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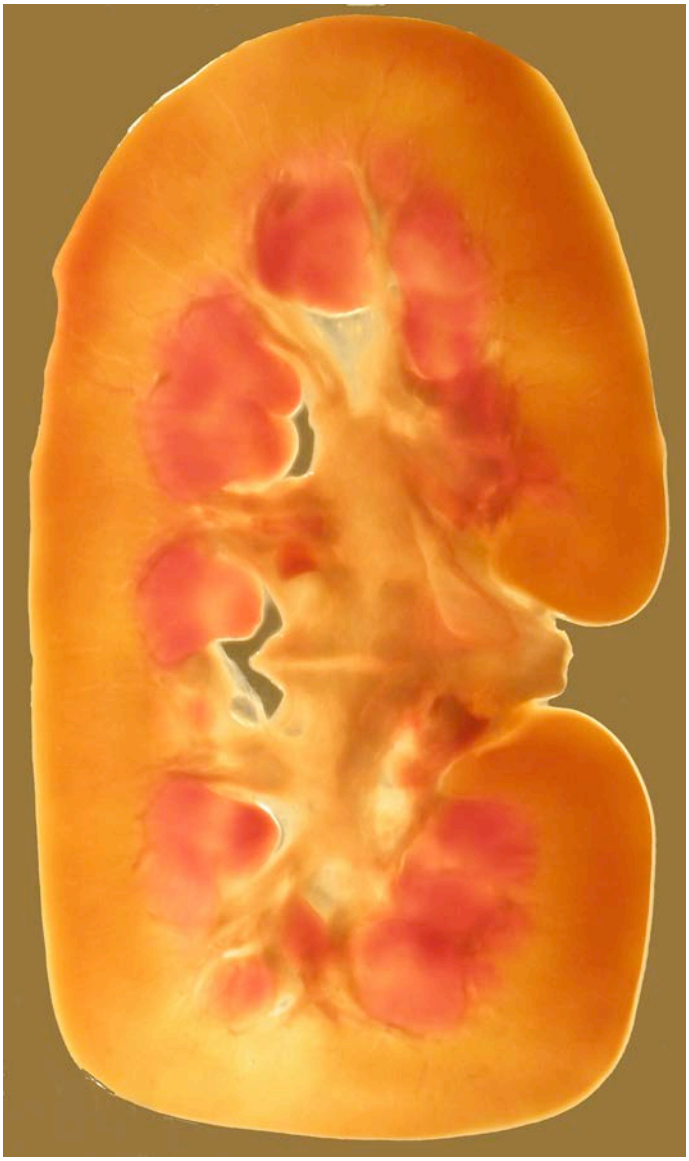
INTERNATIONAL SOCIETY for PLASTINATION



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Manuscripts and figures intended for publication in The Journal of the International Society for Plastination should be sent preferably via E-mail attachment to: **rbreed@utk.edu** or via the post via diskette to: R. B. Reed, 2407 River Drive, Knoxville, TN 37996-4543, USA. Photographs and illustrations should be sent via the postal service along with a hard copy of the text. Manuscript preparation guidelines are on the last pages of this issue.

The Journal of the International Society for Plastination

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Letter from the editor

This 23rd issue of the Journal of the International Society for Plastination will be the final issue. First published in 1987, the JISP was the vehicle for publication for the International Society for Plastination. The Society decided that a change of name for the journal will provide the field of plastination with greater visibility. The new journal of the International Society for Plastination will simply be titled Plastination. The date of publication for issue number 1 of Plastination should be 2010. We hope you will continue to follow the advancements in this field by way of this new journal and hope you find success in your endeavors in the area of tissue preservation.

Minutes of the 14th Meeting of the International Society for Plastination (ISP) Heidelberg, Germany, 20-26th July 2008

Call to Order:
The 14th Biennial Meeting of the society was called to order at 15:45 by the vice-president Andreas H. Weiglein.

Attendance: 31 members

Agenda

Elections:

In the absence of Geoffrey Guttmann the report was given by MC Sora.
The results of the election of officers, which had been held over the internet and ended on July 1st were announced and are as follows:
President: Mircea-Constantin Sora
Vice-President: R. Latorre
Treasurer: Ameed Raoof
Secretary: Barbara Weninger

The next Biennial Meeting 2010 will be hosted by Scott Lazanoff in Hawaii, USA.

Decision: The above board members have been nominated/elected through the email list. A large number of the existing members were not aware of the ballot and didn't receive any email notification. The Board decided to wait until August 30th, 2008 for new nominations and possible re-elections. Otherwise, the above members will continue as the new executive members.

B. Membership:
Members suggested new plans for increasing the ISP membership. Current number is 88. The proposals below have been discussed.

C. The proposals from the Promotion Committee

(1) Backgrounds: the committee
End-users

(2) Challenges:

To increase quantity of manuscripts for the JISP

To help the members, particularly the academic staff, for their research performance

To increase the awareness of plastination

(3) Proposals:

Proposal A - Objective: to increase quality of original research articles
Means: to set up an irregular forum for the end-users
Voted for and approved unanimously.

Proposal B – Objective: to promote plastination to anatomists# societies
Means: to offer free access to the JISP
to offer free access to the plastination propocols
to integrate with annual meetings of various anatomists societies.
Voted for and approved unanimously

4. Journal

Members suggested improving quality of manuscripts; offering the journal online; and changing the journal's title to become: **Plastination**.

Two members: **Selcuk Tunali** and **Carlos Baptista** volunteered to collaborate and have the journal available online, and to enhance the ISP web page.

D. Other discussion issues:

1. Bob Henry read the latest financial statement for the ISP account balance. Current balance is: $24,324.85 details of the report will be sent later.
2. Andreas Weiglein suggested collaborating with the upcoming FASEB meeting in South Africa in August 2009. He encouraged the idea of having a combined ISP and AACA meeting in 2010 in Hawaii.
3. Ming Zhang suggested enhancing the Journals impact factor by improving manuscript quality, and by addressing the academic and technical membership interests.
4. Ameed Raoof suggested enhancing ISP visibility and membership during the upcoming period.
5. Angelina Whalley suggested linking Biodur to the ISP website.

Meeting was adjourned at 5:00 PM.

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Acetone Discoloration of Epoxy Reaction-Mixture

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Abstract: To determine the involvement of acetone in the discoloration of cured epoxy polymer, epoxy reaction-mixture was combined with different concentrations of acetone before being allowed to cure at either one atmosphere or full vacuum. Acetone was found to have a direct impact on the degree of discoloration of cured epoxy reaction-mixture. Curing the epoxy reaction-mixture under vacuum decreased discoloration caused by the acetone.

Key words: **plastination; epoxy; acetone; discoloration**

Introduction

Tissue slices plastinated with epoxy polymer are finding their way into medical research (Guhr et al., 1987; Phillips et al., 2002) and education (Cook, 1997a; Cook 1997b). Being used in these disciplines, it is necessary to minimize artifacts introduced during and after the plastination procedure to ensure accurate assessment of tissue samples. Attempts have been made to increase the transparency of specimens plastinated with epoxy polymer (Mathura and Satyapal, 2000) as well as to decrease the yellowing of cured epoxy following plastination (Latorre et al., 2002). Improved techniques for epoxy plastination have been reported but do not include methods for reducing or eliminating the discoloration of cured epoxy (Fasel et al., 1988; Alston et al., 1997). Shrinkage of tissues plastinated via the E12 technique has been measured and documented (Sora et al., 2002) yet no information is currently available documenting the discoloration of cured epoxy polymer.

Acetone has classically been used as the dehydrating agent for epoxy plastination (von Hagens, 1989; Weber and Henry, 1993; Sora and Cook, 2007; Cook, 2007). Experiments have been performed to determine the optimum method for using acetone as a dehydrating agent for tissue (Holladay, 1988; Brown et al., 2002). However, investigations are lacking in the area of determining possible unwanted side effects of the acetone dehydration on different plastination procedures. This experiment was designed to determine if acetone is involved in the yellow discoloration evident in cured epoxy polymer.

Materials and methods

E1 and E12 were mixed at 95 parts of polymer to 26 parts of hardener by weight as per von Hagens (1989). Glass separator (AE30) was intentionally omitted from the reaction-mixture to eliminate any involvement it may have in the discoloration of epoxy polymer. Forty milliliters of this epoxy reaction-mixture was poured into each of fifteen 50 ml glass beakers. The beakers were randomly divided into five groups of three beakers each. The beakers in group 1 were used as experimental controls. One milliliter of acetone was added to each of the beakers in groups 2 and 3 and stirred into the epoxy reaction-mixture. Eight milliliters of acetone was added to each of the beakers in groups 4 and 5 and stirred into the epoxy reaction-mixture. The beakers in groups 1, 3 and 5 were left uncovered on a counter top for 24 hours.

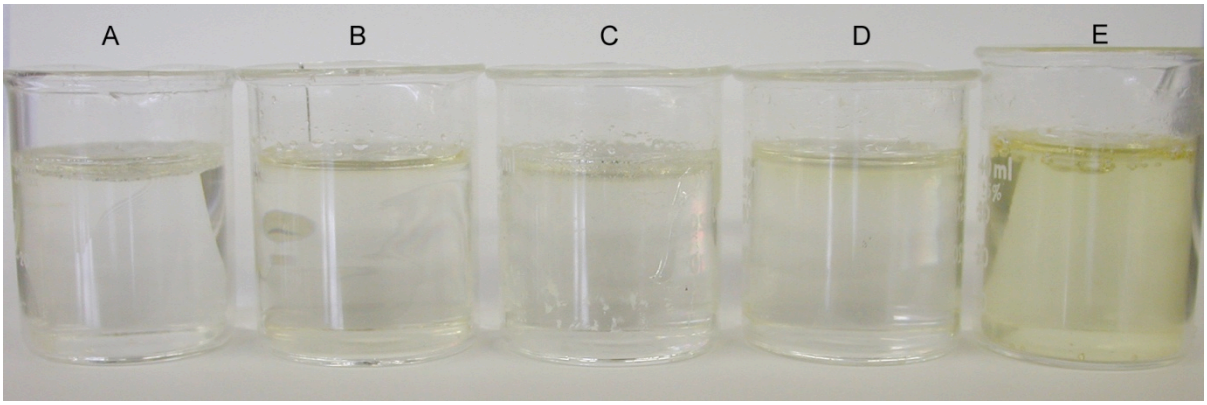

Figure 1. Epoxy reaction-mixture cured for 6 months. Reaction-mixture control (A), reaction-mixture with 1.0ml acetone and subjected to vacuum (B), reaction-mixture with 1.0ml acetone (C), reaction-mixture with 8.0ml acetone and subjected to vacuum (D), reaction-mixture with 8.0ml acetone (E).

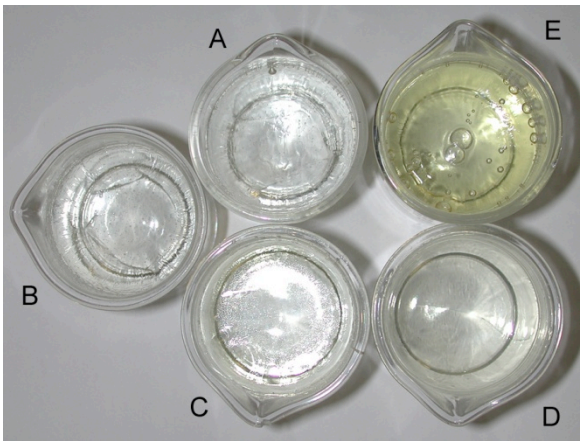

Figure 2. Epoxy reaction-mixture cured for 6 months (same samples as those in figure 1). Reaction-mixture control (A), reaction-mixture with 1.0ml acetone and subjected to vacuum (B), reaction-mixture with 1.0ml acetone (C), reaction-mixture with 8.0ml acetone and subjected to vacuum (D), reaction-mixture with 8.0ml acetone (E).

The beakers in groups 2 and 4 were placed into a vacuum chamber. The pressure in the vacuum chamber was slowly decreased over six hours to the point of full vacuum. The beakers remained at the point of full vacuum for 24 hours. The epoxy reaction-mixture in the beakers of all groups was examined for curing after 24 hours.

The hardened epoxy reaction-mixture in all groups was stored in the dark and evaluated for signs of discoloration at 24 hours, six months and twelve months post experimentation.

Results

The epoxy contents of all beakers had cured after 24 hours. All reaction-mixtures appeared clear 24 hours following curing regardless of the exposure or non-exposure to acetone or vacuum. The control beakers containing epoxy reaction-mixture in the absence of acetone remained clear six months after curing (Figs. 1 and 2).

At six months post-experimentation, all beakers containing the reaction-mixture and 1.0ml of acetone, regardless of whether they were placed under vacuum or not, showed only a very slight yellowing that was best observed when viewing the beaker from the side (Fig. 1). The cured reaction-mixture exhibited a slightly darker yellow discoloration at its surface (Fig. 1). This greater discoloration extended 2.0mm into the cured polymer and was not visible when viewing the cured epoxy from above (Fig. 2).

The beakers containing epoxy reaction-mixture and 8.0 ml of acetone that were exposed to vacuum showed a yellowing of the cured mixture that was greater than that evident in beakers treated with 1.0ml of acetone (Figs. 1 and 2). These beakers also exhibited yellowing at the surface at six months post-experimentation to a greater degree than did the beakers containing 1.0ml of acetone (Fig. 1). The yellowing was first evident at four months post-experimentation. This yellow discoloration extended 6.0 - 8.0mm into the cured polymer. The yellow discoloration of the cured epoxy reaction-mixture was evident in these beakers when viewing

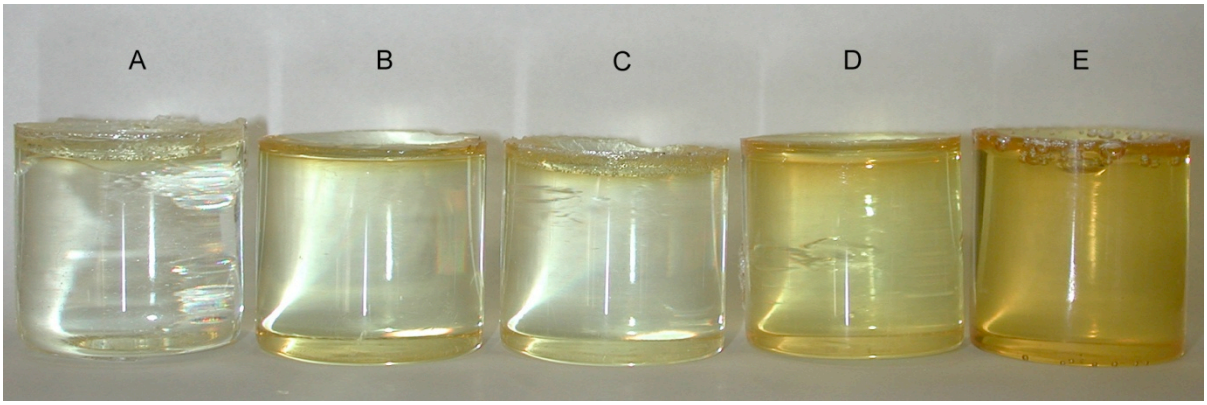

Figure 3. Epoxy reaction-mixture cured for 1 year (same samples as those in figure 1). Reaction-mixture control (A), reaction-mixture with 1.0ml acetone and subjected to vacuum (B), reaction-mixture with 1.0ml acetone (C), reaction-mixture with 8.0ml acetone and subjected to vacuum (D), reaction-mixture with 8.0ml acetone (E).

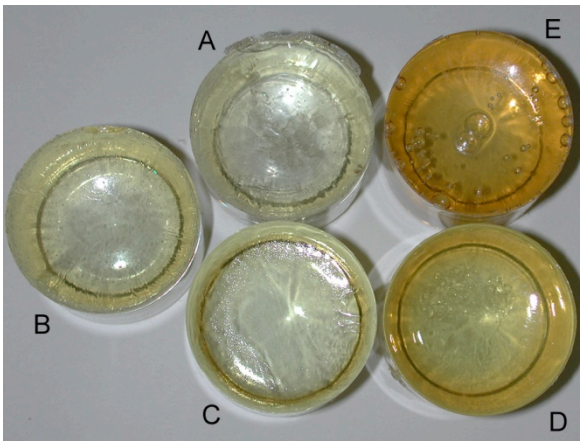

Figure 4. Epoxy reaction-mixture cured for 1 year (same samples as those in figure 1). Reaction-mixture control (A), reaction-mixture with 1.0ml acetone and subjected to vacuum (B), reaction-mixture with 1.0ml acetone (C), reaction-mixture with 8.0ml acetone and subjected to vacuum (D), reaction-mixture with 8.0ml acetone (E).

them from above (Fig. 2).

The beakers containing epoxy reaction-mixture and 8.0mls of acetone and allowed to cure at 1.0 atmosphere exhibited a marked yellow discoloration throughout the extent of the cured epoxy at six months post-experimentation when compared to all other test mixtures. (Figs. 1 and 2). This discoloration first became evident 3 months post-experimentation.

Between six months and one year post-experimentation, the glass beakers began to crack and subsequently fall away from the cured reaction-mixture.

At one year post-experimentation, control beakers and all beakers containing 1.0ml of acetone showed a very light yellowing throughout the cured reaction-mixture (Figs. 3 and 4). The yellowing was slightly less evident in the control beakers when viewed from the side (Fig. 3). The beakers containing the reaction-mixture with 8.0mls of acetone that were placed under vacuum exhibited a deep yellowing of the cured reaction-mixture (Figs. 3 and 4). Beakers containing the reaction-mixture with 8.0mls of acetone that were not subjected to vacuum showed a marked discoloration of the cured reaction-mixture to the point it appeared orange (Figs. 3 and 4).

Discussion

The epoxy reaction-mixture control samples remained clear longer and yellowed to a lesser degree than all the others due to the lack of effects of acetone on the cured mixture. The fact that it did discolor to some degree suggests that acetone is not required to be present for epoxy reaction-mixture to discolor in some manner.

The reaction-mixtures to which 1.0ml of acetone was added cured no differently from one another, whether or not vacuum was applied. When these were compared to the control beakers, they did discolor to a greater degree which can only be attributed to the presence of the acetone. The fact that there was no discernable difference between those beakers to which vacuum was applied and those which were not

subjected to vacuum would suggest that the mere presence of 1.0ml of acetone was enough to discolor the surface of the cured reaction-mixture at six months and discolor the entire mixture at one year whether the acetone was forcibly removed or not. As there was a difference between beakers containing 8.0mls of acetone when subjected to vacuum compared to those that cured at 1.0 atmosphere, we may conclude that the vacuum was able to remove some acetone from the reaction-mixture. In comparing the beakers to which 1.0ml of acetone was added to those with 8.0mls, it appears that vacuum is able to remove acetone to some degree yet not entirely. It cannot be determined from these experiments if the vacuum was unable to remove all of the acetone prior to the acetone affecting the reaction-mixture or if vacuum simply cannot remove all acetone before the reaction-mixture cures and that which is left will then act to discolor the reaction-mixture. Additionally, is vacuum unable to extract acetone once acetone decreases to an undetermined concentration in the reaction-mixture.

The reaction-mixtures to which 8.0mls of acetone were added were markedly discolored by the presence of the acetone regardless of the effects of the vacuum. The results using 8.0mls of acetone clearly demonstrate that if acetone can be removed from the reaction-mixture before it cures, specimens will exhibit less discoloration after the passage of time.

Epoxy reaction-mixture used for impregnation is regularly used for casting the impregnated tissue. As soon as tissue ceases to release acetone bubbles, impregnation is considered complete, however, the reaction-mixture will be saturated with acetone. It would stand to reason that the sooner the impregnation reaction-mixture is used for casting following the completion of impregnation, the greater the probability for epoxy yellowing following curing. In actuality, the time that exists between completion of impregnation and curing of the reaction-mixture is most likely too short for much acetone to escape through the surface of an ever hardening mixture. This technique will encapsulate the tissue slice with reaction-mixture containing acetone which will eventually lead to discoloration of the specimen. If cost is not an issue in specimen preparation, it would be best to use freshly mixed epoxy reaction-mixture which has never been in contact with acetone to plate the tissue slices. This should keep discoloration to a minimum. If a vacuum is applied to reaction-mixture which has been used for impregnation before it is used for casting, it should eliminate some acetone. This application of vacuum will also cause the release of bubbles from the reaction-mixture which are not related to acetone and which should not be confused for a highly acetone saturated reaction-mixture as this bubbling will not cease until the curing reaction-mixture becomes so viscous that bubbles can no longer escape (Reed, 2003).

The cured epoxy reaction-mixtures shown in figures 3 and 4 are not contained within a glass beaker. Over time, the contracting reaction-mixture pulled on the glass and caused the glass beakers to crack and subsequently fall away as there was no glass separator included in the experiment.

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Silicone Plastination Procedure for Producing Thin, Semi-transparent Tissue Slices: A Study Using the Pig Kidney

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Abstract: Kidneys from five month-old mixed-breed pigs were collected and 2-3mm thick longitudinal slices were prepared for viewing sub-gross anatomy as fresh tissue or as routinely plastinated tissue with and without degreasing. Standard cold silicone plastination procedures were used. Sliced fresh tissue and cured plastinated specimens were placed on a glass plate and back-lit to evaluate anatomical detail. All specimens yielded similar anatomical detail. However, degreased, plastinated specimens yielded the most anatomical detail. These thin silicone slices produce a durable permanent record similar to epoxy sections without the need for casting slices.

***Keywords*: plastination; silicone; porcine; kidney; tissue**

Introduction

Plastination of tissue by polymer impregnation is a unique method for preserving specimens in a permanent way (von Hagens, 1986). Sheet plastination is an internationally accepted method for preservation of tissue slices (Weber and Henry, 1993; Sora and Cook, 2007; Weber et al., 2007). It has been shown that sheet plastinated slices are excellent tools for demonstrating the anatomical topography of structures within specimens. The E12 and P35/40 techniques have become the methods of choice for creating 2-5mm or even 8mm semitransparent organ or body slices (Sora and Cook, 2007; Latorre and Henry, 2007). These techniques offer a unique opportunity for radiographic-anatomic-pathologic correlation and facilitation of understanding of complex anatomical relationships (Mc Niesh, 1988).

The S10 technique is best known for preservation of isolated organs or whole bodies (Henry, 1997; de Jong and Henry, 2007). This technique is also used for producing thick slices (0.5 – 1.0cm or more) which are utilized widely for education (von Hagens, 1987; Weiglein, 1997). S10 plastination of entire or halved kidneys is common (von Hagens, 1986; Oostrom, 1998; Ilieski, 2005; Pereira-Sampaio et al., 2007). However, no studies were found in which thin slices of kidneys were prepared and plastinated using the cold silicone S10 process.

Therefore, in order to visualize sub-gross structures of the kidney for anatomical study and research, a protocol was developed which could use the S10 method for producing thin sheet plastinated kidney slices.

Materials and methods

A total of 60 kidneys from five month-old pigs weighing 95kg (mean) were collected from two breeds at the abattoir for this study. Thirty kidneys were from mixed-breed Dalland pigs and 30 kidneys were from mixed-breed Landrace/Yorkshire pigs. The kidneys were removed from the pig carcasses together with the

surrounding adipose tissue in order to preserve kidney shape and size. Upon arrival at the laboratory, the renal fat and capsule were removed and the kidneys were flushed in cold tap water for three hours. The kidneys were then cooled in a refrigerator at +4°C for two hours to firm the tissue for slicing 2-3mm thick longitudinal sections.

Specimen preparation, dehydration and defatting

Tissue slices were cut on a deli slicer (Fig. 1) and divided into three groups: 1. For plastination after fixation and degreasing, 2. For plastination after fixation but without degreasing, and 3. For viewing as fresh tissue (no fixation or plastination).

Group 1. Each slice for plastination was numbered and placed on a wire mesh (Fig. 2). Another wire mesh was placed on top of the slice forming a sandwich of wire mesh/specimen slice/wire mesh/specimen slice/wire mesh, etc. (Sora and Cook, 2007; Henry and Latorre, 2007). The stack of sandwiched slices was placed in a plastic box and the slices were rinsed with flowing cold tap water for one hour to remove remaining blood (Weiglein, 1997; Sora and Cook, 2007). After flushing, the stacked slices were submerged in 3% formalin solution for five days for fixation (Oostrom, 1987). After fixation the sandwiched slices were transferred into in a stainless steel basket and were rinsed with cold tap water overnight to flush out the formalin. Before dehydration, the slices and water were pre-cooled in a refrigerator (+5°C) for five hours. Dehydration of specimens was carried out using the freeze substitution method in pure, cold (-25°C) acetone with a fluid:tissue ratio of 10:1 (Tiedemann, 1988). The basket with slices was removed from the water bath and submerged in the first 100% acetone bath for five days. The slices were then transferred into the second acetone bath for another five days. After transfer of the slices into the third acetone bath for five days, acetone concentration was monitored to make sure that the final acetone percentage was at least 99% for three more days. Acetone purity was monitored with an acetonometer (de Jong and Henry, 2007). After complete dehydration of specimens, the final acetone bath was allowed to gradually warm to ambient (room) temperature for three days to hasten degreasing of the specimens.

Group 2. Slices were produced to compare the clarity of anatomical structures of plastinated pig kidney slices to that of plastinated slices which had not been degreased prior to impregnation. This protocol was the same as for the degreased slices, except they were impregnated when dehydration was complete and not brought out to degrease at ambient temperature. The slices were placed directly into the impregnation polymer from the cold acetone. Impregnation and curing were identical to the degreased plastinated slices.

Group 3. Slices were produced to observe and compare their anatomical structure as fresh tissue slices only and were not plastinated. Their initial preparatory steps were identical to those of slices to be plastinated. However, after the one hour flush to remove any remaining blood and before fixation, their anatomical features were examined using bright, back light illumination similar to viewing of the plastinated slices.

Figure 1. Preparing to cut fresh 2-3 mm longitudinal pig kidney slices on a meat slicer.

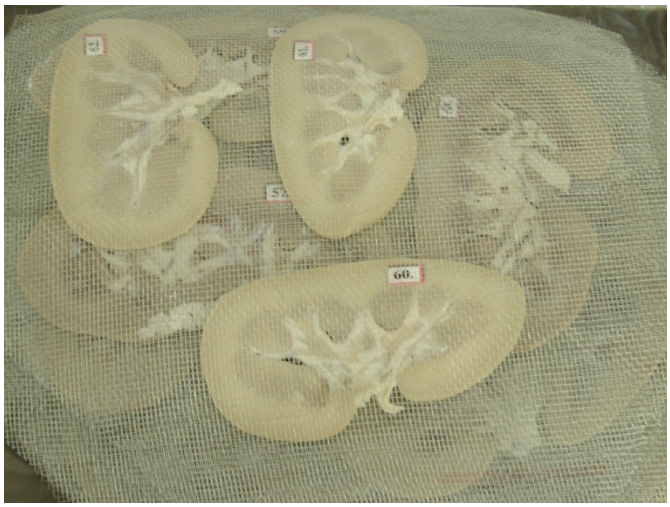

Figure 2. Thin kidney slices, numbered and placed between wire mesh.

Impregnation and curing

Cold impregnation of kidney slices with the silicone reaction-mixture was carried out by continuous impregnation as established by Dr. von Hagens (1986). The dehydrated slices were immersed in a mixture of silicone polymer and catalyst [containing a chain

extender (S10/S3)] at a ratio of 100:0.5 and allowed to sit and equilibrate in the -20°C polymer-mix for three days.

Thereafter, vacuum was applied and pressure was slowly decreased to ~8mmHg over 10 days. The rate of pressure decrease was monitored by observing bubble formation on the polymer surface and then setting the parameters daily for the digital vacuum controller (Fig. 3). The controller consists of a digital manometer and needle valve for pressure increase (open the valve) or pressure decrease (close the valve). The vacuum controller semi-automatically decreases pressure once it has been programmed for that incremental decrease. Prior to a decrease of pressure, the controller will allow an increase in pressure of 10mmHg. This increase in pressure allows tissues to relax and thus release the vaporized acetone which in turn allows better uptake of the S10/S3 mixture into the tissue and hence minimizes shrinkage. Then the controller automatically decreases pressure to the set level to maintain bubble formation and acetone vaporization. The pressure parameters are set daily in conjunction with observation of bubble production.

Figure 3. Digital controller for precise pressure control.

Impregnation was judged complete when bubble production ceased and pressure was stabilized at ~8mmHg. After impregnation, the vacuum chamber and its contents were removed from the deep freezer and placed at room temperature. The pressure was slowly increased to atmospheric pressure over a three day period (Henry and Nel, 1993; deJong and Henry, 2007).

The impregnated slices and grids were removed from the plastination kettle as a unit and the excess coating of polymer was allowed to drain from the slices and screen (Fig. 4). After draining, the slices were placed on paper towels and covered with towels for 24 hours to continue the removal of excess surface silicone (Fig. 5).

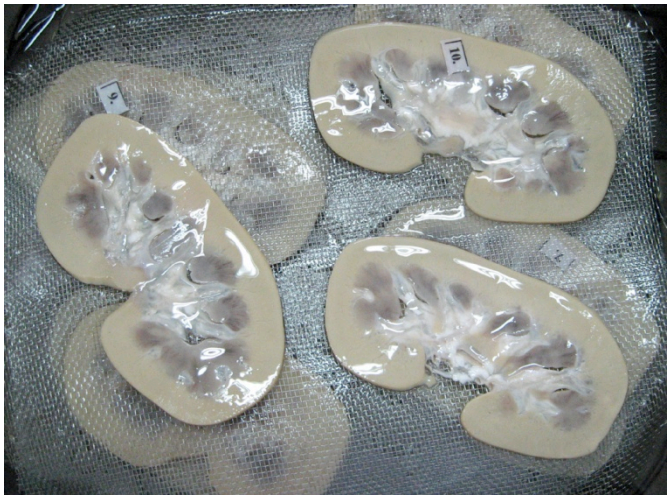

Figure 4. Initial draining of excess polymer after impregnation.

Figure 5. Final draining of impregnated slices.

Curing was carried out in a gas curing chamber in which the specimens were exposed to S6 vapors for five days at room temperature (Fig. 6). An aliquot of $CaCl_2$ was placed in the chamber to control moisture. A small membrane pump was used to bubble air through the S6 to enhance vaporization of the liquid gas cure and hence accelerate curing of the kidney slices (Weiglein and Henry, 1993**;** de Jong and Henry, 2007). After five days exposure to S6, curing of the kidney slices was complete.

Slice evaluation

Each slice was placed on a clear glass plate, at the intended time, and examined and photographed using

the background light (epidiascope) to illuminate the kidney from beneath.

A steps and timetable summary for the S10-technique for thin kidney slices is as follows:

SLICE: cold, non-fixed kidneys
FLUSH; 1 hour cold tap water
FIX: 5 days room temperature formalin (3%)
FLUSH: overnight cold tap water
COOL: 5 hour at +5°C
DEHYDRATE: 15 days at -25°C
DEGREASE: 3 days at room temperature
POLYMER IMMERSION: 3 days at -20°C
FORCED IMPREGNATION: 10 days at -20°C
POST-IMPREGNATION: 3 days at room temperature
GAS-CURING: 5 days at room temperature

Figure 6. Gas curing of impregnated kidney slices.

Results

Thin slices of the kidney were produced using a modified S10 plastination protocol along with and without degreasing. The degreased plastinated slices were of good quality and semitransparent. Plastinated slices yielded clarity of sub-gross anatomy. They were thin and semitransparent and fine detail of many anatomical structures could be observed. The slices were flexible, dry, and odorless with smooth surfaces, were easy to handle and to evaluate. They were prepared with relative ease and limited expense and are suitable for storage at room temperature. Neither noticeable shrinkage nor distortions were observed in the slices.

Anatomic detail down to the wall and lumen of interlobar arteries (a. interlobaris) was clearly delineated from the surrounding kidney tissue (Figs. 7, 8, 9) as were the arcuate arteries (a. arcuate) (Fig. 10). Similarly, most sub-gross anatomy of the plastinated S10 pig kidney slices was easily recognized. Distinction between renal cortical structures and renal medullary structures was evident (Figs. 7, 9). The renal cortex was light colored and the renal medulla, represented by pyramids, was dark in color. The renal pyramids were triangular in form with discrete radial structures and their base was directed toward the outer cortical surfaces of the kidney (Fig. 7). The conical renal papilla of a pyramid was surrounded by a renal calyx that was cup shaped (Fig. 7).

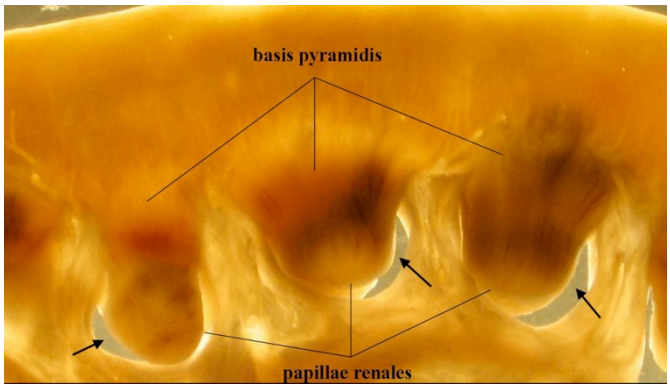

Figure 7. Detail of degreased, plastinated pig kidney slice. Renal papillae surrounded by renal calicies (arrows).

Figure 8. Anatomical detail of degreased, plastinated pig kidney slice. Interlobar arteries(*).

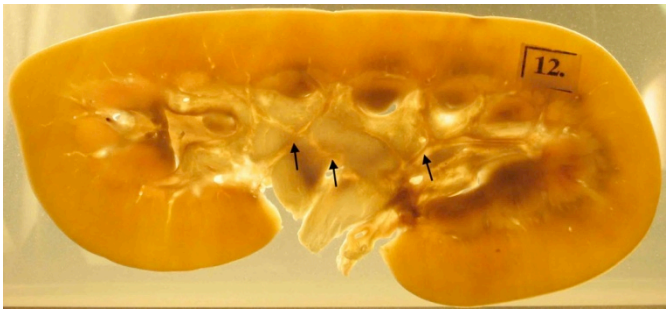

Figure 9. Anatomical detail of degreased, plastinated pig kidney slice. Segmental arteries (arrows) in renal sinus.

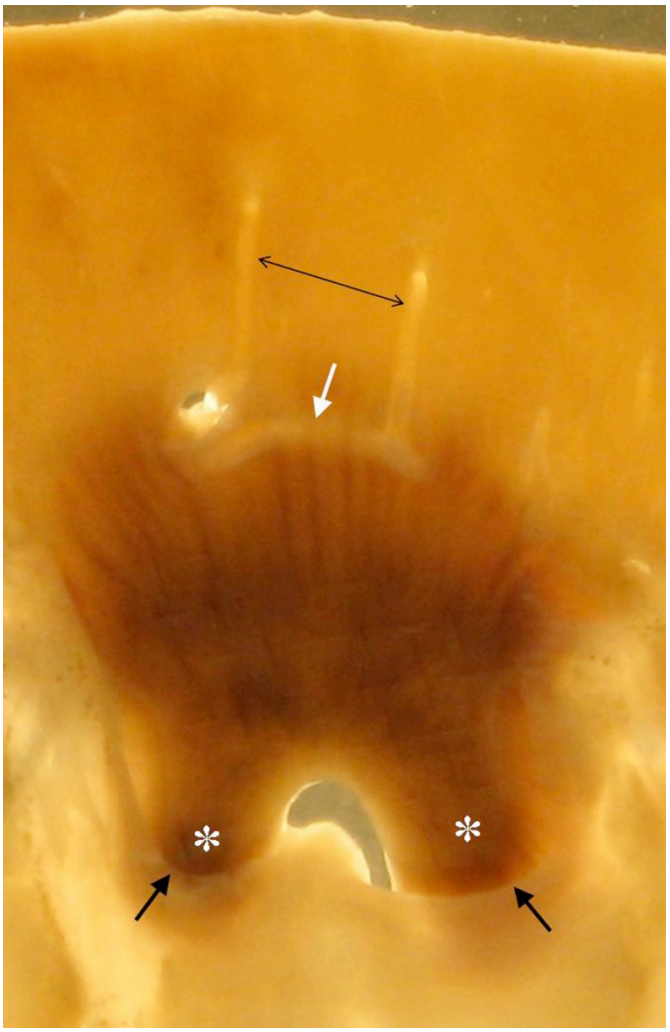

Figure 10. Anatomical detail of degreased, plastinated pig kidney slice. Arcuate artery (white arrow), interlobular arteries (black, double headed arrow), perimeter of calyx (black arrows), renal papillae (*).

Fresh tissue slices had wet surfaces and their anatomical subdivisions were loose and difficult to handle and evaluate. Hand manipulation of these specimens was difficult since the component relationships were easily distorted or disrupted. With background lighting, the medullary structures were reddish colored with no clear demarcation from surrounding lighter colored cortical tissue (Fig. 11).

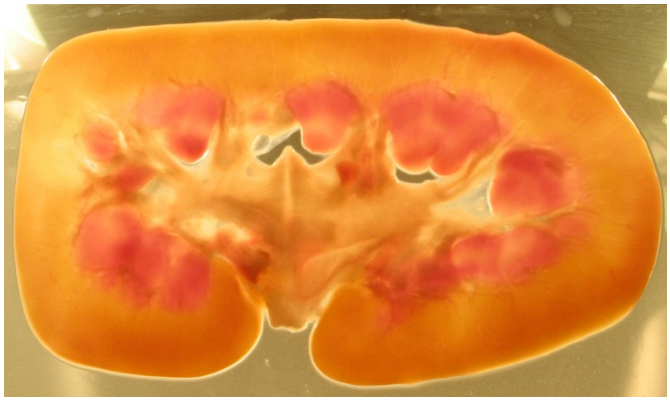

Figure 11. Fresh pig kidney slice.

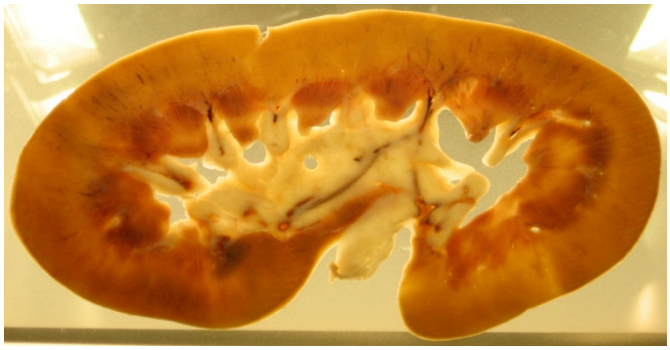

Figure 12. Dehydrated pig kidney slice.

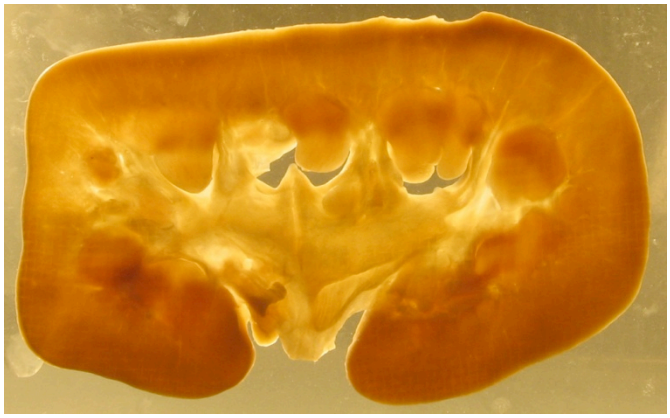

Figure 13. Dehydrated and degreased pig kidney slice.

The dehydrated but not degreased slices showed distinction between the renal structures. However, finer details such as the lumen of some segmental arteries within the parenchyma were not clearly delineated. These slices were less transparent with less delineation between the blood vessels inside the kidney parenchyma primarily due to the presence of fat (Fig. 12).

The dehydrated and degreased slices showed clear distinctions between the renal structures down to the finer detail of sub-gross structures (Fig. 13).

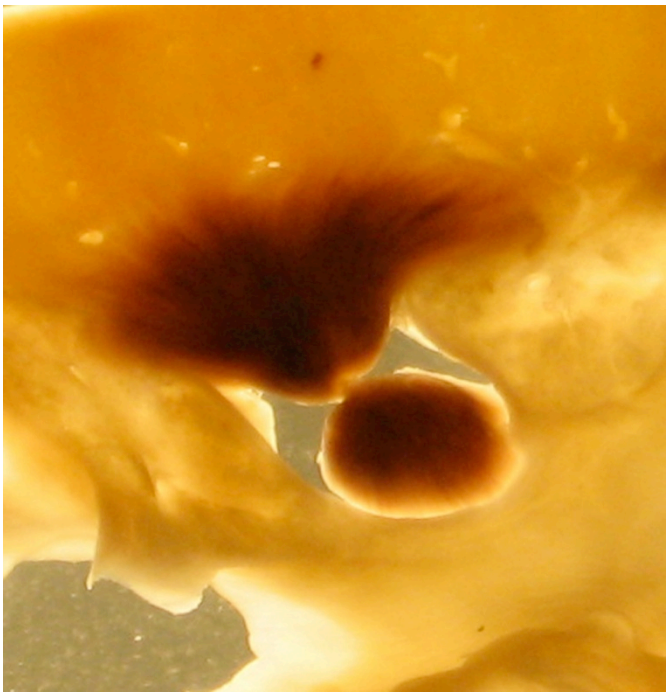

Figure 14. Anatomical detail of degreased, plastinated pig kidney slice.

Discussion

Our goal of producing thin slices of the kidney using a modified S10 plastination protocol which would aid anatomical study was successful. Clarity of sub-gross anatomy was similar to specimens produced by the E12 or P35/40 methods. This is primarily due to the thinness of slices and resultant transparency which yielded anatomical detail of many structures.

To date, epoxy and polyester plastinated slices have no flexibility and are brittle and often fracture if dropped. Classically, silicone plastinated specimens have a degree of flexibility. To provide maximal flexibility of kidney slices, only one half of the S3 catalyst was used to make the reaction-mixture. This along with slice thinness assured that the silicone slices had good flexibility.

As was expected, shrinkage was minimized by cold dehydration. Allowing the slices to equilibrate both when loaded into the reaction-mixture and upon conclusion of impregnation before decreasing or increasing pressure respectively is also known to minimize tissue shrinkage. In addition, the planned incremental decreases of pressure by the vacuum controller, allow uniform decreases of pressure until pressure is reduced to 8mmHg. This uniform decrease of pressure along with alternate timed 10mmHg increases in pressure allows tissues to relax and thus release the vaporized acetone which in turn allows better intake of the S10/S3 mixture into the tissue and hence minimizes shrinkage.

Cook (1997) reported that E12 plastinated kidney sections when viewed in situ body slices yielded an informative profile of the capsule, cortex, medulla and pyramids. Similarly, the sub-gross anatomy of the plastinated S10 pig kidney slices was easily recognized and apparently observed in yet more detail.

One of the main features of E12 or P35/40 specimens is the transparency of the specimen slice (Steinke, 2002; Sora and Cook, 2007; Weber et al., 2007: Henry and Latorre, 2007). Bringing the last acetone bath to room temperature for a few days produced a satisfactory degree of transparency in S10 slices by reducing the fat accumulation in the renal sinus. Likely more transparency of the renal sinus could be obtained by a few more days of room temperature acetone defeating. However, the dehydrated but not degreased slices and the fresh slices both showed less transparency and hence less distinction between renal structures. Medullary structures were dark colored and could be recognized from the surrounding cortical tissue yet segmental arteries in the parenchyma and their lumina were not clearly delineated from the surrounding kidney tissue due to the high lipid content of the renal sinus. This work demonstrates that thin S10 semitransparent kidney slices can contribute to future research activities. The aim of our next study will be to explore the segmental arterial structure in pig kidney.

Being able to visualize the sub-gross anatomy in situ on the thin S10 plastinated slices, should have a positive effect in undergraduate and postgraduate teaching. Students provided with sagittal sections of the kidney can visualize a complete overview of renal anatomy. Besides the great educational value that S10 thin specimens will have, these kidney slices for a research investigation. As well, the possibilities of three dimensional reconstruction of the thin plastinated kidney will allow many opportunities for further investigation, one being counting of the number of renal pyramids per kidney in both breeds and to analyze the variations in the way they unite and way they open into the renal pelvis.

The degreased S10 plastinated pig kidney slices prove to be an excellent teaching and research tool in anatomy. The kidneys plastinated in thin slices by this technique are safe for student handling and use. Students can handle these slices and reconstruct the pig kidney. This will aid their understanding of the specific anatomical detail of the 2-3mm thick specimens; as well as help them bridge the gap between gross and histological structures.

Due to the possibility for analysis of the space relationship between the renal papilla inside the renal calyx (Fig. 12), the thin plastinated slices will be used

for future research activities in endourology where a three dimensional view may aid locating kidney stones inside the lumen of the calyx. We believe this will contribute to development of new or a modification of available techniques for stone disease treatment. The knowledge of anatomy based on thin plastinated kidney slices will also assure more accurate interpretation of diagnostic CT or MR scans.

We can conclude that the S10 technique may be used for producing 2-3mm organ slices. As well, sub-gross anatomy is distinct and these slices are a better aid from which to study and record the various aspects of anatomy in the kidney than fresh slices or non-degreased slices. The method that we applied is easy to follow and uses materials that are found in the most basic plastination laboratory.

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Evaluation of Imidazole for Color Reactivation of Pathological Specimens of Domestic Animals

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Abstract: Formalin-fixed pathologic specimens were impregnated via two methods to evaluate color reactivation quality. 1. The classic S10 procedure and reaction-mixture or 2. The classic S10 procedure followed by a modified S10 process enhanced by adding one part imidazole to the classic reaction-mixture. To record specimen color and size, all specimens were photographed after fixation and again after plastination. Image ProPlus 4.2 software was used to analyze the images for color change and shrinkage. Lungs and kidneys treated with the imidazole additive in the reaction-mixture preserved the characteristics of lesions and the original color. However, statistically, neither group showed a significant difference for either parameter, color or shrinkage ($p>0.05$). The negligible difference of shrinkage was an important finding since shrinkage is often a byproduct of plastination. Plastination is an alternative method to preserve anatomopathologic specimens, particularly with the use of imidazole which yields little shrinkage and preserves original pathological color.

***Keywords*: plastination; S10; imidazole; pathology; specimens**

Introduction

Each day, the use of animals for educational purposes in the laboratory becomes more difficult in veterinary medicine. Amid somewhat routine autopsies on domestic and wild animals in daily practice, an occasional non-frequent injury or a disease that is rare to our geographic zone is observed. Conservation of such specimens that maintain diagnostic characteristics over time allows many students and professors to observe and to learn from these archived specimens. Demonstration of complete pathologic specimens or thick slices of such has worldwide acceptance and plays a very important role in medical science education and also in pathology, anatomy and zoology (Bickley et al., 1981; Hermes, 2006; Latorre et al., 2007). Such specimens allow recognition of structures in their three-dimensional disposition. For hundreds of years, numerous attempts have lead to numerous techniques to maintain intrinsic characteristics of specimens. Plastination was a development which produced durable specimens that were easy to handle (von Hagens, 1987). Nevertheless, the traditional silicone plastination technique has three important disadvantages: loss of color, diminished consistency and shrinkage. Presently, there is no one ideal methodology that recovers the total natural appearance of plastinated organs.

The objective of the present work was to introduce

the technique of reactivation of color in fixed lungs and kidneys with demonstrable pathology. Following impregnation with a traditional silicone reaction-mixture, imidazole was added to the traditional S10 plastination reaction-mixture and specimens were submerged in the mix and vacuum was lowered incrementally again. These re-impregnated specimens were evaluated for color saturation and shrinkage.

Materials and methods

Specimen preparation

Twenty domestic swine lungs and twenty kidneys of domestic dogs were collected from necropsy.

The twenty sets of lungs consisted of eight cases of suppurative bronchopneumonia, five cases of fibrinosuppurative bronchopneumonia, two cases of fibrinosuppurative pleurobronchopneumonia and five cases showing pathologies including pulmonary congestion, hemorrhage and edema.

The dog kidneys were from a variety of breeds and ages. These organs displayed various pathological conditions including four cases of glomerulonephritis of undetermined origin, three cases of hydronephrosis and hydroureter, three cases of urolithiasis, two cases of polycystic kidney disease and one case each of cystic carcinoma, transitional cell carcinoma, metastatic mammary gland carcinoma, metastatic lymphoma, mesothelioma, renal dysplasia, acute renal infarct and kidney atrophy.

All organs were rinsed with tap water and the lungs also via the trachea until excess blood was removed. The lungs were insufflated slightly to drain excess water. The trachea was cannulated with appropriate sized tubing and later infused with 4% buffered formalin and stored in 4% buffered formalin for approximately 48 hours (von Hagens, 1986). The kidneys were submerged in 4% formalin for two days. Following 48 hour fixation, the lungs and kidneys were rinsed with tap water to dilute and remove excess formalin. The lungs were also flushed with water intra-tracheally. After flushing, the organs were stored in cold water for 12 hours in a cold room. The organs were dissected to remove most fat and excess connective tissue. After cleaning of excess tissue, the specimens were photographed with a Nikon A100 camera, at a distance of 55cm from the organ, on a dark background with two 60 watt lamps placed at 45°. A centimeter scale was included for reference. The standardized images were used for lung measurements: a) length right lobe; b) length left lobe; c) bifurcation of the trachea to the tip of the right middle lobe, and d) bifurcation of the trachea to the tip of the cranial left lobe. Kidney measurements were: a) longitudinal axis, b) length of cranial pole, and c) length of caudal pole (Fig. 1).

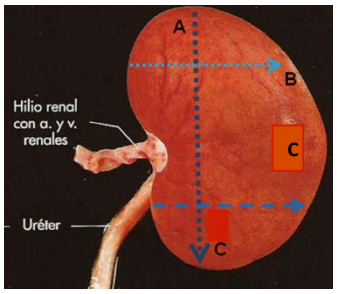

Figure 1. Kidney measurement.

Plastination

All specimens were dehydrated in cold (-20ºC) 100% acetone (freeze substitution). Each third day acetone concentration was measured with an acetonometer. When acetone percent was stable, the specimens were placed in new acetone. Four to six changes of acetone were carried out over an eight week period. The specimens were impregnated using the traditional S10 silicone (Biodur^{TM}) and S3 catalyst (Biodur^{TM}) in a proportion of 100:1 (deJong and Henry, 2007). Pressure decrease was regulated in the vacuum chamber by incremental closure of the valve until bubble formation ceased and pressure had been lowered nearly one atmosphere. The impregnation process took four weeks. The impregnated specimens were removed from the silicone impregnation reaction-mixture. One half of the impregnated specimens (10 lungs and 10 kidneys) were placed in disposable bags and stored for two weeks at 4º C. A color reactivation-mixture was prepared for the other one half of the impregnated specimens by preparing a saturated solution of imidazole/ethanol using a ratio of 1:3. One part of the imidazole-mix was mixed with 100 parts of the classic reaction-mixture and placed in a stainless steel container inside the cold vacuum chamber. The remaining half of impregnated specimens (10 lungs and 10 kidneys) was submerged in the silicone/catalyst/imidazole reaction-mixture in the vacuum chamber in the freezer. Initially pressure was lowered rapidly to 20mmHg (-20º C) and then incrementally lowered to the end point of 5mmHg over a four week period.

Subsequently, all the organs impregnated with silicone/catalyst or with silicone/catalyst plus imidazole were removed from the refrigerator and vacuum chamber, respectively, and allowed to drain at room temperature. The specimens were wiped of excess impregnation-mixture with paper towels and adjusted to proper anatomical position. After draining, the specimens were placed in a closed container and saturated with SH06 gas cure (Biodur^{TM}) using a continuously running aquarium pump. The curing of

specimens was complete between 1 and 4 weeks. The cured lungs and kidneys were photographed and measurements of organs were recorded. The black background of the pictures was changed to white so the image analyzer program could measure the color changes of the organ. Each one of the images of the lungs and kidneys plastinated with and without imidazole were analyzed to evaluate the color, saturation and hue using the program Image Pro Plus, version 4.2®. In order to evaluate the significance of color preservation and the degree of shrinkage of the organs, the Student t test was used from the program SPSS 10 for Windows.

Results

It was observed that lungs and kidneys with imidazole displayed a reddish coloration and pathology was more easily differentiated (Figs. 2-7). Dysplastic kidneys showed a marked increase in red coloration (Fig. 7). Lungs with edema did not show much color differentiation. Specimens plastinated without the addition of imidizole demonstrated the classic bleaching of natural colors (Figs. 8-11). Nevertheless, no significant differences ($P>0.05$) were observed in the values of saturation and hue of color between plastination S10 and the plastination S10/imidazole in lungs and kidneys (Tables 1, 2). The length/width measurements from lung and kidneys, before and after, with plastination reaction-mixtures showed no significant difference in the degree of shrinkage ($P> 0.05$) (Tables 3, 4). The percentage of shrinkage in lungs and kidneys for both techniques was <3% ($P>0.05$). Specimens exposed to 4 weeks of S6 (gas cure) were firmer than those with shorter exposure.

Discussion

In the present work, the lungs and kidneys plastinated using the classic S10 method preserved the characteristics of the represented pathology (Meuten, 2002; López, 2007; Newman et al., 2007). However, pathologic lesions were more evident in lungs plastinated with S10/imidazole-mix, which agrees with the work by Sakamoto et al. (2006), who used the Shin Etsu silicone polymer KE-108 with CAT-108 technique plus imidazole in one week formalin-fixed organs. Lungs with edema did not conserve the original color well, likely because the pathology (increased fluid content) was removed by dehydration. Additionally, the partial or total absence of erythrocytes in the edematous tissue block provided no hemoglobin or myoglobin for the imidazole to form complexes of hemochromogens resulting in the absence of the natural red color (Sandhyamani, 2005).

The results in both lung groups classic S10 plastination and S10 modified with imidazole showed no significant differences in shrinkage percentage which was similar to the findings of Sakamoto and co-workers (2006). Shrinkage of both lung and kidney specimens impregnated with and without imidazole was comparable to specimens treated with Shin Etsu silicone polymer KE-108 with CAT-108 plus imidazole which showed 2 to 5% shrinkage. The absence of significant shrinkage is a valued characteristic of specimens preserved by the plastination technique, especially structures as the nervous system. Impregnation is the fundamental step in the plastination process with or without substances for preservation and restoration of color. If an organ is not impregnated in its totality, it tends to shrink and acquire a dark color (Miklosová et al., 2004; Henry et al., 2006).

A natural red color was observed in kidney specimens impregnated with imidazole yet no significant difference was observed between the two different techniques. With an addition of imidazole, an organ may acquire an intense red coloration which was noted in kidneys with renal dysplasia. This intense red is not natural. It is known, that the histological composition of the organ influences the ferrohemochromogen concentration and the color (Sandhyamani, 2005).

A disadvantage associated with S10 plastinated specimens with imidazole is that over an extended period of time the surface of the organ looses color due to oxidative changes on the surface. Color changes from bright red to dark brown are likely due to surface contact with atmospheric oxygen (Sandhyamani, 2005). Another common alternative for enhancing specimen color is Biodur^{TM} stain which is added in the last acetone bath, prior to impregnation with silicone. However, only the organ's surface is stained with the pink color. Therefore, if the surface of the organ is damaged or removed the color is lost (Henry et al., 1997). With color reactivation, the color is restored throughout the entire specimen.

Both decreased fixation time and percentage of formalin, decrease color loss. Therefore, we chose to fix the kidneys and lungs with buffered 4% formalin for only 48 hours to aid partial preservation of natural color. Specimens dehydrated with acetone to >98% and with temperature control (-20ºC), permitted an adequate interchange of acetone with the silicone reaction-mixture under appropriate vacuum. These properly impregnated pieces did not suffer changes during the curing as shown by Miklosová et al. (2004).

Flexibility and hardness of the specimens obtained from this plastination process were influenced by the time of contact with the cure gas agent and

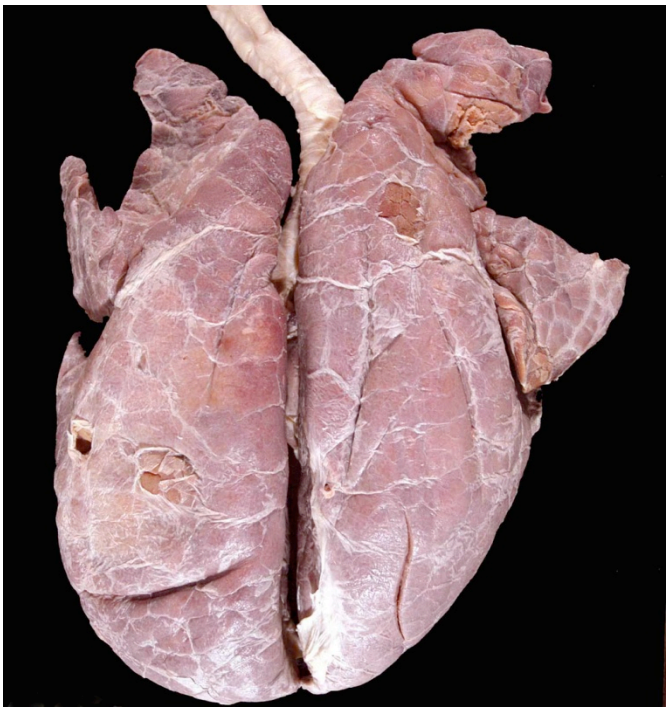

Figure 2. Plastinated porcine lung with imidazole.

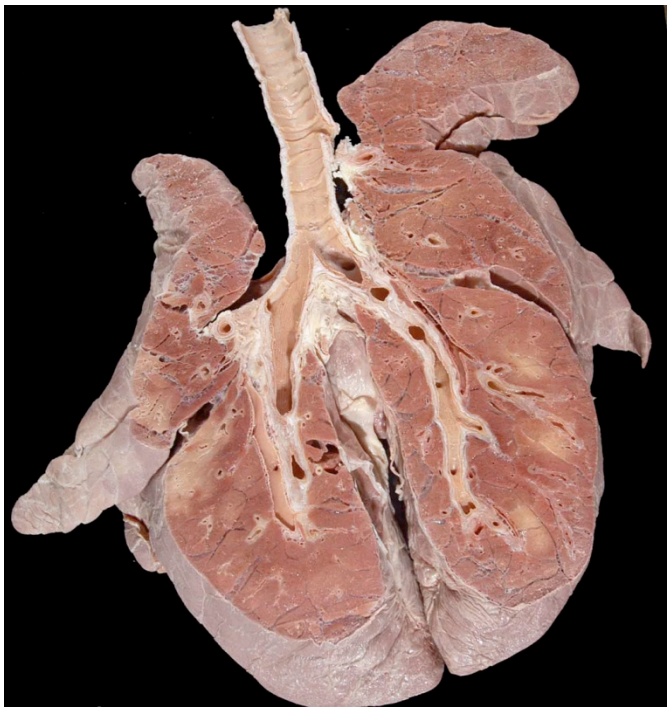

Figure 4. Plastinated porcine lung with imidazole.

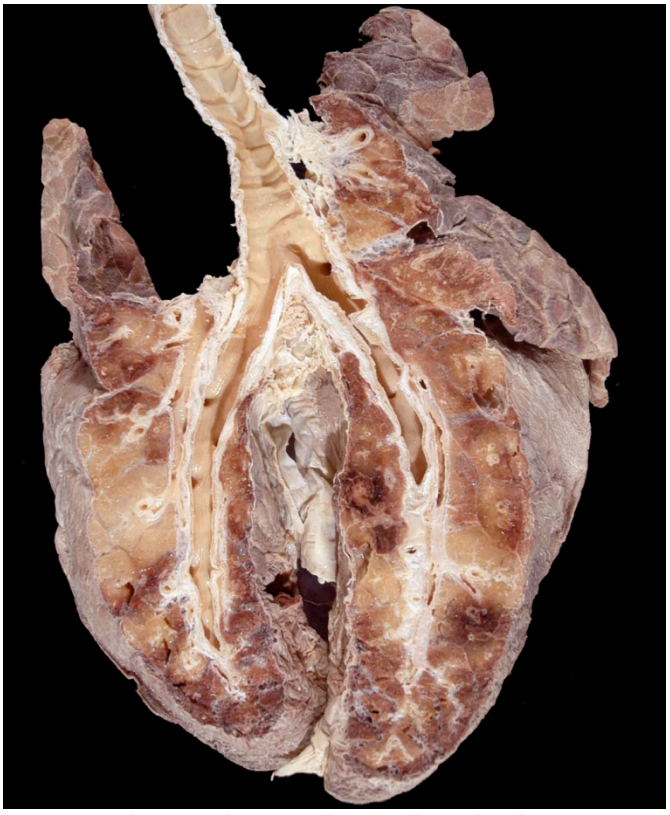

Figure 3. Plastinated porcine lung with imidazole.

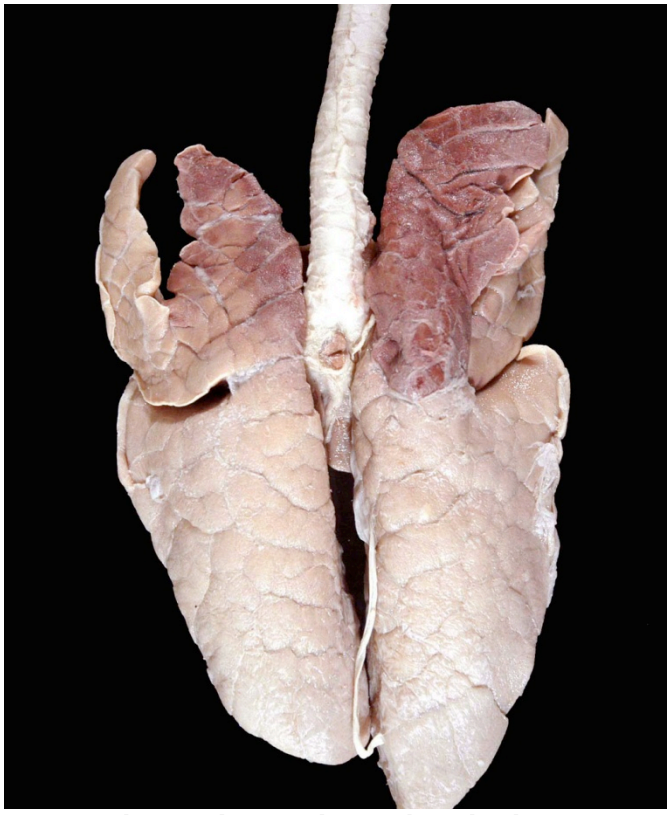

Figure 5. Plastinated porcine lung with imidazole.

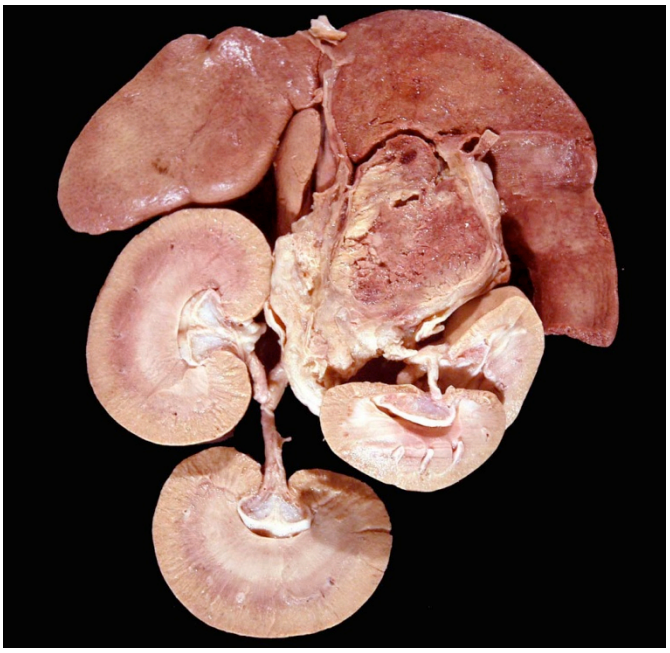

Figure 6. Plastinated canine viscera with imidazole.

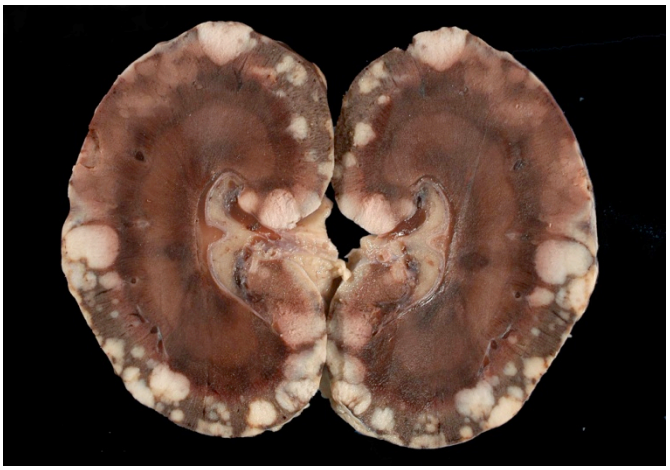

Figure 7. Plastinated canine kidney with imidazole.

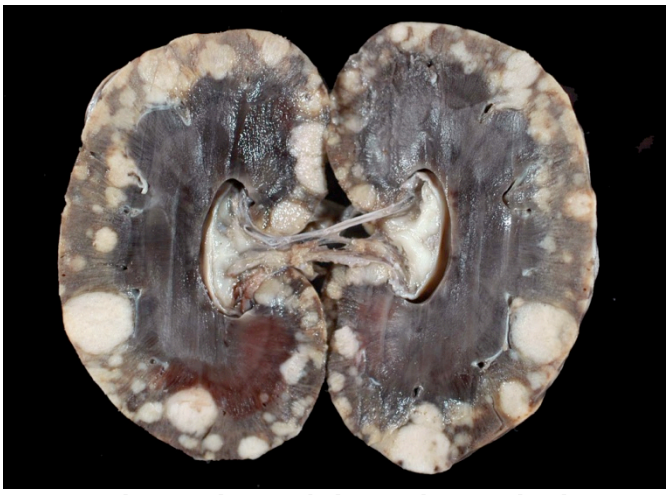

Figure 8. Plastinated canine kidney without imidazole.

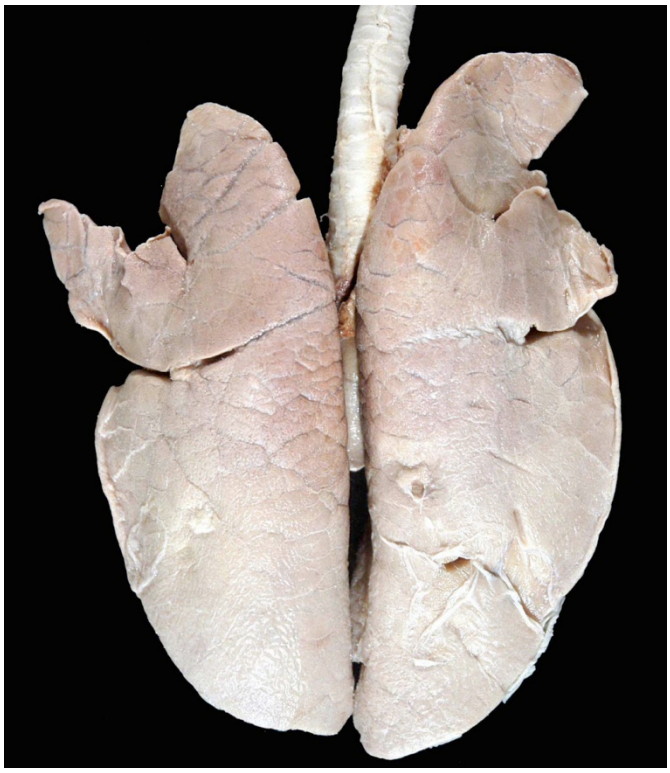

Figure 9. Plastinated porcine lung without imidazole.

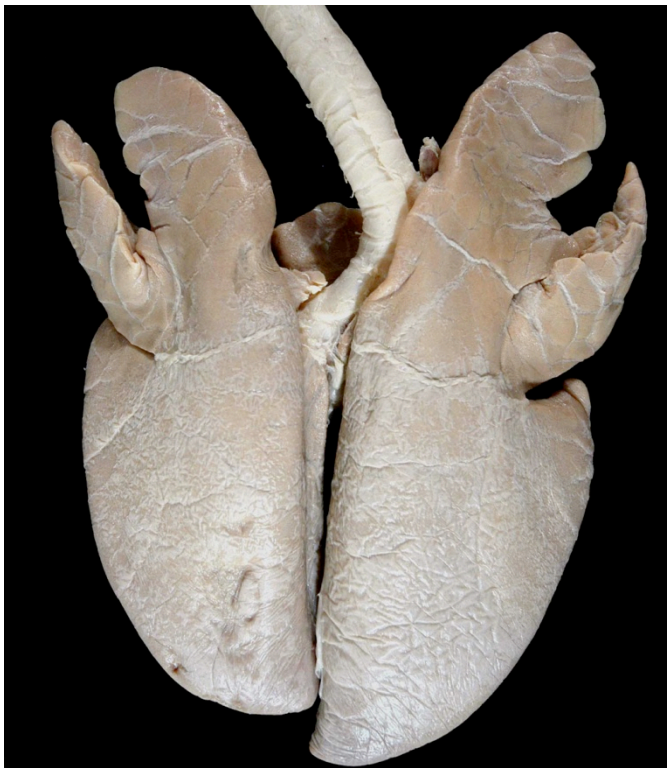

Figure 10. Plastinated porcine lung without imidazole.

Item	*Measurements*	Mean		SE
		before	after	
S10				
	Hue	201.39	204.42	4.91
	Saturation	39.96	44.33	5.76
	Value	123.71	116.94	8.95
S10 plus Imidazole				
	Hue	199.52	209.56	5.81
	Saturation	44.33	44.49	10.24
	Value	115.99	112.75	9.08

Table 1. Evaluation of color in lungs of pigs with pathology, before and after S10 plastination and S10 plastination plus imidazole.

Item		Mean		*SE*
		before	after	
S10				
	Hue	209.03	217.74	8.26
	Saturation	23.70	24.18	4.57
	Value	134.34	149.42	11.40
S10 plus imidazole				
	Hue	207.23	216.55	8.78
	Saturation	32.49	37.29	7.42
	Value	133.42	133.52	12.93

Table 2. Evaluation of color of kidneys of dogs with pathology, before and after S10 plastination and S10 plastination plus imidazole.

Item	*Measurements*	*Mean*		*SE*
		before *cm*	**After** *cm*	
S10	**Length right lobe**	20.74	20.29	3.61
	Length left lobe	21.58	21.33	3.74
	Bifurcation of trachea to right middle lobe	19.74	9.77	10.38
	Bifurcation of trachea to left cranial lobe	9.66	9.73	1.60
S10 plus imidazole				
	Length right lobe	20.09	20.31	2.56
	Length left lobe	22.61	22.92	2.63
	Bifurcation of trachea to right middle lobe	10.22	10.33	1.36

Table 3. Measurements of lungs of pig with pathology, before and after S10 plastination and S10 plastination plus imidazole.

Technique	**Mean**		
S10	before *cm*	after *cm*	SE
longitudinal axis	6.81	6.76	0.42
cranial pole length	3.69	3.66	0.34
caudal pole length	3.84	3.75	0.32
S10 plus imidazole			
longitudinal axis	5.50	5.59	0.77
cranial pole length	3.17	3.29	0.40
caudal pole length	3.57	3.49	0.60

Table 4. Measurements of dog kidneys, before and after the S10 plastination process and S10 plastination plus imidazole.

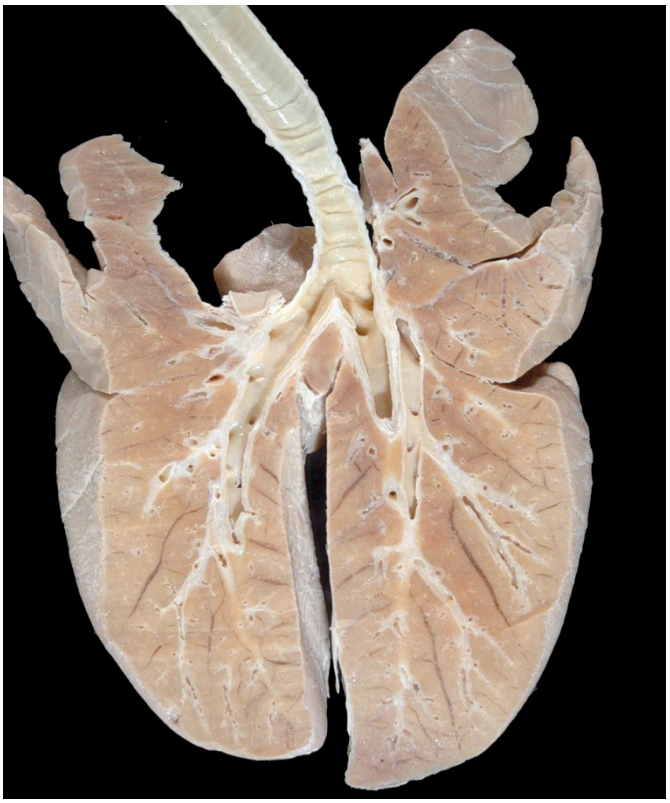

Figure 11. Plastinated porcine lung without imidazole.

polymerization of the silicone chains. Longer contact with the gas cure produced harder specimens.

It will be beneficial to continue experimentation with different techniques of plastination of pathological samples because of the great variety of lesions presented in domestic animals which may significantly change its original histological composition and color.

The results of the present study suggest that the technique of plastination with addition of imidazole in organs with pathology is a good option, because it aids visualization of the pathology. Specimen shrinkage is similar to that of traditional plastination methods. Better results are obtained in compact organs like the kidney. It is recommended not to excessively wash the organs, to help retain the blood present in the organ which reacts with imidazole.

Acknowledgements

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Curing Times of P40 Exposed to Different Light Sources

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Abstract: Glass casting chambers were filled with P40 resin and placed under different light sources to record curing rates and temperatures reached during the process. Results were used to assess the effects of different light sources and the method of application of that light on the curing of P40 resin. It was found that curing of P40 resin was achieved by exposure of the resin to sunlight, artificial UV-A light and mercury lighting. Fluorescent lighting had no effect on P40 resin. Curing rate was increased as was maximum temperature reached during the process when light sources were allowed unregulated interaction with the resin.

Key words: **plastination; P40; light; ultraviolet; UVA; sunlight; mercury; curing**

Introduction

P40 polyester plastination was designed to produce permanent preservation of tissue slices within a rigid, durable sheet of cured resin (von Hagens, 1994). These polyester plastinated specimens are for educational and research purposes (Barnett, 1997; Weiglein and Feigl, 1998; Henry and Weiglein, 1999; Sora et al., 1999; Latorre et al., 2002). Although designed for plastination of brain slices (Henry and Latorre, 2007), P40 plastination may also be used for plastination of other tissues (Latorre et al., 2004) as well as body slices (Latorre et al., 2007). P40 plastination relies on ultraviolet radiation to cure the resin. This study will examine the effects of different sources and delivery of UV light on the curing times and temperatures during curing of P40 resin.

Materials and methods

Glass casting chambers were constructed as described by Henry and Latorre (2007). P40 resin, without catalyst, was poured into the chambers and the chambers were left open on one end. These chambers filled with P40 resin were exposed to different light sources until cured. All chambers were monitored for temperature changes and curing time during the experiment. Curing was monitored by inserting a wire through the open side of the chamber and into the resin. The three delivery methods of the light sources used for exposures included the following: uncontrolled, direct exposure; uncontrolled, indirect exposure; exposure under controlled conditions. Light source/light exposure combinations tested in this experiment included direct sunlight, indirect sunlight, controlled exposure to direct sunlight, controlled exposure to artificial UV-A light, fluorescent light, mercury light, controlled exposure to mercury light and exposure to combined fluorescent and mercury lighting. Temperatures during experiments were recorded and regulated when required using an Omega CN4400 thermo-regulator. Curing of the P40 resin was monitored by probing the chamber contents with a wire and by external visual observations.

Experiment 1: Chambers exposed to direct sunlight were placed outdoors in sunlight on a non-cloudy day in June in Tennessee (30 ^{o}C) and left to cure. The

temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes.

Experiment 2: Chambers exposed to indirect sunlight were placed outdoors in the shade on a non-cloudy day in June in Tennessee (30 °C) and left to cure. The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes.

Experiment 3: Chambers under controlled exposure to direct sunlight were placed in the sun on a non-cloudy day in June in Tennessee (30 °C). The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes. When the surface temperature of the casting chamber reached or exceeded 30°C, the chamber was moved into the shade and cooled with a fan to 25°C at which point it was returned to the direct sun.

Experiment 4: Chambers with controlled exposure to artificial UV light were placed 28.0cm from four 40 watt UV-A light bulbs (two above and two below) and cooled with a fan during the entire experiment (Fig. 1). The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes. When the surface temperature of the casting chamber reached at least 30°C, the light source was turned off until the temperature of the chamber reached 25°C. At that point, the bulbs were turned on once again.

Experiment 5: Chambers exposed to fluorescent lighting were placed on a table top and exposed to fluorescent lighting in a room with no windows. The fluorescent bulbs were located 150.5cm from the casting chamber. The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes.

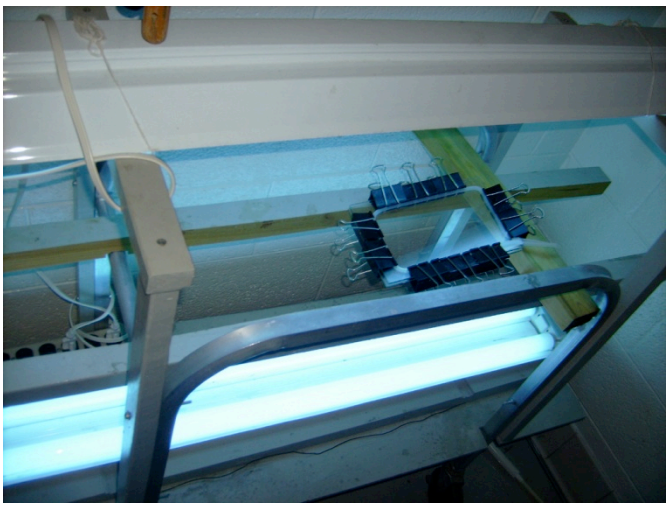

Figure 1. Typical UV-A light source used to cure P40 resin.

Experiment 6: Chambers exposed to direct mercury light were placed within a cardboard shield 58.4cm beneath the mercury bulb. The shield was used to keep out fluorescent light which was also present within the room. The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes.

Experiment 7: Chambers exposed to direct mercury light were placed within a cardboard shield 58.4cm beneath the mercury bulb (Fig. 2) and cooled with a fan during the entire experiment. The shield was used to keep out fluorescent light which was also present within the room. The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes. When the surface temperature of the casting chamber reached at least 30°C, the light source was turned off until the temperature of the chamber reached 25°C. At this point, the mercury light was turned on once again.

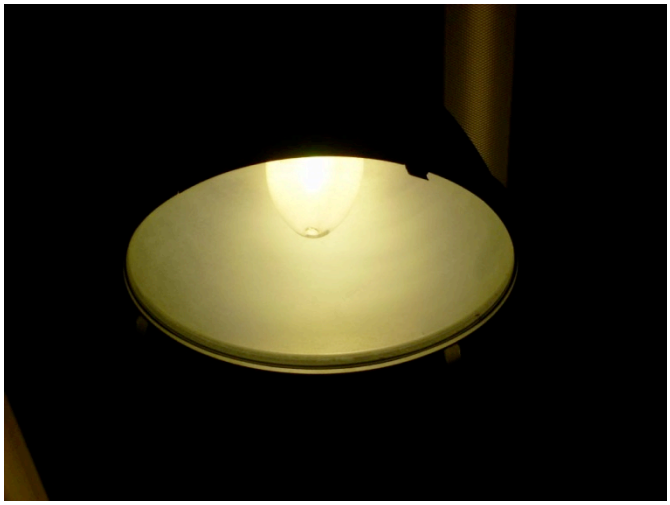

Figure 2. Mercury light source used to cure P40 resin.

Experiment 8: Chambers exposed to combined fluorescent and mercury lighting were placed on a table top 233.7cm beneath the mercury bulb while also exposed to light from fluorescent bulbs within the room. The temperature of the surface of the glass chamber and the progress of curing were monitored every 10 minutes. The chamber was 302.3cm from the fluorescent lights.

It should be noted that curing of all chambers was considered complete when all but the top 5mm of P40 resin was hard enough to resist the insertion of a metal wire. The P40 resin at the air-interface of an open chamber will not cure due to the resin being in contact with air.

Results

Experiment 1: The P40 resin in chambers exposed to

direct sunlight with no temperature regulation formed a solid gel by 20 minutes of exposure and cured completely within 40 minutes. The maximum temperature recorded during the curing process was 68°C and resulted in breakage of the glass used to form the casting chamber.

Experiment 2: The P40 resin in chambers exposed to indirect sunlight with no temperature regulation formed a solid gel by 40 minutes of exposure and cured completely within 80 minutes. The maximum temperature recorded during the curing process was 44°C.

Experiment 3: The P40 resin in chambers exposed to direct sunlight under controlled conditions formed a solid gel by 30 minutes of exposure and cured completely within 40 minutes. The maximum temperature recorded during the curing process was 40°C.

Experiment 4: The P40 resin in chambers exposed to artificial UV light under controlled conditions formed a solid gel by 30 minutes of exposure and cured completely within 40 minutes. The maximum temperature recorded during the curing process was 36°C.

Experiment 5: The P40 resin in chambers exposed to fluorescent lighting never exhibited any signs of curing or the onset of curing by 40 hours into the experiment. The resin remained liquid in form and no increase in surface temperature of the glass chamber was detected at any time. These chambers were exposed to fluorescent lighting for eight to nine hours per day for an additional month post experimentation and curing of the P40 resin was never initiated and the resin remained in liquid form.

Experiment 6: The P40 resin in chambers exposed to mercury lighting with no temperature regulation at close proximity formed a solid gel by 70 minutes of exposure and cured completely within 90 minutes. The maximum temperature recorded during the curing process was 34°C.

Experiment 7: The P40 resin in chambers exposed to mercury lighting under controlled conditions in close proximity to the light source formed a solid gel by 70 minutes of exposure and cured completely within 90 minutes. The maximum temperature recorded during the curing process was 60°C.

Experiment 8: The P40 resin in chambers exposed to fluorescent lighting and mercury lighting, at the increased distance, formed a solid gel by 80 minutes of exposure and cured completely within 180 minutes. The maximum temperature recorded during the curing process was 24°C.

A summary of findings is listed in tables 1 and 2.

Light source	Cure times
Direct sunlight	30 minutes
Indirect sunlight	80 minutes
Controlled exposure to direct sunlight	40 minutes
Artificial UV-A light	40 minutes
Fluorescent lighting	did not cure
Mercury lights	90 minutes
Controlled exposure to mercury lights	130 minutes
Mercury and fluorescent lighting combined	180 minutes

Table 1. Curing times of P40 resin when exposed to different light sources.

Light source	Maximum temperature
Direct sunlight	68 °C
Indirect sunlight	44 °C
Controlled exposure to direct sunlight	40 °C
Artificial UV-A light	36 °C
Fluorescent lighting	N/A
Mercury lights	60 °C
Controlled exposure to mercury lights	34 °C
Mercury and fluorescent lighting combined	24 °C

Table 2. Maximum temperatures reached during curing when P40 resin is exposed to different light sources.

Discussion

As expected, ultraviolet light sources provided the fastest cure rates of P40 resin as they are the suggested method of initiating the curing process. Cure rates were similar for both natural sunlight and artificial ultraviolet light sources when the P40 resin was placed directly within the light source. Curing P40 outdoors in the shade doubled the time for curing when the times were compared to direct exposure to UV sources regardless of temperature regulation. P40 resin was not exposed to the artificial source of ultraviolet light in the absence of temperature regulation as it would result in the breaking of the glass of the chamber. It was discovered that placing the curing chambers in direct sunlight without temperature control would result in the breaking of the glass of those chambers as well.

Direct exposure to mercury lighting produced a curing time of P40 resin similar to that of outdoor shade. Temperature control during curing of P40 with mercury lighting increased cure time by approximately 45% when compared to direct mercury lighting. When

the distance from the mercury bulb was increased, the curing time was markedly increased.

Fluorescent lighting was found to have no effect on the curing of P40 resin. As no increase in temperature of the glass chamber was observed, no exothermic reaction as occurs in curing of P40 resin was present. The P40 resin used in this stage of the experiment was derived from the same stock used to fill all other casting chambers and should have cured if fluorescent lighting had an effect on P40 resin. After it was determined that fluorescent lighting would not cure the resin as it is not ultraviolet in nature, we set those casting chambers in the sun. The P40 resin in those chambers that did not respond to fluorescent lighting did cure in sunlight. This trial with fluorescent lighting was included within the experiment as it would be a common source of light within a laboratory setting.

It has been suggested that P40 slices be cured a minimum of 1 hour (Henry and Latorre, 2007). While there was some relatively small variation in cure times, each of the ultraviolet light source trials (artificial or natural) reached the peak of the exothermic process within 70 minutes (range was 30 to 70 minutes). This variation can likely be explained by dissimilarities in sample positioning, time of day, atmospheric conditions and efforts to control excessive temperatures. The P40 resin was often cured to the point of resisting the insertion of metal wire prior to peak of the exothermic reaction. These trials confirm that approximately 1 hour of curing should be appropriate for ultraviolet light exposure to cure P40 resin.

The sample exposed to the combined mercury and fluorescent light sources (normal laboratory lighting in the institution hosting the experimentation) cured in approximately three hours. The increased curing time was due to the increased distance of the glass chamber from the source of light. Given the results of the fluorescent lighting trial, it is unlikely that the fluorescent source contributed any to the progress of the reaction. In order to expose P40 to mercury light only, the cardboard isolation shroud not only placed the glass chamber in close proximity to the source of light but also increased the temperature in and around the glass chamber due to the shroud holding in any heat produced by the mercury bulb or the exothermic curing reaction. Thus, the maximum reported temperature for the unregulated exposure to mercury lighting is most likely artificially increased. It would be unnecessary for one to use such a shroud to cure P40 as mercury lighting will cure at a great distance. It would not be proper to use artificial UV light to cure P40 at a distance as the exposure of the light to one's eyes is detrimental. For the combined mercury and fluorescent lighting trial, the glass chamber was simply placed on a work table in the middle of the lab in order to approximate normal conditions likely to be encountered during assembly, sample positioning, etc. Although the mercury light source should be producing the same amount of ultraviolet radiation in both trials, the waves will be spread over a larger surface area at the increased distance. As a result, the concentration of ultraviolet waves per unit area in the combined trial would be less. The inverse square law can be used to determine the influence of distance on ultraviolet ray exposure.

$$\textit{Intensity} = \text{original intensity} \times \frac{\text{New distance}^2}{\text{Old Distance}^2}$$

Mercury lights will also lose intensity as they age so if one were to use such a source, cure rates should be expected to increase as the mercury bulb ages.

While it is unlikely that anyone would use one of these alternative light sources to cure P40 samples, knowledge of their respective cure rates, and that of ultraviolet sources, is of practical value. Given the absence of an effect of fluorescent lighting on P40 resin, it is reasonable to avoid rushing during sample preparation. Experimenters should be mindful of any mercury lighting which may be present within a laboratory or of any sunlight entering a window whether it be a direct ray or merely ambient lighting. Additionally, the knowledge of expected cure time is useful when planning monitoring frequency. One may also wish to use mercury lights for curing of P40 resin should an artificial UV light apparatus be cost prohibitive or not allowed due to safety concerns.

The times recorded for the solid gel formation within the resin should be heeded as once this gel has formed, any tissue samples that may have slipped within the resin (i.e. to the edge of the chamber or into a second tissue sample within the chamber) may not be repositioned.

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Design of a silicone gasket with an iron core for polyester and epoxy sheet plastination. ***Borzooeian Z, A Enteshari***. *Plastination Laboratory, Department of Anatomy, Natural History and Technology Museum, Azadegan Street, Modarres Blvd, Shiraz University, Shiraz, Iran.*

Plastination is a unique method for permanent preservation of biological tissues. Sheet plastination has become a popular method for the production of semitransparent sheets for studying sectional anatomy. In sheet plastination, the impregnated slices of organs and whole bodies are usually positioned and cured between glass plates with a gasket of tubing placed between the glass. This presentation demonstrates the unique design of a silicone gasket which includes a central iron core surrounded with silicone (1.5mm). A mold is constructed of glass, the central iron core is placed in the mold and the mold is partially filled with silicone to surround the iron. The sheets produced, using this type of gasket, have smooth, squared edges and are rectangular in shape. The advantages of using this type gasket are: Easier casting of the specimens, especially those which have several pieces; Easier positioning of specimens, because there is not glass in the way; and Ease of reaching and removal of bubbles.

Obesity and its determinants in Adult Lebanese: A cross-sectional survey. ***Jurjus A, A Gerges, H Daouk, HHI Abdallah, J Saliba, R Jurjus***. *Lebanese Health Society - Faculty of Medicine, American University of Beirut, Beirut, Lebanon.*

Obesity, defined as body mass index (8805); 30 kg/m2, is a very important health problem world-wide and particularly in Lebanon. This study's aim was to assess the prevalence of overweight and obesity in adult Lebanese and their associated determinants. Methods: Cross-sectional survey on obesity in a representative sample of 593 individuals, 20 years and older was employed. Interviews using a pretested questionnaire were carried out and waist circumference was measured. The questionnaire included questions on awareness, knowledge, attitude and practices. The WHO criteria for obesity classification were adopted. Results: The results show the prevalence of overweight to be 53.5% and obesity to be 18.16%, which is very close to previous reports although slightly higher. The overall BMI of the total population was in the overweight category 26.37 ± 11.12 kg/m2 with males slightly higher than females 27.5 vs. 25.2 kg/m2. These same respondents attained, in time, a maximal BMI of 28.33 kg/m2, in the upper case of overweight and desired a BMI of 23.83 kg/m2 within the normal weight range. All three classes of obesity were encountered Class I=14.16%, Class II=2.36%, and Class III=1.52%. Taking visceral fat as an indicator of obesity shows that 3.92% of males are obese with waist circumference of 102 cm, and 50.52% females are obese with waist circumference of 88 cm. As for the most proper indicator for obesity, people agreed on overweight (88.03%) and waist circumference (80%). Male respondents showed an underestimation of their own weight, while females perceived themselves more overweight than they really were. The majority ranked obesity, the fifth among a list of eight most important health problems in the country. Less than half (49.7%) believed that personal measures like diet and body activity could control obesity. The eating and drinking habits of interviewees were in favor of fatness. The greatest majority were not aware of their daily caloric intake. Physical activity was assessed in terms of intensity, frequency and duration, and was considered as inadequate. On the other hand, almost two thirds watch TV on a daily basis for two (36.27%), four or more hours (35.08%). This is an indication of a sedentary life style. To treat overweight and obesity, 67.5% to 74.54% selected options related to better food quality and less quantity, and exercising more. Interventions like the use of medications were selected by only 23.3%, followed by use of a combination regimen of medications and life style modification by 18.05%. Other interventions like liposuction 16.7%, bariatric surgery 7.25%, or hypnotherapy 6.3%, were not popular; these were more favored by females. Conclusion: Overweight, obesity and visceral fat showed high estimates. Their prevalence is comparable to neighboring countries.

Plastination and solvent recycling. ***Camiener GW***. *CBG Biotech, Ltd., Naples, FL, USA.*

Plastination requires the continuous purchase of new solvent, and the continuous disposal of hazardous waste solvent. The purchase and disposal aspects are very expensive, and there additionally are many serious ancillary problems related to biohazards, flammability, safety, storage and ventilation, as well as accountability and reporting issues with the EPA, OSHA and Certification groups. Materials and methods: Recycling is performed with Plastination Solvent Recyclers employing fractional distillation, microprocessor-controlled, LCD displays, air-cooled (no water connections), electrical fill pump, multiple redundant safety controls and either 110, 220 or 440 v electrical

connections. The recycling of waste solvents is performed either "batch wise" or "continuous" depending upon the amount of solvent that is recycled. In "batch-wise" recycling, the boiling tank is filled with waste solvent and the "start" button is pushed. The remainder of the process is fully automatic. The process consists of a controlled boiling of the waste solvent with vapors being directed upward through a reflux column where the solvent vapors are purified and separated from other volatile components. The solvent vapors are then condensed and the purified recycled solvent is collected. At the end of the cycle, all that remains in the boiling tank is a mixture of lipids, water, salts and tissue debris (LWST). This LWST waste is automatically drained from the boiling tank at the end of the cycle. In the continuous process, an AutoFill device automatically adds additional waste solvent to the boiling tank as purified solvent is removed. The boiling process continues until the boiling tank is nearly full of waste, at which time the recycler automatically shuts down and the LWST is automatically drained from the tank. Results: Solvent recovery from the waste approaches 100%, and purity of recycled solvent typically exceed 99% as measured by gas chromatography. Appearance-wise, recycled solvent is completely clear, haze-free and completely colorless, and the solvent can be reused indefinitely. Laboratory safety is improved as much as 80-90% as measured by the reduced storage of new and waste solvents, and by the number of daily trips to and from the laboratory to the central storage and dock areas. Infectious agents in waste solvents (such as bacteria, fungi, spores, viruses and prions) are effectively killed and/or inactivated by moist-heat sterilization, especially in the presence of solvents. In the recycling process, infectious agents are exposed to at least 5-7 hours of temperatures ranging up to 100^{o}C, and additional sterilization can be achieved simply by keeping the tank contents at 100^{o}C for longer periods of time. Solvent recycling can reduce EPA paperwork and reporting, it provides safer working conditions, and it meets the certification requirements of CAP.

The history of the association between capital punishment and anatomy. ***Hildebrandt S***. *Division of Anatomical Sciences, Office of Medical Education, University of Michigan Medical School, Ann Arbor, MI, USA.*

Anatomical science has used the bodies of the executed for dissection over many centuries. As anatomy has developed into a vehicle of not only scientific but also moral and ethical education, it is important to consider the source of human bodies for dissection and the manner of their acquisition. In the beginning of scientific anatomy, the bodies of the executed were the only legal source of bodies for dissection and anatomical dissection became part of the legal punishment, until legislation made the bodies of unclaimed persons available. With the developing abolition of the death penalty in many countries around the world and the renunciation of the use of the bodies of the executed by the British legal system, two different practices have developed in that there are anatomy departments who use the bodies of the executed and those who do not. The story of the use of bodies of the executed in German anatomy during the National Socialist regime is presented as a paradigm for the potential abuse inherent in the practice of the use of the bodies of the executed in a context of injustice. Contemporary use of the bodies of the executed, whether unclaimed or donated, is rarely well documented but exists. The intention of this review is to initiate an ethical discourse about the use of the bodies of the executed in contemporary anatomy.

New applications of APL (Acrylic Protection Layer) for anatomical specimens. ***von Horst C***. *HC Biovision, Mainburg, Germany, Europe.*

Sheet plastinates provide excellent anatomical insight not only for medical research and education but also for various fields of lay instruction. Still, the use of plain sheet plastinates in practice is restricted because sheet plastinates get scratched, start yellowing and are not well manageable in many situations. Casting sheet plastinates between acrylic layers solves most of these problems. Since the introduction of APL (Acrylic Protection Layer) there have been various requests to adapt the method to different applications. Methods: The general approach of covering anatomical specimens with acrylic layers was modified in different ways to meet the needs of various applications. This included the renovation of old scratched and yellowed conventional sheet plastinates, the use of new grinding, polishing and laser engraving methods, the addition of handling aids to specimens and the application of APL to other specimens apart from epoxy sheet plastinates. Results: The following modifications could be developed: A. Re-embedding of conventional sheet plastinates: Specimens that had been plastinated for research projects in the past could be used for Tissue Tracing and for casting them between APL. The resulting specimens showed excellent detail visibility and could hardly be distinguished from new plastinates. B. Practice Line Sheet Plastinates: To meet the needs of e.g. farriers and equine practitioners the APL specimens were additionally equipped with a soft edge to further

improve handling abilities and to secure the APL from getting scratched. C. Exhibition specimens: Adding laser engravings, facets and decorative stands improved the appealing look and the attractiveness of the specimens for their use in museums or as decorative items. D. Other applications: Casting other specimens like corrosion casts, color preserved sheets and silicone plastinated specimens between APL opened new fields to use these rather delicate specimens also in challenging environments like schools and public hands-on presentations. Conclusions: In addition to using the APL together with the Tissue Tracing Technique (TTT) for the visualization of complex anatomical structures, the APL opens a large variety of further applications, generally resulting in visually attractive, stable and manageable specimens. Providing individually adapted ways of presenting the specimens in different ways.

Plastination of embalmed and freshly preserved specimen of heart. ***Dhingra R, R Kumar****. Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.*

Plastination is a unique technique of tissue preservation developed by Dr Gunther von Hagens at Heidelberg University, Germany. In this technique water and lipids in biological specimens are replaced by a curable polymer which is then subsequently hardened. These specimens are dry, odorless, nontoxic, durable, portable and easy to handle and store. Plastination involves four basic steps: Fixation, Dehydration, Forced impregnation and Curing. Fixation is done to prevent autolysis. A 10% solution of buffered formalin serves as a suitable fixative. Higher concentrations of formalin and prolonged exposure results in shrinkage and less flexible specimens. In our department the specimens chosen for plastination were obtained from pre embalmed cadavers used for undergraduate teaching. Higher concentration of formalin used for embalming could act as a deterrent for the flexibility and thus a poorer quality specimen. Thus this study was planned to plastinate fresh hearts and compare the results with embalmed hearts. Five embalmed hearts were washed extensively under tap water and transferred to methanol and then a bleaching solution. Next specimens were dehydrated by freeze substitution in cold (-25^{o}C) acetone for 6 weeks. Once dehydrated, vacuum impregnation was carried out using a mixture of S10 (polymer) and S3 (Catalyst/chain extender) Biodur for four weeks at -20^{o}C. The excess polymer was then drained at room temperature and the specimens were kept in the curing chamber and exposed to S6 (cross-linker) vapors. Five fresh specimens of heart were obtained from postmortem cases at the mortuary of AIIMS. They were washed, cleaned and dilated using a perfusion pump. The specimens were fixed in 5% formalin for a week and then 10% formalin for another week. Overnight rinsing with running tap water was followed by the acetone dehydration at -25^{o}C for five weeks. Eosin dye was added at the last step of dehydration to give a pink color to the cardiac muscle. These hearts were then impregnated with S10/S3 mixture under vacuum and cured with S6 vapor. Results: The fresh heart specimens were more flexible and of superior quality as compared to the hearts from embalmed specimens. We acknowledge and thank All India Institute of Medical Sciences for the financial assistance provided for this project.

Varied methods of disposition for plastinated specimens. ***Liquori N, D Peterson, C Wacker, B Schmitt****. Donated Body Program, University of California, Davis, Loma Linda University, University of California, Sacramento, CA, USA.*

The University of California at Davis, School of Medicine has recently identified plastinated specimens that are no longer useful to its teaching mission and has subsequently requested that the University's Donated Body Program find an appropriate method of disposition. Appropriate, according to the University's System-wide Anatomical Materials Review Committee, is one in which the specimens are disposed in compliance with University policy and all applicable laws and is respectful to anatomical donors. In addition, we have been charged with identifying which methodology may have the least environmental impact. Plastinated specimens are virtually indestructible. Finding a method of disposition in which the molecules break down into simpler, non-toxic substances is the primary goal of this experiment. With the task at hand, an inquiry for disposition advice yielded no suggestions, although some literature advises disposition by ground burial. Materials and methods: Specimens plastinated with the S10 technique were selected for disposal using the following criteria: duplicative, crudely preserved/dissected, or damaged due to use. A representative test sample was isolated from the group and the specimens subjected to a combination of solvation by alkaline hydrolysis and standard cremation in an effort to gather information about the effects that these methods of disposition have on plastinated specimens. Their effects were evaluated by visual observation. Results: In this investigation, the specimens subjected to solvation showed color change and some breakdown of the outermost layer of polymer but virtually no breakdown of the tissue or underlying

polymer, those subjected to solvation and subsequent cremation resulted in charred specimens that self-ignited and burned without additional fuel. The methods of disposition explored have proved to be incapable of complete breakdown of the plastinated specimens involved. Conclusion: Due to the subjective nature of this investigation, a comprehensive investigation that considers air emissions and solvation residue is in order. If no traditional or non-traditional disposition methodologies are found, disposal by burial according to legal and ethical standards is recommended.

Three dimensional construction for the anatomical dissection of the brain. ***Atanesyan J, PV Roy, A Michotte, S Provin, JP Clarijs***. *Vrije Universiteit Brussel, Experimental Anatomy Department, Brussels, Belgium, Europe*.

The aim of the study was to make a special 3-D construction of the brain and develop dissection techniques for obtaining discrete areas of the human brain. The principal difference of this dissection is that 3-D construction has three possibilities to move and cut several areas and parts of the brain in order to visualize the inside of the hemispheres (limbic system, thalamus, epithalamus, fornix, hippocampus, pellucid septum and more) and show all the parts together within the network of other parts of the brain. Using the atlas of Pellegrino et al. as a guide, stereotaxically defined areas were removed from coronal, sagittal and horizontal sections prepared with sectioning stages constructed of photo slides. During dissection, all the flats were used at the same time. After anatomical dissection and eventually plastination, all the internal structures of the brain stay 3-D and together. The result of the brain dissection was helpful, it is better understood in the 3D location and it is expected to be used as an educational tool for medical and paramedical students and docents.

Plastinated heart specimens for transesophageal echocardiography education programs: Acquiring specimens. ***Pizzimenti M, E Wilkens***. *University of Iowa, Carver College of Medicine, Department of Anatomy and Cell Biology, Department of Anesthesia, Iowa City, IA, USA*.

To capture clinical transesophageal echocardiography (TEE) images, precise placement of the ultrasound probe is necessary. However, interpreting these images requires that the physician must "mentally rotate" an anatomical image to determine the view as seen on the monitor. To assist beginners in interpreting TEE images, we undertook this project to create plastinated heart samples that reflect three standard views. The purpose of these specimens was to integrate clinical imagery and actual anatomy in educational programs. Materials and methods: Dissection was completed on formalin-fixed human heart tissue that was acquired from the University of Iowa's Deeded Body Program. Heart specimens were prepared and sectioned to demonstrate the following views: mid-esophageal (ME) four-chamber, and trans-gastric (TG) basal short-axis and TG mid short-axis. Standard cold acetone dehydration was used. Polymer (Neat 295) with added S3 was infused into the specimen under vacuum at a temperature of -20°C. The final three days under vacuum were carried out at 21°C. Gas cure methods were used to maintain limited tissue flexibility. Results: Representative sections of the specimens were adequate to demonstrate the TEE images. The specimens maintained limited flexibility so that actions of the chordae tendineae and atrioventricular and semilunar valves were demonstrable. In the ME four-chamber view, ventricular wall thickness, atrioventricular valves, papillary muscles, and chordae tendineae were evident in the plastinated specimen. The specimen view corresponded nicely with the observed anatomy in a captured TEE image. Sectioning for the TG basal short-axis provides a view of the mitral valve leaflets. Imaging this region is helpful to determine complete valvular anatomy, particularly in patients with valve dysfunction. Our section was a few millimeters too superior and resulted in transecting the anterior mitral valve leaflet. Papillary muscle and ventricular wall anatomy are often viewed using the TG mid short-axis. This view coupled with the plastinated specimen clearly demonstrates the necessary anatomy to link image, specimen, and organ. Conclusions: Plastinated specimens that are sectioned to demonstrate typical TEE views are helpful in understanding clinical images. These specimens (and others) will be used during a future resident training session. At this session, we plan to evaluate the effectiveness in assisting novice sonographers in linking anatomical knowledge with clinical images. We present these standard views as discussion for incorporating plastinated specimens in educational programs.

Combined methods of plastination and partial dissection and corrosion of hepatic parenchyma, after injecting the vasculo-ductal system with plastic. ***Matusz P***. *Department of Anatomy, Faculty of Medicine, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania, Europe*.

The modalities of preserving anatomic casts underwent major improvement in recent years. These improvements addressed both: Lowering toxicity of the substance used for conservation as well as, assuring

more durable casts which remain in better condition. The method of plastination allows the conservation of human organs for an unlimited period of time, also maintaining their normal external shape and anatomic relationships. Making corrosion casts by injection of plastic into the vasculo-ductal system (mainly for the parenchymal organs), followed by parenchyma corrosion, allows exposure of the vasculo-ductal system and the unmodified intraparenchymal relationship. Each of these two methods brings distinct information regarding, on one hand, the external shape of the organ and, on the other hand, the internal vasculo-ductal distribution. This paper proposes the combination of the two methods to study liver anatomy, mainly in analyzing the relationship between the planes of the hepatic fissures and their contents. The protocol to make these casts is: 1. Inject the intrahepatic vasculo-ductal system with plastic (Technovit 7143), while maintaining the liver's anatomical form and intraparenchymal relationships by floating the liver in water during injection. 2. Parenchymal fixation. 3. Partial parenchymal dissection of the four divisions of the liver, maintaining a parenchymal layer of 5-7 mm at the level of the hepatic fissures (right, middle and umbilical fissure). 4. Completion of parenchymal removal via corrosion with hydrochloric acid. 5. Plastination of the liver cast. The results show: the plane of the right portal fissure overlaps the trunk of the right hepatic vein; the plane of the main portal fissure overlaps the trunk of the middle hepatic vein and that of the umbilical fissure is over the terminal part of the left hepatic vein. Although considered avascular, the planes of the portal fissures often present anastomoses between the segmental branches of the hepatic portal vein. The application of this combined method can be very useful in training medical professionals interested in liver surgery. (Supported by CEEX 175/2006).

Plastination of the human heart in systole and diastole forms. ***Raoof A, L Marchese, A Marchese, A Wischmeyer***. *University of Michigan Medical School, Ann Arbor, MI, USA.*

Through the use of plastination, the study of anatomy has allowed students to learn gross anatomy beyond the two dimensional level. Plastination has provided students with a priceless tool. Students now have accurate three-dimensional aids which they can hold and manipulate, real specimens. However, understanding the intricacies of organ functioning is still difficult to understand and is crucial to medical students as they prepare to enter the professional world as doctors. The heart, a vital organ of the human body, is particularly difficult for students to study and understand how its form corresponds with its function. As a result, it is imperative that a model be created in order to show which valves correspond to systole and diastole forms. Materials and methods: Multiple hearts were dissected at the coronary sinus level prior to the plastination process. Rubber corks were strategically placed in the various valves that were desired to be kept open. Valves that were intended to remain closed were sealed with sutures. The hearts were then sutured at the coronary sinus level, to keep it in its original exterior form. Lastly, the specimens were plastinated using the room temperature method. Once the dissection and plastination process had been completed, the sutures were removed and a hinge was put into place in order to keep the heart as one whole unit. Results: This method proved to be very successful. The specimens were very maneuverable which allowed us to be able to feel and make precise cuts above the lunar valves. The final product gave us our desired outcome. It permitted us to see the systole and diastole forms while still allowing the superficial structures of the heart to be intact. Conclusion: With the use of hinges, we were able to create an animated model of the heart that could allow students not only to study the heart from the outside, but also to gain a better understanding of how the heart pumps. This allows students to see inside the heart and examine both the systole and diastole forms.

Classification of pig kidney collecting system: Anatomic study for pelvi-caliceal drainage. ***Pendovski L[1], V Ilieski[1], B Trpkovska[2], V Petkov[1]***. *[1]Department of Functional Morphology, Faculty of Veterinary medicine - Skopje,[2]Institute for Anatomy, Medicine Faculty - Skopje, Macedonia.*

Previously, the physiology of the pig kidney has been studied in detail. The anatomy of the pig kidney is also described in some publications but those data are generic and usually don't offer detail concerning the pig collecting system. In recent literature, a few specific studies were found in which the morphology of the pig's pelvicaliceal system was well described. But there is still a shortage of studies in which the drainage pattern of the minor calices and its position in the kidney is analyzed. The results of a detailed anatomical study are presented on the pelvicaliceal system in pig kidneys. Classification of the pig collecting system based on drainage of minor calices into renal pelvis was the aim of this study. Materials and methods: Fifty three kidneys were harvested from adult mixed breed Dalland pigs that were slaughtered at 150-155 days of age with a mean weight of 95 kg. Endocasts of silicone (S 10) were prepared to study this three-dimensional system. Flexible PVC tubing was ligated in the ureter. A

mixture (10-15ml) of S10/S3 (silicone polymer/catalyst), ratio 100:1, was prepared and colored yellow. Five percent of the S6 hardening agent was mixed with the polymer/catalyst mix. This mixture was injected into the ureter to fill the kidney's collecting system. After injection, the kidneys were placed in appropriate anatomical position for 24 hours to ensure deep curing of the silicone mix. The injected kidneys were immersed in a bath of concentrated commercial hydrochloric acid for 48 hours or until total corrosion of the organic matter was achieved. The endocasts of the collecting system remained. Results: Each collecting system in pig kidneys was composed of a renal pelvis and two major calyces or infundibulae (one cranial and one caudal) in which minor calices opened. The number of minor calices per collecting system ranged from 5 to 17 (mean 9.02) and significantly more minor calices were associated with the cranial pole than with the caudal pole ($p<0.05$). Perpendicular minor calices that drained directly into the dorsal or ventral surface of the renal pelvis were found in 33.95% of the casts. Based of drainage into the mid-zone of the renal pelvis, the collecting system was classified into two major groups. Group I: In 55.48%, drainage into the renal pelvis mid-zone was into two discrete areas: Directly into the cranial infundibulum or the caudal infundibulum. Both of these polar groups, separately and simultaneously, drain into the mid-zone of the renal pelvis. Group II kidneys: In addition to infundibular drainage, the remaining 44.52% independently drain into the mid-zone of the renal pelvis. These independent units enter on the lateral margin of the pelvis over its entire length. Conclusions: The existence of minor calices draining perpendicularly into the surfaces of collecting system will help for future interpretation of pyelograms. Also, the results in this study will increase the knowledge about collecting system in pig kidneys for its future application in experimental endourology. S 10 silicone, was used successfully as the injection medium for preparing replica casts, demonstrated the anatomic features for study of the drainage pattern of the pelvicaliceal system of the pig.

Changing the focus from passive to active learning of gross anatomy. ***Gest TR***. *Division of Anatomical Sciences, University of Michigan Medical School, Ann Arbor, MI, USA.*

Over the past 8 years, web based educational materials have been developed that comprise an integrated, comprehensive presentation of gross anatomy. Concurrent with the development of computer based learning materials, a "LectureLite" strategy was adopted to promote an active learning environment. Lectures were reduced to 20-30 minute overviews of dissections. Four years ago, the medical school adopted a new curriculum organized around organ systems. The traditional discipline based courses (gross anatomy, histology, biochemistry, etc.) were replaced with systemic sequences (musculoskeletal, cardiovascular, etc.) presented as a series of integrated lectures. Performance was measured on an entire sequence, and there was no minimum passing score for each discipline within a sequence or across the year. Performance in the gross anatomy labs suffered a steady decline subsequent to the removal of a passing standard for gross anatomy. Last year, in order to focus student effort on the gross dissections and increase active learning, lectures were eliminated completely. Performance on the gross anatomy component of the curriculum did not change following the elimination of lectures. This year, adjustments were made that seem to have increased the quality of student dissections and lab performance. A system of dissection evaluations was implemented. Faculty members use a standard form to evaluate the quality of each day's dissections on four criteria. Although these dissection evaluations can no longer be used as a factor in the sequence score, students have dramatically increased the quality of their dissections. The other adjustment made this year was to modify our system of peer presentations and their evaluation. With relatively simple schedule adjustment and random faculty evaluations of peer presentations, a dramatic improvement in the quality of these presentations has been witnessed.

Plastination of poorly preserved non-human animal specimens for biology education. ***Ostrow B***. *Department of Biology, Grand Valley State University, Allendale, MI, USA.*

Students in zoology courses traditionally examine representative specimens of invertebrate and vertebrate taxa. Animal specimens are usually stored in alcohol or formalin preservative in glass jars or plastic buckets or are frozen. Because curation of collections is time-consuming and expensive, plastination of specimens is a sensible solution for long-term preservation. However, many collections have not been well maintained such that the preservative has evaporated. This study investigates the feasibility of plastinating poorly preserved specimens. Over decades, animal specimens have been collected, purchased from biological suppliers, and donated to Grand Valley State University (GVSU) for use in laboratory courses. Using a room temperature method, 48 specimens from the GVSU biology holdings were plastinated that ranged in quality from new and freshly fixed to old and poorly

preserved. Some of the older specimens were completely dried and coated in a residue that was presumed to be dehydrated formalin. Water was added to those jars to rehydrate the preservative and specimen. The collection for plastination included whole and dissected earthworms (Lumbricus sp.), grasshoppers (Romalea sp.), a shark embryo (Squalus sp.) with attached yolk sac, a bowfin fish (Amia sp.), adult and tadpole bullfrogs (Rana sp.), a sheep embryo (Ovis sp.), a pig embryo (Sus sp.) that had been cleared previously in potassium hydroxide/glycerol, deer fetuses (Odocoileus virginianus), and a bat embryo (Phyllostomus discolor). Also latex-injected squid (Loligo sp.), dogfish sharks (Squalus sp.), and mudpuppies (Necturus sp.) were plastinated. On the whole, plastination of these specimens was successful in that the majority of the plastinates turned out nicely. However, the legs and wings of the plastinated grasshoppers broke off easily from the body and had to be glued back in place. There was no difference in results between specimens that had been preserved in formalin or alcohol nor between new or old specimens as long as the specimen was not completely dried out. Dried specimens were rehydrated satisfactorily in water and returned to their original size. However, they did shrink appreciably upon plastination but not as extensively as when they were found with their preservative evaporated completely. Acetone dehydration during plastination completely shriveled the cleared pig embryo such that it was not recoverable. Plastination of cleared specimens is not recommended. Quality of the final plastinate depends greatly on the quality of the initial specimen. Thus plastination of poorly preserved specimens is less satisfactory than plastination of new and freshly fixed specimens. Still, plastination is a viable and beneficial endeavor for long-term preservation of most all specimens.

Achieving integration in practice as well as in name by the use of cases and plastination labs. ***Brzezinski D****. Division of Anatomical Sciences, University of Michigan Medical School, Ann Arbor, MI, USA.*

At the University of Michigan School of Dentistry, we have recently moved from teaching multiple, distinct disciplines, to teaching a systems-based curriculum. This new curriculum teaches relevant aspects of the gross anatomy, histology, embryology, physiology, pathology and pharmacology of a particular organ system #side-by-side#. While students learn, for example, cardiac anatomy with cardiac pathophysiology at the same time, they still struggle to truly integrate the material. While they may understand the relevant concepts from anatomy and pathophysiology, they have a hard time bringing everything together. In an attempt to facilitate student learning (of important knowledge, skills and attitudes), we are currently modifying our teaching approach. We are utilizing case studies at the end of sequences so that students will actively engage the material in contrast to passive learning in the classroom setting. We are also creating "Medical Science Laboratories" where students will actively work through assignments while using plastinated specimens (both normal and pathologic). Finally, during assessment students are tested via essays which require them to bring all concepts together. The "bar" is set higher and students are required to remediate anything they miss, no matter what their final score (high or low). The implementation of novel teaching methodologies utilizing modern technology has improved student performance, as well as interest in the material.

The pig heart anatomy on thin S10 tissue slices. ***Ilieski V[1], L Pendovski[1], B Bojadzieva[2], V Petkov[1]****. [1]Department of Functional Morphology, Faculty of Veterinary Medicine - Skopje,[2]Institute for Anatomy, Medicine Faculty - Skopje, Macedonia.*

For teaching gross anatomy of the pig heart and acquiring comprehensive knowledge, pictures, charts, diagrams and models are needed. It has been shown that sheet plastinated slices are excellent tools for demonstrating the topography of internal anatomical structures. The E 12 and P 35/40 plastination techniques are known as the methods of choice for creating 3-5mm or even 8mm semitransparent or transparent organ slices. These techniques also required equipment and resins that not all plastination laboratories have. In order to display structure distinctly for study and research, a protocol was developed in which the S 10 method was utilized to produce sheet plastinated slices. Materials and methods: One pig heart taken from a mixed-breed landrace-yorkshire was the subject for S10 sheet plastination. The fresh pig heart was dilated using tap water under hydrostatic pressure to relax the muscle and to remove any remaining blood from its chambers. The dilated heart was fixed by immersion in a 3% solution of formaldehyde for one week. After fixation, the slices of pig heart were cut with a meat slicer into 5mm slices. Each slice was marked with its serial number and placed between two stainless steel grids in a stainless steel basket. The basket with slices was rinsed with cold tap water overnight to remove fixative. Dehydration was carried out in pure acetone -25^{o}C with a tissue:fluid ratio of 10:1. The basket with slices was submerged in the first 100% acetone bath for five days. After the first bath, the slices were transferred in second acetone baths

for another five days. The slices were removed from the last, third acetone bath, when the acetone concentration remained at least 99% for three consecutive days. For forced impregnation, the slices were submerged in a fresh S10/S3 mixture (100:0.05) at -25° C and allowed to equilibrate for three days. Vacuum was applied and over a two week period pressure was decreased (by discontinuous method) slowly until 5 bars were reached. At this pressure impregnation was complete and pressure was returned to atmosphere. The specimens were left submerged in silicone bath at room temperature for three days. The slices were removed from the vacuum chamber for curing. The surface of each slice was wiped of excess silicone-mix. Finally, the gas curing method was applied for two days, and the heart slices were cured. Results: The S10 sheet plastinated pig heart slices were produced over a period of five weeks. The color of cardiac muscle was maintained and shrinkage was not evident. The slices are elastic, easy to orientate and offer an abundance of anatomic detail. The heart muscle fibers are seen individually and their shape can be followed for the entire length of the slice. Anatomical structures, i.e. ostia, maintain their anatomical form. The chordae tendonae are seen attached to the ventricular surfaces. As well, the valves (tricuspid and bicuspid) can be seen originating form the papillary muscles. A three-dimensional view of the atria and ventricles can be imagined, as well as their shape and size. Data was recorded on the daily decrease of vacuum with the aid of a Biodur digital vacuum controller. These data were used to understand the relationship between the decrease of vacuum and the speed of silicone impregnation. Conclusion: The S10 plastinated pig heart slices proved to be a perfect teaching tool in anatomy. The students can view and handle a three-dimensional pig heart, and also be able to better distinguish specific anatomical details of all anatomical structures. The knowledge of anatomy based on thin plastinated heart slices will aid interpretation of diagnostic CT/MRA scans in the clinical setting. The S10 technique demonstrated its usefulness as a method for producing organ slices. This method is relatively easy to carry out and uses materials that are basic for the plastination process.

Color restoration during silicone impregnation. ***Sakamoto Y[1], H Ueki[1], H Najita[2], RW Henry[3]***. *[1]Medical Museum, Kawasaki Medical School, Kurashiki, Japan. [2]Department of Universal Design, Faculty of Health and Welfare Services Administration, Kawasaki University of Medical Welfare, Kurashiki, Japan. [3]University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, Knoxville, TN, USA.*

One of the major deficiencies of silicone plastinated specimens is loss of color, both from formalin fixation and acetone dehydration. For over a century medical museums have been preserving and reactivating color. Recently a method of reactivating color in wet museum specimens has been modified and adapted for silicone Plastination. Imidazole an oxidizing agent is incorporated as a part of the polymer impregnation-mixture. Silicone is mixed at its standard ratio with the catalyst or catalyst/chain extender (100:1-3). This impregnation mixture is mixed 100:2 with a mixture of imidazole and ethanol. The prepared imidazole:ethanol mixture ratio is 1:3. After thorough mixing of the polymer and imidazole, the dehydrated specimens are submerged in this imidazole polymer reaction-mixture. During the impregnation process the hemochromogen reaction occurs from the presence of imidazole around and within the specimens. When the specimens are removed from the polymer imidazole-mixture, the hemoglobin has been reactivated to its bright reddish color. Impregnation is carried out in the cold (-15°C). When specimens are left in a room environment for prolonged periods, there is color loss. However, if specimens are kept in display cases when not in use, the color remains for many years.

Alternative curing methodology of the polyester resin, P40. ***Henry RW***. *University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, Knoxville, TN, USA.*

Polyester resin P 40 was introduced a decade ago as a less cumbersome alternative to the P 35 resin which has become the gold standard for production of brain slices. The advantages of P 40 resin is that no additives are needed for curing, hence an indefinite pot life. Ultraviolet light serves as the catalyst and P 40 may be used on slices from any region of the body. For curing the resin using the standard P 40 protocol, the impregnated slice, surrounded by the resin, is sealed within a flat glass chamber. Both surfaces of the glass enclosed impregnated slice are exposed to ultraviolet light, from lamps, to serve as the catalyst. The unit is cooled using a ventilator (fan) during curing to dissipate the heat generated by the exothermic reaction of curing. A series of tests were conducted to determine if alternative sources of light could be used to cure the cast slices and if sealing of the flat chamber was necessary for curing. Alternative sources used were: a. Daylight out doors and indoors using exposure to sunlight directly or exposure in the shadow only; and b. Electrical sources: Mercury vapor lighting plus

florescent lighting, Florescent lighting, and Ultraviolet light from lamps. Exposure to any of the above served as the catalyst. Direct sunlight, even with a ventilator blowing across both surfaces, may cause too intense of a reaction leading to fracture of one or both glass plates and/or drying of the tissue. Results of the exposure to natural light in a shaded area, with a ventilator blowing air on the cast slices, were similar to those of the standard protocol exposure using ultraviolet lamps. Using the standard protocol, the top of the flat chamber is sealed to: Prevent resin leakage when the chamber is laid horizontal between the lamps; Aid positioning and prevent drifting of the slice; and Prevent exposure to air which interferes with the curing of P 40 resin. For curing, the casts may be left vertical or turned 15^{o} from horizontal without sealing the top gasket. All but <0.5mm of the resin along the perimeter of the created sheet cured except with florescent light cure. When UV exposure to the downside is limited, it is recommended to turn the flat chamber over after 15 minutes of exposure to UV light to assure adequate exposure and curing of the down side. Upright positioned casts may not need to be turned. Mercury vapor light exposure at a distance of five feet causes a slow but productive reaction. Florescent light alone took several days to cure and a 3 - 4 mm perimeter of resin remained liquid. Any of the above techniques appear to be useful for polymerizing the P 40 resin.

Alternative curing methodology of the polyester resin, P40. ***Henry RW****. University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, Knoxville, TN, USA.*

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P40 brain and body slice production. ***Henry RW****. Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

The brain is removed and fixed for several weeks until hard, in 10 - 20 % formalin. For slicing, a deli slicer is used and the fixed brain is flushed for at least 24 hours to remove some of the formalin. A desired thickness is selected and the guide stop set appropriately. The brain is transected in the desired plane and slices produced. The slices are placed on heavy filter paper or fly screen and then on grids. The grids are stacked in a basket. Stacks may be tied together for ease of transfer. Brain slices are flushed in flowing tap water for overnight. The stacked, flushed slices are transferred into the first cold acetone (-25^{o}C) dehydration bath with a tissue:fluid ratio of 1:5-10. After two days, the slices are transferred into the second cold dehydration bath. Acetone purity is checked on the 3rd and 4th days. If the purity of acetone is >98%, the specimens are transferred into the P 40 polyester resin impregnation bath and placed in a vacuum (impregnation) chamber (plastination kettle). A dark cover must be placed over

the glass port to darken the kettle. Ultraviolet light is the catalyst for P 40. The vacuum pump is started and allowed to warm to working temperature. Vacuum is applied to the plastination kettle and the port is sealed. Pressure is lowered one half an atmosphere quickly. From this point forward, pressure is lowered slowly using the manometer and by watching bubble production in the resin. The pressure is slowly lowered to10mm Hg over a 12 - 24 hour period. Pressure <10mm results in the polyester resin to begin to break down. Once impregnation is completed, the kettle pressure is returned to atmosphere and the container of slices with the polyester resin is removed to dark storage. Individual slices are placed in flat chambers constructed from appropriate sized window glass, silicone gasket and large folder clamps. The glass chamber is filled with polyester resin. Trapped air bubbles are allowed to rise and burst. The slice/resin filled chamber is exposed to ultraviolet light for curing. A fan (ventilator) is positioned to blow air across both surfaces of the glass during exposure to UV light. In one to two hours, the resin is hardened. The flat chamber can be dismantled and the slice wrapped in plastic wrap (foil). Later the slices may be sawed to an appropriate size.

Tracheobronchial cast preparation. ***Henry RW***. *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Fresh lungs are collected from a cadaver of choice. Do not cut the lungs. The trachea is cannulated with an appropriate size cannula (tubing) and flushed gently with tap water. Do not over inflate. Trim the excess/extraneous tissue (esophagus, pulmonary vessels, fat, mediastinal structures) from the tissue block. Slowly inflate the lungs with laboratory air until the lungs rise to anatomical position. Use continual air flow until the lungs are dry, usually two to four days. The dry lungs will feel very light, like Styrofoam and will not collapse when removed from the air flow. After lungs are dry, mix an RTV silicone with its catalyst and fill the airways with the polymer-mixture until the polymer can be visualized on the surface as a patchwork of small rosettes (small airways filled). The lung will feel heavy. For better visualization of the bronchial tree, it is better to use a smaller amount of the polymer-mix. Let the preparation stand overnight at room temperature to assure curing of the polymer-mix. After polymerization, the lungs are boiled for 24 - 48 hours to remove most of the tissue. The lung tissue may also be removed by chemical corrosion. Once the majority of the tissue is removed, the cast may be cleaned with a hydrogen peroxide solution (10 - 20%). The results are an exact replica of the airways.

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Plastination – quixotic adventure to public science. ***Whalley A***. *Institute for Plastination, Heidelberg, Germany.*

Plastination, a vacuum-forced impregnation technique with reactive polymers for biological specimens, was invented and patented by Gunther von Hagens at Heidelberg University in 1977. But plastination technology only gained wide acceptance after further progressive developments which included the development of suitable polymer systems, 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 and plastination of large specimens including whole body specimens. The 1st International Congress of Plastination was organized by Harmon Bickley in 1982 at Texas Tech University in San Antonio, 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.

Principals of silicone (S10) plastination. ***De Jong KH***. *Department of Anatomy & Embryology, Academic Medical Centre, University of Amsterdam, Netherlands.*

Plastination is defined as the replacement of tissue water and fat with a curable polymer, either silicone, polyester or epoxy. As all 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 grade of dehydration of >98.5% is reached. After sufficient dehydration the specimen is placed in a receptacle filled with silicone monomer mixed with a combination of catalyst and cross-linker, which is called the impregnation- or reaction mixture. As acetone and reaction mixture are soluble in each other, acetone will diffuse in the reaction mixture. The receptacle with the specimen and reaction mixture is placed in a vacuum-chamber and the pressure is slowly decreased (vacuum is increased). This will cause that the acetone, dissolved in the reaction mixture will evaporate, and new acetone will dissolve from the specimen in the reaction mixture, resulting in a negative pressure in the specimen. This negative pressure will “drag” the silicone molecules into the specimen. By gradually increasing the vacuum slowly all the acetone will be replaced by silicone. This part of the Plastination-process is called forced impregnation When the forced impregnation is complete, the specimen is brought to atmospheric pressure, taken out the reaction mixture and left to drain. When the excess silicone is drained off, the specimen is exposed to a gaseous cross-linking agent, which will form a 3D meshwork of the silicone monomer molecules inside the specimen, first at the surface, making it safe to handle, later also deeper inside the specimen. This process is called curing. Processing time is dependent on the size and tissue of the specimen, and varies from 6 weeks to several months.

Room-temperature impregnation with cold-temperature silicone products. ***Henry RW***. *University of Tennessee, Department of Comparative Medicine, Knoxville, TN, USA.*

Background: The Biodur S10/German/cold-temperature plastination process and products have been used nearly thirty years and have become the gold standard for tissue preservation. Like any good product, alternatives, whether good or bad, are often developed. Such is the case for silicone plastination, both alternative products and techniques are now available. A true room-temperature impregnation process has been developed. Most silicone products for plastination produced today likely could be used in either methodology(cold or room temperature), therefore little is truly unique concerning these “new” products and methodologies. Methods: The main difference of the Room-temperature/Dow™/Corcoran methodology is in the sequence in which basic plastination chemicals are combined and used. The impregnation-mixture is polymer plus cross-linker (instead of polymer plus catalyst and chain extender). This polymer/cross-linker combination is stable at room temperature, while the

polymer/catalyst combination is reactive and is usually kept in the cold at all times. Results: The Biodur chemicals have been used successfully with this room-temperature methodology. Similar polymer/ additive ratios of chemicals were used. Specimens plastinated by this methodology are of similar quality to those produced by the currently recommended products available for room-temperature impregnation. Conclusion: Silicone polymer and additives, designed for cold-temperature impregnation, can be used for room temperature impregnation. However, specimens plastinated by the room-temperature impregnation technique, regardless of products used, are not equal to the gold standard quality of specimens produced by the cold temperature technique.

Degreasing by benzene in silicon10 technique. ***Basset Aly AE, A Abdel Aziz, S Abdel Aziz, H Nosiur, M Konsowa, KZ Soliman***. *Plastination Laboratory, Faculty of Veterinary Medicine, Zagazig University Zagazig, Egypt.*

Background: The aim of this work is to reduce the costs of plastination in Zagazig Plastination Laboratory. During our preparation of plastinated specimens by S10 technique for teaching of Veterinary Anatomy (subject arthrology), we had a stifle joint of a horse contained large amount of adipose tissue. Degreasing process would require a huge amount of acetone.Benzine was used as a degreasing agent, which was 20 times cheaper than acetone in Egypt and almost same results as acetone. Material and Method: After routine fixation by formalin 10% and prosection, the stifle joint was immersed in Benzin (commercial 80) for 3 weeks, changing the benzene every week at room temperature. The fat was degreased, then the specimen was washed in running water for one day. Dehydration: Routine dehydration in cold acetone. Forced Impregnation: Routine forced impregnation (Biodur® S10 &S3). Curing: Routine gas curing (Biodur® S 6). Results: The results were quit good but slight yellow coloration from benzene was observed. Conclusion: The use of benzene (commercial 80) reduced the costs of plastination and allowed our plastination laboratory to produce more joints specimens for teaching of Veterinary Anatomy.

A low-temperature dehydration/ room-temperature impregnation protocol for brain tissue using Biodur S10/S3. ***Adds PJ***. *Division of Basic Medical Sciences (Anatomy), St George's, University of London.*

Background: The standard method for plastination with Biodur S10/S3 silicone involves low-temperature dehydration in a volatile intermediary solvent (acetone or methylene chloride) followed by forced impregnation under vacuum at -15°. However, some institutions have been reluctant to install low-temperature impregnation equipment because of health and safety concerns. A room temperature set-up has the advantages of low cost and simplicity of set-up, and avoids the potential safety hazards associated with low-temperature impregnation. At its best, this method can yield specimens equal to those produced by low-temperature impregnation, but it is not suitable for brain tissue because of the degree of shrinkage. We have trialed a low-temperature dehydration/ room-temperature impregnation method for plastination of brain slices. Method: Formalin-fixed brains were cut into transverse or coronal 1.5cm slices, washed first in 50% ethanol then in water, pre-chilled to 4° C then dehydrated in sealed containers of acetone at -30° C weekly for 3 weeks. The brain slices were then placed in Biodur S10/S3 100:1 and allowed to equilibrate overnight to room temperature. The specimens were then impregnated, drained and cured at room temperature. Measurements of the length and width of each slice were taken after slicing and post-impregnation. Results: The quality of the plastinated brain slices was good, with clear definition of white and grey matter. Shrinkage was less than 5%, and the value of the slices as teaching aids was not impaired. Conclusion: Room temperature plastination is an attractive option for reasons of speed, low setting-up costs and avoiding health and safety hazards, and can give comparable results to the low-temperature process for most tissues except CNS. By combining low-temperature dehydration in sealed containers with room-temperature impregnation, brain slices can be plastinated at room temperature with minimal shrinkage.

Effects of cold and room temperature impregnation on morphological features of the heart during plastination. ***Dhingra R, DN Bhardwaj, K Ilavenil, R Kumar***. *Department of Anatomy, All India Institute of Medical Sciences, , Ansari Nagar, New Delhi, India.*

Background: Plastination has evolved as a new tool for preservation of specimens and can be used as a valuable adjunct for teaching medical students as it offers many advantages over the traditional formalin fixed specimens. The process of plastination involves fixation, dehydration, impregnation and curing. The impregnation is done under vacuum and is the central step of plastination. It may take 2 to 5 weeks to carry out the forced impregnation depending on the temperature. In the present study we aimed to compare the process of impregnation at -20°C with that of at room temperature(25-30°C) and see if room temperature impregnation could minimize the time

spent and financial burden of deep freezers. Material and methods: A total of 20 hearts obtained from autopsy cases being done at department of Forensic medicine, AIIMS were used for the study. Each heart was subjected to dilatation, fixation, dehydration, impregnation and curing. The hearts were divided into two groups (A and B) and each comprised of 10 hearts. All the steps of the procedure were same in the two groups except the impregnation under vacuum. The impregnation was carried out at room temperature (25-30°C) for group A hearts and at cold temperature (-20°C) for group B hearts. The procedure of pre-curing and curing was same for both the groups. Results: The time taken for forced impregnation for group A hearts [room temperature (25-30°C)] was 12 -14 days and was completed at 75-100 mm Hg of pressure, whereas in group B the cold impregnation was complete at 5-10 mm Hg and the time taken was 30-37 days. The morphological features were evaluated on the basis of shrinkage percentage, color, dilatation, flexibility and preservation of external and internal features of heart. The mean shrinkage percentage of group A hearts (room temperature impregnation) was 4.54+ 1.76 and for group B hearts (impregnation at -20°C), it was 4.97+2.67. The difference in shrinkage percentage was statistically insignificant. The other parameters like color, dilatation, flexibility and morphological features were well preserved in both the groups. Conclusion: The rate of impregnation was faster at room temperature however this did not distort the anatomical features of hearts and saved the cost of freezers and electricity. The impregnation done at room temperature has a disadvantage of reduction in the life of the polymer mixture which can be partially overcome by storing the polymer mixture in a freezer in between impregnation cycles.

The plastination Biodur S10 technique applied in teaching the male genital organs in veterinary anatomy. ***Diz A, J García-Monterde, E Agüera, J Vivo, JL Morales, JM Navas-Lloret***. *Department of Comparative Anatomy and Pathology, Faculty of Veterinary Sciences, University of Córdoba, Spain.*

Background: In the teaching program of the Veterinary School of Córdoba, the Gross Anatomy is included in two of its subjects, Embriology and Sistemic Anatomy -1st academic year- and Neuroanatomy and Topographic Anatomy -2nd academic year-. In the lab sessions of the first one, canine dissected specimens are mainly used. In the program of the second academic year subject a part is extensively devoted to the learning of regional and topographical anatomy by species (carnivores, horse, ruminants, pig and birds). To help to a better understanding fresh, formalin-fixed and plastinated specimens are used in the dissection room. In the above two mentioned subjects the male genital organs must be thoroughly analyzed. An Veterinary Anatomy Museum is regularly used by students to complete the objectives of theoretical and practical lectures. The aim of the present study was to describe the advantages of using plastinated specimens compared to fixed and fresh pieces in the teaching of the male genital organs in Veterinary Anatomy Materials and methods: During necropsy male genital organs of domestic animals whose death was not due to infectious cause were removed. After that, they were cleaned, fixed with formalin (5-10%) and thoroughly dissected to make clear the structures of anatomical interest. Then, the specimens were plastinated by using the Biodur S-10 Technique. Finally, part of them were destined for its study in the dissection room and the rest were located in the Veterinary Anatomy Museum to be freely used by the students. Results: Plastinated specimens of male genital organs were well accepted by the students in the dissection room. The main advantages are that they are dry without toxicity and odor and a special equipment, like gas extractor, is not necessary, when comparing to the traditional fresh and fixed pieces. Those destined to the museum are exhibited in glass cases at free disposal of the students. Conclusion: The Biodur S-10 technique of plastination is useful in the understanding and learning the male genital organs in Veterinary Anatomy. Compared to fresh and fixed specimens, plastinated pieces have no toxicity and are durable, which allow not only their use in the dissection room but they may also be exhibited and studied in the Anatomy museums.

Use of first whole body plastination by S10 technique adjunct to dissection in Iran. ***Asadi, MH, H Bahadoran, GR Hassanzadeh***. *Department of Anatomy Baqiyatallah University of Medical Sciences, Tehran, Iran.*

Background: Despite the proliferation of medical schools in Iran the scarcity of cadavers for anatomic dissection continues to be aggravating problems. For this reason at Baqiyatallah University of Medical Sciences, a whole body plastination has become an essential component of medical anatomy education for past two years. The aim of this study has been to provide suitable specimen that reflect the essential concepts in anatomy in order to promote students learning and progress. Material and Methods: The cadaver was fixed by formalin. After fixation, teaching protocols were prepared for each region of anatomy. Then this region were carefully dissected and prepared

for plastination. Following dissection specimen were dehydrated by various grade of cold acetone. Forced impregnation was done by S10 and S3 mixture at –20°C. Then the specimen was kept at room temperature for 5 weeks to drain the excess polymer. In final stage, the specimen was subjected to gas curing for ten days at room temperature. Results: Use of whole body plastination adjunct to dissection in anatomy laboratory enable us to present a complete visual and physical guide to the human body in a revolutionary way, which making anatomy more interesting, easier to learn and more relevant to the students future career objectives. Also it was very helpful in learning the spatial relationships of important anatomical structures. Conclusion: Use of whole body plastination, adjunct to dissection are planned for a wider use in future education program to assist other university and students in Iran.

Creative dissection for plastination. ***Von Hagens G***. *Gubener Plastinate GmbH, Guben, Germany.*

Compared with other dry preservation methods such as freeze drying or paraffinization, plastination leads to specimens with highest mechanical strength. This is due to the high mechanical properties of reactive polymers such as polyester or epoxy resin which are used for the manufacture of plastinated body slices and for the manufacture of dissected silicone rubber specimens up to the size of whole human bodies or even large animals such as a horse and a giraffe. Due to their hardness plastinates can be regarded as combined muscle-organ-bone skeletons. Soft tissue such as muscles can take over the holding function and keep organs or even joints in place. Stainless steel bars, inserted into the long bones and bridging joints can further increase the stability of plastinates. This stability of plastinates makes new kinds of dissections possible not seen before the advent of plastination. As for whole human body specimens the following new specimens became possible: (1) Specimens with opened body doors, (2) Display of organs and organ systems side by side such as a man with his skin or skeleton beside his own muscles in identical poses, (3) Fragmented specimens where organs, muscles or bones are shifted apart from one another, creating visible space in between. Especially the complex anatomical features of joints and of the human head could be dissected in new and favorable ways. Twenty of those new dissections will be demonstrated and their advantages discussed. As examples; the demonstration of the rotary cuff of the shoulder joint with a spilt humeral head and exposition of the glenoid fossa, a human head opened in door-like dissected tissue layers, and a layer dissection of the gluteal region. The dissected specimens can be evaluated during the visit to the Plastinarium in Guben.

Frankincense plastination after honey fixation. ***Elhag AH.; AM Al-Wahaibi***. *Sultan Qaboos University, Alkhodh, Sultanate of Oman.*

Background: In the scientific community's urge to find safer scientific techniques, utilizing natural recourses around us might proof to be efficient in providing safety, less cost, and quality. Honey was used as a fixative to replace the widely used Formalin. Reported formalin hazards increased the awareness to limit its use or even to replace it. The choice of honey, as a fixative, was supported by our earlier findings in using it as a histological fixative. The use of Frankincense or as better known locally Luban, as an impregnation and embedding medium, was supported by the fact that it contains 60-70% resins according to literature, this might qualify it to for purposes mentioned above. Material and Methods: Four "Goat" kidneys and three hearts were collected, rinsed thoroughly in running tap water. Two of the kidneys were bisected; the heart chambers were cut open to facilitate fixative penetration. Specimens were fixed in 20% honey solution, washed in running tap water, dehydrated in acetone, and finally impregnated with Frankincense solution under vacuum. Control specimens were processed using the standard formalin fixation and Silicon resin impregnation (S10). Result: Formalin fixed and silicon impregnated kidney (control) exhibited small flexible with natural color. Formalin fixed and Frankincense impregnated kidney (specimen 2) is not flexible, dry in texture and darker in color compared to the control. Honey fixed and Silicon impregnated kidney (specimen 3) is more flexible, darker in color and shrunken in size compared to the control. Honey fixed and Frankincense impregnated hearts (specimen 4, 5 & 6) were more flexible (initially) darker in color compared to the standard Silicon impregnated heart which is less flexible with natural color. Conclusion: Both methods; honey fixation and Frankincense impregnation experimented in this study were found to be; economic, time saving, safer on health and environment and produced specimens of good quality. All results are true for the tissue types used in this study. The project is to be continued for further refinement of the method.

Comparison of gross morphological features in freshly and old embalmed human hearts using plastination. ***Kumar R, K Ilavenil, R Dhingra***. *Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India.*

Plastination is a technique of tissue preservation in which water and lipids are replaced by polymers which are subsequently hardened. The plastinated specimen becomes dry, odorless and durable. Plastination of various organs is being tried world over using old embalmed human organs, However for the lifelike appearance and better preservation, we have tried to plastinate freshly fixed(obtained during autopsy) organ the human heart and compared its morphological features with that of old embalmed heart. A total of twenty freshly fixed hearts and six old embalmed hearts were taken for the study. Each was subjected to the standard technique of plastination using S-10 method. The process of plastination of heart involved dilatation of heart, fixation with 5%formalin, dehydration by giving 4 changes with cold acetone (-20˚C), impregnation in S3, S10 mixture and gas curing at room temperature. Old hearts, since they were already formalin fixed could not be dilated as the fresh fixed ones. But their morphological details were preserved. In the freshly fixed hearts the morphological features such as color dilatation flexibility were better visualized. The internal features such as AV, pulmonary and aortic valves were very clearly visible. They did not collapse and shrink as compared to the old embalmed hearts. Also the handling and aesthetics were better in these hearts. They preserved their lifelike appearance as compared to old embalmed hearts which looked darker due to long preservation and were relatively non-flexible. Thus the features were more lifelike, better preserved and clearly visible in freshly fixed hearts as compared to old embalmed hearts.

Shrinkage of renal tissue after impregnation via the cold Biodur plastination technique. ***Pereira-Sampaio MA[1,2], FJB Sampaio[1], RW Henry[3]***. *[1]Urogenital Research Unit/UERJ, Rio de Janeiro, Brazil, [2]Morphology/UFF, Niterói, Brazil, [3]Comparative Medicine/UT, Knoxville, TN, USA.*

Background: Thorough dehydration is a key for good plastination and invariably it leads to shrinkage. Shrinkage during plastination has been studied minimally. This study quantifies the shrinkage for each of these activities. Methods: Total tissue shrinkage was studied on ten pig kidneys including regional shrinkage of the kidney (cortex, medulla, sinus) and at which stages of the process (dehydration, impregnation, curing) shrinkage occurred. Kidneys were fixed by perfusion of 10% formalin solution via the renal artery. Next, the vessels and ureter were filled with colored E RTV Silicone. The fixed, injected kidneys were cut into one centimeter transverse slices. The classic cold von Hagens' method was used to plastinate two slices of each kidney. The slices were dehydrated via freeze substitution (-20oC) and impregnated with silicone at -15oC. At the end of each stage of the plastination process, slices were photographed using the same focal length with a digital camera. Slice surface area was determined by a point-counting planimetry method. Results: Total shrinkage of kidney area was 10.2 % post-dehydration and 10.1% post-impregnation. After completion of plastination, total area of kidney slice shrinkage was 19.7%. Cortical area shrunk 12.8% after dehydration and 13.2% after impregnation. After plastination, cortical area had shrunk 24.3%. No significant shrinkage occurred in the medulla and sinus. Shrinkage has been reported with dehydration. Our results demonstrate that kidney shrinkage during impregnation is as intense as during dehydration. Significant shrinkage occurred in the renal cortex but not in the medulla and sinus. Conclusion: This demonstrates that different tissue types, even in the same specimen, may have different rates of shrinkage during dehydration and impregnation.

Renal tissue shrinkage: comparison of 3 classic dehydrants when used with the cold or room temperature Biodur plastination technique. ***Pereira-Sampaio MA[1,2], FJB Sampaio[1], RW Henry[3]***. *[1]Urogenital Research Unit/UERJ, Rio de Janeiro, Brazil, [2]Morphology/UFF, Niterói, Brazil, [3]Comparative Medicine/UT, Knoxville, TN, USA.*

Background: Usually an acceptable percent shrinkage occurs in plastination. This study examines the effects of alcohol and acetone dehydration on shrinkage of the kidney. Methods: Renal vessels and collecting system of 25 fixed porcine kidneys were filled with ERTV silicone and 5 kidneys received no ERTV. All kidneys were sliced transversely at 1 cm intervals. ERTV slices were divided into 4 groups for dehydration using methanol, 2-propanol, ethanol, and ethanol followed by methylene chloride and then subdivided into 2 more groups for impregnation (via cold and room temperature). Non-ERTV slices were divided into 3 random groups (5 slices each) and dehydrated using graded series of either ethanol, ethanol with a final methylene chloride bath, or acetone and impregnated in cold temperature. All slices were photographed after fixation, dehydration, impregnation and curing. A grid was placed over each picture to determine slice area by the counting point planimetry method. Renal tissue shrinkage was calculated after each plastination step, as well as, shrinkage for the entire kidney and each renal part (cortex, medulla and sinus). Results: All kidneys shrank (6.1% to 62.5%). Shrinkage occurred during each plastination step: dehydration (4.2% to 11.7%),

impregnation (7.2% to 34.5%) and curing (1.7% to 4.3%). However, shrinkage was significant only during impregnation. The renal cortex shrank the most (6.6% to 83.3%). Shrinkage was minimal and not significant in 5 groups: Methanol ERTV (room impregnation), Propanol ERTV (room and cold impregnation) and Acetone without ERTV (room and cold impregnation). With Ethanol ERTV there was significant shrinkage after cold impregnation in the cortex ($p \leq 0.01$) (mean 65.3%), medulla ($p \leq 0.05$) (mean 57.8%) and entire kidney ($p \leq 0.01$) (mean 54.7%). Nevertheless, there was no significant shrinkage with room temperature impregnation, even for this group. Significant cortical shrinkage (mean 20.9%) and of entire kidney (mean 17.9%) occurred during impregnation ($p \leq 0.05$) in Ethanol without ERTV. After impregnation Methylene Chloride without ERTV had significant shrinkage ($p \leq 0.05$) in the cortex (mean 26.2%) and the entire kidney (mean 26.4%). Concerning room temperature vs. cold impregnation, there was no significant shrinkage in both room temperature and cold impregnation with Propanol ERTV. There was no significant shrinkage in Methanol ERTV and Methylene Chloride ERTV room temperature. However, both Methanol ERTV and Methylene Chloride ERTV cold temperature had shrinkage over the entire process. Conclusion: Shrinkage in renal tissue was the greatest in the cortex and during impregnation. For renal tissue, if measurements are to be carried out, propanol dehydration was the best as there was no significant shrinkage, while ethanol dehydration had the most shrinkage. Shrinkage was greater with cold impregnation. This is likely a reflection of the direct effect of cold on the solvent boiling point.

Principles of epoxy plastination technique (E12). ***Sora M-C****. Center for Anatomy and Cell Biology, Medical University of Vienna, Austria.*

Background: The E12 plastination process is a well-established preservation technique used for demonstration in teaching and also in research (von Hagens et al., 1987). Material and Methods: Material and Slicing: For E12 plastination we usually use fresh tissue which is frozen at –80°C for one week. In the next step slices with an average thickness between 3 and 5mm are cut. The slices were stored at -25°C overnight. Dehydration and Degreasing: The acetone used for dehydration is cooled at –25°C. Each slice will be placed between soft plastic grids in order to allow better circulation of the dehydration fluid. The acetone was changed once after 3 days at a concentration of 96% (AC1), by using technical quality acetone. The final concentration of the dehydration bath was 99% (AC2). When dehydration is finished 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 (MCL) for degreasing. Degreasing is finished after 7 days. Impregnation: Impregnation is performed at +5°C using the following epoxy (E12) mixture: E12/E1/AE10 (95:26:10 pbw) (von Hagens, 1985). The slices were 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 mm 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 casted between two sheets of tempered glass and a flexible gasket is used as a spacer (4 mm). 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 3 mmHg 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° and left like that for the next 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 4 days. 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 offered a lot of anatomical details down to the submacroscopical level. The transparent loose areolar and adipose tissues contrasted perfectly with the muscle tissues and all epithelial parenchyma. Conclusion: The E12 technique was and still is the elected 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 advance training programs (CT and MRT).

Sheet plastination with the E13 technique. ***Von Hagens G****. Gubener Plastinate GmbH, Guben, Germany.*

Plastination of transparent body slices has been accepted as providing superior teaching specimens. The newly introduced E 13-technique shall serve as an alternative to the E 12-standard technique as described in the Heidelberg Plastination Folder 1985. The strengths and the weaknesses of both techniques will be discussed. The following table shall serve as an

overview and will be discussed during the presentation. Sample slices will illustrate the results.
Technique E12-technique E13-technique
Manufacturing time 2 – 3 days 2 – 3 weeks
Long time yellowing yes no
Separation foil polyester foil silicone foil
Separation from glass plates with release agent no
Number of components two
Transparency of fat high reduced
Property of hardener ammonia smell to be heated up

Advanced exploration of anatomical concepts using E12 sheet plastination. ***Cook PR***. *Department of Anatomy with Radiology, University of Auckland, Auckland, New Zealand.*

Background: The E12 plastination technique is a unique means of achieving precise human sectional anatomical specimens that correlate well with radiographic imaging techniques such as magnetic resonance imaging and computed tomography. E12 plastinated sections are typically 2.5mm thick, smooth, semi-translucent, durable and offer a very high degree of anatomical detail not usually seen in traditional cadaveric wet specimens or in other plastination techniques, and can often surpass the detail available with current radiographic imaging techniques. Methods: The standard E12 process of sawing, dehydration by freeze substitution, degreasing, forced impregnation, casting in flat chambers and heat curing is followed according to established protocols using Biodur epoxy polymer methodology. Results: The unique properties of the E12 process offer an exceptionally vivid survey of the human body in any given plane that in addition to presenting the structural layout in situ also allows significant highly detailed views of any given region right down to the sub-macroscopic level. Standard microscopic teaching and research glass slides providing detail of a specific structure within predetermined parameters are often dictated by the physical limitations of the size of the actual microscope slide itself. E12 sections provide a high degree of detailed anatomical orientation whilst most importantly retaining the in situ structural integrity of the entire region in a complete and uninterrupted state. Conclusions: The detail within E12 sections may be enlarged considerably under high magnification television equipment and even under light microscopy and have proven vital in linking several disciplines namely gross anatomy, pathological anatomy, radiology and microscopic anatomy all from the same specimen.

Excellent brain and tissue slices in one week using P40. ***Henry RW***. *University of Tennessee, Department of Comparative Medicine, Knoxville, TN, USA.*

Background: The Biodur® P40 plastination process and products have been used for a decade and a half and have become the standard for convenient preservation of tissue slices. This product was developed to be a less complex technique for production of brain slices. Vivid sectional anatomy slices, from various regions, are produced and demonstrated using the Biodur® P40 technique. Methods: Two to three millimeter slices of brain or body slices are produced on a band saw from frozen tissue (at least -40°C) is recommended. Slices are cleaned of saw dust and submerged in cold acetone (-25°C) and thoroughly dehydrated. Dehydrated slices are impregnated at room temperature or in the cold room in an immersion bath of P40 or P40 plus an activator. After impregnation overnight, the slices may be stored or removed and placed in glass curing chambers. The slices are cured using UV-light from bulbs or in the shadow of the sun. After curing the slices are removed from the glass chambers, wrapped in foil, and the excess perimeter of polyester is sawn off. Results: High quality polyester sheets with the specimen incorporated within are produced. Anatomical detail is superb. Discussion: Slices from any region of the body may be produced and are excellent educational aids. There use as aids for interpreting images produced using modern diagnostic imaging techniques is unparalleled. Impregnation resin without additives may be reused thus reducing the cost per unit. Natural UV-light may be used as the catalyst. While the Polyester - P35 technique remains the gold standard for brain slices and the epoxy - E12 technique remains such for body slices; both techniques require voluminous effort (time, hands and materials) to prepare slices using these techniques. The major benefit of the P40 technique is the comparative minimal effort needed to produce high quality sections.

Sheet plastination of anatomical objects preserving the natural shape. ***Starchik D, F Kucher***. *International Morphological Centre, St. Petersburg, Russia.*

Background: The sheet plastination of thin slices with epoxy resin allows to demonstrate all the anatomical structures which are in area of section only. Our topic is to demonstrate the topographic spatial anatomy of complex organs using series of slices. The distinctive features of our technique are the opportunity to preserve natural form of a whole organ or part of the body and determine the level of the section. Methods: The process of sheet plastination was carried out as follows: the 6 longitudinal slices (10 to 30 mm thickness) of human foot were made using a high-speed band saw

with the thinnest blade. Then the slices were dehydrated and degreased in acetone. The impregnation procedure was divided into two steps. The first step provides the complete substitution for an acetone in tissue by resin impregnation-mix. The slices were put into epoxy resin with hardener and impregnated as usual. After extraction from impregnation bath they were put between two organic glass plates for two weeks till the resin becomes firm. Then they were polished with the purpose to achieve smooth and congruous surfaces. The skin on each slice was covered with paraffin. The second impregnation with reaction-mixture allows to achieve complete removing of air bubbles and to produce flat and smooth surfaces. On the closing step paraffin was removed from hardened slices by putting them in warm water with final cleaning of surfaces with solvent. Results: All the developed slices had enough clarity to be studied in passing and reflected light. Because of smooth and congruous surfaces of the slices the model of the foot can be easily reconstructed. Preliminary injection of colored-silicone into the arteries makes demonstrative properties of the slices much better. Conclusions: The plastinated slices allowed getting an impression not only about geometry of studied organ but to determine the level of section exactly. This method is useful for creating wedge-shaped slices in order to study topographic anatomy of anatomical structures in any part of studied organ. Besides, this way minimizes amount of wasted tissue and can be considered as practicable.

Thin flexible sheet plastination of human brain. ***Hajian M, A Rabiei, A Fatollahpour, E Esfandiary***. *Isfahan University of Medical Sciences, Isfahan, Iran.*

Plastination is a modern technique that stops decay and deterioration of natural tissues. This new preserving technique includes substituting water and fat with synthetic polymers, such as silicon resins, epoxy resins, acrylic resins and unsaturated polyester resins. In this work, a human brain was fixed in 10% formalin solution for about 1 month. The specimen was then, cut into 1mm thick sections by sausage cutter machine. The sections were dehydrated by cold acetone for a few days and were forced impregnated by P 87, our new soft and flexible polymer, which recently was made in our department.

Enabling sheet plastination with minimum effort and equipment. ***Von Horst C[1], RW Henry[2]***. *[1]HC Biovision, Mainburg, Germany, [2]Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, USA.*

Background: Medical, veterinary and biological institutions, museums and practitioners from various fields worldwide are using sheet plastination specimens. Nevertheless only a few places are able to perform sheet plastination themselves. This is mainly due to the large amount of equipment and experience needed in comparison to silicon plastination and other preparation methods. The goal of our study was to enable as many institutions as possible to prepare their own sheet plastinates with minimal effort and equipment and still receive the highest quality specimens. Method: We used the Tissue Tracing plastination Technique (TTT) invented by Dr. von Horst. Compared to a regular series of parallel sheets of constant thickness, the TTT method allows one to follow anatomical structures within one sheet and to adjust the thickness in different regions of the same specimen for enhanced visual detail and contrast. Apart from these advantages, we chose the TTT method because it allows a division of work between institutions and a professional plastination service provider. Results: The main key to simplifying the process was dividing the work into two parts: 1. The preparation of pre-plastinates by the institutions themselves, 2. Tissue tracing and embedding (with or without acrylic layers) by HC Biovision. This reduces the equipment needed to the following:

- household freezer
- manual bone saw
- buckets of formalin solution and Acetone
- epoxy or polyester plastination resin
- a simple vacuum setup (exsiccator + vacuum pump)

The different steps are:

- deep freezing of the specimen,
- cutting slices of ca. 20mm with a manual bone saw,
- immersion of the slices in 4% formaldehyde solution,
- immersion of the slices in acetone at room temperature,
- changing the acetone 5 times in a 1:10 relation (specimen : acetone),
- vacuum impregnation in an exsiccator,
- curing at room temperature,
- sending the pre-plastinates to HC Biovision for Tissue Tracing and embedding.

Discussion: With the new approach laboratories without special sheet plastination equipment and experience can avoid the most critical steps in sheet plastination by preparing pre-plastinates for further processing by HC Biovision. Most advantages arise from the thickness of the pre-plastinates: an electric bone saw with cooled guide stop is not needed, saw dust does not have to be removed, handling of specimens at any stage is very easy. Critical steps like putting specimens in a perfect shape without bubbles and inclusions and giving them a final polish are taken over by experienced specialists.

Finally the TTT method achieves excellent anatomical visualization by adjusting the thickness of the various tissues for optimal clarity and following structures through the tissue.

Root canal obturation and alveolar ridge augmentation in dental surgery evaluated by sheet plastination. ***Weiglein AH[1], B Weninger[1], L Kqiku[2]***. *[1]Institute of Anatomy, [2]Dental Clinic, Medical University, Graz, Austria.*

During the last four years we have established a dental research laboratory based on polyester plastination and plastination micromorphology. The central equipment for this program is the P35 plastination lab plus the ultra thin slicing and grinding system comprising a diamond band saw (Exact 310 CP) and a diamond grinding system (Exact 420 CL) which allow 1) to produce thin sections (500-100 micron) of P35 impregnated specimens at the exact level of interest and 2) to produce ultrathin sections (100-10 micron) for histological evaluation. The 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 remodeling in dental implantology. The later allowed us to deal with large serial implant specimens, which is not possible with the standard methacrylat protocol. Moreover, osseo-integration was evaluated by plastination histology and Micro- CT to study the accuracy and comparability of both methods. Parallel to the bone remodeling study we also studied the regeneration of the inferior alveolar nerve after injury during implant placement. In all studies the results are excellent and permanent thin sections of the desired region, which allow studying the quality of root canal obturation methods, of implant-osseointegration and of nerve regeneration in series. Thus, the study proves that polyester plastination is an excellent replacement for standard histology embedding methods (e.g.: methacrylat) with the special advantage of being much less expensive.

Thin slice plastiantion and 3D reconstruction. ***Sora M-C***. *Center for Anatomy and Cell Biology, Medical University of Vienna, Austria.*

Background: The E12 method of plastination is usually used to create 2.5 - 5 mm transparent slices. If thinner slices, 0.5 - 1.5 mm, 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 1 mm epoxy plastinated slices. Only by using high temperature is the polymer thin enough to penetrate into the middle of the processed specimen. Materials and Methods: One male unfixed human cadaver ankle was used for this study. The distal third of a limb was cut and the foot positioned in a 90° dorsal flexion. A tissue block containing the ankle was cut starting 40 mm distally to the tip of the lateral malleolus and finishing 50 mm proximal. The tissue block was dehydrated, degreased and finally impregnated with aresine 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 are loaded into WinSURF and traced from the monitor. After all contours are traced, the reconstruction is rendered and visualized and the model was qualitatively checked for surface discontinuities. Results: An E12 block was produced that was hard and transparent. Thin, <1 mm slices produced from this block were transparent and hard with good optical qualities. The finished E12 slices provided anatomic detail to the microscopic level. Conclusion: Thin slices <1 mm 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.

Morphometry of fish muscle fibers in thin epoxy sections: comparison of two processing protocols. ***López-Albors O[1], MD Ayala[1], F Asensio[2], E Abellán[3], J Albarracín[1], J Arredondo[4], R Latorre[1]***. *[1]Veterinary Anatomy and [2]Microscopy Service, University of Murcia. Spain. [3]Spanish Oceanographic Institute, [4]Autonomous Univ. of the State of Mexico, Mexico.*

Background: In commercial fish knowledge of the size of muscle fibers is important to assess the texture of the flesh. The average size of muscle fibers can be estimated by measurement of the cross-sectional area or diameter of a representative number of muscle fibers. When traditional histological protocols are used it is almost impossible to obtain a complete cross-section of the fillet which guarantees that the fibers used for morphometry are really representative. Plastination can

overtake this limitation since the whole cross-section of the fillet can be embedded in an epoxy resin mixture. The obtained block is then cut in thin slices which, after polishing, can be viewed by light microscopy. However, since the plastination process may affect the size of muscle fibres, a comparison between plastinated and non-plastianted sections should be done before validating plastination as an appropriate tool for muscle morphometry. We have investigated this purpose by using either the traditional methodology of muscle tissue processing (cryopreservation) or formaline fixation, both before epoxy impregnation. Methods: Two commercial size sea bass (Dicentrarchus labrax, L.) were used in this study. Two consecutive crosssections (1cm thickness) of the trunk musculature were done in each specimen. Both cranial sections were trimmed in 8 equal blocks of (1x1x1 cm), then frozen in isopentane cooled over liquid nitrogen and finally sectioned in cryostat. The caudal section of one specimen was frozen in cooled isopentane (without trimming) and the other fixed in 10% formalin. Both caudal sections were then plastinated (E12-E6-E600, Biodur®) and cut with a contact point diamond band saw. After polishing, the final thickness of the slices was 80-90 µm. The area and diameter of 800 muscle fibres, equally distributed over the trunk cross-section were recorded in both the cranial and caudal sections. A comparison of the size of the fibers was done for a statistical significance of 95%. Results: The caudal section which, before plastination, had been previously frozen in cooled isopentane was not useful for morphometry of muscle fibers. The dehydration process had altered the structure of muscle fibers and their limits were not clearly observed. Contrarily, in the formalin fixed section, despite of evident shrinkage, the structure of muscle fibers was preserved. The comparison of the size of muscle fibers in this plastinated section and the corresponding cranial section of the same specimen demonstrated that shrinkage was 9,77% for the area and 8,5% for the diameter of muscle fibers. Conclusion: Unfixed, snap-frozen fish muscle was not useful for morphometry purposes after epoxy plastination. Formalin fixation preserves the structure of muscle fibers, however excessive shrinkage limits the use of thin epoxy plastinated slices for fish muscle morphometry.

A new perspective from sheet plastination examination: the longitudinal coat and anal glands. ***Zhang J-J[1], H Han[1], M-C Sora[2], M Zhang[3]***. *[1]Anhui Medical University, Hefei, China, [2]Medical University of Vienna, Vienna, Austria, [3]University of Otago, Dunedin, New Zealand.*

Background: Current understanding of the aetiology of idiopathic anorectal sepsis is based on the concept of infection of the anal glands. The key to this theory is the microanatomy of the anal glands (AGs) and their relation to the surrounding structures, particularly the internal and external anal sphincters and the conjoint longitudinal coat (CLC). One of challenges to reveal such relations is how to demonstrate the structures at both macroscopic and microscopic levels. The aim of this project was to use the ultra-thin E12 plastinated slices to identify the CLC and AGs. Methods: A total of 10 adult (4 females and 6 males, aged 37-81 years old) and 6 infant (1 female and 5 males, aged 4 days - 3 months old) cadavers were used in this study. The 200µm-thick transverse or longitudinal sections were collected from three cadavers and examined under a stereomicroscope microscope or confocal microscope. The remaining cadavers were prepared as 5µm-thick sections stained with H.E. or van-Gieson or Verhoeff's elastin staining. Results: (1) The CLC, AGs and anal sphincters were clearly demonstrated in the E12 slices and infant and adult histological sections. The best histological features were present in the infant specimen. The epithelial layer was often damaged during preparation of both E12 slices and adult histological sections. (2) Compared to the histological sections, the ultra-thin E12 slice revealed a much better configuration of the CLC and its relation to the AGs and sphincters. (3) The CLC is superiorly continuous with skeletal muscle fibers of the pelvic diaphragm and inferiorly fans out, penetrating both internal and external anal sphincters. (4) There is no clear evidence to show that the CLC anchors onto the AGs and mucosal layer or skin. Conclusion: The CLC is the tendinous portion of the pelvic diaphragm and seems not associated with the AGs.

Fascia and sheet plastination. ***Zhang M***. *Department of Anatomy & Structural Biology, University of Otago, Dunedin, New Zealand.*

Background: Terminologia Anatomica (TA) recommends that the term "fascia" is a sheet of fibrous tissue that envelops the body beneath the skin and also encloses and separates muscles, whereas the term "tendon" is a fibrous band that is the part of the muscle, which connects the fleshy (contractile) part of the muscle with its bony attachment or other structures. However, such definitions may be overstated and may represent figments of various anatomists' imagination. In the case of the bicipital aponeurosis, for instance, this tendinous structure fans out and eventually continues with the deep fascia of the forearm. In other words, the deep fascia of the forearm must at least partially consist

of tendinous fibers. The aim of this presentation is to explore the relationship between the fascia and muscular structures in the various regions of the body. Methods: Retrospective analysis of the studies that were undertaken by our group to investigate fascia configuration in the various regions of the body using the sheet plastination technique [1-9]. Results: (1) Nature of the so-called fascia is much more complicated than what we thought before. The major difficulty in studying configuration of fibrous 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. The sheet plastination provides a new approach to elucidate the configuration of the connective tissue at macroscopic and microscopic levels. (2) There are a number of direct evidence to demonstrate that the majority of fibers in the fascia are derived from the muscle tendon. The key difference between a fascia and a tendon is the compactness of connective fibers rather than their origins and/or locations. Conclusion: The sheet plastination technique provides a novel approach to verify the configuration of the fascia in a given region of the body.

3D fluoroscopy reconstruction of plastinated specimens. ***Latorre R[1], F SUN[2], O López-Albors[1]; MD Ayala[1], F Gil[1]; S Losilla[2], M Orenes[1], RW Henry[3]***. *[1]Veterinary Anatomy, Univ. of Murcia, Spain, [2]Minimally Invasive Surgery Centre, Cáceres, Spain, [3]College of Veterinary Medicine, Univ. Tennessee, USA.*

Background: Radiology has been routinely used for evaluation of bony structure and soft tissue in imaging diagnosis. However the superimposition of adjacent osseous structures and difficulties in positioning for multiple views of complex anatomy regions make difficult the interpretation of the radiographic views. Conventional radiography on cadavers has been used in gross anatomy courses to offer a radiographic-anatomic-pathologic correlation and to facilitate understanding of complex anatomical relationships. Teachers of anatomy and radiology would agree that the use of gross tissue specimens in the laboratory is an invaluable aid for radiographic study of normal and diseased structures. However, the preparation and handling of such material is difficult. In this sense, plastinated specimens provide the potential for infinite use as tools in radiology courses. In this project we show the three-dimensional (3D) fluoroscopy imaging properties that can be obtained from plastinated specimens. Material and methods: Five thoracic and five pelvic limbs from embalmed dogs were dissected prior to plastination. The specimens were prepared and plastinated according to the standard S10 Biodur silicone procedure. Several 3D images from the limbs were acquired using a C-arm (BV Pulsera 3D-RX Option. Philips. S. A.). The C-arm rotates continuously through 200º in a 30 second period while acquiring a large set of 450 high-definition fluoroscopic images. The complete set of images was integrated to create a high-quality 3D volume reconstruction. Results: 3D reconstruction of the different regions and joints allowed an easy identification of the main muscles and bones. In order to provide good anatomical detail of the different bony structures, subtraction of the soft tissue was possible. Also, rotation in the three planes permitted a clear viewing of the relationship between different bony structures. Conclusion: Silicone plastinated specimens can be a useful tool to teach and understand the radiographic images. Also, if radiographs are taken before plastination, then the plastinated specimen can be used for comparison.

Computerized 3D anatomical modeling using plastinated anatomical material. ***Tunali S[1,2], M Farrell[2], S Labrash[2], BK Lozanoff[2], S Doll[3], S Lozanoff[2]***. *[1]Hacettepe University, Ankara, Turkey, [2]University of Hawaii, Honolulu, HI, USA, [3]University of Heidelberg, Heidelberg, Germany.*

Background: Computerized 3D anatomical models are routinely used for instructional purposes in the medical education classroom as well as the clinic for explaining diagnosis and treatment options. The advantage of this technology is that complicated three-dimensional morphology can be visualized providing important insights into anatomical spatial relationships. However, the process of obtaining these models remains laborious, complex, and expensive since they are typically generated from 2D section material whose images are proprietary. Even if a suitable conversion program is identified, the process of generating the rendered model requires highly sophisticated equipment unavailable to the typical educator. Plastinated specimens on the other hand, provide a ready source of anatomical material suitable for rapid and reliable modeling. The purpose of this study was to develop a simple system for generating 3D computerized models using plastinated specimens. Methods: Anatomical specimens were obtained and plastinated using the Dow/Corcoran method at room temperature. Specimens were dehydrated in a graded acetone series at -20°C, dehydration time was 2 to 3 weeks depending on the size of the specimen. Then specimens were immersed in

COR-TECH TM PR-10 silicone polymer (Corcoran Laboratories) mixed with 7% cross linker (CR 22 TM) and vacuum was applied. After 3 days of vacuum, no acetone bubbles were observed, impregnation was complete and vacuum was discontinued. Specimens were drained of excess polymer for two days and then brushed with catalyst (CT 32 TM). They were kept in a closed pot containing some drops of catalyst and water for one week. Then, they were put in zipper plastic bags for further curing for one more week. Finally specimens were ready for three-dimensional modeling. Specimens were digitized using a hand held scanner (Polhemus), exported in .obj format into Maya software where they were edited, and exported as .dxf files. Subsequently, they were converted to .xdf files and read into WinSURF (SURFdriver Software), partitioned into individual objects and saved. An icon-driven interface called SURFviewer, was developed and implemented providing a simple desktop application for viewing the models. Results: Models were viewed and qualitatively compared to the actual plastinated specimen and showed close correspondence. The names anatomical structures on the models were successfully viewed and heard via audiovisual files. Conclusion: It is concluded that this system provides a simple yet effective reference tool for anatomical education in multiple languages. Further work will be directed at increasing the database and implementing it in conjunction with an electronic laboratory guide.

3D multidetector CT reconstructions of a diencephalon and brain stem, plastinated with Biodur using the standard S10 technique. ***Cerqueira EP, CAC Baptista, CC Campi, AF Silva***. *University of São Paulo, São Paulo, Brazil.*

In clinical and anatomical practice, comparison between plastinated specimens and Computed Tomography (CT) and Magnetic Resonance (MR) examinations are normally used. However, the direct examination of specimens by CT may be performed for evaluation of its internal and external structures, and to ascertain whether their integrity can be an important value in gross anatomy teaching. It was utilized a Toshiba Aquilion 64-multdetector CT scan, at Radiology Department of the Heart Institute – University of São Paulo/Brazil, to evaluate a diencephalons and brain stem specimen, plastinated in 1986, by Biodur S10 standard technique. It was obtained several cross-section images from the specimen with 0.5- mm slice thickness and 0.5-mm image reconstruction interval. 3D images were reconstructed through MIP (Maximum Intensity Projection) and VR (Volume Rendering) techniques at Aquarius Net Viewer Workstation of TeraRecon Company. Also, it was measured the rate of CT attenuation coefficient (UH) of specimen images and compared with those obtained from white and grey matter of a live brain CT scan images. As far as the anatomical aspect is concerned, the internal and external morphological structures were preserved, especially inside the third ventricle and outside the midbrain, after twenty-two years of its plastination. 3D reconstructions of the specimen showed high spatial resolution, with great detail - state of art - of their anatomical structures. By the Radiological imaging view, the specimen showed an increased attenuation rates, compared with the grey and white matter in live brain images, but less than the attenuation values of any kind of calcification. It was not possible, by CT, to recognize the grey and white matter of specimen. CT scan is an excellent method for assessing plastinated specimens, especially to reveal and evaluate either inner or outer surfaces, but not to differentiate their wall structures. Also, the Biodur S10 alters the CT attenuation rates of specimen.

3D multidetector CT reconstructions of a heart, plastinated with Biodur using the standard S10 technique. ***Cerqueira EP, CAC Baptista, CC Campi, AF Silva***. *University of São Paulo, São Paulo, Brazil.*

In clinical and anatomical practice, comparison between plastinated specimens and Computed Tomography (CT) and Magnetic Resonance (MR) examinations are normally used. However, the direct examination of specimens by CT may be performed for evaluation of its internal and external structures, and to ascertain whether their integrity can be an important value in gross anatomy teaching. It was utilized a Toshiba Aquilion 64-multdetector CT scan, at Radiology Department of the Heart Institute – University of São Paulo/Brazil, to evaluate a heart specimen, plastinated in 1986, by Biodur S10 standard technique. It was obtained several cross-section images from the specimen with 0.5- mm slice thickness and 0.5-mm image reconstruction interval. 3D images were reconstructed through MIP (Maximum Intensity Projection) and VR (Volume Rendering) techniques at Aquarius Net Viewer Workstation of TeraRecon Company. Also, it was measured the rate of CT attenuation coefficient (UH) of myocardium specimen images and compared with those obtained from myocardium of a live heart CT scan images. As far as the anatomical aspect is concerned, the internal and external morphological structures were preserved, especially inside where the valves, ridges and bridges (trabeculae carneae), fibrous threads (chordae tendineae) and papillary muscles where greatly

represented, after twenty-two years of its plastination. 3D reconstructions of the specimen showed high spatial resolution, with great detail - state of art - of their anatomical structures. By the Radiological imaging view, the specimen showed an increased attenuation rates, compared with the myocardium in live heart images, but less than the attenuation values of any kind of calcification. It was not possible, by CT, to recognize the layers of myocardium wall of the specimen. CT scan is an excellent method for assessing plastinated specimens, especially to reveal and evaluate either inner or outer surfaces, but not to differentiate their wall structures. Also, the Biodur S10 alters the CT attenuation rates of specimen.

Visitor reactions to plastination. ***Moore CM[1], CM Brown[2]***. *[1]University of Texas Health Science Center at San Antonio, Texas, USA, [2]Trinity University, San Antonio, Texas, USA.*

Background: Until the advent of plastinated cadavers, few outside the medical professions have had firsthand experience with human corpses. Such opportunities are now available at the Body Worlds exhibits of Gunther von Hagens and other traveling plastination exhibits. This report examines philosophical and religious responses of visitors to several Body Worlds exhibits around the globe to illuminate cultural issues surrounding this new format for the scientific education of the public. Materials and Methods: We limited our examination of visitor responses to comment books available to the public exiting Body Worlds exhibits. We perused over 2500 comments from books in London, Toronto, Singapore, Cleveland, Houston, and Denver. The Institute for Plastination provided us with copies of consecutive but random pages containing approximately 400 responses from each exhibit. We analyzed the responses with an eye to themes of general cultural, philosophical, and religious significance. Results: A large variety of overlapping issues emerge from the comments. Four overarching themes in the form of questions encompass the greater part of visitor comments and reflect major ongoing tensions in society at large: (1) What is life in its relation to death? (2) What do these bodies reveal about our relations with others and our place in the universe? (3) Are these plastinates freak displays or sacred relics? (4) What is the origin of these complex machines we know as our bodies? Under each of these questions we found thoughtful— and not so thoughtful—meditations on personal and social identity, on the individual's relationship to the universe and/or to God, and on the meaning and purpose of life. Religious concerns frequently permeated the comments, although not always in expected ways. Religiously conservative visitors in the United States, for instance, frequently thanked the creators of Body Worlds for providing evidence for the Master Creator and had little problem with the "nudity" of the plastinates as this is how God created humans in the Garden of Eden. However, no consensus emerged on sensitive cultural issues regarding abortion, evolution and intelligent design, and the existence of a soul apart from the body. For those visitors who commented on our relation with others, however, there was broad agreement that there is an underlying unity that transcends race and gender. Conclusion: Visitor comments form a microcosm of social debates on emotionally-charged subjects such as evolution and creationism and pro-life issues. While most responses represent visceral reactions to the plastinates with little understanding of the underlying science or of the scientific method, the exhibits are clearly a way for laypersons to learn about their body. The responses from various cultural, ethnic and socially diverse regions of the world are surprisingly uniform, and overall very positive, regardless of religious and philosophical persuasions.

Ethics in plastination. ***Whalley A***. *Institute for Plastination, Heidelberg, Germany.*

With the Invention of Plastination, a new classification for permanent anatomical specimens was introduced. However, an accord on the ethical use of specimens has been far from officially established. Before plastination, anatomical specimens had never been so accessible to the general public. At the same time, the use of plastinates in the medical field, for teaching and training is growing at exponential levels. The popularity of Plastination has started a wave of universal discussion by ethicists, religious leaders, the public, media, members of the scientific community and most recently, lawmakers. Established standards for ethical display and use of plastinated specimens, have at this time, only been set at the discretion of the scientists working in the lab. Origins of specimens, cultural concerns, human dignity and the profits generated from Plastination, have all led to discussions based on values and opinions. It is important to recognize ethical standards that can and have been adhered to by leaders in the world of Plastination; and at the same time it is necessary to examine cases where ethical standards are not in place, and discuss the necessity for setting controls.

Plastinates in medical education - a new approach at the medical faculty in Mannheim. ***Kriz W***. *Ruprecht-Karls- University Heidelberg, Medical Faculty Mannheim, Germany.*

Two years ago the Medical Faculty in Mannheim, University of Heidelberg started a new preclinical curriculum that follows a strict modular system. Thus, the curriculum is subdivided into functionally defined units, e.g. a module dealing with the locomotor system, with the respiratory system, with the endocrine system etc, in total eight modules. Within each module the relevant anatomical, physiological and biochemical facts were taught in strict integration. Such a system does not correlate with a classic dissecting course. Therefore dissections were greatly reduced in time and placed toward the end of the term close to the final examine. Within each module anatomy was presented with the help of "objects", i.e. anatomical models and plastinates. Together with the Department of Plastination in Heidelberg the teaching rooms (for maximally 12 students) were equipped with models and a rich spectrum of plastinates including silicone specimens and transparent epoxy slices. To give an example: for the module "locomotor system" the inventory of plastinates consisted of plastinated specimens of the main joints, of both the upper and lower extremities, of whole body plastinates and of transparent cross sectional slices through all major regions of both extremities. Evaluation: This kind of modular teaching was well received by the students. Together with the models, the plastinates were extremely helpful in demonstrating the three dimensional arrangement of muscles, tendons, fascia, nerves and arteries. According to our experience the plastinates should show the major structures of topographically relevant regions. Details, such as cutaneous nerves may be omitted; they can equally be well studied in models. Transparent slices were included in the lessons starting from the very beginning; they produced a surprisingly great success. In contrast to the three dimensional specimens, the slices contain every detail in unaltered topography. Learning Anatomy with the sheets stimulated the students to think about anatomical relations finally to think about topography. This led among students to vivid discussions, which essentially contributed to the learning effect. Combining transparent slices with CT or MR pictures increased the interest of the students and will probably be very useful for their future duties. Summary: Teaching anatomy predominantly with plastinates and models is successful and in several aspects superior to teaching anatomy along with dissection, Nevertheless, a dissection course, even if only a short one, is desirable and is strongly wanted by the students. We placed this course close to the final exam taking advantage from the advanced anatomical knowledge of the students that made the dissections extremely fruitful for them.

Plastinated, museum-based prosected specimens and web assessabled images are essential infrastructure for modern education in human gross anatomy. ***Pang SC, C Reifel, R Easteal, LW Mackenzie, R Hunt***. *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

Human Gross Anatomy is an integral part of medical education. However, over the past 10-15 years, there has been tremendous pressure in the North American medical curricula to reduce the amount of basic science teaching, including Gross Anatomy and Histology, in order to accommodate topics such as Cell and Molecular Biology and to lengthen the time for clerkships. Furthermore, as the patient population in Kingston, Ontario is unable to provide the essential experience for increasing numbers of medical students, it is necessary to expand our campus to include hospitals in the surrounding vicinities. The Department of Anatomy and Cell Biology at Queen's University has an extensive collection of prosected human gross anatomy specimens housed in its Anatomy Learning Centre. In order to be able to deliver our learning materials on anatomical subjects to students with various anatomy backgrounds and to those located at distant sites, the Department established a web-based Gross Anatomy and Histology Image Catalogue (GAHIC). Students can gain access to these learning materials anywhere in the world and at any time. The Anatomy Learning Centre remains the main site for self-directed learning (SDL), problem-based learning (PBL) and team-based learning (TBL) modules. Over the past 15 years, the demand for anatomy specimens has increased with the surge in numbers of students enrolled in the Medical, Nursing, Rehabilitation Therapy and Life Sciences Programs. As a result, the numbers of body donations to Queen's University have become insufficient to keep up with these demands, and we have produced plastinated gross anatomy specimens in order to reduce the cost in preparation of cadaveric specimens and extend their life span. In our Anatomy Learning Centre using both wet-prosected and plastinated specimens as well as a web-based image resource; we have been able to serve approximately 2000 professional and undergraduate Life Sciences students with an annual body donation number of approximately 20. Most plastinated gross anatomy specimens have a life span between 5-7 years, and have become an essential infrastructure of a modern anatomy learning facility.

Clinical relevance in teaching neuroanatomy - integrated course based on PBL and plastinated specimens. ***Weiglein AH***. *Institute of Anatomy, Medical*

University Graz, Austria.

For the student neuroanatomy is usually considered to be the most difficult topic in anatomy. One reason for this is that structures and functions do not simply correspond like in other organ systems. A second reason is that learning morphology by itself does not initiate comprehensive understanding and clinical reasoning. To overcome these problems, neuroanatomy at the Medical University Graz is thought in a five weeks integrated course covering macromorphology, micromorphology, developmental and functional anatomy. Since the neuroanatomy course follows immediately after the musculoskeletal course, the peripheral nervous system is thought at the beginning of the neuroanatomy module. After an introduction on the brachial and lumbosacral plexus demonstrated by P-35 plastination procedure the major landmarks are discussed and the students are requested to place wax cords representing the major peripheral nerves into the musculoskeletal specimens they have dissected during the musculoskeletal course. The correct placement is checked and common peripheral nerve lesions (e.g.: radial palsy after humeral shaft fracture, carpal tunnel syndrome etc.) are discussed based on the topographical anatomy. Morphological and functional systems of the central nervous system are taught parallel. After three weeks of introductory lectures, the students study brain and spinal cord models and plastinated brains and brain slices. To put more emphasis on clinical applicable knowledge, the students are viewing CT and MRI scans parallel with the brain slices. Since we teach 180 students parallel in one course, the quantity of P-35 brain slice sets was overcome by a trick. One series of axial P-35 brain slices was digitized and multiply printed on transparencies. The transparencies were then mounted between two Plexiglas plates and finally cut to the desired size and format. After studying the models and slices the students dissect a brain to enhance three-dimensional comprehension. To facilitate clinical reasoning the course ends with a PBL-seminar discussing and solving common clinical neurological problems. The evaluation of the course validates the integration of structure and function and the introduction of sectional anatomy and problem case. In the open questions (what was best?) both the practical course and the PBLseminar are mentioned most frequently: „practical realization of neuroanatomy in brain dissection and studying plastinated brain slices enhance comprehension"; „PBL-seminar at the end (not at the beginning) improve clinical understanding, since what we have heard and learned becomes more comprehensible and lively, clinical correlations become clear".

Exploring new horizons of plastination applications in medical education. ***Raoof A, L Saab, H Zhao, L Liu***. *Plastination Lab, The University of Michigan Medical School, Ann Arbor, Michigan, USA.*

Background and Methods: New horizons for the use of plastinated specimens in anatomy education have been explored at the university of Michigan. These included the addition of structured lab visits in the undergraduate anatomy course syllabus; involving student in the preparation of selected plastinated specimens for the gross anatomy course; using colors to highlight neurovascular pathways; and encouraging students to work on a plastination research project that would enhance their anatomy and plastination knowledge. Results and Conclusion: The lab visits during the undergraduate anatomy course received a high approval by students as a significant tool in understanding complex anatomical concepts and their clinical correlation. The visit have been rated high in the regular course evaluation questionnaire, 79% of the students agreed that lab visits were useful in understanding essential concepts; 87% agreed that review sessions using plastinated specimens were helpful; and 83% agreed that the use of plastinated specimens during lab visits was useful. Similarly, the plastination lab has been accepting more students to participate in doing dissections and plastination for the gross anatomy course. The use of color in identifying neurovascular pathways on plastinated specimens has become a routine procedure in preparing effective educational tools to students. It is becoming increasingly evident that plastinated specimens are constituting a significant role in medical education.

Education in veterinary anatomy by plastination. ***Basset Aly A E***. *Plastination Laboratory, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.*

Zagazig Plastination Laboratory was established at Faculty of Veterinary Medicine, Zagazig University, Egypt to enhance the education of Anatomy for both Veterinary and Human medical students.

The Plastination Laboratory was supported by a Project from Higher Education Enhancement Program Fund (HEEPF, 2nd cycle 2004, Code B-053-To). At this stage the Laboratory is designed for preparation of Plastinated specimens by Silicon 10 technique and P40. 200 Plastinated specimens were prepared and displayed in Plastination exhibition. In addition a web- based photo gallery was established. Results have been quite acceptable but the costs of polymer are very high. The Plastinated specimens were subjected to internal evaluation by students and staff members. In addition to

external evaluation by experts from other countries were consulted as peer reviewers. The analysis of evaluation sheets was positive. Accreditation is the aim to ensure that our faculty and graduates are recognized, compete world-wide and to meet society demand for veterinary services. Website for the plastination laboratory was established and can be used by students And staff members (zu.edu.eg./plastination).

Clinical plastination of the human heart for education in a cardiac center. ***Starchik D, S Marchenko, M Didenko***. *International Morphological Centre, Military Medical Academy, Saint-Petersburg, Russia.*

Background: Clinical plastination is of particular interest in field of cardiovascular surgery because it provides variety of opportunities for widening of clinical manner of thinking using natural plastinated specimens. Methods: For creating demonstrative specimens we used standard plastination techniques of hearts from cadavers with retaining its original shape but for particular clinical requirement and needs. The fixed hearts were undergone to impregnation with silicone or epoxy resin as well as in combination with corrosion techniques. Prosthetic heart valves, rings, electrodes and lights were implanted by dissection or after curing. Results: Innovative approaches to enhance the quality of educational process have been implemented. Several modifications of clinical plastinated specimens were developed: 1) modified types of surgical dissections to expose the valves, great vessels, coronary arteries and conduction system; 2) hearts with pathological changes in the to demonstrate tumors, congenital and acquired heart diseases, hypertrophy or dilatation of the left ventricle, atherosclerotic plaques in aorta and coronary arteries; 3) slices of the heart injected with colored silicone according to the long and shot axis ultrasound views as well as MRI projections; 4) hearts with mechanical, biological valves implanted as well as rings and electrodes. Conclusions: In addition to the traditional methods the clinical plastination technique has to be available in clinical centers as it allows to improve effectiveness of teaching of ultrasound, radiographic and surgical anatomy, procedures and techniques. It is necessary to combine routinely used diagnostic and surgical procedures with heart slices and plastinated specimens as it gives better understanding even to the specialists in terms of clinical necessities.

Plastinates and modern learning practices. ***Cunningham MDF, MK Chuang, LW Mackenzie, RA Easteal***. *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

Background: It is well established that plastinated specimens are extremely useful in the teaching of gross anatomy. Because of the availability of a large number of plastinated human specimens we were able to instigate a new approach to anatomy labs. In our anatomy department, where about six hundred students go through anatomy labs every week, time is clearly of the essence. How best to utilize that limited time for each student – how do you optimize the learning environment to fit the needs of each student? Two separate studies are presented. The first is an analysis of student learning modalities in two large anatomy classes using the VARK modality survey. The second shows the results of using Team Based Learning (T.B.L.) in an anatomy laboratory (T.B. Labs). The VARK study analyzed the distribution of learning proclivities in these classes; while the T.B. Labs investigation measured the efficacy of using T.B. Labs. What was hoped was that the introduction of T.B. Labs would allow students with disparate learning styles to maximize acquisition and retention of the material. Methods: 1. VARK Study – VARK measures learning modalities based on four styles of learning preferences, Visual, Auditory, Reading-writing and Kinesthetic (V.A.R.K). The study was conducted using the on-line version of the VARK survey and results were analyzed according to the established protocol. 2. T.B. Labs Study – Data were analyzed comparing students' marks from a year without the T.B. Labs and a year with the T.B. Labs. The analysis was performed using the Statistical Package for Social Sciences (SPSS). Results: The VARK study indicated that among the 280 students 72% were multimodal learners, of this group 64% were quadric-modal, 17% were tri-modal and 19% were bimodal. Of the uni-modal group (28%), 24% were visual learners, 18% were auditory learners, 25% were read/write learners and 34% were kinesthetic learners. T.B. Labs data indicated a significant positive effect of T.B. Labs methods over traditional laboratory methods. Conclusion: 1. The large number of plastinates enables us to provide hands-on specimens for the 6 teams in any given lab. We need 6 teaching specimens of each structure covered - a lot of specimens. Without plastinates we would not have attempted this initiative. 2. T.B. Labs enabled students with ANY learning modality to be accommodated; the auditory and read/ write learners would be on equal footing with the visual and kinaesthetic learners – not usually the case in anatomy. 3. The VARK survey allowed the students to become aware of their own limitations and advantages, which they could then optimize in the nurturing environment of their team.

Teaching anatomy in the Military Medical School of Mexico. ***Alva M***. *Escuela Medico Militar, Mexico City, Mexico.*

This paper shows the methods and resources used for teaching Human Anatomy in the Military Medical School in Mexico City, Mexico. Historical documents and graphic materials were integrated and converted to a DVD with short descriptions that allows a quick general view of how students and professors interact in the teaching-learning process. During and after the elaboration we concluded that the methodology and resources utilized in the teaching of Human Anatomy in the Military Medical School are good enough for the students to learn (as demonstrated through multiple evaluations), but we want to achieve higher levels, modernizing our methods and adding means (plastinated materials, imaging, endoscopy, etc.) in the search of a better knowledge of this important science.

The exclusive use of plastinates at the New York University Dental College. ***Diwersi N***. *Institute for Plastination, Heidelberg, Germany.*

In 2004, the New York University, College of Dentistry, largest dental school in the country, was the first US school to introduce a 100% non-dissection anatomy curriculum to use plastinated specimens exclusively as an educational tool. Two kinds of plastinates were to be used: dissected silicone plastinates and transparent body slices. At first the acceptance of the dissected plastinates was higher than that of the body slices, but after some familiarization it soon turned out that the plastinated body slices became first choice for problem based learning due to their detective value. Anatomy teachers as well as students appreciated the individual anatomical features of the slices and felt challenged to name all structures of the serial cut slices. Four years after their introduction, plastinated specimens have transformed the way anatomy is taught at NYUCD. Student evaluations proof that the use of plastinates has made anatomy classes more attractive. Statistics also show that there was a significant increase in performance on national board exams by those students that were the first to participate in the non-dissection anatomy curriculum with plastinated specimens.

The use of plastination (Biodur S10 technique) in teaching the large intestine of the horse. ***Diz A, JL López-Rivero, A Martínez-Galisteo, F Miró, MV Roddríguez-Barbudo***. *Department of Comparative Anatomy and Pathology, Faculty of Veterinary Sciences, University of Córdoba, Spain.*

Background: The knowledge of the large intestine of the horse is of great interest in Veterinary medicine as diagnosis of colic pain and post-mortem necropsy are very frequent in Veterinary practice. Due to its complexity and volume the teaching of the large intestine anatomy in Veterinary schools has not been an easy task. Fresh and formalin-fixed preparations has been traditionally used with the inconvenience of its large weight, flaccidity and difficulty in handling. The aim of the present study was to analyze the use and advantages of a plastination technique (Biodur S-10) in the teaching of the large intestine of the horse in Veterinary Anatomy. Materials and methods: The large intestine of three seven-month-old horses were obtained from the slaughterhouse. They were cleaned by means of water and fixed by using formalin (5 %) during a week. Then, the method of plastination with Biodur S10 technique was followed, but they were inflated and placed in an anatomical position previous to curing. Finally, the preparations were used in the teaching sessions of Gross Anatomy in the Veterinary School of Córdoba. Results: The plastinated preparations obtained by using the S-10 Biodur technique were a turning point in the teaching of the large intestine anatomy. Comparing to the use of traditional specimens the morphology and topography of this part of the digestive system were easier understood. It was observed that the students identified faster than before the cecum and the different parts of the colon in the necropsy room. Conclusion: Plastinated preparations of the large intestine are very useful in teaching the Anatomy of the horse. They have advantages compared to the traditional specimens such as fresh and formalin-fixed preparations. These are their less weight, the facility of handling, and, in addition, the lack of toxicity.

A learning model for cardiac catheterization. ***Tomasome J, W Kong, R Hunt, SC Pang***. *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

Cardiovascular disease is a leading cause of morbidity and mortality in modern societies and according to the World Health Organization (WHO) by the year 2015, an estimated 20 million people worldwide will die from cardiovascular diseases, mainly from heart attacks and strokes. One of the greatest advances to date in the management of cardiovascular disease has been development of a procedure to diagnose abnormal coronary circulation that is referred to as cardiac catheterization. This has lead to other procedures like percutaneous coronary intervention with angioplasty and coronary stenting which can be used to treat symptoms of cardiac ischemia and also be used in the acute treatment of heart attacks. Cardiology residents

require extensive training and experience in order to become proficient in cardiac catheterizations. However, hands-on learning models for cardiology residents to practice catheterization before performing this procedure on a living patient are limited. In this investigation, we sought to create a cardiac catheterization learning model using plastinated cadaveric material that would assist in the understanding of the anatomy involved, and the dexterity required, to perform this procedure. The heart, aorta and associated arteries were excised from an embalmed cadaver and cleared of all adhering connective tissue. In order to remove clotted blood, a longitudinal incision was made on the posterior aspect of the thoracic and abdominal aorta and then sutured closed prior to plastination. An aneurysm was present in the abdominal aorta just proximal to the bifurcation of the common iliac arteries. As the femoral artery is the most common entry point of cardiac catheterization, both of arteries were included in the model. The dissected specimen was bleached in 6% H2O2 solution, fixed in 5% formaldehyde, and stuffed with gauze to maintain the patency of all vessels prior to plastination. Following plastination, the specimen was mounted onto preformed plexiglass. Sheathed or diagnostic catheters (e.g. 6F JL 4 or 6F JR 4) were used for practice catheterizations. Angiograms were provided to illustrate the position and manipulation of the catheter in the specimen. This model specimen has been placed in the Anatomy Learning Centre at Queen's University to be used as a tool for teaching cardiology residents the skill of cardiac catheterization.

Classification of pig kidney collecting system: S10/S3 corrosion casts study. ***Pendovski L[1], V Ilieski[1], D Lazarova-Tosovska[2], B Trpkovska[2], V Petkov[1]***. *[1]Department of Functional Morphology, Faculty of Veterinary medicine – Skopje, R. Macedonia, [2]Institute for Anatomy, Medicine Faculty – Skopje, R. Macedonia.*
Background: The pig kidney has been well studied in terms of physiology. In recently published publications the anatomy of pig kidneys is described, but those data are generic and didn't offer details concerning the pig collecting system. The shortage of studies in witch the drainage pattern of minor calices is analyzed, according the literature, has an influence for applicability of pig kidneys in experimental procedures in urology. In this work we present detailed anatomical findings on pelvio-calice system in pig kidneys with aim classification of collecting system based of the drainage on minor calices into renal pelvis. Material and method: The material was consisted of 53 kidneys taken from adult mixed-breed Dalland, slaughtered at age 150- 155 days and weighing 95 kg (mean). The pig collecting system was studied on three-dimensional silicone-S10 corrosion casts. After removing the renal capsule and surrounding fatty deposit on each kidney a short flexible PVC-tube was ligated into ureter. Through the tube a mixture of silicone S10/S3 in ratio 100:1(volume 10-15ml) colored with yellow ink (2-3% of silicone mass) was injected into ureter. As a hardener, added to the silicone was S6 in a proportion of 5% injected silicone. After injection, each kidney was placed in appropriate anatomical position for 24 hours to ensure depth curing of silicon. The corrosion of injected kidneys was achieved by immersion in a bath of concentrated commercial hydrochloric acid for 48 hours until a complete organic matter was decomposed leaving only endocasts of the collecting system that had been injected. Result: The renal pelvis and two major calyces or infundibulae (one cranial and one caudal) were founded in all investigated pig kidney collecting systems. The number of minor calices per collecting system was ranged from 5 to 17 (mean 9.02) and the cranial pole presented significant more minor calices then the caudal pole ($p<0.05$). Based of drainage into mid-zone of renal pelvis, the collecting system was classified into two groups. Group I was composed of kidneys (55.48% of cases) in which the drainage of renal pelvis mid-zone was performed by two major caliceal groups. The first group was drained directly into cranial infundibulae and the other major caliceal group was opened into caudal infundibulae. The both groups separately and simultaneously could drain into mid-zone of renal pelvis. Besides appointed two major groups of calices, in group II of kidneys (44.52% of cases), we found minor calices that were opened on the lateral margin for entire length of the renal pelvis. Those calices could drain independently and simultaneously with major groups of minor calices into mid-zone of renal pelvis. Conclusion: Except in plastination process, the silicon S10 showed that could be used as an injection material for preparing corrosion cast. The obtained casts are imperishable and flexible with well preserved anatomical details. The yellow ink added to the silicon mixture enable a good color visualization of casts allowing for better impetration of anatomical structures. The pelvio-caliceal S10 endocasts showed that are reliable copy image of original on witch the anatomical features at pelvio-caliceal system as its drainage pattern can be investigated. The classification of pig pelvio-caliceal system could be used for diagnostic imagining during interpretation on pyelograms, kidney ultrasound images and MR or CT kidney scans. The obtained data will increase the knowledge about the collecting system in

pig kidneys and will have influence for their future application in experimental endourology.

Body donation, management & museum techniques in Nigeria. ***Azu OO***. *National Postgraduate Medical College of Nigeria, Lagos, Nigeria.*

The practice of body/tissue donation is still seen as an alien tradition in Nigeria. The collection and display of human body parts in museums have been a common practice all over the world. Due to the low level of awareness, cultural beliefs, religious and ethical factors, most Nigerians are ignorant of the benefits of or otherwise of body parts in the training of personnel in the medical discipline as well as for research purposes. This has been responsible for the scarcity of cadaveric materials for use in the teaming medical schools and research institutes who are pressed to seek for cadavers by different ways. There is the belief by some Nigerians that the dead should be accorded their resting place-at the grave. Hence, when the mention of autopsy is done for deceased persons, relatives are the first to object to this, claiming that it dismembers and renders the deceased incomplete for burial. At the National Postgraduate Medical College of Nigeria, body parts used for museum demonstration are basically obtained via donations from tertiary hospitals/centers spread across the country. These donations cover a diverse range of specimens/tissues on pathology (surgical and post-mortem), anatomical dissections, skeletal materials as well as rare collections of relics, reptiles and objects of relevance for postgraduate teaching and research. The museum of the College handles the preparation of these specimens to the final presentation/use in pots or jars. With no plastination center in the West African sub-region, there is the urgent need for the establishment of a plastination laboratory in the museum of the College to provide a rallying point for the future training of experts in the field of plastination. We expect also the greater sensitization of anatomists to take part in the surging public interest in the issue of body donation and the formulation of necessary laws to guide body/organ use in Nigeria.

Suitable equipment for plastination. ***Von Hagens G***. *Gubener Plastiante GmbH, Guben, Germany.*

Proper equipment in Plastination is a must for the manufacture of high quality plastinates. This presentation will present equipment and auxiliaries for silicone and sheet plastination. The pros and cons of alternatives in vacuum chambers, vacuum pumps, separation foils and the like will be discussed. Safety requirements for health and fire hazards will complete the presentation.

Possible pitfalls and improvements of the standard silicone technique. ***Von Hagens G***. *Gubener Plastiante GmbH, Guben, Germany.*

Thirty one years after its invention the most common and the most dangerous pitfalls in plastination can clearly be named. The greatest challenge is to minimize or even prevent overall shrinkage during dehydration, impregnation and curing. In order to achieve this goal a proper diagnostic has to be made, because the reasons for shrinkage can be manifold. The main reasons are insufficient fixation, incomplete dehydration, over-long degreasing and insufficient curing. Improvements have been made by using various surface treatments with the aim of upgrading the final appearance of the specimens. The presentation will show the pitfalls and improvements of standard plastination techniques in pictures. The original plastinates can be evaluated during the visit to the Plastinarium in Guben.

A plastinated human cadaveric model as a realistic simulator for oral endotracheal intubation skills training. ***Ammerata A, R Hunt, C Reifel, R Easteal, L Mackenzie***. *Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada.*

Background: Oral endotracheal intubation is a critical care procedure that requires competent acquisition of skills by the trainee. Simulation in medical education provides an interactive, hands-on option for the trainee to practice technical procedures prior to performing the procedure on live patients. Although simulation as a teaching strategy in clinical education has proven successful, ongoing concern is use of simulators of varying fidelity. Synthetic simulators are available (i.e. rubber manikins, plastic and wood models), as well as animal models. In this study we have embarked on a project to prepare a plastinated human specimen for simulation of oral endotracheal intubation. Materials & Methods: An embalmed cadaver was chosen for the model based on the esthetics of the head and neck dissection (i.e. clearly visible muscles of the face and neck, open mouth, eyelids closed). Since only the head, neck, and superior mediastinum were required, the remainder of the body was removed. The posterior thoracic cage proximal to vertebrae T6 was maintained to provide the model with structural support. The superior mediastinum was dissected to show the trachea, bifurcation of the trachea, proximal portions of the primary bronchi and esophagus. The dissected specimen was bleached in 6% H2O2 solution and fixed in 5% formaldehyde prior to further dissection. To provide the mandible with mobility, the masseter muscles and parotid glands were removed, as well as any tissue beneath the zygomatic arch. The temporalis,

medial and lateral pterygoid muscles were left intact to maintain stability. Additionally, the posterior neck muscles were removed to the layer of the splenius capitis and splenius cervicis muscles, as well as both clavicles. To provide maximal mobility to the temporomandibular joint, the lateral temporomandibular ligament and fibrous capsule of the temporomandicular joint were cut and the condylar processes of the mandible were removed. A polytetrafluoroethylene tube was inserted into the trachea and esophagus to maintain their patency prior to plastination. The specimen was plastinated using the standard S10 method. Results: The plastinated, dissected human cadaver model presents realistic anatomy of the upper airway. The model allows for all of the movements to perform the procedure of oral endotracheal intubation, i.e. neck flexion, occiput extension, mandible elevation and depression, and lateral displacement of the tongue necessary for the visualization of the epiglottis and vocal cords. The instruments for intubation can be positioned and manipulated using this model, and it allows for proper intubation of the trachea, as well as incorrect intubation of the esophagus. Conclusion: The plastinated human model provides a realistic human intubation simulator. Trainees can practice and acquire the skills and dexterity to perform this procedure without risk to the patient. This learning model is effective and practical for training.

Using plastination specimen in laboratory for co-medical students. ***Taguchi M***. *Dept. of Anatomy, School of Allied Health Sciences, Kitasato University, Sagamihara, Japan.*

In the human anatomical lab, human specimens are indispensable. But co-medical students in our university do not dissect human body. Accordingly our students dissect fetal pig instead of human body to learn body structures. Our using fetal pig is about 30cm (head to hip) and 1500g. We prepared plastinated fetal pig specimen for student who dissect animal for the first time of their life to show how to dissect step by step. Several patterns of specimen are prepared, e.g. showing the nervous system, the muscle system, the abdominal viscera and etc. Moreover the students who have failed to dissect certain part structure can review once more. Plastinated specimens help the students to understand body structure.

Teaching the anatomical structures of the hand: a comparative study using prosected plastinates (S10) and dissection. ***Baptista CAC, CAC Bennett-Clarke, RD Lane, M Thorpe, C Shriner***. *University of Toledo, College of Medicine, Toledo, Ohio, USA.*

The importance of dissection in the human anatomy labs to teach the fundamentals of the human body is emphasized by the fact that 97% of medical schools in US require their students to participate in cadaver dissection. Although most anatomists consider dissection to be essential to anatomy, many departments are under pressures to reduce/eliminate dissections from the curriculum. The time pressure in medical school programs, reduction in the number of qualified faculty and advances in technology have also called into question the time vs. value for cadaver dissections. Another challenge to this teaching method has come directly from the AAMC as they encourage self-directed and student-centered pedagogies. These strategies emphasize problem-solving and development of clinical reasoning rather than memorization of content. At UT COM, we have the opportunity to examine the impact of plastinates on efficiency of student learning in the anatomy lab. However, the faculty would like to ensure that plastinates will have a positive impact on student learning before we adopt them in our course. For this study human hands were plastinated by the S 10 standard cold-temperature technique using North Carolina NCSX/NCXIII polymers. Students were asked to participate in the study which included one lab session (3 hours). The lab was conducted in the gross anatomy laboratory and replaced the regularly scheduled lab for those students. Prior to the start of the lab session all students completed an eight question pretest, similar in format to a standard practical examination. Students were asked to identify "tagged" structures using a multiple choice question format. Students were given 8 minutes to complete the pretest. Students were randomly placed in two groups. Group 1 (N= 10) completed the standard dissection of the hand following the instructions provided in the lab dissector and the lab instruction packet. The students in Group 2 (N=9) were given all of the same resources during the lab period, however, they used plastinated prosected hands to learn the lab content. When the students finished their instruction/dissection they completed a post-test in the same format of the pretest (8 multiple choice questions). Students also filled out a brief survey regarding the time investment and the satisfaction of the learning accomplished during the session. Comparision of pre- and posttest mean scores for both groups showed that there was no significant statistical difference between the groups. There is no negative impact on student learning when students use plastinates instead of cadaver dissections. Less time was required to complete lab assignments if students use plastinates to support cadaver dissections in the anatomy lab. Students found

plastinated specimens easy to work with and important structures were easy to locate.Overall students felt that time spent in lab was more productive when using plastinates.

Anatomical collection as a teaching and learning method in riga stradins university: a review of the evidence. ***Kazoka D, M Pilmane, J Vetra***. *Institute of Anatomy and Anthropology, Riga Stradins University, Riga, Latvia.*

Background: Human anatomy is complementary basic medical science, which is generally taught to medical students, physiotherapists, nurses, students of certain biological sciences, medical doctors and doctors working in some diagnostic specialties. Alternatively, human bodies can be preserved for human body exhibits to help others understand the complexities of the body by direct reference and observations. The aim of this study was to analyze anatomical collection as a teaching and learning method in Institute of Anatomy and Anthropology in Riga Stradins University. Methods: The collection of distinguish Latvian surgeon and oncologist Pauls Stradins (1896 – 1958), who has collected material from patients during surgery, was opened on 31st January in 2003. It is located in the building of Institute of Anatomy and Anthropology in Riga. This collection contains over 1000 high quality anatomical, 89 wax preparations, slides and photographs illustrating different diseases, injuries and realities of abnormal childbirth and animals. The author of wax models is famous sculptor – Janis Stradins and his collaborators. Generally, the same collection is created in the process of scientific research work of the Institute teaching staff and students. The majority of collection is organized in systems format and in regional format, in macroscopic and microscopic levels. The main part of all anatomical preparations (dissections) are in closed glass boxes (in 5% formalin). Results: The collection is valuable source of information for students of Medical faculty, Dental faculty, Medical History and public service organization at the Riga Stradins University. Students learn gross anatomy from models, photographs, lectures and tutorials. The study of gross anatomy using collection requires students to utilize two different learning strategies, the memorization of a large and complex medical vocabulary and the visual recall of three-dimensional structural relationships within the body. The study of microscopic anatomy (or histology) can be aided by practical experience examining histological preparations (or slides) under microscope; and in addition, students generally also learn anatomy with practical experience of dissection and inspection of collection. Dissections develop both learning techniques, and collection-aided teaching provides an important alternative pedagogical tool. In addition to these educational functions, physicians and students use collection as sources of illustrations for lectures, articles, and books. Conclusion: This unique collection is a basis of significant teaching and learning, research work and demonstrates an excellent exposition. It represents knowledge and understanding of the functional and structural changes in disease.

Computed tomography imaging of the equine temporormandibular joint: a sheet plastination study. ***Rodríguez MJ[1], A Agut[1], O López-Albors[2], J Arredondo[3], JM Vazquez[2], G Ramirez[2], R Latorre[2]***. *[1]Medicine & Surgery, and [2]Veterinary Anatomy, University of Murcia, Spain, [3]Fac Veterinary Medicine & Zootech., Autonomous Univ. State of Mexico.*

Background: Pathology of the temporomandibular joint (TMJ) is a challenge for clinicians due to its complex anatomy. This joint has recently gained high significance in a number of clinical problems (oral disorder and poor performance) therefore, several anatomical and biomechanical studies have been currently performed. Radiography has been the standard imaging method for the TMJ, however the interpretation of the images is difficult mainly due to overlapping of adjacent osseous structures. In human beings, CT has demonstrated a high sensitivity and specificity in assessing TMJ bone components. Furthermore, thanks to the recent development of helical multi-slice CT, three-dimensional re-formatted images can be obtained, this allowing a very realistic and spatially accurate reconstruction of TMJ structures which provides relevant information for a better planning and effectiveness of treatments. However, CT imaging interpretation requires a precise knowledge of sectional anatomy of the TMJ region. The aim of the study was to describe the normal crosssectional anatomy of the equine temporomandibular joint by using CT images and plastinated sections as anatomical references. Methods: CT evaluation was performed on eight temporomandibular joints from four pure-bred Spanish immature horses within the 2 hours of euthanasia to minimise post-mortem changes. A helical CT scanner (CT HiSpeed CT/e Dual; General Electric®) was employed to obtain contiguous 1 mm transverse slices. CT images were then re-formatted into sagittal and dorsal planes, transferred to an image analysis workstation (GE Advantage Workstation 3.1) and used to generate a three-dimensional model of the joint. Afterwards, the heads were firstly frozen at -30ºC for 48 hours and then cut off in blocks containing only

the TMJ. These blocks were frozen at -70ºC for 1 week to obtain 3 mm-thick transverse, sagittal or dorsal cryosections which were photographed and plastinated using the E-12 plastination method. Results: CT images and the corresponding anatomical sections were compared to achieve an accurate identification of the anatomy structures of the TMJ. Clinically relevant structures could be identified and labelled in both the CT images and the corresponding anatomical section. The best anatomical-CT depictions were acquired with the transverse images; they provided a detailed evaluation of the articular surfaces (articular cartilage and subchondral bone) of the zygomatic arch of the temporal bone and the mandibular condyle, and the relationship between the TMJ and the masticatory muscles, middle and inner ear. Conclusion: Plastination is an excellent method which provides full anatomic detail of structures of the TMJ in the horse to compare with novel imaging methods such as CT images from our study. This information may be a useful reference to assist clinicians in the interpretation and following diagnosis of the equine TMJ disorders.

Three dimensional reconstruction of the temporomandibular joint of a feline model by means of epoxy plastinated sections. ***Arredondo J[1], MD Ayala[2], O López-Albors[2], A Agut[3], JM Vázquez[2], F Asensio[4], E Latorre[2]***. [1]
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Background: Pathologies involving the articular and/or periarticular structures of the temporomandibular joint (TMJ) have been described in the cat. In most cases, surgery is necessary, however surgical approach of the TMJ is difficult due to complexity of the anatomical structures related to this joint. The use of epoxy plastinated slices allow accurate descriptions of complex anatomical regions and, in the end can be used for three dimentional reconstructions (3D). The aim of this work was setting up a 3D computer model of the anatomical structures of the TMJ of the cat from plastinated thin sections. This model will facilitate learning of this anatomical region in felines and assist surgeons when planning and carrying out surgical procedures. Methods: One TMJ of a cat that had undergone euthanasia for reasons other than temporomandibular joint problems was used in this study. The cadaver was frozen at -30ºC for 48 hours and a block containing the TMJ removed. The block was plastinated by epoxy impregnation E12-E1-E600 (Biodur®) and then cut into 0.4- 0.6 mm thick slices with a contact point diamond band saw. The plastinated slices were scanned and the images uploaded into 3D reconstruction software. Results: The thin plastinated slices provided a good anatomical detail of the TMJ structures and related. In the 3D model the osseous structures were particularly well reproduced. Subtraction of specific structures was possible; so all the elements in the model could be displayed in groups or as a whole, as well as rotated in the simulated 3D space. This facility increased the understanding of the anatomy of the TMJ and may be useful to assess surgical or clinical problems in this joint. Conclusions: The 3D model of the TMJ of the cat is a reliable tool to study this joint and could become a useful tool to plan standard and alternative surgical approaches in this or other feline species.

Epoxy plastinated slices of the temporomandibular joint of the cat are used to assess high resolution computed tomography. ***Arredondo J[1]; O López-Albors[2], A Agut[3], F Gil[2], M Soler[3], MJ Rodriguez[3], R Latorre[2]***. [1]*Faculty Veterinary Medicine & Zootechny, Autonomous Univ. of the State of México,* [2]*Veterinary Anatomy and* [3]*Medicine and Surgery, University of Murcia, Spain.*

Background: The study of sectional anatomy is a prerequisite to interpret image diagnosis techniques as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography. High resolution CT can provide slices of approximately 1 mm thickness from a body region. Thin plastination can even go further, as 0.5 mm thick slices may be obtained from a band saw after epoxy embedding. The aim of this study was to correlate thin epoxy plastinated slices with high resolution CT images of the temporomandibular joint (TMJ) in the cat. This joint has a number of clinical problems that require a precise anatomical knowledge for a correct imaging diagnosis. Methods: The TMJ from one adult cat was used in this study. The cat had undergone euthanasia for reasons other than TMJ problems. A high resolution CT study was carried out within one hour of euthanasia. Transversal tomographic images were obtained and multi-planar reconstruction in the sagittal plane performed. After CT examination, the cadaver was frozen at -30ºC for 48 hours and a block containing the left TMJ removed. The block was plastinated by epoxy impregnation E12-E1-E600 (Biodur®) and then cut longitudinally into 0.4-0.6 mm thick slices with a contact point diamond band saw. A comparative description between the plastinated slices and the CT images was made. Results: In the CT reconstructed model the bonny structures of the TMJ were precisely reproduced. Muscles related to the TMJ

were also identified with this imaging technique. Similarly, in the thin plastinated sections the anatomy of the TMJ was observed in detail. Thus, an accurate anatomical correspondence between the plastinated sections and the high resolution CT images was found. Conclusions: Thin plastinated slices are a good alternative to correlate high resolution CT images of the TMJ in the cat. It is likely that they will also be useful to asses MRI images but, in this sense further studies are required.

The pig heart anatomy on thin S10 tissue slices. ***Ilieski V[1], L Pendovski[1], B Bojadzieva[2], V Petkov[1]***. *[1]Department of Functional Morphology, Faculty of Veterinary medicine – Skopje, R. Macedonia, [2]Institute for Anatomy, Medicine Faculty – Skopje, R. Macedonaia.*

Background: The gross anatomy teaching requires pictures, charts and models for the comprehensive knowledge of pig heart. It has been proved that sheet plastinated slices are excellent tools for demonstrating the topography of anatomical structures inside the specimens. E12 and P35/40 techniques in plastination are known as methods of choice for creating 3-5 mm or even 8 mm semitransparent or transparent organ slices. Those techniques also required equipment and polymers that not any plastination laboratory has. Specific in this investigation was measuring of decreased vacuum in vacuum chamber on daily basis. In our work we try to make relationship between level of decreasing of vacuum and speed of silicone impregnation. In order to display structure distinctly for study and researches we develop a protocol in which S10 method was applied to produce sheet plastinated slices. Material and Methods: One pig heart taken from pig mixed breed landras/jorksir was subject of S10 sheet plastination. The process of sheet S10 plastination was carried out as following: The fresh heart from pig was dilated with tap water under hydrostatic pressure to relax the muscle and to remove the remaining blood off its chambers. The dilated heart was fixed by immersion in 3% solution of formaldehyde for one week. After fixation, the slices of pig heart were cut with meat slicer into 3-5 mm thin slices. Each slice was market with serial number and placed between two stainless steel grids in a stainless steel basket. The basket with slices was rinsed with cold tap water overnight to get rid off excessive fixative. The dehydration of specimens was carried out with pure acetone in ratio 10:1 at –25°C. The basket with slices was submerged in first 100% acetone bath for 5 days. After first bath, the slices were transferred in second acetone baths for another 5 days. In the last, third acetone bath, the slices were kept until acetone concentration was at least 99% for three following days. For forced impregnation, the slices were submerged in a fresh S10/S3 mixture (100:0.05) for 3 days at –25°C. In a period of two weeks, the vacuum via a non-continuous method, was slowly increased until 5 bars was reached. When the impregnation was ended, the vacuum was decreased at atmospheric pressure and the specimens were left for another 3 days submerged in silicone bath at room temperature. In curing stage, the slices were removed from the vacuum chamber, and each slice was smoothed in towel so the silicone on the slice surfaces was removed. Finally, the gas curing method in gas chamber was applied and for another 2 days and the heart slices were completely cured. Results: The S10 sheet plastinated pig heart slices were obtained for a period of 5 weeks. The color of muscles on slices was maintained and the shrinkage was not evident. The slices are elastic, easy to orientated and offer a lot of anatomical details. The heart muscle fibers are clearly separated from each other and their shape can be followed for entire length of slice. The anatomical structures like different vessel ostia maintain their anatomical form. The chord tendons attached on ventricular surfaces at valves (tricuspid and bicuspid) can be seeing as originating form papillary muscles. Three-dimensional view of atria and ventricles can be completely reconstructed for their shape and size. Due to the thicknesses of slices and its transparency, the details of all anatomical structures can be reached. Conclusions: The S10 plastinated pig heart slices showed to be an excellent teaching tool in anatomy. With this slices students can view separate anatomical structures and they can handle reconstructed pig heart. Also they will be able better distinguish specific anatomical details in the internal structure of the level of 3-5 mm thick specimens. The knowledge of anatomy based on thin plastinated heart slices will help for better and more accurate interpretation of diagnostic CT / MR scans in clinical reveal. On other side, the S10 technique showed that could be used as method for producing the sheet organ slices. The method that we applied is relative easy to follow and uses affordable materials that are basic for plastination process.

Plastination of reptiles for veterinary education. ***Wendel H, R Stark, J Plendl***. *Faculty of Veterinary Medicine, Institute for Veterinary-Anatomy, Freie Universität Berlin, Germany.*

Background: Reptile medicine encompasses the medical needs of more than 7000 vertebrate species. So far, limited formal training for veterinaries is offered concerning anatomy of reptiles. Therefore a program was started dealing particularly on reptile anatomy,

comprising 14 one-hour lectures once a week. For these purposes whole-body specimens of different turtles, non-toxic snakes and saurians were plastinated at our institute. Method: Formalin (4%) was injected via a tube through the body orifices as well as via injection needles through the skin. After a fixation period of 4 weeks, specimens were dehydrated in 3 or 4 successive baths of acetone at a temperature of -25°C. Duration for each bath was 10 days. Subsequent degreasing in acetone or methylene chloride at room temperature was performed for a short period of time (10 days). Impregnation was carried out in a bath of silicone starting at a vacuum of 7,5 mm Hg; final vacuum was in the range between 2 and 0,5 mm Hg. After removing specimens from the impregnation bath and after a subsequent relaxation at standard pressure for 24 hours, curing with Biodur S6 in a gas hardening chamber followed. At first the specimens partly hardened at room temperature on grids. For the final cure, several injection needles were placed through the skin of the reptiles in hidden areas of the body. Gas curing was complete when the body surface appeared dry. Results and conclusions: The S10-technique according to Von Hagen (1985) was used successfully to plastinate different reptiles. Concerning methodology, some specifics have to be observed for plastination of reptiles: 1) Due to the thick and scaly skin of reptiles liquids and gas does not pass easily. Therefore immersion of the specimen into the fixative is not sufficient. Also, for the final cure several injection needles should be placed through the skin. 2) In case of long snakes it is important to place the body during the fixation period in the exact position requested. 3) Degreasing period has to be short because snakes have massive fat pads in their body cavity which need to be preserved.

Analysis and standardization of morphological types of intraparenchymal spatial distribution of the caudate branches of the portal hepatic vein. ***Matusz PL, E-C Hordovan, AM Pusztai***. *University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania.*

Terminologia Anatomica (1998) homologates the caudate branches of the portal hepatic vein (PHV) having the origin from the transverse portion of the left branch. Lately, studies on corrosion casts performed at the University of Medicine and Pharmacy “Victor Babes” from Timisoara found caudate branches of the PHV having different origin. This paper analyses and standardizes the morphological types of intra-parenchymal spatial distribution of these branches, considering all their points of origin. The study material was represented by 100 hepatic corrosion casts. They were made by injecting with plastic (paste AGO II and TECHNOVIT 7143) of the vasculo-ductal system, followed by parenchyma corrosion with hydrochloric acid. On the study material we found 297 caudate branches of the PHV, having their origin in: the PHV trunk (0.33%), the PHV bifurcation (1.35%), the left branch of PHV (89.23%), the right branch of PHV (5.39%), the anterior branch (3.37%) and the posterior branch (0.33%). In order to study some of the morphological factors that favor the PHV caudate branches with peculiar origins, we analyzed the branching modalities of the PHV trunk. In 97% cases we found the modal type, with the bifurcation of the trunk into the left branch and the right branch; in 3% cases we found the trifurcation of the PHV trunk into the left branch, the anterior branch and the posterior branch. We found 15 morphological types of origin of the PHV caudate branches, with the number of portal caudate branches varying between 1 and 6. In 11% casts we found one caudate branch, in 32% - 2 caudate branches, in 18% casts – 3 caudate branches, in 29% - 4 caudate branches, in 8% - 5 caudate branches, and in 2% cases – 6 caudate branches. In 65% cases we found caudate branches originating only in the left branch of the PHV. The trifurcation of the PHV trunk leads to peculiar origins of the caudate branches (from the anterior branch, the posterior branch, the bifurcation and the trunk). Knowing the peculiar origins of the caudate branches of PHV is very useful for the surgeons performing liver resections. (Supported by CNMP 4.1-092/2007)

Modalities of branching of the vascular elements of liver’s afferent pedicle. study on corrosion casts. ***Matusz PL, AM Pusztai***. *University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania.*

Liver’s vascular elements of the afferent pedicle are the portal hepatic vein (PHV) and the proper hepatic artery (PHA). They are accompanied by elements of the biliary ducts system. Branching of the vascular elements in the superior portion of the hepatic pedicle presents morphological aspects with surgical significance. This paper analyses the branching modalities of PHV and PHA trunks on one side, and on the other side the peculiar morphologic relationship of the branching vascular elements. The study material consisted of 100 liver corrosion casts. They were made by injecting with plastic (AGO II paste and TECHNOVIT 7143) of the vasculo-ductal systems, followed by hepatic parenchyma corrosion with hydrochloric acid. PHV presents 3 morphological types of branching. In 97% cases the PHV trunk forks into the

right branch (RB) and the left branch (LB). In turn, RB forks into the anterior branch (ABr) and the posterior branch (PBr); LB gives birth to lateral branches (LBr) and to medial branches (MBr), from the umbilical portion. In 2% cases the PHV trunk trifurcates into: LB, ABr and PBr. In 1% cases the PHV trunk bifurcates into LB and PBr, with ABr starting from the transverse portion of the LB. RB and LB of the PHA were analyzed separately, according to the branching modalities. Thus, RB gives birth, in 84% cases, to the anterior branch (ABr) and to the posterior branch (PBr); in 5% cases it continues only with PBr; and in 11% cases it gives birth to 3 branches: ABr, Pbr and the medial branch (MBr), which crosses the plane of the main portal fissure. LB gives birth, in 87% cases, to LBr and MBr; in 13% cases it continues only with LBr. By analyzing the variability of branching of liver's afferent vascular elements we notice that PHV presents a modal aspect in 97% cases (with 3% variability). PHA presents a modal aspect in 74% cases (with 26% variability). The aspects of variability of PHV were accompanied by the modal distribution of the PHA branches. Only in one case the origin of MBr of PHA from the RB was associated with a major variant of branching of PHV trunk (trunk trifurcation into LB, ABr and PBr). These morphological aspects are very useful for surgeons when planning liver resection and transplant surgery.

The Journal of the International Society for Plastination

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