The P35 technique has previously shown to offer consistently good results for plastination of thin brain slices when used on fresh brain material fixed in formalin (Weber and Henry, 1992; Weber, 1994;

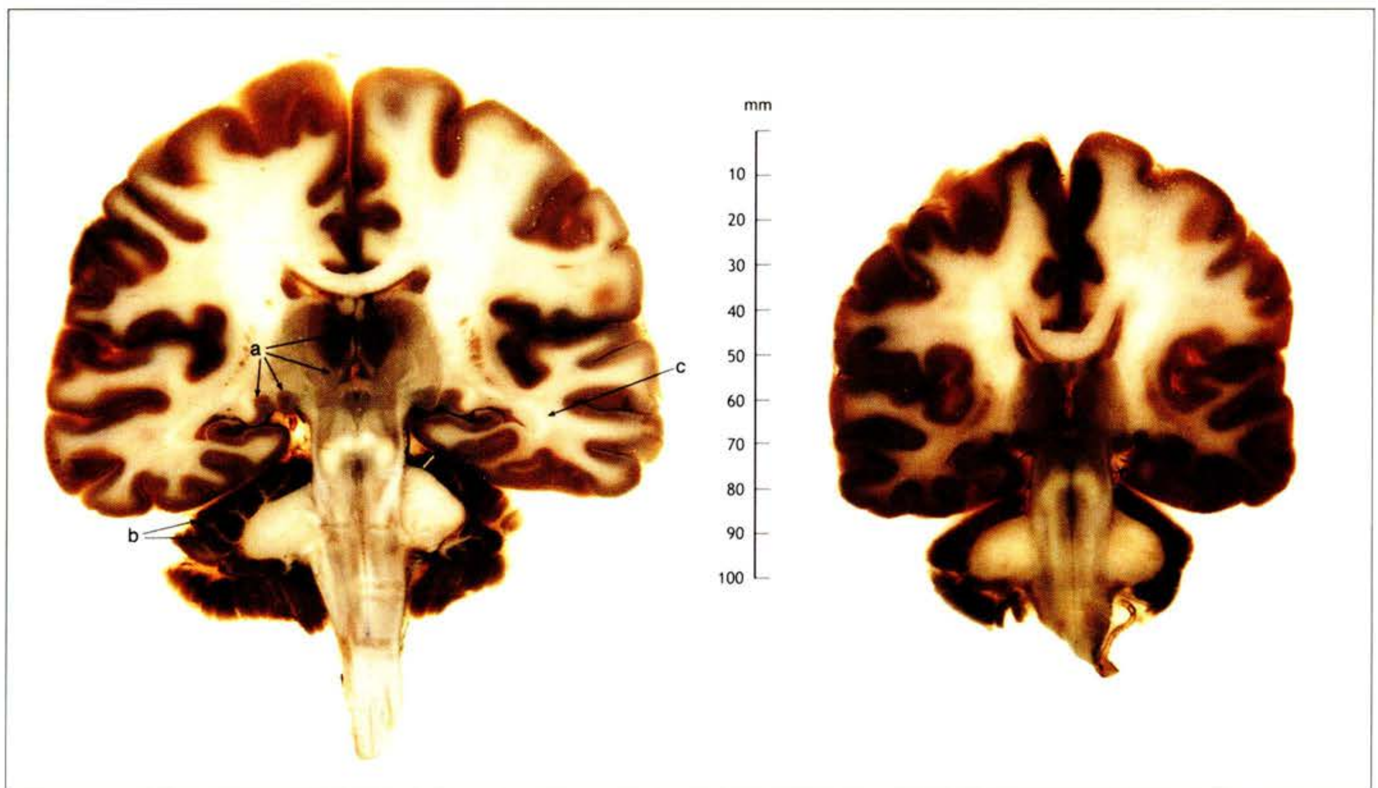

Figure 1. Human brain slices plastinated via the P35 process. Section on left embalmed with Dodge arterial mix. Section on right embalmed with Crosado mix. Thalamic nuclei (a), white matter of cerebellar folia (b), dense optic radiation (c).

Weiglein, 1996). This report demonstrates the successful use of P35 on brains which have been embalmed with either an alcohol-based (Crosado mix) or formalin-based (Dodge anatomical mix) embalming fluids.

Of the two embalming fluids tested, the formalin-based mix resulted in better anatomical definition within the P35 plastinated slices than did the alcohol-based mix. As well, the Dodge brain retained a more normal size.

The alcohol-based Crosado embalming fluid was prepared and tested in our department in order to eliminate the exposure of students and staff to phenol and to reduce exposure to formalin. The Crosado mix has also been superior to the 10% formalin-based Dodge anatomical mix in controlling growth of mould on stored cadavers. However, for P35 slice-plastination purposes, it seems somewhat less successful in retaining the finer anatomical detail in the plastinated brains than is the Dodge Anatomical Mix.

We have stopped routine embalming with Dodge mix due to mold problems. However, we still embalm bodies with Dodge mix specifically for use in research and plastination and have found that it gives excellent results with most plastination techniques, including P35.

The level of shrinkage was not determined in either of the brains used for this study. The brain fixed with Crosado mix was smaller upon removal than the Dodge arterial mix brain. This smaller size likely indicates that the Crosado mix embalmed brain, which contains 60% alcohol, shrunk prior to removal. Previous studies have shown shrinkage occurs during dehydration and impregnation (Tiedemann and Ivic-Matijas, 1988; Henry et al., 1998; Sora et al., 1999). Measurement of shrinkage before and after plastination would have been beneficial to note whether both embalming methodologies yielded similar shrinkage. There was no apparent visual distortion of anatomical structures due to shrinkage in either brain, thus providing us with a suitable resource for teaching neuroanatomy.

The ability to use brains from embalmed cadavers to produce P35 slices has now guaranteed a continued supply of brain slice sets for use in teaching neuroanatomy.

Acknowledgments

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Surface Detail Comparison of Specimens Impregnated using Six Current Plastination Regimens

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Abstract: Numerous silicone impregnated specimens were produced for many years to increase our specimen inventory. Six current methods of silicone plastination were used with the intent to evaluate the resultant products. These included the classic von Hagens' (S10, German, Biodur™) cold method and five modifications of this method: Chinese, Corcoran, VisDocta™, North Carolina A and B. All of these methodologies use polymer, catalyst, chain extender, and cross-linker which are commonly used in today's silicone polymer industry. Cold acetone was used on most specimens; however, room temperature acetone was used on some specimens. The classic impregnation process, using decreasing pressure, was utilized in each process to exchange the intermediary solvent (acetone) with the polymer. The difference in these techniques was primarily the number of reactants and how they were combined and utilized in the plastination process. Specimens produced by the classic S10 method (using an impregnation reaction-mixture of polymer, catalyst and chain extender, with later cross-linking) and similar copies of this process (VisDocta™, North Carolina A) routinely yielded exquisite surface detail of the specimen. Combining the cross-linker and polymer as the impregnation reaction-mixture with later catalyst application (Corcoran) produced specimens with a granular semitranslucent surface. The impregnation-bath consisting of polymer alone (China, NC B) yielded good surface detail but occasionally yielded specimens with small, dry semitranslucent areas on the surface if catalyst and/or cross-linker were added. However, all specimens produced by all methods were deemed useful. The combination of ingredients is the key factor in the differing results.

Key words: **plastination; silicone; polymer; evaluation; surface; detail**

Introduction

The plastination process for impregnation of biological tissues has remained nearly the same since its inception twenty-seven years ago by von Hagens (von Hagens, 1979a, 1979b, 1980, 1981, 1985; Bickley et al., 1981, 1987; Tiedemann and von Hagens, 1982; Henry, 1987, 1995, 1998; Oostrom, 1987b; von Hagens et al.,

1987; Nicaise et al., 1990; Henry and Nel, 1993; Weiglein and Henry, 1996; von Hagens and Whalley, 2000). The various polymers, catalysts, chain extenders and cross-linkers used for plastination are all products currently used in the silicone polymer industry (Holladay et al., 2001; Henry et al., 2002b; Henry, 2004). There are at least six methodologies widely used today: S10 (von Hagens', Biodur™), Corcoran (Dow), Chinese (Su-Yi), VisDocta™ (Italy), North Carolina A (NC A) and B (NC B). The major difference in these processes is how the components are combined and used during the process. Combination of polymer and catalyst (S10, VisDocta™, NC A) yields an unstable impregnation reaction-mixture which may be semi-stabilized and hence the rate of thickening (chain elongation) remarkably retarded if the reaction-mixture is stored at -20°C or less. Impregnation is recommended at -15°C but may be carried out at slightly lower or much higher temperatures. However, the combination of polymer and cross-linker (Corcoran) yields a stable impregnation reaction-mixture even at room temperature and hence impregnation with such a product is routinely done at room temperature (Glover et al., 1998; Henry et al., 2001; Latorre et al., 2001; Raoof, 2001). Generally, China and NC B use only the polymer for impregnation (a stable impregnation bath at room temperature) and later a catalyst/cross-linker may be added. However, on hairy specimens it is desirable to use no additives. Any of these combinations have been recognized to produce useful, durable specimens (von Hagens 1979a, 1979b, 1980, 1981a, 1982, 1985; Bickley et al., 1981, 1987; Tiedemann and von Hagens, 1982; von Hagens et al., 1987; Weiglein, 1996; Zheng et al., 1996, 1998b, 2001; Glover et al., 1998; Henry, 1998; von Hagens and Whalley, 2000; Henry et al., 2002a, 2004; Latorre et al., 2002). However, it has been noted that surface clarity appears altered in specimens produced using cross-linker and polymer as the impregnation-mixture (Henry et al., 2001; Latorre et al., 2001; Henry, 2004). However, surface clarity is altered in specimens produced using cross-linker and polymer as the impregnation-mixture (Henry et al., 2001; Latorre et al., 2001; Henry, 2004). This problem continually appeared in routinely plastinated specimens (Fig. 1). Therefore, it was decided to have attendees of the 12th International Congress on Plastination evaluate the surface of representative specimens from the six plastination methodologies. The results of these evaluations are presented in this paper.

Materials and methods

Over a five year period, various specimens from many species (human, domestic animals and exotic animals) were prepared in Knoxville, Tennessee and Murcia, Spain using classic techniques (prosection, dilation, vascular injection, etc.) for rendering anatomical specimens suitable for plastination (Oostrom, 1987; Henry et al., 1997) and each method recorded. Fresh rather than embalmed tissue was used in preparation of most specimens. Fresh tissue was usually fixed in five to ten percent formaldehyde solution for a short time either prior to, during or after prosection. A smaller number of embalmed specimens (primarily brains embalmed in 10% formaldehyde solution) were collected and prosected. After fixation and prosection were completed, fixative was rinsed from the tissue via flowing tap water. Cool specimens were placed into either room temperature acetone (Brown et al., 2002) or cold acetone (-15°C) (Schwab and von Hagens, 1981; von Hagens, 1985; Tiedemann and Ivic-Matijas, 1988; Ripani et al., 1994; Henry et al., 1998) of at least 80% purity. Weekly changes into a higher percent acetone were performed until >99% purity was maintained. After dehydration in acetone, specimens from each type of processing were placed into plastination (vacuum) chambers containing one of the six silicone impregnation recipes for forced impregnation (von Hagens, 1985; Bickley et al., 1987; Henry and Nel, 1993).

Six recipes and techniques were carried out at various times over a period of five years in order to compare the quality of the finished plastinated specimens, as well as, provide new specimens for our collection. The six formularies were as follows:

1. The classic cold S10 (Biodur™, German, von Hagens) method and chemicals were used. Silicone polymer (SR 10) and catalyst and chain extender (SH 03) at a ratio of 100:1 were thoroughly mixed for the impregnation reaction-mixture (von Hagens, 1985; Bickley et al., 1987; Henry and Nel, 1993). Impregnation, at both -15°C and room temperature (25°C), was carried out. When the room temperature reaction-mixture was not in use, it was stored at -20°C to retard elongation of the silicone molecules and hence thickening of the reaction-mixture. After impregnation, excess polymer was drained from the specimens and the specimens remained at room temperature for several days to allow any remaining surface polymer to drain from them. Once drainage was deemed complete, the specimens were placed in a contained environment and gas cure (SH 06) was added to the environment and vaporized for 10 minutes a day with an aquarium pump (Weiglein and Henry, 1993). Specimens were manicured daily until all weeping had ceased and their surfaces were dry.

2. The VisDocta™ (Italy) method and chemicals

were used: Silicone polymer (SH1) and catalyst (SH1/D) at a ratio of 100:1 for the impregnation reaction-mixture. After thorough mixing, impregnation was carried out at -15°C and room temperature (25°C) (Shahar and Pace, 2003). When the room temperature reaction-mixture was not in use, it was stored at -20°C to retard elongation of the silicone molecules and hence slow thickening of the reaction-mixture. After impregnation, the excess polymer was drained from the specimens and the specimens remained at room temperature for several days to allow excess polymer to drain from them. Once drainage was deemed complete, the specimens were placed in a contained environment and gas curing agent (SH1/G) was added to the environment and vaporized for 10 minutes a day with an aquarium pump. Specimens were manicured daily until all weeping had ceased and their surface was dry.

3. The Corcoran (Dow™) method and chemicals were used. Silicone polymer (PR 10) and cross-linker (CR 22) at a ratio of 100 to 8 were thoroughly mixed for the impregnation reaction-mixture. Impregnation at room temperature (25°C) was carried out (Glover et al., 1998; Latorre et al., 2001; Raoof, 2001). When the room temperature reaction-mixture was not in use, it was stored at room temperature as this mixture is stable perhaps forever but for certain at least 8 years with no thickening of the reaction-mixture. After impregnation, the excess polymer was drained from the specimens and the specimens remained at room temperature for a few days to allow excess polymer to drain from them. Once drainage was deemed thorough, the specimens were misted with catalyst (CT 30), wrapped with foil/plastic wrap for twenty-four hours, and then inspected for extent of curing. Usually more curing time was needed. If so, the specimens were misted or wiped with more catalyst and rewrapped with foil until the next day. Their surface was checked to assert they were dry.

4. The Chinese (Su-Yi) method and chemicals were used: Silicone polymer (SuYi) alone was used for the impregnation bath (Zheng et al., 1996, 1998a, 1998b, 2001). Impregnation was carried out at room temperature (25°C). When the room temperature impregnation bath was not in use, it was stored at room temperature as this bath is stable perhaps forever but for certain at least 3 years with no thickening of the impregnation bath. After impregnation, the excess polymer was drained from the specimens and the specimens remained at room temperature for several months to allow excess polymer to drain from them. At a later time some of these specimens were placed in a contained environment and the accompanying Chinese chemical (chemical composition unknown by authors but is likely a catalyst and/or cross-linker) was added to the environment and vaporized for 10 minutes a day with an aquarium pump or wiped onto the specimen. Specimens were manicured daily to determine if the damp, oily texture would decrease and yield a dry surface. The remaining specimens (Chinese) were not exposed to the catalyst/cross-linker but were allowed to remain a semi-damp.

5. The North Carolina A method and chemicals were used: Silicone polymer (Neat 285 or 295) and catalyst (S3) were combined at a ratio of 100:3. After thorough mixing, impregnation was carried out at -15°C or at room temperature (25°C). When the room temperature reaction-mixture was not in use, it was stored at -20°C to retard elongation of the silicone molecules and hence thickening of the reaction-mixture. After impregnation, the excess polymer was drained from the specimens and the specimens remained at room temperature for several days to allow excess polymer to drain from them. Once drainage seemed complete, the specimens were placed in a contained environment and 2.0ml of chain extender (S7) was added to the environment daily for one week and vaporized for ten minutes a day with an aquarium pump. Specimens were manicured daily to alleviate weeping polymer. Then the specimens were exposed to gas cure (S6). Three milliliters of gas cure were added daily to the environment and vaporized for ten minutes a day with an aquarium pump. Specimens were manicured daily until all weeping had ceased and their surface was dry.

6. The North Carolina B method (Henry et al., 2002, 2004) and chemicals were used: Silicone polymer (Neat 285 or 295) by itself was used for the impregnation bath. Impregnation was carried out at room temperature (25°C). When the room temperature impregnation bath was not in use, it was stored at room temperature as this bath is stable perhaps forever but for certain at least five years with no thickening of the impregnation bath. After impregnation, the excess polymer was drained from the specimens and the specimens remained at room temperature for several months to allow excess polymer to drain from them. Once drainage appeared complete, some specimens (NC B_1) were placed in a contained environment and 2.0 ml of super-catalyst was added to the environment and vaporized for 10 minutes a day with an aquarium pump. Most specimens were manicured daily until all weeping had ceased and their surface was dry. The remaining specimens (NC B_2), primarily hair covered, were not exposed to catalyst or cross-linker but were allowed to remain semi-damp.

As a group of specimens completed the plastination process, the cured and non-cured specimens were stored in appropriate cabinets and used routinely as needed for teaching, demonstration or display.

The surfaces of the cured specimens, aged from four and one half years to ten days, were graded by attendees of the 12th International Congress on Plastination at Murcia, Spain. For this evaluation, the specimens were assigned and tagged with random numbers and placed on tables for voluntary participation by attendees. The evaluators were asked to examine the surface of the specimens for quality and clarity of surface detail. They were asked to disregard obvious blemished areas due to handling, shipping or abuse of the specimens. They were to record their results as follows: ***B** - For best surface detail*, ***G** - For good surface detail* and ***O** - For okay surface detail*. The results were recorded for each specimen followed by the number of responses for each category of evaluation grade.

In addition, the authors described the surfaces of the specimens which were graded in this study to provide the reader with an idea of what the evaluators were seeing and upon which they presumably based their evaluations. The authors did not participate in the evaluation at the 12th International Congress on Plastination. The authors also described the surfaces of these specimens in their non-cured state prior to the evaluation that occurred in Spain.

Figure 1. Plastinated camel fetuses. The fetus at top was plastinated using the classic S10 process. The fetus on bottom was plastinated using the Corcoran process.

Results

Plastination procedures

Occasionally a cured specimen would weep polymer and need to be wiped and exposed to additional curing agent to complete the curing process. Specimens with no catalyst or curing agent added needed to be wiped of weeping polymer. All specimens prior to curing exhibited great surface detail. Room temperature vs. cold dehydration of specimens and room temperature impregnation vs. cold impregnation of specimens yielded no discernable difference in surface quality. Between one week and one month after exposure to catalyst, a distortion of the surface of specimens impregnated with the polymer cross-linker reaction-mixture was noted on all of this type specimen. The decrease in clarity of surface detail peaked by three months post impregnation and then remained at a constant level. This decrease in clarity was due to polymer invading the surface of the specimens produced via this plastination methodology. This resulted in a dull, gritty and/or blistered appearance to the surface of these specimens. The process in which specimens were impregnated with polymer only and not exposed to any catalyst/chain extender/cross-linker was especially user friendly for hair covered specimens. Because the polymer bath contained no additives, the polymer remained fluid and drained freely from the hair and prolonged or extra manicuring was not necessary.

Specimen evaluation

All classic S10 method, VisDocta™ method and North Carolina A method specimens exhibited excellent surface clarity at any time throughout the process.

The serosal surface of the bovine uterus (Figs. 2, 3) and North American opossum liver (Fig. 4) plastinated using the classic S10 method are smooth and exhibit surface detail as is seen on fresh tissue. The margins of the liver are sharp and prominent (Fig. 4).

The VisDocta™ specimens have clear smooth margins with no excessive accumulation of polymer allowing clear visualization of all structures (Figs. 5a, 6, 7). The canine heart has a transparent epicardium such that the myocardium is visualized (Fig. 6). Brain exhibited sharp surface detail (Fig. 7).

The North Carolina processes also produced specimens with surface detail representative of fresh tissue. A feline stomach and liver display clear surface detail with sharp margins (Fig. 8). The surface detail of a porcine uterus (Fig. 9) and a canine heart (Fig. 10) is remarkable. Individual muscle fibers of the muscularis mucosa are visible through the serosa of tubular organs (Fig. 11).

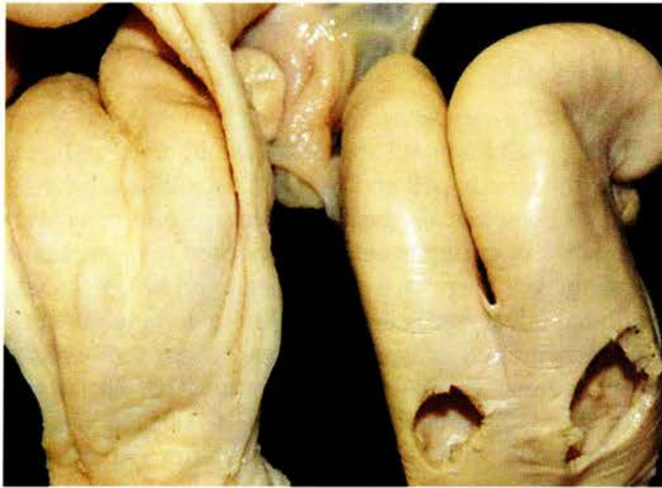

Figure 2. Dorsal view of bovine uteri. The specimen on the left was plastinated using the Corcoran process. The specimen on the right was plastinated using the classic S10 process.

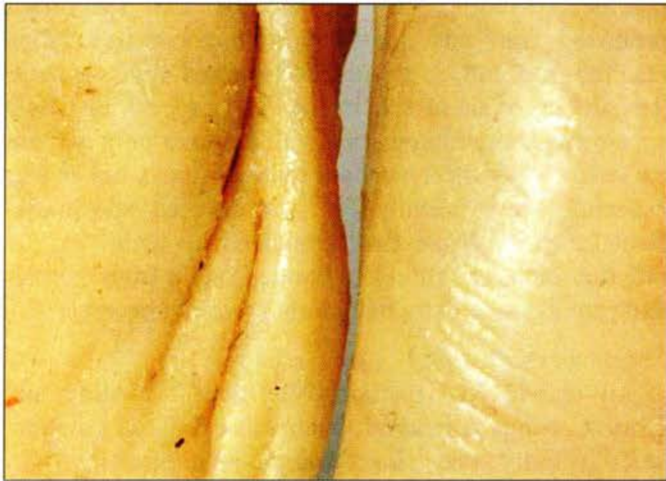

Figure 3. Close up of serosal surface of bovine uteri from figure 2. The specimen on the left was plastinated using the Corcoran process. The specimen on the right was plastinated using the classic S10 process.

Figure 4. Surface of North American opossum liver plastinated using the classic S10 process.

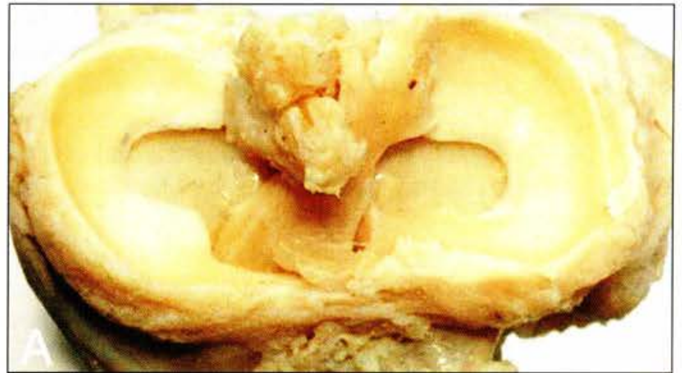

Figure 5. Canine tibial plateau with menisci and ligaments plastinated using the VisDocta™ process (A). Canine tibial plateau with menisci and ligaments plastinated using the Corcoran process (B).

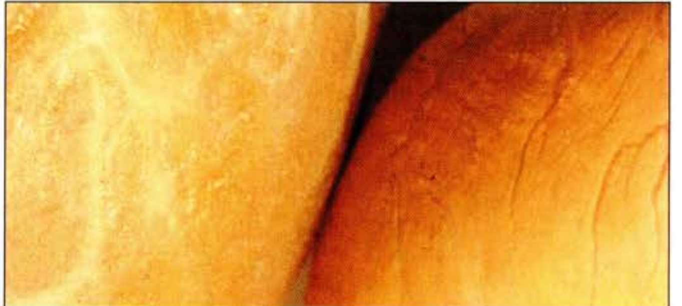

Figure 6. Plastinated canine hearts. Specimen on left was plastinated using the Corcoran process. Specimen on right was plastinated using the VisDocta™ process.

Figure 7. Mid-sagittal view of equine brain plastinated using the VisDocta™ process.

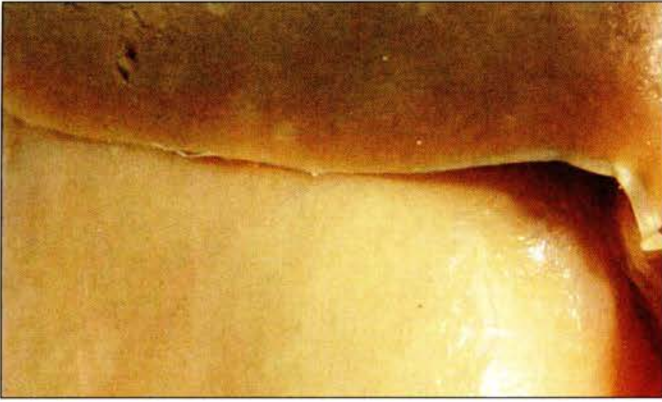

Figure 8. Feline liver (top) and stomach plastinated using the North Carolina A process.

Figure 9. Serosal surface of porcine uterine horn plastinated using the North Carolina A process.

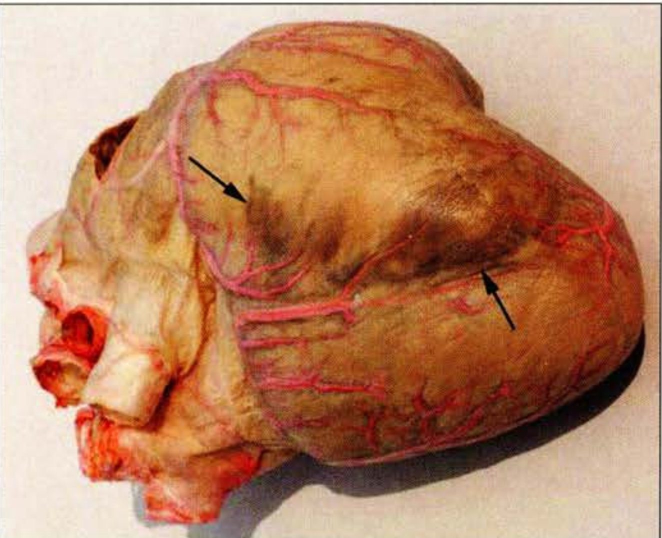

Figure 10. Canine heart plastinated with the North Carolina A process. Dark regions at arrows are blood remaining in tissue.

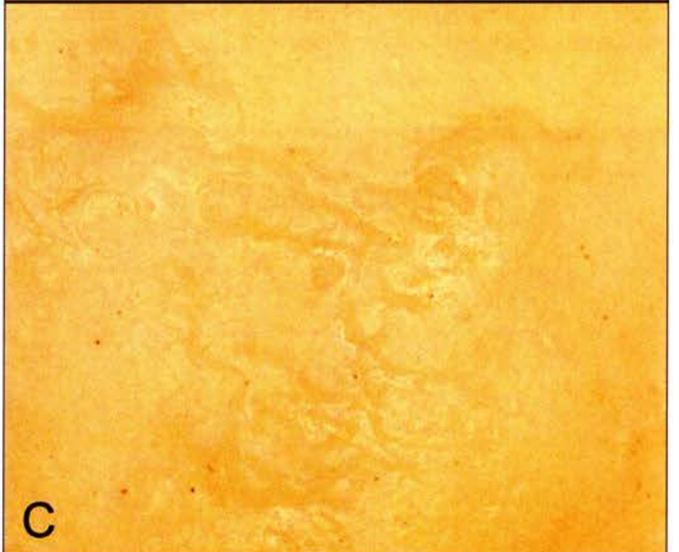

Figure 11. Serosal surface of tubular organs plastinated using the classic S10 process (A), VisDocta™ process (B) and the Corcoran process (C).

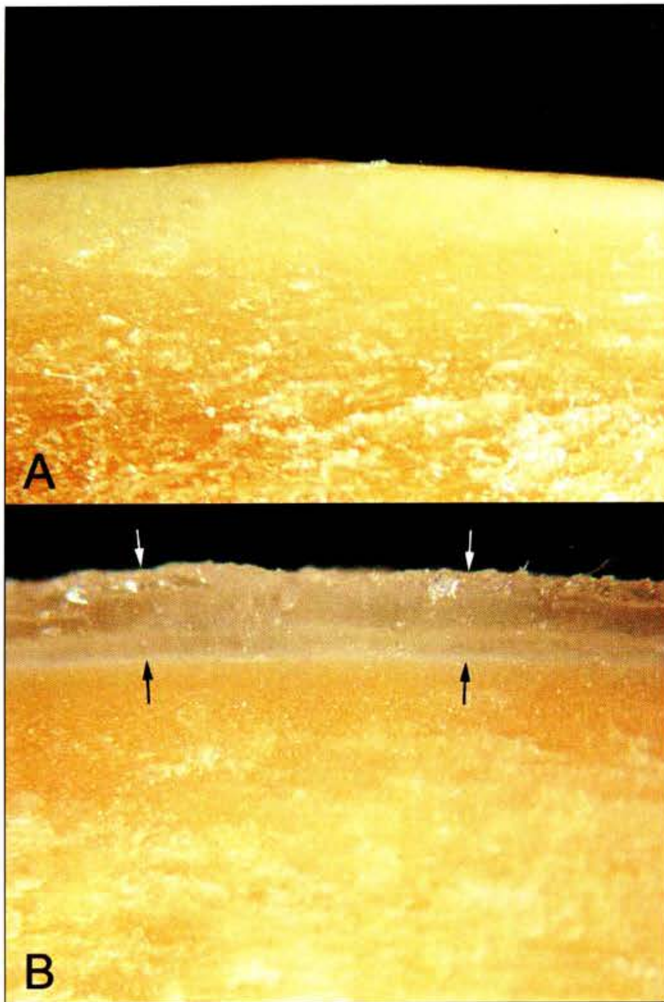

Figure 12. Cross section of canine heart plastinated with the classic S10 process (A). Cross section of canine heart plastinated with the Corcoran process (B). Subserosal accumulation of polymer between arrows. Outer surface of myocardium at black arrows. Epicardium at white arrows.

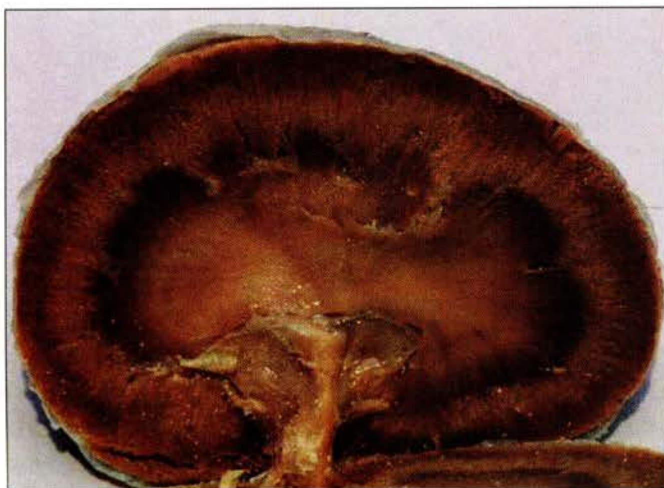

Figure 13. Feline kidney plastinated using the Chinese process.

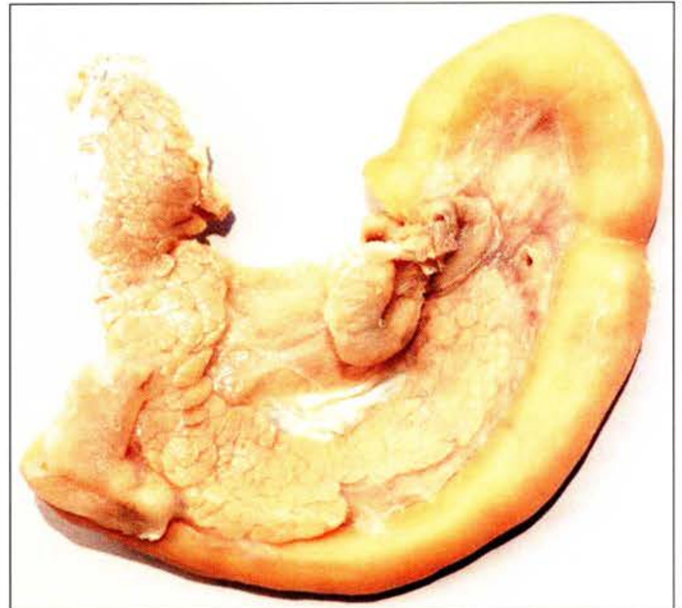

Figure 14. Canine duodenum and pancreas plastinated using the Chinese process. White area on left lobe of pancreas is a curing artifact.

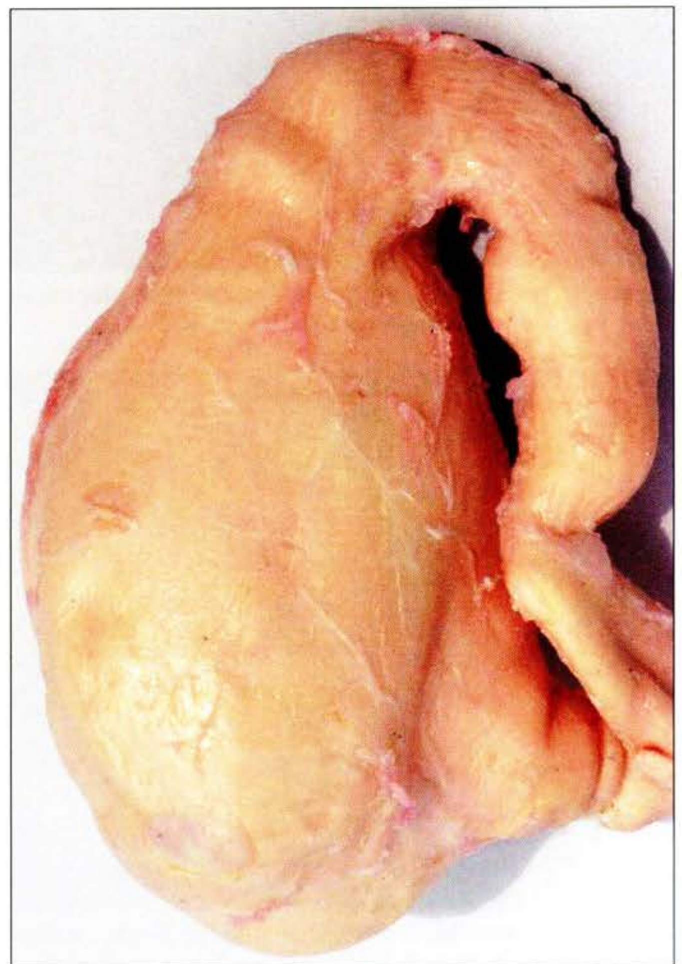

Figure 15. Feline stomach plastinated using the North Carolina B_1 process.

Figure 16. Rainbow trout (longitudinal and transverse section views) plastinated using the North Carolina B_1 process.

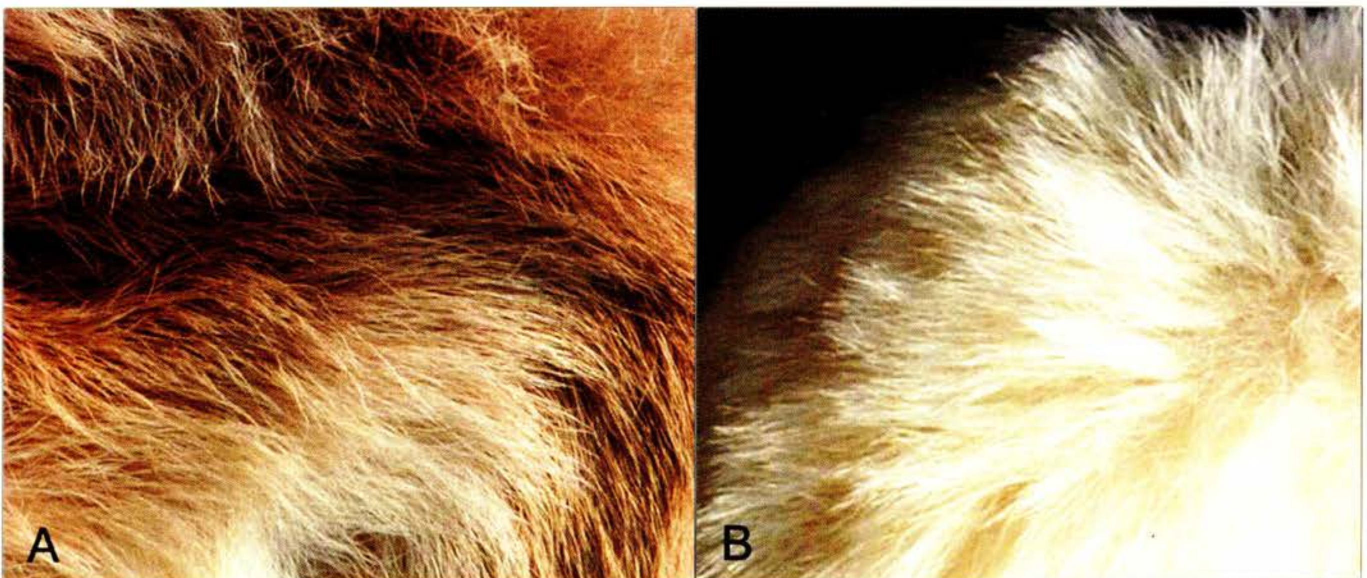

Figure 17. Hair on Jersey calf (A) and alpaca limb (B) which were plastinated using the North Carolina B_2 process with no additives.

All plastinated specimens produced with the Corcoran process exhibited altered clarity of surface detail with a slightly granular, translucent appearance after completion of polymerization (Figs. 2, 3, 5b, 6, 11). Raised, lumpy areas appeared on some specimens (Fig. 1). On transverse section, a heart is seen with subserosal accumulation of cured polymer (Fig. 12b). In many instances, surface detail is hidden by this subserosal accumulation of polymer (Figs. 6, 11). Nothing similar to this was found on any specimens produced using the other procedures of this study.

The Chinese process produced specimens on which surface detail appeared as it would on fresh tissue (Figs. 13, 14). The demarcation between the cortex and medulla on the cut surface of a feline kidney was excellent (Fig. 13). Occasionally, dry, white areas would appear on specimens produced with this method (Fig. 14). The surfaces of all specimens produced using the Chinese process remain slightly damp or oily feeling even three and one half years after impregnation.

The North Carolina B_1 and B_2 processes produced specimens that exhibit remarkable surface detail (Figs. 15, 16, 17). One NC B_1 specimen had two (2 x 3mm) raised areas of cured polymer which had oozed and not been wiped prior to curing. This common curing artifact detracted from that portion of the surface. The surface of NC B_2 specimens, which received no curing, had great surface detail but remain slightly damp to touch.

Using the process in which hair covered specimens were impregnated with polymer only and not exposed to any catalyst/chain extender/cross-linker produced specimens with hair that looked and felt like it would on a living animal (Fig. 17). The drawback to this method

Plastination method	*Tissue*	# B's	# G's	# O's
Classic S10	bovine uterus (figures 2 and 3)	15	8	0
Classic S10	male opossum genitalia	12	11	0
Classic S10	porcine uterus	13	10	0
Classic S10	canine heart	15	8	0
Classic S10	opossum liver (figure 4)	17	6	0
VisDocta	canine limb (figure 5a)	15	8	0
VisDocta	bovine kidney	12	12	1
VisDocta	equine brain (figure 7)	6	21	2
VisDocta	canine heart (figure 6)	12	11	0
VisDocta	feline stomach	8	14	1
Corcoran	canine limb (figure 5b)	1	6	16
Corcoran	bovine uterus (figures 2 and 3)	0	7	16
Corcoran	male opossum genitalia	0	5	18
Corcoran	porcine stomach	0	7	16
Corcoran	canine brain	0	6	17
Corcoran	canine heart (figures 6 and 12b)	0	7	16
China	feline spleen	3	12	8
China	feline duodenum/pancreas (figure 14)	6	15	2
China	feline kidney (figure 13)	12	7	6
China	feline liver	11	10	2
North Carolina A	porcine uterus (figure 9)	7	14	2
North Carolina A	canine heart (figure 10)	11	11	1
North Carolina A	feline spleen	12	10	1
North Carolina A	feline liver/stomach (figure 8)	4	17	2
North Carolina B	feline stomach (figure 15)	5	12	6
North Carolina B	feline kidney	9	13	1
North Carolina B	rainbow trout (figure 16)	15	8	0
North Carolina B	feline stomach	12	10	1
North Carolina B	feline liver	4	11	8

Table 1. Results of specimen evaluation by attendees of the 12th International Congress on Plastination, Murcia, Spain. Evaluators were asked to evaluate the quality of surface detail using the following key: B (best) = surface detail is distinct, G (good) = surface not quite as clear as those ranked B, O (okay) = surface detail not clear (distorted). These results are recorded for each specimen followed by the number of responses for each category of the evaluation grades. Evaluated specimens appearing in figures are labeled accordingly.

is the specimens will remain damp for approximately one year.

Most of the finished specimens were used routinely as needed for teaching or demonstration prior to their use in this evaluation and hence some specimens exhibited small use/trauma artifacts.

The results of the evaluation by the attendees of the 12th International Congress on Plastination, Murcia, Spain are listed in table 1.

Discussion

All specimens produced during this project period were of good to superior quality and durable. Each of the three general types of impregnation has their own unique qualities and attributes. Since its invention, the classic S10 method is the most reliable method for production of high quality plastinated specimens. As well, the generic copies of the classic S10 method (VisDocta™ and North Carolina A) provide equally as high quality plastinated specimens. Room temperature vs. cold dehydration of specimens and room temperature impregnation vs. cold impregnation of specimens yielded no discernable difference in surface quality or durability. Hence these specimens were not distinguished as a separate group for evaluation at the ISP congress.

The other two general types of plastination [impregnation reaction-mixture of polymer and cross-linker (Corcoran) and polymer only in the impregnation bath (Chinese and North Carolina B)] also produced good to high quality specimens. However, the quality of specimens produced by these processes seems to be less predictable and yet all have their bright spots including a shortened impregnation time, a decreased need for

freezer space for impregnation as well as the ease of polymer drainage and manicuring. The benefits in the ease of polymer drainage were most evident on hair covered specimens. The hair on specimens produced by other processes needed to be wiped excessively to remove the polymer and, even so, the hair of these cured specimens often contained some cured polymer giving the hair a matted, unnatural appearance. Currently, curing of the polymer is less predictable with these types of impregnation. If the specimens are left with a damp surface, they may not be as aesthetically pleasing to some. However, the benefit of life-like hair is great for hairy creatures.

The Chinese method recommends not to cure the impregnated specimen. Attempts to cure a few Chinese specimens by vaporizing the intended product as well as wiping it on the specimen mixed with polymer were not successful. The feline pancreas/duodenum was an example of this curing method and the surface detail was obscured in a few areas. Zheng and colleagues (1998a) recommend to mix old polymer with 1-5% hardener and wipe it onto the specimen surface to produce a dry specimen. This methodology was not used in our studies as it yields specimens with a high sheen and coat of polymer on the surface.

The negative feature of the Corcoran method (polymer cross-linker impregnation reaction-mixture) is that there is poor clarity of surface detail of the impregnated specimens after curing. The appearance of the semi-translucent to opaque surface accumulation of polymer after polymerization to date has not been explained. This granular surface detracts from the beauty of the specimen and renders less prominent anatomical detail more difficult to visualize. Beyond this, nothing else has been observed that decreases the usefulness of these specimens.

The evaluation by the attendees of the 12th International Congress on Plastination tends to support the findings of the authors. However, there is a high number of "G's" (mid-grade) among all types of specimens. This is a bit worrisome, but it may be that many felt more comfortable staying near the median rather than committing to either extreme. Also, it was not known that this evaluation was for part of a publication as we did not want to bias the evaluators. Additionally, some specimens had some "wear and tear" from usage which we hoped would not be included for or against the specimen during its evaluation. If we were to do a similar evaluation again, to narrow the choices to two or to widen to five choices may have moved the "G's" to one pole or the other. However, the high number of "G's" may support the fact that surface clarity may not be a major issue for the person new to plastination or even to an established plastinator.

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Abstracts - 7th Interim Conference on Plastination - June 11-15, 2001

A series of S10 or Su Yi plastinated specimens to demonstrate the normal and pathological anatomy of the cardiovascular system. ***Olry R[1], G Grondin[1], TZ Zheng[2].*** *[1]Université du Québec à Trois-Rivières, Trois-Rivières, Qc, Canada; [2]Shanghai Medical University, Shanghai, China.*

Plastination of the heart and the vascular system has been of great interest since the very beginning of the utilization of the plastination process in anatomy as well as pathology. A series of plastinated human as well as animal hearts are presented. These specimens present interior cavities of the heart as well as coronary arteries and cardiac veins. Some of these specimens (human and bovine) were prepared with Biodur S10 using the standard technique while the others (porcine) were plastinated at room temperature with the Su Yi silicone polymer developed in China. A series of aortic specimens showing various degree of arterial sclerosis, calcification and aneurisms are also presented.

Plastinated prosections: An aid to understanding sectional neuroantomy. ***Martín-Alguacil N, R Martín-Orti, J Camón Urgel, RW Henry.*** *Department of Anatomy and Embriology, School of Veterinary Medicine, Universidad Complutense de Madrid, Spain. [1]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, USA.*

Understanding the spatial organization of various structures of the brain can sometimes be difficult for students. However it is important to visualize how neuroanatomic structures are oriented within the brain in order to interpret diagnostic images. The use of plastinated specimens can certainly assure a better understanding of the three-dimensional geometry of the brain and its parts. Herein, we present examples of S10 plastinated brain prosections and epoxy and polyester brain sections to visualize structural detail and spatial relationships within the brain. A silicone plastinated dog brain, with the vascular system injected with colored latex and the cranial nerves colored, is used as a general reference to identify the level of the particular section to be studied. Brain prosections of deep structures like the caudate nucleus and hippocampus as they project dorsally from the floor of the lateral ventricle are seen. As well, the internal capsule, rostral and caudal colliculi, thalamus and geniculate bodies are exposed. All these dissected structures can be identified on various sections. Combined plastinated prosections and sections of the brain can generate models to identify structures of different types and size. Using these specimens, students can study sequential sections by taking them apart or building them up and/or comparing them with the prosected specimen. By using plastinated specimens, useful three-dimensional models can also be generated for teaching neuroanatomy.

The Su Yi Chinese silicone for doing plastination at room temperature. ***Zheng TZ.*** *Shanghai Medical University, Shanghai, China.*

In 1996 a new silicone was developed named Su-Yi Chinese silicone. As well, an intermittent vacuum procedure was introduced. Ten thousand specimens have been plastinated using this process at the Nanjing Plastination Factory. Both human and animal specimens were immersed in 7% formalin solution at room temperature for ten days and then kept in a 5% formalin bath for one month. Specimens were then dehydrated in a graded series of acetone. After dehydration, specimens were submerged in the Chinese silicone mixed with 0.1% hardener and allowed to remain in the solution for five to seven days prior to applying vacuum to allow normal vaporization of the acetone. The specimens were turned daily to let acetone and polymer reach equilibrium. After this pre-impregnation, the specimens and silicone were placed in a vacuum chamber and vacuum was applied and pressure slowly reduced during the work day over a two week period to 10mbar (7.5mm). Pressure decrease was monitored by observing bubble formation. After impregnation was complete, the specimens were allowed to remain in the polymer and equilibrate for five days. Two curing methods are used. 1. Slowcure: specimens are allowed to cure on their own for 2 months at room temperature. This yields a flexible specimen with a slight oily feel. 2. Add hardener: place the impregnated specimens into a silicone bath containing 3.0% hardener for 5 to 7 days, remove and smear curing agent onto the surface of each specimen. This method yields a hard dry surface. All specimens remain preserved and useful.

Restoration of a human fetal teaching collection with subsequent examination using magnetic resonance imaging (MRI). ***Lyons GW[1], CW Reifel[1], R Smith[2], R Temkin[1], D Situ[1], SC Pang[1].*** *Department of Anatomy and Cell Biology[1] and Department of Radiology[2], Queens University, Kingston, Ontario, Canada.*

Between the 1940's and 1960's, Queens University's medical school acquired several normal and abnormal human fetal specimens. With the changing medical curriculum of the late 1960's, the collection was relocated to storage. Recently, this collection was retrieved as part of our museum collection and an MRI investigation of morphological defects in the specimens was undertaken. After prolonged storage in formalin, some specimens showed signs of dehydration. To facilitate rehydration, the specimens were placed under running tap water for 24-48 hours and transferred to 10% NBF. After one month, specimens with major defects (sirenomelia, dicephalic iliothoracopagus, etc.) were prepared for scanning. The specimens were washed overnight in tap water, surface dried, mounted on plexiglass, sealed in 6 ml. plastic cocoons and scanned with a GE Signa, 1.5 Tesla magnetic resonance imager using 5.4.2 software. T2 weighted, 2D FSE images were obtained and evaluated. Following scanning, specimens were mounted and displayed in plexiglass jars containing 5.0% NBF. The revamping of this collection and the non-invasive nature of MRI scanning allowed us to reconstruct a teaching asset and afforded us with an excellent method of investigating these specimens for defects of internal organs and structures.

Abstracts - 8th Interim Conference on Plastination - July 5-11, 2005

Oral presentations

History of plastination. ***Sora M-C.*** *Plastination Laboratory, Department for Sytematical Anatomy, Centre for Anatomy and Cell Biology, The Medical University of Vienna, Austria, Europe.*

Plastination was developed for teaching as well as for research. In 1977, at the department of Anatomy of Heidelberg University, Dr. von Hagens invented plastination as a groundbreaking technology for preserving anatomical specimens with reactive polymers. The processes were patented between 1978 and 1982. This method has proved to be the superior method for preservation of gross specimens. Vienna was the first place to introduce this new method in the late 70's. Presently, the method is applied in more than 250 institutes for Human Anatomy, Clinical Pathology, Biology and Zoology worldwide. The "International Society for Plastination" was founded in 1986. The first issue of the Journal of the International Society of Plastination appeared in 1987. Optical properties, opaque or transparent, and mechanical properties, smooth and flexible or hard, can be chosen by appropriate composition of plastination resins. Plastination allows for preservation of specimens with completely visible surfaces and high durability. Plastinated specimens are odorless, non-toxic and mechanically resistant to a high degree. Plastination is a procedure during which water and fat of gross specimen are replaced by a polymerizable resin. In the past years, the use of plastinated slices has become an interesting research tool. Thin plastinated slices are essential if the histology or morphometrical investigations are to be studied on plastinated slices or if 3D reconstruction is desired. Histological examination can be performed up to a magnification of 40X. The major advantage of this method is that the structures remain intact and the decalcifying of bony tissue is not necessary.

Overview and General Principles of Plastination Procedures. ***Weiglein AH.*** *Institute of Anatomy, Medical University Graz, Graz, Austria.*

Decay is a vital process in nature but an impediment to morphological studies, teaching, and research. This is particularly true for biological specimens that shrink considerably when exposed to normal atmospheric conditions. Therefore, it has always been a goal to find suitable preservation techniques, especially for anatomists. 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. The class of polymer used determines the optical (transparent or opaque) and mechanical (flexible or firm) properties of the impregnated specimen. Silicone is used for whole specimens and thick body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices. Polyester-copolymer is exclusively used for brain slices to gain an excellent distinction of gray and white matter. The technique consists of four main steps: fixation, dehydration, forced impregnation and hardening (curing). Fixation can be accomplished by most conventional fixatives. Dehydration is achieved mainly by acetone because acetone also serves as the intermediary solvent during impregnation. Forced impregnation is the central step in plastination. Vacuum forces the acetone out of and the polymer into the specimen. Hardening (curing): finally the impregnated specimen is hardened by exposing it to a gaseous hardener (silicone) or by UVA-light and heat (polyester, epoxy). Plastinated specimens are perfect for teaching, particularly for neuroanatomy. Silicone plastinated brains are useful because they can be grasped literally and they are almost everlasting. Polyester plastination of brain slices provides an excellent distinction of gray and white matter and thus a better orientation. Plastination is carried out in many institutions worldwide and has obtained great acceptance particularly because of the durability of the plastinated specimens, the possibility for direct comparison to CT- and MR-images and the high teaching value of plastinated specimens. *Silicone (S10) standard procedure:* The S10 technique is the standard technique in plastination. Specimen impregnation with Biodur™ S10 results in opaque, more or less flexible and natural looking specimens. The procedure consists of the four main steps of plastination in addition to specimen dissection and preparation. Fixation can be achieved by all usual fixatives as formaldehyde solution, Kayserling solution etc. Hollow organs must be dilated during fixation as well as during dehydration and gas curing. Dehydration removes the specimen fluid as well as some fat. In this step, tissue fluid is replaced with an organic solvent. Either alcohol or acetone may be used as a dehydrating agent for plastination. Acetone is used in most cases because acetone also serves as the intermediary solvent during the next step - forced impregnation. To

minimize specimen shrinkage, dehydration is done in cold (-15°C to 25°C) acetone. If the removal of fat is also desired, the dehydrated specimen must be kept in acetone at room temperature for some time (this however, must not be done with nervous tissue, particularly with brains). An acetone amount of 10 times the specimen weight is best for good results. Dehydration is finished when the water content of the specimen is less than 1%. Equipment needed for dehydration includes a deep freezer (explosion proof or motor and compressor removed and placed in a different room) and an acetonometer to measure the content of water. Forced impregnation is the central step of plastination. In this step, the intermediary solvent (acetone) is replaced with a curable polymer (Biodur™ S10). The silicone polymer S10 is mixed with a curing agent Biodur™ S3 (1 part S3 to 100 parts S10) which commences the process of end-to-end linkage of the molecules. This linking is enhanced at room temperature; however, it is very slow when kept in the freezer at -15°C to -25°C. The dehydrated specimen is submerged in the cold 8-15°C to -25°C) polymer mixture. After some days of immersion, vacuum is applied to it. Vacuum is increased gradually to boil the intermediary solvent (acetone), which has a lower boiling point (56°C) out of the specimen. Impregnation is monitored by watching the bubble formation on the surface of the mixture and by a vacuum gauge. Vacuum is complete when the pressure is around 5mm Hg. Equipment needed for forced impregnation includes a deep freezer (explosion proof or motor and compressor removed and placed in a different room), vacuum chamber (e.g. Heidelberg plastination kettle) and a vacuum pump with a pumping speed of 1.5 m^3/min. for a 15:l polymer mixture or 3m^3/min. for a 30:l polymer mixture. Finally, the polymer inside the specimen has to be cured (hardened). This is achieved by exposing the impregnated specimen to a gaseous hardener (Biodur™ S6). S6 is a liquid that vaporizes at room temperature. The impregnated specimen and a bowl filled with S6 are placed in a tightly closed chamber for several weeks. To keep the environment for curing dehumidified a bowl with a desiccant (e.g. calcium chloride) is also placed in the curing chamber. To enhance the curing procedure, air may be bubbled through the fluid S6. For complete curing inside the specimen, the specimen should be kept in a plastic bag for several weeks. Equipment needed for curing includes a plastic or stainless steel box, stretch foil and membrane (aquarium) pump. *Epoxy (E12) procedure:* The E12 technique allows processing of fixed and unfixed fresh specimens. Kayserling solution is recommended for fixation. Others work as well. The specimens are initially frozen in a deep freezer. For best results, an ultra low deep freezer (-70°C) is recommended. With a special band saw, body parts are sliced into 2.5mm thick slices. After removing the saw dust, the slices are transferred to plastic grids. The grids containing the slices are placed into a steel basket for further procedures. The basket of slices is placed in acetone at -25°C. Three or four acetone baths are used subsequently to achieve complete dehydration. The basket of slices is transferred to a fresh E12 mixture (95pbw E12, 5pbw AT30, 20pbw AT10, 26pbw E1) and placed under vacuum for 24-48 hours at 0-10°C. The vacuum is increased until 5mm Hg is attained. The basket of slices is then removed from the vacuum chamber. Individual slices are placed between two glass plates. A silicone gasket is placed between the outer edges of the sheets and then clamped in position using fold-back clamps. The glass chambers containing the specimens are filled with a fresh E12 mixture (95pbw E12, 5pbw AT30, 26pbw E1). Following 2-3 days precuring at room temperature, the glass chambers are placed in a well-ventilated oven at 50°C for another 2-3 days. When curing is completed, the cold glass chambers are dismantled and the sections are trimmed on a band saw. After sawing, the edges are smoothed using a belt sander. *Polyester (P35) procedure:* Fresh brains are fixed the usual way with 10% formaldehyde for 4-6 weeks. Specimens which have been fixed by other methods should be avoided for this procedure because other fixation methods may interfere with the P35 reaction. After embedding in 20% gelatin (Barnett et al., 1980), brains are cut with a meat slicer into 4mm slices. To prevent disintegration of the slices after cutting and during subsequent handling, they are placed on a piece of wet filter paper before being transferred to stainless steel grids. The grids containing the slices are placed into a stainless steel basket for flushing. The basket of slices is rinsed with cold tap water overnight and cooled to 5°C before proceeding. The basket of slices is placed in 100% acetone at -20°C for 3 days. The basket of brain slices is placed in a bath containing a mixture of P35/A9 (100 parts P35 polymer and 2 parts A9 hardener) for one day at 5°C. This bath might be the 2nd immersion bath mixture of a previously run group of specimens. The basket of brain slices is placed in a second immersion bath containing a mixture of P35/A9 (100:2) for a further 24 hours at 5°C. This bath might be the bath used for forced impregnation during for a previously run group of specimens. The basket of brain slices is transferred to a fresh P35/A9 mixture (100:2) and placed under vacuum for 24 hours at room temperature. The vacuum is increased until 10-15mm Hg is attained. The basket of slices is removed from the

vacuum chamber. Individual slices are placed between two sheets of glass plates. Each sheet consists of an outer piece of safety glass and an inner sheet of float glass, the latter sheet facing the brain slices. A silicone gasket is placed between the outer edges of the sheets and the clamped in position using fold-back clamps. The double glass chambers containing the specimens are filled with a fresh P35/A9 (100:2) mixture. After casting, the double glass chambers are exposed to UVA light for 3 hours. During this procedure, it is necessary to cool the chambers either by ventilators or by blowing compressed air over both sides of the double glass chamber. Following light curing, the double glass chambers are placed in a well ventilated oven at 45°C for 5 days. When curing is complete, the glass chambers are dismantled and the sections are trimmed on a band saw. After sawing, the edges are smoothed using a belt sander. *Polyester (P40) procedure:* This technique has advantages over the others. P40 resin has a lower viscosity than the P35 resin. The advantages of using this technique are: 1. The same polymer can be used for immersion, impregnation, and casting of specimens. 2. Only single float glass chambers are necessary when casting specimens as compared with the expensive double glass chambers, containing safety glass, as in the P35 method. 3. P40 is cured by UVA light only; therefore, there is no requirement for an expensive ventilated heat cabinet as in the P35 method. 4. P40 can be used for production of transparent body slices as well as brain slices. The disadvantages associated with the P40 procedure occur during the curing process. UVA curing may be incomplete in brain tissue and will appear as orange discolored regions within the gray matter.

The S10 - standard procedure for beginners.
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The S10 procedure is the most widely used application in plastination. Specimen impregnation with Biodur™ S10, or similar products from other companies (Cor-Tech™ PR-10, Syl-Tech B, VisDocta™ SH-1) results in opaque, more or less flexible and natural looking specimens. The procedure consists of the four main steps of plastination: fixation, dehydration, forced impregnation and curing. Other steps may be added for special results. Specimens are fixed the usual way with 5-10% formaldehyde or other fixatives. Old, wet specimens can be used for the S10 procedure provided they are thoroughly washed with tap water. Hollow organs must be dilated with the fixative. Specimens are dissected as desired. Some cuts may better be done after curing to obtain smooth surfaces. Slicing can be done now, but may preferably be done after plastination (see step 4b). Hollow organs must not be cut; if windows are desired they may be cut after curing. Specimens are rinsed with cold tap water overnight and cooled to 5°C. This step is both important to get rid of the fixative and to pre-cool the specimens before dehydration. Additives (activator, cross-linker, thinner) and procedures (time, temperature, and method of application) may vary depending on the brand. Flexibility mainly depends on specimen thickness. Stomachs, intestines and thin tissue layers are more flexible than thick, parenchymal organs such as liver and kidney. Specimens are submerged in usually three subsequent baths of pure acetone at -20°C (10:1 per 1kg specimen). Each dehydration bath should last about one week; however, the dehydration period may be extended to several weeks. Dehydration is finished when the acetone concentration is at least 99%. Complete dehydration at -20°C is necessary to avoid shrinkage. Dehydrated specimens are submerged in the S10/S3 mixture (1 part S3 hardener to 100 parts S10 polymer) for at least one day (up to several weeks) at -20°C. This additional step again prevents shrinkage. The longer specimens are immersed in the S10/S3 mixture, the shorter the following impregnation time will be. The submerged specimens are set on vacuum for three weeks at -20°C. Impregnation starts when large bubbles (=acetone) start to pop up to the surface of the silicone in the vacuum chamber (usually at about 150mm Hg). From this point on the vacuum must be gradually increased to 0mm Hg within the three weeks. The slower one goes, the better are the results. Fast impregnation causes shrinkage mainly in thick specimens because the polymer cannot be forced into the cells as fast as the acetone leaves them. After removal of excess polymer, the specimens are placed on a grid in an air tight chamber and exposed to S6 gas cure for about 7 days at room temperature. The vaporization of S6 is enhanced by bubbling air through the fluid S6 by means of small aquarium pumps or by the use of ventilators. This causes fast curing of the surface. To cure the polymer inside the specimen it is necessary to store the specimens in air tight plastic bags for up to two months. A small container with calcium chloride in the curing chamber collects moisture, which otherwise may cause white spots on the brain's surface. When the whole procedure is finished, the S10 plastinated specimens can be sliced with a band saw or a strong rotation meat slicer. After slicing the slices may be smoothed by sanding the slices on a belt sander, ideally with addition of water.

Dissections before, during and after plastination

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Novice plastinators may assume that dissection of a specimen must be completed prior to commencement of the plastination process. However, further dissection is possible both during and after plastination which often results in enhancement of features chosen for display. The purpose of this presentation is to provide a range of examples of dissection techniques of plastinated material at various stages of the process. The importance of dissection prior to plastination is paramount because the final appearance of the specimen is dependent on the time, effort and technical expertise of the dissector. All extraneous connective tissue must be carefully removed by the prosector. Underwater dissection magnifies the connective tissue which can then be carefully removed to produce a smoother surface with muscle fascicles enhanced. Stents can be used to maintain the shape and size of vessel lumen and orifices. Other devices are used to maintain the correct relationships between structures during the process. Some of these devices can be retained while others are removed during the plastination process. Use of compressed air during the plastination process produces organs with a realistic size and shape, such as the lungs, stomach and intestines. Sections of organs can be produced after plastination from complete organs. As an example, brain slices can be produced from whole brains, often producing slices of better quality than the alternative of slicing the brain prior to plastination. In terms of presentation of plastinated specimens, there is definite benefit in using a range of techniques to highlight specified features and to maintain the correct appearance of structures that tend to collapse during the plastination process.

Silicone impregnation and curing.

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Impregnation is the exchange of a volatile intermediary solvent [acetone or methylene chloride (dichloromethane)] for a curable polymer and is the key point in the plastination process. Impregnation in the classic S10 procedure is carried out over several weeks. Dehydrated, solvent-saturated specimens are submerged in a polymer reaction-mixture inside the vacuum chamber. This reaction-mixture is made by mixing silicone polymer (S10) and the catalyst/chain extender (S3) at a ratio of 100:1. Submerged specimens are allowed to equilibrate with the reaction-mixture for a day. The next day pressure is decreased two-thirds of an atmosphere by closing the port and needle valves and starting the vacuum pump. When pressure has decreased two-thirds of an atmosphere (25cm Hg, 10 inches Hg), pressure is stabilized by opening the valve(s) slowly until pressure remains at this level. Small air bubbles that were mixed into the reaction-mixture will rise to the polymer surface while pressure is lowered. The next day, the needle valve is closed a portion of a turn to decrease pressure 5cm Hg (2 in.). The pressure reading will be around 20cm Hg (9 in.). Each day pressure is decreased 2.5cm Hg (1 in.) and allowed to stabilize overnight. When pressure is near 8cm Hg (3 in.), bubbles will likely be seen rising to the surface and bursting continually. These bubbles are from acetone vaporizing and being pumped off. At this vacuum level, when bubbles are forming and rising to the top, do not decrease pressure. When no bubbles are rising, decrease pressure 1cm to enhance bubble formation (acetone vaporization). Vacuum is continued until near zero pressure is maintained for a few days and bubbles are very large or diminished in formation. End point time depends on volume of specimens and pump speed. When impregnation is complete, the vacuum pump is turned off and the plastination kettle with the specimens is allowed to return to ambience. After a few hours or days, the specimens are drained of excess polymer and brought to room temperature to allow the conclusion of draining of excess polymer. After the excess polymer has been drained from the specimens, specimen surfaces are wiped or blotted dry. The next and final step is curing. Curing (polymerization, hardening) is the process which changes the liquid polymer reaction-mixture into a solid. Two variations of curing may be used, slow cure and fast cure. Slow cure allows the chain extender of the polymer reaction-mixture time to create longer silicone molecules which will likely create a more flexible specimen. The catalyst has prepared the silicone polymer molecules to react with the chain extender and cross-linker. The specimen is placed on absorbent toweling, wiped of excess or oozing polymer and left at room temperature and environment for weeks to months. During this phase, hollow organs must be distended with air or absorbent material, as well as, all specimens must be molded into their best anatomical form. Fast cure uses daily applications of the cross-linker (S6) in a closed environment to begin the cross-linking of the silicone polymer molecules. This transforms the polymer from a liquid into a solid. During this time it is important to wipe excess polymer and drips or runs from the surface and crevices of the specimen. As well, hollow organs must be dilated and

all specimens must be molded into their final anatomical position. After the surface is dry, the specimen may be used but should be kept in a closed environment (bag) for several weeks to assure curing into the depths of the organ.

Vacuum and vacuum monitoring during silicone plastination. ***Henry RW.*** *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, USA.*

Vacuum is the key mechanism for the plastination process. Vacuum is read and recorded two ways: 1) Absolute pressure (AP) or 2) Vacuum. What these readings represent, when measuring the pressure (vacuum) or changes in pressure, varies depending on the type of instrumentation. Commonly, changes are measured via a Hg column, gauge or manometer. A column of Hg or vacuum/pressure gauge yields a higher reading as absolute pressure is decreased and is referred to as gauge pressure. However, a manometer yields a lower number as AP decreases and is read as absolute pressure. The manometer utilizes the difference in two columns of Hg. The vacuum gauge or column of Hg uses atmospheric pressure as point zero while the manometer uses total vacuum as point zero. Hence, the readings are at opposite poles of the 760mm scale (atmospheric pressure at sea level). Because most manometers used in plastination laboratories utilize two columns of Hg whose difference in height is 20cm or less, only the lower 1/3 of atmospheric pressure (<25cm) may be measured. Hence, a vacuum gauge or Hg column is necessary to monitor changes in absolute pressure (vacuum) in the earlier stages of impregnation (first two-thirds atmosphere decrease). Pressure is read in milli/centimeters or inches of Hg. Torr is another unit used to measure pressure. One Torr equals 1/760 of an atmosphere. Why/how vacuum for plastination? During tissue processing for electron microscopy, polymer penetrates only 1-2mm. Plastination of large specimens needs penetration of several cm. Vacuum lowers the vapor pressure of the solvent so that the solvent can be regulated and extracted slowly over a period of time even at a lower temperature. This slow release of solvent from the cells allows time for the more viscous polymer to enter the cells, thus replacing the evaporating solvent. Two solvents fit these criteria: acetone and dichloromethane (methylene chloride). The saturated vapor pressure (similar to boiling point) of dichloromethane is higher than that of acetone; 32.5mm Hg vs. 14.8mm at -25°C and 78.0mm vs. 35.9mm at -10°C respectively. Hence, methylene chloride will vaporize at a higher AP and be extracted before acetone at any given temperature or pressure. This shows that the greatest extraction of acetone will not occur until near 3.5cm AP. When using deep vats of silicone for impregnation, remember; pressure is proportional to depth. This results in the pressure being greater at the bottom of the polymer than on the surface of the polymer. At -15°C, acetone will remain in a specimen which is submerged 15 to 20cm below the surface of the polymer longer than a specimen near the top. The gauge reads surface pressure. When contemplating construction or purchase of a plastination kettle, the force generated when absolute pressure is decreased one atmosphere is 15 pounds per square inch (6.45cm^2). At total vacuum, the one foot (30cm) cube desiccator used for plastination has 2,160 pounds (lbs) of force on its walls while a 46cm x 76cm (18 in. by 30 in.) medium size plastination kettle has 8,000 lbs of force and a 50cm x 127cm (20 in. x 50 in.) vacuum chamber has 15,000 lbs per square inch (6.45cm^2).

Scientific potential of plastination and high-tech equipment. ***Weiglein AH.*** *Institute of Anatomy, Medical University Graz, Graz, Austria.*

To evaluate the scientific impact of plastination the New Plastination Index- Online (http://www.uqtr.ca/plastination/) is a helpful tool. During the years 1978-2005, a total number of about 1100 manuscripts have been published in more than 180 scientific journals and books. Most of the 553 original articles in peer-reviewed journals, 429 communications at scientific congresses and 87 other articles deal with plastination technology, teaching, public exhibition/ethics and sectional anatomy. The plastination method with the highest educational value is the most widely used silicone procedure. The sheet plastination techniques have the highest scientific impact in both scientific teaching and research in sectional anatomy. The scientific application of sheet plastination usually requires high-tech equipment such as equipment for special staining techniques (plastination histology) and slicing devices before impregnation (ultra low temperature deep freezers, stainless steel band saws, and rotation meat slicers) and after impregnation (diamond band and diamond wire saws). One major application of sheet plastination is the study of tissue patterning because the delicate structures particularly of connective and muscular tissue are easily damaged or altered during dissection and histological examination is limited by the sample size. Sheet plastination on the one hand does not destroy the tissues and on the other hand the sample size is not limited as in histology. Thus, sheet plastination offers a new approach to study tissues at both macroscopic and microscopic levels and thus provides a tool to close

the gap between macro- and microstructure.

Epoxy and polyester sheet plastination. ***Cook PR.***
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As sophisticated diagnostic imaging technology has become commonplace in the clinical environment, it has never been more imperative for the medical student, clinician and researcher alike to understand and further explore the cross sectional approach to the human body. Sheet plastination has proven to be a vital tool in the enhancement and clarification of anatomical concepts and relationships previously often difficult to appreciate. Sheet plastination is a means in which thin slices of organs, extremities, brain or even whole *in situ* sections may be specially processed and encapsulated within a clear, smooth resin sheet. Sections may vary in thickness from 2mm to 6mm depending upon the region, type of tissue and the desired result. Specimens are cut, dehydrated and vacuum impregnated with a polymer of either epoxy or polyester base and are subsequently processed between two glass plates using either heat or ultra violet light. The finished specimens offer excellent clarity, providing a vantage point to the submacroscopic level easily observed with the naked eye. Sections may also be further magnified using either a light microscope or CCD closed circuit video system. When viewing extremely fine vascular or nervous detail under a close-up video system, whole body sections may be viewed first in their entirety, then magnified at various levels to display anatomical relationships without the need for the interconnected tissues to become physically separated. Sheet plastination maintains the entire anatomical plane in a complete and uninterrupted state, thus retaining the overall structural integrity. Brain sections are greatly enhanced by a superb differentiation of the white and the gray matter. By closely observing a number of key plastination protocols, the individual serial sections display an exceptionally vivid degree of anatomical detail not previously visible in traditional cross sections, wet gross specimens or even, in some cases, radiographic images. Sheet plastinated specimens are an ideal link between three disciplines; namely cross-sectional anatomy, radiology and microscopy from just the one specimen.

Expanding the role of plastination in anatomy education. ***Raoof A, C Baumann, K Falk, N Hendon, L Liu, A Marchese, L Marchese, R Mediratta, N Mirafzali, J Munch, C Parres, M Wells, H Zhao.***
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Since the introduction of plastination about three decades ago, anatomical specimens preserved in silicone have been broadly used in medical schools as a valuable resource to gross anatomy education. At the University of Michigan Medical School, plastinated specimens have become an essential part of medical, undergraduate and dental anatomy education. The newly implemented, system-based, integrated medical curriculum necessitated the introduction of a different set of specimens that are more relevant to the new curricular approach. The aim has been to demonstrate systemic and essential concepts in anatomy in order to promote students' independent learning. In the undergraduate "*Introduction to Human Anatomy*" course, laboratory visits were introduced into the syllabus where pertinent plastinated specimens were displayed. During the visits, faculty using these specimens explained the anatomical and the clinical relevance of the related systems. In the dental anatomy sessions, there has been more reliance on the use of plastinated specimens that demonstrate essential and inaccessible areas in the head and neck region. Medical and undergraduate students participated in preparing these specimens. Innovative approaches to enhance the quality of plastinated specimens were implemented such as coloring neurovascular pathways and casting hollow viscera to facilitate learning. The validity of these specimens was regularly tested through surveys administered to students. Also, a pilot study was conducted whereby the performance of a test group of medical students, who reviewed anatomy using the new set of specimens, was compared to that of a control group using the traditional set of specimens. Results indicated an overall acceptance of the new specimens as a valuable resource for learning anatomy. The reliance on plastinated specimens in education is certainly on the rise. Efforts in producing more relevant specimens for that purpose are focused on the exposure and coloring of essential neurovascular pathways. The new approach in preparing specimens is planned for a wider application of plastination in the future to facilitate comprehension of anatomical knowledge and to assist faculty and students in the effective utilization of the time allocated to anatomy.

Teaching with plastinated specimens in veterinary medicine. ***Henry RW.***
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Plastination is touted as one of the great teaching innovations of the 20th century. It has numerous qualities of which tissue preservation is of foremost

importance. Many hope that plastinated specimens do not replace cadaver dissection. Prior to a major curricular revision seven years ago, plastinated specimens were used primarily to supplement information on areas which are difficult to dissect, and to understand with professionally prepared plastinated dissections that would enhance understanding of these topics. A library of a wide range of specimens, as well as novel preparations and finds, birth defects, exotic animal preparations and diseased specimens were routinely plastinated. Plastinated specimens were usually placed in the laboratory after a region or system had been dissected. Two reasons: 1. This might assure more thorough dissection. 2. These specimens would be used primarily as a review of previously dissected areas. This approach works particularly well if you use the student dissections as a major portion of their examination. Since curricular change reduced laboratory time, such that even preferred dissections had to be removed or limited, plastinated specimens were seen as a mechanism to help resolve this situation. One such project was preparation and plastination of the proximal limbs of the horse and cow. Fifteen thoracic and pelvic limb preparations were dissected and plastinated to emphasize important structures of the proximal limbs, as well as, serve as the connection between the trunk and the distal limb. Neuroanatomy is now taught primarily with prosected plastinated brains of various species. Plastinated specimens may be pinned and/or photographed and labeled to highlight salient landmarks and a key prepared for the pinned or marked structures as well as suggested items that the student should be able to identify. Plastinated specimens are available 24 hours a day for student use. Students are asked to handle the plastinated specimens with clean hands (not gloves) and not to place plastinated specimens on wet dissection tables. Specimens are used frequently by upper classmen and clinical faculty to review the anatomy of an area prior to treatment of a clinical case. Specimens are used for public relations as in tours as well as for clients to demonstrate the problematic area of their pet. Plastinated specimens are used as props for educational talks by faculty or students at area schools or civic organizations. Plastinated specimens are also building a library of exotic or unusual anatomy that serves to document anatomical information of various species, pathology and anomalies.

Thin slice plastination and 3D reconstruction. ***Sora M-C.*** *Plastination Laboratory, Department for Sytematical Anatomy, Centre for Anatomy and Cell Biology, The Medical University of Vienna, Austria, Europe.*

The E12 method of plastination is usually used to create 2.5-5mm transparent slices. If thinner slices, 0.5-1.5mm, are desired it is necessary to use the thin-slice plastination method. Using this method, the specimen must be first plastinated as a block and then cut into thinner slices. The impregnation temperature is the key element to obtain a proper impregnation of the desired tissue block and contrary to all other plastination methods high temperature is used. The main goal of this paper is to describe the use of high temperature for processing 1mm epoxy plastinated slices. Only by using high temperature is the polymer thin enough to penetrate into the middle of the processed specimen. One male unfixed human cadaver ankle was used for this study. The distal third of a limb was cut and the foot positioned in a 90° dorsal flexion. A tissue block containing the ankle was cut starting 40mm distal to the tip of the lateral malleolus and finishing 50mm proximal. The tissue block was dehydrated, degreased and finally impregnated with arsine mixture E12/E6/E600. Using a band saw, Exact 310 CP, the E12 block was cut into 1mm slices. Once scanned, these images of the plastinated slices were loaded into WinSURF and traced from the monitor. Once all contours were traced, the reconstruction was rendered and visualized and the model was qualitatively checked for surface discontinuities. An E12 block was produced that was hard and transparent. Thin, <1mm slices produced from this block were transparent and hard with good optical qualities. The finished E12 slices provided anatomic detail to the microscopic level. Thin slices <1mm are essential if the histology is to be studied on plastinated slices or if 3D reconstruction is desired. These thin slices can only be cut from a solid E12 block. Therefore, knowledge of controlling temperature and percent of accelerator in the thin-plastination method is essential. Histological examination can be performed up to a magnification of 40X. The major advantage of this method is that the structures remain intact and the decalcifying of bony tissue is not necessary.

The new plastination index. ***Grondin G.*** *1765 rue Charon, Trois-Rivières, Qc G8Y 2L3, Canada.*

The "New Plastination Index" has been online since one year and is continuously growing. The author will review and explain the various sections of the index. Participants will also be invited to comment on their perception of the utility of the index and to suggest additions or modifications to it in order to make it as useful as possible for every person involved in

plastination. The address is: www.uqtr.ca/plastination.

Plastinated specimens for bronchoscopy. ***Latorre R, F Soria, F Gil, J Usón, MD Ayala, S Climent, O López-Albors, RW Henry.*** *Anatomy and Embryology, University of Murcia, Spain, and Minimally Invasive Surgery Centre, Cáceres, Spain.*

Specially designed plastinated organs aid the teaching-learning process when training to learn minimally invasive surgical techniques. These specimens allow a real life training opportunity using endoscopic techniques and skills prior to using a living patient. These experimental models should be used for the basic steps of specialized training and learning programs of minimally invasive surgery procedures. The morphological and physical characteristics of plastinated specimens are excellent to study the topographic and clinical anatomy of the bronchio-alveolar tree. Fixation of fresh tissue specimens was by perfusion of 5% formaldehyde through the trachea with a peristaltic pump for 8 hours. Later, fixative was rinsed from the tissue via flowing tap water. Dehydration was achieved by tracheal perfusion of 90% acetone for 24h. A weekly change into a higher percent acetone was performed until an acetone percent of >99% was maintained. North Carolina Silicone polymer (Neat 285 or 295) was used first to fill the lungs and then for impregnation at room temperature. After impregnation, the specimens remained at room temperature dilated with circulating air for several months to allow excess polymer to drain. Once drainage appeared complete, specimens were placed in a curing chamber and super-catalyst was added to the environment until all weeping had ceased and the lung surface was dry. Plastinated cardiopulmonary blocks and isolated lungs of dogs were produced to practice: 1. Respiratory endoscopic exploration of the trachea and bronchi; 2. Diagnostic and therapeutic techniques (cytology, biopsy, tracheal and bronchio-alveolar suction, endoscopic placement of tracheal stents in the stenotic model and 3. Selective intubations (endotracheal tubes of Robert-Shaw and bronchial brockade Univent).

Poster presentations

An investigation of renal artery and its extrarenal distribution in sheep. ***Acer N, N Ekinci, T Ertekin, K Aycan.*** *Mugla University Mugla Health School, Mugla, Turkey. Department of Anatomy, Faculty of Medicine, Kayseri, Turkey.*

In the present study, the anomalies relating to the origin and number of renal arteries which provide the major source of circulation to the kidneys were investigated in sheep. There are several studies covering renal vessels of pigs, dogs, camels and other animals. However, little is known about the branching pattern of the sheep renal artery as compared with other animals. The purpose of this investigation was to describe the renal blood vessels in sheep. The purpose of this study is to establish the incidence and characteristics of variations of renal arteries in 28 Karaman sheep of both sexes (total of 56 renal arteries) in the Kayseri Region of Turkey. The material was fixed in a 5% formalin solution for 9 weeks. The renal vessels were then prepared and photographed. All examined renal arteries arose from the aorta as a single vessel. The renal artery was then seen to branch into two vessels in 44 of 56 vessels (78.6%) and into three branches in 12 of 56 vessels (21.4%). Our findings were analyzed morphologically and the data was evaluated in the light of related literature. The gross renal arterial system of the sheep was compared to, and contrasted with, renal vascular anatomy of other species.

Introductive report to plastination of Catholic Institute for Applied Anatomy in Korea. ***Lee UY, BU Hong, JY Lee, SH Han.*** *Catholic Institute for Applied Anatomy, Department of Anatomy, College of Medicine, The Catholic University of Korea, Seoul, Korea.*

Many researchers interested in morphology have known that plastination is a unique technique of tissue preservation producing durable, dry and handleable specimens. However, there were few attempts to produce plastinated specimens in Korea. For the first time in Korea, the Catholic Institute for Applied Anatomy succeeded in producing plastinated specimens using the silicone S10 standard technique in July, 2003. Since then, our institute has plastinated 80 specimens and introduced the results of these specimens and future plans to the International Society for Plastination. Of the 80 plastinated specimens, 60 specimens were obtained from human cadavers and 20 specimens from plants and animals. All specimens were plastinated using the S10 technique, (48 specimens were treated with Biodur™ S10 and the others were with silicone made in Korea). During these 2 years, our institute gained experience with the S10 technique using varying specimens such as digestive organs, hearts, brains, bone, muscle, regional material (hemisected head or shoulder region), fetuses, pathologic specimens, animals and plants. Many problems were encountered and we selected three major problems to be solved for better products. The control of hardness, the maintenance of morphologic characteristics and the minimization of color change. Presently, to solve these

problems, all records about material characteristics, work history and quality of plastination are entered into a database. On the basis of this database, one may find the value of silicone viscosity, modified dehydration and curing method according to material characteristics. Also, our institute is preparing additional plastination methods using epoxy and polyester resin.

Morphological study of human proximal femur: gender and regional differences. ***Chen H, S Shoumura.*** *Department of Anatomy, Gifu University Graduate School of Medicine, Gifu, Japan.*

Femoral neck fractures are a major cause of morbidity and mortality in elderly humans. The weakening bone is reflected by morphological changes. In the present study, we apply histo-morphometric methods and scanning electron microscopy to compare the proximal femoral structure of different regions of males and females. Proximal femurs were obtained from 13 male cadavers (mean age 80.2±10.3 years) and 14 female cadavers (mean age 80.8±13.5 years) during the dissection practice. Proximal femurs were fixed in 70% ethanol and embedded in methylmethacrylate without prior decalcification. Cross sections of femoral head and neck were cut with a low-speed saw. Each section was divided into quadrants and the regional histomorphometry was performed. Some samples were observed with scanning electron microscopy. The mean diameter of female femoral heads was smaller than that of the male. The subchondral bones of anterior and posterior regions in female femoral heads were thinner when compared with those of males. The percentage area of cancellous bones in the anterior and posterior regions of female femoral head was significantly lower than that of males. The total cross section area of the femoral neck was significantly lower in females compared to males. The cortical bone of the female femoral neck was thinner than that of the male. There was an increased intracortical porosity in female femoral necks, especially in the anterior and posterior regions. The trabecular width in female femoral necks was significantly less than that of males. The female cancellous bone of both the femoral head and neck exhibited an increased resorbing surface in the anterior and posterior regions. These findings indicate that the female proximal femur, particularly in the anterior and posterior regions, was brittle. We consider that the decreased subchondral bone and cancellous bone of the femoral head, increased cortical porosity, decreased cortical thickness and trabecular width of femoral neck are involved in the fragility of the proximal femur.

The interest of plastination for pedagogical and display purposes: plastination of soft organs of a dog and installation within the skeleton. ***Grondin G[1], C Guintard[2], E Betti[2], B Chanet[2].*** *[1]1765 rue Charron, Trois-Rivières, QC, G8Y 2L3, Canada, [2]École Nationale Vétérinaire de Nantes, Unité d'anatomie comparée, Nantes, France Europe*

The plastination technique offers a unique advantage over all the other techniques as it permits the display of internal organs that are dry and close to the living state. These organs can be placed into skeletons to enhance their value. Internal organs from a dog were plastinated according to the standard S10 technique. The skeleton of the dog was also cleaned and mounted. The plastinated organs were afterward positioned within the skeleton. This type of presentation allows people to see the size, shape and exact location of all the organs within the body. They can also enhance the interest for the various skeletons displayed in museums. We believe that this new kind of exhibit is worthy of interest for museums as well as pedagogical purposes.

Evaluation of shrinkage on pig kidneys with S10 technique: study before and after dehydration and impregnation. ***Ilieski V, L Pendovski, I Ulcar.*** *Department of Functional Morphology, Faculty of Veterinary Medicine-Skopje, University St., Cyril and Methodius, Macedonia, Europe.*

Plastination is a method that offers an ideal visual presentation of anatomical structure. This technique is widely and extensively used in teaching and in researching purposes. Plastination allows production of anatomical specimens that appear lifelike in state without many noticeable changes. Shrinkage of tissue is one of the disadvantages to plastination. Many researchers deduce that the shrinkage of specimens occurs during the stages of dehydration and impregnation. However, there are sufficient studies which evaluate the influence of plastination on the shrinkage of the tissue during the plastination procedure. The aim of this study was to evaluate the value of shrinkage that occurs during dehydration and impregnation using the standard S10 technique for plastination. Thirty kidneys obtained from adult mixed breed Landrace farm pigs were plastinated according to the standard protocol described by Biodur™. The kidneys were removed between 130-150 days of age when the body weight ranged from 50-70kg. Before plastination, each kidney was labeled with a plastic ring with an identification number. The kidneys were quantitatively evaluated considering the following measurements: greatest longitudinal length (LL), cranial pole width (CRP), caudal pole width (CDP) and weight (W). The qualitative evaluation of these parameters was performed before and after dehydration and silicone

impregnation using a digital caliper ruler and a digital balance with a precision of 0.01g. Statistical analysis of each parameter was performed by t-test for dependent samples. The kidney morphometric measurements before and after dehydration revealed the following means: longitudinal length (LL) 9.82cm before and 9.77cm after ($p=0.49$), cranial pole width (CRP) 5.09cm before and 5.08cm after ($p=0.86$), caudal pole width (CDP) 4.63cm before and 4.61cm after ($p=0.66$) and the weight (W) 90.99g before and 84.01g after ($p<0.00$). The results showed that the dehydration process influences tissue shrinkage but with no significant differences ($p>0.05$) except for the weight where there was a significant decrease of kidney weight ($p<0.05$). The results obtained after silicone impregnation reveal the following means: length (LL) 9.45cm, cranial pole width (CRP) 4.75cm, caudal pole width (CDP) 4.36cm and weight (W) 98.55g. Compared with measurements made of kidneys before and after dehydration, these results clearly indicate significant differences ($p<0.05$) in all parameters in the period of impregnation. During our research, the shrinkage of kidneys was apparent and a significant change was recorded in the stage of impregnation. The level of shrinkage that occurred suggests that the most precise macroscopic morphological investigations of anatomical specimens and structures should not be carried out on plastinated specimens. The specimens obtained provided an excellent tool for demonstration of kidney morphology and can be used as a teaching model because the kidneys displayed anatomical details suitable for morphological studies.

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The Journal of the International Society for Plastination

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Submissions and all correspondence should be addressed to the editor.

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