



Electron Microscopy in Glomerulonephritis

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Abstract

Electron microscopy has been in use as a research tool for many years, and as such has helped to demonstrate and elucidate the fine structure of many organs, including that of the kidney. The intricate pedicel structure of the renal glomerular epithelial cell, the glomerular endothelial fenestrae (Fig. I) and the varying cytological arrangements in the tubular part of the nephron were all unknown before the advent of electron microscopy. As a development of this anatomical function of the microscope, the technique began to be applied to diseased tissues in an attempt to analyse further the relation of disordered structure to disordered function. This type of investigation held the inherent difficulty that human tissues to be examined in this way had, of necessity, to be biopsy material, since autolytic changes in post-mortem material are so gross that cytological ultrastructure is significantly destroyed.

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ELECTRON MICROSCOPY IN GLOMERULONEPHRITIS

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Electron microscopy has been in use as a research tool for many years, and as such has helped to demonstrate and elucidate the fine structure of many organs, including that of the kidney. The intricate pedicel structure of the renal glomerular epithelial cell, the glomerular endothelial fenestrac (Fig. 1) and the varying cytological arrangements in the tubular part of the nephron were all unknown before the advent of electron microscopy. As a development of this anatomical function of the microscope, the technique began to be applied to diseased tissues in an attempt to analyse further the relation of disordered structure to disordered function. This type of investigation held the inherent difficulty that human tissues to be examined in this way had, of necessity, to be biopsy material, since autolytic changes in post-mortem material are so gross that cytological ultrastructure is significantly destroyed.

Investigations were carried out in many human diseases where biopsy material was available, and in particular several diseases of the renal glomeruli became capable of more thorough description, classification and clinico-pathological correlation. It is probably not too much to say that the concept of glomerulonephritis itself was revolutionised.

EARLY CONCEPTS OF GLOMERULONEPHRITIS

Before the advent of the electron microscope and the technique of renal biopsy the usually accepted classification of glomerulonephritis was that of Ellis, proposed in 1942; this had superseded the old classification elaborated by

Volhard and Fahr, which divided the disease into three stages, acute, subacute and chronic. Ellis' classification, more clinically acceptable, described two separate disease processes. One was named Type I glomerulonephritis; this was of acute nature, clinically and pathologically, and usually resolved with complete clinical recovery. It might, however, progress to a chronic, fibrosing stage in which the patient developed chronic renal failure and died. This condition was accepted as being of post-streptococcal nature. Type II glomerulonephritis was a completely different disease, in which the patient presented with a slowly developing nephrotic syndrome; this eventually progressed to chronic renal failure in the majority of cases, although in a few complete recovery ensued. The pathology of this second type, according to Ellis, was basically a thickening of the glomerular capillary walls, although other complicating and often inflammatory features were described in some cases. The cause of the capillary wall thickening was quite unknown, and likewise the aetiology was obscure. It must be appreciated that all the histological material on which this extremely perspective concept was based was post-mortem in nature.

The more complex and subtle types of glomerulonephritis were at that time almost unrecognised. Most cases of glomerular disease were put into one or other of the two main categories if no generalised systemic condition such as diabetes mellitus was present. Focal glomerulonephritis was not well appreciated as a clinical or pathological entity, and

the existence of a functional disease of the glomerular capillary basement membrane unassociated with gross structural glomerular changes was unsuspected. It was at this historical point, in the middle 1950's, that the electron microscope became functionally translated from anatomy to the pathological field, and began to answer some of the questions which light microscopy had created but had been unable to solve; and it was at approximately the same time that renal biopsy became practical, safe and an accepted part of investigation of renal disease.

"ELLIS TYPE II" GLOMERULONEPHRITIS

REAPPRAISAL OF CLASSIFICATION

The first real impact of electron microscopy on human renal disease was in the field of what was then known as Ellis Type II glomerulonephritis. It was becoming obvious that Ellis

had, in fact, included in this group a number of different conditions, some of which were basically inflammatory; many cases which clinically could be placed in this group showed histological features which were in fact those of Type I glomerulonephritis, with the addition of some glomerular capillary wall thickening and focal hyalinisation of the tufts. The development of such progressive cases of acute glomerulonephritis could now be followed by means of serial renal biopsy.

In addition, increasing knowledge of the effect on the kidneys of some systemic diseases of the so-called "collagen disease" group, for example, disseminated lupus erythematosus, had revealed that clinical states ranging from acute glomerulonephritis to a pure nephrotic syndrome could be produced by these conditions, and a corresponding variety of histological features was possible, including glomerular capillary wall thickening.



Fig. 1. This figure shows part of a normal human glomerulus; two capillary walls are seen, and the epithelial pedicel structure is well demonstrated. x 15,000

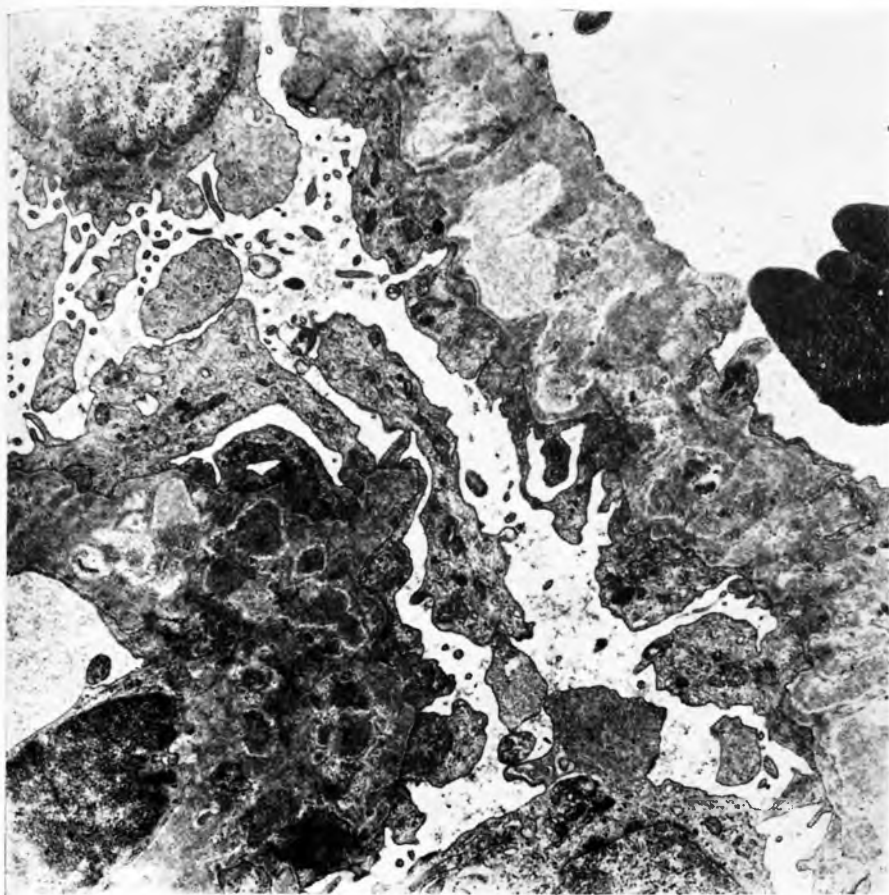


Fig. 2. This micrograph is from a case of membranous glomerulonephritis. Two glomerular capillary walls are seen, and it is obvious that their structure is quite abnormal. Vacuoles are present in the basement membrane, which is thickened and irregular, and some dense, granular deposits are also visible in this layer. The epithelial layer is continuous over the basement membrane.
x 8,000

As a result of these advances, it became obvious that when all cases which could be placed in other categories were removed, the group of patients with Type II glomerulonephritis consisted of individuals exhibiting clinically a pure nephrotic syndrome and showing on renal biopsy no inflammatory or proliferative changes of any significance in the glomeruli. Specimens from these patients showed either a diffuse thickening of the glomerular capillary walls, or, surprisingly, no glomerular abnormality of any importance. It became clear that the development of the former lesion was towards increasing capillary wall thickening and obliteration of normal glomerular structure, with glomerular ischaemia and progressive renal failure. It is perhaps natural that at this time the type of case in which no capillary wall thickening or other glomerular lesion was evident was thought to be simply an early stage of the con-

dition, and that given time it would progress to a stage at which the capillary wall thickening became visible on light microscopy.

PATHOLOGICAL CHANGES

Electron microscopic investigation of these cases, by this time usually referred to collectively as early and late stages of Type II glomerulonephritis, now began to throw light on the structural changes involved and on the relationship of the conditions, changing completely some of the ideas hitherto accepted without question. The hyaline thickening of the capillary walls seen in the so-called late stage of the condition was found to be accounted for by a complicated change in the structure of the capillary walls and in the relationship of their three main components. (Fig. 2). The basement membrane itself, which, as will be recalled, is the middle layer of the capillary

wall, had become of irregular thickness, and often much thicker than normal: very dark patches or deposits were visible between it and the epithelium, and the latter had completely lost its pedicel structure, lying in a continuous layer over the uneven basement membrane. The endothelium was often thickened, and the cells occasionally showed evidence of slight proliferation. Vacuoles tended to appear in the basement membrane, and in later cases the capillary walls could be seen to be composed of a confused mass of basement membrane material and fragments of epithelial or endothelial cytoplasm, with little or no evidence of the original three distinct layers. Such changes, or modifications of them, were always seen in cases in whom the renal biopsy had shown glomerular capillary wall thickening by light microscopy.

In the other "early" group of cases, that is, these patients in whom renal biopsy on light microscopy had revealed no significant change in glomerular structure, electron microscopy

showed that the basement membrane was apparently normal in thickness and composition, but that again the epithelial cells had lost their pedicel structure, and the epithelium lay in an almost continuous layer over the basement membrane. (Fig. 3). As in the group of cases previously described, the endothelial cells sometimes showed a little proliferation.

Recalling that this latter type of case was thought to be an early stage of the condition, and that the fully developed state involved marked basement membrane changes visible on the electron microscope, it will be understood why the two types of cases were now referred to as early and late stages of *membranous glomerulonephritis*. It was considered, both from the structural and functional points of view, that in this disease, involving as it did proteinuria as its main feature, the glomerular capillary basement membrane must be the site of the basic lesion. The position of the epithelial cell changes in the aetiology was not well understood, but it was felt that the loss



Fig. 3. This shows part of a glomerulus in a case of minimal lesion glomerulonephritis. The basement membrane of the capillaries appears normal, but the epithelium has lost its pedicel structure, and lies in a continuous layer over the basement membrane. x 6,000

of pedicel structure might be in fact secondary to the proteinuria, constituting an attempt to "seal off" the points of leakage in the basement membrane.

MEMBRANEOUS AND MINIMAL LESION GLOMERULONEPHRITIS

However, this relatively simple concept of membranous glomerulonephritis was then undermined by a fact which became apparent only as the years went by, and more patients were followed up both clinically and by biopsy examination. The obvious corollary to the idea that there were histologically distinguishable early and late stages of membranous glomerulonephritis would be the recognition of the transition of patients from one stage to the other, and up to the present this has simply not been proved to occur. In addition, patients in the so-called early stage are found to have proteinuria and oedema as severe as those in the "late stage". Patients suffering from the type of disease in which no glomerular abnormalities are visible on light microscopy may continue for a long time excreting protein and yet the light microscopy appearances of the glomerular capillaries do not become those of the "late stage", and in fact change very little, if at all. Likewise these patients have a good prognosis clinically, and while it is not possible to dogmatise on this subject, it appears that they do not progress to renal failure and hypertension as do the patients who exhibit histologically the more severe lesion. These findings cast grave doubt upon the advisability of regarding the two types of case as different stages of the same condition, and the idea has gradually evolved that they might be, in fact, two different diseases. The term "membranous glomerulonephritis" has naturally been reserved for the type in which the capillary basement membrane is visibly abnormal, and the somewhat awkward name "minimal lesion glomerulonephritis" was coined for the condition in which glomerular capillary wall thickening is not visible on light microscopy. These terms are now well established as the nomenclature for the two conditions, and more and more clinical workers are apprehending the distinction between the diseases, and are appreciating the fact that the prognosis in minimal lesion glomerulonephritis is good, whereas that in membranous glomerulonephritis is bad, although the disease may be of long duration.

THE ROLE OF STEROID THERAPY

The common method of dealing with cases of both minimal lesion and membranous glomerulonephritis is therapy by steroids. It would therefore be a matter of great difficulty to collect and compare series of cases of the two conditions in which repeated renal biopsy had been performed and in which the course of the disease had not been influenced by steroids. In fact, the majority of reports, relating to the investigation of such cases by electron microscopy in recent years, have referred to patients who had been given steroids at some time. It does seem probable, from observation of occasional untreated cases, that the natural history and microscopic appearances in membranous glomerulonephritis are in general the same, whether or not steroids have been given: while there is an impression that such treatment may slow up the process, the light and electron microscopic appearances undoubtedly still show a continuous, although in some cases very slow, progression towards disruption of the normal structure of the capillary walls, with thickening and eventual obliteration of capillary lumina.

In minimal lesion glomerulonephritis, on the other hand, it is obvious that while some patients show spontaneous recovery without the aid of steroid therapy, in many individuals proteinuria will continue unless steroids are given, and will then cease, often within a very short time. Indeed, some patients can be shown to be highly sensitive to steroids, the proteinuria appearing and stopping as these drugs are withdrawn and reintroduced. The interesting fact in this connection is that in most cases electron microscopic investigation of the glomeruli, at a time when the patient has no proteinuria due to steroid therapy or to spontaneous cure, shows more or less complete restoration to normal of the capillary wall ultrastructure, with re-appearance of pedicels.

Basically a more important point, however, arising from the widespread use of steroid therapy, is the possibility that it could be this form of treatment which prevents cases of minimal lesion glomerulonephritis from progressing to membranous glomerulonephritis. Without going into any detail, it may be said that there is a significant amount of evidence militating against this concept.

The aetiology of minimal lesion and membranous glomerulonephritis is quite obscure up to the present time, and indeed there is

no real evidence that they are the result of the same cause, or even related causes. As might be supposed, it has been suggested that the lesion is basically an immune reaction, possibly of "auto-immune" type: there is some positive evidence that this is so. However, no definite concept of the aetiology of these conditions has yet been evolved.

"ELLIS TYPE I" GLOMERULO-NEPHRITIS

When we turn to the condition which Ellis described as Type I glomerulonephritis, and which was, in his view, a hypersensitive reaction of the glomeruli to the streptococcus, we find that here the electron microscope itself has made a less basic and categorical contribution: however, it is true to say that the advent of renal biopsy, with a combination of light and electron microscopic analysis, has transformed our appreciation of the significance of pathological glomerular changes in this condition, or rather, as soon became obvious, this group of conditions.

The essential lesion in what was then called Type I glomerulonephritis was a generalised proliferation of the endothelial cells of the glomerular capillaries with endothelial swelling, and consequent partial or complete blockage of the capillary lumina. This led to glomerular ischaemia, to which is due many of the important clinical features of the disease. This endothelial cell change is responsible for the fact that the name now usually given to this condition is *proliferative glomerulonephritis*. It was realised that many cases of this type of glomerulonephritis recovered completely, and that a few cases continued or recurred, developing in a clinically subacute fashion to a condition of hypertension and chronic renal failure, which always proved fatal. It must be appreciated once again that virtually all the pathological material available for examination in this respect was post-mortem in nature.

PATHOLOGICAL CHANGES

When it became possible to visualise the changes going on in the kidney during life, some important and hitherto unrecognised points were discovered, and a much more complex classification began to evolve. In the first place, it was found that in renal biopsies from patients who presented with one or more of the clinical features of an

acute inflammatory glomerular lesion, the lesions encountered were very varied. Some cases might show the characteristic, obvious endothelial cell proliferation with perhaps polymorph infiltration of the glomeruli: others showed only a very slight degree of this lesion, and in several cases the endothelial cell proliferation was very focal, occurring only in some glomeruli, and only in parts of these glomeruli. In addition, some biopsies showed small, scattered foci of necrosis in glomeruli; in other cases, endothelial cell proliferation was associated with localised thickening of the capillary walls, and occasionally this thickening had a bright, hyper eosinophilic, refractile appearance. Other, more subtle characteristics were picked up as more series of biopsies were examined, and gradually composite pathological pictures began to be associated with certain clinical conditions. There is no doubt that we are yet far from a complete and satisfactory classification of the acute inflammatory lesions of the renal glomeruli, but we now possess an overall understanding of clinico-pathological correlation which is much greater than anything we have known before.

CLASSIFICATION

In general, proliferative glomerulonephritis can now be divided into *diffuse* and *focal* types. Many of the cases of the *diffuse type*, in which virtually all of the glomerular tissue is involved, are obviously post-streptococcal in nature from a clinical point of view, and present the usual clinical features of this condition. Other patients, however, exhibiting similar histological features on renal biopsy, are not clinically diagnosable as definite cases of the post-streptococcal syndrome. In this situation, electron microscopy appears to offer some help. Examination of biopsy material by this means in several centres has suggested that in true post-streptococcal cases, a characteristic feature can be found in the glomerular capillaries when the disease has been present for some time. This consists of a few, scattered, large, localised granular deposits of darkly osmiophilic material lying on the epithelial surface of the basement membrane, pushing deeply into the epithelium which has often lost its pedicel structure in this region. Whether this feature will be found to be sufficiently pathognomonic to be used as a parameter for diagnosis of post-streptococcal disease remains to be seen.

Focal glomerulonephritis has become a fairly common diagnosis in renal biopsy material;

patients exhibiting this pathological condition may present with a variety of clinical features including haematuria, proteinuria, oedema, hypertension. The focal lesions in the glomeruli may be simply of endothelial cell proliferation, or may include small patches of necrosis or focal capillary wall thickening. Some of these cases are found on examination to be quite definite, but mild, cases of post-streptococcal glomerulonephritis; but many others turn out to be in fact cases of so-called "collagen disease" of some type, usually disseminated lupus erythematosus or polyarteritis; Henoch-Schönlein disease also can be associated with such a histological picture. While in many cases electron microscopy is not able to distinguish between these conditions, a diagnosis of disseminated lupus erythematosus may be strongly supported by the finding of thin, dark, layered patches of a finely granular material, lying between the glomerular capillary basement membrane and the endothelium; this feature is very frequently seen in proved cases of the condition. Such supportive evidence

for the presence of disseminated lupus erythematosus may be of great help in dealing with a case otherwise difficult to diagnose.

CONCLUSION

There is no doubt that we are still only scratching at the surface of the problem of glomerulonephritis, in spite of the very numerous specialised techniques now being used in its investigation. The cause of minimal lesion and membranous glomerulonephritis, the complex question of the reaction of the glomerulus to the streptococcus, the reasons for the varying types of development of cases of acute proliferative glomerulonephritis, the problem of proteinuria in the presence of a basement membrane normal to ultrastructural examination — all these, and many more difficulties, must be elucidated before we can claim to have a rational insight into the diseases which affect the glomerulus, and before we can evolve a really intelligent mode of therapy for these conditions.

FURTHER READING

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EXPERIENCE

"Our own Experience is but a narrow field to walking. Man's life is short, nor is even that little spent in Medicinal Enquiries, so that it is a pity so useful a part of knowledge should have no wider Bounds; but even limited as it is we tread surer when we rely on it than trust to the Experience of others. What a man has himself seen he is much more certain of than what he is told by others; the pleasure which the success or the pain which a disappointment may have given him, strike him more deeply, and make him for the future more bold or more cautious as his case requires."

(Extract from one of the earliest Dissertations in the library, dated 1751.)