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Letter from the Editor

Dear readers,

The next volume of The Journal of the International Society for Plastination to be published, volume 22, will contain invited review articles covering all aspects of plastination. It is intended to be a plastination reference manual with all of the procedures described in one volume including current information on the advancements in the field. Subjects covered will range from specimen preparation to new methods for curing polyester. It will undoubtedly be the premier plastination publication for those new to plastination as well as investigators familiar with plastination since its inception.

In my last letter, I asked for nominations to the journal's editorial board. I have not yet received any nominations. If you would like to be a member of the editorial board, please contact me at rbreed@utk.edu. If you know of someone you believe would make a good reviewer, please nominate them and we will contact them.

Yours sincerely,
Dr. Robert Brackin Reed, Jr.

Letter from the President

Dear fellow plastinators,

On behalf of the International Society for Plastination - I would like to thank you, who responded to our invitation to participate in the 13th International Conference of Plastination in Vienna, Austria 2006. Especially, I would like to thank all of you, who actively contributed to the success of this conference by giving oral and/or poster presentations. The Center of Anatomy and Cell Biology of the Medical University of Vienna is proud to have hosted this scientific meeting of such a high level and of international prestige. Platform and poster presentations dealing with new applications of plastination techniques both in teaching and in research, demonstrate that plastination is going beyond the borders of medical schools, addressing an increasingly wider community.

ISP members voted for the upcoming meetings and as a result we will hold an Interim Meeting 2007 in Ann Arbor, Michigan, USA, hosted by Dr. Ameed Raoof. The next ISP Biennial Meeting, 2008, will be hosted by Dr. Gunther von Hagens in Heidelberg, Germany, Europe.

I would like also to welcome all new members of the ISP. You did an important step by applying for membership in the society. You will not only get price reductions on conference, meeting and workshop fees, you will also get the ISP journal and 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 on line 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 in 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

The 13th International Conference on Plastination

The majestic, imperial city of Vienna was the venue for the 13th ISP congress. Hosted by the Faculty of Medicine, Anatomisches Institut, Medical University of Vienna, the grandeur of the city's past and present was alive during the 13th International Congress on Plastination. Sixty participants from twenty countries enjoyed the presentations and posters and between session exchange. Constantin and his excellent colleagues made us feel like royalty which was appropriate for the venue. The bountiful array of typically Austrian cuisine and "Heuriger - new wine" at the restaurant Bachhengl was a festive end to the second day of great fellowship and scientific exchange in the near by wine growing area of Grinzing. The scientific exchange ended with a sumptuous Gala Dinner at the Strudelhof. Our travel through the Burgenland with visits to Eisenstadt, Rust (The Iron City) and the Neusiedler See raised ones imaginations of the glory, in by gone days, of the dynamic Austro-Hungarian Empire and the present beauty of the area. Thank you to the Medical University Anatomy Faculty.

The 9th Interim Congress on Plastination
July 8 - 13, 2007
University of Michigan Medical School, Ann Arbor, Michigan

Workshops: July 8 - 12

- Silicone Plastination
- Polyester Plastination
- Anatomical Donation Program
- Painting Neurovascular Pathways
- Corrosion Casting

Interim Meeting: July 10 - 13

- Platform & Poster Presentations
- Gala Banquet and Henry Ford Museum Tour

Early registration - April 27, 2007
Final registration - June 30, 2007
Deadline for abstracts submission - May 15, 2007

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& Biennial Meeting of the International Society for Plastination

in Heidelberg & Guben, Germany
with sojourns in Dresden
July 20 - 26, 2008

The 14th International Conference on Plastination, a six day immersion in the evolving and advancing science of plastination, will take place this summer in Germany.

The Conference aims to create best practice standards in the field of plastination by featuring authoritative lectures by the world's leading plastination practitioners, insightful demonstrations and open meetings, and plenary and peer debates.

The conference this year will convene in Heidelberg, the birthplace of plastination, and will be hosted by the Institute for Plastination and sponsored by Biodur-pioneers in the science of plastination.

Part 1 - July 20-23 Plastination Symposium in Heidelberg
Lectures, audio visual presentations and plenary peer reviews and discussions of current work and experiments in plastination, a tour of the Institute for Plastination facilities hosted by Gunther von Hagens and sightseeing tour of Heidelberg.

Part 2 - July 24-26 Demonstration & Sightseeing, in Dresden and Guben
July 24 Depart from Heidelberg to Dresden
Dresden: rest of the day - visit of the Museum of Hygiene "Deutsches Hygiene-Museum" (http://www.dhmd.de) or Frauenkirche (famous church), overnight stay.

July 25 Depart from Dresden to Guben,
Visit the PLASTINARIUM for anatomical exhibition, demonstration of plastination techniques, and discussions of new applications in plastination; departure to Berlin, overnight stay in Berlin.

July 26 Depart for home from Berlin.

For more details on the conference,
please visit www.plastination2008.com (online in June 2007)
or e-mail: conference2008@plastination.com.

Conference Organizer:
Institute for Plastination, Rathausstrasse 11, 69126 Heidelberg, Germany
Tel. 49 6221 331154 - Fax 49 6221 331124

A New Polyester Technique for Sheet Plastination

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Abstract: A cape dolphin cadaver was used to evaluate a new polyester sheet plastination technique in this experiment. The dolphin was divided into two regions, the head and the body. After freezing the tissue at -7°C, the head was cut into forty-three 3.0mm thick sagittal slices with a high-speed band saw. The body of the dolphin was cut into 348 transverse slices of the same thickness. All slices were fixed using 10% formaldehyde and bleached using 5% dioxogen. The slices were dehydrated in a cold acetone bath and degreased in room temperature acetone. The slices were impregnated with Hoffen polyester (P45, China) and cast between two glass plates. Tissue slices were cured in a heated water bath instead of UV-light. The finished polyester slices cured properly and exhibited detailed anatomical information.

Key words: **plastination; polyester; sheet; curing; dolphin; Hoffen**

Introduction

The rapid development of imaging technology used for medical examination and diagnosis such as magnetic resonance imaging, computed tomography, and ultrasonography have required an in depth study of sectional anatomy. Sheet plastination of specimens, a technology for tissue preservation, provides material for the study of sectional anatomical structure (von Hagens et al., 1987). This technology has been improved by researchers to produce an improved final product (Weiglein et al., 1993; deBoer-van et al., 1993; Weiglein, 1996; Cook, 1997). In our experiment, a new polyester polymer (Hoffen polyester P45, Dalian Hoffen Bio-technique Co. Ltd, room 301, number 32, Lixian Street, Hi-Tech Zone, Dalian, China) and a new curing method of sheet plastination were applied for the preservation of tissue slices for a cape dolphin (*Delphinus capensis*). The purpose of the present study was to find an easier and more cost effective method for sheet plastination.

Materials and methods

Slicing

A female cape dolphin, approximately two meters in length, was found dead on a beach and was collected by a natural history museum (Fig. 1). The dolphin was frozen at -70°C for two weeks. The dolphin was cut into two sections with a hand saw at a point 55cm from the tip of the beak. In order to facilitate the cutting of the trunk of the specimen along predetermined lines, the specimen was placed in a wooden box, positioned appropriately and embedded in polyurethane foam. The head of the dolphin was cut into forty-three 3.0mm thick sagittal slices with a high-speed band saw. The tissue lost between adjacent slices due to the cutting process was approximately 1.0mm in thickness. The trunk was cut into 348 transverse slices which were also 3.0mm in thickness (Fig. 2). The slices were placed in anatomic order on polyethylene grids with a piece of wire screen between the slice and the grid. The saw dust was removed with a small stream of running water. The

grids with screen and slice were then placed in small stacks and tied with twine to hold each stack as a unit. All units were labeled and placed into square polyethylene containers for fixation and bleaching.

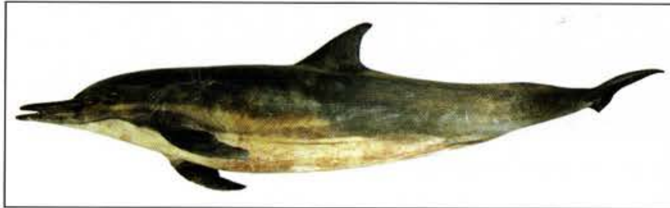

Figure 1. Cape dolphin used for sheet plastination.

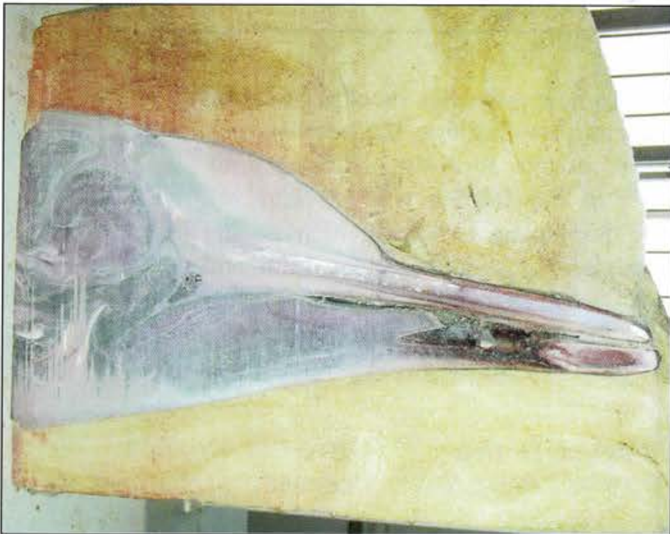

Figure 2. Sagittal section of dolphin head embedded in polyurethane foam.

Fixation and bleaching

The slices were fixed in 10% formaldehyde for two weeks at room temperature. The polyurethane was easily removed from the tissue slices following submersion in the formaldehyde solution. After fixation, the tissue slices were rinsed in cold running water overnight to remove excess formaldehyde. Slices were then immersed in 5% dioxogen overnight to improve tissue color and transparency.

Dehydration

After bleaching, the slices were dehydrated using the freeze substitution method (von Hagens, 1985). The slices were precooled to 5°C to avoid ice crystal formation and to minimize shrinkage upon placement into cold acetone. Tissue slices were placed in the first bath of 100% acetone at -2°C they remained for seven days. Slices were then transferred into a second bath of 100% acetone at -1°C for ten days. Finally, they were put into 100% acetone for degreasing at room temperature for one week. Following final degreasing of the tissue, the slices were finally submerged in fresh 100% acetone at room temperature where they were held for one week awaiting impregnation. The concentration of acetone was monitored with an acetonometer each day during dehydration and degreasing. Once the concentration of acetone remained unchanged for three consecutive days, the slices were moved to the next dehydrating solution.

Casting and forced impregnation

The casting chamber was prepared from two plates of 5.0mm tempered glass, flexible 4.0mm latex tubing, and several large fold back clamps. This has classically been referred to as a flat chamber (Weber and Henry, 1992; Weber and Henry, 1993) (Fig. 3). The slices were removed from the final acetone bath and placed into the chambers. The chambers were filled with P45 polyester resin mixture (Hoffen polyester, China) via a funnel. The components of Hoffen polyester P45 were mixed at 1000 ml of polyester P45 resin to 10g of P45a to 30ml of P45b to 5g of P45c. The P45a and P45c were used as plasticizer and the P45b is the hardener for sheet plastination. The resin reaction-mixture is mixed immediately prior to casting since it thickens over time. Refrigeration of the reaction-mixture will retard this thickening.

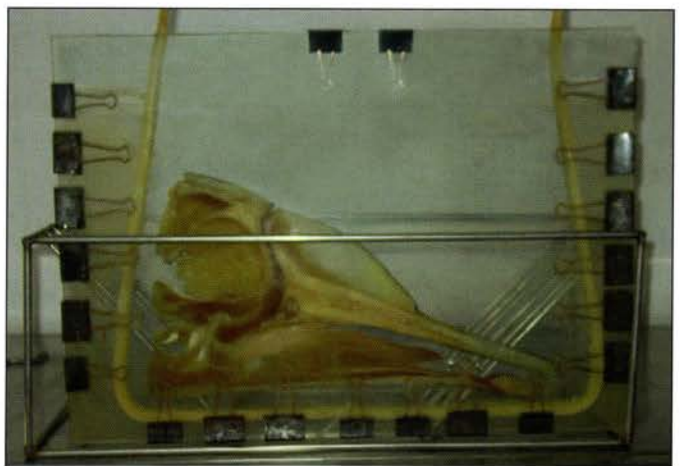

Figure 3. Chamber used for casting with tissue in place.

Once the casting chambers were filled, large air bubbles were removed from the casting chambers manually using a piece of 1.0mm stainless steel wire. The chambers were then placed upright into a vacuum chamber for impregnation at room temperature. The absolute pressure in the vacuum chamber was slowly decreased to 20.0mm Hg, 10.0mm Hg, 5.0mm Hg, and finally to 0.0mm Hg according to the slow release of bubbles from the tissue slices. A pressure of 0.0mm Hg was maintained until all bubbling ceased. The duration of impregnation was slightly greater than eight hours.

Curing

After the vacuum had been released, the casting chambers were rechecked for the presence of any air bubbles which would need to be removed prior to curing. The alignment of the tissue slices was also checked and repositioneded with the steel wire if necessary. Lastly, the latex tubing was closed across the top of the casting chamber and held in place by clamps.

The sheets were cured in a vertical position in a heated water bath at 40°C for 3 days (Fig. 4). A small circulatory pump was used to circulate the water within the bath to equilibrate the temperature of the water at all points within the chamber.

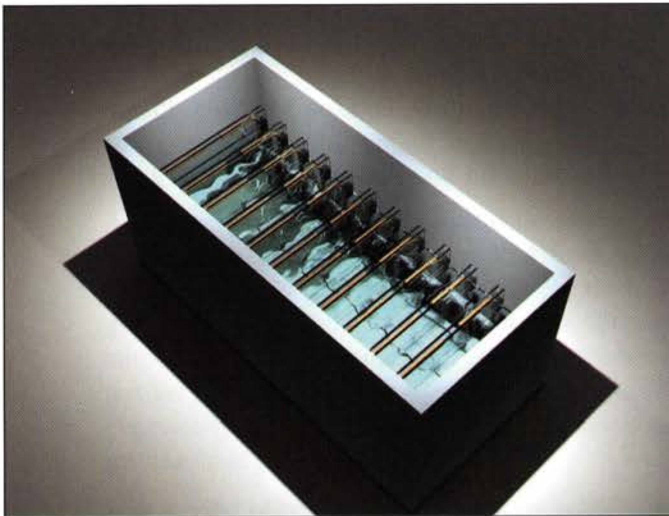

Figure 4. Representation of water bath used for curing.

Cutting and sanding sheets

After curing, the sheets were removed from the bath and cooled to room temperature on a rack. The casting chambers were dismantled and the slices removed and covered with plastic wrap for protection. A band saw was used to cut and trim the polyester along the outside edges of the tissue slices at a distance of approximately 1.0mm from the tissue. A wool sander was then used to remove the sharp edges of the slices. Following sanding, the plastic wrap was removed and the slices were placed into protective plastic bags to avoid scratching. The sheets were then ready for use or storage.

Results

The tissue sections produced using the new polyester polymer and new curing method yielded specimens which exhibit clear delineation between different tissue types (Fig. 5). The sections provide visually clear information about the morphology of dolphin. The polyester sheets and impregnated specimens cured completely resulting in clear, cured tissue slices within a protective polyester sheet.

Figure 5. Sheet plastinated dolphin head.

Discussion

The traditional P35 and P40 plastination techniques are unique means for tissue slice preservation. The P40 technique was designed as an improvement over the P35 technique as the former has a shorter processing time and uses less resin. Specimens produced using either P35 or P40 show excellent distinction between gray and white matter for brain specimens (von Hagens et al., 1987, 1994; Barnett, 2005). With the polyester technique, sections of specimens were more detailed, durable, and easier to handle than previous technique (Weber and Henry, 1992). Furthermore, these slices were also used for interpretation of medical diagnostic images such as MRI and CT scans and offered excellent reference material.

However, the curing process for the P35 and P40 techniques uses UV-light (von Hagens, 1994) which is an added expense. The process requires close monitoring of the exothermic reaction during curing and ventilators are used to keep temperatures from rising too high during curing.

In the present experiment, the sheet plastination of the dolphin was completed with the Hoffen polyester P45 technique. The slices were cured with a water bath instead of UV-light. With this new polyester for sheet plastination and new curing method, the vertical positioning of the casting chambers reduced the space required for curing specimens while the water bath provided equal dispersion of temperature during curing. The water bath keeps temperatures from rising too high as well as keeping temperatures uniform throughout the slices. The water bath also provides a more cost efficient and safer method for each specimen produced when the effects of UV-light production are taken into account.

The benefits provided by the Hoffen polyester P45 technique can effectively circumvent the disadvantages

associated with the P35 and P45 plastination techniques. It is believed that the new Hoffen polyester P45 and the new curing method would facilitate sectional anatomy study and provided a good approach for sheet plastination.

The slices of this dolphin are on public display at Dalian Natural Science Museum, Dalian, China.

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Chemically Reactivated Plastination with Shin-Etsu Silicone KE-108

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Abstract: Since von Hagens developed the plastination technique in 1978, plastination has gained world wide popularity. During this time, new techniques have been developed and an array of experiences has been accumulated. Plastinated specimens are invaluable for teaching and learning gross pathology and anatomy. However, for gross pathology, not only their natural shape but also the authentic color of normal and diseased tissues is desirable. Unfortunately information is scarce and not very helpful on how to prepare such plastinated specimens. In this report, a new technique for chemically reactivated blood color in plastinated specimens using Shin-Etsu Silicone KE-108 and imidazole is presented.

Key words: **plastination; silicone; KE-108; imidazole; color**

Introduction

Since Kaiserling (1896) reported the development of a technique to reactivate wet mount specimens to enhance color, other techniques have been developed and reported. Romhanyi's technique has proven to be one of the best techniques to preserve specimen color (Romhanyi, 1956; Sandhyamani et al., 2005). This technique, based on the hemochromogen reaction, uses pyridine and nicotine as bases and sodium dithionite as a reducing agent to restore a somewhat natural red color to formalin-fixed gross specimens. We modified this technique by adding sodium dithionite and imidazole, which are a reducing agent and a base (Sakamoto et al., 1993), to the mounting medium fluid (water, formalin, glycerin and sodium acetate). Specimens which were recolorized with this modified and improved Romhanyi technique have remained stable and continue to display a natural red color for over 20 years. Using this basic reaction as the key to color restoration, this wet technique was modified for plastination of specimens using only imidazole of the wet mount ingredients.

Shin-Etsu silicone polymer KE-108 combined with CAT-108 (catalyst plus cross-linker) and room temperature vulcanizing (RTV) thinner has been used for over a decade for plastination (Miyake et al., 1990; Sakamoto et al., 1995). KE-108 polymer is a transparent, colorless, two-component RTV silicone. Industrially, KE-108 polymer is used for potting (sealing) electrical/electronic equipment and optical equipment. Its curing mechanism is via condensation polymerization similar to other popular plastination silicones.

Materials and methods

Fixation: Pathological specimens used for this project were fresh tissues fixed in 10 to 20% buffered formalin for one week at room temperature. As well, longer term fixed specimens in varying formalin solutions were

plastinated. In preparation for dehydration, specimens were washed in running tap water for 24 hours and then photocopied to record specimen size. The perimeter of each of the resulting images was traced and the area of each was calculated using the image measuring system (IBAS). After photocopying the specimens, they were precooled to +5°C.

Dehydration: Dehydration of specimens was carried out using the classic freeze substitution method in 100% cold (-25°C) acetone (Tiedemann and Ivic-Matijas, 1988). Specimens were immersed in acetone bathes and at least three weekly changes were performed. After dehydration was complete, more lipid was removed from lipid-rich specimens by placing the specimens in a fresh acetone bath at room temperature for at least one week.

Forced impregnation: A polymer reaction-mixture was prepared by mixing 100 parts Shin-Etsu silicone polymer (KE-108), 2 parts catalyst (CAT-108) and 10 parts RTV thinner, and 2 parts of an imidazole-ethanol mixture (polymer reaction-mixture ratio 100:2:10:2). An appropriate quantity of imidazole-ethanol mixture was prepared in advance using a ratio of 1:3 by weight. Ethanol was decanted and stirred with a stick into the imidazole.

This polymer/catalyst/thinner/imidazole reaction-mixture was poured into a disposable acetone-resistant bag. The dehydrated specimens were submerged in this polymer reaction-mixture inside the bag. The bag was loosely closed and placed in the vacuum chamber in a -25°C freezer. A small weight was placed on top of the bag to keep specimens submerged in the polymer and the chamber was closed.

Two defined stages of impregnation were used. Stage one was carried out at -25°C. The vacuum pump was turned on and pumped at full vacuum (no throttling via an intake valve) and pressure was lowered to approximately 1mm Hg by the end of day one. At the end of the day, the pump was turned off and pressure was returned to ambience. Two acetone traps placed in line between the vacuum chamber and the vacuum pump condensed and collected the vaporized acetone. Traps were emptied as needed. Stage one of impregnation varied from one week for thin specimens to one month for large specimens and was judged complete when no acetone was collected in the traps. In stage two, the vacuum chamber and its contents were brought out to room temperature during the day. As in stage one, the pump was turned on in the morning and the pressure lowered to approximately 1mm Hg. At the end of the day, the pump was switched off and the chamber and its contents were placed back in the freezer over night. This routine was repeated for usually 4 days or until no acetone was collected in the traps. Stage two time varied from 1 day for thin specimens to 4 days for large thick specimens (liver and brain).

Curing: After forced impregnation, the specimens were removed from the vacuum chamber and polymer reaction-mixture and allowed to drain at room temperature. Daily, specimens were gently wiped of excess polymer and adjusted to a desired form. Curing took two to three days at room temperature. If any areas of the specimen remained sticky, CAT-108 was applied to the area to finish curing. The remaining polymer reaction-mixture usually was not reused but allowed to cure inside the bag at room temperature and discarded.

After the specimens had been cured, they were photocopied to record size. The perimeter of each of the resulting images was traced and the area of each was calculated using the image measuring system (IBAS). After photocopying, the specimens were used many different ways: Exhibition in room atmosphere or in display cases in the museum, Used for demonstration during lecture in the lecture hall, or placed in plastic bags for storage.

Results

After plastination, the specimens retained their original shape and some flexibility. Their grayish formalin color appeared authentically red. Areas of hemorrhage and erythema were visible (Figs. 1, 2, 3, 4). Hemorrhagic edema of the lungs was demonstrable (Figs. 5, 6). Red colored erythrocytes were visible in vessels (Fig. 7). Comparison and analysis of images of post-fixed specimens with images of specimens after curing showed shrinkage of most specimens ranged from 2 to 5%. Whole brain shrinkage was less than 10%. After many years of exposure to the atmosphere, some specimens lost surface color (Fig. 8). However, the reactivated color was maintained inside the specimen.

Discussion

Plastinated specimens have become a great asset in teaching pathology and anatomy (von Hagens et al., 1987; Bickley et al., 1981; Nicaise et al., 1990; Hirokawa et al., 1994; Latorre et al., 2001; Alpár et al., 2005). Modifications of the original plastination equipment and process have been varied (von Hagens, 1979; Miyake et al., 1990; O'Sullivan and Mitchell, 1995; Smodlaka et al., 2005). However, loss of color is a distraction from the effectiveness and aesthetics of plastinated specimens (Alpár et al., 2005). Surface stains have been used with minimal success (Henry et al., 1997). This process demonstrated a method for restoration of specimen color during the plastination process.

During forced impregnation, a hemochromogen reaction occurs from the presence of imidazole within the specimens. It reacted with the hemoglobin to change

Figure 1. Visceral surface of formalin fixed/ stored liver with multiple tumors.

Figure 2. Visceral surface of liver shown in figure 1 after reactive plastination. Note the more natural appearance of the organ and the hemorrhagic area of the left lobe.

Figure 3. Opened lumen of formalin fixed/stored colon with psuedomembranous colitis.

Figure 4. Opened lumen of colon with psuedomembranous colitis shown in figure 3 after reactive plastination. The hemorrhagic colitis is demonstrated.

Figure 5. Anterior view of formalin fixed/stored lungs with hemorrhagic edema.

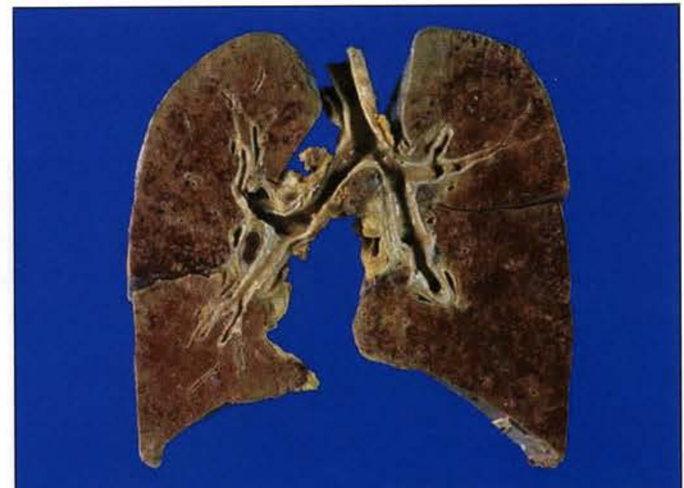

Figure 6. Anterior view of formalin fixed/stored lungs with hemorrhagic edema shown in figure 5 after reactive plastination. Observe the nearly uniform hemorrhagic edematous nature of the lungs.

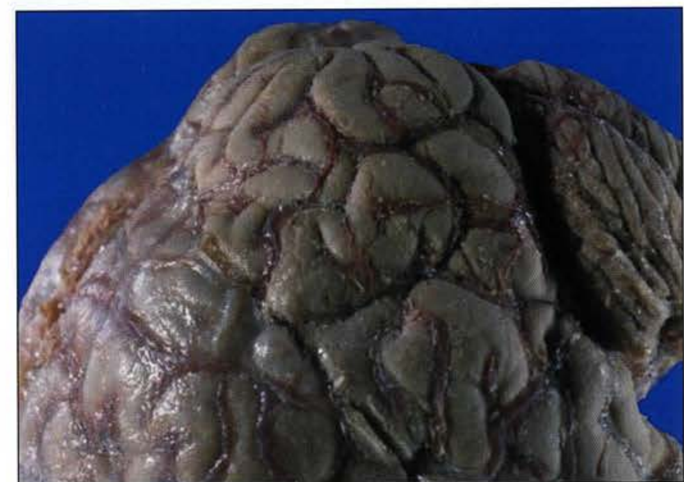

Figure 7. Left lateral view of brain after reactive plastination demonstrating red blood in the vessels.

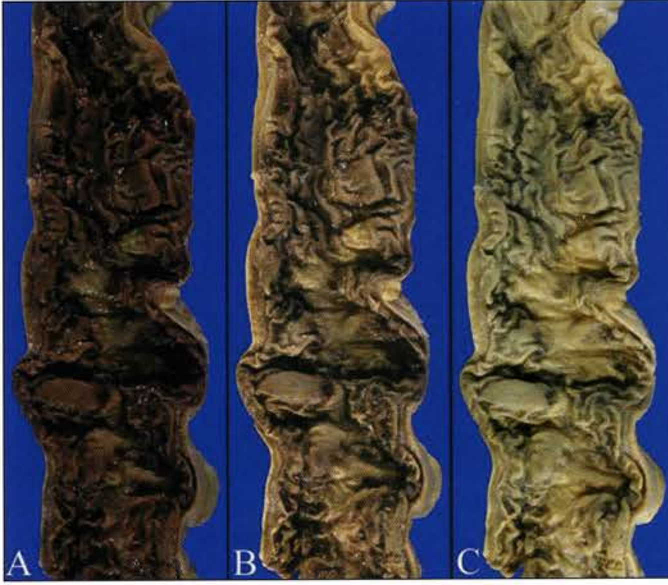

Figure 8. Pictoral history of a reactivated specimen: Chemically reactivated-plastinated, 1996 (A); demonstrating color loss after continual room environment display, 1998 (B); near complete color loss with continued display, 2006 (C).

Figure 9. Section of specimen shown in figure 8c demonstrating sub-surface color retention.

the dark color due to oxidation to a reactivated more normal red. This color is similar to the color obtained in restored wet specimens. Thus, specimen color appeared authentic. Unfortunately, this active red color on the surface, over time, will revert back to the dark color if plastinated specimens are kept in the atmosphere. On the surface of the plastinated organ, the ferrohemochrome/hemochromogen (bright red color) may be easily oxidized and revert to ferrihemochrome/parahematin (burnt umber) when exposed to air. However internally, the reactivated color remains. This reverting back to the fixed color can be slowed dramatically by exhibiting the plastinated specimens in display cases or storage in bags, when not in use. Using these precautions, has kept the renewed red color in plastinated specimens more than three years. Research on this subject should be continued to find a method to preserve and/or restore the surface activated blood color longer term. If a more suitable reducing agent can be identified, the resulting color could likely be improved to be even more life-like.

Other silicones have not been used for this process. However, we believe other silicones could be used. It is possible that specimens preserved by specialized preservation solutions other than formalin alone might interfere with this reactivation process. Plastination with chemical reactivation offers advantages over both routine plastinated and wet mount specimens. There is no odor or oozing and the specimens may be handled without gloves. In addition, the specimen is preserved in a more natural color tone. This methodology results in excellent specimens for teaching and exhibition.

Plastination of color reactivated wet mounted specimens does not work for reactivation of the color. When the specimen is removed from a display container and reintroduced to air to flush away chemicals and to dehydrate, the reactivated color is lost. Once the reactivated color is lost (oxidized), it is difficult to reactivate the color again using current techniques. Also the presence of glycerin, sodium dithionite and sodium acetate in the wet mounted specimens would likely leave residue on the surface of the plastinated specimens as previously reported, unless they undergo ethanol bathes and flushing (von Hagens, 1985).

Importing the Biodur silicone polymer series, to determine if plastination of our museum specimens would be feasible, was expensive and needed a lot of time and effort to obtain such. Therefore, we researched locally available Japanese silicones to see if one might meet the conditions necessary for plastination: Room temperature vulcanizing (RTV) type silicone, Colorless and transparent, Specific gravity similar to water, Room temperature cure, Flexibility after curing, and No known curing inhibitor. KE-108 met these criteria and was chosen to use for plastination of the museum specimens. CAT-108 (catalyst) was the standard curing agent for this polymer. Its condensation type curing of RTV silicone has no known curing inhibitors, such as water, organic solvents, organic metal salts, sulfur, phosphorus, nitrogen compounds, etc.

The recommended mixture of catalyst (catalyst and hardener) with the KE-108 polymer is 5:100. To ensure

adequate impregnation time, the rate of cure needed to be prolonged. Therefore, only 2 parts of catalyst were used. To decrease the polymer reaction-mixture viscosity, 10 parts of RTV thinner was added to the 100 parts of the KE108 polymer. This decreases polymer viscosity from 700 centipoise to around 600 cps. RTV thinner is nonvolatile and was used to dilute the KE-108 polymer. Polymer dilution enhances the influx of polymer into the specimen and may extend the workable time of the polymer reaction-mixture. The workable time for this reaction-mixture, which contains both catalyst and cross-linker, is over three months when kept at -25°C. However at room temperature, pot-life is decreased to a few days. Impregnating specimens in a bag within the vacuum chamber served several purposes: Kept specimens submerged, Reduced the amount of reaction-mixture needed for each load, Served as liner for the vacuum chamber, Prevented aspiration of exploding silicone bubbles into the vacuum line and pump which can clog them when it hardens, Ease of removing specimens and polymer reaction-mixture from the vacuum chamber, and Ease of disposal of thick silicone.

It was necessary to add imidazole to the polymer-mixture to reactivate the natural color of the blood. Imidazole is not soluble in silicone. Therefore, an ethanol-saturated mixture was prepared and used in the polymer reaction-mixture. Imidazole remained suspended in the reaction-mixture. Shin-Etsu silicone polymer KE-108 mixed with CAT-108 is a useful plastination mixture to impregnate specimens. Measured shrinkage of 2 - 10% was less than the previously reported 8 - 29% by Brown and colleagues (2002). Part of this discrepency is likely due to the difference of units used: Brown's group used volume as opposed to area in this project. Imidazole is the key to preparing dry specimens with natural coloring. The exact minimal amount of imidazole to specimen volume has not been determined. However, at the determined level (0.5 pts imidazole/100 pts KE-108 polymer) it is likely to be an adequate amount for impregnation of more than one load of specimens. We chose not to reuse the mixture because of the increased viscosity of the polymer reaction-mixture after one load of specimens had been impregnated. Much higher concentrations of imidazole will likely yield a much darker unnatural red and is much more expensive.

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Design of a Silicone Gasket with an Iron Core for Polyester and Epoxy Sheet Plastination

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Abstract: Plastination is a unique method for permanent preservation of biological tissues. Sheet plastination has become popular for the production of sectional aids for studying sectional anatomy. In sheet plastination, the slices of organs and whole bodies are cured between glass plates with a gasket placed between the glass. This uniquely designed silicone gasket has a central iron core which is covered with silicone (1.5mm). The sheets produced using this type gasket have smooth, squared edges and are rectangular in shape. The advantages of using this type gasket are ease of casting of specimens, especially those which have several pieces; ease of positioning of specimens because there is no glass in the way; and ease of reaching and removal of bubbles.

Key words: **ring silicone gasket, iron core, plastination laboratory**

Introduction

Plastination is a unique method of permanently preserving tissue in a dry state. In this process, tissue fluid and lipids are replaced with a curable polymer which is subsequently hardened, resulting in dry, odorless and durable specimens. (von Hagens, 1979; von Hagens, 1985; Weiglein, 1997).

The basic steps for preparing either entire bodies or portions thereof with silicone or thin slices using epoxy or polyester are similar (von Hagens et al., 1987). Sheet plastinated specimens have a high acceptance and are frequently used for studying sectional anatomical detail often in conjunction with imaging modalities (Henry et al, 1997; Latorre et al. 2001).

The sheet plastination technique is a mechanism for preservation of thin (2-8mm) slices of organs or whole bodies. In this technique after specimen preparation, dehydration and forced impregnation, the slices are cured between tempered glass plates (Weber and Henry, 1992) with a gasket placed between the glass or the slices can be stacked between heavy sheets of polyester foil (von Hagens et al., 1987; Weiglein, 1997).

Gaskets, used in plastination laboratories, are flexible (Weber and Henry, 1992). Such gaskets are round and do not conform to a rectangular shape. Consequently the produced specimen sheets do not have squared edges but rather the edges are irregular and concave. Therefore, the sheets need to be cut to the desired shape and polished in order to have a smooth, regular, geometrical configuration (Fig. 1).

The purpose of this study is to present a design for a rigid rectangular silicone gasket. This gasket is placed near the perimeter of the bottom glass of the flat chamber. A thin layer of epoxy or polyester resin is placed in the flat chamber and allowed to cure. After this layer of resin has cured, the specimen is positioned on the cured resin and the chamber is filled with resin covering the specimen while in a horizontal position. Bubbles are allowed to rise and burst and then the top glass is put into place. Fold-back clamps are positioned around the perimeter over the glass and gasket to seal the glass to the gasket.

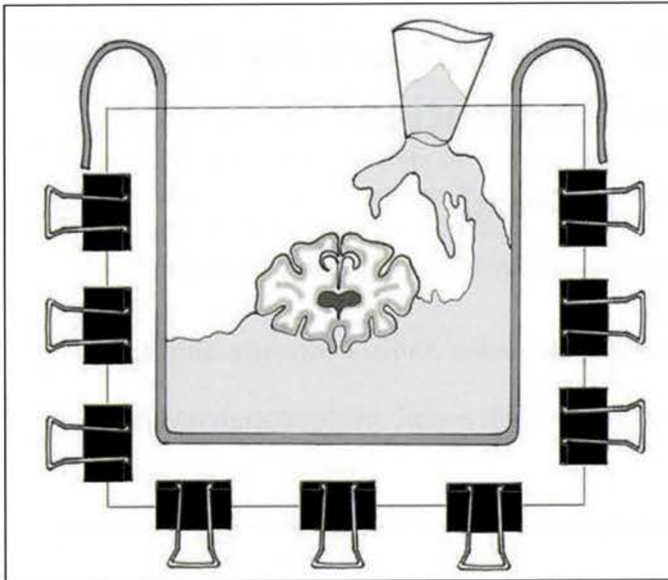

Figure 1. Gasket for a typical flat chamber for epoxy or polyester plastination.

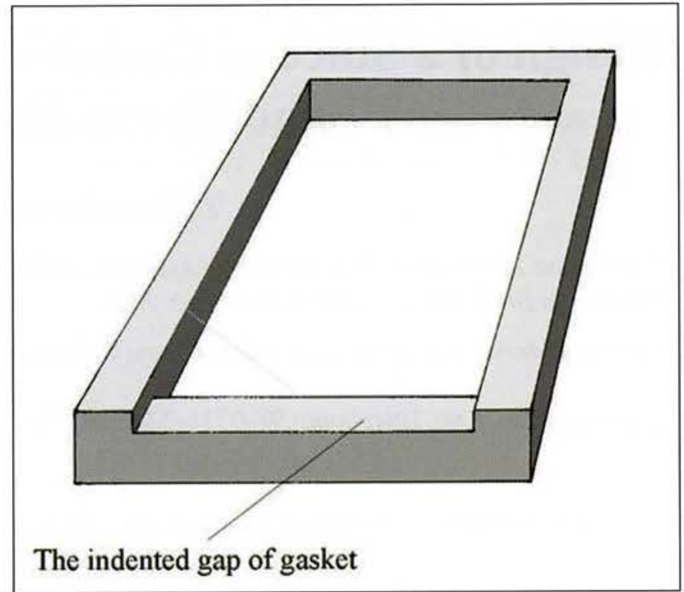

Figure 2. Gasket constructed with a gap for access to the slice inside the flat chamber.

Materials and methods

Rectangular iron frames of desired sizes, to serve as gasket cores, were made from four pieces of iron by welding them together. The dimensions of these pieces of iron were 6.0mm thick by 10.0mm wide. To provide a 3.0mm gap on one side of one end of the gasket, the thickness of that one iron piece was only 3.0mm.

Dimensions of the frames were designed to fit the dimensions of the various sizes of glass plates chosen to accommodate a variety of specimen sizes. The iron frame serves as the core of the silicone gasket.

In preparation to construct a mold to encapsulate the iron core with silicone, 2.0cm wide strips of 3.0 to 6.0mm thick glass were cut. For the outer mold wall, the four strips were cut to approximate moat length for the two sides and two ends of intended size glass plates and to allow for a 1.5mm gap on the outer surface of the iron frame. For the inner mold wall, the four strips were cut approximately 13.0mm shorter. The strips were glued onto a glass plate using *Allplast* adhesive to form the gasket mold. The internal dimensions of the constructed mold were 13.0mm wide x 20.0mm high x the appropriate length to accommodate the iron core. To create the slot in one side of one end of the gasket, a 13.0mm wide strip of 3mm thick glass was cut to the appropriate length and glued in to fill the bottom of the moat (Fig. 2).

The internal surface of the cast was coated with a thin layer of polyester wax (separator used in the fiberglass industry) for easy separation of the gasket from the mold. Four small pieces of 2mm thick silicone were placed equidistantly on the moat floor as spacers under the iron core to create a space for the silicone to coat the under side of the core. The iron core was placed in the center of the moat leaving a gap of 1.5mm on each side (Fig. 3). The width of the moat will be the dimension of the intended gasket.

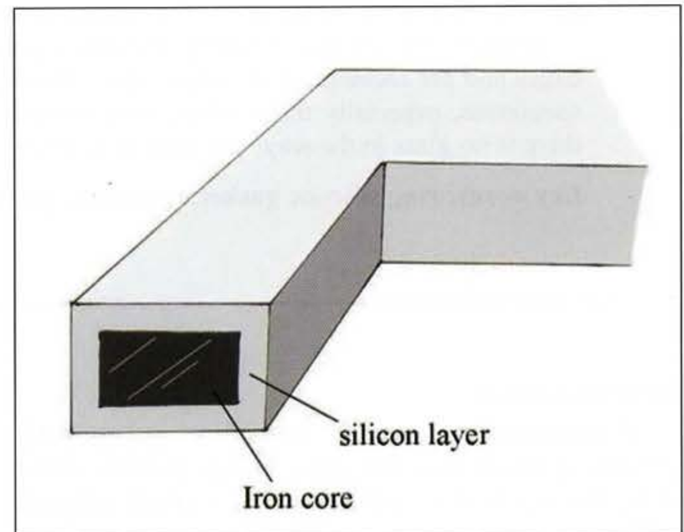

Figure 3. Cut away of gasket with an iron core demonstrating its smooth parallel surfaces.

An appropriate volume of silicone RTV2 (Rhodia Silicones, Lyon, France) was mixed and poured into the moat and allowed to surround the iron core and fill the mold 5.0 to 10.0mm from the top. After curing for twenty-four hours, the gasket was separated from the glass mold (Fig. 4).

Results

Semi-rigid gaskets were constructed which allowed for ease of specimen manipulation and air bubble

Figure 4. The increased weight, flexibility and smoothness of the silicon gasket prevents the formation of micropores between the gasket and glass.

removal with a wire and filling with the resin. As well, there was no leakage of resin between the gasket and the bottom glass plate of the flat chamber while lying in a horizontal orientation with the top glass removed and no clamps utilized (Fig. 5).

As well, the chamber may be held vertically (slotted end up) to add resin to the flat chamber, to manipulate trapped bubbles with a wire thus encouraging them to rise and to manipulate or adjust the slice after clamping the three sides to create a seal (Figs. 2, 6). The hardened slices have smooth, parallel sides with ninety degree corners (Figs. 6, 7).

Discussion

Gaskets routinely used in plastination laboratories for sheet plastination are very flexible and do not create a desired geometrical shape. Thus, the produced sheets have irregular concave edges which are often not parallel. In order to have an aesthetic, smooth and rectangular shape, the sheets need to be cut with a saw and be sanded.

The iron core gasket, with its smooth parallel sides, eliminates the need to trim the edges of the hardened slice. As well, the iron core, encapsulated in silicone, increases gasket firmness and weight which aids its ability to easily seal. This not only keeps the gasket from deformation but also makes the gasket heavy enough to adhere to the glass and to allow no leakage of resin between the gasket and lower glass when the top glass and clamps are not in place. This is possible because the silicone encapsulating the steel core is soft and smooth enough to form a seal with the glass without the clamps being applied when lying in a horizontal position. Therefore, the specimen can be placed in the open horizontal flat chamber and manipulated for proper positioning. As well, the chamber can be filled with resin allowing trapped bubble to be freed easily and removed.

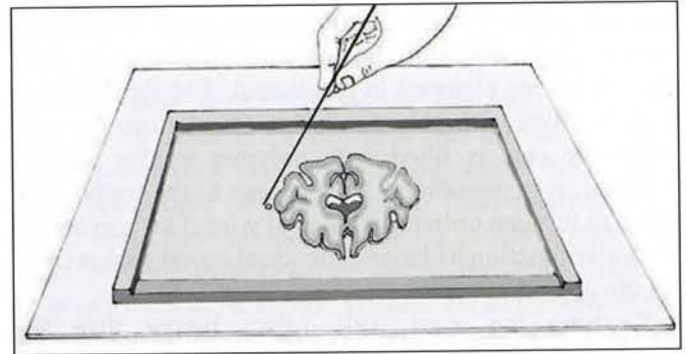

Figure 5. Demonstration of the possibility for slice positioning, air bubble removal and filling the open flat chamber with polymer (top glass removed).

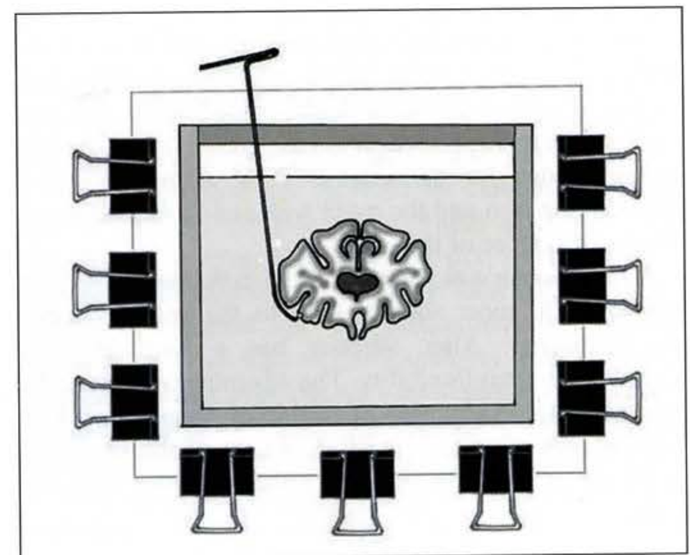

Figure 6. Demonstration of slice adjustment via the gasket gap on one side.

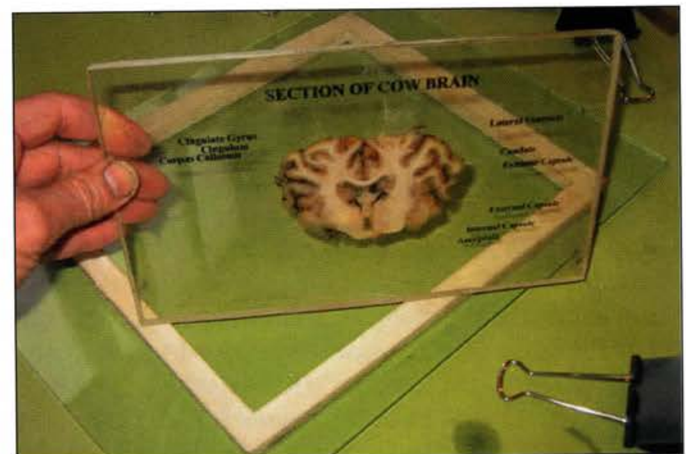

Figure 7. The finished, rectangular sheet with smooth, squared edges.

Slices which have several pieces or have been fractured and need special positioning while casting, may be adjusted and fitted together in proper position

because of their accessibility. The 3.0mm slot in the gasket allows access to the specimen after the top glass plate has been clamped in positioned. The flat chamber may be filled with polymer in this orientation. As well, when the cast is filled with polymer in this vertical position, it is possible to have access to the tissue slice through this gap utilizing a piece of wire. This gap serves a similar function as having the usual round gasket open on the up end.

Creating the moat wall 1.0cm higher than the anticipated gasket thickness allows ample space for the viscous silicone to pool until it flows around and under the iron for optimum coating of the iron core. It also serves as a reservoir for bubbles which rise to the top and eventually burst.

The wax coating provided ease of separation of the gasket from the internal surface of the mold. The four pieces of silicone, 2.0mm thick, placed under the iron core were incorporated into the gasket and created enough space for the viscous RTV silicone to flow between the iron and the mold wall and completely coat the down surface of the iron core.

RTV silicone was chosen for this gasket because of its resistance to most solvents and to the heat produced during curing. Also, silicone has a long life and maintains it great flexibility. The advantages of using the iron core gasket are: ease of casting of specimens which have several pieces, ease of alignment of the specimen and ease of reaching and removal of bubbles from the resin.

Acknowledgments

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plastinated. In preparation for dehydration, specimens were washed in running tap water for 24 hours and then photocopied to record specimen size. The perimeter of each of the resulting images was traced and the area of each was calculated using the image measuring system (IBAS). After photocopying the specimens, they were precooled to +5°C.

Dehydration: Dehydration of specimens was carried out using the classic freeze substitution method in 100% cold (-25°C) acetone (Tiedemann and Ivic-Matijas, 1988). Specimens were immersed in acetone bathes and at least three weekly changes were performed. After dehydration was complete, more lipid was removed from lipid-rich specimens by placing the specimens in a fresh acetone bath at room temperature for at least one week.

Forced impregnation: A polymer reaction-mixture was prepared by mixing 100 parts Shin-Etsu silicone polymer (KE-108), 2 parts catalyst (CAT-108) and 10 parts RTV thinner, and 2 parts of an imidazole-ethanol mixture (polymer reaction-mixture ratio 100:2:10:2). An appropriate quantity of imidazole-ethanol mixture was prepared in advance using a ratio of 1:3 by weight. Ethanol was decanted and stirred with a stick into the imidazole.

This polymer/catalyst/thinner/imidazole reaction-mixture was poured into a disposable acetone-resistant bag. The dehydrated specimens were submerged in this polymer reaction-mixture inside the bag. The bag was loosely closed and placed in the vacuum chamber in a -25°C freezer. A small weight was placed on top of the bag to keep specimens submerged in the polymer and the chamber was closed.

Two defined stages of impregnation were used. Stage one was carried out at -25°C. The vacuum pump was turned on and pumped at full vacuum (no throttling via an intake valve) and pressure was lowered to approximately 1mm Hg by the end of day one. At the end of the day, the pump was turned off and pressure was returned to ambience. Two acetone traps placed in line between the vacuum chamber and the vacuum pump condensed and collected the vaporized acetone. Traps were emptied as needed. Stage one of impregnation varied from one week for thin specimens to one month for large specimens and was judged complete when no acetone was collected in the traps. In stage two, the vacuum chamber and its contents were brought out to room temperature during the day. As in stage one, the pump was turned on in the morning and the pressure lowered to approximately 1mm Hg. At the end of the day, the pump was switched off and the chamber and its contents were placed back in the freezer over night. This routine was repeated for usually 4 days or until no acetone was collected in the traps. Stage two time varied from 1 day for thin specimens to 4 days for large thick specimens (liver and brain).

Curing: After forced impregnation, the specimens were removed from the vacuum chamber and polymer reaction-mixture and allowed to drain at room temperature. Daily, specimens were gently wiped of excess polymer and adjusted to a desired form. Curing took two to three days at room temperature. If any areas of the specimen remained sticky, CAT-108 was applied to the area to finish curing. The remaining polymer reaction-mixture usually was not reused but allowed to cure inside the bag at room temperature and discarded.

After the specimens had been cured, they were photocopied to record size. The perimeter of each of the resulting images was traced and the area of each was calculated using the image measuring system (IBAS). After photocopying, the specimens were used many different ways: Exhibition in room atmosphere or in display cases in the museum, Used for demonstration during lecture in the lecture hall, or placed in plastic bags for storage.

Results

After plastination, the specimens retained their original shape and some flexibility. Their grayish formalin color appeared authentically red. Areas of hemorrhage and erythema were visible (Figs. 1, 2, 3, 4). Hemorrhagic edema of the lungs was demonstrable (Figs. 5, 6). Red colored erythrocytes were visible in vessels (Fig. 7). Comparison and analysis of images of post-fixed specimens with images of specimens after curing showed shrinkage of most specimens ranged from 2 to 5%. Whole brain shrinkage was less than 10%. After many years of exposure to the atmosphere, some specimens lost surface color (Fig. 8). However, the reactivated color was maintained inside the specimen.

Discussion

Plastinated specimens have become a great asset in teaching pathology and anatomy (von Hagens et al., 1987; Bickley et al., 1981; Nicaise et al., 1990; Hirokawa et al., 1994; Latorre et al., 2001; Alpár et al., 2005). Modifications of the original plastination equipment and process have been varied (von Hagens, 1979; Miyake et al., 1990; O'Sullivan and Mitchell, 1995; Smodlaka et al., 2005). However, loss of color is a distraction from the effectiveness and aesthetics of plastinated specimens (Alpár et al., 2005). Surface stains have been used with minimal success (Henry et al., 1997). This process demonstrated a method for restoration of specimen color during the plastination process.

During forced impregnation, a hemochromogen reaction occurs from the presence of imidazole within the specimens. It reacted with the hemoglobin to change

Figure 1. Visceral surface of formalin fixed/ stored liver with multiple tumors.

Figure 2. Visceral surface of liver shown in figure 1 after reactive plastination. Note the more natural appearance of the organ and the hemorrhagic area of the left lobe.

Figure 3. Opened lumen of formalin fixed/stored colon with psuedomembranous colitis.

Figure 4. Opened lumen of colon with psuedomembranous colitis shown in figure 3 after reactive plastination. The hemorrhagic colitis is demonstrated.

Figure 5. Anterior view of formalin fixed/stored lungs with hemorrhagic edema.

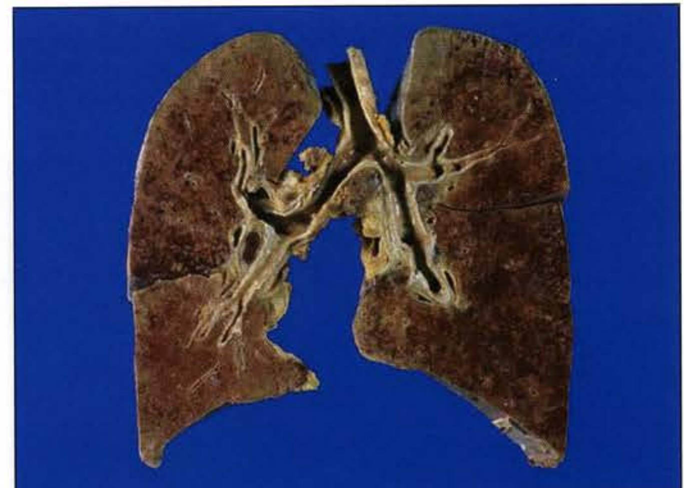

Figure 6. Anterior view of formalin fixed/stored lungs with hemorrhagic edema shown in figure 5 after reactive plastination. Observe the nearly uniform hemorrhagic edematous nature of the lungs.

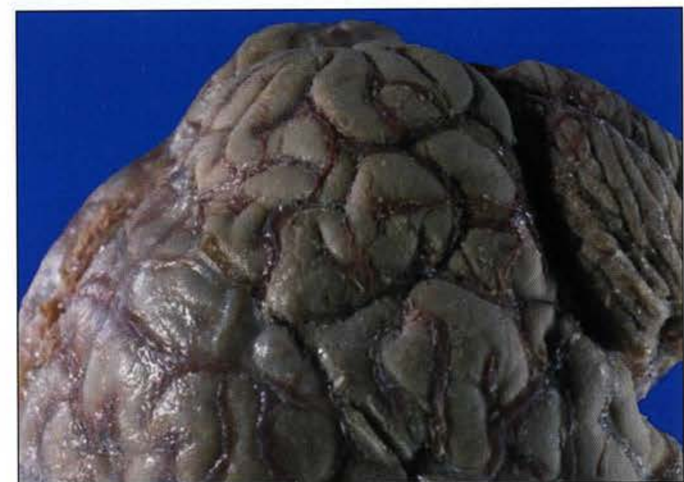

Figure 7. Left lateral view of brain after reactive plastination demonstrating red blood in the vessels.

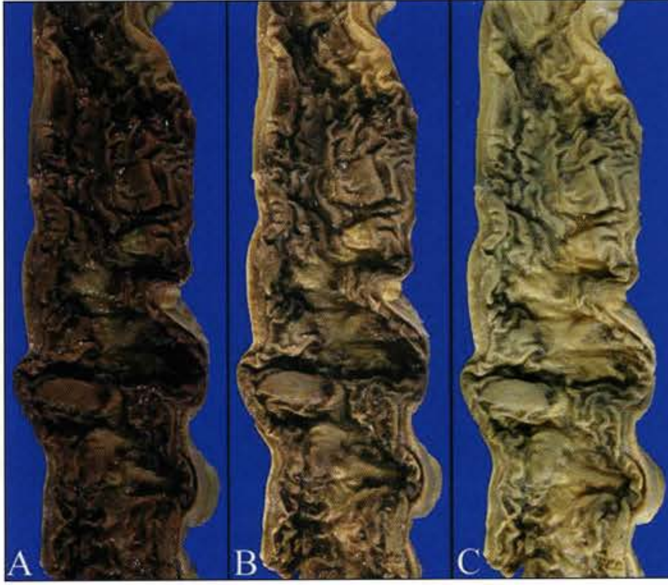

Figure 8. Pictoral history of a reactivated specimen: Chemically reactivated-plastinated, 1996 (A); demonstrating color loss after continual room environment display, 1998 (B); near complete color loss with continued display, 2006 (C).

Figure 9. Section of specimen shown in figure 8c demonstrating sub-surface color retention.

the dark color due to oxidation to a reactivated more normal red. This color is similar to the color obtained in restored wet specimens. Thus, specimen color appeared authentic. Unfortunately, this active red color on the surface, over time, will revert back to the dark color if plastinated specimens are kept in the atmosphere. On the surface of the plastinated organ, the ferrohemochrome/hemochromogen (bright red color) may be easily oxidized and revert to ferrihemochrome/ parahematin (burnt umber) when exposed to air. However internally, the reactivated color remains. This reverting back to the fixed color can be slowed dramatically by exhibiting the plastinated specimens in display cases or storage in bags, when not in use. Using these precautions, has kept the renewed red color in plastinated specimens more than three years. Research on this subject should be continued to find a method to preserve and/or restore the surface activated blood color longer term. If a more suitable reducing agent can be identified, the resulting color could likely be improved to be even more life-like.

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The recommended mixture of catalyst (catalyst and hardener) with the KE-108 polymer is 5:100. To ensure

adequate impregnation time, the rate of cure needed to be prolonged. Therefore, only 2 parts of catalyst were used. To decrease the polymer reaction-mixture viscosity, 10 parts of RTV thinner was added to the 100 parts of the KE108 polymer. This decreases polymer viscosity from 700 centipoise to around 600 cps. RTV thinner is nonvolatile and was used to dilute the KE-108 polymer. Polymer dilution enhances the influx of polymer into the specimen and may extend the workable time of the polymer reaction-mixture. The workable time for this reaction-mixture, which contains both catalyst and cross-linker, is over three months when kept at -25°C. However at room temperature, pot-life is decreased to a few days. Impregnating specimens in a bag within the vacuum chamber served several purposes: Kept specimens submerged, Reduced the amount of reaction-mixture needed for each load, Served as liner for the vacuum chamber, Prevented aspiration of exploding silicone bubbles into the vacuum line and pump which can clog them when it hardens, Ease of removing specimens and polymer reaction-mixture from the vacuum chamber, and Ease of disposal of thick silicone.

It was necessary to add imidazole to the polymer-mixture to reactivate the natural color of the blood. Imidazole is not soluble in silicone. Therefore, an ethanol-saturated mixture was prepared and used in the polymer reaction-mixture. Imidazole remained suspended in the reaction-mixture. Shin-Etsu silicone polymer KE-108 mixed with CAT-108 is a useful plastination mixture to impregnate specimens. Measured shrinkage of 2 - 10% was less than the previously reported 8 - 29% by Brown and colleagues (2002). Part of this discrepency is likely due to the difference of units used: Brown's group used volume as opposed to area in this project. Imidazole is the key to preparing dry specimens with natural coloring. The exact minimal amount of imidazole to specimen volume has not been determined. However, at the determined level (0.5 pts imidazole/100 pts KE-108 polymer) it is likely to be an adequate amount for impregnation of more than one load of specimens. We chose not to reuse the mixture because of the increased viscosity of the polymer reaction-mixture after one load of specimens had been impregnated. Much higher concentrations of imidazole will likely yield a much darker unnatural red and is much more expensive.

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Design of a Silicone Gasket with an Iron Core for Polyester and Epoxy Sheet Plastination

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Abstract: Plastination is a unique method for permanent preservation of biological tissues. Sheet plastination has become popular for the production of sectional aids for studying sectional anatomy. In sheet plastination, the slices of organs and whole bodies are cured between glass plates with a gasket placed between the glass. This uniquely designed silicone gasket has a central iron core which is covered with silicone (1.5mm). The sheets produced using this type gasket have smooth, squared edges and are rectangular in shape. The advantages of using this type gasket are ease of casting of specimens, especially those which have several pieces; ease of positioning of specimens because there is no glass in the way; and ease of reaching and removal of bubbles.

Key words: **ring silicone gasket, iron core, plastination laboratory**

Introduction

Plastination is a unique method of permanently preserving tissue in a dry state. In this process, tissue fluid and lipids are replaced with a curable polymer which is subsequently hardened, resulting in dry, odorless and durable specimens. (von Hagens, 1979; von Hagens, 1985; Weiglein, 1997).

The basic steps for preparing either entire bodies or portions thereof with silicone or thin slices using epoxy or polyester are similar (von Hagens et al., 1987). Sheet plastinated specimens have a high acceptance and are frequently used for studying sectional anatomical detail often in conjunction with imaging modalities (Henry et al, 1997; Latorre et al. 2001).

The sheet plastination technique is a mechanism for preservation of thin (2-8mm) slices of organs or whole bodies. In this technique after specimen preparation, dehydration and forced impregnation, the slices are cured between tempered glass plates (Weber and Henry, 1992) with a gasket placed between the glass or the slices can be stacked between heavy sheets of polyester foil (von Hagens et al., 1987; Weiglein, 1997).

Gaskets, used in plastination laboratories, are flexible (Weber and Henry, 1992). Such gaskets are round and do not conform to a rectangular shape. Consequently the produced specimen sheets do not have squared edges but rather the edges are irregular and concave. Therefore, the sheets need to be cut to the desired shape and polished in order to have a smooth, regular, geometrical configuration (Fig. 1).

The purpose of this study is to present a design for a rigid rectangular silicone gasket. This gasket is placed near the perimeter of the bottom glass of the flat chamber. A thin layer of epoxy or polyester resin is placed in the flat chamber and allowed to cure. After this layer of resin has cured, the specimen is positioned on the cured resin and the chamber is filled with resin covering the specimen while in a horizontal position. Bubbles are allowed to rise and burst and then the top glass is put into place. Fold-back clamps are positioned around the perimeter over the glass and gasket to seal the glass to the gasket.

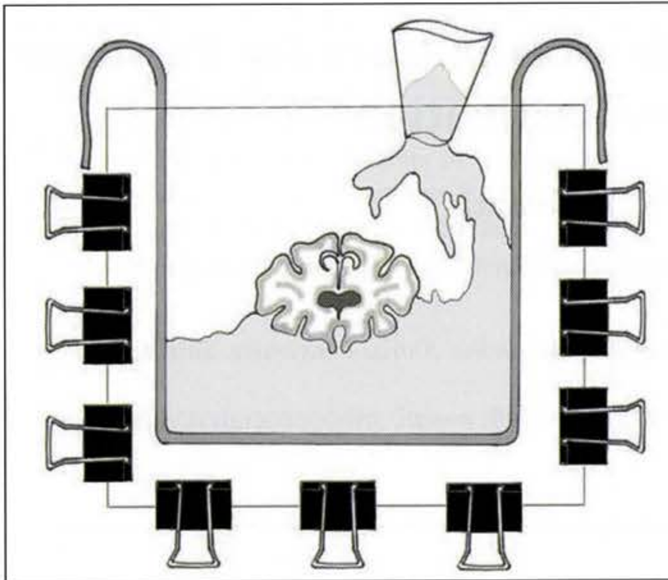

Figure 1. Gasket for a typical flat chamber for epoxy or polyester plastination.

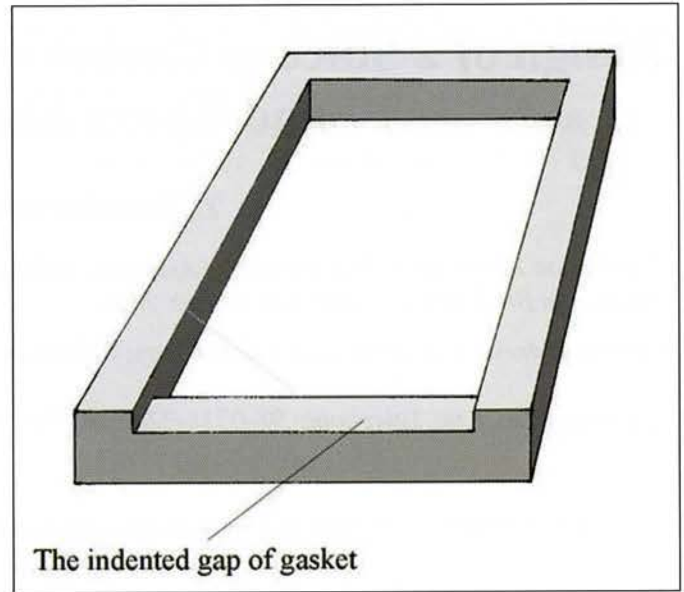

Figure 2. Gasket constructed with a gap for access to the slice inside the flat chamber.

Materials and methods

Rectangular iron frames of desired sizes, to serve as gasket cores, were made from four pieces of iron by welding them together. The dimensions of these pieces of iron were 6.0mm thick by 10.0mm wide. To provide a 3.0mm gap on one side of one end of the gasket, the thickness of that one iron piece was only 3.0mm.

Dimensions of the frames were designed to fit the dimensions of the various sizes of glass plates chosen to accommodate a variety of specimen sizes. The iron frame serves as the core of the silicone gasket.

In preparation to construct a mold to encapsulate the iron core with silicone, 2.0cm wide strips of 3.0 to 6.0mm thick glass were cut. For the outer mold wall, the four strips were cut to approximate moat length for the two sides and two ends of intended size glass plates and to allow for a 1.5mm gap on the outer surface of the iron frame. For the inner mold wall, the four strips were cut approximately 13.0mm shorter. The strips were glued onto a glass plate using *Allplast* adhesive to form the gasket mold. The internal dimensions of the constructed mold were 13.0mm wide x 20.0mm high x the appropriate length to accommodate the iron core. To create the slot in one side of one end of the gasket, a 13.0mm wide strip of 3mm thick glass was cut to the appropriate length and glued in to fill the bottom of the moat (Fig. 2).

The internal surface of the cast was coated with a thin layer of polyester wax (separator used in the fiberglass industry) for easy separation of the gasket from the mold. Four small pieces of 2mm thick silicone were placed equidistantly on the moat floor as spacers under the iron core to create a space for the silicone to coat the under side of the core. The iron core was placed in the center of the moat leaving a gap of 1.5mm on each side (Fig. 3). The width of the moat will be the dimension of the intended gasket.

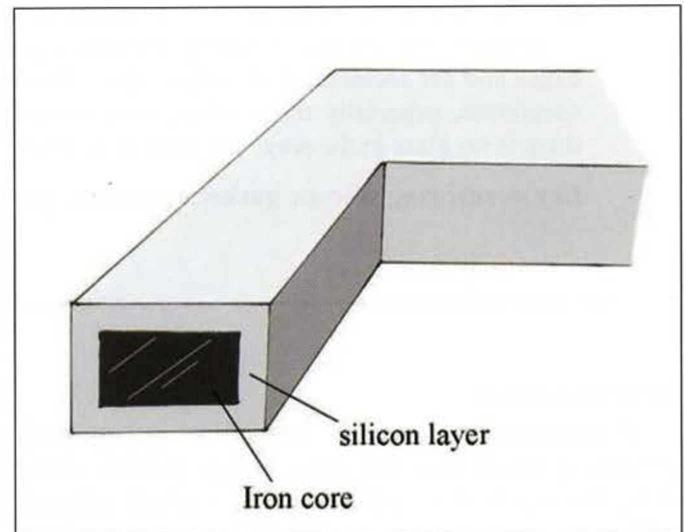

Figure 3. Cut away of gasket with an iron core demonstrating its smooth parallel surfaces.

An appropriate volume of silicone RTV2 (Rhodia Silicones, Lyon, France) was mixed and poured into the moat and allowed to surround the iron core and fill the mold 5.0 to 10.0mm from the top. After curing for twenty-four hours, the gasket was separated from the glass mold (Fig. 4).

Results

Semi-rigid gaskets were constructed which allowed for ease of specimen manipulation and air bubble

Figure 4. The increased weight, flexibility and smoothness of the silicon gasket prevents the formation of micropores between the gasket and glass.

removal with a wire and filling with the resin. As well, there was no leakage of resin between the gasket and the bottom glass plate of the flat chamber while lying in a horizontal orientation with the top glass removed and no clamps utilized (Fig. 5).

As well, the chamber may be held vertically (slotted end up) to add resin to the flat chamber, to manipulate trapped bubbles with a wire thus encouraging them to rise and to manipulate or adjust the slice after clamping the three sides to create a seal (Figs. 2, 6). The hardened slices have smooth, parallel sides with ninety degree corners (Figs. 6, 7).

Discussion

Gaskets routinely used in plastination laboratories for sheet plastination are very flexible and do not create a desired geometrical shape. Thus, the produced sheets have irregular concave edges which are often not parallel. In order to have an aesthetic, smooth and rectangular shape, the sheets need to be cut with a saw and be sanded.

The iron core gasket, with its smooth parallel sides, eliminates the need to trim the edges of the hardened slice. As well, the iron core, encapsulated in silicone, increases gasket firmness and weight which aids its ability to easily seal. This not only keeps the gasket from deformation but also makes the gasket heavy enough to adhere to the glass and to allow no leakage of resin between the gasket and lower glass when the top glass and clamps are not in place. This is possible because the silicone encapsulating the steel core is soft and smooth enough to form a seal with the glass without the clamps being applied when lying in a horizontal position. Therefore, the specimen can be placed in the open horizontal flat chamber and manipulated for proper positioning. As well, the chamber can be filled with resin allowing trapped bubble to be freed easily and removed.

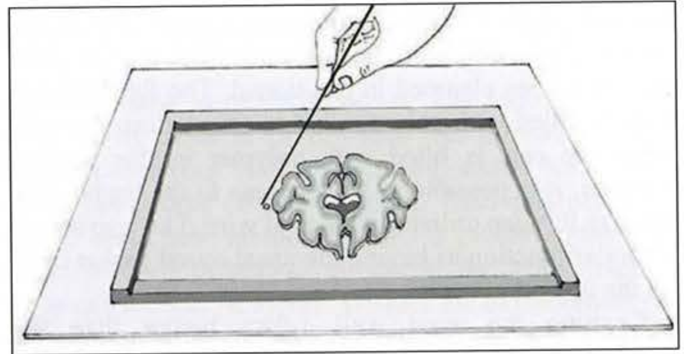

Figure 5. Demonstration of the possibility for slice positioning, air bubble removal and filling the open flat chamber with polymer (top glass removed).

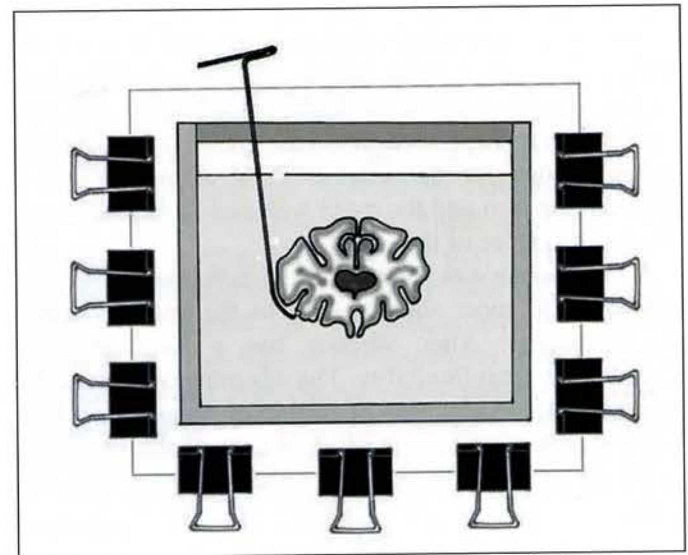

Figure 6. Demonstration of slice adjustment via the gasket gap on one side.

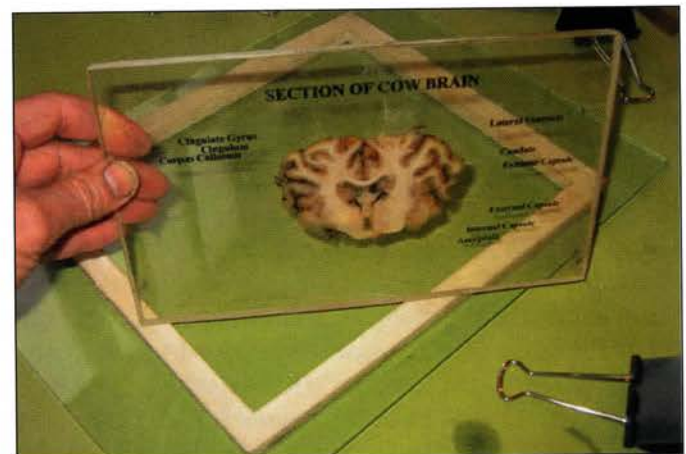

Figure 7. The finished, rectangular sheet with smooth, squared edges.

Slices which have several pieces or have been fractured and need special positioning while casting, may be adjusted and fitted together in proper position

because of their accessibility. The 3.0mm slot in the gasket allows access to the specimen after the top glass plate has been clamped in positioned. The flat chamber may be filled with polymer in this orientation. As well, when the cast is filled with polymer in this vertical position, it is possible to have access to the tissue slice through this gap utilizing a piece of wire. This gap serves a similar function as having the usual round gasket open on the up end.

Creating the moat wall 1.0cm higher than the anticipated gasket thickness allows ample space for the viscous silicone to pool until it flows around and under the iron for optimum coating of the iron core. It also serves as a reservoir for bubbles which rise to the top and eventually burst.

The wax coating provided ease of separation of the gasket from the internal surface of the mold. The four pieces of silicone, 2.0mm thick, placed under the iron core were incorporated into the gasket and created enough space for the viscous RTV silicone to flow between the iron and the mold wall and completely coat the down surface of the iron core.

RTV silicone was chosen for this gasket because of its resistance to most solvents and to the heat produced during curing. Also, silicone has a long life and maintains it great flexibility. The advantages of using the iron core gasket are: ease of casting of specimens which have several pieces, ease of alignment of the specimen and ease of reaching and removal of bubbles from the resin.

Acknowledgments

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Abstracts - 13th International Conference on Plastination - July 2 to 7, 2006

Oral presentations

History of plastination. ***von Hagens G.*** *Institut für Plastination, Heidelberg, Germany, Europe.*

Plastination, a vacuum-forced impregnation technique with reactive polymers for biological specimens, was invented by the author at Heidelberg University in 1977. But plastination technology only gained wide acceptance after further progressive developments which included:

- The technique of gas curing for silicone impregnated plastinates.
- The creation of polymerizing emulsions for hard and opaque plastinates.
- Sheet plastination resulting in transparent body slices, patented in 1982.
- Sheet plastination of brain slices.
- Blood removal by plain water of thoracis-abdominal en bloc specimens.

The 1st International Congress of Plastination was organized by Harmon Bickley in 1982 at The University of Texas in San Antonio, Texas, USA. Martin Lischka, at the Anatomical Institute of the University in Vienna, Austria was an early exponent of plastination and the first to implement it outside of Heidelberg in 1977. The International Society for Plastination was founded in 1986 and the inaugural issue of Journal of the International Society of Plastination was published in 1987. In 1990, the process extended the frontier of biological specimen preservation with the plastination of a whole human body. The first public exhibition of whole human body plastinates and the juxtaposition of healthy and diseased organs was shown in Tokyo, Japan in 1995. Currently, plastination is performed in over 40 countries at 400 institutes of Anatomy, Pathology, Biology and Zoology. Since 2003, public exhibitions of human plastinates have been presented worldwide, and several entities have emerged to provide polymers and equipment for plastination.

Preservation and plastination. ***Weiglein AH.*** *Institute of Anatomy, Medical University Graz, Graz, Austria, Europe.*

For over 3,000 years, efforts have been made to stop postmortem decay and to keep the human body intact to preserve the mortal frame for coming back to life sometime later (e.g., ancient Egyptian mummification in 1550 BC and cryopreservation in the 20th century). Interest in morphology made it necessary to preserve human tissue to investigate its anatomy. The most important step in preservation was the introduction of formalin by Blum in 1896, which was followed by the colour preserving embalming solutions of Kaiserling (1900) and Jores (1930). In 1992, Thiel published an article on a new method of colour preservation and, based on this technique, produced the Photographic Atlas of Practical Anatomy. Besides the development of embalming solutions, which allow for the preservation of lifelike colour and flexibility for student dissection and the teaching of surgical techniques, other methods were developed for the demonstration of human anatomy in museum specimens. For this later purpose, paraffin impregnation was introduced by Hochstetter in 1925. Embedding of organic tissue in plastic was introduced in the 1960s. In 1977, Gunther von Hagens invented plastination. This technique utilizes both impregnation and embedding in which water and lipids of biological tissues are replaced by curable polymers (silicone, epoxy or polyester). These polymers are subsequently hardened, resulting in dry, odourless and durable organ specimens or body slices. Thus, this method is excellent for the production of museum specimens to teach human gross anatomy. Furthermore, sheet plastination provides an excellent tool for use in sectional anatomy and for research. Plastinated specimens are perfect for teaching because they show the real specimen, they are easy to handle, almost everlasting and need a minimum of maintenance.

Principles of silicone plastination techniques. ***de Jong KH.*** *Department of Anatomy and Embryology, Academic Medical Centre, University of Amsterdam, The Netherlands, Europe.*

Plastination is defined as the replacement of tissue water and fat with a curable polymer, either silicone, polyester or epoxy. As these polymers are not soluble in water, tissue water has to be replaced with a volatile intermediate solvent, which is subsequently replaced with the polymer of choice. Replacement of water with the intermediate solvent (dehydration), preferably done with acetone, is performed by placing the specimen in subsequent baths of 100% acetone until a dehydration of >98.5% is obtained. To prevent excessive shrinkage dehydration is performed between -15°C and -20°C (freeze substitution). During dehydration, due to the physical properties of acetone, a certain degree of defatting of the tissue is obtained. Before starting the impregnation the silicone S10 is mixed with the chain extender/catalyst S3 in a 100/1 ratio pbv. Chain extension is delayed in the cold; the S10/S3 mixture is stored in a deep freezer. When dehydration is

completed, the specimen is submerged in the S10/S3 mixture and placed in a vacuum chamber. By applying increasing vacuum (decreasing pressure) the acetone is extracted from the specimen and replaced with silicone (forced impregnation). When all acetone is extracted and impregnation is considered to be complete the specimen is removed from the S10/ S3 mixture and left to drain. If performed at room temperature the silicone chains will start to elongate due to the presence of the S3 in the mixture (pre-curing). When the specimen is successfully drained the final polymerisation can be performed (curing). Therefore the specimen is placed in an air-tight closed container in which a vaporised cross-linker (S6) is added. The gaseous cross-linker will diffuse into the specimen and cause a cross-linking of the elongating silicone chains, thus hardening the specimen from the surface to the depth. During the beginning of the curing process the surface of the specimen has to be manicured carefully to avoid the development of dimples of silicone oozing out of the specimen. After the curing the specimen is placed in a well ventilated place to let the excess of S6 evaporate from the specimen. In this presentation also the basic equipment for silicone plastination will be shown.

Results of silicone polymer impregnation with no additives. ***Henry RW[1], RB Reed[1], R Latorre[2], H Smodlaka[3].*** *[1]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA, [2]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe, [3]College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA.*

Hair covered domestic and exotic animal specimens were impregnated using a silicone impregnation/ reaction-mixture which contained no additives. This rational was used to impregnate hairy specimens which results in: Preservation in a shortened period of time and Polymer which drains quicker from the hair with virtually little to no wiping of polymer form the surface. Low viscosity (40 - 80 centistokes) polymer at room temperature decreases the impregnation time and yields a shortened draining and manicuring time with less labor involved. A philosophy of not curing these specimens has evolved. These noncured specimens seem more life-like and curing is a bit sporadic. For five years, two curing regimens were used to cure the specimens: **1.** Specimens are exposed to the vaporized cross linker (S6) for 2 - 4 days then S3 (catalyst/chain extender) is wiped onto the surface of the specimen twice at 24 hour intervals. **2.** Specimens are exposed to the vapor of a new Supercatalyst for 2 days then are exposed to a volatilized chain extender (S7) and finally to a cross-linker (S6). Both of these methods result in polymerization and specimen quality seems similar to specimens produced by the classic von Hagen's method. However, a small percent of these specimens may remain partially uncured and a small percent become very hard. Currently, the two biggest advantages of using no additives to the impregnation bath are: 1. Polymer runs freely off of hair-covered specimens eliminating the tedious task of manicuring due to thickened silicone when the cold process is used. 2. Impregnation is carried out at room temperature.

Comparison of plastinated specimens prepared using six regimens. ***Smodlaka H[1], RB Reed[2], R Latorre[3], O López-Albors[3], JM Hervás[4], R Cuellar[4], RW Henry[2].*** *[1]College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA; [2]Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA, [3]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe, [4]Departmento Anatomía Veterinaria, Universidad Nacional de Aguas Calientes, México.*

To assess quality of surface detail of silicone impregnated specimens and increase our specimen inventory, numerous specimens were prepared using six current methods of silicone plastination. These included the classic von Hagens' (S 10, 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 for the exchange of the intermediary solvent (acetone) for 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 S 10 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.

Plastinated head as a guide for computed tomography diagnosis in goat. ***Basset A[1], AE Mervat[1], MH Konsowa[1], HM Imam[2].*** *[1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Egypt, [2]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.*

The present work investigated the relationship between plastinated cross sections and CT images of Goat heads. It was carried out on five adult apparently healthy Goats of both sexes. Three of them were used for CT Scans. Each animal was anaesthetized, placed in a prone position (with the head extended and held in place with the help of a cushion) for conducting transverse axial computed tomography (soft tissue window) using 1.5 Tesla, Philips Gyro-scan S15 system (Faculty of Medicine, Suez Canal University). Transverse axial CT images were taken with 10-15 mm intervals; at 120 Kv, 200 MAs, F 3HF/S and W 200+64; from the level of the nostrils up to the level of the eyes. The other two goats were transported to the Zagazig Plastination Laboratory. They were euthanized and perfused with formalin 10% via the common carotid artery and left for one week to complete the fixation. The fixed heads were frozen and cut by a hand saw into serial cross sections, about three centimeters in thickness. The sections were plastinated by the Silicone 10 technique at Zagazig Plastination Laboratory. The obtained results revealed that: both the plastinated cross sections and the electronic CT images portrayed a combination of soft tissues, cartilages, bones, and nasal air passages that provided a good anatomical view and good diagnosis of the pathological and malformations of Goat nasal cavity. In our study, we used plastinated cross sections as an aid for interpretation of CT images.

Room-temperature impregnation with Biodur S10/S3: Towards a quantitative evaluation. ***Adds PJ.*** *St George's, University of London, London, United Kingdom.*

Room-temperature silicone plastination has the twin advantages of reduced cost and simplicity of set-up. At its best it can yield specimens which are the equal of those produced by low-temperature impregnation. However, problems encountered include increased shrinkage of the specimen, as well as gradually increasing viscosity of the S10/S3 (polymer/catalyst/chain extender) mixture (reaction-mixture). The shortened life of the polymer leads inevitably to wasted silicone and, therefore, increased costs. By comparing the viscosity of room-temperature and low-temperature reaction-mixtures, this study aims to quantify the hidden extra costs of consumables resulting from the use of a room-temperature impregnation set-up. Over a period of six months the viscosity of successive mixes of Biodur S10/S3 were recorded both at -30°C and at room temperature in the laboratory. The ambient maximum and minimum laboratory temperatures were also recorded. Specimens plastinated during the test period were monitored closely for quality. The subjective cut-off point when the polymer was thought to have become too viscous for use was recorded. By comparison with the cold-temperature polymer, the shortened life of the room temperature mixture can be quantified. From this the added overhead costs of consumables can be calculated. Results show that the room temperature mixture has a significantly shorter usable life when compared to -20°C. The polymer becomes so viscous that specimen quality is adversely affected. Ultimately, the polymer becomes unusable. Room-temperature impregnation appears to be an attractive option for reasons of speed and set-up costs, and this is the method currently employed at St. George's in London. The best specimens produced early in the cycle are of comparable quality to those produced by low-temperature impregnation. However, there are significant cost implications in opting for a room-temperature procedure. The shortened shelf-life of the S10/S3 and S15/S3 mixtures results in added polymer usage and therefore increased production costs.

Using acrylic paints on neurovascular pathways to enhance the educational value of plastinated specimens. ***Raoof A, K Falk, A Marchese, L Marchese, N Mirafzali.*** *Division of Anatomical Sciences, Office of Medical Education, The University of Michigan Medical School, 3808 Med. Sci. II Bldg., Ann Arbor, Michigan, USA.*

In attempts to enhance anatomy education through plastination, a section of the research has been devoted to painting the finished neurovascular pathways. The introduction of coloring to plastination has proven to be a useful practice in improving the study of anatomy. When painting specimens, blood vessels and nerves are color coded to provide better visual models for students from which to learn. This way, not only can students view and handle three-dimensional specimens, but also they will be able to better distinguish specific anatomical patterns. It was with this new educational device that excessive handling became an issue and the paints often became chipped or damaged. This has

become the focus of our research to determine a more durable painting method so students can benefit from an ideal specimen without fear of damaging its quality. Specimens plastinated using the room temperature method and colored with conventional application of acrylic paints showed a significant deterioration of paint following continued handling over time. Originally, Tamiya® Acrylic paints had been used to color the specimen with a simple brush application. This method did not yield permanent coloration since the paints did not adhere firmly to the silicone surface of the specimen. The paint chipped and flaked only after moderate handling and manipulation of the painted areas. Similar results were demonstrated with models students used during a lab practical exam at the University of Michigan Medical School. Students studied specimens colored with Tamiya® Acrylics that had been painted the previous year and again an hour prior to an exam after which many noted the paint came off on their gloves. After testing different paints, solvents, and base coats, we found that the acrylic paint applied to the specimen prior to catalyst application and with ethyl silicate (Silbond-40®) coated on top of the paint, the new application provided a strong and durable paint that withstood experimental vigorous handling. We conclude that the aesthetic and hopefully educational value of specimens could be augmented by this method of acrylic paint application. The method we applied is relatively easy to follow and uses affordable materials. We are working on a pilot study using medical students to test the durability of these new coloring methods versus the old coloring methods.

Modified plastination technique for viewing deeper structures and their interrelationships *in situ*. ***Kumar R, V Tarnikanti, R Dhingra.*** *Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.*

Traditional anatomy teaching needs a lot of effort and imagination on the part of the teacher and those being taught. Classical anatomical teaching aids require diagrams, charts, models, cadaver specimens and dissection for the comprehensive knowledge of the human body. With the advent of computer generated diagrams and charts, this problem has been partly overcome. With an increase in the number of medical institutions, there is always a shortage of human specimens and cadavers. Therefore, plastination of human specimens is gradually becoming an invaluable teaching resource in Anatomy. Although plastinated, specimens are dry & non toxic, they cannot be manipulated and the deeper structures cannot be viewed as may be done with formalin-fixed specimens. In the present study, we have utilized a simple method, which after plastination, the superficial structures may be easily retracted, to view the deeper ones and their inter relationships can also be easily appreciated. For this we chose the popliteal fossa. The popliteal fossa was formalin fixed and separated from thigh and leg. The fossa was carefully dissected to define its boundaries and contents. The specimen was dehydrated using cold acetone. Forced impregnation was done by Silicone S10 and S3 mixture at -20°C. Then the specimen was kept at room temperature for a week to drain the excess polymer. At this stage small sheets of aluminium foil were inserted between various muscles forming the boundaries of popliteal fossa. Along with aluminium foil the popliteal fossa specimen was subjected to curing for seven days. After curing, the aluminium foil was removed. After removing the aluminium foil, the superficial structures, i.e. heads of gastrocnemius muscles could be easily separated from deeper muscle i.e. soleus. Similarly the semimembranosus and semi-tendon could be easily separated. The popliteal artery could be easily separated from popliteal vein and the tibial nerve. The inter relationship of the muscles, popliteal vessels and tibial nerve was maintained in situ even after removal of the aluminium foil between them after plastination. Thus the limitation of manipulation of plastinated specimens has been partially overcome by the above procedure which also maintains the inter relationship of the structures.

Effect of stepwise dehydration by acetone and temperature on plastinated brain section length following Mulligan staining. ***Asadi MH, A Azami, F Mohammadzadeh, F AbouAli, Z Zare, M Jalali-Monfared, Y Yousofi.*** *Department of anatomy, Bighlatollah University Medical Sciences, Tehran, Iran.*

Mulligan-stained brain sections have been used in teaching neuroanatomy for a long time. Plastinated preparations have proven to be an additional valuable tool due to the ease of handling and durability compared to that of wet specimens. In this paper, we discussed the effect of dehydration by acetone and temperature on the length of plastinated brain sections. Cow brains were used for this study. After fixation, four brains labelled A, B, C and D, were prepared. Each brain was cut in two similar median sagittal sections. The cerebellum and pons were separated by a section between the pons and midbrain. After that, each sagittal section was sliced in seven parasagittal sections (~10.0mm). Maximum length of each section was measured in millimeters through three phases (fixation, dehydration and curing). We defined "difference of reduction" as the percentage of difference of length between the first and

second phase. Staining was performed according to Tompsett's modified Mulligan-staining procedure. After dehydration in: Pure acetone (A @ -20°C and C @ room temperature) and 80% acetone (B @ -20°C and D @ room temperature) with the final bath of 99% acetone, these specimens were then impregnated in a vacuum chamber at -20°C using Biodur S10 silicone mixed with Biodur S3 catalyst and chain extender. Finally, the slices were cured by exposure to S6 vapor at room temperature. Difference of reduction between group A and C was -4.30 with 95% Confidence Interval from -7.67 to -0.98mm. This indicates that the shrinkage in group C was 4.3% more than group A. The difference of reduction between group A and D was -6.35 with 95% confidence interval from -9.66 to -3.04 mm, which indicates the shrinkage in group D is 6.35% more than group A. Since dehydration temperature in both groups (C and D) was at room temperature, it is concluded that the temperature is the main factor of the shrinkage at the dehydration stage.

Microscopic morphological investigation of deplastinated pig kidney sections. ***Ilieski V[1], L Pendovski[1], T Ristoski[2].*** *[1]Department of Functional Morphology, Faculty of Veterinary Medicine, Skopje, Macedonia, [2]Department of Pathology, Faculty of Veterinary Medicine, Skopje, Macedonia.*

Plastination is a method for preservation of biological specimens which allows the specimen to retain its original shape. Microscopic changes which might occur during preservation by plastination have been a subject of interest in years past. Several publications were found where different methods of deplastination were used. Findings show that plastinated specimens maintain their histological structure and that deplastination affects the morphology in different ways. The aim of this study was to determine level of morphological change during preservation with plastination of the kidney structure and to describe the most suitable protocol for deplastination. In this study, we used pig kidneys plastinated via the standard S10 procedure three years prior. The plastinated pig kidney was transversally cut into (0.5cm - 1.0cm - 0.5cm) thick specimens and divided in five groups. For deplastination, absolute alcohol (99%) and toluol solution were used. The first group of specimens were immersed in alcohol for 24 hours, the second was in 48 hours, the third in 72 hours, the forth was immersed for 90 hours and the last group of specimens were immersed for more then 200 hours in alcohol. After that, the slices were transferred in toluol, using the same protocol. The last phase for all specimens was immersion into 10% formalin. At the end, the tissue specimens were embedded in paraffin using standard protocol. For examination by light microscopy, paraffin sections were cut in 5 to 10μm slices and stained with haematoxylin and eosin. The specimens were imaged using Lucia G software. On the specimens immersed in toluol, resin was noticed, which had arisen from the surface of the slices in thin transparent particles. The quantity of particles depended on the protocol used for deplastination. Histological section showed clear distinction between cortex and medulla. On higher magnification, lesions were found on the renal tubules with unclear borders between epithelial cells. On some sections the tubules were disrupted and on other tortuous tubules were observed. Occlusion with cell debris was also identified inside the collecting tubules. The main changes were located in the medulla whereas the cortex was less damaged. Bowman's space, of some renal corpuscles was enlarged with noticeable capillary shrinkage. Our results show that duration of tissue immersion in toluol has important impact for deplastination. Based on histological findings, the best procedure for deplastination of kidney sections was protocol, which immersed specimens 48 hours in alcohol and afterwards ninety hours in toluol. These specimens can be used for optical microscopic studies. The morphology is well preserved and comparable with normal histological structure of kidney. However, structural changes were found and mostly were located in medulla of kidney.

Principles of epoxy (E12) plastination technique. ***Sora M-C.*** *Plastination Laboratory, Anatomical Institute, Medical University of Vienna, Vienna, Austria, Europe.*

The E12 plastination process is a well-established preservation technique used for demonstration in teaching and also in research. Material and Methods: *Material and Slicing:* For E12 plastination, usually fresh tissue used and frozen at -80°C for one week. In the next step slices with an average thickness between 3 and 5mm are cut. Between the sections, 1 mm of tissue is lost due to the thickness of the saw blade. The slices were stored at -25°C overnight. *Dehydration and Degreasing:* The acetone used for dehydration is cooled to -25°C. For dehydration of slices, technical quality acetone is used. Each slice is placed between soft plastic grids in order to allow better circulation of the dehydration fluid. The dehydration time for the slices is seven days; the acetone was changed once after three days at a concentration of 96% (bath 1), by using technical quality acetone. The final concentration of the dehydration bath was 99% (bath 2). When dehydration is complete, the freezer is disconnected. The

temperature increases and after one day room temperature (15°C) is reached. Now the acetone is changed with room temperature methylene-chloride (MeCl) for degreasing. Degreasing is finished after seven days. *Impregnation:* Impregnation is performed at 5°C using the following epoxy (E12) mixture: E12/E1/AE10 (95:26:10 pbw). The slices are submerged in the E12-mixture and placed in a vacuum chamber, directly out of the methylene chloride bath. Pressure is continuously reduced over the next two days down to 2.0mm Hg. Temperature is kept under surveillance in order to avoid E12 crystal formation which would take place if temperature decreases under 0°C. *Casting and Curing:* The slices are cast between two sheets of tempered glass and a flexible gasket is used as a spacer (4mm). The following E12-mixture was used for casting: E12/E1/AT30 (95:26:5). The slices are placed between glass plates, sealed and the flat chambers were filled with casting mixture. Then they are placed for one hour in a vacuum chamber at 3mm Hg to remove small air bubbles present in the resin. Large bubbles are removed afterwards manually. After bubble removal, the flat chambers are placed horizontally inclined at 15° from the horizontal and left for one day. The polymer gets more viscous and sticky and after one more day the flat chambers containing the slices are placed in an oven at 45°C for four days. After the flat chambers cool to room temperature, the glass plates are removed carefully and the sheets are cut as desired. Results: The transparency and color of the slices are perfect and shrinkage is not evident. The finished E12 slices are semi-transparent, easy to orientate and offer a lot of anatomical details down to the submacroscopic level. The transparent loose areolar and adipose tissues contrasted perfectly with the muscle tissues and all epithelial parenchyma. Conclusion: Since the beginning of plastination, the E12 technique was and still is the preferred method for producing transparent body slices. Transparent body or organ slices are used for teaching and research purposes, because they allow studying the topography of all body structures in a non-collapsed and non-dislocated state. In addition, the specimens are useful in advanced training programs in sectional topography (resident training in CT and NMR).

Plastination and vascular anatomy. ***Latorre R[1], W Hernández[2], F Sun[3], F Gil[1], J Arredondo[2], F Martinez[1], O López-Albors[1], E Abellán[3], E Estaca[3], MD Ayala[1], RW Henry[4].*** *[1]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Spain, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad del Estado de México, México, [3]Minimally Invasive Surgery Centre, Cáceres, Spain, [4]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Interventional radiographic procedures are currently used world wide, in almost any clinical specialty, for diagnosis and treatment. In angiography, which is the fundamental technique in interventional radiology, knowledge of vascular anatomy is crucial. The effectiveness of a combined use of a vascular radio-opaque injection and plastination techniques was investigated in this project. The left common carotid artery of a dog cadaver was used for arterial injection of red gelatin and barium sulfate (AG12 Biodur™) with a peristaltic pump. The body cavities and thoracic and pelvic limbs were dissected prior to plastination. The specimen was prepared and plastinated according to the standard S10 Biodur silicone procedure (von Hagens, 1985). This specimen has been used during the Course on Basic Endoluminal and Interventional Radiology Techniques in Veterinary Medicine. The use of high resolution fluoroscopy with digital recording and digital subtraction imaging (C-arm Digital Subtraction Angiography Philips BV 300) shows that this plastinated specimen is a good vascular anatomy reference to be used during clinical instruction. Visualization of plastinated specimens before and during a fluoroscopic examination aids the understanding of the organization of the arterial supply and affords a convenient, easy and accurate method for training the surgeon.

Plastinated sections for demonstration purposes of fascial layers and conduction structures adjacent to the carpal joint of the horse highlighting the carpal flexor retinaculum, carpi radiatum ligamentum and the accessory ligament. ***Probst A, E Polsterer, C Hinterhofer, I Guarda, M-C Sora, HE König.*** *Department for Pathobiology (Anatomy), University of Veterinary Medicine, Veterinärplatz 1, A-1210, Vienna, Austria, Europe.*

The carpal flexor retinaculum has been defined as a fascial reinforcement structure in the veterinary nomenclature. A resulting inflammation and swelling leads to a compression of the structures located within the carpal canal. Decompression can be achieved by a partial surgical tenotomy of the carpal flexor retinaculum. The carpal flexor retinaculum coursing between the accessory carpal bone and the medial located carpal bones has not been exactly defined and described in the literature. Conflicting reports exist regarding the carpal check ligament and regarding the origin of the accessory ligament joining the deep digital flexor tendon. In three adult horses, stratigraphic

preparation of the carpal region documenting every demonstrated layer has been carried out. Particular attention was given to the layer of the carpal flexor retinaculum, the carpal check ligament and the accessory ligament. In two other horses, blood vessels were injected followed by preparation of cross-sections of the carpal region. A series of slices was plastinated using the S10 method and another series using the E12 method. The carpal flexor retinaculum of the horse is subdivided into a complex tunnel system through which run tendons, vessels and nerves separated by fascial layers. The superficial and deep flexor tendon, as well as the median artery and medial palmar nerve pass through a tube like fascial layer. Both flexor tendons share a common carpal flexor tendon sheath. The carpal check ligament is composed of several strong bundles of fibers, which run from the proximal and distal row of carpal bones to the cannon bone and to the medial splint bone. The accessory ligament originates from the middle part of the carpal check ligament which joins the deep flexor tendon at the proximal third of the metacarpus. Despite our intense literature search, the terms "palmar carpal annular ligament" and "carpal canal (tunnel)" are not clearly defined. The term "palmar carpal annular ligament" should include all layers of the flexor retinaculum. The "carpal canal" in the horse is made up of several individual layers, which form a well differentiated tunnel-like system. Not necessarily all anatomical structures in the area of the carpal canal might therefore be affected in the case of the carpal canal syndrome. The carpal check ligament, the accessory ligament and in addition the suspensory ligament play an important role in the suspensory function of the digit. They act as antagonists of the suspensory apparatus of the coffin bone and have a major effect in the pathogenesis of laminitis.

Influence of solvent vaporization in plastination. *von Horst C. HC Biovision, Mainburg, Germany, Europe.*

Incomplete impregnation can be a problem in plastination. Ways to improve the impregnation procedure mainly focus on prolonging the impregnation period, reducing the viscosity of the resin and using low boiling solvents. Achieving a maximum vacuum, close to 0mm Hg, is considered to be another key to good impregnation results. A theoretical investigation was made on how additional factors influence the vaporization of solvents at the relative depth of the specimen within the polymer, referred to as the Point of Impregnation (POI). Acetone served as an example to graphically demonstrate the interactions of different factors. The main additional influences on solvent vaporization are: *Resin column:* The pressure on top of the POI, which results from the weight of the resin column, acts additionally to the pressure on top of the bath. Assuming a specific weight of the resin of around 0.9g/ccm, a 40cm resin column in an impregnation chamber would add about 27mm Hg to what is shown on the manometer. *Temperature:* At a Temperature of -18°C the vapour pressure of acetone is about 24mm Hg. Increasing the temperature to -10°C has approximately the same effect on vaporization like a change in vacuum from 20 to 6mm Hg. *Gas tight barriers:* Tissues can be surprisingly tight barriers for vaporized solvents. Any pressure gradient that builds up between the inside and the outside of structures has to be added to the other influences. Considering these influences, shows that low temperatures in a deep impregnation bath can result in a complete lack of direct vaporization from the specimen. When estimating the vaporization and quality of impregnation the pressure shown on the manometer has to be put into relation to the other influences. Differences in vapour pressure and vaporization can be compensated by adapting the additional factors, e.g. temperature, depth of the impregnation bath, perforation of gas tight barriers, etc. Not only direct vaporization of acetone plays a major role in cold temperature impregnation, but also acetone diffusion through barriers and through the bath upwards until a lower pressure of the resin column allows vaporization. If diffusion of solvents alone can lead to sufficient impregnation in an acceptable amount of time, completely new impregnation procedures seem possible.

Theoretical considerations and preliminary studies on alcohol as an intermediary solvent. *Pereira-Sampaio MA[1], C von Horst[2], BPS Marques-Sampaio[1], H Smodlaka[3], L.A. Favorito[4], F.J.B. Sampaio[4], RW Henry[5]. [1]Department of Morphology, Fluminense Federal University, Niteroi, RJ, Brazil, [2]HC Biovision, Mainburg, Germany, Europe, [3]College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, USA, [4]Urogenital Research Unit, State University of Rio de Janeiro, RJ, Brazil, [5]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Alcohols have been used as a dehydration medium for nearly two centuries and have been the selected dehydrant for selected procedures during this period for biological tissue. However, the vapor pressure of alcohols (vp methanol - 97mm Hg at 20°C, 12mm at -15°C; vp ethanol - 44mm at 20°C, 4mm at -15°C; vp 2-propanol - 32mm Hg at 20°C,3mm at -15°C) is not good for silicone impregnation during plastination, especially in the cold, because they are too low. Alcohols were

used on a small scale in the 1980's and 90's for specimen dehydration for plastination of primarily long-term fixed specimens. However, before impregnation the alcohol was replaced with a solvent with a lower boiling point, methylene chloride (dichloromethane) (vp 375mm Hg at 20°C; 70mm at -15°C) or acetone (vp 175 mm Hg at 20°C; 28mm at -15°C). Classic dehydration for plastination is via acetone. Using MeCl has personal considerations and acetone is often a major hurdle with institutional safety personnel. To quantify shrinkage and determine if an alcohol could be used as the intermediary solvent for impregnation, pig kidneys were collected from local slaughter houses, fixed in 10% formalin by perfusion of the renal artery and then by submerged in 10% formalin. The renal vessels and collecting system were filled with RTV silicone; after silicone had cured, the kidneys were sliced in 1 cm sections, photographed for documentation, dehydrated using a graded series (50, 60, 70, 80, 90, 95, 100) of either ethanol, methanol or 2-propanol. The dehydrated specimens were photographed for documentation, impregnated in a cold or room temperature silicone impregnation reaction-mixture and excess polymer was drained and wiped off. Impregnated specimens were photographed, cured using S7 followed by using S6, and photographed after curing. Two dog kidneys were collected during an autopsy, fixed by immersion into 10% formalin solution, dehydration was via a graded methanol series, impregnation was at room temperature in a silicone reaction-mixture (S10/S3) and curing was via exposure to S6. After three weeks of cold impregnation, the vacuum kettle with the specimens were brought out to room temperature for one week to complete impregnation since vaporization of alcohol was weak and almost nonexistent in the ethanol slices. Room temperature alcohols impregnated well but at a very low pressure (1-3mm Hg) and even then it was difficult to vaporize the alcohols while of the cold temperatures, ethanol did not impregnate well.

Thin slice plastination and 3D. *Sora M-C, B Genser-Strobl. Plastination Laboratory, 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 to 5.0mm transparent slices. If thinner slices, 0.5 to 1.5mm, are desired it is necessary to use the thin-slice plastination method. By 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 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 a resin mixture E12/E6/E600. Using a band saw, Exact 310 CP, the E12 block was cut into 1 mm 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.

Geometry of the pharynx and swallowing difficulty. ***Zhang M.*** *Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand.*

Swallowing involves rapid, precise coordination of numerous muscles and tissue of the head and neck. It includes both voluntary and involuntary processes. The involuntary process starts from the hypopharynx through which food bolus quickly passes into the esophagus. The hypopharynx is bounded by two solid structures: the cricoid cartilage and cervical vertebrae, forming a tunnel-like space ("hypopharyngeal tunnel") that limits food bolus passage. Change of head and neck position alters the integrity of the space, e.g. the geometry and density of soft tissues. Alteration of the head and neck posture has been used in swallowing therapy practice for feeding neurologically impaired patients, such as stroke and Parkinson's disease. We have recently shown that 30% (9/31) of elderly cadavers had a thickened or folded muscular wall at the end of the hypopharyngeal tunnel but the

thickened/folded wall was not found in non-elderly cadavers (0/63). Such structural change may represent a compensation mechanism for the alteration of the tunnel geometry and/or decrease of the tissue compactness during aging, likely causing swallowing difficulty in the living subject. This hypothesis, however, has never been tested. As a first step to test the hypothesis, using gross anatomy dissection, E12 sheet plastination and MR images, we examined the geometrical relationship between the anterior and posterior pharyngeal walls and between the thyroid and cricoid cartilages in the anterior pharyngeal wall. Our preliminary results demonstrated that (1) the posterior surface of the cricoid cartilage and the anterior surface of the vertebral column are not parallel to each other and (2) there are at least three patterns of the geometric relationship between the posterior borders of the thyroid cartilage and the posterior surface of the cricoid cartilage. These results suggest that the passive elements of the hypopharynx may also play an important role during pharyngeal phase of swallowing.

Alar fascia, danger space and retropharyngeal space. ***Nash L[1], M Zhang[2]***. *[1]Department of Anatomy, American University of the Caribbean School of Medicine, St. Maarten, The Netherlands. [2]Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand.*

The deep cervical fascia is conventionally classified as the investing, pretracheal and prevertebral fasciae. This classification is largely based on the classic study by Grodinsky and Holyoke in 1938, in which adult and fetal cadavers were examined using dissection and ink injection methods. Using sheet plastination and confocal microscopy, several recent studies have demonstrated that the configuration of the deep cervical fascia is much more complicated than previously thought. The prevertebral fascia envelopes the deep cervical muscles. Its anterior portion has been considered by some authors to be a double layered structure. The more anterior layer is called the alar fascia; the posterior layer is the prevertebral fascia proper and the space between them, the danger space. Thus, the alar fascia serves as the anterior wall of the danger space and the posterior wall of the retropharyngeal or retrovisceral space. Clinically, however, the danger space and retropharyngeal space are considered together as they are not differentiated radiographically. The key issue to clarify these conflicting views is whether there is the alar fascia. The aim of the present project was to determine the connective tissue configuration in the retropharyngeal region. Three adult cadavers aged from 67 to 89 years old were processed as sets of transverse plastinated sections of the neck (a total of 32 to 33 sections per cadaver) using the E12 sheet plastination technique. Our preliminary results indicated that the region between the vertebral column and the posterior pharyngeal wall had multiple and irregular fascial layers. A more or less constant layer was observed over the anterior surface of the vertebral bodies, anterior longitudinal ligament and deep anterior cervical muscles. This layer mainly contained longitudinally-orientated dense connective tissue fibers, presumably arising from muscular structures. Anterior to this layer were several layers of connective tissue fibers, often observed to be interwoven with each other and to form various patterns of fascia-like structures at different cervical levels. Laterally, this irregular multiple layered fascia continued with the carotid fascia, prevertebral fascia and the fascia around the pharynx and esophagus. These results indicate that there may not be a well-defined alar fascia and thus the danger space and retropharyngeal space may be a part of a single irregular space.

Double balloon endoscopy in dogs. ***Latorre R[1], I Ayala[2], F Soria[3], F Carballo[4], E Pérez-Cuadrado[4], C Sánchez[1], F Martínez[1], G Ramirez[1], E Abellán[3], E Estaca[3], R Henry[5]***. *[1]Department of Veterinary Anatomy and Embryology, Veterinary School, University of Murcia, Spain, [2]Department of Veterinary Surgery and Medicine, Veterinary School, University of Murcia, Spain, [3]Minimally Invasive Surgery Center, Campus Universitario, Cáceres, Spain, [4]Digestive Service, Hospital Morales Meseguer, Murcia, Spain, [5]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Deep insertion of an endoscope into the small intestine is difficult. A new method, double balloon enteroscopy (DBE), has been developed to improve access to the small intestine. The aim of this study was to evaluate the usefulness of this endoscopic system for small intestinal exploration in the dog. Experimental Procedure: This new method uses two balloons, one attached to the tip of the endoscope and the other one at the tip of an overtube. Four dog gastrointestinal tracts obtained from necropsy at the Veterinary Hospital, University of Murcia were plastinated as anatomical specimens. They were explored with DBE to check if the technical conditions of this method were optimal for dogs. The double-balloon endoscope advances through the intestine being held alternatively by the balloon on the endoscope and the balloon on the overtube. Results: It was possible to examine the entire small intestine.

Conclusion: Enteroscopy with the double balloon technique promises to become a standard method for diagnostic and therapeutic endoscopy of the small intestine in dogs without surgical laparotomy.

Principles of polyester P35 plastination technique.
Weiglein AH. *Institute of Anatomy, Medical University Graz, Graz, Austria, Europe.*

The P35-procedure is used to produce thin (4, 6, or 8 mm) and semitransparent slices. It utilizes the ***FOUR MAIN STEPS**** in plastination plus some extra steps especially for the production of the slice. ****FIXATION*:** Fresh brain specimens are fixed the usual way with 5-10% formaldehyde for three to six weeks. Specimens that have been fixed by other methods should not be used for the P35 procedure because fixatives other than formaldehyde may cause unintentional reactions with the polymer. ***Slicing*:** After embedding in 20% gelatin to prevent degradation of the slices the brain specimens are cut with a meat slicer into 4mm (or 6 or 8mm) slices. The slices are placed on stainless steel grids. The grids are piled up in a stainless steel basket. ***Flushing & Precooling*:** The basket of brain slices is rinsed with cold tap water overnight and cooled down to 5°C. Flushing may be extended to 2 days and is used to get rid of the fixative. ****DEHYDRATION*:** Subsequent dehydration in two baths of 100% acetone with an acetone-brain ratio of at least 10:1. The basket of brain slices is submerged in 100% acetone at -20°C (10:l per brain) for 1 to 2 days. The basket of brain slices is submerged in another bath of 100% acetone at -20°C (10:l per brain) for 1-2 days. Dehydration must be as complete as possible for brains - with an acetone concentration 98% or higher. *Caution:* Dehydrated brains become very brittle and breakable - handle with care! ***Immersion*:** The basket of brain slices is submerged in precooled P35/A9 mixture (100:2) for 1 day at 5°C (to -25°C). The basket of brain slices is submerged in fresh precooled P35/A9 mixture (100:2) for 1 more day at 5°C (to -25°C). *Caution:* Immersion baths must be kept in the dark to prevent the reaction mixture from polymerization. *Note:* The first immersion bath should not be reused and be discarded away after use. The 2nd bath may be used as 1st immersion bath for the next procedure. ****FORCED IMPREGNATION*:** The basket of brain slices is submerged in a fresh P35/A9 mixture (100:2) and placed under vacuum for 24 hours at -25°C or, if the vacuum chamber is too large, at room temperature. The vacuum is increased until 1-2mm Hg is attained when impregnating at -25°C or 10-15mm Hg is attained when impregnating at room temperature. *Note:* This bath may be used as 2nd immersion bath for the next procedure. ***Casting/ double glass chambers*:** The slices are removed from the vacuum chamber and individual slices are placed between two sets of glass plates. Each set consists of one outer sheet of safety glass and one inner sheet of float (window) glass, the latter sheet facing the brain slices. A silicone gasket (6mm for 4mm slices) is used to seal the chamber around the edges and fold-back clamps are used to fix the two double glass plates together. Then the glass chambers containing the specimens are filled with a fresh P35/A9 mixture (100:2). For filling the standard size chamber (35 x 45cm) about 700cc polymer mixture is needed. ****CURING:*** is a two step procedure consisting of subsequent application of UVA-light and heat (45°C). *Light Curing:* After casting, the double glass chambers are exposed to UVA-light for a period of 45 minutes to 4 hours depending on the wattage and on the distance of the UVA lamps. During this procedure it is necessary to cool the chambers either by ventilators on both sides or by blowing compressed air over both sides of the double glass chamber. *Caution*: Cooling is important because the UVA-light causes an exothermic that would destroy the specimens if they are not cooled. To prevent cracking of the P35 slices during light curing it is also recommended to use low wattage UVA-lamps and longer curing time. *Heat Curing:* Following light curing the double glass chambers are exposed to 45°C for 4 to 5 days in a well-ventilated oven. ***Finishing:*** After curing is finished the glass chambers are dismantled and the sections are trimmed on a band saw and the edges smoothed using a belt sander.

Epoxy polymer: old and new generations.
Tsabari S, EC Pace. *VisDocta Research Laboratory: Biological material preservation/Advanced polymers, Tignale, Italy, Europe.*

Previous epoxy systems, know as old generation epoxy were compared with two newly developed formulas of epoxy systems. These are labelled as new generation epoxy resins. Epoxy resin and components, hardener component and co-reactant component, were chosen for their specific predetermined characteristics and their suitability for solving common trouble areas in epoxy impregnated specimens. Their physical and chemical parameters were taken into consideration for five different types and combinations of tissues: connective, adipose, glandular, muscular, bone and mixed systems. The goal was to develop resins providing special performance properties that will assist both room temperature and cool temperature processes. The first product enables the plastinator to impregnate specimens and obtain excellent results at low and high profile (slides or cubes) 0.5mm to 1000mm. The second

product is an excellent medium for obtaining casts of vessels by using very low-density products. 0.1mm tubes and 50.0mm cylinders were realized and tested. Both products are characterized by: increased pot-life, reduced viscosity, colour/transparency stability, yellowing and blushing resistance and an insignificant shrinkage using pure elements.

Polyester plastination technique: Specific troubles and problems. ***Henry RW.*** *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

The introduction of polyester resins for sheet plastination two decades ago has revolutionized brain sectional study. Exquisite slices with remarkable differentiation of gray and white matter are generally obtainable if a few simple rules are followed. P35 and P40 are the two widely used polymers. P35 was introduced in the 1980's and yielded brain slices of unparalleled beauty, clarity and definition of white and gray matter. It was a perfect product and likely very difficult to improve upon. P35 is without flaw. If dropped on a hard surface, the P35 slice will likely fracture or crack. If too much UV light is used and/or not enough cooling is provided during curing, the enveloping glass plates may crack. Ten years later P40 was unveiled as a shorter and less cumbersome technique with good contrast of gray and white matter. P40 slices are good quality. However, the P40 polymer is not as problem free at least for the novice. Similar problems may occur during curing as with P35, glass fracture. However a major problem when used on brain tissue may occur: Orange areas in the gray matter appear. To date, the etiology of these spots is not known. Various reasons for the discoloration have been suggested: Incomplete fixation or wrong choice of fixative, incomplete impregnation, and tissue peroxidases. However, there seems to be no way to predict if this will occur and no clear resolution has been provided. To remedy this problem, an additive has been developed. It seems to minimize spots. Shrinkage of 4.5 to 7.0% has been reported, varying from cold to warm temperature impregnation. P40 slice production takes only five or six days to complete, while P35 slice production takes ten to twelve days. P40 slice production generally takes only one half of the glass to make the flat chambers when compared to P35 slices. P35 uses more resin (three immersion bathes) while P40 only one bath. Since catalyst is used in the production of P35 slices in the immersion and impregnation baths, the impregnated slices need to be cast within a few days. However, with the possibility of using no catalyst in the impregnation bath, P40 slices may be held for several weeks to months after impregnation in the impregnation bath before casting. If the P40 impregnated slices remain in the polymer for extended periods of time (months), the slices may adhere to the grid spacers that are used to separate the tissue slices. Since the early 2000's, P40 has been used successfully to produce slices from all regions of the body. Additionally, P35 has been used for head slices. P35 and P40 slices remain fully transparent with no yellowing over time. However, if the P40 sections are too thick or have dense, dark organs and catalyst has not been used, the polyester over this area of the slice may not completely cure or remain weak.

A study of osseointegration and nerve regeneration after dental implantology by means of P35. ***Weninger B, AH Weiglein.*** *Institute of Anatomy, Medical University Graz, Austria.Medical University, Graz, Austria, Europe.*

During the last year, studies have been conducted at the Institute of Anatomy, Medical University Graz to evaluate the osseointegration of dental implants and the regeneration of the inferior alveolar nerve, respectively. To this effect implants of different sizes were placed in the upper jaws of pigs. The lower jaws received only one implant on each side with the special goal of injuring the inferior alveolar nerve. The pigs were sacrificed after different periods of time. Both upper and lower jaws were embedded in P35 using the standard P35 plastination technique. To achieve histological sections of the bone-implant-complexes, the embedded upper jaws were cut with a diamond band saw (Exact 310 CP) at the exact level of interest and afterwards ground to the desired thickness with the diamond grinding system (Exact 420 CL). The lower jaws received the same treatment, only repeatedly so, to achieve a series of ultrathin sections. The pieces of the lower jaws containing the implants underwent MicroCT-scanning as well to allow a comparison with standard histological evaluation. The main advantage of using P35 as embedding medium - instead of methacrylate or Technovit 7200 - is the possibility to use larger objects because of using forced impregnation of P35.

Principles of polyester P40 plastination techniques. Sheet plastination with polyester. An alternative for all tissues. ***Latorre R[1], W Hernández[2], F Gil[1], J Arredondo[2], G Ramírez[1], O López-Albors[1], MD Ayala[1], JM Váquez[1], RW Henry[3].*** *[1]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain, [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad del Estado de*

México, México. [3]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, TN, USA.

Classically, the main application of polyester polymer (P40 and P35, Biodur™) is the production of brain or head slices. Recently semi-transparent body slices were produced using P40. The purpose of this study was to develop a protocol for using P40 to produce slices from all regions of the body. An unembalmed cat cadaver was cleaned and frozen at -70ºC. A modification of the P40 technique (von Hagens, 1994) was used to plastinate the 2.0mm sections. The manufactured tissue slices were semi-transparent. After curing, the polyester around the tissue was transparent and no yellowing was detected in any slices. The fat tissue was semi-transparent, and the other tissues or organs were significantly highlighted against the cleared fat. There were no problems impregnating or curing any type of tissue. All of the slices became hard after curing, even when the surface of the tissue slice was close to the glass of the flat chamber. The cast tissue slices provided a high degree of detail and permitted visualization of the various body structures in their normal topography of the region. Also the semi-transparency of the specimens allows viewing at the submacroscopic level. The results of this work demonstrate that the P40 method (Biodur™) may be used to produce semi-transparent body slices from any region of the body as with the E12 method (Biodur™).

Applications of plastination in dentistry: Evaluation of different antero- and retrograde root canal obturation methods, osseointegration and nerve regeneration after dental implantology. ***Weiglein AH[1], B Weninger[1]; L Kqiku[2].*** *[1]Institute of Anatomy and [2]Department of Conservative Dentistry, Dental Clinic, Medical University Graz, Austria.Medical University, Graz, Austria, Europe.*

During the last two years at the Institute of Anatomy, Medical University Graz, a dental research program based on polyester plastination and plastination micromorphology has been established. The central equipment for this program is the P35 plastination lab plus the Exact Slicing and Grinding System consisting of a diamond band saw (Exact 310 CP) and the diamond grinding system (Exact 420 CL) which allow: 1) Production of thin sections of P35 impregnated specimens at the exact level of interest and 2) Production of ultrathin sections for histological evaluation. These thin sections, at a predefined level, have been used to study the quality of different root canal obturation methods and to study a new methodology for obturation after root tip extraction. The ultrathin histology is recently used for the study of bone-implant-interfaces and bone remodelling in dental implantology. The later allowed large serial implant specimens to be viewed, which is not possible with the standard methacrylate protocol. Moreover, osseointegration was evaluated by plastination histology and Micro-CT to study the accuracy and comparability of both methods. Parallel to the bone remodelling study, regeneration of the inferior alveolar nerve was studied after injury during implant placement. In all studies, the results were excellent and yielded permanent thin sections of the desired region, which allow the quality of root canal obturation methods, implant-osseointegration and nerve regeneration to be studied in series. Thus, the study demonstrated that polyester plastination is an excellent replacement for standard histology embedding methods (e.g.: methacrylate) with the special advantage of being much less expensive.

Split brain CT and P35 data set. ***Weninger B, E Pusch, W Rosmarin, AH Weiglein.*** *Institute of Anatomy, Medical University Graz, Austria.Medical University, Graz, Austria, Europe.*

During the routine neuroanatomy dissection course a split brain - a brain without a corpus callosum was found. The brain was immediately taken to the CT scanner (Siemens Somatom AR.T) and an axial and coronal CT data set was acquired. The data set was used for 3D reconstruction using the 3D Doctor software on a regular PC. Subsequently, the brain was sliced in the coronal plane and the slices processed with the standard P35 method. A series of 4mm thick slices is now available to study the interesting anatomy of a split brain. Patients with absent corpus callosum are known to have no motor, sensory or intelligence deficits. However, the main problem remaining and not necessarily recognized is the fact that the right and left visual fields have no interconnection. Thus, in a short visual signal only to the "minor" (non-dominant) hemisphere, which usually is the right, no verbal response can be obtained and the patient is unaware of the occurrence of the signal. The dominant hemisphere is the verbal, linguistic, mathematical, analytical hemisphere with a direct link to consciousness. Whereas, the non-dominant hemisphere is mostly non-verbal, dealing with music, geometry, spatial comprehension with no positive connections to consciousness.

Plastination of 3.5-5.0 month old human aborted fetuses. ***Esfandiary E, M Mardani, M Naghdi.*** *Anatomical Sciences Department, Isfahan University of Medical Sciences, Isfahan, Iran.*

A collection of human fetuses with ages between 3.5-5.0 months needed both for an objective approach in teaching medical embryology, as well as, for educational purposes in forensic medicine, according to Islamic law. Therefore, fetuses were collected and preserved by a modified-polyester plastination technique. Twelve human aborted fetuses of 3.5-5.0 months of age were collected from university hospitals in Isfahan. These were fixed in a 10% formalin solution, cleared in hydrogen peroxide, if needed. Dehydration was down in acetone. The specimens were impregnated using a polyester resin, P75 with glycerine added. Resin was also injected by positive pressure in anatomical spaces for prevention of shrinkage. A collection of 12 plastinated aborted human fetuses were prepared. The color of specimen were clear except one of them which was an abortus, with black color, that was not treated with hydrogen peroxide, in order to show the color of a missed aborted fetus to the students. These polyester resin plastinated samples compared favorably with silicone plastinated specimens. Both polymers provide similar durability, but flexibility using resin with glycerine added for impregnation was greater than impregnation with silicone. This is probably due to the effect of glycerine in the impregnated polyester. In this project, silicone was substituted for an inexpensive polyester resin called P75, a provided interesting, dry, durable and odorless specimens. The specimens were good for teaching of embryology and for forensic medicine purposes.

The polyester technique for sheet plastination of the common dolphin. *Gao H, J Liu, S Yu, H Sui. Department of Anatomy, Dalian Medical University, Dalian, China.*

In order to display the structure distinctly for study and research, the polyester technique for sheet plastination of the common dolphin was used in the experiment. The process of sheet plastination was carried out as following: A dead common dolphin was divided into two parts, the head and the body. After freezing at -70°C, 43 sagittal slices of the head and 348 transverse slices of the body were made in total on a high-speed band saw. All the slices were fixed in 10% formaldehyde for two weeks, and then bleached using 5% dioxogen overnight. They were precooled in 5°C refrigerator prior to dehydration. The slices were dehydrated in a cold acetone baths, and degreased in an acetone baths gradually warmed to room temperature. Flat chambers were prepared for casting using two plates of tempered glass. Slices were placed between the two glass plates with a new resin mix for impregnation. The slices were impregnated under vacuum in the flat chambers. They were cured with heat in a water bath. After curing, the sheets were cut and trimmed to size and sanded. After the process of sheet plastination, the dolphin sections had excellent contrast between the different tissues. Detailed information on the anatomy of dolphin was provided in each slice. The results of our experiment were satisfying. The polyester technique for sheet plastination of common dolphin was practical.

Sheet plastinates for everyday teaching purposes. ***von Horst C.*** *HC Biovision, Mainburg, Germany.*

Sheet plastinates and pictures thereof can give amazing insights into the topography of anatomical structures. For a number of reasons the plastinates themselves are still not commonly used in everyday teaching. Some of the reasons are the: a) Need for seeing a series of plastinates to visualize complex anatomical structures, b) Risk of scratching and breaking, c) Yellowing of epoxy plastinates and d) Lack of manageability of sheet plastinates in classrooms and courses. We used the Tissue Tracing Technique (TTT) for the ideal display of anatomical structures. First a rather thick sheet plastinate is prepared, which includes all the structures that need to be shown. In a second step, plastinated tissue is taken away by grinding, so that even complex anatomical structures are visible in one single sheet plastinate. The thickness can be adapted at different parts of the plastinate individually. Casting the ground plastinates between Acrylic Protection Layers (APL) added the stability and manageability needed for teaching purposes. The plastinates were tested in practice by vets, farriers, in student classes, in lay education (Lernort Natur) and by a series of tests performed by ourselves. The ability of the plastinate to stand free on a plane surface was a big advantage in classroom teaching, while vets and farriers appreciated the manageable format and durability of the plastinates when taking them to their clients. Scratches which appeared on the APL could be removed by polishing the APL. Dropping the plastinate on a stone surface from 3m height in our tests led to breaking of edges and to limited detachment of APL. The plastinate was still usable for teaching. After severe damage of the APL from repeated hits and falls the TTT-sheet plastinate could be completely detached from the APL and showed no damage at all. After re-embedding between APL the appearance compared to a new plastinate. TTT-sheet plastinates are useful teaching aids. One single sheet plastinate can provide enough insights to add significant value to a teaching course. Its manageability and durability allow real hands-on experience in the class room. Through polishing and re-

embedding between APL, the TTT-sheet plastinates are extremely durable and practically ever lasting. Even old yellowed or scratched conventional sheet plastinates can be renovated with this method.

Plastination, learning styles and teaching strategies.
Easteal RA, L MacKenzie, CW Reifel, SC Pang, RE Hunt. *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

To best optimize a students performance and enjoyment in a Gross Anatomy course it has become increasingly clear that the student optimum learning style should be matched by the availability of teaching methodologies and specimens - especially plastinated specimens.
The three basic learning styles for Anatomy may be listed:
1. VISUAL - think in pictures
2. TACTILE - "hands on" learning
3. AUDITORY - you "see" words
How can modern, integrated, interactive teaching methods be tailored to meet all 3 *learning styles*? What are the educational strategies? At Queen's, we see several options:
1. Traditional lecture/lab
2. Lecture - plus self-directed lab (S.D.L.)
3. Lecture - S.D.L. plus team based learning (T.B.L.)
4. Lecture - peer teaching (P.T.)
We are able to have these options because we have:
1. 800 specimen traditional "wet" specimen museum
2. 600 plastinated "hands-on" teaching specimens
3. GAHIC - a web based atlas - with most of these specimens
4. A hard copy Atlas of our teaching collection
The paper will demonstrate how we match the three learning styles and the four teaching strategies with the specimens, and will emphasize the overriding importance of Plastination to our future plans.

Treasures of our department: The Professor Albert Gellért Anatomy Museum.
Weiczner R, A Mihály. *Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Szeged, Szeged, Hungary.*

In Europe, there has always been a tradition and custom to establish and continuously enrich "anatomical collections" or museums for the benefit of anatomical studies and research. Although the Anatomy Museum of our Department had been set up, around the end of the 19th century in Kolozsvár, its greatest development took place under the Institute leadership of Prof. Dr. Albert Gellért (1894-1967, Chairman of the Department: 1936-1967). After studying the traditional concepts of Frédéricq (1876) and Hochstetter (1927), he developed a new method (first published in Hungarian, 1935) that was successfully applied over 30 years. The freshly prepared demonstrative material was first fixed in formaldehyde-solution, and then dehydrated in alcohol solutions of increasing concentration, over few days-weeks depending on the size. After dehydration, the specimen was bathed in a mix of alcohol and benzene solutions with increasing benzene proportion, later on in pure benzene. After this pre-treatment, the preparates were paraffin-impregnated, at first in soft, than in hard paraffin wax over few days-one week in thermostat incubator. The preparate was chilled in room temperature, positioned, and then remodelling took place. Remodelling was carried out using a hairdresser's fan blowing hot air to soften the specimen. This was followed by careful re-shaping with a hot wax knife, in order to rearrange every detail according to the original characteristics. The specimen was then covered with a layer of varnish, and stained; joint ligaments blue; muscles brown; arteries red; veins blue; nerves yellow. Finally, a second layer of thin varnish was applied to the specimen which had already been mounted on a suitable metal and wood support. Our mission is to use and treasure this collection, the achievement of tremendous human endeavour for teaching and learning. An important step of our traditional anatomy final exam (Rigorosum) is based on the identification and description of certain anatomical relations and structures by using the original preparates of the Professor Gellért Museum.

Scientific potential of plastination: Tissue patterning and sheet plastination.
Zhang M. *Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand.*

The body arranges organs, tissues and cells in special patterns to form distinct structures through a set of developmental instructions that we do not fully understand. Elucidation of the mechanisms of the patterning is necessary to provide practical principles for functional replacements of diseased tissues and organs (e.g. tissue and organ engineering). Patterning at the cellular level and at developmental biology level has been extensively studied for decades. Evidence from a number of studies strongly indicates that the information controlling patterning is localized within the connective tissue. However, although the important role of the connective tissue in controlling the patterning has been well recognized, the patterning of connective tissue itself has been largely ignored, particularly at the tissue and organ levels. The major difficulty in studying the tissue patterning of connective tissue is that its delicate structure lacks a clear

demarcation from the surrounding tissue and thus is damaged or altered easily during dissection. Although histological examination may overcome the problem, application of such method is greatly limited by the size of sample areas. Sheet plastination provides a new approach to elucidate the patterning of the connective tissue at macroscopic and microscopic levels. The objective of this presentation is to use our recent studies on connective tissue patterning in the human cervical region (Johnson et al., 2000; Johnson and Zhang, 2002; Zhang and Lee, 2002; Nash et al., 2005a, Nash et al., 2005b) as an example to demonstrate advantages and limitations of the sheet plastination technique in studying the connective tissue patterning.

Plastinated specimens in a fully integrated teaching model. ***MacKenzie L, R Easteal, C Reifel, S Pang, R Hunt.*** *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

It is well established that students learn more effectively if they interact and are engaged (active learning) in the subject matter. We have adopted strategies focussed on active learning in large laboratory classes that are interactive and practical as possible. Concurrently, we have been able to optimize teaching time for our faculty while maintaining high educational standards for the students. Laboratory exercises of anatomical and morphological sciences are organized into modules and are essential for active learning by students. Each module contains detailed instruction, self-directed learning components and a variety of quizzes and self-assessment learning activities. In addition, our department has established an extensive, well-organized and internationally recognized anatomy museum consisting mainly of wet specimens. Our dissected specimens represent real structures and provide the students with a complete visual guide to the human body. However, handling the specimen is the best way to begin to understand and learn the three-dimensional aspects of the human body. We are actively involved in preparation of specimens using plastination technology. The plastinated specimens complement our collection of wet specimens but more importantly they augment the students learning by providing the physical "hands on" component of active learning. These specimens play a pivotal role in the student's learning whether it be instructor taught, self-directed or team based. As a result, the students have become much more independent and actively involved. Plastinated specimens have allowed us to present a complete visual and physical guide to the human body in a revolutionary way making anatomy more interesting, easier to learn and more relevant to the student's future career objectives. Our innovative approach is receiving excellent reviews, both informal and through formal course evaluations, from students of Medicine, Nursing, Rehabilitation Therapy, Life Sciences, and Physical and Health Education.

Plastinated specimens as an adjunct to dissection: Are they really helpful? ***Raoof A, L Liu, H Zhao, K Falk, T Bodnar, E Dueke.*** *Division of Anatomical Sciences, Office of Medical Education, The University of Michigan Medical School, Ann Arbor, Michigan, USA.*

At the University of Michigan Medical School, plastinated specimens have become an essential component of undergraduate, medical, and dental anatomy education particularly during the past few years. The aim has been to provide suitable specimens that reflect the essential concepts in anatomy in order to promote students independent learning. In the traditional, lecture-based undergraduate anatomy course, visits to the anatomy lab have been introduced where pertinent plastinated specimens are displayed. Members of faculty explain the anatomical and clinical material using plastinated specimens. A practical quiz will follow where students are asked to identify essential anatomical landmarks on those specimens. Innovative approaches to enhance the quality of plastinated specimens have been implemented, such as coloring neurovascular pathways and casting hollow viscera to demonstrate complex anatomical features. The validity of these specimens in facilitating anatomy learning has been assessed through surveys administered both to first- and second-year medical and undergraduate students. Results showed an overall acceptance of the plastinated specimens as a valuable adjunct to dissection. Forty four percent (44%) of the first year medical students believed that plastinated specimens were very helpful in learning the spatial relationships of important anatomical structures. While 33% of the second year medical students thought that coloring neurovascular structures was very helpful. Also, 65% of the undergraduate students strongly agreed that using plastinated specimens during lab visits was useful in understanding essential anatomical concepts. These specimens are planned for a wider use in the future to assist faculty and students in the effective utilization of the time allocated to anatomy.

Plastinated specimens and their use for universal design for education and multimedia for students with disabilities. ***Miklošová M[1], V Lucza[1], Z Daxnerová[1], Z Miklošová[2].*** *[1]Department of Biology and Ecology, Faculty of Science, University of P.J. Safarik,*

Košice, Slovak Republic, [2]University of Veterinary Medicine, Košice, Slovak Republic.

Development of special education software and multimedia products for students with different disabilities using principles of Universal Design and Human Computer Interface will be described. These exist as some original technologies for interaction with users with different levels of disability used in developing special education. The plastinated specimens were used as computer models and for animations created in CD MMS 2004. Presentations were used in common PCs with Windows 98 operation system and higher. The CD presentation contains information, which is usable in learning for the blind and in learning of students with other disabilities. Use of presentations (developed with the aid of plastinated specimens) in the youth centres after lessons is a meaningful application of computers in the schools. The presentations include special test parts with feedback information for students or teacher. Each presentation is developed as freeware. We have developed a special education software and multimedia product with application of previous principles of UDL and HCL. We produced special multimedia CDs. The CDs were tested in different schools for students with disabilities. The programmes have been created not only for education but also for probing, prevention, and rehabilitation of disabilities. The products take individual disabilities, their specific qualities and educating software availability rules into account. The CD includes special educational software with complex of functions: Education - parts of software with education software; Assessment - parts of software with tests; Diagnostics of disability - developing of special diagnostic software needs cooperation with psychologists and special educators; Therapy of disabilities, software in the form of a game or quiz helps students to eliminate their disabilities; Information - database of AT; Motivation - for students in school who work with software and programmers, research, each software contains documentation with own research of UDL and HCI. We intend to apply our experiences in development of new special education software for students with diverse disabilities. The special software and multimedia enable students with disabilities to be better integrated into schools and in social life. (Supported by CEGA Slovakia grant No. 3/ 3006/05).

Poster presentations

Technique for the demonstration of arterial arcades in the jejunum using the E12 technique of plastination, casting resin and silicone rubber injection. ***Mathura G, N Lachman.*** *Department of Human Biology, Durban Institute of Technology, Durban, South Africa.*

Often, the preparation of anatomical teaching and learning aids require innovative use of anatomical techniques that combine standard methods of plastination, resin cast preparation and silicone rubber injection. Arterial arcades of the small intestine are demonstrable features in the comparison between jejunum and the ileum. This study aimed to demonstrate by means of an innovative technique, the arterial arcades of a segment of a jejunum. During a postmortem examination two segments of approximately 120mm were carefully removed with the superior mesenteric artery attached. The harvested specimen were thoroughly washed in luke warm water and submerged in 3% formalin. Injection was achieved via a branch of the superior mesenteric artery. A volume of 50ml red pigmented casting resin was prepared and injected. This was followed immediately by injection of approximately 100 ml of clear resin into the lumen so that the intestine assumed its anatomical shape. The resin was allowed to set at room temperature and left overnight in cold water. On the following day the tissue was subjected to acid maceration in concentrated hydrochloric acid. After 24 hours the cast was washed, dried and varnished for display. The second segment was injected with pigmented silicone rubber via a branch of the superior mesenteric artery and prepared for plastination. Once again the lumen was injected with approximately 100ml clear resin so that the intestine assumed its anatomical shape. The tissue was dehydrated using alcohol at concentrations from 40% to 100%. Forced impregnation in E12 plastination solution was then administered, followed by curing. A purpose-made electrically lit box was constructed to house these specimens to give the desired effect.

Rejuvenation of damaged and post-plastination enhancement of human anatomy specimens for teaching and learning. ***Hunt RE, A Parfitt, LW MacKenzie, RA Easteal, CW Reifel, SC Pang.*** *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

During a regular academic year, more than 1,000 students utilize our teaching facilities per week. Most of these students handle our plastinated human Anatomy specimens to fulfill their learning experience. As a result of extensive use, many of our plastinated specimens have been damaged and therefore became less desirable for learning and teaching. Over the past few years, we have been investigating alternative methods for specimen preparation in an attempt to

increase the durability and to enhance special features of these specimens. Other laboratories have tried several methods to reinforce the orifices of organs but with little long-term success. In our laboratory, we have reinforced boundaries of a variety of orifices using common cotton string prior to plastination. The structural integrity of the orifices in these specimens remained intact for at least 2 to 3 years following extensive use. Moreover, we have also found that dissecting and removing tissue from damaged plastinated specimens could reveal structures that were not visible in the original dissection. Thus, post-plastination dissection can rejuvenate old and damaged specimens into new, structurally and functionally usable materials for learning and teaching. As well, we have recently been experimenting with a technique of post-processing staining (colouring) of plastinated specimens and found that commonly used plastination polymer can be easily tinted with various dyes. When properly applied to correctly-prepared surfaces, post-processing staining provides an enhancement for both old and new plastinated specimens. These simple techniques add longevity and enhancement to plastinated specimens that have become important learning and teaching tools for students and teachers of human Gross Anatomy at Queen's University.

Anatomy of the temporomandibular joint in the cat: A study using P40 sections. ***Arredondo J[1], A Agut[2], M Ayala[3], JM Hervás[3], A Arencibia[4], E Rodríguez[1], W Hernández[1], E Abellán[5], E Estaca[5], R Latorre[3].*** *[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México. [2]Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain. [3]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain. [4]Departamento de Morfología, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Spain, [5]Centro de Cirugía de Mínima Invasión, Cáceres, Spain.*

The temporomandibular joint (TMJ) has received little attention in cats. Its precise anatomy is poorly documented in textbooks and few reports describe it. A detailed anatomical knowledge of the TMJ is a prerequisite for assisting diagnostic techniques such as ultrasonography. The objectives of this work are: 1) To recognize macroscopic structures by using different anatomical techniques and 2) To identify the anatomical structures by ultrasounds. Dissections and synovial and vascular injections were performed in seven adult mixed breed cats. Thin cryosections (2-3mm) were obtained on the transversal and longitudinal planes and preserved using the P40 plastination method. The ultrasonographic study was preformed in three adult mixed breed cats, under deep sedation. An 11MHz linear transducer was used over the masseter muscle to make transversal and longitudinal approaches to the joint. A correlation between plastinated sections and ultrasonographic images was made. The condylar process of the mandible, retroarticular process, articular disc, articular space, articular capsule and capsular reinforcements were identified in the macroscopic sections. Also, the relationship between the TMJ and other structures such as the parotid gland, the maxillary artery and vein and their branches was evidenced. Attachments of the masticatory muscles to the TMJ were also observed. The vascular supply of TMJ was described in detail. The use of the plastinated anatomic cross-sections made on the same planes of the sonographic views in the cat TMJ, allowed a correct identification of all structures and to establish a direct correlation with other adjacent structures.

Anatomy of the pancreas of the pig: A study using P40 sections. ***Hernández W[1], S Fei[2], M Ayala[3], F Martínez[3], E Rodríguez[1], E Abellán[2], E Estaca[2], JM Hervás[3], J Arredondo[1], R Latorre[3].*** *[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México, [2]Centro de Cirugía de Mínima Invasión, Cáceres, Spain, [3]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain, [4]Departamento de Morfología, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Spain.*

Pigs have been used as large animal models of many human diseases. The porcine pancreas is similar to the human pancreas, it is almost a retroperitoneal organ and the pancreatic body wraps around the portal vein. Branches from the celiac and the cranial mesenteric arteries supply blood to the pancreas. The pancreatic branches of the splenic artery irrigate the left lobe, whereas the right lobe is irrigated by the cranial and caudal pancreaticoduodenal arteries. The anatomic location and the common anastomoses in pancreatic circulation make difficult its approach by classic surgery. The objective of this work was to study the topography of the normal pig pancreas. Eight juvenile pig cadavers (25kg) were injected with epoxy via the external iliac artery to form a vascular cast of the abdominal aorta. Dissections were performed and thin cryosections (2-3mm) were obtained on the transverse plane and preserved using the P40 plastination method. Branching patterns of the pancreatic arteries were then examined. These anatomical techniques allowed demonstration of the diameters of some pancreatic

arteries. This revealed they were large enough to be entered with endovascular catheters by using interventional radiology techniques. The retroperitoneal topography of the vascular supply of the pancreas makes it almost impossible to approach it by current surgical techniques.

Zinc chloride embalming technique and silicone plastination. ***van Toor I, V Verplancke, F van Glabbeek, M Vandersteen, E van Marck, H Bortier.*** *Human Anatomy and Embryology, Medicine Faculty, University of Antwerp, Belgium, Europe.*

It is known that formalin and phenol vapors are irritants to airways and eyes of man and animals. Personal health of the technician, tutors and students and efficiency considerations led to the adaptation of a zinc chloride embalming technique 6 years ago. Since the year 2000, the anatomy institute has embalmed 180 cadavers using this technique. All embalmed cadavers were used in dissection courses and experimental anatomy. The embalming solution contains 10 liters of Zinc Chloride® and 100ml Arthyl®. Embalming starts with the injection of fluid through the femoral artery within one week of death. Blood is not removed from the cadaver. In order to shorten the embalming period, a pump is fitted to the cannulae and the solution is pumped into the cadaver in three to four hours. After embalming, the cadavers can be stored for over 2 years at four degrees Celsius temperature. Five months after embalming, a cadaver was dissected and several organs and tissues were removed and prepared for plastination using the S10 Silicone technique. No irritation of the airways or the eyes was experienced during dissections by the technician, tutors and the students. Cadavers embalmed with zinc chloride have more flexible joints than cadavers embalmed with formalin and phenol. Natural colors are preserved and odor is low. Microscopic morphology of embalmed cadaver specimens, removed one year post mortem, is comparable with microscopic morphology of specimens at the moment of embalming. The embalmed specimens were compatible with acetone dehydration for plastination. The plastinated specimens of the pharynx, foot, a section of the lower limb, two kidneys, spleen, liver, lung, knee, cerebrum, cerebellum, part of the chest wall, testis and two different hand specimens retained their natural color and macroscopic morphology. After plastination of the specimens, the median shrink percentage was 10 percent. The adapted embalming technique fixes the tissues sufficiently for over a year. Because of flexibility of tissues and joints in cadavers embalmed with the adapted zinc chloride solution, surgical techniques can be exercised. It is possible to plastinate specimens removed from a zinc chloride embalmed cadaver using the S10 plastination technique. The plastinates are used for educational purposes in the medical curriculum at the University of Antwerp.

Plastination laboratory at the Faculty of Veterinary Medicine, Zagazig University, Egypt. Aly AE[1], H König[2], M Sora[3]. [1]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Zagazig University, Egypt, [2]Veterinary Medicine University, Vienna, [3]Medical University, Vienna, Austria, Europe.

The Zagazig Plastination Laboratory was established at the Faculty of Veterinary Medicine, Zagazig University, Egypt, to enhance the teaching of Anatomy for both Veterinary and Human medical students. The Laboratory contains 4 Plastination deep freezers (ca.250 Liters, -25°C), six stainless steel containers for dehydration, two plastination units from Biodur, Heidelberg, Germany and one local made Plastination unit. Two curing units are available, one gas curing and another ultraviolet curing for Polyester 40. At this stage, the Laboratory is designed for preparation of Plastinated specimens by the S10 technique and P40 for sheet plastination of brains. A plastination exhibition is established with more than one hundred plastinated specimens. Students are very happy with this exhibition, which helps them in their studies. International symposium on Plastination was held on February 12, 2006 with more than two hundred Anatomists from Egyptian and Austrian Universities. Now we plan to make plastinated specimens at all Egyptian Universities. The Plastination Laboratory is supported by a Project from Higher Education Enhancement Program Fund (HEEPF, 2nd cycle 2004, Code B-053-To).

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Minutes of the 13th Biennial Meeting of the International Society for Plastination (ISP)
Vienna, Austria, July 5th 2006

The participants were asked to move the business meeting of ISP from Friday, July 7th in the morning to Wednesday, July 5th in the afternoon. Participants consented to do such.

Call to Order:
The 13th Biennial Meeting of the society was called to order at 14:15 by vice-president Andreas H. Weiglein.
A quorum was established.

1. **Reports of the Officers:**

President's Report:

MC Sora mentioned that the past meetings in Murcia, Spain (2004) and in Ohrid, Macedonia (2005) had been very successful ones.
On request (von Horst) and in the absence of the chair of the nominating committee (Geoffrey Guttmann), the president explained once again the election modus was via the internet.
The president furthermore pointed out that he has worked towards increasing membership of the society. Each member was also encouraged to recruit new members. He encouraged members to promote plastination where ever possible but especially among clinicians of which he expects much impact.

Vice-President's Report:

AH Weiglein reported that there were 89 dues paying members, 4 emeritus members and 2 student members in the society. The half day plastination morning meeting at the EACA's conference in Palermo (2005), was very successful and served as promotion of the ISP among attending anatomists and clinicians.

Treasurer's Report:

RW Henry reported the following financial situation:
Balance 1/1/05: $29,366.48
Income 1/1/05 to 30/6/06: $6,441.64
Expenses 1/1/05 to 30/6/06: $4,022.80
Net income 1/1/05 to 30/6/06: $2,418.84
Balance 30/6/06: $31,785.32
Outstanding expenses $4,200.00 (Journal Vol. 20 & postage)
In addition to the Tennessee account, an account is kept in Graz for Europe.
Treasurer's report was moved (Easteal), seconded and accepted.

2. **Reports of the Committees:**

Local Organizing Committee:

MC Sora reported that 60 participants from 22 nations are taking part in this meeting.

Journal Committee:

In the absence of the Editor in Chief Robert Reed, Robert Henry gave the report. We hope that articles currently under review will be completed to publish Volume 21 at the end of December. Members are encouraged to write and submit articles. At the present rate of papers submitted about 1 issue of the journal per year can be published. To hasten the publication process, authors need to follow the journal's "guidelines for authors" accurately. Reviewer questions, asked to clarify unclear items and to provide information which is lacking need to be addressed in a timely and thorough manner. Color production costs are quite reasonable, so consider using more color pictures in your manuscripts when appropriate. Authors should cite all applicable references.
Von Horst - When may articles be sent in. Articles can be submitted anytime, but please follow the guidelines for authors. Asadi - Are their impact points for the ISP journal. Henry - Not so far, but hopefully next year. Dr. Reed is working on this matter. Weiglein - Citing references from the ISP journal is an important factor to achieve impact points.

Promotion Committee:

Ming Zhang reported on the activities of the promotion committee, established at the 12th international meeting of the ISP in Murcia in 2004. The following members made contributions to activities: Weiglein, Sora, Henry, Cook, de Jong, Latorre and Zhang.
The greatest achievement was a symposium entitled "Current Trends in Plastination" which took place at the 8th conference of the EACA in Palermo, 2005. Other contributions included 7 invited speeches at several other conferences (European Association of Veterinary Anatomy, Japanese Association of Veterinary Anatomy, Romanian Orthopaedic Surgery, Australian and New Zealand Association of Clinical Anatomy, Symposium on Plastination in Egypt), as well as, 59 publications related to plastination in 37 different journals.

Nominating Committee:

In the absence of Geoffrey Guttmann, the report was given by AH Weiglein.