Technical method

An electron microscopy method for studying full cross-sections of the human renal glomerulus

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In recent years a better understanding of the natural history of the glomerular nephritides has been achieved due to improved clinicopathological correlation, mediated by interpretation of renal biopsies and using combined techniques of light microscopy, immunofluorescence and electron microscopy.

However, the efficiency of sampling for electron microscopy is seriously diminished by the common usage of mesh grids for mounting ultrathin sections. Their design precludes examination of the full surface of the section due to masking of detail underneath the grid bars. Although sampling problems may be partly overcome by mounting several sections on a grid, for quantitative studies clearly the whole section area needs to be examined.

In the last three years, we have developed in this laboratory a simple method which allows precise cellular identification and quantification of glomerular morphology at any level through the glomerulus.

Material and methods

Tissues emanated from the routine and research EM service the bulk of the samples being renal and tumour biopsies. Blocks for electron microscopy are prepared by standard techniques using glutaraldehyde and osmium fixation and embedding in epoxy resins.¹ Ultrathin sections are cut with glass knives at around 80 nm thickness, after selection of the areas with glomeruli. The outline of the glomerulus is clearly visible on the ultrathin sections floating on the waterbath; an uncoated grid with a central single hole is manoeuvred under the section and lifted so that the section sits over the hole with the glomerulus in its centre. Copper grids (Athene) with holes varying between 200, 375 and 600 μm

were used; the most useful size being 375 μm . After drying, the sections are stained with uranyl acetate (5% in 50% ethanol) and lead citrate (0.25% in 0.4% NaOH) and are photographed on a TEM Jeol 100S, at a magnification of $\times 500$; three or four overlapping fields are photographed to cover the whole area of the glomerulus. The plates are enlarged to a magnification of $\times 2000$ and photographic montages are prepared from the various prints. Cell counts, measurements and quantitative estimations are effected on the photographic montages (Figure).

Discussion

The method requires skill but is easily learned by a trained technician. The quality of the ultrathin sections has to be good though not necessarily excellent. Sections containing deep knife marks or many holes invariably break up in the electron beam. Occasional folds across the sections are eliminated if adequate expansion of the ultrathin sections is performed with chloroform vapour before they are mounted onto the single hole grids. Working at 40 kV using a 60 μm objective aperture with an under-focused lens current gives excellent image contrast.

Precise cellular counts can be given, as well as other quantitative parameters arising from determination of glomerular compartments.

The elimination of the sampling errors usually inherent to electron microscopy allows very precise correlation between light microscopy and electron microscopy appearances. This ability to correlate easily light and electron microscopy is of considerable advantage for teaching and diagnostic purposes. In addition this method will facilitate the application of quantitative light microscopy morphometric methods²⁻⁴ to electron micrographs of the human glomerulus.

Its use extends beyond the study of renal biopsies. We have found it ideal to study lymph node malignancies and anaplastic carcinomas.

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Renal biopsy from a 32-year-old woman. IgA disease. Segmental increase in mesangium is seen at middle left; electron dense deposits were found in this area at higher magnification. If mesh grids had been used this small area of mesangial reaction might have been obscured by the grid bars. Area of glomerular section: $5500 \mu\text{m}^2$. Maximum diameter: $90 \mu\text{m}$; $\times 2000$.

References

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