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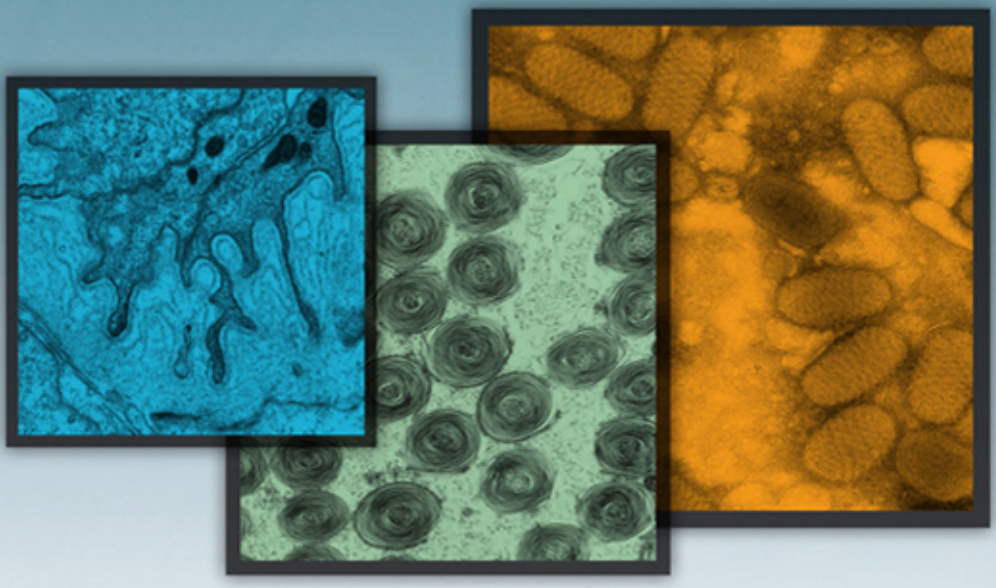
John W. Stirling, Alan Curry and Brian P. Eyden

Diagnostic Electron Microscopy



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Diagnostic Electron Microscopy – A Practical Guide to Interpretation and Technique

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Finally, the editors dedicate this book to diagnostic electron microscopists – wherever they may be – who continue to make uncertain diagnoses more precise as a result of their labours, which, in turn, help clinicians to treat their patients better, the ultimate purpose of our work.

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Preface – Introduction

John W. Stirling, Alan Curry and Brian Eyden

DIAGNOSTIC ELECTRON MICROSCOPY

Science progresses as a result of a variety of factors. Critical to progress, however, is the invention and availability of appropriate tools and techniques that can completely transform our ability to investigate and understand the world around us – without such tools our ability to investigate even basic phenomena would be severely restricted. One such 'transformational' technology is the electron microscope. Although transmission electron microscopy (TEM) is now taken for granted, its application to the biological and medical sciences in the late 1950s and early 1960s ranks as one of the single most important factors that has impacted on our knowledge in biology and medicine. The resolving power of the transmission electron microscope ($\sim$0.2 nm as compared with the light microscope with a resolution of $\sim$200 nm) made two important things possible for the first time, these being the visualisation of: (1) cell organelles and cytoplasmic structures at the macromolecular level (both useful indicators of cell differentiation) and; (2) viruses and microorganisms in general. Thus, TEM gave us new fundamental insights into cell structure and function, histogenesis and differentiation, and, following from this, our understanding of disease and disease processes.

TEM was quickly taken up as a diagnostic tool. In the clinical setting, electron microscopy has been used to improve diagnostic precision and confidence in many fields, including renal disease, neuromuscular disease, microbiology (particularly virology), tumour pathology, skin

diseases, industrial diseases, haematology, metabolic storage diseases and conditions involving abnormalities of cilia and sperm. A number of encyclopaedic atlases of normal and pathological tissues quickly followed the introduction of electron microscopy and the medical literature contains many articles describing diagnostic applications of TEM in a wide range of conditions and specialist areas. Diagnostic TEM reached a zenith during the 1980s; however, since then, the introduction of new methodologies (particularly molecular techniques and affinity labelling systems) has reduced the need for TEM, particularly in tumour diagnosis. Despite this, TEM continues to play a significant and important role in pathology, and techniques continue to develop and improve. For example, the introduction of microwave processing and digital cameras has transformed tissue processing and screening so that 'same-day' reporting is easily achieved.

THE PURPOSE AND USE OF TEM

The purpose of TEM is to diagnose disease based on the ultrastructural features of the tissue. These features include:

1. The presence (or sometimes the absence) of specific or characteristic cellular structures or organelles that indicate cell differentiation
2. The general ultrastructural architecture, including the identity, location and morphology of specific structural features that may be associated with pathology, or indicate disease.

In general, the use of TEM will be predetermined either as a standalone protocol (e.g., CADASIL) or as part of a broad integrated diagnostic strategy (e.g., renal biopsies). However, TEM can also be applied on an *ad hoc* basis whenever there is a chance it will give an improved diagnosis (and therefore better patient care). The general criteria indicating the use of TEM may be summarised simply as follows:

1. When it provides useful (complementary) structural, functional or compositional information in respect to diagnosis, differential diagnoses or disease staging
2. When only atypical features or minor abnormalities are visible by light microscopy despite clear clinical evidence of disease (e.g. some renal diseases)
3. When affinity labelling results are equivocal (e.g. renal disease and tumours)

4. When there is no realistic alternative diagnostic technique or a 'simple' test is not available or feasible (e.g. genetic diseases with multiple mutations such as CADASIL and primary ciliary dyskinesia)
5. The investigation and diagnosis of new diseases and microorganisms
6. When it is time and/or cost effective in respect to alternative techniques.

THE AIM AND PURPOSE OF THIS BOOK

The prime aim and purpose of this book is to summarise the current interpretational applications of TEM in diagnostic pathology. In this respect, we have not attempted to reproduce previous encyclopaedic texts but to provide what we regard as a working guide to the main, or most useful, applications of the technique given the limited space available in a text of this size. In addition, we have also included practical topics of concern to laboratory scientists, including brief guides to traditional tissue and microbiological preparation techniques, microwave processing, digital imaging and measurement uncertainty.

1

Renal Disease

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1.1 THE ROLE OF TRANSMISSION ELECTRON MICROSCOPY (TEM) IN RENAL DIAGNOSTICS

The ultrastructural examination of renal biopsies has made a significant contribution to our understanding of renal disease and is fundamental to accurate diagnosis. For overall tissue evaluation, light microscopy (LM), immunolabelling and transmission electron microscopy (TEM) are generally combined as an integrated protocol. LM is used to make an assessment of overall tissue morphology and to identify the major pathological processes present. Immunolabelling (preferably using immunofluorescence or by the immunoperoxidase technique) is used to determine the composition and location of glomerular immune deposits. Local practices vary, but an antibody panel can contain antibodies directed against IgG, IgA, IgM, complement (C3, C1q and sometimes C4), κ and λ light chains and albumin. TEM can play a major role when LM and immunolabelling findings are normal, only mildly atypical or equivocal and difficult to interpret, particularly in respect to conditions where there may be similar LM or immunolabelling findings. Thus, the technique is particularly useful in the setting of familial disease where the

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structural abnormalities in the glomerular basement membrane (GBM) cannot be resolved by LM (e.g. Alport's syndrome). TEM can also provide critical information not revealed by the other methodologies to identify underlying primary disease and unexpected concomitant disease. Similarly with immunolabelling, the full classification and staging of deposits require ultrastructural analysis. Some transplant biopsies can also benefit from ultrastructural evaluation (see Chapter 2); however, TEM rarely contributes to the diagnosis of tubular, vascular or interstitial disease. Overall, ultrastructural screening is essential; it can change the diagnosis in ~25% of cases and provides 'useful' information in ~66% of cases (Pearson *et al.*, 1994; Elhefnawy, 2011).

1.2 ULTRASTRUCTURAL EVALUATION AND INTERPRETATION

Examination of glomeruli (and other areas, if necessary) should be thorough and systematic with all components being evaluated for possibly significant features or changes. During screening, a range of representative images should be taken. These should include low-power images to show overall glomerular morphology, plus a representative selection of higher power images to show the specific and critical diagnostic features. In some instances, it may also be important to show that certain features are, in fact, absent (e.g. deposits) or normal (e.g. foot processes). The principal elements that should be examined are (i) the location, size and morphology of immune-related deposits and other inclusions; (ii) the thickness, overall morphology and texture of the GBM; (iii) the size and morphology of the mesangial matrix and (iv) the number and morphology of the cellular components of the glomerulus (Stirling *et al.*, 2000). Sclerotic glomeruli should be avoided, and only well-preserved functional (or significantly functional) glomeruli should be examined. It is also important to ensure that the glomeruli screened are representative of the LM findings: this means that, ideally, the choice of glomeruli to be screened (from semithin sections) should be done in collaboration with the reporting pathologist. Finally, it should be stressed that screening should be unbiased, although some knowledge of the pathology and immunolabelling results may be useful if the features expected are minor or uncommon. The vascular pole should be avoided during ultrastructural evaluation as it may contain misleading nonpathologic deposits, and likewise Bowman's capsule which has no real diagnostic value, although the presence of crescents can be confirmed.

Following evaluation, representative images and findings should be communicated to the reporting pathologist, the latter verbally or in a concise written report. If the initial evaluation does not correspond with the LM evaluation (e.g. the electron microscopy (EM) samples only a tiny fraction of the available tissue), then the specimen should be re-examined or additional glomeruli observed to increase diagnostic confidence.

A critical question is 'How many glomeruli should be examined, and for how long?' Unfortunately, there is no definitive answer to this dilemma except to say that enough tissue should be examined to answer the diagnostic question posed and to ensure that no additional or unexpected pathology is present. A single glomerulus (or even part of one) may be adequate in respect to diffuse disease and/or when the glomerulus screened is typical of the disease process identified by LM. In contrast, several glomeruli, or possibly glomeruli from different blocks, may be required to capture the full range of pathological changes in focal disease. Perhaps the final word on this issue is to say that the tissue must be screened thoroughly; it is bad practice to stop screening once the features that were expected have been located because additional findings that affect the accuracy of the diagnosis may be missed.

1.3 THE NORMAL GLOMERULUS

The glomerulus (Figure 1.1) is composed of a tuft of branching capillaries that originate from the afferent arteriole at the vascular pole to form a series of lobules (segments) that ultimately rejoin at the vascular pole and exit the glomerulus via the efferent arteriole. At the core of each lobule is the mesangium which supports the capillary loops; capillary loops are lined by endothelial cells (Figure 1.1). The mesangial matrix principally consists of collagen IV and is populated by mesangial cells (usually 1–3 in normal mesangium) plus a small number of immune-competent cells and rare transient cells of the monocyte–macrophage lineage (Sterzel *et al.*, 1982). The entire capillary tuft is enclosed within Bowman's capsule, the inner aspect of which is lined by a thin layer of epithelial cells (the parietal epithelial cells); a second inner population of epithelial cells (the visceral epithelial cells or podocytes) is closely associated with the capillary tufts, and extensions of these cells form the foot processes (pedicels) that cover the outer aspect of the capillary walls (Figure 1.1). The podocytes are the sole source of the collagen IV $\alpha 3$, $\alpha 4$ and $\alpha 5$ subtypes that form the bulk of the GBM (Abrahamson *et al.*, 2009), and the foot processes play a major role in ultrafiltration and the

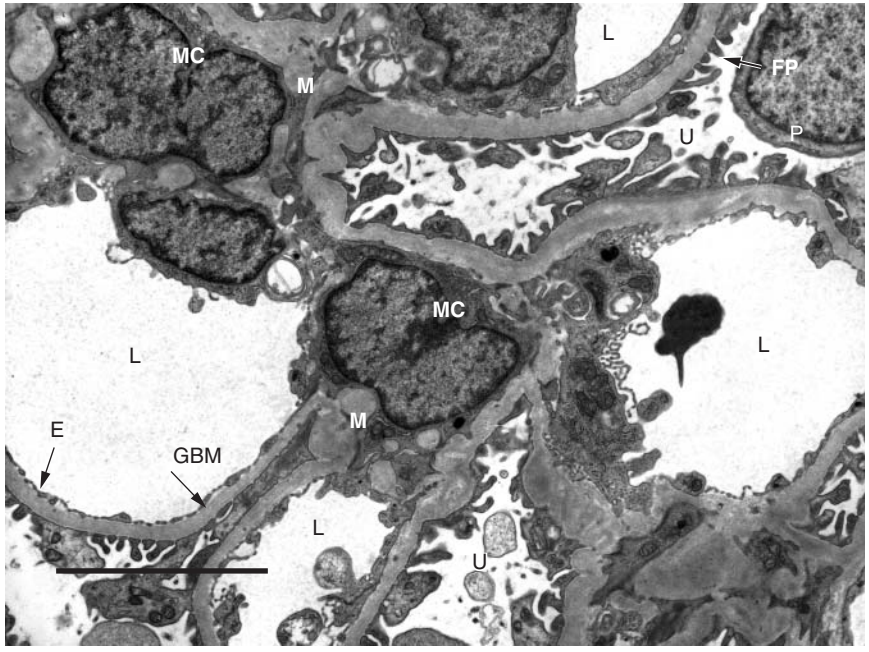

Figure 1.1 Detail of a normal glomerulus. The capillary loops are supported by the mesangium (M). Mesangial cells with nuclei (MC); capillary lumens (L); urinary space (U); podocyte (P) (epithelial cell) and foot processes (FP). Here, the overall width of Overall, the glomerular basement membrane (GBM) averages ~380 nm in width. Loops are lined with fenestrated endothelial cells (E). Bar = 5 μm.

maintenance of the filtration barrier. As a result, podocyte dysfunction plays a major role in a wide range of glomerular diseases (Wiggins, 2007; Haraldsson, Nystrom and Deen, 2008). Opposite the vascular pole, Bowman's capsule is continuous with the proximal tubule which drains filtrate from the glomerulus (the urinary pole). Overall, filtration is said to be a function of size, shape and charge selection, although the nature and contribution of charge selection are debated (Harvey *et al*., 2007; Haraldsson, Nystrom and Deen, 2008; Goldberg *et al*., 2009). The capillary wall as a whole is responsible for the filtration process, and it appears that the capillary endothelium, the GBM and the podocyte foot processes must all be intact for normal filtration to occur (Patrakka and Tryggvason, 2010).

1.3.1 The Glomerular Basement Membrane

The GBM (Figure 1.1) is made of three layers: (i) the lamina rara interna, the electron-lucent layer immediately adjacent to the endothelium;