**Dr J R Tighe and Dr N F Jones**
*(Department of Surgical Pathology, St Thomas's Hospital, London SE1)*

## The Diagnostic Value of Routine Electron Microscopy of Renal Biopsies

For the past three years, electron microscopy has been performed whenever possible on all diagnostic renal biopsies in St Thomas's Hospital. Over 85% of biopsies were found suitable for electron microscopy. This report relates to the first 100 biopsies examined in this way. The indications for biopsy were: Nephrotic syndrome 46, subnephrotic proteinuria 14, unexplained renal failure 33, miscellaneous 11.

The figures total more than 100 as patients occasionally had more than one indication for biopsy, for example, acute renal failure complicating the nephrotic syndrome. The 'miscellaneous group' includes cases of systemic lupus erythematosus (4), polyarteritis nodosa (2), renal potassium wastage (2), single nonfunctioning kidney (1), recurrent hæmaturia (1) and chronic pyelonephritis (1). For the most part, patients with unexplained renal failure had open surgical biopsies and the others had needle biopsies.

All the biopsies were examined immediately with a dissecting microscope to assess their adequacy. Needle biopsies were usually cut lengthwise to provide a sample for electron microscopy. Blocks of 1 $mm^3$ were fixed in 4% methanol-free formaldehyde in cacodylate buffer at 4°C, post-fixed in Palade's osmium tetroxide-sucrose fixative and processed to Epon. Sections 1 μm thick stained with toluidene blue were examined by light microscopy and several representative glomeruli were selected for further examination in the electron microscope in each case. Ultrathin sections, mounted on copper grids, stained with uranyl acetate and lead citrate were carbon coated and examined in a Siemens Elmiskop 1 electron microscope at 80 kV.

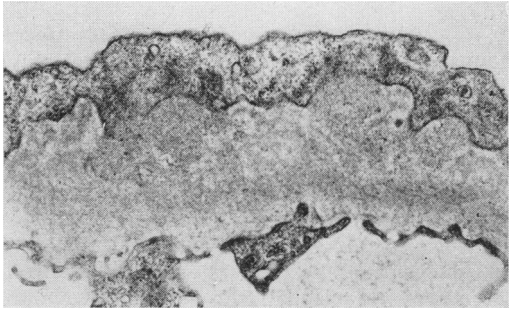

Fig 1 *Membranous glomerulonephritis. The glomerular basement membrane is thickened and of uneven texture due to the subepithelial deposition of granular material. Epithelial cell cytoplasm (top) rests on the basement membrane without foot processes. Endothelial cell cytoplasm (bottom) is normally fenestrated.* × *13,700*

The pathological diagnoses in the 46 cases of nephrotic syndrome were: Membranous glomerulonephritis 12, minimal change (foot process disease) 9, proliferative glomerulonephritis 13, amyloid 7, not diagnostic 5.

The group of 'proliferative glomerulonephritis' included generalized, focal and lobular forms. Four of the 'not diagnostic' cases showed only ischæmic glomerulosclerosis. Clinical selection accounted for the absence of diabetic patients with the nephrotic syndrome.

Of the patients presenting in renal failure, 20 showed the changes of ischæmia or chronic glomerulonephritis and a further 8 showed active proliferative glomerulonephritis.

Salient features of the electron microscope appearances follow.

*Membranous glomerulonephritis:* Irregular dense deposits occur focally within the glomerular basement membrane in less severe cases (Fig 1). In more severe cases, these deposits produce an almost continuous band within the membrane. This results in variable thickening of the membrane, usually detectable by light microscopy but occasionally so slight as to be detectable only by electron microscopy. The visceral epithelial cells, having lost their foot processes, are smudged on to the basement membrane. There is cytoplasmic invagination of the membrane giving it an irregular outline. Within the cytoplasm, particularly in relation to the membranous deposits, there is increased density. The epithelial cells characteristically show microvillous proliferation in the urinary space. There is no increase in glomerular cellularity.

*Minimal change (foot process disease):* There is no basement membrane abnormality but the overlying epithelial cells show loss of foot processes (Fig 2). They do not show cytoplasmic invagination of the basement membrane and microvillous proliferation in the urinary space is less noticeable than in membranous glomerulonephritis. Deposits in or on the membrane are not apparent.

*Proliferative glomerulonephritis:* There is endothelial cell proliferation with narrowing of the capillary lumen. Epithelial cell proliferation in the

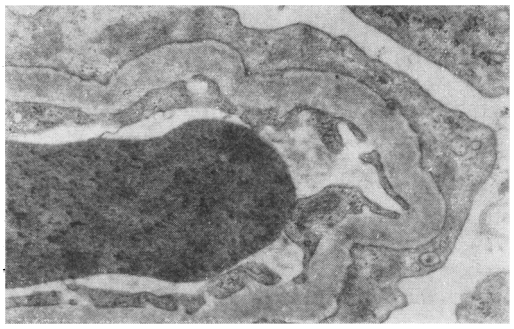

Fig 2 *Foot process disease. Compare the thickness and texture of the basement membrane with* Fig 1. *The three layers of the membrane (lamina rara interna, lamina densa and lamina rara externa) can be identified and there are no deposits. The epithelial cell cytoplasm is smudged on the basement membrane and foot processes have been lost.* × *13,700*

glomeruli is more variable. The glomerular basement membrane is frequently thickened and shows dense deposits, most commonly occurring as a protrusion or 'hump' (Kimmelstiel *et al.* 1962) on the epithelial side of the membrane (Fig 3). Poststreptococcal glomerulonephritis is characterized by homogeneous dense deposits of this pattern sometimes in continuity with the lamina densa and sometimes separated from it by the lamina rara externa (Osawa *et al.* 1966). Overlying these deposits, epithelial cells show focal fusion of foot processes and increased cytoplasmic density. Other deposits, not typical of a streptococcal etiology, may be found within the basement membrane or on its endothelial surface.

*Focal glomerulonephritis:* Focal lesions presented features similar to those of the diffuse form of proliferative glomerulonephritis.

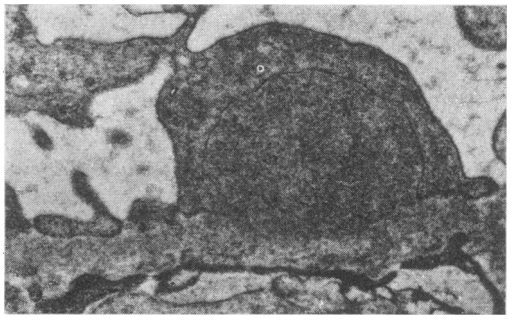

Fig 3 *Proliferative glomerulonephritis. A dense deposit retts on the epithelial side of the glomerular basement. Overlying this deposit, epithelial cell cytoplasm is smudged. Elsewhere, foot processes are preserved.* × *15,500*

*Lobular glomerulonephritis:* Mesangial matrix is increased and scanty collagen fibres are laid down in the mesangium. Mesangial cells are increased in number and show more abundant cytoplasm than normal. There is irregular thickening of the glomerular basement membrane and deposits similar to those of proliferative glomerulonephritis may be found. Visceral epithelial cells show focal fusion of foot processes.

*Systemic lupus erythematosus:* Dense deposits occur within and on the epithelial and endothelial surfaces of the glomerular basement membrane. These deposits are particularly noticeable on the endothelial surface of the basement membrane where they assume a 'woolly' or faintly fibrillary pattern (Fig 4). They correspond to the 'wire loops' seen on light microscopy. The basement membrane may be thickened and the overlying epithelial cells show fusion of foot processes. Focal proliferative endothelial cell changes are common.

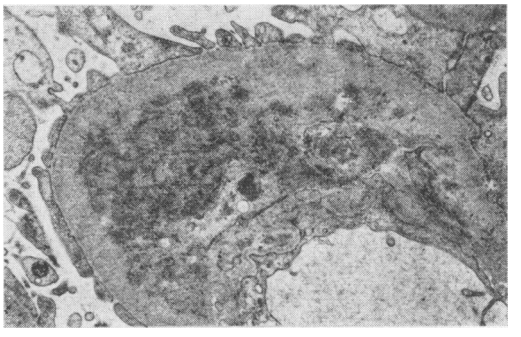

Fig 4 *Systemic lupus erythematosus. Some of the epithelial cell foot processes (top left) are preserved, but others are blunt. A 'woolly' dense deposit separates the basement membrane from the endothelial cell lining the capillary (bottom right).* × *7,900*

*Polyarteritis nodosa:* Glomerular changes of focal glomerulonephritis may be found. In some cases, deposits typical of poststreptococcal glomerulonephritis may be found. Arterioles show proliferation of smooth muscle cells and deposition between the cells of dense fibrillary material, apparently fibrin.

*Ischæmic nephrosclerosis:* Although glomeruli appear sclerosed by light microscopy, electron microscopy reveals that this is mainly due to excess of mesangial matrix. Collagen fibres are generally scanty in these sclerosed tufts, although collagen is plentiful in the proliferating tissue of Bowman's capsule. Mesangial cells remain viable in the sclerosed tuft to an advanced stage of the disease.

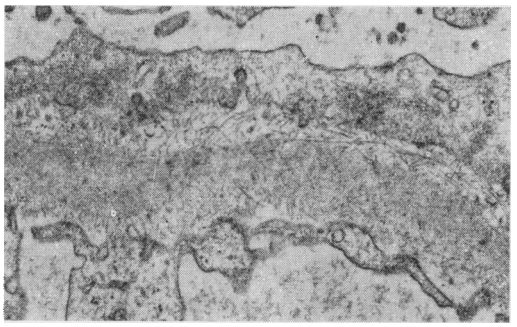

Fig 5 *Amyloid. Fibrils of amyloid infiltrate the glomerular basement membrane. The epithelial cell cytoplasm (top) has lost its foot procesess.* × *21,000*

Minor degrees of ischæmia are associated with focal increase of mesangial matrix, slight glomerular basement membrane thickening and partial fusion of epithelial cell foot processes.

*Amyloid:* When amyloid is scanty, electron microscopy provides valuable confirmatory evidence. Characteristic 10 nm fibrils, with a triple-layered structure, are found infiltrating the glomerular basement membrane (Fig 5), the mesangium, arteriolar walls from endothelium through the smooth muscle coat, and in tubular basement membranes. In the glomerular basement membrane, amyloid fibrils when few in number bear no consistent relationship to either the endothelial or epithelial surface of the membrane.

*Juxtaglomerular cells:* Granules in the juxtaglomerular cells of the afferent arteriole are few in man and difficult to demonstrate by light microscopy. The granules are recognizable in the electron microscope as dense homogeneous bodies surrounded by a single membrane (Fig 6). Electron microscopy provides a valuable means of quantitative assessment of the granularity of these cells.

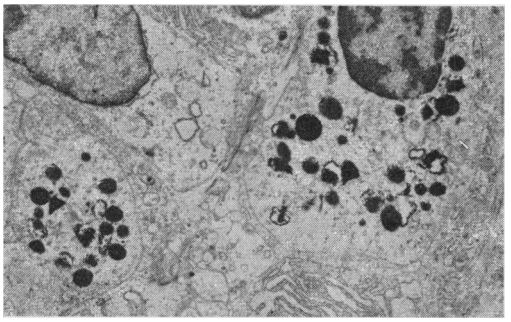

Fig 6 *Juxtaglomerular (JG) cells. The membrane-bound, dense bodies are prominent in the cytoplasm of the JG cells. These bodies are the JG granules and are here increased in number associated with secondary aldosteronism.* × *3,600*

*Tubular changes:* These are not characteristic in the biopsies selected although one patient with secondary aldosteronism showed cytoplasmic vacuolation of the proximal tubular epithelium. A variety of cellular degenerative changes accompanied by tubular basement membrane thickening and peritubular fibrosis accompanies ischæmic damage.

*Comment*

Routine diagnostic electron microscopy has proved to be of greatest value in the differential diagnosis of the nephrotic syndrome. Of the 12 cases diagnosed as membranous glomerulonephritis on electron microscopy, no fewer than 4 were diagnosed on light microscopy as 'no change'. These 4 and 7 of the other 8 cases of membranous glomerulonephritis, failed to respond to a standard course of steroid therapy. In contrast, only one of the 9 cases of foot process disease – a diagnosis which can be established only by electron microscopy – failed to respond to steroid therapy. It is of interest that in this one patient the proteinuria was not as highly selective (ratio of the clearance of IgG to albumin clearance = 0·25) as is usual with the foot process lesion. In this instance the proteinuria selectivity was a better guide to steroid response than histology; however, on other occasions, the morphological appearances proved to be more reliable. In general there is good correlation between the selectivity of the proteinuria and the morphology, membranous glomerulonephritis being less selective than foot process disease.

The limitations of routine electron microscopy are that it is costly and time consuming. The risk of inadequate or unrepresentative areas of the kidney being examined is greater than with light microscopy, but initial optical microscopic examination of 1 μm sections enables the focally involved glomeruli to be selected and examined by electron microscopy. This resulted in this series in diseased glomeruli being examined by electron microscopy in 10 of the 11 cases in which a histological diagnosis of focal glomerulonephritis had been made.

The use of electron microscopy in the precise classification of glomerular disease is established, and it is likely that with increasing knowledge its use as a guide to therapy will increase.

REFERENCES

**Kimmelstiel P, Kim O J & Beres J** (1962) *Amer. J. clin. Path.* **38**, 280

**Osawa G, Beres J & Kimmelstiel P** (1966) *Amer. J. clin. Path.* **46**, 295