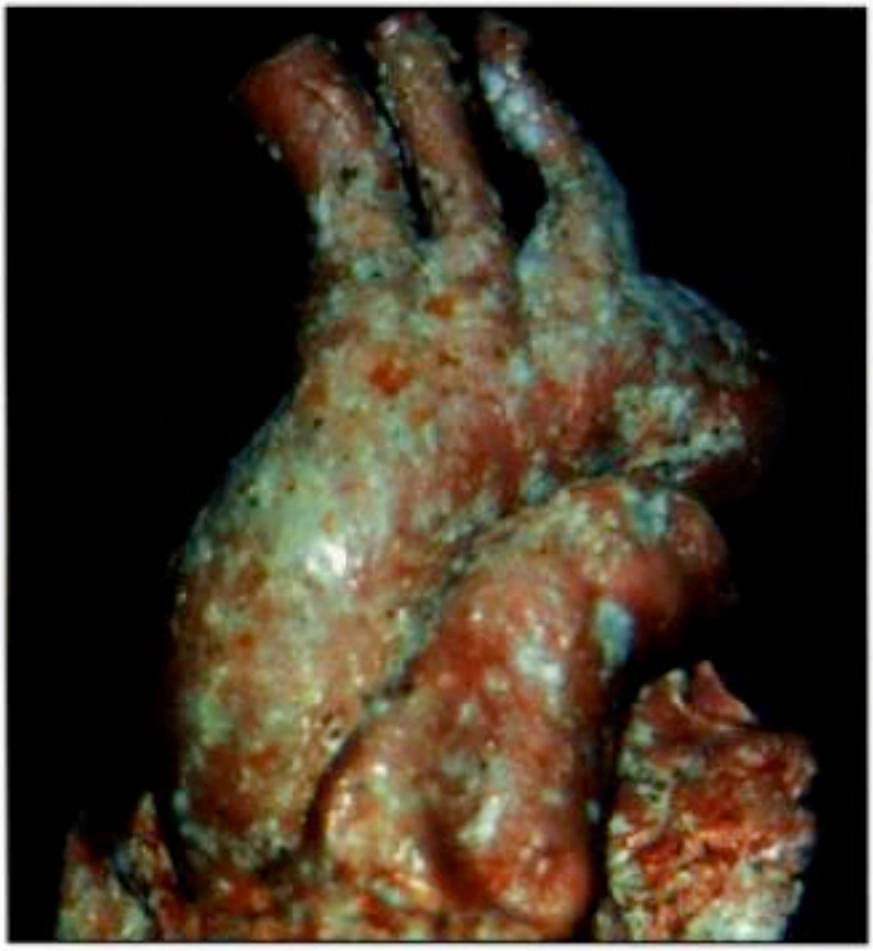
Journal of the INTERNATIONAL SOCIETY for PLASTINATION



VOLUME 14, No 2
DECEMBER 1999

PRESIDENTIAL LETTER

Dear ISP-Members,
Dear fellow plastinators,

We are running towards the end of the 2nd millenium and there are several news taking place in the International Society for Plastination.

* First of all, we will have some amendments of our bylaws which I hope you will accept and vote for. The main amendment enables every member to vote via secret mail ballot in order to give all members the chance to express their opinion independently from their presence at the biennial general meeting. The draft of these amendments has been sent to you by the chair of the bylaws committee, Robert W. Henry, who together with the bylaws committee members did tremendous work for these changes.

* Secondly, we will elect a new executive board and to do that, the chair of the nominations committee, Robert W. Henry asked you for nominations. Each member may nominate one person - including him- or herself - for each Executive Office. Please be sure that the nominated person is willing to serve as an officer and that the person is a member whose dues are paid up to date. If the amendements of the bylaws are accepted by the majority we will have a mail ballot to vote on the Executive Board Members in Spring 2000.

* And thirdly, I regret to inform you that our treasurer, Ronald S. Wade requested to be excused from his office as Treasurer and Executive Board Member of the International Society of Plastination, an office that he held since 1994. Ronn ensured me that it was with no ill will toward any member, issue, action, or reaction that he resigned. He also helped that his resignation created as little disruption as possible to the ISP-members.

At this point I, on behalf of the members of the International Society for Plastination, want to thank Ronald S. Wade for his long term serving the ISP and we wish him all the best for the future!

* The resignation of Ronald S. Wade required several other changes:

1) Wolfgang Weber, Iowa State University, Dept of Veterinary Anatomy, Ames, Iowa, USA was appointed interim treasurer until the elections in 2000. I am happy that Wolfgang Weber accepted to serve as interim treasurer and I am sure that he will meet our expectations and I strongly encourage you to express your acceptance by nominating Wolfgang for the treasurer's office from 2000 to 2002 and vote for him as well, as this is a key position in our society.

2) The ISP-Listserver has to be transferred to Graz. This listserver will work like the one that has been established in Baltimore by Ronn Wade and will start within the next days. All currently subscribed list-members will automatically be subscribed to the new server and you will get the respective information. I am sure that the transition will run smoothly and that we will have our pan-world-communication system running as it did for the last four years, thanks to Ronn Wade.

Finally, I cordially invite you to participate in our society and in particular to participate in the 10th International Conference on Plastination in Saint-Etienne, France. This important meeting will take place from July, 2nd to July 7th, 2000.

With the very best wishes for a happy, healthy and successful start into the year 2000 I remain,

Yours sincerely

Andreas H. Weiglein

LETTERS TO THE EDITOR

ROCHESTER CONFERENCE

Editor
JISP

Well, it has happened again. A large group of diverse people got together and, without any murder or mayhem, managed to learn a lot from each other about a process near and dear to our hearts. (No pun intended.)

Cindy Ryan and the University of Rochester did indeed put on a good conference. While it took a bevy of able assistants to aid her - - and they surely deserve a great deal of credit - - Cindy is the one we know and love and the job couldn't have been done better.

On Sunday and on Monday morning I wasn't sure that Cindy would make it without a large infusion of Valium, but as the week moved on and all systems were functioning near perfect, she smoothed out and was a gracious hostess to us all. She might have fed us a little too well - - - and this comes from a trencherman of some note.

Owing to the reputation of some members of the ISP, it was no surprise that Peter Cook has refrained from bringing his nice wife to a conference. Adoration is indeed a lady and far too good for a rapscallion such as Peter.

There were a lot of new faces and one would hope that Ron Wade held them hostage until they signed their membership applications and paid a membership fee. They won't regret it.

Such a lot of "hands-on" lab time was welcome at this meeting. I suspect that our friends from Michigan made some friends and fostered some potential customers. It was good of them to attend and demonstrate.

Was the trip to the Art Museum and the meal they served us there an elegant occasion or what ? It's not often a bunch of Silicon Stooges get to mix and mingle with the fine arts. (Cindy was wise enough to arrange it so that no one else was around to see her in our company! Good move Cindy.)

In short it couldn't have been better planned or executed. So, it's on to France in 2000. Get ready Marc - - we'll be there and you've got a hard act to follow.

Mr Tim Barnes
135 Grosvenor Hall
Athens OH 45701 U.S.A.

CORCORAN POLYMERS

Last June, July and August, we kept finding on the ISP-L list server many comments about the curing problems with the Corcoran silicone polymer described by some readers in our last issue. All these messages were bringing more questions than answers so this is why we decided not to publish them all but to only publish two of these that, we believe, brought answers to the questions raised on this topic.

Gilles Grondin
Editor

Greetings fellow plastinators,

My first experience with Corcoran polymers was at the 9th International Conference on Plastination. A demo was given and specimens that had been impregnated with Corcoran polymers were on view (who could forget Ron Wade's 2 headed pig).

At the meeting it was suggested that impregnation could be carried out in 24 hrs and was demonstrated that way. To look down at that vacuum chamber with what seemed to be thousands of acetone bubbles emanating from the surface of a heart was incredible (when one is use to hearing, the bubbles are to slowly rise to the surface, gently bursting at the surface). The next day the heart was taken out of the polymer, with no signs of shrinkage, and the catalyst administered.

On my arrival down under I ordered some polymer and processed my first batch of specimens no problems what so ever. The second batch of specimens took longer then usual to cure and the third batch of specimens, I had great difficulty in curing. Pressure was brought down to approx 1mm Hg ON ALL THREE OCCASIONS. This resulted in the cross linker being removed from the polymer (boiled out) during the three impregnations. We all know now, that this is fact.

The method I use, is not to allow the vacuum to fall lower than 15mm, at this pressure crosslinker removal I believe is minimal. I also infiltrate my specimens with the polymer solution before starting impregnation and also during impregnation. This particular polymer is extremely thin so this technique works well. I believe the method of syringe infiltration along with vacuum impregnation of the polymer will deposit enough polymer and crosslinker in the tissue of specimens for the catalyst to do it's job of curing. I either brush my catalyst directly onto the specimen or spray it on and then brush the residual catalyst into all the nooks and

crannies with good results. Placing the specimens into a sealed container is a must.

I place my dehydrated specimens into polymer on Friday and let equilibrate till Monday. I then commence my vacuum regime. Day 1 - 125mm decrease pressure to 15mm generally over a 5 to 6 day period works fine for me. Acetone bubbles are more numerous at a pressure of 125 mm at room temperature then in the cold, I never get concerned about shrinkage as the polymer is so thin that it is filling the voids left by the departing acetone, infiltrating before and during vacuum impregnation helps in this regard.

I have just completed a set of reproductive tracts of the male genitalia successfully in 5 days with no shrinkage of the testicles. Recently I plastinated a sugar glider pup about 1.5 inches long with great results with heavily infiltrating before and during impregnation. The surface detail of the face was tremendous with the fine whiskers visible under the dissecting microscope. I find that once specimens are taken out of the polymer and left to drain for 24 to 48 hrs that catalyst can be applied. The polymer is thin so that the excess drains of the specimen rapidly and have not had a problem with cured polymer spoiling the result and find that surface detail is well preserved, as was the case with the sugar glider.

Recently I injected catalyst directly into the thorax and abdomen of some large insects and crustaceans that were required urgently. The polymer cured within 12 hours with good results.

Regards

Richard Borg M.I.S.P.
Senior Technical Officer
Department of Veterinary Anatomy and Pathology
University of Sydney

Dear fellow-plastinators,

First let me introduce myself. I am Jerry Klosowski, a senior scientist at Dow Corning and I worked on the modified version of tissue preservation since 1993 starting with Wayne Smith of Texas A &M and am one of the patent holders on the modified process. Now on an as-needed basis I do some consulting with Dan Corcoran of Corcoran Laboratories who supplies the preservation chemicals and still some with Smith at Texas A& M who is still looking at preservation of all kinds of things.

Dan asked me to send a note addressing the evaporation of the crosslinker and related issues. If your a chemist, excuse the detailed explanation, I don't mean to insult you. However most people I met in the preservation area are not of the chemical persuasion and thus tend to appreciate the more detailed answers.

In our technique, we mix the polymer with the crosslinker to give a mixture that will react when it is in contact with a catalyst, which we introduce later. But it does not react before it sees the catalyst. This has the inherent advantage of having long shelf life and gives the ability to save and store unused, uncatalyzed materials. This also prevents any premature reactions.

We keep the crosslinker of low molecular weight (small size) so that we can get good pore and cell penetration. In essence we want the crosslinker to go everywhere and the polymer to go where ever its size will let it go. The final chemistry is to react the crosslinker with the polymer and the cell walls. We think that it is the reaction with the cell walls that stops the decaying processes (similar reactions with iron surfaces keeps iron from rusting). The reason for this explanation is so you will realize that we do want a lot of crosslinker in most systems and it must get into the tissue.

Now many of you are rightly concerned that when you pull a vacuum to remove the acetone that you are removing some of the crosslinker. You are correct. First you can try to minimize this loss by applying only a slight vacuum. Just enough to boil the acetone but not the crosslinker. Do just enough that you can see the little bubbles form. The boiling point of the acetone is at least 50°C lower than that of even the most volatile crosslinker. The proper vacuum for you will depend on temperature. Thus your first effort should be to control vacuum.

Now it is generally okay to use extra crosslinker in this part of the process. You can use 3%, 6% or even 10%. However don't be concerned too much about cost. If you put a cold trap in your vacuum line you will trap the material coming off. This will be a mixture of acetone and crosslinker. You could set up a still and distill off the acetone and reuse the crosslinker. You could throw away the first few samples you obtain in the cold trap, since that should be mainly acetone and save the rest of the samples taken from the trap. Determining the trap composition, and if you want to save and reuse it, is most easily done by gas chromatograph. A hydrometer might also work. I will leave it to your more fertile, conservative minds to determine if you want to save the crosslinker and which technique to determine which sample collected from the cold trap you want to save. The important part is that not too much happens to the crosslinker in all of this work, as long as there is no catalyst around. So what you collect and save is generally reuseable.

Once you catalyze the system, don't put that back with anything. You shouldn't probably use a vacuum on the

catalyzation step. Just painting the catalyst on the specimen and letting it penetrate is generally sufficient. Remember it is best to let the catalyst penetrate in a system that is not open to the air. It is also good to remember that after the catalyst has penetrated the system, to put the specimen in the open air to finish the cure. The cure finishes with the moisture in the air reacting with the extra crosslinker in the specimen.

Another thought is that some of you had tacky surfaces on a specimen. It is generally very acceptable to paint crosslinker, mixed with catalyst, on the surface and let it cure in air. That usually dries the surface nicely.

Some thought the surface of some specimens was too hard and shiny with the technique they were using. In such a case you can wipe the surface before you catalyze it. It can be wiped with a towel or lightly rinse it with a solvent. This removes the excess polymer and crosslinker from the surface before the cure starts.

Lastly, as you keep Dan Corcoran abreast of your needs and problems you can also keep me aware of the technical issues.

Jerome (Jerry) Klosowski
Senior Scientist
Dow Corning Corp; DC 4 3C5
2200 Salzburg Rd.; P.O. Box 994
Midland, MI 48686-0994
U.S.A.
phone 517.496.4244; fax 517.496.5956
Mailto:j.m.klosowski@dowcorning.com

TRAINING IN PLASTINATION

Dear plastinators,

I am a 19 year old student in medical biology at the «Université du Québec à Trois-Rivières» and since october, I have the opportunity to be trained at the plastination laboratory of the university. I want to express myself on the various advantages of training students to plastination; for the student himself as well as for the laboratory.

Plastination helps me in different ways. It is an outstanding way to learn anatomy, better than any book because I experience, see and touch what is shown on pictures or summarized in texts. I have to prepare my dissections and choose, with my supervisor Mr. Gilles Grondin, the final aspect of the specimens I work on. This preparation allows me to develop my autonomy and responsability to become, with practice, professional. I discovered plastination at the beginning of the semester and since, I am very interested to learn more in order to prepare attractive and useful specimens. It is the same phenomenon about my « motivation degree» which increases every time I find new ideas for the laboratory or for my personal project. When I work with Gilles, I gain experience that may lead me to participate at the international conference next july, or to write articles in your journal. In fact, plastination helps me in my studies and, with the experience I am gaining, will surely have a positive impact on my future.

Being a trainee offers me the possibility to do a lot for the laboratory. There will eventually be more specimens in the museum. Also, hiring more people definitively increase the popularity of the «science of plastination» and of the laboratory. I can bring new ideas of projects, or complete some already started. Even if, at the begining, Gilles has to take time to teach me the technics of dissection and plastination, it is a good investment of time because I will be able to work alone on some projects while he will work on other things. I think that even if I am a student without much experience, I can help Gilles a lot, as I contribute to expand the museum.

With this letter, I want to encourage you to hire trainees. It has a lot of positive effects on the trainee and on the laboratory. Many students would like to be volunteer and there are specific subventions for the laboratory that want to give a salary to the trainee. I am very grateful to my supervisor, Gilles Grondin, and I am conviced that some students in your university would be very interested to work with you, plastinators, in your laboratories.

Thank you for your interest

Louise Vigneault
Student in medical biology
and plastination trainee
UQTR

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Body Snatchers: the Hidden Side of the History of Anatomy

Regis Olry

Departement de chimie-biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada.

(received November 22, accepted December 20, 1999) ***Key words:*** *Human dissection, body supply, body snatchers, grave robbers, legislation of human dissection.*

Abstract

From the seventeenth to the nineteenth centuries, anatomical knowledge expanded greatly for human dissections were more and more recognized as essential to medical and surgical training. The need for bodies, especially in private medical schools, increased so much that the bodies legally obtained could not meet the demand. This situation gave rise to underground organizations, known as body snatchers or resurrectionists, which supplied anatomists with bodies which were illegally exhumed from their graves. The lure of money even led some of them to murder people. These malefactors heaped opprobrium on the anatomists who enlisted their services. Notwithstanding their motives, they contributed to the progress of human anatomy.

Introduction

Some years ago, the famous anatomical atlas of Eduard Pernkopf was put in the hot seat by people who claimed that the author, an ardent Nazi, used material obtained from Nazi victims to illustrate his book (Williams, 1988; Israel and Seidelman, 1996). It is not my intention to discuss this assertion: I just want to remind the reader of the fact that this practice unfortunately goes back a very long time, especially in Great Britain. In the last centuries, many private medical schools attracted large number of students from everywhere, including from abroad, because of the reputation of their teachers (Persaud, 1997). The pressing demand for human material exceeded by far the supply that was available, giving rise to an illicit trade of bodies. Some of the most famous anatomists of the last centuries received bodies from grave robbers and body snatchers who were making lots of money. This paper aims at summarizing the reason why many celebrated anatomists associated with these rather undesirable people, at the risk of losing their reputation.

The sad fate of condemned persons

Condemned persons were often used to supply laboratories of anatomy with bodies all around the world. As far back as in 1542, an Act of Parliament permitted the British Company of Barbers and Surgeons to use the bodies of four executed "malefactors" for dissection. Over two centuries later, the Parliament Act of 1752 expanded this practice to include the body of any criminal executed in London and Middlesex (Persaud, 1997). In the early seventeenth century, the Jena anatomist Werner Rolfinck was famous for having at his disposal the bodies of many executed people. His reputation as a dissector was so widespread that it terrorized all the inhabitants of the region. As a matter of fact: " The terror that the name Rolfinck filled the population with, led poor sinners to make arrangements not to be "rolfincked" " (Fro'ber, 1996). In Japan, the bodies of condemned people were also used for dissections: in the 1786 "Sanno suke kaibu zu", the illustrators overdid realism to the point of depicting the wound made by the executioner's axe on the anterior aspect of the thigh: the blow had been so strong that the axe sank into the thigh of the condemned man who was executed in a squatting position (Olry and Motomiya, 1996) (figure 1). In Switzerland, Andreas Vesalius dissected the body of Jakob Karrer, a notorious murderer who was beheaded on May 12, 1543 (Olry, 1998). Also in France, the bodies of condemned persons were given to the medical faculties until the late nineteenth century (Chapoutot, 1894). Unfortunately, bodies of condemned persons could not provide for the anatomists' needs: this left the way open to the body snatchers.

Body snatchers: the trade of death

The history of body snatchers was the subject of many historical, biographical and psychological studies (Cohen, 1975: Richardson, 1991; Shultz, 1992; French, 1997). Many gangs were involved in the business: over a period of fifteen

Address correspondence to: Dr. R. Olry, Departement de chimie-biologie, Universite du Quebec a Trois-Rivieres, C.P. 500, Trois-Rivieres, Quebec, Canada G9A 5H7. Telephone: 819 376 5053 / Fax: 819 376 5084. Email: Regis_Olry ©uqtr.uquebec.ca

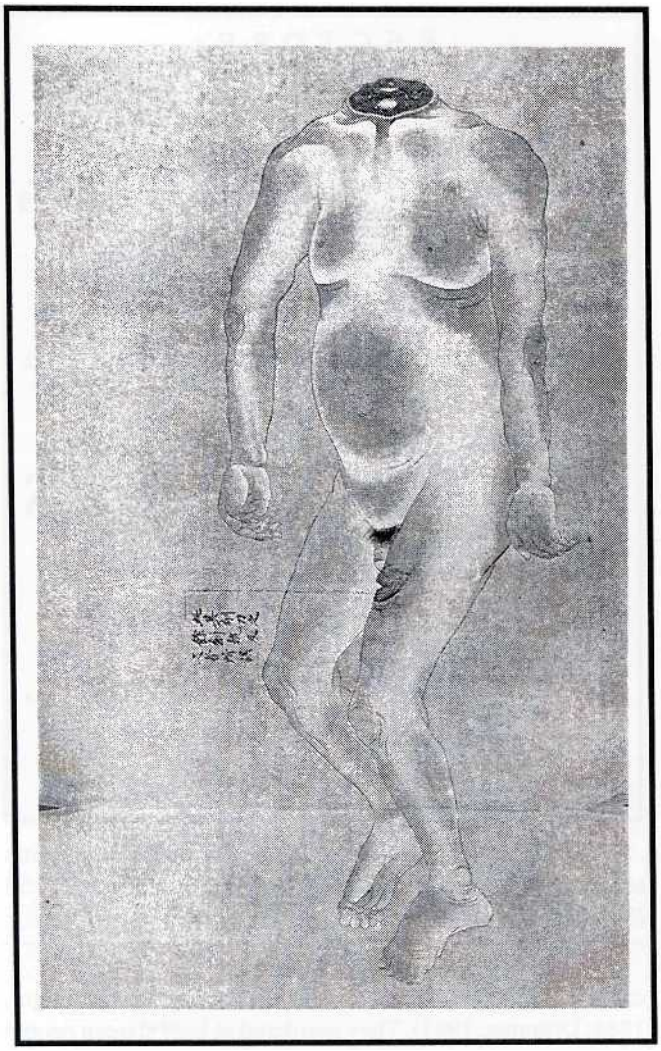

Figure 1. One figure from the Sannosuke-kaibu-zu (1786). The condemned man was in a squatting position for his capital execution; the blow by the executioner was so strong that the axe sank into the ventral aspect of the left thigh after having beheaded the malefactor.

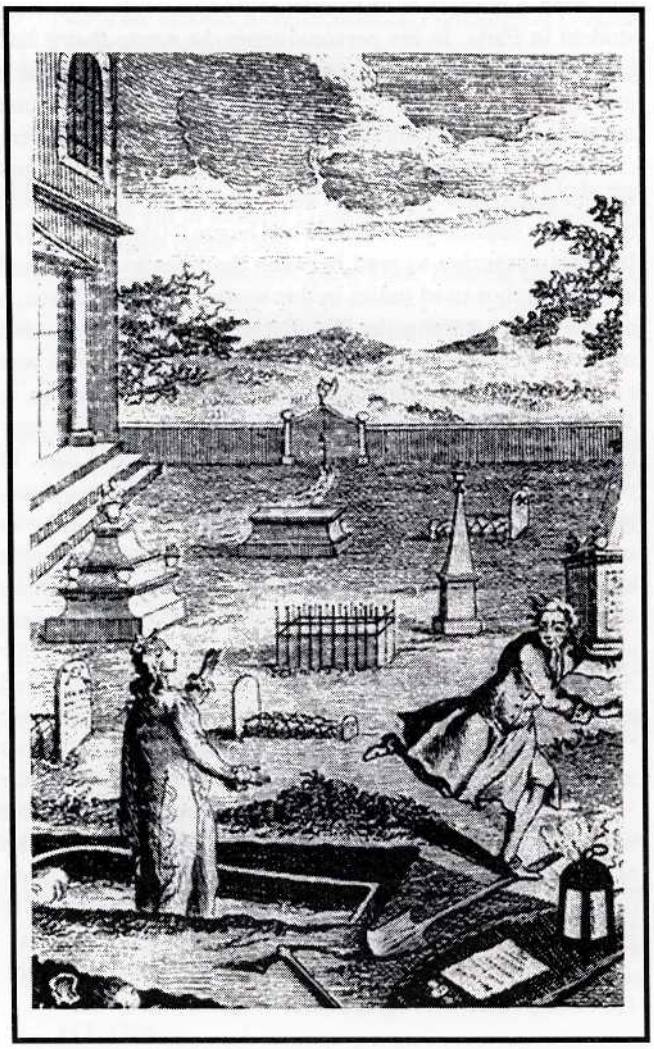

Figure 2. A grave robber who exhumed a prematurely interred person flees in terror (taken from Winslow, 1746).

months in 1830-1831, seven gangs were arrested in London (Desmond, 1989). At that time, the London grave robbers were estimated to be about 200 in number (figure 2). Their only fear was the public hostility, and, to a lesser extent, the law: they found their task increasingly difficult and dangerous, and therefore increased the price of their commodity (French, 1997). Some of the most effective grave robbers were able to exhume discreetly about ten bodies during a single night. It was for them the only way to earn so much money so easily.

Duverney, Haller, Hunter, Knox, Cooper and others

Resorting to grave robbery or body snatching was not a marginal phenomenon. According to Lassek (1958), between 1500 and 2000 bodies were removed annually by grave robbers from the cemetery known as Bully's Acre in Dublin. Dead bodies were exhumed without distinction because it was very profitable: the famous English writer Laurence Sterne died on March 16, 1678. Short after he was buried, his body was removed by grave robbers and delivered to Cambridge where one of his close relations recognized him in the dissecting room (Wolf-Heidegger and Ceto, 1967).

Many celebrated anatomists were involved in these dealings. Guichard Joseph Du Verney, a professor of anatomy at the Jardin Royal, is famous for his contribution to the anatomy of the organ of hearing (Asherson, 1979). For over thirty years (1682-1716), he purchased bodies from gravediggers of the Clamart cemetery in the outer suburbs of Paris," much to the alarm of the population which viewed such spectacles with horror " (Gannal, 1893). Albrecht von Haller, one of the most celebrated anatomists of his time,

had been a witness to the trade of bodies while a medical student in Paris. In his personal diary, he wrote that it had been necessary to pay out ten francs to obtain a body from a gravedigger on October 17,1727 (Hintzsche, 1942). William and John Hunter of the Great Windmill Street School were clients of the grave robbers. In his 1774 masterpiece, William Hunter stated that" the body was *procured* (author's italics) before any sensible putrefaction had begun " (Persaud, 1997). It is not necessary to read between the lines to understand why the author used italics in this sentence. Robert Knox, a pivotal figure among the Edinburgh anatomists, was also involved in the trade of resurrectionists. However, he was exonerated by the Edinburgh columnist Lord Cockburn in 1856: " All our anatomists incurred a most injust and a very alarming, though not an unnatural odium; Dr. Knox, in particular, against whom not only the anger of the populace, but the condemnation of more intelligent persons was specially directed. But tried in reference to the invariable and the necessary practice of the profession, our anatomists were spotlessly correct, and Knox the most correct of them all" (Lonsdale, 1870). In the mid-nineteenth century, Sir Astley Cooper, a very skillful surgeon and dedicated anatomist, obtained illegally some of the bodies he dissected: " Under the encouragement of Sir Astley Cooper and other teachers, who paid high prices for anatomical material, the violation of graves in or near London became a horrible trade " (Ball, 1928). Joseph Constantine Carpue, the founder of the Dean Street Anatomical School, Charles Bell and many others may add to the list of anatomists who turned to graverobbers or to the body snatchers in the last centuries.

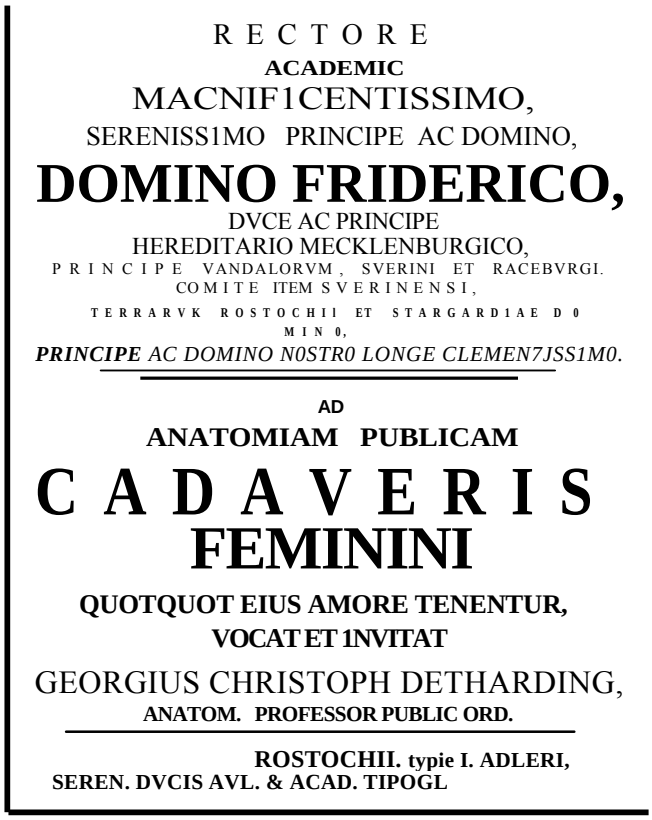
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Figure 3. Invitation to the last public human dissection to be conducted at the Rostock University in 1753 (Schumacher and Wischhusen, 1970, p. 177).

The public's indignation

In the eighteenth century, dissections were conducted not only for medical students, but also sometimes for the public which enjoyed attending human dissections (figure 3). However, the public was outraged by the body snatchers and the grave robbers who removed bodies from their grave: all the more frightening since many people had a phobia about untimely burials (Olry, 1996). In all parts of Great Britain, dissecting rooms were burnt down. In Philadelphia, the opening of an anatomical theatre created great alarm among some of the citizens, and the professor of anatomy and surgery William Shippen had to flee his home more than once in order to avoid bodily harm (Persaud, 1997). In New York City, a riotous mob stormed the New York Hospital for the same reasons, and the " students were confined in the common prison for security against the wild passions of the populace " (Thacher, 1828; cited in Persaud, 1997).

Escalation of horror: Burke and Hare

In the early nineteenth century, William Burke and his companion William Hare were the actors of the " blackest chapter in the black annals of body snatching " (MacGregor, 1884; Drimmer, 1981). They murdered at least sixteen people to supply anatomists with human bodies. The last of these bodies, those of an old woman named Docherty, was discovered in Robert Knox's dissecting rooms by the authorities. In his confession, Burke described how they carried out their heinous murders:" After they ceased crying and making resistance, we left them to die of themselves, but their bodies would often move afterwards, and for some time they would heave long breathings before life went out" (Persaud, 1997).

William Burke was hanged on January 28, 1829, in the presence of a cheering crowd estimated at 30,000 people (figure 4). His body was then dissected by Alexander Monro Tertius who subsequently lectured on his skull and brain from the phrenological point of view (Wright-St. Clair, 1964). His companion William Hare escaped the gallows and returned to his native country (Ireland).

Conclusion

Body snatchers were the result of an inadequacy between

Figure 4. William Burke in his prison cell (Persaud, 1997, p. 181).

the ever growing need for human bodies for dissection, and the impossibility of legally supplying anatomists with enough bodies. Human bodies had a scientific value for anatomists, and a market value for body snatchers: there was something in it for everybody. Without the bodies, which were illegally obtained through grave robbers, the work of many anatomists would not have been possible. The Anatomy Act of 1832 provided an adequate supply of bodies for the teaching of anatomy, gradually putting out of business ressurectionists (Poison and Marshall, 1975): the end stooped to justify the means...

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ABSTRACTS
6th Interim Conference on Plastination
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PLASTINATION - A TEACHING AND RESEARCH TOOL

WeigleinAH Anatomical Institute, Karl-Franzens-University Graz, Austria

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 (silicone, epoxy, polyester) which are subsequently hardened, resulting in dry, odorless and durable specimens. Silicone is used for whole specimens and thick body and organ slices to obtain a natural look. Epoxy resins are used for thin, transparent body and organ slices. Polyester is used for brain slices to gain an excellent distinction of gray and white matter.

The technique consists of four main steps: 1) Fixation, 2) Dehydration, 3) Forced Impregnation, and 4) Hardening. Fixation can be done by almost all conventional fixatives. Dehydration is achieved mainly by acetone because acetone also serves as the intermediary solvent during impregnation. Forced impregnation is the central step in plastination: vacuum forces the acetone out of and the polymer into the specimen. Finally the impregnated specimen is hardened by exposing it to a gaseous hardener (silicone), or by UVA-light and/or heat (polyester, epoxy).

Plastinated specimens are perfect for teaching. Silicone plastinated organs and body parts are excellent teaching tools because they can be grasped literally, they are dry and do not smell and they are almost everlasting. Sheet plastination provides an excellent tool for teaching sectional anatomy.

Particularly the plastination techniques for brain (polyester) and body slices (epoxy) are also used in research, particularly in comparison to CT- and MR-images.

STEP BY STEP INTRODUCTION TO THE BIODUR S10 STANDARD TECHNIQUE OF PLASTINATION

Weber W
Department of Biomedical Science, Iowa State University, College of Veterinary Medicine, Ames, Iowa, U.S.A.

The Biodur S10 standard technique of plastination is useful to preserve all types of teaching specimens in the medical field. It produces specimens that are odorless, dry, durable and lifelike by replacing the water content with silicone polymer. The method is carried out in 4 major steps: Fixation, Dehydration, Forced impregnation and Curing. Fixation with formaldehyde solution deactivates enzymes, halts autolysis, prevents putrefactive odor, and preserves the shape of the specimens. The preferred method of dehydration is freeze substitution with acetone at -19°C. The low temperature fixes the shape of the specimens and minimizes shrinkage. Usually, four bathes of acetone are required to complete dehydration. A hydrometer is used to monitor the acetone concentration. For most specimens an acetone bath at room temperature is recommended for degreasing. Alternately, a series of ethanol bathes allows dehydration at room temperature. Ethanol must be replaced by acetone or methylene chloride prior to the step of forced impregnation. In this step the volatile medium is removed with the aid of vacuum and replaced by a Silicone reaction mixture consisting of Biodur S10 and 1% Biodur Hardener S3. The speed of exchange is controlled by either an inline valve or a bypass valve. The vacuum is adjusted to a slow rising of bubbles in the silicone. This step is usually carried out at -19°C in a freezer in order to keep the reaction mixture reusable. It is considered complete when the manometer reading is 5 mm Hg or less and bubbling has stopped or nearly stopped. The specimens are returned to atmospheric pressure, removed from the silicone bath and warmed to room temperature. They are wiped down and exposed to Biodur Gas Cure S6 inside an airtight container. An apparatus consisting of an aquarium air pump, hose and bottle with Gas Cure S6 speeds up the process by saturating and agitating the atmosphere in the enclosure. Daily wipe downs are required to avoid pooling of oozing silicone. Calcium chloride is used to control moisture. Curing takes from 1 week to 2 months depending on the thickness and type of specimens.

SUBMACROSCOPIC INTERPRETATION OF HUMAN SECTIONAL ANATOMY USING PLASTINATED E12 SECTIONS

CookP,Al-AHS
Department of Anatomy with Radiology, University of Auckland School of Medicine, Auckland, New Zealand

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 utilized 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 delineation of the structural layout *in situ.* 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 utilized 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 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 El2 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.

PRESCRIBED SEQUENCE LABELING METHOD: A STRATEGY FOR MASTERY OF SECTIONAL ANATOMY USING PLASTINATED SPECIMENS

Lane A Triton College,
River Grove, Illinois, U.S.A.

Previous studies (Lane 1998) have demonstrated that a prescribed sequence of labeling method (PSLM) increases the learning rate and comprehension of relationship of structures/features of anatomical photographs acquired from The Visible Human Project. This project was sponsored by National Library of Medicine.

This present study using photographs of plastinated anatomical brain sections correlates and parallels previous studies.

Thirty-two (32) college students enrolled in sectional anatomy participated in this present research. They were randomly separated into two groups 1 and 2. All students were given the same pretest, followed by a study period, and then were issued posttests. The study material for group 1 consisted of an axial brain section, labeled in accordance with the PSLM protocol. Group 2 was issued the identical photograph, labeled randomly. Both groups were instructed to "list and recognize the parts/layers of a transverse brain section, form superficial to deep" by writing their answers in spaces provided as one of two posttests. The second posttest (multiple choice) stressed the adjacent relationship of those same structures. Group 2 was then issued the identical study guide distributed to group 1 initially, and thereafter, a set of posttests. These studies have shown that the PSLM significantly increased a given student's rate of learning and comprehensive of adjacent structures.

Statistical analysis shows a significant difference in group 1 and group 2 on both the written identification of structures and similar questions in a Multiple Choice format.

PSLM in both photographs from The Visible Human project and the photographs from plastinated sections appear to equally accomplish the following

- Learning rate increases
- Relationship of features are comprehended more rapidly and completely
- Well-organized student study system is advanced

For Further Information
Visit the web sites at:

http://www.triton.cc.il.us/inst.depts/biology/NLM
or
www.nlm.nih.gov

PLASTINATION TECHNOLOGY FOR BIOMEDICAL RESEARCH AND STUDIES IN KENYA

Kipchumba Peris Jelagat Kenya Medical Training College, Nairobi, Kenya

In Kenya, preservation of biological tissues is done by embalming and perfusion with formaldehyde and gluteraldehyde. Plastination method was described in 1978, for preservation of tissues in a life-like state. It involves impregnation of biological tissues with a polymer which replaces water and lipids. The information on plastination in the literature is scanty and the method has been used recently in America and Europe.

From a pilot survey, it was found out that awareness of plastination method was very low amongst the Kenyan scientists. Incidentally, some international scientists were also not familiar with it. Compared with conventional methods, where fixation may be inadequate and long term preservation is poor, plastination maintains original morphological and histological integrity of tissues allowing them to be used for research as well as preservation of specimens for long term use. Fumes from acetone and fixatives pose a hazard in conventional and plastination methods. Special equipment is needed for plastination method.

In Kenya we have available human and animal cadavers which are preserved using conventional methods but are replaced when necessary. However, there is need for the knowledge on alternative methods with wide application in biomedical studies such as plastination. This method may also be used for long-term preservation of rare tissues and morphometric studies.

The advocacy of this method of plastination has been gradual in developed countries. The extent to which it may make an impact in biomedical field in developing countries will depend on cost effectiveness and feasibility of implementation as well as provision for training of personnel.

EFFECT OF LEAD ON THE PRENATAL DEVELOPMENT OF THE SPINAL CORD OF RABBIT (LUMBOSACRAL REGION)

El-Mogny Gabr MA, Mohammed RS, Hassan SA, Mohammed DA-A
Department of Anatomy, Assiut College of Medicine, Assiut, Egypt

The histopathological investigations of lead on the developing central nervous system including the spinal cord are scanty. Moreover, investigations about the effect of lead on the prenatal development of CNS in mammals are still deficient.

The aim of this study is to throw light on the normal prenatal development of the spinal cord in Boscat rabbit and to investigate the effects of lead on it.

For this work one hundred sixty rabbits (160) were taken at 12, 14, 16, 18, 20, 24, 26 and 28 days of gestation and newborn rabbits. They were divided into control groups and experimental groups. The samples were prepared for both light and microscopic examinations.

The control groups showed that the development of the spinal cord passes into the following stages.

1. Stage of differentiation of cells of the ventral horn from the germinal epithelium.
2. Stage of appearance of cell columns.
3. Stage of spliting of cell columns into subsidiary columns.
4. Stage of appearance of Nissil granules.

The experimental group showed that the manifestations on the spinal cord development were delayed development, distortion of arrangement of cells, and pericapillary oedema. Experimental group also showed that lead has an effect on cell organelles and nuclei of motor cells, astroglial cells and endothelial cells of blood capillaries.

PLASTINATION AS A CLINICALLY BASED TEACHING AID AT THE UNIVERSITY OF AUCKLAND

CookP
Department of Anatomy with Radiology, University of Auckland School of Medicine, Auckland, New Zealand

As a concerted move toward closer integration of the clinical and pre-clinical aspects of the undergraduate medical curriculum at the University of Auckland, the Department of Anatomy has established a formal link with the Department of Radiology resulting in a structured program of clinically based teaching of gross anatomy to second and third year medical students and several post graduate specialist training programs.

As sophisticated diagnostic techniques and methods of treatment have now become common place, our teaching program has been tailored to accommodate a greater degree of case based learning within the undergraduate course.

Dissecting room demonstration is provided by Radiology, Pathology, Ophthalmology and Surgical registrars, with a number of clinical procedures, pathological observations and diagnostic methods employed during the routine dissection of the cadaver.

The learning process is enhanced and aided on a number of levels through careful integration of several key plastination techniques, yielding a high level of anatomical detail and clinically relevant information in a readily accessible form.

While S10 silicone, P.E.M. polymerised emulsion and E20 injection casting all provide an excellent three dimensional concept of the human body, it is the El2 epoxy method of producing serial sectioned anatomy that has offered significant educational and research opportunities. The E12 epoxy technique has allowed an accurate and highly detailed orientation of the planes of the body, providing the student with a clearer understanding of anatomical structures and pathological anomalies as seen with modern imaging techniques.

VENTRICLE CASTS IN S10 PLASTINATED HUMAN BRAINS

Grondin G, Sianothai A, Olry R
Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada

The aim of this study was to provide students with plastinated specimens showing the three dimensional features and content of the rhombencephalon, mesencephalon and prosencephalon ventricles. The brains were removed from fixed bodies used in dissection course, and stored in 10% formalin at +4°C. To perform the filling of the ependymal cavities with gelatin and silicone, 6 cannulas were carefully introduced through the cortex into the anterior, inferior and posterior cornu of the lateral ventricles. One more cannula was introduced into the fourth ventricle between the posterior aspect of the medulla oblongata and the cerebellum. Injection of water via the cannulas allowed to ascertain that they were in good position. Filling of the cavities was performed either with 10% gelatin mixed with acrylic staining, or with a mixture of S10/S3/S6/S2 died with Biodur colorpaste. Injection was performed via both occipital cannulas until the filling material overflowed via the other five cannulas. The cannulas were then clamped, and the brains stored in cold water (+4°C) for 72 hours before being dissected. For the duration of the dissection, the brains were kept in 10% formalin. The lateral portion of the cerebral hemispheres was

Continued on page 28

partly removed to expose both lateral ventricles and their choroid plexuses. The floor of the third ventricle and one cerebellar hemisphere were also removed to expose both third and fourth ventricles, respectively. The specimens were finally dehydrated by freeze substitution and plastinated according to the standard S10 technique.

OLD ANATOMICAL EMBALMING & PREPARATION METHODS

WadeR
School of Medicine, University of Maryland, Baltimore, Maryland, U.S.A.

This presentation will focus on historical embalming and specimen preparation methods used to prepare and preserve medical teaching specimens. At the turn of the past century, every school had its preparators, who developed various formulas and methods to accomplish the teaching needs. I will describe various solutions and formulas used and methods used to enhance the student's study of gross anatomy. Some of the methods and material are still found in use today, while others have been avoided as health risks. New methods have been developed, such as polymer impregnation of specimens, i.e. Plastination, that have greatly improved the specimen quality.

PLASTINATION OF HUMAN CADAVER LIMB SPECIMENS USED FOR VALIDATION OF MAGNETIC RESONANCE IMAGERY (MRI) AND COMPUTERIZED TOMOGRAPHY (CT) MEASUREMENT OF HUMAN SKELETAL MUSCLE

Lyons GW[1], Mitsiopoulos N[2], Ross R[2]^Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada
[2]School of Physical and Health Education, Queen's University, Kingston, Ontario, Canada

The primary objective of this study was to plastinate cadaver limb sections used for the validation of magnetic resonance imaging (MRI) and computerized tomography (CT) measurement of skeletal muscle. Two limbs (one arm and one leg) were removed from embalmed cadavers, stabilized and fitted with internal orientation markers in preparation for imaging. Contiguous MRI and CT images were obtained every 1 cm over the entire length of each limb. These specimens were subsequently frozen in liquid nitrogen and sectioned at levels corresponding to each of the scans (119 sections). Following sectioning the specimens were carefully cleaned and photographed. Most of the sections were then plastinated using the S10 Biodur polymer method. The plastinated specimens retained excellent detail and provided reference necessary when confirming the identification of various tissues represented in the photographs of the unplastinated specimens. In addition to their utility for reference purposes, the plastinated cross-sections have been used with great success as teaching aids for gross anatomy courses in the Department of Anatomy and Cell Biology.

PLASTINATION OF HUMAN TISSUES FOR USE IN MEDICAL TEACHING

Riederer BM,Dörfl J
Institut de Biologie Cellulaire et de Morphologie, University of Lausanne, Lausanne, Switzerland

The use of silicone has proven a valuable tool to preserve prosected and sliced tissue preparations for demonstrations, dissection courses and for a presentation in lectures. Human corpses were fixed by 17L of a perfusion solution (2% formaldehyde, 2.5% phenol, 5% glycerol, 22% alcohol) at room temperature. Body parts were prosected or frozen at -20°C and sectioned with an electrical band saw into slices of l-2cm thickness. These preparations were siliconized by the S10 silicone procedure described by G. von Hagens (Plastination Folder, Anatomisches Institut, Heidelberg, 1985). The samples were dehydrated at room temperature by increasing alcohol concentrations, and kept for 24 hours in acetone at 4°C. Tissues were completely dehydrated by several incubations in acetone at -20°C. Samples were kept for 24 hours in silicone at -20°C, and subsequently acetone was substituted by silicone using vacuum penetration. The pressure was lowered to 0.002 bar by maintaining a constant and light bubble formation. Specimens were polymerized with a volatile hardener S6 at room temperature. Human brain tissue, prior to plastination was colored by a Prussian blue reaction as described by Le Masurier (Arch Neurol Psychiat 34: 1065, 1935) where gray matter is colored in a Prussian blue tone. The use of plastinated tissue has already proven a valuable tool to be added to the dissection courses and demonstrations, given those specimens can be used over years without any tissue detriment. The dry state of specimens allows their introduction in lectures without having to take too many precautions. A video set-up in the lecture hall allows on-line presentation of specimens. Another advantage is that students and faculty can borrow plastinated tissues for individual learning and studies outside the dissection facilities. From the best samples digitized pictures are currently introduced on the server of our institute (Intranet) which is accessible to faculty and students providing another tool for an individual preparation of courses and exams. However, by making human tissue preparation accessible to a broader audience via Internet some ethical considerations are necessary, such as what and how to present samples, and how to prepare potential viewers to what they will see.

Nevertheless, the Internet allows also to exchange material with other institutions developing similar tools for gross anatomy teaching.

DEVELOPMENT OF TEACHING MODULES FOR HUMAN ANATOMY

Hunt R, Beam R, Brown C, Pang SC
Department of Anatomy and Cell Biology, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada

Self-directed learning has been frequently used as a mode of teaching anatomical subjects in the medical and undergraduate curriculum in many medical schools in Canada. In order to aid the students in learning and reviewing their course materials at their own pace without increasing time commitment of instructors or tutors, we have developed a number of modules for Gross Anatomy teaching. Well dissected wet and plastinated human specimens were used to produce teaching modules. Images showing various aspects and angles of the specimens were captured with a digital camera (Agfa; model e-photo 1680). For wet specimens it was necessary to remove all storage fluid in the specimens prior to photography. Images were stored as "TIF" files, and they were organized and labeled by Power Point software with a Pentium personal computer. Simple animation is possible with this program. We have produced teaching modules for teaching the heart, hand and urinary system and found that this is a very cost-effective way of producing custom-made teaching modules for various courses at many levels of education, i.e. high school, undergraduate and graduate studies. Provided there are enough manpower and infrastructure support in the institute, these teaching modules can be produced with very little cost (approximately $10-15,000). It is also possible to include a number of other media for presentation, e.g. charts, x-ray films and histology slides if a scanner is available. Images of the teaching modules can be printed out as projection slides, overhead transparencies or handouts. Examination questions can be made from these images as well. In conclusion, teaching modules are an efficient and effective way to educate students in programs using self-directed learning as a mode of delivery.

PLASTINATED WHOLE ORGAN SECTIONS AND BONE DENSITY MEASUREMENTS OF THE MIDDLE FACE

Menzler A[1], Sprinzl GM[2], Eckel HE[1], Sittel C[1], Koebke J[3], Jungehiilsing M[1]
^Dept. of Oto-Rhino-Laryngology, University of Cologne, Germany
[2]Dept. of Oto-Rhino-Laryngology, University of Innsbruck, Austria
[3]Dept. of Anatomy, University of Cologne, Germany

Introduction: Modern imaging techniques and the rapid development of cranial endoscopic surgery techniques increase the demand for accurate knowledge of the human skullbase and paranasal sinuses anatomy.

Serial sectioning after embedding with epoxy resins allow for artefact-free sectioning of tissues with marked differences in hardness, as found in the skull-base region. Subsequent to processing, quantitative and qualitative evaluations are delivered via imaging analysis systems.

Materials and Methods: Thirty cadaver heads were used for this study. The fixed specimens were watered, shockfrozen and subsequently dehydrated in cold (-30°C) acetone. The dehydrated specimens were embedded in a mixture of Biodur E12 and El. The resulting blocks were cut into 1 mm thick plane-parallel sections using a diamond wire saw (Firm Well, Mannheim, Germany). By adjusting direction, it is possible to cut the blocks into frontal, axial and sagittal planes. Material loss was not more than 0.3 mm per section. Radiograms of the sections were scanned into a computerized image analyzing system. Finally, data were calibrated against an aluminum wedge reference and then photographed from the monitor. The bone density decreases were colour-coded on a constant scale of red, yellow, green and blue. Another part of the sections were coloured with a special bone colour mixture for later histologic studies.

Results: The architecture of the orbit is shown under qualitative and quantitative aspects. Weak points of the osseous structure are revealed. Bone densities were highest in the region of the supra and infraorbital margins. Additionally, bone density was higher in the lateral wall than in the medial counterpart. Minimum bone densities were located in the cellulae ethmoidales, the orbital floor and in the infraorbital channel. The region of the lamina papyracea is an additional point of weakness and frequently involved in fractures. The dorsal wall of the orbit cannot be assigned to either group.

Summary and discussion: Bone density as well as morphology of fronto-basal and ethmoidal areas are clearly depicted on plastinated specimens. Weak points of the bony structure of the orbita are shown in detailed densitograms. The results provide a detailed and thorough impression of the human skull anatomy. Knowledge of these structures is very important for an early and exact evaluation of traumatic lesions of the midface and for precise planning of endoscopic minimal invasive surgery via transnasal approach.

PLASTINATION IN NEUROANATOMY

WeigleinAH Anatomical Institute, Karl-Franzens-University Graz, Austria

Brain slices may be produced by both the S10 standard plastination technique and the P35 or P40 techniques.

The S10-standard technique is mainly used to plastinate whole brains and brain prosections. The plastinated brains can be sliced after final curing by means of a meat sheer or band saw. This results in smooth surfaces of the slices and thus slices are exactly adjacent to each other. Moreover, these

slices show good differentiation between gray and white matter, due to freezing and thawing during the S10 standard procedure. This technique allows to produce brain slices from 0.5 mm up to several centimeters. Therefore slicing S10 plastinated brains is much better than plastinating pre-sliced brain slices.

For the S10 technique I recommend to add the following steps to the standard procedure: Before starting the forced impregnation start with an immersion period. During this immersion-step the brains are immersed in the S10/S3 mixture for several days at -20°C. The longer the immersion time, the shorter the needed impregnation time will be. Moreover, this will also minimize the shrinkage of the brains. After curing is completed the brains are cut into slices of the desired thickness.

Polyester (P35 or P40) plastinated brain slices provide an excellent tool for teaching and research, because the differentiation between cortex, nuclei and fiber tracts is superior to all other techniques. The thickness of the slices can vary between 4 and 8 mm.

The polyester-procedure consists of the following steps: Fixation - Slicing - Flushing - Dehydration - Immersion - Forced Impregnation - Casting - Curing. P40 impregnated brain slices are cured by UVA-light only. For curing of P35 brain slices a well ventilated heat cabinet is needed. After a short light curing period P35 slices are finally cured at 40 - 50°C.

SILICONE CASTING OF THE AIRWAYS OF THE LUNGS

Grondin G[1], Lane A[2], Henry RW[3] Reed RB[3], Hromis G[3]
^Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada
[2]Triton College, River Grove, Illinois, U.S.A.
[3]College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, U.S.A.

The study of normal anatomy of the lungs is enhanced by viewing tracheobronchial casts. To aid understanding of the branching of the tracheobronchial tree, silicone casts of the lung airways were made from intact lung specimens harvested fresh from various cadavers. Excess tissue was removed and the trachea was cannulated. Lungs were flushed via the trachea with tap water to remove mucous and blood from the airways. Excess water was allowed to drip from the lungs and pressurized air was hooked to the tracheal cannula. Enough air flow and pressure were used to keep the lungs inflated to mimic normal inspiratory, anatomical position until the lungs were dry. Drying time was about 24 hours. After drying, Silastic E RTV polymer (Dow Corning, Midland, MI, 48640) or silicone P45 RTV polymer (Silicone, Inc., High Point, NC, 27261) was mixed with its hardener (10:1 ratio). The reaction mixture was syringed into the airways via the tracheal cannula. Filling was judged complete when the silicone could be seen through the lung parenchyma filling the small airways. The silicone was allowed to harden overnight. After hardening of the silicone, lung tissue was first macerated using boiling water and then completed in 5% hydrogen peroxide. Durable, anatomical duplicates of the conduction system of the lungs were produced.

MEDICAL MUMMIES: THE BURN'S MUSEUM COLLECTION

WadeR
School of Medicine, University of Maryland, Baltimore, Maryland, U.S.A.

At the University of Maryland's School of Medicine, there is a unique collection of human anatomical teaching specimens. Prepared by the eminent Scottish anatomist Burns in the late 1700's, today they exist as medical mummies and specimens, delicately detailing structures. Some of the specimens were prosected with special vascular casts and other unknown methods were used to create and preserve teaching specimens in the early era of medical education. A discussion of the collection's origin, history, and preparation methods will be discussed and a pictorial review of the collection will be presented.

SILICONE CASTING OF THE AIRWAYS OF THE LUNGS AND THE PULMONARY VESSELS

Henry RW[1], Reed RB[1], Grondin G[2], Hromis G[1], Lane A[3]
iCollege of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, U.S.A.
[2]Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada [3]Triton College, River Grove, Illinois, U.S.A.

The study of lung vasculature and its relationship to the airways of the lungs is important. To aid understanding of the pulmonary vasculature and its relationship to the bronchial tree, silicone casts of the lung vasculature and airways were made from fresh en bloc heart and lung specimens from various cadavers. The caudal (inferior) vena cava, the left auricle and trachea were cannulated for flushing the heart and lungs and for later injection of the silicone. Excess water was allowed to drip from the specimen and the aorta and remaining transected vessels of the heart were ligated. Silastic E RTV silicone polymer (Dow Corning, Midland, MI, 48640) or silicone P45 RTV polymer (Silicone, Inc., High Point, NC, 27261) was mixed with its hardener (10:1 ratio) and colored using either blue or red color paste. The colored mix was injected into the cardiac chambers and hence into the pulmonary vessels via the cannulated vena cava and left auricle. Filling was judged complete when the silicone could be seen beneath the surface of the lung filling the small

vessels. To assure that the silicone injected vessels hardened in proper anatomical position, pressurized air was introduced via the tracheal cannula. Enough pressure and air flow was used to keep the lungs inflated in normal inspiratory anatomical position until the lungs were dry and the silicone had hardened (24 hours). The airways were then filled with white or clear silicone mix via the tracheal cannula. Filling was judged complete when the silicone could be seen through the lung parencyhma filling the small airways. After the airway silicone had hardened, tissues was macerated first in boiling water and then complete in 5% hydrogen peroxide. Durable, anatomical duplicates of pulmonary vasculature, airways, cardiac chambers and great vessels were produced. These served as an anatomical tool for understanding the relationship between the pulmonary vasculature and the airways.

ANATOMICAL BASIS FOR THE ENDONASAL APPROACH TO THE NASOLACRIMAL DUCT

Weiglein AH, Feigl G, Wolf G, Muellner K, Szolar D
Anatomical Institute, Karl-Franzens-University Graz, Austria

The main parts of the tear conducting system are the lacrimal sac and the nasolacrimal duct. The membranous duct extends from the lower part of the lacrimal sac to the anterior part of the inferior meatus of the nose. The duct is contained in an osseous canal, formed by the maxilla, the lacrimal bone and the inferior concha. The direction of the lacrimal canal is described by a line drawn from the medial corner of the eye to the second upper molar. It is directed downwards, backwards and laterally or straight downwards.

Stenosis of the lacrimal drainage system leads to epiphora. To improve lacrimal drainage in such cases of dacryostenosis the lacrimal drainage system can be connected to the nasal cavity by a stoma. This procedure, called dacryocystorhinostomy, is usually performed via a skin incision on the lateral aspect of the nose. An endonasal procedure, however, would help to avoid scars. For an endonasal dacryocystorhinostomy the thickness of the surrounding bones is of importance. In order to determine the easiest approach to the nasolacrimal duct we measured the thickness of bone in both plastinated horizontal slices and CT-scans of the nose.

We subdivide the lacrimal drainage system into three parts: 1) the lacrimal sac, 2) the upper part of the nasolacrimal duct lateral to the middle nasal meatus - the meatal part - and 3) the lower part lateral to the attachment of the inferior concha - conchal part.

We found that the surrounding bony structures are of different thickness. The thinnest part of the osseous canal is in its posteromedial quarter and in its lateral aspect adjacent to the maxillary sinus. For endonasal approach only the posteromedial quarter of the meatal part is of relevance. In this part the bony wall of the nasolacrimal canal measures 1 mm and less in thickness. In the meatal part of the right nasolacrimal canal the thinnest wall is in between 5 and 9 o'clock. Further down in the conchal part the thinnest part of the wall wanders a little bit forward, so that it is between 7 and 10 o'clock in the lower third of the nasolacrimal canal.

INJECTING URETERS AND RENAL VESSELS WITH RTV SILICONE FOR PLASTINATION OF THE KIDNEY

Henry RH[1], Reed R[1], Hromis G[1], Grondin G[2], Lane A[3]
^College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, U.S.A. 2Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada
[3]Triton College, River Grove, Illinois, U.S.A.

The study of normal anatomy of the kidney is enhanced by silicone injection of the vasculature and the ureter. Bovine kidneys were obtained from the slaughter house. The perirenal fat was carefully removed and the renal vessels and ureter were isolated and cannulated. Cannula size was selected so that a catheter-tip syringe would fit snuggly into the cannula. Regular syringes for injection have a much smaller outlet which makes it difficult for the silicone mix to be pushed out of the syringe into the kidney. Silastic E RTV polymer (Dow Corning, Midland, MI 48640) was mixed with its hardener (10:1 ratio). The mixture for the vessels was colored using either a red or blue color paste. The colored silicone mix was injected into the renal vessels and the non-colored mix into the ureter. Filling was judged complete when silicone could be seen through the parenchyma, when back pressure on the syringe increased markedly or when silicone ruptured to the surface. After injection, the specimen was submerged in cold 5% formalin solution and the silicone allowed to harden overnight. Later the kidney was prosected to show desired internal anatomy. Freeze substitution was used to dehydrate the specimen and impregnation was via the standard S10 procedure.

SHEET PLASTINATION OF BRAIN SLICES - P35 PROCEDURE

Weiglein AH[1], Henry RW[2], Lyons W[3] 1 Anatomical Institute, Karl-Franzens-University Graz, Austria [2]College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, U.S.A. [3]Queens University, Kingston, Ontario, Canada

The P35 procedure is used to produce semitransparent, thin (4 - 8 mm) brain slices. The brain is fixed thoroughly with formalin for several weeks and then sliced. The slices are rinsed of formalin and cooled to 5°C prior to submerging in cold acetone for dehydration by the freeze substitution method. After dehydration is complete, the brain sections

are immersed in two successive reaction mixture (P35/A9, 100/2) bathes. After the two immersions are complete, they are impregnated with the polyester reaction mixture. After impregnation is complete, the brain slices are placed in a flat chamber. The flat chamber is fashioned from four sheets of glass (two regular glass and two tempered glass), an appropriate diameter gasket (2mm thicker than slice) and clamps. The plastinated brain slice is placed on a double layer of glass. The gasket is placed on the glass, the second set of glass is placed on the gasket and finally clamps are placed around the perimeter of the apparatus. The flat chambers are set upright and filled with the polyester polymer mixture (P35/ A9). Hence, the plastinated slices are incorporated into sheets of the plastination resin. The sheets containing the slices are cured initially using ultraviolet light from UVA lamps and finally in a 45°C oven. They are not merely embedded in the resin. The specimens show marked delineation of white and gray matter and are durable. Using this technique, brain slices show more detail, are more durable and easier to handle than those produced with other techniques.

EDUCATION OF A WIDER COMMUNITY WITH PLASTINATED SPECIMENS

Gubbins B, Ford S
Department of Pathology, Queen's University, Kingston, Ontario, Canada.

Anatomists led the way in using plastinated human tissues for teaching their students, followed by Pathologists and Veterinarians. We have reviewed how the scope of use of these specimens has developed over the last ten years.

With material from hospital autopsies in which permission to use organs for teaching was granted, we have built a teaching museum consisting of almost 900 specimens. The specimens consist of case groupings and individual organs of interest, as well as normals of each organ that are essential for comparison. The museum is indexed and cross reference by organ, disease and case type, both electronically and in hard copy. The museum is locked for security, and specimens have to be signed in and out.

In the first two years of operation, the specimens were used by pathology staff for teaching of undergraduate medical students in seminar settings. Due to the popularity of these demonstrations, each year has shown a wider audience starting with education of allied health professionals and the Hospital Board of Governors.

A meeting with the Heads of the local School Board Science Departments paved the way for use of non-human plastinated specimens in science classes, which was followed by the loan of human specimens. Other school events have included a medical student organized health outreach project, and an annual demonstration to high school law class. Other clients are the local Health Unit who use specimens on long term loan for smoking cessation classes and prison health educators who use the specimens in anti-drug and smoking education.

We continue to seek ways in which we can expand the use of plastinated specimens for education, reaching out into the community, from whence after all, the material originated.

SHEET PLASTINATION OF BRAIN SLICES - P40 PROCEDURE

Henry RW1, Weiglein AH[2] 1College of Veterinary Medicine, The University of Tennessee, Knoxville, Tennessee, U.S.A. 2Anatomical Institute, Karl-Franzens-University Graz, Austria

The P40 procedure is a newer version of the classic P35 technique. Both are used to produce semitransparent, thin (4-8 mm) brain slices. The brain is thoroughly fixed with formalin for an appropriate period and then sliced. Formalin is rinsed from the slices using running tap water. After rinsing, the slices are cooled to 5°C in preparation for dehydration by the freeze substitution method. The slices are submerged in cold acetone which is changed twice at two day intervals. After dehydration is complete, the brain sections are submerged in the polyester polymer for twenty four hours prior to plastination with the polyester reaction mixture (P40). The slices are impregnated either in the cold or at room temperature and in the dark. After impregnation is complete, the brain slices are placed in flat chambers. These flat chambers are fashioned from only two sheets of regular glass (2 mm), an appropriate diameter gasket (2mm thicker than slice) and clamps around the perimeter of the chamber. The flat chambers are set upright, a slice is inserted and the chamber filled with the polyester polymer (P40). Hence, the plastinated slice is incorporated in the plastination resin and is not just embedded in the resin. The sheets are cured using ultraviolet light from UVA lamps. The specimens show marked delineation of white and gray matter and are durable. Using this technique, is a less expensive. Since only one polymer immersion is used, less polymer is required. There are no additives to the P40 polymer and no tempered glass is needed.

MACROSCOPIC INTERPRETATION OF THE DISTAL PART OF THE EQUINE FORELIMB BY USING PLASTINATED SECTIONS (S10 AND COR-TECH PR-10)

Latorre R, Vazquez JM, Gil F, Ramirez G, Lopez-Albors 0, Arencibia A, Moreno F
Departamento de Anatomia y Embriologia, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain.

Six horse digits (distal part of the forelimb) were removed from fresh cadavers. All specimens were injected on both arterial and venous sides. The fetlock joint was

injected by its palmar pouch, and the pastern and coffin joints were injected by their dorsal pouches. The digital sheath was injected between the tendons of the deep digital flexor and the superficial digital flexor. The arteries (aa.), veins (vv.), and synovial formations were injected with red, blue and green colorated latex, respectively. The injection was done with normal manual pressure with a 10 to 20 ml syringe. Specimens were frozen and cross-sectionally sliced with a band saw (1 to 1,5 cm thickness), in a direction to the distal surface, lied from the carpal joint to the hoof. Twenty sections of each horse digits were plastinated according to the standard S10 and COR-TECH PR-10 techniques. Students are using them in the practical lectures with high didactic success. The quality of the material and differentiation of the anatomical structures are quite optimum. This fact let us give to undergraduate students and veterinary professionals a clear and accurate overview of the extension, limits and relationships of anatomical complex structures as the dorsal and palmar pouches of fetlock, pastern and coffin joints; the navicular bursa; the digital sheath; the interosseus "muscle"; the hoof cartilage; the digital cushion; the coronary and laminar dermis; palmar, metacarpal and digital aa. and vv.; lateral and medial palmar nerves, etc. These sections, highly appreciated by veterinary professional because their applicative use, can be correlated with other modern diagnostic imaging techniques such as radiology, CT and MRI.

POSTNATAL DEVELOPMENT OF THE HUMAN PARANASAL SINUSES

Weiglein AH,FeiglG Anatomical Institute, Karl-Franzens-University Graz, Austria

The human paranasal sinuses are the maxillary sinus, the sphenoidal sinus, the frontal sinus, and the ethmoidal air cells. The development of these paranasal sinuses starts during the 10^{h} and 12^{th} fetal week building the ethmoidal air cells. Some ethmoidal cells expand beyond the margins of the ethmoid bone, thus, forming the other sinuses.

To determine which sinus are present at birth ten newborns' heads were plastinated with Biodur™ S10. After plastination five heads were sliced horizontally and five were sliced coronally.

The slices showed that the ethmoidal air cells were all present at birth, however, they were separated by relatively thick connective tissue. Also the maxillary sinus is present at birth. It has the size of a coffee bean with the sagittal expansion bigger than the transversal expansion. A frontal or sphenoidal sinus has not been found in any of the newborns.

Further investigations in 150 skulls of children from newborn to fifteen years showed that the frontal sinus starts to develop in the fourth postnatal year and the sphenoidal sinus starts in the sixth or seventh postnatal year.

The conclusions are:

1) An infant may suffer from maxillary and/or ethmoidal sinusitis; the latter may be dangerous for the eye.

2) The variability in the pneumatization of the sphenoid body and the frontal squama may be due to the late development of the respective sinuses.

PREPARATION AND PLASTINATION OF A SPECIMEN TO DEMONSTRATE THE COURSE OF THE HUMAN FACIAL NERVE

Durand M[1], Grondin G[2], Olry R[2] [1]Laboratoire d'Anatomie, Faculte de Mededne J. Lisfranc, Saint-Etienne, France
[2]Departement de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Quebec, Canada

The head of a 68 year old male was sectioned and injected via both common carotid arteries with 20 ml on each side with a mixture of S10 / S3 / S2 / S6 / AC50. It was kept at 4°C for 48 hours before being frozen at -25°C. The head was sagitally sectioned and stored in a modified Kayserling solution containing *5%* formalin. Dissection of the facial nerve was performed on the left side by a retrolabyrinthin approach and a squeletization of the facial canal. The superficial lobe of parotid gland was removed and the dissection of the facial branches was performed. The sternocleidomastoideus and the platysma were partially removed. The specimen was then dehydrated by freeze substitution and plastinated according to the standard S10 procedure. This plastinated specimen shows the course of the facial nerve from the geniculate ganglion to its ending.

A NEW PROCEDURE IN FINISHING MEDIUM THIN PLASTINATED TISSUE SLICES USING THE BIODUR S10 TECHNIQUE

de Jong KH, Meijer JH, Vinkeles P, Gihaux R
Department of Anatomy & Embryology, Academic Medical Center, Amsterdam, the Netherlands

Medium thin tissue slices are of great value for teaching medical students sectional anatomy in combination with CT and MRI scans because they show depth and allow us to follow structures throughout the specimen. After getting experienced with the plastination of whole organs and larger parts of the human body using the Biodur S10 technique we wanted to produce medium thin slices (1 cm) of the human body using the S10 technique. Major difficulty using the S10 technique was the finishing of the surfaces of the fully cured slices.

Slices were made from the head of a cadaver, fixed and used for normal anatomical dissection courses. After freezing sections were made with a band saw. Dehydration with

acetone and impregnation was performed using the Standard technique described by von Hagens (1978). Impregnated slices were placed onto a glass plate covered with plastic foil. The edges of the foil were lifted, forming a box with the specimen in it. The box was filled with Biodur S1O/S3 mixture until the specimen was submerged and slow-curing was performed.

After curing a block of silicone was obtained, with one side of the slice at the outside of the block. This side was attached onto a flat surface, the other side of the slice (covered with silicone) was carefully milled, using a top-milling machine in a supporting frame to insure that the upper side of the block became exactly parallel with the underside of the block. In this way the silicone covering the slice was removed, hardly any tissue was removed of the slice. If necessary the procedure was repeated at the other side of the slice. Remaining silicone at the circumference was peeled of.

The slice was then attached onto a large wooden shelf, using several steel nails without head. A heavy belt sander was placed on the slice, supported by adjustable wheels wide away from the slice resting on the underlying shelf, insuring the belt sander stayed parallel with the shelf and the surface was polished. This procedure was repeated at the other side of the slice producing two very smooth and exactly parallel surfaces. Loss of tissue was less than 1 mm.

THE RESTORATION OF ANATOMICAL AND ARCHAEOLOGICAL SPECIMENS USING THE S10 PLASTINATION METHOD: WITH SPECIAL REFERENCE TO PRESERVING THE GOOD HEART OF A GOOD PRIEST

Wade R[1], Lyons W[21]School of Medicine, University of Maryland, Baltimore, Maryland, U.S.A.
department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada

Plastination is a process that permanently preserves biological materials using curable polymers that render the tissues and whole specimens dry, odourless and durable. The process involves fixation, dehydration, impregnation and curing. Plastinated specimens retain their original surface relief and cellular identity.

On January 29,1828 his Excellency, the Most Reverend Archbishop of Baltimore, Ambrose Marechal died of distressful asthma. In his will he bequeathed his heart to St. Mary's Seminary. Enshrined in a glass reliquary, it was preserved in whisky and rested in the seminary chapel until 1925. Recently rediscovered within the archives, the Baltimore Archdiocese released the heart (relic) specimen to the Anatomical Service Division of the University of Maryland School of Medicine for plastination. The 166 year old post-mortem heart specimen was received at the lab in June 1993. The specimen's appearance was dark; the tissue was hard, showed signs of shrinkage, and evidenced an odour of distilled alcohol (whisky).

The specimen's treatment began with immersion in a bath of 5% hydrogen peroxide for one week, resulting in a markedly lighter colour and hydration to a more normal appearance and shape. Plastination commenced with freeze substitution in 100% acetone followed by dehydration in three baths of 100% acetone over a 30 day period at -28 degrees Celsius. Forced impregnation in S10 silicone polymer followed. After 7 days the heart was removed from the silicone vacuum chamber, reoriented to its natural shape using polyethylene plastic film for packing the cavities and gum rubber bands to hold the shape, and placed in a curing chamber. Following the curing Archbishop Marechal's heart was now permanently preserved and was returned on October 5, 1993 to be reinstalled in a new reliquary at the St. Mary's Seminary.

The above account outlines one particular use of the S10 technique in restoring anatomical material that may or may not have been properly preserved at the onset of fixation. This technique not only lends itself to the restoration of anatomical materials, but may also play a significant part in preserving archaeological remains such as bone (animal or human), skins (leather) or wood.

This presentation will outline some of the uses and results obtained when applying the S10 plastination technique for these purposes.

PLASTINATION OF THE AORTA FOR USE IN THE TRAINING OF PHYSICIANS IN THE TECHNIQUE OF VASCULAR CATHERIZATION

Tamburlin J, Guterman LR, Sharma M, Minniefield W
Departments of Anatomy and Cell Biology, State University of New York at Buffalo, Buffalo, New York, U.S.A.
and Neurosurgery, Millard Fillmore Hospital, Buffalo, New York, U.S.A.

The training of clinicians in the technique of vascular catherization has been greatly enhanced through the use of anatomically correct plastinated aortic specimens produced in our laboratory. These human teaching specimens have an advantage over other synthetic materials which have been designed to mimic the vascular system in that they are more representative of both the normal anatomical features of the aorta and its branches, as well as pathological changes that may be encountered in performing this technique on patients in the clinical setting.

The aortic specimens were processed using a modification of the procedure outlined by Corcoran Laboratories, Inc., Bay City, Michigan. Previously fixed cadaveric aortic specimens were carefully dissected ensuring that the branches of the aortic arch, as well as the renal and

iliac branches of the descending aorta, were preserved. The vessels were packed with gauze to aid in maintaining the correct anatomical alignment and to prevent their collapse. Specimens were then dehydrated in three changes of acetone at -25°C. They were then immersed in COR-TECH PR-10 preservation polymer containing 3% COR-TECH CR-22 crosslinker. To enhance impregnation, specimens were processed in a vacuum chamber for two days. Following impregnation, the gauze packing was removed from the vessels and the preservation process was completed using COR-TECH CT-32 catalyst.

These plastinated specimens are currently being used in vascular catherization training sessions as follows. The aortic specimens are filled with water and with the aid of a fluoroscope a catheter is guided through the vessel by the physician. An unexpected, yet welcomed, advantage to the use of this type of specimen is that the plastination polymer renders the vessels radio opaque. This allows the physician the advantage of actually being able to visualize the vessels he/she is directing the catheter through. This novel use of plastinated human vascular specimens is currently being perfected. Undoubtedly, it has potential to play a significant role in medical education. Hence, additional uses are currently being explored.

PLASTINATION AS A CONSOLIDATION TECHNIQUE FOR ARCHAEOLOGICAL BONE, WET LEATHER AND WATERLOGGED WOOD

De La Cruz Baltazar V, Lyons W, Murray A, Hanlan J
Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada

Natural and man-made environmental conditions may adversely affect archaeological materials causing them to become weak and fragile. This has been of concern when trying to preserve specimens such as archaeological bone, leather and waterlogged wood.

In order to restore their strength and lengthen their lifespan these objects have often been treated with synthetic adhesives and consolidants. After analysing the effects of these treatments, it was decided to research the possibility of using a method called plastination.

Specimens of bone, leather and wood were prepared using the standard S10 method of plastination (von Hagens, 1985). Control specimens were prepared involving: plastination without dehydration and defatting [bone only], bleaching before plastination [bone only], solvent cleaning before curing the S10 [bone, leather, wood], air-drying [wood and leather only], vacuum freeze-drying [wood and leather only] and vacuum freeze-drying followed by plastination [wood and leather only].

All specimens were evaluated for: weight change, dimensional stability, colour change, hardness [bone and wood], indentation resistance [wood only], flexibility [leather only], removability and clean-up of the polymer and light aging. A chemical analysis of the S10 polymer was also done.

After assessment and evaluation, the S10 was found to be a promising consolidant. Treated specimens were: well penetrated, showed little dimensional change and were aesthetically pleasing. Although the polymer was found to be irremovable, it was valuable for specific applications.

Until further studies are completed assessing its long term stability and effects on specimens, the plastination technique cannot be recommended for consolidation.

LOCAL FLAPS FOR FINGERTIP INJURIES - PLASTINATED HAND SPECIMENS IN SURGERY EDUCATION

Alpar A, Gal A, Kalman M, Patonay L
Department of Anatomy, Semmelweis Medical School University, Budapest, Hungary

Normal, formalin-fixed anatomical specimens are of great use in studying anatomy. They are, however, not hygienic and smell, which greatly prevents their clinical use, although the exact knowledge of anatomical relations is essential in many disciplines, e.g. in surgery. Plastinated specimens in turn are dry, hygienic enough to be stored and used in clinics as well. By this method the discipline of anatomy gains new perspectives in medical education. The present study offers a help for surgeons, who apply local flaps for fingertip injuries.

Fingertip injuries often occur in the general surgical practice. The wide variety of these injuries and the great number of methods in treatment make the choice of the best therapy often difficult. The knowledge of the regional anatomy and blood supply is essential. Unfixed, human hand was used to demonstrate the anatomical basis, the harvesting technic, the three dimensional appearance and the indications of the numerous local flaps published in the literature. The Atasoy, Hueston, Moberg, O'Brien and the Venkatasvami-Subramanian methods were applied. The specimens were dehydrated in acetone at -25°C, and underwent forced impregnation with silicone at -25°C. These specimens can be used to study the anatomical relations in clinics right before operation.

THE MODULAR RESOURCE CENTER - LEARNING RESOURCES FOR THE STUDY OF VETERINARY MEDICINE

Mizer LA
Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, U.S.A.

The Veterinary College at Cornell has implemented a student-centered, tutorial-based curriculum in which learning issues in gross anatomy, histology, genetics and animal

development are brought to light through the use of clinical case scenarios. This curriculum creates an entirely new set of relations between the learner and resources. A key element of this program is the availability of visually oriented resources in "modules" where information has been organized into thematic, mixedmedia presentations for individual or small group study. These presentations are particularly well suited to disciplines in which gross, microscopic, and radiographic anatomy constitute core material.

Modules are self-contained study stations that contain a combination of plastinated and wet specimens, bone preparations, models, illustrations, radiographs, cross-sectional panels, microscope slides, computer-based images, and other materials. Plastinated specimens play a key role in many of these modules. Modules combine 2- and 3-dimensional materials to present a defined set of learning issues augmented by a brief text that helps the students more effectively interact with the specimens. The modules are clustered by body system, body region or by discipline and permanently housed in rooms that are available at all times to faculty, staff, residents, interns and veterinary students.

COR-TECH PR-10 Silicone: Initial Trials in Plastinating Human Tissue.

James Arnold Baker

The National College of Chiropractic, Lombard, IL, USA

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Key Words: Room temperature impregnation, Human tissue preservation, COR-TECH PR-10 silicone

Abstract

For our beginning plastination laboratory the attraction for us of COR-TECH PR-10 silicone was its easily availability for U.S.A. customers as it is locally formulated and supplied and, especially, that its claimed ability to impregnate objects could be done at room temperature, rather than at the -25°C required for standard S10 technique. We could find nothing in the published literature about this new silicone. In order to obtain some indication of its utility for a wide selection of tissues, we first plastinated with COR-TECH PR-10 portions of several organs (kidney, pancreas, gall bladder, brain cortex, muscle, and bone) following verbal instructions from the supplier. In a second trial with the same silicone we plastinated a brain stem and heart. With the cautious, slow plastination of our first specimens no detectable shrinkage had been found (linear, measured between two pins). In our second trial, in spite of fast impregnation at maximum vacuum throughout, the brain stem and heart appeared unchanged after plastination, and shrinkage for the brain stem was only on the order of 3%, compared with as high as 10% in the literature on standard technique. The heart demonstrated 1% shrinkage by the same method of measurement. All of the specimens were usable. Discussion compares and contrasts this silicone process with standard S10, and describes pertinent aspects of our procedures, errors and successes. Future plans are noted. Although the described examples are few, we conclude that this new polymer can easily be used at room temperature. It appears to be faster than standard technique, has minimal shrinkage-even for brain tissue, and is worthy of further exploration.

Introduction

This report covers two trials of COR-TECH PR-10, each with multiple tissues. First, to gain experience with a wide selection of tissues, we began by plastinating several specimens, viz. a half kidney, a gall bladder, articulated carpal bones, a parasagittal section of lumbar spine, and portions of brain cortex, pancreas, and gastrocnemius muscle. Second, although our plastination laboratory was still under creation, we were implored to plastinate an excellent dissection of a human brain stem in time for the next teaching trimester. The specimen included the diencephalon, pons, and medulla. In addition, the internal capsule and corona radiata were displayed on the left side, and the insula was displayed on the right side.The vasculature, including the circle of Willis, and all cranial nerves were also preserved. We included a heart to be plastinated under the same conditions.

Our primary interest was to try the fast room-temperature impregnation described as possible with the new Corcoran silicone polymers (Corcoran Laboratories Inc., 1405 North Johnson Street, Bay City, MI 48708-5487, U.S.A.). These products were attractive because they were locally available and if they proved usable at room temperature would make low temperature equipment unnecessary for the impregnation step of plastination.

We wanted to examine shrinkage of items plastinated with this silicone because verbal discussions with experienced plastinators warned that brain tissue, especially, suffers in this regard. Long, carefully monitored periods of impregnation time were considered necessary to prevent major shrinkage (von Hagens, 1986; von Hagens et al., 1987). Shrinkage measurement by a linear process has been reported before (Suriyaprapadilok and Withyachumnarnkul, 1997; Sora et al., 1999). To gain an estimate of shrinkage we elected to fix two pins in specimens at a known distance and measure changes in this distance.

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Address correspondence to: James Arnold Baker, D.C., The National College of Chiropractic, 200 East Roosevelt Road, Lombard, IL 60148-4583, U.S.A. Tel: 630 889 6852 / Fax: 630 495 6664. Email: JBAKER@NATIONAL.CHIROPRACTIC.EDU

Materials and Methods

Equipment

With the room temperature plastination possible with the Corcoran polymer we were able to use simple, inexpensive equipment. We modified a small counter-height home-type refrigerator by enclosing it in 4 1/2 inches (11.4cm) of polystyrene insulation (Owens Corning Foamular®) (figures 1 and 2), removing all spark-producing contacts to the outside of the refrigerator, and installing an external industrial thermostat with its eight-foot (2.4m) capillary tube leading to the sensing bulb inside (Johnson Controls, Inc. Model A19ABC-24C). A household indoor-outdoor thermometer gave temperature comparisons. An externally controlled 13 watt box-type fan from an old computer circulated internal air. With these modifications we were able to achieve a temperature of -20°C. We rebuilt an existing, but broken, vacuum pump (Gast Mfg. Co. 755 N. Edgewood Wood Dale, IL 6091-1254. New equivalent pump model 2565V2A). Our vacuum vessel was a four-quart Presto® pressure cooker with a gasketed 3/8 inch (9.5mm) thick Lexan® plastic lid (Baker, 1998). We had a large Bourdon-tube-type vacuum gauge to judge applied vacuum.

Fixation

All our specimens were from cadavers from our gross anatomy teaching laboratory which had been fixed in a standard anatomic embalming solution (6% formalin, 4% phenol, <2% glycerin, and water), then stored in 2.5% formalin for varying periods up to approximately eight months. After dissection the specimens were stored in 2.5% formalin for one week, until preparations for plastination were completed.

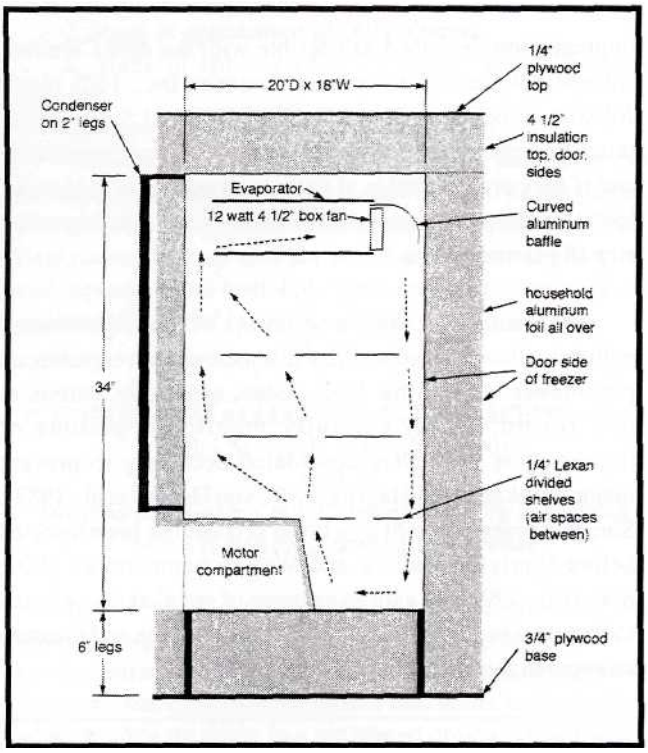

Figure 1. Side View of freezer showing air circulation and construction.

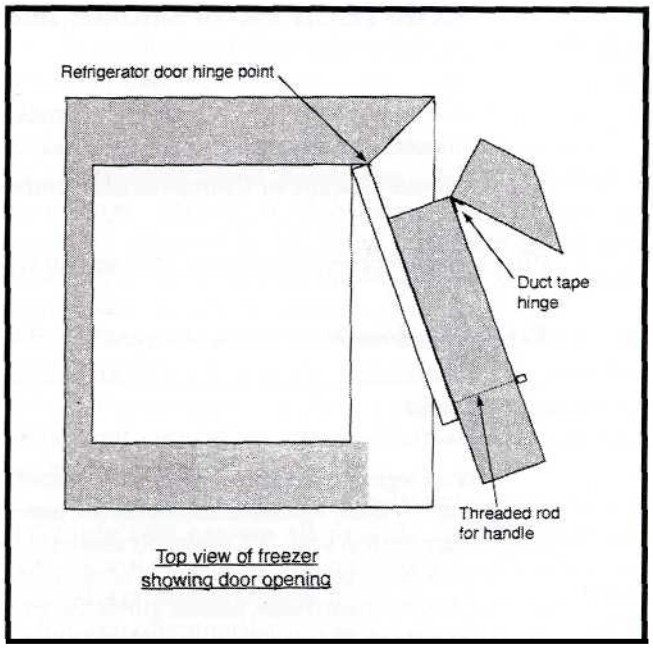

Figure 2. Top view of freezer showing door opening.

Dehydration

Specimens were drained then placed in cold 100% acetone. All specimens were dehydrated by the freeze substitution method (von Hagens et al., 1987). In an attempt to estimate shrinkage, pins were placed in the specimens and measured prior to dehydration. On each specimen two pins were located as far apart as possible on the largest available reasonably flat surface.

Plastination

Corcoran silicones and associated crosslinkers are supplied in several varieties. At the recommendation of the supplier, we chose to begin with COR-TECH PR-10 and COR-TECH CR-22 crosslinker, said to be easy to use, transparent, of near-water consistency, and producing a reasonably flexible product (Dan Corcoran, personal communication). We used the recommended "preservation catalyst CT-30," a somewhat viscous clear liquid.

Impregnation:

Impregnations were all performed at ambient temperature (24°C).

Trial 1. The half kidney, a gall bladder, articulated carpal bones, a parasagittal section of lumbar spine, and portions of brain cortex, pancreas, and gastrocnemius muscle were all impregnated simultaneously in our one vacuum chamber shown in figure 3. Specimens were kept immersed by an overlying bronze window screen (folded into several thicknesses to increase its weight) in a mixture of 97% COR-TECH PR-10 silicone polymer and 3% COR-TECH CR-22 crosslinker (this ratio was initially recommended by the supplier). We very slowly began to apply the vacuum, just enough to keep a small amount of bubbles flowing from the specimens. Bubbles were easy to see due to the clarity of the polymer-crosslinker mixture. Bubbling stopped when we reached a pressure of 50mm Hg inside the impregnation chamber. We do not yet have a Bennert type manometer so the pressure was calculated from the stable maximum vacuum applied. Impregnation was then considered complete and took 12 days. The specimens were then taken out to drain for one more day, ready for curing.

Trial 2a. The heart was placed in the same vacuum chamber as used in trial 1. However, we had added sufficient COR-TECH CR-22 crosslinker to the mixture to increase its percentage from 3% (as in trial 1) to 5% because the supplier informed us that this would decrease impregnation time (Dan Corcoran, personal communication). Since the specimen floated, a screen was again placed on top of the specimen to hold it below the surface of the liquid.

As opposed to our first experiment, the vacuum was immediately turned up to the maximum. Furious bubbles appeared immediately, both large (air) and small (acetone). Within eighteen hours, bubbling had settled down to minor, regular fine bubbles; this condition persisted with decreasing activity over time, until no more bubbles appeared, at which point the specimen was removed to have its surface wiped off, then it was drained for a day. The final pressure was calculated to be 50mm Hg. Total impregnation time was fifty-four and one half hours.

Trial 2b. The brain stem was impregnated immediately after the heart in the same 95%/5% silicone-crosslinker solution as the heart. As with the heart, vacuum was immediately turned up to the maximum, with frantic bubbling. Residual end-point pressure was 50mm Hg. Total impregnation time was forty-two and one quarter hours. The longer impregnation time of the heart than the brain stem represented interruptions due to other duties rather than the needs of the specimen. The brain stem was drained for two and one quarter days before curing. Because of the delicate structure its surface was not wiped before draining. To suspend the brain stem in a closed polyethylene container for draining, we made a U-shaped wire frame from one sixteenth inch diameter (1.6mm) type 304 stainless steel gas-type welding rod and attached it by a small copper wire looped around a straight pin inserted through the specimen (figure 4).

Curing:

Curing was also done at ambient temperature (24°C).

Trial 1. When we completed the first batch of specimens we had understood that curing was effected by placing them in a closed container with an open dish of COR-TECH CT-30 catalyst so the vapor would begin the curing process (Dan Corcoran, personal communication). We did this for several days, using a closed Rubbermaid™ container, shown in figure 5. Nothing happened. While we were trying to sort out the curing process, we performed an experiment with a few drops of polymer in open petri dishes together with an open petri dish of catalyst, all in a closed container. Each day for 10 days we removed a dish of polymer to discover whether it

Figure 3. Trial 1 specimens in vacuum chamber.

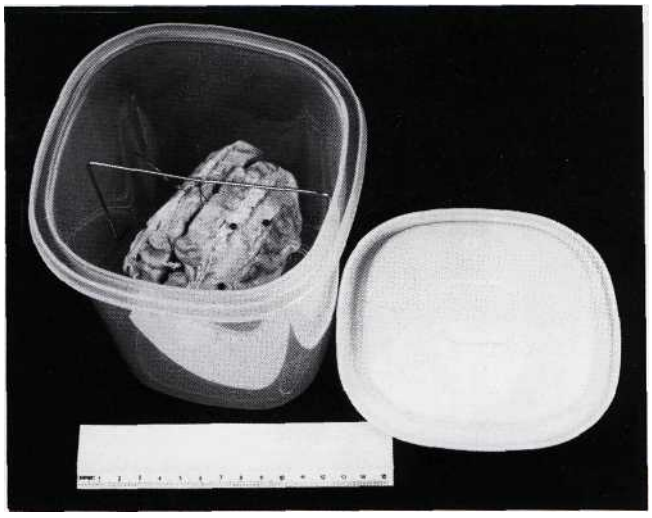

Figure 4. Method of suspending brain stem for drainage.

had cured. None did, so we abandoned trying to cure the polymer with only vapor contact. We phoned the supplier again and found that we had missed the fact that an initial topical application of curing agent was necessary. We applied catalyst with a cloth, being very careful not to contaminate our catalyst with polymer, and waited. Finally, at eleven days the specimens appeared to be cured, but they still had a greasy surface residue which we wiped off with acetone. After eight more days, curing was deemed to be complete, as determined by dry, odorless surfaces.

Trial 2a. Subsequent to trial 1 we were informed that a good way to apply catalyst without the danger of contamination of catalyst by contact with silicone was to spray it on from a common hand sprayer (Dan Corcoran, personal communication). So, after draining was complete, we attempted, without success, to spray COR-TECH CT-30 catalyst on the specimen with a common hand sprayer with an adjustable nozzle. We tried with five different sprayers. The problem was that the catalyst came out of sprayers in a solid stream. We finally squirted catalyst on and carefully wiped off the excess catalyst liquid and droplets using paper towels and light tissue.

We were also told to place the specimen in a common self-seal plastic freezer bag. When air is excluded the process is said to occur much more rapidly (Dan Corcoran, personal communication). So, after wiping off the excess catalyst we placed the heart in a freezer bag, squeezing out as much air as possible before sealing the bag. After forty-one hours we opened it to find polymer cured on the surface of the heart in many spots where the bag had contacted the heart. With care and persistence, it was possible to remove these white spots. Further phone conversation made it clear that we had misunderstood the instructions by leaving it in the bag too long. Twenty four hours is the maximum recommended time (Dan Corcoran, personal communication).

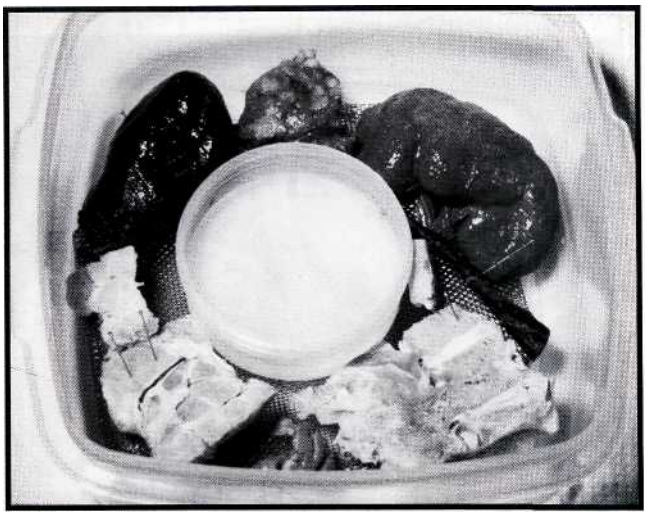

Figure 5. Trial 1 specimens in unsatisfactory curing attempt.

Table 1. Distance (mm) between the two pins and Shrinkage %

	Before	>C3H60	>PR-10	End	%
Trial 1					
Half kidney	19	18.5	19	19	0
Pancreas	16	16	16	17	+6
Brain cortex	18	18	17.5	18	0
Carpal bones	11	12	12	11.5	+5
Lumbar spine	14*	-	-	-	
Gastroc.	11	11	11	11	0
Gall bladder	12	11	12	11	0
Trial 2a					
Heart	46.5	45	45	46	1
Trial 2b					
Brain stem	37.5	36	36	36.5	3

*One pin fell out.

Trial 2b. The brain stem was cured by the same procedure as the heart (in a plastic freezer bag with most of the air squeezed out before closing, as noted above, except that, learning from our experience with the heart, we removed the brain stem from the bag after twenty hours. The bag was opened and the surface wiped. Polymer that had been loosely catalyzed on the surface where the bag contacted it was easily removed. A sheen of polymer showed on a few surfaces where the plastic bag had contacted it. Most of these were easily wiped off. Finally, the brain stem was left suspended in open air (in the container, but with the lid removed) at room temperature. In five days, the surface was dry and the catalyzing process was considered complete enough so the brain stem could be handled.

Results

Shrinkage of our samples by the two-pin method we used is shown in Table 1. Beginning and ending values are probably the most accurate. For the first batch, plastinated in a relatively long procedure (12 days of impregnation), there was essentially no shrinkage. The increase for the brain tissue and carpal bones is probably measurement error. For the heart and brain stem, fast-as-possible plastination produced 1% and 3% shrinkage, respectively. It would appear that, for these two, most of the shrinkage took place during dehydration. The only difference in time between all the various trials was in impregnation.

Figures 6a to e show some of the finished specimens from our first trial. The plastinated heart is shown in figure 7, and figures 8 and 9 show the plastinated brain stem. None of the specimens were unusable, or significantly altered in color, shape or general appearance. The brain stem was immediately put into use for teaching purposes where it still is being used. The heart was put aside for the time being. We had no air to expand the heart chambers or vessels, so the great vessels were somewhat folded. They were fairly rigid, but the heart muscle was not. The brain cortex sample was somewhat brittle. The pancreas tissue and half kidney were barely flexible. The gall bladder was highly flexible, as was the gastrocnemius muscle. The carpal bones were moveable, approximating their state before plastination. Samples of the brain cortex and kidney were taken for microscopic examination, with initial promising results (e.g. cellular organelles and capillaries were easily seen).

Discussion

The two-pin method we used for measuring shrinkage is admittedly not rigorous, and should be considered an approximation only. Many of the first specimens were too small to use multiple measurement points. For example, three pairs of pins placed in lines orthogonal to each other would give more accurate information. Volumetric procedures, where applicable, could be used to good advantage.

Dehydration is not different from that used for the S10 technique.

Impregnation is the major difference from standard S10 technique. Impregnation with the COR-TECH-xx series of silicones may be done at room temperature, whereas S10 requires minus 25°C for impregnation (von Hagens, 1986). Room temperature impregnation means that low temperature facilities are needed only for dehydration, thus reducing equipment cost.

A second difference is that with the one we have used, COR-TECH PR-10, rising acetone bubbles are easy to see, as the polymer is crystal clear. As well, the bubbles rise quickly, since this polymer is near water consistency, whereas S10 polymer is "highly viscous" (von Hagens, 1986). This facilitates faster impregnation time.

Curing for COR-TECH PR-10 appears to be a gas-cure, similar to S10, however an initial topical application of catalyst must be made, followed by enclosing the specimen in a plastic bag with as much air as possible excluded for a maximum of twenty-four hours. Initially excluding air in the curing process is said to allow a high concentration of catalyst vapor pressure to "drive" the catalyst deep into the specimen (Dan Corcoran, personal communication).

Surface curing proceeds rapidly. If there is any remaining polymer on the surface, this will cure against the bag or in droplets, leaving white cured drops or a sheen of cured plastic where the bag touched. If the specimen is taken out soon enough, these surfaces may be wiped clean before complete curing has set in. After removing the item from the bag and wiping off any excess, it should be left to air cure at ambient temperature.

We had wondered how inaccessible crevices in the highly convoluted brain stem would cure when they could not be directly painted with catalyst. This turned out not to be a problem because curing seems to proceed throughout the specimen, once started. The end point of curing may be considered to be when the surface of the specimen is dry.

Any problems we had were due to our newness to the process of plastination and to our misunderstanding of the verbal instructions. We made several procedural errors, notably not making an initial topical application of catalyst and keeping it in the plastic bag for longer than 24 hours. Since we processed the above samples, a summary set of instructions has been made available (Corcoran Laboratories, Inc., 1998). This describes the polymer we used as well as the several others which are available.

After phone discussion with the supplier we chose to start with COR-TECH PR-10 for all the described specimens. It is stated to have "easy penetration with some flexibility, fastest impregnation time" (Corcoran Laboratories, Inc., 1998). The great vessels of the heart we plastinated are more stiff than we had expected. This may be due to reported firmness of some tissues, as intestine, if left too long in acetone (von Hagens, 1986). We plan to experiment with COR-TECH PR-12 polymer, which is stated to have more flexibility " easy penetration with more flexibility that COR-TECH PR-10. A little longer penetration time." (Corcoran Laboratories, Inc., 1998). With the above noted reservation, the plastinated heart is otherwise excellent.

From this limited trial it is unclear exactly what results may be expected from varying the silicone crosslinker ratio. We noticed no difference that could be specifically attributed to the two concentrations we used. We intend to experiment with varying amounts of crosslinker, and varying amounts of directly applied catalyst to obtain a better conception of appropriate curing parameters. Sheet plastination with this silicone remains to be explored.

It is imperative not to contaminate either the catalyst or polymer, such that one's stock of raw material begins to catalyze. To this end it is suggested to spray ("mist") catalyst onto the surface of the specimen, thus avoiding physical contact. We understand that catalyst COR-TECH CT-32 can

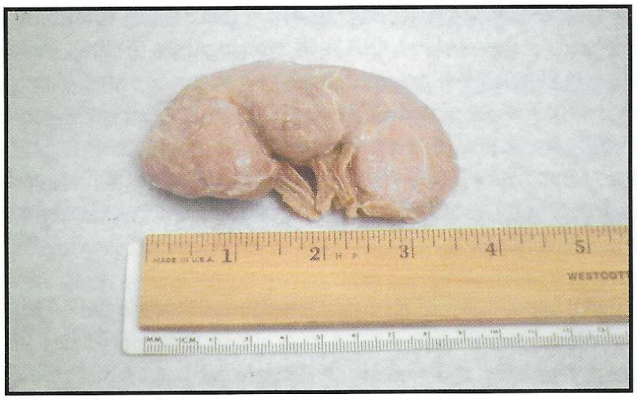

Figure 6 a

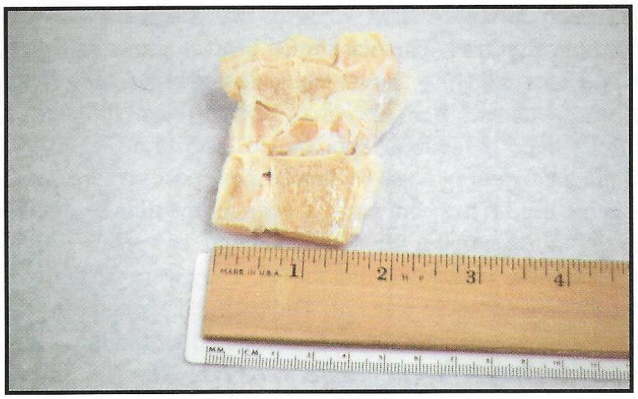

Figure 6 b

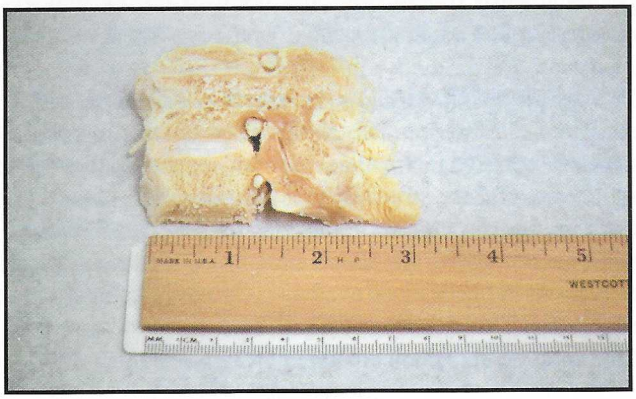

Figure 6 c

Figure 6 d

Figure 6 e

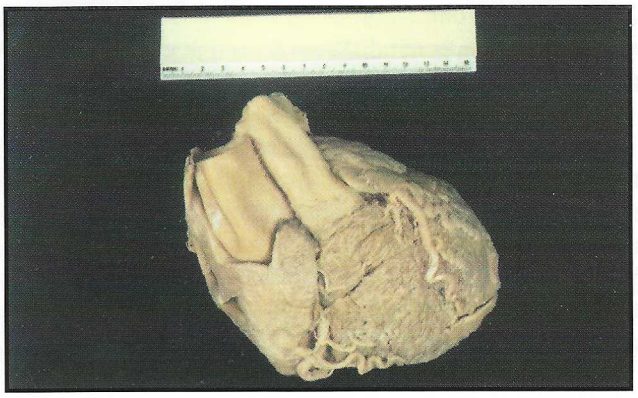

Figure 7

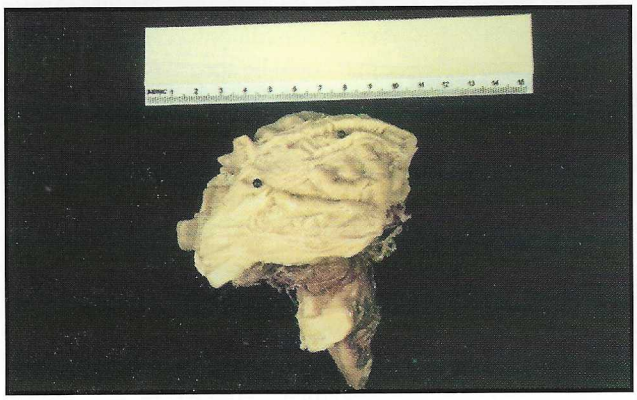

Figure 8

Figure 9

be sprayed from a common hand sprayer with an adjustable nozzle (Dan Corcoran, personal communication). We intend to try this on a future specimen.

The plastination materials (polymer, crosslinker and catalyst) are Dow Corning products (Midland, MI), and labels list multiple constituents. Reasonable precautions need to be taken with regard to handling the three components, e.g. gloves and proper ventilation, especially when spraying catalyst. Of these three components, the crosslinker is the only one which was shipped with a "flammable liquid" label and a "poison" warning, possibly because it contains methanol.

Conclusion

In spite of our inexperience and the dearth of information about the new silicone, we had no disasters with the above-noted specimens. Our initial specimens remain unchanged in appearance or consistency after a year. All were usable as planned. Rapid application of the vacuum for impregnation of the heart and brain stem appeared to cause no problems. The small amount of shrinkage might have been reduced even further by increasing the vacuum gradually, but the shrinkage was well within our expectations. We did not have any difficulty with handling silicone, cross-linker or catalyst. We conclude that COR-TEC PR-10 is an excellent, easy-to-use silicone for plastination of the materials for which we have used it, including brain stem tissue. It may be used at room temperature and, importantly, allows quick preparation of specimens. We realize that the limited examples cited do not compose a rigorous examination of the use of this silicone for plastination, but we hope that our experience may benefit those who also wish to explore using this material.

Acknowledgements

1. Thanks to Dan Corcoran, Roy Glover, Gilles Grondin, and Ronald Wade, who responded patiently to our many phoned questions with helpful information.

2. Thanks to Kyle Worell, Diener, for the excellent brain stem dissection, and Shahar Kenin, Pre-Doctoral Fellow, Department of Anatomy, for logistic support.

3. Thanks to Linda Puckette for editorial assistance.

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Figures 6 a - e: Trial 1 finished specimens, a. External surface of coronally sectioned kidney b: Articulated carpal bones c. Parasagittal section of lumbar spine d. Brain cortex (with fragments broken off in assessing flexibility) e. Portion of gastrocnemius muscle.

Figure 7. Plastinated heart.

Figure 8 and 9. Plastinated brain stem.

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Fungal Contamination of Plastinated Specimens

Rafael Augusto Dantas Prinz[1], Joao Antonio Pereira Correia[1], Aurea Maria Lage Moraes[2], Almir Lobo da Silva[1], Susanne Queiroz[1] and Lucia Helena Antunes Pezzi[1]

'Department of Anatomy, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[2]Department of Micology, Fundagdo Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

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Key words: Fungi, Fungicide, Fungal Contamination.

Abstract

Fungal contamination of plastinated specimens has not been previously reported in literature. However, during a tropical rainstorm period, last summer, we have been surprised by a massive fungal infestation of our plastinated specimens collection. The intent of this report is to discuss the probable causes, consequences and prevention of this misfortune, as well as to present an efficient, harmless and low-cost fungicide method that can be used in plastinated specimens.

Introduction

Last summer, after a tropical rainstorm period, there was an overflow in our Plastinated Specimens Facility. After that misfortune, progressive, fast growing white, green and black spots could be seen over almost all the plastinated specimens (figure 1) as well as over the wooden shelves (figure 2). Before the overflow, the specimens were stored in a low humidity environment (Correia et al., 1998) and all have been plastinated according to the standard S10 technique (von Hagens, 1985).

Mycological analysis revealed fungal contamination by several different species. Therefore, we tried to develop a fungicide process that would be efficient, nonexpensive and harmless for the plastinated specimens.

Materials and Methods

Mycological analysis of plastinated specimens

Eight specimens that had the largest surface infestation (1 heart, 1 specimen with the muscles of rotator cuff, 1 kidney, 1 abdominal sagital section, 1 abdominal transverse section, 1 cerebellum and brain stem, 1 hand, 1 stomach) were selected for mycological evaluation at the Mycology Department from Fundacao Oswaldo Cruz.

In that Institution the specimens were slightly scraped and the fragments were sed in Petri dishes with Potato Dextrose Agar (PDA - Difco), which is a standard medium for isolation of a wide range of fungi (Moraes et al., 1998), and incubated at room temperature (+/- 28° C). During the first ten days, daily examinations were carried out in order to observe fungal growth (figure 3), followed by examinations every 3 days until the 21st day.

The isolated colonies were subcultured in Malt extract and Czapeck-dox (Difco) medium for identification. The microscopic characteristics produced by the species were studied using the technique of culture on slide (Rivalier and Seydel, 1932). The material was colored with 10% KOH for the representative with dark pigmentation and with Amann's Lactophenol with cotton blue for the hyaline isolated representatives and observed under a Nikon model Labophot light microscope. Species identification was made according to Pitt (1979, 1985), Raper and Fennell (1965), Samson (1979) and Klich and Pitt (1994).

Samples of deeper tissues from the specimens were also evaluated, as well as the Biodur S10 silicone rubber and the silicone hardeners Biodur S3 and S6.

Both Rafael Augusto Dantas Prinz and Joao Antonio Pereira Correia contributed equally to this work. Address correspondence to: Rafael Augusto Dantas Prinz, Rua Gurupi 105, Apartamento 301, Grajaii Rio de Janeiro - RJ, Brazil, CEP: 20561-100. Tel: 55 21 290 0587 / Fax: 55 21 562 6394. Email: prinz@netrio.com.br

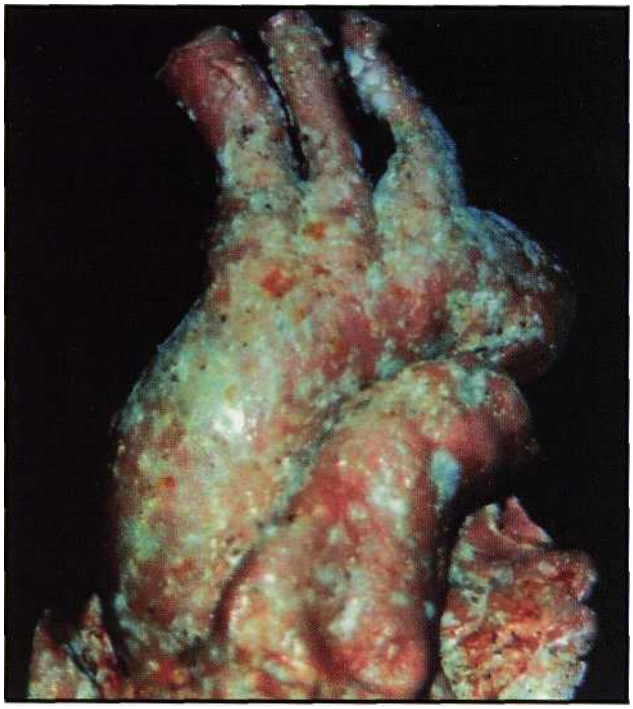


Figure 1. After a period of excess humidity environment, progressive, fast growing white, green and black spots could be seen over almost all the plastinated specimens.

Fungicide protocol

Contaminated plastinated specimens have undergone a fungicide protocol developed by us, which consists of the following steps:

1. Immersion of the plastinated specimens in a 10% formalin solution at room temperature for 5 minutes;
2. Rinsing and manual surface brushing, in cold running tap water, for 5 minutes;
3. Immersion in a alcohol-chlorine solution (lOOmg of granular chlorine, as the one used in pools, dissolved in 100 ml of absolute alcohol) for 20 minutes, at room temperature. The solution must be shaken about every 5 minutes;
4. Rinsing and manual surface brushing, in cold running tap water, for 5 minutes;
5. Rinsing in distilled water for 5 minutes;
6. Drying in a low humidity, clear and clean environment. The process may be fastened by using an electric fan to ventilate the specimens.

The Plastinated Specimens Facility shelves have undergone a similar protocol, as follows:

1. Wash the shelves with a 10% formalin solution. Let the solution act for 5 minutes;

Figure 2. The wooden shelves of our Plastinated Specimens Facility were covered by white spots.

2. Wash and brush them with cold tap water;
3. Wash the shelves with the same composition of the alcohol-chlorine solution used before for 20 minutes;
4. Wash and brush the shelves with cold tap water;
5. Use an electric fan to dry the shelves.

Mycological analysis of the plastinated specimens after application of the fungicide protocol

The specimens selected for the first mycological analysis went through the fungicide protocol and after 2 months were once again evaluated by the Mycology Department from Fundagao Oswaldo Cruz.

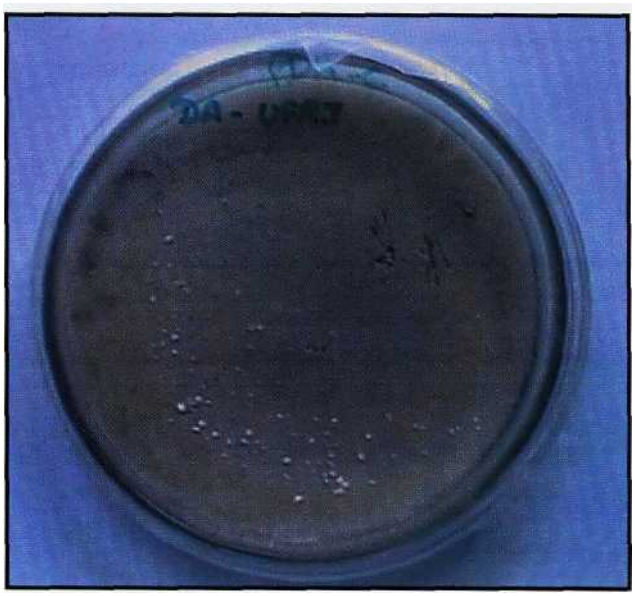


Figure 3. Culture media from surface fragments of plastinated specimens showing *Aspergillus fumigatus* growth (white spots).

Table 1. Different fungi species identified over the plastinated specimens

PLASTINATED SPECIMENS	FUNGI SPECIES
Kidney, Cerebellum and Brain stem	*Penicillium janthinellum*
Abdominal sagital section, Stomach, Hand	*Penicillium corylophilum*
Muscles of rotator cuff	*Aspergillus niger*
Heart	*Aspergillus flavus*
Abdominal transverse section	*Aspergillus fumigatus*

Results

The cultures of plastinated specimens superficial fragments showed significant growth of different fungi species (Table 1).

The Biodur products and deep tissues analysis showed no fungal contamination.

The specimens which had undergone our fungicide Protocol did not show fungi infestation anymore.

Discussion

Based on the fact that the specimens had been properly fixed before being correctly plastinated, according to the S10 standard technique, as well as stored in a low humidity, clean environment and considering that the mycological analysis of the Biodur products showed no contamination, we can conclude that the sudden environment humidity increase was the cause of fungal infestation, since it is well-known that such environment predisposes to the development and growth of habitual ambient fungi.

Since the plastinated specimens have been free from contamination 2 months after the fungicide protocol application, we can affirm that such protocol was efficient in eradicating the contaminating fungi. Up to this date (10 months after the fungicide protocol application) we have not detected any kind of macroscopic fungi growth in our specimens. The products used in the protocol were choosen since they are widely cited in literature being efficient for all kind of surfaces and live tissues, like insect cuticles and plants, without causing any harm (Hakwsworth, 1977), besides they are cheap products, such as formalin, granular chlorine and alcohol, easily found in supermarkets as well as in Anatomy departments and easy to use and prepare in the laboratory routine.

All the contaminated plastinated specimens (more than 500) went through this fungicide sequence and none has been harmed (figures 3,4 and 5), except for some white spots that could be seen in a few specimens, due to chlorine deposition, easily removed by brushing.

Concerning the consequences of fungal infestation, the process proved to be superficial and left superficial white, black and brown spots in some plastinated specimens (figure 6). These spots could be the result of the probable use of plastinated specimens as a substratum for parasites development. The promptly use of the fungicide protocol may reduce the chances of such consequence.

For prevention, we suggest the use of devices that reduce environmental humidity in the plastinated specimens storing room, such as air conditioners, specially in tropical countries. The Plastinated Specimens Facility should present a good infra-structure in order to prevent leakage and inundations, conditions that predispose to fungal growth.

The isolated genera, *Aspergillus* and *Penicillium,* are ubiquotous fungi. They are found worldwild in the most different hosts and substrats (Raper and Fennel, 1965; Pitt, 1979). So we suspected that they would be isolated from the material that were exposed to the high humidity that the water overflow caused and the results obtained, confirmed our expectations.

Figure 4. Plastinated stomach covered by *Penicillium corylophilum* before (left) and after (right) undergoing fungicide process.

Figure 5. Plastinated cerebellum and brain stem covered by *Penicilliumjanthinellum* before (left) and after (right) undergoing fungicide process.

Figure 6. Plastinated heart covered by *Aspergillus flavus* before (left) and after (right) undergoing fungicide process .

Figure 7. Plastinated head (sagital section) after undergoing fungicide process, showing superficial brown spots caused by fungal contamination.

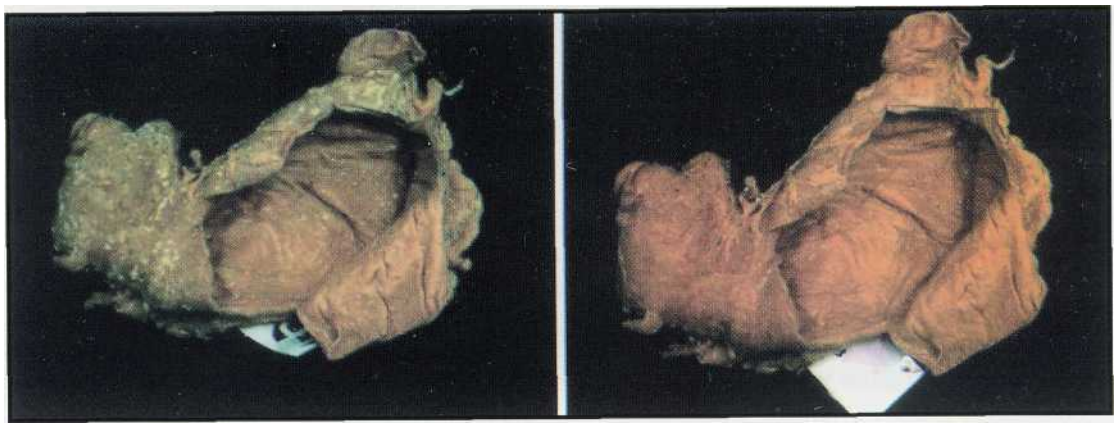
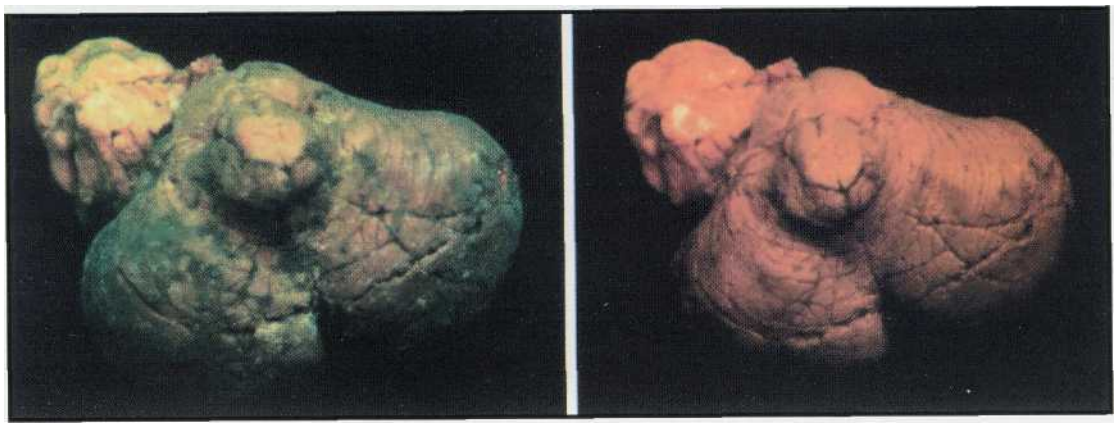


Figure 4



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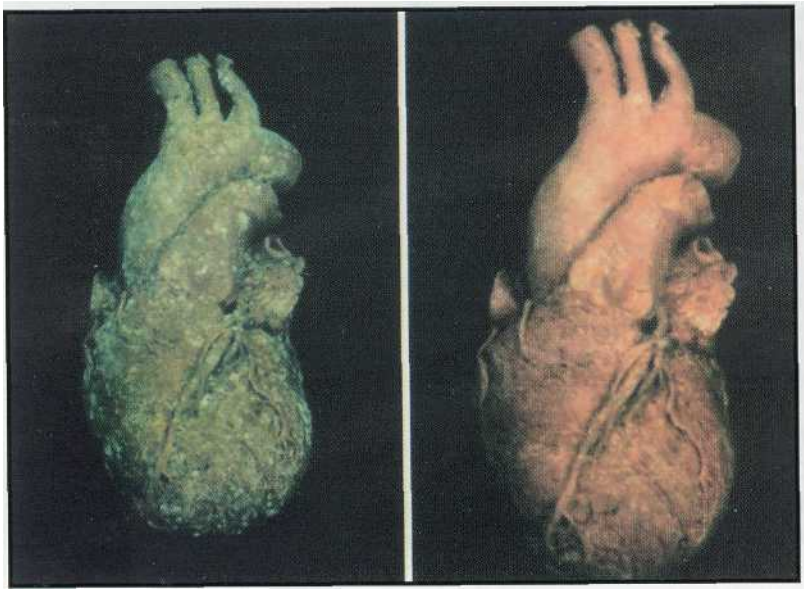

Figure 6

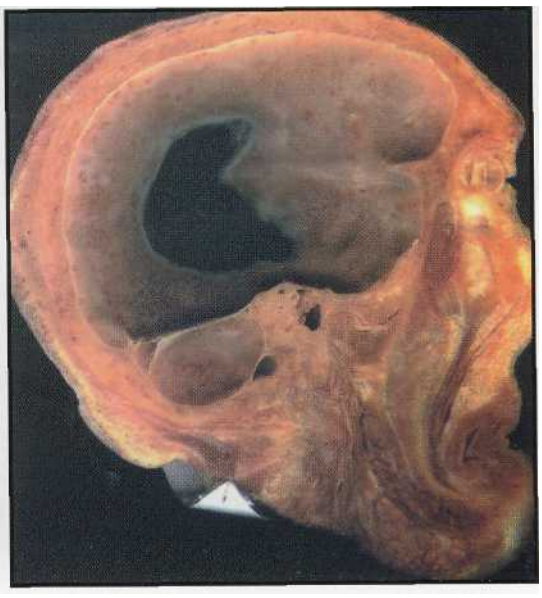

Figure 7

In relation to the pathogenic capacity of the fungi species, we should focus on *Aspergillus. Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* can be responsible for allergic bronchopulmonary aspergillosis in immunocompetent hosts, especially in individuals with an increased responsiveness of trachea and bronchi to various stimuli (asthma), chronic sinusitis and colonization of preexisting pulmonary cavities (Aspergilloma) (Bennet, 1998; Hamill and Hollander, 1997). In immunodeficient people, however, the infection is considered severe since the patient can develop invasive aspergillosis (Lortholary et al., 1993). This disease has a mortality rate well above 50%. The *Penicillium* infection is not clinically important in immunocompetent hosts.

We strongly suggest the use of gloves and masks during the fungicide process.

Acknowledgment

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THESIS REVIEW

Plastination as a Consolidation Technique for Archaeological Bone, Waterlogged Leather and Waterlogged Wood

Author: V. De La Cruz Baltazar, Department of Art,
Queen's University, Kingston, Ontario, Canada, 1996

In April 1996, Vera De La Cruz Baltazar submitted a thesis to the Art Conservation Program in the Department of Art in conformity with the requirements for the degree of Master of Art Conservation. This is the fourth thesis to be reviewed in the Journal of the International Society for Plastination. The first one was devoted to the comparison of thin plastinated human slices and magnetic resonance images (Olry, 1997). The second one aimed at introducing a basic knowledge of plastination procedures in French scientific literature (Olry, 1998a). The third one dealt with the use of plastinated specimens for surgical training in otorhinolaryngology (Olry, 1998b).

De La Cruz Baltazar's 177-page thesis includes 76 tables, 36 figures and a bibliography of 121 references (2 from our Journal).

The first chapter (pp. 1-2) gives the objectives of De La Cruz Baltazar's thesis. These were to monitor some of the changes that plastination produced in samples (archaeological bone, waterlogged leather and waterlogged wood), to improve the physical appearance of the samples, to compare the plastination treatment with air-drying and vacuum freeze-drying, and finally to gain some understanding about the chemical composition and stability of the substances used in plastination.

The second chapter (pp. 3-46) is a literature review of the composition, decay and consolidation of bone, leather and wood. It gives a very interesting and well-documented summary of the consolidation or conservation methods used in the last decades.

Chapter 3 (pp. 47-53) is a literature review of silicone polymers and plastination technique.

Chapter 4 (pp. 54-65) deals with the experimental procedure: origin of samples and experimental design (standard plastination technique, plastination without dehydration and defatting, bleaching before plastination, solvent cleaning before curing, air-drying, vacuum freeze-drying, and vacuum freeze-drying followed by plastination). The weight changes, dimensional stability, colour changes, hardness, indentation resistance, flexibility, removability and surface clean up, and light aging were analyzed by means of electronic digital scale, Craftsman calipers, spectro-photometer, durometer, etc.

Chapter 5 (pp. 66-95) contains the results. Many interesting informations are to be found in this chapter: the weight gain of plastinated bones is around 25% of its original weight, no change of colour could be observed on plastinated bones (standard technique), the hardness of plastinated bones decreased (2 to 12 points), etc.

Chapter 6 (pp. 96-107) analyses the results and chapter 7 (pp. 108-111) summarizes the conclusions.

The bibliography occupies pp. 112-125. The last pages are devoted to appendix 1 (tables of results, pp. 126-159), appendix 2 (MS-GC, IR and NMR spectra, pp. 160-170), appendix 3 (data sheets, pp. 171-176), and the author's vita (p. 177).

This thesis is the result of a minutely detailed study of the potential of plastination in archaeology. The methodology is by far the most scientific one I never found in a research devoted to plastination. I would advise the author to submit a summary of her thesis to the Journal of the International Society for Plastination.

Regis Olry Vice-
President ISP

References

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