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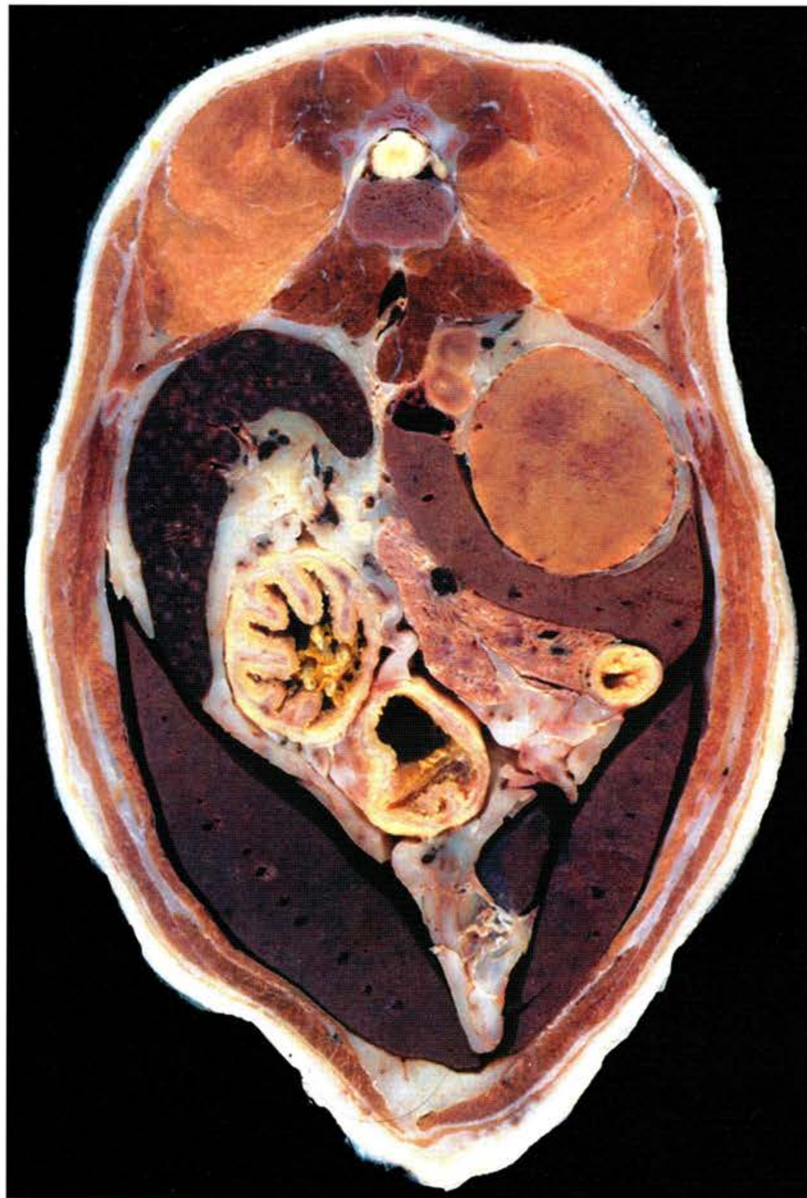
journal of the INTERNATIONAL SOCIETY for PLASTINATION



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International Society
for **Plastination**

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Minutes of the 9th Biennial Meeting of the International Society for Plastination Murcia, Spain, 16 July 2004

Call to Order:

The 9th Biennial Meeting of the society was called to order at 11:15 by President Andreas H. Weiglein. With the absence of the secretary, B. Weninger was appointed and approved to record the minutes. A quorum of 29 members was established. The agenda was presented, moved, seconded and approved.

Minutes of the 2002 Meeting: as printed in the 2002 ISP journal were moved (Sora) to be accepted as published, seconded and passed.

Report of the Officers:

President's Report:

The meeting in San Juan, although nice, was overshadowed by a near financial disaster caused by a fine of $18000, which was averted due to negotiations by the president and treasurer. Thus the congress broke even.

With Robert Reed and Robert Henry as editors, the journals quality has been improved.

October, 2002, a conference planning committee (R. Latorre, R. Henry) was appointed. Conference guidelines have been updated.

January, 2004 G. Guttmann chaired the nominating committee. There was only one application for each post.

Weiglein announced that after 8 years of a stable presidency, it was time for some fresh air. He will assist the new president in his new post as vice president. He gave his thanks to the society and especially to R. Henry for helping to resolve past problems.

Treasurer's Report:

Balance 1/1/03: $25,433.08

Income 1/1/03 to 6/30/04: $7,861.97

Expenses 1/1/03 to 6/30/04: $3,455.89

Net income 1/1/03 to 6/30/04: $4,406.08

Balance 6/30/04: $29,839.16 - unpaid expenses of $2,000, anticipated balance 12/31/04 $27,839.16

Proposal to change the accounting basis to a fiscal year basis: moved, seconded and accepted.

Proposal to keep two accounts (Graz for Europe and Tennessee): moved, seconded and accepted.

Acceptance of Treasurer's report: moved (Sora), seconded and accepted.

Reports of the Committees:

Local Organizing Committee:

R. Latorre reported: 88 congress participants from 25 nations, 29 were ISP members. There were 77 presentations (17 conference and 29 oral presentations, 31 posters and 6 exhibitions). Seventeen participants from 14 nations took part in the precongress plastination workshop.

Membership Committee:

M.C. Sora reported: a decline in society membership from 140 (2001) to 100 (2003). The list server has more members (225). He encouraged becoming a society member especially with such a reasonable biennial fee ($75).

Journal Committee:

In the absence of Editor in Chief R. Reed, R. Henry reported: updating guidelines to give the journal a more scientific appeal. Authors were encouraged to cite all relevant publications from the journal to increase the number of impact points of our journal. New reviewers are needed and encouraged to nominate themselves. Standards achieved up to now will be kept. Authors are encouraged to submit papers for further issues.

Bylaws Committee:

R. Henry reported: updated bylaws include electronic notification of important issues by e-mail (for those who have an address) or by post (for those who don't). A second notification will be in postal form, if no answer is received. E-mail ballots will be used by the nomination committee. Information will be published on the list-server. Members were encouraged to respond to information sent out to them.

Conference Committee:

A.H. Weiglein reported: members prefer universities as conference sites over hotels. It was suggested that the conference fee should not exceed $350 and accomodations should be offered in various price categories (with

$50 to $70 as a standard). The Interim Meeting 2003 in Colombia was canceled due to difficulties in the country. A letter of apology by the local organizers was read and apologies accepted.

Nominations Committee:

In the absence of Geoff Guttmann, the report was given by A.H. Weiglein:

1. Nominations for officer started in January 2004. Since there was only one nomination for each position, there was no need for election and the executive would be confirmed by acclaimation. The nominations are as follows:

President: Mircea-Constantin Sora, Vice President: Andreas H. Weiglein, Treasurer: Robert Henry, Secretary: David Hostler

2. ISP members are invited to the Interim Meeting 2005 in Ohrid, Macedonia hosted by Dr. Vlatko Ilieski, Dean of the Faculty of Veterinary Medicine.

3. The next Biennial Meeting 2006 will be hosted by Mircea-Constantin Sora in Vienna.

Announcements:

There have been no proposals to host the 2008 Biennial Meeting. Members were encouraged to apply for hosting the conference.

The death of Carlos Kordjian, Australia was reported and a standing, moment of silence was observed in his memory.

The first **ISP Travel Award** was presented to: Lazo Pendovski, V Ilieski and G Nikolovski, Department of Anatomy, Faculty of Veterinary medicine-Skopje, Macedonia for their presentation "Green iguana plastination with the S 10 technique in Macedonia".

Old Business: None

New Business:

A updating of the journal's website was proposed. Riederer: suggested having articles online as in PubMed. Henry: suggested that it should be done in a professional manner. Sora: mentioned that the homepage was currently located in Canada with Grondin, with the archives at his disposition. A potential problem: adequate server space. Sora will contact Grondin and clarify this matter.

Henry: suggested publishing an issue of the journal dedicated entirely to the different plastination techniques, to have a standard procedure for new plastinators to use. Riederer: each method should be dealt with by a group of authors and the booklet should contain tips and tricks not included in normal protocols. Weiglein: mentioned a booklet he had prepared for a plastination workshop in Macedonia in 2004 which had at least 2 different authors for each technique. Zhang: such an issue would be cited often and would increase the impact factor of the journal. Sora: the standardized procedures be accessible in pdf-format on the homepage after publication in the journal. For each technique, specialists should be appointed and mentioned on the homepage as such, which would make it easier for outsiders and beginners to get information. De Jong: will access to the protocols on the net be limited to members only. Weiglein: the standardized protocols to be published first before putting them on the net.

Henry: mentions the emeritus statute in the bylaws - is it necessary to have such a status and how to decide to whom it should be awarded. Sora: suggests retired members should apply for emeritus status and the executive council should decide on the matter. Henry: Emeritus status should only be available for members of the society in good standing.

Zhang: suggests that plastination techniques should be promoted outside the medical world, because of little understanding there. Nielsen: sees a wide sector, like education and research, where the technique could be promoted. Henry: contacts at professional societies are needed. Zhang: is there an existing strategy on the matter of promoting the techniques. Weiglein: points out that promotion of the technique is the duty of every member, but suggests that a committee for promotion of plastination would be a good idea. The proposal was moved, seconded and accepted. The new committee will be chaired by Ming Zhang who accepted the duty.

Sora: Make a world wide list of plastination labs and share experiences and help each other in producing good specimens. Weiglein: good idea.

Weiglein: extends an invitation to the 2005 meeting of EACA in Palermo, Italy, and promises to encourage the organizers to have a special session on plastination.

Adjournment:

It was moved, seconded and accepted that the meeting be adjourned at 1:00 pm on Friday, 16th of July 2004.

The 12th International Conference on Plastination

Magnificent southern Spain, Murcia, was the venue for the 12th ISP congress. Hosted by the Veterinary Faculty, at the University of Murcia, the days were filled with fellowship, new faces, aroma of delectable Mediterranean cuisine, perfect weather and most of all lasting memories and friendships. The breath taking 14th century Alhambra, likely the most beautiful fortress ever built, rising above mystic Granada, was the mid-day retreat for the Plastination Pilgrimage by the attendees in route to visit a great exhibition of plastinated specimens and other treasures in the Parque de las Ciencias, Granada. Seventy-seven scientific presentations and posters filled any eager mind. One hundred participants from 25 countries enjoyed a superb conference and were pampered by Rafa and his excellent colleagues. Likewise, the precongress workshop was a "bubbling" success. Seventeen participants from 14 countries learned silicone and polyester plastination techniques and refreshed skills. Thank you to the Murcia Veterinary Anatomy Faculty.

The 13th International Congress on Plastination

July 2 - 7, 2006, Vienna, Austria

Center for Anatomy and Cell Biology

Medical University of Vienna

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A-1090 Wien, Austria

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http://www.meduniwien.ac.at/plastination2006/start.htm

Phone: 43 1 4277 61150

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Preliminary call for abstracts

Dr. Mircea-Constantin Sora

Oral presentations - invited speakers

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

Background: After death, biological tissues decompose by autolysis or putrefaction. 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; later on, interest in morphology made it necessary to preserve human tissue in order to investigate its anatomy. The most important step in preservation was the introduction of formalin by Blum in 1896. Formalin consolidates tissue and quickly stops the decomposition processes. Disadvantages to formalin fixation include unnatural hardening and discoloration of tissues. The introduction of formalin was followed by the color-preserving embalming solutions by Kaiserling (1900) and Jores (1930). In 1992, Thiel published an article on a new method of color preservation that preserved the human body in lifelike condition (color and flexibility). In addition to the development of embalming solutions that allow preservation for dissection and particularly for surgical training and other invasive procedures, methods were developed for demonstration of human anatomy in museum specimens. In museum specimens preservation of flexibility is not essential. 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. Materials and methods: This technique utilizes both impregnation and embedding, transforming the tissues into plastic with the respective mechanical properties of the polymer. Thus, plastinated specimens are more or less inflexible. 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. Results: Silicone is used for whole specimens (organs, body parts and whole bodies) and thick body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices excellent for comparison to scans produced by medical imaging techniques. Polyester is used for brain slices to gain an excellent distinction of gray and white matter. Conclusion: Plastinated specimens are perfect for teaching because they show the real specimen, they are easy to handle, almost everlasting and need a minimum of maintenance.

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

Twenty six years ago the first publication on a process for "Impregnation of large specimens with polymers" was issued in a German journal (*Verhandlungen der Anatomischen Gesellschaft*). A year later, in 1979 an article on "Impregnation of soft biological specimens by thermosetting resins and elastomers" was published in the *Anatomical Record* and later on in 1979 the term "plastination" appeared for the first time in "Emulsifying resins for plastination" *(The Preparator)*. The first conference on Plastination was held in San Antonio, Texas in 1982. Since then plastinators from all over the world meet every even year for an international conference. Interim meetings, which are primarily workshop based and usually take place in the USA, were started in 1989 in Knoxville, Tennessee. In 1987 the first issue of "The Journal of the International Society for Plastination" was published. In 1996 the first issue of the Current Plastination Index - an index listing all publications dealing with plastination - was issued and updated in 2000. This CPI 2000 lists almost 800 publications dealing with plastination in more than 80 different journals. However, it was not before 1996 that the International Society for Plastination (ISP) was officially launched during the meeting in Graz. Since then, the ISP serves as a forum for the exchange of information about Plastination, which is accomplished by the publication of the journal, by holding conferences, meetings and workshops on a regular basis and by our web page and list server.

Principals of plastination. ***Henry RW.*** *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*
Plastination, the exchange of tissue fluid for a curable polymer, is an intriguing method for preserving biological tissue in a cost effective manner. Plastination has four basic steps: Specimen preparation, Dehydration, Impregnation and Polymerization. To get the best specimen for your effort, plan the final product before production commences. Have a road map. The raw materials (specimen) likely should undergo some major renovations during the specimen preparation stage. Do not attempt to show every feature on one specimen. Once the specimen is prepared or during preparation it may be fixed in 2 - 20% formaldehyde solution. After fixation for a desired period, the fixative is flushed out to prevent contamination of the acetone and hence the distillation unit with paraformaldehyde. Dehydration is best carried out with cold (-25°C)

acetone - "Freeze substitution". Consistently, the best specimens will result from this type of cold dehydration. Cold room or room temperature acetone may be used with good results. For large specimens a higher percentage graded acetone series may be utilized with success. Your favorite graded series of alcohol may also be used for dehydration. However, excessive shrinkage occurs and a suitable intermediary solvent will need to be used for the polymer exchange, since alcohol is not an appropriate solvent for this exchange. The exchange of the solvent for the polymer is called "forced impregnation". Impregnation takes place in a vacuum chamber and will occur if the solvent boiling point is low enough or vapor pressure is high enough such that it will sufficiently and steadily vaporize as the pressure is lowered in the plastination kettle. As well, the polymer must have a boiling point that is high enough that the polymer will not vaporize. Acetone and methylene chloride (dichloromethane) (MeCl) both are suitable solvents for the impregnation process. However, MeCl is not a dehydrant. Therefore it can only be used after the specimen has been dehydrated. Silicone, epoxy and polyester are the commonly used polymers. Silicone is generally used to plastinate whole or portions of the body or organs. It may also be used for preserving sections from various regions of the body. Both epoxy and polyester are used primarily to produce thin sections (2-6 mm) of the body. Classically, the main use of polyester has been for head and brain slices. Special processes for both epoxy and polyester polymers maybe used for producing thin sections, <1mm. Rendering the polymer ready for polymerization is accomplished by adding a catalyst. In the case of epoxy and silicone, the catalyst is recommended to be mixed with the polymer prior to impregnation. While polyester's catalyst is UV light and a catalyst does not have to be mixed in the impregnation polymer. The reactivity of the catalyzed polymer is retarded by cold temperature. Epoxy and polyester both yield a hard sheet. The plastinated slice is placed between two plates of glass separated by a gasket and the remaining space is filled with polymer. Silicone, on the other hand, produces a specimen with some degree of flexibility. Flexibility of silicone specimens seems to be dependent on the thickness of the tissue and possibly tissue density. Flexibility may be enhanced by the addition of a chain extender which may be added to the impregnation reaction-mixture or to the specimen after impregnation. Plastination polymers are not inherently flexible and durable after curing when compared to RTV silicone. RTV contains fillers that provide its great flexibility. However, any of the plastination silicone polymers yield similar finished products if the ingredients are added in a similar sequence. The standard Biodur method of combining polymer and catalyst in the impregnation mixture consistently yields excellent specimens. Polymers used today for plastination are routinely used in the polymer industry. The biggest difference in silicone polymers is their viscosity which ranges form 40 to 1,000 cstk. Polymerization (hardening or curing) of the polymer is promoted by adding a hardening agent that promotes cross-linking of the silicone chains. The Dow/Corcoran method adds the cross-linker and catalyst in the reverse of the classic method and specimens generally have poorer surface detail. Thorough mixing of ingredients is a necessity. Patience and accuracy will yield a durable specimen.

Dissections before, during and after plastination.
Boyes R, V Kippers. *School of Biomedical Sciences and Plastination Laboratory, Faculties of Bological & Chemical Sciences and Health Sciences, University of Queensland, Brisbane, Australia.*
Novice plastinators may assume that dissection of a specimen must be completed prior to commencement of the plastination process. However, further dissection is possible both during and after plastination, which often results in enhancement of features chosen for display. The purpose of this presentation is to generate discussion about dissection techniques of plastinated material, seeking the input of plastinators who can share their experiences and provide tips for their colleagues. The presentation will briefly discuss pre-dissection techniques for the benefit of novice technicians. The importance of dissection prior to plastination is paramount because the final appearance of the specimen is dependent on the time, effort and technical expertise of the dissector. All extraneous connective tissue must be carefully removed; underwater dissection magnifies the connective tissue which can then be carefully removed to produce a smoother surface with muscle fascicles enhanced. Stents can be used to maintain the shape and size of the lumen of vessels and orifices. Other devices are used to maintain correct relationships between structures during the process. Some of these devices can be retained while others are removed during the plastination process. Use of compressed air during the plastination process produces organs with a realistic size and shape, such as the lungs, stomach and intestines. Sections of organs can be produced after plastination of complete organs. As an example, brain slices can be produced from whole brains, often

producing slices of better quality than the alternative of slicing the brain prior to plastination. In terms of presentation of plastinated specimens, there is definite benefit in using a range of techniques to highlight specified features and to maintain the correct appearance of structures that tend to collapse during the plastination process.

Principles of silicone plastination techniques. ***de Jong KH.*** *Academic Medical Center. Anatomy and Embryology. Meibergdreef 15, 1105AZ, Amsterdam, N.H., The Netherlands, Europe.*

Since 1979 different techniques of plastination are in use in many institutes all over the world. These techniques can be divided in two groups: sheet plastination using epoxy or polyester and silicone plastination. Although the basic principles are similar for both procedures, differences exist for each polymer used and the flexibility of the resulting specimen. Most people starting with plastination will begin their career as a plastinator using the silicone technique due to factors as: easy to learn, relatively low starting costs, easy obtainable equipment, etc. Therefore this technique will be explained and discussed in this presentation and special attention will be paid to tips, tricks, and pitfalls in each step of the procedure. The main steps in silicone plastination are: fixation, dissection, dehydration, (forced) impregnation, and curing. **1. Fixation:** this step is not necessary. Both formalin or otherwise fixed specimen or fresh specimen can be plastinated. **2. Dissection:** special care must be given to dissection, only the best dissected specimen will yield a nice looking plastinated specimen. **3. Dehydration:** is performed by submerging the specimen in subsequent acetone baths (100%) until the remaining amount of tissue water is <1%. Dependent on the desired degreasing effect of the acetone, the specimen can be left in the last acetone bath longer than desired for the dehydration. **4. (Forced) impregnation:** is performed by submerging the specimen in a bath of monomer silicone with an increasing vacuum, thus boiling the acetone out of the specimen and dragging the silicone into the specimen. The rate of impregnation is monitored by watching the amount and size of the escaping acetone bubbles. **5. Curing:** is a two-step procedure. First elongation of the monomer silicone molecules, caused by a "chain elongator" and second cross-linking of the elongated silicone molecules, caused by the "cross-linker". The latter step forms a 3-D spatial meshwork of flexible molecules throughout the specimen, thus producing a lifelike, dry, odorless and easy to handle anatomical specimen.

Silicone plastination, room temperature methodology: Basic techniques, applications and benefits for the interested user. *Glover R. Director, Plastination Laboratory, University of Michigan, Ann Arbor, MI, USA.*

In July 1998, Dow Corning introduced a room temperature preservation process to those attending the Ninth International Conference on Plastination in Trois-Rivieres Quebec, Canada. This was a significant event in the history of the society because, up until that time, Biodur S10 methodology was the only method available to those interested in doing tissue impregnation with silicone. Prior to the Quebec meeting, Dow Corning, through its chemical distributor Corcoran Laboratories, selected three active plastination laboratories and provided them with chemicals and a description of their newly patented process. The laboratories were asked to experiment with the process and evaluate it to see if it was effective and had significant technical benefits that might be of interest to the society as a whole. The laboratory at the University of Michigan, Ann Arbor, Michigan, was one of the three laboratories invited to experiment with the new process. Dow Corning's invitation was accepted. Since, the Biodur S10 process had been used in the lab for over six years, both processes and chemicals could be compared and evaluated. This presentation will present the highlight of six years of experience using exclusively the room temperature process in our laboratory since its introduction. The focus will be on: the significant benefits inherent in the room temperature process - the things that make it a flexible, time efficient and a cost effective approach to silicone preservation. We hope that this information will give conference attendees who are familiar with the room temperature process an opportunity to understand its basic methodology thus enabeling them to decide whether or not to adopt this process for use in their own laboratories.

Maximizing anatomical concepts with sheet plastination. ***Cook PR.*** *Department of anatomy with Radiology, University of Auckland, Auckland New Zealand.*

Background: As sophisticated diagnostic technology has become commonplace, our teaching program has been tailored to maximize clinical relevancy of the learning experience, with practical laboratory demonstration routinely provided by radiology, ophthalmology, orthopaedic, otorolaryngology, surgical registrars and an anatomical pathologist. Clinical procedures, pathological observations and diagnostic methods are as integral a part of the education process

as is the gross structure of the human body. Plastination has proven to be a vital tool in the enhancement and broadening of anatomical concepts. Materials and Methods: While the University of Auckland utilizes all current Biodur based plastination techniques - S10 silicone, P.E.M. polymerized emulsion and E20 injection casting - to provide an excellent three dimensional realization of the human body, it is the E12 epoxy method of producing serial-sectioned anatomy that has provided significant educational opportunities. Whole cadavers or regions were cut into 2.5mm thin transverse, coronal or sagittal sections, depending upon the desired result. Sections were dehydrated, degreased, impregnated, cast and cured according to standard epoxy plastination protocols. The completed sections are smooth, semi-transparent, durable and offer an exceptionally clear degree of anatomical detail not seen in traditional cross sections or wet gross specimens. Results: The E12 epoxy method of sheet plastination permits, the finished sections to be utilized for correlation and clearer interpretation of similar magnetic resonance images (MRI) and computer tomography (CT). When used in student microscopy laboratories, E12 anatomical structures may be magnified considerably to the submacroscopic level allowing an important conjunct between microscopic anatomy and macroscopic anatomy. Whereas standard histological sections provide detail only within predetermined parameters dictated by the physical limitations of the dimensions of the actual microscopic slide itself. E12 sections contain a high degree of detail whilst, importantly, retaining the in situ structural integrity of the entire region in a complete and uninterrupted state. Conclusion: The unique properties of the E12 process results in the linking together of three distinct disciplines, namely cross-sectional anatomy, radiology and histology from just the one specimen.

Principles of polyester plastination techniques.
Weiglein AH. *Anatomical Institute, Medical University Graz, Graz, Austria, Europe.*
Background: Plastination of brain slices can be done by the standard silicone technique or by the polyester techniques. The silicone technique is used to produce thick, opaque and natural looking brain slices. The polyester techniques (P 35 or P 40) are used to produce thin semitransparent brain slices. Materials and methods: For polyester plastination the recommended procedure is as follows: *Fixation*: Brains are fixed the usual way with 10% formaldehyde alone, since other fixatives may interfere with this procedure. *Slicing*: Brains are sliced with a meat slicer into 4-8 mm thick slices. To avoid disintegration, a piece of wet filter paper trimmed to the size of the brain is placed on top of the brain slice before slicing. The slices are placed on stainless steel grids and the grids are put together into a stainless steel basket and stored in cold fresh tap water. *Flushing*: The basket of brain slices is rinsed with cold tap water overnight and thereby cooled down to 5°C. *Dehydration*: The basket of brain slices is submerged in 100% acetone at -20° for three days. *Immersion*: The basket of brain slices is removed from the acetone and immediately submerged in the polyester-mixture for 1-2 days at 5°C. *Forced impregnation*: The basket with brain slices, submerged in the polyester-mixture is exposed to vacuum for 24 hours either at room temperature (not below 10 mm Hg) or at -20°C (not below 2 mm Hg). *Glass chambers*: The impregnated slices are enclosed in 5-10 mm wide glass chambers. Two glass plates are separated by a silicone gasket and held together by fold-back clamps. The glass chambers are filled with the polyester mixture. *Curing*: The glass chambers are exposed to UVA-light for 3 hours (400 Watts at 25 cm distance from the chamber). During this procedure the chambers must be cooled either by ventilators or by blowing compressed air over both sides of the chambers. P 40 slices are cured by UVA light only, whilst P 35 slices need additional heat curing. Following light curing, the P 35 glass chambers are exposed to 45°C for 5 days in a well-ventilated oven. After curing is finished the slices are dismantled, trimmed by a band saw and the edges smoothened by a belt sander. Results: Polyester plastination of brain slices results in 5-10 mm thick, easy to handle plates enclosing the impregnated brain slices. The semitransparent brain slices are particularly superb in gray and white matter distinction. Conclusion: Due to the detailed anatomy shown in polyester brain slices and due to their comparability to CT and MRI scans, they are excellent for teaching neuroanatomy.

Polyester plastination techniques: Specific troubles and problems.
Henry RW. *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*
Polyester plastination has been a popular and convenient method to preserve brain slices for nearly two decades. It involves slicing thin (2-6 mm) slices of well-fixed brain, dehydrating, soaking in polymer, impregnating with polymer and finally placing the slice in a flat chamber with polymer to make the final sheet. In the resulting slice, gray and white matter are clearly

differentiated. Two widely used polymers have been used, P35 and P40. P35 was introduced first and yielded brain slices of unparalleled beauty, clarity and definition of white and gray matter. P40 was introduced 10 years later and is a shorter and less cumbersome technique. It also yields a remarkable differentiation of white and gray matter. However, the P40 polymer has a specific problem when used on brain tissue, orange spots in the gray matter and when uniformly distributed throughout the gray matter, it may resemble the coloration of P35 slices. No predictable mechanism has been developed to determine if the spots will or will not develop in brain slices. Some theories of the cause or reason for the orange spots have been offered (tissue peroxidase, fixation or fixatives, incomplete impregnation) but no clear resolution has been offered. An additive has been developed to correct this problem and it is seemingly beneficial to minimize spots. Shrinkage of 4.5 to 7% has been reported depending on whether cold or warm temperature impregnation was used to produce P40 slices. P35 slice production takes ten to twelve days, while P40 slices take only four or five days to complete. P35 slices use twice as much glass as P40 slices. Catalyst must be used in the immersion and impregnation baths of P35, therefore more polymer is used and impregnated slices should be cast within a few days. However, P40 slices can be held for several weeks after impregnation since there is the possibility of using no catalyst in the impregnation bath. Recent results show that P40 can be used successfully to produce slices from all regions of the body. P35 and P40 slices remain fully transparent with no yellowing over time. If the P35 or P40 impregnated slices remain in the polymer for extended periods of time, the slices may adhere to the grid spacers that are used to separate the tissue slices.

Principles of epoxy plastination technique (E12).
Sora M-C. *Plastination Laboratory, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria, Europe.*
Background: The E12 plastination process is a well-established preservation technique used for demonstration in teaching and also in research. Material and Methods: Materials and slicing: For E12 plastination, fresh tissue which is frozen at -80°C for one week is used. Next slices with an average thickness of 3 to 5mm are cut. Between the sections, 1 mm of tissue is lost due to the thickness of the saw blade. The slices are stored at -25°C over night. Dehydration and Degreasing: Cold technical quality acetone (-25°C) is used for dehydration of slices. Each slice is placed between plastic grids to allow better circulation of the dehydration fluid. Dehydration time for the slices is 7 days. The acetone is changed after 3 days and its concentration will have dropped to around 96%. After 4 days in new acetone, the final concentration of the acetone dehydration bath should be 99%. When dehydration is complete, the freezer is disconnected and the temperature allowed to increase to room temperature (+15°C). Next the acetone is changed for room temperature methylene-chloride (MeCl) for degreasing. Degreasing is finished after 7 days. Impregnation: Impregnation is performed at +5°C using an epoxy reaction-mixture (E12). The slices are submerged in the E12 reaction-mixture and placed in a vacuum chamber, directly out of the methylene-chloride bath. Pressure is continuously reduced over the next two days down to 2mm Hg. Temperature is kept under surveillance in order to avoid E12 crystal formation which may take place if temperature decreases below 0°C. Casting and curing: The slices are cast between two sheets of tempered glass and a flexible gasket is used as a spacer (4mm). The following E12 reaction-mixture is used for casting: E12/E1/AT30 (95:26:5). The slices are placed between glass plates, sealed with a gasket and clamps, and the chambers filled with the casting mixture. After filling, they are placed in a vacuum chamber at 3 mmHg for one hour to remove small air bubbles present in the resin. Large bubbles are removed afterwards manually. After bubble removal, the flat chambers are placed nearly horizontal (inclined at 15°) and left until the next day. The polymer becomes more viscous and sticky and after one more day the flat chambers containing the slices are placed in an oven at 45°C for 4 days. After removal from the oven, the flat chambers are cooled to room temperature. The glass plates are removed carefully and the epoxy sheets are trimmed as desired. Results: The transperancy and color of the slices are perfect and shrinkage is not evident. The finished E12 slices are semi-transparent, easy to orient and offer a lot of anatomical detail down to the submacroscopic level. The transparent areolar and adipose tissues contrast perfectly with the muscle tissue and epithelial parenchyma. Conclusion: Since the beginning of plastination, the E12 technique is still the selected method for producing transparent body slices. Transparent body or organ slices are used for teaching and research, because they allow study of the topography of all body structures in a non-collapsed and non-dislocated state. In addition, the specimens are

useful in advanced training programs in sectional topography (resident training in CT and NMR).

Tips and tricks in plastination. ***Weiglein AH.*** *Institute of Anatomy, Medical University Graz, Graz, Austria, Europe.*

Practically every step in plastination can be improved to save time and/ or money or to improve the production of better plastinated specimens. A good specimen can only be the result of a well planned **dissection**: It is advisable to leave structures longer than they should be in the final specimen. E.g. blood vessels, nerves, muscles and tendons can be trimmed after the plastination process, which results in smooth sections. Hollow organs (lung, heart, stomach, intestines, large blood vessels) must be dilated during the whole plastination process, at least during dehydration and curing to keep their form. Several studies have shown that specimen shrinkage is lowest when cold acetone is used for dehydration. **Dehydration** at room temperature with acetone or methanol can result in up to 50 % shrinkage (20-25%) compared to dehydration at minus 25°C (10-15%). For improvement of the dehydration process, the basis for dehydration must be understood: It is a dilution process during which the water concentration of the specimen is minimized to zero by diluting the water inside the specimen with acetone. Basically, a specimen could be dehydrated in one step by submerging it in pure acetone 100 times the volume of the specimen. E.g.: one human brain weighs 1,3 kg which contains approximately 1.0 L of water. If this brain is submerged in 100 L of pure acetone this would result in a 99% concentration over a period of time. To reduce the amount of acetone needed it is recommended to work with a 1:10 specimen-acetone ratio. In the same case (1 human brain ~ 1 L water) the first acetone bath of 10 L pure acetone results in a 90 % concentration, the second in 99 % and the third in 99.9 %. Thus, it is recommended to use three baths of pure acetone each with 10 times the specimen's volume. This procedure is the fastest possible, but also the most expensive one. To save money, acetone of lower concentration (down to 80%) can be used to start dehydration; the last bath, however, would have to be pure acetone to get complete dehydration. Acetone of lower concentration (below 75 %) can be recycled with commercial or self made solvent recyclers. To minimize **shrinkage** it is recommended: To use cold (-20 to -25°C) acetone for dehydration, To dehydrate to 99-100%, To impregnate completely (down to 0-5 mm Hg), To use fast curing, and To dilate hollow organs. High flexibility can only be reached in thin organs; e.g. in the stomach and the intestines. If a specimen is too dark after plastination it can be brightened up by wrapping it in cloth soaked in 10 % peroxide for several minutes or hours. To keep some **flexibility** it is recommended: To use no hard fixation (no formalin), To use less S3 (hardener / chain-extender), To dilate hollow organs, To place plastic foil in between anatomical layers, and To wipe and move the specimen during curing process.

Equipment and costs for plastination. ***Diz A.*** *Departamento de Anatomía y Anatomía Patológica Comparadas, Faculty of Veterinary Sciences. University of Córdoba, Córdoba, España, Europe.*

The following review is suggested for beginners and will give you a general overview of necessary equipment and cost of a silicone plastination laboratory using the standard S-10 technique and sheet plastination (E-12 and P-35/P-40 techniques). The proportions of the equipment depend on the kind, size and number of specimens to be plastinated and on the plastination technique being used. Generally it is advisable to start with the standard silicone technique at a low level and to expand the laboratory set-up after some experience has been gained. All of the various methods of plastination have the common principle: the use of a vacuum chamber and a vacuum pump to extract the solvent saturated within the specimen. A means of regulating and monitoring the amount of pressure is also vital to the success of the plastination process.

Basic equipment for silicone plastination :

3+ plastic (polyethylene or polypropylene) (PVC or polyester are not recommended), aluminum or stainless steel containers corresponding to size of specimens for fixation, dehydration and degreasing, but large enough to hold the specimens and dehydration agent at a ratio of 1:10.

1+ deep freezers large enough to hold at least 2 of the dehydration containers and 1 vacuum chamber corresponding to size of specimens.

1 Grid basket (made of acetone resistant plastic, aluminum or stainless steel) to contain the specimens for dehydration and forced impregnation.

1 Acetonometer 0-100% and 1 acetonometer 90-100% to monitor dehydration. Ideally no more than 1-2% at the most of water, is allowable to remain in the specimen.

1 Vacuum chamber of stainless steel corresponding to size of specimens with 3 connecting ports and a glass plate on top for a lid and for visual control of the impregnation process.

1 Rotary vane vacuum pump, suitable for solvents, suitable for size of vacuum chamber and vacuum tubing. The pumping speed needed in plastination depends on volume of impregnation bath. Usually pumping speed is $1m^3$/h per 10 liters of impregnation bath. If in doubt, a lower pumping speed is advisable.

1 Separator for oil and solvent in the pump exhaust (not indispensable): a condensation and collecting vessel for extracted solvent and oil vapor given off during forced impregnation. Placing it in the deep freezer allows to recover almost 100% of extracted solvent. At room temperature recondensation is only partial.

1+ vacuum adjustment valves: needle valve for manual control of forced impregnation via admission of external air. System of two parallel-grouped valves allows finer adjustment of vacuum than one valve.

1 Mmanometer to monitor vacuum during the first two thirds of forced impregnation.

1 Bennert-Manometer to monitor vacuum during the last third of forced impregnation.

Vacuum tubing to connect vacuum chamber to: vacuum control and adjustment devices, to vacuum pump and to separator for oil and solvents.

1 Gas curing unit (for curing silicone impregnated specimens) corresponding to size and number of specimens, consisting of a plastic, aluminium or stainless steel container provided with sealable lid, 1 or more closeable glasses for gas curing and desiccator agent, 1 small membrane pump to vaporize silicone hardener (S6) and 1 draining grid. Moreover, an air compressor is recommended to dilate hollow organs and a distillation unit for recycling acetone.

For sheet plastination, all the equipment described above is necessary except the gas curing unit; moreover, the following equipment should be available:

1 Rotary meat slicer for organ slices

1 Band saw with coolable guide stop for frozen specimens and body specimens containing bony structures.

1 Thickness guage.

Sheets of polymer gauze and polymer or stainless steel grids and nets for stacking and stabilizing of body and organ slices during dehydration, degreasing and forced impregnation.

Equipment for flat chambers: Toughened glass plates (3-4 mm. thick, free of scratches), silicone gaskets as spacer for flat chamber (thickness depending on thickness of slice), foldback clamps for assembly of flat chambers, 1 or more flat funnels to fill flat chambers with resin, wire in order to positioning the slices.

Oven (50°C) for curing of specimen slices according to E-12 and P-35 technique (for P-40 technique it is not necessary).

1 U.V.A. light curing unit for curing of plastinated slices with P-35 or P-40 Techniques, constructed with two standard fluorescent light fittings equipped with fluorescent U.V.A. tubes. It must integrate a fan for cooling and time switch.

In a plastination lab, remember that acetone fumes are an explosion danger, so all precautions be taken to minimize the risk of explosion. Great care must be taken to isolate the electrical elements in the lab. A general hint in plastination is that if a household deep-freezer is used, the interior lighting has to be removed. Moreover, the compressor and motor should be removed and re-situateded outside the freezer in the adjoining room for maximum hazard control. In case the compressor and motor are not dismounted, the freezer should be disconnected from power supply before handling of acetone inside the freezer. The vacuum pump must be sited outside the lab for the same reason, and an acetone fume detector should be installed in the plastination lab. In order to reduce costs in plastination, much of the equipment required for the setting up of a small to medium sized plastination lab is often readily available in most universities and other institutions, so major capital expense can be negligible. It is desirable to acquire most of the equipment from local suppliers and to recycle used acetone, polymers and other chemicals in the lab. Finally, a great deal of talent and imaginative flair can often produce good quality plastinated specimens with limited means.

Plastination: A tool for education: ***Lozanoff S.*** *Department of Biomedical Sciences, University of Hawai'i, School of Medicine, Honolulu, HI, and SURFdriver Software, Kailua, HI, USA.*

Background: Problem based learning (PBL) pedagogical strategies have become more prevalent in medical school curricula. PBL focuses on small group interaction and experiential, case-based learning. The clinical problem is the vehicle for learning and it is peer-taught and tutor-mediated. A specific case scenario is developed and then the small group works through the case to establish a differential diagnosis in a logical, hypothesis driven process. PBL also provides the opportunity to introduce virtual reality (VR) teaching tools into the curriculum that includes anatomically relevant clinical conditions. The purpose of this paper is to review the efficacy of VR presentations using anatomical animations generated from plastinated tissues. Materials and Methods: A PBL case involving an epidural hematoma patient was

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Computerized Reconstruction of a Plastinated Human Kidney Using Serial Tissue Sections

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Abstract: Computerized reconstruction of anatomical structures is becoming very useful for developing anatomical teaching modules and animations. Although databases exist consisting of serial sections derived from frozen cadaveric material, plastination represents an alternate method for developing anatomical data useful for computerized reconstruction. The purpose of this study was to describe a method for developing a computerized model of the human kidney and ureter using plastinated tissue. A human kidney was obtained, plastinated, sectioned and subjected to 3D computerized reconstruction using the WinSURF modeling system (SURFdriver Software). The kidney was generated rapidly and rendered easily on a Windows laptop machine in real time. Qualitative observations revealed that the morphological features of the model were consistent with those displayed by typical cadaveric specimens. Morphometric analysis indicated that the model did not differ significantly from a sample of cadaveric specimens. These data support the use of plastinated tissue for generating tissue sections useful for 3D computerized modeling.

Key words: **plastination; E12; kidney; computer; modeling; SURFdriver**

Introduction

Gross Anatomy consumes a large portion of the first year of training in U.S. Medical Schools requiring approximately 150 hours of contact time (Drake et al.,2002). Human dissection continues to be seen as the primary means of learning human anatomy and this should be a priority for medical students (Aziz et al., 2002). However, new modalities are emerging as a means to supplement certain experiences not easily simulated with cadaveric material. For example, Project TOUCH (http://hsc.unm.edu/touch) is a collaborative effort between the University of Hawai'i and the University of New Mexico medical schools aimed at developing problem based learning (PBL) cases that can be distributed via the National Computational Science Alliance' Access Grid (http://archive.ncsa.uiuc.edu/alliance/access-dc/) simultaneously across large distances (Jacobs et al., 2003). The objective of this project is to generate a virtual patient that can be treated simultaneously by students at remote locations within a PBL context (Caudell et al., 2003). Electronic anatomical models are becoming increasingly important as they can be transmitted across the internet within the case (Lozanoff et al., 2003).

Computer models and animations of anatomical features are becoming increasingly attractive as a means

to communicate complex spatial relationships and concepts effectively (Dev et al., 2002). Although many educational animations are based on artistic renderings (Johnson and Whitaker, 1994; Habbal and Harris, 1995; Gould, 2001), more recent applications are using virtual representations derived from actual cadaveric material (Neider et al., 2000; Lozanoff et al., 2003). Within the medical curriculum, anatomical specimens can be used to develop animations and to incorporate into didactic lectures providing insight into function and spatial relationships not easily conveyed through static 2D images (Trelease et al. 2000; Neider et al., 2000). However, anatomical modeling traditionally has been difficult for the individual instructor due to the limited availability of low cost software that can be implemented on an individual desktop or laptop computer. Similarly, the quantity of cross sectional image data useful for three-dimensional modeling is limited.

The Visible Human Male (VHM) and Visible Human Female (VHF) datasets developed through the National Library of Medicine (NLM) provide a valuable resource of image data even to the individual instructor with only desktop computer modeling capabilities (Spitzer and Whitlock, 1998; Jastrow and Vollrath, 2003). Even though these data sets are extremely impressive and useful, representation has been a problem for some organs and tissues. For example, no attempt was made to infuse hollow organs to prevent their collapse and thus certain structures are difficult to reconstruct accurately.

The purpose of this paper is to present the use of serially sectioned plastinated tissues for generating anatomical reconstructions using a simple desktop modeling system. The kidney was selected as the subject for its potential use in simulated renal failure problem based learning cases within a medical school curriculum (Jacobs et al., 2003).

Materials and methods

Tissue Processing

One human kidney (right) was removed from a fresh unfixed cadaver (female, 80 years old with no apparent pathology), measured and finally plastinated as described by von Hagens (1985). Measurements taken were the length of the kidney from superior to inferior poles (TRH), width of the anterior surface of the kidney at the level of the hilus (WH), width of the anterior surface of the kidney at 1/3 and 2/3 of TRH (W1/3H and W2/3H) and height of the hilus (HH) (Fig.1). Kidney thickness was measured at the point at which the kidney appeared thickest (MD) (Fig. 1). The needles which were used to indicate the initial measurement

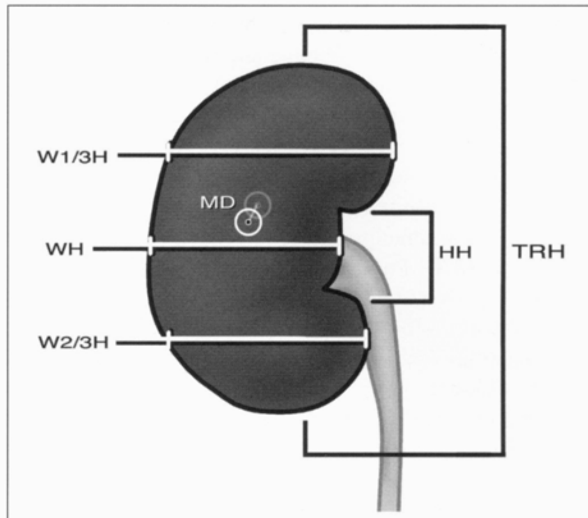

Figure 1. Locations of measurements recorded from the kidney reconstruction and cadaveric specimens. TRH, total renal height; HH, hilum height; MD, maximum depth; WH, width at hilum; W1/3H, width at 1/3rd height of kidney; W2/3H, width at 2/3rds height of kidney.

points were left in place to be used again following plastination for accurate re-measurement of the kidney for determination of tissue shrinkage. The kidney was kept frozen at -80°C for one week before being placed into a freezer at -25°C for 2 days. The 25 liters of technical quality acetone used for dehydration of the kidney was cooled to -25°C in a freezer. The acetone was divided into three aliquots to form three sequential dehydration baths. The kidney was allowed to dehydrate in the first acetone bath for four weeks. The lengths of the second and third dehydration baths were three and two weeks respectively. The concentration of the three acetone aliquots at the end of the three baths was measured to be 92%, 97% and 99% respectively (Table 1).

	Temperature	Days
Fresh	-80°C/-25°C	7/2
AC1	-25°C (96%)	28
AC2	-25°C (97%)	21
AC3	-25°C (99%)	14
MCl	15°C	28
Impregnation	30°C/60°C	4/1
Curing	65°C	4

Table 1. Plastination conditions for the E12 block.

With dehydration complete, the freezer was disconnected and the specimen reached room temperature (15°C) after 24 hours. The acetone was changed with room temperature methylene-chloride (MeCl) for degreasing of the specimen. Degreasing was

deemed sufficient when the MeCl bath appeared clear instead of yellow at the change and the adipose tissue in the specimen became transparent. The dehydrated, degreased specimen was removed from the MeCl bath and submerged in a E12/E6/AE600 mixture (100/50/0.2by volume) (von Hagens, 1985). This was placed in a Heraeus VT 6130 M vacuum drying oven (Haraeus Instruments, Kendro Laboratory Products GmbH) at 30°C. Pressure in the oven was decreased to 350mm Hg overnight to commence penetration of the E12 mixture into the specimen. The following day, impregnation was continued at 30°C using a second epoxy mixture of E12/E6/E600 (100/50/0.2) (von Hagens, 1985). Pressure was continuously reduced to the level of 2mm Hg within five days. Temperature was held at 30°C for the first four days. The temperature was increased to 60°C on the fifth day.

Once impregnation was complete, the specimen was removed from the vacuum and placed in a rectangular mold built of styrofoam and lined internally with polyethylene foil. The mold containing the impregnated specimen and embedding medium was returned to the oven at 65°C for 4 days. After the block cooled to room temperature, the mold was carefully removed. The E12 block measured 150x100x100mm. The E12 block was serially sectioned using a diamond blade band saw (Exact 310 CP; Exact Apparatebau GmbH, Norderstedt, Germany). The average thickness of sections was 0.6mm. Due to the thickness of the saw blade, 0.4mm of specimen was lost between sections. Each section was coated with polymer reaction mixture E12:E1 (100:28) (von Hagens, 1985; Weber and Henry, 1993) and cast between two layers of polyester foil. The foil plates containing the laminated slices were allowed to cure for 24 hours at room temperature after which they were placed in an oven at 45°C for 24 hours. This procedure provided the sections with a smooth, refinished polymer surface devoid of refractive artifacts caused by cutting with a saw.

To determine shrinkage resulting from the plastination process, the kidney was remeasured following plastination.

Computer Modeling

Laminated slices were scanned using an EPSON GT-10000+ Color Image Scanner at 600 dpi. Placement of slices on the scanner bed occurred with the inferior surface of the laminate resting on the glass of the bed. A ruler (mm) was included with every scan as a calibration marker. Although the scanned images could have been used directly for computer modeling, we decided to include an extra step involving manual tracing so that alignment could by more closely controlled. Scanned images of the tissue slices were printed and manually traced. Alignment guides were transferred to each tracing. Each manual tracing was placed on a scanner so that fiducial points aligned with a base alignment tracing attached to the scanning bed. Once scanned, these images (jpeg format) were loaded into WinSURF (SURFdriver 4.0; http://www.surfdriver.com) and traced from the monitor. Features used in the reconstruction, defined as objects, included the kidney parenchyma (cortex and medulla) and ureter. Each object was traced and numbered accordingly. Once all contours were traced, the reconstruction was rendered and visualized and the model was qualitatively checked for surface discontinuities in the renal cortex, renal medulla and ureter by rotating the model and viewing. Kidney and ureter textures were applied using SURFdriver maps (www.surfdrivermaps.com). System execution times and data array features were recorded as an index of program efficiency.

Once rendered, the measuring tool available in WinSURF was used to record height, width and depth measurements from the model. Measurements, TRH, WH, W1/3H, W2/3H, HH, MD, were also recorded from 12 cadaveric specimens using a Helios Caliper (0.1mm). Average values and their standard deviations were also recorded for the cadaveric specimens. The corresponding measurements taken from the model were used and a weighted mean difference was calculated (t_s). Statistical significance was determined at the $p < 0.05$ level using a sample observation compared to a population mean test (Sokal and Rohlf, 1981:229-231).

Results

Prior to plastination, the length of the kidney from the superior pole to the inferior pole was 10.21cm while the width in the middle of the anterior surface was 4.72cm. Following plastination, the length of the kidney measured 10.16cm while the width was measured to be 4.69cm. Hence, the amount of overall shrinkage of the specimen was calculated to be 5.23%.

Figure 2 shows the transparency and color of the laminated slices. The renal parenchyma was easily identified and the borders could be easily copied onto tracing paper (Fig. 3). Following this manual procedure, the traced contours were scanned and loaded into WinSURF and automatic edge detection was used to quickly collect tissue borders or contours (Fig. 4). These contours were visualized on the tissue borders and showed close continuity with the edges of the objects (Fig. 5). The rendered kidney showed a distinct parenchyma with calyces emptying into the ureter (Fig. 6). Renal fat was not reconstructed so the hilar region was clearly visible from the medial aspect. The kidney

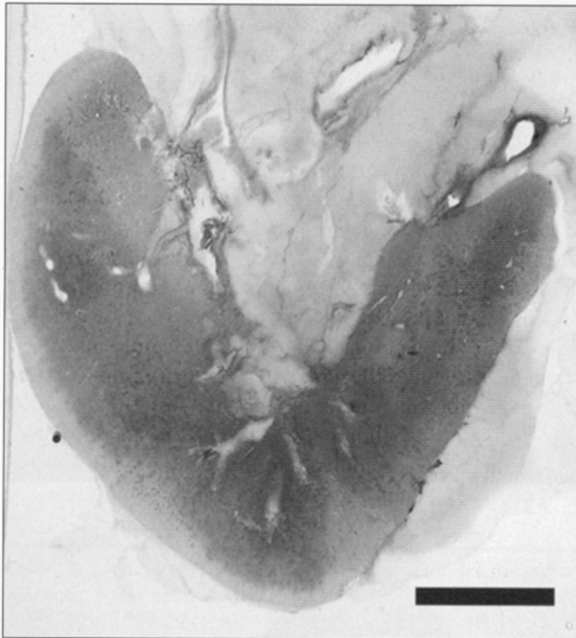

Figure 2. A representative section of the plastinated kidney. Scale = 1.5 cm.

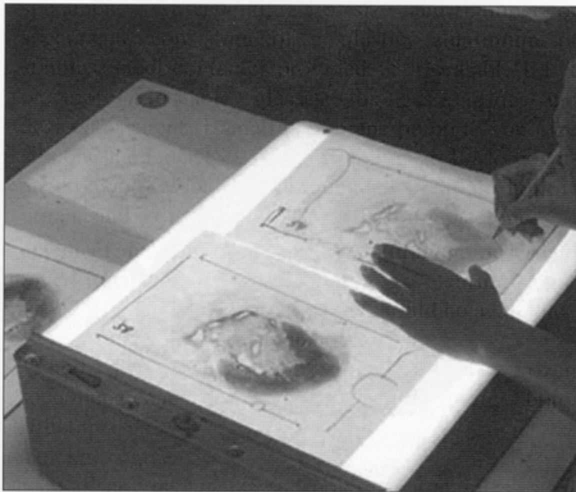

Figure 3. Manual tracing of plastinated specimens.

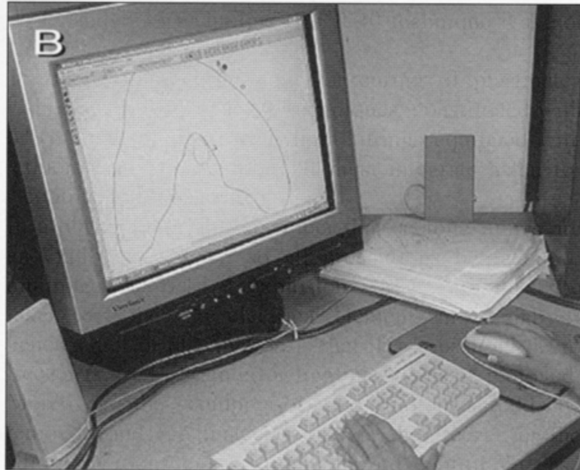

Figure 4. Collection of tissue contours from WinSURF generated images of scanned specimen tracings.

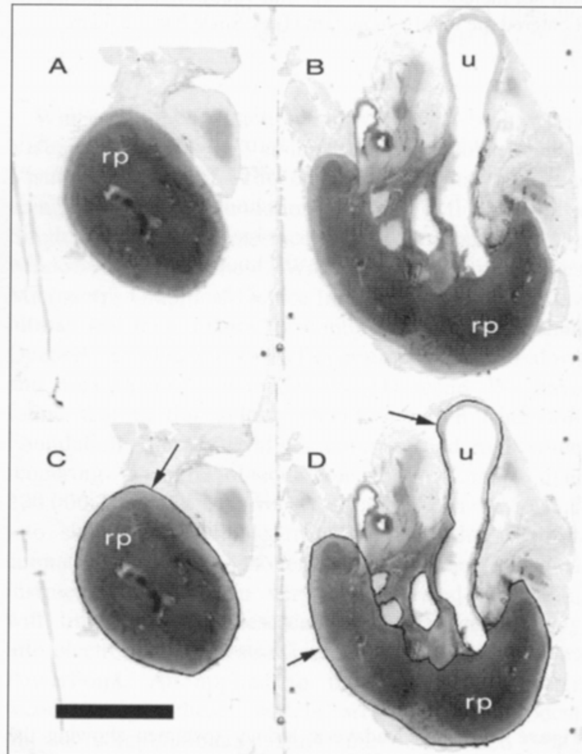

Figure 5. Representative tissue sections from superior (A) and middle (B) regions of the kidney. Tissue contours were traced (arrows in C,D) for the ureter and its branches (u) and renal parenchyma (cortex and medulla, rp) for all sections. These contours provided the data for the model. Scale bar = 2.0 cm.

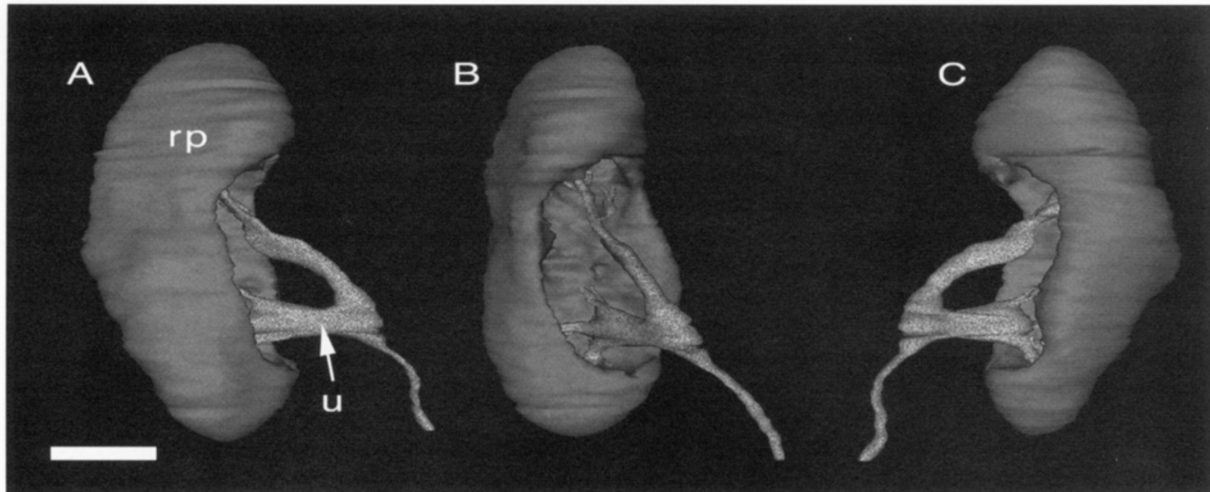

Figure 6. Rendered kidney viewed from the anterior (A), medial (B) and posterior aspects (C). Perirenal adipose tissue was not included in the model thus revealing the ureter (u) as it entered the renal parenchyma (rp). Scale bar = 2.0 cm.

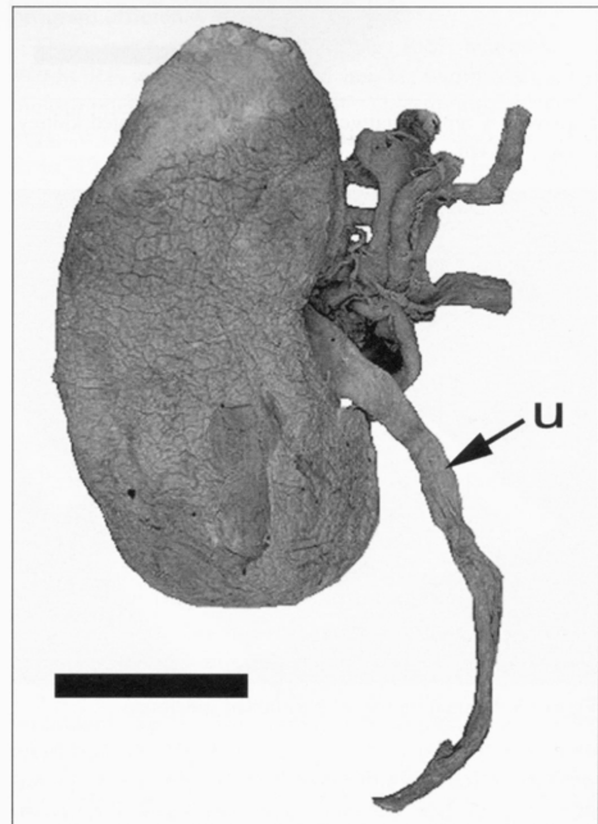

Figure 7. Typical cadaveric kidney specimen showing the ureter (u). Scale bar = 3.0 cm.

model was rendered and rotated in real time and it compared well with a representative cadaveric kidney using qualitative observations (Fig. 7).

The digitally rendered kidney consisted of 9992 triangles on 4952 vertices while the ureter comprised 3553 triangles on 1730 vertices (Fig. 6). Triangulating the surface from the contours took 4.5 seconds on a 1.5 GhZ machine with 512Mb RAM and an ATI Radeon Graphics card. Rendering of the surfaced objects using OpenGL was virtually instantaneous, with or without the graphics card enabled. Thus, the model was moved smoothly in real time with the mouse pointer.

Specimen Number	THR	HH	WH	MD	W1/3H	W2/3H
1	10.72	2.68	4.06	4.25	5.03	3.46
2	10.08	3.05	4.53	5.52	6.06	4.04
3	7.79	3.54	3.62	3.61	4.03	3.67
4	7.64	3.98	4.14	3.53	4.74	3.00
5	10.50	3.96	4.50	4.80	4.50	4.37
6	10.24	3.49	5.00	4.90	4.67	4.86
7	8.43	2.36	4.10	4.22	4.80	3.88
8	9.41	4.10	4.16	3.50	3.76	4.22
9	9.83	4.63	5.00	5.36	5.45	4.16
10	10.82	4.50	5.49	4.88	4.77	4.90
11	10.07	3.05	4.44	4.58	4.72	4.29
12	9.51	3.64	4.29	4.03	4.26	4.84
Mean (st dev)	9.59 (1.09)	3.58 (0.70)	4.44 (0.51)	4.43 (0.69)	4.73 (0.61)	4.14 (0.58)
Model Value	9.42	4.81	4.25	4.34	4.31	3.30
t_s	-0.15	1.68	-0.37	-0.13	-0.67	-1.39
sig. level	ns	ns	ns	ns	ns	ns

Table 2. Comparison of the single kidney model measurements with the corresponding measurements recorded from the cadaveric renal sample (n=12). Measurements in cm; t_s = weighted difference of means and the observed value (Sokal and Rohlf, 1981: 229-231); ns = not significant at $p < .05$

Quantitative measurements showed that the overall morphology was retained (Table 2). Weighted difference of the means and observed values differed very slightly with the greatest difference recorded for hilum height (HH, 1.68) and the smallest for maximum depth (MD, -0.13). Coefficients of variation for the six variables ranged from a low of 11.3% to 19.5%. None of the variables recorded from the model were significantly different from the corresponding values measured from cadaveric specimens at the $p < .05$ level.

Discussion

The system described here relies on relatively inexpensive hardware including a EPSON GT-10000+ scanner and Dell Laptop computer. The WinSURF reconstruction package from SURFdriver Softwere © (*surf*ace reconstruction *driver*), was developed expressly for use in three-dimensional anatomical reconstruction and it is a simple icon-driven system (Moody and Lozanoff, 1998). It has been shown to provide accurate and precise computer generated anatomical models (Lozanoff, 1992; 1999). Minimum requirements for the software are extremely modest and include a 200 MHz Intel Pentium processor, Windows 2000, Windows XP, Windows NT, 24 MB of free available systems RAM (64 MB recommended), 50 MB of available disk space, 1024 x 768, 16-bit color display, CD ROM drive and 3 1/2" floppy or 100 MB Zip Drive, and a Microsoft Mouse or compatible pointing device; all of which comes with standard IBM equipment.

WinSURF reconstruction software was built using Visual Studio C++ Version 6.0, and subsequently Visual Studio .NET. The code was originally written using Microsoft Foundation Classes (MFC) with a Single Document Interface (SDI) to manage the windowing environment. Within MFC, WinSurf uses Microsoft's Graphical Device Interface (GDI) to display bitmap and jpeg images for contour tracing rather than OpenGL, as OpenGL's pixel operations were too slow, and uses OpenGL to render the 3D scene. We have found that in this setting OpenGL within Microsoft Foundation Classes exhibits choppy rendering when rendering OpenGL objects consisting of more than 200,000 triangles. As well, the output can be loaded into shareware available from the web that permits animation of complex movements. Thus, the individual instructor can develop very sophisticated animation with little effort and these can be incorporated directly into electronic presentations utilizing such programs as PowerPoint. As applied to the kidney and ureter reconstruction, these models are relatively modest objects computationally since they comprised 9992 triangles and were easily handled by the system for real time manipulation and display.

The kidney model generated displays a morphology that corresponds qualitatively to the actual cadaveric specimen. Perirenal fat was not reconstructed so that the hilus would be easily seen from the medical aspect of the model. This adipose probably could be reconstructed in subsequent experiments to achieve a realistic appearance for the model. Similarly, the ureter

and major and minor renal calyces apparently collapsed and could be depicted more effectively if the collecting duct system had been dilated prior to plastination. Quantitative analysis of the model was based on a comparison of the reconstruction to a sample of cadaveric specimens. The coefficients of variation for each cadaveric specimen variable were relatively large (ranged from 11.3% to 19.5%) which probably contributed to the lack of significance. A larger cadaveric sample would probably provide a greater measure of accuracy for the model. Nonetheless, the model shows close morphometric correspondence to the cadaveric specimens with all variables not significantly different from the actual kidneys. Results generated from this analysis demonstrate that plastinated material can be used effectively to generate tissue contours suitable for computerized three-dimensional reconstruction.

A logical advantage of models is that they provide a greater sense of realism for the student and thus should permit the student to learn more information while reducing the time required. One could hypothesize that this realism stimulates students to generate learning issues more effectively and possibly with a greater sense of urgency (Jacobs et al., 2003; Caudell et al., 2003). This effect remains largely untested and exists as a ripe area for educational investigation.

A major problem with existing anatomical databases is the low resolution for smaller anatomical structures. Large features are easily visible and can be modeled successfully for anatomical animations. However, many of the smaller structures such as arteries and nerves are not easily visible. Plastination provides a useful alternative for generating anatomical databases (Lozanoff et al., 2003, Qiu et al., 2003). These preserved tissues are significantly easier to cut, stain, and handle compared to fresh frozen tissue since they are much more durable owing to the silicon infiltrate. Plastinated tissues may provide much greater resolution compared to fresh frozen tissue sectioning, yet this remains to be tested. Although the scanned plastinated images could have been traced directly within the WinSURF editing window, it was decided to include an extra step involving manual tracing so that alignment could be more closely controlled. This step increased the processing time, but decreased postprocessing. Nonetheless, plastinating specimens locally enables anatomical computer models to be generated achieving very specific educational course objectives. Future work will be directed at developing datasets utilizing plastinated tissue and determining their usefullness for 3D reconstruction.

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Ascaris Plastination through S10 Techniques

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Abstract: Ascaris Lumbricoides is the largest of the common nematode parasites of the human digestive system. It has a world-wide distribution and is very common in regions with poor sanitation. Because of this association with man, Ascaris' impact on human health is taught in medical institutions around the world. Different techniques are used to prepare Ascaris for educational purposes in medical colleges. One method is immersion and storage of the nemotode specimens in formaldehyde. A second less common method is preservation in alcohol. Histological cross sections are also utilized in some teaching laboratories. The disadvantages to teaching with specimens stored in formaldehyde or alcohol is that they are wet and these solutions are associated with noxious odors and potential health problems in humans. Additionally, specimens stored in formaldehyde or alcohol often present problems in transportation for use outside institutions. The disadvantage of histologic specimens is their limitation in scope. The purpose of this study was to perform plastination of Ascaris Lumbricoides specimens using the S10 technique. This procedure produced specimens which are dry, odorless and easy to handle while maintaining the natural shape of the specimen. Ascaris Lumbricoides preserved in this manner can be easily used for demonstration in educational courses working with medical students as well as public health education of the general population.

Key words: **plastination; silicone; S10; Ascaris**

Introduction

Infection with Ascaris lumbricoides continues to be a significant heath problem throughout the world (Lynne and Garcis, 2001). Ascaris occurs in about 22 percent of the world population (Muller and Baker, 1990). Dependent upon poor sanitation for its spread, human Ascarids have been described as a household and backyard infection (Bogitsh and Cheng, 1998). Ascaris is contacted by ingesting mature eggs from contaminated soil which occurs most often among children who play on the ground. Food, mainly vegetables, and drinking water may be contaminated and become the source of infection (Tsieh, 1999). In educational courses in medical colleges, specimens of Ascaris are utilized for demonstration purposes. The form most widely used is formalin fixed worms. This approach to teaching has some disadvantages, among the most undesirable are odor and dampness. This investigation was designed to employ S10 method instead of formalin to produce more desirable teaching specimens. It was assumed that using the S10 plastination technique would produce specimens which are dry, odorless, and preserves the natural shape and appearance of the parasite.

Materials and methods

The Ascarid specimens used in this study had been fixed in 5% formaldehyde for an extended period of time. These specimens were rinsed in cold tap water to remove as much formaldehyde as possible. The specimens were subsequently cooled to 5°C prior to

dehydration. Several specimens of Ascaris were submerged in at least three baths of one hundred percent acetone at -20ºC. The ratio volume of acetone to the specimens approximately was 10:1. The low temperature fixes the shape of specimens. When the acetone concentration remained at 99%, after approximately three weeks, dehydration was deemed complete. The Ascarids were then submerged in a mixture of S10/S3 (100:1) for at least one day at -20ºC. Longer immersion at this stage serves to shorten the impregnation time for the specimens and also reduces the shrinkage of the worms' cuticles. The volume ratio of silicone to the specimens was 3:1. In this stage the specimens immersed in silicone were placed under vacuum for 14 to 18 days at -20ºC. The pressure was slowly decreased to 5mm Hg. Ascaris cuticles are somewhat impermeable to large molecules and surrounds a very small visceral space. For this reason, a slow decrease in pressure helps to prevent shrinkage of the specimens (Table 1). After forced impregnation, the Ascarids were removed from the polymer and excess polymer was drained from their surfaces for three days. The specimens were then placed in a single layer on a grid in the Biodur HH_{10} gas curing unit and exposed to S6 gas cure vapors for 3 days at room temperature (von Hagens, 1985).

pressure	time
85mm Hg	1-2 days
75mm Hg	2 days
65mm Hg	1-2 days
55mm Hg	2 days
45mm Hg	1-2 days
35mm Hg	2 days
25mm Hg	1-2 days
15mm Hg	2 days
5mm Hg	2 days

Table 1. Impregnation schedule.

Results

After plastination, the Ascarid specimens retained their original shapes (Fig. 1). The Ascarids retained some flexibility. The plastinated specimens are odorless, non-toxic and dry to the touch (von Hagens et al., 1987).

Discussion

Plastination of animal and fish are reported in some articles (Asadi, 1998; Zhong et al., 2000), but in this research, it was the first trial of plastination in parasitology. Not only were the resulting specimens dry and odorless, but the color of the plastinated worms was more aesthetic than that of specimens stored in formaldehyde. Because of the good results for teaching, it was suggested that in the future that plastination in the teaching of parasitology be considered as an alternative to traditional preservation methods. It should be mentioned that S10 plastination is recommended for considerably large size parasites like Ascaris and Teania and it may not be suitable for smaller nemotodes.

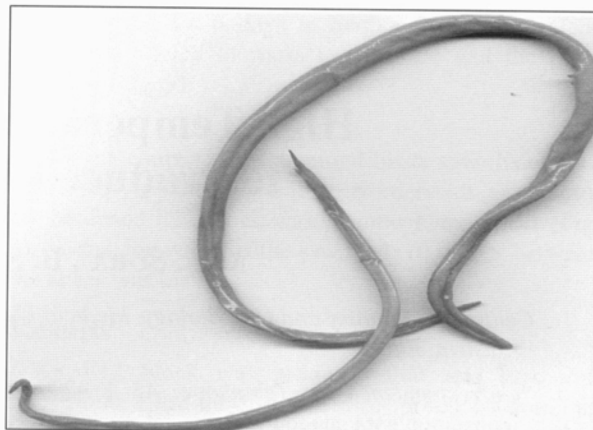

Figure 1. Plastinated Ascarid.

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High Temperature E12 Plastination to Produce Ultra-thin Sheets

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Abstract: The E12 method of plastination classically is used to create thin 3 - 5 mm transparent slices. If thinner slices, 0.5 - 1.0 mm are desired, it is necessary to use an ultra-thin slice plastination method. By using this method, the specimen is first plastinated as a block and then cut into the thinner slices The impregnation temperature and the percent accelerator (E600) are the key elements necessary to obtain proper impregnation of the desired tissue block and contrary to all other plastination methods high temperature (30 to 60°C) is desired. The main goal of this paper is to describe the use of high temperature for processing ultra-thin (1 mm) epoxy plastinated slices. Only by using high temperature is the polymer thin enough to penetrate into the middle of a large processed specimen.

Key Words: **plastination; epoxy; E12; E6; impregnation; temperature; ultra-thin; slices**

Introduction

One of the critical factors of plastination is temperature. Usually, during dehydration, degreasing or E12 impregnation of slices, the temperature does not exceed room temperature values. High temperature, up to 45°C is only used during curing. The question is: "Why high temperature is not used?" High temperatures are not used during dehydration and degreasing because high temperature increases shrinkage and during impregnation the E12/E1 impregnation mixture becomes very viscous within hours, preventing complete impregnation. The exception is E12/E1 block impregnation with curing using E6 hardener which utilizes high temperature (60°C).

The usual steps in E12 plastination are: preparing thin slices (3-5mm) of the desired specimen, cold dehydration, degreasing, impregnation and finally curing (von Hagens, 1985; Weber and Henry, 1993; Cook, 1996; Cook, 1997; Fasel, 1988; Ann, 1999; Lane, 2000; Sora, 2002; Sora, 2004). However, ultra-thin slice plastination produces slices with a thickness of 1 mm or less after inpregnation (Fritsch, 1991; Seibold, 1991; Sittel, 1996; Johnson, 2000; Windisch, 2001). Therefore, after the specimen has been dehydrated, it is impregnated, cured and finally sawed to obtain the ultra-thin E12 slices. The main goal of this paper is to present the utility of high temperature in processing and creating cured E12 blocks for sawing ultra-thin plastinated slices. One of the greatest problems during plastination of bigger tissue blocks is to get the E12 polymer into the middle of the block. At room temperature, the E12 polymer is a liquid but gets thicker when temperature is decreased. If temperature is increased, the E12 polymer gets thin, almost like water. However, once theE12/E1 reaction mixture is prepared, increased temperature hastens the reaction of the E1 hardener with the E12 and after a short time the polymer mixture becomes too viscous to impregnate tissue. Therefore a different hardener, E6, is used in

making E12 blocks. The Biodur E6 hardener is an anhydride-based hardener of low viscosity (von Hagens, 1985) which permits impregnation of the specimen at 30°C to 60°C with no immediate thickening of the polymer reaction mixture.

Materials and methods

Material, dehydration and degreasing:

One male unfixed human cadaver ankle was used for this study. The foot and the distal third of the tibia were removed. The foot was positioned in 90° dorsal flexion and frozen at -80°C for one week. Next, a tissue block containing the ankle was produced for plastination by removal of the tissue 4cm distal to and 5cm proximal to the tip of the lateral malleolus. The ankle block was placed into a -25°C freezer for two days and then submerged in 25 liters of cold (-25°C) technical quality 100% acetone for dehydration. This dehydration bath was changed after 4 weeks and had been diluted to a concentration of 92%. The second dehydration bath was for 3 weeks and the final concentration was 97%. The third and final dehydration bath was for two weeks and had a final reading of 99%. When dehydration was finished, the freezer was disconnected allowing the temperature to increase to room temperature (15°C). The warmed acetone was changed for room temperature methylene-chloride (MeCl) for degreasing. Degreasing was complete when the adipose tissue became transparent after 4 weeks (Table 1).

Impregnation:

The dehydrated/degreased specimen was removed from the methylene chloride bath and submerged in E12 impregnation mixture [E12(polymer)/E6(hardener)/ E600(accelerator) (100/50/0.2)] (von Hagens, 1985) in a Heraeus VT 6130 M vacuum drying oven (Haraeus Instruments, Kendro Laboratory Products GmbH) at 30°C. No vacuum was applied until the next morning in order to allow equilibration and penetration of the E12 mixture. To commence impregnation, the next day vacuum was applied and stabilized at 40cm Hg pressure at +30°C. From this point, pressure was continuously reduced, 8cm Hg daily, over five days until 2 mm Hg was reached. Temperature was kept at 30°C the first four days and on the last day the temperature was increased to 60°C (Table 1).

Casting and curing:

After impregnation, the specimen (Fig. 1) was removed from the vacuum oven and placed in a mold built of Styrofoam and lined with polyethylene foil (Fig. 2). The mold was filled with the polymer reaction-mixture [E12/E6/E600 (100/ 50/ 0.2)]. The filled mold, containing the impregnated specimen, was placed in an oven at 65°C for 4 days to harden the polymer. The block was cooled to room temperature and the mold removed (Fig. 3).

Slicing:

Using a contact point diamond blade saw, Exact 310 CP (Exact Apparatebau GmbH, Norderstedt, Germany), the hardened E12 block was cut into 1 mm slices (Fig. 4). Tissue, the width of the saw blade (0.4mm), between the slices was lost.

Results

An E12 block was produced that was hard and transparent. Ultra-thin, <1 mm slices produced from this block were transparent and hard with good optical qualities. The finished E12 slices provided excellent anatomic detail down to the microscopic level.

Discussion

Impregnation is one of the main steps in plastination. The main goal is to impregnate the specimen thoroughly. This requires that the resin/hardener-mixture be thin enough to penetrate the specimen and also have a processing time of sufficient length to penetrate the depths of the specimen. The viscosity of epoxy resin varies markedly with temperature. At low temperature the viscosity of the resin is high, while high temperature yields a resin of low viscosity. During impregnation at low temperatures, 5°C or less, epoxy becomes viscous. However, low temperature prolongs the processing time. This prolonged processing time with the standard E12 method (E12 polymer mixed with E1 hardener) is the reason that impregnation is preferably performed at 5°C for 2 days. But by using this method, only slices of an average thickness of 3-5mm can be impregnated because of the rapidly increasing viscosity of the reaction-mixture.

The epoxy technique described in this work produces slices by sawing 1 mm (ultra-thin) plastinated plates from an E12 specimen block. Therefore, a large tissue block, with a thickness of 10cm, is impregnated rather than thin, 3-5mm slices. In order to impregnate such a tissue block, the reaction-mixture must have a low viscosity and the impregnation time must be prolonged. This is achieved by using the E6 hardener and by increasing the temperature during impregnation. The processing time of the E12/E6 reaction-mixture depends on the quantity of accelerator E600 and on the temperature. High temperature and increased quantity of E600 both lead to faster polymerization and a decrease in the processing time. Before starting impregnation, the dehydrated and degreased tissue block is submerged in the reaction-mixture for at least 8 hours, to allow the E12/E6/E600 mixture to equilibrate

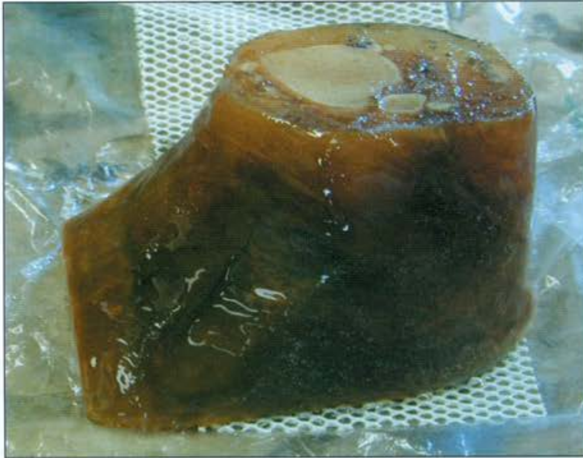

Figure 1. Impregnated E12 ankle specimen.

Figure 2. Building up an adequate mold for the specimen.

Figure 3. Cured E12 block.

Figure 4. Cross section at the level of the tibio-fibular joint.

and begin penetration into the tissue block. As well, some volatile intermedium (methylene chloride) escapes from the specimen and lowers the viscosity of the reaction-mixture. The viscosity of the E6 hardener is high, and for this reason the E12/E6/E600 mixture is quite viscous at room temperature. Therefore, impregnation is started at 30°C. At this temperature, the reaction-mixture viscosity remains low for the next few days, since viscosity decreases with increase of temperature.

The first four days, impregnation is performed at 30°C and on the fifth (last) day the temperature is increased to 60°C to aid extraction of the remaining volatile intermedium from the specimen and to aid influx of the polymer reaction-mixture. Since increasing the temperature thins the E12/E6/E600 reaction-mixture, extraction of MeCl from the impregnation bath is easier. As well, the lower viscosity impregnation mixture penetrates the tissue block easier.

This kind of temperature/vacuum regulation is performed easily in a vacuum drying oven, where simultaneous adjustment of vacuum and regulation of temperature can be performed. The use of 60°C on the last day must be monitored carefully. At this temperature, the E12/E6/E600 mixture at first becomes thinner; but after several hours the polymer-mixture becomes thicker and bubbles rise slower and splash intensely marking the onset of polymerization.

Polymerization of E12 is dependent on the chosen hardener, temperature and percent of accelerator. For the impregnation of large tissue blocks, the E6 hardener was used which provides a longer impregnation time. Hence, by varying the amount of accelerator used, the length of time available for impregnation can be

significantly altered. Using 0.2% of accelerator E600 in the E12/E6 mixture was sufficient to assure proper impregnation and curing of the block. By omitting the E600 from the E12/E6 mixture, polymerization of the block will not occur, even if temperatures of 60°C are maintained for several months (von Hagens, 1985). The plastination folder is the only source which lists, in a table, the reaction time and hardening of E12/E6/E600 mixtures. All suggested percentages of E600 have been tested and are correct. The end product, using this protocol, was an E12 block that was firm and transparent. Such blocks are necessary to cut ultra-thin slices (<1 mm).

Ultra-thin slices <1 mm are essential if the histology is to be studied on plastinated slices or if 3D reconstruction is desired (Sha, 2001; Qiuet, 2003). These ultra-thin slices can only be cut from a solid E12 block. Therefore, knowledge of controlling temperature and percent of accelerator in the ultra-thin sheet plastination method is essential. Histological examination can be performed up to a magnification of 40X. Greater magnification is not possible due to the thickness (>300 microns) of the specimen (Sora, 2002). Slides can be stained by the usual methods for histological specimens (Gruber, 2001). The major advantage of this method is that the structures remain intact and the decalcifying of bony tissue is not necessary. Plastination allows the topography of structures to be studied in a non-collapsed and non-dislocated state. Therefore, morphological measurements can be performed easily and accurately.

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Influence of Formaldehyde/phenol Fixation on MRI of the Stifle Joint and Correlation with Plastinated Slices

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Abstract: To evaluate the effect of embalming and plastination on the magnetic resonance image quality of tissue, a human cadaveric knee was examined was subjected to these processes prior to imaging. Sagittal images were obtained using 4 different pulse sequences: T1-weighted spin echo; T2 weighted spin echo; T2-weighted turbo spin echo and proton density images. Images were made prior to embalming, post embalming and following plastination. Embalming was found to cause considerable swelling of the soft tissues and induced fluid accumulation in joint spaces. Moreover, a general decrease of image quality was noted in all pulse sequences caused by a loss of tissue contrast (homogenization of the image). However, when compared to the sheet plastinated corresponding slices, no important anatomical information was lost. Although there is a notable effect, the embalming process does not significantly affect the interpretation of gross anatomical structures on magnetic resonance imaging.

Key words: **plastination; formaldehyde; phenol; MRI; stifle**

Introduction

Three-dimensional multi-planar imaging techniques, such as magnetic resonance imaging (MRI), have become indispensable tools for the diagnosis of disease. MRI is a technique based on the relative response of specific protons to absorb radio frequency energy. Like conventional radiography the obtained image is a function of density, in this case protons. However, the image is influenced by other physical factors such as the ability to re-emit the absorbed radio frequency signal and flow phenomena. Moreover, no ionising radiation is involved.

As a result of the implementation of these techniques, anatomy lessons at medical schools focus more often on the knowledge of cross-sectional anatomy. This is important as most medical students will have to interpret these images in their future profession. The introduction of sheet plastination has provided an opportunity to combine modern cross-sectional imaging techniques with corresponding plastinated slices of human tissue (von Hagens, 1987; McNiesh and von Hagens, 1988; Hussain et al., 1996; Cook and Al-Ali, 1997; Entius et al., 1997; Magiros et al., 1997) and animal tissue (Henry et al., 1997).

Due to a relative shortage and associated bio-hazards of working with fresh cadaver material, it would be beneficial if embalmed material could be used for these purposes. However, MRI depends on proton densities and it is yet unknown whether and how embalming might affect MRI results. Batista and colleagues (1990) performed an MRI study on fixed tissues and effects of plastination and curing on MR images but did not take pre-embalming

images. The aim of this study is to compare MR images obtained prior to and after embalming with their corresponding sheet plastinated slices to determine if fixation alters the MR image.

The genual joint was chosen because it contains many distinct and spatially separate anatomical structures which are clinically important and we believe will best demonstrate any changes due to embalming.

Materials and methods

Specimen preparation

A fresh cadaver lower limb, cut 20 centimeters above the genual joint, was obtained within 48 hours after death. The specimen was mounted on a perspex plate. Two plastic needle markers, which are visible on the MR images, were drilled into the midline of the distal femur and proximal tibia (Fig. 1). Using these pins as markers, enabled obtaining identical imaged to be made both prior to and after embalming. The initial MR images were produced following placement of the markers and before embalming.

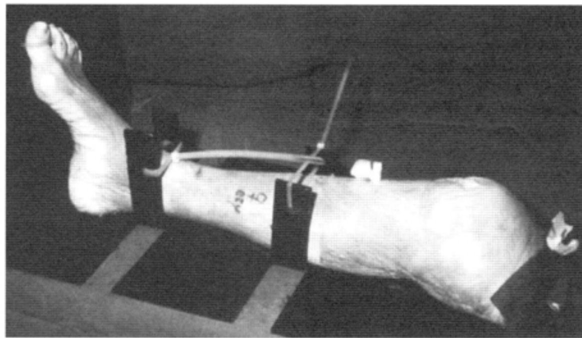

Figure 1. Specimen mounted on a perspex plate with cod-liver oil markers in place.

The specimen was then embalmed by vascular perfusion of the superficial femoral vessels within 60 hours of death using a solution containing: 50 g Phenol 99%, 20g $MgSO_4$, 20 g $NaSO_4$, 10 g NaCl, 60 ml formaldehyde 37%, 60 ml glycerine, H_2O ad 1000 ml (Kleinrensink, 1995). After embalming, a second set of MR images was produced. Following the production of the second set of MR images, the specimen was cut with a bandsaw into 2mm thick sagittal slices along the MRI scanplane. Subsequently the slices were dehydrated in 2 changes of cold aceton. After complete dehydration the slices were plastinated according the von Hagens E12 technique described by von Hagens (von Hagens, 1985; von Hagens, 1987; Weber and Henry, 1993). The exact position of the slices that are presented in this study (mid-sagittal and lateral) is depicted on a three-dimensional CT model (GE pro-Speed scanner GE Medical Systems Milwaukee, Wis.USA) Slice thickness was 3 mm, pitch 1.0, 120KV, 100 mA and 2 second scan time.

Anatomical drawings were made by scanning the plastinated slices on a commercially available flatbed scanner followed by computerized elaboration using computer software (Coreldraw)

Magnetic Resonance Imaging

Contiguous MR images were produced with a slice thickness of millimetre along the plane defined by the markers in the specimen, prior to and following embalming, with a Philips Gyroscan NT 1.0 Tesla (Philips Medical Systems, Best, the Netherlands). Four different sequences were used. These sequences were chosen either because of their ability to demonstrate anatomical detail or because they are commonly used in clinical practice. The sequences parameters were: 1. Spin echo technique, T_1 weighted (TR 550, TE 16): anatomy; 2. Spin echo technique, T_2 weighted (TR 1965, TE 80): pathology especially edema and effusions; 3. Spin echo technique, proton density (TR 1965, TE 15): pathology, especially menisci and ligaments and 4. Turbo spin echo technique, T_2 weighted (TR 3750, TE 95, TSE factor 9): identical to technique 2 only with significantly shorter acquisition times (in this study, 3 versus 13 minutes).

Pre- and post-embalming MR images were compared with the plastinated slices. Overall signal intensity and tissue contrast was assessed, with special attention to anatomical structures such as cruciate ligaments, menisci, cartilage and the ability of MRI to distinguish these structures from the surrounding tissues before and after embalming.

Results

In this study, the plastinated tissue slices were compared with the corresponding MR slices at 2 levels, mid-sagittal and lateral (Fig. 2).

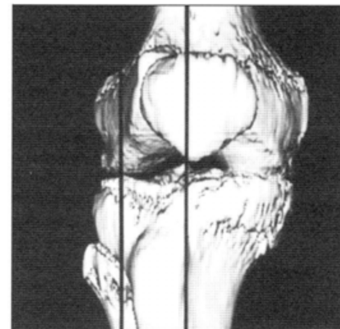

Figure 2. Three dimensional computed tomography image of the knee. The origin of the two slices is demarcated with a line.

Figures 3 and 4 demonstrate the grossly visible structures located within these two regions: plastinated tissue slices (Figs. 3a, 4a) and MR images prior to and

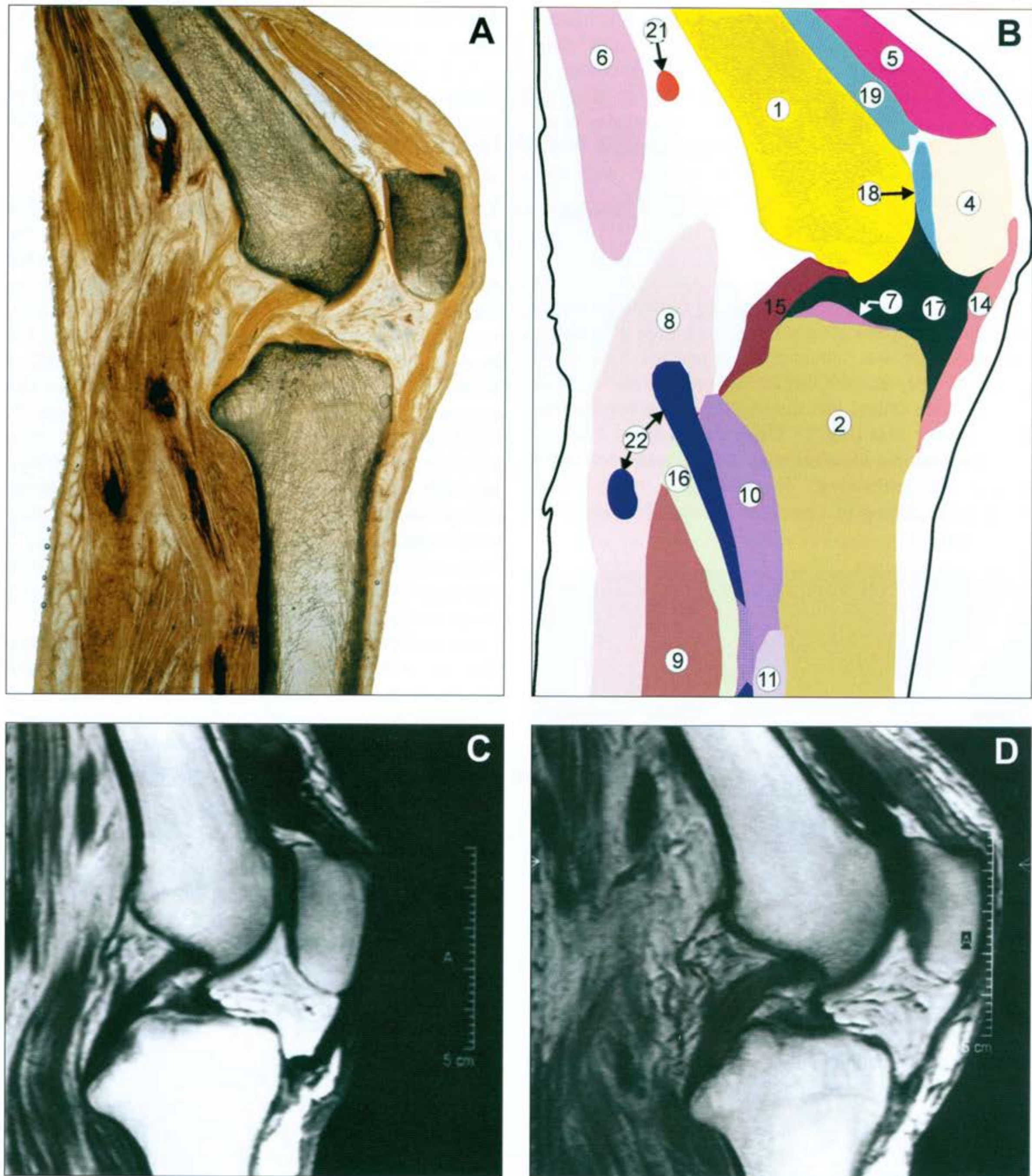

Figure 3 a. Plastinated mid sagittal slice, **b.** anatomical drawing: 1. femur, 2. tibia, 3. fibula, 4. patella, 5. tendon of m. quadriceps femoris, 6. M. semimembranous, 7. M. plantaris, 8. M. gastrocnemius (lateral head), 9. M. soleus, 10. M. popliteus, 11. Posterior tibial muscle, 12. Anterior tibial muscle, 13. Vastus lateral muscle, 14. Patellar ligament, 15. Posterior cruciate ligament, 16. Anterior cruciate ligament, 17. Infrapatellar fat body, 18. Suprapatellar bursa, 19. Adipose body, 20. Lateral meniscus, 21. Popliteal artery, 22. Popliteal vein, **c.** T_1 weighted spin echo image prior to embalming, **d.** T_1 weighted spin echo images after embalming.

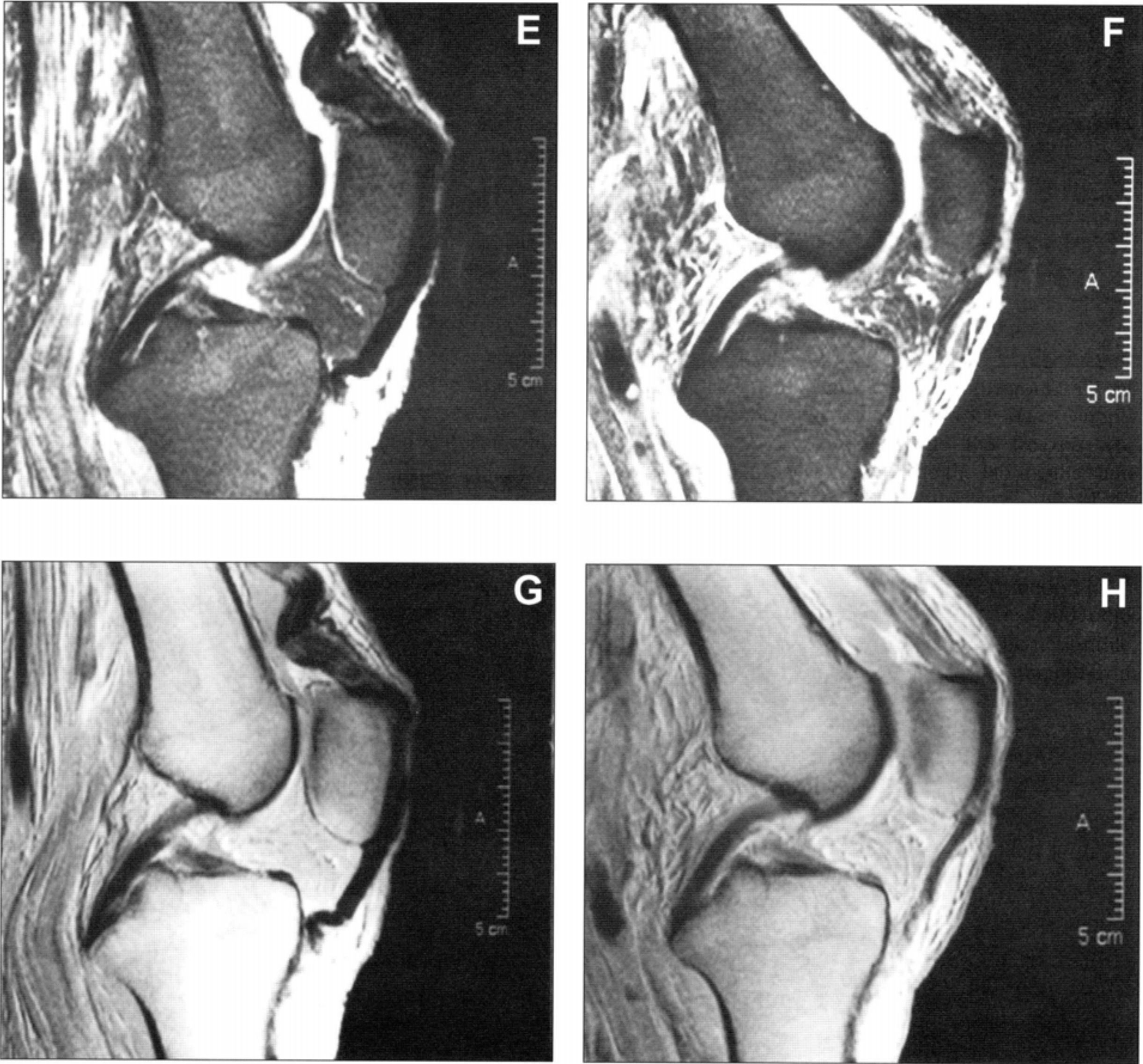

Figure 3 e. T_2 weighted spin echo image prior to embalming, **f.** T_2 weighted spin echo image after embalming, **g.** proton density spin echo image prior to embalming, **h.** proton density spin echo image after embalming.

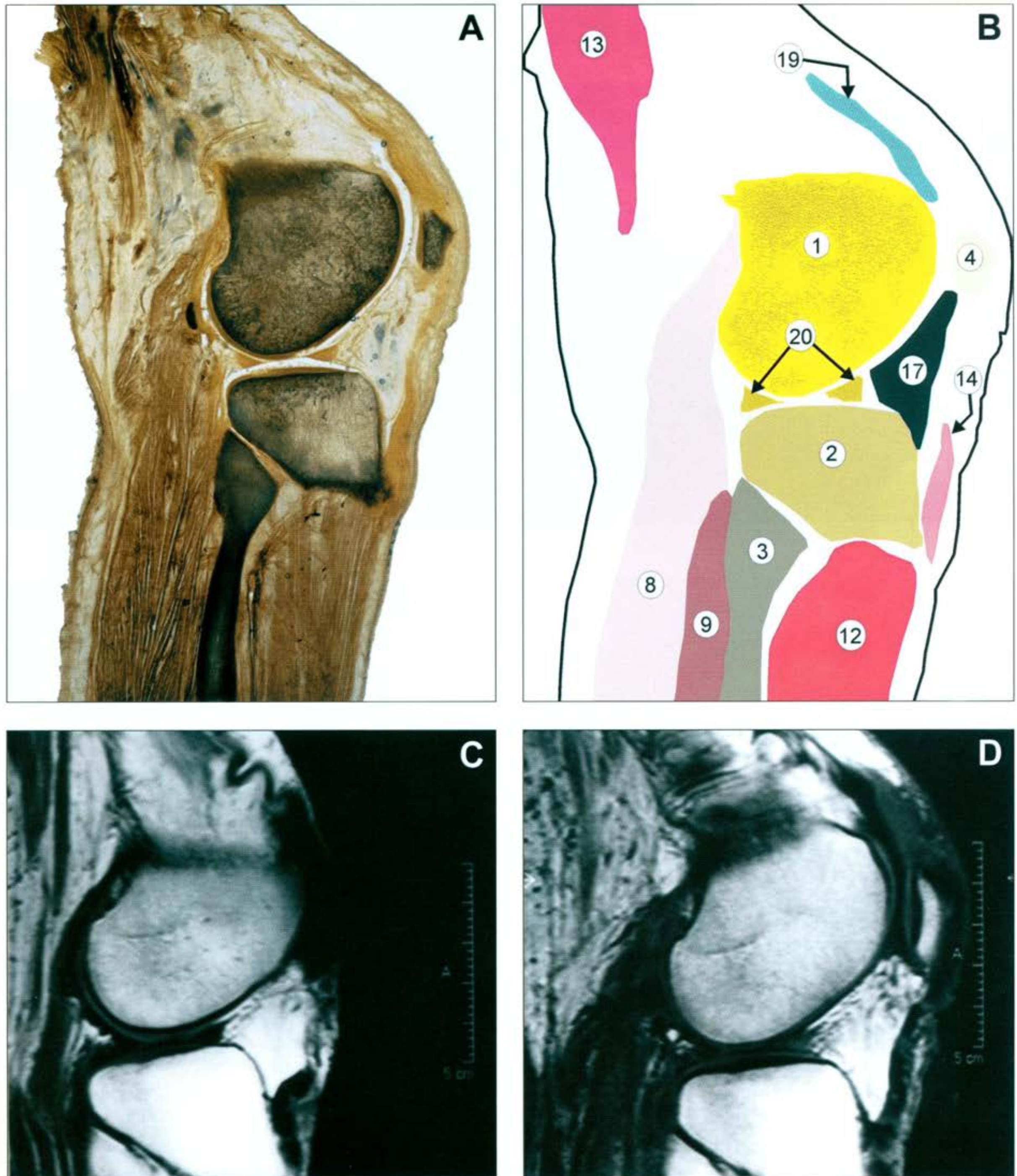

Figure 4 a. Plastinated lateral sagittal slice, **b.** anatomical drawing: 1. femur, 2. tibia, 3. fibula, 4. patella, 5. tendon of m. quadriceps femoris, 6. M. semimembranous, 7. M. plantaris, 8. M. gastrocnemius (lateral head), 9. M. soleus, 10. M. popliteus, 11. Posterior tibial muscle, 12. Anterior tibial muscle, 13. Vastus lateral muscle, 14. Patellar ligament, 15. Posterior cruciate ligament, 16. Anterior cruciate ligament, 17. Infrapatellar fat body, 18. Suprapatellar bursa, 19. Adipose body, 20. Lateral meniscus, 21. Popliteal artery, 22. Popliteal vein, **c.** T_1 weighted spin echo image prior to embalming, **d.** T_1 weighted spin echo images after embalming.

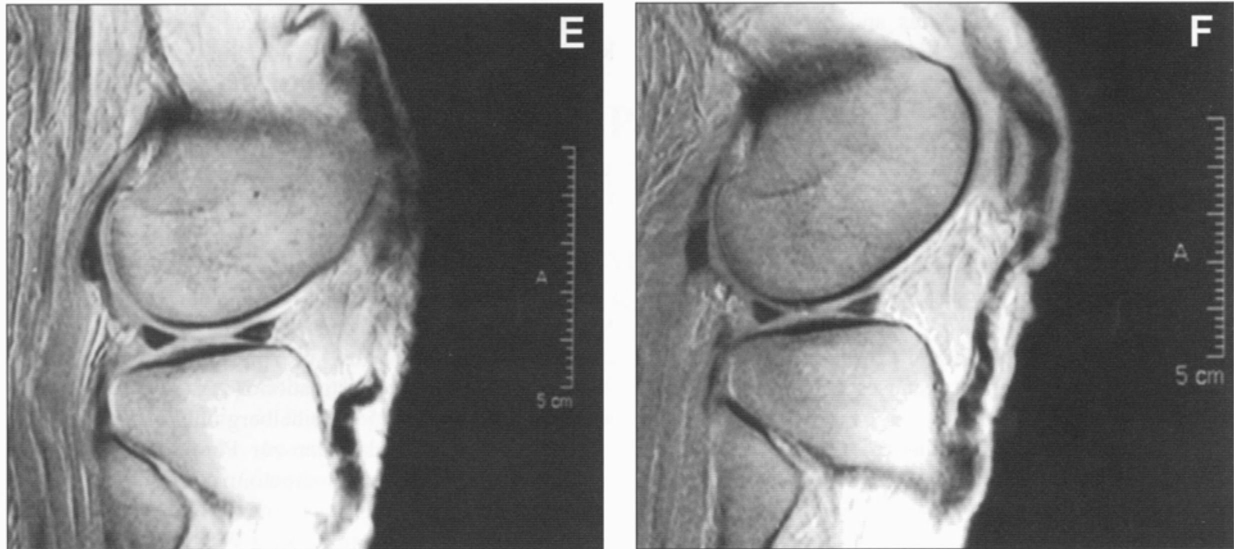

Figure 4 e. proton density spin echo image prior to embalming, **f.** proton density spin echo image after embalming.

after embalming (Fig. 3c-h, 4c-f). The most striking effect was a significant swelling of the soft tissues that occurred due to the embalming technique. This caused some flexion of the knee, despite tight fixation on the perspex plate (Fig. 3c-h, 4c-f). Additionally, the amount of fluid in the suprapatellar recess of the knee joint increased leading to stretching of the tendon of insertion of the quadriceps muscle (Figs. 3e and f). While the diameter of the cruciate ligaments increased, however, their position and general shape remained unchanged. Moreover, signal intensity of the cruciate ligaments increased on T1-weighted images and proton density images (Fig. 3c-d, g-h). The musculature around the knee showed significant swelling but differentiation between muscle and surrounding soft tissues was still possible (Fig. 4c-d). The menisci showed some increase in signal intensity on T1-weighted and proton density images but otherwise its appearance did not change (Fig. 4c-f).

As a result of embalming T_2 weighted spin echo and T_2 weighted turbo spin echo images showed a diffuse increase in signal intensity. T_1 weighted spin echo images and proton density images showed a diffuse decrease in tissue contrast both resulting in a deterioration of image quality compared to the pre-embalming images. The image quality in all four techniques decreased due to a homogenization of signal intensity.

For this study we present three techniques which show either an increase, a decrease or no change in signal intensity (T_2 weighted spin echo are not shown as they resemble T_2 weighted turbo spin echo images). Figures 3 and 4, c through h, show MR images prior to and after embalming. For clarity line drawings are presented of the anatomical region according to a method we previously presented (Figs. 3b, 4b) (Entius, 1997).

Discussion

The embalming process deteriorated the quality of the MR images in four pulse sequences. However, for teaching purposes this degradation was not significant. Also correlation with plastinated slices was still possible. Our results suggest that embalmed material can be used in correlation studies and for educational purposes.

The difference in flexion of the knee before and after embalming was caused by diffuse swelling of the soft tissues around the knee joint, caused by the embalming process in which a large quantity of fixating fluid is administrated through the femoral artery. In our opinion this did not affect the comparison between pre- and post-embalming images.

Although the degradation effect of the embalming process on the MR images does not significantly affect the interpretation of gross anatomical structures in the musculoskeletal system, further research is necessary to study the effect of embalming on microstructures and on other organ systems.

For educational purposes with regard to multi-planar visualization embalmed material can be imaged with MR imaging without significant loss of information compared to in vivo anatomy.

Acknowledgments: The authors wish to thank Teun Rijsdijk for his excellent assistance in preparing the photographs.

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Sheet Plastination with Polyester: An Alternative for All Tissues

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Abstract: Classically, the application of polyester polymer in plastination (P40 and P35, Biodur™) has been the production of brain or head slices. Recently semi-transparent body slices have been produced in our labs using P40. No literature was found on the production of body slices using P40. The purpose of this study was to develop a protocol for using P40 to produce slices from all regions of the body. Two millimeter slices were cut from all regions of the body of a cat. The slices were processed using a modified P40 technique. Compared to the E12 method, the P40 technique offers not only comparable results, but also some extra advantages. Slices do not yellow, the method is easier for the beginner and an indefinite pot life of the impregnation bath. The P40 plastinated body slices yielded excellent anatomical detail of all tissues that were observed. In addition, they have been excellent aids for teaching and research in our class rooms and laboratories.

Key words: **plastination; polyester; P40, sheet; section**

Introduction

Classically the epoxy E12 (Biodur™) method has been used to produce body slices (von Hagens, 1979; 1985; von Hagens et al., 1987; Weber and Henry, 1993). However, the epoxy method has two main limitations: 1. Short period of time available to cast the impregnated slices (von Hagens, 1985; Latorre et al., 2002 a, b; Reed et al., 2002) and 2. Yellowing of E12 plastinated slices (Latorre et al., 2002a). P40 is used routinely for preserving brain sections (von Hagens, 1994; Barnett, 1997a, b; Henry, 1998; Sora et al., 1998a; Weiglein and Feigl, 1998; Henry and Weiglein 1999; Sora et al., 1999) and one use was for production of three-dimensional peripheral nervous tissue specimens, the brachial plexus (Sora, 1998; Sora et al., 1998b). Some authors suggest that P40 may also be used for production of transparent body slices (Barnett, 1997b; Weiglein and Feigl, 1998) but no protocol or examples were given. However, using P40 for producing body slices (other than head slices) has not been reported until recently when Latorre and colleagues (2002b) prepared equine tarsal joint slices using P40.

In this work, a technique is described to produce slices of any portion of the body using P40. Hence P40

is a viable alternative to the E12 method, for preserving semitransparent sections of the body.

Materials and methods

Tissues

An unembalmed feline cadaver had the hair clipped, was cleaned and frozen at -70°C. After freezing, it was divided into five regions (head, neck, thorax, abdomen and pelvis) (Fig. 1). Serially, two transverse slices, two millimeters thick and one transverse slice, one centimeter thick were cut from each block in a repetitive manner. The number of slices for each region is detailed in figure 1. Slices were placed on aluminum grids and sawdust was removed by submerging them in cold acetone and scraping or flushing with a stream of cold water prior to submersion in cold acetone. A modification of the P40 technique (von Hagens, 1994) was used to plastinate the 2mm sections. The 1cm sections were Plastinated using the standard silicone technique (von Hagens, 1985; von Hagens et al., 1987; Henry and Nel, 1993; Weiglein and Henry, 1993).

Dehydration

Dehydration was by freeze substitution. Cleaned slices on their grids were initially submerged in 90% acetone at -15°C. The approximate acetone to specimen ratio was 10:1. Two changes of 100% cold acetone were carried out at weekly intervals.

Forced Impregnation

After dehydration, the slices were impregnated using P40 (Biodur™) at room temperature. The specimens and grids were immersed into P40 polymer with no hardener or additives. After 24 hours, the immersed specimens and grids were placed in the vacuum chamber and pressure was decreased over a 24 hour period to 10mm Hg while maintaining a rapid boil. Vacuum was held at 10mm Hg for 5 hours until bubbling had greatly diminished. Vacuum was released and the submerged specimens returned to ambience. During the entire period of using P40, the polymer and the polymer with the slices were protected from light by using covered dark containers and a dark cover on the

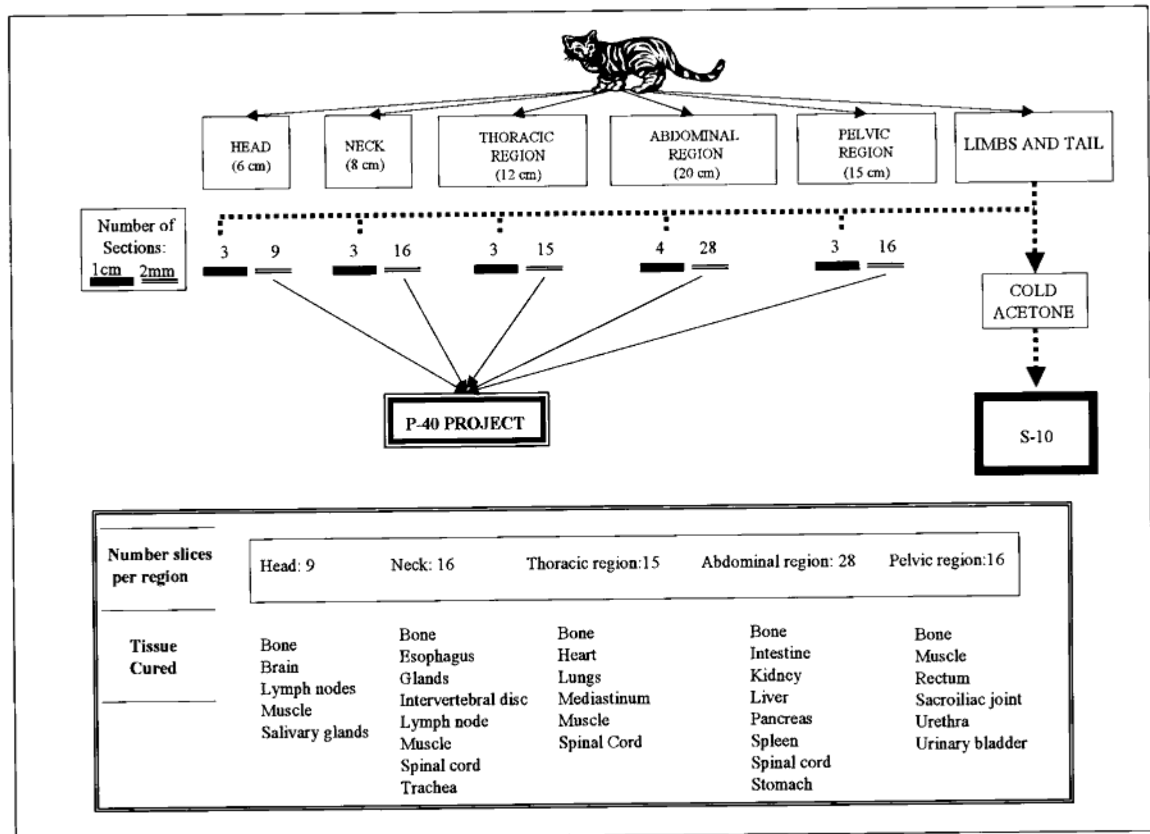

Figure 1. Regional schema of transverse slices indicating number of slices plastinated by both techniques (silicone - 1cm; polyester - 2mm) after cold acetone dehydration and chart indicating tissue cured in each region.

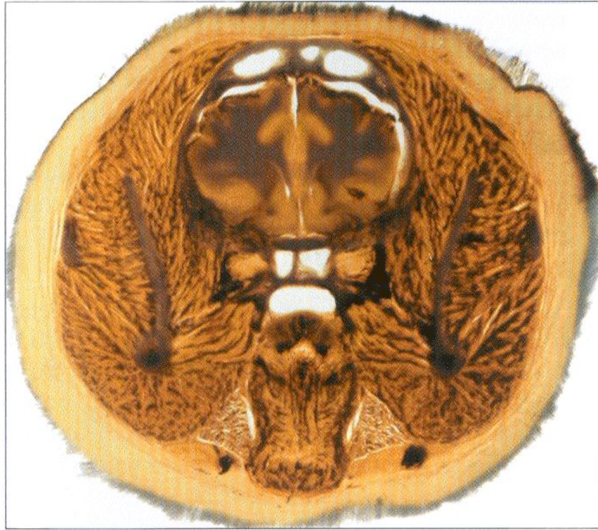

Figure 2. Polyester head section. Caudal view.

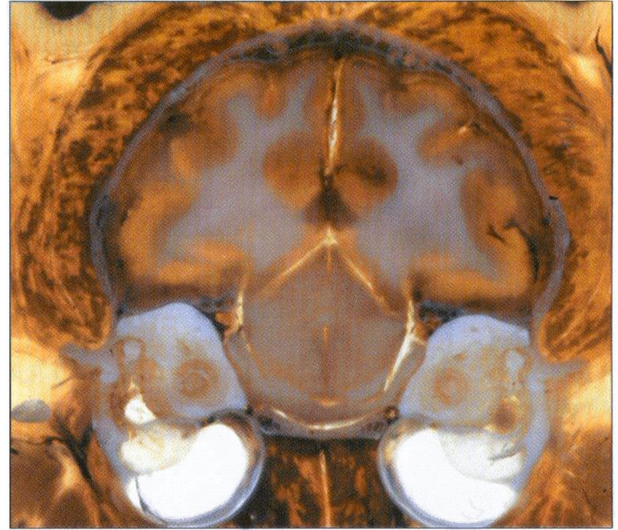

Figure 3. Close-up of polyester head section. Caudal view.

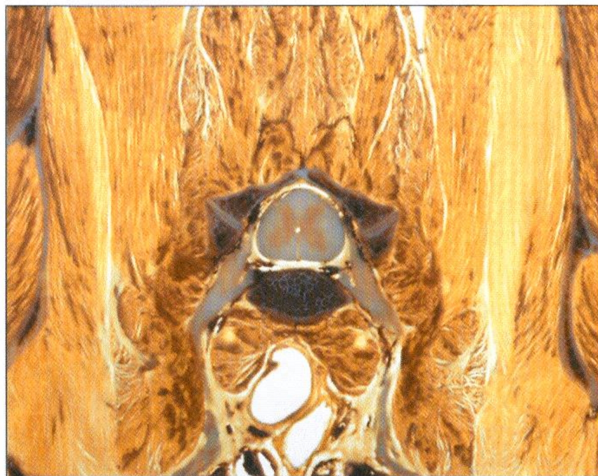

Figure 4. Polyester cervical region section. Cranial view.

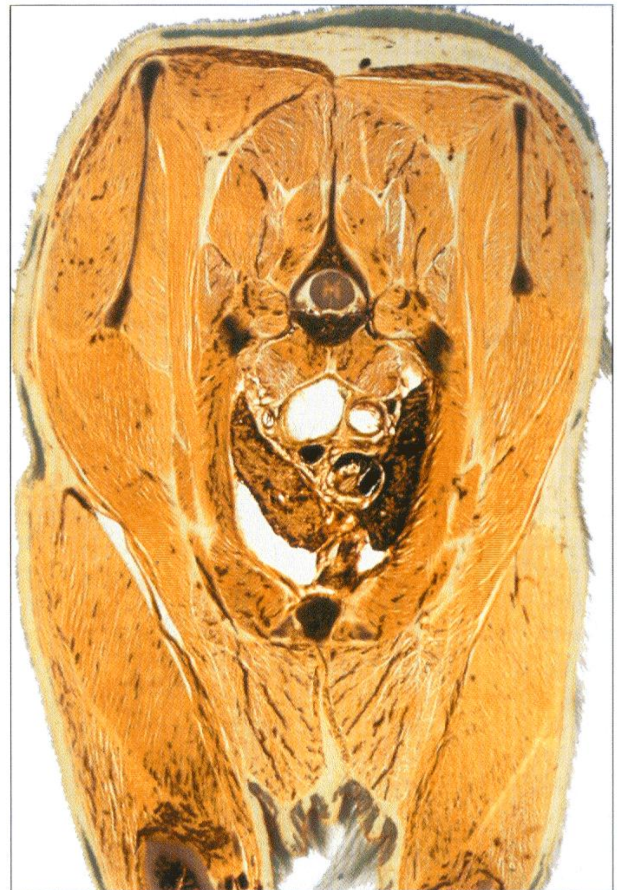

Figure 5. Polyester cranial thoracic region. Caudal view.

vacuum chamber. The container with the impregnated slices was returned to the freezer (-15°C) to hold until casting.

Casting and curing

Flat chambers were made from 2 sheets of 2 or 3mm tempered (hardened) glass, 6mm silicone gasket (HS06-1 Biodur™) and fold back clamps. Each impregnated slice was removed from the impregnation bath and placed in a flat chamber. Two ball bearings (3mm) were inserted into each chamber. The chambers were filled with fresh P-40, the gasket closed across the top of the flat chamber, and sealed with Biodur sealant (HS 80 Biodur™). The slices were centered in the sealed flat chambers by using a magnet to push the bearings against the slice (Barnett, 1997a) thus pushing the slice to the desired position. The bearings were parked at the perimeter by the gasket. The slices were laid flat (horizontal) on a supporting rack between two UV-A light units. Each UV light unit had 2 x 40 watt bulbs such that 2 bulbs were on each side of the flat chamber. The UV light serves as the catalyst for polymerization of the polyester polymer. To avoid excess heat build up during the curing phase, a thermostatically controlled switch was used to switch off the UV-A lights when the surface of the chambers reached 30°C. A fan (ventilator) was used to blow air continually over the chambers to cool them. Curing time was 30-60 minutes. After curing was completed, the clamps and the gaskets were removed and the glass chambers dismantled. The cast slices were wrapped in foil and the excess polyester was trimmed from the edges using a band saw.

Results

The manufactured thin (2mm) tissue slices were semi-transparent and yielded excellent anatomical detail (Figs. 2, 3, 4, 5, 6, 7, 8). After curing, the polyester around the tissue was transparent and no yellowing was detected in any slices two and one half years later. The fat tissue was semi-transparent, and the other tissues or organs were significantly highlighted against the cleared fat. There were no problems impregnating or curing any type of tissue. All of the slices hardened after curing, even when the surface of the tissue slice was close to the glass of the flat chamber.

All P40 and silicone (Fig. 9) plastinated body slices yielded excellent anatomical detail of all regions of the body that were observed. The main organs and tissues checked in each region were the following:

Head slices: bone, brain, lymph nodes, muscle, salivary glands.

Neck slices: bone, esophagus, glands, intervertebral disc, lymph nodes, muscle, spinal cord, trachea.

Thoracic region: bone, heart, lungs, mediastinum, muscle, spinal cord.

Abdominal slices: bone, intestine, kidney, liver, pancreas, spleen, stomach, spinal cord.

Pelvic slices: bone, muscle, rectum, sacroiliac joint, urethra, urinary bladder.

These slices have been excellent teaching aids in our class rooms and aids in our research and laboratories.

Discussion

The cast tissue slices provided a high degree of detail and permitted visualization of the various body structures in the normal topography of the region. Also the semi-transparency of the specimens allows viewing at the submacroscopic level. These characteristics are similar to those described for tissue slices produced by the E12 technique (Entius et al., 1993; Cook, 1997).

Methylene chloride was not used as an additional step for the lipid extraction. Even so, much of the fat tissue was semi-transparent in the polyester slices. This was likely due to the thinness of the slices, 2mm, which allowed much of the lipid to be extracted in the cold during the two-week dehydration. Similar to recent literature, impregnation used only P40 polymer (von Hagens, 1994; Barnett, 1997a; Sora, 1998; Latorre et al., 2002b) and no A4 hardener was added as currently recommended (Henry, 1998). By using no hardener or additives with the polyester, there is no time limit for casting the impregnated slices. Also the impregnation solution may be reused for another impregnation. With respect to the impregnation temperature, Sora and coworkers (1998, 1999) found that slices processed at -25°C showed 2.48% less shrinkage than slices impregnated at room temperature. For such an anatomical project as this, the percent shrinkage is deemed small enough to use room temperature impregnation as other authors have (von Hagens, 1994; Barnett, 1997a; Sora, 1998a; Sora et al., 1999; Latorre et al., 2002b) rather than the more cumbersome cold impregnation (von Hagens, 1994; Sora et al., 1999). The lowered pressure used in this work was similar to that used by various authors (von Hagens, 1994; Barnett 1997a; Sora, 1998; Latorre et al., 2002b). Pressure was not allowed to go lower than 10mm Hg, to avoid extraction of monomeric styrene from the impregnation bath polymer (von Hagens, 1994). Our results show that it is not necessary to use the hardener, A4, within the casting solution for any area of the body. This is similar to the results of Barnett (1997a), Sora (1998), Sora and colleagues (1999), and Latorre and co-workers (2002b). Upon introduction of P40 to plastination, only UV-A light was used to cure the polyester. After a few years, a hardener, A4, was introduced to assure curing of P40

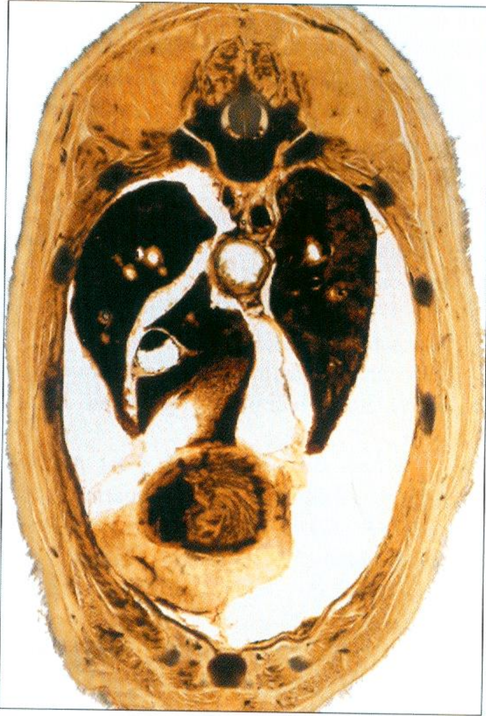

Figure 6. Polyester caudal thoracic region section. Cranial view.

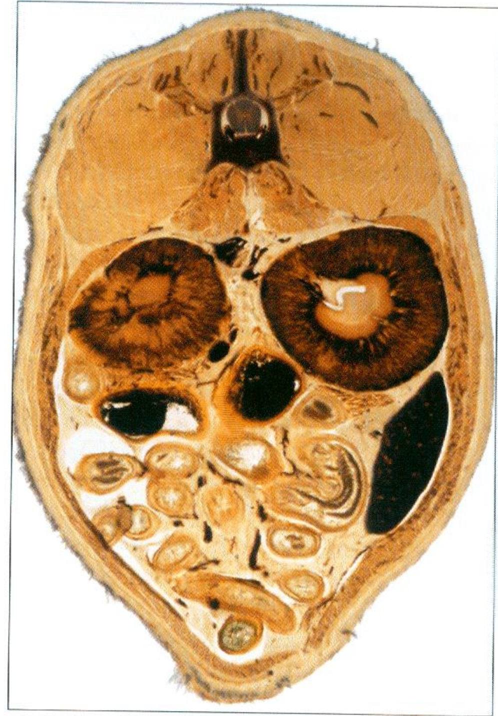

Figure 7. Polyester caudal abdominal region section. Cranial view.

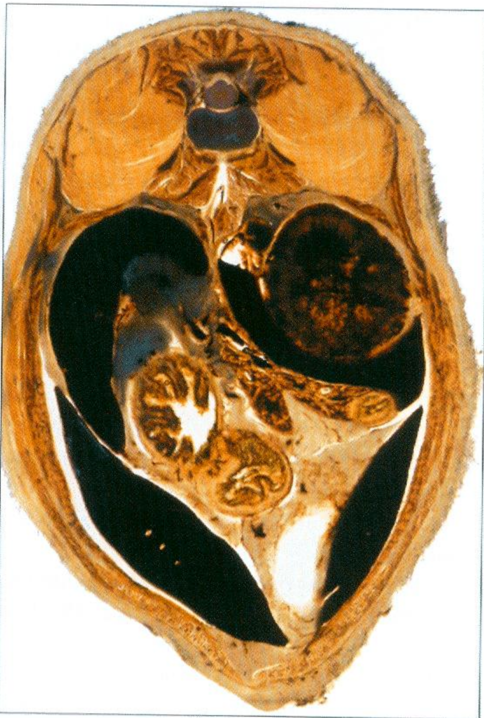

Figure 8. Polyester cranial abdominal region section. Caudal view.

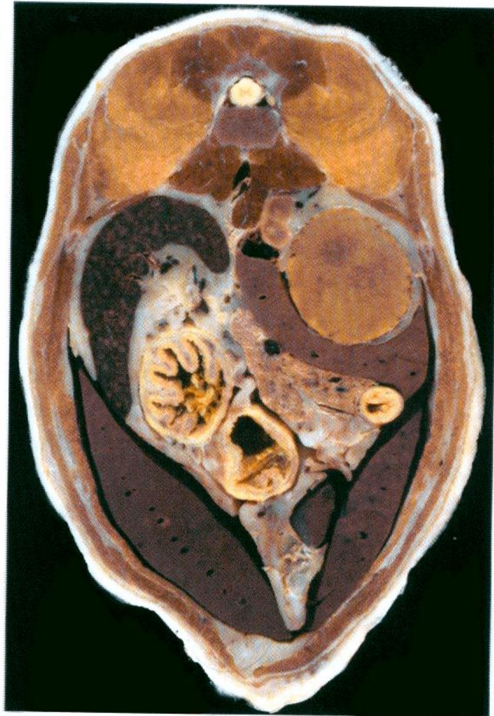

Figure 9. Silicone cranial abdominal region section. Caudal view.

polymer in dense, dark tissues that might not cure uniformly using only UV-A light as the catalyst. Using this chemical activator within the casting solution has produced similar results (Henry, 1998). Both methods have continued to yield acceptable manufactured sheets for several years. The main advantage of using no A4 in the impregnation bath is that both the impregnation time and the casting time may be prolonged indefinitely. For this reason, we chose not to use the A4 hardener. The reported exposure time to UV-A light for curing is variable from 15 minutes to two days [15 minutes (Sora, 1998), 3 hours (Sora et al., 1999), 45 minutes to 4 hours (Weiglein and Feigl, 1998), 5 hours (Sora, 1998), 2 days (Barnett, 1997a). Weiglein and Feigl (1998) suggest that exposure time depends on wattage and on distance of the UV-A lights. Our exposure of 45 minutes of light from four 40-watt UV-A tubes at a distance of 20cm provided adequate exposure time for curing. The problems during light curing (too rapid curing and excess heat build up) reported by other authors (Weiglein and Feigl, 1998) were not encountered. Also the orange spots found in the cortex of the brain slices by others authors (Barnett, 1997a; Henry, 1998; Weiglein and Feigl, 1998) did not appear in our slices.

Compared to the E12 technique the advantages and disadvantages of the P40 technique are:

Advantages:

- There is no time limit in which to cast the impregnated slices.
- Polymer used for immersion and impregnation can be reused.
- Bubbles rise faster to the top of the chamber during casting.
- Curing is via UV-A light.
- Curing time can be shorter and no heat cabinet is necessary.
- Plastinated slices do not become yellow.

Disadvantages:

- P40 is sensitive to both light and warm temperature. Therefore, it is necessary to keep P40 in a cool, darkened environment.
- Orange spots may occur in the brain.
- Flat chambers must be sealed for curing.
- Exposure of polyester to oxygen prevents curing.

The results of this work demonstrate that the P-40 method (Biodur^{TM}) may be used to produce semi-transparent body slices from any region of the body as with the E12 method (Biodur^{TM}). Furthermore, using P40 eliminates the disadvantages associated with the classic E12 technique.

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Silicone Plastination of a Malpositioned Long-term Formalin-fixed Green Iguana

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Abstract: Plastination of a malpositioned, long term-formalin fixed green iguana *(iguana iguana)* was performed to attempt a repositioning of the specimen once impregnated with silicone. The impregnated specimen was able to be brought into proper anatomical position during the curing period. The iguana was preserved without noticeable shrinkage and appeared very natural. The resulting specimen is now a nice representation of its genus.

Key words: **plastination; silicone; S10; formalin; iguana; reptile**

Introduction

The green iguana *(iguana iguana)* is the most common pet lizard whose popularity as a domestic pet is increasing. Caring for a green iguana is difficult because they have special husbandry considerations such as appropriate humidity, temperature and diet. Iguanas often suffer from metabolic disease due to inadequate husbandry. Preservation of green iguanas using plastination is an excellent method for preparation and storage of a reference collection for and indefinite period.

Often, iguana specimens which have been improperly positioned before fixation with preservatives are donated to herpetological collections. Formalin fixed biological tissues are not very pliable and are resistant to repositioning. Specimens in extreme states of distortion tend to make poor examples of living animals and validates the desirability to prepare specimens appropriately before fixation (Henry et al., 1997).

The iguana cadaver selected for this study had been formalin fixed in a contorted position for a period of more than three years. Its head was ventro-flexed such that it was positioned below and between its forelimbs. The iguana's trunk was wrapped with its tail. The dorsal spinous scales were flattened against the trunk. The challenge was to correct the position of iguana after impregnation with silicone before the curing stage to assess if silicone impregnated-formalin fixed tissue is more pliable to bending and repositioning without disruption of the normal anatomy of the biological specimen.

This work describes the complete plastination and repositioning procedure of a green iguana carried out in the Department of Anatomy, Faculty of Veterinary Medicine in Skopje, Macedonia.

Materials and methods

Fixation

The green iguana was obtained from the Main Veterinary Hospital in Skopje. The fresh specimen was fixed by immersion in 3% formaldehyde solution and was kept in this solution for more than three years. A midventral incision of abdominal wall that had been made previously during an operation had been closed with suture. This port provided entrance for fixative solution into the body cavity and hence good preservation of the tissues.

After this extended period of fixation, the iguana was rinsed with cold tap water for one week to remove the majority of formalin. Before dehydration, the iguana was cooled to 5°C. Table 1 lists the stages of plastination, their durations and the temperature under which the stages occurred.

Step	Time	Temperature
Fixation	>3 years	room temperature
Flushing	1 week	10°C
Cooling	24 hours	5°C
Dehydration I	31 days	-25°C
Defatting/ Dehydration II	5 days	room temperature
Immersion	7 days	-20°C
Forced impregnation	3 weeks	-20°C
Pre-curing	5 days	room temperature
Gas-curing	12 days	room temperature
Post-curing	>3 months	room temperature

Table 1. Steps and timetable of iguana plastination.

Dehydration

The iguana was dehydrated using cold acetone (freeze substitution method) (Tiedemann and Ivic-Matijas, 1988; Weiglein, 1997). The iguana was submerged in the first acetone bath of 100% acetone for two weeks at -25°C. The second acetone bath (100% acetone) was for ten days at -25°C. Finally, the iguana was transferred to the third 100% acetone bath for seven days at -25°C. The purity of each acetone bath was monitored after warming to 20°C using an appropriately calibrated acetonometer. When the acetone purity of the third bath remained over 98%, the iguana was transferred to a fourth acetone bath (100%) at room temperature for five days for defatting and to complete dehydration. The acetone concentration of the acetone following the last acetone bath measured 99.5% at which point dehydration was considered complete.

Forced impregnation

Forced impregnation was by continuous impregnation (von Hagens, 1986; Henry and Nel, 1993). However, at the end of each working day, the vacuum pump was turned off and the vacuum sealed such that the pressure inside the vacuum chamber remained constant. The iguana was submerged in a mixture of silicone polymer (S10) and catalyst/chain extender (S3) combined at a ratio of 100:0.5 for one week at -20°C. After one week of immersion, vacuum was applied for three weeks and pressure was slowly decreased to 3mbar. Vacuum was controlled via a vacuum controller and monitored via formation of acetone bubbles at the surface of polymer. The impregnation process was considered complete when bubbles ceased and the pressure stabilized at 3mbar. During the next three days, the pressure was slowly increased to atmospheric pressure. The iguana was then placed at room temperature and left submerged in silicone for an additional day.

Figure 1. Final cosmetic repositioning using nails, tacks and towels of silicone impregnated green iguana prior to curing.

Figure 2. Gas curing of green iguana. S6 was introduced via tube into the iguana's body cavity.

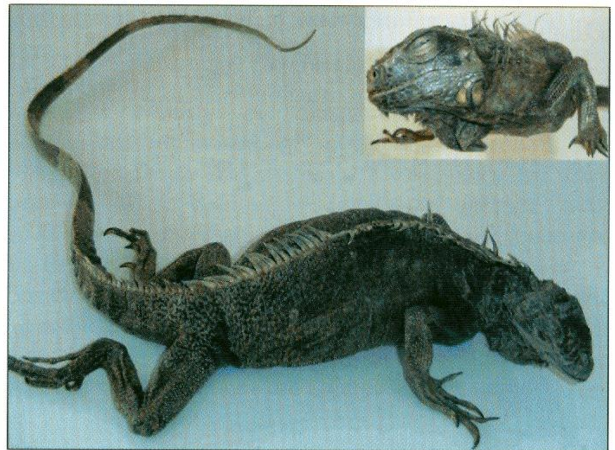

Figure 3. Views of plastinated green iguana.

Curing

In preparation for curing, the iguana was removed from the silicone and placed on a grid to drain excess

polymer from its surface for five days at room temperature. Following draining, the iguana was posed in a desirable position using large nails and a supporting board to fix the tail, legs and head (Fig. 1).

To keep the spines of the back and tail upright, towels were placed adjacent to the spines and fixed by pins to the body of iguana to support the spines. Little pieces of towel were placed between the eyelids to keep the eyes open. Finally, the fast gas curing method was used for curing the specimen.

The specimen was placed in a gas curing chamber and exposed to S6 vapors for five days at room temperature. To prevent white precipitate from forming on the surface of the iguana, $CaCl_2$ was added to the gas curing chamber. A small membrane pump was used to bubble air through the S6 to accelerate the curing of the surface of the iguana. After five days, the surface of the iguana was dry (cured) but the silicone inside the body wasn't polymerized. Therefore, a tube with attached needle was attached to the S6 vapor bottle and inserted into the body cavity of the iguana (Fig. 2). S6 vapor was introduced into the abdomen for seven days. Following gas curing, the iguana was stored in a sealed plastic bag for three months to assure curing of the entire specimen.

Results

The green iguana was preserved well with the slightly altered S10 standard plastination technique. A lower concentration of S3 was chosen in an attempt to obtain a more flexible specimen. No noticeable shrinkage was observed in the plastinated iguana. The position into which the iguana was placed was retained following the removal of the specimen from the board (Fig. 3).

Discussion

The plastinated iguana looked natural. The color of the skin was darker than usual but was likely due to the very long period of fixation in formalin. Slow increases of vacuum were used to prevent shrinkage of the iguana. When the vacuum pump was turned off at the end of the day, the vacuum was sealed and held constant inside the vacuum chamber. This also helped to prevent shrinkage of the specimen. Repositioning the iguana prior to the curing stage was the crucial step in plastination of the iguana because the impregnated, twisted specimen needed to be manipulated and fastened to a retaining board. Following impregnation, the twisted shape of the iguana was reformed even after having been fixed for three years in an inappropriate position. This suggests that poorly shaped formalin fixed specimens can be reshaped with proper impregnation and gentle manipulation.

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developed. An adult human brain was obtained, dehydrated, impregnated (P40), cast and serially sectioned. Cross sections were obtained and a base illustration was prepared to represent an epidural and subdural hematoma. Interpolated frames were established based on a linear transformation between 28 key frames emphasizing compression of the parietal region and cranial nerve III ipsilaterally. The animation was presented within the context of a child who fell sustaining a head injury. It was posted on a website and a questionnaire was developed and circulated to medical students participating in the PBL case. Results: The evaluation showed that the animation aided the students' understanding of the health care problem (average score of 1.36; SD = 0.59; n = 19, on a scale of 1-5 with 1 being "Strongly Agree"). Numerous additional comments were presented indicating that the epidural hematoma provided a useful PBL resource. Conclusion: Plastination provides a powerful technique to develop supplemental learning tools for VR delivery of PBL concepts. Supported, in part, by OAT HHS 2 D1B TM 00003-02.

Plastination, a useful tool in teaching clinical anatomy. ***Riederer BM. E Musumeci[1], B Duvoisin[2], FJW Lang[1]***. *Department de Biologie Cellulaire et de Morphologie, University of Lausanne, Service [1]d'OLR et [2]Radiologie, Lausanne, Switzerland, Europe.*

Plastination is essential in teaching several courses at the University hospital at Lausanne. Previous reports outlined the necessity to reduce the number of courses and dissection hours in teaching human gross anatomy in the 2nd year of medical studies at the DPCM. In addition, a report on another module, where plastination is advantageous for preservation due to a repetitive use of specimens. One module, such as the lower limb, usually consisted of 27 hours of dissection, which was distributed over 9 afternoons, but for the novel, the course had to be reduced to three blocks of two hours of self-directed learning. Assistants predissected specimens several months in advance which familiarized them with the teaching material, since they were to supervise and guide the students and furthermore gave them a good opportunity to learn topographical anatomy in more detail. This allowed ample time for plastination of the dissected specimens by the standard S10 procedure. Further details on the technique, references and links can be obtained on our web site: (http://www-ibcm.unil.ch/teaching/anatomie/plast/indexplast.html). During the two first hours of the self-directed teaching module, cutaneous innervation and articulations were studied; followed by the topography of the thigh and popliteal region, and finally lower leg and foot were studied. The plastinated specimens supplemented the teaching material, which consisted in part of wet specimens, usually kept in 50% alcohol solution between courses. Unfortunately, delicate nerve structures of different cutaneous regions resisted only for a short time the inquisitive approach of our students. Even nerves sutured to the tissue were soon torn apart. Therefore, the use of plastination resulted in more resistant specimens. This module will now be adapted for 1st year medical students which is a challenge because of the large number of student (over 400 students). The plan is to include plastinated specimens in this new module, because of the previous good experience. Furthermore, the plastinated specimens were also very welcome by the students, especially for individual study before exams. For a second teaching course for 5th year medical students on the maxillary sinuses, samples were prepared and plastinated. Each year, human heads obtained from the local donation program had to be prepared again, therefore, these permanent preparations are very welcome. Six heads were prepared acording to the traditional protocol, in addition red silicone color with radio-opaque material (Lipiodol) was injected into the internal carotid artery. Several surgical interventions (unciformectomy, frontomeatotomy, ethmoidectomy or antrostomy) were applied to some blocks, furthermore some arteries were prepared prior to plastination. The maxillary blocks were subjected to CT scans before and after plastination to determine shrinkage effects due to the plastination process. Bony structures, although fragile, were far less prone to shrinkage than sinus mucosa. To illustrate the trajectories of several arteries and to illustrate surgical interventions an endoscopic approach was chosen. However, the endoscopic examination proved sometimes more difficult due to the increased rigidity of plastinated specimens. In conclusion, advantages of introducing dissected and plastinated tissues are manifold. Less bodies are used because of a reduced necessity to prepare new specimens every year. Furthermore, delicate structures become more resistant and well dissected tissues is preserved for a long time. In addition, plastinated samples can be used also outside the dissection hall. The introduction of plastinated samples adds another tool to teaching clinical and applied gross anatomy.

Plastination: A tool for research: ***Zhang M.*** *Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand.*

Background: Since its innovation by von Hagens in

1979, the plastination technique has been widely and extensively used in teaching. There are also increasing number of researchers who take advantage of the sheet plastination technique for 3-D reconstruction and CT/MRI correlation. However, the application of plastination for other research is not very common. For instance, the PubMed search for “Sheet plastination“ reveals that up to April, 2004, there are 80 publicatons, over 70% of which were related to teaching, 3-D reconstruction and technical development. Even less hits (5-6 papers) were generated for "Sheet plastination". Moreover, the areas covered by these published studies were relatively limited. Based on those published studies and our own experience, we believe that the potential of the plastination, particularly E12 sheet plastination, as a tool for morphological research should be further and widely explored. Materials and Methods: A literature review. Results: It was found that sheet plastination techniques are extremely useful for the investigation of the spatial relationships between fine structures and the macroconfiguration of connective tissue structures, e.g. tendons, ligaments, fascia, sheathes and septa. With pre- or post-treatment, the sheet plastination technique can be modified to meet specific objectives, such as localization of micro-venous valves and collagen components, as the prepared specimen can be examined under optical, electron and confocol microscopes. On the other hand, however, there are some limitations, for instance, it is time-consuming and expensive. These limitation may limit the size of samples and prolong the study. The tissue loss due to slicing may also obliterate vital information skewing results of those studies on an unevenly distributed structure. Conclusion: The plastination technique, particularly sheet plastination, is a unique tool for the morphological investigation at the macro- and microscopic level.

Plastinated specimens in the minimally invasive surgery center. ***Sánchez Margallo FM.*** *Minimally invasive surgery Center, Cacerés, España, Europe.*

Introduction: Now days, surgery is a multidisciplinary specialty that involves different technological fields. Surgeons should be able to perfectly understand and use the latest technological advances. The Minimally Invasive Surgery Centre (MISC) has always promoted totally practical surgical training programs, where experimental models such as plastinated organs play an essential role, especially in the basic steps of the surgeon’s learning curve. The advantages of minimally invasive surgery over conventional approaches are already proven. One of the main problems of minimally invasive procedures is that their clinical application must be preceded by a highly specialized training program. This protocol should be divided in different levels of progressive difficulty, where experimental models should be used on the basic steps. Over the last years, plastinatioin has proved its usefulness in the fields of anatomy, pathology, surgery and radiology. This report is intended to discuss the advantages of the use of plastinated organs as adjunct tools for research and teaching in minimally invasive surgical techniques. Advances in plastination over the last years have allowed the development of new research areas in the fields of anatomy and on several diseases. Simulation of clinical situations would allow clinicians to profit from practical training courses. From our point of view, the development and clinical validation of new training protocols that include the use of plastinated organs is of the greatest importance. This would decrease time and costs of the learning curve while minimizing risks for both patients and clinicians. Materials and Methods: Plastinated models of the canine digestive system were prepared at the Veterinary Anatomy Department of the University of Murcia. These organs were used as experimental models on the Theoretical and Practical International Course on Digestive Endoscopy, held at the MISC. Attendees of the course were trained on both basic diagnostic and therapeutic endoscopic procedures, such as esophagogastroduodenoscopy, colonoscopy, biopsy, cytology, polipectomy and foreign body retrieval, using plastinated organs as a step prior to the "in-vivo" procedures on experimental animals. Finally, plastinated organs were compared with experimental animals in terms of effectiveness and usefulness for surgical training programs. Results: The course attendees agreed on the usefulness of plastinated organs, especially in the first steps of their learning curve. Endoscopic evaluation of the gastrointestinal mucosa, as well as basic therapeutic techniques were successfully carried out on plastinated models. Intimate knowledge of the canine gastrointestinal anatomy was easily learned in the plastinated organs, which is a basic prerequisite for the performance of "in-vivo" endoscopic explorations. Training on therapeutic endoscopic techniques (cytology, biopsy, polipectomy and foreign body retrieval) using plastinated models provided the attendants with the necessary basic practice for safe "in-vivo" endoscopic procedures. Conclusions: The use of plastinated organs is a useful tool for surgical training. Plastinated organs exhibit detailed anatomy and allow for an adequate basic training prior to "in-vivo" practice. Attendees of our trianing course have widely accepted the use of

plastinated organs due to their ease of handling and high training value. We conclude that the combination of plastinated organs and minimally invasive surgical techniques (laparoscopy, thoracoscopy, endoscopy, interventional radiology, etc.) constitutes a useful tool for both anatomical studies and basic surgical training.

The journal for the international society for plastination. ***Reed RB, RW Henry.*** *Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*
After delays, the 2003 issue of the JISP has gone to press and will be presented at the 12th ISP congress. In an attempt to produce a journal of the highest quality, it was necessary to postpone the printing of JISP volume #18 until enough appropriate articles were submitted and reviewer's comments answered approgriately. We would like to have a minimum of five manuscripts for each volume. Manuscripts are currently being reviewed for volume #19 of the JISP which will go to press winter of 2004. This issue will also contain the abstracts from the 2001 interim meeting in China as well as the abstracts from the 2004 meeting in Spain. Volumes 17,18 and 19 will be submitted to Science Citation Index for consideration and inclusion in their list of scientific journals. In order to attain for our journal the respect of the scientific community, we will need to publish manuscripts which demonstrate new findings or novel ideas.

Oral presentations

A new method for preservation of anatomical specimens. ***Arnautovic I, R Henry[1], H Pobric, R Avdic, V Cutahija, M Tabakovic.*** *Department of anatomy, Faculty of Veterinary Medicine, University of Sarajevo, Sarajevo, Bosnia-Hercegovina, [1]Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA.*
For hundreds of years desiccation of biological tissue has been a useful means of preservation. As well, drying of hollow organs has been an inexpensive method of preservation of anatomical specimens. However, hungry insects have a predilection for such dried organs. A mechanism to prevent infestation of the dried specimen with insects and thus prevent the destruction of the dried organs was necessary to be developed. This process has been called internal plastification and consists of two stages. **1.** The preparation progresses by cleaning the organ and flowing room temperature air through the organ, as has been done historically. The organ is first dilated to the desired degree of inflation and then the exhaust port is regulated to maintain inflation. Depending on the size of the organ, drying takes three to four days. The stomach (monogastric and ruminant), small and large intestines, lungs and uterus and vagina have been preserved by this method. **2.** The second phase commences after drying is completed and consists of gradual injection of plastic expanding foam, [Tekapur (Bosnia-Hercegovina) or Great Stuff (USA)]. The plastic expands in volume in all directions and hardens gradually. Depending on the product used, it enlarges two to five times. Next, more foam is injected at sites that are devoid of foam to assure complete lining and filling of the organ. The hardening time is eight hours to one or two days depending on the size of the organ. The organs filled with foam are lightweight and the foam lining protects the internal surface against insects. The external surface is protected by varnishing and named regions may be painted for delineation of such region. This method produces a specimen that maintains normal anatomical structures, is durable and is inexpensive. We believe this foam could be used to dilate plastinated hollow organs prior to polymerization.

Ascaris plastination using the S 10 technique. ***Asadi MH, A Mahmodzadeh, H Haydari.*** *Department of Anatomy, Baghiatollah University of Medical Sciences and Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Ascaris lumbricoides is one of the most common Parasites of the human digestive system. It has a world-wide distribution and is particularly very common in regions with poor sanitation. Ascaris is the largest of the common nematode parasites of the human. Different techniques are used to prepare Ascaris for educational purposes in medical colleges. The common method employing formaldehyde fixed produces specimens which are wet and with an annoying odor. The purpose of this study was to perform plastination using the S10 technique. This procedure produced specimens which are dry, odorless, and easy to handle. Meanwhile, the natural shape of the specimen is preserved and specimens can be easily used for demonstration in educational courses while working with medical students.

Role of hydrogen peroxide in plastination of upper and lower limbs with injection of colored polymer into the arteries. ***Dashti GR, AR Sabahi, M Hajian, E Esfandiari, A Saki.*** *Department of Anatomical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.*

Plastination is a unique technique of tissue preservation. The aim of this study was to prepare plastinated specimens of upper and lower limbs of good quality with colored polymer injected into the arteries using hydrogen peroxide to lighten muscle tissue. Materials and Methods: This study was carried out on a human body. The colored polymer was prepared by mixing colored paste with polymer, cobalt (accelerator) and peroxide (hardener). The polymer was injected into the body through femoral artery by a hydraulic pump. Dissection, dehydration, bleaching with hydrogen peroxide, defatting, impregnation and curing were carried out. Results: The plastinated specimen produced showed clearly the bright appearing muscle tissue which had fine stature, flexibility and good traction which was accessed by the universal test DARTC (England) apparatus in the medical physics department, school of medicine. Conclusion: The specimen obtained provided an excellent opportunity to demonstrate that hydrogen peroxide, an oxidizing agent, can play a significant role in preventing tissue from becoming dark or black in color during the course of plastination of tissue which may be due to impurities of chemicals.

Whole body plastination with a polyester resin. ***Esfandiary E, M Hajian, A Rabiei****. Department of Anatomical Sciences, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*

In Iran due to the religious culture, body donation for dissection is very rare. There is an acute shortage of cadavers for medical schools. In order to compensate for this shortage, cadavers can be preserved with the new preservation technique of plastination. This reality led us to find a cheap way to plastinate the specimen with a polyester resin instead of silicone. Materials and Methods: A new cheap polymer for plastination of cadavers was made and called P75 in Isfahan University of Medical Sciences. The specimens were dehydrated in room temperature acetone. A vacuum chamber was made for impregnation of the specimen. Results:The product obtained was flexible, natural colored, dry and durable. This plastinated specimen is comparable with world standard plastination. Conclusions: We hope plastination techniques become a routine work in every anatomy department of our country to save both sophisticated dissected bodies and time of the dissectors.

Refurbishment of plastinated joints for interactive clinical teaching. ***Easteal R, RE Hunt, SC Pang.*** *Department of Anatomy and Cell Biology, Queen's University, Kingston, Canada.*

In teaching of clinical anatomy it is important for students to handle human specimens. Plastinated specimens are by far the most student friendly means of affording hands-on experience to these clinical students. However, when using plastinated joints, eventually the ligaments, even plastinated ones, will tear rendering them useless for interactive teaching. Materials and methods: However, using a little ingenuity and some items found in most hardware stores, we have developed artificial ligaments for these worn-out specimens, rendering them again functional. It is important that the artificial ligaments (especially in the knee) are placed exactly in the correct anatomical location with the proper elastic coefficient. Results and conclusion: These two functions completely return the specimens to their former use as correctly operating joints, with anatomical and mechanical integrity.

India ink enhances the coloration and teaching effectiveness of plastinated specimens. ***Glover R, M Wells.*** *Undergraduate Research Opportunities Program, University of Michigan Medical School, Ann Arbor, MI, USA.*

The use of acetone to dehydrate tissues in the plastination process and their saturation with silicone polymer after impregnation appear to significantly interfere with permanent coloration of plastinated specimens. Therefore, it was determined to develop a method for the coloration of plastinated tissues that would withstand both of these necessary conditions. Formalin- fixed limb tissue blocks were washed, partially dried and their arteries, veins and nerves were selectively colored with several coats of India ink using a fine camel's hair paint brush. The gyri of several brain specimens were colored in the same manner. Before of painting, as much connective tissue as possible was removed from the surface of structures to prevent India ink from bleeding color into their immediate surroundings. Painted tissues were dehydrated in acetone and checked daily for loss of color. Impregnation with silicone, under vacuum, and the application of catalyst were carried out in the usual manner. India ink colored tissues did not fade but withstood acetone dehydration, normal vacuum impregnation and curing without loss of color. Equally important, their color was not adversely affected by normal use and handling. The availability of an effective coloration methodology for plastinated specimens is desirable for many reasons. For educators, the ability to selectively color plastinated specimens should markedly improve their effectiveness in a variety of different teaching/learning settings.

Preparation and application of a new UV stabilized polyester in plastination in human anatomy. ***Hajian M, E Esfandiari, AA Rabiei.*** *Department of Chemistry and Anatomical Department of Medical School of Isfahan University, Isfahan, Iran.*

Many synthetic resins such as polyesters, silicone and epoxy resins are used for prolonged preservation of organs and tissues of man and animals without need for storage in formalin or ethanol. In this project a typical UV stabilized unsaturated polyester resin was prepared from two kinds of glycol (propylene and ethylene glycol), phthalic and maleic anhydride, a UV stabilizer (2-2 di hydroxyl- 4 methoxy), a cross-linking agent (a styrene monomer) and a hardener (cobalt naphthanate). The polyester was used for plastination of a sliced human brain and a whole body. The stages of fixation, dehydration, defatting, forced impregnation and curing were used. Important properties of the plastinated specimens, UV stability, chemical resistance, durability and mechanical properties, were investigated and compared with the specimens prepared at the institute of plastination in Germany.

Continued studies on impregnation with silicone polymer and no additives. ***Henry RW, Reed RB, Latorre R[1], H Smodlaka[2].*** *Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA, [1]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, [2]Department of Anatomy and Radiology, College of Veterimary Medicine, Univeristy of Georgia, Athens, GA, USA,.*

The time tested silicone technique introduced by Dr. von Hagens (Biodur) uses a reaction-mixture of S10/S3 and has been used for impregnation and production of high-quality, aesthetic specimens for 26 years. The Biodur process is most often used at cold temperatures, but may be used at room temperature. The one drawback of using this reaction-mixture is thickening after several months. Six years ago, a reaction-mixture that is stable at room temperature was introduced. This room temperature process (Dow/Corcoran) yielded good quality specimens and reduced plastination time by nearly one half. One problem of this method is a detracting granular looking film which develops on the surface of the specimens. In an attempt to decrease plastination time and have a polymer that is easily removed from fur and yet still produce the highest quality specimens, impregnation of silicone without catalyst/chain extender or cross-linker is being used on a variety of specimens. Impregnation time ranges from one day to two weeks. The low viscosity (40 - 80 centistokes) of the polymer provides a shortened draining and manicuring time. Two regimes are used to cure the specimens: **1.** Specimens are exposed to the vaporized cross-linker (S6) for 2 - 4 days then S3 (catalyst/chain extender) is wiped onto the surface of the specimen twice at 24 hour intervals. **2.** Specimens are exposed to the vapor of a new Supercatalyst for 2 days. After sitting for 2 days, specimens are exposed to a volatilized chain extender (S7) for 2 days. Finally, two days later they are exposed to the volatilized cross-linker (S6) for 4 days and allowed to remain in the closed environment as long as necessary (2 to 4 weeks). S6 exposure is repeated if deemed necessary. Both methods result in polymerization of the outer layer of the specimen within several days. When specimens are sectioned two and four weeks later, the interior of most of the specimens contains not totally cured polymer. Some specimens take several weeks for the polymer to cure throughout the depths. Some may take months to cure. The quality of the specimens appears to be similar to that of specimens produced via the classic Biodur method. Currently, the biggest advantage is the polymer runs freely off of hair-covered specimens, which eliminates the tedious task of manicuring which is associated with hair covered specimens produced by the classic method. In time this may prove to be another useful method for producing specimens more rapidly and with out the need of refrigeration.

Plastination of the bronchial tree with RTV silicone at different developmental stages: Dehydration with alcohol at room temperature. ***Jimenez Mejia R, O Isaza Castro.*** *Department of Morphology, Faculty of Medicine, University of Antioquia, Colombia, South America.*

The difficulties associated with the process of obtaining and managing acetone in Colombia have driven a search for alternatives to the various steps of plastination, among them dehydration of anatomical specimens, which is recommended to be carried out at low temperatures with acetone. One alternative to aid in describing development of the bronchial tree from the embryonic process up to childhood stage and allow demonstration of the successive dichotomist division in the lung is by the injection of a flexible silicone. Materials and Methods: Five different ages fetuses and corpses pulmonary blocks were fixed with 10% formalin for four 4 weeks. After fixation they were dehydrated at room temperature in increasing concentrations of isopropyl alcohol (70 %, 75 %, 90 %, 95 % and 100 %) for a period of one week in each

concentration. After dehydration, the tracheobronchial tree was injected through the trachea with RTV silicone. The polymer in the injected specimens was allowed to cure for three days. After curing, the specimens were placed in an autokey at 100°C for 30 minutes. Subsequently they were washed with water at a high pressure to remove respiratory tissue and the tissue remains were manually eliminated. Digital images were taken. Results: This technique allowed the dehydration of the specimens and the injection of a flexible silicone to yield flexible tracheobronchial tree preparations of different stages of development. The developmental progression of the tracheobronchial tree was readily visible in this series of lungs. Conclusions: This work demonstrates the efficiency of the dehydration with isopropyl alcohol at room temperature. The successful utilization of a flexible silicone in hollow structures demonstrates overcoming shortages that are present in our society and the difficulties generated partly by problems as grave as the drugs traffic.

Sheet plastination with Polyester: An alternative for all tissues. ***Latorre R, JM Hervás, A. Arencibia[1], F Gil, M Rivero[1], G. Ramírez, JM Váquez-Autón, RW. Henry[2].*** *Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, [1]Departamento de Morfología, Facultad de Veterinaria, Universidad de Las Palmas, de Gran Canaria, España, Europe, [2]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Classically, the main application of polyester polymer in plastination (P40 and P35, Biodur™) is the production of brain or head slices. Recently semi-transparent body slices have been produced in our labs using P40. No literature was found on the production of body slices using P40. The purpose of this study was to develop a protocol for using P40 to produce slices from all regions of the body. Two millimeter slices were cut from all regions of the body of a cat. The slices were processed using a modified P40 technique. Compared to the E12 method, the P40 technique offers not only great optical and anatomical detail, but also some extra advantages: Slices do not yellow, Ease for the beginner, and Indefinite pot life of the impregnation bath. The P40 plastinated body slices yielded excellent anatomical detail of all tissues that were observed and all tissues cured in a routine manner. In addition, the slices have been excellent aids for teaching and research in our class rooms and laboratories.

Evaluation of plastinated organs as a resource [...] improvement of the teaching-learning proce[...] ***Latorre R, MP García-Sanz[1], F Gil, M Moren[...] Agut[3], JM Quiñonero[4], E Lozano[5], J Herrero[6] Hernández-Pina[1], H Fenandes- Seródio[7], R He[...]*** *Departamento de Anatomía y Anatomía Patológica Comparadas, Anatomía Veterinaria, Universidad de Murcia, [1]Departamento de Métodos de Investigación y Diagnóstico en Educación, Universidad de Murcia, [2]Departamento de Ciencias Morfológicas, Anatomía Humana, Universidad de Murcia, [3]Departamento de Patología Animal, Cirugía Veterinaria, Universidad de Murcia, [4]I.E.S.Alfonso X El Sabio, Murcia, [5]I.E.S. Ingeniero de la Cierva, Murcia, [6]I.E.S.Saavedra Fajardo, Murcia, España, Europe, [7] Anatomia Veterinária, Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal, Europe, [8]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, USA.*

Plastinated specimens can be considered didactic materials. The general goal of this investigation consisted of evaluating the use of plastination as a resource towards innovative teaching, being capable of incrementing the quality of the education-learning process in high school and university, in the region of Murcia. For this investigation, teachers and students from three levels of education in the region of Murcia were involved: High School Education, Univeristy Education and Postgraduate Education. The decision of which plastinated specimens should be used was reached by consensus, in response to the needs of the different teaching levels. We worked through an interdepartmental approach. The evaluation instruments consisted of observation techniques combined with inquires and previous knowledge and output examination examinations. For this study, four types of instruments were selected:

* A 3-grade numerical estimation scale, registered by the teachers, after attentive observation while using the plastinated specimens.
* A closed questionnaire using a 3-grade Lickert-type scale, filled out by the students gathered information referring the effectiveness of the didactic resources.
* An output examination made up in order to evaluate the quantity and quality of the knowledge and skills acquired as a consequence of the use of specific didactic material.

The results show that plastinated specimens are very useful as a new educative method in secondary schools and university from Murcia region. Supported by SENECA Foundation, Project:PC/2/FS/99.

Plastination workshops: Four years experience in Spain.
Latorre R, O López-Albors., JM Hervás, E Abellán., JM Vázquez, M Orenes, C Sánchez, F Martínez., A Diz[1], R Henry[2]. *Anatomia y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe, [1]Departmennto d Anatomia Patológica Comparadas, Universidad de Córdoba, Córdoba, España, Europe, [2]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Since 2000, the Anatomy Department of the University of Murcia has been promoting plastination techniques by conducting two-day workshops once per year. The workshops gave hands on experience primarily with the silicone cold and room temperature processes. RTV silicone and polyester polymers were also demonstrated. A maximum of 15 participants were admitted to each workshop, most of participants were from the various regions of Spain. As workshops were taught in Spanish, participants were attracted from various countries of both Central and South America. At the end of each workshop all participants filled out an evaluation sheet. Most participants considered these workshops a very valuable tool for them to gain the experience to begin the plastination technique and to set up a plastination lab in their University. In fact, ten (six are working and four are starting up) new plastination labs have been set up around Spain in the last five years. These additions to the plastination community are directly related to participation in Murcia´s workshops.

S-10 and P-40 plastination techniques for anatomical studies of the Sea Bass (*Dicentrarchus labrax*, L.; Teleost, Fish).
López-Albors O, MªD Ayala, R Cuéllar[1], I Abdel[2], M Orenes, R Latorre. *Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, [1]Dept. Anatomía Veterinaria, Universidad Nacional de Aguas Calientes, México, [2]Instituto Oceanográfico de Mazarrón, Murcia, España, Europe.*

Plastination is very useful to study the external and internal anatomy of fish. However, its use and application is still scarce. The sea bass is an intensively reared teleost and highly appreciated in Mediterran ean countries. Anatomical studies of this species are relevant for related histological and physiological investigations. In this work, plastination techniques were used on prosected and serially sectioned specimens. Two whole specimens with lateral dissection of the body wall, two transversally sectioned and two horizontally sectioned specimens were plastinated according to the standard S-10 and P-40 techniques. The combined use of prosections and cross-sections was very useful to describe the gross anatomy of sea bass. Cross-sections at the level of the head allowed the study of internal organs which are difficult to view by dissection: encephalon, eye-ball and related structures. Transverse and horizontal sections allowed a detailed description of the topography of the viscera. The P-40 thin sections allowed evaluation of anatomical structures at submacroscopic level. A common problem with the S-10 technique was that the muscle tissue tended to display a final orange appearance, which may be due to the high fat content of farmed fish.

Curing influences on tissue preservation of silicone plastinated organs.
López-Albors O, F Gil, M Orenes, MªD Ayala, R Henry[1], R Latorre. *Departamento de Anatomía y Anatomía Patológica Comparadas, Anatomía Veterinaria, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe, [1]Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.*

Deplastination of silicone plastinated specimens has been proposed as a valid technique for histological studies. Despite some structural changes deplastinated/paraffin embedded tissue maintains most of its structural features. Fixation and deplastination have been designed as the most critical steps when plastination is used for histological purposes. However, the influence of the curing on the tissue structure of plastinated organs has not been evaluated yet. With this objective, heart, lung, kidney, liver, uterus, omasum and rumen of several domestic mammals were plastinated according to three standardized protocols: Biodur S-10, Corcoran PR-10 and Vis-Docta HS1. Tissue samples of these organs, before and after curing were deplastinated with 5% sodium methoxide in methanol for 48 h. Afterwards the samples were washed in methanol, routinely processed for paraffin embedding and sectioning and finally stained with hematoxylin-eosin. Results showed that the curing process influences the tissue preservation, however with no significant differences between the three plastination techniques. Uncured samples had little or unappreciable tissue damage, whereas cured samples showed histological change. To find out whether these findings are only produced by the curing process or also related to other processing steps such as the

deplastination methodology or the paraffin embedding deserves further studies.

Fine configuration of the connective tissue in the posterior atlanto-occipital region: An E-12 sheet plastination and confocal microscopy study. ***Nash L.***
Clinical Anatomy Lab, Department of Anatomy & Structural Biology, School of Medical Science, University of Otago, Dunedin, Otago, New Zealand.

Aberrant kinematics of the dura-muscular and dura-ligamentous connections located in the posterior atlanto-occipital (PAO) region are widely thought to transmit mechanical forces from the intervertebral joints to the pain-sensitive spinal dura generating pathologies such as cervicogenic headaches. However, little information exists regarding the anatomical arrangements of the main structures, i.e. rectus capitis posterior minor (RCPm), PAO membrane, nuchal ligament, and spinal dura. The aim of this study, using plastination and confocal microscopy, was to examine the morphology of the fibrous structures and to determine the anatomy of the dura-muscular and dura-ligamentous connections in the PAO region. Materials and Methods: Twenty-two embalmed adult human cadavers were used. Thirteen were prepared by sheet plastination. Plastinated tissues were examined macroscopically using of a light box and dissecting microscope (x1.25) and microscopically with a confocal microscope. Two dissection procedures were performed on nine cadavers. The first involved conventional mid-sagittal sections from the occiput to C7. The second entailed a lateral approach in the atlanto-occipital region. Results: Direct evidence from cadaveric and plastinated material revealed: 1. Anteroinferiorly orientated tendon fibers from the medial and deep aspects of RCPm directly connected with the spinal dura within the PAO region. 2. The POA membrane was formed by the RCPm fascia and vertebral vascular sheath and inferiorly was fused with the spinal dura. 3. No direct connection between the nuchal ligament and the posterior cervical dura matter was found in the PAO region. Conclusions: The deep layer of the RCPm fascia and the perivascular sheath of the external vertebral plexuses are the main connective tissue structures of the PAO membrane between RCPm and the spinal dura in the PAO region. The morphological features of the RCPm tendon and fascia indicate that they have an important role in the maintenance of posterior craniocervical stability, the prevention of the dural infolding during extension, and the generation of cervicogenic headaches.

Plastinated organs as models for endoscopic training. ***Oppitz M, A Kirschniak, U Drews.***
Department of Experimental Embryology, Institute of Anatomy, University of Tübingen, Tübingen, Germany, Europe.

For training medical doctors skills for endoscopic surgery, a variety of procedures and equipment have been established. Proposals range from artificial phantoms to surgery training on animals. However, these procedures have the disadvantage of lacking the normal human topographical anatomy. In our clinical anatomy training unit, cadavers for training surgical endoscopy were preserved. Apart from whole body preservation with ethanol/glycerol and fixation after Thiel, plastinated organs of the gastrointestinal (GI) tract are used for training of flexible endoscopy. Materials and Methods: Phantoms were prepared from formalin-fixed human cadavers using classical anatomical and appropriate surgical techniques. Blood vessels were filled with colored silicone. After fixation of organs and dehydration in acetone at -20°C, the preparations were impregnated with silicone for 4 to 8 weeks depending on size and left to polymerize for 4 to 7 days. Results: The procedure described produced plastinated organ preparations of the gastrointestinal tract that simulate clinical human endoscopic anatomy in a close-to-reality setting. The disadvantage of reduced tissue softness in plastinated organs is compensated by the realistic detail such as excellent visualization of the course and topography of the blood supply as seen from the endoscopist's view. Conclusion: Endoscopic training with realistic simulation of anatomical detail is important for medical personnel when having to deal with emergencies such as upper GI-bleeding of peptic ulcers or esophagus varices. In addition to hands-on experience, the detailed knowledge of anatomical detail gained from endoscopy of plastinated organs will be of great help for stopping GI- hemorrhage.

Enhancing the educational value of plastinated specimens in the gross anatomy curriculum: Adding color to human neurovascular pathways. ***Raoof A, C Parres, J Blythe.***
Anatomical Division, Medical Education, University of Michigan Medical School, Ann Arbor, MI, USA.

In recent years, plastinated specimens have become invaluable assets to the gross human anatomy curriculum, furthering the development of students' identification and diagnostic skills of anatomical structures. Students often have difficulties, however, in conceptualizing important neurovascular pathways and

relationships using these virtually colorless specimens. An applicable method for improving plastinated specimens as learning tools involves the use of acrylic paints. Nerves, arteries, and veins of plastinated specimens are actually painted by hand, yielding detailed, colorful specimens and providing a more holistic representation of the neurovascular picture. Methods: Plastinated upper limb, brachial plexus, pelvis, and two lower limb specimens were selected for coloring. Nerves, arteries, and veins of each specimen were cleaned using dissection tools. Each nerve/vessel was carefully painted the appropriate color and then allowed to air-dry for twenty-four hours. Questionnaires regarding the effectiveness and applicability of the colored specimens were administered to eleven students enrolled in a 2003 summer pre-matriculation anatomy course at the University of Michigan. Results: Life-like, durable, and anatomically accurate models representing in color major neurovascular pathways in human upper and lower limbs were created for use in the gross anatomy medical education curriculum. Questionnaire responses were overwhelmingly positive - 81.8 % of the students supported that "the painted specimens would reinforce the value of dissection and would be beneficial in a teaching environment." Discussion: Colored plastinated specimens proved to be effective and engaging learning tools for medical students. If put to use in the gross anatomy curriculum, these specimens would lend to a fuller understanding of neurovascular relationships and of important physiological processes of the body. A few problems to work through: Time-consuming work to hand-paint the specimens and more experimentation should be done to provide better paint durability over time. Advantages of this coloring technique over others such as casting: Specimens chosen for casting are usually hollow visceral organs while our coloring technique is applicable to almost any specimen.

Ultrasonographic anatomy of the horse temporomandibular joint: A study by P-40 sections. ***Rodríguez MJ, A Agut, F Gil[1], M Rivero[2], E Martínez[1], R Latorre[1].*** *Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad de Murcia, España. [1]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España. Anatomía y Embriología, Facultad de Veterinaria, Universidad de Las Palmas, de Gran Canaria, España, Europe.*

The aim of our study was to establish a protocol of the ultrasonographic evaluation for the equine temporomandibular joint (TMJ), to describe the normal macroscopic and ultrasonographic anatomy with cross-sections made on the same planes. Materials and Methods: Twenty-four TMJ from twelve horse heads were used for ultrasonographic studies. The dorsal synovial pouch was injected via its caudolateral compartment. Specimens were frozen at -80°C and sectioned with a high-speed band saw at desired thicknesses (2 mm). The cut surfaces of each section were rinsed and numbered before fixation. Photographs of both surfaces of each section were taken before and after fixation. Sections were plastinated using the P-40 technique. Results: Our ultrasonographic and anatomic results suggest that three sonograms from lateral, rostral and caudal approaches are necessary to provide a correct and complete ultrasonographic evaluation of the equine TMJ. The structures that could be evaluated were the articular surfaces, articular cartilage in yearlings, articular disc and its caudal fibrocartilaginous expansion, intra-articular fat tissue or retro disc tissue, caudolateral compartment of the dorsal synovial pouch, articular capsule, parotid gland and subcutaneous tissue. However, the ligamentum laterale and ligamentum caudale, the vascular and nervous structures and the ventral synovial pouch could not be identified. Conclusion: The use of the anatomic cross-sections made on the same planes as the sonographic views in the equine TMJ allowed correct identification of all structures and established a direct correlation with other adjacent structures.

Clearing and plastination of anatomical sections: An alternative method to study the murine vascular system. ***Ruberte J, C Llombart, M Navarro, G Ramírez[1], MD Ayala[1], R Latorre[1].*** *Unidad de Anatomía y Embriología. Facultad de Veterinaria. Universidad Autónoma de Barcelona, España, [1]Anatomía y Embriología, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe.*

Murine blood vessels are too small to study by dissection, but too large for satisfactory histological work. Injection of blood vessels and clearing the tissues have been classical methods to analyze small vessels (Tompsett, 1970). However, long term exposure to vapours can be hazardous, when the Spalterholz clearing technique (1914) is used. Moreover, preservation of specimens requires much attention, when glycerine is used for transparency. The aim of this work was to preserve cleared sections of mice with sheet plastination techniques, in order to avoid toxicity and conservation problems. Materials and Methods: after intravascular injection of colored latex through the

thoracic aorta, mice were frozen at -80°C and sectioned transversely with a high-speed band saw at desired thicknesses (2-4 mm). Two different techniques were used for clearing. With the Spalterholz technique, after dehydration, impregnation with methyl salicylate and benzyl benzoate was necessary for a complete transparency of the tissue. With the glycerine technique (Staples and Schnell, 1964), initial clearing was accomplished by soaking the sections in an aqueous solution of potassium hydroxide; after selective staining of calcified bones with Alizarin Red, complete transparency was obtained by immersion in a mixture of glycerine and benzyl alcohol. Sections were plastinated using the P-40 and E-12 techniques. Different dehydration protocols were used before impregnation. Results and Conclusion: The best results were obtained in Spalterholz cleared sections impregnated in E-12, following the regular protocol of the technique, without previous steep in acetone. P40 impregnated sections showed less transparency than E12 impregnated sections. A proper adaptation of the sheet plastination technique allows preserving of cleared sections.

Plastination and animation: Pedagogic-didactic proposal for study of fetal circulation in ruminant fetuses, as a paradigm for the study in other mammals. ***Seródio HC, J Gouveia[1], JM Lameiras[2], F Gil[3], G Ramirez[3], R Latorre[3]***. *Anatomia Veterinária, Departamento de Zootecnia, Universidade de Trás-os-Montes e Alto Douro, Vila Real Portugal, [1]Metodologia de Intervenção Educativa, Escola Superior de Educação Paula Frassinetti, Porto. [2]Departamento de Engenharia Biológica e Ambiental, Universidade de Trás-os-Montes e Alto Douro, Vila Real Portugal, [3]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe.*

Reasons that justify teaching of fetal blood circulation to veterinary and medical students are: 1. Analysis of patterns of venous blood flow in fetal vessels, as a way of detecting fetal distress; 2. Interpretation of congenital malformations associated with the retention of fetal circulatory structures; 3. Study of the effect of drugs which combat these malformations. Based on the need of pedagogic methods that allow understanding (as opposed to simple memorization), self-studying, as well as heightening the interest of the subject, the authors conceived a pedagogic-didactic sequence designed to be used in the classroom. Students, who intend to work and develop their own knowledge, have free-access to this resource. Material and Methods: Two animations were developed using Macromedia Flash™, two self-study guides and plastinated bovine fetuses (Biodur® S10). The pedagogic sequence consisted of previous observation of an animation, which shows the trajectory of circulating blood on a photograph, followed by a second animation that invites the student to fill in an equivalent diagram, and presents new information as it compares photographs with echographic images. Additionally, the student has access to fetuses, specifically dissected and plastinated for this purpose. Results and Conclusions: The first animation gives thevstudent a reasonable perspective of the topographical anatomy of the dissected fetus and the variation of blood oxygen and nutrients at different sites in the body. The second animation allows the student to consolidate the concepts presented in the first animation and expand them, as it compares the anatomy of newly presented anatomic structures with those of echographic images. Plastinated fetuses demonstrate significant morphology and are considered crucial resources. They allow manipulation and three-dimensional topographical analysis. Fetuses bring reality to the subject, demystifying it and developing the student's self-confidence as they experience anatomical facts that will serve as basic knowledge for future problem solving.

Comparison of plastinated specimens prepared using five silicone polymers and five techniques. ***Smodlaka H, RW Henry[1], RB Reed[1], R Latorre[2], O Lopez-Albors[2], JM Hervás[2], R Cuellar[3]***. *Department of Anatomy and Radiology, College of Veterinary Medicine, Univeristy of Georgia, Athens, GA, USA, [1]Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN, USA, [2]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe. [3]Departmento Anatomia Veterinaria. Universidad Nacional de Aguas Calientes, México.*

The impregnation technique for plastination of biological tissues has remained nearly the same since its inception twenty-six years ago. The polymers, cross-linkers, chain extenders and catalysts are all products currently used in the silicone polymer industry. The major differences in the Biodur, China, Corcoran, Italian and North Carolina A (NCa) and B (NCb) processes is in how the components are combined: Biodur, Italian and NCa combine polymer, catalyst and chain extender in the impregnation bath and after impregnation cross-linker is applied. China and NCb uses only the polymer and later a catalyst/cross-linker

may be added. Corcoran combines polymer and cross-linker and later the catalyst is added. Specimens were prepared using all six recipes and techniques in order to compare the final product. Specimens will be presented at the 12th International Congress on Plastination for evaluation. Results will be presented at the conclusion of the congress.

Sectional anatomy of dog knee joint: A study by MRI, Ultrasonographic and plastinated sections. ***Soler M, A Agut, F Gil[1], JM Vázquez[1], A Arencibia[2], R Latorre[1].*** *Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad de Murcia, España. [1]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España. [2]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, España, Europe.*

The purpose of this study was to use a combination of P-40 plastinated slices, ultrasonographic and magnetic resonance (MR) images to better understanding the anatomy of the normal canine stifle joint. Materials and Methods: Ultrasonographic study was performed using a 7.5 MHz linear transducer. Magnetic resonance images of the stifle joints were made in sagittal, dorsal and transverse planes using a 0.5 Tesla GE magnet and a solenoidal human extremity coil. A T1-weighted spin echo and T2*-weighted gradient echo images sequences were used. The canine stifle specimens, were frozen at -80°C after intraarticular injection with latex and sectioned either transversely or sagittally with a high-speed band saw at desired thicknesses (2-3 mm). At least twenty sections were obtained from each specimen. Both surfaces of each section were rinsed and photographed before and after plastination. Sections were plastinated using the P-40 technique. These plastinated slices were used to facilitate an accurate interpretation of the anatomical structures. Results: The normal anatomic structures that could be consistently visualized ultrasonographically included: normal cartilage on the femoral condyles and the trochlear ridges; the patellar ligament that was hypoechoic with a hyperechoic peritendon; medial and lateral menisci, cranial and caudal cruciate ligaments, and the tendon of the long digital extensor muscle all of which were hypoechoic compared to the patellar ligament. MR images showed ligaments, tendons and menisci that were seen with a low intensity signal. Articular cartilage had a similar low signal in T1-weighted SE sequence, but a very high intensity signal in T2*-weighted gradient echo images. The various plastinated anatomic sections were used to identify the structures of the stifle joint as well as correlate anatomic structures with the MR images. Conclusions:The combination of sheet plastination and imaging techniques as described in this study allow students, radiologists and anatomists to gain better insight into the three-dimensional relations of anatomic structures of certain regions, in this case the canine stifle joint. Sheet plastinated anatomic sections are a valuable tool to teach the interpretation of the ultrasonographic and MR images of the canine stifle joint.

High temperature in plastination. ***Sora M-C, B Strobl.*** *Plastination Laboratory, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria, Europe.*

The E12 method of plastination is usually used to create 2.5 - 5 mm transparent slices. If thinner slices, 0.5 - 1.5 mm, are needed, it is necessary to use the epoxy thin-slice plastination method. By using this method, the specimen is first plastinated as a block and then cut in thin slices. The impregnation temperature is the key element in order to obtain complete impregnation of the desired block and contrary to all other plastination methods uses high temperatures. The main goal of our presentation is to describe the use of high temperature for processing 1 mm epoxy plastinated slices. Only by using high temperature is the polymer thin enough to penetrate into the middle of the processed specimen.

P35 Plastination - Experiences with delayed impregnation. ***Üzel M, A Weiglein[1].*** *Vocational School of Health Services, Istanbul University, Istanbul, Turkey, [1]Institute of Anatomy, Medical University Graz, Graz, Austria, Europe.*

During an educational demonstration of P35 technique, brain slices which had stayed immersed in P35 resin in a cooling room (5° Celsius) for approximately two years were used. During the procedure, it was observed that the resin was quite sticky and it was very difficult to take the steel basket with the brain slices from it. After removal of the basket from the immersion bath, the: a) Slices were rigid, fragile and difficult to handle, b) Filter paper between the slices was almost united with the slices, and c) Some resin remnants stuck onto the metal grids. Because of their fragility, it was difficult to put the slices onto the glass plates to set up the double glass chambers without the slices breaking. After the double glass chambers were assembled, the chambers were filled with P35/A9 mixture and slice position was corrected with a wire. The hardening

procedure was the usual UVA-light - heat combination for P35. Despite the long immersion period and the problems encountered with removal of the slices, the final result was satisfactory from an optical point of view. As a conclusion, the time for the production of brain slices may be extended up to several months by leaving the slices in the cold immersion bath; however, periods of too much length need to be avoided because the slices start to cure where they are in direct contact with the steel grid.

Plastination and sectioning of the cranio-cervical junction. ***Weninger B****. Institute of Anatomy, Medical University Graz, Graz, Austria, Europe.*

The aim of this study was to visualize aberrant joints between the occipital bone and the dens of the axis and to preserve them in a stable form. Materials and Methods: Heads with necks still attached were used and preserved after Thiel. To determine which heads showed the desired characteristics, CT-scans were made of the atlanto-occipital region in advance and heads were selected accordingly. The heads were placed in a -70° C freezer. Of two frozen heads, one with latex injected vessels and the other without, squares with edges about 3 cm long were cut, which contained the occipital condyles, the atlas and the axis. These squares were dehydrated in cold acetone (-25°C). Subsequently, the squares were plastinated using E12. The cured squares were then cut with a diamond-wire saw. Results: The plastinated squares could be cut into very thin slices without much loss of material and with the advantage of reaching the desired plane of section gradually and exactly under supervision. After appropriate sectioning, the joint was clearly visible and stabilized for further examinations. Conclusions: The technique described is a valuable means of visualizing the structure of joints in sections without the disadvantage of dislocating the head and cavity of the joint, be it normal or - as in this case - an aberration.

Posters presentations

Nervous tissue - Plastination versus Embalming: The UKM experience. ***Azian AL, AG Norzana, HS Farihah, AR Fairuz, G Hairi, O Faizah****. Department of Anatomy, Medical Faculty, Universiti Kebangsaan Malaysia,Kuala Lumpur, Malaysia.*

Anatomical study requires long-lasting preservation of the cadaver. Arterial embalming which has been used since the 17th century allows for a three-dimensional study of the human body. However, in a tropical climate, the cadavers need more meticulous care and they require space for storage tanks. As well, formalin may pose as an occupational hazard. Due to the shortage of cadavers, specimens need to be preserved for a longer duration while retaining its natural and realistic appearance. With the advent of plastination as a tool for research and teaching, some of the problems encountered with embalming can be overcome. However, plastinated tissues at the UKM have some shortcomings when compared to embalmed specimens. Comparisons were made between brain and spinal cord specimens either whole or cross-sectioned preserved using the embalming solution only and plastination post-embalming. Results showed that brain tissues using the plastination technique shrunk between 30 - 50% compared to the arterial embalmed specimens. These plastinated tissues were hard, brittle and not pliable enough to show the sulci and gyri of the brain. Blood vessels and cranial nerves looked wiry and broke easily. Cross-sectioned specimens were darker in color, thus rendering it difficult to differentiate between gray and white matter. Apart from shrinkage, spinal cord specimens fared better and structures were maintained using plastination techniques. Various factors contributed to the poor results including specimens being prepared at room temperature, improper concentration of acetone during dehydration (final concentration of < 100%), insufficient dehydration period, a higher concentration of hardener (1%) and inappropriate pressure during the forced impregnation process. Based on these experiences, steps have been taken to rectify the mistakes and improvement of the techniques involved in order to obtain more favorable results.

Plastination of head and neck, posterior wall of trunk, and spinal cord with injection of colored polymer into the arteries. ***Dashti G, A Sabahi, H Ghaffary****. Anatomy Department, School of Medicine, Isfaha University ofMedical Sciences, Isfahan, Iran.*

The aim of this study was to prepare plastinated specimens of the head and neck, posterior wall of trunk and spinal cord by injecting colored polymer into the arteries. Materials and methods: After fixation of a human cadaver, colored polymer was injected into the femoral artery by the hydraulic pump. After a few days dissection, dehydration, defatting, impregnation and curing were carried out. Results: Plastinated specimens obtained were comparable with specimens prepared in Heidelberg, Germany, for their stature, flexibility and traction as done by the universal test Darte(England) apparatus. Conclusions: According to this study, p-

value was greater than 0.05, which shows no significant difference statistically. The specimens obtained were clear, dry, safe, durable and provided an excellent opportunity to demonstrate and study the dissected areas.

Plastination of fungi as an aid in teaching botanic classification. ***Diz A, A Martínez -Galisteo, M Sánchez-Rodríguez[1], A Conde-Pérez[2].*** *Department of Comparative Anatomy and Pathology. Faculty of Veterinary Sciences. University of Córdoba, España, [1]Department of Animal Production, Faculty of Veterinary Sciences, University of Córdoba, España, [2]Sanitary District Jaen Sur. Andalussian Health Service, Junta de Andalucia, España, Europe.*

Picking fungi is a growing hobby in south Spain. When eating is the intended purpose, greater security is necessary to avoid intoxication including death. The aim of this study was to preserve fungi picked during the different seasons of the year, and make a collection of the more frequently collected fungi of south Spain which would aid in teaching amateur people the botanic classification of fungi in the growing local Mycological Societies. Materials and methods: Country fungi were picked in the various stages of growth. The fungi were frozen as rapidly as possible to maintain their shape until transport to the lab. A GPS location detector was employed to localize the point where each fungus was picked. The standard S-10 silicone technique was applied for permanent preservation of fungi. To preserve color, no species were mixed during dehydration. The fast gas curing method was used to try and minimize shrinkage. The fungi were classified and placed in padded boxes to facilitate transport. Results: Fungi were successfully plastinated using the S 10 method. Our results were mixed and some problems were identified: Different degrees of shrinkage, shape changes, and discoloration. Plastination is a valid method to preserve fungi collections for teaching mycological classification all year long. However, the current results suggest that they are best used as complementary material to fresh, photographic and bibliographic sources. Conclusion: Plastination of fungi with the standard S-10 silicone technique is a valid method of preservation of fungi and has become a well accepted method of preservation of fungi by local Mycological Societies. Plastinated fungi are used to complement the teaching of the classification of fungi to amateur people. However, improved techniques must be found for our plastination lab in order to obtain the better results.

Plastination of exotic animals in veterinary medicine. ***Diz A, J Vivo, F Miro, JL Morales, JM Molero.*** *Department of Comparative Anatomy and Pathology. Faculty of Veterinary Sciences. University of Córdoba, España, Europe.*

Plastination is used more and more every day around the world as a process for preservation for whole body, body parts and organs of domestic animals in veterinary medicine. In developed countries, it is more frequent use exotics animals as pets. Anatomical and clinical knowledge is growing daily in veterinary schools. The aim of this study was to preserve by silicone plastination reptiles, amphibians, fishes and invertebrate animals. These zoological groups are known as exotic species in veterinary medicine. Materials and methods: Specimens were acquired from various places (necropsy rooms, markets, and dead animals found in the soil). This material was plastinated using the Biodur S-10 standard technique develop by Dr. von Hagens. Results: Reptiles, amphibians and fishes are specimens which are preserved well by this technique. Although those with vivid colors may result in loss of coloration to varying degrees. Moreover, they may shrink depending on their structure and consistency. Shrinkage can be reduced by injecting polymer-mix from the forced impregnation bath by mouth, anus and corporal cavities. Plastination of invertebrates had similar results although it is important to consider the great diversity of this zoological group. Conclusion: Plastination is an appropriate method to preserve zoological specimens different from mammalians. This technique allows the formation of collections of exotic animals, from both healthy and pathological origins, for study in veterinary medicine.

Some aspects of fungi plastination. ***Diz A, A Martínez -Galisteo, J Berlango[1], A Conde-Pérez[2].*** *Department of Comparative Anatomy and Pathology. Faculty of Veterinary Sciences. University of Córdoba, España, [1]Reina Sofia University Hospital. University of Córdoba, España, [2]Sanitary District Jaen Sur. Andalussian Health Service, Junta de Andalucia, España Europe.*

The aim of this study was to determinate if plastination is a valid preservation technique to obtain permanently preserved fungi as an aid to its botanic identification. Materials and methods: Fresh fungi were picked in the Sierra Morena hills, selecting young and healthy specimens. They were frozen and maintained in a household deep freezer until transport to the lab. Fungi were plastinated following the standard S-10 silicone technique without fixation. Before dehydration, fungi

were arranged loosely into an acetone resistant plastic basket and covered with a polymer mesh which was fixed to the basket to prevent floating. Dehydration was made in successive (3-4) baths of 100% cool acetone (-25°C) until no more than 1% residual water was present. Great care must be used in not mixing different species to preserve color. After dehydration, forced impregnation of S 10/S 3 was carried out. After impregnation and prior to curing, the fungi were placed in a vertical position (12-24 h.) in the freezer to drain the excess polymer. The fast gas curing method was applied to reduce shrinkage. Specimens were wiped of oozing polymer throughout the curing process. Results: Plastinated fungi, with great interspecies differences, discolor and shrink to some degree depending on the variety of the fungus. Nevertheless, they are preserved permanently, permitting identification of poisonous and edible fungi at any moment. Conclusion: Plastination of fungi with the standard S-10 silicone technique is a very valuable method for fungi preservation, although shrinkage, shape changes and partial damage of colour is observed.

Computerized reconstruction of a plastinated human kidney using serial tissue sections. ***Doll F, S Doll, M-C Sora[1], M Kuroyama, E Neufeld[2], S Lozanoff.*** *Department of Biomedical Sciences, University of Hawaii, School of Medicine, Honolulu, HI, USA, [1]University of Vienna, Vienna, Austria, Europe, [2]University of Saskatchewan, Saskatoon, SK, Canada.*

Baskground: Computerized reconstruction of anatomical structures is becoming very useful for developing anatomical teaching modules and animations. Although databases exist comprising serial sections derived from frozen cadaveric material, plastination represents an alternate method for developing anatomical data useful for computerized reconstruction. The purpose of this study is to describe a method for developing a computerized model of the human kidney and ureter using plastinates. Materials and Methods: A human kidney was obtained, plastinated, sectioned and subjected to 3D cmputerized reconstruction using WinSURF modeling system (SURFdriver Software). The kidney was generated rapidly and rendered easily on Windows laptop machine in real time. Results: Qualitative observations revealed that the morphological features of the model were consistent with those displayed by typical cadaveric specimens. Morphometric analysis indicated that the model did not differ significantly from a sample of cadaveric specimens. Conclusion: Although further experiments will be required to minimize tubular compression of the ureter, these data support the use of plastinates for generating tissue sections useful for 3D computerized modeling. Supported by BRIN NIH Grant Number RR-164676.

Latex injection for the arterial vascular patterns of the Ethmoidal arteries. ***Erdogmus S, F Govsa.*** *Ege University Faculty of Medicine, Department of Anatomy, Ege University, Izmir, Turkey.*

Ethmoidal arteries are damaged in endonasal surgical interventions and in operations performed on the inner wall of the orbit. Ligation of the anterior and posterior ethmoidal arteries causes severe nasal bleeding and mortality. The aim of this study is providing ligation with the appropriate intervention thus reducing the rate of damage to these arteries. Materials and methods: Vessels supplying blood to the orbit were examined by dissecting 16 cadavers that were fixed with a 10% formaldehyde solution. Red latex was injected into the internal carotid arteries before dissection. The size and distribution of the vessels were investigated and the primary supplying vessels were identified. Results: The anterior ethmoidal artery was observed in all specimens except one. The diameter of the artery, 0.92 ± 0.2 mm on the right and 0.88 ± 0.15 mm on the left, was thicker than the posterior ethmoidal artery. The branching of the anterior ethmoidal artery from the ophthalmic artery was determined to be four different types. The posterior ethmoidal artery was observed in all cases. The diameter of the posterior ethmoidal artery was 0.66 ± 0.21 mm on the right and 0.63 ± 0.19 mm on the left. Conclusion: Knowledge concerning the variations in the anatomy of the ethmoidal arteries is important for surgeons. This data emphasizes the importance of a sound anatomical understanding of the arterial system of the ethmoidal cells in relation to the surgical procedures to be done.

Plastination of musculoskeletal system: A useful tool in teaching. ***Faizah O,, HS Farihah, AR Fairuz, G Hairi, AL Azian.*** *Department of Anatomy, Medical Faculty, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.*

Interest in the human body leading to the dissection of corpses has been documented sine the 15th century. Although the study of cell has reached molecular levels, macroscopic anatomy remains as an indispensable component of the medical training. The musculoskeletal system being the backbone of Orthopedic Surgery need to be learnt thoroughly in the early years of the course. Pictures and images alone

may be inadequate to achieve this objective. Hands on experience is still seen as one of the best methodology in learning the musculoskeletal system. However, wet and smelly specimens deter effective learning. Plastination techniques allow specimens to be kept longer, dry and easy handling with minimal problems of storage and transportation. Regions of the upper and lower limbs were carefully dissected to reveal superficial and deep structures. These dissected parts were then processed according to standard plastination procedure which is dehydration, forced impregnation and gas curing. Muscles tendons, cartilages and bones plastinated with silicone rubber retained their natural appearance and colour within the acceptable limits of shrinkage. Although the consistency were rather hard, the results obtained were comparable to the exhibits shown in Body Worlds. However, comparing to the formalin preserved specimens, the neurovascular bundle were more brittle. Plastination technique provided more opportunity for students' self-directed learning since specimens are easily accessible, dry and odourless. In order for the specimens to achieve more flexibility, the concentration of the hardener has been reduced. Steps in dehydration and impregnation are strictly followed in order to obtain specimens which looked natural and realistic and at the same time more durable in view of shortage of cadaver in the near future.

Pitfalls of plastination: Solid and hollow organs. ***Farihah S, O Faizah, AR Fairuz, G Hairi, AL Azian.*** *Department of Anatomy, Medical Faculty, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.*

In teaching Gross Anatomy, internal organs are an invaluable teaching aid especially when cadavers are becoming increasingly difficult to obtain. Current methods of preservation, embalming with a solution with formaldehyde as the main preservative, exposes individuals to a variety of hazardous materials and causes irritation to mucous membranes of the eyes and respiratory tract. Embalmed organs remain wet with a fairly strong odor of formalin and there is a tendency for fungal growth in a humid environment. The plastination technique of silicone impregnation allows preservation of organs making them durable, dry and odorless thus facilitating handling. However, a number of technical problems were encountered with the plastination method used in the UKM Medical Faculty. Solid and hollow organs which were plastinated include the liver, lungs, heart and stomach. Results showed that all the organs shrunk between 10 - 30% with hollow organs shrinking more when compared to solid organs. Lungs and stomachs appeared unnatural as they collapsed and become hardened. Plastinated organs from embalmed cadavers were darker in color. Chambers of the heart often contained hardened polymer and were collapsed and difficult to visualize. Multiple technical errors have been identified which gave rise to these results: 1. Improper temperature (warm) and concentration of the acetone during dehydration, 2. Use of a higher concentration ratio of chain extender to polymer, 3. Inappropriate pressure and duration during forced impregnation, 4.Hollow organs were not inflated at the beginning of the plastination process. Hydrogen peroxide may be used to decolorize darkened specimens following the initial embalming process. Other corrective measures have been taken to improve the quality of the specimens.

Basis of latex injection techniques in arterial features of the posterior maxillary segment in relation to the Le Fort I osteotomy. ***Govsa F[1, 2], O Zuhal[2], Y Pinar[1].*** [1]Ege University Faculty of Medicine, Department of Anatomy, and [2]Ataturk Health Care College, Ege University, Izmir, Turkey.

The most common site of hemorrhage in maxillary osteotomies is the posterior maxilla. Better understanding of the vascular anatomy in posterior maxilla may minimize vascular complications. The aim of this study was to investigate the posterior maxillary region and establish safety guidelines for the Le Fort I osteotomy. Materials and methods: Intra-arterial injection of colored latex and dissection of the vessels running to the palate was performed in 30 male cadavers. The size and distribution of the vessels were investigated and the primary supplying vessels were identified. Results: The maxillary artery enters the pterygopalatine fossa approximately 16.2 mm superior to the nasal floor and gives off the descending palatine artery. The descending palatine artery travels a short distance within the pterygopalatine fossa and then enters the greater palatine canal. It travels approximately 10 mm within the canal in an inferior, anterior and slightly medial direction to exit the greater palatine foramen in the region of the second and third molars. Conclusion: Injury to the descending palatine artery during Le Fort I osteotomy can be minimized by not extending the osteotomy more than 35 mm posterior to the piriform rim in males. Pterygomaxillary separation should be made by closely adapting the cutting edge of a curved osteotome or right-angled saw to the pterygomaxillary fissure while avoiding excessive angulation. Furthermore, the superior cutting edge of the osteotome or saw blade should be less than

10 mm superior to the nasal floor. This study establishes that while it is possible to conserve the descending palatine arteries intact during Le Fort I maxillary osteotomies, injury to them leads only to moderate and transient ischemia of the palate thanks to vascular substitution which comes from the ascending palatine arteries and pharyngeal branches running through the soft palate.

Starting plastination in the 2000's. ***Guintard C, E Betti, J-C DesFontis, G Grondin[1].*** *École Nationale Vétérinaire de Nantes, Unité d'anatomie comparée, Nantes, France Europe, [1]Université du Québec à Trois-Rivières, Département de chimie-biologie, Trois-Rivières, Qc, Canada.*

Since the introduction of the technique in 1979 by Dr. von Hagens, plastination laboratories have been developed around the world. Each time a project for a new laboratory was presented, two important questions were raised: What equipment is necessary and how much will it cost? These two questions still appear today when someone decides to start plastination. The design and starting cost of plastination laboratories have been reported in the literature in past years (Lischka and Prihoda, 1987; Gubbins, 1990; Bore and Boyes, 1994; O'Sullivan and Mitchell, 1995; Briggs et al., 1997) but has not been updated since 2002. The present work reports on the starting of a plastination laboratory in the summer 2003 at the "École Nationale Vétérinaire de Nantes" with a listing of the equipment purchased for this project and its cost. The selected location for this laboratory will be described along with the modifications that were necessary to make the laboratory safe and functional. The cost of these modifications is also listed.

Sheet plastination of human brain slices: Comparison between two staining methods. ***Jareonsuppapuch E, U Tankittiwat, P Padungchaichot.*** *Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.*

Sheet plastinated brain slices are excellent teaching specimen in medical schools. Sheet plastinated brain slices utilizing resin produced in Thailand were prepared and stained. The aim of our study was to stain 2-6 mm thick sheet plastinated brain slices with Luxol fast blue and Astra blue staining methods. The result demonstrated that both stains clearly differentiate grey and white matter. The grey matter was white and the white matter was greenish blue in the Luxol fast blue staining method whereas the grey matter had blue color and the white matter showed white in the Astra blue staining method. In addition, the shrinkage of the two different stained sheet plastinated brain slices was insignificant.

Study of brain evolution in human and animals using the S/10 plastination method. ***Joghataei MT; MH Asadi, A Mohammadzade, F Negahdar, G Izadi-Mobarakeh.*** *Iran Medical University, Baqiyatallah University of Medical Science, Department of Anatomy, Tehran, Iran.*

During the course of evolution, the area of brain that shows the most change is the cerebral hemispheres. The more recently evolved animals have a larger proportion of the brain taken up by the cerebral cortex. In the "higher" animals, the surface of the cerebral cortex becomes folded resulting in sulci and gyri. Material and Methods: This study used the S/10 plastination method on human, monkey, cat, cow, sheep, chicken, mouse, and fish brains. Results: Different neuroanatomical parts of the brains were compared. Also, sulci and gyri of mammal brains were compared. The results of the study are presented in a comprehensive table. Conclusion: Plastinated brains are available for demonstration of the evolutionary changes.

Computer image demonstrations combined with plastinated specimens: Applications for topographic lectures of head and neck clinical anatomy. ***Klepá ek I, I Helekal[1], J. Lexa[2].*** *Department of Anatomy, Kuwait University, Kuwait, [1]Higher Professional School of Applied Art and Secondary School of Applied Art, Prague, Czech Republic, [2]Biomedical Engineering Unit, Kuwait University, Kuwait.*

The dissection of human cadaver materials may not provide all the topographical relations between human body structures. Because of our 2004 curricular schedule, it appears beneficial to establish an efficient method for lecture presentations to be oriented toward the postgraduate students. Lectures are followed up with clinically oriented anatomy. Here a combination of techniques are used which combine plastinated specimens with images of anatomical structures and computer applications. The dissection of the human material only does not always give correct topographical relations among human body structures. It appears that it is necessary to establish an efficient method for the lecture presentations oriented to the postgraduate students (due to our curriculum for 2004). We follow up the teaching process of clinically oriented anatomy by combining pictures of the presented topic, plastinated specimens and computer

viewed applications. Materials and methods: Original pictures of the head and neck regions were made using the method of American retouch. The plastinated specimens (Kuwait and Prague anatomy collections; the Silicone S 10 procedure) and dissected organs served as master specimens. Using computer equipment and digital audio/video system, the animated series of pictures and 3-D imagination were produces using software (ACDsee, Adobe-Photoshop, 3-D Anamaker). Results: Picture sets (based on curricular materials) were combined into four mutually "dissolving picture sets". These sets (15 pictures each) displayed structures from surface to depth. Photos of the plastinated specimens showing details of the selected topographic areas were incorporated into these sets. The following regions were illustrated: a) The face and orbit. b) The infratemporal fossa. c) The submandibular area. d) The neck triangles. Conclusion: Desired relations among head and neck structures are presented using picture sets, which are made as if seen by the physician eyes (if he is leaning towards patient). This is crucial to understand and remember mutual relationships, visual learning and an easy understanding of the external projection of the structures. Materials & Subjects & Methods: Original images of the head and neck regions were made utilizing American retouch technology. The silicone (S10) plastinated specimens from the Kuwait and Prague anatomy collections and dissected organs served as the master models. An animated series of photos and 3-D imaging were produced using computer equipment, digital audio/video systems and production software (ACDsee, Adobe-Photoshop, 3-D Anamaker). Results: Based on curriculum materials and needs, image sets were combined into mutually "dissolving image sets". Each set contained 15 images and were programmed to display anatomic structures from superficial to deep. Photos of the plastinated specimens showing details of the selected topographic areas were incorporated to the sets. The following regions were illustrated: 1. The face and orbit, 2. The infratemporal fossa, 3. The submandibular area, 4. The neck triangles. Conclusion: The programs are shown from the perspective of a physician leaning toward and viewing the patient. The sets show the relationships between head and neck structures. This methodology is an important way to reinforce the relationships of structures, visual learning and to understand the projection of deep structures under the surface.

The impact of plastination on the teaching of neuroanatomy. ***López-Solerm M, O Roda-Murillo, A Roda-Murillo, I Ramírez-Ortiz.*** *Department of Human Anatomy and Embryology, Medicine Faculty, University of Granada, España, Europe.*

The development of plastination has allowed the introduction of new tools into the teaching of anatomy. Specimens obtained by this method are dry, odorless, long-lasting and non-toxic. The present study compared the degree of satisfaction of students with the utilization of plastinated versus formalin preserved specimens for the study of neuroanatomy. Materials and methods: Analysis of variance was used to determine whether there was a relationship between the type of preservation technique (formalin or plastination) as an independent variable and the degree of satisfaction as the dependent variable. Whether there was equality of means for the two techniques was investigated. Results: Equality of means was not observed. The independent variable (type of technique) significantly influenced the dependent variable (degree of satisfaction). The mean obtained for the plastination technique was higher than that for the formalin technique. Therefore, there was a greater degree of satisfaction with plastinated versus formalin preserved specimens. Conclusion: The plastination technique positively influences the degree of satisfaction of the student.

Plastination of a canine encephalon with hydrocephalus. ***Martín -Alguacil N, R Martín -Orti.*** *Department of Anatomy and Comparative Pathologic Anatomy, School of Veterinary Medicine, Universidad Complutense de Madrid, España, Europe.*

It is important to visualize the three-dimensional features of the ventricular system in order to understand how some pathological processes such as hydrocephalus can occur. Hydrocephalus is an increase in the volume of the cerebrospinal fluid, resulting in an extreme expansion of all the ventricles. Brain prosections and brain slices were plastinated to study the ventricular system of the dog encephalon. Sections of a dog brain with hydrocephalus were plastinated as well. All specimens were plastinated with silicone using the Biodur S 10 technique. In order to minimize shrinkage of the brain tissue, the standard procedure was slightly modified as recommended by Weiglein (2000). Prior to forced impregnation, the brains were immersed in a mixture of S10/S3 at -20°C for three days. Good quality specimens were obtained after plastination of all tissues. A very good correlation between anatomical structures was seen, both before the specimens were fixed and after they were plastinated. Plastinated specimens of central nervous system provide the student with a useful tool to

understand the dog ventricular system.

Anatomy of the mammary gland of the goat using S10 plastinated specimen. ***Martín -Alguacil N, R Martín-Orti, A García, A Gómez, L Gallego.*** *Department of Anatomy and Comparative Pathologic Anatomy, School of Veterinary Medicine, Universidad Complutense de Madrid, España, Europe.*

Understanding the anatomy of the mammary gland is important not only for veterinary students but also for practitioners while undertaking surgery on that region. The aim of this project was to provide a plastinated model to study the anatomy of the mammary gland. The tissue was obtained from adult goats. To avoid blood clots, the goats were heparinized prior to euthanasia. The vascular system was washed by perfusion of saline solution and then the arteries and veins were injected with latex colored with red and blue pigments. The collecting sinuses were dilated due to the presence of milk. The tissue was frozen and cut in 2.5 mm sections. The slices were fixed in 5% formaldehyde for two weeks. The mammary gland sections were dehydrated in increasing baths of acetone and plastinated following the standard S10 plastination technique. Nice specimens were obtained as a result. There was a good correlation between fresh and plastinated tissue.

Use of plastinated specimens to visualize the vascularization and inervation of the thorax. ***Martín-Orti R, N Martín-Alguacil.*** *Department of Anatomy and Comparative Pathologic Anatomy, School of Veterinary Medicine, Universidad Complutense de Madrid, España, Europe.*

Plastination is a great tool to use in teaching veterinary anatomy. It is also of a great value for student self-directed learning. Students can freely access the prosection and visualize the different structures without spending a lot of time preparing a dissection. Herein we present a plastinated thorax from a foal that was dissected to study the thoraic vascular distribution and innervation as well. Special attention was given to show the thoracic autonomic nerves. Among these structures sympathetic trunk and ganglion, cervicothoracic ganglion, vagosympathetic trunk, recurrent laryngeal nerve and cardiac autonomic nerves were dissected and preserved. The thorax was plastinated using the S-10 technique with slight modifications. A very good quality specimen was obtained.

Room temperature degreasing body sections. ***Moreno M., H Abellán[1], MD Ayala[1], C Sanchez[1], R Latorre[1].*** *Anatomia y Embriologia, Facultad de Medicina, Universidad de Murcia, [1]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, España, Europe.*

The main objective of this work was to compare two room temperature degreasing processes, a short one (3 weeks) and a long one (6 months). A whole fresh human body was frozen at -40C and transversely cut into sections of 1cm thickness. Fixation was done by immersion in a 10% formalin solution and dehydration according to the regular system in cold acetone. Sections from head to cranial part of thoracic cavity were degreased in room temperature acetone for 3 weeks. The other sections were degreased in room temperature acetone for 6 months, (until acetone baths did not turn yellow). The short degreasing process had no shrinkage but the fat tissue had a bad appearance and the sections a bad smell. Sections from the long degreasing process had transparent odourless fat but more shrinkage than short time process.

Sheet plastination of the human midbrain slices demonstrating dopaminergic neurons. ***Padungchaichot P, E-O Jareonsuppapuch, U Tankittiwat.*** *Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand.*

Parkinson's disease is one of the major neurodegenerative disorders. The pathology of the disease is a specific degeneration of dopamine neurons in the midbrain. This was an applied study which utilized the sheet plastination technique for permanent preservation of immunohistochemically processed 1-2 mm thick-midbrain slices demonstrating dopaminergic neurons. Either DMSO or citrate buffer solution was applied to reverse the loss of antigenicity that occurs with some epitopes in formalin-fixed human brain. Primary antisera against tyrosine hydroxylase were used to demonstrate dopaminergic neurons in the midbrain. The results showed dark stained dopaminergic neurons in the immunohistochemically processed midbrain. In addition, the stained neurons could be permanently preserved by the sheet plastination technique.

Green iguana plastination with the S 10 technique in Macedonia. ***Pendovski L, V Ilieski, G Nikolovski[1].*** *Department of Anatomy, Faculty of Veterinary medicine-Skopje, Macedonia, [1]Department of Internal Disease, Faculty of Veterinary Medicine-Skopje, Macedonia, Europe.*

Green iguanas *(iguana iguana)*, the most popular

common lizard, has grow in popularity as a domestic pet. Preservation of the green iguana using the silicone plastination method is an essential process for storing the specimen as a reference collection for a long time. This work describes the complete process for plastination of an iguana preformed by the standard S 10 silicone technique, according to von Hagens. The green iguana's thick skin provides an opportunity to prevent the process of impregnation with silicone from penetrating the skin. Also the iguana's body was fixed in 3% formalin and in a bad body position for more than three years. Its body was enveloped by its tail and its head was positioned below and between its forelimbs. One of the main goals was to acquire more correct anatomical position of the iguana, especially during the curing stage. The green iguana was dehydrated by the freeze substitution method using three acetone baths (100%) at -25°C over a four week period. The fourth acetone bath was at room temperature for the period of five days. When the acetone purity in the last acetone bath remained over 99.5%, dehydration and defatting were considered complete. Forced impregnation was preformed, after the iguana was submerged in a mixture of silicone S10/S3 (100:0.5) in a freezer at -20 °C, by continuous pumping for three weeks. The vacuum was slowly increased to assure better penetration of polymer-mix and hence prevent shrinkage of the iguana. The impregnation process was considered complete when the bubbles ceased and the pressure was stabilized at 3 mbar. Finally, the iguana was cured by exposure to S6 gas cure. After 5 days, the surface of the iguana was cured, but to assure that the depths of the iguana cured, a tube with syringe was inserted in the abdomen of the iguana and S6 was conducted via this into abdomen for more than seven days. The iguana was perfectly preserved without noticeable shrinkage. The penetration of silicone trough iguana skin was very successful in spite of its thickness. Compared with other plastinated specimens, there wasn't a big difference considering the skin thickness. During the curing stage, the iguana shape was reformed even after being fixed for over three years in an inappropriate position. The plastinated iguana looked very natural and can be easily carried and manipulated with less risk of damage.

Branches of the facial artery of the perioral region: Latex injection study. ***Pinar Y, O Bilge, F Govsa.*** *Ege University Faculty of Medicine, Department of Anatomy, Ege University, Izmir, Turkey.*

There are numerous techniques for reconstruction of lip defects. The use of flaps requires a detailed knowledge of local vasculature. New flaps for the region around the mouth can be devised if the surgeon is aware of the distribution of the perioral arterial branches. Materials and methods: Vessels supplying blood to the perioral region were examined by dissecting 25 cadavers that were fixed with a 10% formaldehyde solution. Red latex was injected into the common carotid arteries before dissection. The size and distribution of the vessels were investigated and the primary supplying vessels were identified. Results: The facial artery (FA) was symmetrical in 17 of the 25 heads (68%). It terminated as an angular facial vessel in 11 (22%), a nasal facial vessel in 30 (60%), a superior alar vessel in 6 (12%) and a superior labial vessel in 2 facial halves (4 %). It terminated as a hypoplastic type of FA in 1 facial half (2%). The average external diameter of the superior labial artery at its origin was 1.6 mm. The beginning point of the superior labial artery (SLA) was above the angle of mouth in 34 out of 47 specimens (72.3%), and at the angle of mouth in 13 out of 47 specimens (27.7%). Three of the specimens were not evaluated because in 2 of these 3 the SLA was the continuation of LA and the other was a hypoplastic type. The SLA supplied the columellar branches in all samples except for the hypoplastic type (49 specimens). Columellar branches were classified according to their number or type. In 5 specimens (10 %), there was no inferior labial artery (ILA). In the other specimens, the bifurcation point of the ILA varied between the corner of the mouth and the inferior margin of the mandible. ILA arises from the FA above the angle of mouth in 4 (8.8%) specimens, under the angle of mouth in 9 (20 %) specimens and, at the angle of mouth in 30 (66.6%) specimens. Conclusion: We suggest that knowledge of the artery's location with respect to easily identifiable landmarks will help to avoid complications.

The experience of setting up a plastination laboratory. ***Reina-De-La Torre F, A. Rodríguez-Baeza, JM Doménech-Mateu, M Querol-Jiménez, I Delgado-Calvarro.*** *Department of Morphological Sciences (Anatomy and Embryology Unit), Faculty of Medicine, Autonomous University of Barcelona, Barcelona, España, Europe.*

Contrary to intuition, to perform the basic techniques of plastination does not require a significant economical investment. The technical equipment can be obtained with a limited budget. The present work reports the recent experience in the design and setting up of a laboratory to plastinate anatomical preparations using the standard S-10 silicone technique. The different

ways used to finance the cost of the facility and the technical equipment will be explained. Materials and methods: The plastination laboratory was designed in accordance with the International Society of Plastination guidelines and with the technical support of the Plastination Laboratory of the University of Murcia. The financial resources came from the Faculty of Medicine, the Anatomy and Embryology budget or from external grants for increasing safety in research laboratories. The design of the plastination laboratory was carried out in order to start up the standard S10 silicone technique. Results: The laboratory occupies a total surface of 12.5 m^2. The laboratory has a forced extraction system for acetone vapors. The financial resources allocated to the plastination laboratory were 10,418 euros. These resources were used as follows: 21.85% for the facility, 61.75% for technical equipment and 16.40% for laboratory materials. Conclusion: The setting up of a plastination laboratory does not need to be a high economical effort. On the contrary, the advantages of the technique justify the investment. It reduces the exposure to toxic fumes, improves the preservation of teaching preparations and promotes techniques in self-directed learning of anatomy.

Preliminary results with the S10 standard technique: An experience of the future in the Faculty of Medicine of the Autonomous University of Barcelona. ***Reina-De-La Torre F, A. Rodríguez-Baeza, JM Doménech-Mateu, M Querol-Jiménez, I Delgado-Calvarro.*** *Department of Morphological Sciences (Anatomy and Embryology Unit), Faculty of Medicine, Autonomous University of Barcelona, Barcelona, España, Europe.*

According to the collection of technical leaflets for plastination from Heidelberg, the easiest and cheapest plastination methodology is the standard S10 silicone technique. With the technical support and collaboration of the plastination laboratory of the University of Murcia, a plastination experience was initiated in the Faculty of Medicine. The two main objectives were to initiate the elaboration of an anatomical collection of plastinated specimens in order to promote self-learning techniques in human anatomy and to reduce exposure to carcinogenic toxic fumes. The present work shows the preliminary results. Materials and methods: Specimens of various anatomical regions or systems (central nervous system, heart, hand, topographical slices, facial region and bones) from human cadavers previously fixed in Cambridge solution were processed according to the standard S10 silicone technique. Results: The obtained plastinated specimens were optimum with regard to preservation, color and durability. The processing time ranged from 1.5 to 4 months. Specimens with the most difficulties for dehydration and impregnation were the facial and hand regions. Vascular injection with colored latex complimented the process. The use of plastinated specimens for practical activities in the medicine curriculum has been received by the students enthusiastically. Specimens can be manipulated without disruption, they are odorless and they may be used outside the programmed sessions. Conclusions: The standard S10 silicone technique is a very standardized method, not difficult from a technical point of view, and offers a great range of possibilities to improve the teaching of anatomy: It reduces the exposure of teachers and students during practical activities to carcinogenic toxic fumes, It better preserves teaching specimens, It renders access to such specimens by students, and It promotes techniques in self-directed learning. There is no doubt that the technique will have an important role in the future changes of higher education curricula.

Plastination in teaching neuroanatomy. ***Roda-Murillo O, López -Solerm M, A Roda-Murillo, I Ramirez-Ortiz.*** *Department of Human Anatomy and Embriology, Medicine Faculty, University of Granada, España, Europe.*

The difficulty of obtaining human brains for practical classes in Human Neuroanatomy and the fragility of this material has led to the development of new preservation techniques to obtain more resilient human brain specimens that are faithful to reality and suitable for handling by students. The present study compared the value of plastinated versus formalin preserved specimens in neuroanatomy teaching. Materials and methods: A questionnaire was administered to students of neuroanatomy on the use of formalin preserved and plastinated specimens. It asked: Which technique better preserved and allowed longer use of the specimen, Which specimens were more faithful to reality and better to handle, and Which technique was preferred by the students. The results were subjected to statistical analysis. Results: 90.8% of the students considered plastination preserved specimens better when compared with formalin preservation. 93.3% believed plastinated specimens to be faithful to reality. 40.8% thought they were more realistic than formalin preserved specimens, 45.4% thought they were equally realistic, and only 13% described the plastinated specimens as less realistic. All of the students believed that the handling was better with plastinated versus formalin preserved

specimens. 93.6% preferred plastinated specimens for the study of neuroanatomy and 6.4% preferred formalin preserved specimens. Conclusion: Plastination is a very valuable tool for teaching of neuroanatomy.

Plastination and mimimally invasive surgery. *Sáchez Margallo FM, J Usón, F Soria, F Gil[1], R Latorre[1].O López -Albors[1], M Sarasa[1], S Climent[1]. Minimally invasive surgery Center, Caceres, Spain, Europe. Medicina y Cirugia Animal, Facultad de Veterinaria, Universidad de Murcia, España. [1]Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, Murcia, España, Europe.*

Plastination is a unique technique of tissue preservation. Plastinated organs are useful as didactic resources when teaching minimally invasive surgery. The morphological and physical characteristics of plastinated specimens are excellent to study topographic and clinical anatomy. Specially designed plastinated organs aid the teaching-learning process of minimally invasive surgical techniques, as they allow training of endoscopic techniques and skills. These techniques will be shown in a DVD presented. Digestive system: Whole or partial gastrointestinal tracts of the dog were specially designed for exercises in digestive endoscopic explorations (esophagogastroscopy, duodenoscopy and colonoscopy) and plastinated. Visceral topography and the endoluminal aspects of plastinated organs are real and their flexibility allows exploration with a regular digestive endoscopic. Cardiopulmonary System: Plastinated cardiopulmonary blocks and isolated lungs of dogs, were designed to practice respiratory endoscopic explorations (bronchoscopy, cytology and tracheal suction and bronchial flushing). Isolated and plastinated hearts were used as a vascular model for endoluminal and interventional radiology techniques. Whole cardiopulmonary casts, bronchial tree casts and colored silicone pulmonary vessels casts of pigs and sheep were used as didactic tools for thoracoscopic surgery. Reproductive System: Plastinated female genital tracts of dogs and sheep and male genital tracts of dogs and pigs, suitable for gynecological laparoscopy surgery, were prepared and used as anatomical didactic models. Urinary System: Kidneys, ureters, urinary bladder and urethra in abdominal and pelvic cavities of the dog, pig and sheep were vascular injected and dissected. These plastinated specimens were used for teaching experimental urological laparoscopy.

Embalming technique and dissection procedure of human bodies at the University of Antwerp, Belgium. ***Van Toor I, H Bortier.** Human Anatomy and Embryology, Dpartament of Medicine, University of Antwerp, Antwerpen, Belgium, Europe.*

Personal health of the technician and the students as well as environmental considerations led to the adaptation of this embalming technique and the dissection procedure for human bodies. It is known that formalin and phenol vapours cause irritation of human airways and cause environmental concerns. Second, there is only one technician at the institution to embalm all of the human bodies. Third, the educational developers of the new curriculum asked whether it would be possible to work in smaller groups. For all these reasons the embalming technique and the dissection procedure were adapted as follows. Materials and methods: An ethanol solution is injected as the embalming fluid via a catheter in the A. femoralis. Second, in order to shorten the embalming period, a pump is fitted to the catheter. Third, during the dissection course, six first year medical students who are coached by at least one medical student dissect one body. Results: According to the comments of the technician, the students, the environmental adviser and the medical coaches, there is less irritation of the airways, the human bodies smell less and the department has taken care of the environmental concerns. Second, the technician is happy that embalming a human body only takes three to four hours. Third, the medical students and the curriculum developers are pleased that there is more space to work and that they are coached better. Conclusions The embalming technique and the dissection procedure of human bodies at the University of Antwerp were successfully adapted to the problems and the questions put forward.