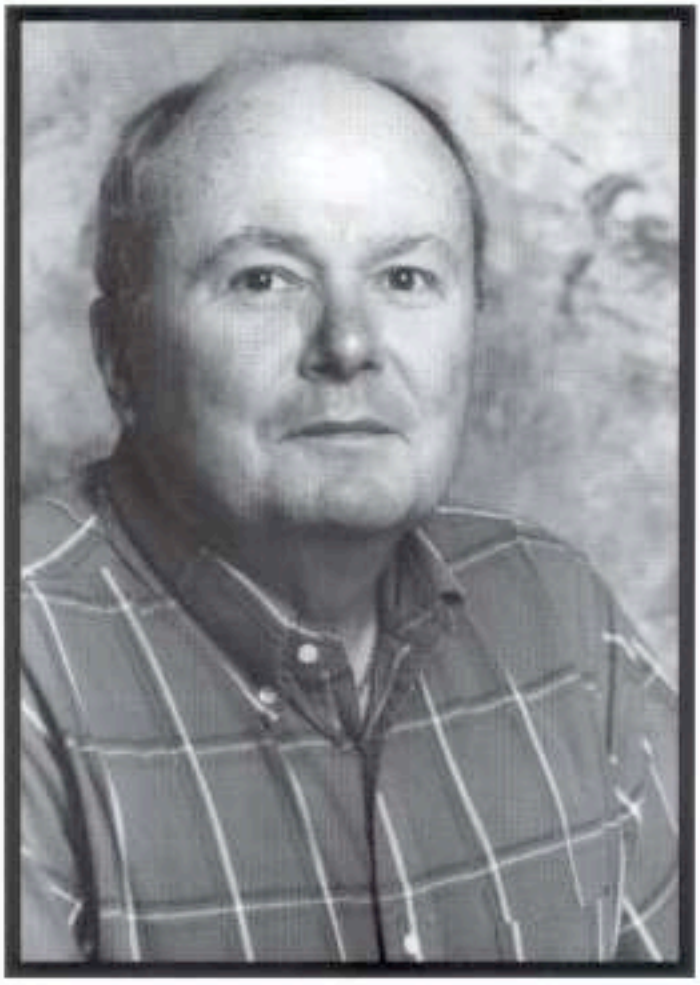
journal of the

INTERNATIONAL SOCIETY for PLASTINATION

This volume is dedicated in memory of Dr. Harmon Bickley



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Letter from the President

Dear fellow plastinators,

Since our meeting in St. Etienne last summer, several changes have taken place with our journal, all of them intended to enhance the scientific quality of the journal to the best possible level.

The Editorial Board (EB), a group of thirteen internationally renown scientists from all over the world, has set up guidelines for authors (attached at the end of this issue), reviewers and editors. The objectives of these guidelines and of the EB are to professionally assist you, the authors, with your publication and to ensure that your articles are published in a high quality journal.

Robert W. Henry, who besides serving the ISP as treasurer, accepted the challenge to chair this committee until an editor is appointed. He has done a tremendous job both in overseeing the establishment of *Guidelines for Authors* and the production of this *volume 16 of the JISP*. The obvious high quality of our new journal is due in part to his merit and the merit of Dr. Robert B. Reed, Jr., Assistant Editor.

I do hope that, with the increase of both scientific and layout quality of the new JISP, you are encouraged to submit high quality research and education papers to ensure that the JISP will flourish.

The search for the new editor is just about to come to an end. The JC will soon suggest the candidate to be appointed editor of the Journal of the ISP. The editor's charge will be to ensure continuity of what has begun with our journal and to continue its improvement.

Moreover, you will shortly be asked for nominations of officers - i.e. president, vice-president, secretary and treasurer – please begin to think about nominees and also about your own willingness to serve as an officer for a two-year term.

I am looking forward to meeting you and to exchange ideas and news during the 11th International Conference on Plastination in San Juan, Puerto Rico from July 14th through 19th, 2002. Start now to plan your trip to Puerto Rico!

Finally, I need to address a sad circumstance. The father and first Executive Director of this Society and the father and first Editor of this Journal, our Honored Member Dr. Harmon Bickley, passed away on February 19, 2001, in Ludington, Michigan following a period of declining health. To honor his work for this society, this volume is dedicated in memory of Dr. Harmon Bickley. *Requiescat in pace!*

With the kindest regards from Graz, Austria.

Andreas H. Weiglein
ISP-President

Journal of the International Society for Plastination 16:3-4 (2001)

A Tribute to the Father of Plastination in the USA

Dr. Harmon Bickley

May, 1930 - February, 2001

"Fixation! Dehydration! Defatting! Impregnation! and Curing!"

How many times did we hear him say it? For this writer it was numerous times and each time Harmon conveyed a sincerity that can come only from the true believer.

Most of us knew that Harmon Bickley was in declining heath but the notification of his death was a jolt none the same. One immediately conjured up the pleasant face, the fringe of hair, the unique voice pattern and the purposeful bearing that said "we have things to do and we should get to them".

I first met Harmon in San Antonio in the spring of 1986. My institution had received notification of a meeting to be conducted there concerning preservation of teaching specimens for medical education. The illustration on the brochure showed a cross-section of a torso seemingly submerged in a liquid with bubbles issuing from the specimen. This was surrounded by a rectangle and we took it to be an embedment of a cross-section. Our anatomy course coordinator thought that it would be a good thing for us to have the capability of manufacturing similar teaching aids and arrangements were made for me to attend the meeting. At the end of the first morning of the meeting I called home and told him "This isn't an embedding class - - IT'S A WHOLE LOT BETTER !".

And that is what Harmon realized from his first experience with plastination. It was a WHOLE LOT BETTER. But then, Harmon had been at this business of a WHOLE LOT BETTER for years. He was dedicated to teaching healthcare professionals and always on the lookout for better and innovative ways to do so. Even in the midst of a plastination meeting in Heidelberg I was talking to Harmon in the atrium of the Pathology Institute and he reached in his pocket and pulled out an unusual specimen. It was a hologram that he had recently made and was exploring the possibilities of its use in medical education.

Another example of Harmon's dedication to education and innovative methods occurred at the plastination meeting at Mercer University. Most of us that attended the meeting were aware of a new curriculum that was beginning to be implemented at a few places and Mercer University had embraced it to the fullest. "Problem-Based Curriculum" had a somewhat sinister name that both intrigued and repelled some of us. As the meeting progressed, questions about the curriculum were repeatedly raised and Harmon finally said that the purpose of the meeting was plastination and not problem-based learning but he would be willing to address that subject if we were willing to return in the evening for an unstructured session. As I recall, the space that Harmon had chosen for the meeting proved to be too small and we needed to move to a larger room to accommodate those who returned to hear him talk about this new method. (When plastinators elect to listen to education philosophies rather than sample the local nightlife and places of alcohol consumption, you know that something big is up and the speaker is highly respected.) There was nearly 100% attendance at this *ad hoc* meeting. Calmly, coolly and capably Harmon talked about the curriculum and in his typical way was willing to talk about the faults as well as the positive aspects of a method in which he believed. Typically too, he was very positive about the important part plastinated specimens were playing in the curriculum. The extent to which they were used can be pointed out when a question was raised about some aspect of dissection and Harmon turned to another staff member and said "Do we still have a cadaver upstairs?". At this I sat bolt-upright and several others shifted uneasily in their seats as we could not imagine a medical school that did not engage in an extensive dissection

experience. But Harmon calmly allayed our concerns and went on to talk extensively about how it all happened. It was a long session - - and yet another example of Harmon's mission in life - - - to educate.

In reviewing Harmon's curriculum vitae, his list of publications, his memberships, his offices held, his land-mark activities, one gets a further sense of Harmon's dedication to education. The published obituaries gave me some insight into Harmon's life that I had not known and told me who most poignantly mourn his passing. Well, we are going to miss him too. Who else will be able to initiate a plastination conference by saying "fixation, dehydration, defatting, impregnation and curing" and have quite the same sincerity ? Oh, we will find someone and they will do a commendable job of it. But it won't have the "Harmon touch".

By: Mr. TIM BARNES, SECRETARY ISP

Addendum: For those of you who are new to ISP and did not have the privilege to know or meet Harmon Bickley: Harmon was instrumental in bringing the concept of plastination to North America. He worked closely with Dr. Gunther von Hagens and also brought the involved technology to North America. In 1982 he hosted the first workshop, which has now gone on to become what we know as the International Conferences on Plastination. He hosted the next three conferences. He was the founder of the International Society for Plastination and Harmon served as the executive director. From this organization, this journal was born and Harmon served as the first editor.

Journal of the **International Society for Plastination 16:5-8 (2001)**

Using a Room-Temperature Plastination Technique in Assessing Prenatal Changes in the Human Spinal Cord

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Abstract: This work examines the efficiency of a modified room-temperature plastination technique for preserving prenatal spinal cord morphology by assessing changes in prenatal cord length in human fetuses before and after plastination. Changes in cord length were correlated to fetal age, gender and ethnicity. Gross morphological features of the spinal cord preserved by this technique were clearly recognizable. Statistically significant shortening of spinal cords ($p < 0.05$) resulted when using this plastination technique. Differences in spinal cord and crown-rump lengths in relation to age were also significant. However, crown-rump length did not change significantly after plastination. Also, length changes in relation to gender and ethnicity were not significant.

Key words: **human fetus; room-temperature plastination; spinal cord**

Introduction

The introduction of plastination as a technique for tissue preservation by Dr. Gunther von Hagens has broadened the horizon in anatomy education all over the world (von Hagens, 1987). In this process, curable polymers replace water and lipids in biological tissues. The polymer is subsequently hardened, resulting in dry, odorless and durable specimens (Weiglein, 1997). This room-temperature plastination technique is a modification of the original techniques described by von Hagens in 1978 and Glover et al. in 1998. Specimens are dehydrated in a graded acetone series at room temperature. After dehydration, specimens are immersed in liquid silicone polymer mixed with a cross linker. Vacuum is applied and when impregnation is achieved, specimens are treated with a catalyst to initiate curing.

Since its introduction four years ago, experimentation with the process continues to ensure that specimens produced are of a reasonable quality in terms of color, flexibility and clarity of detail. In search of new applications and ways to validate this technique, the spinal cord was chosen. The length of the spinal cord in human fetuses at different ages was measured before and after plastination to assess variations in cord length. The aim of this work is to examine the efficiency of another room-temperature plastination technique by demonstrating morphological features and growth changes in the spinal cord using human fetal specimens as models.

Materials and Methods

Thirty human fetuses (14 female and 16 male; 5 black and 25 white) between 16.5 and 38 weeks of age (mean = 25.2 weeks) were obtained from the Patten Embryology Collection, The University of Michigan. The fetuses were from stillbirths and miscarriages and were kept in formalin for 15 years. Spinal cords were

exposed by doing laminectomies on the inferior thoracic and all lumbar vertebrae. Crown-rump (CR) and spinal cord lengths were measured. Spinal cord length was measured from the base of the skull to the tip of the conus medullaris. A pin inserted deep into the back muscles marked this level. CR length, considered here as a rough guide of the vertebral column length, was measured between the vertex of the skull to the midpoint between the apices of the buttocks as suggested by Sadler (1990). All measurements were taken pre and post dehydration and after curing.

Specimens were washed in running water for two days and then dehydrated in a graded acetone series at room temperature. Specimens were placed initially in 90% acetone for 24 hours, and then transferred to two consecutive pure acetone baths. Specimen/acetone ratio was 1:10. Dehydration was deemed complete when the concentration of acetone measured with a hydrometer was stable at 99% during the last three days. Dehydration time, five days, was the same for all the specimens.

Specimens were immersed in COR-TECH™ PR-10 silicone polymer (Corcoran laboratories) mixed with 7% cross linker (CR 22™) and vacuum was applied. Vacuum was increased slowly for 24 hours until final vacuum was achieved (28 to 29 inches of Hg). After a total of 35 hours of vacuum, no acetone bubbles were observed, impregnation was complete and vacuum was discontinued. Acetone was reclaimed in two dry ice cooled traps (Welch Vacuum #545003) (Fig. 1). About 2.8 liters of dry ice slurry (dry ice and alcohol) was used to fill each trap. Usually the holding time of the dry ice was about 4-5 hours. However, dry ice stayed for longer times when trapped acetone became less in volume during the later stages. Locally made Styrofoam® jackets to ensure better insulation and a longer holding time covered the traps. Acetone was drained from the traps through the lower port after closing the valves to maintain the vacuum while pumps were running. Trap volume was 3 liters. The level of acetone in the traps was monitored through the clear acrylic plastic lid. The initial and final volume of the polymer-cross linker reaction mixture was estimated.

Specimens were drained of excess polymer for 24 hours and then sprayed and brushed with catalyst (CT 32™). They were wrapped in plastic wrap for 6 hours, unwrapped, wiped of any excess polymer and left to cure in moist room temperature.

After curing, the spinal cord and the crown rump length of each specimen were measured. Initial measurements and those made after room temperature processing were compared. Student's *t* test was calculated using the SPSS statistical package.

Results

Impregnation took 35 hours. Specimens were nearly cured and ready to handle 24 hours after the initial application of catalyst.

No gross morphological changes particularly in flexibility and firmness were observed in the fetal specimens following the impregnation and curing. General spinal cord features, e.g. the cauda equina, the dorsal and ventral roots of spinal nerves and dorsal root ganglia were clearly preserved in all specimens (Figs. 2, 3, 3a). The tip of the conus medullaris, taken as the point of spinal cord termination, was at the level of the second to third lumbar vertebra in younger fetuses and more superior (LI - L2) in fetuses approaching term. After dehydration, no shrinkage was measured. However, after impregnation and curing, mean length of spinal cords was 17.8% shorter than before plastination. The difference in mean length was statistically significant ($p < 0.05$). Also, there is a significant statistical correlation between fetal ages and spinal cord and CR mean lengths ($p < 0.05$). Changes in spinal cord mean length showed no significant correlation to gender and ethnicity (Table 1, Fig. 4). Crown-rump mean length did not change significantly after plastination.

Acetone collected in the dry ice-cooled traps was 12.5 liters. The volume of the polymer-cross linker reaction mixture used was 10 liters.

Discussion

Our work confirms the findings of Baker (1999) in that this room-temperature plastination technique yielded reasonable clarity and flexibility on whole and on portions of human bodies. Various gross morphological features of the spinal cord, cauda equina and dorsal and ventral roots were clearly preserved and demonstrated using this technique.

Prenatal changes in spinal cord mean lengths with age were demonstrated earlier both in animal and human models without the use of plastination (Barson, 1970; Icten et al., 1995). It is interesting that no significant decrease in cord length was observed after dehydration. The significant shortening of spinal cord mean length after curing using this room-temperature plastination technique suggests the need for further experiments. This includes, among other things, using a slower impregnation rate. Of particular interest will be the comparison of such data with those obtained using the original low-temperature as well as the original room temperature plastination techniques. Apart from the extensive research work done on the plastination of the brain (Suriyaprapadilok and Withyachumnarnku,

1997; Weiglein, 1997; Sora et al., 1999), no reports were found applying any of the plastination techniques to the human spinal cord.

Our specimens were stored in formalin for many years and most likely some degree of shortening had already taken place. It will be interesting to perform similar investigations on fresh specimens and see if the degree of shortening differs.

Our results on non-significant changes in spinal cord mean length in relation to gender and ethnicity have been reported by previous researchers (Icten et al., 1995).

This room-temperature plastination technique appears to have a promising potential for further morphological applications due to its flexibility and reliability in demonstrating different anatomical features.

Acknowledgements: The author wishes to thank Dr. Roy Glover, Director of The Plastination Lab, for his help and guidance during this work, Dr. Bruce Carlson, ex-chairman of the Department of Cell and Developmental Biology and Dr. Alphonse Burdi, Department of Cell and Developmental Biology, The University of Michigan Medical School for providing the specimens.

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N=30	Spinal Cord Mean Length (mm)	Crown-Rump Mean Length (mm)
Before Plastination	118.3 ± 32.6	218.5 ±62.3
After Plastination	97.2 ±25.1	200.5 ±51
Correlation Before & After Plastination	S	NS
Correlation with Age	S	S
Correlation with Gender	NS	NS
Correlation with Ethnicity (B/W)	NS	NS

Table 1: Fetal specimen data using room-temperature plastination technique. N = Number of specimens, S = Significant ($p < 0.05$), NS = Non-significant.

Fig. 1: Insulated acetone trap.

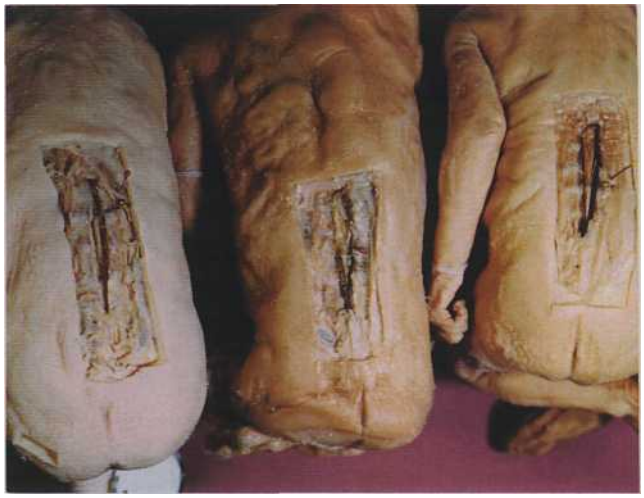

Fig. 2: Laminectomy sites on fetal specimens.

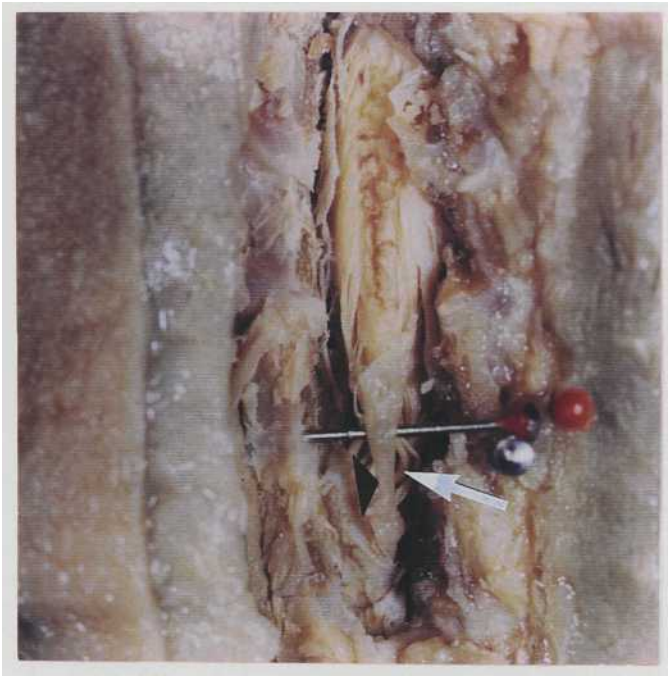

Fig. 3: Plastinated 26 week old fetus with spinal cord exposed showing filum terminale (arrowhead and elevated on pin) and cauda equina (arrow on a fiber of cauda equina).

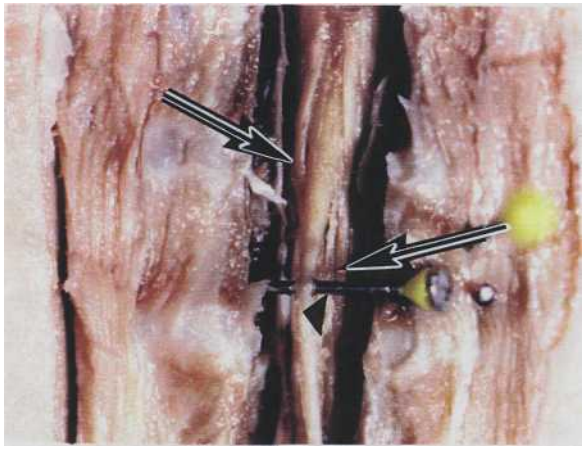

Fig. 3 a: Close up of figure 3.

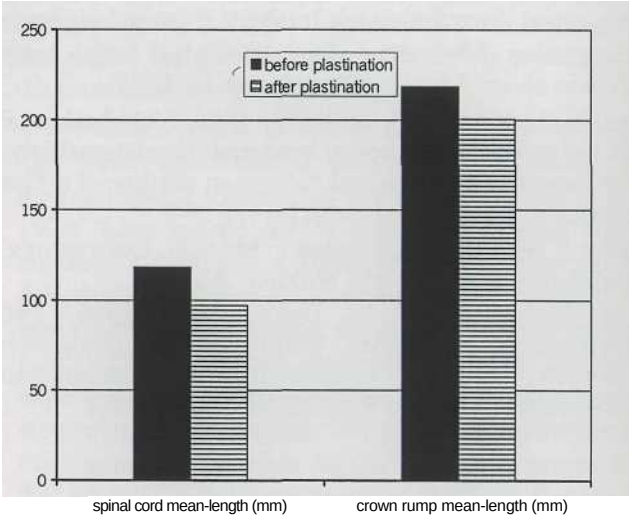

Fig. 4: Comparison of mean spinal cord and crown-rump lengths before and after room-temperature plastination.

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Risk Factors Associated with Plastination: I. Chemical Toxicity Considerations

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Abstract: The technique of plastination requires the handling of chemical agents not normally encountered by anatomic preparators. Material safety data sheets that come with these chemicals provide limited information regarding potential toxic effects following skin, inhalational or other exposures to these compounds. Biodur™ SlO-type silicones that are commonly used in plastination are polydialkyl siloxanes and are widely used in industry. A review of chemical safety sheets from industrial sources that produce such siloxanes suggests limited toxic effects associated with handling these agents. In contrast, a variety of adverse chemical effects including skin hypersensitivity-type responses have been associated with use of Biodur™ S6 and Biodur™ S3-like compounds. Epoxy resins commonly used by plastinators are well-recognized skin, eye and mucous membrane irritants and have been associated with allergic skin responses. Such toxic effects associated with the more common plastination chemicals are discussed in this paper.

Key words: **chemical safety; plastination; siloxane**

Introduction

Health concerns associated with the plastination procedure are most commonly divided into two areas: 1. possible toxic effects of the chemicals used by plastinators and 2. risk associated with infectious agents contained in the tissues handled by plastinators. Regarding chemical toxicity, the proprietary nature of plastination chemicals has resulted in limitations on available data. Specifically, the precise chemical identity of plastination components (e.g., S10 silicone; S6 gas cure, S3 catalyst/chain extender) is not public information, thus restricting literature searches for relevant information on undesirable effects from exposure to these compounds. Material Safety Data Sheets (MSDSs) have been available but are limited at best in terms of information provided. Nonetheless, sufficient information is available for many of these chemicals to provide plastinators with expanded information on potential chemical toxicity. Silicone monomers and associated catalysts and hardeners and epoxy resins are common agents used for tissue impregnation during plastination. These chemicals are the focus of this paper,

Background: Chemistry of Silicone Plastination

Some understanding of silicone chemistry as it relates to plastination is helpful before discussing potential adverse effects of these agents. Biodur™ S10 is the most commonly used plastination chemical for tissue impregnation. This silicone product is a polyalkyl siloxane (Biodur™ S10 MSDS, Nov. 1998). Many

closely related siloxanes are commercially available and widely used with S10-like compounds typically having methyl groups as the alkyl substitutions. However, the nature of the S3 hardener (a tin compound: Biodur™ S3 MSDS, Jan. 1999) suggests the reactive end groups on the S10 siloxane monomer are hydroxy 1 groups. Together these observations suggest the polyalkyl siloxane making up Biodur S10 is likely to be a hydroxyl-terminated polydimethyl siloxane or very similar silicone monomer. The chemical structure of this molecule is shown below (Fig. 1).

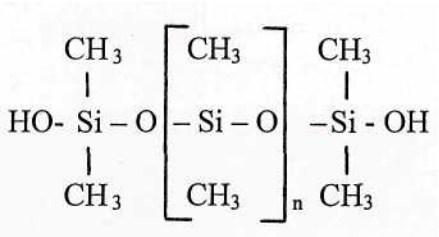

Figure 1. Structure of hydroxyl-terminated polydimethyl siloxane. [Si - O] represents a basic silicone molecule.

Monomeric chain length determines the viscosity of SlO-type silicones. The S3 catalyst joins monomer chains end-to-end, resulting in increased viscosity of the S10 silicone. This reaction is somewhat autocatalytic, thus S10 tends to become more viscous as time passes, even without addition of S3 hardener. Cold storage temperatures inhibit both the autocatalytic or catalyzed chain-lengthening reaction. The S6 gas cure is a chemical cross-linker that joins monomer chains side-to-side and end-to-side. This final reaction is sometimes referred to as "proceeding to ultimate molecular weight" in that all of the siloxane monomer present is joined by the cross-linker to form one large molecule.

Toxicity of Polydimethyl Siloxane, Catalyst, and Cross-linker:

The Biodur™ S10 MSDS provides limited toxicity data for this compound. The following first aid measures are indicated:

After eye contact: Rinse with plenty of water. A doctor should be contacted immediately.

After skin contact: Remove product mechanically.

After swallowing: Seek medical advice.

We were surprised to find that industrial MSDSs for polydimethyl siloxanes similarly lacked specific information regarding chemical toxicity. A representative sheet obtained from United Chemical Technologies, Inc. (Bristol, PA, USA), which produces siloxanes including hydroxyl-terminated polydimethyl siloxanes, simply indicated, "No toxicity information available" for this silicone product. However they recommended that adequate ventilation, impervious gloves and safety glasses be used. Suggested first aid procedures were nearly identical to measures listed on the Biodur™ S10 MSDS and included: *After eye contact: Rinse with plenty of water. A doctor should be contacted immediately.*

After skin contact: Remove product mechanically.

After swallowing: Seek medical advice.

Given the large-scale industrial use of polydimethyl siloxanes for the past 20 or more years, these minimal cautions on the MSDSs suggest that limited toxicity has been observed in individuals working with polydimethyl siloxanes. The possibility of topical hypersensitivity (allergic) responses in siloxane-exposed individuals, such as may occur in individuals who become sensitized to formaldehyde, has been a concern expressed by some plastinators. This potential toxicity was not mentioned on MSDSs we obtained with the exception of one sheet from Imperial, Inc. (Green Bay, WI, USA) for a room temperature vulcanizing (RTV) silicone product that was 70-80% hydroxyl-terminated polydimethyl siloxane. This particular product contained low levels of other organosilanes, as well as carbon black. The MSDS indicated that the product may enhance allergic conditions in certain people. We contacted company representatives regarding this possible effect and requested additional information about the specific component of their product (siloxane or carbon black) that may lead to allergic responses. Unfortunately, Imperial, Inc. declined our request for more detailed information about the potential allergenicity of their product. In that carbon black is a suspected allergen (Anderson et al., 1992), the Imperial, Inc. MSDS reference to allergic responses may be a reflection of this constituent of this particular siloxane product.

In a response to an e-mail inquiry about hypersensitivity responses following skin contact with siloxanes, a chemist and technical manager at United Chemical Technologies, Inc. indicated, "to our knowledge none of these materials show allergic responses in animals or humans." It is interesting to

note that Silly Putty® (Dow Corning, USA), a putty-like toy sold in the USA for the past 30 years, contains 65% hydroxyl-terminated dimethylsiloxane polymers (ref: http://www.thebuzz.net/ingredie.htm). Large numbers of children and others have handled this product without reports of skin allergic responses or other adverse health effects. It should also be pointed out that considerable human and animal data exist regarding possible adverse health effects from internal exposure to polydimethyl siloxane, in the form of silicone breast implants. The postulated relationship between breast implants and increased risk of autoimmune disease has generated intense medical and legal interest during the past 10 years. It now appears probable that these implants do not increase the risk of aberrant immune responses in humans (Janowsky et al., 2000). Thus, the available data suggest limited adverse immune or other toxic responses from exposure to silicone monomers similar to those used in plastination. Biodur™ gas cure S6 is described as an alkyl-silicate (S6 MSDS, Feb. 1999). Under the heading "Regulatory Information," the MSDS indicates S6 is harmful by inhalation and irritating to the eyes and respiratory system. Under the "Information on Toxicity" section, S6 is described as slightly irritating to eyes and moderately irritating to skin. Although the precise chemical nature of gas cure S6 is not provided, ethyl silicate (Fig. 2) is a physically similar, highly effective, common and inexpensive chemical used to cross-link polyalkylsiloxanes.

$$Si\text{-}(O\text{-}CH_4\text{-}CH_4)_4$$

Figure 2. Structure of ethyl silicate. Si - refers to the silicone nucleus, which is covalently linked to four ethoxy groups.

Other related cross-linkers (e.g., ethyl polysilicate) may be used with equal success and show similar toxicity to ethyl silicate. The MSDSs provided by the U.S. Occupational Safety and Health Administration (OSHA; http://www.osha-slc.gOv//SLTC/healthguidelines/ethylsilicate/recognition. html#healthhazard) summarizes potential toxic effects in humans exposed to ethyl silicate as follows:

Ethyl silicate is an eye, mucous membrane and respiratory irritant in humans. By analogy with effects seen in animals, it may also cause liver and kidney damage, central nervous system depression and anemia. At concentrations of 3,000 ppm, ethyl silicate causes extreme and intolerable irritation of the eyes and mucous membranes; at 1,200 ppm, it produces tearing of the eyes; at 700 ppm, it causes mild stinging of the eyes and nose; and at 250 ppm, it produces slight irritation of the eyes and nose (Hathaway et al., 1991).

The effects described by OSHA at the lower end of these exposures (i.e., 250 - 700 ppm) may be experienced by individuals who use S6 and/or ethyl silicate with inadequate ventilation. These effects are clearly avoidable with proper use of hoods. It should be remembered that cross-linkers of this type are highly reactive to available hydroxyl groups (i.e., H-OH) such as those in moisture on the surface of the eyes, mucus membranes and respiratory tract. Therefore, nearly all water must be removed from tissues before impregnated S10 silicone can be hardened. If not, the cross-linker will readily bind available water molecules on one or more of its four cross-linking arms instead of binding silicone monomer. This inhibits the silicone hardening reaction. In like manner, inhaled cross-linker will react with water in the lungs, and may thereby contribute to pulmonary silicosis. Ethyl silicate can cause severe damage to the eyes should the liquid form (as compared to gas vapors) be inadvertently splashed into the eyes. The MSDS provided by United Chemical Technologies, Inc. for ethyl polysilicate cross-linker indicates, for such eye contact, eyes should be flushed with clean water for at least 15 minutes and medical attention should be sought immediately.

Acute exposure to high levels of ethyl silicate may cause respiratory difficulty, tremor, fatigue, narcosis, nausea and vomiting (Sittig, 1991). Such exposures should be unlikely in plastinators who practice reasonable levels of caution while handling S6 cross-linker. Prolonged or repeated exposure of the skin to ethyl silicate has been reported to cause dermatitis (Genium, 1989). In summary, the available data suggest that plastinators should take care to limit their exposure to S6 vapors. These vapors may irritate the eyes and potentially deposit silicone into the lungs. Contact with the S6 liquid on skin or, especially, eyes should also clearly be avoided.

As we mention above, Biodur™ hardener S3 is described as a tin compound, specifically a dialkyl tin ester. A compound that has these characteristics and is used to join polyalkyl siloxane monomer chains end-to-end is dibutyltin dilaurate (Fig. 3).

Under the heading "Regulatory Information", the MSDS indicates S3 may be harmful if inhaled or swallowed and is irritating to eyes. It is also indicated that S3 can be handled without risk to health if used properly according to specification and if usual precautions of industrial hygiene are observed. The precise chemical nature of S3 is again unknown. However, relatively high-level exposure to related tin catalysts has been reported to produce hematopoietic (Subramoniam et al., 1994) and central nervous system (Alam et al., 1988) effects in laboratory rodents. The MSDS provided by OSHA (http://www.osha-slc.gov/ChemSamp_data/CH_271900.html) for dibutyl-tin dilaurate indicates health affects that include irritation to the eyes, nose, throat and skin. Acute exposure to high doses of dibutyltin dilaurate (which again should not be a problem in a typical plastination setting) may cause headaches, vertigo, eye irritation, psycho-neurological disturbance, sore throat, coughing, abdominal pain, vomiting, urine retention, paresis, focal anesthesia, skin burns and pruritis. Thus, available data for S3-like tin catalysts suggest that eye and respiratory exposure to vapors should be avoided as should skin contact with the liquid agent. Similar to S6, this suggests that these agents should be used under a hood while wearing protective gloves and eye-cover.

$[CH3(CH_2)_{10}C0_2]_2$-Sn-$[(CH_2)3CH_3]2$

Figure 3. Structure of dibutyltin dilaurate

Various epoxy resins such as Biodur™ E12 are used for preparing thin transparent body or organ slices. The E12 resin is hardened by addition of polyamine compounds (Biodur™ Hardener El MSDS, Jan. 1999). The nature of the amine mixture that is used to harden El2 is unknown and difficult to predict, however it should be considered that amines can be highly toxic compounds. The MSDS cautions that El is an irritant and poisonous if swallowed, inhaled or absorbed by skin contact. The following are recommended: *Eye contact: Rinse immediately with plenty of water and seek medical advice. Skin contact: Wash immediately with plenty of water and soap. In case of accident or sickness: Immediately contact a doctor.*

Prior to hardening of E12 epoxy resin using El polyamines, the skin, eyes or respiratory tract of the preparator may be exposed to the epoxy resin or to resin vapors. The Biodur™ El2 MSDS (May, 1998) again provides limited toxicity data for this plastination compound. The following are listed as first aid measures after contact by various routes:

After inhalation: Supply fresh air and, for safety reasons, call a doctor.

After skin contact: Wash immediately with water and soap and rinse thoroughly. After eye contact: Immediately rinse opened eye for several minutes under running water. Consult a doctor immediately.

After swallowing: In case of persistent symptoms, consult a doctor.

The acute toxicity information listed is based primarily on the acute toxicity of the bisphenol A (epichlorhydrine) component which has an oral LD50 of > 10,000 mg/kg (rat) and a dermal LD50 of >2,000 mg/kg (rat). However, bisphenol A is an estrogenic compound, and as such, potential endocrine disrupting effects have recently become of concern (Ashby and Odum, 1998). Thus, limiting skin exposure to Biodur epoxy should be recommended.

Regarding personal protection, MSDS recommends the following for individuals working with El2:

Respiratory protection: Not necessary if room is well-ventilated.

Hand protection: Plastic gloves.

Eye protection: Tightly sealed safety glasses.

Body protection: Protective work clothing. Epoxy resins are well-recognized skin, eye and mucous membrane irritants. We obtained MSDSs for epoxy resins from a variety of industrial sources, and all indicated that the resins might cause skin and eye irritation. In addition to primary irritation, allergic sensitization may also occur after skin contact with epoxy resins. This potential for an allergic skin reaction was less commonly mentioned on MSDSs we surveyed. However, there are several literature references documenting both skin irritation and allergic dermatitis associated with epoxy resins. Wyoto et al. (1976) found that workers who came into contact with epoxy resins and their hardeners in the workplace had increased positive skin tests and skin lesions with time of employment. Kanerva et al. (1997) described allergic contact dermatitis as well as allergic rhinitis and an immediate contact skin reaction from a component of epoxy resins, methylhexahydrophthalic anhydride. Jolandi et al. (1990) found that allergic contact dermatitis was induced by several components of epoxy resins including amine hardeners, epoxy acrylates and

the diglycidyl ether of bisphenol A. Beyond allergic dermatitis, numerous reports have described occupational asthma resulting from inhalation of epoxy resin vapors (Nielsen et al., 1989; Liss et al., 1993; Grammer, et al., 1994; Yokota et al., 1997; Bernstein, 1997). For the latter reports, various anhydride components of the epoxy compounds were generally regarded to be the allergic components. These collective reports suggest that it is prudent to avoid inhalation of vapors while working with epoxy resins. We believe these collective data are sufficient to recommend that Biodur™ epoxy be used under a hood rather than simply in a well-ventilated room.

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Risk Factors Associated with Plastination: II. Infectious Agent Considerations

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Abstract: The technique of plastination often requires handling organs and tissues of human or animal origin. An ongoing concern associated with the plastination process has been risk of contracting infectious pathogens as the preparator works with such tissues. Human pathogens, including the agent causing Creutzfeldt-Jakob disease (CJD) or the human immunodeficiency virus (HIV), and animal pathogens that affect humans (e.g., rabies virus, *E. coli* bacteria) have been of particular concern. This paper provides an overview of several viral and bacterial pathogens that may be of concern to plastinators as well as recommended methodologies for avoiding infection by these agents.

Key words: **Creutzfeldt-Jakob; HIV; pathogen; plastination; risk**

Introduction

Individuals handling fresh human or animal tissues must continually be mindful of infectious agents that may be contained in these tissues. The risk of contracting disease from such tissue handling should be quite low with adequate safeguards. However, due to the potentially serious consequences of some of these diseases, potential risk cannot be overlooked. This paper is by no means an exhaustive review of infectious agent health risks associated with plastination. Instead, it is meant to provide a general overview of pathogens that may be encountered by individuals practicing plastination. This information has been available in the literature for some time and has not greatly changed in recent years. For the convenience of journal readers, we review bacterial and viral pathogens that may be of particular concern to preparators handling human or animal tissues. Concern regarding the Creutzfeldt-Jakob pathogen prion has increased in recent years, thus this agent is also discussed.

Infectious agent concerns related to plastination:

A major concern of tissue handling associated with plastination is exposure of the preparator to pathogens. The risk of exposure to viable pathogens is greatest early in the preparation process, when tissues are still fresh and body fluids are still liquid. For low-risk as well as high-risk organisms in both human and veterinary fields, simple and common sense practices involving cadaver and tissue handling serve to greatly reduce exposure to, and therefore infection by, pathogens. Such procedures include: wearing of protective items such as gloves, surgical masks, eye protection and aprons whenever handling fresh tissue; avoidance of aerosolization of body fluids by careless handling or use of bone saws outside of hoods; taking care to avoid accidental cuts or needle sticks and paying prompt attention to such injuries when they do occur; careful cleaning and disinfection of surfaces and instruments; and proper disposal of unused tissues and

tissues intended for plastination.

One report described successful use of a formic acid-formalin technique for histological tissue preparation in both inactivating the infectious agent in CJD-infected mouse brains and preparing excellent-quality slides (Brown et al., 1990b). This treatment was described as effective in "almost completely eliminating infectivity in sections that were histologically indistinguishable from formalin-fixed material." This method was suggested as "virtually eliminating the risk of handling infectious material in neuropathologic processing of tissues from patients with CJD" (Brown et al., 1982). However, caution dictates careful consideration of the wording "almost completely." Further, note must be taken that this near-complete elimination of infectious agent took place while processing 4-5 mm slices of tissue, rather than large blocks of tissue or entire organs. The important question remains whether this fixation protocol would also be effective when processing larger blocks of tissue and/or entire organs, as is the normal situation in gross anatomical preparation. Indeed, Brown et al. (1990a) confine their remarks to fixation of histological materials. Prudence thus dictates that no cadaver of an individual diagnosed with CJD ever be used as a source of organs or large body parts for teaching in any mode. Further, the best degree of caution would be to avoid using tissues from any individual diagnosed with even non-specific neurologic signs.

It should be comforting that, for all of the above organisms except that causing CJD, handling the finished plastinated product would be expected to be associated with essentially no risk of disease transmission. This is true because the plastination process sequentially exposes tissues or organs to multiple procedures that independently or conjointly serve to render such pathogens non-infective.

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contamination with chemical germicides of all used instruments and surfaces should follow all procedures. Of course, the waste generated during these procedures should be properly identified as biohazardous as well as properly disposed. While simple, these procedures are extremely effective for minimizing risk of exposure and infection by most human pathogens that may be encountered during the early stages of specimen preparation (Rutala, 1995).

Embalming fluids, which contain formalin, completely inactivate HIV (CDC, 1987). Thus, assuming complete fixation via good perfusion and/or adequate contact time, formalin fixation should render tissues safe. The HIV virus demonstrates no inordinate stability in the environment and is subject to rapid inactivation by drying in ambient air. Thus, extraordinary procedures are unnecessary for de-contamination of potentially contaminated surfaces and instruments. Standard sterilization procedures are adequate for sterilizing instruments and surfaces contaminated with body fluids including blood from individuals infected with HIV (CDC, 1987). Sodium hypochlorite (household bleach) also effectively inactivates the HIV virus. The most important caveat here relates to the degree of contamination of the surface or instrument with organic material (since organic contamination renders hypochlorite less effective) (Rutala, 1995). Solutions between 500 and 5000 ppm (1:100 to 1:10 dilution) of household bleach are effective, depending on the degree of contamination of the object.

Hepatitis B virus is another pathogen of particular concern. Like HIV, exposure to infected blood is the single most important means of transmission of this disease (CDC, 1989). Sterilization/disinfection agents with activity ranked "intermediate to high" have been shown to be effective against HBV (Bond et al., 1983). Due to similarities in transmission and sensitivity to sterilization agents, adoption of the same barrier protection and sterilization procedures recommended for HIV will also minimize exposure to and infection by HBV. However, one critical difference between these two diseases is that an effective vaccine (90% effective for at least 7 years following immunization) for HBV is available. Indeed, the CDC as well as other groups recommends that individuals routinely exposed to human blood receive immunization for HBV. This extends beyond health care workers to include public safety personnel such as firefighters, law-enforcement and correctional-facility personnel (Handsfield et al., 1987; CDC, 1989). Individuals routinely involved in anatomical preparation of human tissues may of course also benefit from such protection.

Other human hepatitis viruses (non-A and non-B) are also currently of potential relevance to plastinators. Because these viruses are not reliably culturable, categorical statements as to the effectiveness of various disinfectants or sterilants are difficult to make. Nonetheless, the current consensus is that these viruses are not particularly resistant to disinfectants (Favero and Bond, 1991).

The single organism that departs significantly from the above generalizations is the agent causing Creutzfeldt-Jakob disease (CJD). This disease is caused by an unconventional virus-like organism that causes a spongiform encephalopathy. Though the incubation period may vary from months to many years, once clinical signs present, the disease is inevitably fatal. Neither treatment nor vaccine is available for this disease. Furthermore, CJD is uniquely resistant to nearly all routine sterilization procedures. Thus, CJD should be considered separately from all other pathogens.

Although the CJD agent resides mainly in and causes its main clinical signs related to tissues of the central nervous system (including the optic nerve and the cerebrospinal fluid), many other organs carry sufficient levels of pathogen to be infectious to humans (Rosenberg et al., 1985). Such tissues include liver, lung, lymph nodes, kidneys, corneas, white blood cells, whole blood and urine (Manuelidis et al., 1977; Gajdusek et al., 1977; Manuelidis et al., 1985; Tateishi, 1985). The CJD agent presents extreme resistance to denaturation or destruction. Indeed, transmission of the disease has been effected following exposure to formalin-fixed and paraffin-embedded human brain tissue routinely prepared for histopathological examination (Brown et al., 1986). The infectious agent has long been shown to be resistant to most fixatives, including formaldehyde (Zlotnik and Stamp, 1965; Gajdusek et al., 1976; Brown et al., 1982; Brown et al., 1986). Indeed, formalin fixation actually stabilizes the pathogen against autoclaving (Taylor and McConnell, 1988; Brown et al., 1990a). Routine disinfectants and sterilants as well as extreme heat are also ineffective against the agent. Procedures that are effective against the CJD agent include steam autoclaving at 132°C for one hour, immersion in IN sodium hydroxide at room temperature for one hour (Rosenberg et al., 1985) and exposure to high levels of phenol (far exceeding those used in fixative solutions) (Hunter et al., 1969; Kingsbury et al., 1983). Exposure to a 5000 ppm hypochlorite has also been reported as effectively inactivating the pathogen (Brown et al., 1982). Although these techniques are effective for instruments and surfaces, they are incompatible with treatment of

tissues intended for plastination.

One report described successful use of a formic acid-formalin technique for histological tissue preparation in both inactivating the infectious agent in CJD-infected mouse brains and preparing excellent-quality slides (Brown et al., 1990b). This treatment was described as effective in "almost completely eliminating infectivity in sections that were histologically indistinguishable from formalin-fixed material." This method was suggested as "virtually eliminating the risk of handling infectious material in neuropathologic processing of tissues from patients with CJD" (Brown et al., 1982). However, caution dictates careful consideration of the wording "almost completely." Further, note must be taken that this near-complete elimination of infectious agent took place while processing 4-5 mm slices of tissue, rather than large blocks of tissue or entire organs. The important question remains whether this fixation protocol would also be effective when processing larger blocks of tissue and/or entire organs, as is the normal situation in gross anatomical preparation. Indeed, Brown et al. (1990a) confine their remarks to fixation of histological materials. Prudence thus dictates that no cadaver of an individual diagnosed with CJD ever be used as a source of organs or large body parts for teaching in any mode. Further, the best degree of caution would be to avoid using tissues from any individual diagnosed with even non-specific neurologic signs.

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Anatomy of Synovial Sheaths in the Talocrural Region Evaluated by Sheet Plastination

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Abstract'. Tendon sheaths are complex tubes wrapped completely around tendons. They are placed between two movable tissues to diminish friction when tendons pass either under ligaments or retinacula or pass through fascial slings or osseofibrous tunnels. The aim of this study was to show that the El2 plastination technique is a useful research tool for demonstrating different anatomical structures, e.g. tendon sheaths in the talocrural region. Tendon sheaths were injected with Xantopren® blue and plastinated via the E12 plastination procedure. Finally, the cured specimens were cut with a diamond wire saw into slices of 200um thick. We found that tendon sheaths in the talocrural region do not enclose tendons like a cylinder. They mimic an elongated bursa that minimizes friction between tendons and retinacula.

Key words: sheet **plastination; talocrural region; tendon sheaths**

Introduction

Tendon sheaths are similar to bursae in structure. They develop in response to friction between tendons and bones or tendons and ligaments, or fascial slings (Hartman, 1896). Bursae are closed connective tissue sacs underlying a tendon and can easily be converted into a tendon sheath simply by extension around the tendon. Hence, tubes are formed which wrap completely around tendons and consist of two layers. The structure of the fibrous layer shows a tight collagenous tissue that encloses the tendon like a tube. Therefore, it is able to reduce friction against bones (Hollinshead and Rosse, 1985). In the area of the tendon sheaths of fingers and toes, the outer layer has compact fibers surrounding the tendon called cruciate or annular ligaments.

The synovial surface is a closed double-walled cylinder and consists of two layers. The visceral layer is closely attached to the surface of the tendon. The second layer, surrounding the cavity, is known as the parietal layer. These two smooth, glistening layers, for the most part are separated from each other by synovial fluid and are continuous with each other at the end of tendon sheaths (Lovell and Tanner, 1908).

The best medium to produce ideal conditions for slicing plastinated specimens with a diamond saw is the epoxy (El 2, Biodur™) plastination technique. Xantopren® blue was chosen to inject the tendon sheaths due to its fluid nature. Xantopren® blue cures without damaging the sheath and does not shrink. These slices become translucent and provide detailed information about anatomical structures (McNiesh and von Hagens, 1988) in the talocrural region. Furthermore, with the thin slices, we were able to visualize tissue differences with the naked eye (Cook and Al-Ali, 1997; Skalkos et al., 1999). Also, tendon sheath structure was found to be different from classic

descriptions.

Materials and Methods

Xantopren® blue (Bayer Dental, D-5090, Leverkusen, Germany) is used by dentists to get an impression of teeth to fit dental braces. Before starting the plastination procedure with El2, the compatibility of Xantopren® blue was checked by placing 10 ml Xantopren® blue into two containers. Fifty milliliters of acetone was added to one container and 50 ml of epoxy resin was added to a second. These mixtures were left together for 1 month to confirm the compatibility of Xantopren® blue with the E12 technique (Biodur™).

Five feet from human cadavers, aged between 34 and 76 years, were studied. All of the cadavers were embalmed by Thiel's method (Thiel, 1992). Dissection layer by layer was performed until the deep fascia of the leg and the dorsal fascia of foot were reached. Using a 10 ml syringe and a small needle, a mixture of Xantopren® blue and hardener [elastomer activator (Bayer Dental, D-5090, Leverkusen, Germany)] was injected into the upper end of the sheaths of the tibialis posterior m., extensor digitorum longus m. and flexor hallucis longus m. where the muscle fibers join the tendon. This must be done very precisely and with low pressure to avoid damage to the sheath. It also must be done quickly, to prevent the Xantopren® blue-hardener mix from curing within the syringe.

To be certain that only tendon sheaths were injected, and not fibers of the tendon or the surrounding loose peritendineum, our injections were verified with standard histology sections (Figs. 1, 2). Five human feet were prepared for routine histological examination. Tandler gelatin was injected via a 10 ml syringe and small needle into the tendon sheaths of the above mentioned muscles. Specimens were dehydrated in a graded alcohol series from 60% to 100% over a five-week period. All specimens were embedded in paraffin. Histological sections of 13 to Hμ™ were cut and evaluated.

Freezer and Band saw:

Feet with injected tendon sheaths were put into a freezer at -70°C for three days. Two-centimeter thick frontal sections were made with the band saw. To prevent warming of the specimen and slices, the band saw was equipped with a cooling chamber filled with cold water and bottles of frozen glycerine.

Dehydration and degreasing:

Cold acetone (-25°C) was used for dehydration. Water content was monitored regularly with the acetonometer. After three weeks, water content had decreased to 2% (purity of acetone 98%) and dehydration was deemed complete. To ensure extraction of lipids, degreasing in room temperature acetone was carried out for two weeks.

Immersion, impregnation and embedding:

The dehydrated slices were immersed into a mixture of Biodur™ E12 (epoxy resin) and Biodur™ E6 (hardener) (18%) over night for 12 hours. The specimens, in the original resin, were placed in the vacuum chamber and sealed with a glass plate. The slices were impregnated under vacuum ranging from 100 - 10mm Hg for 24 hours at -4°C. Pressure was monitored with a manometer. For embedding, slices were placed into a plastic form. The form was filled with a mixture of E12 and E6. To prevent them from floating and drifting, Plexiglas® plates were laid on the slices. Trapped bubbles were removed with a fine wire.

Pre-curing and curing:

It took one day for the epoxy resin to gelate at room temperature. The plastic form with the slice was cured in an oven for 48 hours at +45°C.

Band saw and diamond wire saw:

The plastic form with cured specimen was sawed into pieces measuring 7 x 15 cm. The pieces were fixed with plastic screws on blocks of wood in readiness for the diamond wire saw. This saw is equipped with a special bath filled with a mixture of water and petroleum to prevent the wire from gumming up with resin dust. The blocks were clamped on the guiding table and 200μm thick slices were made.

Results

After one month, Xantopren® blue showed no reactivity with either acetone nor epoxy polymer. For our plastination project, Xantopren® blue proved to be an excellent material. As well as being compatible with the El2 polymer and acetone, it was fluid enough to inject into the tendon sheaths and fill them without damage. The translucent nature of these slices combined with the Xantopren® blue mass enabled differentiation of the various tissues and spaces. The blue filled only the cavity between tendons and retinacula, as seen on the sections (Figs. 3 - 5). It should be noted that Xantopren® blue was confined to only one side of the tendon. None of the injected tendon sheaths demonstrated the typical double-walled cylinder type sheath.

Histological sections showed tendon sheaths with Tandler gelatine lying on one side of the tendon like a pillow, hanging down with its corners to the medial and lateral side (Figs. 1, 2).

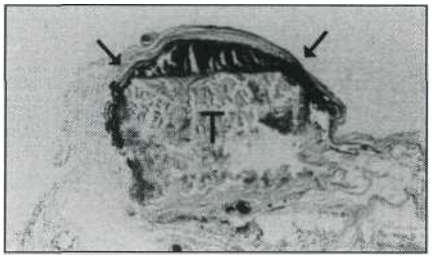


Figure 1. Histological section through a single tendon. *Arrows* showing Tandler gelatin injected tendon sheath, *T* - Tendon.

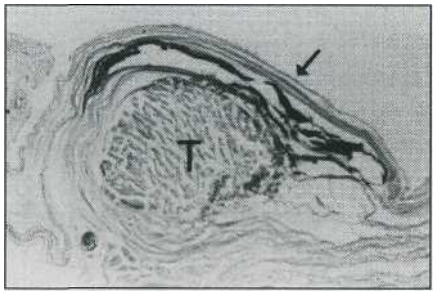


Figure 2. Histological section through a single tendon. *Arrows* showing Tandler gelatin injected tendon sheath, *T*- Tendon.

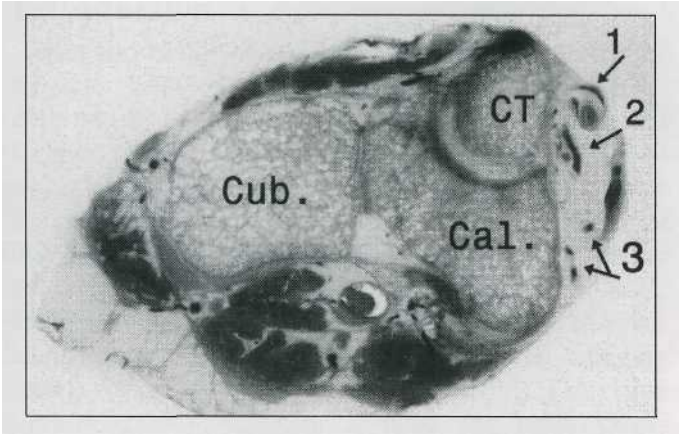
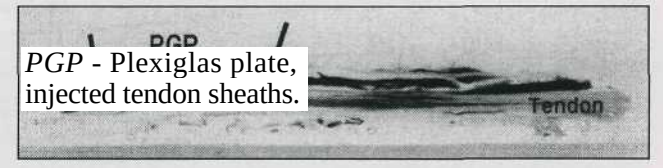


Figure 3. Frontal section through the collum tali *(CT),* calcaneus *(Cal)* and cuboid bone (CM) of right foot. *Arrow 1* - Xantopren® blue injected tendon sheath of tibialis posterior muscle, *Arrow 2* - Tendon sheath of flexor digitorum muscle. Both tendon sheaths lie on tendons, *Arrow 3* - Vessels and nerve.



PGP - Plexiglas plate, injected tendon sheaths.

Figure 4. Sagittal section through a single tendon, late. *Arrows* - Xantooren® **blue**

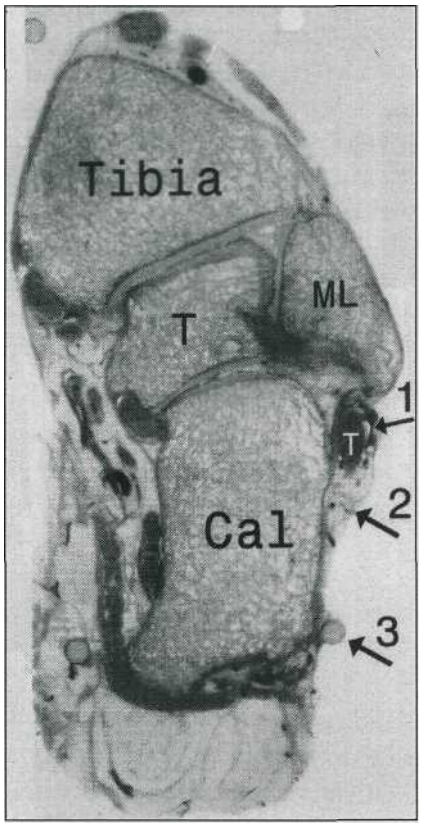


Figure 5. Frontal section through right ankle joint. *Arrow 1* - Injected tendon sheaths, Arrow 2 - Flexor retinaculum, *Arrow 3* - Plexiglas screw. *T (white)* - Tendon, *T (black)* - Tendon, *ML* - lateral malleolus.

Discussion

The aim of this work was **to** study the anatomy of synovial sheaths in the talocrural region by the use of the E12 plastination method as described by Weber and

Henry (1993). The advantage of plastination is that plastinated slices preserve the relationship of one structure to another. With Xantopren® blue, we found a new material which proved to be very useful for this special plastination technique. Xantopren® blue was not reactive to the chemicals used in the E12 technique and filled the tendon sheaths without damaging them. Also, the 20(^m sections allowed us to distinguish dense connective tissue from loose tissue and to differentiate adjacent structures with the naked eye similar to the findings of Cook and Al-Ali (1997). The E12 technique may be used as an alternative method to the standard histological technique.

With the aid of the E12 plastination technique, we demonstrated a new method to delineate anatomical structures and function of tendon sheaths in the talocrural region. The results demonstrate that tendon sheaths of the tarsal region, indeed, show fundamental differences in the structure of the sheath from those usually embraced. These results were verified with standard histology sections, which showed that tendon sheaths are not wrapped completely around tendons (Figs. 1, 2). Since these tendons are held down by retinacula, the friction occurs between the tendon and the retinacula. Therefore, sheaths develop just in between these two movable tissues.

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Teaching Anatomy of the Distal Equine Thoracic Limb with Plastinated Slices

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Abstract: Tendons and ligaments of the equine distal limb have a distinct anatomy with important functional and clinical implications. This article reviews the descriptive and topographic anatomy of this region using fresh-fixed and plastinated sections. The polymers used to plastinate the specimens were PR-10 from Corcoran Laboratories™ and SIO from Biodur™ Company. The results demonstrate that plastination is an adequate preservation procedure for soft tissue structures of the equine thoracic limb. The specimens plastinated with SIO polymer were better for anatomical detail.

Key words: **equine thoracic limb; foot-anatomy; ligaments-articular; plastination; sectional-anatomy; tendons-anatomy**

Introduction

The knowledge of the normal anatomy of the equine thoracic limb is essential for understanding foot deformities or injuries (Denoix, 1994; Dyson and Denoix, 1995). A thorough knowledge of the sectional anatomy of thoracic limb structures and their interrelationships is crucial for accurate interpretation of diagnostic images as seen with ultrasonographic examination (Nicoll et al., 1993; Denoix et al., 1996; Denoix et al., 1997). The increased use of imaging technologies such as magnetic resonance imaging (MRI), computed tomography (CT) and ultrasonography suggests a need for more cross-sectional anatomic studies. Unfortunately, body slices prepared by traditional methods are often unpleasant to handle and prone to deterioration. The technique of plastination (von Hagens, 1985) produces dry, odorless, non-toxic and durable specimens that retain their original surface and cellular detail (von Hagens et al., 1987). Traditional polymers suitable for plastination can be used at room temperature but must be stored in a freezer. Most plastination laboratories perform the impregnation process in the freezer to insure a longer pot-life of the polymer reaction mixture. The recent introduction of polymers that can be used and stored at room temperature eliminates the need of a freezer for impregnation and polymer storage. In addition, processing specimens at a warmer temperature may make them less rigid and may increase their flexibility (Glover et al., 1998).

The aim of this paper is to explore the use of plastinated transverse sections as a model for understanding the tendons, ligaments, arteries, veins and other soft tissue structures in the distal horse limb. A comparison between the use of polymer from Biodur (Heidelberg, Germany) and from Corcoran Laboratories (Bay City, MI) was also done.

MATERIALS and METHODS

Injection:

Equine thoracic limbs were obtained from horses at a slaughterhouse. Their ages ranged from one to three years and they were of random sex. The specimens were cleaned and shaved. The arteries, veins and synovial structures of the thoracic limb were injected with red, blue and green latex, respectively. White latex was colored using pigment paste (2% ppv): AC50 (red), AC52 (blue) and AC54 (green) (Biodur™, Heidelberg). The injection was done by manual pressure with 10 or 20 ml syringes. Arterial injection was carried out via the brachial artery in full-length limb preparations or via the median artery when specimens were separated at the radial carpal joint. When the latex mixture started to ooze from the radial artery and the palmar branch of the median artery, they were ligated. Injection was continued until red latex also oozed from smaller arteries and the pressure on the syringe increased. After arterial injection, venous injection was performed via one of the two palmar digital veins, as distal as possible to avoid filling defects due to interference by valves. The proximal ends of the veins were left open until blue latex started to ooze from them. The fetlock joint *{Articulationes metacarpophalangeae)* was injected via its palmar pouch. Both the pastern joint *{Articulationes interphalangeae proximalis manus)* and coffin joint *{Articulations interphalangeae distales manus)* were injected via their dorsal pouches. The digital sheath of the deep digital flexor and the superficial digital flexor tendons was injected between the tendons.

Slicing:

After latex injection, specimens were frozen for several days at -25°C. The frozen limbs were sectioned transversely from the radius to the hoof with a high-speed band saw at the desired thicknesses (0.5 to 1.5 cm.). Twenty sections were made from each thoracic limb. The cut surfaces of each section were rinsed with water and numbered before fixation. Photographs were taken of both surfaces of each section before and after fixation.

Fixation:

The sections were fixed in a 10% formaldehyde solution for 15-30 days at room temperature. After removal from the fixative, they were washed under running tap water for one day.

Dehydration:

Dehydration was performed by freeze substitution in cold acetone (96 to 100% purity). The sections were submerged in plastic vats containing ten times the specimen volume of acetone at -20°C. Acetone was changed weekly until the water content was less than 1%. Dehydration time for each specimen was four to six weeks.

Forced Impregnation:

After slicing, fixation and dehydration, the slices were plastinated according to either the standard cold S10 technique (Biodur™, Heidelberg) or a room temperature process, PR-10 technique (Corcoran Laboratories™, Bay City, MI). *Group 1 reaction mixture: Polymer SR10 + Catalyst SH03 (1% ppv) (Biodur™, standard S10 procedure, at -20°C) (von Hagens, 1985). Group 2 reaction mixture: Polymer Cor-Tech PR-10 + Cross linker CR-22 (5% pbw) (Corcoran Laboratories™, room temperature).*

Vacuum was applied to the immersed sections and gradually increased for both groups until a pressure of 1-5 mm Hg was attained and bubbling had ceased.

Curing:

The sections were removed from the polymer reaction mixtures. Polymer was allowed to drip from the slices. The slices were wiped clean of excess polymer. The impregnated specimens were placed in appropriate airtight containers and each group was exposed to their respective curing medium at room temperature.

Group 1: Cross linker SH06 (Biodur™, standard SI 0 procedure) (von Hagens, 1985). The specimens were placed into a curing chamber with S6, which was vaporized ten minutes daily for one week. During this period, it was necessary to wipe the specimens three to four times a day. Finally, the specimens were placed in a plastic bag for 2 months to contain the S6 vapors around the specimens and allow curing of the center of the specimen.

Group 2: Catalyst CT-30 Corcoran Laboratories™). The specimens were either placed in a curing chamber and catalyst was evaporated for one week or catalyst was sprayed onto the specimens and then the excess catalyst was wiped off. When the spray method was used, the specimens were placed in a plastic bag for 24 hours with most of the air removed. It was necessary to repeat this spraying and bagging process until the specimens were completely dry (two to three days). Clean material (cloth or paper) was always used to wipe the catalyst onto the specimens.

Display:

Each specimen was mounted onto a piece of methacrylate using fast gum (cyanocrylato). Some of the mounted slices were stored and displayed in a

manufactured transparent methacrylate box (Fig. 1).

Results

Plastination aspects:

S10 impregnation (group 1) took at least two weeks and one week was needed for curing. Sections of group 2 (PR-10, Corcoran technique) were impregnated in three to four days. Curing was faster in group 2 when the catalyst was sprayed directly onto the specimen (two to three days). However, curing by evaporation took a minimum of five days.

Specimens plastinated via the S10 technique displayed better anatomical detail, while PR-10 specimens displayed an excess of cured polymer build up on their surface during and after curing. To remove this deposit, it was necessary to brush the slices several times. However, surface quality of specimens was improved if the catalyst (CT-30) was evaporated and the liquid not applied directly onto the specimens. With this modified curing technique, it was necessary to cure at least five days to stop oozing of polymer, but the results were better than when the catalyst was applied directly to the slice.

The final color of plastinated specimens in both groups was similar to that of fixed specimens. The latex color in the vessels and synovial structures darkened significantly two or three months after plastination in both groups.

The final plastinated sections of both group 1 and group 2 showed the exceptional potential of plastination to preserve anatomical structures. No apparent differences in preservation quality were observed between the SR10 (group 1) and the PR-10 (group 2) specimens, but the anatomical detail was sharper in S10 specimens.

Anatomical aspects:

Excellent correlation of anatomical structures can be seen in all sections, both before fixation and after plastination (Figs. 2 - 6). The relationships between muscles, tendons and vessels at the carpal joint are easily seen (Figs. 2, 3). The oval tendon shape of the extensor carpi radialis m. is observed near the axis of the limb as it crosses the dorsum of the carpus. The common digital extensor t. is seen on the craniolateral aspect of the radius and carpus while the lateral digital extensor m. is noted on the lateral aspect. The small extensor carpi obliqus m. *(M. abductor digiti I longus)* (Fig. 2) crosses the dorsal aspect of the carpus from lateral to medial. Its insertion is near the lateral collateral ligament of the carpus.

In the metacarpal and digital regions (Figs. 4 - 6), the superficial digital flexor tendon, the deep digital flexor tendon, the accessory ligament of the deep digital flexor tendon and the suspensory ligament *(M. interosseus)* are readily observed. The tendon of superficial digital flexor crosses the palmar aspect of the carpus and the fetlock joint. It is nearly round in the carpal region (Fig. 3), becomes flattened in the metacarpal region (Fig. 4), and forms a ring around the deep digital flexor tendon as it crosses the fetlock joint. The tendon of the deep digital flexor lies deep to the superficial digital flexor tendon and is round in the metacarpal region becoming oval at the fetlock joint. The accessory ligament of the deep digital flexor (inferior/distal check ligament) is rectangular at its origin and lies deep to the deep digital flexor tendon (Fig. 4). The suspensory ligament is prominent in the metacarpal region between the second and fourth metacarpal bones (Fig. 4) having a rectangular shape. In the proximal metacarpal region it is located between the accessory ligament of the deep digital flexor muscle and the third metacarpal bone. The tendon sheath of the digital flexors encircles the superficial and deep digital flexor tendons (Fig. 4). The navicular bursa *{bursae podotrochleares manus)* (Fig. 6) is visible between the deep digital flexor tendon and the distal sesamoid bone.

Blood vessels can be readily identified on the plastinated section. For example, the median artery is seen on the caudomedial surface of the forearm (antebrachium), passing through the carpal canal with the flexor tendons. The palmar ramus and the radial artery (Fig. 3), which contribute to the palmar and dorsal metacarpal arteries are visible. The paired digital arteries (Fig. 5) pass over the abaxial surfaces of the proximal sesamoid bones.

Discussion

With the S10 technique (group 1), a longer processing time is needed than with PR-10. There is little published information available concerning the use of Corcoran laboratories polymer (PR-10). Impregnation and curing are both faster with the Cor-Tech method. This is in agreement with Glover et al. (1998). However, the final quality of sections plastinated with this room temperature process (Corcoran) must be researched and improved to produce specimens of comparable quality to the S-10 technique. Polymer build up on the surface of slices is a problem with this room temperature process. The evaporation of the catalyst (CT-30), rather than spraying it onto the specimens, during the curing of sections improves the quality of the specimens. This additional time renders this curing time similar to that of the Biodur technique.

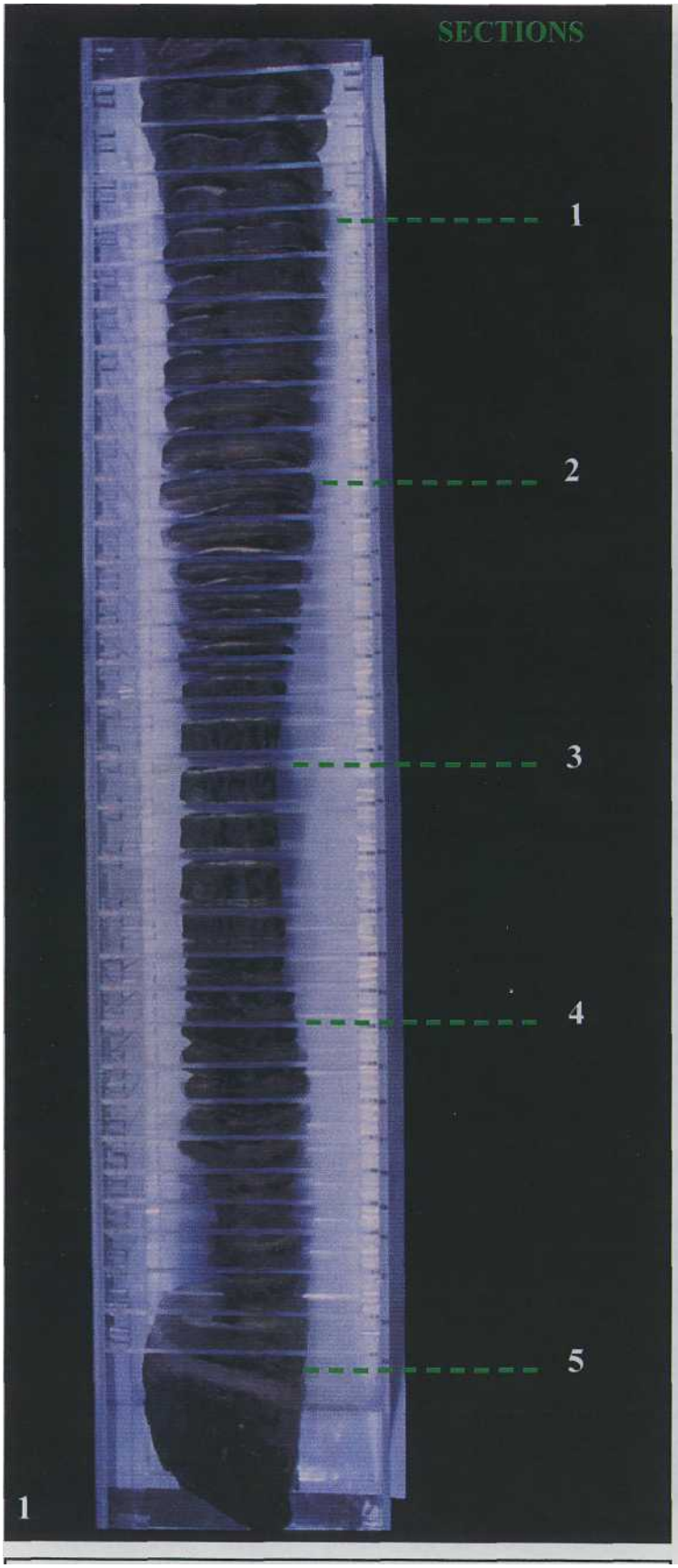

Fig. 1: *Transverse sections of right thoracic limb at different levels (specimens mounted on transparent methacrylate sheets and held in a transparent box).*

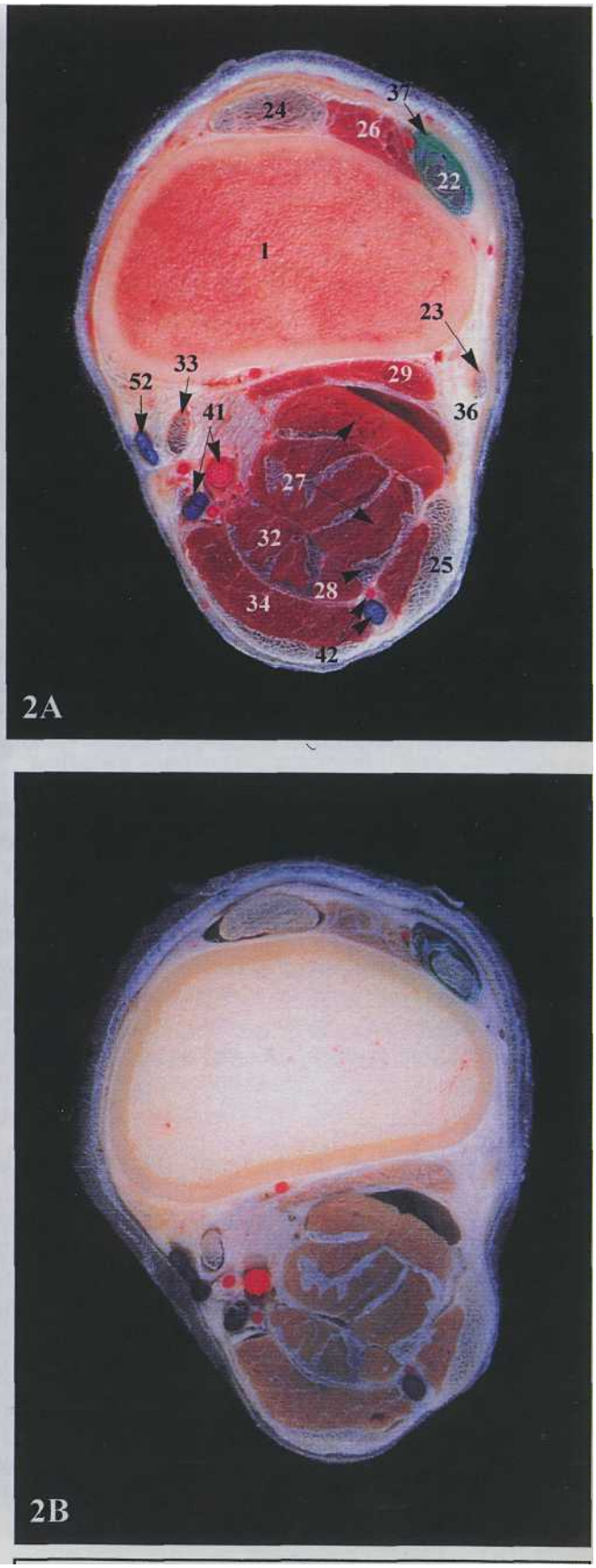

Fig. 2: *Fresh (A) and S-10 plastinated (B) section at the level of the distal radius (proximal view).*

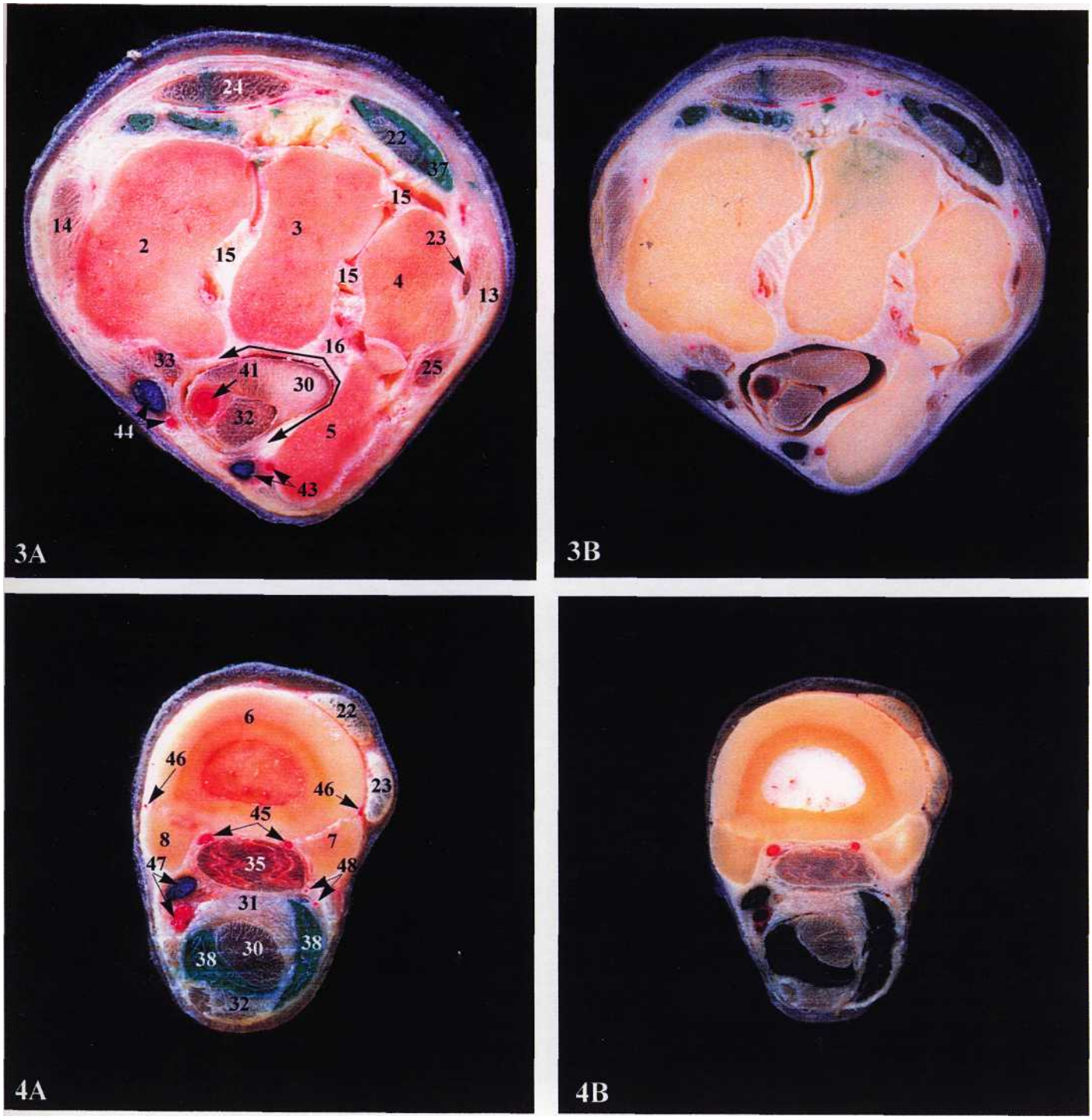

Fig. 3: Fresh specimen (A) and S10 plastinated specimen (B) at the level of the right proximal row of carpal bones (proximal view).

Fig. 4: Fresh section (A) and S10 plastinated section (B) of the right mid-metacarpal region (metacarpal bones, proximal view).

Legends figures 2, 3 and 4: 1. Distal radius, 2. Radial carpal bone, 3. Intermediate carpal bone, 4. Ulnar carpal bone, 5. Accessory carpal bone, 6. Cannon bone (Os metacarpal III), 7. Lateral splint bone (Os metacarpal IV), 8. Medial splint bone (Os metacarpal II), 13. Lateral collateral ligament of the carpus, 14. Medial collateral ligament of the carpus, 15. Intercarpal ligaments, 16. Carpal canal, 22. Common digital extensor m., 23. Lateral digital extensor m., 24. Extensor carpi radialis t., 25. Extensor carpi ulnaris m. *{ulnaris lateralis),* 26. Extensor carpi obliqus m., 27. Deep digital flexor m., humeral head, 28. Deep digital flexor m., ulnar head, 29. Deep digital flexor m., radial head, 30. Deep digital flexor t, 31. Accessory ligament of deep digital flexor m., 32. Superficial digital flexor m., 33. Flexor carpi radialis t., 35. Suspensory ligament, 34. Flexor carpi ulnaris m., 36. Antebrachial fascia, 37. Tendon sheath of common digital extensor t., 38. Tendon sheath of digital flexors, 41. Median a. & v., 42. Collateral ulnar a. & v., 43. Palmar branch a. & v., 44. Radial a. & v., 45. Palmar metacarpal arteries II & III, 46. Dorsal metacarpal arteries, 47. Medial palmar a. & v. *{A & V. digitalis palmaris communis II),* 48. Lateral palmar a. & v. *{A & V. digitalis palmaris communis III),* 52. Cephalic vein.

Also, to produce good specimens in successive batches, it is necessary to add 2% (p.b.w) of cross linker to the Corcoran polymer reaction mixture prior to impregnation of a new group of specimens. Baker (1998) reported shrinkage percent with Cor-Tech PR-10 polymer to be 3% or less in most tissues. We did not measure specimen shrinkage for either technique. This would be interesting to do in future work.

The methacrylate storage box offered a convenient solution for storage of the slices in an orderly manner. As well, mounting the specimens on clear methacrylate allowed both sides of each numbered section to be studied.

The quality of our injections suggests that the use of anticoagulant and lavage prior to injection of the vascular system, as described by Martin-Orti et al. (1999), is not necessary in large animal specimens excised post mortem at the slaughterhouse. The vascular and microvascular anatomy of superficial and deep digital flexor tendons could also have been studied by using colored latex with barium sulphate as reported by Kraus-Hansen et al. (1992) Kraus et al. (1995). However, when observing our plastinated transverse sections in sequence, the vascularity of the tendons was also readily observed. The latex inside the smallest vessels or synovial structures became darker after two or three months. Pigment was mixed into the latex just prior to injection. Possibly the pigment was not stable in at least one phase of the plastination process. Grondin and Olry (1996) used a mixture of Biodur S10/S3/S6/S2 and colored it with AC50 for plastination and obtained good color as well as long-term stability of color.

The most common injuries in the metacarpal and digital regions affect four anatomical structures which are all visualized in the plastinated specimens (Figs. 4 - 6): the superficial digital flexor tendon, the deep digital flexor tendon, the accessory ligament of the deep digital flexor tendon (distal check ligament) and the suspensory ligament. Under clinical conditions, a knowledge of normal anatomy of these complex areas is critical for accurate diagnosis of tendon or ligament injuries (Denoix et al., 1996). Tendons or ligaments normally change in size and shape from their origin to insertion. Because of this variation, equine veterinarians appreciate plastinated specimens which demonstrate important anatomical structures. Also, plastinated anatomical sections have high clinical value when used together with other diagnostic imaging techniques such as radiology, MRI and CT (Baptista, 1989; Fritsch, 1996). For example, ultrasonographic observation of the flexor tendons and ligaments in transverse and sagittal planes of the metacarpal region of horses provides quantitative data relating to the macroscopic sections (Nicoll et al., 1993).

On the other hand, undergraduate anatomy students should find it easier to comprehend sectional anatomy using these specimens. They should have a clear and accurate overview of the expanse, limits and relationships of complex anatomical areas (Marigos et al., 1997; Latorre et al., 1998). Our students are using these sections in practical lectures with high didactic success. This type of material also offers numerous possibilities for education and for surgical practice and training.

Acknowledgements: This work was supported by the project: 0075/CV/99 (Ayudas para la realization de proyectos de investigation de la Consejeria de Cultura y Education. Comunidad Autonoma de la Region de Murcia).

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Legends figures 5 **and** 6: 6. Cannon bone (Os metacarpal III), 9. Proximal sesamoid bone, 10. Middle phalanx, 11. Distal phalanx, 12. Navicular bone *(os sesamoideum distale),* 17. Dorsal pouch of fetlock joint, 18. Collateral ligament, 19. Palmar ligament, 20. Dorsal pouch and 21. Palmar pouch of coffin joint, 22. Common digital extensor t., 23. Lateral digital extensor t., 30. Deep digital flexor t., 32. Superficial digital flexor t., 35. Suspensory ligament, 39. Navicular bursa, 40. Digital cushion *[Tela subcutanea tori (pulvinus digitalis)],* **49.** Medial digital a. & v. *(palmaris propia IT),* 50. Lateral digital a. & v. *(palmaris propia III),* 51. Terminal arch.

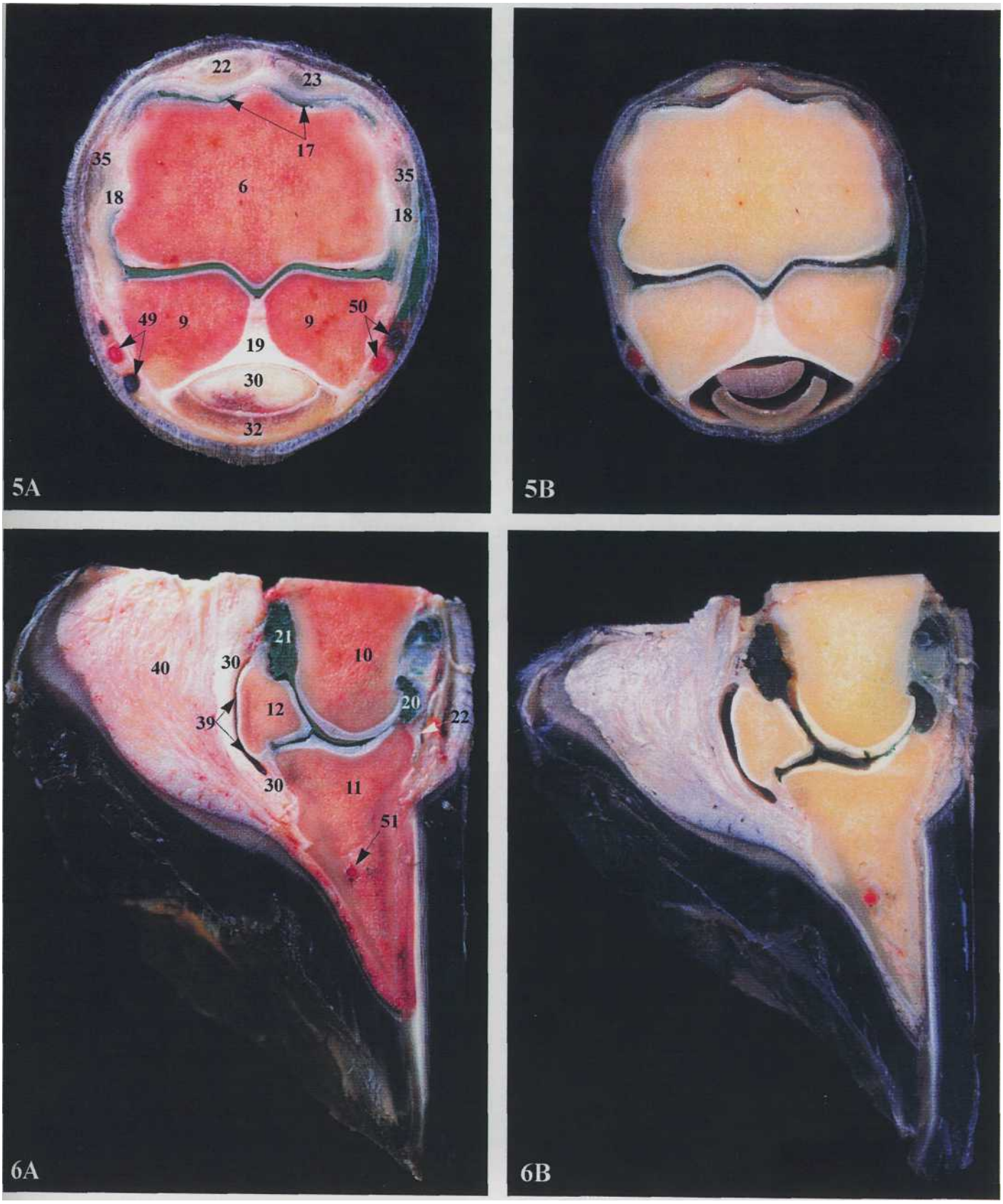

Fig. **5:** Fresh (A) and SIO plastinated (B) sections at the level of the right fetlock joint (proximal view).

Fig. 6: Fresh (A) and SIO plastinated (B) sagittal sections at the level of the right coffin joint (axial view).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Local Flaps for Fingertip Injuries: A Plastinated Model

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Abstract: This paper demonstrates preparation and plastination of local flaps, commonly used in fingertip injuries, for use as clinical models. Additionally, it reports on the introduction of plastination in Hungary and demonstrates an application of the plastination method in the clinical field. During the last two years, over 150 plastinated specimens were prepared and made available for student use in the Teaching Museum of Anatomy, Semmelweis University of Medicine. Our present goal is to prepare plastinated specimens that can be used in our clinics. Local flaps for fingertip injuries were prepared and plastinated. Four current techniques (Atasoy, Hueston, Venkataswami-Subramanian and O'Brien flaps) were carried out on an unfixed, right cadaver hand. After preparation, the hand was plastinated according to the S10 technique. Although shrinkage was significant enough to distort the natural appearance, the specimen maintained the shape of the prepared flap. Curing was not uniform. Non-dissected parts of the specimen covered by skin did not cure properly.

Key words: **clinical anatomy; digital flaps;** finger; **plastination; trauma**

Introduction

Plastinated specimens have proved to be of great use in morphological disciplines (von Hagens et al., 1987). In the last two years over 150 specimens were prepared in the Department of Anatomy, Histology and Embryology of the Semmelweis University of Medicine. In contrast to traditional specimens, which are stored in fixative solutions, the plastinated specimens are dry and hygienic. Students readily use these specimens that are housed in the Teaching Museum of Anatomy. Plastinated specimens do not replace the traditional dissection and investigation of formalin-fixed, flexible specimens, but instead serve as supplements to student studies. Additionally, specimens are plastinated for other Institutes of Anatomy and Pathology and for the National Institute of Traumatology. Our goal was to prepare plastinated specimens to serve as clinical models. Their physical properties make them useful for clinical demonstration (von Hagens, 1985; von Hagens et al., 1987). Use in the operating room is possible after proper sterilization (gas or gamma-ray). The project was started by preparing one local flap (Atasoy, Hueston, Venkataswami-Subramanian or O'Brien) which was dissected on each finger and thumb (Digitus primus) on an unfixed, right cadaver hand (Hueston, 1966; Atasoy, 1970; Venkataswami and Subramanian, 1980). These prepared flaps were subsequently plastinated using the S10 technique (von Hagens, 1985).

Materials and Methods

An unfixed right hand was used to prepare the four

flaps (Atasoy, Hueston, Venkataswami-Subramanian or O'Brien) (Fig.l). The hand was cut off just proximal to the wrist. A different procedure was prepared on each finger of the hand. The fingertips were cut off with strong surgical scissors (forfex). Normal surgical scalpels and forceps were used to prepare the four flaps. The pedicle of the local flap contains the neurovascular bundle. Therefore, the neurovascular bundle needed to be isolated and dissected first. To view the vessels with ease, they were rinsed with saline and injected with blue ink. The following flaps were prepared.

Atasoy flap: After a V-shaped incision on the palmar side of the distal phalanx, the mobilized tissue is shifted distally. The vessels of the flap are the fine subcutaneous vessels of the finger pulp (Figs. 1, 2).

Hueston flap: Elevated by a L-shaped incision, a mid-lateral incision is performed from the fingertip to the proximal interphalangeal (PIP) flexion crease where it turns medially. The neurovascular axis of the neurovascular bundle is from the opposite side (Figs. 1, 2).

Venkataswami-Subramanian flap: This is an oblique triangular flap, which is applied, in oblique amputations of the fingertip and thumb. A mid-lateral incision and an oblique incision are made from the PIP flexion crease to the opposite side of the proximal margin of the defect. This flap includes the neurovascular bundle of the incision side (Figs. 1, 2).

O'Brien flap: From the middle phalangeal flexion crease, a quadrangular flap is dissected distally to the defect. The neurovascular bundle is prepared to insure the proper neurovascular supply. This technique is typically applied on the thumb (Figs. 1, 3).

Without fixation, the hand was dehydrated by freeze substitution with acetone of -25°C. Four changes of acetone were carried out over an eight-week period. After dehydration, the specimen was impregnated in a reaction mixture of SRI0 polymer (Biodur™) with 1% Catalyst SH03 (Biodur™). Vacuum was adjusted to a slow rising of bubbles in the silicone. Bubbles lasted four weeks. The hand was returned to atmospheric pressure, removed from the silicone bath, warmed to room temperature and prepared for curing with Gas Cure SH06 (Biodur). Prior to curing, the flaps were lifted and absorbent paper placed under the flap, such that the flaps were cured in a half-elevated position. The specimen was placed in a closed atmosphere, which was saturated with gas cure using an aquarium pump working continuously for seven hours per day the first two days. Calcium chloride was used to control moisture in the curing chamber. The specimen was wiped down every two hours the first day. Thereafter, it was kept in the closed container for seven days.

Results

Local flaps were prepared on the palmar surface of the fingers and thumb and then plastinated. After impregnation, the characteristics of the flaps were clearly recognized. The neurovascular bundles were evident. After curing, however, significant shrinkage was observed, especially on the edges of the flaps. We tried to maintain their shape by strategically placing folded paper and small sticks but with little effect. The flaps and the neurovascular bundles seemed to have dried out and were distorted during this step. However, the features of the flaps remained demonstrable (Figs. 2, 3). The flaps remained moveable to some extent. The fingers of the specimen completely cured. However, other parts of the specimen covered by skin (metacarpal and carpal regions), where no dissection had taken place, were only partially cured. On the cut surface of the wrist, where the tissues were not covered by skin, the curing was complete.

Discussion

This was our first attempt at plastinating an unfixed specimen. There are two reasons for not using fixed materials: /. Soft pliable tissue is desirable and needed to prepare these elaborate local flaps. The skin of the fingertips thickens and dries out very fast on fixed specimens. Even if we found a fixed hand with suitably soft fingertips, it would likely have deep wrinkles, especially in the interphalangeal spaces that interferes with and distorts the dissection. *2.* We wanted the experience of plastinating unfixed tissue. For plastination, the unfixed hand was processed similar to fixed specimens.

In preparation of the flaps, the fingertips were cut off. This often is the case after finger injuries to remove damaged, dead and/or infected tissue.

In general, the flaps maintained their characteristic shape in all but the last phase of the plastination procedure. Deformation of the flaps and neurovascular bundles remained negligible during dehydration and impregnation. But during curing, shrinkage and drying were significant enough to distort the natural appearance of the specimen. Placing folded papers and sticks strategically during curing did not halt distortion. Even though the thin neurovascular bundles and flaps dried out and became deformed, they remained somewhat moveable. This flexibility made investigation of the

specimens easier. Most probably drying and distortion occurred because these pieces of tissues were very thin. The difficulty with curing in the non-dissected parts of the specimen may be due to the thick skin that might have hindered the penetration of acetone, silicone and gas cure. However, it is undesirable to remove the skin on the whole hand because this would result in an unnatural appearance. Two possibilities arise which could be of use in plastinating future unfixed specimens. First, the skin could be removed completely from the whole dorsal surface. This could facilitate penetration without disfiguring the dissected palmar region. As well, the skin could also be punctured on the palmar side. Puncture may result in shrinkage of the surrounding skin, especially if the slits are not small enough. Secondly, both acetone and silicone could be injected beneath the skin. Using the slow cure method might have helped decrease distortion. If problems can be eliminated, such specimens can be of use in the clinics, in both the undergraduate and postgraduate studies along with the other specimens housed in the Teaching Museum of Anatomy (Fig. 4).

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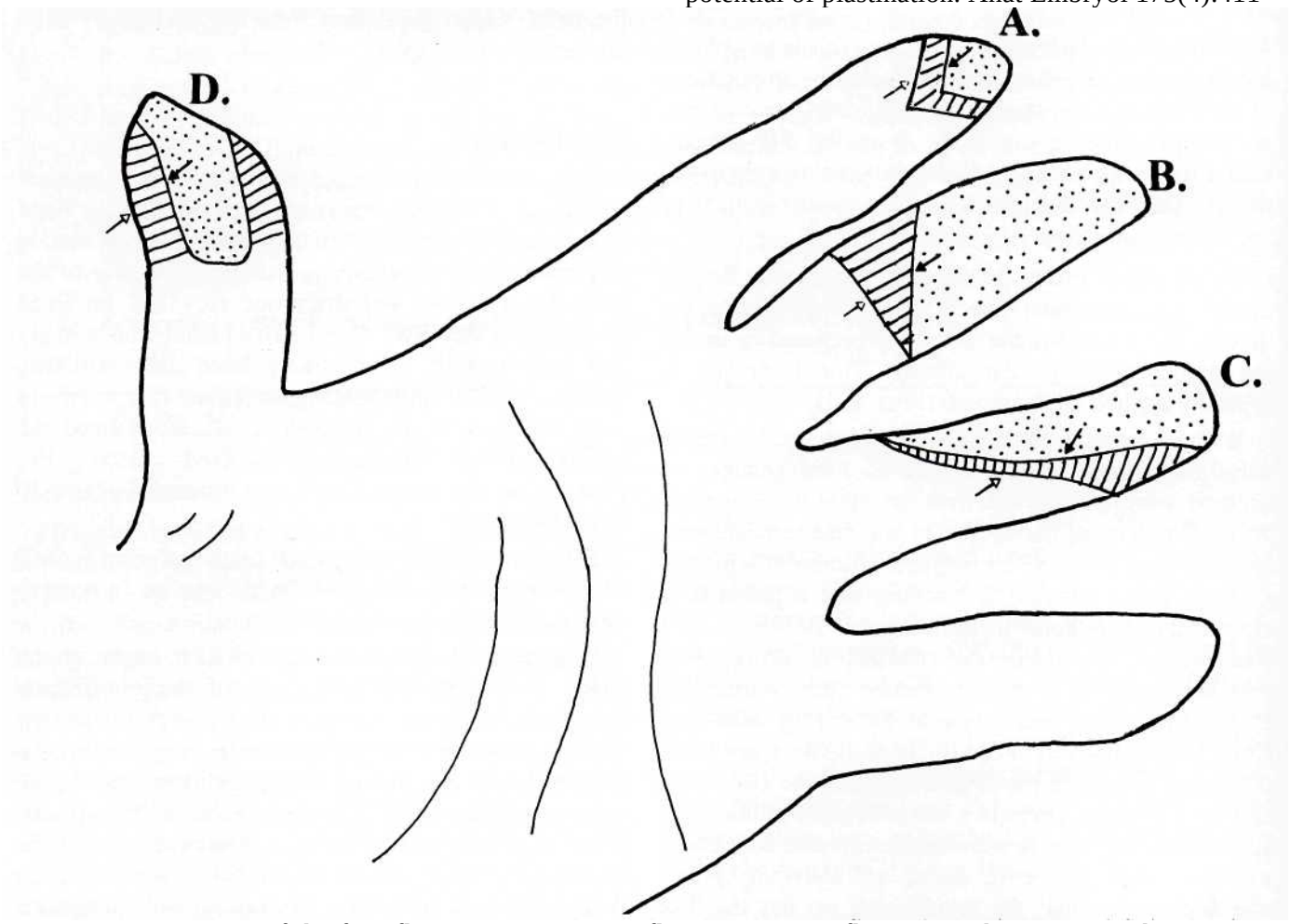

Figure 1. Drawing of the four flap types: A. Atasoy flap, B. Hueston flap, C. Venkataswami-Subramanian flap, D. O'Brien flap. Flaps (dotted area) are pulled distally onto the injured surface of the finger leaving a gap (lined area). Open arrow - Line of surgical incision, Closed arrow - Proximal margin of flap. Redrawn after: Atasoy et al. (1970), Hueston (1965), O'Brien (1968) and Venkataswami & Subramanian (1980).

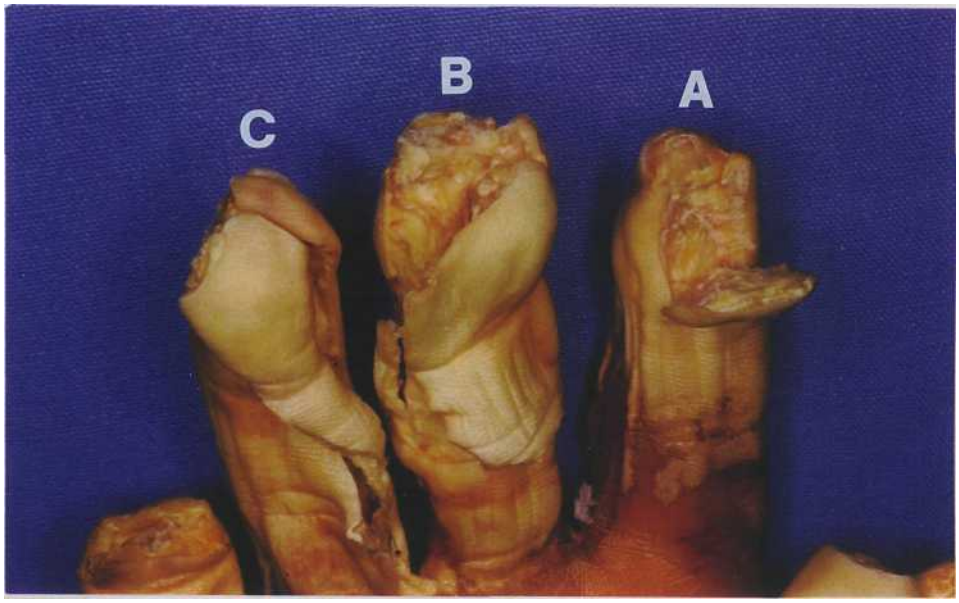

Figure 2. Plastinated hand showing fingers with: A. Atasoy flap, B. Hueston flap, C. Venkataswami-Subramanian flap.

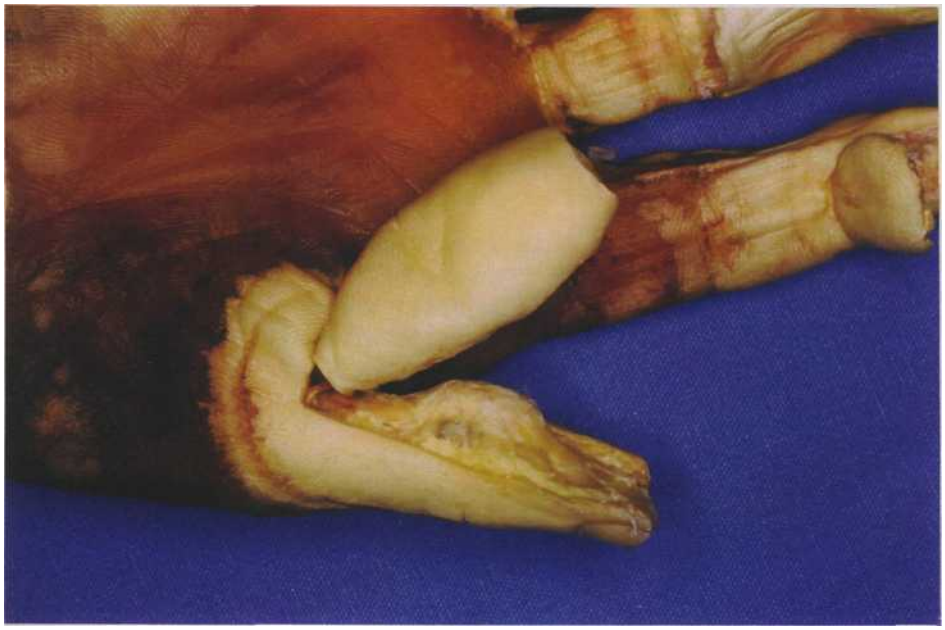

Figure 3. Plastinated hand showing thumb with an O'Brien flap.

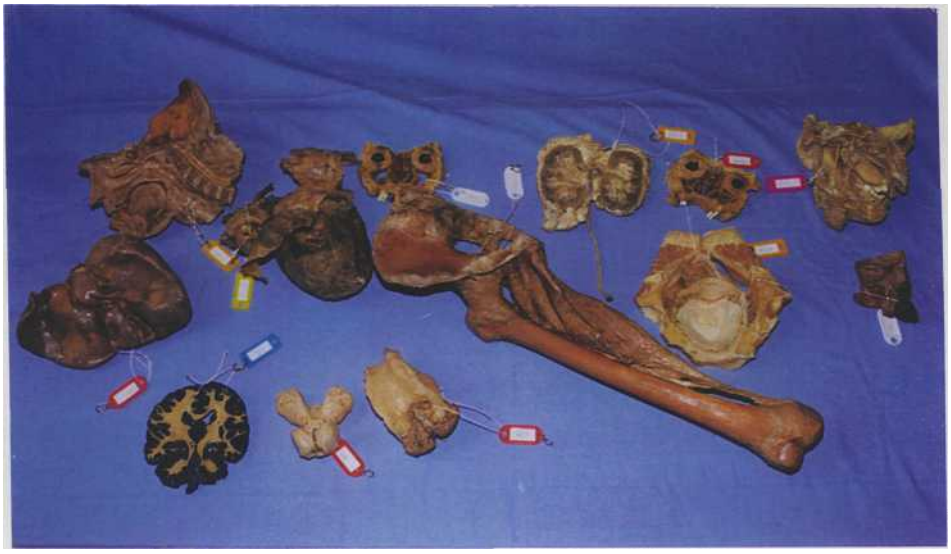

Figure 4. Plastinated specimens in the Department of Anatomy, Histology and Embryology of Semmelweis University for Medicine.