

## 9th Interim Conference on Plastination - July 8-13, 2007

**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);  $30 \text{ kg/m}^2$ , 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  $26.37 \pm 11.12 \text{ kg/m}^2$  with males slightly higher than females 27.5 vs. 25.2  $\text{kg/m}^2$ . These same respondents attained, in time, a maximal BMI of 28.33  $\text{kg/m}^2$ , in the upper case of overweight and

desired a BMI of 23.83  $\text{kg/m}^2$  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°C, and additional sterilization can be achieved simply by keeping the tank contents at 100°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°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°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 diluted 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°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<sup>1</sup>, V Ilieski<sup>1</sup>, B Trpkovska<sup>2</sup>, V Petkov<sup>1</sup>.* <sup>1</sup>Department of Functional Morphology, Faculty of Veterinary medicine - Skopje, <sup>2</sup>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 Daland 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<sup>1</sup>, L Pendovski<sup>1</sup>, B Bojadzieva<sup>2</sup>, V Petkov<sup>1</sup>.* <sup>1</sup>*Department of Functional Morphology, Faculty of Veterinary Medicine - Skopje,* <sup>2</sup>*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°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 from 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.**  
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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

fluorescent 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° 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.

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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°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 3<sup>rd</sup> and 4<sup>th</sup> 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 to 10mm 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.

hydrogen peroxide solution (10 - 20%). The results are an exact replica of the airways.

**Tracheobronchial cast preparation. Henry RW.**  
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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