Rapid processing of renal glomeruli for electron microscopy

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Recently we reported a new fine-needle aspiration biopsy method to obtain single glomeruli for electron microscopy in diagnostic and investigative pathology of the kidney. The small size and fragmentary nature of the specimen obtained by this technique necessitated a special device for collecting and processing the tissue for electron microscopy (Pasternack et al., 1978). A new and simpler device has now been constructed, which allows the method to be applied to routine laboratory work and clinical diagnostics. With this new device it is possible to carry out the whole process from fixation to polymerisation of the specimen.

Having used conventional processing methods for electron microscopy with the new device, we were tempted to try out previously described rapid processing methods (Estes and Apicella, 1969; Bencosme and Tsutsumi, 1970; Hayat and Giaquinta, 1970; Johannessen, 1973; Rowden and Lewis, 1974) for two reasons. Firstly, the fine-needle aspirate is small enough to guarantee adequate penetration of fixatives, buffers, and embedding materials in a shorter time than is needed when conventional methods are used. Secondly, drainage of the processing fluids allows a thorough exchange of the processing medium. This is thought to render the processing more effective, thus reducing further the need for more time-consuming schedules.

We describe below the processing device and report on its use with the fine-needle aspiration method and rapid processing.

Methods

To examine the effects of the use of this device together with rapid processing on glomerular ultrastructure, several biopsies were taken from exposed kidneys of anaesthetised New Zealand White rabbits. After this pilot study the same method was used to investigate a wide range of human diffuse glomerular diseases. Both the rabbit and the human biopsies were carried out according to previously described techniques (Pasternack et al., 1978). Immediately after aspiration of the biopsy specimen, the aspiration syringe was filled with the primary fixative. The aspirates were then, after completed prefixation, transferred from the syringe to the processing device. This was constructed by connecting a Beem capsule with a polyethylene adapter1 fitted to the syringe. A small piece of nylon sieve (100 μm mesh opening) was attached to the Beem capsule after the tip had been cut to make an open end of about 1 mm2 (Fig. 1). The specimens were processed by letting the solutions drain through the sieve, which retained glomeruli and other tissue fragments. With the exception of prefixation, the whole procedure was carried out in the device, including polymerisation of the Epon. For processing, the following method, modified according to Hayat and Giaquinta (1970) and Johannessen (1973), was used:

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1Manufactured by Kodin Muovi, Helsinki, Finland. Not commercially available
**Technical methods**

Fig. 2 Glomerular capillary wall of rabbit. Electron micrograph with normal ultrastructure. BM = basement membrane; CL = capillary lumen; US = urinary space. (× 92 400).

Fig. 3 Electron micrograph of normal glomerular epithelial cell showing good preservation of the subcellular details. Aspiration biopsy of human kidney. (× 13 500).
Fig. 4  Electron micrograph of glomerular capillary loop. Marked thickening of glomerular basement membrane (BM) with electron dense deposits (arrows) in intramembranous position. Aspiration biopsy in lupus nephropathy. US = urinary space; CL = capillary lumen; En = endothelial cell. (× 13709).

1 Prefix with cacodylate-buffered 4% glutaraldehyde at pH 7.4 for 30 minutes.
2 Wash with 0.1 M cacodylate buffer, pH 7.4, for 15 minutes.
3 Postfix with 2% cacodylate-buffered osmium tetroxide at pH 7.4 for 30 minutes.
4 Repeat step 2.
5 Stain en bloc with aqueous uranyl acetate for 5 minutes.
6 Dehydrate in acetone series: 30%, 60%, 90%, and 100% for 10 minutes each.
7 Infiltrate with a mixture (1:1) of Epon 812 and absolute acetone for 15 minutes followed by pure Epon for 20 minutes; steps 1 to 6 should be carried out at room temperature.
8 Embed in fresh Epon for 1 hour at 100°C; the mixture of Epon used was: 4 ml A + 6 ml B with 0.15 ml DMP-30 (Fluka AG, Buchs, Switzerland). The Epon was allowed to cool for at least 15 minutes and the Beem capsule was then removed together with the sieve. Silver or grey sections were cut off the blocks with a diamond knife on an LKB
Fig. 5  Electron micrograph of glomerulus showing proliferation of mesangial cells (MC), increase of mesangial matrix (MM), and deposition of electron dense material (arrows). Aspiration biopsy of a patient with previous poststreptococcal glomerulonephritis. (× 6200).
Ultratome III, mounted on 150-mesh formvar-coated or 300-mesh uncoated copper grids, stained with lead citrate and uranyl acetate, and examined under the JEM 100C electron microscope. Before ultrathin sectioning 0.5 μm sections were cut for light microscopy and stained with periodic acid-silver methenamine.

Results and comments

Neither the handling of the aspirated tissue sample in the processing device nor the rapid processing had any harmful effect on the glomerular ultrastructure. This was shown for both normal rabbit and normal and diseased human glomeruli. Some of the results are illustrated in Figures 2-5. No difference has been noted in structural integrity between normal and moderately or severely altered tissue in a series of 65 human fine-needle aspiration biopsies. The good structural preservation seems to be favourably influenced by the small size of the aspirate: large specimen size is a known source of fixation and infiltration artefacts (Hayat and Giaquinta, 1970).

The blocks produced with this method had good cutting qualities. The silver to grey coloured sections, mounted on coated or uncoated grids, were stable in the electron beam. The fine-needle aspiration biopsy of the kidney used together with a rapid processing schedule offers a safe, technically simple, and time-saving alternative to conventional biopsy methods in the investigation of human glomerular ultrastructure. The clinical value of the method has been discussed in a previous publication (Pasternack et al., 1977).

References


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Letter to the Editor

Recovery of spores from impregnated filter paper

Drs Annear and Green misquote us (Journal of Clinical Pathology, 1979, 32, 93).

Far from stating that quantitative recovery of spores from spore papers was difficult, we described a simple method for doing just that.

It was Kelsey, writing in 1961, who stated that 'Despite repeated attempts no such quantitative recovery technique could be devised'. We referred to this only to show that now at least it is no longer true.

P. H. EVERALL and C. A. MORRIS
The Royal Shrewsbury Hospital, Shropshire

Reference


Book reviews


Most pathologists face recurrent questions of nomenclature, classification, and pathogenesis which they feel should be at their fingertips. Their guilt is now assuaged. With two new editors, 24 contributors, 356 pages (including index), and a paperback production the latest Recent Advances in Histopathology takes care of many of these problems. In this one volume you can get to grips with the evidence for the neuroectodermal origin of the APUD system; compare the sea of classifications
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