## PLEXIGLASS MOUNTED S10 PLASTINATED THIN BRAIN SLICES

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

## CONGRUENT CT-SCANS AND PLASTINATED SLICES

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

## SILICONE CAST OF THE CHAMBERS OF THE HEART

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

## PLASTINATION OF BRAIN DISSECTIONS AND BRAIN SLICES

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

teaching are:

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

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

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

## THE P-40 TECHNIQUE SCALED DOWN

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

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

## SUBMACROSCOPIC INTERPRETATION OF HUMAN SECTIONAL ANATOMY USING PLASTINATED E12 SECTIONS

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

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

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

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

## SCALING DOWN THE P35 TECHNIQUE

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

1) it is far more conservative of resin.

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

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

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

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

## SHEET PLASTINATION OF BRAIN SLICES ACCORDING TO THE P35 PROCEDURE

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

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

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

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

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

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

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

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

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

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

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

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

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

## A BRIEF CHRONOLOGY OF PLASTINATION

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

What we can date with assurance are the international

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

## THE INTERNATIONAL MEETINGS

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

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

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

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

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

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

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

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

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

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

## INTERIM MEETINGS

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

## THE INTERNATIONAL SOCIETY FOR PLASTINATION

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

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

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