The Preparation of Sections and Museum Specimens of Eyes Using a Polyester Resin


Examination of the eye histopathologically has long been recognized as a difficult procedure, due mainly to the fact that it is composed of several mixed hard and soft tissue layers which tend to separate during cutting. Furthermore, since a section of the entire globe is desirable this results in a large sized block which due to its hardness does not always permit even sections to be cut unless the embedding medium is an excellent one. An ideal embedding medium therefore is one that (a) does not crumble under the impact of knife on sclera and lens, (b) preserves the cytologic detail of the retina and other tissues, (c) permits a large block of tissue to be cut evenly and completely, (d) permits reasonably thin sections to be cut, and (e) gives a completed section in a reasonable time. While paraffin embedding will occasionally satisfy most of these criteria it usually crumbles on cutting or gives uneven incomplete sections. Ester wax described by Steedman is more resilient than paraffin wax but in our hands has not proved successful for much the same reasons as paraffin wax, and the tissue does seem unduly brittle after processing. Low viscosity nitrocellulose (I.V.N.) gives reasonably good sections, being similar to celloidin in all respects except that processing is complete in 10-12 days, and sections have a great tendency to crack during staining.

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even when oleum ricini recommended by Moore is added. Celloidin and Nickoloidin, the procedure for which can be found in Culling's textbook, satisfy all but the last two criteria and have been the method of choice in most institutions for many years.

It was in an attempt to satisfy all five criteria that a method of plastic embedding was sought.

Bohatirchuk (1957) was able to cut sections of undecalcified bone on a "bone microtome" after embedding in Wards' Bio-Plastic. Kuhn and Lutz, in 1958, described a polyester embedding medium (C.M.E. tissue support resin) which seemed to give reasonable results with a variety of tissues, and it was decided to use this method or a modification of it.

Of the methods presently available the only one which, in our hands, satisfies the five criteria laid down above is the plastic embedding technique.

The Embedding Medium

The plastic used for embedding was first prepared for use as described by Kuhn and Lutz. Prepared in this manner the plastic proved impossible to cut on a routine sliding or rotary microtome. It was then decided to experiment with the plastic to see if it could be softened to such a degree that it could be cut reasonably well on a sliding microtome. Kuhn and Lutz noted in their paper that the hardness of the plastic could be modified by varying the plasticizer resin ratio. A series of experiments were designed whereby we added to the complete resin varying amounts of plasticizer. We found

* Obtainable from Natcol Laboratories, Redlands, Calif.
that a mixture of 1 part of soft resin to 20 parts of the plasticizer gave the best consistency for section cutting. This mixture gave a plastic similar to a very hard celloidin and with somewhat the same physical characteristic.

Preparing the Plastic Embedding Medium

The following apparatus is necessary: Materials required: one 50 ml. beaker; one boiling waterbath; one stirring rod (15 cm. long, 3 mm. in diameter).

The resin is prepared by mixing 20 ml. of plasticizer and 1 ml. of resin in a small beaker. The small beaker is then placed in a boiling waterbath and the mixture stirred. Three drops of catalyst No. 1 and three drops of promoter No. 2 are added at this stage and stirred in thoroughly with a glass rod until all the hair lines disappear. The higher the temperature to which the plasticizer-resin mixture is heated at this stage the more rapidly the jelling occurs. The hardening time can also be controlled by varying the amount of catalyst and promoter; i.e., if the mixture is jelling too rapidly, the amounts can be reduced by 1 drop of each.

Preparation and Fixation of Specimen

The surgical specimen is put into fresh formol saline and left overnight. The next step is to bisect the eye, which is done in such a manner that the macula and the optic nerve are in that half of the eye which is to be sectioned. If there are other features such as a tumor present this is, of course, taken into account when sectioning the globe and the section is so located as to include the lesion. To give the eye increased rigidity, when bisecting it, it should be frozen for one-half hour in a mixture of crushed ice and salt.

Dehydration of Specimen

After fixation of the eye as described above, it is transferred to 50% ethyl alcohol for two hours and then to 70% ethyl alcohol where it is left for four hours, after which it is put into 90% ethyl alcohol where it is left overnight. The next morning it is transferred into absolute alcohol for two hours and then into fresh absolute alcohol where it is left for a further three hours. It is now placed in ether and left for three hours, after which it is transferred to a pre-embedding simple plasticizer-resin mixture (resin plasticizer mixture—without heat, catalyst or promoter) where it is left overnight. The next morning the final embedding process is undertaken.

Embedding the Specimen

The prepared plastic embedding medium is poured into a convenient type of mold, such as those used for paraffin wax embedding; we use small wax cartons of the type used by catering establishments. The eye is introduced into the embedding plastic with the cut surface uppermost to make sure no air bubbles are trapped. When all air bubbles have been released, the eye is turned over using a glass rod so that the cut surface of the eye is face down in the plastic. If the specimen tends to float it should be gently pressed down with the stirring rod; if it immediately rises it will be found that by waiting a short time the resin will become more of a gel, and the specimen can then be pushed down and held for a second or two after which it will remain in position. It is then put into an oven at 37 C. Overnight the plastic will harden. It should be noted that this resin-plasticizer mixture does not harden satisfactorily at room temperature. If a report is urgently required the resin may be hardened more rapidly by placing it in a 56 C oven instead of a 37 C oven. This will make the plastic set more rapidly but has a tendency to make it harder than if it were left to cure at the lower temperature. The glassware that has been used for making up the plastic must be soaked in a strong detergent solution promptly, otherwise it will be very difficult to clean. We use a product known as Sparkleen.
Cutting the Block

When the plastic has set hard, the waxed container is removed by slitting down one side and peeling it off. The block is trimmed in a similar way to that in which paraffin blocks are trimmed, with a sharp knife or a fine saw; the saw is more efficient. At least one-quarter inch of the plastic should be left on all sides of the specimen except the face which is to be cut. It was found that when the block containing the eye was clamped in preparation for cutting, it bowed upward causing uneven sections, and when the clamp was released a concavity resulted. This was overcome by preparing a block of hard plastic, using pure resin, and the soft block containing the specimen was set into this and the resin block allowed to harden, the microtome clamp being applied to the hard block during cutting.

We found that the plasticizer-resin mixture could not be cut on the rotary microtome with any great success. It can be cut reasonably easily, however, on a sliding microtome or the giant sledge type microtome. The knife is of great importance and must be extremely sharp to get thin even sections. It is our experience that knives which have been sharpened on the mechanical sharpener alone are not sufficiently sharp, although this may be due to limitations of the particular knife sharpening machine that we use. In our hands they need to be hand-honed and stropped. The wedge knife should be used to minimize vibration. The block, having been put into the microtome holder, is orientated so that the cut surface of the eye is parallel to the plane of the knife and the excess plastic is removed with a microtome knife kept for trimming. The thickness gauge for this purpose is set at 20µ to 30µ, so that large shavings are removed during this trimming process. When the complete section is being cut the knife is replaced by the sharp knife and the thickness indicator set to 10µ; at this thickness sections can be obtained reasonably easily. Depending on the type of material which is being cut, this thickness can be reduced; in our better specimens we cut sections of 5µ. However, it is not felt that a thickness below 8µ is necessary for diagnostic pathology. During the whole of the cutting process it is advantageous to flood the knife with 70% alcohol at intervals. The cut sections are kept in 70% alcohol in an airtight jar. Better sections are cut by having the knife set at an angle of 135 degrees to 140 degrees to the direction of travel instead of the conventional 90 degrees, and by slightly reducing the tilt of the knife.

Staining the Sections

The plastic sections should be stained in the same manner as celloidin sections, that is they are floated freely in the successive staining solutions as described by Culling. They are transferred from solution to solution either by a glass hockey stick (which is a piece of glass rod bent at the bottom at an angle very much like a hockey stick) or by the flat type of section lifter. The technique described will be that for hematoxylin and eosin. There appears to be no reason why any other staining technique cannot be employed if the fact is borne in mind that the staining times have to be greatly increased.

1. Transfer sections directly from 70% alcohol to Ehrlich’s or Harris’ hematoxylin and leave overnight at room temperature.
2. Next morning the sections are removed from the hematoxylin and transferred to a dish of distilled water where the excess hematoxylin is washed off.
3. The sections are then transferred to 1% acid alcohol (1% hydrochloric acid in 95% alcohol), where they are left for 5 to 6 minutes, and rinsed in distilled water.
4. Transfer to an alkaline solution. (This alkaline solution is prepared by dissolving 1.5 gm. of sodium bicarbonate in tap water.)
5. Leave for 15 to 30 minutes until they appear to be quite blue, having been pink in color when removed from the acid alcohol.
6. When it is felt that they have attained a maximum blue color they are transferred
to a large dish of water from which they are floated onto a slide.

7. With the slide held by two fingers, the section is brought up to the surface of the slide and held there with a dissecting needle while the slide is withdrawn bringing the section with it. This is then examined under the microscope to ensure that nuclear staining is adequate and differentiation is complete. Differentiation is the removal of the excess hematoxylin stain from the plastic and from the cytoplasm of the cells leaving clearly differentiated nuclei. If differentiation is not complete the section is returned to alcohol and Stages 3 to 7 repeated.

8. Transfer the section to 5% eosin for 30 to 60 minutes (depending on the specimen).

9. Wash in a large dish of tap water, gently agitating the fluid until the eosin is differentiated. This will take from one to five minutes.

10. Transfer to a dish of 70% alcohol for one to two minutes and then into a dish of 95% alcohol—this time in a rather large Petri dish. Note: This dish will need to have sufficient depth for a microscope slide to be completely immersed when put in at an angle of about 45 degrees. A clean grease free slide should be used. Such slides may be produced by storing them in 1% alcohol and wiping on a clean cloth before use. The larger slides (3 in.×1½ in.) should be used to mount the specimen.

11. The section is orientated on or near the surface of the alcohol and the clean slide is inserted beneath it. By slowly withdrawing the slide, and holding the section to it with the hockey stick or needle as the slide is withdrawn, the section will be withdrawn with it and should be perfectly flat on the slide. This is now laid between clean fine filter paper and blotted firmly. A few drops of absolute alcohol are dropped onto it and it is again blotted. A few drops of xylene are dropped onto the center of the section; it is immediately blotted flat and 2 or 3 drops of H.S.R. resin (the normal Harleco synthetic resin mountant used in the laboratory) are put into the center of the section and the cover slip lowered onto it. These last stages must be carried out rapidly before any wrinkles can develop. Any air bubbles present are easily removed by stroking the cover slip from the center with the end of a pencil or other clean instrument when they escape to the side. It will be found that an excess of mountant is an advantage at this stage, this excess being removed when the slide is completely dry after a day or two. The slide is now ready.

Fig. 1.—Full section of eye ×3.
for examination under the normal light microscope (Figs. 1, 2, 3, and 4; photographs of eye sections ×3, ×100, ×300, and ×800 magnification).

The Block as a Museum Specimen

The resin embedded eye may also be used as a museum specimen after it has been trimmed and polished. If further sections are required the specimen can be removed from the museum and fresh sections cut without impairing its appearance (Fig. 5).

The specimen block is first trimmed with a fine toothed saw in a manner that will best display the specimen concerned. The back and sides are then sandpapered until quite smooth (first with Grade "0" and then "00"); the cut surface should be perfectly smooth and polished by the cutting.

The specimen is finally polished by rubbing it on a cloth saturated with metal polish or by using an electric buffing wheel with a buffing compound.

It can be labeled with India ink on a roughened area or a number painted onto it using a plastic enamel.

Summary

A method of plastic embedding of eyes is described which gives sections in four to five days. The plastic used is known as C.M.E. tissue support resin and used as
described has a consistency very similar to, although slightly harder than celloidin, and may be cut in a similar manner. The cut sections must be handled as celloidin sections; they are stained by floating from solution to solution. The resulting sections resemble paraffin sections very closely. The block of plastic containing the eye from which the sections have been cut can afterwards be used as a museum specimen. There seems no reason why this method of embedding cannot be used for any hard tissue or hard and soft tissue combinations. It is our impression that the above technique satisfies the five criteria for an ideal embedding medium postulated earlier in the paper. The staining method described is that of hematoxylin and eosin although there is no reason why other stains cannot be employed with similar success. At present we are working on this phase of the problem.

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Fig. 4.—One ciliary process. Reduced 8% from mag. X 800.

Fig. 5.—A museum specimen of an eye embedded in a polyester resin after the cutting of the pathology sections shown in Figure 1.
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REFERENCES


