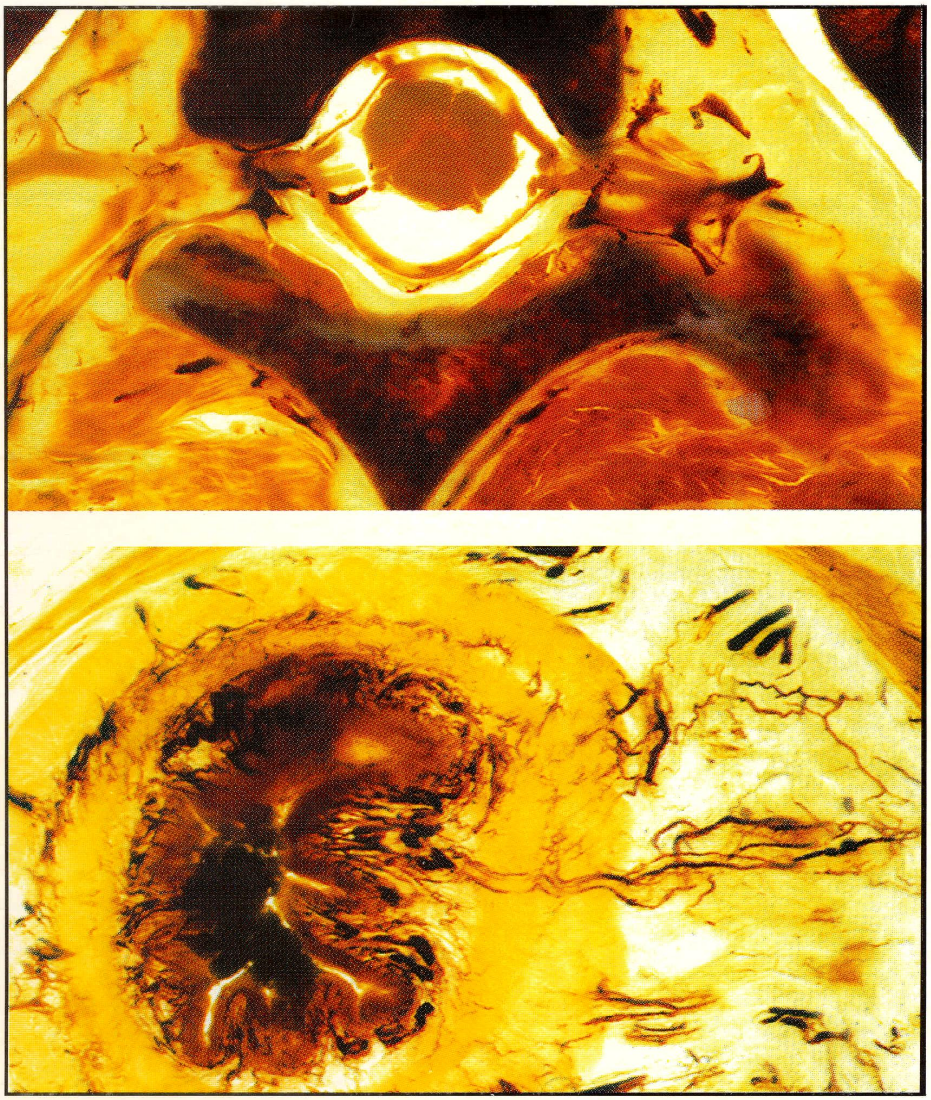
Journal of the INTERNATIONAL SOCIETY for PLASTINATION



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Presidential Letter

Dear fellow plastinators,

What a great meeting and what a superb workshop we had at The University of Tennessee in Knoxville!

A package of informative lectures and posters and lots of practical sessions where packed together by Bob Henry and Larry Janick. 48 experienced plastinators and newcomers from Austria, Australia, Canada, Korea, Mexico, New Zealand and the USA met in Knoxville for the Plastination Workshop and the 5th Interim Conference of the ISP. After many acetone bubbles and orange spots everyone could not only take home the new experience with the main plastination procedures, but also a sample of S10 stomach, E12 head slice and P35 brain slice.

A picnic and hayride at Cades Cove in the Smoky Mountains and a visit to the body farm guided by Dr. Bill Bass of the UT Anthropology Forensic Investigation Team completed the impressions of five wonderful and warm days in Tennessee.

In behalf of all the participants and of myself I want to thank Bob and his wife Carol for their endeavor to make this an unforgettable meeting.

I am looking forward to meeting you all in Trois-Rivières in July 1998.

Yours sincerely,

Andreas H. Weiglein
President

Paolo Mascagni, Ernest Alexandra Lauth and Marie Philibert Constant Sappey on the Dissection and Injection of the Lymphatics

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(received August 23 , accepted September 9,1997)

Key words: *Lymph vessels, Lymph nodes, Preparation, Injection*

Abstract

In the last centuries, many anatomists tried to describe and to understand the course and the function of the lymphatic system. Technical difficulties explain the numerous controversies which stand out as milestones in the history of lymph vessels and nodes. This paper aims at summarizing the main steps in the discovery of the lymphatic system, and at describing the important contributions of pivotal figures in this field of vascular anatomy: Paolo Mascagni, Ernest Alexandra Lauth and Marie Philibert Constant Sappey.

Introduction

There are a number of steps in the history of the lymphatic system. Discovered by chance, misunderstood for a very long time, lymphatics were the subject of much controversy up to the early twentieth century, when their accurate description was deemed necessary to promote advances in oncology (Rouviere, 1932). The purpose of this paper is to summarize the history of the discovery of the lymphatic system and the differents methods used by three pivotal figures in the history of lymphatics: the Italian Paolo Mascagni in the late eighteenth century and two Frenchmen, Ernest Alexandre Lauth and Marie Philibert Constant Sappey, in the nineteenth century.

The Forerunners

The most famous Alexandrian anatomists observed lymph vessels in animals. But Erasistrate mistook them for arteries and Herophile mistook them for veins. However, the latter also referred to «glandular bodies», which must be regarded as the current lymph nodes.

In the sixteenth century, Nicolas Massa described briefly some lymph vessels of the human kidneys (1536), and Bartolomeo Eustachi (1564) made the first accurate description of the thoracic duct in the horse, which he called « Vena alba thoracis». However, he only observed its thoracic course and its connection with the subclavian or internal jugular vein.

In the seventeenth century, Gaspare Aselli, Thomas Bartholin, Olof Rudbeck, Jean Pecquet and Frederik Ruysch made important contributions to the knowledge of the lymphatic system.The Italian surgeon Gaspare Aselli (1581-1626) had been a pupil of his famous compatriot Gabriele Falloppio. On July 22 (or 23), 1622, he discovered the lacteal vessels while displaying the abdominal viscera of a dog at an anatomical demonstration. As it happened, the animal had been fed shortly before the dissection, and Aselli could therefore observe milky "fibers" running on the mesentery. He first mistook them for nerves and decided to cut them in the course of his demonstration. He then observed a whitish liquid flowing out of these "fibers" and came to the conclusion that they were vessels. Some days later, he began to verify his observations in other animal species: cats, lambs, cows, pigs, and even a horse he bought only for this purpose (Sappey, 1869). Unfortunately, Aselli could not confirm the existence of lacteal vessels in humans before his death in 1626. Aselli's book was published post-

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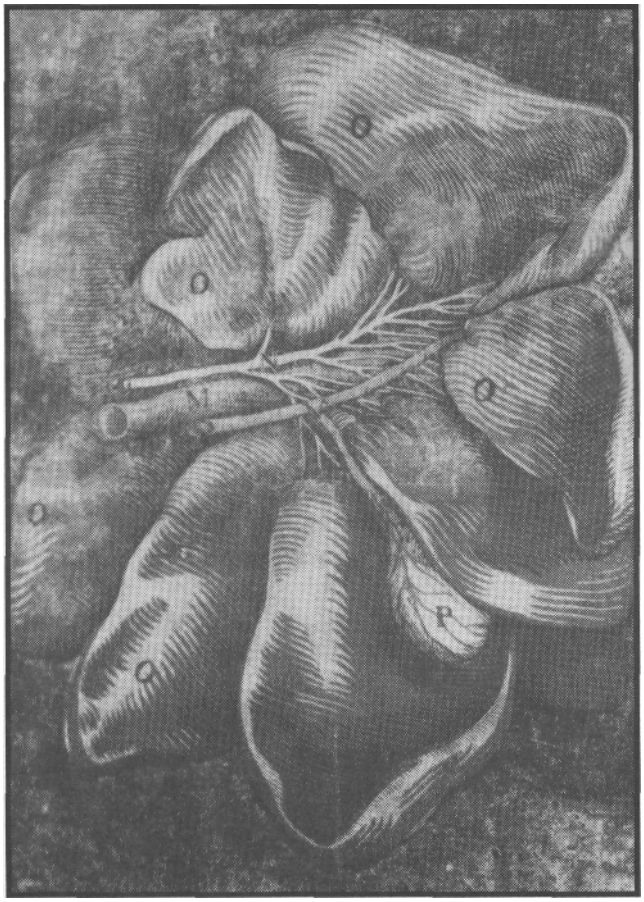

Figure 1. Aselli's plate of the lacteal vessels (1627). M - portal vein, N - lymphatic vessels, O - hepatic lobes, P - gall bladder, V - bile duct.

humously under the editorship of Alessandro Tadini and Luigi Settala (1627). In addition to its historical contribution to the history of the lymphatic system, this book also has the distinction of including the first anatomical plates printed in colour (figure 1), probably engraved by Cesare Bassano (Choulant, 1852; LeFanu, 1976; Norman, 1994). The Danish anatomists Thomas Bartholin (1616-1680) and Olof Rudbeck (1630-1702) published numerous studies on lymphatics and disagreed over their distribution (for a review, see Hagelin, 1989). Jean Pecquet (1622-1681) described the confluence of abdominal lymph trunks and the cysterna chyli at the origin of the thoracic duct in the dog (Pecquet, 1651). Eleven years later, Frederik Ruysch (1638-1731) established the existence of valves in the lymphatic vessels (figure 2) in a small book dedicated to his three much-admired teachers, Franciscus De Le Boe, Johann van Home and Florentius Schuyl (Ruysch, 1665; Luyendijk-Elshout, 1964).

Paolo Mascagni

The Italian anatomist Paolo Mascagni (1755-1815)

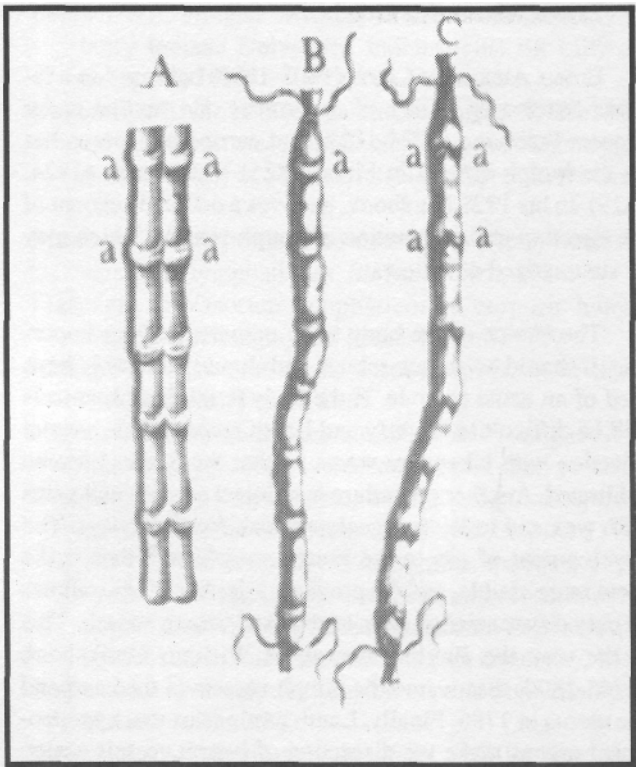

Figure 2. Valves in the lymphatics vessels (Ruysch, 1665). A - a lenghtwise cut lymphatic vessel, B-C - two lymphatic vessels full of water, so that the location of valves become visible, a - semilunar valves.

studied at Siena under Pietro Tabarrini (1702-1780). His most successful research was on lymphatics, and led first, to the submission of preliminary results (1784), to which the French Academy of Sciences awarded a prize, and second, to the publication of one of the most striking atlases of the eighteenth century (1787), illustrated by Giro Santi. By using a very simple method (a tubular needle bent at right angle), he was able to discover about fifty percent of all the lymphatic vessels now known (Norman, 1978). Moreover, he established that every lymph vessel must in its course enter at least one lymph node, disproved the existence of arterial and venous lymph vessels, and concluded that the lymphatic system originates from all the cavities and surfaces of the body.

Mascagni's procedure for the injection of superficial lymph vessels was widely used in the following century. In order to avoid filling the deep lymph vessels during the injection of the superficial ones, Mascagni recommended injecting glue in the arteries first, then cooling the specimen. In this way, all lymph vessels (superficial and deep) became collapsed. For the selective injection of the superficial vessels, he warmed up the surface of the specimen so that the glue could soften, therefore making the superficial lymph vessels permeable again.

Ernest Alexandra Lauth

Ernest Alexandre Lauth (1803-1837) belonged to a famous Strasbourg family of anatomists. He studied under Vincent Fohmann (1794-1837) and carried out researches on the lymph system in birds (1825) and in man (1824, 1829). In his 1829 handbook, he gives a detailed account of the injection and preparation of lymph vessels, which may be summarized as follows:

The choice of the body to be prepared is very important. It should be young, robust, and should preferably have died of an acute episode. If the body is fat, lymph vessels will be difficult to identify and Lauth recommends arterial injection with lukewarm water, so that the tissues become infiltrated. Another procedure is to inject arteries and veins with wax and to let the specimen soak for some days. The development of gas in the lymph vessels will then make them more visible, and the previous injection of wax allows an easy differenciation from arterial and venous vessels. This is the way the English anatomist William Cruikshank (1745-1800) discovered the lymph vessels of the heart and the uterus in 1786. Finally, Lauth reminds us that hypertrophied organs make the dissection of lymph vessels easier. According to Lauth, the lymph vessels of a gravid uterus are thicker in diameter than the feather of a crow!

In the nineteenth century, the lymph vessels were usually injected with mercury. Lauth stresses the fact that mercury must be as pure as possible, and must in any case be filtered through a piece of chamois leather. To check the purity of the mercury, he put a drop of mercury on an inclined plate. If the drop slid without dirtying the plate, the mercury was pure enough for the injection. If not, it meant it contained traces of lead or tin and must not be used for anatomical preparations. But other products could also be used. For the injection of thick lymph vessels (thoracic duct or right lymphatic duct), Lauth writes that plaster yields good results provided one takes care of the orientation of the valves. In addition, he reminds us that his colleague Andre Marie Constant Dumeril (1774-1860) made successful injections of lymph vessels with milk, provided the specimen was not intended to be preserved by drying. Other anatomists injected black ink by blowing into a tube connected with a thin glass needle. This procedure was used for the first study of lymphatics in Japan (Husiya, 1805).

To inject mercury into the superficial lymph vessels, Lauth removed carefully a small square of skin, located a vessel, made a small incision in its wall with a lancet, and introduced a thin glass or steel tube into the vessel. Owing to the high number of anastomoses, three injections are sufficient to inject all superficial lymph vessels of the lower limb: one injection on the hallux, a second on the fifth toe, and a third one behind the medial malleolus. For the same reason, three or four injections in various parts of the hand will fill all superficial lymph vessels of the upper limb (here he is at variance with Mascagni, who wrote that at least twenty injections are necessary for a whole limb).

Marie Philibert Constant Sappey

The French anatomist Marie Philibert Constant Sappey (1810-1896) conducted numerous studies on the lymphatic system (1843,1847,1874-75) (figure 3). In the second edition of his treatise on anatomy (1869, vol. 2: pp. 809-812), he gives a summary of the important points that have to be taken into consideration for a successful injection of the lymphatics.

Sappey differs with Lauth regarding the choice of body. Sappey recommends the body of man who died of a chronic disease because it will be sufficiently emaciated. The body of a child should only be used to study the lymphatics of the head, the tongue, the soft palate, the gums or the scrotum. Regardless of the specimen, however, Sappey thought the

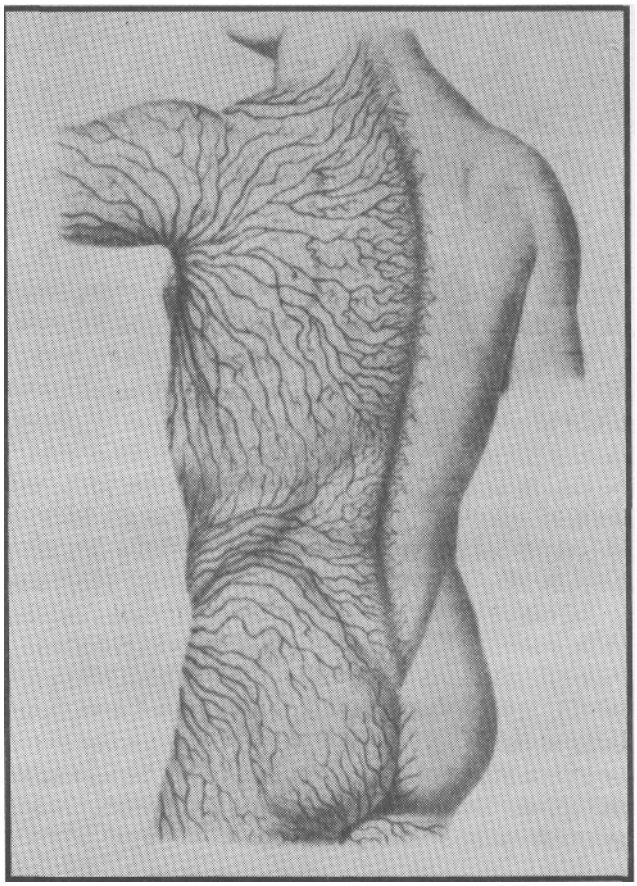

Figure 3. Superficial lymphatics of the trunk (Sappey, 1874-75).

injection of lymphatics should always be carried out in summer because a high temperature makes the progression of mercury easier.

Putrefaction also has an influence on the procedure. Early putrefaction helps the injection of superficial lymph networks (finger, toes and scalp, especially), but makes the main lymph trunks unusable.

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Development of an Anatomical Technologies Laboratory

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Key words: Preservation, Plastination, Laboratory design, Cost, Equipment

Abstract

This paper documents the procedures followed in establishing an anatomical technologies laboratory for the preparation of anatomical and plastinated specimens, in the Department of Anatomy and Cell Biology at the University of Melbourne. Funding for the laboratory was obtained through a Faculty of Medicine teaching development and innovation grant, supplemented by funds from the Department. Next to the embalming room, an existing space, which was formerly used for the storage of cadavers, was converted into two suitable work areas. One a preparation room and the other a spark-proof area housing the freezer with an isolated vacuum pump and refrigeration unit. Part of the funds obtained from the Faculty have been used to employ a technical officer who has dedicated responsibility to the facility and its day-to-day operation. The laboratory has been fully operational for over a year and a large number of plastinated specimens have been prepared during that time. We are in the process of developing 'sheet plastination' and have a project under-way with radiologists from the Royal Melbourne Hospital correlating cross-sectional plastinated specimens with CT-scans and magnetic resonance images. It is our intention to direct most of our work towards the Department's teaching requirements, however we also intend using the laboratory to train technicians in anatomical techniques and encourage honours and higher degree students in the area.

Introduction.

The Department of Anatomy and Cell Biology at the University of Melbourne, like Anatomy departments universally, has extensive teaching commitments. In any given year we provide instruction to over 1500 undergraduate students in medicine, physiotherapy, dentistry, optometry and science, as well as to a variety of postgraduate groups and professional organisations. Our undergraduate teaching programs continue to run along traditional lines, comprising lectures, demonstrations using prosected wet specimens, and student dissection. Students receive practical demonstration of material presented in lectures through a program known as 'wet specimen workshops'. The term 'wet' refers to the manner of storage of such specimens - immersed in a bathing solution of 99% ethanol:water (1:3). Prior to such workshops, specimens are removed from a number of storage tanks and displayed on tables in the laboratory, where students preview, under supervision, the anatomy of a structure or region before proceeding to dissect it for themselves. We also have a large and well-stocked Anatomy museum but unfortunately specimens are mounted, unlabelled, in perspex containers behind glass cabinets. Structures are often difficult to see and identify and as a result our museum has been progressively underutilised in recent years.

Due to large student numbers, increased demand on the Department's limited stock of suitable prosected material has meant greater wear and tear and an increased rate of attrition of such specimens in recent years. Ideally we should be producing greater numbers of specimens to increase the variety and range of those already available. However, at present we are unable to produce enough to maintain our existing stock. As S10 silicone fully cured specimens are durable (Weiglein and Henry, 1993) and do not require 'wet' storage, plastination provides us with the opportunity both to extend the life of this material, and additionally maintain effective student/specimen interaction. Therefore in 1994, following a visit by one of the authors (CB) to Heidelberg in Germany to examine Von Hagen's plastinated materials, as well as visits to anatomy departments in North America and the United Kingdom (CB and BK) to thoroughly re-

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search the approaches to anatomy teaching taken by those departments, we decided to investigate means of extending the life of cadaveric material by plastinating our own anatomical specimens.

The S10 technique (Von Hagens, 1986) is highly reproducible and nearly always results in a satisfactory, durable specimen. The methodology can be applied to older formalin fixed specimens (Cannas and Fuda, 1991) which effectively extends the life of our museum stored specimens, and has the added bonus of making them much more user friendly as they can now be handled by both teachers and students. Our initial challenge was to find the funds to establish a laboratory, as well as the space in an already crowded building to house one. With the assistance of a Teaching Development and Innovations Grant from the Faculty of Medicine at the University and additional funds from the Department we were able, at the end of 1994, to start planning the facility.

Laboratory Construction

In addition to the traditional prosected anatomical specimens, techniques are available to cast anatomical cavities, viscera and blood vessels, assemble articulated skeletons and display joints and their ligaments (Tompsett, 1970). New methods of preparing anatomical specimens are also appearing in the literature (Ocello, 1997). Because we intend to experiment with a number of these techniques the laboratory was named the Anatomical Technologies Laboratory rather than merely Plastination Laboratory. However, plastination has become the major focus. The laboratory was constructed within an area occupying approximately 45 square metres of floor space in the 'tank room', a room in the basement of the Medical Centre adjacent to our embalming room, where cadavers were formerly stored immersed in large tanks of alcohol, water and formaldehyde. The tanks were made of reinforced concrete, lined with stainless steel and connected to the floor. It was a major undertaking to remove the tanks and make good the floor (figure 1).

This task and the overall refurbishment of the space, including sealing-off a 3-phase electronic lifting system, upgrading the air conditioning, making selected power outlets and light fittings spark proof and altering the space to conform to safety standards, cost over $Aus.40 000. The final plan of the laboratory is illustrated in figure 2.

By early 1995 this task was achieved and we then started to acquire equipment to get the plastination process under way. This phase of the project could not have happened as quickly as it did without the appointment, again funded from the grant, of a Technical Officer, a science graduate with training in human anatomy, who has taken on

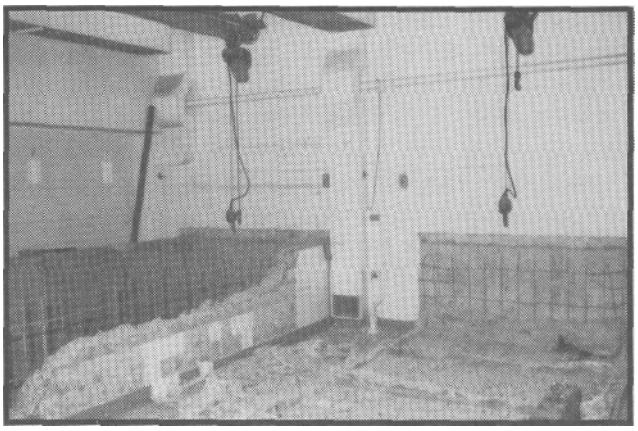

Figure 1. Preparation of Anatomical Technologies Laboratory. Existing space during demolition.

responsibility for day-to-day operation of the laboratory.

Our initial objective was to quickly produce S10 specimens and to this end we sought a freezer sufficiently large to have several specimens simultaneously under preparation, a vacuum system for acetone removal and impregnation, and a curing chamber. With considerable help and advice from Mr Robbie Boyes at the University of Queensland we found local producers for all major items of equipment (details available on request). The overall cost of fitting out the laboratory was approximately $Aus. 10 500, the freezer including remote unit (2000x700x1000 cm) and vacuum pumps being the most expensive items ($Aus.5 000

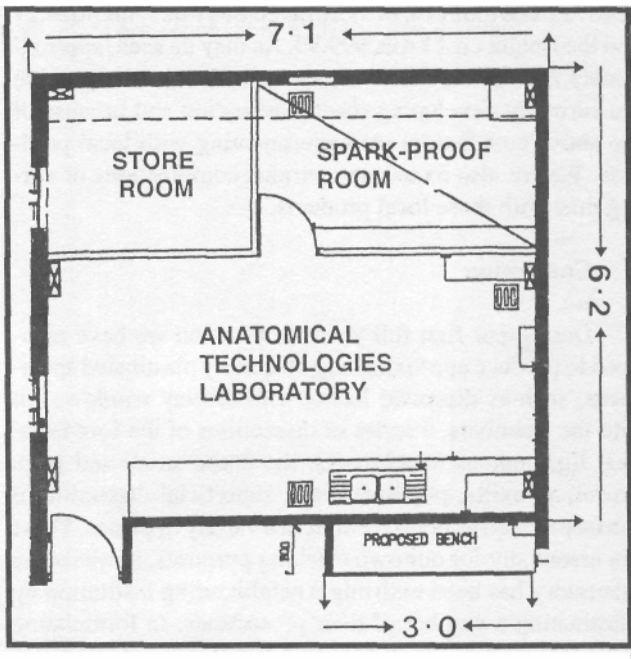

Figure 2. Plan of the Anatomical Technologies Laboratory *(all measurements in metres).*

and $Aus.2 600 respectively). The freezer has been especially built for our purposes and is capable of fitting four large barrels for dehydration. It possesses an easily removable lid and has no ignition sources. The walls contain an extensive coiling system allowing rapid cooling, and they are insulated in a fashion that allows the freezer to remain below zero (Celsius) for a period of 24-48 hours in the event of a power failure. Using a normal household unit the freezer will draw and hold -40° Celsius. The vacuum pump is a RV3 rotary van pump, 220-240V, 50/60 Hz single phase Edwards pump (Edwards High Vacuum International, Manor Royal, Crawley, West Sussex, RH102W, U.K.) which has warranty certificates and good servicing facilities. Maintenance is simple and requires minimal time. The pump is capable of holding a vacuum in many large chambers and is therefore suitable for current or future needs should we further expand the laboratory. We did not investigate the use of reconditioned parts but they are undoubtedly available.

To get us started we required S10, hardener and other products from Biodur, which significantly increased costs (by an additional $Aus.5 600 in the first year). The initial purchase consisted of Biodur S10: 15 kg ($Aus.915 / DM1005), Biodur Hardener S3: 0.15 kg ($Aus.l6.14/ DM16.50), Biodur Hardener S6: 1L ($Aus.65.52 / DM72), acetonometer: 90-100% ($Aus.lOO / DM106), Biodur Fix A: 10.4 kg ($Aus.327 / DM360), Biodur Fix T20: 2L ($Aus.61.88 /DM68), Pine Oil: 2L ($Aus.72.80 / DM80). To the above will shortly be added a purchase of Biodur S10: 50 kg ($Aus.2 935.42 / DM 3 000), Biodur Hardener S3:500ml ($Aus.50 / DM55) and Biodur S6:1L ($Aus.70.45 /DM72). The total cost of these purchases was $Aus.4614.21 and the freight cost $Aus.999.43. As may be seen, approximately 20% of our funds are spent on freight charges. We are currently developing sheet plastination and because of the above cost factors are experimenting with local products. We are also examining various combinations of curing time with these local products.

Conclusion

During our first full year of operation we have managed to produce approximately 60 quality plastinated specimens, such as dissected hearts with display windows cut into the chambers, a series of dissections of the foot to reveal ligamentous attachments, the entire small and large bowel, an axilla, popliteal fossa, superficial dissection of the face as well as brain sections in a variety of planes. These are essentially for our own teaching purposes, however the laboratory has been assisting a neighbouring institution by plastinating a number of their prosections. In formulating the successful funding proposal for this innovation, we costed the preparation of a wet specimen (including academic and technical time) at between $Aus.350-500, depending on size and complexity of the structure or region. With an average life span for a wet specimen of only three to five years, we anticipate making significant cost savings after three years, through the use of plastinated rather than wet specimens. The added benefit will be a large and diverse number of durable plastinated specimens which we anticipate using for many years to come. In addition, we have instituted a research project with radiologists from the neighbouring Royal Melbourne Hospital to develop correlated MRI and CT-scans for teaching purposes. We also intend using the laboratory to train technicians in anatomical techniques and encourage honours and higher degree students in the area. Student and teacher reaction to the availability of plastinated specimens has been favourable. We are currently preparing plastinated preparations of both normal and pathological abdominal and pelvic viscera. These will also be photographed and the images stored in CD-ROM format with appropriate labelling and text which the students may access in the dissecting room and in the museum. In addition, neurosciences teaching staff have requested multiple sets of sagittal, coronal and transverse views of the brain for neuroanatomy teaching purposes.

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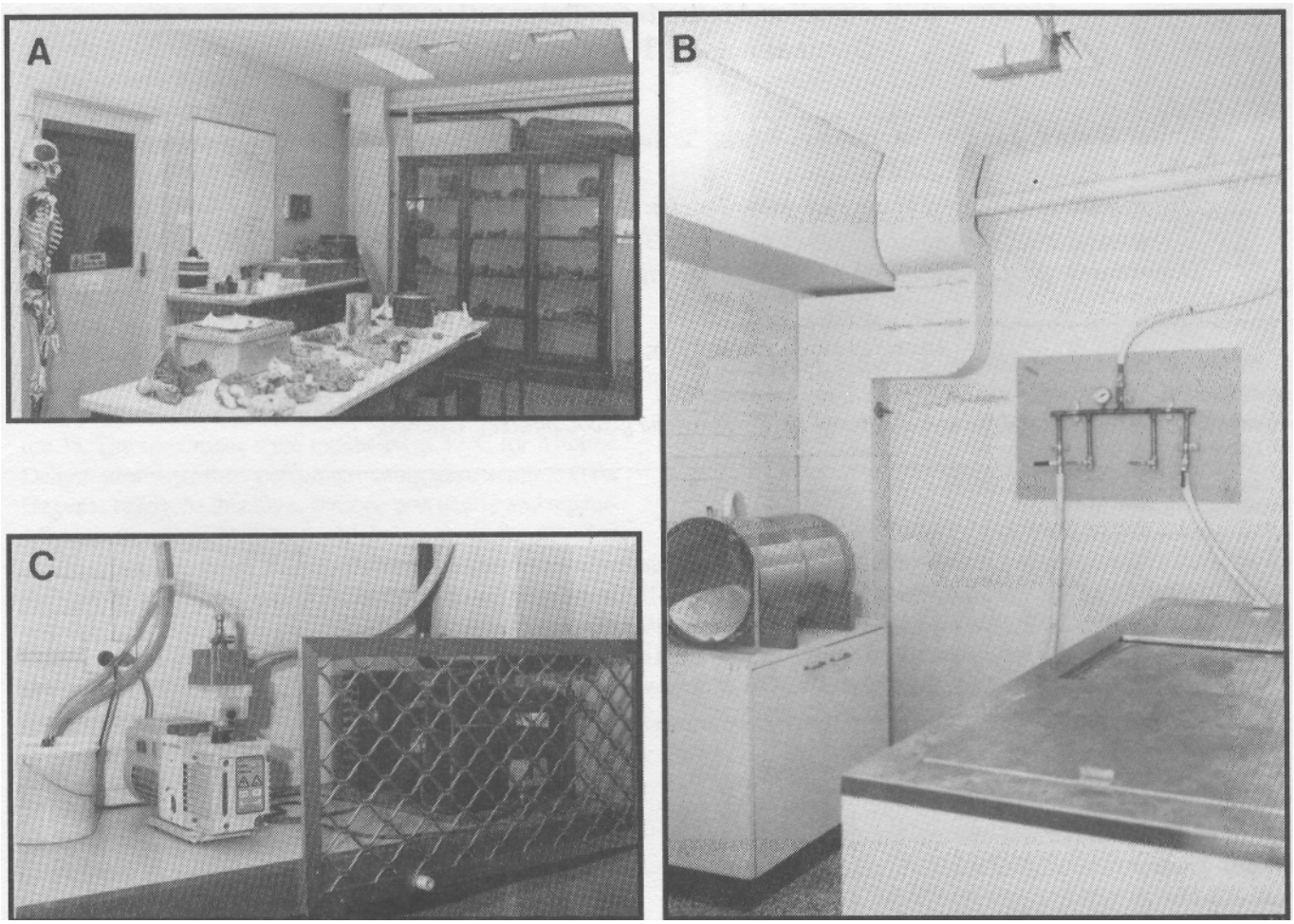

Figure 3. Laboratory in current use: A. Preparation room. B. Spark proof room with vacuum distribution apparatus. C. External bench with remote units.

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Combined Plastination Methods for Preparation of Improved Ophthalmologic Teaching Models

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(received November 6,1996; accepted March 10, 1997) ***Key***

words: *Ophthalmology, Polymer-SlO, Polyethylene glycol, Surgery, Teaching*

Abstract

Pig eyes were prepared, using a combination of Silicone S10 infiltration and polyethylene glycol impregnation. The vitreous body (corpus vitreum) was liquified, withdrawn and replaced by silicone S10. The wall of the eyeball (bulbus oculi) was then impregnated with polyethylene glycol. This first attempt to combine two chemically-different polymers for the plastination of teaching specimens successfully resulted in soft and life-like eyes. The specimens plastinated with this technique are safe for student handling and use during anatomical study. They are also unique for practising delicate surgery procedures.

Introduction

It is not always possible for the ophthalmology students' and post-graduate students' education to take place in real clinical settings (with live patients) because of the danger of injuring or even loosing eyesight as a result of the trainees' unskilful manipulations. Practicing on dead human or animal material is more complicated because of the irreversible postdeath changes in the organic components which occur under the influence of microorganisms and proteolytic enzymes contained within the tissues. Thus there is a need for developing improved techniques for preparing eye specimens suitable for training of ophthalmology students.

After several centuries of more or less successful attempts to develop techniques for preserving the organic matter (reviewed by Kurz, 1993) the plastination technique (von Hagens et al., 1987) has been widely acclaimed for preparing anatomically-accurate teaching specimens from human or animal tissues, and represents a major step forward over previous techniques. This technique involves solvent dehydration and subsequent impregnation of the actual animal tissue to be studied or manipulated. It largely preserves the anatomic integrity of the tissue. Polymers selected for the impregnation vary according to desired physical characteristics of the final product (Sivrev and Kayriakov, 1995), and commonly include methyl methacrylate, polyethylene glycol, epoxy resins and silicone derivatives (Whalley, 1994). This is the first report in which two chemically-different polymers have been successfully used to plastinate tissue for teaching purposes.

Materials and Methods

Pig eyes (which are close in size and structure to human eyes) were selected for model development. Fixation was performed by immersion at room temperature.The fixative solution used (Formaldehyde 10%, Ethanol 40%, Water 50%) (von Hagens, personal communication) preserved the specimen colour relatively similar to the natural one. The specimens were stored in fixative solution until ready for plastination. They can remain in this solution for unlimited time. The experiments carried out with 5% formaldehyde solution; Kaiserling solution (Kaiserling, 1896); and the Plasdoform based embalming fluid (Dodge Chemical Co., Cambridge, Massachusetts, USA) led to greater darkening of the specimen.

Following fixation, eyes were washed in running tap water. Blood traces remaining on them were then removed

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by the use of 0.5 - 3% solution of hydrogen peroxide. This procedure takes a few minutes. The whole process was controlled visually and discontinued immediately after the disappearance of the blood traces, so that a change in the tissue colour was avoided (lower concentration of hydrogen peroxide and longer duration of exposition produced better results). Afterwards, the specimens were once again washed in tap water. They were ready to be cleaned of excess tissues by dissection to levels suitable for intended practice surgery procedures.

Hylase (Farma Dessau, Gbm, Germany) 300 UI was injected through the pars plana into the vitreous chambers to help liquification and removal of the vitreous body (figure 1). The specimens were incubated at 37°C for 2 hours. Dehydration was then performed using pure acetone (von Hagens, 1985). At this time, acetone was gently and repeatedly injected into, and then withdrawn from the vitreous chambers of eyes using a syringe and a broadlumen needle. Following several minutes of this procedure the acetone withdrawn from the vitreous chambers became clear, indicating that the hylase-digested vitreous body had been removed. If successful liquification had been achieved, only 3 changes of acetone were generally sufficient. Excess acetone was then drained from the vitreous chambers, after which Biodur S10/S3/S6 (100:1:0.7) silicone (von Hagens, 1987) was injected into the chambers until they were full. Specimens were then placed in the polyethylene glycol 400 (PEG 400) and stored at 5°C to permit the curing of the silicone.

The silicone filled specimens were afterward impregnated at room temperature under vacuum with the solution of PEG 400 (Steinmann, 1986) which impregnated the wall of the eyeball and associated structures (figure 2). The process lasted approximately 12 hours at a constant pressure of 11.5 to 19 mm Hg inside the impregnation chamber. The disappearance of the bubbles indicated the end of the impregnation process. After complete impregnation, the specimens were removed from the vacuum chamber and dried for several weeks by placing them on filter paper in a room protected from direct sun light (figure 3).

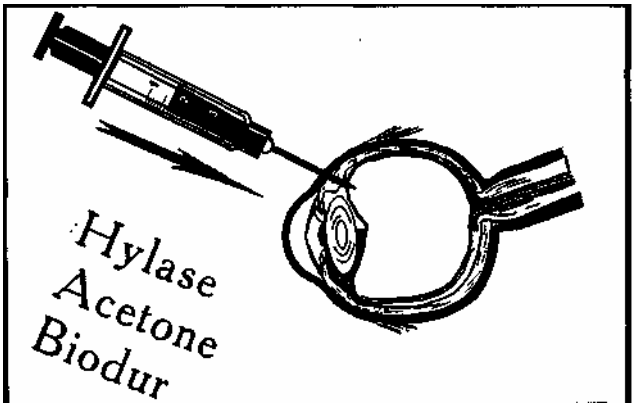

Figure 1. Steps of liquification and removal of the vitreous body with Hylase; dehydration with acetone and infiltration with silicone S10 are all performed by injection through the pars plana.

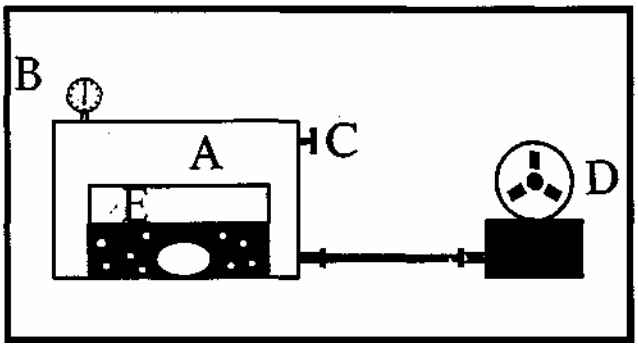

Figure 2. Impregnation is performed under vacuum at room temperature. A- vacuum chamber, B - Vacuum gauge, C - Adjustment tap, D - vacuum pump, E - impregnation container.

Results

After drying, the specimens were soft, life-like and suitable to be used in the educational process. In addition, the reversibility of the PEG impregnation process permits the correction of the specimens that were not properly impregnated. In these cases, there appeared shrinking of the specimens. The eyes were then completely immersed in water at

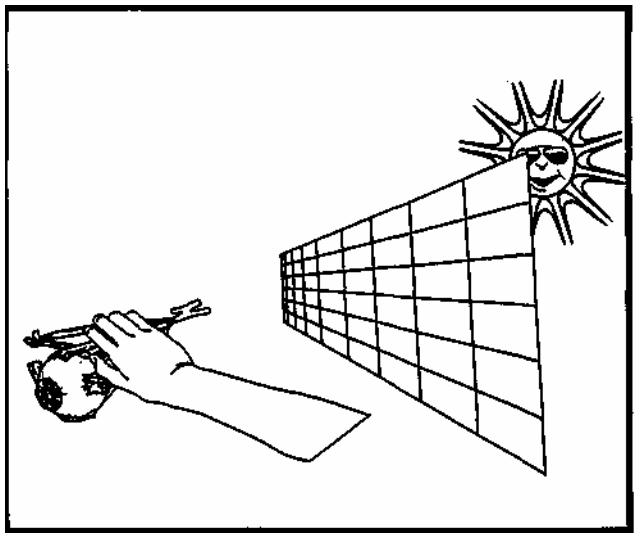

Figure 3. Curing is performed at room temperature, protected from direct sun light over a period of several weeks.

room temperature. PEG dissolved and the entire impregnation process was repeated until we achieved satisfactory specimens (e.g. re-impregnation was performed). The appearance of the so-plastinated specimens is similar to their natural condition. In spite of a light darkening of the lens, these specimens proved to be excellent models for the ophthalmology-surgical educational process. Students and post-graduate students in ophthalmology may practise operating techniques on these specimens until the techniques are mastered, without any danger of eyesight loss due to any mistakes by the trainees as could happen with living patients.

Discussion

The vitreous body is constructed of interlaced filaments, and the space between them is filled with liquid consisting of 99% water and 1% hyaluronic acid, as well as hydrophilic polysaccharides. This colourless and amorphous mucilaginous mass was replaced by Biodur S10. The cured silicone protects internal structures, maintains the shape of the eye and prevents the retina from detaching when the eye is placed under vacuum during the subsequent phases of the impregnation process. This makes the pig eye surgery models more representative of living human eyes. Eyes plastinated by this new technique are safe for student handling and use. They were prepared with a relative ease and limited expense, and are suitable for storage at room temperature in boxes lined with filter paper, rather than requiring submersion in formaldehyde-containing fixatives in glass jars. We found the combination of the two chemically different polymers (Silicone S10 and Polyethylene Glycol 400) an excellent method for the preparation of this new type of specimens. Eyes prepared by this new technique appear to represent improved specimens for teaching certain surgery procedures to ophthalmology students as compared to previously used eyes in our laboratory. Mastering of complicated techniques in the eye operations have been greatly improved in our laboratories by the use of these exceptional specimens. We are at the present time experimenting on the removal of the vitreous body by using the "pars plana-vitrectomy" method (Majdrakova et al., 1994). We expect it to be a more efficient method for the extraction of the vitreous body.

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An Acetone-Vapor Reducing Method for Freeze-Substitution

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Key Words: Dehydration, Equipment, Safety

Abstract

Use of acetone as a common volatile intermediary for the plastination process entails problems in containing its flammable vapors. During the freeze-substitution stage of the plastination process, acetone vapors can be contained by using multiple heat sealed polyethylene bags containing heavy duty polyethylene boxes in which the specimens are placed.

Introduction

The plastination process requires the replacement of tissue water with a miscible organic solvent acting as a volatile intermediary for the plastination compounds. One such common solvent is acetone (von Hagens, 1985; Henry, 1991). However, acetone is considered highly flammable and explosive in its vapor state. Attempts to set up a plastination laboratory in the department of Anatomy, Lund, Sweden, were stopped by the faculty due to the acetone vapor problem. A method for storing and handling acetone and for freeze-substituting with acetone with a minimum of acetone vapor escaping into freezers and the laboratory atmosphere is described.

Materials and Methods

Acetone (99%) was purchased in soft plastic one-liter bottles from a local paint and chemical store. The bottles deformability reduced the risk of accidents which can occur with the standard glass bottles when stored in the cold or handled when cold.

Three-liter capacity heavy duty polyethylene freezer bags (not the zip-lock type, though these may serve well too) were purchased from a local grocery store, as were 3 two-liter high density polyethylene ice cream boxes. The plastic bags were tested for leaks by either filling with water, or by blowing them up by mouth and squeezing the airfilled bag. Leaking bags were discarded.

Specimens chosen were: a small intestine with mesentery (volume: 850 cc); a colon (volume: 700 cc) and a stomach together with a caecum-ascending colon specimen (combined volume: 250 cc).

Each acetone bottle was placed in a plastic bag, excess air was gently squeezed out of the bag, and the open end of the bag heat sealed with an Impulse Sealer (TEW(QS), type TISH(QS)300, O. Mollerstrom AB, Gothenburg, Sweden) set at 50%, for 2 seconds. Each bagged acetone bottle was then placed in a second plastic bag which was also heat sealed. The process was repeated a third time, for a total of 3 bags enveloping each bottle. All work done with acetone and the Impulse Sealer was done in a draft cupboard. The acetone bottles were then stored in a freezer chest at -25°C. for 1 month. The freezer and heat seals were checked for acetone vapor leaks on a weekly basis. As no leakage could be detected, work with specimens was initiated.

The 3 specimen groups were each placed in a plastic freezer bag, each of which was placed in a polyethylene box. A bottle of the -25°C. cooled acetone was emptied into each specimen bag. The top of the specimen bag was adjusted so it hung over the edge of the box. The opposite side of the box was held slightly lower while air in the bag was gently pressed out, taking care not to get any acetone over the edge of the box! The protruding part of the bag was heat sealed promptly (figure 1). The heat sealed specimen bag was then completely tucked inside the box and the box's lid pressed down in place. The box was then placed in a plastic bag, which was also heat sealed. This was in turn placed in an outer plastic bag, which was also heat sealed. All three specimen boxes were processed in the same manner and

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stored in the same freezer chest as the acetone bottles. The freezer and outer plastic bag's heat seals were checked once every week for acetone vapors.

Acetone was changed every third week. The outer plastic bags surrounding each specimen box were cut open at the heat seal. A funnel was inserted into the neck of an empty acetone bottle, a 5 cm hole was cut below the heat seal of the specimen bag and the waste acetone drained into the bottle. The specimen was then swiftly transferred to a new bag and placed in its box. To minimize acetone evaporation, the old acetone soaked specimen bag was immediately placed in a plastic freezer bag which was subsequently placed in a plastic trash bag whose neck was twisted tight after each acetone change. Next, a bottle of acetone precooled to -25°C was added to each specimen bag, which was then heat sealed as described above. Each specimen box was finally placed in double heat sealed bags as described above and stored in the freezer chest. In all, 4 changes of acetone were made on each of the 3 specimen boxes. More changes were deemed unnecessary, as a tissue volume to acetone ratio of 1:5 has been shown to be adequate for dehydration (Tiedemann and Ivic-Matijas, 1988).

The water content of the waste acetone could not be measured since such instrumentation was unavailable.

Results

A total of 12 acetone changes were made during the course of the experiment. No acetone vapors were detectable in the freezer. A 1 week period should give a leak adequate time to make itself noticeable. No acetone vapor leakage was detectable from the outer bags surrounding the polyethylene containers.

Pre-cooling acetone to -25°C, which is below acetone's flash point of -18°C (Budavari S, 1996), minimizes shrinkage of tissues (von Hagens, 1985). Pre-cooling thus also reduces acetone evaporation during acetone changes, which is important since the heat sealer uses electricity. During acetone changes, the lower evaporation rate of cold acetone was taken advantage of to minimize vapor formation by swiftly transferring the used specimen bags to a fresh plastic bag for disposal.

At each acetone change, the inside of each polyethylene box was checked for acetone leakage. In 2 cases acetone had leaked out of the specimen bag into the box. Careful inspection of the heat seals of the leaking bags showed that one had not sealed properly due to doubled up wrinkles at one point across the seal. Wrinkles crossing the line of sealing were carefully avoided thereafter. The other leaking specimen bag had a half-mm hole in one end of the seal where the plastic had melted excessively during sealing. However, the outer bags surrounding the polyethylene boxes had successfully prevented acetone vapors from escaping into the freezer, which was the principal reason for devising this method of freeze-substitution.

Unfortunately, the experiment could not be brought to its conclusion, ie- S-28 plastinated specimens, due to the closure of the anatomy department with cessation of all work with human specimens.

Conclusion

This method, if meticulously applied, should permit freeze substitution in any freezer without risking acetone vapor escape, fire or explosion. For larger specimens, custom sized bags could be made from heavy gauge polyethylene sheeting with an appropriate heat sealing apparatus designed for such purpose. However, an adequate draft cupboard for any specimen size and acetone quantity is a must when changing or otherwise working with acetone in the open.

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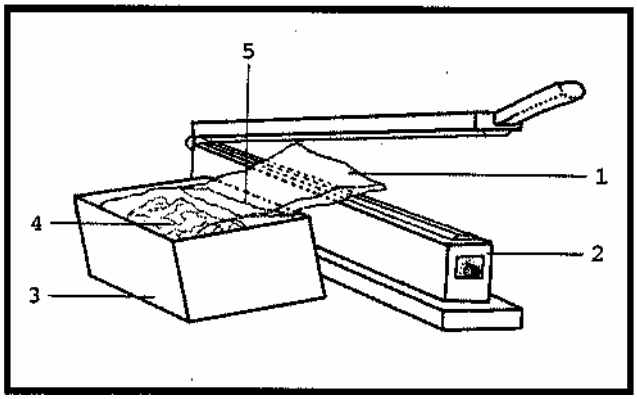

Figure 1. Heat-sealing of the specimen bag. 1 - specimen bag, 2 - heat sealer, 3 - polyethylene specimen box (note tilting of box away from heat sealer, preventing acetone spillage), 4 - specimen in bag, 5 - acetone level in bag. (figure by the author)

Submacroscopic Interpretation of Human Sectional Anatomy using Plastinated E12 Sections

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Key words: *Submacroscopy, Sectional Anatomy, Histology, Polymer E12*

Abstract

The E12 epoxy method of sheet plastination for preparing thin, transparent and serially sectioned cadaveric teaching specimens has been utilised primarily for studying sectional anatomy and correlation of MRI and CT radiographic images. The unique properties of the E12 process offer an exceptionally vivid survey of the human body in any given plane resulting in precise deliniation of the structural layout in situ. Maximum detail of sections is attained by large scale lipid extraction which enhances transparency.

Sections were prepared in sagittal, horizontal and coronal planes from different regions of the body including head and neck, trunk, inguinal region, limbs and joints. The macroscopic structures within these sections correspond precisely with images of the same structures obtained radiologically.

By introducing E12 sectional anatomy specimens to the anatomy teaching laboratory, the transition between gross anatomy and histology has been made possible by studying the one specimen. When utilised in our combined topographic anatomy and histology teaching laboratories, anatomical structures of thin and transparent slices can be magnified considerably. Standard histological slides providing detail of only a small area within predetermined parameters are often dictated by the physical limitations of the microscope slide itself. E12 sections provide a high degree of detail whilst retaining in situ structural integrity of the entire region in a complete and uninterrupted state.

One specimen provides students with significant gross detail down to the Submacroscopic level thus linking three disciplines, cross-sectional anatomy, radiology and histology. E12 plastinated sections have long been recognized as an ideal teaching aid in conjunction with radiological correlation, but it is in the microscopy laboratory that a valuable new dimension of this multi-disciplinary plastination technique has recently been realised.

Introduction

The E12 epoxy method of plastination provides a number of opportunities to study the morphology of the human body in various forms and at various levels while using the one plastination technique (von Hagens, 1985). The development of the E12 method of sheet plastination was primarily an aid to conceptualise the human body in anatomical planes. Providing the undergraduate medical students with anatomical sections to aid their understanding of modern diagnostic imaging procedures such as computed tomography (C.T), magnetic resonance imaging (M.R.I), positron emission tomography (P.E.T), ultrasound and even gamma knife radiosurgery is an essential component of the curriculum in the Department of Anatomy with Radiology at the University of Auckland.

The unique characteristics of the E12 plastination technique has led to new and exciting directions in the teaching of anatomy to both undergraduate medical students and clinicians preparing for final fellowship examinations. Introduction of E12 sections to the student microscopy teaching laboratory, aided by closed circuit television, provides a transition between histology, radiology and clinically based gross anatomy all from one specimen (Cook, 1997). Sections are able to be viewed in their entirety and then may be magni-

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fied to various levels to display morphological relationships between contiguous structures ensuring an accurate representation of the morphology without the need for interconnected tissues to be physically separated. At high magnification E12 specimens correlate well with routine histological slides viewed by each student using conventional light microscopes. Joints, nerves, vessels, individual muscle fibres, the vasculature within nerves and even the vasculature within bone is easily identifiable, providing the student with views that have not been possible by conventional means.

Materials and Methods

Two cadavers of medium body weight with no widespread metastases, one male and one female, were used. Though not a prerequisite to the E12 technique, embalming was carried out using one of the techniques established in our laboratory (Cook and Dawson, 1996). In both cases arterial perfusion using the common carotid and femoral arteries in conjunction with venous drainage from the jugular and femoral veins were performed. A pre-injection vascular conditioner comprising of metaflow and rectifiant (Dodge catalogue, 1990) was perfused into the carotid arteries of the cadavers prior to embalming. Upon leaving the pre-injection mixture within the closed circulatory system for up to 30 minutes, vascular obstacles such as blood clots are rendered diffusible and non-coagulating allowing a more even distribution of embalming fluid. Following pre-injection, anatomical embalming was performed. The fixative solution of cadaver one consisted of mold x (500ml), phenol (1000ml), formalin (1500ml), chromatech (1000ml), monopropylene glycol (3000ml), methylated spirits (6000ml) and water (5000ml). The fixative solution of cadaver two consisted of metaflow (4000ml), rectifiant (4000ml), metasyn accelerated (5000ml), mold x (2500ml), icterine (25ml) and water (3500ml). Following embalming the cadavers were stored under refrigeration for several months. In readiness for serial sectioning each cadaver was cut into regions; head and thorax, abdomen, pelvis and extremities. To facilitate sectioning, these regions were frozen to -80°C for 4 days to ensure maximum firmness of the tissue. The frozen regions were serially sectioned using an AEW 350 high speed meat cutting bandsaw (AEW Engineering, Norfolk, England) equipped with a 4 tooth per inch Shark band blade (Alston et al., 1997). The bandsaw guide stop was cooled to -30°C for 4 hours prior to the commencement of cutting to retard early thawing of the frozen tissue. The guide stop was set for 2.5 mm cutting depth (figure 1).

The male cadaver was sectioned in transverse planes. Care was taken to ensure consistently even sections of an overall thickness of 2.5 mm to 2.8 mm. The pelvis, abdomen and half thorax of the female cadaver were sectioned

Figure 1. A whole head specimen was frozen to -80°C to facilitate sawing into thin 2.5 mm slices using an industrial meat cutting bandsaw.

sagittally at 2.5 mm increments, while the head and remaining half thorax and shoulder were sectioned in coronal planes. Following sectioning, each slice was carefully cleaned, scraped free of tissue sawdust and laid flat on fibreglass screen mesh. The mesh supported sections were secured within special polypropylene grid baskets and immersed in the first of three acetone baths for dehydration by freeze substitution. Total dehydration time was 5 weeks. Purity of the final acetone bath remained at 99% for 3 days.

After complete dehydration of specimens, the final acetone bath was allowed to gradually warm up to ambient room temperature of 18°C to initiate degreasing. Yellowing of the acetone was an indication of lipid extraction from the sections thus requiring a change of solvent. The initial trial batch of four coronal head sections was degreased using only room temperature acetone as the choice of solvent. As transparency of the tissue is essential for obtaining the highest possible optical quality and differentiation of components within the finished sections, all subsequent specimens were then transferred from the yellow acetone to a pure bath of rnethylene chloride (dichloromethane) for a more thorough lipid extraction (Weber and Henry, 1993). Immersion in two baths of methylene chloride lasted for 8 weeks.

Due to the large number of sections to be processed, forced impregnation was carried out in batches of between 10 to 30 sections in the grid basket at any one time. Forced

impregnation was carried out according to standard principles with the reaction mixture consisting of 95 p.b.w. (parts by weight) of E12 epoxy polymer, 5 p.b.w. of AE30 glass separator, 20 p.b.w. of AE10 plasticiser and 26 p.b.w. of El hardener (von Hagens, 1989). To retard early gelation of the reactive polymer mixture, temperature within the forced impregnation chamber was maintained at between zero and 5°C with a thermostat control built into the impregnation chamber. The impregnation chamber was sited within a standard household chest freezer.

Unlike other plastination methods where careful control of vacuum speed is essential, forced impregnation for E12 was rapid with a relatively vigourous bubbling of the volatile intermedium, which in this case was methylene chloride. After 48 hours of impregnation, absolute pressure was less than 1 mm Hg and bubbling had dropped off considerably. Vacuum was then discontinued (Cook and Barnett, 1996).

In readiness for the casting and curing stage, toughened 5 mm thick glass plates were washed in a laboratory dishwasher at 95°C to remove previous deposits of hardened epoxy resin. A series of styrofoam (polystyrene) blocks were positioned with glass plates, silicone gasket and foldback clips. The impregnated sections were removed from the grid baskets and excess El2 polymer was briefly allowed to drain back into the reaction mixture (figure 2). Some impregnation reaction mixture was deemed suitable for re-use in casting and was filtered to extract tissue fragments and lumps of clotted blood contaminents before being added to fresh casting resin as a cost saving measure.

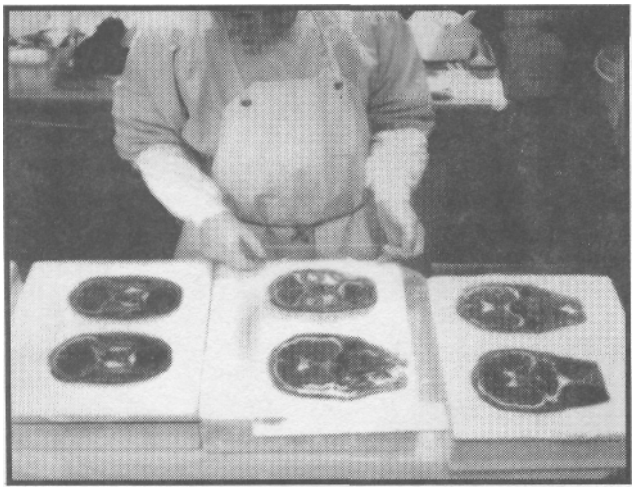


Figure 2. El2 impregnated coronal sections of the head are laid on sheets of glass in readiness for the casting and curing stage of the process.

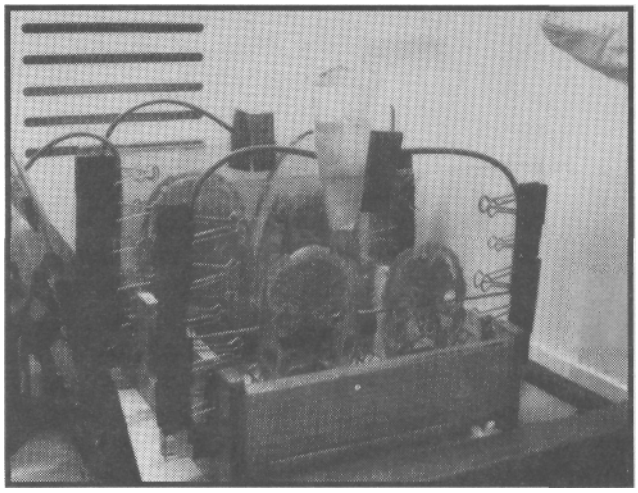


Figure 3. Processed sections are positioned between sheets of glass forming a flat chamber. A special rubber gasket provides sufficient clearence between the section and each glass plate allowing resin to be poured around the slices.

Casting was carried out by 2 methods. Sections that were structurally sound enough to withstand the flat chamber assembly and filling stage, for example the head, pelvis and extremity sections were cast according to the flat chamber technique between two sheets of glass separated by a gasket of slightly greater thickness than the tissue slice to permit equal volume of resin to surround and stabilize the section on both surface areas (figure 3). For our purposes, the distance between the two sheets of glass was 8 mm, thus providing at least 2 mm coverage on either surface of the section. For the sections which could not structurally withstand the vertical orientation of the flat chamber casting process, such as abdominal slices where loops of bowel and projections of organs no longer attached to mesentary, fat or body wall were more than likely to collapse under their own weight, it was imperative to use the draining method (von Hagens, 1987). Each section was carefully sandwiched horizontally between two sheets of hostaphan foil.

Assembled flat chambers containing sections were filled with filtered, used impregnation resin mixed with fresh E12/ El resin.The ratio of used resin mixed with fresh resin was usually between 10% and 50%. Chambers were thoroughly checked for leaks and placed under vacuum for 45 minutes to extract air bubbles which became trapped under structures during the casting stage. Filled and deaerated flat chambers were then placed at an oblique angle and allowed to pre-cure for 24 to 48 hours. Following pre-cure (gelation), the flat chambers were placed in a laboratory heat cabinet at 45°C for 5 days.

Sections set up according to the draining method required removal of air bubbles by rolling and pressing bubbles to the edge of the foil or by extraction with a syringe and large bore spinal needle. These sections were kept flat and of even thickness with weighted sheets of glass and allowed to slowly gelate and cure at room temperature for 24 to 48 hours. Once cured, they were re-cast using flat chambers after removal of the foil.

Results

Average tissue loss between sections from sawing was less than 1.0 mm. The total number of cut sections selected for subsequent processing amounted to some 175 sections. The finished E12 sections are semi-transparent, easy to orientate, correlate superbly with radiographs and above all else offer more detail right down to the submacroscopic level than is possible with any other plastination method or gross dissection in the anatomy examination laboratory. Successful results with the E12 technique are wholly dependent on specific parameters of section thickness and lipid extraction.

Comparisons between degreasing using only acetone, and degreasing using acetone followed by methylene chloride showed a marked difference in extent of detail and general appearance of the sections. In the case of methylene chloride adipose tissue assumed a high level of translucency with a notably clear appearance while degreasing with only acetone tended to give a pronounced yellowing to the tissue and inadequate clarity to the extent we require. Monitoring of E12 sections prepared using only acetone as both dehydration and degreasing mediums has tended to show a definite yellowing, or fading of both the tissue and the actual E12 resin itself over a prolonged time frame of several years. Alternatively, E12 processing using acetone only as the dehydration medium followed by methylene chloride as the degreasing medium has produced markedly better results which to date have not demonstrated any changes within the actual resin or the tissue section itself.

Examination of the head

Close examination of a coronal section through the frontal portion of the head (figure 4) offers a clear overview of the structures of the deep facial area. When magnified, (figure 5) the right eye from the specimen seen in figure 4 reveals a considerable amount of detail due to the plane of sectioning, thus preserving major components of the eye in an undisturbed state.

Close examination of the nasal cavity reveals the extensive vascularity of the conchae, which may not be readily observed in the dissecting room or histological specimen (figure 6). With an extraordinary degree of structural detail, E12 has distinct advantages over gross anatomy, radiology and histology in clarity of anatomical components.

The structure of the optic nerve, its meningeal covering and blood supply can be observed without any special preparation. The tongue, when seen in coronal section, provides a level of detail not readily seen using traditional dissection methods. The pattern of muscle fibres within the substance of the tongue clearly shows the different groups with a vividly defined intermingling of extrinsic and intrinsic muscles as well as the branches of the hypoglossal nerve and lingual vessels. Within the same E12 section, detailed components of the roof of the oral cavity (mucosal lining, palatine glands, hard palate and floor of the nasal cavity) may be clearly seen. Although not expressly intended for

Figure 4. The first in a series of 25 serial coronal sections of the head. Visible are the eye (E), tongue (T), nasal cavity (NC), hard palate (HP) and mandible (M).

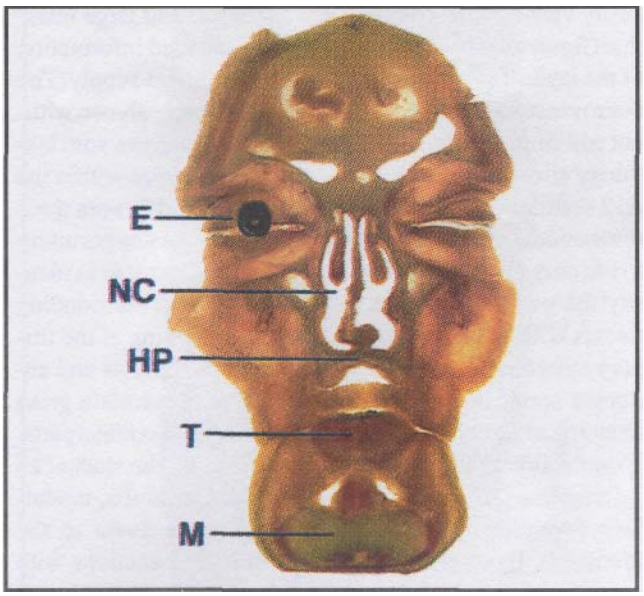
Figure 5. The right eye, magnified from figure 4 reveals considerable information due to the plane of sectioning, thus preserving the organ in situ. Visible are the pupil (P), iris (I), ciliary processes (CP), the sclero-corneal layer (SC) and orbicularis oculi (OM) muscle.

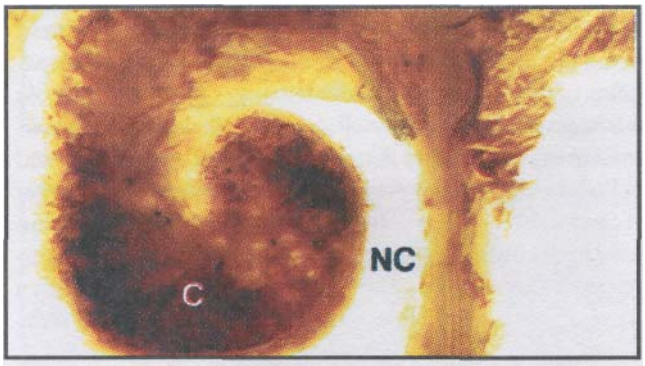
Figure 6. The nasal cavity (NC) when examined in coronal section reveals the extensive vascularity of the concha (C).

Figure 7. Transverse section through the thorax showing the layers of the body wall, lungs (L), heart (H), aorta (A), thoracic vertebra (V) and pectoralis major muscle (PM).

Figure 8. Coronal section of the shoulder region and thoracic wall showing the rotator cuff (RC) fusing with the capsule of the shoulder joint, ribs (R), intercostal muscles (I), lung (L), deltoid muscle (D), scapula (S) and head of the humerus (H).

Figure 9. A close view of the thoracic wall magnified from figure 8 revealing rib (R), lung (L) and the arrangement of the external (EX), internal (IN) and innermost (IM) intercostal muscles. The superficial lymphatics of the lung are delineated with carbon deposits (CD) evident, just deep to the visceral pleura.





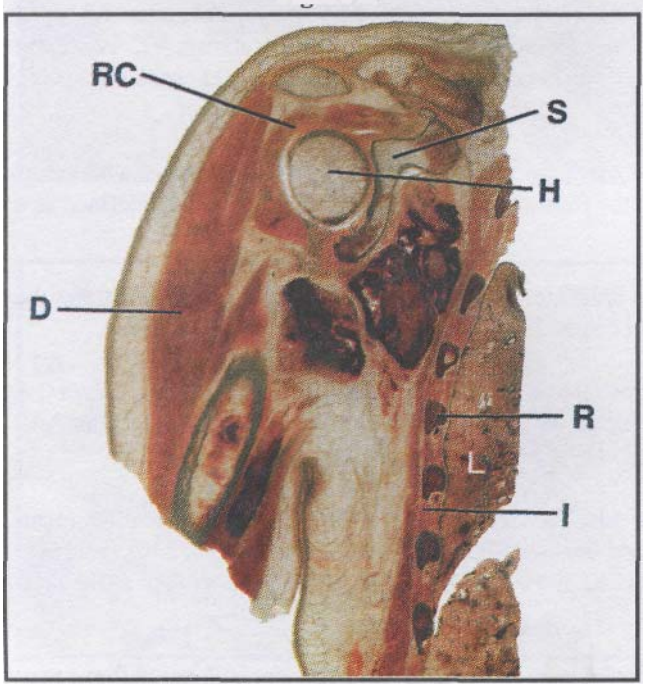


Figure 4.

Figure 6.

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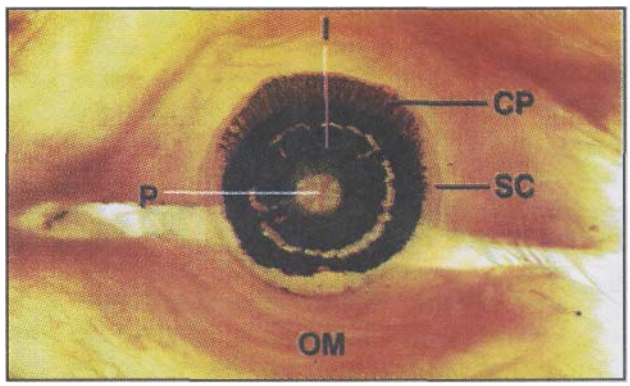
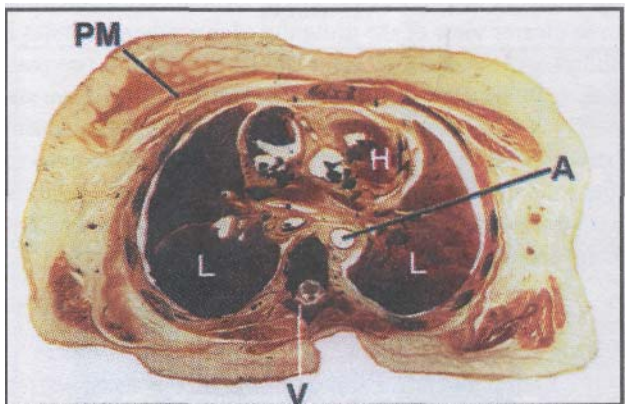




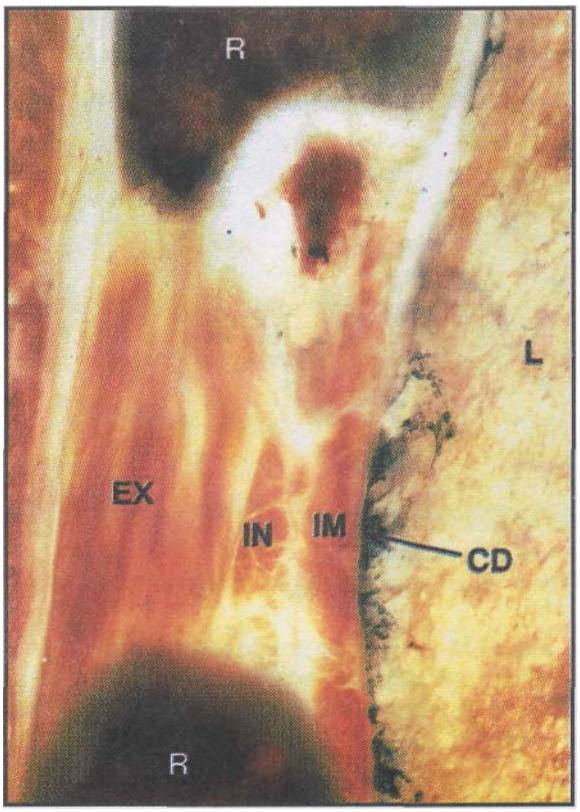


Figure 5.

Figure 7.

Figure 9.

plastination of the brain, the E12 method of sheet plastination provides a clear demarcation of the grey matter from the white matter. The column like arrangement of the actual fibre system of the cerebral cortex is not easily seen under routine light microscopy but may be observed well within E12 sections.

Examination of the thorax

A full E12 transverse section through the thorax (figure 7) allows orientation with various layers of the body wall, the vertebral canal, heart, lungs and great vessels. When examining the same area by way of a coronal section through the shoulder and thorax (figure 8) students are afforded an even clearer view of the grouping of intercostal muscles in addition to the shoulder joint and its relations. When magnified, students are able to truly appreciate the relative size of the wedge shaped external intercostal muscle in comparison with the significantly smaller mass of the internal and innermost intercostal muscles. This section (figure 9) also shows the superficial lymphatics of the lung with carbon deposits visible just deep to the visceral pleura.

Examination of the abdomen and pelvis

A multitude of anatomical information is obtained from the full sagittal section through the female pelvis and abdomen (figure 10). The vertebral canal and its contents, the stomach, transverse colon, duodenum, jejunum, various loops of ileum, rectum, vagina, uterus and bladder are all easily viewed. Close up study of the small and large intestine (figures 11,12,13) provides highly detailed information of the layers of the wall and pattern of its blood supply. The microvascular submucosal plexuses are clearly shown without additional Colour enhancement. In comparison with histology slides, the relative thickness of the tissue within the E12 sections allows structures to retain a readily seen three dimensional aspect. This allows the student an opportunity to not only identify individual components, but also to identify the pathways of structures in relation to surrounding tissues with great accuracy (figure 11). Looking at the urinary system as seen in a sagittally sectioned pelvis and abdomen opens up excellent opportunities to correlate gross anatomical structures with their histological counterparts. Examination of the kidney for example gives the student an informative profile of the capsule, cortex, medulla, medullary pyramids and renal microvasculature down to the glomeruli. Examination of the ureter in E12 sections with magnification reveals the characteristic folding of the epithelium of the ureter which is identical to histology slides as seen in the microscopy laboratory with the added benefit of not having been removed from the abdomen. E12 preparation of the urinary bladder demonstrates not only the whole thickness of the bladder wall, but the interlacing pattern of smooth muscle bundles that run longitudily, transversely and obliquely. To fully appreciate the fine line between gross anatomy and histology, we are able to clearly identify and compare the actual mucosal glands within the epithelial lining of the sigmoid colon in an E12 sectioned pelvis with a similar histological slide of the same organ with remark-

Figure 10. Median sagittal section through the female pelvis and abdomen demonstrating the gut, its relations and blood supply. The female internal genitalia and its relations with the urinary bladder and rectum are also clearly shown. VC - vertebral column, L - liver, S - stomach, LI - large intestine, SI - small intestine, B - urinary bladder, U - uterus, AC - anal canal, PB - pubic bone.

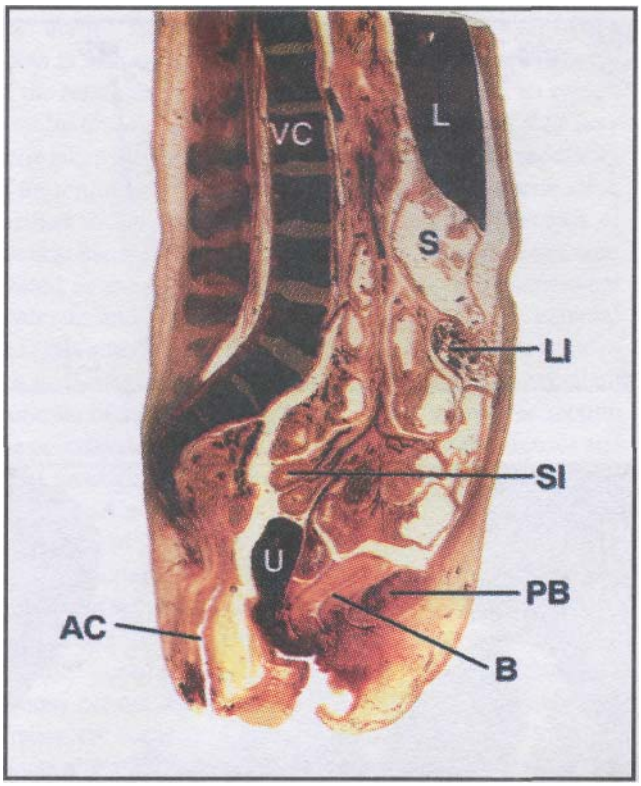
Figure 11. In this loop of ileum enlarged from figure 10, we can examine the external blood supply, in the form of a branch of mesenteric vessel (MV). The internal distribution of blood vessels in the form of a submucosal plexus (SP) is well defined as a distinct ring within the ileum itself. MW - muscular wall.

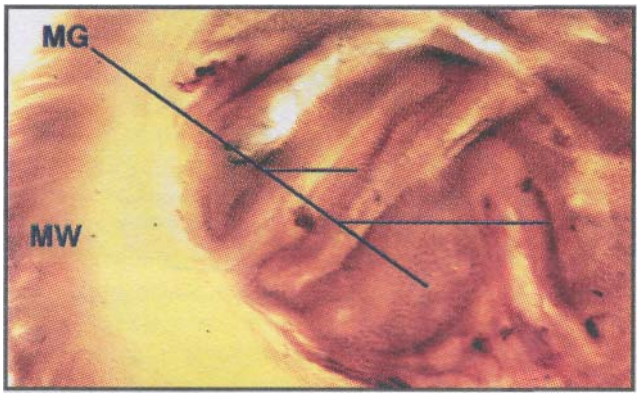
Figure 12. The fine line between gross anatomy and histology is illustrated in this example which clearly demonstrates the actual mucosal glands (MG) within the epithelial lining of the sigmoid colon. The muscular wall (MW) is also observed.

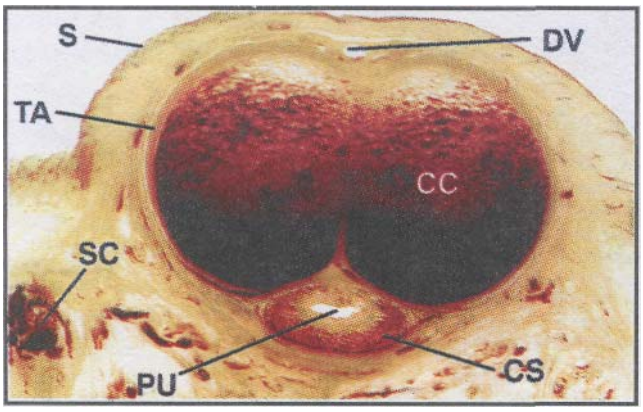
Figure 13. The complete structure of the anal canal may now be realized with the E12 process. In this close view, lumen (L), mucosa (M), submucosa (SM) rich in vasculature, internal anal sphincter (IAS) (smooth muscle fibres) and the external anal sphincter (EAS) (skeletal muscle fibres) are observed.

Figure 14. A complete profile of the external male genitalia is provided with the El2 plastination technique. The constituents of the penis including the various fascial layers between the skin (S) and the tunica albuginia (TA) are presented. Also visible are the corpora cavernosa (CC), penile urethra (PU), corpus spongiosum (CS), the dorsal vein (DV) of the penis as well as the spermatic cord (SC).

Figure 15. Sacroiliac joint showing articular cartilage (AC), synovial joint cavity (JC) and hematopoietic tissue (bone marrow) of the sacrum (S) and the ilium (I).







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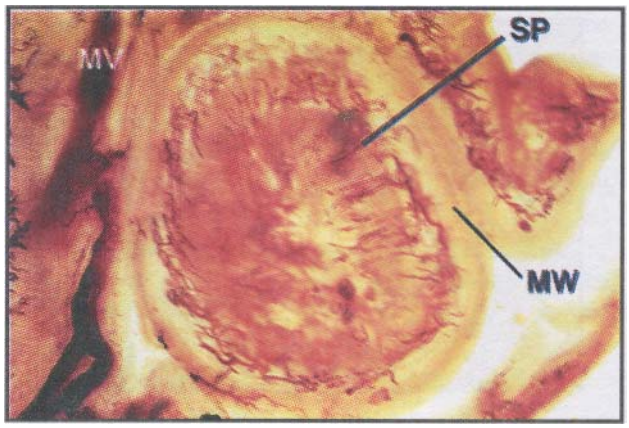

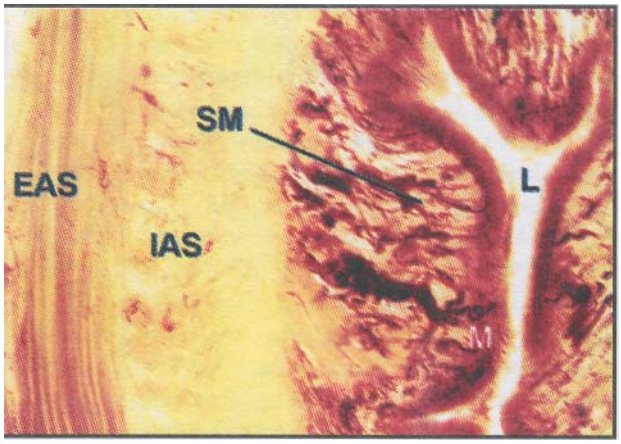

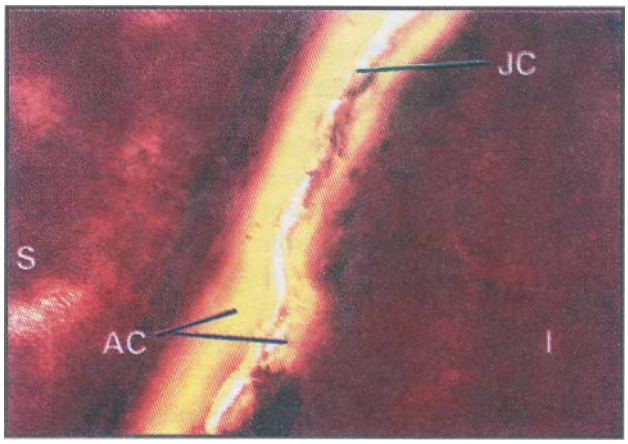

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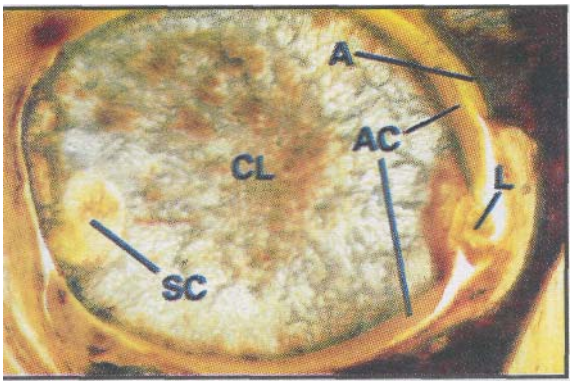

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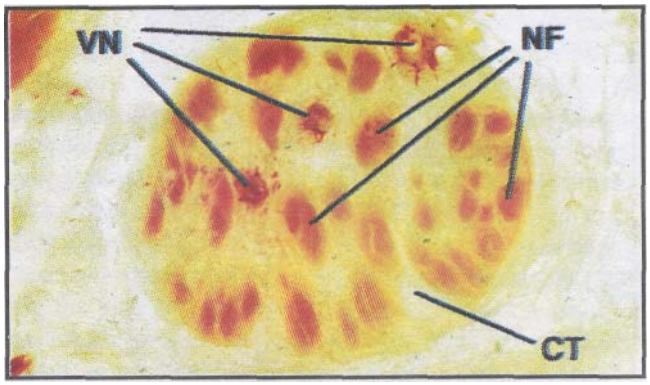

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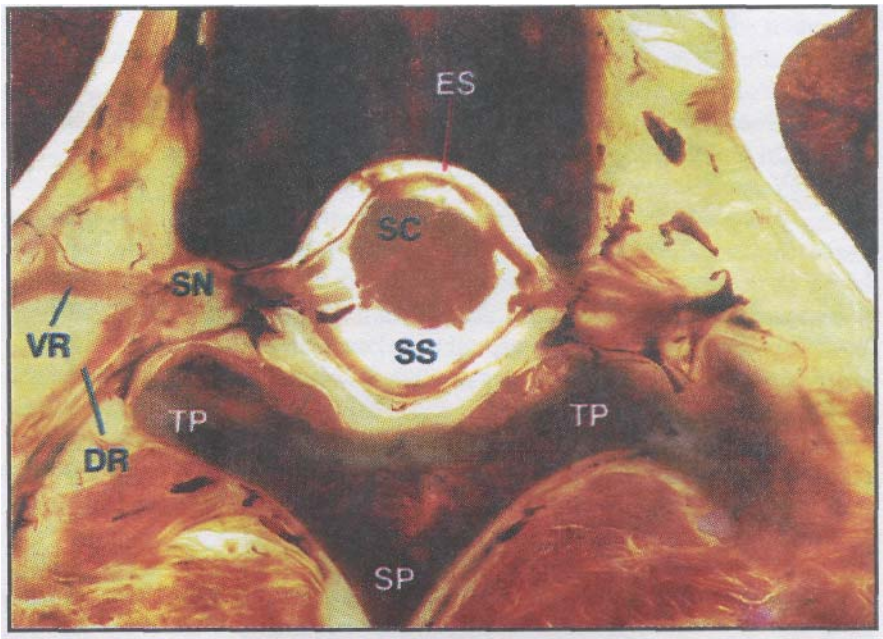

Figure 18.

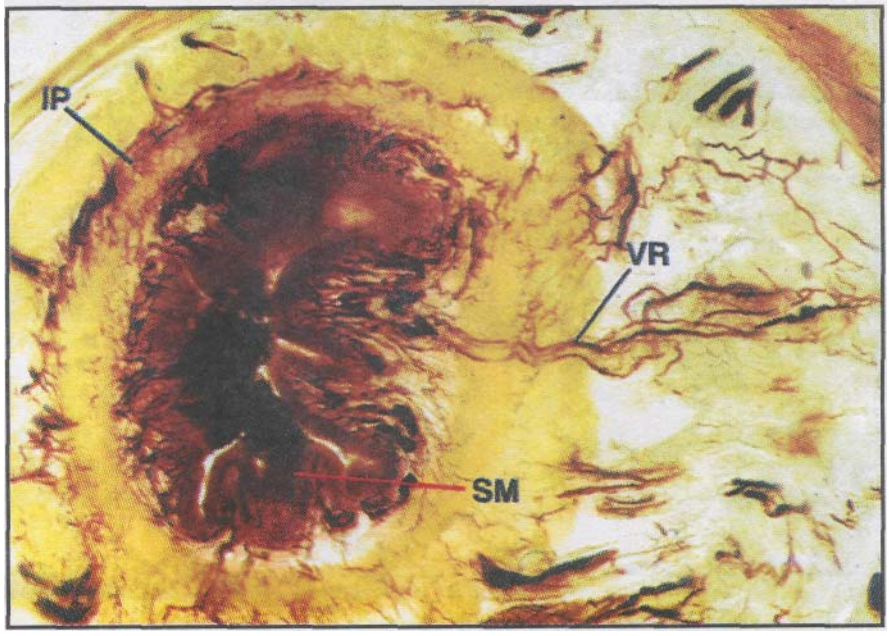

Figure 19.

able clarity. Additionally, the smooth muscle wall of the colon is also well presented (figure 12). No better concept of the relationships of tissues within the ano-rectal region may be realized than in the series of transverse E12 sections taken through the male pelvis. The intense vascularity of the region becomes readily apparent with this plastination method such as the mucosal and submucosal plexuses of the anal canal. The differing muscle types are no better contrasted as the smooth muscle making up the internal anal sphincter and the skeletal muscle making up the external anal sphincter (figure 13). The appearence of the prostate in the sections produced in the E12 technique is an excellent example of submacroscopy. In standard transverse section the prostatic urethra, the urethral crest, prostatic sinuses and even prostatic concretions, in the form of small spherical granules of glycoprotein in the prostatic glandular tissue, are observed. Posterior to the bladder and the prostate an excellent internal profile of the seminal vesicles and the ampulla of the ductus deferens in situ are seen. Further study of the male reproductive organs are well presented by the spermatic cord in which we may identify the pampiniform venous plexus, testicular vessels and the ductus deferens. Transverse sections of the perineum at the level of the male external genitalia demonstrates most accurately the structure of the penis. The various fascial layers between the skin and the tunica albuginia, corpora cavernosa, penile urethra, corpus spongiosum and the dorsal vein of the penis are illustrated (figure 14).

Examination of the musculoskeletal system

Transverse sections through the pelvis, at the level of the hip joint offer a considerable degree of detail with numerous anatomical landmarks such as the gluteal muscles, the ischium, sciatic nerve and even examples of differing bone consistency, spongy and compact, within two examples of the femur. Bone diseases, for example osteosarcoma of the tibia, and its effects on surrounding tissues can be readily examined. Joints present superbly, with certain joints gaining a level of visibility not possible without the E12 method. By magnifying an area of transversely sectioned pelvis, students may gain an insight into the synovial cavity of the sacro-iliac joint whereby hematopoietic tissue (bone marrow), articular cartilage, joint cavity and internal features of the adjacent bone become clearly visible (figure 15). On subsequent transverse sections, observations of the hip joint at the level of the mid head of the femur are unique in as much as they clearly demonstrate not only the joint cavity, ligament of the head of the femur, acetabulum and articular cartilage, but also the blood supply to the head of the femur by way of the foveolar artery situated within the ligament of the head of the femur. In addition to normal anatomy, a lesion or cyst present in the bone can be readily seen (figure 16).

Examination of the spinal cord and nerves

Nervous tissue is well represented in cross sections and compares precisely with histological slides, yet retaining the complete, uninterrupted attributes which are the foundation of the E12 method. A close view of the sciatic nerve as seen in the full transverse section shows the extensive amount of connective tissue separating the individual nerve fascicles (figure 17). Additionally the vasa nervorum, the vasculature within the nerve, can also be identified as easily as in histological form. When magnified under the television system in the teaching laboratory, the complete profile of the spinal cord and vertebral canal is readily accessible. Fine details of the cross section of the cord, including the meningeal coverings, spinal nerve roots and rami are all visible in one section (figure 18).

Discussion

Since its inception some eighteen years ago, plastination has grown and found its place among many mostly science based institutions around the world. The four major methods of plastination (S10, P.E.M, P35 and E12) collectively provide a cost effective means for preserving and presenting perishable biological specimens with each of these primary techniques having either generalized applications, or limited specific applications (von Hagens et al., 1987). The amount of anatomical information which can be attained is highly variable according to the characteristics of the indi-

Figure 16. Transverse section of the hip joint demonstrates the acetabulum (A), articular cartilage (AC), ligament of the head (L), cancellous bone lamellae of the head (CL) and in this case a subchondral cyst (SC) laterally.

Figure 17. The sciatic nerve, as part of a transverse pelvic section, reveals the individual nerve fascicles (NF) separated by extensive connective tissue (CT) and the blood supply, vasa nervorum (VN) within the nerve itself.

Figure 18. In this close view of figure 7 we can see the transverse processes (TP), spinous process (SP), epidural space (ES), subarachnoid space (SS), the spinal cord (SC), ventral ramus (VR) and dorsal ramus (DR) traceable to the spinal nerve (SN).

Figure 19. The arterial vasa rectae (VR) penetrating the wall of the large intestine. SM - submucosal vascular plexus, IP - intermuscular vascular plexus.

vidual class of polymer and method of processing. The S10 technique is an all purpose means for preserving whole or dissected tissues while retaining their natural shape, contours and to some extent colour (Henry and Nel, 1993). P.E.M specimens typically, though not exclusively, are cross sectional specimens of considerable thickness and offer a better degree of detail of cut surfaces than is possible with the S10 method (Lischka and Prihoda, 1987). P35 is only suitable for preparing relatively thin brain slices using the sheet plastination method. Detail in P35 specimens is extremely precise and sectioned neuroanatomical structures are considerably better defined than is possible with either S10 or P.E.M though the use of this method is limited (Weiglein, 1996).

It has been reported that tissue sections prepared according to the E12 process may be made to more than 5 mm in thickness and processed with satisfactory results (Whalley, 1994). In our experience tissue sections of greater than 2.5 mm in thickness will exhibit a distinct loss in clarity with many structures superimposed and difficult to discern. With attention to obtaining and demonstrating very fine submacroscopic detail within our finished E12 specimens, it was critical that the uniformity of section thickness be maintained at no greater than 2.5 mm in thickness throughout the ongoing E12 program. Though most plastinators engaged in sheet plastination use a sliding cutting table on the bandsaw, we prefer to use a fixed table bandsaw for reasons of safety and hygiene whilst still achieving accurate sections during the sawing phase.

The removal of lipid content from already sawed body slices is an essential procedure of the success of the E12 technique. Acetone, as well as being the recommended medium for dehydration by freeze substitution, serves as an adequate degreasing solvent when subjected to room temperature or slightly higher. Although not mandatory, our experiences demonstrated that by far the highest optical quality of El2 sections were achieved from tissue sections that had been degreased in acetone at room temperature, then transferred to methylene chloride for more extensive lipid extraction.

The casting of abdominal slices by the draining method has proven to be vital to avoid dislodging of structures that would otherwise not survive the standard flat chamber process. Sections prepared according to this method had no supporting layer of polymer on either side. It was neccessary to re-cast them using the flat chamber method to ensure a uniform thickness of the finished specimens. The hostaphan foil was simply peeled away from the sections revealing a fully cured specimen thus eliminating any risk of collapse or damage to the delicate structures. The sections could now be re-cast to the desired thickness in between glass plates according to the flat chamber technique.

The E12 process, although only suitable for thin cross sectional specimens prepared according to the sheet plastination method, has in effect filled a void in undergraduate teaching. Students are provided with a clear, unimpeded overview of the planes of the body seen with a whole section. Specific regions are then partially magnified to relay a concept of the relationships of the surrounding tissues not present in histological slides. Finally, specific structures are highly magnified enabling submacroscopic observation of components of these structures. They closely correspond to histological features as seen by the student using traditional microscopic slides. By combining traditional, as well as modern teaching techniques, the student is provided with an altogether clearer concept and relevance of microanatomy, clinically based gross anatomy and radiology.

With experience with virtually every plastination method at the University of Auckland, the E12 technique has proven itself to be by far the most beneficial to the student in terms of providing a firm link between macroscopic and microscopic anatomy. Student appreciation and understanding of microscopic anatomy has been enhanced by the easily accessible and above all else, in situ presentation of relevant anatomical information which is able to be attained from an individual E12 section.

It is our aim to further develop the E12 process to include even thinner sections down to 1 mm or less, of specific organs and pathological anomalies. Investigation of injuries, diseases or primary lesions on target organs and the effects on other organs and structures such as bones, joints, vessels and nerves further removed from the site of the lesion, at both gross and submacroscopic levels is possible. Anatomic pathology can be extended to submacroscopic histopathology from a single section where we can study tumours of the paranasal sinuses and their effects on the surrounding region. Various diseases of the gut, for example the formation of the colonic diverticular diseases, could be substantiated through E12 submacroscopy. The arterial vasa rectae penetrate the wall of the gut resulting in muscular weakness, thus leading to a possible diverticular herniation of the colonic mucosa and sub mucosa (Cotran et al., 1994) (figure 19). Injuries to the vertebral column which could include vertebral disc prolapse and the effects on nerve roots and the spinal cord are good examples of the types of pathology that may be studied in these sections. By employing whole cross sections from all regions of the human body, students now are able to appreciate and better understand anatomical features, both generalised and specific (figure 20). With a ready supply of bequeathed cadavers and access to their relevant clinical information, we are endeavouring to take 'submacroscopic'

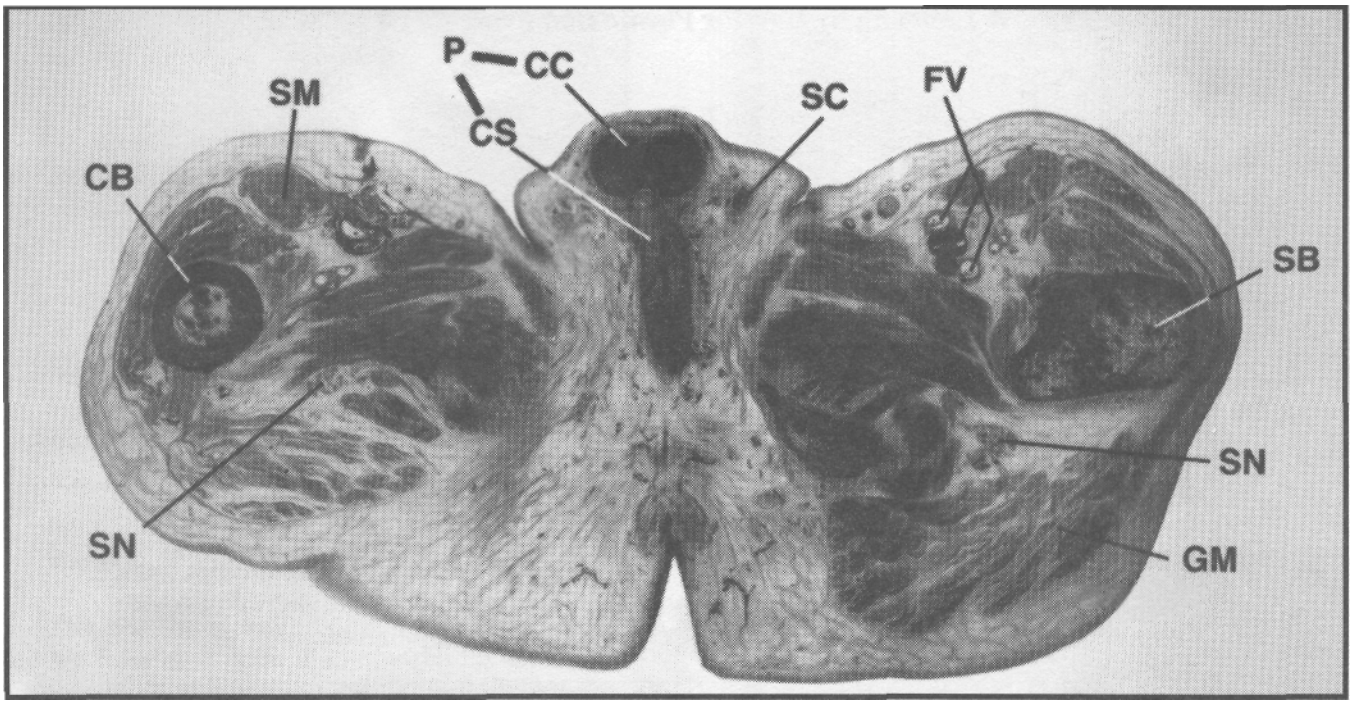

Figure 20. The male perineum and gluteal region seen in marginally oblique transverse section showing spongy (SB) and compact (CB) bony landmarks of the femur. Also visible are the corpus cavernosum (CC) and the corpus spongiosum (CS) of the penis (P), the spermatic cords (SC), gluteal muscles (GM), the sciatic nerves (SN), femoral vessels (FV) and sartorius muscle (SM).

study to exciting new levels for both teaching and research opportunities.

Acknowledgments

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A Labeling System for Plastinated Prosections

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Key Words: *Equipment, Specimen Labelling*

Abstract

The aim of this study was to devise a practical and inexpensive labelling system for plastinated prosected specimens. Letters printed in various colours are punched in either a round or a pointed shape by means of a specially constructed punch and subsequently glued onto the specimen. The final product is clearly labelled and valuable for purposes of learning or examination.

Introduction

Development of Technology is always followed by a vast number of challenges. Plastination of prosected material has left the technician with the challenge of how to identify or label the different structures (Jackson, 1987). The aim of this study was to develop a labeling system for prosected plastinated specimens.

Materials and Methods

Alphabet letters are printed on 0,5mm thick Poly vinyl chloride (PVC) sheating. This is done by using a hot lead block containing an alphabet. Pigment stamping foil is used to print the letters onto the PVC (Brune, 1987). The PVC is cut to a size of 150mm x 70mm to make handling easier. The letters are spaced 5mm apart and are 3mm high from top to bottom.

A special punch was made as can be seen in figure 1. The letters are visible through the top die plate (figure 2) of the apparatus and the letter may be centralized before punching. The punching is an easy process. The punch moves from below and pushes the letter through the die plate to the top (figure 3). The round punch used here has a diameter of 4mm. To produce arrows a pointed punch was made. These pointed markers use the same alphabet and are used to indicate small structures without obscuring them (figure 4).

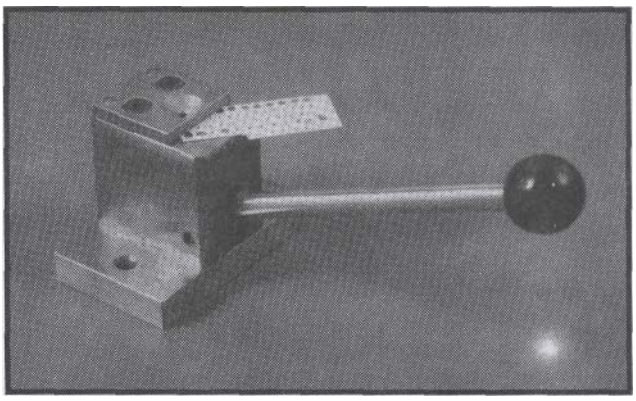

Figure 1. The punching apparatus with the die plate on top for easy centralizing of the letters.

The lettered discs are secured onto the prosection using household clear silicone. A thin layer of silicone coates the top surface of the disc as well. Because some specimens are large with more structures, more than one alphabet may be required and the solution to this problem was to print alphabet-letters of various colours, using different coloured pigment stamping foil on the white PVC.

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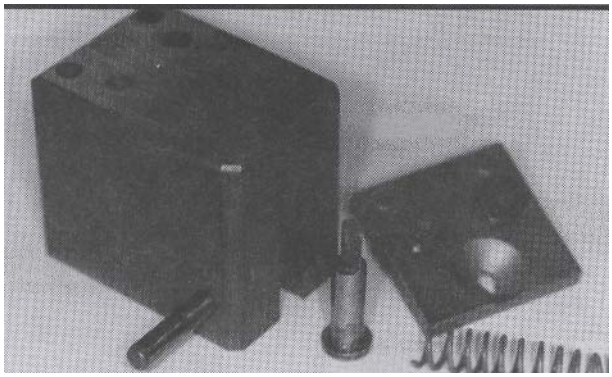

Figure 2. The top die plate (right hand side) and the punch (centre of photo) for pointed markers.

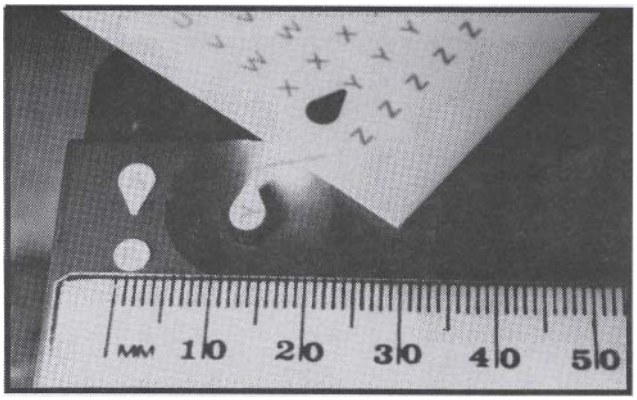

Figure 3. The punch moves from below and pushes the letter through the die plate to the top.

Results

This labelling system was found to be very practical and we even used these discs (labels) on the bottled prosections (wet specimens). Cyanoacrylate glue was used in the case of bottled specimens to attach the labels onto the prosection. The letters are easily identifiable and, due to the small dimension of the disc, it seldom obscures structures. The glue is of lasting quality and very few cases of re-application have occured.

Conclusion

Because only letters and arrows are used on the prosected specimens, without names, these specimens may also be used for spot tests. Next to the specimen in the study centre (museum) is the legend enabling the student to identify the structure he is looking at (figures 5 and 6). Finally, a specialised engineering shop will be able to manufacture these punches and apparatus at a very low cost, making this identification method very cheap.

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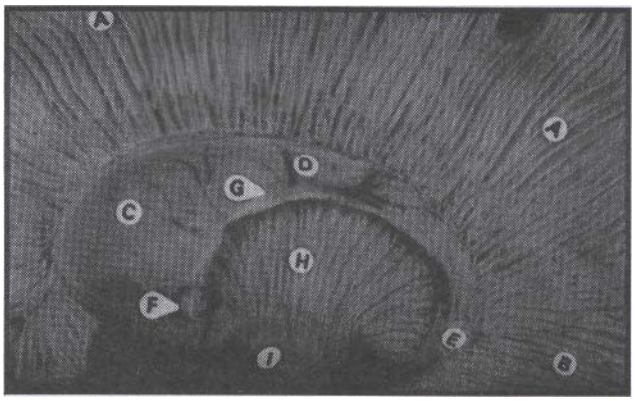

Figure 4. Both markers use the same alphabet. The pointed markers are mounted next to the small structures without obscuring them.

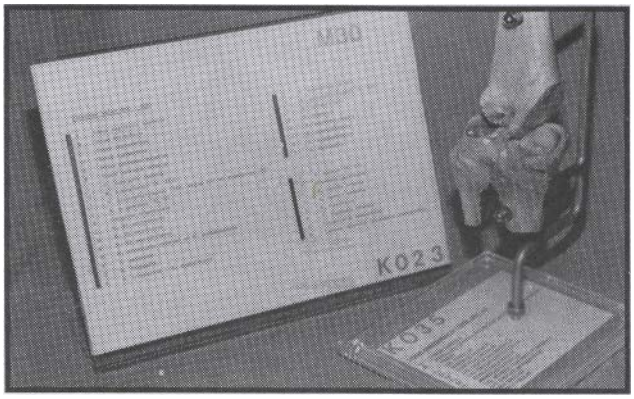

Figure 5. The legend can be taped to the transparent base. Alternatively, the legend is mounted in a perspex stand.

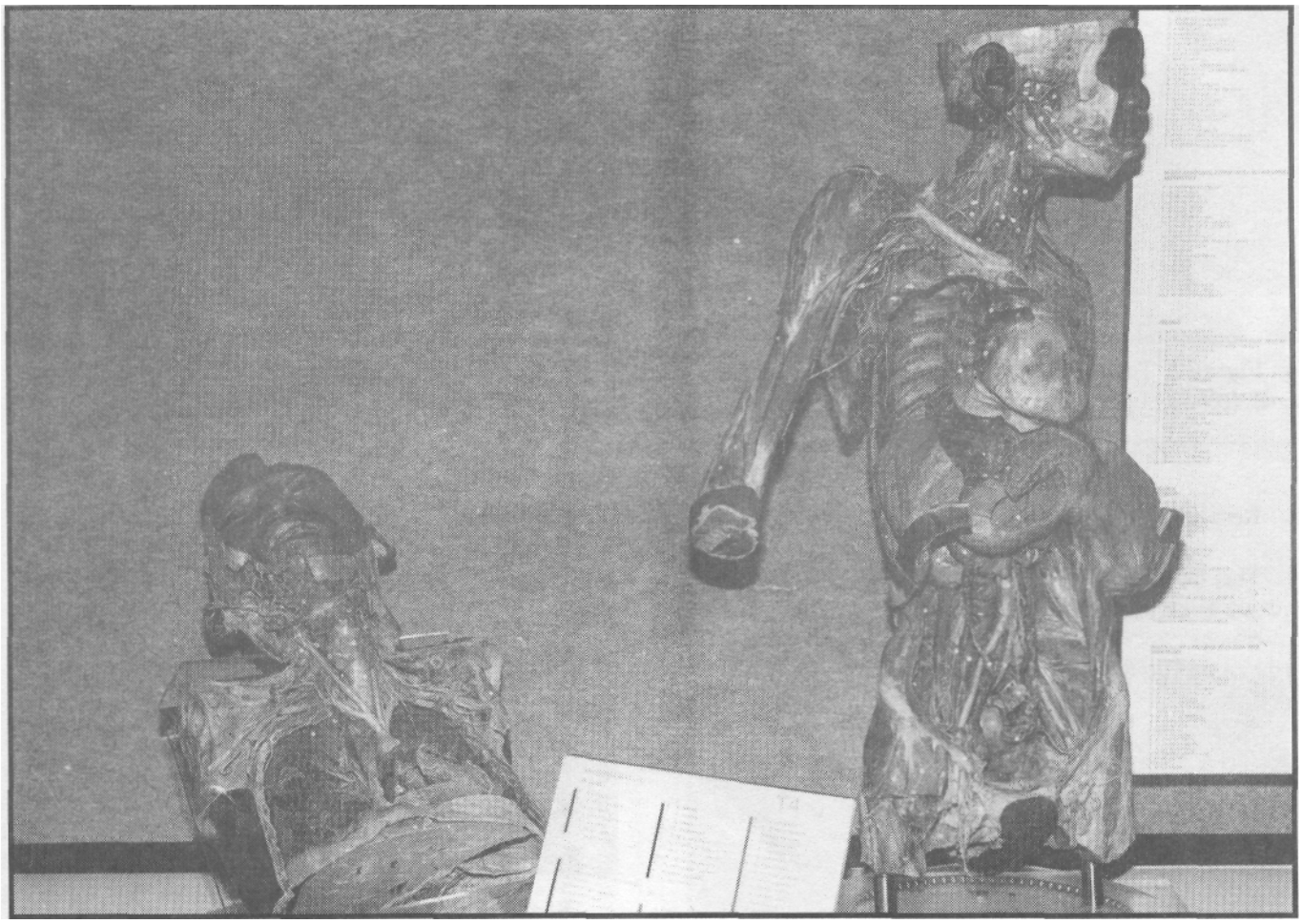

Figure 6. Two large prosections with their legends as used in the study centre.

The S10 Process as a Solution for the Preservation of Cartilage in the Thoracic Skeleton

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Historically, serious difficulties were experienced in respect of the preservation of the articulation of the thoracic skeleton, and in particular, the cartilage of the thorax. Attempts at replacing the cartilage with either rubber plastic or wire and putty produce somewhat inferior imitations.

Several anatomy departments in South African Medical schools and individuals made different attempts at solving this problem, which were acceptable until plastination appeared on the scene. Most of these applications were intended to replace the cartilage. Attempts at our University entailed the use of a section consisting of sternum, inclusive of the cartilage, which was covered in plaster of Paris (plus or minus 1cm thickness) and left to dry for a period of 3 months. After removing of the plaster of Paris by tapping the cast with a lightweight object, the thoracic skeleton was then scraped clean and varnished before again being attached to the ribcage. The plaster prevented changing of the shape while drying.

Although slightly shrunken this method produced fairly accurate dimensions of the actual structure (figure 1). The only problem being the brittle nature thereof, being at risk of easy breakage if not handled with care.

However, since the introduction of plastination, the following technique is being practiced with great success. The sternum, with cartilage, is removed from a previously embalmed cadaver and plastinated according to the standard S10 technique (von Hagens, 1985). Dehydration is performed in cold acetone for 1 month and impregnation for 3 weeks. Care should be taken to secure the structure's dimensions with wire as the cartilage has a tendancy to change shape.

To great advantage is the fact that it does not produce a bulky mass and has small dimensions. Therefore it dehydrates and impregnates very well and it is a relatively cheap procedure, particularly due to the small mass and dimensions. Cleaning and scraping is done after fast curing. This structure is then attached to the ribs with fishing line.

The final product (figure 2) is exceptionally flexible, and more resistant to breakage. The dimensions are very similar to the actual structure and give the students much better insight into the human anatomy.

Unlike the original or versions previously mentioned which require continuous maintenance, the plastinated specimen can be expected to be maintenance free.

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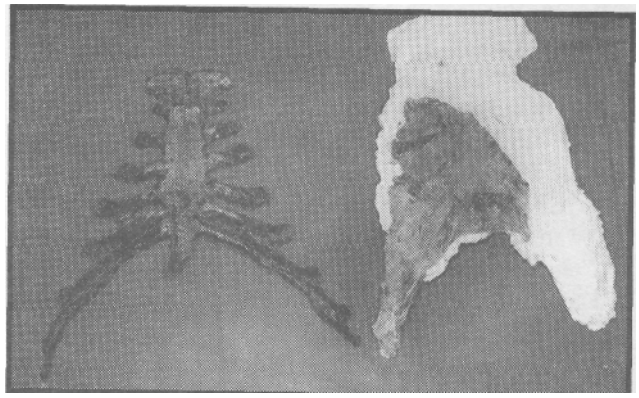

Figure 1. Dried cartilage and plaster of Paris shell

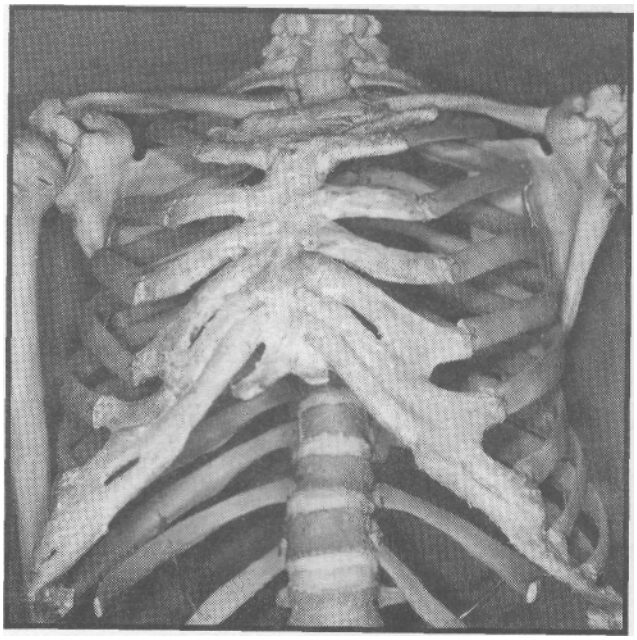

Figure 2. Plastinated cartilage

Current Plastination Index - Updating

Gilles Grondin and Régis Olry

You will find in the following updating of the Current Plastination Index the complete list of the papers and textbooks that were brought to our attention since the last updating. It includes the papers published in the last issue of Acta Anatomica (Vol. 158, number 1) dedicated to plastination. We have also received many Japanese references from Akiyoshi Matsumura, Kaoru Motomiya and Yumi Sakamoto. We wish to thank them particularily for their precious collaboration. We also added the address of the Web page of the museum of the Kawasaki Medical School. This page shows pictures of plastinated specimens and describes the plastination process, using the KE 108 silicone polymer. An English version of this page should be available soon.

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THESIS REVIEW

An Educational Comparison of Thin Cadaveric Sections and Magnetic Resonance Images

Author: M. Magiros, Department of Anatomy and Histology, The University of Sydney, Australia, 1996.

Our Australian colleague Marisa Magiros submitted in November 1996 a thesis entitled "An Educational Comparison of Thin Plastinated Cadaveric Sections and Magnetic Resonance Images" (Bachelor of Medical Science, University of Sydney). We were delighted to receive a copy of her well illustrated thesis, which deserves a review in our journal.

The aims of the study are:

1. To determine which plastination technique (PEM 11, PEM 27 or E 12) will produce the most suitable specimens for student testing,
2. To show that thin plastinated cadaveric sections can be accurately correlated with Magnetic Resonance (MR) scans,
3. To determine whether cross-sectional anatomy can be learnt more effectively from a thin plastinated cadaveric section, from an MR scan or from simultaneously using a corresponding MRI and plastinated section, and
4. To evaluate students' preferences for learning anatomy.

The first chapter (pp. 1-25) summarizes the early preservation techniques, the four steps of plastination, the characteristics of the polymers used in this study (PEM 11, PEM 27, and E 12), the physics of MRI, and the main trends in anatomical pedagogics.

The second chapter (pp. 26-62) describes in detail the materials and methods: scanning of heads, preparation and plastination of specimens (only the PEM 11 and E 12 techniques were used), and the organization and statistical analysis of student testing (Test A: Dentistry 2 students, n = 47; TestB:n=15).

The third chapter (pp. 63-72) correlates plastinated sections and MRIs.

The fourth chapter (pp. 73-120) gives a detailed account of the student testing. The statistical analysis is especially rigorous and will serve as a model for all future researches on the place of plastination in anatomical pedagogics. The results of the tests are of outstanding importance: they show the benefit gained by using plastinated sections and MRIs simultaneously, and the type of plastinated sections the students prefer.

One of the most important points of the conclusion (pp. 121-123) is the place of PEM 11 plastinated sections in anatomical and radiological training. The bibliography includes 96 references, and the last fourteen pages are appendices.

To my knowledge, Ms. Magiros is the third student to choose plastination as the subject of a thesis. Her two predecessors were Mr. Poncot (France, 1993) and Ms. De La Cruz Baltazar (Canada, 1996). A fourth thesis on plastination is about to be submitted in France by Mr. Durand, probably in November 1997 (plastination in otorhinolaryngology). This trend confirms the importance of plastination in current morphological sciences and its breakthrough in radiological and surgical fields.

In conclusion, Ms. Magiros's thesis is to date the best one ever written. I firmly encourage the author to submit a summary of the fourth chapter of her thesis to our journal, so that every one may take advantage of her results and her exemplary methodology.

R6gis Olry, Vice-President Universite
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PLEXIGLASS MOUNTED S10 PLASTINATED THIN BRAIN SLICES

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The aim of this study was to produce serial thin slices from a S10 plastinated brain. The brain was taken from an embalmed body, and the surrounding blood vessels and meninges were carefully removed. After an additional fixation of 18 months, the brain was plastinated according to the standard S10 procedure, followed by an 8 months post-cure in a plastic bag. In order to make sectionning easier, the brain was embedded in a gelatine block, and subsequently frozen at -20°C. The optimal thickness of slices was 7 millimeters, but it is noteworthy that the depth of brain tissue was still sticky, in spite of the rather long period of post-cure. One side of the brain slices was smeared with a mixture of S10-S3-S6-S2, mounted on plexiglass, and left to cure again. Both P35 or P40 methods bring out a better differenciation between grey and white matters; however, our method provides good results, and could therefore be regarded as an alternative for plastination laboratories using only the standard S10 technique.

CONGRUENT CT-SCANS AND PLASTINATED SLICES

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Ever since the methods of tomography have been developed it is the aim of slice atlases to show exactly correlating anatomical slices and CT-scans. However, most of the atlases show more or less similar slices and therefore allow no direct comparison of anatomical and tomographical slices. About 10 years ago new methods for slice plastination have been developed in Heidelberg. Plastination with epoxy resins (Biodur El 2) has been developed to produce thin (2-4 mm) and transparent body slices. Plastination with polyester resins (Biodur P35 and P40) has been developed to produce semitransparent brain slices. However, both epoxy and polyester resins may be used for body slices, although polyester plastination, particularly the plastination procedure using P35 is more expensive than epoxy plastination. There have been many considerations as how to get congruent or at least comparable plastinated and CT slices. However, none of these considerations led to a fully satisfactory result. Thus, the simplicity of the solution is stunning: a simple X-ray of the slice that will be plastinated or that has already been plastinated results in a clear, distinct and congruent "CT scan" of exactly the same slice. However, due to the thinness of the slices a high resolution film - e.g. mammography film - and low voltage is needed to get the desired results. Radiographs of the already plastinated slices, only allow production of "bone-window" CT-scans, because all the soft tissue has got the same density through impregnation. Thus only osseous structure can be seen in these radiographs. If "soft tissue window" CT-scans are desired, that show also the soft tissues, the slices have to be roentgenized before impregnation. By this simple method it is possible to produce identical anatomical slices and CT scans for teaching sectional anatomy and radiology.

SILICONE CAST OF THE CHAMBERS OF THE HEART

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Orientation of the overlapping chambers of the heart is difficult for first year veterinary medical students to conceptualize and confounding when attempting to determine ventricular volume using imaging techniques. To better visualize and understand the spatial relationship between the ventricles, silicone casts of the heart and great vessels were made from unembalmed sets of heart and lungs. The major vessels of the heart were either ligated or cannulated for silicone injection. Silastic E RTV (Dow Corning, Midland, MI, 48640-0994 USA) was activated, colored and injected until the cardiac chambers were filled. After hardening, the specimens were first macerated in boiling water and maceration was completed in 5% hydrogen peroxide. A highly durable, anatomically precise replica of the cardiac chambers, valves and great vessels was thus obtained for student instruction and image analysis.

PLASTINATION OF BRAIN DISSECTIONS AND BRAIN SLICES

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Plastination is a unique technique of tissue preservation developed by Dr. Gunther von Hagens in Heidelberg, Germany in 1978. In this process, water and lipids in biological tissues are replaced by curable polymers which are subsequently hardened, resulting in dry, odorless and durable specimens. In neuroanatomy silicone (Biodur S10) and polyester (Biodur P35 or P40) resins are used. Silicone rubber is used for whole brains and brain dissections resulting in natural looking specimens. Polyester resin is used for plastination of brain slices resulting in an excellent distinction of gray and white matter. P35 and P40 procedures offer comparable results. However, the P40 technique is less expensive because less resin and no safety glass is needed for P40 compared to P35. The advantages of plastinated specimens in neuroanatomy

teaching are:

1. Plastinated brain and brain slices are convenient to use because they are dry, odorless, and nontoxic.

2. Plastinated brains and brain slices are an important step against the lack of fresh brain tissue for teaching because they are durable and almost everlasting.

3. P35 or P40 plastinated brain slices demonstrate very fine anatomical detail and have the best possible distinction between gray and white matter.

THE P-40 TECHNIQUE SCALED DOWN

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The P-40 polyester is used for the plastination of thin brain and body slices yielding specimens of excellent instructional value. It was found that in the case of brains, thorough and even fixation was vital to the final quality of the slices and in particular to prevent the occurrence of orange spots in the cortex, suspected to be peroxidase.

Over recent years our department has experienced difficulty in obtaining fresh brain material for teaching. Although this problem has been alleviated, to some extent with the plastination of a large variety of brain specimens with Biodur silicone S10, loosing specimens to experimentation was a consideration. For this reason, and due to the difficulty first experienced with the P-40 technique, it was convenient to process 1 slice at a time until sufficient data was gathered. Then, as results became more consistent, production was increased to 3 brain slices at a time. Processing slices in a small number also minimised the health risk, made use of additional equipment in the lab and allowed the technique to fit into a busy schedule.

SUBMACROSCOPIC INTERPRETATION OF HUMAN SECTIONAL ANATOMY USING PLASTINATED E12 SECTIONS

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The E12 epoxy method of sheet plastination for preparing thin, transparent and serially sectioned cadaveric teaching specimens has for the most part seen the finished sections utilised in the correlation of MRI and CT radiographic images. The unique properties of the E12 process offer an exceptionally vivid survey of the human body in any given plane resulting in precise deliniation of the structural layout *in situ*. Maximum detail of the sections is attained by way of transparency through large scale lipid extraction producing easily discernible detail of anatomical structures within a gross specimen.

Sections were prepared in sagittal, horizontal and coronal planes from different regions of the body including head and neck, trunk, inguinal region, limbs and joints. The macroscopic structures within these sections correspond precisely with images of the same structures obtained radiologically.

By introducing E12 sectional anatomy specimens to the anatomy teaching laboratory, the transition between gross anatomy and histology has been made possible by studying the one specimen. When utilised in our combined topographic anatomy and histology teaching laboratories, anatomical structures of thin and tansparent slices can be magnified considerably. Standard histotogical slides providing detail of a specific structure within predetermined parameters, are often dictated by the physical limitations of the microscope slide itself. E12 sections provide a high degree of detail whilst retaining *in situ* structural integrity of the entire region in a complete and uninterrupted state.

Students are provided with significant detail of all components to the submacroscopic level from any one specimen thus linking the three disciplines, namely cross-sectional anatomy, radiology and histology using a single E12 slice. E12 plastinated sections have been recognized as an ideal teaching aid in conjunction with radiological correlation, but it is in the microscopy laboratory that a valuable new dimension of this multi-disciplinary plastination technique has recently been realized.

SCALING DOWN THE P35 TECHNIQUE

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Polyester (P35) plastination of brain slices results in specimens with excellent instructional potential. It is also valuable for certain kinds of research. If a person has extensive teaching and administrative duties, however, the conventional, high-output, P35 technique, is far too time-consuming. I have been using an attenuated (scaled-down) P35 procedure that may be of interest to others who have extensive responsibilities but would still like to turn out useful specimens. In addition to requiring smaller increments of time, miniaturizing the P35 technique offers an assortment of other advantages. For example:

1) it is far more conservative of resin.

2) it is easier to maintain resin hygiene, and,

3) it diminishes the release of styrene vapor (just to men tion a few).

The key to miniaturization is standardization of all aspects of the process. more than 300 ml of resin is mixed at one time, permitting the production of 1 or 2 slices per day, at most. Small food-storage vessels are used for mixing and

processing and are cleaned immediately after use. Castings are prepared using smaller standard-size glass plates which accommodate only 1 slice per chamber. Reuseable gaskets for casting chambers are fabricated from heavy-gauge wire covered with plastic tubing. Degassing of castings is accomplished by returning them to the vacuum chamber. The finished specimen is trimmed to a standard size, labelled and stored in specially constructed standard carriers. Although not intended for maximum specimen output, this technique permits the busy teacher to plastinate and still attend to his or her other duties.

SHEET PLASTINATION OF BRAIN SLICES ACCORDING TO THE P35 PROCEDURE

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The P35 procedure is used to produce thin (4, 6, or 8 mm) and semitransparent slices.

Fixation: Fresh brain specimens are fixed the usual way with 5-10% formaldehyde for 3 to 6 weeks. Specimens that have been fixed by other methods should not be used for the P35 procedure because fixatives other than formaldehyde may cause unintentional reactions with the polymer.

Slicing: After embedding in 20% gelatin to prevent degradation of the slices, the brain specimens are cut with a meat slicer into 4 mm (or 6 or 8 mm) slices. The slices are placed on stainless steel grids. The grids are piled up in a stainless steel basket.

Flushing & Precooling: The basket of brain slices is rinsed with cold tap water overnight and cooled to 5°C. Flushing may be extended to 2 days.

1. Dehydration: The basket of brain slices is submerged in 100% acetone at -20°C (25 1 per brain) for 1-2 days. Cau tion: Dehydrated brains become very brittle and breakable - handle with care!

2. Dehydration: The basket of brain slices is submerged in another bath of 100% acetone at -20°C (25 1 per brain) for 1-2 days. Note: The remaining water in the acetone must be lower than 2% measured with an acetonometer. If this is not reached the dehydration procedure must be extended until dehydration is complete.

1. Immersion: The basket of brain slices is submerged in precooled P35/A9 mixture (100:2) for 1 day at 5°C (to -25°C). Caution: Immersion baths must be kept in the dark to prevent the reaction mixture from polymerization. Note: This bath must be discarded after use.

2. Immersion: The basket of brain slices is submerged in fresh precooled P35/A9 mixture (100:2) for 1 more day at 5°C (to -25°C). Note: This bath may be used as 1st immer sion bath for the next procedure.

Forced impregnation: The basket of brain slices is submerged in a fresh P35/A9 mixture (100:2) and placed under vacuum for 24 hours at -25°C or if the vacuum chamber is too small at room temperature. The vacuum is increased until 1-2 mm Hg are attained when impregnating at -25°C or 10-15 mm Hg are attained when impregnating at room temperature. Note: This bath may be used as 2nd immersion bath for the next procedure.

Casting/double glass chambers: The slices are removed from the vacuum chamber, and individual slices are placed between two sheets of glass plates. Each sheet consists of one outer sheet of safety (tempered) glass and one inner sheet of float (regular) glass, the latter sheet facing the brain slices. A silicone gasket (6 mm for 4 mm slices) is used to seal the chamber around the edges and fold-back. Clamps are used to fix the two double glass plates together. Then the double glass chambers containing the specimens are filled with a fresh P35/A9 mixture (100:2). For filling the standard size chamber (35 x 45 cm) about 700 cc of polymer mixture are needed.

Light Curing: After casting, the double glass chambers are exposed to UVA-light for a period of 45 minutes to 4 hours depending on the watts and on the distance of the UVA lamps. During this procedure it is necessary to cool the chambers either by ventilators on both sides or by blowing compressed air over both sides of the double glass chamber. Caution: Cooling is important because the UVA-light causes an exothermic reaction that would destroy the specimens if they are not cooled. To prevent cracking of the P-35 slices during light curing it is also recommended to use low watt UVA-lamps and longer curing time.

Heat Curing: Following light curing the double glass chambers are exposed to 45°C for 4-5 days in a well ventilated oven.

Finishing: After curing is finished the glass chambers are dismantled and the sections are trimmed on a band saw and the edges smoothed using a belt sander.

A BRIEF CHRONOLOGY OF PLASTINATION

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I suspect that there is no official "Birthday of Plastination". As most of you know, the concept was the brainchild of Gunther von Hagens, who one day decided that, instead of embedding a specimen in plastic, the plastic should be induced to enter the specimen and then cured, in situ. This led to the concept of forced impregnation and, after many years, to the technology, the International Society for Plastination and the meeting schedule we now enjoy. If I had to assign some kind of a date for its origin, I would suggest that the intellectual process leading to Plastination began sometime during the year 1975 - but I would have to check with Gunther to be sure or more specific.

What we can date with assurance are the international

meetings, so let's use these as an approach to our chronology.

THE INTERNATIONAL MEETINGS

Let's begin with what we are calling (in retrospect) the "First International Conference on Plastination".

The "First International Conference on Plastination" was actually entitled "Preservation of Biological Materials by Plastination". It was convened in San Antonio, Texas, USA on Friday, April 16, 1982 and lasted only one day. Eighty people were registered, all from the United States. It wasn't very formal and it wasn't really international. But we're counting it anyway.

The "Second International Conference on Plastination" was held in San Antonio during April of 1984. It seems that it was hardly more formal than the first since my files contain no examples of brochures or other mailings. As I remember, attendance was close to 100 and even included some from outside the US. The need for this kind of conference expressed by those in attendance encouraged us to do a better job on the next one.

The 'Third International Conference on Plastination" was held in San Antonio on April 21-25th, 1986. Anticipating a strong response, it was publicized widely in both North America and Europe. As a result, attendance was excellent and the meeting finally began to take on an international character. With this conference, a five-day meeting format was adopted: two days of lectures dealing with the principles of plastination, one day of informal gatherings and two days of papers related to advanced topics. It was at this meeting that the International Society for Plastination was formed and plans were made for publication of the journal. Volume 1, Number 1 of the Journal of the International Society for Plastination was released in January of 1987 and contained many of the papers presented at this meeting.

The "Fourth International Conference on Plastination" was held at Mercer University School of Medicine, Macon, Georgia, USA, March 21-25th, 1988, again employing the 5-day format. Judging from both attendance and comments, it was a resounding success.

The "Fifth International Conference on Plastination" was one of the highlights of our brief history. It was particularly significant since it was held at Heidelberg, the "Birthplace of Plastination". The dates were July 22-27,1990, a change from the usual springtime interval. It was well-publicized throughout the world and attendance was the best ever.

The "Sixth International Conference on Plastination" was held at Kingston, Ontario, Canada, in 1992. Again, July dates (26-31) were selected since this seemed to accommodate more of us who had teaching duties. The meeting was thoroughly enjoyable and introduced many new people to plastination.

The "Seventh International Conference on Plastination" was held at Graz, Austria in the summer of 1994. It was well-attended and featured, in addition to a lot of great technology, a night of over-alimentation and revelry at the Landhaus - very memorable.

The "Eighth International Conference on Plastination", was held at Brisbane, Australia, July, 1996. We couldn't have chosen a nicer or more interesting place to meet as this turned out to be just another great meeting.

The "Ninth International Conference on Plastination" will be held at the University du Quebec aTrois-Rivieres, Quebec, Canada, July 5-10,1998. It promises to be another outstanding experience.

INTERIM MEETINGS

Interim meetings (those held during the off-year intervening the international conferences) popped up quite spontaneously. The initiative for holding them was provided by members who wanted an opportunity to serve as host for a Plastination meeting. They have been held at a number of interesting places such as Knoxville, Tennessee; Rancho Cucamonga, California; Mobile, Alabama; and Columbus, Ohio; all in the United States. Although not advertised as international meetings they have gradually become quite international in composition. They tend to emphasize the "hands-on" rather than the didactic approach.

THE INTERNATIONAL SOCIETY FOR PLASTINATION

As mentioned above, the International Society for Plastination was organised at the 3rd International Conference on Plastination. It was conceived as a means of defining plastination as an area of professional activity and serving as an agency for disseminating information relative to the art and science of plastination. Its role was envisioned as consisting of five principal services:

1. to identify an international community of scientists and technologists interested in plastination.
2. to serve as a forum for the international exchange of information about plastination through the periodical publi cation of a journal.
3. to organise and conduct regular regional and interna tional workshops and meetings.
4. to maintain an international registry of laboratories and technologists skilled in the performance of plastination.
5. to define plastination as an area of professional activ ity and provide a means of learning and practicing plastination as a carrer.

Well, that's the whole story in outline. As you can see we've come a long way since the first silicone elastomer was forced into a specimen. Our organization has been simple and pragmatic, but it seems to be working well.

REPORT

" THE 1996 OSAKA PLASTINATION EXHIBITION"

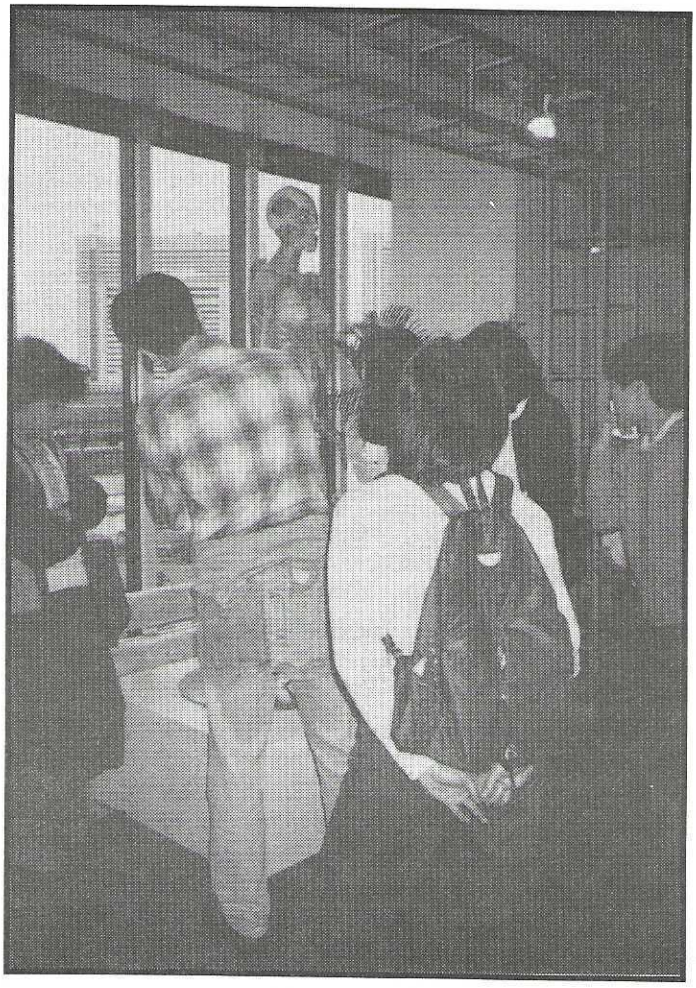
A wonderfull exhibition of plastinated specimens was held last year in Osaka. It was the fourth event of this kind in Japan: the first, organized by the Japanese Association of Anatomists, took place at the Tokyo University Museum (March 30-April 2, 1995); the second one was held by the Japan-America Student Conference at the Sendai Science Museum (July 27-30, 1995); the third one occured during the centenary of the Japanese Association of Anatomists at the National Science Museum of Tokyo (September 15-November 26, 1995).

The Osaka exhibition was entitled "Plastination. The wonder of human body". It was held from July 20 to October 31, 1996 at the Shin-Umeda City Museum, Osaka, Japan. This event was sponsored, among others, by the Sankei Newspapers Publishing Company, the Osaka Newspaper Publishing Company and the Heidelberg Institute for Plastination. The local exhibition staff included Toshitugu Oda, professor emeritus at the Tokyo University, Wataru Mori, president of the Japan Medical Society, and some fifteen members. The artist Noriyuki Tanaka was responsible for the space design.

This exhibition was very successfull: it drew a crowd of 250 000 people who could enjoy about hundred human plastinated specimens: whole bodies, bones and muscles preparations, hearts and vessels, brains, digestive, respiratory, urinary and reproductive systems. Numerous pathological specimens were included in each of these categories: cerebral haemorrhage, hydrocephalus, liver metastasis, smoker's lung, bronchial carcinoma, tuberculosis, myocardial infarction, aneurysm of aorta, nephritis, and breast carcinoma. Some embryology specimens, including for example a striking transparent slice of a pregnant woman, were also displayed. A lavishly illustrated catalogue of the exhibition was published, with explanations in English by Angelina Whalley.

The success of this exhibition brings two important facts to light. First, plastinated specimens are not to be restricted to medical and allied sciences audience: general population, including many children, also shows a great interest in human anatomy, and the exhibition of pathological specimens may get people to ask themselves about their own way of live (smoker's lungs, alcoholic cirrhosis). Secondly, it becomes obvious since many years that North America and Europe are not the only two centers of plastination on the world: our Australian colleagues organized an unforgettable conference last year, some African countries show an ever increasing interest in the plastination procedure, and Asia proved the potential of plastination in popularization of anatomical knowledge. This point will be to be taken into consideration for the choice of our future international conferences.

Regis Olry, Vice-President ISP, Canada
Kaoru Motomiya, Japan



Ninth International Conference on Plastination
July 5-10, 1998
Trois-Rivières, Québec
Canada

Neuvième conférence internationale sur la plastination
5 au 10 juillet 1998
Trois-Rivières, Québec
Canada

The 9th International Conference on Plastination

and

Sixth Biennal Meeting of the International Society for Plastination

will be held July 5 to 10, 1998 at the Université du Québec à Trois-Rivières, Trois-Rivières, Québec, CANADA.

Program Outline

Sunday July 5	PM	Registration; Welcome reception
Monday July 6	AM	Plastination with Silicone, Principles and Techniques
	PM	Plastination with Epoxy, Principles and Techniques
Tuesday July 7	AM	Plastination with Polyester, Principles and Techniques
	PM	Plastination Laboratoty design and Equipment
Wednesday July 8	Free day	- visit to the University - visit to the plastination laboratory - conference dinner
Thursday July 9	AM	New developments in Plastination Plastination applications in Teaching
	PM	Plastination applications in Research ISP meeting
Friday July 10	AM	Plastination applications in Research Group discussions - "Ask the Experts"
	PM	Special techniques - vascular injection - casting
		Close

PAPERS

The organisers invite you to pass on your experiences and ideas by presenting a paper to the conference. We propose to schedule papers with 15 minutes for presentation and 10 minutes for discussion.

POSTERS AND EXIBITS

During the whole week, a room will be available for posters and exibits display. Participants are encouraged to bring examples of their own work.

ACCOMPANYING PERSONS PROGRAM

Many half-day or full-day tours will be made available during the conference week.

FOR ADDITIONAL INFORMATION:

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Université du Québec à Trois-Rivières

Ninth International Conference on Plastination

Sixth Biennal Meeting of the International Society for Plastination

1998 Abstract Form

Smallest Recommended Type Size: 10 Point

Sample:
9th International Conference
Trois-Rivières,
July 5-10, 1998

Check one:

Paper ☐

Poster ☐

According to the demand, your choice may have to be modified by the organizing committee

Postmark Deadline:

Monday, March 30, 1998

Provide full name, address and phone number of first author on abstract (please type or print in black ink).

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E-mail: _____ Fax: _____

Special Requests
(audio/visual requirements)

INSTRUCTIONS TO AUTHORS

Aims

The Journal of the International Society for Plastination (ISSN 1090-2171) is an international forum for the diffusion of the plastination technique among scientists interested in preservation of biological specimens for teaching and research. The Journal permits communication of every new application or development of the plastination technique, as well as any other innovating complementary preservation technique, applicable to animal or plant specimens.

Submission

All manuscripts are subject to peer review. The acceptation of an article implies the transfer of the copyright from the authors to the publisher. It is the author's responsability to obtain permission to reproduce illustrations, tables, etc from other publications. The Journal will accept articles, brief communications as well as reviews. Editorials may also be accepted. They are generally invited, but unsolicited editorials will be considered. Letters may also be considered for publication if they are judged of general interest.

Format

Manuscripts should be written in English. They should be submitted in triplicate (with 2 sets of illustrations), typewritten double-spaced on one side of the paper with 2,5 cm margins. Lines should be numbered on the margin and all pages should be numbered.

Title Page

Title page should include title, the author's names and the institute where the work was conducted, and full address of the authors. Please, also supply Phone and Fax numbers, as well as e-mail address of the author to whom correspondence should be sent.

Key Words

For indexing purpose, provide a list of up to 5 keywords.

Abstract

Each paper needs an abstract of up to 20 lines, summarizing the essential new information communicated. Abbreviations and citations should be avoided in the abstract.

Subheadings

Each paper should include an

INTRODUCTION
MATERIALS AND METHODS
RESULTS
DISCUSSION OR CONCLUSION
BIBLIOGRAPHY

Numbers and abbreviations

Arabic numerals should be used wherever digits are needed except at the beginning of a sentence. Abbreviations of weights and measures as given in standard dictionaries are usually acceptable. Other abbreviations should be followed by an explanation (within parentheses) the first time they are mentioned.

Nomenclature

The nomenclature used should conform, wherever possible, to the current edition of the *Nomina Anatomica* or the *Nomina Anatomica Veterinaria.*

Tables and illustrations

Tables and illustrations (both numbered in Arabic numerals) must be cited in the text and should be prepared on separate sheets. Tables require a heading and illustrations a legend, also prepared on a separate sheet. They should be one column (8.4 cm) or two columns (18 cm) width. On the back of each illustration, indicate its number, the author's name, and "top". For the reproduction of illustrations, only good drawings and original photographs can be accepted.

References

References to published works, abstracts, personnal communication and books should be limited to what is relevant and necessary. Citations in the text should be given in parentheses; e.g., (Bickley et al., 1981; von Hagens, 1985; Henry and Hayes, 1989) except when the author's name is part of a sentence; e.g., "von Hagens (1985) reported that..." When references are made to more than one paper by the same author, published in the same year, designate them as a. b. c. etc.

Bibliography

The bibliography should include only the publications which are cited in the text. References should be listed alphabetically using abbreviated journal names according to the Index Medicus.

Examples are as follows:

Bickley HC, von Hagens G, Townsend FM: An improved method for preserving of teaching specimens. Arch Pathol Lab Med 105: 674-676, 1981.

Henry R, Haynes C: An Atlas and Guide to the Dissection of the Pony, 2nd Ed. Edina, MN: Alpha Editions, pp 25-88, 1989.

von Hagens G: Heidelberg Plastination Folder: Collection of all technical leaflets for plastination. Anatomische Institut 1, Universität Heidelberg, Heidelberg, Germany, 1985.

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