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.

References

- 1 Lawler W, Tarpey P, Williams G, Acheson EJ, Mallick NP. Diseases and histological normality of the renal glomerulus: a clinicopathological study. *J Clin Pathol* 1976;29:380-97.
- 2 Janes RB. A review of three resin processing techniques applicable to light microscopy. *Med Lab Sci* 1979;36:249-67.
- 3 Hayat MA, Giaquinta R. Rapid fixation and embedding for electron microscopy. *Tissue and Cell* 1970;2:191-5.
- 4 Lillie RD. *Histopathologic technic and practical histochemistry*. 3rd ed. New York: McGraw-Hill, 1965:270.
- 5 Lendrum AC, Fraser DS, Slidders W, Henderson R. Studies on the character and staining of fibrin. *J Clin Pathol* 1962;15:401-13.
- 6 Jones DB. Nephrotic glomerulonephritis. *Am J Pathol* 1957;33:313-29.

Requests for reprints to: Dr W Lawler, Department of Pathology, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, England.

Detection of asbestos fibres by dark ground microscopy

K R JAMES, T B BULL, B FOX *From the Department of Histopathology, Charing Cross and Westminster Medical Schools, London*

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.

Fig 1 (a) Lung digest: bright field illumination showing coated fibres.

Fig 1 (b) Same field as 1 (a). Dark ground illumination showing coated fibres together with numerous uncoated fibres.

Fig 2 (a) Lung section stained by Perls's method: bright field illumination showing coated fibres (arrow).

Fig 2 (b) Same field as 2 (a). Dark ground illumination showing both coated and uncoated fibres (arrows).

$$\frac{a}{b \times c} = \text{fibres/g of tissue}$$

Both coated and uncoated fibres can also be seen in unstained and Perls's stained paraffin sections using dark ground illumination (fig 2). Over the past three years we have found that dark ground illumination microscopy gives similar results to those obtained by phase-contrast microscopy and has three advantages. The equipment required is cheaper, it is simpler to set up and use, and most important, it is much less tiring when counting fibres. The method of preparation of the lung tissue is simple, and we have found that it is

suitable for electron microscopy and for electron microprobe analysis.

We thank RS Barnett for the photography.

Reference

- 1 Davis JMG, Glyseth B, Morgan A. Assessment of mineral fibres from human lung tissue. *Thorax* 1986;44:167-75.

Request for reprints to: Dr KR James, Department of Histopathology and Experimental Pathology, Charing Cross and Westminster Medical School, St Dunstan's Road, London W6 8RF, England.