Technique for Embedding Brain Slices in Plastic

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This project was carried out in the Department of Veterinary Anatomy, Division of Veterinary Medicine, Iowa State College, Ames, Iowa, during the years 1953-54. The purpose in developing this technique was to provide demonstration material for the neuroanatomy courses offered to the veterinary students and to graduate students in other fields. Experience in the past showed that brain slices prepared and preserved in 10 per cent formalin were too fragile for routine use in the classroom. It was also difficult to see many of the structures after the sections had been preserved for some time.

Other soft tissues and organs have been embedded in plastic in the Department of Veterinary Anatomy during the past seven years using the technique described for brain section embedding. Serial sections of ovaries, various portions of the reproductive tract and the kidneys and other organs all can readily be embedded in plastic thus making useful teaching aids.

MATERIALS AND METHODS

Many sources were drawn upon in order to form a basis for this study. The technique follows the general scheme that Kampmeier[1] of the University of Illinois used in his embedding. Various modifications were made to suit the materials on hand and the different type of tissue preservation. Previous to this work, experiments were carried out in the department as to which plastic would be the most suitable for this type of embedding. The type of plastic used was Selectron Resin, No. 5003** and the catalyst used was Duoprox***. A catalyst called Cumen Hydro peroxide **** was used in the latter part of our work. It, too, was very satisfactory and was used in a 1% mixture with the plastic.

Brains for the work were obtained from dogs destroyed by desanguination. The whole brain was removed immediately following death. The dura mater and the arachnoidia were removed and the surface of the brain was then washed with cold water. The brain was fixed in a 10 per cent formalin solution for ten days to two weeks. This did not complete the fixing of the brain but it was hardened sufficiently to be handled, and there was no subsequent interference with the technique from autolysis or decomposition. The slices were fixed more fully at a later stage in the process. The slicing technique was developed through the aid of Dr. J. Graca, formerly of the Department of Physiology and Pharmacology. It is a modification of a technique developed by Dr. Graca for the slicing of mouse brains used in tissue respiration studies.

I. PREPARING BRAIN FOR SECTIONING

The slicer (Fig. 1) is composed of three parts: (a) a glass cylinder 8 cm. in diameter. This was obtained from the Chem-

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**Pittsburg Plate Glass Co., Pittsburg, Pa.

***Thalco, 765 S. Harvard Blvd., Los Angeles 5, California.

****Hercules Powder Co., Wilmington, Delaware.

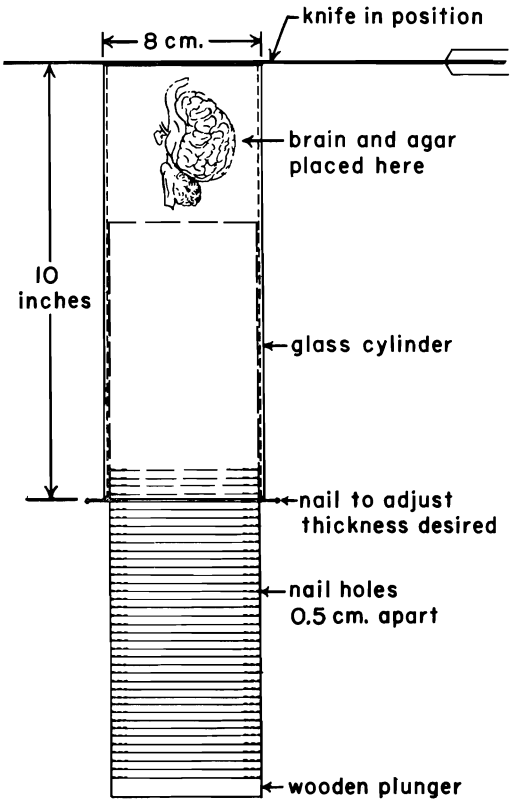

Fig. 1. Glass cylinder slicer.

istry Stores on the campus. Both ends of the cylinder were fired to remove rough edges, and one end was ground in order to provide a square cutting edge. The cylinder was 10 inches in length, but may vary in length; (b) a wooden plunger, made of a piece of 4" x 4" pine lumber, was turned down on a lathe to the desired diameter, which was slightly less than the inside diameter of the cylinder to allow for free movement. Rings were cut into the plunger on the lathe every .5 cm. Holes were then drilled on opposite sides of the plunger in these rings. Two small finishing nails were used to move up and down in these holes to obtain the desired thickness of the slices. It must be cautioned that the plunger will swell if wet. Therefore, it was found necessary to coat the plunger with paraffin; (c) the third part of the slicer is the knife. Various types of knives were tried, but the most satisfactory was found to be a very thin, double-edged knife about a foot long.

The slicing was accomplished as follows: A 2 per cent agar agar solution was prepared and cooled in a water bath to about 40° C. The whole brain was removed from the formalin and rinsed with water. It was then held in place in the cylinder with the medulla downward. The agar was then poured around the brain and the operator held the brain in place until the agar jelled. As soon as the agar would support the brain, the brain, slicer and all were placed in a refrigerator for 30 minutes. Cutting was accomplished by grasping the knife by both ends and pushing away with a diagonal motion, beginning with the tip of the knife and finishing at the hub. The slices were left in place until a number were cut, thus helping to hold the brain and agar in place. As the agar does not penetrate the brain, it was readily peeled off. After the brain was sliced, the sections were again placed in 10 per cent formalin, thus completing the fixing of the tissue. Care must be taken to keep the slices in proper sequence.

II. STAINING THE BRAIN SECTIONS

The Berlin blue method, using LeMasurier's modification of Landau-Sincke technique[3] was used to stain the macroscopic brain sections. The materials used in the staining process are as follows:

a. Mulligan's phenol solution (4 per cent phenol crystals, 0.5 per cent $CuSO_4$ and 0.125 per cent HCL dissolved in distilled water);

b. 1 per cent solution of ferric chloride in distilled water;

c. 1 per cent solution of potassium ferrocyanide in distilled water. These solutions were placed in wide-mouth pint jars with caps.

The staining technique is similar to Kampmeier's with some modifications: A slice is washed in running water for 12-14 hours and is then transferred to distilled water for one hour. This is changed 3 times. After this, it is placed in Mulligan's phenol solution in a quantity sufficient to cover the specimen to a depth of 6-7 cm. for 2 minutes. The slice is now immersed in a large volume of cold tap water for one minute, then in a 1 per cent ferric chloride solution for 5 minutes, and washed in running water for 5 minutes.

Following this it is treated with a 1 per cent solution of potassium ferrocyanide until the desired blue color is attained. This may take 5-8 minutes. After staining is completed the slice should be washed in running tap water for 24 hours and then stored in 10 per cent formalin for at least 24 hours before embedding.

In this study it was found that the last washing process and subsequent storage in formalin produced considerable fading, and it was sometimes necessary to restain the slices. It was found that good contrast between the white and grey matter could be produced by just running the slices through the process to, and including, the ferric chloride. The grey matter then took on a light tan color.

III. EMBEDDING BRAIN SECTIONS IN PLASTIC

The container used to hold the liquid plastic and brain was constructed as follows: Window glass was cut into 7 cm. squares for the base of the box, and strips 2.25 cm. wide and 7.2 cm. long were used for the sides. The parts were held together with drafting tape. Upon completion of the block, the tape was removed and the glass was knocked off with a wooden stick. At times, the base layer may prove difficult to remove, and in this case one should use a sharp object to pry it off.

Large amounts of plastic were heated at 60° C. in a paraffin oven. Half-pint milk bottles were used. The proper amount of catalyst to use with the plastic was discovered by trial and error. We were not able to use the directions of Kampmeier[2]. It is believed that the age of the catalyst or the plastic may affect the results obtained. Our dilutions were 25-30 drops of catalyst to 100 cc. of plastic, as compared to Kampmeier's 4-8 drops per 100 cc. of plastic.

The first base layer was poured into the box to form a layer .75 cm. thick. This layer was allowed to harden at room temperature for at least 48 hours. However, it can be stored for two weeks before use if protected from dust. It is important that no extraneous material is on this layer, as this is the layer through which the slice will be viewed.

The second or casting layer contains the brain slice. The slice is removed from the formalin and placed between the layers of a paper towel. It is allowed to dry until no further moisture can be blotted from it. The surface that is to be viewed is then rubbed with plastic, using a finger. It is then centered in the box and pressed against the base layer. There will be a number of air bubbles formed, but by pressing with the fingers these can be removed. One can see when all the air bubbles are gone by inverting the box and observing through the plastic. As soon as all the air bubbles have been removed, set the box upright and pour the plastic over the slice so that it is slightly covered. Remove any air bubbles that may form in the plastic with a teasing needle or a small probe. Allow this layer to harden 4-8 hours at room temperature.

The third or capping layer can be left clear, as the other layers, or a dark or colored background can be used. In this study, it was found that black gave a desirable contrast. A bioplastic dye was mixed with the plastic just before it was poured. The dye used was Ward's bioplastic color (black)*. It is important to place enough dye in the plastic to make it jet black; otherwise, a streaking effect may result if there is an insufficient amount of dye. This capping layer is .5 to .75 cm. thick. It is allowed to harden for 24 hours at room temperature. Nothing is placed over the top of this layer; however, it is important all through the process to prevent dust from settling on the plastic.

One of the authors, Dr. R. Getty, following a discussion with Dr. Kampmeier, learned that the hardening process may be speeded up following the use of ultra-violet light. After the base layer has been poured and hardened, the tissue is placed on the base layer and a casting layer is poured. This layer is then exposed to ultra-violet light for about three hours. The light should be about two and one-half feet above the block. An ultra-violet lamp can be purchased at almost any hardware store. After applying the light, allow the

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casting layer to harden an additional 12-15 hours. Then the capping layer is poured. This layer is exposed to the light for about 6-10 hours depending on the thickness of the layer and the amount of wrinkling that occurs. When the plastic is hardened rapidly with the light, the surface tends to become wavy. This may be prevented to some extent by applying the light at intervals or having the source of light farther from the block. The ultra-violet light gives the free surface a much harder finish than any method discovered by us so far. It may be slightly wavy, but it is completely transparent and does not distort the specimen greatly. We feel that the advantages outweigh the disadvantages. Follow by curing block in an oven at 40°-50° C.

DISCUSSION

Reports by other workers frequently state that the plastic will not produce a hard surface unless it is hardened against a smooth object such as glass, wood, or cellophane. However, in this work it was found that after the curing process the black surface was sufficiently hard to allow handling, and since nothing was being viewed through it, there was no need for a smooth, glossy finish.

The following technique should be followed if a hard, smooth surface is desired. After the curing process, which will be described later, the glass box is removed from the block. The edges of the plastic will be raised in a meniscus. Part of this can be cut off with a knife. This must be done upside down so that the particles of plastic do not fall on the soft surface. Then obtain a piece of glass one inch wider and longer than the block and pour a "puddle" of plastic on it containing more catalyst than was used in the block. Invert the block onto this puddle. Frequently, air bubbles are formed which may prove difficult to remove. If, however, one keeps moving the block in back and forth motion the bubbles will work out. Allow this preparation to harden at room temperature for 24 hours, and then place in the curing oven for 24 hours. After the curing, remove the glass plate and cut off the protruding edges of plastic with a saw. The edges are then sanded and buffed.

The plastic is not completely hardened until it has been cured. This is accomplished by placing the blocks in a paraffin oven at 40° to 50° C. for 24 hours. It is very important that the temperature does not exceed 50° C., as greater heat tends to cause fading of the stain.

The completed block is removed from the oven and allowed to cool slowly at room temperature. The glass box can then be removed. The edges of the meniscus on the black layer should be cut off and the corners and edges should be sanded or buffed to prevent chipping. Each block should be inscribed with a number designating its position in the series. This may be done by using a sharp dissecting needle and scratching it into the black layer.

Other techniques have been developed since the original inception of this work[4] however it is not the intent of this article to discuss the advantages and disadvantages of various methods but rather describe the method that has been used satisfactorily in the department of Veterinary Anatomy at Iowa State University for the past seven years.

SUMMARY

In summary it can be stated that this article is not written with the intent or thought that it is a completely documented "research article." Rather, it is hoped that the availability of a rather detailed description of the essential steps necessary in embedding soft tissues in plastic may be of use to others interested in this aspect of plastic embedding. Frequently the second author is asked to present such information by letter and thus the availability of reprints describing the steps should expedite the descriptive process.

It should also be stated that the technique described has produced clearly visible, permanent mounts of brain slices which have been used for seven years in routine classwork in neuroanatomy in the Department of Veterinary Anatomy at Iowa State University.

It can also be emphasized that other soft tissues and visceral organs may be embedded in plastic using a similar technique.

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A RATION FOR GROWING PIGS HAVING ENTEROTOXEMIA, OVEREATING DISEASE, OR GUT EDEMA

Enterotoxemia, overeating disease, or gut edema cause considerable death loss in growing pigs each year. The etiology of this disease has not been explained but it is associated with too much feed. The disease is not new and has been recognized by practitioners for more than thirty years.

The period before and after vaccination is a particularly critical time. Hogs should be fed one-half to two-thirds of the normal amount of feed for a week before vaccination and preferably for two weeks after vaccination.

The following is a ration designed to prevent overeating disease:

Ground corn	500 pounds
Ground oats	900 pounds
Alfalfa meal	200 pounds
Wheat middlings	200 pounds
Tankage	100 pounds
Soybean meal	50 pounds
Linseed meal	25 pounds
Steamed bone meal	40 pounds
Salt	10 pounds
Trace mineral	7 pounds
Antibiotics (Aureomycin)	50 grams

When overfeeding occurs, reduce the total amount of feed fed and increase the amount of oats and alfalfa in the ration. The farmer will probably object, but it is better to have the pigs reach market weight a week or two later than to lose them for overeating.

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[1] **Kampmeier, Otto F. and Emil W. Hospodar. Mounting of stained serial slices of the brain as wet specimens in transparent plastic. *Anatomical Record,* Vol. 110, no. 1, May 1951, pp. 1-15.**

[2] **Kampmeier, Otto F., Thomas Haviland, Max M. Strumia, J. Ivan Hershey. Plastics: Mounting of Biological Specimens. *Medical Physics,* Vol. II, 1950, pp. 719-728.**

[3] **LeMasurier, H. E. Simple method of staining macroscopic brain sections. *Arch of Neur. and Psychiat.,* 34:1065-1067, 1935.**

[4] **Brody, Harold and John E. Wirth. A Staining and Plastic Embedding Technique for Macroscopic Brain Sections. *Anatomical Record,* Vol. 127, 1957, pp. 65-73.**