other lengthy rigorous cytochemical techniques.

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Rapid method for stained renal biopsy specimens embedded in epoxy resin

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Embedding small biopsy specimens in epoxy resin (by preparing 1.5 µm sections and staining them with a variety of techniques) enhances optical resolution and accuracy of histopathological diagnosis.

For many years, all our renal biopsy specimens, routinely embedded in paraffin wax, were stained with haematoxylin and eosin, periodic acid Schiff, Martius scarlet-blue and periodic acid-methenamine silver methods,¹ which we were anxious to retain after switching to plastic embedded specimens. Epoxy resin was found to be preferable to acrylic resin, mainly because it could be partially removed to allow for good staining. Some technical modifications were necessary as thin plastic sections require more intense staining. We found that some stains benefitted from being heated while others required their times extending; and phloxine replaced eosin as it gave similar but stronger results.²

Since 1979, all routine, non-urgent renal biopsy specimens in this department have been embedded in epoxy resin. Until now, this procedure has taken at least four days, because specimens had to be hand

processed and the resin required polymerisation overnight at 60°C. The drying (overnight at 35°C) and staining of sections were also time consuming, making the process unsuitable for urgent cases (patients with rapidly deteriorating renal function and most transplant biopsy specimens).

During the summer of 1986, an automatic "Lynx electron microscopy Tissue Processor" was purchased for all the various users of the electron microscopy suite of our department, the following method was devised to combine the benefits of plastic embedding with the availability of stained sections within 24 hours of biopsy.

Methods

After collection the specimen is processed overnight on the tissue processor with a sufficient delay in 10% buffered formalin to permit adequate fixation. For needle cores, the programme takes five hours and is as follows:

- 1 35% alcohol for 30 minutes
- 2 75% alcohol for 30 minutes
- 3 95% alcohol for 30 minutes
- 4 100% alcohol I for 30 minutes
- 5 100% alcohol II for 30 minutes
- 6 Propylene oxide I for 15 minutes
- 7 Propylene oxide II for 15 minutes
- 8 50:50 resin:propylene oxide for 60 minutes at 40°C
- 9 Neat resin for 60 minutes at 40°C.

When larger pieces of tissue are submitted (such as small open wedge biopsy specimens), processing times

are extended to a total of 10 hours:

- 1 70% alcohol for 60 minutes
- 2 95% alcohol I for 60 minutes
- 3 95% alcohol II for 60 minutes
- 4 100% alcohol I for 60 minutes
- 5 100% alcohol II for 60 minutes
- 6 100% alcohol III for 60 minutes
- 7 Propylene oxide I for 30 minutes
- 8 Propylene oxide II for 30 minutes
- 9 50:50 resin:propylene oxide for 90 minutes at 40°C
- 10 Neat resin for 90 minutes at 40°C.

With this particular tissue processor, specimens larger than 10 × 5 × 2 mm cannot be accommodated and have to be divided.

The tissue is then embedded in medium "E-mix" resin (low viscosity epoxy resin, "EM-SCOPE Ltd"), and allowed to polymerise for 75 minutes at 95°C (variables modified according to Hayat and Giaquinta³). Sections are then cut at 1.5 µm on a "Reichert Jung Autocut" microtome and dried on a hot plate at 60°C for 60 minutes. Before staining all sections are treated with a saturated solution of sodium hydroxide in absolute alcohol at room temperature for 15 minutes followed by several changes of absolute alcohol. The sections are then taken to water and stained as follows. All reactions take place at room temperature unless otherwise stated.

HAEMATOXYLIN AND PHLOXINE

- 1 Stain in Harris's haematoxylin for 10 minutes at 40°C
- 2 Rinse in water
- 3 Differentiate and blue
- 4 Stain in 0.5% phloxine in 0.5% aqueous calcium chloride for 10 minutes
- 5 Rinse in water
- 6 Dehydrate, clear in xylene, and mount.

PERIODIC ACID SCHIFF

The Schiff reagent was prepared by the cold Schiff method described by Lillie.⁴

- 1 Treat with periodic acid for 10 minutes
- 2 Rinse well in distilled water
- 3 Treat with Schiff reagent for seven minutes
- 4 Rinse in distilled water
- 5 Wash in tap water for 10 minutes
- 6 Stain nuclei with haematoxylin
- 7 Blue
- 8 Dehydrate, clear, and mount.

MARTIUS SCARLET BLUE⁵

- 1 Stain with celestin blue for 15 minutes at 40°C
- 2 Rinse in tap water
- 3 Stain with haematoxylin for 10 minutes at 40°C
- 4 Rinse in tap water
- 5 Differentiate in 1% acid alcohol

- 6 Rinse in 95% alcohol
- 7 Stain with 0.5% Martius yellow in 95% alcohol to which 2 g of phosphotungstic acid has been added, for five minutes
- 8 Rinse in tap water
- 9 Stain with 1% brilliant crystal scarlet in 2% glacial acetic acid for 10 minutes
- 10 Rinse in tap water
- 11 Treat with phosphotungstic acid for five minutes
- 12 Rinse in tap water
- 13 Stain with 0.5% methyl blue in 1% acetic acid for 10 to 30 minutes until the required depth of staining is reached
- 14 Rinse in tap water
- 15 Dry on hot plate or blot sections
- 16 Clear and mount.

PERIODIC ACID-METHENAMINE SILVER⁶

The silver solution is made up as follows: to 50 ml of 3% hexamine add 2.5 ml of 5% silver nitrate; mix well; add 3 ml of saturated aqueous borax; warm the solution to 60°C before use.

- 1 Rinse sections with distilled water
- 2 Treat with periodic acid for 10 minutes
- 3 Rinse in distilled water
- 4 Place sections in 5% chromic acid for 45 minutes
- 5 Wash well in tap water followed by several changes of distilled water
- 6 Place sections in preheated silver solution at 60°C for 50 to 70 minutes until membranes are adequately stained
- 7 Rinse well in distilled water
- 8 Treat with 0.2% gold chloride for two minutes
- 9 Rinse in distilled water
- 10 Treat with 3% sodium thiosulphate for two minutes
- 11 Rinse in distilled water and wash well in tap water
- 12 Counterstain with 1% light green for one to two minutes
- 13 Rinse in tap water
- 14 Dry on hot plate or blot
- 15 Clear and mount

Discussion

Adequately stained thin plastic sections of renal biopsy material provide better cellular morphology than routine paraffin sections and therefore provide more accurate histopathological diagnoses.

Existing methods for processing, cutting, and staining sections embedded in epoxy resin are both prolonged (taking at least four days) and time consuming; the techniques described in this paper (using an automatic tissue processor) enable stained embedded sections to be available for examination

Technical methods

within 24 hours of biopsy.

We have been using these techniques for renal biopsy specimens (including transplants) for four months, during which time we have encountered no problems and have found the methods described here to be both reliable and reproducible. We have used other stains (including Sirius red and sulphated alcian blue for amyloid, toluidine blue, and Miller's stain for elastic tissue) when appropriate, and these have also proved reproducible. Attempts with methyl green-pyronin were only partly successful: while RNA was undoubtedly pyroninophilic, the nuclei did not stain; this problem has been overcome by using haematoxylin as the nuclear stain.

Stained sections can be provided within 24 hours of biopsy because an automatic tissue processor (which allows the specimen to be fixed and processed overnight) is used. If no such instrument is available processing can be done by hand once the tissue submitted is fixed; the whole procedure of processing, cutting, and staining will therefore take two working days, but this is still a considerable improvement on the current time.

We believe that this method represents a considerable improvement in the provision of sections of renal biopsy tissue embedded in epoxy resin, and we are now investigating its application to small biopsy specimens from other organs and tissues.

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Detection of asbestos fibres by dark ground microscopy

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The identification and quantification of asbestos fibres from human lung tissue is important in the assessment of the role of asbestos in the pathogenesis of disease, especially in medicolegal cases.¹ Most studies have used phase-contrast microscopy for the counting of fibres at light microscopy level.¹ We describe a method using dark ground illumination that has distinct advantages over the phase-contrast method.

Method

TISSUE DIGESTION AND PREPARATION OF LIGHT MICROSCOPY SLIDES

- 1 A piece of lung about 1 cm³ is gently blotted to remove excess moisture and weighed. Do not dry the specimen.
- 2 Cut the lung into small pieces and place in a 10 ml centrifuge tube.
- 3 Fill the tube with 5% aqueous sodium hydroxide, seal, and place in an oven (60°C) for 24 hours. Agitate occasionally during the day.
- 4 Centrifuge at 500 g or more for 10 minutes.
- 5 Carefully remove most of the supernatant and resuspend the deposit in distilled water. Mix to wash

and leave for two to three minutes.

6 Repeat steps 4 and 5 twice. It is important to wash away the sodium hydroxide or crystals will form which interfere with the microscopy.

7 Centrifuge again and remove supernatant.

8 Resuspend the deposit in 1 ml distilled water.

9 Using a glass pipette drop a measured amount of suspension on to a clean microscope slide and evaporate on a hot plate. Examine with dark ground illumination. If fibres appear very scanty add one or more further drops of suspension to the same area of the slide and dry. If fibres are so numerous as to make counting difficult make a fresh preparation diluting the drop of suspension on the slide with distilled water before drying.

10 Mount preparation under a coverslip using any routine mountant.

11 Examine using dark ground illumination for both coated and uncoated fibres.

DARK GROUND ILLUMINATION

To a routine microscope with a good light source, objectives $\times 10$ to $\times 40$ magnification and $\times 10$ eyepieces, a dark ground condenser is added with a numerical aperture the same as or higher than that of the objective. Click-stop models that permit rapid switching from normal to dark ground illumination are available from most leading microscope manufacturers. Fibres, both coated and uncoated, appear bright and shining against a dark background (fig 1).

QUANTIFICATION

a = all fibres counted on slide; b = volume of suspension on slide (taken from an original 1 ml); c = weight (g) of tissue digested.