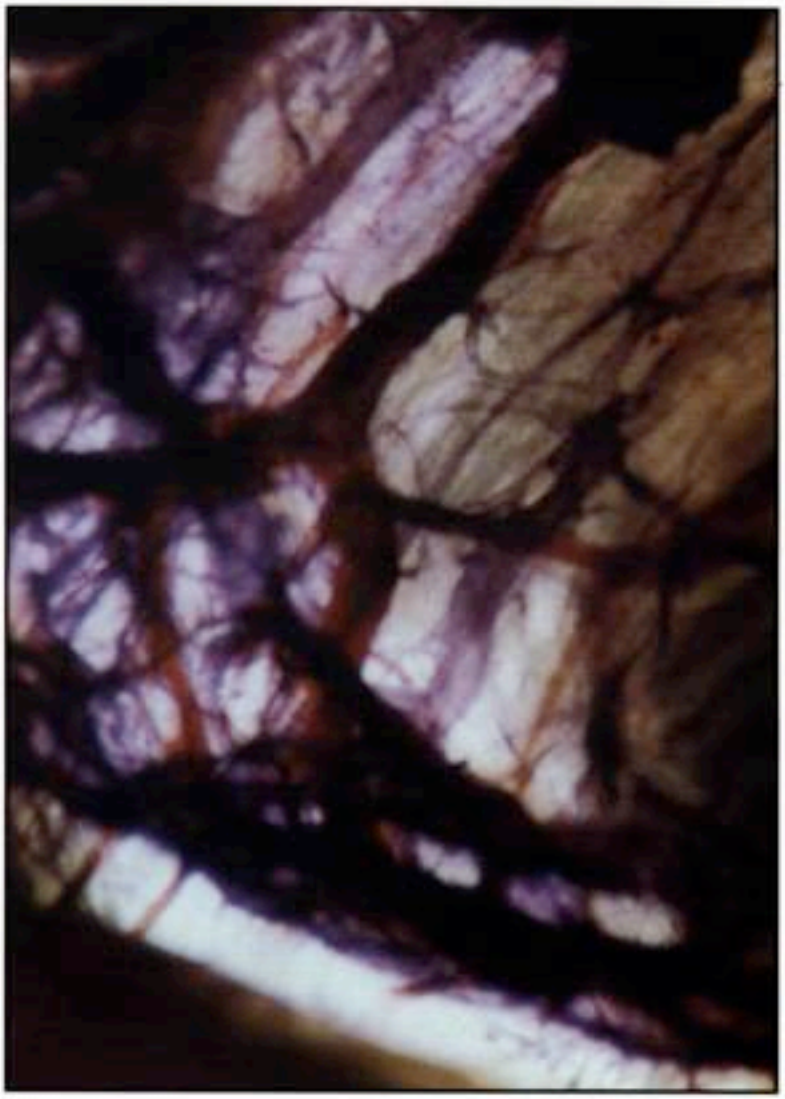
Journal of the INTERNATIONAL SOCIETY for PLASTINATION



VOLUME 14, No 1
JUNE 1999

The Journal of the INTERNATIONAL SOCIETY for PLASTINATION (ISSN 1090-2171)

EXECUTIVE BOARD OF THE SOCIETY:

official publication of the International Society for Plastination

TABLE OF CONTENTS

(continued on page 2)

COVER PAGE: Illustration of the subarachnoid space prepared and plastinated according a modified E12 technique (see paper page 12)

TABLE OF CONTENTS

ANNOUNCEMENTS

PRESIDENTIAL LETTER

Dear fellow plastinators,

WELCOME TO ROCHESTER, NEW YORK!

Thanks to the efforts of Cynthia Ryan, we are looking forward to a very well organized and hopefully well attended interim meeting - the last meeting of the members of the ISP before the millenary.

Platform and poster presentations dealing with new applications of the plastination techniques both in teaching and in research show that plastination is going beyond the borders of medical schools, increasingly addressing a wider community.

Basic lectures will introduce all the plastination procedures and the specialty of the interim meetings - the hands-on sessions - will complete a week of experiences.

This time four special topic workshops are offered, that should attract both freshman and expert plastinators: the not so commonly used techniques of sheet plastination of body slices (epoxy) and brain slices (polyester) are offered as well as completely new workshops on color injection casting and on room temperature plastination.

I wish Cindy and her team at the University of Rochester School of Medicine and Dentistry a successful 6th interim meeting and I am convinced that all the participants will enjoy a week of intensive work and discussion resulting in new experience and new friendships.

With the kindest regards from Europe, where we hope to meet you next time in July 2000 in St. Etienne, France.

Yours sincerely

Andreas H. Weiglein

MESSAGE FROM THE EDITOR

Dear readers,

We witnessed last January (18 to 22) to a particular series of messages through the ISP-L list regarding the Journal. It all started when one person asked informations about an article that appeared in the vol 13 no 2. It quite fast degenerated to become nothing but a complain's line against the Journal. We found in some of these messages sentences like:

"...I have also noticed the lack of specifics in a number of journal articles but I have no answer to the problem..."

"...some items can not be reproduced that appear in our journal..."

"...we need to get a handle on what gets accepted as article for the Journal..."

"...Journal and conferencees are showcases for the Society. These should excude excellence, not calamity..."

and even:

"...I have receive e-mail concerning a number of presentations at Trois-Rivieres that were
1. not related to plastination
2. of lousy quality (slides of unidentifiable objects)
3. exceeding time allocation..."

This last sentence coming from the fact that ONE participant complained to the wrong person (neither Dr Olry nor myself ever received this kind of complain) refering to ONE single poster presentation that according to him was below any standards.

All these exagerations rapidly took the appearance of a "group therapy" more than an exchange among scientists. This king of fast exchange even makes difficult to find who wrote what and when?

As mentioned by Mr. Ronn Wade, the ISP-L list is an open list. Open to members and non-members. This means that non-members can be signed-on and read all these messages. Peoples that have never seen one copy of your Journal or never attended one plastination conference may judge our group according to what they read in the ISP-L list. This can give a quite negative image of our Journal and Society. It will never be possible to measure the negative impact that such a story could have had on our Society and Journal but I strongly believe that it certainly had no real positive impact.

Your Journal is prepared according the following rules:

1. All articles submitted are sent for review to 2 members of the editorial board. In some particular situations, the editor also have the opportunity to ask external reviewers when it is considered that these persons could have a particular interest in the subject of the reviewed paper. When the 2 reviewers do not aggree on the paper submitted, the editor will ask a third opinion before taking the decision to accept or reject the paper.

2. The editor will then send the reviewers' comments to the authors asking them to answer the questions and to modify their paper to comply with the reviewers comments.

3. The authors will send back the corrected paper to the editor for publication. When major corrections have been asked by the reviewers, the paper may be sent to them again for a second review.

All this process is a "double blind processs" meaning that the reviewers do not know the names of the authors of the paper they review (until that paper is published) and the authors will never know the names of the reviewers who evaluated their papers.

The final decision regarding the publication belongs to the editor and the authors always remain responsible for their writing. In my opinion, the members of the editorial board always did their best and I am sure they will keep on doing so to ensure that a paper published in your Journal will respond to the criteria of a scientific paper but we can not guarantee the complete exactitude of everything included in an article. We do not have the task nor the possibilities to reproduce any experiment described in an article to ensure that everyone will be capable to reproduce exactly what is described. Some processes described may need to be modified or adapted to be reproduced in other laboratories.

Any reader who do not understand or agree with an article appearing in the Journal is welcomed to address his (her) critics and questions directly to the authors. This is why you always find the complete mailing address of the authors on the first page of the papers published. Any reader can also choose to address a letter to the editor who will transmit it to

the authors and publish in a following issue of the Journal, the letters from readers and the answers from the authors. This can help to clarify articles or add details that may have been omitted in the first publication.

The letters that will be judged of general interest, clearly identified and signed will be published in the Journal. I do believe that this is the best way to improve the content of your Journal and to answer all your questions. I hope that you will take the habit to express your opinions regarding the Journal and it's content because you are the persons to whom the Journal is dedicated. Any reaction, negative or positive, is worth being shared with other readers but in a way that will benefit to everyone. We must remember that less than one half of the members of the Society are signed to the ISP-L list but everyone receives the Journal.

You will find in the present issue 2 novelties. The "*Current Plastination Index - Updating*" section will now include abstracts of the new papers, in addition to the complete references.

In spite of the problems related at the beginning of my present message, I also decided to follow the suggestion received from many of you and to add at the end of the "*Letters to the Editor*" section a section of "*Questions and Answers*" reprinted from the ISP-L. I hope that you will consider these 2 additions to the content of your Journal as an improvement of the quality as well as the quantity of the information that we want to bring to you.

Finally, I would like to welcome Mr Vincent DiFabio and Mr Geoffrey D. Guttmann who offered to join the Editorial Board.

Thank you,

Gilles Grondin

LETTERS TO THE EDITOR

PLASTINATION AT ROOM TEMPERATURE

Dear Editor:

In J Int Soc Plastination (Vol 13, No 2: 21- 25, 1998) Zheng et al. are describing a procedure of intermittent forced impregnation and a design of an acetone gas filter system.

Intermittent forced impregnation is described as follow: "Daily vacuum was established.....Before the end of each working day the vacuum pump was turned off and the vacuum released. The chamber was then opened to allow the specimens to be moved around to relax and further equilibrate with silicone....." Intermittent impregnation took 12 to 15 days and additional 3 to 4 days of continuous vacuum. Intermittent impregnation supposedly aids the exchange of acetone and silicone particles in deep layers of the tissues. It supposedly also "reduces significantly the work of the pump."

Intermittent impregnation subjects the pump to 12 to 15 cycles of warm up and removal of the air above the silicone level. Removing large volumes of air at initially atmospheric pressure is the most damaging operation mode for a rotary van vacuum pump because the air is compressed inside the pump chamber creating compression heat. The pressure that builds up is contained by the vanes which move slightly in the rotor's slots. This movement under high pressure is causing friction and wear. A continuous impregnation limits the number of such cycles to one.

In addition the daily "equilibration" will at least for the first 4 or 5 cycles allow acetone to seep out of the specimen and collect on the surface of the silicone bath. After removing the air the pump will have to deal with a high concentration of acetone which contaminates and thins the pump oil. A continuous vacuum would subject the pump to one phase of high acetone concentration after establishing the initial vacuum. The gas ballast is sufficient to deal with the continuous but reduced flow of acetone vapors.

The article did not show a comparison of specimens impregnated under continuous vacuum vs. intermittent vacuum. Experiences with the rather viscous Biodur S10 when impregnating at -20° centigrade under continuous vacuum show that incomplete penetration and resulting shrinkage is only a problem if the impregnation is run at an excessive speed i.e.: to many bubbles. I conclude that intermittent impregnation with a less viscous silicone is without merit and even damaging to the vacuum pump.

Zheng at al. also describe an acetone filter where three stainless steel containers are installed between the vacuum chamber and the pump. The middle container is filled with water and the containers at either side are empty and serve to catch possible spills. I was prompted by an e-mail discussion to look at this design with a critical eye.

It appears to me that the designers were guided by good intentions (to protect the vacuum pump) rather than technical knowledge. Apparently, the system works at room temperature and since it is water operated it cannot be at a temperature below freezing. I am at a loss trying to find any forces that would keed the acetone in the water inside the middle tank. I believe the vapor pressure will make the acetone change into it's gaseous state as vacuum is applied. It will not matter whether the acetone is contained in silicone or in water. This design has no merit other then making the acetone bubble twice: once in the silicone and then again passing through the water. The only way I can think of catching acetone in line would be by means of a liquid nitrogen operated trap. Such a system would not be feasible because it would require to maintain an ultra low temperature over an extended period of time.

While the authors intended to protect the pump they may in reality cause damage. First, the 3 containers add air volume to the vacuum system which imparts stresses to the moving parts when the vacuum is established. Second, as soon as the vacuum is low enough and all acetone is evapored the substance ranking next in terms of vapor pressure will change into the gaseous state. This may be a component of the silicone mixture or more likely the water in the filter.

Vacuum pumps have very limited water tolerances. Water does not mix with the oil and it cannot evaporate at a pump temperature of only 85 centigrade. Water droplets will be squeezed by the moving parts interrupting the lubricating oil film. Dr. Zheng finishes impregnation at a pressure of 7.5mm Hg. That may have kept the water inside the tank and saved the pump.

I very much appreciate the contributions by Dr. Zheng et al. Tinkering with processes and running afoul has lead to many advancements in technology. I admire Dr. Zeng's spirit and energy. I am sure at some point in time his inventive mind will produce a widely accepted advancement in plastination.

Sincerely,

Wolfgang Weber
Anatomical preparator
Iowa State University
Ames, Iowa
U.S.A.

The Answer

Dear Mr. Weber,

Greeting from China. I received the letter that you sent to Mr Gilles Grondin, editor of the Journal of ISP. I am very glad of your interest in my paper "Plastination at Room Temperature". Thank you for your comments regarding the intermittent forced impregnation and the acetone gas filter system. Your opinions and suggestions will help me to improve my technique and equipment to perform plastination at room temperature.

For the intermittent forced impregnation, I am just finishing a paper: "Intermittent Vacuum Forced Impregnation for Plastination". It is specially prepared to discuss this particular topic. You conclude that "intermittent impregnation with a less viscous silicone is without merit and even damaging to the vacuum pump". Please notice one key point that we had to rebuild the commercial vacuum pump by replacing the plastic parts and common rubber with metal material and silicone rubber. So in my plastination factory after these modifications simple commercial rotary vane vacuum pumps have been used for more than two years without any problems. According to my practice the cheaper commercial rotary vane vacuum pumps are very easily damaged by compression heat that comes from long time continual work. During the intermittent vacuum procedure the vacuum pump only works 6-8 hours then get 16-18 hours to relax and cool down. That will protect the pump to avoid damaging. The cheaper commercial rotary vane vacuum pump made in China is also good enough for intermittent vacuum procedure. After rebuilding the commercial vacuum pump, it also can resist the high concentration of acetone that contaminates and thins the pump oil. I have practiced the intermittent forced impregnation more than 6 years (since 1993, when I worked in Iceland). There is also a very important key point in my plastination practice. It saves time, saves money (needn't expensive vacuum pump) and also gets very good results. In my Plastination Factory in Nanjing no workers are on duty at night, and we close the workshop on weekends and holidays.

For your second opinion about the acetone gas filter system, you mentioned that it was not possible for the acetone to be trapped in the water because the pressure in the water receptacle is the same than the pressure in the impregnation chamber and that the acetone will boil from the water as well as it boils from the impregnation chamber. I think you may be right on this point, but please notice that my acetone gas filter system consists of two phases. In the first phase, there is vacuum inside all the three stainless containers, but in the second phase there is no vacuum inside the exhaust tube. There is not only water inside the first phase, there are also some special structure designed inside the three stainless containers. Compared to the total volume of our vacuum chamber (1.9M by 0.8M), 3 containers do not add much air volume to the vacuum system. Now there are six 1.9M long vacuum chambers in Nanjing plastination factory. We use two sets of acetone gas filter systems to protect our vacuum pumps and decrease air pollution since more than one and half years. The results and efficiency are perfect.

Now I would like tell you the story why I designed the acetone gas filter system and how the system works. When I worked in Hong Kong, I used a container with many tubes inside as acetone gas filter, but it was not efficient. Fortunately, the vacuum pump in Hong Kong was very good one. It was of high quality and it could resist acetone, but it was also very expensive. In 1996 while working in Hong Kong, I started to set up the first plastination laboratory in Canton, China. We had not enough money to import expensive vacuum pumps and had to use Chinese pumps. Chinese pump is not expensive, but it is not acetone resistant. Some rubber pads are very easily damaged by acetone vapors. More than 6 Chinese pumps were damaged in 3 months. So I designed and set up the first phase of the acetone gas filter system to protect the vacuum pump. It works very well and protects the Chinese vacuum pump effectively. For decreasing air pollution, I also designed and set up the second phase of the acetone gas filter system and it also works well. Every month the public health department of our city government checks the air two times around our factory. If they find out acetone pollution, our plastination factory will be closed.

I hope to make my equipment system more clear in theory and have more scientists to accept it. So I urgently need your help to improve my system. My main purpose is to use plastinated specimens instead of formalin preserved specimens (say good-by to formalin) and to keep formalin out of the classrooms and benefit to the health of students and teachers. China is a big country, we need big amount of plastinated specimens to replace formalin preserved specimens in the near future. If you have different opinions, ideas or suggestions, please directly write to me. I would like to discuss with you and welcome you to visit my plastination factory in Nanjing and plastination laboratory in Shanghai, China. After your visit you will better understand how I am doing plastination at room temperature and what are the results of our equipment for plastination at room temperature.

Thank you again,

With best regards,

Prof. Zheng Tian Zhong
Department of Anatomy
Shanghai Medical University
138 Yi Xue yuan Road
Shanghai 200032
CHINA

ROOM TEMPERATURE DEHYDRATION

Dear Editor,

Being a keen reader of the mail that's put out on the ISP e-mail list and the ISP journal it has become apparent to me that there is a great diversity in the amount of knowledge that plastinators possess.

It seems that plastinators range from highly educated academics to those who, like myself, have far less formal, theoretical training.

I wonder then, if plastinators can be broadly grouped into two 'classes' of operators?

Those who have an academic interest in the technique and are in a position (as well as having the time and resources) to research this area and those, who like myself, are employed by an institution to perform specific tasks.

Technicians such as I, have of necessity, a 'cook book' mentality. All I ask is that given the appropriate equipment, the required reagents and the prescribed method then the results are assured. I can then do my job for which I am paid. But to do this I must have the recipe. A recipe that, given no change to the equipment, reagents and method, will repeatedly produce the same results. For this I rely on published papers by scientific minds for their information and take it pretty much for granted that stated facts are facts (especially where it concerns technique and their outcomes).

So confusion reigns when, something I've accepted as fact (and has been repeated so often in the literature) is contradicted. An example of this is seen when looking at acetone as a dehydration medium and it's application in the room temperature versus cold temperature environment.

My understanding has been that cold acetone dehydration (freeze substitution) has been the method of choice because of it's minimal effect on specimen shrinkage. It has been repeatedly claimed that room temperature, acetone dehydration produces unacceptable levels of specimen shrinkage (up to 40%). With this in mind our laboratory has been developed, at some considerable cost, to employ freeze substitution with all the attendant safety precautions in place. The latest issue of our journal (Vol 13, No 2) has a paper describing room temperature, stepwise dehydration with acetone. It claims shrinkage to be less than 5%.!! What am I to make of this?

Yours sincerely

Mr. Richard Krumins
Department of Anatomy
Anatomy School of Vet. Studies
Murdoch University
Murdoch, 6150
WESTERN AUSTRALIA

The answer

Since the introduction of the plastination technique, many dehydration methods have been proposed. Some used freeze-drying; ethanol dehydration at room temperature followed by a methylene chloride bath as an intermediary solvent; and the freeze substitution method that was judged the most efficient and recommended since many years. It was adopted by almost all the plastination laboratories. More recently acetone dehydration at room temperature was described. A quick glance in the Current Plastination Index and in the abstracts of the last conference in Trois-Rivières will permit you to find the references of all these works. This shows us that the plastination technique is not a static thing. It does progress and we hope it will keep on doing so for the benefit of all the actual and future plastinators. The object of our Journal is to inform the members of all these new methods that are proposed. Even if we think that most of us will continue to use the proved methods, it is important that we keep our mind open to novelties and it is to each plastinator to evaluate if he or she wants to change his or her method.

Gilles Grondin
Editor

PLASTINATION IN CHINA

At the present time, most specimens in the biological laboratories and museums are fixed by formalin and kept in the glass containers. Manipulation of these specimens involves protective equipment like gloves and often gaz masks. Formalin is irritating for mouth and nose, and it's toxicity is recognized. So storage of biological specimens in formalin is certainly not the best method.

Plastination is a relatively new method developed in the middle of the 80's for permanently preserving biological specimens in a lifelike state. Through a vacuum procedure, the tissue water is replaced by curable polymer (silicon). Once the polymerization (curing) has taken place inside the specimens, silicone fills and supports the tissues. Plastinated biological specimens retain their original surface relief and cellular identity down to the microscopic level. They are dry, odorless, durable, of natural appearance and not toxic.

The plastination technique is one of this century's most welcomed and exciting preservation method. The plastinated biological specimens will replace the formalin preserved specimens and will be widely used for teaching and research in the next century.

In China, we started plastination quite late, but during the recent two years we developed it very quickly. Our method is distinct from the one used in west countries. Contrarily to

Continued on page 28

Antonio Pacchioni and Giovanni Fantoni on the Anatomy and Functions of the Human Cerebral Dura Mater

Regis Olry

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

(received March 22, accepted June 2,1999) *Key words:*

Meninges - Arachnoid granulations - Antonio Pacchioni - Giovanni Fantoni.

Abstract

The western anatomical descriptions of the human cerebral meninges still available date back to the mid-fourteenth century. Since that time, numerous anatomists made more or less accurate descriptions of the human meninges and tried to understand their functions. Unfortunately, the belief in animal spirits led to esoteric interpretations which hindered the understanding of these structures up to the early eighteenth century. This paper summarizes the contributions of Antonio Pacchioni and Giovanni Fantoni to the anatomy and functions of the meninges, respectively.

Introduction

Though Galen probably outlined the anatomy of the meninges and its sinuses in the early first millennium (Dumont, 1894), the description of this part of neuroanatomy remained for a long time marked with esotericism (see Clarke and Dewurst, 1984 and Corsi, 1990 for review). One of the very first anatomical illustrations depicting the human meninges is to be found in Guido da Vigevano's manuscript "Liber notabilium" (1345): four plates show the chest of a trephined body and both dura and pia mater are outlined (Olry, 1997). Subsequently, many anatomists tried to describe the structure of the meninges and to understand their functions. Johannes Dryander (1536, PI. 4) described two meningeal layers (probably the dura and pia mater). Some years later, Andreas Vesalius (1543) depicted the dura mater with its vessels (sinuses and middle meningeal artery) and the pia mater. In the mid-seventeenth century, Jean Riolan denied the existence of both layers of the cerebral dura mater (meningeal and endosteal, respectively), but their existence was confirmed by Isbrand van Diemerbroeck (1695) and Philippe Verheyen (1708).

Antonio Pacchioni

Antonio Pacchioni (1665-1726), a friend and pupil of Marcello Malpighi, was particularly concerned with the anatomy and function of the dura mater (Kemper, 1905; Norman, 1983; Eimas, 1990). In his 1701 treatise, he described in detail the tentorial incisure (Pacchionian foramen) and the structure of the falx cerebri, including its radiate fibres (figure 1). Unfortunately, he mistook them for muscle and tendon bundles which were supposed to contract the falx and therefore compress the cerebral cortex. According to Pacchioni, this compression was intended to make the cerebral glands secrete the animal spirits. The dura mater was therefore regarded as an "encephalic heart" (De Smet, 1986), and a dozen years later, the medial and lateral longitudinal striae (Lancisi, 1713) were believed to be the marks of the regular impacts of the falx on the superior surface of the corpus callosum.

In a later dissertation (1705), Pacchioni made the decription which made him find his place in the history of anatomy: he described the arachnoid granulations which are called "Pacchionian glands" since that time. These structures had been previously depicted by Andreas Vesalius (1543, Plates 7 and 66), but the author did not pay much attention to them. The first plate of Pacchioni's dissertation was drawn by D. Moratori and engraved by N. Oddi. It depicts the superior sagittal and transverse sinuses, and many arachnoid granulations are to be seen in the lumen of the superior sagittal sinus (figure 2). However, Pacchioni believed that the function of these granulations was to secrete the cerebrospinal fluid.

Address correspondence to: Dr. R. Olry, Departement de chimie-biologie, Universite du Quebec a Trois-Rivieres, C. P. 500, Trois-Rivieres, Quebec, CanadaG9A5H7. Telephone: 819 376 5053 /Fax: 819 376 5084. Email: Regis_Olry@uqtr.uquebec.ca

Antonio Pacchioni made therefore accurate anatomical descriptions, but misunderstood the functions of the dura mater and arachnoid granulations.

Giovanni Fantoni

Some decades later, Giovanni Fantoni (1675-1758) corrected both Pacchioni's mistakes: on the one hand, he showed that the dura mater does not contain any muscular bundles, and on the other hand he asserted that the arachnoid granulations are in charge of the reabsorption, and not secretion, of the cerebrospinal fluid: "The humoral flow is sent to the superior sagittal sinus rather than to the hemisphere convexity. This is more in line with the laws of nature, and the sinus itself, and not the meninges, is irrigated by this liquid, and the blood will therefore be diluted" (1738).

Discussion

Though he misunderstood the nature of the fibrous tracts in the falx cerebri and the real function of the arachnoid granulations, Antonio Pacchioni has to be regarded as a pivotal figure in the history of the anatomy of human meninges. He described the tentorial incisure and the arachnoid granulations which had only been mentioned by his predecessors, and his name rapidly appeared in the studies of his contemporaries (Heister, 1719). That is why Antonio Pacchioni became eponymous in the medical profession.

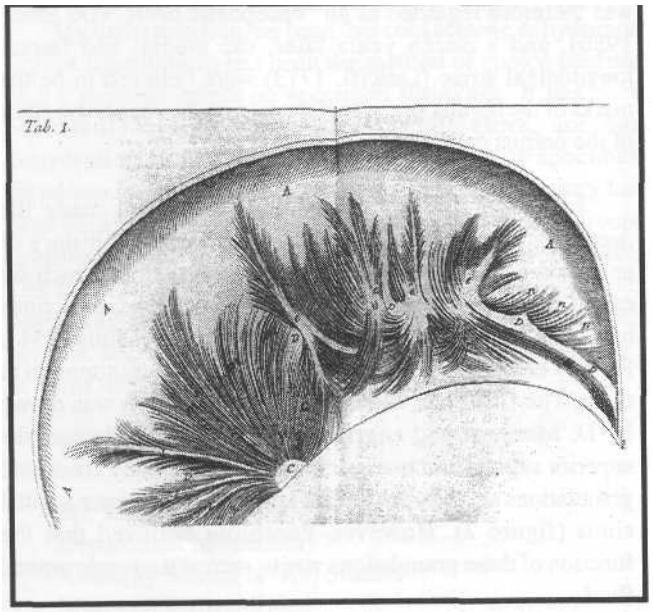


Figure 1. Plate 1 of Pacchioni's 1701 treatise. The radiate fibres of the falx cerebri were believed to be muscular bundles.

Bibliography

Clarke E, Dewurst K: Histoire illustree de la fonction cerebrale. Paris: R. Dacosta, 1984. Corsi P: La decouverte du cerveau. De l'art de la memoire aux neurosciences. Milan: Electa, 1990. De Smet Y: La neuropsychologic "pre-corticale". Histoire de la localisation et de la constitution des "fonctions mentales superieures" de Thales de Milet a Franz Josef Gall. Bruxelles: Ciaco editeur, 1986. Diemerbroeck I van: L'anatomie du corps humain. Lyon: Anisson & Posuel, 2 volumes (French translation by J. Prost), 1695. Dryander J: Anatomia capitis humani. Marburg: E. Cervicornus, 1536. Dumont J: Les sinus posterieurs de la dure-mere et le pressoir d'Herophile chez l'homme. Nancy: A. Voirin et L. Kreis, 1894.

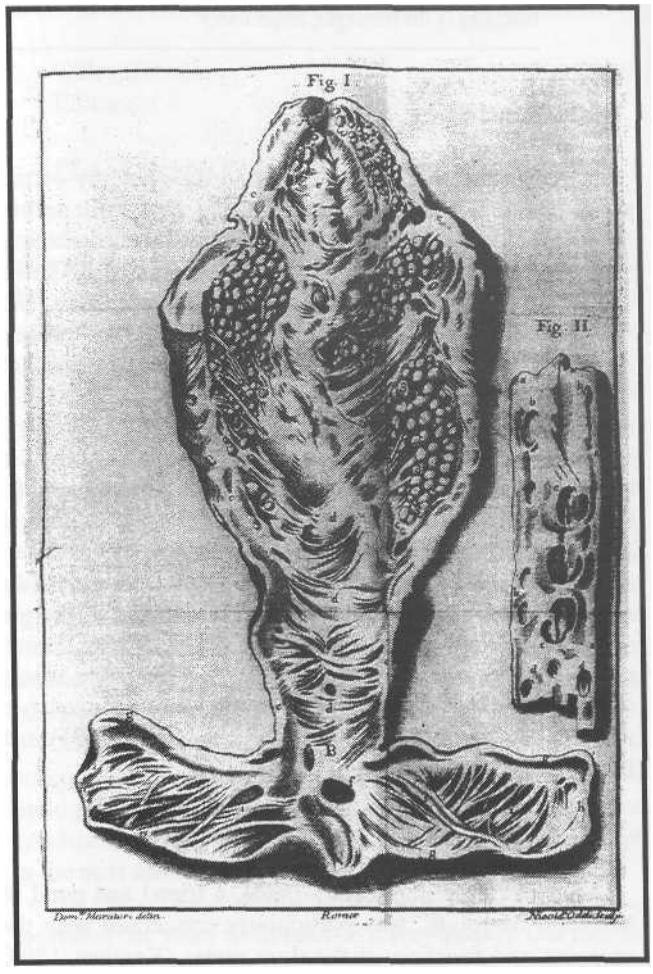


Figure 2. Plate 1 of Pacchioni's 1705 dissertation. The superior sagittal and transverse sinuses are open, and many arachnoid granulations are depicted.

Eimas R: Heirs of Hippocrates. The Development of Medicine in a Catalogue of Historic Books in the Hardin Library for the Health Sciences, the University of Iowa, 3rd Ed. Iowa City: University of Iowa Press, 1991.

Fantoni G: Diss. de structura & motu durae membranae cerebri, de glandulis ejus, & vasis lymphaticis piae meningis. In: Opusculamedicaetphysiologica. Geneva: Pellissari, 1738.

Heister L: Compendium anatomicum. Altdorf & Nuremberg: Kohlesiano et Adolphiano, 1719.

Kemper GWH: The World's Anatomists. Philadelphia: P. Blakiston's Son & Co., 1905.

Lancisi GM: Diss. II. quarum prior est de physiognomia, altera de sede cogitantis animae. Venice, 1713.

Norman J: Medicine and the life sciences. Catalogue thirteen. San Francisco: J. Norman & Co., 1983.

Olry R: Medieval Neuroanatomy: the Text of Mondino dei Luzzi and the Plates of Guido da Vigevano. J Hist Neurosci 6 (2): 113-123, 1997.

Pacchioni A: De durae meningis fabrica & usu disquisitio anatomica. Rome: typis D. A. Herculis, 1701.

Pacchioni A: Dissertatio epistolaris ad Lucam Schroeckium de glandulis conglobatis durae meningis humanae, indeque ortis lymphaticis ad piam meningem productis. Rome: Giovanni Francisco Buagni, 1705.

Verheyen P: Anatomie oder Zerlegung des menschlichen Leibes. Leipzig: Thomas Fritsche, 1708.

Vesalius A: De humani corporis fabrica libri septem. Basle: J. Oporinus, 1543.

Vigevano G da: Liber notabilium illustrissimi principis Phiippi septimi, Francorum regis, a libris Galieni per me Guidonem de Papia, medicum suprascripti regis atque consortis ejus indite Johanne regine, extractus, anno Domini millesimo CCC° XLV°, papa vivente Sexto Clemente. Latin manuscript No. 569, Muse'e Cond6 de Chantilly, 1345.

J Int Soc Plastination Vol 14, No 1:12-17, 1999

A Technique for Preserving the Subarachnoid Space and its Contents in a Natural State with Different Colours

Po-Chung An, Ming Zhang

Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand

(received February 11, accepted April 16, 1999)

Keywords: subarachnoid space, sheet plastination, cisternal anatomy

Abstract

The subarachnoid space consists of a number of distinct compartments called subarachnoid cisterns. Knowledge of cisternal anatomy is very important not only for anatomists but also for clinicians, particularly neurosurgeons. This paper reports a technique which combines the traditional E12 sheet plastination method with several special treatments so that the subarachnoid space, transcisternal arteries and veins, cranial nerves and arachnoid trabeculae are preserved in a relatively natural state and shown with different colours. This technique should greatly facilitate cisternal anatomy studies and provide a new approach for examining structures in the subarachnoid space at both macroscopic and microscopic levels.

Introduction

Compartmentalisation of the subarachnoid space (SAS) into subarachnoid cisterns by arachnoid trabecular walls has been widely described (Yasargil et al., 1976; Matsuno et al., 1988; Brasil and Schneider, 1993; Vinas et al., 1994,1996a,b). Most of the intracranial operations for intracranial aneurysms, brain tumours and disorders of cranial nerves are directed through the subarachnoid cisterns. These cisterns provide a natural pathway through which the major intracranial arteries, veins and cranial nerves can be approached. Thus cisternal anatomy (the anatomical relationship of arachnoid trabecular walls with vascular and neural elements) has significant importance not only to anatomists but also to clinicians, especially neurosurgeons.

Current knowledge of cisternal anatomy mainly comes from two sources: anatomical dissection and clinical observation, such as radiological examination and intracranial operation. However, the fine and delicate arachnoid trabeculae are easily destroyed in anatomical dissection when brain is removed from its cranial cavity during preparation. Also, pathological changes in the brain may affect clinical observation. Thus, in order to obtain reliable information about cisternal anatomy, a method that can preserve the SAS and its contents in a natural state without above drawbacks is needed.

Sheet plastination is a recently developed technique in which water and lipids of tissues are replaced by curable resin on a cellular level. The sheet plastination technique has been widely applied to human brain studies to demonstrate neuroanatomy (see Grondin and Olry, 1996 for review). However the subarachnoid cisterns and their contents can not be adequately demonstrated by this technique. In this study, we have modified the traditional techniques so that the SAS, arachnoid membranes, transcistemal arteries and veins can be preserved in a natural state and stained with different colours. Our modified technique should greatly facilitate further cisternal anatomy studies, and provide a new approach for examining structures in the arachnoid cisterns at both macroscopic and microscopic levels.

Materials and Methods

Materials

Cadavers of three female humans aged from 75 to 85, and 8 sheep were used in this study. The experiments using sheep were approved by the University of Otago Committee on Ethics in the Care & Use of Laboratory Animals.

Fixation

The right femoral artery and vein of a cadaver were

Address correspondence to: Dr Ming Zhang, Department of Anatomy and Structural Biology, University of Otago, PO. Box 913, Dunedin, New Zealand. Telephone: 64 3 479 7378 / Fax: 64 3 479 7254 Email: zhang.ming@stonebow.otago.ac.nz

cannulated and blood was washed out by saline. Ten percent formalin followed to fix the whole body. The body was then stored in the same solution until required.

Pretreatment

Arachnoid mater staining:

In order to completely stain the whole arachnoid mater, ten burr holes of 0.5 mm in diameter were drilled into the skull. The locations of these holes are shown in figure 1. Catheters of 14 gauge were properly inserted into the SAS with the aid of a stylet with dura puncture. The burr holes were then sealed using epoxy glue. Each hole was perfused with 10 ml of Gill's haematoxylin No.2. The SAS was stained for 2-3 minutes and then irrigated using distilled water to wash out the staining solution. The staining was made blue with the Scott's tap water for 2-3 minutes, 40 ml for each hole. Then the SAS was irrigated with the same amount of distilled water.

Vascular fillings:

The internal jugular veins and internal carotid arteries were exposed and cannulated at both sides and perfused with blue and red coloured epoxy resin E20, respectively.

SAS perfusion:

After arachnoid mater staining and vascular filling, the SAS was perfused with 400ml of 10% gelatine solution, 40 ml for each hole. The cadaver was left at room temperature for 24 hours to allow the gelatine to become solid.

Variations for pretreatment

In order to achieve optimal results for staining of arachnoid trabeculae and fillings of vessels and SAS, eight sheep heads were used in our preliminary experiments. Table 1 and Table 2 summarise the materials which were used for the arachnoid mater staining (Table 1) and the SAS and vessel fillings (Table 2).

E12 sheet plastination

Slicing:

The head of a cadaver was disarticulated at the second cervical vertebra level (C2), embedded in 20% gelatine solution and frozen at -30°C to make a gelatine block. Then the gelatine block was frozen at -80°C for 24 hours. The block was sectioned in a thickness of 2.5 mm by a butcher's bandsaw and the cutting surfaces of slices were cleaned with tap water.

Dehydration:

The slices were laid between mesh and stacked in a grid basket. The basket was immersed in ascending concentration

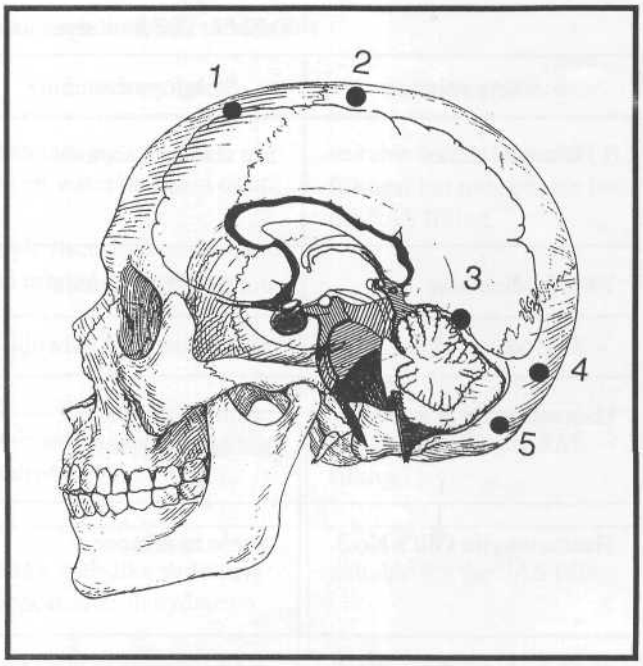


Figure 1. A lateral view of the skull showing the location of the burr holes. On each side, two burr holes on the vault (1&2) cover the frontal and superior regions of the subarachnoid space (SAS) and two burr holes (3&4) above and one (5) below the nuchal line cover the superior and inferior tentorial regions of the SAS, respectively.

of acetone (95% to 100%) at -30°C. The acetone was replaced every week with higher concentration. The dehydration was completed when the water content in the acetone was less than 1%. The dehydration process took 6 weeks.

Degreasing:

After dehydration, the specimens were left in final acetone bath for degreasing. The temperature of acetone bath was kept at 22-24°C. The degreasing was completed after 2 weeks at which time the fat tissues appeared translucent.

Forced impregnation:

The degreased slices were immersed in a polymer mixture and placed in a vacuum container at 0°C for 36 hours (see "Plastination Workshop", 1997). The polymer mixture was Biodur E12/E1/AE10/AE30 (BIODUR; Rathausstrasse 18, 69126 Heidelberg, Germany) in a ratio of 100:28:20:5 pbw (parts by weight).

Hardening:

The impregnated slices were laid between 0.25mm A.P.E.T (Amorphous Polyethylene Terephthalate, Progressive Plastic Ltd, 31-37 Fraytt St, Dunedin, New Zealand) plastic sheets and cured at 32°C for one week and then placed in an oven at 45°C for another week. Then the oven was switched

Table 1. Different dyes used for the arachnoid mater staining

Staining solution	Stability of staining	Results	Comments
0.1% cresyl violet	not stable in acetone	the resin becomes milky white due to acidity	the SAS needs to be acidified by 10% acetic acid.
1% toluidine blue	not stable in acetone	the staining fade away during dehydration	
Haematoxylin & eosin	eosin staining is not stable in acetone.	blue-stained tissue	
Haematoxylin Gill's No.3	stable in acetone	black blue-stained tissue	hard to control the incubation time as it was less than 1 minute
Haematoxylin Gill's No.2	stable in acetone	purple blue-stained tissue	relatively easy to control

off and allowed to cool down gradually before the slices were removed.

Results

Initial method

The SAS is located between the arachnoid mater and pia mater and mainly contains cranial nerves, arachnoid trabeculae, transcisternal arteries and veins. Our initial method for preserving the SAS and its contents was based on E12 sheet plastination protocol (von Hagens et al.,1987; Cook, 1997). Figure 2a shows a specimen which was processed with a standard E12 sheet plastination technique after the arteries were perfused with red epoxy resin E20. The fine vascular structures were very well preserved in the brain tissue but the SAS and its contents were completely destroyed. It is impossible to distinguish the pia mater, arachnoid mater and arachnoid trabeculae from surrounding structures in the specimen which was processed using the standard sheet plastination technique.

Modified method

In order to overcome the above problems, 8 sheep heads were used in the preliminary experiments. Tables 1 and 2 summarise the results and the recommended procedures have been described in the Methods section. With this modified E12 sheet plastination technique, the SAS (transparent with a few fine yellowish web-like lines), transcisternal arteries (red) and veins (blue), the cranial nerves and the arachnoid trabeculae (purple-blue) were well preserved in a relatively natural state and stained with different colours (figure 2b).

Discussion

In this study, for the first time, we provide a method for using E12 sheet plastination technique with several special treatments to preserve the SAS, transcisternal arteries and veins, the cranial nerves and the arachnoid trabeculae in a relatively natural state with different colours. Our method should greatly facilitate further cisternal anatomy studies and provide a new approach for examining structures in the SAS at both macroscopic and microscopic levels.

The protocol that we have established is based on a combination of the traditional E12 sheet plastination technique (von Hagens et al. 1987; Cook, 1997) and a technique of SAS perfusion and staining (Brasil and Schneider, 1993). However, there were several limitations for a simple combination. For example, according to the Brasil and Schneider's method, the SAS needs to be acidified in order to stain SAS with cresyl violet. However, the acidification makes translucent epoxy resin become milky white after its injection. Thus the structures in the SAS can not be identified in this situation. Therefore, we first tried to find a suitable dye which would stain the arachnoid trabeculae, which would not need acidification of the SAS, and would not fade in acetone during the dehydration procedure. We found that haematoxylin Gill's No.2 or No.3 stain was very stable in acetone. However, Gill's No.3 is such a strong staining solution that all the structures in the SAS

Table 2. The fillings of the subarachnoid space (SAS) and vessels

Materials	Advantage	Disadvantage	Comments
epoxy resin E20	translucent and similar to E12	becomes milky white when water and acid exist. high viscosity and needs to be diluted with acetone	suitable for the vascular fillings, but not suitable for the SAS filling.
20% gelatine	soluble in water	yellowish after dehydration	not suitable for the SAS filling
20% gelatine with 5% Arabic gum	soluble in water	becomes a white mass after dehydration	not suitable for the SAS filling.
10% gelatine	transparent and soluble in water	a few web-like structures appear after dehydration	suitable for the SAS filling
5% gelatine	transparent and soluble in water	not solid at room temperature	not suitable for the SAS filling

were stained as dark blue in a very short incubation period (less than 1 minute). Thus the structures in the SAS became indistinguishable. Haematoxylin Gill's No.2 is optimal for the arachnoid trabeculae staining because its incubation time is easily controlled and it is stable during E12 sheet plastination procedures.

Before plastination, it is also important to fill the SAS with a transparent material to hold the structures in a natural state and prevent any damage to these structures during the sheet plastination procedures. Epoxy resin E20 and 5,10 and 20% gelatine solutions with or without Arabic gum have been tried in this study. Translucent epoxy resin E20 was supposed to be an ideal SAS filling material because red-coloured epoxy resin E20 was used to perfuse arteries in our initial trial and demonstrated a very good vascular filling (figure 2a). However the viscosity of pure E20 is very high and needs to be diluted in acetone. The acetone diluted epoxy resin became milky white in the SAS when cured. This was caused by the residual water in the SAS and the water extracted by the acetone in the diluted epoxy resin. Moreover, it seems that the change of translucent epoxy resin is irreversible. The cured milky white resin in the SAS of the specimen still showed milky white after dehydration in acetone. A mixture of gelatine and Arabic gum recommended by Brasil and Schneider to fill the SAS lost its transparency after dehydration. Twenty percent gelatine solution could provide only a semitransparency after dehydration. Five percent gelatine solution offers an ideal transparency but it will not become solid at room temperature and, most importantly, cannot provide sufficient support for the contents of the SAS. Based on these findings, we found that 10% gelatine solution is the best selection for SAS filling. It can preserve the SAS in a relatively natural state, provide enough protection for the contents of the SAS during the plastination procedures and still show a satisfactory transparency after dehydration. Under a stereomicroscope, a few fine web-like structures can be seen in some regions of the SAS (figure 2b) and are presumably derived from the gelatine. However, these fine artifactual structures can be easily distinguished from the arachnoid trabeculae as they appear yellowish while the stained arachnoid trabeculae show purple-blue.

The purpose of perfusing vessels with coloured filling materials was to distinguish the small transcisternal arteries and veins from the fine arachnoid trabeculae. As a result of our modification, the small arteries appear red, the small veins blue, and the arachnoid trabeculae purple-blue. These coloured structures are readily distinguishable under the stereomicroscope. It was found that colored epoxy resin E20 is suitable for vascular filling. However, gelatine was found to be unsuitable: upon injection of gelatine into the vascular lumen, many small gaps occurred due to gelatine shrinkage and breakage after dehydration. This may explain why the small perforating vessels are not observable in brain tissue that has been perfused with colored gelatine.

A minor technical point is perhaps worthy of note. Rather than washing out the venous blood prior to fixation, it may be preferable to leave the blood in the veins, so that after

plastination, the small transcisternal veins appear almost black and thus easily differentiated from fine arachnoid trabeculae and transcisternal arteries.

We believe that the technique reported in this paper, which combines E12 sheet plastination with the special treatments described will greatly facilitate cisternal anatomy studies and provide a new approach for examining structures in the subarachnoid space at both macroscopic and microscopic levels.

Acknowledgment

The authors wish to thank Mr. Russell Barnett for his help in E12 sheet plastination technique, Mr. Brynley Crosado for his help in cadaver fixation.

Bibliography

Brasil AVB, Schneider FL: Anatomy of Liliequist's membrane. Neurosurgery 32(6): 956-961, 1993. Cook P: Sheet plastination as a clinically based teaching aid at the University of Auckland. Acta Anat 158: 33-36, 1997. Grondin G, Olry R: Current Plastination Index 1996.
Publication of the International Society for Plastination. Universite du Quebec a Trois-Rivieres, Quebec, Canada, 1996.

Matsuno H, Rhoton Jr AL, Peace D: Microsurgical anatomy of the posterior fossa cisterns. Neurosurgery 23(1): 58-80, 1988.

Vinas FC, Fandino R, Dujovny M, Chaverz V: Microsurgical anatomy of the supratentorial arachnoidal trabecular membranes and cisterns. Neurological Research 16: 417-424, 1994.

Vinas FC, Dujovny M, Frandio R, Chavez V: Microsurgical anatomy of the infratentorial trabecular membranes and subarachnoid cisterns. Neurological Research 18: 117-125, 1996a.

Vinas FC, Dujovny M, Frandio R, Chavez V: Microsurgical anatomy of the arachnoidal trabecular membranes and cisterns at the level of the tentorium. Neurological Research 18: 305-312, 1996b.

von Hagens G, Tiedemann K, Kriz W: The current potential of plastination. Anat Embryol 175: 411-421, 1987.

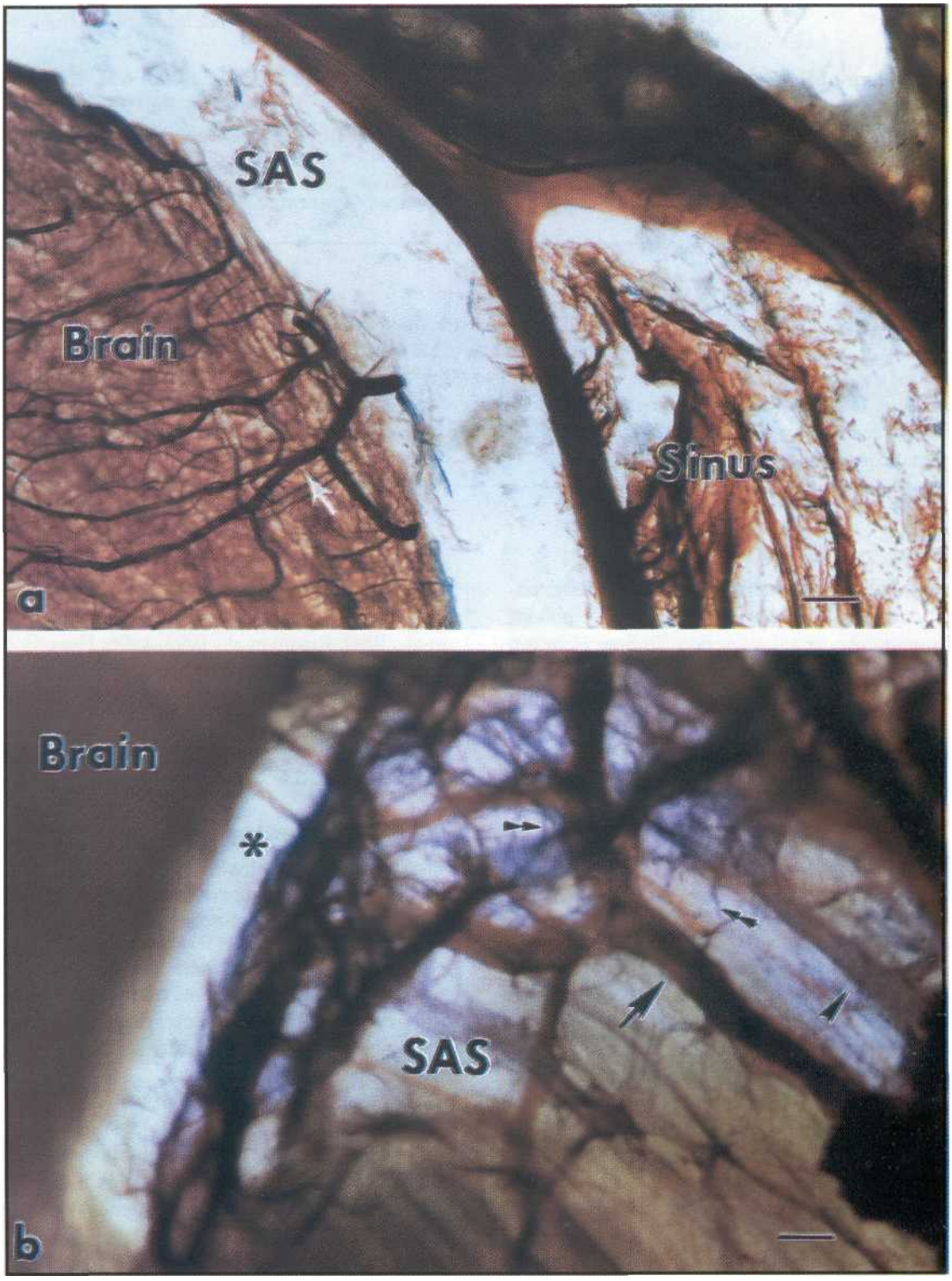
Yasargil MG, Kasdaglis K, Jain KK, Weber HP: Anatomical observation of the subarachnoid cisterns of the brain during surgery. J Neurosurg 44: 298-302, 1976.

Plastination Workshop and 5th Interim Meeting of the International Society for Plastination. The University of Tennessee, Knoxville, TN. Page 22, 1997.

Figure 2. Two plastinated slices prepared with the traditional E12 sheet plastination technique (a) and with the technique developed in this study (b). Bar =1 mm.

(a) shows that the brain tissue shrank, the subarachnoid space (SAS) enlarged, and the vessels in the brain were preserved well (arrow) but the most SAS contents were destroyed.

(b) demonstrates that the transcisternal arteries (arrow), veins (arrowhead), and the arachnoid trabeculae (double arrowheads) were held in their natural positions. The SAS was also well preserved in its natural size although an artifactual gap (asterisk) appeared underneath the pia mater due to the brain tissue shrinkage.


SAS
Brain
Sinus
a
Brain
SAS
b

The E12 Technique as an Accessory Tool for the Study of Myocardial Fiber Structure Analysis in MRI

E. Skalkos[1], G. Williams[1] and C.A.C. Baptista[2]

Department of Radiology' and Pathology[2], Medical College of Ohio, Toledo, Ohio, U.S.A.

(received March 1, accepted May 3, 1999)

Key Words: Heart, Magnetic Resonance Imaging, E12 Technique, Myocardial Fibers

Abstract

This paper documents use of the plastination E12 techique to analyze myocardial fiber arrangement and compare its pattern of distribution to magnetic resonance (MR) images. Human hearts were embed in a "plastic block" consisting of gelatin and polyethylene glycol and scanned using a General Electric Superconducting Magnet (Signa). After scanning the hearts were sectioned and processed for plastination. The E12 plastinated heart sections allowed visualization of the 3-dimensional details of the heart, vessels and myocardial bundles for comparison with the MR images. The myocardial fibers seen in the MR images showed similar gradient directions and details to the anatomical heart sections.

Introduction

The timing and adequacy of reperfusion of an infarcted-related artery (Croisille et al., 1999) determine patient prognosis in acute myocardial infarction. To limit ischemic myocardial injury, it is important to differentiate viable from infarcted (non viable) myocardium. Assessment of viability is based on either stress echocardiography or trace techniques; however, those methods have limited spatial resolution. For a review on predicting myocardial viability by MRI see Higgins (1999).

Magnetic Resonance Imaging (MRI) has the ability to quantify myocardial 3D deformation and strain precisely and non-invasively. In order to document that the MR (magnetic resonance) permit a true comparison between the generated images and the anatomy of the scanned organ, hearts were scanned and then sectioned so that anatomical details of each slice could be compared to the details in the image. The use of plastinated specimens seemed ideal for this purpose. The acquired transparency of the specimens as final result of the E12 technique permitted a 3D analysis and true comparison to the images generated by the MRI. Plastinated specimens can be stored for long-term use, and do not give off fixative fumes. Plastination is also a relatively simple and easier method to obtain clear specimens when compared to traditional methods such as Spaltholz (1924) and Tompsett (1956).

Since the birth of plastination in the mid-80s, sever authors have successfully used plastinated specimens to correlate morphology with MRI in research, education and clinical medicine (McNiesh and von Hagens, 1988; Baptista et al., 1990; Ripani et al., 1993, 1996; Hussain et al. ,1996; Magiros et al. ,1996,1997; Cook, 1997; Entius et al., 1997).

This paper introduces the E-12 technique as a tool for the comparative analysis of myocardial fiber distribution study using MRI.

Materials and Methods

Materials

Several human hearts were used for this study. The hearts were removed from cadavers and washed in tap water to remove blood and blood clots from the chambers, coronary arteries and cardiac veins. After the blood was removed, the heart chambers were filled in with cotton to maintain the cavities in the dilated state.

The coronary arteries were injected with a mixture of 30% gelatin and 0.1ml (0.05mmol)/kg of gadopentetate dimeglumine (Magnevist® - Berlex Laboratories, Inc., Wayne, NJ 07470, USA). Eosin (Sigma-Aldrich, 3050 Spruce Street, St Louis, MO 53178, USA) was added to the mixture for color.

Address correspondence to: C.A.C. Baptista, M.D. Ph.D., Medical College of Ohio, Department of Pathology, 3035 Arlington Avenue, Toledo, Ohio 43614-4804 U.S.A. Telephone: 419 383 6326 /Fax: 419 383 3066. Email: cbaptista@mco.edu

The hearts were then immersed in a 10% formalin solution for 24 hours. After fixation the hearts were prepared for MRI scanning.

Preparation of the heart for scanning

The hearts were immersed in a solution of gelatin (15 gm/100ml) and polyethylene glycol 1000 (lOml/lOOml of gelatin solution) using the method described by McCormick (1961). This plastic/gelatin solution was transparent and after solidifying maintained the position of the heart during the MRI scan and during physical sectioning.

Scanning

MRI images were obtained using the General Electric Superconducting Magnet (Signa) operating at 1.5 Tesla with a corresponding resonance frequency for protons of 63.9 MHz.

The scan protocol is a balanced matrix spin echo gated acquisition, (TR=2000 msec, TE=20 and 60 msec) for oblique, sagittal and/or coronal short and long axis views of the myocardium. Two images were generated at each anatomic level: one was formed from the first spin-echo (TE=20 msec) and the other from the second echo (TE=60 msec). The MR scan slice thickness was 3.0 mm with a skip distance of 3.0 mm. The images were archived to magnetic tape and transferred to the imaging workstation for analysis. Medical College of Ohio (MCO) software on the DEC microVax workstation allowed display, image processing and texture analysis of the images.

Image processing and mathematical codes have been written on the DEC microVax Workstation to analyze texture on the intensity distributions of the tissues scanned in the MR scanner.The cardiac slices were preserved by plastinau'on for future study and permanent storage.

Sectioning

The "plastic block" (gelatin and polyethylene glycol) was sectioned using an electric circular meat slicer. Three millimeters (3 mm) sections were made parallel to the long axis of the left ventricle. Each slice was placed between plastic grids and immersed in cold acetone (-25°C) for dehydration.

Dehydration

Freeze substitution as described by von Hagens (1985) and von Hagens et al. (1987) was used for dehydration. Three changes of acetone were necessary, with each bath containing a volume of acetone 5-10 times the volume of the specimens. After dehydration, the heart sections were immersed in methylene chloride for defatting. Two weekly changes of methylene chloride were necessary. After defatting the sections were impregnated with epoxy.

Impregnation

Impregnation using an epoxy based reaction mixture (El2) technique was performed at room temperature. The epoxy reaction mixture was: E12/AT30/AT10/E1 (95:5:20:26 pbw).

The specimens were submerged in the reaction mixture and placed in a vacuum chamber, directly from the methylene chloride solution. Pressure was reduced rapidly to lOmmHg. The rapid boiling out of the methylene chloride caused the temperature of the mixture to be reduced drastically. This helped to control the exothermic reaction taking place (Weber and Henry, 1993). Impregnation was accomplished in 24 hours.

Casting

The heart sections were cast between two sheets of tempered glass and a flexible gasket (Parker O-Ring - Zatkoff Seals & Packing, 8929 Airport HWY. Holland, Ohio 43528-9604, USA) was used as spacer. The following mixture was used as the casting resin: E12/AT30/E1 (95:5:26 pbw).

The specimens were placed between two glass plates, sealed and the molds filled with the casting mixture. After the molds were filled in they were placed inside a vacuum chamber to remove small bubbles present in the resin. This took place in 45 minutes to 1 hour. Larger bubbles were afterwards removed manually. After bubble removal, the mold was placed at horizontal 15° incline to assure correct positioning of the heart slice in the mold. When the polymer showed more viscosity and was tacky (2 to 3 days) the specimens were placed in an oven at 40°C for 10 days. Hardening was completed when "Newton" rings were seen in the casting molds. The glass plates were removed carefully and the sheets were cut and trimmed as desired.

Cutting and sanding the molds

A bend saw (Sears Roebuck and Co., 3333 Beverly Road, Hoffman Estate, IL 60179, USA) was used to cut and trim the plastic along the edges of the heart slices. Belt and disc sanders from Sears Roebuck and Co. were used to remove sharp edges from the plastic slices.

Photographic images of the 3.0 mm anatomical sections and MR scan slices were compared.

Results

In order to evaluate the quality of cardiac imaging of the GE MR scanner at the Medical College of Ohio, and to verify anatomical details on the MR images, several cadaver hearts were imaged and sectioned at 3 mm intervals. The heart images were processed using zoom, filters contour and cut modules. The maximum gradient images were then obtained and filtered. The gradient images were subtracted from the original images of the myocardium using an absolute subtraction method, to enhance the direction and structure of the muscle bundles in the myocardium. The heart sections processed by the E12 technique were correlated with the MR images showing that the myocardium can be identified in the computer-processed images. Maximum gradient processing emphasizes the direction of the muscle bundles and such images show similar gradient directions and details seen with the anatomical heart sections. Examples of the resulting anatomical slices and an intermediate scanned image are shown in figure 1.

Discussion

Magnevist® (that was injected in the coronary arteries mixed with gelatin and red dye) is a paramagnetic metal ion chelate used in clinical diagnostic procedures as an injectable enhancement agent to enhance signal intensity, and thus visual contrast, in magnetic resonance imaging.

McCormick's method (1961) to obtain cross sections of fresh tissue was helpful to align and section the heart similar to the coordinates used for MR scanning. Positioning the heart in the mixture of gelatin and polyethylene glycol 1000 ensured stability during the scan as well as during sectioning. Marking the MR scan lines on the container facilitated sawing the specimen in the same planes as the scans. The resultant images were closely related to the scan sections.

Parker O-Ring from Zatkoff was an ideal locally available product to use for the gasket in the plastination mold. It is of a flexible base polymer, nitrile and is supplied in a variety of diameters (3 mm to 10 mm). In our case the thickness used was 3 mm.

The El2 sheet specimens allowed visualization of the 3-dimensional detail of the heart, vessels and myocardial bundles for comparison with the MR images. Maximum gradient processing emphasizes the direction of the muscle bundles and such images show similar gradient directions and details with the anatomical heart sections.

Acknowledgement

The authors wish to thank Dr. Robert W. Henry, College of Veterinary Medicine, The University of Tennessee, for his critical review of this manuscript, and Tina Kinkaid and Carol Okenka, Department of Radiology, Medical College of Ohio for their assistance with the MRI scanning.

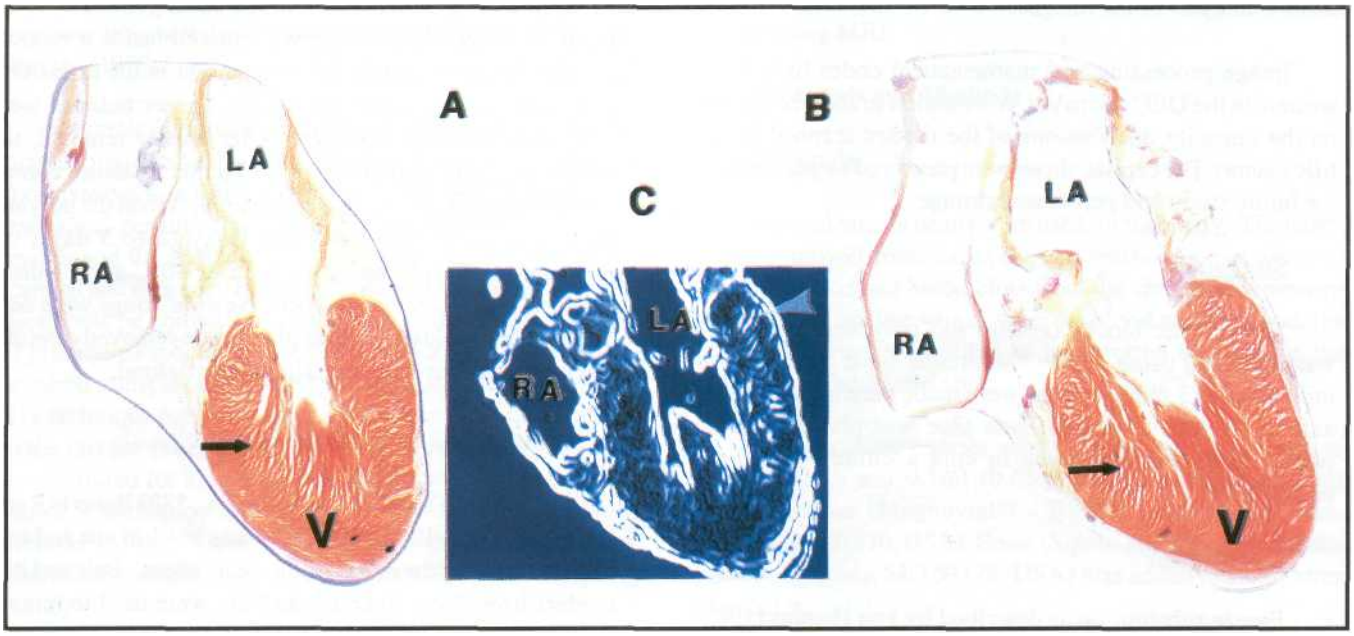


Figure 1: Photographs of plastinated heart sections (A, B) and an intermediate magnetic resonance image (C) showing the following structures: left atrium (LA); right atrium (RA); left ventricle (V) and myocardial bundles an fibers (arrows).

Bibliography

Baptista CAC, Henry RW, Williams G, Brinker R: Magnetic resonance imaging of plastinated specimens: study of tissue characteristics and evaluation of the hardening process of the S10 technique. J Int Soc Plastination 4:3, 1990.

Cook P: Sheet plastination as a clinically based teaching aid at the University of Auckland. Acta Anat 158(1): 33-36, 1997.

Croisille P, Moore CC, Judd RM, Lima JAC, Arai M, Me Veigh ER, Becker LC, Zerhouni EA: Differentiation of viable and nonviable myocardium by the use of three-dimensional tagged MRI in 2-day-old reperfused canine infarcts. Circulation 99: 284-291,1999.

Entius CAC, van Rijn RR, Holstege JC, Stoeckart R, Zwamborn AW: Correlating sheet plastination slices, computed tomography images and magnetic resonance images of the pelvic girdle: a teaching tool. Acta Anat 158(1): 44-47,1997.

Higgins CB: Prediction of myocardial viability by MRI. Circulation 99: 727-729, 1999.

Hussain SM, Stoker J, Entius CAC, van Rijn RR, Zwamborn AW, den Hollander JC, Lameris JS: Endoanal magnetic resonance imaging of the anal sphincter complex: correlation with sheet plastination slices and histology. J Int Soc Plastination 11(1): 5, 1996. Magiros M, Kekic M, Mills FR, Cheyne-Macpherson S, Huand M, Doran GA: Magnetic resonance imaging (MRI) versus thin plastinated cadaveric sections as an anatomic learning tool. J Int Soc Plastination 11(1): 12, 1996.

Magiros M, Kekic M, Doran GA: Learning relational anatomy by correlating thin plastinated sections and magnetic resonance images: preparation of specimens Acta Anat 158(1): 37-43, 1997. McCormick JE: Serial Sections of Gross Anatomy. Arch Path 72 (6): 93-95,1961. McNiesh LM, von Hagens G: The diagnostic imaging characteristics of plastinated anatomical specimens. J Int Soc Plastination 2(1): 24-39, 1988. Ripani M, Bassi A, Perracchio L, Boccoa ML, Tomaselli G, Giacomelli L, Messinett S, Marinozzi G: Comparative analysis of a plastination specimens and clinical diagnostic images. J Int Soc Plastination 8(1): 12-13, 1993. Ripani M, Di Cosimo E, Franco T, Crecco M: Comparative study of NMR-CAT images within series plastinated sections. J Int Soc Plastination 11(1): 22-23, 1996.

Spaltholz W: Das Durchsichtigmachen als biologische Arbeitsmethode. Handb. Biol. Arbeitsmethoden, Abt. 9 Berlin. Wien, 1924. Tompsett DH: Anatomical Techniques. Edinburgh, London: E & S Livingstone, 1956. von Hagens G: Heidelberg Plastination Folder: Collection of all technical leaflets for plastination. Anatomische Institut 1, Universitat Heidelberg, Heidelberg, Germany, 1985. von Hagens G, Tiedmann K, Kriz W: The current potential of plastination. Anat Embryol 175: 411-421,1987.

Weber W, Henry RW: Sheet plastination of body slices - E 12 technique, filling method. J Int Soc Plastination 7:

-J lnt Soc Plastination Vol 14, No 1: 22-24, 1999

P40 Plastination of Human Brain Slices: Comparison between Different Immersion and Impregnation Conditions

Mircea-Constantin Sora[1], Peter Brugger[1] and Hannes Traxler[2]

'Department of Anatomy 2, Anatomical Institute, Vienna University, Austria
department of Anatomy 1, Anatomical Institute, Vienna University, Austria

(received September 30, 1998; accepted February 24, 1999)

Key Words: Brain, Polymer P40, Impregnation, Temperature

Abstract

One human brain was used for this study. The brain was fixed in 5% formalin for two months, rinced and cut in two halves on the sagital plane. Both brain halves were sagitally sliced at a tickness of 4 mm. From each brain half we selected 8 slices and plastinated them with P40 using different immersion and impregnation conditions. Two points were marked on each slice and subsequently an imprint of the slices was drawn on transparency film. After dehydration in -25°C acetone, the slices of the left brain half were immersed at -25°C, for two days in P40 and then impregnated for 24 hours. The slices of the right brain half were immersed at +5°C for two days and impregnated at room temperature at+15°C for 24 hours. All impregnated slices were cured with UV light. The imprints of the fixed brain slices were scanned into a computer, as well as the plastinated slices. By using a Kontron KSA 400 v. 2.0 (ZEISS) software we calculated the area of the plastinated brain slices as well as the area of the scanned imprints. By comparing the obtained data we were able to determine the shrinkage rate of the slices. The slices processed at -25°C showed a shrinkage rate of 4.41%. In comparison the slices immersed at +5°C and impregnated at +15°C showed a shrinkage rate of 6.96%.

Introduction

Brain slices plastinated with the P40 technique (von Hagens, 1994) are shrinking and sometime this shrinkage is very annoying. In order to find out the shrinkage degree we used two different immersion and impregnation conditions. One set of slices was immersed and impregnated at -25°C and an other set of slices was immersed at +5°C and impregnated at room temperature (+15°C). These temperature conditions being both described (von Hagens, 1994; Barnett, 1997), the purpose of this study was to find out the most suitable plastination conditions for brain slices.

Materials and Methods

Fixation and Sectioning

A human brain was obtain at post-mortem from a cadaver. The brain was fixed in 5% formalin for two months. Before being serially sectioned the brain was washed in running tap water for two days.

The brain was first cut in two halves. By using a meat slicer each half was sliced in 4 mm thick sagittal slices. Sixteen slices were selected for this study: 8 slices from the left brain half and 8 slices from the right brain half. As we intended to find out the shrinkage degree of the slices we placed in each slice two plastic markers, one anterior and one posterior (figure 1). The distance between the marker was measured and subsequently an imprint of each slice was drawn on transparency film. The imprints drawn from the fresh slices (figure 2) were scanned into the computer and the area was calculated by using a Kontron KSA 400 v.2.0 (ZEISS) software. In a next step the plastinated brain slices were scanned into the computer and the area of the plastinated slices was determined by using the same Kontron KSA 400 v.2.0 (ZEISS) software. The distance between "A" and "B" was necessary in order to calibrate the scanned images. From those brain slices where the cerebellum was not attached to the slice, imprints were made only from the cerebrum. The marker placed anterior was named "A" and the marker placed posterior was named "B". The obtained slices were placed into two grid baskets and into distilled water at +5°C overnight (von Hagens, 1994).

Presented in part at the 9th International Conference on Plastination, Trois-Rivieres, Canada, July 5-10, 1998. Address correspondence to: M.-C. Sora, MD, Anatomical Institute, Vienna University, Währingerstrasse 13/3, A-1090 Vienna, Austria. Telephone: 43 1 4277 611 50 / Fax: 43 1 4277 611 42. Email: mircea-constantin.sora@univie.ac.at

Dehydration

Both sets of slices were placed into an acetone bath at -25°C (Barnett, 1997). After two days the grid baskets containing the slices were moved into an other acetone bath, also at -25°C. Dehydration was completed after one week.

Immersion

The slices of the left brain half were placed with the grid basket into the immersion bath of P40 at -25°C for 2 days. The slices of the right brain half were placed with the grid basket into the immersion bath of P40, at +5°C, for 2 days.

Impregnation

The grid basket containing the left brain slices was taken from the immersion bath (-25°C) and placed in cold P40 at -25°C in the vacuum chamber. The vacuum chamber was then placed in a freezer at -25°C. Impregnation was undertaken for 12 hours and completed at a pressure of 2mm Hg (von Hagens, 1994).

The grid basket containing the right brain slices was taken from the immersion bath (+5°C) and placed in an impregnation bath at +5°C. The impregnation bath was placed into a vacuum chamber at room temperature (Cook and Barnett, 1996). Impregnation was undertaken for 12 hours and completed at a pressure of 10mm Hg (von Hagens, 1994).

Casting and Curing

The casting and curing conditions were the same for both brain halves. All slices were casted in flat chambers and the chambers cured with UV lights for 3 hours.

Results

In order to calculate shrinkage, the distance between the marker "A" and "B" was measured on all slices before and after plastination. One dimensional shrinkage could be calculated by comparing the distance between "A" and "B" on fresh and plastinated slices. At -25°C the average shrinkage is 1.92% and at room temperature it is 2.60%. By comparing the area of the fresh and of the plastinated slices a two dimensional shrinkage was calculated. On slices impregnated at -25°C we found a shrinkage of 4.41% and on slices impregnated at room temperature the shrinkage was of 6.96%.

Discussion

The aim of the present study was to compare 2 conditions of immersion and impregnation. Although we all know that dehydration leads to shrinkage (Schwab and von Hagens, 1981; Holladay, 1988; Tiedemann and Ivic-Matijas, 1988) we did not measure the shrinkage following dehydration for 2 reasons. First all 16 slices were dehydrated in the same way and second we considered that taking the slices out of the acetone to measure them could have produce drying of the slices, increasing of the shrinkage and could have damaged them.

The study suggests that immersion and impregnation of brain slices should occur at -25°C. Shrinkage can not be appreciated only by comparing linear distances. Precise appreciation of the degree of shrinkage can be obtained by area comparison or better by volumetric comparison. The shrinkage values determined in the present study represent only a two dimensional shrinkage. We all know that the total shrinkage of a specimen after plastination is three dimensional so the values for the real shrinkage will be greater than our findings.

The finding that the degree of shrinkage is about 4.41 % after immersion and impregnation at -25°C is excellent. P40 plastination is in our opinion the best method to preserve brain slices. Furthermore we think that the P40 technique is much better than the S10 standard technique for plastination of brain slices, where shrinkage can reach up to 10% (Suriyaprapadilok and Withyachumnarkul, 1997).

Even if the degree of shrinkage is 6.96% at room temperature the method could be used if a -25°C impregnation is not possible. Both shrinkage values are acceptable as we know that unsaturated polyester resins can usually shrink 5 - 8% during the transition from the liquid to the solid state (Arpe et al., 1992; Kirk-Othmer, 1996).

Table 1. Plastination conditions of the brain slices and corresponding percentage of shrinkage.

	RIGHT HALF	LEFT HALF
DEHYDRATION	-25°C, one week	-25°C, one week
IMMERSION	+5°C, 48 hours	-25°C, 48 hours
IMPREGNATION	+15°C, 24 hours	-25°C, 24 hours
LINEAR SHRINKAGE (mm)	2.60%	1.92%
SURFACE SHRINKAGE (mm^2)	6.96%	4.41%

Bibliography

Arpe H-J, Schulz G, Elvers B ed.: Ullmann's Encyclopedia of Industrial Chemistry. 5th Ed., Weinheim, VCH publishers, vol 21, pp 217-225, 1992. Barnett RJ: Plastination of Coronal and Horizontal Brain Slices using the P40 technique. J Int Soc Plastination 12 (1): 33-36, 1997. Cook P, Barnett R: Practical applications in plastination. Instructional videotape, Departments of Anatomy, University of Auckland and University of Otago, New Zealand, 1996. Holladay SD: Experiments in dehydration technique. J Int Soc Plastination 2 (2): 17-20, 1988.

Kirk-Othmer: Encyclopedia of chemical technology. 4th Ed., New York, Wiley, vol 19, pp 654-677, 1996. Schwab K, von Hagens G: Freeze substitution of macroscopic specimens for plastination. Acta Anat 111: 139-140, 1981. Suriyaprapadilok L., Withyachumnarnkul B: Plastination of stained section of the human brain: Comparison between different staining methods. J Int Soc Plastination 12 (1): 27-32, 1997. Tiedemann K, Ivic-Matijas D: Dehydration of macroscopic specimens by freeze substitution in acetone. J Int Soc Plastination 2 (2): 2-12, 1988. von Hagens G: Plastination of brain slices according to the P40 procedure. A step-by-step description, pp. 1-23, 1994.

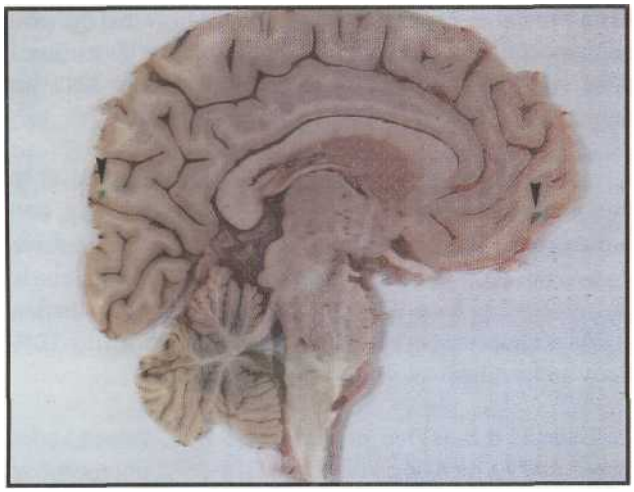


Figure 1. Fixed brain slice illustrating the markers (arrows) that were placed to measure the linear shrinking during the process.

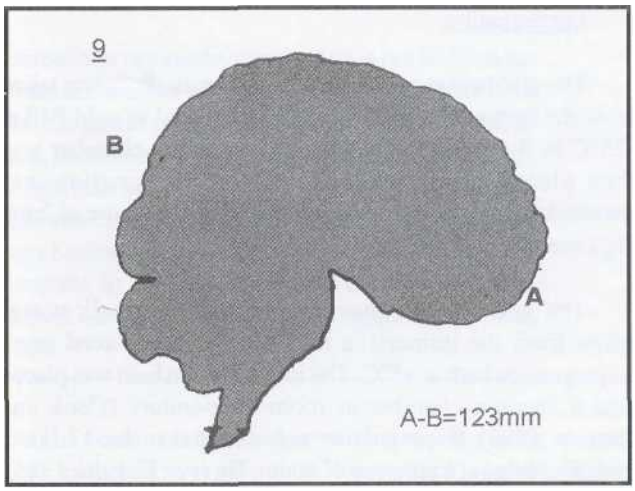


Figure 2. Scanned imprint from a brain slice used to calculate the surface of each slice before and after plastination in order to measure the shrinkage of the whole surface.

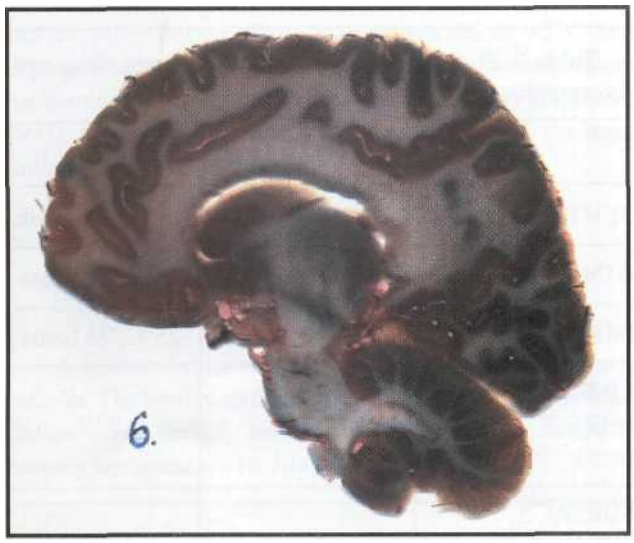


Figure 3. P40 plastinated brain slice after immersion at -25°C and impregnation at -25°C.

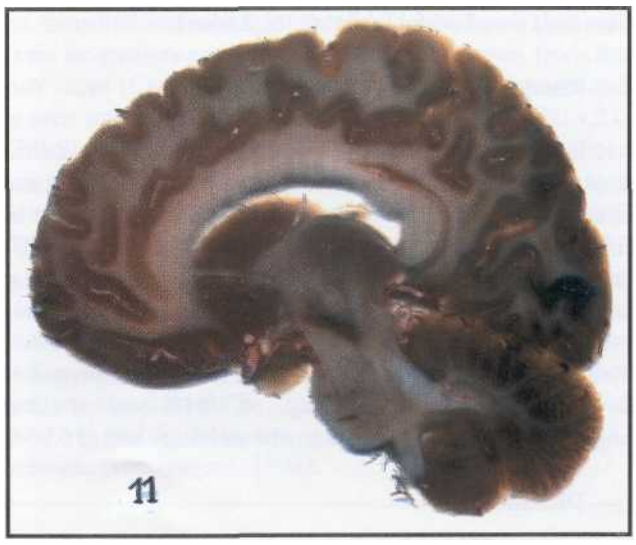


Figure 4. P40 plastinated brain slice after immersion at +5°C and impregnation at +15°C.

The Journal of the International Society fo Plastination: Assessment and Future Prospects

Regis Olry

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

(received March 22, accepted May 26,1999)

Key words: Journal of the International Society for Plastination, Full-length papers, Bibliographical references, Internationalization

Abstract

The Journal of the International Society for Plastination was founded twelve years ago by Harmon Bickley. The aim of this study was to assess its evolution through its first seventeen issues by analyzing different data of the full-length papers.

Introduction

The Journal of the International Society for Plastination (JISP) was founded in 1987 by Harmon Bickley. Since that date, it went through 13 volumes, for a total of 17 issues. Four editors have followed one another: Harmon Bickley, USA (Vols. 1-2), Robert W. Henry, USA (Vols. 3-7), Dale Ulmer, USA (Vols. 8-11), and Gilles Grondin, Canada (Vols. 12 and following ones). Wayne Lyons, Canada, was assistant editor for vols. 9-11.

Methodology

This study intended assessing the first seventeen issues of the journal (Vol. 1, number 1 to Vol. 13, number 2), and bringing out some future prospects. In that aim, only the full-length papers published in the JISP were taken into account. All other content was excluded from this study (editor's notes, presidential letters, minutes and abstracts of the biennial meetings or international conferences, editorials, annoucements, thesis reviews, exhibition reports, letters to the editor, short communictions). The data analyzed in each issue were the number of pages, the number of full-length papers, their average number of pages, of authors, of bibliographical references and the number of them taken from the JISP, and finally their geographical origin (continent and country).

Results

Owing to the variable number of issues per volume (2 issues for vols. 1-2, and 12-13; one issue for all other volumes), the results of this study have been expressed per issue and not per volume.

The average number of pages per issue is 39.6 (31-49). However, it has to be pointed out that the size of characters used by the editors changed as the years went. Moreover, the first issue of Vol. 1 was the only one to be printed in one column (all following issues were printed in two columns). These parameters explain the relative disparity from the average.

The average number of full-length publications per issue is 6.7 (5-11). However, it was sometimes difficult to acknowledge some articles as full-length publications in some issues. Aside from Vol. 10, number one, the number of full-length papers varies between 5 and 8.

The average number of pages per publication is 4.1 (1.6-7.4). As for the first section of the results, the size of characters may have influenced this parameter.

The number of authors per publication is 2.2 on average (1.4-3.6). It becomes stabilized between 2 and 2.6 since Vol. 11, number 1.

Address correspondence to: Dr. R. Olry, Departement de chimie-biologie, University du Quebec a Trois-Rivieres, C P. 500, Trois-Rivieres, Quebec, CanadaG9A5H7. Telephone: 819 3765053 /Fax: 819 3765084.Email: Regis_Olry@uqtr.uquebec.ca

The average number of bibliographical references per publication is 5.8 (1.0-13.0), including 1.2 (0.0-3.8) references taken from previous issues of our journal (figure 1).

The geographical origin of the full-length papers is summarized on Table 1. The total number of publications in this table (128) is higher that the real total number of full-length papers published in the journal (114) as some publications were written by two (or more) authors from different countries. However, it is much more interesting to analyze the chronology of the distribution (figure 2). From 1987 to 1992 (Vol. 1, number 1 to Vol. 6, number 1), all the papers published were written by European or American authors. In 1993 (Vol. 7, number 1) was published the first article from Africa. In the following issues appeared the first publications of Oceanian and Asian origins, in 1994 (Vol. 8, number 1) and 1996 (Vol. 11, number 1), respectively. If we analyze the geographical origin of the last four issues of the JISP, we can see that about 50% of the publications were written by non-American non-European authors.

Table 1. Geographical origins (continent and country) of the 114 full-length papers published in the JISP from Vol. 1, number 1 to Vol. 13, number 2.

Continent	Country	Number of publications
Africa	South Africa	9
America	Brasil Canada USA	1 12 40
Asia	China Iran Japan Malaysia Thailand	3 1 1 2 1 2
Europe	Austria Bulgaria Germany Italy The Netherlands Norway Sweden Switzerland	7 1 19 6 6 1 1 1
Oceania	Australia New Zealand	3 8

Discussion

This paper aimed at analyzing the content (full-length papers) of the first seventeen issues of the JISP.

The number of papers is scarcely sufficient to publish two issues a year. Each member of the society should plan at least one manuscript every two or three years (this is a standard for some other international journals). This would lead every one to take advantage of the experience of his (her) colleagues and relieve the stress of the editors.

As can be seen on figure 1, the papers published since Vol. 12, number 1 are more well-documented in the general and specific literatures. This trend seems to be related to the publication of the Current Plastination Index in 1996. It is obvious that this bibliography on plastination, though rarely cited in the publications (3 times, including 1 editorial), proved very helpful to researchers.

In conclusion, the Journal of the International Society for Plastination is establishing its pedigree slowly but surely. This trend is the fruit of the editors' labour, and has to be supported by each member of the society.

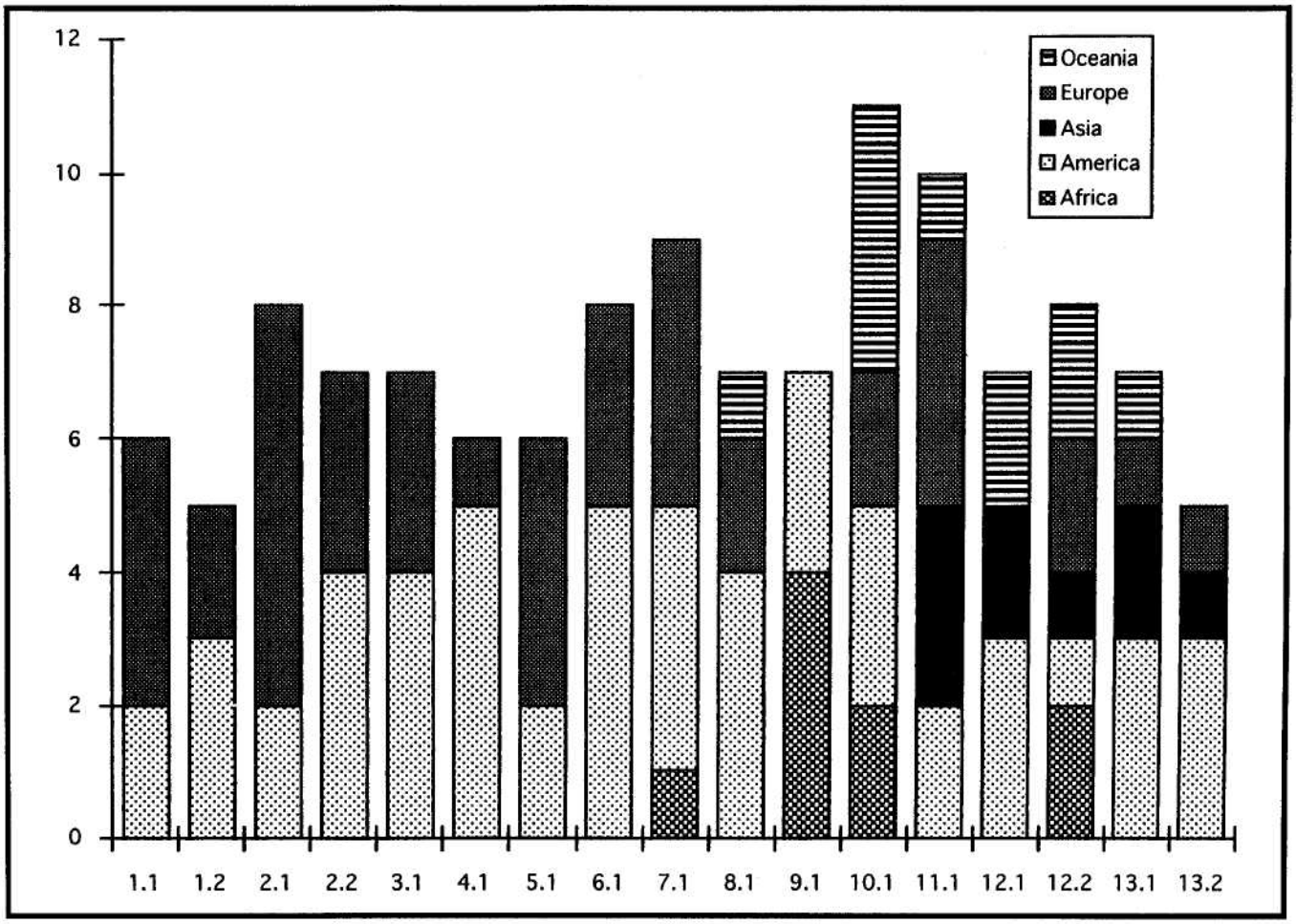


Figure 1. Number of publications per continent in each issue of our journal. Internationalization of the articles is obvious in the last 6 issues.

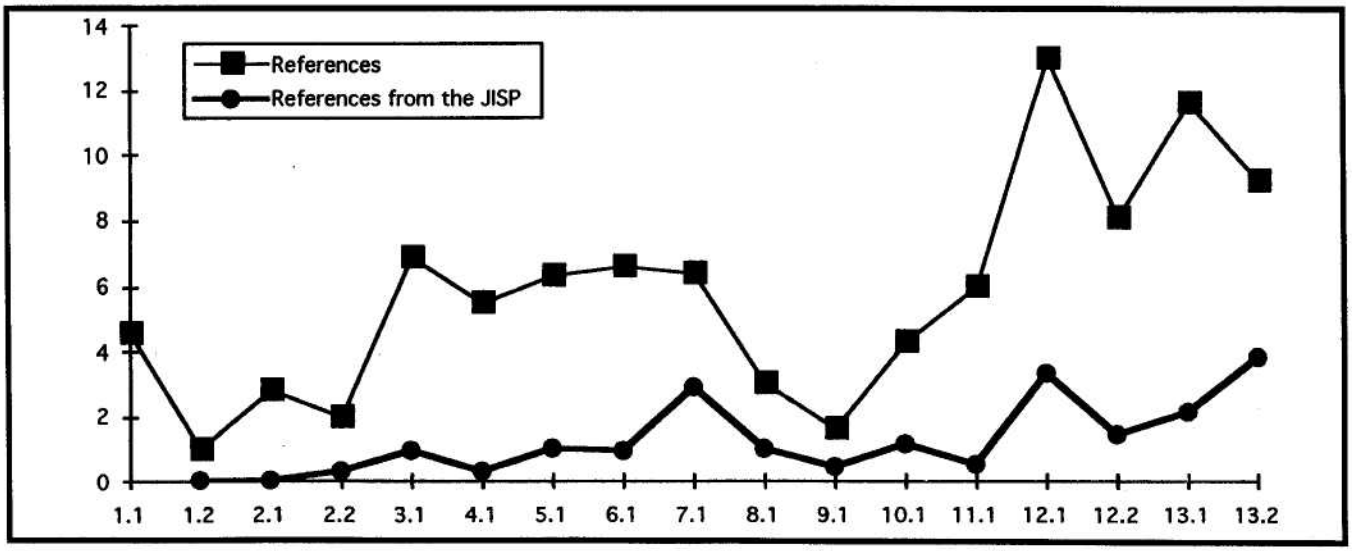


Figure 2. Evolution of the bibliographical references cited in the full-length papers: average number per publication (black squares) and average number of them taken from our journal (black disks). The Current Plastination Index was made available to plastinators between Vols. 11 and 12.

LETTERS TO THE EDITOR...Continued

the low temperature (-25°C to -30°C) technique, the standard plastination procedures, originally developed and described by Dr von Hagens from University of Heidelberg, Germany (von Hagens, 1985; von Hagens et al., 1987), the plastination laboratories in Canton, Nanjing, Shanghai, Hong Kong and Taiwan use the room temperature technique developed by Professor Zheng from the Shanghai Medical University (Zheng et al., 1996; 1998a, b). Professor Zheng started doing plastination research in 1990 when he was in Iceland (Zheng, 1995). He was the first Chinese to do plastination research. Professor Zheng have been able to develop plastination procedures which successfully allow all the four basic steps (Fixation-Dehydration-Forced Impregnation and Curing) to be carried out at room temperature (15-30°C).

Observing that at room temperature the silicone retains a much lower viscosity than at -25°C, permitting faster penetration, and easier acetone gas bubbles escape, professor Zheng has also developed an intermittent vacuum procedure at room temperature that replaces the continued forced impregnation procedures at low temperature. All of these improvements reduce capital costs (expensive spark proof deep-freezer) and improve safety by reducing the risk of explosion that acetone vapour could generate in an enclosed space. The intermittent vacuum procedure also reduces capital costs of high quality vacuum pump. In the Chinese plastination laboratories set up by Professor Zheng, many large and small anatomical specimens have been plastinated in this way and they have remained in good condition and retained stable color for many years.

At the beginning of March 1999, more than 25 specimens were sent for supporting the exhibition "Der Mythos Mensch" in Vienna. These include one of the plastinated mummies (Zheng et al., 1998a) and many prosected bodies showing muscular, squeletal, circulatory, nervous, respiratory and reproductive systems. There are also many body slices and normal as well as pathological organs.

All the equipments and silicone used in China for plastination laboratories are made in China. The first chinese plastination factory with 4000m² workshop has been set up in Nanjing and may be the biggest plastination factory in the world. There are six 1.9m long vacuum chambers equiped with the gas acetone filter system. The Nanjing factory is also equiped with an acetone recycling system. In addition to producing various biological specimens, the factory also produces the Su-Yi Chinese silicone for plastination that is different from the Biodur S10 silicone produced in Germany. The Su-Yi Chinese silicone has a faster penetration and more elasticity. Besides being used in all the chinese plastination laboratories, the Su-Yi Chinese silicone is also used in some west countries. Therefore China do not need to import equipment and silicone. This will permit plastination to develop widely in China.

Recently two ancient corpses died 400 years ago have been successfully plastinated in Nanjing and Canton (Zheng et al., 1998a). All the plastination procedures were carried out at room temperature (20°C). We also used the intermittent vacuum procedure and the Su-Yi Chinese silicone. After plastination the ancient corpses retained their original shape but weight has increased. The colors of the ancient corpses are much better than before plastination. The soft tissues remained flexible and the specimens presented no smell and no toxicity. The surfaces are dry, present no oozing of remnant silicone and can be touched by bare hands. The ancient corpses can now be preserved easily for long time without special care. During the histological study performed before and after plastination, we found some red cells in the lung tissue of one of the ancient corpses. The morphology of these 400 years old red cells have been preserved as fresh ones. This use of the plastination technique and the successful results obtained show that the Chinese plastination technique has already reached an advanced level in the world.

Xin Ya Shen
Liu Cai Dong
Wang Jie
Chen Xia Di

Department of Anatomy
Shanghai Medical University
138 Yi Xue yuan Road
Shanghai 200032
CHINA

References

von Hagens G: Heidelberg Plastination Folder. Anatomisches Institut, Universität Heidelberg, Heidelberg, Germany, 1985.

von Hagens G, Tiedemann K, Kriz W: The current potential of plastination. Anat Embryol 175 (4): 411-421, 1987.

Zheng TZ: The acetone gas detector for plastination. 7th Int Conf Plast, Graz, Austria, 1994. Abstract in J Int Soc Plastination 9 (1): 25, 1995.

Zheng TZ, Weatherhead B, Gosling J: Plastination at room temperature. J Int Soc Plastination 11 (1): 33, 1996.

Zheng TZ, Xuegui Y, Jingren L, Kerming Z: A Study on the Preservation of Exhumed Mummies by Plastination. J Int Soc Plastination 13 (1): 20-22, 1998a.

Zheng TZ, Jingren L, Kerming Z: Plastination at Room Temperature. J Int Soc Plastination 13 (2): 21-25, 1998b.

In order to give as much information as possible to all our members, particularly those who are not signed to the ISP-L group discussion, it was decided to include in the Journal some ***Questions and Answers*** reprinted from this list. If you wish to join the ISP-L list, just send a Email to Mr Ronn Wade at : "rwade@umaryland.edu" and ask him to sign you on.

All the messages that you will find in this section are reproduced with the permission of their authors.

THICKENED SILICONE

DATE: Fri, 05 Feb 1999

Hello Plastinators!

This is the first time I've stuck my head above the bunker with regard to plastination problems - I've certainly been gaining some vicarious thrills reading about the "goings - on" out there in plastination land in the last few months though!

Well, I have a problem now. Owing to very tight deadlines for some material we are preparing, when the refrigeration compressor for our freeze dryer/plastination chamber packed up on the first day of processing, I have had no option but to continue processing the material at room temp. in Biodur S10. This, of course, is not helpful for polymer life, or even infiltration in the later stages as the polymer thickens, especially as our summer temp. can reach 38-40°C for a few days at a time. The polymer is, I believe, showing signs of thickening now after 2 weeks, and I would estimate another few days to go to eliminate all acetone.

I expect to have maybe 25 litres (liters ok?) of this thickened polymer left over after processing - a considerable waste if it can't be utilised, I'm sure you'll agree.

What I'd like to know is: If I finish this processing asap, to reduce the overall thickening of the S10, then throw it (the S10!) into a freezer to hopefully halt the curing process, can I then add some of this used polymer to fresh S10 (in limited quantities) in future processing? I realise that any bath containing such a mix would have less than optimum infiltration properties, but would a small increase in viscosity make much difference? You will understand that I really don't want to waste such an expensive commodity.

If anyone out there can help with the appropriate info, or has found themselves in a similar situation, I'd be grateful for some advice.

Regards to all,

Jamie Stuart

The aswers

DATE: Fri, 05 Feb 1999

I once took a course at Heidelberg where they talked about this. If your polymer starts to thicken, you have a couple of choices.

1. Use it to impregnate hard specimens like joint/ligament/bone, that are resistant to shrinkage, then you can be reckless with the vacuum and really suck the stuff in.

2. Dilute the old mix with fresh mix. You can do this no problem.

They also said that the mix will slowly but surely harden at -20°C, and that to stop the thickening process totally if you go on holidays or similar you have to put the mix into -70°C. However, in previous messages on the list people have said that in their experience this is not a problem and that the mix will remain fine for very long periods at -20°C.

Hope this helps,

David Griffiths, B. V. Sc.,
Section of Anatomy,
The Norwegian School of Veterinary Science,
Post Box 8146 DEP.,
N-0033 Oslo,
NORWAY

DATE: Mon, 08 Feb 1999

Jamie,

All my plastination experience has been at room temperature although, of course, we store the S10 in a freezer. I also keep the acetone and S10 infiltration steps in a refrigerator rather than on the countertop overnight...however, the vacuum chamber is not cooled. I have mixed viscous "old" S10 into fresh solution and do not find any significant impairment of the process. It all depends on how viscous and how much you decide to use. Try it; it will probably extend the life of your S10 stock.

Marita L. Nelson, Ph.D.
Professor of Clinical Anatomy
Dept. of Pathology
John A. Burns School of Medicine
University of Hawaii, Manoa
1060 East-West Rd., Honolulu HI 96822, U.S.A.

DATE: Tue, 09 Feb 1999

Hi Jamie, David, Marita and anyone else out there....

I concur with David and Marita on the 'thickened silicone' situation. The same has happened to me and I also found that mixing it in with fresh S10 with Xylene helps.

I routinely use Xylene in the S10 anyway (usually between about 10% and 30% depending on the bulk of the specimen and the condition of the polymer, and mostly on my frame of mind at the time). I tend to soak the specimens for anything from 1 day to several weeks prior to impregnation and have found that this extremely dilute silicone infuses very well, particularly into dense specimens even before vacuum impregnation has begun.

Don't throw it away... it still has some life in it.

Regards,

Peter Cook
ISP Secretary
Department of Anatomy with Radiology
School of Medicine
University of Auckland
NEW ZEALAND

CURING COR-TECH

Date: Fri, 11 Jun 1999

Corcoran users, can you help?

I have plastinated some human brain, half hemispheres with COR-TECH PR-12, CR-20 and CT-32. I found an air-brush to be ideal for applying an even mist of catalyst to the specimens surface after which they were placed in a sealed container for 24 hours. The following day they were sprayed again and left out of the container. After 4 days the surfaces were still tacky so the specimens were placed back in a sealed container with a little of the crosslinker, in a pettry dish, beside them. The next day the specimens were tack free and left in the open air again. Everything up to this point seemed to be very quick and straight forward. However, after 2 months silicone is still oozing out of these specimens and I can only assume that, because brain tissue is quite dense, they are not fully cured on the inside.

Any suggestions what to do from here?

Thanks,

Russell Barnett
Dept. Anatomy and Structural Biology.
P.O. Box. 913
Dunedin
NEW ZEALAND

The aswers

Date: Mon, 14 jun 1999

Russ,

What I did recently with a small number of specimens that were taking a longer than usual time to cure is first wipe them down thoroughly and then paint their surfaces with some excess crosslinker using a small paint brush. Within a day or two these specimens were completely cured. Granted these were not brain specimens but I do think this treatment with a little excess crosslinker is the solution to most slow curing problems. I also added a little more crosslinker to my polymer/crosslinker just before I started impregnating my last group of specimens. These are ready to come out this week and I'll keep you informed on how the curing of these goes. The load contains all kinds of different specimens, a dissected torso, a few brains, hearts, limb cross sections, lungs, you name it and I think it's in there. We'll get to the bottom of this oozy problem yet. Roy

Roy A. Glover, Ph.D.
Associate Professor
Department of Anatomy and Cell Biology
Director; Plastination Laboratory
The University of Michigan Medical School
5732 Medical Science II
Ann Arbor, MI 48109-0616
U.S.A.

Date: Mon, 14 Jun 1999

Thanks folks for all your replys about curing Cor-Tech.

The first experimental brain specimen I processed with PR-12 was impregnated to a vacuum pressure of 5mm Hg. This brain was leaking for 2 weeks after curing and is still doing so after 2 months. Those of us that have been using S10 have been used to taking the vacuum quite low and this may be the problem when changing to a different polymer.

After checking the information, I then processed a second batch of brain specimens and was careful not to let the vacuum pressure fall below 10mm Hg, hopefully, to avoid boiling out the cross-linker. All these brains specimens are still leaking after I month.

Overall, I have processed a about 21 small specimens with my first batch of Cor-tech and have returned the residue, each time, to the one 20lb. container that I purchased originally. If the cross-linker is being boiled out of the polymer what is the suggested amount to be re-added?

I will do as Dan has suggested and return the brain specimens to a sealed container, with some cross-linker, and keep you all posted as to the outcome.

This polymer is extremely easy to use so I am hopefull of 100% success with it.

Regards to all,

Russell Barnett
Dept. Anatomy and Structural Biology.
P.O. Box. 913
Dunedin
NEW ZEALAND

Date: Thu, 17 Jun 1999
Hi folks,

Just thought I would give you an update after following the advise and placing those oozing brain specimens in a sealed container with some crosslinker for 2 days.

There were five specimens in total. Two half hemispheres were still very wet on the underside when I removed them. Two small infant brains appear to be cured but the meninges has become hard and crisp and when it is touched it breaks and flakes off. A half cerebellum with brain stem appeared to be cured before I exposed it to the crosslinker, I did so just to be sure. Now I'm sorry I did that, all the external detailed features (nerves, blood vessels) are hard and brittle and easily broken.

Brain specimens are extremely hard for us to obtain so I won't be risking anymore until someone has clear evidence that this polymer is better than S10 and the protocol for using it is alot clearer.

I have had messages from 2 other users to say they have had similar problems.

Regards,

Mr. Russell Barnett
Dept. Anatomy and Structural Biology.
P.O. Box. 913
Dunedin
NEW ZEALAND

Current Plastination Index - Updating

Gilles Grondin and Régis Olry

In order to supply the most complete and useful information, this review will now include the abstracts of the new papers involving plastination every time these will be available. We are thankful to the various Editors and Publishers who granted us the permission to reproduce their work.

Eckel HE, Koebke J, Sittel C, Sprinzl GM, Pototschnig C, Stennert E: Morphology of the human larynx during the first five years of life studied on whole organ serial sections. Ann Otol Rhinol Laryng 108 (3): 232-238, 1999.

Abstract:

The morphologic development of the human larynx during the first years of life is poorly understood to date. This study used plastinated whole organ serial sections to determine the growth and structure of the infant larynx. The larynges of 43 children 1 to 60 months old were plastinated. Whole organ serial sections were obtained by cutting the resulting specimen with a diamond band saw. The slices were then submitted to computer-assisted morphometric investigation. We found that the subglottic airway rapidly increases in size during the first 2 years of life. Further growth follows a linear mode. The relative proportion of the mucosal lining decreases likewise. In contrast to that in adults and comparable to that in most mammals, the cartilaginous glottis accounts for 60 % to 75% of the vocal folds' length at <2 years. No sexual dimorphism of the larynx exists during childhood. This study supplies detailed morphometric data on the growth and structure of the human larynx during the first years of life. It is the first to use plastinated whole organ serial sections for morphology of the pediatric larynx. Therefore, this study provides quantitative anatomic data of clinical interest that have not been available to date.

Reidenbach MM: Topographical Anatomy and Oncologic Implications of the Anterolateral Surface of the Arytenoid Cartilage. Eur Arch Oto-Rhino-L 255 (3): 140-142, 1998.

Abstract:

The normal topographic relationships of the anterolateral surface of the arytenoid cartilage (ALSAC) were reinvestigated with special regard to possible implications involving the extensions of local cancer, Serial plastinated whole-organ sections of 34 normal adult larynges were examined. A cartilaginous crest subdivided the ALSAC into a cranial triangular fossa and a caudal oblong fossa and provided attachment to the lateral fibers of the vocal cord. The thyroarytenoid muscle was mainly anchored within the oblong fossa, but a few lateral muscle fibers extended further cranial ward along the ALSAC. The triangular fossa was the area of fixation of the vestibular ligament and was filled with adipose tissue as well as mucous glands grouped together by strong collagenous fiber septa. These septa revealed a craniocaudal orientation medially and a horizontal anteroposterior orientation laterally. These findings suggest that the septa guide early local cancer extension parallel to their own arrangement, acting as barriers that cannot be easily crossed. Correspondingly, cranial and horizontal-lateral cancer spread along the ALSAC has been reported in the clinical literature. (C) Copyright Springer-Verlag.

Reidenbach MM: Aryepiglottic Fold - Normal Topography and Clinical Implications. Clin Anat 11 (4): 223-235, 1998.

Abstract:

The aryepiglottic folds extend between the arytenoid cartilage and the lateral margin of the epiglottis on each side and constitute the lateral borders of the laryngeal inlet. They are involved in physiologic closure mechanisms of the larynx and in pathologic conditions such as inspiratory strider. Information on the normal topography of the aryepiglottic folds is poor and controversial. Therefore, this region was reinvestigated in serial whole-organ sections of 25 plastinated normal adult human larynges. Dorsally, the right and the left aryepiglottic folds are separated by the interarytenoid notch and comprise the corniculate and cuneiform cartilages, as well as numerous groups of mucous glands. Ventrally, the aryepiglottic folds are adjacent to the peri-epiglottic adipose tissue. Both regions are clearly separated by several layers of transversely oriented collagenous fiber layers. The muscular constituent of the aryepiglottic folds is only poorly developed, and no muscle fibers insert at the epiglottis. A coherent quadrangular membrane representing a ligamentous 'skeleton' of the aryepiglottic folds is absent. A conspicuous collagenous fiber layer is found only to strengthen the free dorsal margin of the fold. Both muscular and ligamentous components may render the aryepiglottic folds sufficiently tense as to resist inspiratory inward suction in normal cases. However, pliability must be preserved to guarantee adequate folding in approximation of the aryepiglottic folds during

deglutition. Thereby, the posterior part of the laryngeal inlet is closed, whereas the anterior part is probably closed by independent inward bulging of the peri-epiglottic adipose tissue. (C) 1998 Wiley-Liss, Inc.

Reidenbach MM: The Muscular Tissue of the Vestibular Folds of the Larynx. Eur Arch Oto-Rhino-L 255 (7): 365-367, 1998.

Abstract:

The muscular tissue of the vestibular folds was investigated in plastinated serial sections of 32 normal adult larynges. Three muscular systems could be distinguished. A posterolateral muscle layer was found to be developed at the lateral margin of the posterior part of the vestibular fold. Its fibers extended in a sagittal direction, and their contraction probably resulted in an adduction of the entire tissue of the vestibular fold towards the midline. Within the anterior part of the vestibular fold, an anterolateral muscle sheet was seen to attach to the thyroid cartilage. An anteromedial muscular system consisted of scattered groups of muscle fibers situated medially and dorsally to the laryngeal ventricle and saccule. These fibers were presumed to exert a downward pressure on the vestibular folds, in addition to an adductor function. According to clinical experience, adductor movements of the vestibular folds can be trained, even in cases with a recurrent laryngeal nerve lesion, in order to produce a compensatory voice. Thus, the muscles of the vestibular folds are probably innervated by the superior laryngeal nerve. (C) Copyright Springer-Verlag.

Reidenbach MM: Anatomical bases of glottic widening surgery related to arytenoidectomy. Clin Anat 12 (2): 94-102, 1999.

Abstract:

Derived from arytenoidectomy, different surgical techniques have been developed for widening the glottis in cases of bilateral vocal cord paralysis. Their anatomical bases were reinvestigated in plastinated serial sections of 25 adult human larynges. At the anterolateral surface of the arytenoid cartilage, blood vessels crossing the crista arcuata may cause bleeding complications. The arytenoid cartilage is related to three major histologic complexes which must be taken into account during surgery. The dense connective tissue complex consists of the cricoarytenoid ligament and the conus elasticus, which are connected ventrocaudally. The cricoarytenoid ligament and the vocal cord are separated by the cartilaginous inscription of the vocal process. The muscular complex consists of the transverse arytenoid muscle, which is the posterior wall of the glottis, and the thyroarytenoid muscle, which is intimately fixed to the conus elasticus near the arytenoid cartilage. The loose connective tissue complex is represented by the vestibular fold, containing adipose tissue, mucous glands, few collagenous fiber septa, and at its posterior end, a small cranial extension of the vocal cord. For glottic widening surgery, the arytenoid cartilage must be regarded as an integrated component of an extended fibro-cartilaginous framework supporting the laryngeal ah-way. Shrinking processes of the dense connective tissue elements may complicate surgical interventions. Iatrogenic lesions of the posterior glottis should be avoided to prevent the development of synechia or insufficient closure of the larynx during swallowing. (C) 1999 Wiley-Liss, Inc.

Bleyl U: And what we are, that you will be - the dignity and inviolability of the plastinated dead. Pathologe 19 (3): 171-175, 1998.

Goyette V: La Plastination Bien Accueillie. Le Nouvelliste: 8, 9 juillet 1998.

Martin Y: The Good that can come from Death - Donating your Body to Science. The Dominion, April 17: 9, 1998.

Thompsom M: Plastic Bodies. University of Auckland News 28 (2): 24-25, 1998.

Caron M, Grondin G, Bourassa JP: Approche expérimentale en vue d'établir une clé d'identification tridimensionnelle pour des espèces de Microchiroptères du Québec, à l'aide de la technique de plastination S10. Rapport de projet de fin d'étude, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada, 1998.

THESIS

Durand M: Plastination et angle ponto-cérébelleux. Thèse de Doctorat en Médecine, Saint-Étienne, France, 1998.

WEB PAGES

http://www.rzuser.uni-heidelberg.de/~b54/plastination/frames.htm

http://www.univie.ac.at/anatomie2/plastination.html

New homepage for the plastination laboratory at the Vienna University. One can find some useful information about plastination and also some pictures of plastinated specimens, using all plastination techniques.

LOCATION

Saint-Etienne is located in the south of France in the historic region "Le Massif Central". It is about 200 000 inhabitants. Our region offers many possibilities for holidays. It is less than 2 hours by car from "Les Alpes" or "La Côte d'Azur". Activities will be scheduled for the accompanying persons every day during the conference week.

LODGING

Rooms will be reserved for participants in various hotels of the city and on the University campus. Easy transportation will be organized to the location of the conference.
Reservation will be possible through the Tourist Information Center in Saint-Etienne.

INSTRUCTIONS TO AUTHORS

Aims

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

Submission

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

Format

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

Title Page

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

Key Words

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

Abstract

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

Subheadings

Each paper should include an

INTRODUCTION
MATERIALS AND METHODS
RESULTS
DISCUSSION OR CONCLUSION
BIBLIOGRAPHY

Numbers and abbreviations

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

Nomenclature

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

Tables and illustrations

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

References

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

Bibliography

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

Examples are as follows:

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

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

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

Correspondence

All correspondence should be addressed to the editor:

Gilles Grondin
Département de Chimie-Biologie
Université du Québec à Trois-Rivières
C.P. 500
Trois-Rivières, Qué.
CANADA
G9A 5H7
Tel: 819 376 5053
Fax: 819 376 5084
E-mail: gilles_grondin@uqtr.uquebec.ca