Technical methods

A routine method for embedding animal tissues in Spurr resin for electron microscopy

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The epoxy resin epon 812 has been used in this department to embed a variety of normal and pathological tissues for a number of years. Unfortunately during the past 18 months sectioning qualities of polymerized epon blocks have become variable in an unpredictable manner. It is possible that the batch variation in the quality and composition of the dodecynl succinic anhydride (DDSA) could be responsible.

In view of the unpredictable sectioning qualities of the epon currently in use it was decided to investigate the possibility of replacing the epon mixture with Spurr's (Spurr, 1969) resin.[1] This resin mixture is considerably less viscous than epon mixtures and consequently easier to dispense. The resin is also hydrophobic and wetting of the block face is not liable to occur.

After several trials of embedding kidney in Spurr's resin it was apparent that it would be necessary to modify our sectioning and staining techniques. Of the number of drawbacks encountered with Spurr's resin, possibly the most serious was the poor staining of ultrathin sections. Thick (2 μm) sections for light microscopy would not adhere to glass microscope slides while being stained. Similarly ultrathin sections would not adhere to copper grids if these were used as received from the supplier.

After extensive trials we were able to overcome these initial difficulties and use the methods described below.

Method

Following conventional fixation techniques for electron microscopy, rinse blocks in distilled water after the osmium tetroxide stage.

Two per cent aqueous uranyl acetate for 30 minutes at room temperature. This fixes and stains some of the tissue proteins giving a valuable increase in the overall contrast of ultrathin sections.

At all the following stages vials containing tissue blocks and fluid are placed on a slow mechanical rotator to ensure complete penetration of the dehydration fluids and the resin mixtures: (1) 70% acetone, two changes of five minutes; (2) 90% acetone, two changes of five minutes; and (3) 100% acetone, two changes of 15 minutes.

SPURR RESIN

It should be noted that this resin is toxic by skin absorption, especially of the epoxy resin ERL 4206. Adequate precautions to avoid all contact with this resin should be taken. Accidental contamination of the skin with the mixed resin or its components should be removed with soap and water, not solvents.

The resin is made up as follows:

Weigh sequentially into a disposable polythene beaker.

ERL 4206	10g
DER 736	6g
NSA	26g

Mix these components with a variable speed electric stirrer. Avoid stirring air into the mixture. Add 0·4 ml SI accelerator drop by drop.

For impregnation take 1 part acetone/1 part Spurr resin for 30 minutes; 1 part acetone/ 3 parts Spurr resin for 30 minutes; Spurr resin for at least two hours.

The blocks are transferred to oven-dried gelatin capsules filled with fresh resin. The resin is polymerized overnight at 70°C.

THICK SECTIONING

Sections 1-2 μm thick, are obtained in the usual way. They are then placed on a drop of gelatin adhesive on a glass microscope slide.

The gelatin adhesive stock solutions are: (*a*) 0·5% gelatin solution and (*b*) 0·5% chrome alum in distilled water. For use take equal parts of *a* and *b*. Fresh adhesive is made daily. Heat the drop of adhesive by holding the slide over a small bunsen flame until the section is seen to flatten. Dry in a 70°C incubator.

Thick sections are stained as follows: (1) one per cent periodic acid in distilled water for one minute at room temperature; (2) rinse in distilled water; (3) mix equal quantities of 1% methylene blue in 1% in borax and 1% azure II in distilled

[1]Spurr resin, stains etc, TAAB Laboratories, Kidmore End Road, Emmer Green, Reading.

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water, and filter into a Coplin jar which is kept in a 70°C incubator. Stain the slide for three minutes at 70°C.

If the stain is too intense 70% ethyl alcohol may be used for differentiation.

ULTRATHIN SECTIONING

Ultrathin sections are made in the usual manner. The wrinkles formed in ultrathin sections during the cutting stage are removed with chloroform vapour as the sections float on the surface of the water. During the flattening process the interference colour of the sections alters to make the sections appear thinner. In view of this apparent change of thickness ultrathin sections are cut slightly thicker than required.

The alcoholic uranyl acetate used to stain sections made from Spurr resin frequently removes the sections from the grid. To stop this from happening grids are held in watchmaker's forceps, sprayed with Inhibisol from an aerosol spray (Penetone Co),[2] and allowed to dry. The grid is then dipped into a dilute solution of wetting agent (eg, Teepol in distilled water) and used immediately. The wetting agent reduces the surface tension of the water which would normally cause the sections to slip off the grid.

Ultrathin sections are stained as follows: (1) Stain the sections in 5% uranyl acetate in methanol for five minutes. Use a covered staining well to prevent evaporation of the methanol.

(2) Wash the grid in methanol and allow to dry.

(3) Stain in Reynold's lead citrate for five minutes.

(4) Wash in distilled water and allow to dry in a dust-free atmosphere.

The grid is now ready for examination in the electron microscope.

Results

Using the methods described we now embed a wide range of human and animal tissue in Spurr resin. Blocks from this resin are considerably easier to section than epon and ultrathin sections of high quality are routinely obtained.

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References

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Spurr, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *J. ultrastruct. Res.*, 26, 31-43.

[2]Inhibisol spray, Penetone Co Ltd, Cramlington, Northumberland, England.

A method for preparing and staining the platelet-collagen interaction for microscopic observation

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Interactions *in vivo* involving circulating platelets and subintimal collagen have been reported to be fundamental in the formation of a thrombus (Hovig, Jorgensen, Packham, and Mustard, 1968). Most microscopic investigations concerning the adhesion of platelets to collagen employ *in-vivo* methods, such as histological examination of exposed intimal collagen and adhering platelets after induced arterial trauma (Warren, 1971). In contrast to *in-vivo* methods this *in-vitro* method offers a controlled procedure for preparing and differentially staining the platelet-collagen interaction. This procedure makes possible the investigation of different parameters affecting both platelet-collagen adhesion and the aggregation of free platelets to collagen-adhering platelets.

Methods

A 0·1 ml aliquot of human tendon suspension, prepared according to Hovig (1963), is added to 1·0 ml of human platelet-rich plasma, obtained by the method of MacKenzie, Blohm, and Auxier (1971). The suspension is gently agitated for three seconds, and, at the fifth second, the interaction between platelets and collagen is stopped by the addition of 3·0 ml of 4/3% glutaraldehyde solution. Centrifugation at 90 *g* for 10 minutes precipitates the platelet-collagen masses from the suspension. The supernatant is discarded, 3·0 ml of Tyrode's solution added to the precipitate, and the mixture gently agitated. After 20 minutes the suspension is centrifuged for 10 minutes at 90 *g*, the supernatant discarded, and the platelet-collagen masses are resuspended in 1 ml of Tyrode's solution. A slide smear is prepared with two drops of the suspension, and allowed to dry in air.

After the slide is dry, it is fixed for 2·5 minutes in the glutaraldehyde-ethanol fixative, rinsed in distilled water, and stained by the following sequence.

1 Stain in the acid fuchsin-sirius red stain for 20 minutes.

2 Stain in 25% Wright's stain (in distilled water) for five minutes.

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