**INTRODUCTION**

The use of transmission electron microscopy (TEM) is an essential diagnostic tool (Stirling & Woods 2002). The requirement for TEM is usually unpredictable and tissue is collected and processed specifically in order to optimise ultrastructural detail (Bretschneider et al. 1981). However, in some cases the requirement for TEM may not be anticipated, or the tissue collected for TEM may be inadequate (for example, when there are no glomeruli in the TEM sample of a renal biopsy).

In instances where the opportunity to collect material specifically for TEM has passed, all is not lost! Useful information can be gleaned from reprocessing either (1) formalin-fixed, paraffin-embedded tissue (Woods & Stirling 2002) or (2) sections prepared for light microscopy (LM) using the ‘pop-off’ technique (Bretschneider et al. 1981, Woods & Stirling 2002). Here we describe two cases where the use of reprocessed tissue for ultrastructural studies contributed additional ultrastructural information, thus facilitating the diagnosis.

**METHOD**

**Overview**

First, the tissue or section is dewaxed (in the case of an existing slide the mountant and a variety of deposits (D). A TEM sample of a renal biopsy (Fig. 3). The glomerular basement membrane (GBM) contains a variety of deposits (D). In some areas the GBM is thickened (TGM) in keeping with diabetic nephropathy. Capillary lumens (CL) are prominent. Bar = 10 µm.

Immunofluorescence

Direct immunofluorescence (IF) with a monoclonal antibody against IgG showed linear C3 deposition, but possible granular reactions in some loops. Labelling for loops and lambda light chains was positive (CL) as also granular in some loops (Fig. 5).

Transmission electron microscopy

No glomeruli were seen in the tissue submitted for TEM, however, the positive basement membrane immunoperoxidase (Fig. 4). Electron microscopy showed that glomeruli were displaced from the wax block (Fig. 5) and reprocessed for TEM evaluation.

Ultrasound examination - reprocessed tissue

Some loops appeared to be thickened, keeping with diabetic changes.

**CASE STUDY 1**

**WAX EMBEDDED TISSUE - RETINAL BIOPSY**

A 25-year-old male presented with retinopathy, the nephrotic syndrome and renal failure (glomerular filtration rate of 18 ml/min/1.73 m² with a proteinuric level of 0.10 mmol/L). The renal physician questioned the possibility of diabetic nephropathy.

Light microscopy

Glomeruli showed nodular glomerulosclerosis and a diffuse increase in mesangial matrix (M) and capillary wall thickening. Capillary loops similar to those marked* in Fig. 4 are shown in Figs 5 and 6. Bar = 100 µm.

**Immunofluorescence**

No material was available for IF labelling. Immunoperoxidase labelling for IgG, IgM, light and C3 was negative.

Transmission electron microscopy

No glomeruli were evident on Thermanox cover slip (Fig 2), preferably coated in a section of 0.09 mmol/L and hypertension. The renal physician questioned the possibility of IgA nephropathy.

Light microscopy

Four glomeruli were found, one was obliterated. Glomeruli were mildly or moderately enlarged, a small number of slightly prominent mesangia were also present (Fig 5). In sections containing these there was a suggestion of subtle GBM abnormality, primarily variable membrane thickness.

**DIAGNOSTIC COMMENTS**

The LM and IF data suggested diabetic nephropathy and possibly membranous glomerulonephritis or immune complex-mediated disease. The ultrastructural evidence confirmed diabetic glomerulonephritis with stage II/III, focally stage IV, membranous GN.

The presence of subepithelial and intramembranous deposits in GBMs indicated thickened GBM was consistent with diabetic nephropathy. The presence of subepithelial and intramembranous deposits in GBMs indicated thickened GBM was consistent with diabetic nephropathy.

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**TISSUE SECTION - RETINAL BIOPSY**

A 55-year-old male presented with haematuria, albuminuria, a plasma creatinine level of 0.10 mmol/L, and hypertension. The renal physician questioned the possibility of IgA nephropathy.

Light microscopy

Four glomeruli were found, one was obliterated. Glomeruli were mildly or moderately enlarged, a small number of slightly prominent mesangia were also present (Fig 5). In sections containing these there was a suggestion of subtle GBM abnormality, primarily variable membrane thickness.

**Immunofluorescence**

No material was available for IF labelling. Immunoperoxidase labelling for IgG, IgM, light and C3 was negative.

Transmission electron microscopy

No glomeruli were evident on Thermanox cover slip (Fig 2), preferably coated in a section of 0.09 mmol/L and hypertension. The renal physician questioned the possibility of IgA nephropathy.

Light microscopy

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The LM and IF data suggested diabetic nephropathy and possibly membranous glomerulonephritis or immune complex-mediated disease. The ultrastructural evidence confirmed diabetic glomerulonephritis with stage II/III, focally stage IV, membranous GN.

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- The presence of subepithelial and intramembranous deposits in GBMs indicated thickened GBM was consistent with diabetic nephropathy.
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**REFERENCES**


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**CASE STUDY 2**

**REPROCESSED FORMALIN-FIXED, WAX-EMBEDDED TISSUE CAN BE USED FOR DIAGNOSTIC ELECTRON MICROSCOPY**

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