Preservation of Biological Tissue: Yesterday -Today -Tomorrow. ***Weiglein, AH.*** *Anatomical Institute, Karl-Franzens-University, Graz, Austria.*

After death, biological tissue decomposes by autolysis or putrefaction. Ultimately, it disintegrates into its inorganic elements. Ever since the beginning of human life, efforts have been made to stop decay and to keep the body intact, primarily to keep the mortal frame for coming back to life sometime later (e.g. Egyptian mummification in 1550 BC and cryo-preservation in 20th century). Later on, interest in morphology made it necessary to preserve human tissue in order to investigate its anatomy. Fixation and preservation work against this decomposition made biological tissues insoluble, firm and protected them against deterioration. Fixation is done to keep a specimen in a specific lifelike state; preservation is done for indefinite maintenance of the condition obtained by fixation. The first preservation technique was done by nature: skeletons resist putrefaction and thus may be found in nature. The most important step in preservation was the introduction of formalin by Blum in 1896. Formalin consolidates tissue and stops the rapid decomposition processes. Disadvantages to formalin fixation include unnatural hardening and dis- or decoloration of the tissues. The introduction of formalin was followed by the color-preserving embalming solutions by Kaiserling (1900) and Jores (1930). These solutions contained mainly formalin, potassium nitride, and potassium acid. After fixation the specimens are submerged in alcohol. Formalin changes hemoglobin to brown colored met-hemoglobin, which is changed to red-colored kat-hemoglobin by submersion in concentrated alcohol. Formalin, however, is still mainly used for preservation of cadavers because of its preservation properties. In 1992, Thiel published an article on a new method of color preservation and based on this preservation technique, he produced a photographic atlas of practical anatomy. This atlas showed the excellent results of his new embalming technique that preserved the human body in a lifelike state. Besides the development of embalming solutions that allow preservation of lifelike conditions (color and flexibility) for student dissection and training of surgical techniques, other methods were developed for demonstration of human anatomy in museum specimens. In museum specimen preservation, flexibility is not necessary as they are made just for display. Paraffin impregnation was performed by Hochstetter in 1925. Embedding of organic tissue in plastic was introduced in the 1960's. In 1978 Gunther von Hagens invented plastination. This technique utilizes both impregnation and embedding. The central step of this method is the forcing of polymers into the cells of specimens using vacuum. Three different polymers are used for this technique: silicone for obtaining natural looking specimens, epoxy for transparent body and organ slices, and polyester for semitransparent brain (and organ) slices. Development of different methods of preservation have supported and will continue to support both medical research and teaching. Without these methods, we would lack most of today's knowledge in medicine.

Plastination - Idea, Procedures, Results. ***Weiglein,*** *AH. Anatomical Institute, Karl-Franzens-University, Graz, Austria.*

Dr. Gunther von Hagens in Heidelberg, Germany developed plastination, a unique technique of tissue preservation in 1978. In this process, water and lipids in biological tissues are replaced by curable polymers (silicone, epoxy or polyester). These polymers are subsequently hardened, resulting in dry, odorless and durable specimens. Silicone is used for whole specimens and thick body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices. Polyester is used for brain slices to gain an excellent distinction of gray and white matter. Plastination consists of four main steps: 1) Fixation, 2) Dehydration, 3) Forced Impregnation, 4) Hardening (Curing). Fixation can be achieved by the use of almost all conventional fixatives. Dehydration is achieved mainly by acetone because acetone also serves as the intermediary solvent during impregnation. Forced impregnation is the central step in plastination: vacuum forces the acetone out of and the polymer into the specimen. Finally the impregnated specimen is hardened by exposing it to a gaseous hardener (silicone) or by UVA-light and heat (polyester, epoxy). Plastinated specimens are perfect for teaching, particularly for neuroanatomy. Silicone plastinated brains are useful because they can be handled and they are almost everlasting. Polyester plastination of brain slices provides an excellent distinction of gray and white matter and thus a better definition of the two areas. The plastination techniques for brain (polyester) and body slices (epoxy) are also used in research, particularly in comparison with CT and MRI images.

History and Principles of Dehydration. ***Henry, RW.*** *Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Dehydration of specimens for histological preparation commenced in the 19th century. However, dehydration for preservation of biological tissues predates 6000 B.C. The concept of dehydration for plastination is to replace tissue fluid with a volatile

intermedium that can be replaced with a curable polymer (plastination). Cold (-15 to -25°C) acetone has emerged as the gold standard dehydrant for plastination. Acetone dehydrated specimens have yielded excellent silicone impregnated specimens for over two decades. Room temperature acetone or **a** standardized graded series of alcohol may be used with good success. Cold acetone dehydration reduces shrinkage, especially when processing brain tissue. The dehydration procedure involves placing prepared specimens in a series of acetone baths. Classically, three changes of 100% acetone have been used at 3, 2, and 1 week intervals. Weekly changes of 100% acetone may be used as well. Specimens may be started in a lower percent acetone (70 - 80%) and moved into higher percentages weekly until 100% is maintained. The percent of acetone vs water is measured with a hydrometer (acetonometer). A hydrometer is temperature dependent. It is helpful to monitor acetone purity at the end of each dehydration period. After the specimens have passed through 3 or 4 changes of acetone, they should be dehydrated. At this time the specimens should be brought out to room temperature in 100% acetone for a few weeks to aid in defatting. Fat does not impregnate well and may ooze lipid for many years. Thin specimen slices may be dehydrated in cold or room temperature acetone. One bath of higher percent acetone and two bathes of 100 % or three bathes of 100% acetone changed at 2 - 3 day intervals is usually sufficient. Complete dehydration is central to the plastination process.

Dehydration: 6,000 B.C., 19th Century, First Quarter Century of Plastination, and the New **Millennium.** ***Henry RW, Brown MA, Henry CL, Reed RB.*** *Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Dehydration has been used to some extent for centuries. As early as 6,000 B.C., Egyptians used wine and salt to partially dehydrate and preserve the viscera in the mummification process. Salt has been used to dehydrate and preserve food and hides for centuries. In the 19th century, dehydration of histological tissue came into vogue. Since its inception, dehydration for plastination has been in cold (-20°C) acetone. This process has provided excellent dehydration and hence resulted in superbly impregnated specimens for nearly the last quarter of the 20th century. Alcohol has also been used since the inception of plastination and it provides a reasonable alternative to acetone dehydration. Classically a general rule for acetone dehydration has been some form of three changes of acetone over a 4 week period of time. Recently, room temperature acetone dehydration, which could be a more hazardous situation, has been suggested and utilized. Claimed benefits include faster dehydration, decreased color loss, and increased flexibility. Vacuum dehydration has been suggested to decrease dehydration time, however, no published data was found. Recent studies show this procedure actually increases dehydration time. The present study was done in an attempt to define the approximate time needed to completely dehydrate specimens in acetone. Specimens of various sizes and densities were dehydrated in both cold and room temperature acetone. The dehydration periods and acetone percentages were recorded and the specimens were plastinated via the classic von Hagens' method. The time needed to dehydrate specimens of varying sizes in acetone was 4 - 6 days in cold or room temperatures. Following dehydration, specimens were impregnated using the classic cold von Hagens' silicone method. Upon completion of plastination, no significant differences were observed between specimens.

History and Principles of Silicone Impregnation. ***Henry*** *RW. Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Silicone plastination for preservation of biological specimens has been used for nearly 25 years. Production of anatomical and pathological teaching specimens has seen the most use. However, archeological artifact preservation is also enhanced by plastination. Silicone plastination was invented in Heidelberg, Germany, around 1978 by the physician and anatomist, Dr. Gunther von Hagens. Several institutions, scattered throughout the World routinely use the process and a few specimens are available commercially. Silicone plastinated specimens are clean, dry and free from irritating formaldehyde vapors. They are esthetically pleasing to students, offer a convenient mode for reviewing anatomy, and are an excellent tool for public education. In brief, silicone plastination is the process of replacing fluid in a specimen with a curable silicone polymer. The first and most crucial step is specimen preparation. The specimen must first be prepared to demonstrate the desired features. This generally includes fixation in a low percent formaldehyde solution. After any fixative is removed by running tap water, tissue fluid must be removed by dehydration in preparation for introduction of the silicone polymer into the tissue. Cold acetone has emerged as the classic dehydrating agent. Once the specimen is dehydrated, a volatile intermediary solvent is needed for exchange with the silicone polymer. This solvent's boiling point must be sufficiently different from the polymer so that the solvent can be extracted

from the specimen. Acetone possesses these properties as does methylene chloride (dichloromethane). Therefore, if acetone is used for dehydration, acetone serves as the volatile intermediary. Exchange of the volatile intermediary for the silicone polymer is called impregnation. As the solvent is extracted slowly, a void is created in the tissue that will allow the silicone polymer reaction mixture (silicone/catalyst/chain extender) to move into the tissue void. This is accomplished by decreasing pressure to the point where the intermediary solvent vaporizes and boils out of the specimen. At -15°C, this pressure is 2 - 3 cm and at room temperature 5 - 7 cm pressure. The solvent in its gaseous form is extracted from the vacuum chamber in the exhaust of the vacuum pump. The polymer enters the specimen over a period ranging from 24 hours to weeks depending on the thickness of the specimen and the viscosity of the reaction mixture. Polymerization (hardening) is the remaining step. A cross-linker is exposed to the polymer reaction mixture filled specimen to cause the polymer to harden (polymerize) and stay inside the specimen. Upon completion, the specimen is forever preserved. Silicone impregnation has classically been done in the cold. However, room temperature impregnation has been practiced since the inception of plastination.

Using the Room Temperature Plastination Technique to Assess Human Prenatal Growth of the Vertebral Column and Spinal Cord. ***Raoof*** *A[1]*, ***Glover R[1]***, ***Jurjus A[2]***. *'Department of Cell & Developmental Biology, The University of Michigan Medical School, Ann Arbor, MI, USA. [2]Department of Human Morphology, The American University of Beirut, Lebanon.*

This work describes morphometric variations in the length of the spinal cord and vertebral column in 30 (14 female and 16 male; 5 black and 25 white) human fetuses between 16.5 and 38 weeks of age. It has been traditional to run similar investigations on freshly dissected specimens preserved in formaldehyde (Icten et al., 1995). However, plastination techniques are more favorable for this type of work since they yield permanently preserved specimens that are easier to process and handle (von Hagens et al., 1987). Specimens processed using room temperature plastination techniques employing COR-TECH PR-10 (CST 70) silicone polymer yielded a clear visualization of the spinal cord and allowed for more precise morphological measurements. Most importantly, specimens showed no significant shrinkage in crown rump length during dehydration, impregnation and curing. However, spinal cords were significantly shortened. Also, an interesting correlation was observed between spinal cord length and crown rump length. Sex and ethnic differences were observed but without statistical significance.

"Classic" Silicone Processed Specimens vs "New formula" Silicone Plastinated Specimens: A Two Year Study. ***Henry RW, Reed RB, Henry CL.*** *Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.* With the expiration of certain patents of the silicone plastination process (Biodur), variations in the plastination process have been introduced. The most publicized change is in sequence that the components of the silicone polymers are combined. To compare some of the various products, a group of specimens was prepared and dehydrated in a similar manner for impregnation using four methods and three polymers: 1. The classic cold -15°C von Hagens' (Biodur™/ Heidelberg) method; 2. The new Dow™ (Corcoran) method (room temperature); 3. The classic room temperature von Hagens' method; 4. Generic silicone polymer and hardeners at room, cold room (5°C), or freezer temperatures (-15°C). The classic von Hagens' method and polymers consistently produced the best specimens. The Dow™ method specimens were good specimens. However, after a few weeks, all Dow™ specimens commenced to have a polymer build up on the surface. This build up of polymer on the surface detracts from the beauty of the specimen and often is under the serosa. The generic polymers produced specimens similar to the classic von Hagens' method and polymers. However, the generic room temperature and cold room impregnated specimens had a slightly drier look. All specimens seem to be durable and possess a similar degree of flexibility. Thinner specimens continue to have more flexibility.

How to Make Flexible Lung Specimens Using the Biodur S10 Technique, ***de Jong K.*** *Department of Anatomy & Embryology, Academic Medical Center, Amsterdam, the Netherlands.*

When I started plastination 10 years ago, my first specimens were a heart and a lung obtained from an embalmed body. When finished, the heart specimen was acceptable, but the lung specimen was too dark, too solid, too heavy and too small. The next lung was of the same poor quality and I was eager to improve the quality of these specimens. By accident, I acquired a fresh lung, which was rinsed through the trachea, then fixed in formaldehyde, dehydrated and impregnated using the standard S10 technique described by von Hagens (1985). The result was a specimen of a far better color, due to the rinsing out of the blood. However, weight, size and flexibility were still not good enough. When comparing microscopic slides of lung

tissue with tissue of any other organ, the most striking difference is the amount of hollow structures (airways, alveoli and vessels) in lung tissue. When a plastinated lung is examined all hollow structures should be free of silicone to provide maximal flexibility. Thus, the main challenge when plastinating lung tissue is not "how to get the silicone in", but "how to get the silicone out". Furthermore, all contractile tissue in the lung should be expanded prior to and during fixation. To achieve this, the next lung to be plastinated was cannulated and rinsed with running tap water until the water flowing out of the lung was clear, indicating that most of the blood was rinsed out. Next, a 5% formaldehyde solution was pumped through the lung by the tracheal cannula for 5 hours using an adjustable pumping speed. Pumping speed was controlled by looking at the size of the lung. Further fixation was achieved by leaving the lung in a 5% formaldehyde solution for 1 week. Dehydration was performed by pumping used acetone (60%) through the lung for 5 hours, followed by 100% pure acetone. Again, pumping speed was controlled by looking at the size of the lung. Hereafter, dehydration was completed in the usual way by immersion in 100% pure acetone. The lung was then perfused through the tracheal cannula with a mixture of Biodur S10/S3 (60 pbv.) and xylene (40 pbv.) under a fume hood for 5 hours. Again, pumping speed was controlled by looking at the size of the lung. The normal vacuum impregnation was then performed, but the pressure was not brought lower than 15mm Hg to avoid evacuation of the xylene. After impregnation, the specimen was suspended by the tracheal cannula to let the excess polymer drip off. After this, the free polymer in the lung was pumped out, using the tracheal cannula and the same pump, but now pumping in reverse direction. This caused the specimen to shrink a lot, but this shrinking was reversible. When no more polymer was coming out, the specimen was placed under a fume hood suspended by the tracheal cannula. The cannula was then connected to compressed air, using silicone tubing, and inflated. The rate of airflow was monitored by the size and form of the specimen. When the correct size and form was obtained, Biodur S6 gas cure agent was slowly injected directly in the silicone tubing, thus performing fast curing from the inside of the specimen. This was repeated until the outside of the specimen was cured. After removal of the tracheal cannula, the outer side of the trachea and the main bronchi were finished by coating them several times with a solution of fully pre-cured S10/S3 (10 pbv.) in methylene chloride (90 pbv.). Finally, the specimen was gas-cured.

Colored Plastinates. ***Steinke H, Koitzsch C, Schmidt W.*** *Institut für Anatomie, Universitat Leipzig, Germany.*

Understanding a macroscopic region is often difficult because of the complex three-dimensional anatomy of arteries, veins and nerves. The distinct demonstration that is helpful for students and laymen can be obtained by giving color to specific structures in a plastinated specimen. When ordinary colors are applied before the plastination procedure, the steps of dehydration and defatting with acetone and methylene chloride will remove the colors. When the color is added after plastination, in a dry condition, the color easily becomes "flaky" under tension of the Plastinate. For this reason, we modified the technique of Gyermek (1918) who used chemical color reactions in the presence of the tissue under study. The obtained color remained stable during dehydration, defatting and impregnation with silicone resin. We added different types of chemical solutions one after the other and displayed nine differentially colored structures in e.g. red, blue, yellow and black. The chemical reaction that causes the permanent response is partially explained.

Preparation of Plastinated Specimens of the Human Central Nervous System for use in Teaching of Medical and Dental Students. ***Baeres FMM, Wamberg J, Metier M.*** *Institute of Medical Anatomy, University of Copenhagen, Denmark.*

Due to a dramatic fall in the autopsy rate in Denmark, the Health Science Faculty at the University of Copenhagen has found it increasingly difficult to obtain human brains for the teaching of medical and dental students. However, the durability of plastinated specimens has provided us with a satisfactory alternative in our teaching compared to our use of fresh preparations. We plastinate whole human brains, dissected human brains, and stained or unstained coronal or sagittal brain sections. After removal of the brains from the skull, the specimens are fixed in 4% paraformaldehyde on 0.1 phosphate buffer for 3 months. The brains are then washed in tap water for several days and then dehydrated in a series of acetone baths at -20°C for several weeks. The specimens are then impregnated in Biodur S10 with 1% Biodur S3 hardener under vacuum at -20°C for 3 weeks. After impregnation, the specimens are left for 12 hours at room temperature to allow the silicone to drip off the specimens. This is followed by a fast gas-cure with Biodur S6 vapor for 2 weeks. CaCl2 powder is placed in the gas-cure box to obtain a dry atmosphere. After the gas-cure, the specimens are dried for several weeks in a tight box. These procedures result in hard durable specimens with good visualization of fiber tracts as well as good contrast between gray and white matter. The coronal or sagittal 0.5-1 cm thick brain sections are cut in a Bizerba meat slicer. Some sections are stained

according to Mulligan with a solution of phenol, cuprisulfate in 0.1% HC1 followed by ferrichloride and potassium-ferrocyanide. The Mulligan-stained sections are dehydrated and impregnated in Biodur S10 as described above. A satisfactory bluish-green Mulligan-staining is present after the impregnation procedure. The sections can be handled in the classroom without protective covering. In summary Biodur S10 impregnation of brain specimens and sections has been a satisfactory alternative for use in teaching of medical and dental students compared to fresh brain specimens and sections.

Plastination: Application for the Conservation of Natural History Collections. *Marechal JP[1,2], **Grondin G[3], Clique A[2], Durand M[4], Maigret J[1].***
'Museum National d'Histoire Naturelle, Grande Galerie de VEvolution, Service Conservation-Collections, Paris, France. [2]Station de Biologie Marine du Museum National d'Histoire Naturelle et College de France, Concarneau, France. [3]Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Qc, Canada. [4]Laboratoire d'Anatomie, Faculte de Medecine Jacques Lisfranc, Saint Etienne, France.

The Collection-Conservation Department of the "Grande Galerie de l'Evolution" of the National Museum of Natural History (Paris, France) is developing a research program for the technical preparation of scientific collections in museums and exhibitions. This program will establish the optimal conservation practices that are essential for the preservation of the specimens constitutive of the collections. The increasing number of exhibitions and the requirement for sophisticated scenarios lead to a reflection about the technical preparation of various zoological groups (molluscs, annelids...). Although many advances have been made in the technical preparation of the taxidermy of mammals and birds, many gaps remain for invertebrates and plants. Only few a methods have been developed to study these groups, and the techniques have not changed since the 19th century. This is in spite the emergence of many chemical products, which could be used for solving different problems in the effort to conserve these organisms. According to the requirements of the specimens, their utilization in exhibitions requires various techniques, such as formalin tank, moulding, wax, resin embedding and models. Plastination can be used for museological applications (handled specimens, realistic presentations). The S10 technique was tested on 15 marine organisms (molluscs, crustaceans and fishes). Specimens were frozen (-25°C) and then dehydrated using cold acetone or methanol (-25°C). Specimens were divided in two groups and forced impregnations were carried out at room temperature (20°C) for one group and at -25°C for the second group. We have obtained promising results, with the exception of a fish showing skin distortion due to the lack of permeability of the epidermis. Problems were encountered prior to forced impregnation. Acetone bleaches out color differently according to the zoological group and specimen. The goal of the research should be the conservation of shape (with specific methods for the different zoological groups) and pigments (with new intermediary solvents). Plastination could be potentially used for the conservation of organisms like medusa worms.

Plastination of *Astacus leptodactylus* **(freshwater crayfish) with the S10 Technique.** *Asadi MH, **Joghatai MT.** Iran University of Medical Sciences, Tehran, Iran.*

The exoskeleton of the *Astacus leptodactylus* is relatively impermeable. Plastination depends upon the ability of formalin, acetone and polymer to penetrate into the specimen. In this paper, the procedure for *Astacus leptodactylus* plastination with S10 will be stated. The specimens (weight 90+/-5 gr.) were fixed in 5% formaldehyde. Dehydration occurred via freeze substitution in acetone. For impregnation, a low viscosity S10/S3 solution was used. First, the specimens were placed at room temperature and atmospheric pressure in a mixture of S10/S3 (100:1) for 24h. During the forced impregnation step, specimens were placed under vacuum at -25 °C and pressure was decreased slowly down to 5mm Hg. Curing was done in two stages. In the pre-curing stage, the excess polymer was drained from the specimen surfaces and they were placed in an oven at 40°C for one day. In the gas curing stage, they were exposed to S6 vapors for 3 days at normal room temperature.

Principles of the P35/P40 Polyester Procedures for the Plastination of Brain Slices. ***Barnett RJ.** Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand.*

The P35 and P40 procedures are used to produce thin 4, 6 and 8 mm semitransparent slices of brain tissue. Brain slices produced with these two techniques possess excellent instructional potential giving distinctive differentiation of white and gray matter and beautifully highlighting blood-filled vessels. Both the P35 and P40 polymers can also be used for the production of thin body slices. Although these two polymers are slightly different in their physical nature and the way they perform during manufacture, most of the stages of the technical procedures are comparable. Brains are fixed by immersion in 5% formalin at 5°C for 3 months. A meat slicer is used to slice the brains into 4, 6 or 8mm

slices and the slices are laid on grids that are stacked in a basket and secured. The basket of slices is rinsed with tap water overnight then precooled to 5°C before being submerged in 100% acetone at -20°C for 1-2 days. The basket is submerged in a 2nd bath of 100% acetone at -20°C for another 1-2 days and this bath should stabilize at 99% purity. For immersion in P35, two baths of P35/ A9 mixture are used at 5°C in the dark. Immersion for P40 only requires one bath at -20°C in the dark (both the P35 and P40 polymers are cured with UV-A light so care must be taken to prevent early polymerization). For both techniques, impregnation can be undertaken at room temperature, in the dark, for 24 hours to a residual vacuum pressure of 10-15mm Hg. Single walled glass chambers, suitable for a single brain slice, can be used for casting both the P35 and P40 slices. Curing is initiated by UV light, P35 requiring 45 minutes to 4 hours and P40 requiring 3 to 24 hours. Glass chambers containing P35 slices are heat cured in an oven at 45 °C for 4-5 days after light curing. P40 slices do not require heat curing. After cooling to room temperature, the chambers are dismantled, the cast slices are sawed to size, and edges polished.

Transparent Whole Body Slices Plastinated with the E12 Technique of Plastination. The Video: Practical Applications in Plastination. ***Barnett RJ[1], Cook P[2].*** *'Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand. [2]Department of Anatomy with Radiology, School of Medicine, University of Auckland, Auckland, New Zealand.*

The E-12 procedure preserves cross sections of the body that are detailed, transparent, projectable, durable, and easy to handle. These specimens are proving to be extremely useful for teaching and in particular assisting in understanding the modern clinical radiographic diagnostic tools such as CT, MRI and ultrasound. Potential for this technique in the research area is becoming well recognized with a number of papers published in the Journal of the International Society for Plastination to date. Practical Applications in Plastination is an informative and comprehensive video tape presenting each of the major plastination techniques: S10 Standard Method, E12 Transparent Serial Sections, P40 Brain Slice Method.

Polyester Plastination (P40) of Body Slices. ***Henry RW, Reed RB, Henry CL.*** *Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Thick plastinated body slices have been produced since near the inception of plastination using silicone polymers or PEM (Biodur™). Thin body slices have been routinely produced since the early 1980's. Thin slices were first impregnated with epoxy and later with polyester. With the advent of MRI, CT and ultrasonography an understanding of sectional anatomy is desirable. Thin slices have been an ideal tool to aid their understanding. When producing epoxy slices, a hardener is added to the epoxy resin. This limits impregnation time and casting to about 48 hours. If a large number of slices are being produced, it rushes one to get all specimens cast before the mix becomes too thick. Over a period of time the epoxy sheet yellows. The P40 technique (Biodur™) has been used successfully for producing thin brain sheets for several years. The P40 technique has no hardener added. If kept cool and from light, the impregnated slices may remain in the impregnation bath for long periods of time prior to casting. This is advantageous if one has a busy schedule and/or limited help. Slices of 3 to 4 mm were cut from frozen specimens of which some were fixed and some unfixed. Slices were placed on grids and sawdust was removed by scraping and/or a gentle water stream. Three changes of -15°C acetone (90, 100, 100%) over 5 - 7 day periods were used for dehydration. The dehydrated specimens were submerged in the P40 resin. Impregnation was carried out for 24 - 48 hours at room temperature. Final pressure was not allowed to go below 1 cm Hg. Either safety or tempered (hardened) glass plates, silicone gasket of the appropriate diameter, and clamps were used to construct the flat chambers for casting of the slices. The junction at the ends of the gasket was sealed with Biodur™ sealant (HS80). Four hours of exposure to ultraviolet light was used to harden the cast slices. Over a 2 month period, slices were cast and cured as time permitted. P40 slices provide an excellent means to study sectional anatomy.

Plastination with a Limited Budget. ***Grondin G.*** *Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada.*

Plastination is considered as one of the best techniques for the preservation of biological specimens for teaching and research in Biology, Medical Sciences and Veterinary Sciences. One of the major concerns when someone wants to start plastination is the cost related to this project. You may believe that such a project is very expensive but we will see that it is possible to start plastination even with a limited budget. This presentation will describe the minimal essential equipment and chemicals required to plastinate. We will also look at some modifications that can be made to usual pieces of equipment found in every laboratory to recycle these into plastination tools.

The History of Plastination in China. ***Zheng TZ[1], You X[2], Cai L[2], Liu J[3].*** *'Department of Anatomy,*

Shanghai Medical University, Shanghai, China. [2]*Ministry of Education of Jiang Su province, China.* [3]*Nanjing Su Yi Plastination Factory, Nanjing, China.*

Plastination was introduced in the Chinese province of Jiang Su in 1997. The enormous needs for plastinated specimens in various levels of Chinese education institutions were easily identified but the cost of importing the equipment and polymers rendered plastination at a large scale impossible in China. A project was then elaborated in collaboration with the ministry of Education of the Jiang Su province in order to identify all the essential equipment and research was started to produce these locally in order to reduce the costs. The standard protocols had to be modified, "three steps impregnation" and "intermittent forced impregnation" were elaborated. All the equipment (vacuum chambers, vacuum pumps, etc..) used in our laboratories was fabricated in China thus eliminating the cost of importation. After six months of work, a Chinese research group developed a new silicone suitable for plastination, which was produced locally. Many thousands of specimens ranging from small animals to whole human bodies have been produced using Chinese techniques and polymers are now used in 20 Chinese schools and universities. Two different types of plastinated specimens are produced with the Su-Yi Chinese silicone, dry and rigid or soft and oily. Until now, we need to make a choice between a dry but hard and soft but oily specimen. Our research continues in order to improve our silicone and plastination technology to produce the ideal type of soft plastinated specimens with dry surfaces. The first plastination laboratory in China was created in Canton in 1996 and the Nanjing Plastination Factory was opened by the end of 1997 and has produced since then thousands of specimens used in many Chinese schools and universities. Plastination laboratories were also opened in some universities in China. We will continue to work to create more plastination laboratories all over China and to supply all the Chinese schools and universities with plastinated specimens. This is certainly a very long term project that is estimated to take 20 to 30 years. We finally hope that our experience will serve as an example for other developing countries and promote plastination to as many countries as possible.

Plastination: Salvation for Curricular Cuts. ***Reed RB, Henry RW, Henry CL.*** *Department of Animal Science, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Small animal gross anatomy and large animal gross anatomy were combined into a one year long comparative course in the professional curriculum at the University of Tennessee, College of Veterinary Medicine. Student laboratory time was decreased by 20 percent in the resulting course. Without adequate time for each student lab group to dissect both a dog and a horse during the academic year, each group dissected a canine cadaver. After each assigned segment of dissection was completed on the canine, students used prosected, plastinated specimens as well as fresh tissue for study of large animal species. Plastinated specimens were prepared via the classic von Hagens' (Biodur™) method. Study of these plastinated specimens was generally done on days in which the canine cadaver was not in use. This measure ensured that students did not spend the majority of their time dissecting and studying the canine species and only giving the plastinated large animal specimens a cursory look. The use of prosected, plastinated large animal specimens allows students access to competently prepared, long lasting study material. By not having to dissect an entire large animal cadaver, students were prevented from rushing through dissection of the equine and bovine species to compensate for the reduction in laboratory time. This approach allows sufficient time for students to study the various species within the time frame dictated by the curricular revision.

Plastination of a Human Torso and Hearts for Emergency Medical Service Education. ***Hostler D, Barnes*** *T. Department of Biomedical Sciences, Ohio University, Athens, Ohio, USA.*

Emergency medical service personnel typically receive little or no laboratory training in anatomy. It is often difficult and expensive to arrange for time to dissect cadaver specimens or even view prosected specimens. The primary objective of this project was to plastinate a prosected torso and a number of human hearts in a manner that would be useful to emergency medical service personnel. Four human hearts were prosected to demonstrate the normal anatomy including the chambers, valves, papillary muscles, and coronary arteries. One human cadaver was prosected to demonstrate areas typically injured after trauma. This prosection included evisceration and removal of the limbs. The lungs were removed to demonstrate the size of the pleural cavities and the thickness of the thoracic walls. Care was taken to expose the vessels of the mediastinum and a laminectomy was performed on the lumbar spine. The materials were plastinated using the standard S10 method. Briefly, the embalmed specimens were dehydrated at room temperature by increasing alcohol concentrations. Degreasing was accomplished in acetone. Prior to impregnation, the vessels of the mediastinum and the heart chambers were filled with gauze to maintain shape and orientation. Specimens

were then impregnated at -20°C with S10 silicone polymer. These specimens have been used with great success in a local paramedic education program. They are easy to transport and can be used as part of an outreach program for both emergency medical service training and continuing education programs. Additionally, they will be utilized in the undergraduate, physical therapy, and medical gross anatomy programs at our university for teaching and examination. Large specimens which are to be plastinated require extensive preparation in terms of both the time for the prosection and the plastination itself. The torso specimen required a 36x24x24" container and large volumes of chemicals for complete submersion. However, the initial cost in time and supplies is justified for specimens which can be utilized year round in multiple venues.

Prescribed Sequence Labeling Method: A Strategy for Mastery of Sectional Anatomy Using Plastinated Specimens. ***Lane A.*** *Triton College, River Grove, Illinois, USA.*

It is general knowledge that every day there is more information and less time to be informed, thus the need to increase the rate of learning. Studies have shown that a prescribed sequence labeling method for cadaver sections including clinical images significantly increases the learning and retention rates. Previous studies (Lane 1998) have demonstrated that a prescribed sequence of labeling method (PSLM) increases the learning rate and comprehension of relationship of structures/features of anatomical photographs acquired from The Visible Human Project. This project was sponsored by National Library of Medicine. This present study using photographs of plastinated anatomical brain sections correlates and parallels previous studies. Thirty-two college students enrolled in sectional anatomy participated in this present research. They were randomly separated into two groups, 1 and 2. All students were given the same pre-test, followed by a study period and then were issued post-tests. The study material for group 1 consisted of an axial brain section labeled in accordance with the PSLM protocol. Group 2 was issued the identical photograph labeled randomly. Both groups were instructed to "list and recognize the parts/layers of a transverse brain section, from superficial to deep" by writing their answers in spaces provided as one of two post-tests. The second post-test (multiple choice) stressed the adjacent relationship of those same structures. Group 2 was then issued the identical study guide distributed to group 1 initially, and thereafter, a set of post tests. These studies have shown that the PSLM significantly increased a student's learning and retention rates as well as comprehension of adjacent structures. Statistical analysis shows a significant difference in group 1 and group 2 on both the written identification of structures and similar questions in a multiple choice format. PSLM in both photographs from The Visible Human project and the photographs from plastinated sections appear to equally accomplish the following: learning and retention rates increase. Relationship of features are comprehended more rapidly and completely. Well-organized student study system is advanced.

A Modified Technique of Plastination in Medical School of Isfahan. ***Esfandiary*** *E. Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran.*

In this method, specimens were fixed by immersion in a 10% formaldehyde solution for 6 months and then were dehydrated by placement in acetone for 20 days at room temperature. A new polymer mixed with a hardener and an accelerator was added for impregnation. After impregnation was complete, the specimen (a human larynx) was cured only by waiting a few days for drying. In this method, impregnation and curing stages were integrated. The plastinated specimen was soft rather than hard.

Plastination and Self-directed Learning, an Integrated Approach to Gross Anatomy. ***Easteal RA, Lyons GW, Pang SC.*** *Department of Anatomy and Cell Biology, Queen's University, Kingstom, Ontario, Canada.*

For about a decade, Queen's University Anatomy Department has extensively used plastination in the teaching of Gross and Neuro Anatomy. Due to the increase in enrollment in theses courses, we have found it necessary to incorporate self-directed learning (SDL) into our teaching methodology. We believed at the outset that SDL would not disadvantage the learning process. We also believed that SDL would be enhanced by the user-friendly nature of plastinated specimens. Gross Anatomy at Queen's is taught to undergraduate students in two half-courses, Musculoskeletal Anatomy in one term and Visceral Anatomy in the second. Each course has an enrollment of 250 students. We have a museum with 800 specimens in jars, 400-500 plastinated specimens and 200 models as well as 12 dissecting tables. To test the hypothesis that SDL did not disadvantage the learning process, a group of 40 students was randomly selected for each course. These students were not allowed to participate in regular laboratory work and were given no formal laboratory teaching. They were allowed to attend lectures and were given a brief orientation at the start of each course. These then were the SDL groups. Examination results were tabulated and analyzed. The mean marks for the

SDL students were not statistically different although the range and standard deviations were greater. The results indicated that SDL does not adversely affect the majority of student, benefits motivated students and perhaps disadvantages the less motivated ones.

Plastination in Medical Teaching. *Beat Riederer. IBCM, Faculte de Medecine, Lausanne, Switzerland.*

In the search of new tools for teaching human gross anatomy, the introduction of plastinated specimens proved a valuable addition. This is even more important when body donations are scarce or when the number of cadavers for dissections are limited. Preparation of specimens for demonstration purposes are time consuming and when specimens are also used in practical courses they need to be robust and sustain an inexperienced handling by students. Delicate tissues such as nervous tissue suffers most and advantages of tissue plastination are obvious - tissues become by a polymer impregnation more robust, inoffensive and odorless and are preserved permanently. Here, I would like to report our experiences in plastinating brain samples for a Neuroscience course and making them accessible on our WEB site. During a course, brains are cut in front of the students and regions are identified. The number of intact brains are limited, therefore individual hemispheres and brain slices were selected for plastination by the standard method in order to introduce them in following courses. In short, tissues were dehydrated with alcohol, followed by acetone, and Silicone S10 was introduced by a forced impregnation under vacuum. Curing was done with S6. Surfaces were polished and scanned with a regular scanner. Image files were treated by Adobe Photoshop and prepared by Adobe Image Styler for a transfer on the WEB. The images were used without labeling or with selected structures indicated. We use an 02 workstation, with silicone graphics and a Fast Track 2.01 server program. When preparing a WEB site, it seems essential to have a clear concept of how to organize the site and to facilitate moving from one page to another. In conclusion, advantages of tissue plastination are two-fold. Firstly, brain tissues are less abused and reusable. Secondly, students can now use the plastinated samples during the course and later consult our WEB site to revise and consolidate what they have learned. However, plastinated tissue samples and setting up a WEB site will not replace lectures or practical courses but rather supplement and enrich the traditional teaching. So far we have a very positive response from the students. Making plastinated and digitalized material available on the WEB is definitively an avenue that is worth being elaborated.

Liliequist's Membrane is a Fold of the Arachnoid Mater: A Study with sheet Plastination and Scanning Electron Microscopy. ***Zhang M, An P-C.*** *Department of Anatomy and Structural Biology, University of Otago, Dundin, New Zealand.*

The subarachnoid space consists of a number of subarachnoid cisterns. They are separated from each other by incomplete arachnoid walls with openings of various sizes or complete walls without openings. It has never been investigated whether these two types of the walls have the same property. Liliequist's membrane is an arachnoid wall in the basilar cisterns. Descriptions of its attachments, subdivisions and relationship with surrounding structures are very conflicting. This study, using the modified E12 sheet plastination method and scanning electron microscopy, investigated the property of Liliequist's membrane. Thirty-eight cadavers were used, 3 for the plastination and 35 for the gross anatomy dissection, two of which were further examined under the scanning electron microscope. The results indicate (i) Liliequist's membrane is an avascular fold of the arachnoid mater; (ii) the carotid-chiasmatic walls, which separate the chiasmatic cistern and carotid cisterns and had been considered to be parts of Liliesquit's membrane, are vascular and incomplete trabecular walls, have a close relationship with the perforating arteries and should not be considered as a part of Liliequist's membrane, and (iii) Liliequist's membrane does not directly attach on temporal lobes and oculomotor nerves.

A Series of S10 Plastinated Specimens for the Teaching of Human Neuroanatomy. ***Grondin G, Olry R.*** *Deparement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada.*

Plastination is known to be a very useful teaching aid in human and comparative neuroanatomy. Although the polyester techniques (P35, P40) are acknowledged as the best methods to contrast gray and white matters, they are not the easiest plastination techniques, and new plastinators usually start their laboratory with the S10 procedure. This presentation aims at showing the potential of S10 plastinated specimens (human central nervous system with meninges and vessels) in the teaching of human neuroanatomy.

Surgical Anatomy of the Infratemporal Preauricular Approach for Skull Base Tumors. ***Prades JM, Timochenko A, Guillin G, Durand M, Martin C.*** *Laboratory of Anatomy, Department of Otorhinolaringlogy, Head and Neck Surgery, Bellevue Hospital, University of Saint-Etienne, France.*

The infratemporal fossa, ITF, can be defined as the area under the floor of the middle cranial fossa. The

styloid diaphragm divides the ITF into the prestyloid region (PSR) and the retrostyloid region (RSR). The PSR contains the parotid gland, the facial nerve (VII) and the mandibular nerve (V3). The retrostyloid region (RSR) gives passage to the internal carotid artery and the internal jugular vein. The aim of this study is to photographically demonstrate the surgical technique of combined transmandibular, transzygomatic infra-temporal fossa approach using a plastinated specimen. Six adult cadaver heads were dissected by using magnification of 6x to 40x. Colored latex was injected into the arteries. A teaching plastination specimen was obtained using the S10 technique. The cutaneous preauricular incision is curved below to the angle of the mandible extended superiorly in a frontotemporal scalp. The temporal fascia is elevated for the muscle and protected the frontotemporal branch of the facial nerve (VII). The zygomatic arch is removed and the temporalis muscle is transected inferiorly which makes superior reflection of the muscle possible. The ramus of the mandible is removed. The mandibular nerve and the maxillary artery are dissected. A general view of combined superior and inferior approach to the ITF is possible. The appropriate surgical approach to ITF should provide maximum exposure with minimal morbidity so as to preserve the quality of life. The combined transmandibular, transzygomatic infra-temporal fossa approach appears as a versatile procedure for wide access to ITF when operating skull base high risk lesions.

E12 Sheet Plastination of Isolated Human Aorta: Preparation for the Qualitative and Quantitative Study of the Aortic Wall. ***Barnett R, Quennell JH, Zhang M.*** *Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand.*

Different parts of the aorta have different embryological origins, e.g. the ascending, arch and descending aorta deriving from the endothelial heart tube, aortic roots and paired endothelial vessels, respectively. There is little study on the comparison of the basic components of the aortic wall between its different parts. In this study, we reported a method which allowed us to random-systematically sample sets of slices from an isolated aorta. One set of the slices was prepared by E12 sheet plastination technique and the adjacent set of the slices was used for routine histology or immunohistochemistry study. Three adult cadavers were used. After the aorta was removed from the body and embedded in gelatin, ten to twelve 2.5 mm thick sections were selected by random systematic sampling and cut from each gelatin block. The sections were plastinated with E12 technique as previously described. After hardening, each slice was trimmed to a size which can fit on the mobile stage of the confocal microscope. This preparation enabled us to qualitatively and quantitatively investigate the fine architecture of aortic wall at both macroscopic and microscopic levels and has the potential to study a structure at molecular level.

Anatomy of Synovial Sheaths in the Talocrural Region Evaluated by Plastination. *Windisch* ***G, Weiglein*** *AH. Institute of Anatomy, Karl-Franzens-University, Graz, Austria.*

Tendon synovial sheaths are usually described as closed double-walled cylinders lying around tendons when they pass under ligamentous bands, retinaculae, through fascial slings or osseofibrous tunnels. The various tendons in the talocrural region are said to be enclosed in synovial sheaths to minimize friction of the tendons against the bones. However, indeed the tendons crossing the talocrural joint are deflected from a straight course, and are hence held down by retinacula. Thus, the friction occurs in between the tendons and the retinacula. Infiltration of the talocrural tendon sheaths was followed by plastination by both the S 10 and the E 12 procedure. Tendons with infiltrated synovial sheaths were plastinated with silicone. Whole ankle joints with the infiltrated synovial sheaths were cut slices of 2 cm thickness and plastinated with epoxy resin. After curing the specimen was sliced into 200 µm thick slices by means of a diamond wire saw. The results show that the synovial sheaths in the talocrural region are not double walled cylinders. They are just developed in between the tendons and the retinacula, thus more or less representing a long stretched bursa, which minimized the friction in between tendons and retinaculae.

Valves are Abundant in Small Superficial Veins of the Lower Limb. ***Phillips MN[1]*[2], Zhang M[1], van Rij Aftfl.*** *1 Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand. ^Department of Medicine & Surgery, University of Otago, Dundin, New Zealand.*

Venous valves are important for the prevention of blood reflux in veins. This is especially true for the veins of the lower limb where the effect of gravity is the greatest. Venous valves in large superficial veins of the lower limb have been extensively studied. Very few studies have investigated the valves in the intermediate and small superficial veins of the human lower limb. Common anatomical texts state that no valves are present in veins of smaller than 2 mm in diameter. In this study, using microdissection, E12 sheet plastination, and resin scanning electron microscopy, venous valves have been identified in small superficial

veins and venules as small as l0microm in diameter in the subcutaneous tissue of five human lower limbs. Sixty-eight percent of the valves (808/1190) were in postcapillary venules (10-50µm in internal diameter) and collecting venules (51-150µm). Using resin cast in 16 sites (2x2cm), the mean density of valves in veins smaller than lmm in diameter was 10.67±4.1 per cm^3. The presence of valves in small veins and venules implies a mechanism for the forward propulsion of blood in these veins.

The Confocal Microscopy and El 2 Sheet Plastination. *Phillips MN, Burnett R, Zhang M. Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand.*

For the past 20 years, plastination of human specimens has been used for anatomy teaching and research. This paper demonstrates a technique for the combination of E12 sheet plastination and confocal laser scanning microscopy (CLSM). Three formalin fixed cadavers were prepared for this investigation. The blood vessels were stained by the injection of Gill's Haematoxylin #1 into the femoral artery. The skin flaps of the lower limbs were removed and processed for E12 sheet plastination. The resulting plastinated specimens were viewed using conventional light microscopy and CLSM. The results from this modified technique of El2 sheet plastination, along with the use of CLSM, show that clear two-dimensional autofluorescence images are obtainable from the El2 plastinated sections. The CLSM allows visualization of very sharp optical sections through the plastinated specimen due to the autofluorescence of collagen. CLSM in conjunction with conventional light microscopy imaging of the stained blood vessels has enabled very clear detailed inspection of the vascular and microvascular structures. This procedure holds possibilities for further research into areas of anatomy.

Forced Impregnation in our Laboratory. *Meiko Taguchi. Department of Anatomy, Allied Health Sciences, Kitasato Japan.*

The S10 standard technique of plastination is a method that is easier than the other plastination technique and is suitable to preserve all types of specimens we usually use in our practice for co-medical students. The method is carried out in four steps: fixation, dehydration, forced impregnation and curing. In our laboratory, three steps except forced impregnation, are carried out according to the standard method, i.e. fixation with formaldehyde, dehydration with acetone at -25°C and gas cure with the Biodur S6. In the normal forced impregnation, when the manometer reads under 8mm Hg and bubbles had stopped, it is considered that acetone has been completely replaced by silicone. However, our equipment does not permit us to reduce the pressure to a high degree. Therefore, we tried to impregnate by decreasing the pressure very slowly and by observing the bubbling down to 100mm Hg. We tried this procedure using a fetal pig (about 20cm long from the head to the hip and about 20cm of ventral girth). After enough curing of the surface, the whole body of the pig was cut into halves by median longitudinal plane. Next, the cut surface was cured again. It came out that by means of this forced impregnation method, a specimen of this size was impregnated.

Plastination of Non-Human Tissues. *Weninger B, Weiglein AH. Institute of Anatomy, Karl Franzens University, Graz, Austria.*

Plastination is not only a method of preservation for human tissue, but also a method of preserving other biological tissues. In plastinating other than human tissues, however, we encountered several tissue-specific problems. Plastination of plants (Bellis perennis, Ficus elastica, Galanthus nivalis, Primula elatior) and fruits (Citrus limon) was difficult to accomplish because of two specific problems. 1) Loss of color: Particularly green plants loose lots of chlorophyll when submerged in room temperature acetone. This process can be minimized but not completely stopped in cold acetone (-25°C). 2) Penetration of polymer: The cuticula - the wax cover of these tissues - was difficult or even impossible to be penetrated with silicone S10. This problem was partially solved by submerging the fruits into acetone at room temperature after dehydration as is done for defatting human tissue and by cutting them. Plastination of mushrooms: 1) Color loss was a minor problem and solved by using cold acetone only for dehydration. Plastination of fish, lobster, slugs: l)This was easy to accomplish by the above mentioned methods.

Plastination of Nervous Tissue. *Weiglein AH. Institute of Anatomy, Karl Franzens University, Graz, Austria.*

For teaching neuroanatomy we produce both brain prosections and brain slices. Brain prosections, as well as whole and half brains are plastinated with silicone rubber. For the Biodur S10 technique, we use a slightly changed protocol of the S10 standard procedure. Before starting the forced impregnation, we add an immersion period. During this immersion step, the brains are immersed in the S10/S3 mixture for several days at -20°C. The longer the immersion time, the shorter the time for impregnation. Moreover, this helps to minimize the shrinkage of the brains. For silicone brain slices, we cut the brains into - Continued on p 47

slices of the desired thickness after curing is completed. For cutting, we use a band saw or for very thin slices (down to 100 microm) a diamond wire saw or a diamond band saw. This results in smooth surfaces of the slices and thus slices are exactly adjacent to each other. Brain slices are plastinated with polyester (Biodur P35) because the differentiation between cortex, nuclei and fiber tracts is superior to all other techniques. The thickness of the slices can vary between 4 and 8 mm. The polyester-procedure consists of the following steps: Fixation - Slicing - Flushing - Dehydration - Immersion - Forced Impregnation - Casting - Curing. For curing P35 brain slices a well ventilated heat cabinet is needed. After a short light curing period P35 slices are finally cured at 40 - 50 °C.

A Simple Acetone Distillation Apparatus at Nancy Medicine Faculty, France. ***Arnoux JM, Braun M, Gombar E, Lascombes E.*** *Faculte de Medecine Nancy I, Nancy, France.*

Acetone cost, stock and elimination process are three majors drawbacks of the plastination procedure. Two of these may be dramatically reduced by recycling the old acetone produced by the dehydration process. We present a distillation procedure commonly used in the industry and devoted to acetone and alcohols.

Development and use of Plastination as a New Education Method for Secondary Schools, Universities and Postgraduate Teaching Centers of Murcia (Spain). ***Latorre R, Vazquez JM, Gil F, Ramirez G, Lopez-Albors O, Moreno F.*** *Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain.*

The aim of this project (0075/CV/99) is to transfer and spread the plastination of biological specimens as a new educative technology in secondary schools, universities and postgraduate teaching centers of Murcia. Most of the biological specimens (organs) for teaching in different sciences have to be preserved currently in fixative liquids (formaldehyde), which are toxic and have several limitations of use and manipulation. Plastination techniques (S-10) are, essentially, a vacuum process where all original tissue fluids are slowly extracted and replaced by special curable polymers. This process permits one to obtain biological specimens in a completely real, clean, dry and permanent state. Beside, this material can be handled and examined without gloves and do not need any special treatment or conditions of storage. Moreover, the use of these plastinated specimens prevent the daily exposure of teachers and students to toxic products because they are odorless and free of toxic products such as formaldehyde, phenol and alcohol. The centers of private and public teaching which are involved in the project will work as Testing-centers where these techniques will be developed and applied. They will also contribute to spread the results to other educational centers. This project will result in an advantageous situation for the students since this technology will clearly increase the quality of their practical lectures in Biology, Anatomy, Pathology, Surgery, Ophthalmology, Radiology, etc. We also try to improve the background of postgraduates in Medicine, Veterinary, Biology, etc. Thus, many studies have demonstrated the validity of the use of plastinated material to improve the knowledge in diagnostic imaging techniques such as endoscopy, arthroscopy, echografy, magnetic resonance, computed tomography, etc. This work was supported by the project: 0075/CV/99 (C.A.R.M.).

Relationship of Female Dog Reproductive Organs with Other Anatomical Structures. A Study by Plastinated Specimens. ***Latorre R, Gil F, Moreno F, Lopez-Albors O, Arencibia A, Orenes M.*** *Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain.*

A dog specimen of organa genitalia feminina (ovarium, uterus, vagina and vestibulum vaginae) was removed from the carcass with the organa urinaria (ren, ureter, vesica urinaria, urethra feminina), peritoneum parietale, aorta abdominalis, aa. iliacas, vena cava caudalis, etc. Each ovarium was suspended within the lig. suspensorium ovarii, cranial part of the mesovarium. The specimen was submerged two weeks in formaldehyde solution at room temperature. Dehydration took three weeks in cold acetone and three weeks into room temperature acetone to removal of lipids. The preparation was preserved attending the S10 technique (Von Hagens, 1987). The vessels were injected with color before removing the preparation. The final specimen permits one to understand the normal position and relation of bitch organa genitale feminina with the others organs or important vessels as: a. renalis, a. ovarica, a. mesenterica caudalis, a. circumflexa ilium profunda, a. pudenda interna, v. renal, v. ovarica sinistra, v. ovarica dextra etc. The different portions of lig. latum uteri and its relation with the excavatio rectogenitalis and the excavation vesicogenitalis were also visible. This work was supported by the project: 0075/CV/99 (C.A.R.M.).

The Stomach of the Ruminant (Goat). A Precise View of Internal Anatomy through Plastinated Specimens. ***Latorre R, Vazquez JM, Gil F, Ramirez G, Arencibia A, Orenes M.*** *Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain.* Three adult goat stomachs were taken from recently

euthanized animals and flushed with water until all contents were removed. The specimens were dilated with a 10% formaldehyde solution and submerged two days in a 2% formaldehyde solution at room temperature. Dehydration of specimens took three weeks in cold acetone and three weeks in room temperature acetone for removal of lipids. Two specimens were impregnated at cold temperature with S10/S3 mixture (Biodur) and the last one with COR-TECH PR10/CT22 mixture (Corcoran) at room temperature. The quality of the material and differentiation of the anatomical structures were quite optimum. These specimens gave to students and veterinary professionals a clear and accurate overview of the extension, limits and relationships of anatomically complex structures as the communication of the rumen and reticulum with the esophagus and omasum through the sulcus reticuli (labium dextrum and labium sinistrum); even the papillae unguiculiformes in the ostium reticulo-omasicum. These specimens showed different internal anatomical structures at the rumen. It was easy to identify the papillae ruminis at the tunica mucosa, ruminal pillars: pila longitudinalis dextra, pila longitudinalis sinistra, pila cornaria dorsalis and pila cornaria ventralis dividing saccus dorsalis and saccus ventralis (dorsal and ventral major sacs) or the saccus cecus caudodorsalis and saccus cecus caudoventralis (caudal blind sacs). The communication between rumen and reticulum (ostium ruminoreticulare) was also open over the plica ruminoreticularis (ruminoreticular fold). The tunica mucosa had a precise aspect also in reticulum: cellula reticuli, crista reticuli, papillae reticuli; omasum: papilla omasi, lamina omasi, recessus interlaminares; and abomasum: plicae spirales abomasi, vela abomasica. Students are using them in the practical lectures with high didactic success. This work was supported by the project: 0075/CV/99 (C.A.R.M.). **Plastinated Anatomical Specimens Applied in Medical Teaching.** ***Wang H[j], Liu fi.*** [jJ] *Department of anatomy, Nanjing Medical University, Nanjing, China, 2 Nanjing Su Yi Plastination Factory, Nanjing, China.*

Anatomy is an important fundamental course and the specimens are the safeguard to improve teaching quality. But for a long time the specimens were fixed and preserved by a traditional method - formalin that was a notorious poisonous material. So plastination is an iconoclastic and new method for preservation of anatomical specimens. In the last two years, we, with Nanjing Su Yi plastination factory, have successfully made 1000 plastinated anatomical specimens and applied these in the practice of anatomical teaching. A good effect was obtained. The advantage as fowling as: 1. It is propitious to carry to a new and higher level of anatomical teaching. Because the specimens are innocuous and odorless, students are more likely to look at and feel these specimens. Undoubtedly, they more deeply understand the structure of human body and the form of various organs. 2. It is propitious to assure the health of the teachers and students. If one comes into contact with formalin over a long period of time, he may develop illness of the respiratory or digestive system and so on. When the plastinated specimens are used, the unhealthy affects of formalin will be avoided. 3. It is propitious to protect environment.

A Comparison between Epoxy Resin and Histological Sections in the Study of Spinal Connective Tissue Structure. ***Johnson G, Zhang M, Barnett R.*** *Department of Anatomy & Structural Biology, University of Otago, Dundin, New Zealand.*

A comparative study of spinal connective tissue and its structural arrangement using tissue preserved in epoxy resin plastinated slices and paraffin embedded sections was carried out. The results showed that the 2.5mm horizontal plastinated slices have the advantages of providing a more complete overview of connective tissue arrangement in the spine including fiber arrangement, bony attachment sites and continuity with the neighboring structures compared with 7μm paraffin embedded standard histology sections. Distinctive macroscopic definition of connective tissue fiber arrangement of spinal tendons, ligaments and fascia was obtainable in the plastinated sections. The quality of information obtained regarding connective tissue arrangement makes use of epoxy plastinated sections an attractive alternative to conventional histology in spinal research on selected dense connective tissue structures.

Surgical Anatomy of Combined Transmandibular Transzygomatic Infratemporal Fossa Approach for Skull Base Tumors. ***Prades JM, Timochenko A, Durand M, Martin C.*** *Laboratory of Anatomy, Department of Otorhinolaringlogy, Head and Neck Surgery, Bellevue Hospital, University of Saint-Etienne, France.*

The infratemporal fossa (ITF) can be defined as the area under the floor of the middle cranial fossa. The ITF is limited anteriorly by the maxillary tuberosity, superiorly by the greater wing of the sphenoid bone and a part of the squamous temporal bone, medially by the lateral pterygoid plate and the lateral wall of the pharynx, laterally by the skin covering the parotid gland, the zygomatic arch, the masseter muscle and the mandible, inferiorly by the horizontal plane passing through the inferior border of the angle of the mandible, posteriorly by the cervical prevertebral fascia. The

styloid diaphragm divides the ITF into the prestyloid region (PSR) and the retrostyloid region (RSR). The PSR contains the parotid gland, the facial nerve, the terminal branches of the external carotid artery. The RSR contains major vascular structures as internal carotid artery, internal jugular vein and the initial exo-cranial portion of the lower cranial nerves because of its concealed localization, tumors of ITF, primary tumors or contiguous tumors may remain unnoticed for some time. Modern imaging techniques are of the great help for careful planning of surgical excision. The appropriate surgical approach will provide maximum exposure with minimal morbidity so as to preserve the quality of life. There are a variety of surgical approaches to the ITF: the lateral trans-mandibular approach was described by JJ Conley (1956) and the transzygomatic approach by FJ Barbosa (1961). LN Sekhar (1987) advocated subtemporal preauricular infra-temporal fossa approach to large, lateral and posterior cranial base neoplasms. The aim of the study is to photographically demonstrate the surgical technique of combined trans-mandibular, transzygomatic infra-temporal fossa approach. As large skull base lesions involving ITF are treated with more aggressive surgical therapy, refinement of the approaches to this region is inevitable. However with versatile resection of mandibular ramus and zygomatic arch combined transmandibular, trans-zygomatic infra temporal fossa approach provide excellent visualization of the whole extracranial ITF with good superior and inferior facial nerve preservation. This combined approach appears as a versatile procedure for wide access to the ITF.

Anatomical Landmarks of **Ethmoidal Labyrinths.** *Prades JM, Veyret C, Durand M, Martin C. Laboratory of Anatomy, Department of Otorhinolaringlogy, Head and Neck Surgery, Bellevue Hospital, University of Saint-Etienne, France.*

A complete description of the fundamental structures of the ethmoid was given by anatomists at the beginning of this century, notably by E. Zukerkandl (1895) and J. Mouret (1922). The present quality of CT scan and the advent of endoscopic surgery account for the rediscovery of this anatomy. Despite this modern ethmoidectomy by endonasal route under endoscopic monitoring may give rise to major complications. Cerebrospinal fluid leak, intra cranial or orbital hematoma, direct lesion of the optic nerve, severe nasal bleeding, nasolacrimal duct stenosis. Variation of ethmoidal pneumatization constitutes one of the important CT scan findings before operating. Surgical advance must be guided by constant landmarks. CT scan and endoscopy permit optimal analysis of the constant anatomical landmarks in the ethmoidal labyrinth. These are: The root of attachments of the middle nasal concha and the superior wall of the ethmoidal labyrinth. Uncinate process and ethmoidal bulla. The septal root of the middle nasal concha. The superior wall, properly so-called, of the rhomboidal labyrinth, situated in continuity with that of the sphenoidal sinus.

The Necessity of **Plastination** in **the Education of** Medical Sciences in France. *Sheibanifar M. Rennes, France.*

Plastination is a unique method of preserving tissue in a lifelike state. These specimens are dry, odorless, durable, last indefinitely and can literally be grasped. By this way, plastinated specimens can be used and studied everywhere. But, in spite of this fact, some of the professors and students do not believe in that. In their ideas, it limits the field of activity for the students and students should feel and dissect the real specimens. To make a good judgment, I will try to explain the broad spectrum of the applications of plastination in the medical sciences as following: 1. Anatomical sciences (including micro- and macro-anatomy): It's the main field of plastination. But the most important point is that plastination does not replace the dissection. In fact, it acts as a complement for the students to learn the anatomical sciences much better. Additionally, it can open the new horizons in the anatomical researches, especially in the field of embryology and also sectional anatomy. 2. Clinical sciences (especially the different branches of surgery): In the hospitals, when the residents and interns want to review their knowledge of anatomy or when a physician wants to remind them the anatomy of a special region, they use books, atlases, slides and probably plastic specimens. By this way, there is always a gap between clinical sciences and anatomy, as anatomy is one of the most important parts of basic sciences. But plastination can fill this gap and brings in touch the natural specimens in the hospitals without any problem. 3. Radiology (especially CT and MRI): There is the same problem in this section but it's more prominent here, that's to say, the interpretation of the images of CT and MRI needs a good knowledge of sectional anatomy. Here, it's sheet plastination that can facilitate and improve the process of residency training thanks to the transparent and opaque slices of any size. As the final conclusion, I should say that plastination is a real necessity of education not only of anatomy but also of medicine as a whole. Unfortunately in France, only a few faculties of medicine are active in this field. A more detailed discussion is given in the original paper.

The use of Dog Complete Gastrointestinal Tracts to Teach the Basic External and Internal Anatomy Necessary **for Flexible Endoscopic Training.** ***Latorre R, Uson J, Climent S, Sanchez-Margallo F, Vazquez JM, Gil F, Moreno F. 1*** *Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain. 2 Centre de Cirugia de Minima Invasion, Universidad de Extremadura, Cdceres, Spain.*
The aim of this experience was to evaluate the use of plastinated specimens during postgraduate courses about flexible digestive endoscopic training in dogs. Three complete gastrointestinal tracts with the arteries injected (red latex), omentum majus and minus, lien, etc. were prepared to be used for endoscopy, attending the S10 technique (Henry, 1997). Also, esophagus-ventriculus (stomach)-duodenum pieces and rectum-colon descendens-colon transversum - colon ascendens-cecum- ileum pieces were used. These specimens were used in the Minimally Invasive Surgery Center (Caceres, Spain) during three courses for postgraduate students about digestive flexible endoscopic technique. The results show the utility of these specimens to understand the normal endoscopic anatomy of the gastrointestinal tract (gastroduodenoscopy and colonoscopy). Their use to study the relation between endoscopic anatomy and external anatomy was perfect. The clinic students used the plastinated specimens to train for the standard manipulation of endoscopes during a normal gastrointestinal inspection. They understood perfectly why they need to move the endoscope in each part of gastrointestinal tract and what were the normal movements in the retroflexion etc. This work was supported by the project: 0075/CV/99 (C.A.R.M.)

Shrinkage During El 2 Plastination. *Sora M-C[1], **Brugger P*, Traxler H[2] Bareck J. 1** Department of Anatomy 2, Anatomical Institute, Vienna University, Austria. 2 Department of Anatomy 1, Anatomical Institute, Vienna University, Austria.*

The goal of this study was to determine the shrinkage rate, which occurs during E12 plastination. Fresh human pelvis slices, 3.5mm thick, were digitalized and then area measurements were done. After marking the slices, they were processed using the standard E12 plastination procedure. Special measurements were done after each plastination step. The measurements were determined by using the IMAGE TOOL v. 2.0 software. By comparing the data obtained, we were able to determine the shrinkage rate of the slices in each plastination step, but also the general shrinkage rate, which occurred after El2 plastination.

Toxicity of Plasticizers on Osteogenisis of Mice Skeleton. ***Abdel Malek AK, Shehata R, Hassan S, Abdel Aziz H.***

Sixty-six pregnant mice were classified into 11 groups and were given a single oral dose of phtalate ester (plasticizer) for each member on one of the days from 6-16 of pregnancy. Group 12, pregnant mice, were given olive oil and used as control. Newborn mice were examined using Alizarin red stain. Newborn mice of treated mothers showed defective ossification of the skull bones, defective ossification of the cervical and caudal vertebrae, and delayed ossification of bones of the fore and hind limbs. Ossification defects were most pronounced in newborns of pregnant mice which received phtalate esters during the period from 6-8 days of pregnancy. The most sensitive day for toxicity was the 7th day. This period is most probably the critical period of skeletal genesis in mice.

Cat Central Nervous System and Circle of Willis Demonstrated by the S10 Technique. ***Joghatai** MT 1, **Asadi MH, Negahdar F.** *Iran University of Medical Sciences, Tehran. Iran, Baghiatallah University, Tehran, Iran.*

In this paper, the complete procedure for producing 3D models of the cat CNS and circle of Willis will be stated. The cats were fixed by perfusion of 5% formaldehyde for fixation. After removal of the calvaria, the brains and spinal cord dissections were begun from the upper end of the vertebral column to its lower part. During the dissection, we tried to save most of the peripheral nerves. Dehydration was performed by the freeze substitution method. Specimens were submerged in a mixture of S10/S3 (100:1) for 24 hours and then were forced impregnated at -25°C for five weeks. The pressure was slowly decreased down to 5mm of Hg. They were precured for two weeks after removing of the excess of polymer from the specimen surfaces. Finally, in the gas curing stage, they were exposed to S6 vapors for three days at normal room temperature. The produced 3D models can be used as useful pedagogical instruments to help university students in various majors to understand the nature of the CNS.