Use of tissues embedded in epoxy resin for routine histological examination of renal biopsies

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SYNOPSIS The production of 0.4 μ thick, full-length sections from renal tissue taken by needle biopsy is achieved by formalin and osmic acid fixation and embedding in epoxy resin as for ultramicrotomy. The marked improvement in histological clarity by using this technique is illustrated by comparison with material embedded in paraffin.

Histological clarity by light microscopy depends not only on the optical qualities of the microscope but also on the thickness of the tissue sections examined. Techniques for producing ultrathin sections were developed for electron microscopy (Newman, Borysko, and Swerdlow, 1949), but the embedded specimens are so small that they are often unrepresentative of the tissue of origin. Methods for the successful preparation of thin sections for light microscopy have been described (Cathey, 1963; Ashley and Feder, 1966) but until recently (Lynn, Martin, and Race, 1966) there have been no reports of the employment of these techniques for routine processing of surgical biopsies.

The object of this paper is to describe a method for the preparation of very thin, yet adequately stained, full-length sections from renal tissue taken by needle biopsy and embedded in epoxy resin.

MATERIALS AND METHODS

FIXATION The cylinder of renal tissue obtained by percutaneous needle biopsy is immediately transferred to 10% buffered formol saline (pH 7) at room temperature. The period of formalin fixation should not be less than 12 hours but may be much longer: fixation for several days does not appear to have adverse effects (Baker and McCrae, 1966). When fixed, the specimen is placed in a 15 ml glass tube from which it need not be removed until the stage of embedding. It is washed in phosphate-buffered saline (0.145M NaCl, 0.01M phosphate, pH 7.1) for at least 30 minutes with two changes of fluid and, if desired, it may be left overnight at 4°C in the buffered saline. After the washing, the tissue is post-fixed in a solution of 1% osmium tetroxide (equal parts of the phosphate buffer and 2% osmium tetroxide in distilled water) for 90 minutes. This is followed by further washing

in phosphate-buffered saline for at least 30 minutes and until the odour of the osmium fixative is dissipated.

DEHYDRATION This is achieved by adding increasing concentrations of acetone to the tube. The buffered saline is decanted and replaced by 30% acetone which is followed at half-hourly intervals by a single change of 70% acetone and six changes of 100% acetone.

EMBEDDING The acetone is replaced by 30% Araldite (Ciba Ltd, Basle) in dry acetone, penetration being assisted by gentle mechanical agitation for 30 minutes at room temperature. A 70% mixture is then substituted for the 30% mixture and agitation continued for 30 minutes at room temperature and 30 minutes in a 60°C oven. Finally, the 70% mixture is replaced by 100% Araldite and the specimen kept at 60°C with intermittent gentle stirring for a further 30 minutes. Embedding is completed by leaving the specimen in 100% Araldite at 60°C for 24 to 48 hours, by which time polymerization of the resin will have taken place. The embedding chamber must consist of polypropylene or some similar substance which will not adhere to the hardened Araldite and can be easily peeled off (Anderson and Doane, 1967). Almost any plastic cap provided for many commercial containers will serve.

SECTIONING Araldite blocks, on removal from the embedding chamber, are trimmed with a small handsaw, leaving the block surface to be more carefully prepared with scalpel or razor blade. An ultramicrotome is essential for cutting sections less than 1 μ in thickness but the less elaborate models are best suited to the production of full-length sections. An A. F. Huxley pattern, Cambridge ultramicrotome was used to produce the sections described below. This instrument has a simple specimen holder which is easily replaced by an improvised modification designed to hold the linear blocks in a vertical position (Fig. 1). A glass knife is used: it can be made by hand (Latta and Hartmann, 1950; Pease, 1964a) or more

FIG. 1. Araldite-embedded specimen and modified specimen holder. The block is held vertically above the glass knife edge and attached water trough. $\times 1.25$.

conveniently on a knife-making machine (LKB Sweden, 1963). Final trimming of the block before sectioning is carried out on the ultramicrotome and the knife is changed for making the thin sections. These can be floated off in the same way as for electronmicroscopy, by using a small metal water trough behind the cutting edge of the knife (Fig. 1) to catch the sections (Pease, 1964b). Full-length sections as thin as 0.2μ can be obtained but optimal staining is achieved with sections in the region of 0.4μ thick. The section is removed from the water trough with a fine glass rod and placed onto the meniscus of a drop of water on the microscope slide. Adequate adhesion of the section to the slide is achieved by drying the slide on a hot-plate which is thermostatically controlled to maintain a temperature of 65°C .

STAINING Sections prepared in this manner are often difficult to stain satisfactorily. This is due primarily to the barrier afforded by the embedding resin and secondarily to the extreme thinness of the tissue section itself (Pease, 1964c). Adequate results, however, are obtainable with metachromatic dyes, or the commercial preparation Paragon (Paragon C & C Co. Inc. N.Y.). The method of staining is a modification of a technique used by Charles and Meek (Pease, 1964d). The section is covered with 1 drop of the dye and 3 drops of a 2% borax solution ($2\text{g Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in 100 ml distilled H_2O). The slide is again heated in the manner described above, for 15 to 30 seconds. After washing with distilled water the

section is dried once more on the hot-plate for five minutes, dipped in xylol and then mounted in neutral D.P.X.

Examples of glomerular histology as seen in 0.4μ sections taken from 10 renal biopsies are described to illustrate the value of the method in a variety of conditions. Two postmortem specimens were also processed; the first was from a normal kidney removed and perfused with formalin within 10 minutes of death; the second was from routine formalin-fixed tissue severely affected by amyloidosis. The biopsies were formalin-fixed specimens and were bisected on arrival at the laboratory to permit comparison of the Araldite sections with conventional paraffin processing. Paraffin-embedded sections were cut at 4μ on a Leitz-Minot microtome and stained with haematoxylin and eosin, the periodic-acid-Schiff technique and Masson's trichrome stain. All sections were examined and photographed with a Leitz Ortholux microscope. The Araldite-embedded specimens were especially suited to high-power oil immersion examination, but the paraffin sections are too thick for similar study and are best viewed through lower power dry objectives. The comparative photomicrographs were taken under the microscopic conditions which proved most informative for the paraffin-embedded sections this was at a magnification of $\times 400$ using $\times 40$ dry objective and for the Araldite-embedded tissues at a magnification of $\times 1,000$ using the $\times 100$ oil immersion objective.

RESULTS

NORMAL KIDNEY The clarity of detail afforded by the Araldite embedding and sectioning technique is demonstrated in Figure 2. Endothelial and epithelial cells can be readily distinguished by their relative sizes and their relationship to the capillary loop. The larger epithelial cells embrace the outer aspect of the loops with extensions of cytoplasm and numerous foot processes. Only occasionally can these cells be identified with certainty in the paraffin-embedded material (Fig. 3). In the resin-embedded section the mesangial areas, consisting of mesangial cells and matrix, are clearly defined; in the paraffin section they are seen only as slight focal thickenings of the basement membrane so that positive identification is not always possible.

MINIMAL LESION One example of such a lesion was studied. The patient presented with the features of the nephrotic syndrome, renal biopsy being subsequently performed on two separate occasions. No significant renal lesion was observed on examination of any of the paraffin-embedded material (Fig. 4). Examination of the Araldite sections (Fig. 5) confirmed the absence of basement membrane changes but the glomerular epithelial cells were seen to have lost their clear cytoplasmic borders and there was fusion of foot processes in several areas. These features were subsequently confirmed by electron microscopy.

FIG. 2. Normal glomerulus. Araldite-embedded section. Paragon stain $\times 3,000$.

FIG. 3. Normal glomerulus. Paraffin-embedded section. PAS $\times 1,070$.

FIG. 4. Minimal lesion. Paraffin-embedded section. PAS $\times 1,070$.

- CL = capillary loop
- Ep = epithelial cell
- En = endothelial cell
- BM = basement membrane
- M = mesangial cell
- N = large nodular deposition
- FP = foot processes
- D = deposit within the basement membrane
- RBC = erythrocytes
- WBC = leucocytes
- BC = Bowman's capsule

FIG. 5. Minimal lesion. Araldite-embedded section. Paragon stain $\times 3,000$.

FIG. 6. Systemic lupus erythematosus kidney. Paraffin embedded section. PAS $\times 1,060$.

FIG. 7. Systemic lupus erythematosus kidney. Araldite embedded section. Paragon stain $\times 3,000$.

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FIG. 8.

FIG. 8. *Membranous glomerulonephritis. Case 1. Paraffin-embedded section. Arrow indicates areas of rarified basement membrane density. PAS $\times 1,060$.*

FIG. 9.

FIG. 9. *Membranous glomerulonephritis. Case 1. Araldite-embedded section. Paragon stain $\times 3,000$.*

FIG. 10. *Membranous glomerulonephritis. Case 2. Paraffin-embedded section. PAS $\times 1,060$.*

FIG. 11. *Membranous glomerulonephritis. Case 2. Araldite-embedded section. Paragon stain $\times 3,000$.*

FIG. 12. Membranous glomerulonephritis. Case 3. Paraffin-embedded section. PAS $\times 1,060$.

CL = capillary loop
Ep = epithelial cell
En = endothelial cell
BM = basement membrane
M = mesangial cell

FIG. 13. Membranous glomerulonephritis. Case 3. Araldite-embedded section. Paragon stain $\times 3,000$.

N = large nodular deposition
FP = foot processes
D = deposit within the basement membrane
RBC = erythrocytes
WBC = leucocytes

BC = Bowman's capsule

SYSTEMIC LUPUS ERYTHEMATOSUS One case was examined, the diagnosis being made on paraffin-embedded material (Fig. 6), which showed a generalized but irregular increase in the thickness of the basement membrane and 'wire-looping' in the peripheral parts of the glomerular tuft. In addition to these changes the Araldite sections (Fig. 7) revealed distinct nodular deposits within the basement membrane, partial occlusion of the capillary spaces by endothelial cell debris and polymorphonuclear leucocytes, foot process fusion, and disintegration of epithelial cells.

MEMBRANOUS GLOMERULONEPHRITIS Of the three biopsies which were ultimately classified as membranous glomerulonephritis, only two were recognized as such on examination of the paraffin-embedded material.

The first case showed marked thickening of the glomerular basement membrane which was clearly

visible in the paraffin sections (Fig. 8). Examination of Araldite sections (Fig. 9) confirmed this and in addition revealed both dense and rarified zones within the thickened membranes, the low density areas resembling vacuoles. These could be identified on retrospective examination of the paraffin sections (Fig. 9). Other features, recognizable only in the 0.4 μ sections, were early degenerative changes of the epithelial cells with lipid droplet accumulation and fusion of the foot processes.

Examination of paraffin-embedded sections from the second case revealed insufficient changes to account for the clinical picture (Fig. 10). In the Araldite sections (Fig. 11), several important lesions were noted: the basement membrane was thickened in places up to three times the normal; dense deposits, mainly of linear distribution on the epithelial aspect of the capillary loops, were present in most areas and were accompanied by epithelial cell degeneration and foot process fusion.

FIG. 14. Diabetic glomerulosclerosis. Paraffin-embedded section. H & E $\times 1,060$.

FIG. 15. Diabetic glomerulosclerosis. Araldite-embedded section. Paragon stain $\times 3,000$.

Similar changes were also seen in the third case, although the degree of basement membrane thickening was so marked and uniform in its distribution that the diagnosis was readily apparent on examination of the paraffin sections alone (Fig. 12). The resin-embedded sections showed deposits and thickening of the membrane by at least four times (Fig. 13).

PROLIFERATIVE GLOMERULONEPHRITIS Diagnosis of the single case examined was based on glomerular hypercellularity, digitation of the tuft, capsular adhesions, and occasional crescent formation as observed in the paraffin-embedded sections. An increase in the PAS-positive material was also noted in the mesangial areas. These observations were confirmed on examination of the Araldite sections, which revealed additional features, namely, enlarged mesangia appearing to enclose several nuclei of apparently endothelial origin, basement membrane thickening confined to portions of capillary loops adjacent to the mesangia, and degenerative changes in epithelial cells.

RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS Two cases were included under this heading on the basis

of clinical data and advanced glomerular fibrosis. The resin-embedded material did not contribute significantly to the information already obtained from examination of the paraffin sections.

DIABETIC GLOMERULOSCLEROSIS Paraffin-embedded material from a single case (Fig. 14) showed both nodular and diffuse lesions characteristic of diabetic glomerulosclerosis. The Araldite sections (Fig. 15) did not contribute further information but it was apparent that they could help in the detection of lesser thickening of the basement membrane.

AMYLOIDOSIS Although staining reactions are reduced with resin-embedded section, metachromasia is still obtained and the fine distribution of amyloid deposits is readily analysed.

COMMENT

There are several advantages in preparing very thin sections for routine histological diagnosis. Histological detail is better than with conventional thicker sections and they less often require special stains for the detection of marginal degrees of basement membrane thickening. They also provide a useful link with the ultrastructural picture afforded by

electron microscopical examination. Ultrastructural studies are usually restricted to an individual glomerulus, but the 0.4μ sections permit whole biopsy examination including vessels, tubules, interstitium, and perhaps several glomeruli, at any one time.

The use of formalin as the basic fixative avoids the complications of inexperienced theatre and ward staff handling a variety of special fixatives for different purposes. It has been previously shown (Baker, 1965) that formalin-fixed tissues are adequate for some ultrastructural studies which means that once the tissues have been embedded in an epoxy resin further sections can be prepared later for electron microscopical examination should these be required. The post-fixation period with osmium tetroxide in the laboratory helps to clarify the cytoplasmic details, cell boundaries, and membranes: it must not be omitted, especially if ultrastructural investigations are ultimately contemplated.

The technique is not restricted to renal biopsies

but can be employed for a much wider range of tissues.

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REFERENCES

- Anderson, N., and Doane, F. W. (1967). *Stain Technol.*, **42**, 169.
 Ashley, C. A., and Feder, N. (1966). *Arch. Path.*, **81**, 391.
 Baker, J. R. (1965). *J. roy. micr. Soc.*, **84**, 115.
 —, and McCrae, J. M. (1966). *Ibid.*, **85**, 391.
 Cathey, W. J. (1963). *Stain Technol.*, **38**, 213.
 Latta, H., and Hartmann, J. F. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **74**, 436.
 LKB, Sweden (1963). *Science Tools*, **10**, 59.
 Lynn, J. A., Martin, B. S., and Race, G. J. (1966). *Amer. J. clin. Path.*, **45**, 704.
 Newman, S. B., Borysko, E., and Swerdlow, M. (1949). *J. Res. Nat. Bur. Stand.*, **43**, 183.
 Pease, D. C. (1964a). *Histological Techniques for Electron Microscopy*, 2nd ed., p. 149. Academic Press, New York.
 — (1964b). *Ibid.*, p. 165.
 — (1964c). *Ibid.*, p. 256.
 — (1964d). *Ibid.*, p. 260.

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