THE DIABETIC KIDNEY

A study of diabetic microangiopathy by light and electron microscopy examination of percutaneous renal biopsies

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SUMMARY

Although there is an extensive literature concerning diabetic renal involvement, disagreement regarding the diffuse and nodular glomerular lesions, and uncertainty about their relationship to pyelonephritis and arteriolosclerosis, have obscured the pathogenesis of renal disease in diabetes. The general belief, however, that diabetics are more prone than non-diabetics to atheroma and other less specific changes in larger blood vessels in no way detracts from the concept that it is involvement of small blood vessels, and of capillaries in particular, which characterises the diabetic lesion.

To investigate this problem a light and electron microscopy examination has been made of renal tissue obtained by percutaneous biopsy from selected groups of patients representing different stages or clinical categories of diabetes and from healthy non-diabetics. Since reliable quantitative methods must be applied when electron microscopy is used to demonstrate cellular ultrastructure, and because the value of data based upon small biopsy samples is limited unless it can be shown that the tissue obtained is representative of that individual's glomerular population, statistical methods appropriate to small samples and free of assumptions concerning distribution have been used. Thus such non-parametric methods as the Mann-Whitney 'U' test or the Kolmogorov-Smirnov analysis of cumulative frequency distribution have been applied to both the measurement of glomerular capillary basement membrane thickness and to a technique of assessing on

a quantitative basis, within the glomerular mesangium, the ratio of basement membrane substance to cellular cytoplasm which has been designated the Mesangial Index.

Using these methods the mean glomerular capillary basement membrane thickness in nine non-diabetic subjects was 2,200 Å (Angstrom Units) and although the findings in four newly diagnosed juvenile diabetics were not significantly different from normal either in terms of basement membrane thickness or mesangial index, significant basement membrane thickening was found in seven of ten patients having diabetes secondary to haemochromatosis, chronic pancreatitis or pancreatic carcinoma. One of ten age-and duration-matched long-standing diabetics had no evidence of glomerular pathology after 23 years of insulin dependence. All other nine patients had unequivocal basement membrane thickening, however those without diabetic retinopathy had significantly lower mesangial indices than patients having proliferative retinopathy. Thus in the latter patients there was attenuation of mesangial and endothelial cells with massive basement membrane accumulation. This aspect was studied further in six diabetics having advancing proliferative retinopathy by obtaining renal biopsy tissue before and one to two years after pituitary surgery. Successful pituitary ablation was followed by significant reduction in the thickness of the glomerular capillary basement membrane, restoration of atrophic endothelial and mesangial cells to their normal appearance, persistence of morphological features suggesting over-

activity of the epithelial cells and no improvement in the arteriolar lesion.

Other light and electron microscopic features of the diabetic kidney have been reviewed and the above findings considered in relation to various clinical factors of significance in the diagnosis and assessment of diabetic renal disease. Current aspects of genetic, metabolic, immunological, endocrine and other factors of possible inportance in the pathogenesis of diabetic small blood vessel disease have been considered in relation to the findings in this study.

It is concluded that although the nodular glomerular lesion of Kimmelstiel and Wilson remains for the light microscopist the hallmark of diabetes in the kidney, usually it is found only in association with the more common diffuse glomerular changes, and with afferent and efferent arteriolosclerosis. Accompanying changes in the tubules and interstitial tissue which often in the past have been interpreted as chronic healed pyelonephritis probably represent the end result of diabetic vascular lesions.

It is suggested that the excessive basement membrane material found in the glomerular capillaries probably represents varying degress of its epithelial production or impaired mesangial turnover. The absence of glomerular lesions in newly diagnosed juvenile diabetics and the demonstration of basement membrane lesions in secondary diabetes suggests that the diabetic lesion is independent of the genetically determined diathesis to idiopathic diabetes but is due to some metabolic derangement common to both primary and

secondary diabetes. The possibility that some components of diabetic microangiopathy can occur only in and be due to some as yet undefined aspect of the idiopathic disorder is, however, not excluded by these observations. Thus it is probable that diabetic nephropathy results from several separate though interrelated lesions each of which may be variously influenced by the metabolic disturbance, the inherited diabetic diathesis or pituitary activity.

Although the idea of a specific diabetic microangiopathy is widely accepted, it is concluded that this simple concept probably conceals the complexity of the factors concerned in the pathogenesis of the various arteriolar and capillary lesions.

CHAPTER 1

INTRODUCTION.

> "Diabetes is a wonderful affliction, not very frequent among men, being a melting down of the flesh and limbs into urine.....Life is short, disgusting and painful, thirst unquenchable.....It is not improbable, also, that something pernicious, derived from other diseases which attack the bladder and kidneys may sometimes prove the cause of this affliction".
>
> Aretaeus the Cappadocian,
> 2nd century A.D.

Thus diabetes mellitus has been known since antiquity. Yet few would now accept this original definition of the disorder despite the clarity with which Aretaeus set down his unaided observations. Certainly definitions are not eternal scientific truths, but temporal evaluations limited by the general structure of medical thought. How much has advance in medical knowledge changed our views about diabetes? Undoubtedly the consequences of pancreatic removal described by von Mering and Minkowski (1890) and the isolation of insulin by Banting and Best in 1922, by demonstrating the importance of hormonal deficiency in diabetes mellitus, dramatically altered medical thinking.

Yet the insulin era, with its brave promise of cure for diabetics, brought fresh uncertainties and setbacks. First Houssay and Biasotti (1926) showed the importance of the anterior pituitary, besides the pancreas, in diabetes. To the present time it is still uncertain how much diabetes is a consequence of defective insulin secretion and how much it is due to insulin

antagonism mediated by growth hormone, fatty acids or other hormones and substrates.

Then in 1936, Kimmelstiel and Wilson described spherical hyaline masses in the glomeruli of eight patients who died of renal failure following illnesses characterised by albuminuria, nephrotic oedema and hypertension. The glomerular lesions were attributed to diabetes because seven of the patients were known to have had the disorder. Although many would accord Kimmelstiel and Wilson a place in medical history on account of their description of a new syndrome, the fundamental importance of their observation lay in the interest it stimulated in the possibility of a specific small blood vessel disorder in diabetes. The subsequent demonstration, in autopsies on diabetics, of diffuse glomerular lesions (Fahr, 1942), and of associated hyaline arteriolosclerosis (Bell, 1942), together with renewed interest in diabetic retinopathy (Ballantyne and Loewenstein, 1944), led to the concept of a specific and widespread diabetic microangiopathy (Lundbaek, 1954).

The general belief that diabetics are more prone than non-diabetics to atheroma and other less specific changes in larger blood vessels in no way detracts from the concept that it is involvement of small blood vessels, and of capillaries in particular, which characterises the diabetic lesion. Although there is an extensive literature concerning diabetic renal involvement, disagreement regarding the diffuse and nodular glomerular lesions, and uncertainty about their relationship to pyelonephritis, arteriolosclerosis and other vascular lesions, have obscured the pathogenesis of renal disease in diabetes (Fig. 1).

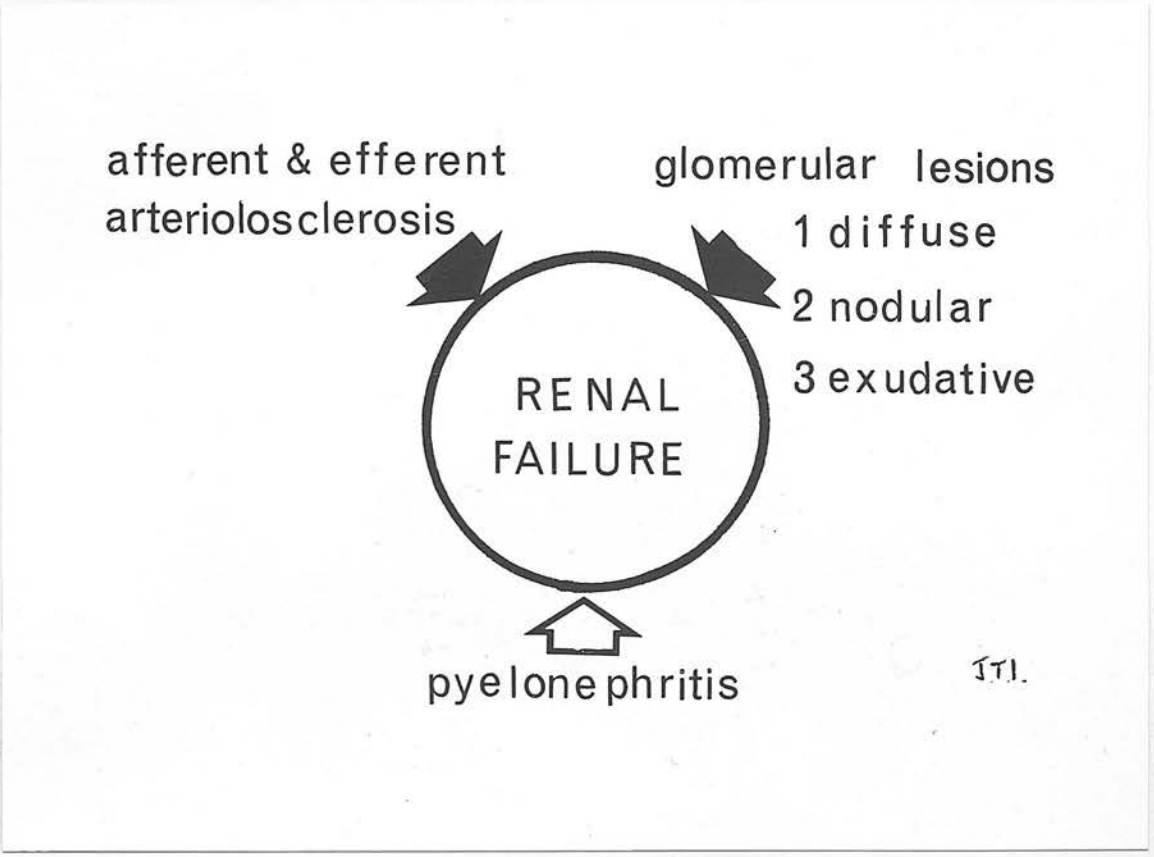


Fig. 1. Diabetic nephropathy.

Some of these difficulties have been overcome by the use of electron microscopy, which has shown, by sharpening the focus on glomerular and other capillaries, that homogenous thickening of their basement membranes distinguishes diabetes from other causes of capillary disease. As a result "basement membrane thickening" has become the new catch-phrase of diabetic vascular pathology. Whether this lesion is a morphological consequence of a metabolic or genetic defect, or an immunological response to exogenous insulin, to other antigens, or some other defect remains undecided. That the problem is not only of academic interest is emphasised by the fact that more than half of those who develop diabetes in childhood die of renal failure or its consequences (Warren, Le Compte and Legg, 1966).

Sadly the observations of Aretaeus largely remain true. Despite the isolation and free availability of insulin, life may still remain shorter and more painful for the diabetic than for others. Moreover, one cannot contemplate, without dismay, the sombre truth that the insulin era has afforded ample opportunity to witness, but so far not to avert, diabetic small blood vessel disease.

The purpose of this study has been to investigate the natural history and pathogenesis of diabetic small blood vessel disease by light and electron microscopy examination of renal tissue obtained by percutaneous biopsy from diabetic patients. In essence, selection of clinical material, percutaneous renal biopsy, electron microscopy examination and evaluation of the data by statistical methods appropriate to the nature of the samples, have been the basis of the methodology.

Instead of attempting to examine a cross-section of a diabetic population, selected groups representing different stages and clinical categories of diabetes have been chosen for comparison with healthy non-diabetic subjects. Thus biopsies have been obtained at the onset of clinical diabetes and after 20 years of insulin dependence. However in the latter group patients were chosen either because of absence of clinical evidence of diabetic retinopathy and other features of diabetic angiopathy on the one hand, or because of the presence of advanced proliferative diabetic retinopathy in age and duration-matched diabetics on the other. Both extremes are rare. Yet it was hoped that this approach might etch in sharper relief the morphological features associated with freedom from angiopathy and those associated with advanced stages of small blood vessel disease. Some aspects of a possible influence of genetic factors in the pathogenesis of diabetic angiopathy have been examined by comparing tissue obtained from patients having diabetes secondary to chronic pancreatitis, pancreatic carcinoma or haemochromatosis

with that of those having the genetically determined disorder. The relationship of the anterior pituitary to the pathogenesis of diabetic small blood vessel disease remains uncertain. This problem has been studied by comparing renal biopsies obtained from diabetic patients before and after pituitary destruction in the treatment of advanced proliferative retinopathy.

Undoubtedly percutaneous renal biopsy has brought a new dimension to the study of renal disease. In trained hands the technique is safe, provided that patients are carefully selected, screened and properly prepared. However the value of data based on small renal biopsy samples is limited unless it can be shown that the tissue obtained is representative of that individual's glomerular population. Moreover reliable quantitative methods must be applied when electron microscopy is used to demonstrate cellular ultrastructure. Thus statistical methods appropriate to small samples and free of assumptions concerning distribution must be applied. Such non-parametric methods (Siegal, 1956) as analysis of variance are ideally suited to the problem of demonstrating whether or nor a small biopsy sample of three or four glomeruli is representative of a patient's glomerular population. The ranking methods of the Mann-Whitney 'U' test or the more powerful Kolmogorov-Smirnov test are well suited to comparisons between clinical groups of patients (Siegal, 1956).

However such non-parametric statistical tests are dependent upon absence of bias in measuring electron micrographs.

Accordingly great care has been taken in designing methods of measurement free of bias. Not only have appropriate methods of measuring glomerular capillary basement membrane thickness been developed, but in order to relate changes in basement membrane structure to alterations in associated cellular cytoplasm a technique of assessing on a quantitative basis, the ratio of basement membrane substance to cellular cytoplasm has also been introduced.

Before proceeding to the details of this study, the appearances of the diabetic kidney on light microscopy (Chapter 2) and electron microscopy (Chapter 3) are reviewed.

CHAPTER 2

The macroscopic and light microscopic features of the kidney in diabetes mellitus.

"Cases are described which show a striking hyaline thickening of the intercapillary connective tissue of the glomerulus. Evidence is presented which indicates that the change is degenerative in nature and suggests that arteriosclerosis and diabetes may play a part in its causation. The lesion is therefore termed intercapillary glomerulosclerosis".

Kimmelstiel and Wilson, 1936.

The gross appearance of the diabetic kidney has been mentioned by Kimmelstiel and Wilson (1936), Allen (1941, 1951), Spühler and Zollinger (1943), Rifkin et al. (1948, 1952) and others. On the whole the appearance is unremarkable though there may be slight enlargement (Rifkin et al. 1952).

On light microscopy examination lesions of the following structures may be found:

1. Arteries of the kidney.
2. Afferent and efferent arterioles.
3. Glomeruli.
4. Tubules.
5. Interstitial tissue.

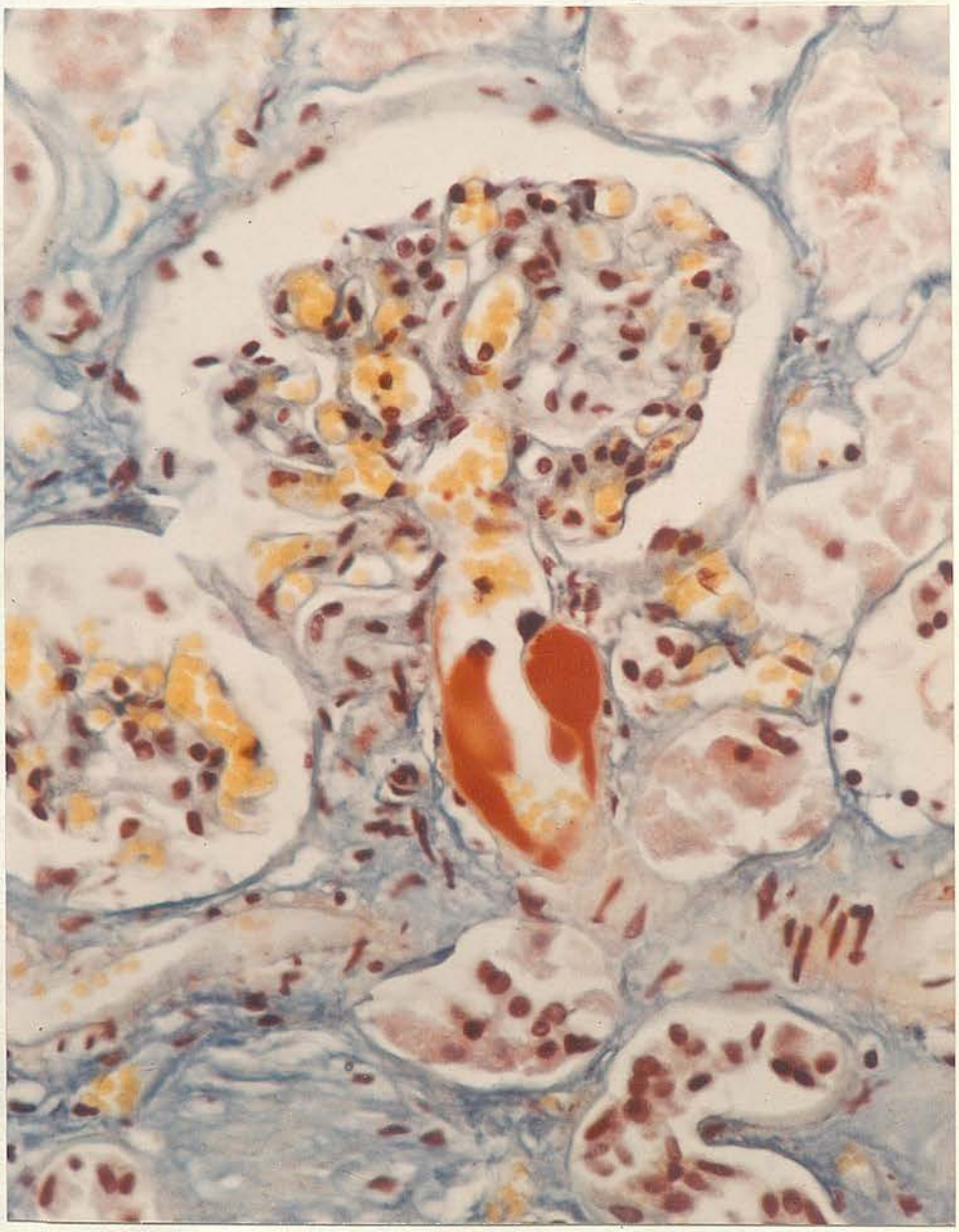
1. Arteries

Although atheroma is said to be more common in diabetes there is no accurate documentation of any increase in its incidence in the larger renal vessels of diabetics in autopsy or other studies of age-matched subjects. On the other hand, diffuse intimal fibrosis in these vessels, which is believed to be almost universal after the age of 50, has been found to be more frequent and advanced in autopsies on diabetics by comparison with non-diabetics (Bell, 1952; Warren et al. 1966).

2. Arterioles

Although mentioned by Kimmelstiel and Wilson (1936) and Allen (1941), it was Bell (1953) who established the importance of hyaline arteriolar lesions in diabetes (Colour plates 1 and 2). He emphasised that there was efferent arteriolar involvement besides afferent arteriolosclerosis in diabetes and showed that although hypertension was common in the 1,465 diabetics examined by him at autopsy, a large percentage without hypertension had these lesions. Indeed efferent arteriolar involvement is sufficiently characteristic of diabetes to be of aid to the light microscopist in concluding that associated glomerular disease is diabetic in origin.

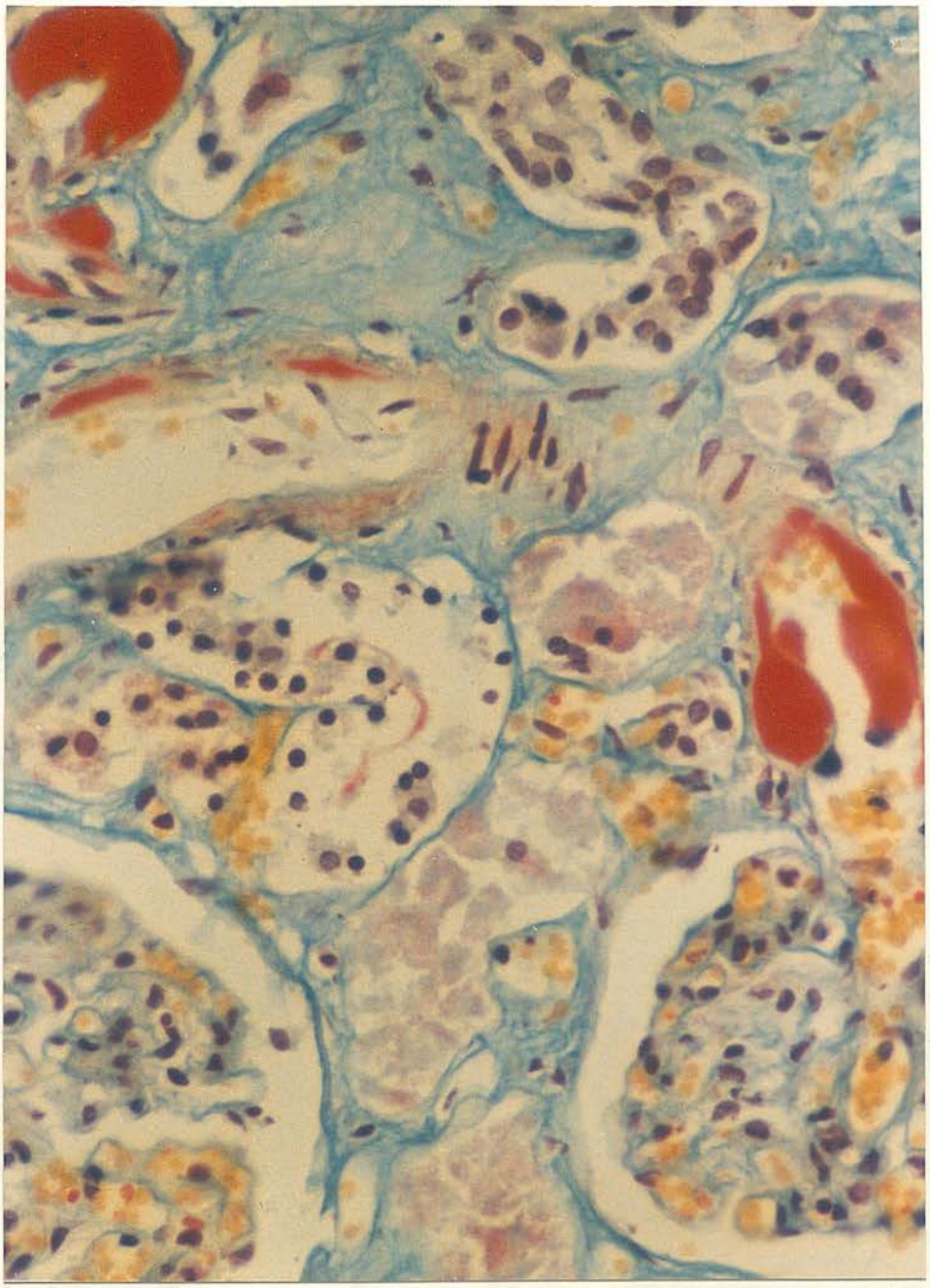
Blumenthal et al. (1962) have described a proliferative arteriolar lesion with excessive deposition of PAS-positive material, involving the endothelial cells and occurring more commonly in the renal arterioles of diabetics than non-diabetics.



Colour plate 1: Scarlet stain in hyaline arteriolar lesion.

Diabetic P.M. Case 36.

M.S.B. stain (appendix 1) X 500 magnification.



Colour plate 2: Scarlet stain in afferent and efferent arteriolar lesion. Diabetic. P.M. Case 36.
M.S.B. stain. X 500.

The same workers have reported similar lesions in many other sites (Blumenthal et al., 1960, 1961, 1963) and have suggested an immunological relationship to exogenous and endogenous insulin; yet this lesion has rarely been noted by other observers (Berkman and Rifkin, 1966). Likewise the suggestion of Lendrum (1963) that both the arteriolar and glomerular lesions of diabetes should be regarded as a form of "plasmatic fibrinosis" has not found general acceptance.

3. Glomerulus

Under the general heading of diabetic glomerulosclerosis the following lesions have been recognised by light microscopy:

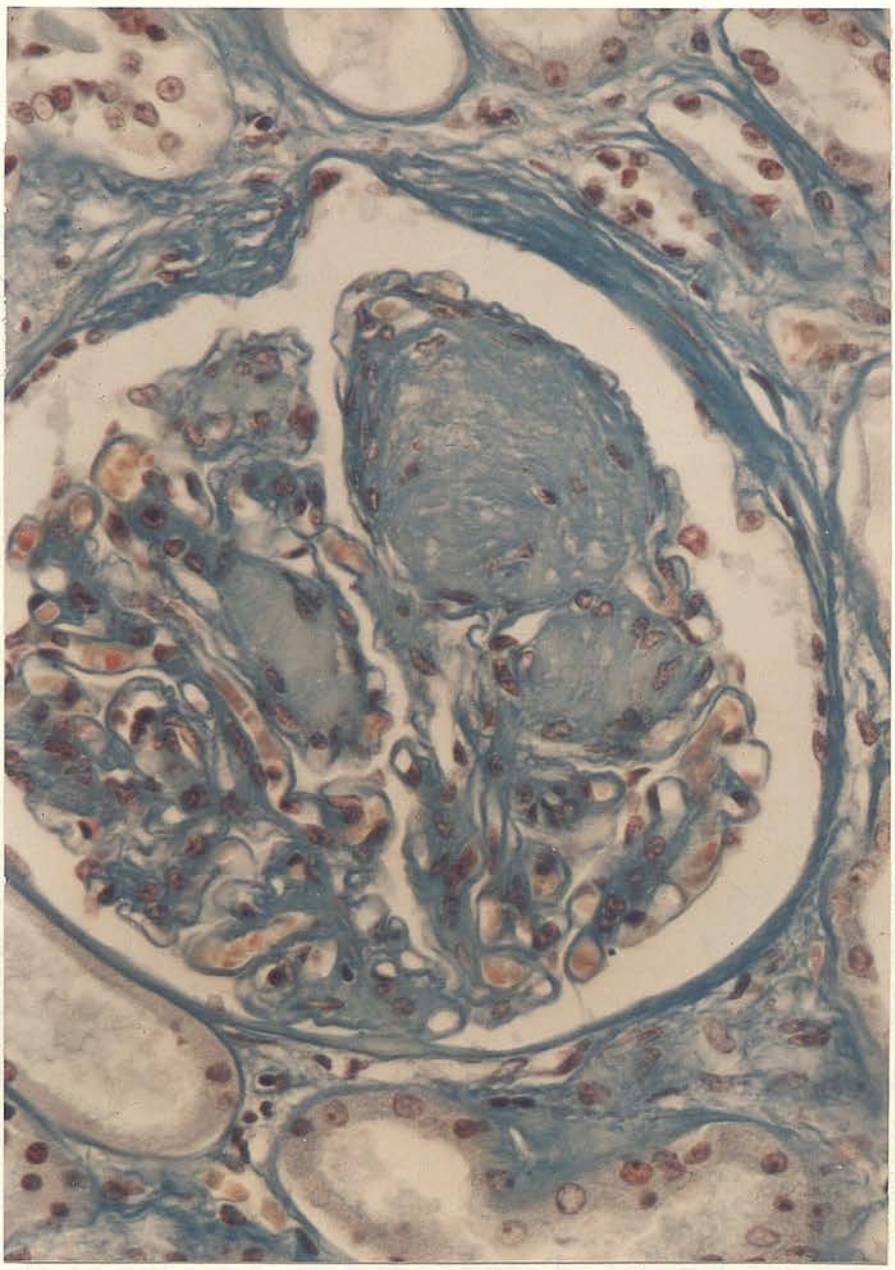
(i) Nodular

(ii) Diffuse

(iii) Exudative lesions.

(i) Nodular glomerular lesions: (Colour plates 3, 4 and 5)

In their original description, Kimmelstiel and Wilson (1936) were struck by the great regularity with which hyalinisation of the glomerulus in diabetes was confined to the centre of one or more lobules. They regarded the hyaline mass as representing a broadening of the intercapillary connective tissue, particularly at the hilum. A high degree of arteriosclerosis with fatty degeneration of the arterioles was present in most cases and the hyaline material was seen to be continuous with the vasa afferentia. They did not, however, regard the intercapillary hyalinization as

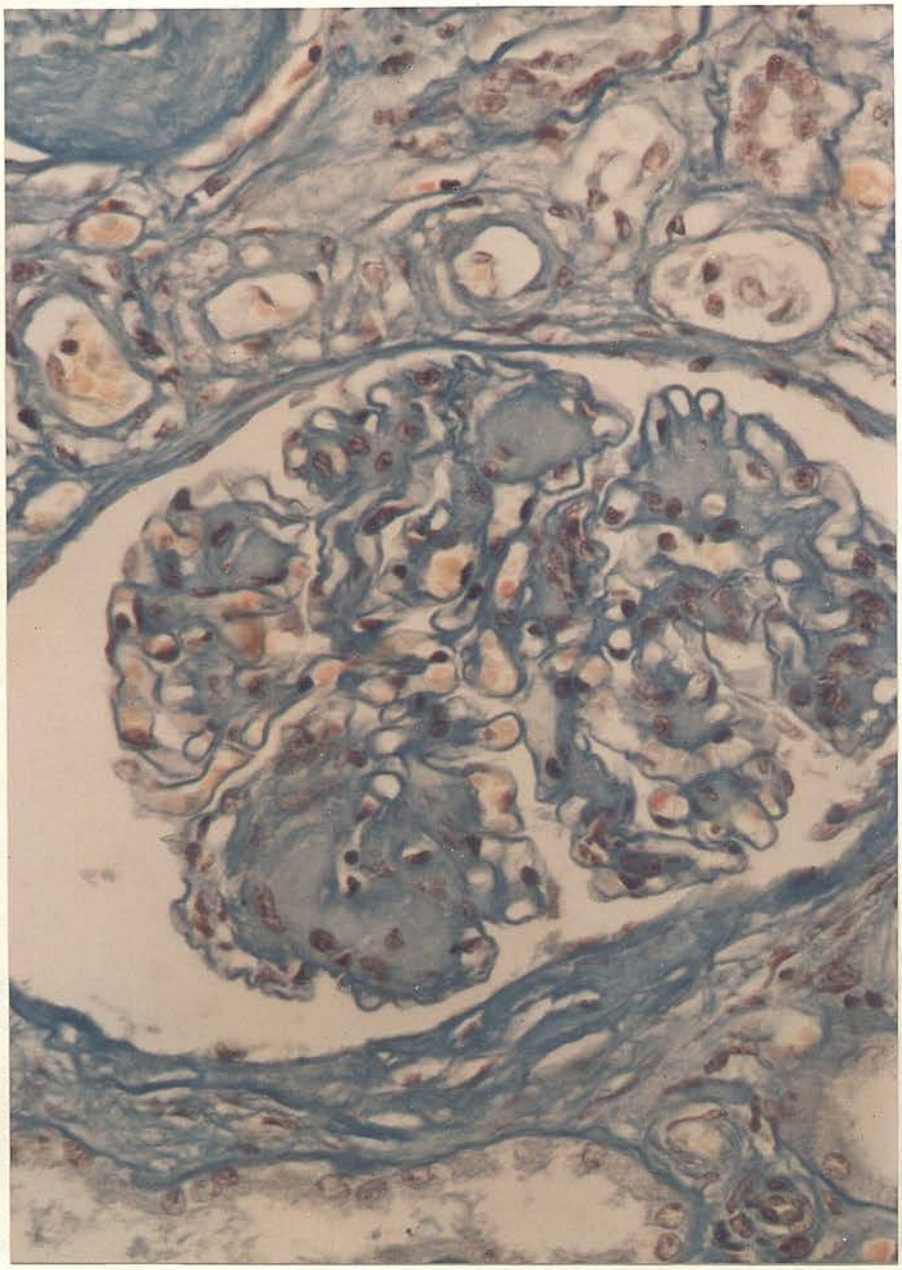


Colour plate 3: Nodular diabetic glomerulosclerosis.
P.M. Case 36. M.S.B. stain. Compare blue laminated nodule with arteriolar lesion, note absence of scarlet stain.
X 500.

an extension of the degenerative process from the vas afferens since it was also found in glomeruli where the vas afferens was normal. Although not infrequently similar to appearances which in non-diabetics they had regarded as an ageing process, the axial thickening was much more striking and massive. They also noted that the endothelial nuclei showed pyknosis and were finally entirely embedded in a homogenous hyaline mass indicative of a regressive process.

Kimmelstiel (1966) has emphasised that in the past confusion has arisen through failure to define the morphology of the nodular lesion. Within an affected glomerulus, which may be normal in size or enlarged, nodules occupy the centres of single or multiple peripheral glomerular lobules (Colour plate 3). The fully developed lesion may be an almost spherical, homogenous, vacuolated, fibrillar or lamellar mass often having a patent or distended capillary running over its surface (Allen, 1941; Farquhar, Hopper and Moon, 1959). The histological lesion most commonly confused with the diabetic nodule is the lobular form of glomerulonephritis. However, the latter can usually be distinguished because the lobules are more uniformly affected, the peripheral capillary is less distinct and the afferent arteriole is not involved as it is in diabetes.

It is now agreed that the nodule is confined to, and pathognomic of, diabetes mellitus (Rifkin, Leiter and Berkman, 1962) and that early reports to the contrary were a consequence of mistaking either lobular nephritis or exudative glomerular lesions



Colour plate 4: Diffuse and nodular diabetic glomerulosclerosis. Note early nodules in peripheral lobules and widespread thickening of capillary walls. P.M. Case 36. M.S.B. stain. X 500.

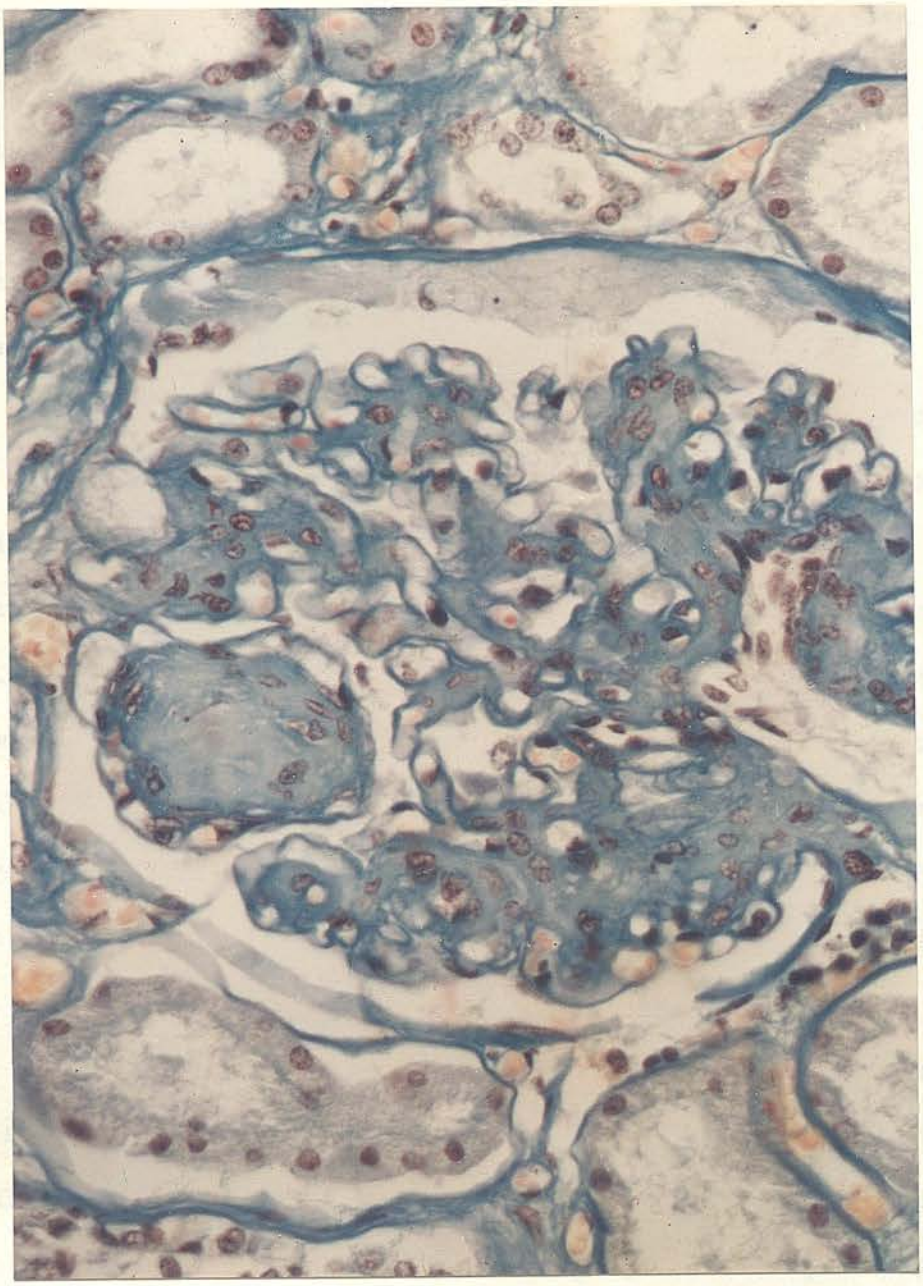
with the nodule, or of a failure to detect clinically mild disturbances of carbohydrate metabolism in so-called non-diabetics.

In considering the ultrastructure (Chapter 3) and pathogenesis of the nodular lesion (Chapter 11) it is worth emphasising its three main features: namely that it is focal, centrolobular and has an acellular centre.

(ii) Diffuse glomerular lesions: (Colour plates 4 and 5).

Although diffuse diabetic glomerulosclerosis was first described by Fahr (1942), and subsequently mentioned by Spühler and Zollinger, (1943), Laipply et al. (1944) and Bell (1953), the lesion received little attention until Gellman et al. (1959), as a result of a careful clinico-pathological study of biopsy material, showed that it was twice as common as the nodular lesion. They found, moreover, that the severity of the features of renal failure and nephrotic syndrome correlated with the extent of diffuse rather than nodular glomerular involvement.

The diffuse diabetic lesion usually begins with thickening of the whole circumference of the wall of peripheral capillaries of the glomerular tuft (Gellman et al. 1959). With increasing severity the lesion becomes diffuse within the glomerulus and generalised in the kidney. Further progression leads to narrowing of the capillary lumina and eventually to complete hyalinization of the glomerulus. Gellman emphasised that nodules occur only in glomeruli involved by the diffuse process; nonetheless he regarded the two lesions as separate entities

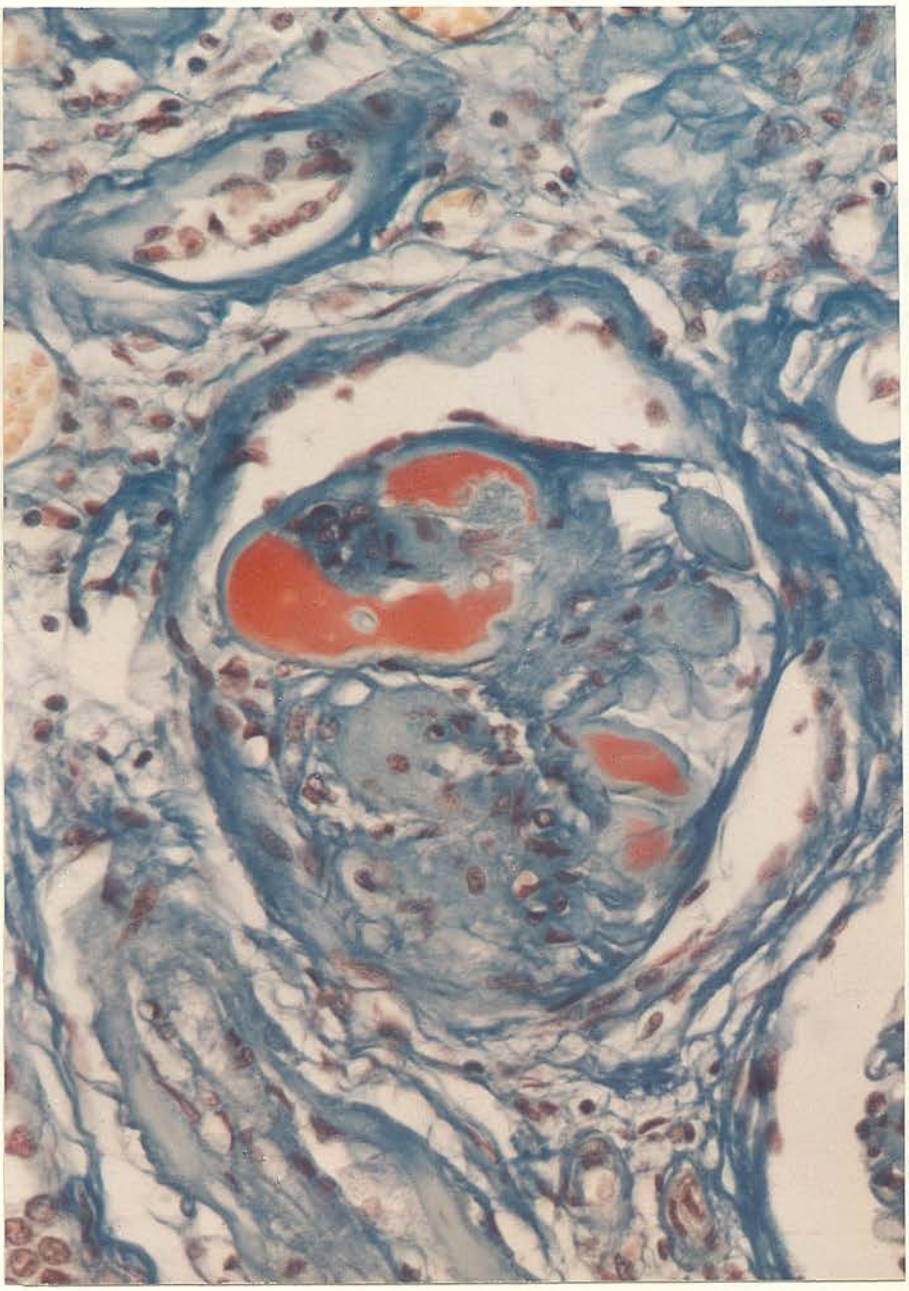


Colour plate 5: Diffuse and nodular diabetic glomerulosclerosis. P.M. Case 36. M.S.B. stain. X 500.

whereas many other observers (Farquhar et al. 1959; Bloodworth, 1963; MacDonald, 1966) believe that the nodule simply represents a more advanced stage of the diffuse lesion. Bell (1953) recognised the separate identity of the diffuse and nodular lesions, but also described an intermediate stage, chiefly affecting the centrolobular region which he termed "diffuse intercapillary glomerulosclerosis".

Often being unable to distinguish between diffuse lesions due to diabetes and such other causes as membranous glomerulonephritis or amyloidosis, the light microscopist has been reluctant to regard the diffuse diabetic lesion as a specific entity. This uncertainty has led many observers to make the paradoxical and confusing statement that the specific diabetic nodule arises in glomeruli involved by, and is a more advanced stage of, the non-specific diffuse lesion. The application of examination by electron microscopy as well as light microscopy has, to a large extent, resolved these difficulties.

Bell (1953) first emphasised the parallel association between the severity of afferent arteriolosclerosis and diffuse and nodular glomerular involvement. This has led some to conclude that the diabetic glomerular lesions might be a consequence of the arteriolar disease. This seems unlikely, however, since arteriolosclerosis in the absence of diabetes usually leads to ischaemic atrophy of the glomerular tuft with thickening of the capillary wall at the hilus and shrinkage of the remaining vessels instead of the diffuse and nodular changes.



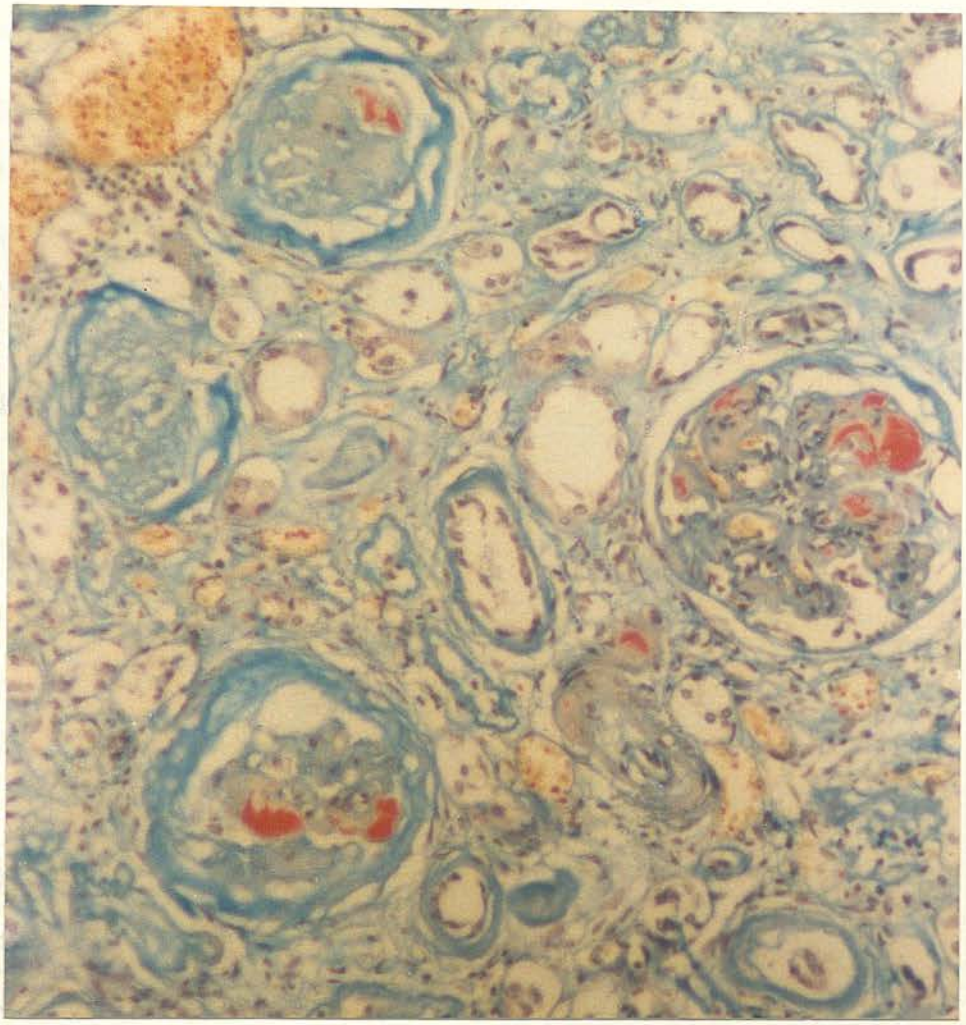
Colour plate 6: Exudative lesion. Note "acellular hyaline" or "fibrin" staining scarlet with M.S.B. stain. P.M. Case 36. X 400.

(iii) Exudative glomerular lesion: (Colour plate 6).

The exudative lesion is the least significant of the three glomerular changes in diabetes. It also occurs in various non-diabetic glomerular disorders associated with renal failure, and in diabetics only in the late stages of nephropathy. Also known as "hyaline-fibrinoid", "acellular hyaline", "fibrin cap" and "capsular drop", the exudative lesion usually consists of rounded and crescentic deposits of either homogenous or vacuolated, intensely acidophilic material without nuclei. It may form a cap over a glomerular capillary loop or may be attached to the inside of Bowman's space, and represents a mixture of various proteins and fibrinoid (Altshuler and Angevine, 1949) which has leaked into the capsular space. Alternatively the material may be within the glomerular capillary lumen.

The lesion represents non-specific glomerular damage and it is only of importance because it has been confused with the Kimmelstiel-Wilson nodule leading to doubts that the latter is pathognomonic of diabetes. Conversely, steroid-induced lesions in the glomeruli of experimental animals which resemble the exudative lesion have been interpreted erroneously as specifically diabetic (Bloodworth and Hamwi, 1956).

As a consequence of the above lesions, an increasing number of glomeruli become completely hyalinized in advanced cases (Colour plate 7). Moreover, in many renal lesions with vascular changes, whether diabetic or not, some glomeruli become ischaemic. In these the glomerular tuft shrinks with fibrous thickening of the inner



Colour plate 7: Glomerular hyalinization, periglomerular fibrosis. Scarlet stain in exudative and arteriolar lesions. P.M. Case 36. M.S.B. stain. X 200.

surface of Bowman's capsule. However some glomeruli contain traces of a fibrin cap and are rarely contracted. In others periglomerular or pericapsular fibrosis, in which there is marked thickening of Bowman's capsule by connective tissue fibrils laid down concentrically round the capsule, may be evident (Colour plate 7). Although originally regarded as evidence of pyelonephritis (Weiss and Parker, 1939) in diabetics the changes may be ischaemic in origin (Thomsen, 1965).

4. Tubules

Although various lesions may be found in the tubules of diabetics, few are of specific significance and in general they are secondary to glomerulosclerosis, ischaemia, pyelonephritis or long-standing electrolyte disturbance.

PAS-positive thickening of both the proximal and distal tubular basement membrane has been reported frequently in diabetics (Allen, 1941; Fahr, 1942; Horst, 1947; Koss, 1952; Kimmelstiel, 1956) leading some to regard this as further evidence of "widespread basement membrane disease in diabetes". However there are no reports of data comparing thickness in diabetic and non-diabetic tubular tissue examined by electron microscopy; furthermore, the PAS-positive changes may also be found in the tubules in the presence of other diseases causing ischaemia or in pyelonephritis.

The "Armanni-Ebstein lesion", or "glycogen nephrosis", first described in 1877, consists of glycogen-laden vacuoles in the tubules of the cortico-medullary region. It was a common

post-mortem finding in the pre-insulin era and is still occasionally found in those who die following uncorrected hyperglycaemia, acidosis and dehydration. Severe electrolyte and circulatory distances in profound diabetic keto-acidosis may also cause acute tubular necrosis (Oliver, MacDowell and Tracy, 1951).

Large, clear, "empty" vacuoles may be found in the proximal convoluted and collecting tubules at biopsy in diabetics having severe potassium depletion (Relman and Schwartz, 1958). Electron microscopy examination shows degeneration of the epithelial cytoplasm, and in diabetics the lesion may be confused easily with the fine hydropic degeneration which accompanies glucose-induced osmotic diuresis (Muehrcke and Rosen, 1964).

5. Interstitial Tissue

Pyelonephritis

Many believe that diabetics are more prone than others to infections in general and pyelonephritis in particular. In autopsies on diabetics, changes interpreted as chronic pyelonephritis were frequently reported to be found in association with diabetic arteriolosclerosis and glomerulosclerosis. Indeed, some observers were sufficiently impressed by the association to conclude that the diabetic lesions might be a consequence of chronic pyelonephritis. However, the more experienced observer now appreciates that most of the changes which resemble healed chronic pyelonephritis in the interstitial tissue and tubules are secondary to ischaemia and the glomerular lesions of diabetes.

Kimmelstiel (1961) was one of the first to doubt the validity, in the presence of ischaemia, of accepting many of the criteria upon which the diagnosis of healed chronic pyelonephritis might otherwise be made. He concluded that pronounced lymphocytic and plasma cell infiltration of the intertubular tissue, periglomerular fibrosis and increased connective tissue probably resulted from ischaemia alone. Heptinstall (1967), in a perceptive review of the limitations of diagnosis in chronic pyelonephritis, found it painfully obvious that many pathological processes can produce a similar end result. He stressed that the most certain evidence in favour of an infective cause of interstitial damage is the presence of associated inflammatory changes in the pelvi-calyceal system.

Nonetheless, there are several factors which may predispose the diabetic to urinary tract infection. These include the effect of diabetic autonomic neuropathy on bladder function favouring stasis, the possibility of catheterisation still being practised in the management of keto-acidosis, and that infection is more likely in renal tissue affected by such vascular lesions as arteriolosclerosis and glomerulosclerosis.

Attempts to assess in diabetics the incidence of acute and chronic pyelonephritis on the basis of quantitative bacteriological examination of the urine, radiology, renal biopsy or other methods have produced conflicting results. Whereas renal biopsy is ideally suited to the assessment of specific diabetic renal involvement which is essentially diffuse, the procedure

may produce misleading results in such a focal disorder as chronic pyelonephritis. Reviewing many studies based on quantitative examination of the urine, Thomsen (1965) concluded that, with the exception of the elderly diabetic female, urinary infection was not more frequent in diabetic patients. Velsgaard (1966) also found a significantly higher incidence of infection in diabetic women but no significant difference between diabetic and non-diabetic men.

Nevertheless, in diabetics having glomerulosclerosis and renal failure, the possibility that the latter is being aggravated by either acute or chronic pyelonephritis (Fig. 1) should always be considered, particularly since infection is more amenable to treatment than the diabetic lesion.

Renal papillary necrosis.

This usually results from acute infection in which the tips of medullary tissue become necrotic and is reported to be more common in diabetics (Whitehouse and Root, 1956; Lagergren and Lindwall, 1958; Zollinger, 1960; Rutner and Smith, 1961; Abdulhayoglu and Marble, 1964). In diabetics the lesion may be exacerbated by ischaemia due to disease of the long thin vasa recta though compression of the submucosal vessels by infective oedema may further compromise papillary blood supply. Radiological examination is of value in making the diagnosis by demonstrating a "moth-eaten" appearance of the calyces as contrast media penetrates necrotic tissue, while the "ring-shadow" corresponding to necrotic

papillae is believed to be pathognomonic. With improved antibiotic therapy the condition is reported in diabetics less frequently, though phenacetin abuse is becoming increasingly recognised as an alternative cause in non-diabetics (Lindeneg, 1958; Harvald, 1963).

Finally it should not be overlooked that diabetics are by no means immune to renal tuberculosis or any other non-diabetic renal disease.

CHAPTER 3

Electron microscopy of the normal and diabetic glomerulus.

"A jungle of glomerular raspberries".

R.E. Fishbein. JAMA 1969.

The application of electron microscopy has led to many recent advances in our knowledge of the normal anatomy of the glomerulus and of its appearance in diabetes. Accordingly the structure of the glomerular capillaries will be reviewed with particular reference to those details relevant to diabetes.

Normal anatomy: The afferent arteriole divides into several branches to form the glomerular capillaries which are grouped into lobules (Fig. 2). Thus two or more capillaries become arranged about a centrolobular region known also as the axial zone, the mesangium or the intercapillary space. The central zone lies closest to the origin of the capillaries from the afferent arteriole. The peripheral capillary loops branch out from the central zone to face Bowman's space. The epithelium which lines Bowman's capsule is reflected over the capillaries of the glomerular tuft and attached by a system of interdigitating foot processes (Plates 1 and 4). The epithelial cytoplasm contains vesicles concerned with filtration (Farquhar, Wissig and Palade, 1961) and well developed rough-surfaced endoplasmic reticulum (Plate 2) suggesting that protein synthesis is an important function.

Although PAS (period acid-Schiff reagent) had been used to demonstrate that the epithelium and endothelium of the capillary walls were separated by a clear glycoprotein basement membrane

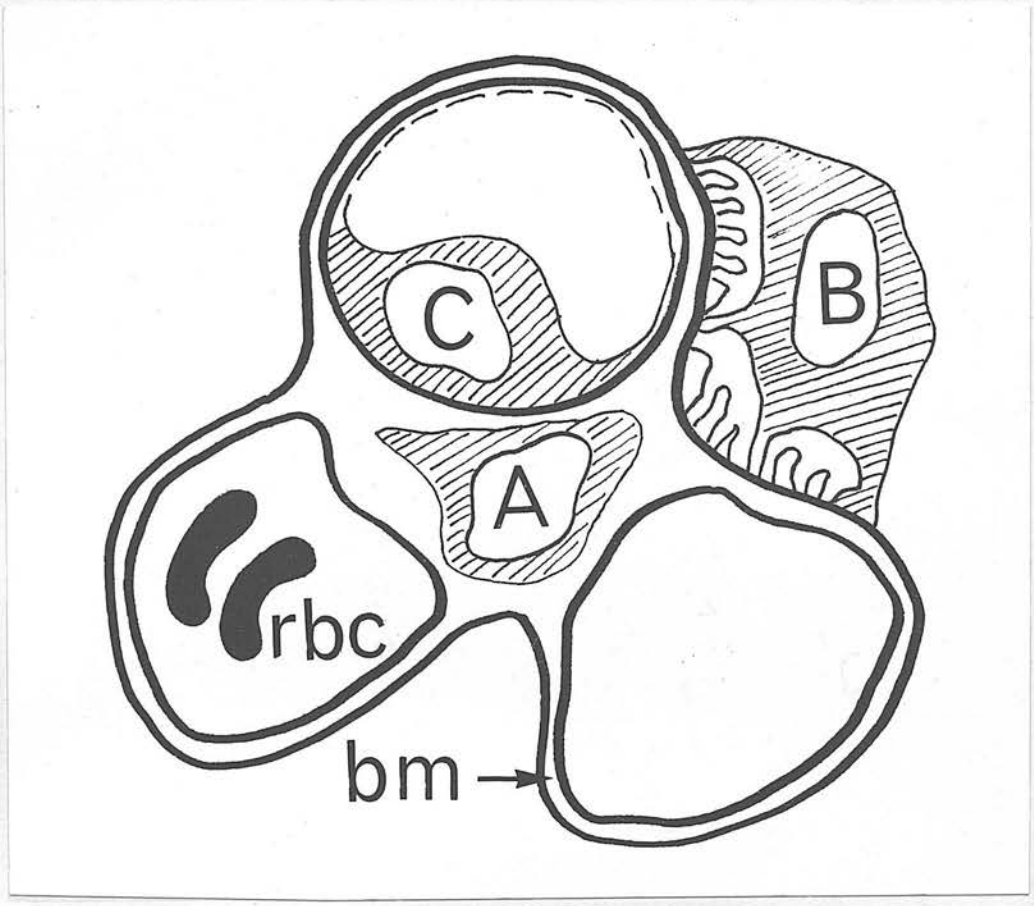


Fig. 2: Diagram of glomerular capillary lobule.
A Mesangial cell in centrolobular region. B. Epithelial cell in Bowman's space. C. Endothelial cell within capillary. rbc Red blood cells within capillary lumen. bm Continuous basement membrane of capillary wall.

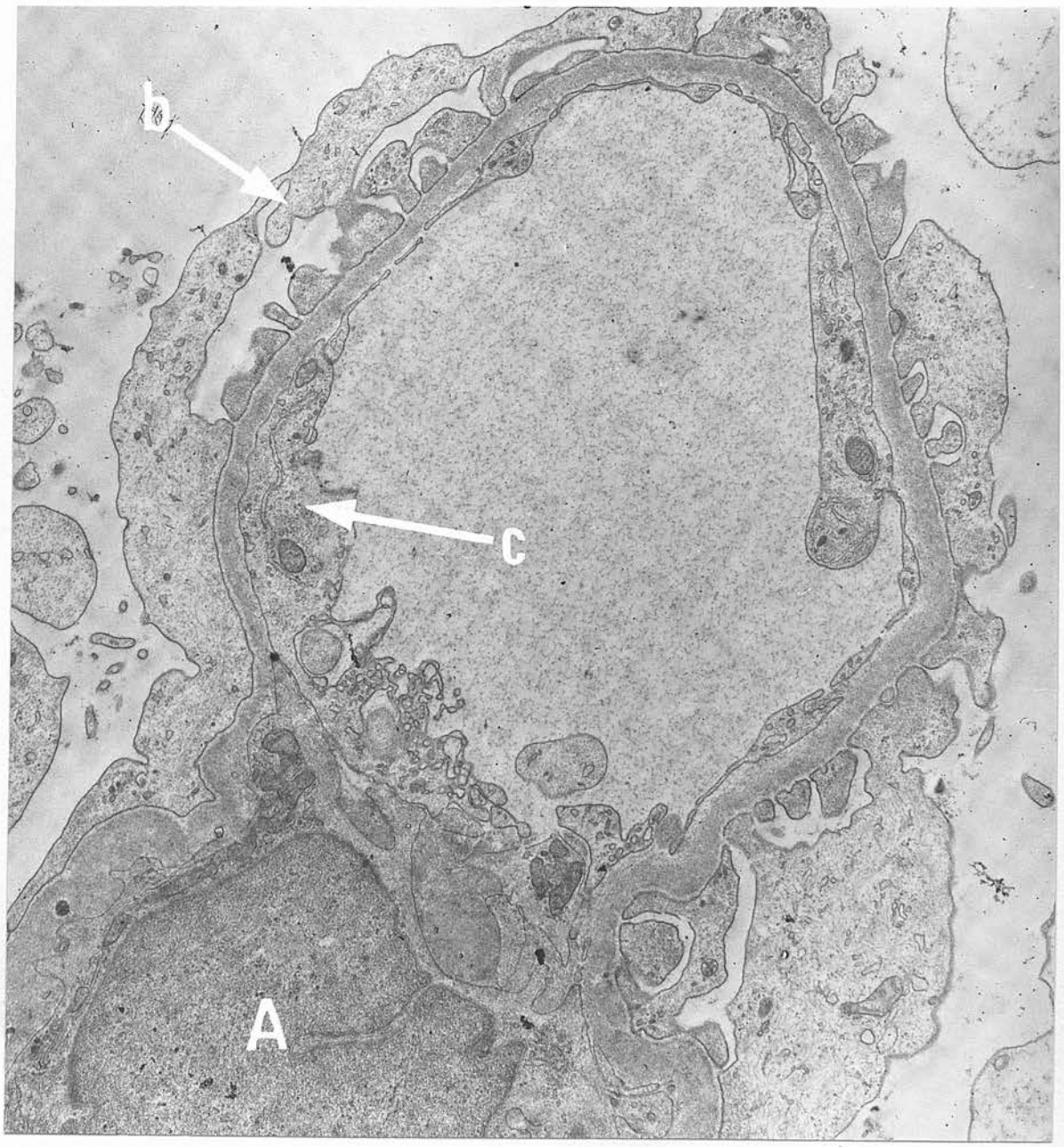


Plate 1: Glomerular capillary loop from normal subject. Axial or mesangial zone A containing mesangial cell. Epithelium (b) attached to basement membrane by system of foot processes. Endothelium (c). Case 8. X 16,000.

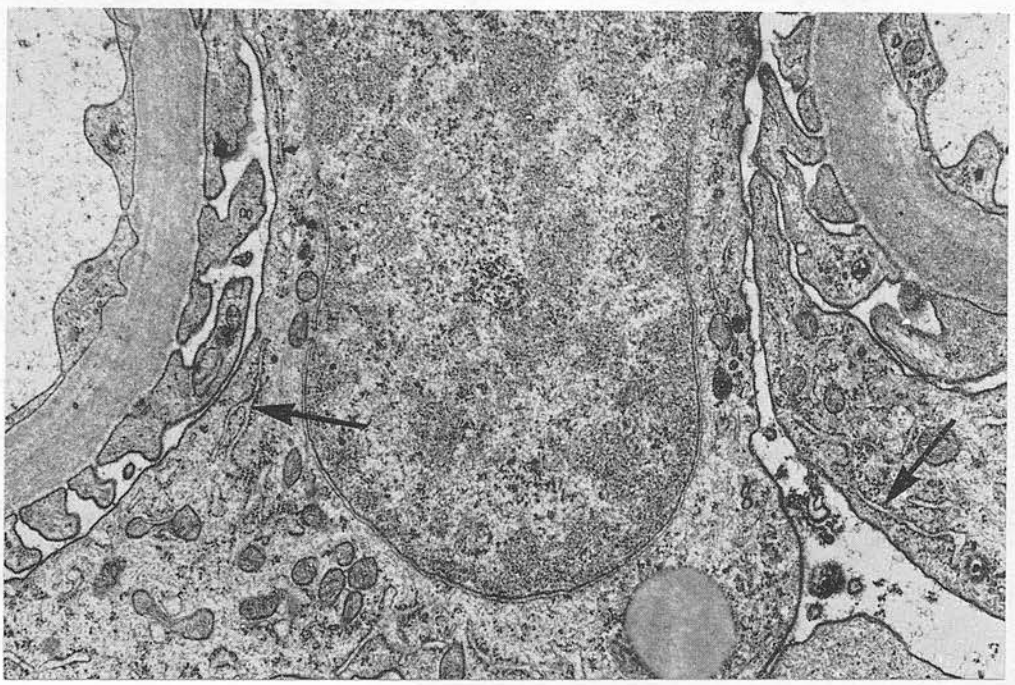


Plate 2: Peripheral glomerular capillaries from patient having diabetes secondary to chronic pancreatitis (Case 10). Thickened capillary walls (right and left) separated by epithelial cell having prominent mitochondria and RNA-studded endoplasmic reticulum (arrowed). X 20,000.

(McManus, 1950), the uniform and homogenous nature of the basement membrane was only revealed by electron microscopy (Plates 1, 2 and 3). The endothelial nuclei usually lie in the proximity of the central, axial or mesangial zones which contain a third type of cell known, because of their situation, as axial, mesangial or deep endothelial cells. Although frequently disputed in the past, the evidence of Farquhar and Palade (1962) not only confirmed the separate identity of these cells but suggested for them an important function in phagocytosis of filtration residues, plasma proteins, lipid droplets and antigen-antibody complexes. The mesangial cells are separated from the endothelial cells by loose-textured basement membrane and can be identified by their darker nuclei (Plate 3). Moreover, on account of their situation they have been likened to the pericytes in other capillaries. However, even in the normal glomerulus, the capillaries are extremely tortuous, thus the three-dimensional distribution of the cells in the axial or mesangial zone is difficult to visualise on electron microscopy of sectioned material.

In contrast, the peripheral capillary loops have only three distinct components: the endothelium, the basement membrane and the epithelium. The basement membrane is the only continuous layer. The endothelium is composed of the attenuated periphery of the endothelial cells and is perforated by large pores or

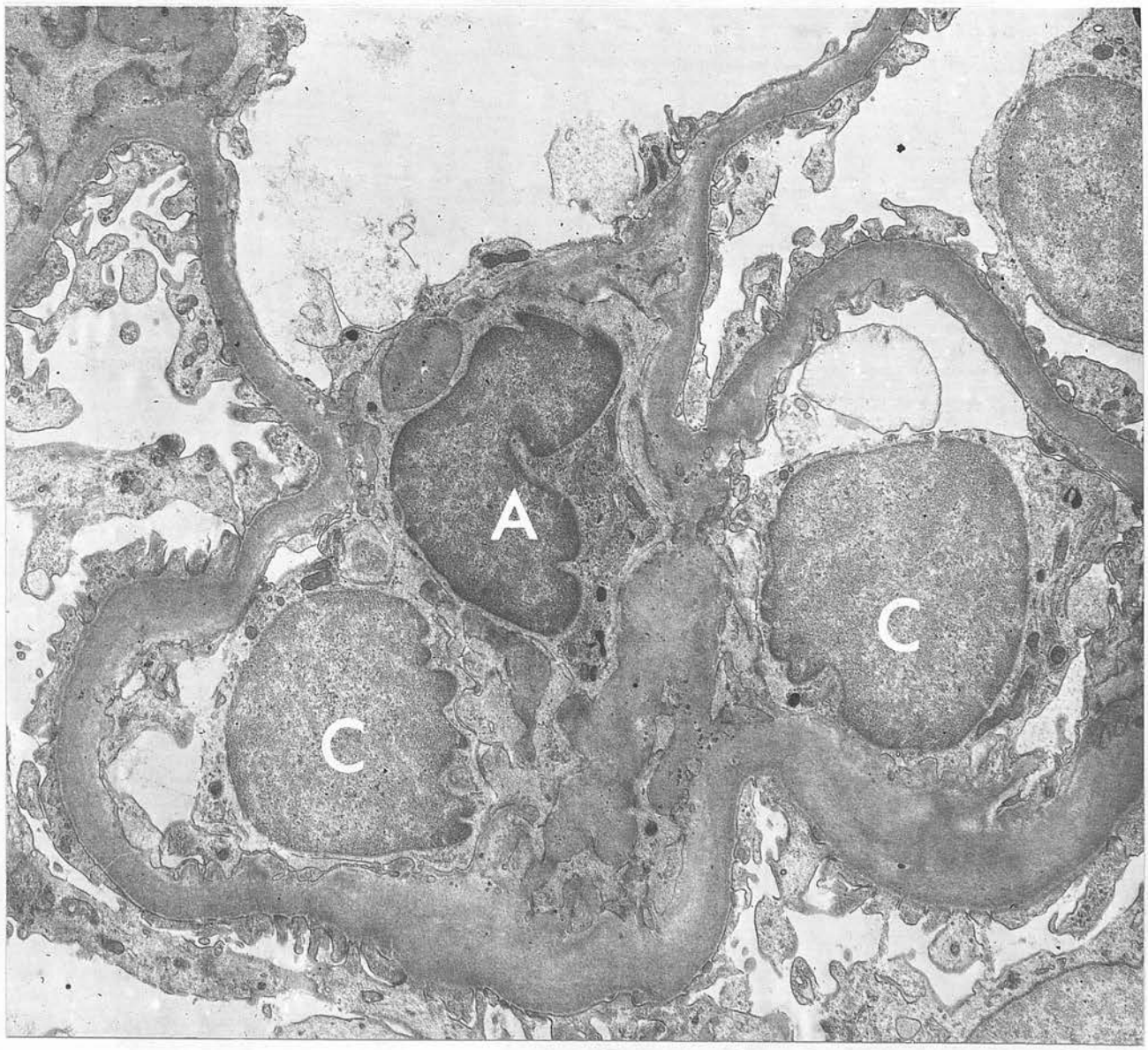


Plate 3: Glomerular capillary lobule from long-standing diabetic without retinopathy. Mesangial cell (A) separated by loose-textured basement membrane substance from endothelial cells (C). Diffuse basement membrane thickening. Case 42. X 10,000.

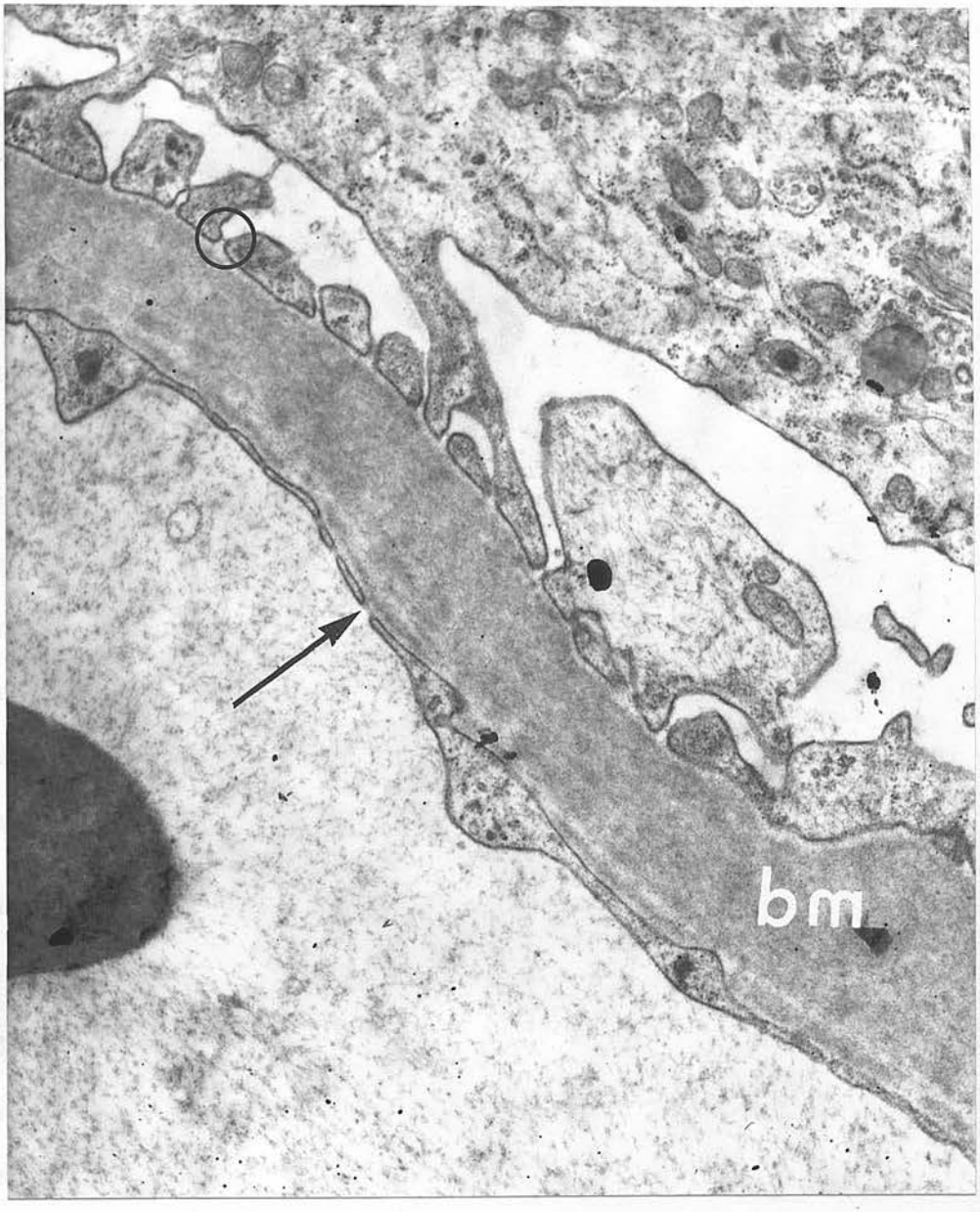


Plate 4: Detail of capillary wall. Basement membrane (bm) separating endothelium perforated by pores (arrowed) from epithelial foot processes. Note slit membrane between foot processes (circled). Diabetic. Case 42.

X 35,000.

'fenestrae' measuring 500-1000 Å (50-100 nm) in diameter (Plate 4). The epithelium lies over the free surface of the capillaries and is attached to the basement membrane by foot processes which are usually separated by a gap of about 250 A (25 nm) bridged by a thin line: the slit membrane (Plate 4).

Relevant to their special function, the glomerular capillaries differ in several significant aspects from those elsewhere in the body. Moreover, the unique features of the epithelium and endothelium of the peripheral loops are of advantage to the electron microscopist in assessing basement membrane thickness. Thus, where either the epithelial foot processes are sectioned longitudinally or where the pores in the endothelial cytoplasm occur as spaces, the examiner can be confident of having obtained a true cross-section of the capillary basement membrane (Plate 4).

The glomerulus in diabetes.

Early electron microscopy studies of renal biopsy tissue obtained from diabetic patients having clinical evidence of nephropathy, confirmed that diffuse glomerulosclerosis was due to marked thickening of the glomerular capillary basement membrane (Farquhar, Hopper and Moon, 1959; Bergstrand and Bucht, 1959). The peripheral capillary loops were universally, though not uniformly, affected, while the axial or mesangial zones contained large accumulations of basement membrane material (Plates 5, 6 and 7).

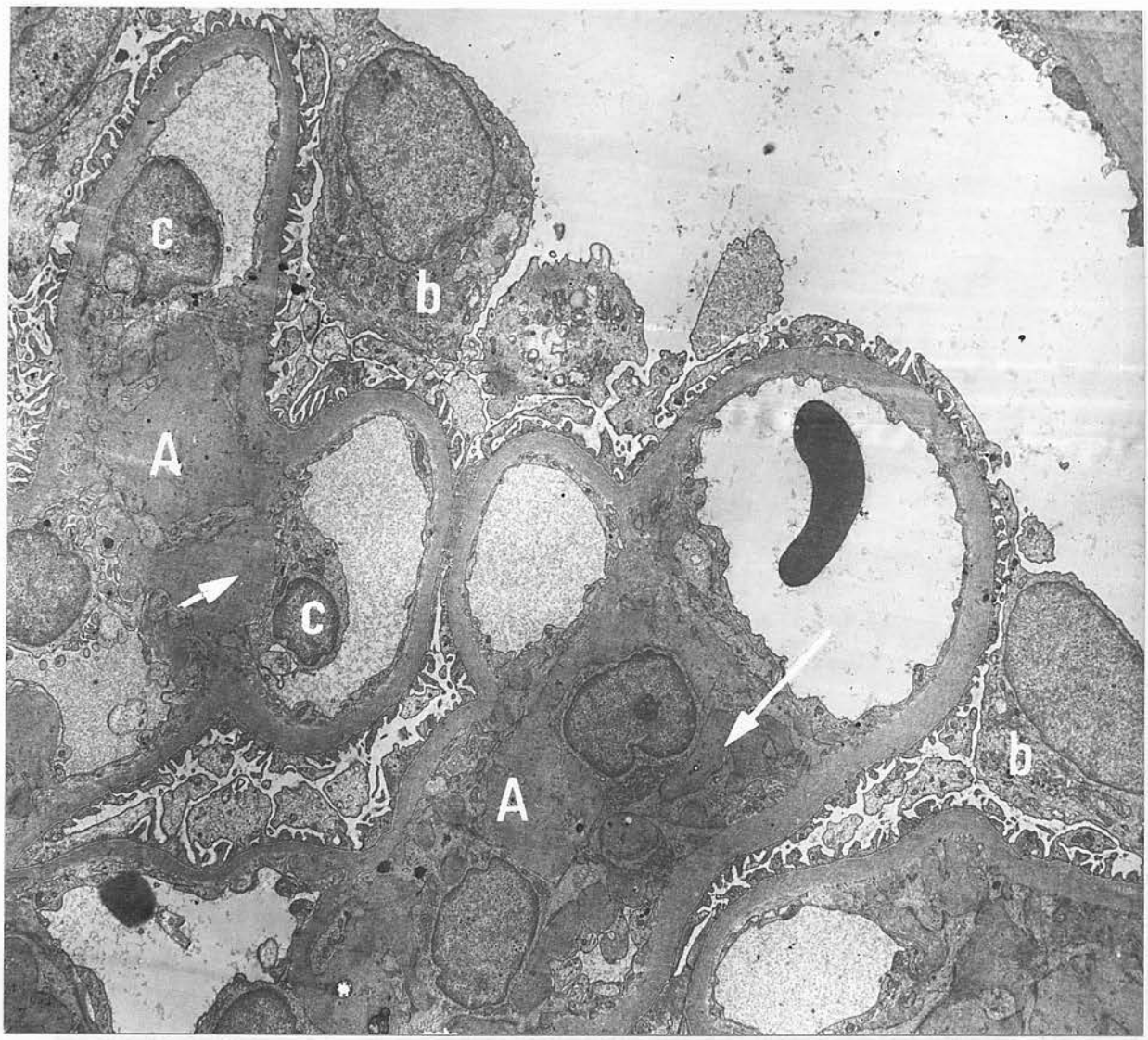


Plate 5: Diffuse thickening of basement membrane of glomerular capillaries from idiopathic diabetic with retinopathy. Mesangial regions (A) having excess basement membrane (arrowed) Epithelial cytoplasm (b) Endothelium (C). Case 37. X 5,000.

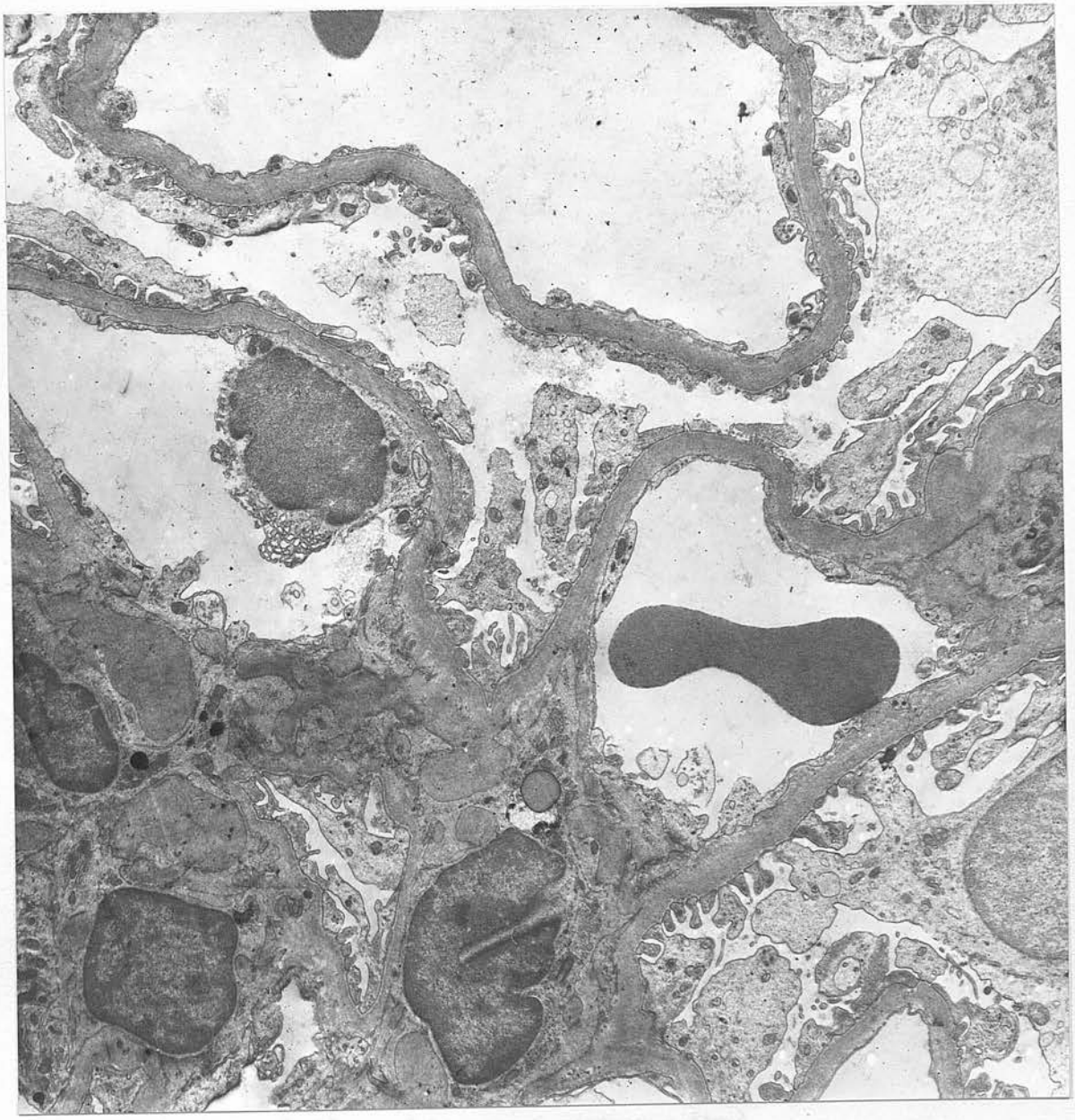


Plate 6: Diffuse thickening of glomerular capillaries. Idiopathic diabetic without retinopathy. Note R.B.C. in capillary lumen. Case 42. X 6,000.

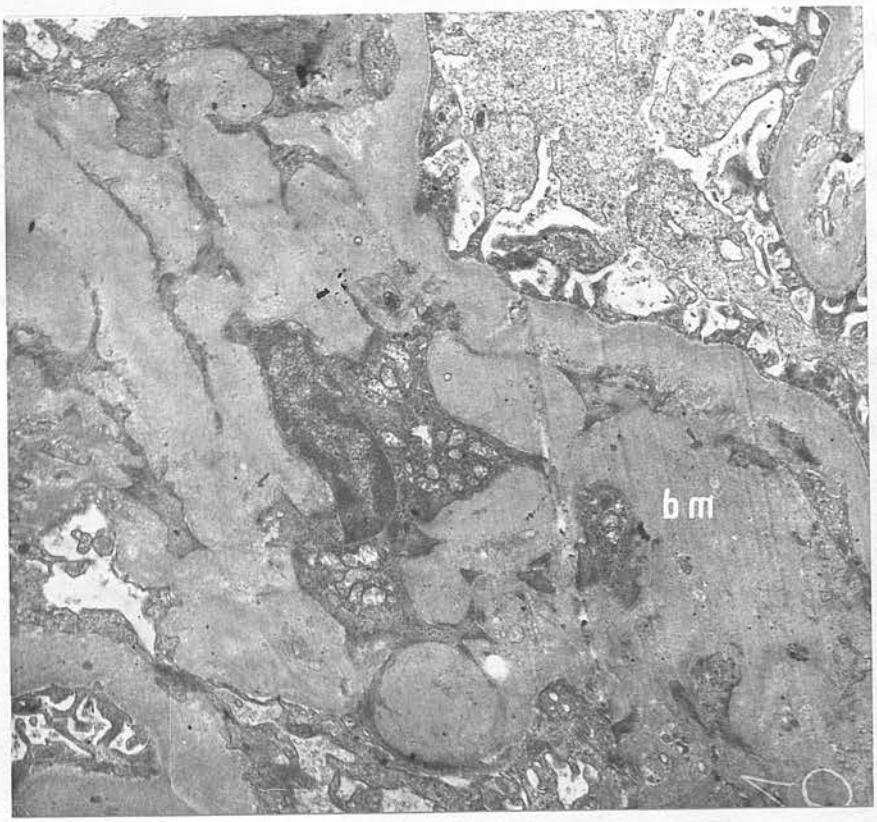


Plate 7: Accumulation of basement membrane (bm) within mesangium. Case 39. X 8,000.

Many subsequent reports confirmed these observations (Bloodworth, 1963; Dachs, Churg, Mautner and Grishman, 1964; Lannigan and Blainey 1964; MacDonald, 1966), and showed also that there was a generalised increase in quantity of basement membrane leading to folding and fusion of capillary loops, massive axial enlargement and capillary obliteration. Thus it appeared to most observers that the nodular lesion of light microscopy terminology represented focal, centrolobular exaggeration of diffuse basement membrane thickening. Yet Kimmelstiel (1966) strongly opposed this view by arguing that the nodule may arise from the centrolobular (intercapillary) zone without diffuse capillary thickening being present.

It is probable that the pathogenesis of diabetic glomerular capillary disease is more complex than either of these opposing views. This problem is taken up in detail in Chapter 11.

CHAPTER 4.

Materials and Methods.

"Statistically analysed data, commonly accepted in other areas of medical research, have not been adequately applied in previous reports on diabetic glomerulosclerosis".

Paul Kimmelstiel, M.D.

Editorial, "Diabetes". 1966.

This section deals with the following aspects:

1. Clinical material.
2. Percutaneous renal biopsy.
3. Light and electron microscopy methods.
4. Methods of measuring electron micrographs.
5. Statistical procedures.

1. Clinical Material.

The patients included in this study were selected, except where otherwise indicated, either from those attending the Diabetic & Dietetic Out-Patient Department, the Royal Infirmary, Edinburgh and associated peripheral diabetic clinics in Galashiels, Haddington, Dunfermline and Kirkcaldy or the Diabetic Clinic, Southern General Hospital, Glasgow. This provided a large clinical pool of more than 4,000 well-documented cases of diabetes in the Edinburgh area and a further 1,000 patients attending the Southern General Hospital, from which to select particular clinical groups for study. Despite the numbers available it often proved difficult to find sufficient patients fulfilling the criteria of the highly selected groups described in subsequent

chapters.

Specific data concerning the patients studied are detailed in each section. However, certain investigations, common to all patients are detailed here. Before contemplating renal biopsy each patient suitable for study was examined and investigated to exclude the possibility of:

1) Any bleeding diathesis.
2) Hypertension.
3) Myocardial ischaemia or infarction.
4) Evidence of non-diabetic renal disease.

Thus the following investigations were routinely carried out:

i) Clinical examination including cardiovascular system, B.P., peripheral pulses and optic fundi.
ii) Haemoglobin, blood indices, white cell count, platelet count, E.S.R., prothrombin activity, blood group.
iii) If the prothrombin activity exceeded that of the control by more than 2 seconds, or if the platelet count was less than 100,000 per cu.mm., the bleeding and clotting times (Lee and White) were estimated (Biggs and MacFarlane, 1966).
iv) 12-lead electrocardiograph.
v) Chest X-ray.
vi) Intravenous pyelogram.
vii) Blood urea and electrolytes.
viii) 5-hour urea or 24-hour creatinine clearance.
ix) Urinary protein.
x) Microscopy and culture of 2 mid-stream urine samples.

2. Percutaneous Renal Biopsy.

Since the reports of Perez (1950) and Iversen and Brun (1951) percutaneous biopsy of the kidney has been regarded as a safe procedure provided that patients are carefully selected, properly prepared and that the operator is skilled and experienced in the technique.

Because the full consent and co-operation of the patient is essential, the nature and purpose of the procedure was explained to the patient and any who declined or who were of an unduly nervous disposition were excluded. In this context, however, diabetic patients were remarkably willing to co-operate. Probably as a consequence of the chronic nature of the disorder, such patients are well accustomed to doctors and hospitals; moreover, insulin-dependent patients, having mastered the technique of self-injection, usually are unafraid of needles.

Haemorrhage is the outstanding risk in percutaneous renal biopsy. Thus elimination of factors which might precipitate or accentuate haemorrhage is the essence of preparatory screening (vide supra). All patients were admitted to hospital for biopsy and 2 pints of blood were routinely held in reserve.

Authors technique (based on standard practice)

The technical problem of locating the lower pole of the kidney and obtaining a biopsy from the relatively narrow cortex was dealt with as follows:

i) The lower pole of the right (or left if more accessible) kidney was identified on an intravenous pyelogram film and a cross marked on the X-ray plate (Fig. 3). The distance from point 'X' to the lumbar spinous processes, the iliac crest and the 12th rib were measured (1, 2 and 3 on Fig. 3).

ii) The details of the procedure were explained to the patient who, recently having emptied the bladder, lay prone on his or her bed.

iii) In order to compress the kidney upon the lumbar muscles, a cardiac-arrest board covered by a rolled woolen blanket was placed between the patient and the top sheet so that the blanket compressed the abdomen in the transpyloric plane.

iv) The lumbar spinous processes, the right 12th rib and the iliac crest were identified by palpation and point 'X' in the lower pole of the kidney mapped on the skin surface with skin pencil by reference to the measurements obtained from the X-ray plate.

v) Thereafter with full sterile precautions the marked area of skin was infiltrated with 2% plain Lignocaine local anaesthetic.

vi) Using a 6 inch long 22 gauge exploring needle, the kidney was penetrated with the patient holding their breath in full inspiration.

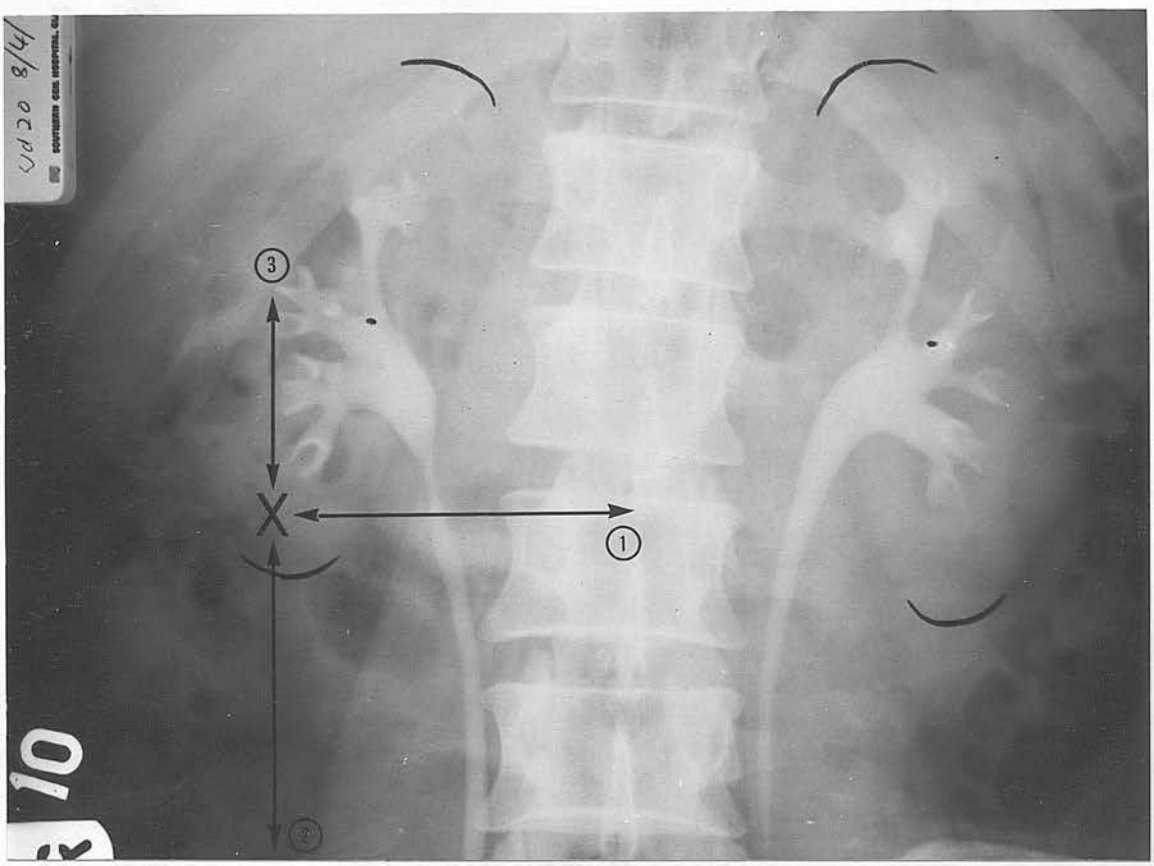


Fig. 3: X-Ray of intravenous pyelogram. Point X in cortex of lower pole of right kidney. 1, 2 and 3: measurements from X to lumbar spinous processes, iliac crest and 12th right rib respectively.

vii) The patient was then asked to breathe in and out, since the swing of the probing needle in the long axis of the body during respiration is good evidence of renal penetration, On the other hand, a sharp flick of the needle, synchronous with arterial pulsation suggests that the needle is immediately adjacent to, but not within, the kidney.

viii) Having located the kidney, the needle was withdrawn to the renal capsule, the depth of the needle noted, and while infiltrating its course with local anaesthetic, the probing needle was withdrawn slowly.

ix) Using a scalpel a 5 mm. bistoury incision was made in the skin wheal.

x) A core of renal cortex was obtained with the Franklin modified Vim-Silverman needle (Plates 8 and 9) by carrying out the following manoeuvres deliberately and quickly while the patient again held their breath in full inspiration: the needle was inserted into the renal cortex, the stylet removed, the Franklin cutting prongs inserted to their full depth, with the cutting prongs fixed the outer needle sheath was pushed down to the tips of the prongs to cut the core of tissue free from the kidney, the needle was then removed from the patient's back.

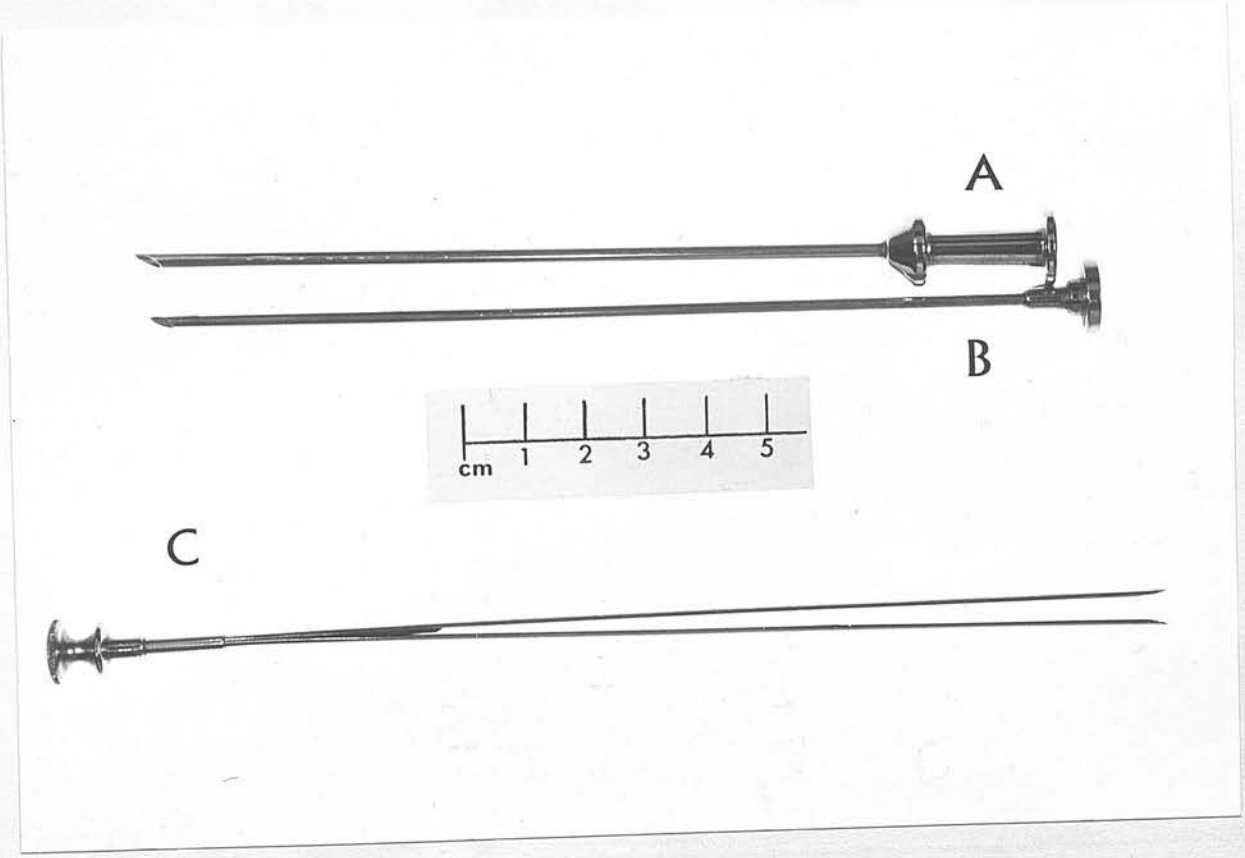


Plate 8: Franklin-modified Vim-Silverman biopsy needle. A: Outer needle sheath. B: Stylet. C: Franklin cutting prongs.

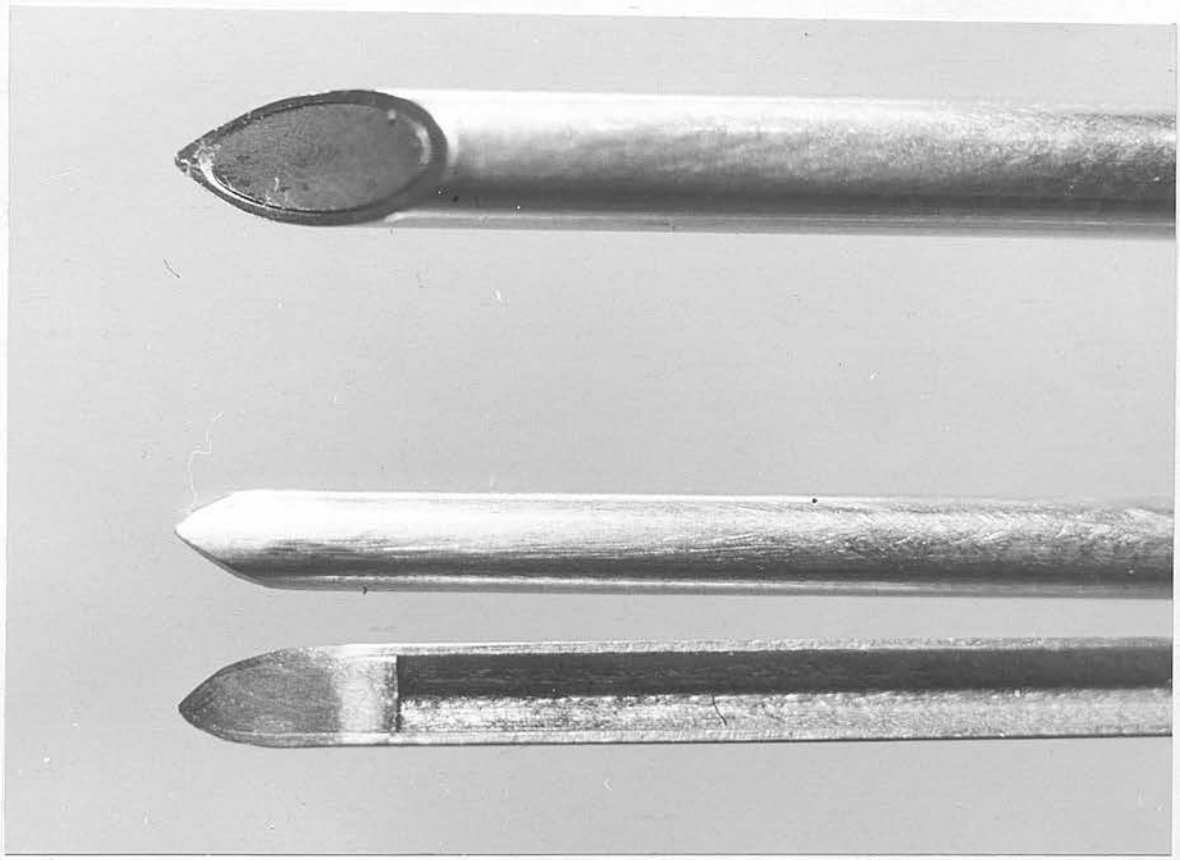


Plate 9: Detail of Franklin-modified Vim-Silverman needle. Above, bevelled blades of needle and stylet. Below, cutting prongs showing guttering in which renal biopsy is held.

xi) The core of renal cortex was removed from the needle and on a sterile watch-glass approximately 2-3 mm. cut cleanly from each end of the biopsy and placed in Osmium tetroxide fixative for electron microscopy examination and the remaining centre portion placed in corosive formal fixative for light microscopy. The time was noted.

xii) Procedure(x)was repeated to obtain a second core of renal tissue.

xiii) Pressure was applied to skin incision, a cotton swab was then fixed with elastoplast bandage.

xiv) With the patient remaining as still as possible the cardiac-arrest board and blanket were removed, and the patient turned onto their back.

xv) The nursing staff maintained a $\frac{1}{2}$ hourly pulse and hourly B.P. record for the subsequent 24 hours with advice to call the resident house physician if there was significant tachycardia or fall in B.P.

xvi) The patient was seen and examined after 24 hours and allowed home if there was no evidence of discomfort or other untoward features.

3. Light and Electron Microscopy Methods

Tissue for light microscopy examination was fixed in primary or secondary formal-sublimate. Thin paraffin sections (5 micron) were routinely stained with haematoxylin and eosin

(H & E) and Periodic-acid-Schiff reagent (P.A.S.). The colour plates reproduced in Chapter 2 and elsewhere were stained by the Martius, Scarlet Blue (M.S.B.) method of Lendrum et al., (1962), which is detailed in Appendix 1.

Tissue for electron microscopy was cut into approximately 1 mm. cubes, fixed in 2% Osmium tetroxide in Sörensen buffer (Appendix II) and embedded in ARALDITE, Ciba Ltd., (Appendix II).

Araldite embedded cubes of renal cortex were sectioned in the early stage of the study on a Sorvall MTI ultramicrotome and latterly using an automatic Sorvall MT2 ultramicrotome and steriomicroscope (Appendix III). ½-1 micron sections were cut, stained with 1% Toluidine Blue in 1% borax and examined under phase-contrast microscopy. This procedure was repeated until a glomerulus was identified. The same glomerulus was then marked on the face of the Araldite block and a pyramid trimmed so that the glomerulus alone occupied the cutting face (Appendix III, pages 2 and 3, photographs 1, 2 and 3 (circled)).

Using a glass knife freshly prepared from high quality 6-7 mm. glass strips cut in an L.K.B. 7800B knifemaker, ultra thin sections from the Araldite embedded glomerulus were prepared in ribbons (Appendix III, page 3). The sections were then stained with uranyl acetate (Appendix II) and treated with lead citrate contrast enhancement agent (Appendix II) before being transferred to copper grids  . Thereafter the sections on the copper grids were examined and photographed (vide infra) using an A.E.I.

(Metropolitan Vickers) E.M.6. electron microscope.

The above procedures have been dealt with in summary. However, no amount of elaboration would alter the fact that they are completed successfully only when the individual concerned has had considerable practice and maintains meticulous attention to detail.

4. Methods of measuring electron micrographs

Since reliable quantitative methods are an essential pre-requisite of data based on small biopsy samples and electron microscopy, close attention has been given to this aspect.

i) Peripheral glomerular capillary basement membrane thickness.

Whereas the electron microscopist has an outstanding advantage in measuring glomerular, as opposed to other capillaries, bias must be avoided in selecting measuring points. As indicated in Chapter 3, the peculiar anatomy of the peripheral capillary loop of the glomerulus permits accurate assessment of true cross-sectioning, i.e. where either the epithelial foot processes are sectioned longitudinally or the pores in the endothelial cytoplasm occur as spaces. However this advantage is lost in the mesangial zone, moreover in both normal and diabetic tissue there is a tendency for the basement membrane to become thicker in the proximity of the mesangial zone. To eliminate bias in selecting the junction between the mesangium and the peripheral parts of the capillary loop, cross-section measurements were made only in

the peripheral half of the capillary loop (Fig. 4). A magnifying micrometer was placed at right angles to the plane of the basement membrane at regular intervals (as represented by lines aa, bb and cc in Fig. 4). The thickness of the basement membrane was measured between the bases of the epithelial foot processes and the endothelial membrane. Moreover, the areas of glomeruli examined were unselected apart from suitability for photographic reproduction. Thus on viewing with the electron microscope, areas of glomerular tufts were examined and photographed at magnifications of 1,000 to 2,500 times (Plate 10) and when peripheral loops were identified they were photographed at higher magnifications of 6,000 to 16,000 times. The latter were used for measuring basement membrane thickness because electron microscopy magnifications could be more accurately calibrated in this range and the ultrastructural details more easily identified. Approximately 10 measurements were obtained from each capillary loop. By examining 10 loops from each of three glomeruli in a biopsy sample, approximately 300 measurements were available in each biopsy.

The large number of photographs necessary to obtain such measurements provided an opportunity to study cellular morphology in detail.

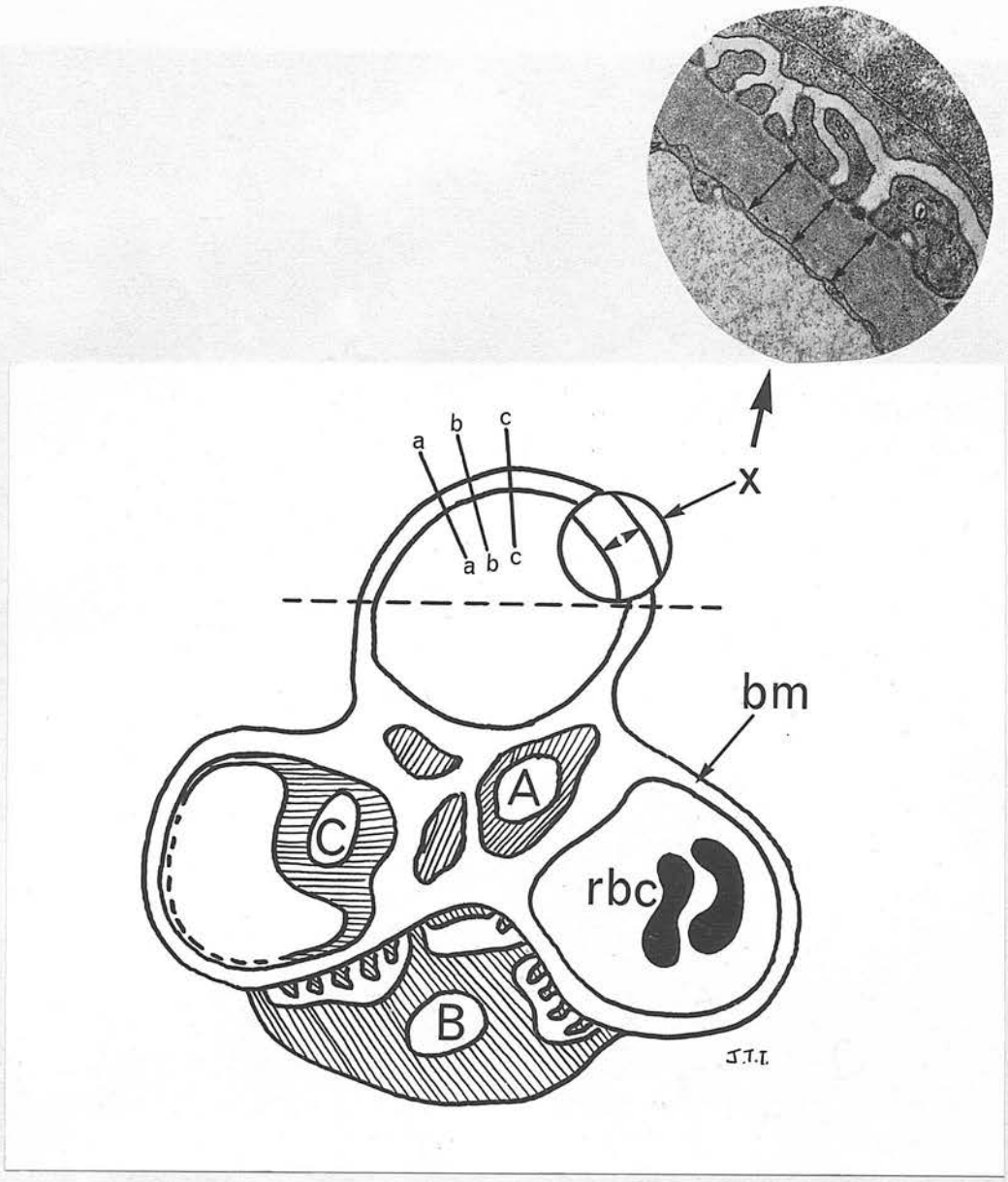


Fig. 4: Diagram of glomerular capillary lobule.

rbc: red blood cells with capillary lumen to demonstrate scale of diagram. bm: basement membrane.

A: Mesangial cell. B: epithelial cell. C: endothelial cell.

X (inset): method of measuring basement membrane thickness in peripheral half of capillary loop, (above dotted line). Measurements obtained at intervals indicated by lines aa, bb and cc.

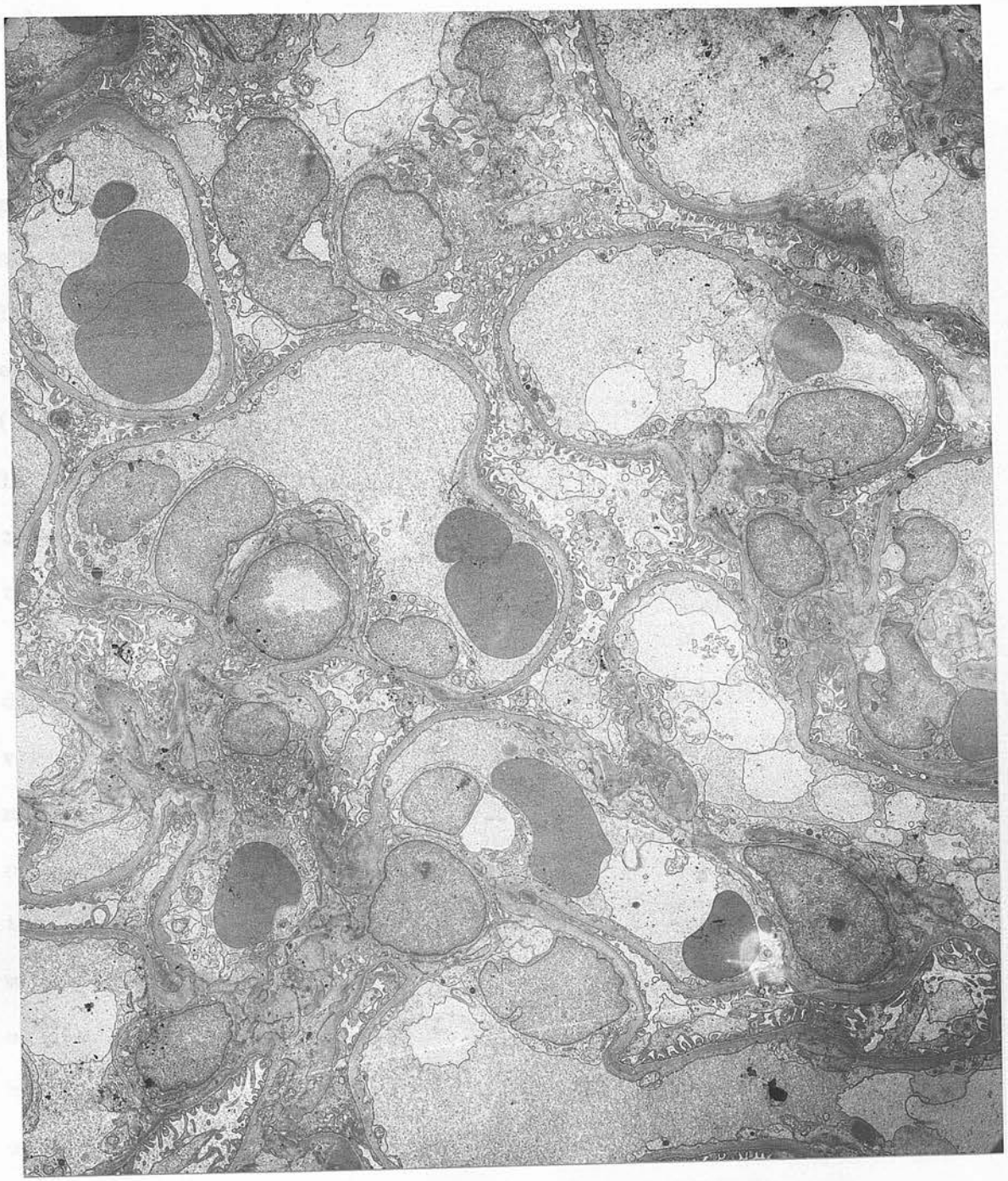


Plate 10: Low power view of lobules in glomerular capillary tuft. Long-standing idiopathic diabetic without retinopathy. Case 44. X 2,000.

ii) Mesangial Index

Examination of the anatomy of the glomerular capillary lobule (Fig. 4) will show that the above method of measuring peripheral capillary basement membrane thickness, arbitrarily excludes the mesangial zone and other cellular details. The mesangium, in particular, cannot be ignored in any valid evaluation of diabetic glomerular capillary disease (Kimmelstiel, 1966). Indeed in assessing early diabetic lesions one cannot overlook the possibility that the mesangial zone might be a more rewarding area of examination than the remainder of the glomerular capillary lobule.

In the mesangium, however, the three-dimensional distribution of the cells and basement membrane is difficult to visualise on electron microscopy of sectioned material. Without multiple serial sections, the size of the mesangial zone and its cellular complement cannot be estimated accurately. However, the absence of any linear distribution of the structures in the mesangial zone can be used to advantage, and the problem of obtaining a true cross-section on electron micrographs eliminated by measuring the ratio of basement membrane material to cellular substance. Massive accumulation of basement membrane (MacDonald, 1966), and attenuation of mesangial cytoplasm is characteristic of the advanced diabetic lesion (Chapter 8). Thus the ratio of basement membrane to cells, which the author has arbitrarily designated the "Mesangial Index", may be used as a parameter of diabetic involvement in the mesangium.

Method of measuring the "Mesangial Index"

i) On electron microscopy, glomerular capillary tufts were examined at magnifications of 1,000 to 2,500 times (Plate 10) and the mesangial zones identified (Fig. 5).

ii) The centre of the mesangial zone was aligned with the centre markings on the electron microscopy viewing screen.

iii) Photographs were obtained at magnifications of 2,500 to 4,000 times so that at no point was the capillary lumen within 2.0 cm. of the centre of the image.

iv) The electron micrograph negative was illuminated on a horizontal viewing box. A 100 square grid (1" x 1") was placed centrally on the negative to approximate to the position of the centre markings on the electron microscopy screen at the time of viewing (Fig. 5, Plate 11). Thus having aligned the grid to the anatomical centre of the mesangium, its composition, in terms of cell cytoplasm or basement membrane substance, was of no consequence in selecting the area of examination.

v) The number of squares covering basement membrane substance and mesangial cell components were counted separately and the Mesangial Index expressed as the percentage of basement membrane within the area examined.

The risk of significant error from inadequate sampling was minimised by measuring at least 10 mesangial zones from each of three glomeruli in individual renal biopsy samples. Variance between glomeruli within samples or the differences between

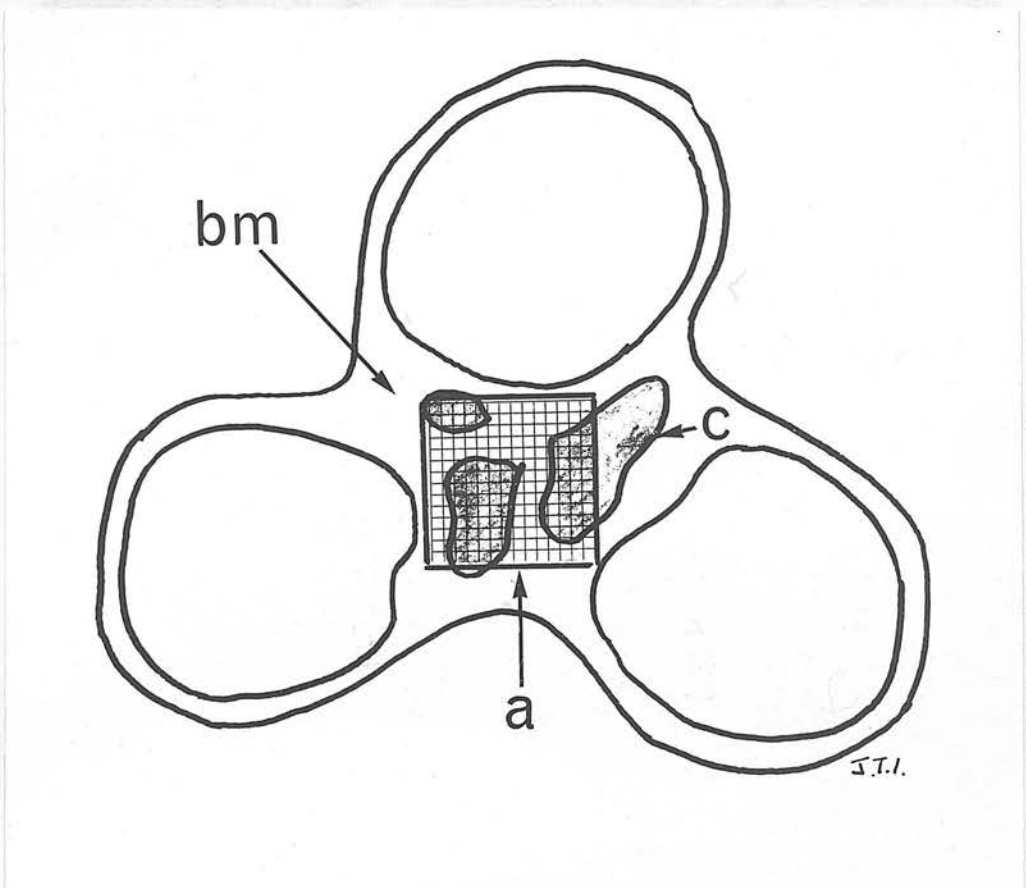


Fig. 5: Method of measuring "mesangial index"

a: Grid placed in mesangial zone and ratio of basement membrane substance (bm) to meaangial cytoplasm (c) estimated.

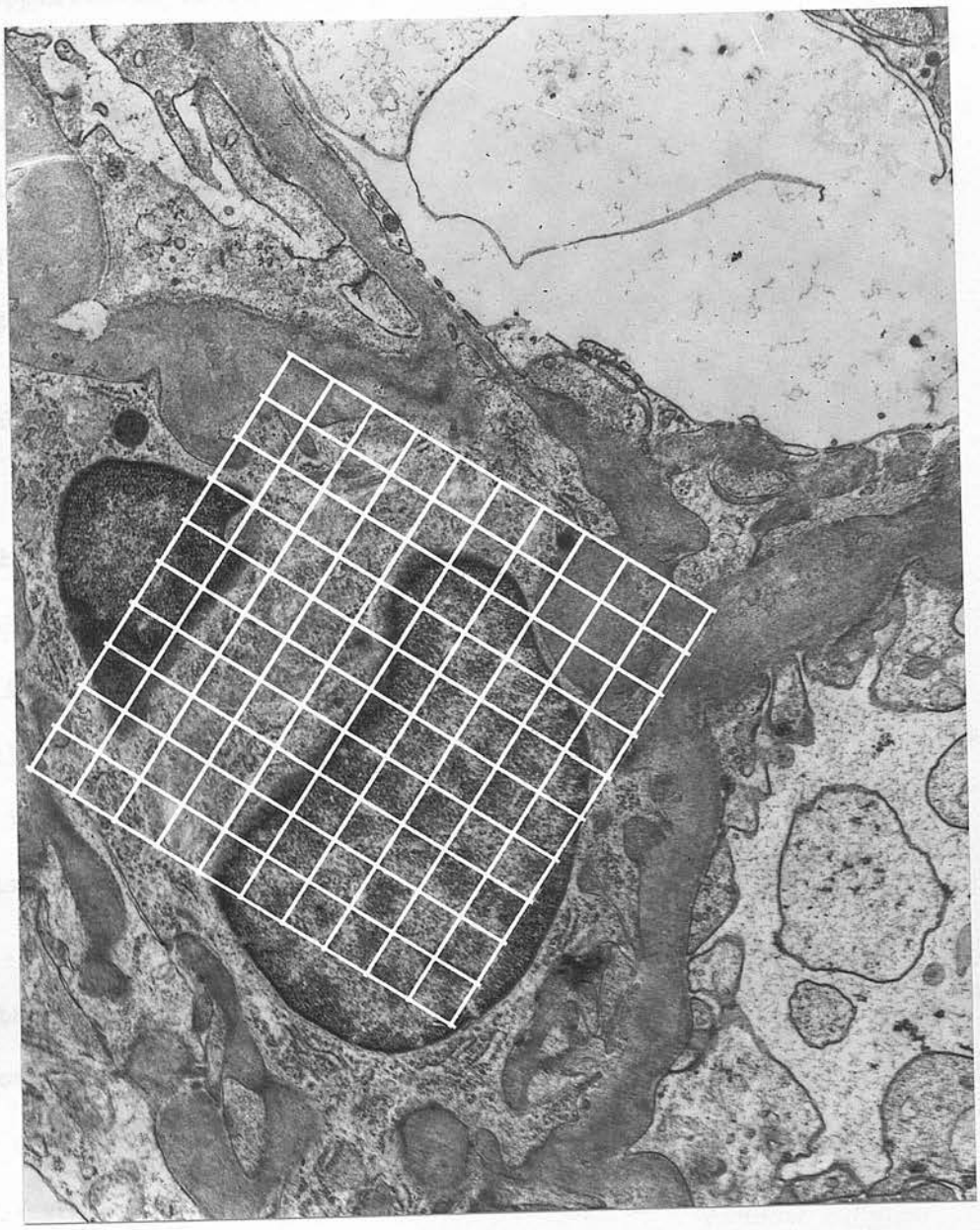


Plate 11: 100 square grid placed centrally in mesangial zone as in Fig. 5. Diabetic. Case 44. X 20,000.

samples or groups of samples could be analysed statistically by the application of such non-parametric methods as the Mann-Whitney 'U' test and the Kruskal-Wallis analysis of variance (vide infra). It is worth emphasising that, although electron microscopy magnifications are less easily calibrated at the magnifications used, the method of expressing the "Mesangial Index" is independent of the magnification of the electron micrograph.

5. Statistical methods

To demonstrate that a small biopsy sample is representative of that individual's glomerular population presents a formidable problem. Although this difficulty may be tackled by the application of statistical inference, which implies drawing conclusions about a large number of events on the basis of a small portion of them, it is essential that the methods used are entirely relevant to the test situation.

The most commonly used statistical methods, the parametric tests, are dependent upon assumptions concerning the distribution of events or biological data. However, definite data concerning the distribution of basement membrane thickness in either normal subjects or diabetics is not available. Thus tests free of assumptions concerning distribution should be applied to this problem. Moreover, parametric tests are dependent upon calculations involving addition, division and multiplication; in using such processes distortion of the data may be introduced.

For these reasons, non-parametric methods (Seigal, 1956) which are not only free of assumptions concerning distribution,

but also are ideal for small samples, have been used exclusively. Indeed, in sample sizes as small as 6 or less, there is no alternative to using a non-parametric statistical test unless the nature of the population distribution is known exactly. Whereas parametric tests focus on the differences between means, the non-parametric tests focus on medians. Nonetheless, it is often simpler for the reader to be shown mean values. Thus in many of the tables in subsequent sections, both mean values and standard deviations have been given despite the fact that the data have not been used in that form for statistical calculation.

In determining or stating probability, obectivity must be maintained. Thus conclusions should be reached by methods which are public and the statistical model, in which the nature of the population and the manner of sampling have been stated, should be clear. In subsequent sections, therefore, when a p-value is given, the test used is also stated. The tests most commonly applied have been:

i) The ranking method of the Mann-Whitney 'U' test (Seigal, 1956). This is ideal for small samples and can be best described as the non-parametric equivalent of the Student '**t**' test. Although requiring ordinal measurement there are no restrictions about distribution.

ii) The Kruskal-Wallis one-way analysis of variance (Seigal, 1956) is well suited to deciding whether independent samples are from different populations. Thus having approximately 10 estimations of the "Mesangial Index" from each of 3

glomeruli in a biopsy sample, it is possible by this method to decide whether differences among the values signify true differences, or whether they represent merely chance variations which may be expected among several random samples from the same population.

iii) The Kolmogarov-Smirnov Test (Seigal, 1956). This is a most powerful non-parametric test since it uses the size of readings rather than just ranking distribution. To apply the test, a cumulative frequency distribution is made for the control and test groups (i.e. normal and diabetic or between 2 groups of diabetics). For each interval, one step function is subtracted from the other. The test focuses on the largest of the observed deviations. However, equal numbers are required in each group, thus this test could not be applied in all cases.

All the above methods assume the absence of bias. Bias has been avoided in the nature of the studies as follows:

i) Percutaneous renal biopsy ideally provides a random sample of glomeruli.

ii) The nature of electron microscopy sectioning prevents selection of capillary tufts from glomeruli.

iii) Care has been taken in the methods of measuring capillaries to avoid selection (vide supra).

CHAPTER 5.

Glomerular ultra-structure in normal subjects.

"Many problems regarding the glomerular basement membrane remain to be solved. We do not yet know if one single cross-section is truly representative of the whole glomerulus, or whether one glomerulus can be accepted as representative in any single patient".

R. Østerby-Hansen, 1965.

Although "basement membrane thickening" is the characteristic diabetic lesion, the phrase loses all meaning unless judged against the fundamental yardstick of normal tissue similarly prepared and examined. In this section the findings in renal biopsy samples from nine non-diabetic subjects are reported and discussed.

Materials and Methods:

The opportunities of obtaining biopsies from healthy volunteers are limited, nevertheless the subjects examined were all healthy with the exception of Case 9 who, although he had chronic pancreatitis, was without abnormality in glucose tolerance (Table 1). No subject was hypertensive, and none had clinical or biopsy evidence of non-diabetic renal disease. Renal tissue was obtained by percutaneous biopsy using the Franklin-modified Vim-Silverman needle (Chapter 4). In each case three glomeruli were examined by electron microscopy; the basement membrane thickness was measured, and the "Mesangial Index" estimated as described in Chapter 4.

TABLE 1

Nondiabetic subjects: Clinical data, light and electron microscopy findings

Case no.	Age	Sex	Clinical status	Light microscopy findings in renal biopsies	Electron microscopy measurement of peripheral glomerular capillary basement membrane thickness (Angstrom units)							
					Glomerulus 1		Glomerulus 2		Glomerulus 3		Mean of all measurements obtained	
					Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.		± 1 S.D.
1	15	M	Normal	Normal	2,275	350	2,680	290	2,575	315	2,540	350
2	17	M	Normal	Normal	1,450	225	1,420	250	1,370	210	1,400	250
3	19	F	Normal	Normal	1,200	300	1,525	225	1,550	215	1,450	300
4	19	M	Normal	Normal	2,055	370	1,975	280	2,310	355	2,150	380
5	38	F	Normal	Normal	1,980	335	2,300	250	2,550	285	2,310	400
6	41	M	Normal	Normal	2,570	350	2,815	400	2,775	430	2,750	450
7	44	M	Normal	Normal	2,240	420	2,410	470	2,130	420	2,260	470
8	52	M	Normal	Normal	2,725	400	2,650	330	2,600	360	2,700	400
9	78	M	Chronic pancreatitis	Normal	1,875	340	2,340	275	2,510	325	2,210	390

Table 1: Non-diabetic subjects. Clinical data, light and electron microscopy findings.

Results:

The results of measuring peripheral glomerular capillary basement membrane thickness in individual glomeruli are detailed in Table 1, and summarised alongside the "Mesangial Index" in Table 2. The mean glomerular capillary basement membrane thickness ranged in individual cases from 1,400Å to 2,750Å (see footnote*), the mean value for the group being 2,200Å. Although the mean basement membrane thickness varied considerably between individuals, there was absence of significant variance between glomeruli within biopsy samples which suggests that the results obtained were representative of the glomerular population of that individual. The large number of measurements obtained in individual cases are demonstrated best by plotting them as a frequency distribution curve. Thus in Fig. 6, the frequency distribution curve of measurements obtained from each of three glomeruli from one patient (Case 8) illustrates the lack of variance. The shape of the curve shows that the distribution of measurements is not a normal Gaussian shape but is skewed towards thickness. This is the mathematical representation of irregular thickening seen even in the normal glomerulus. Because the curves are skewed, the modal points are less than the mean values.

The "Mesangial Index" ranged from 14 to 30 with a mean value of 21. There was no positive correlation between mean basement membrane thickness and the "Mesangial Index" (Fig. 7). Nonetheless,

* Note: 1 Angstrom Unit (Å) = 10^{-10} meter
10 Angstrom Units = 1 millimicron (nm)

PERCUTANEOUS RENAL BIOPSIES FROM NONDIABETICS

Case	Age	Sex	Peripheral glomerular capillary basement membrane thickness (Angstrom units)		Mesangial Index	
			Mean of 3 glomeruli	±ISD	Mean	±ISD
Nondiabetic						
1	15	M	2540	350	14	4
2	17	M	1400	250	18	4
3	19	F	1450	300	19	6
4	19	M	2150	380	26	7
5	38	F	2310	400	22	5
6	41	M	2750	450	14	6
7	44	M	2260	470	20	7
8	52	M	2700	400	30	9
9	78	M	2210	390	28	6

Table 2: Non-diabetic subjects. Mean glomerular capillary basement membrane thickness (mean of all measurements made) and mesangial index (mean of all measurements made).

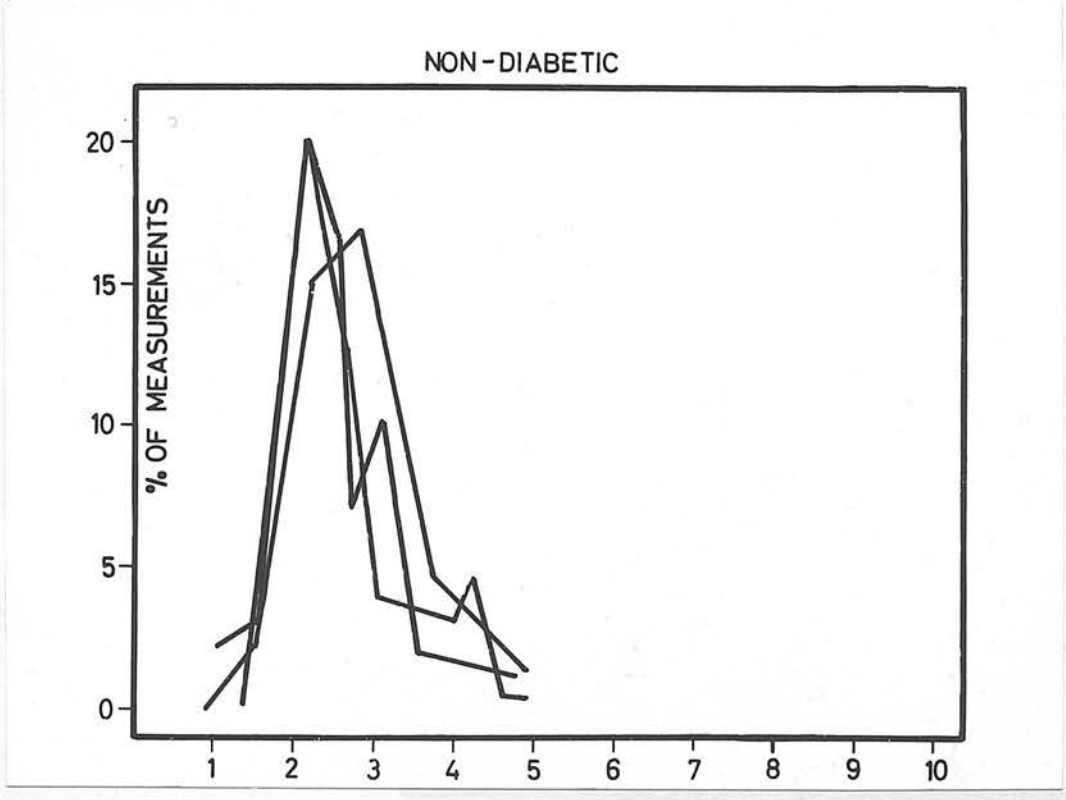


Fig. 6: Frequency distribution curve of measurements obtained from each of 3 glomeruli from Case 8.

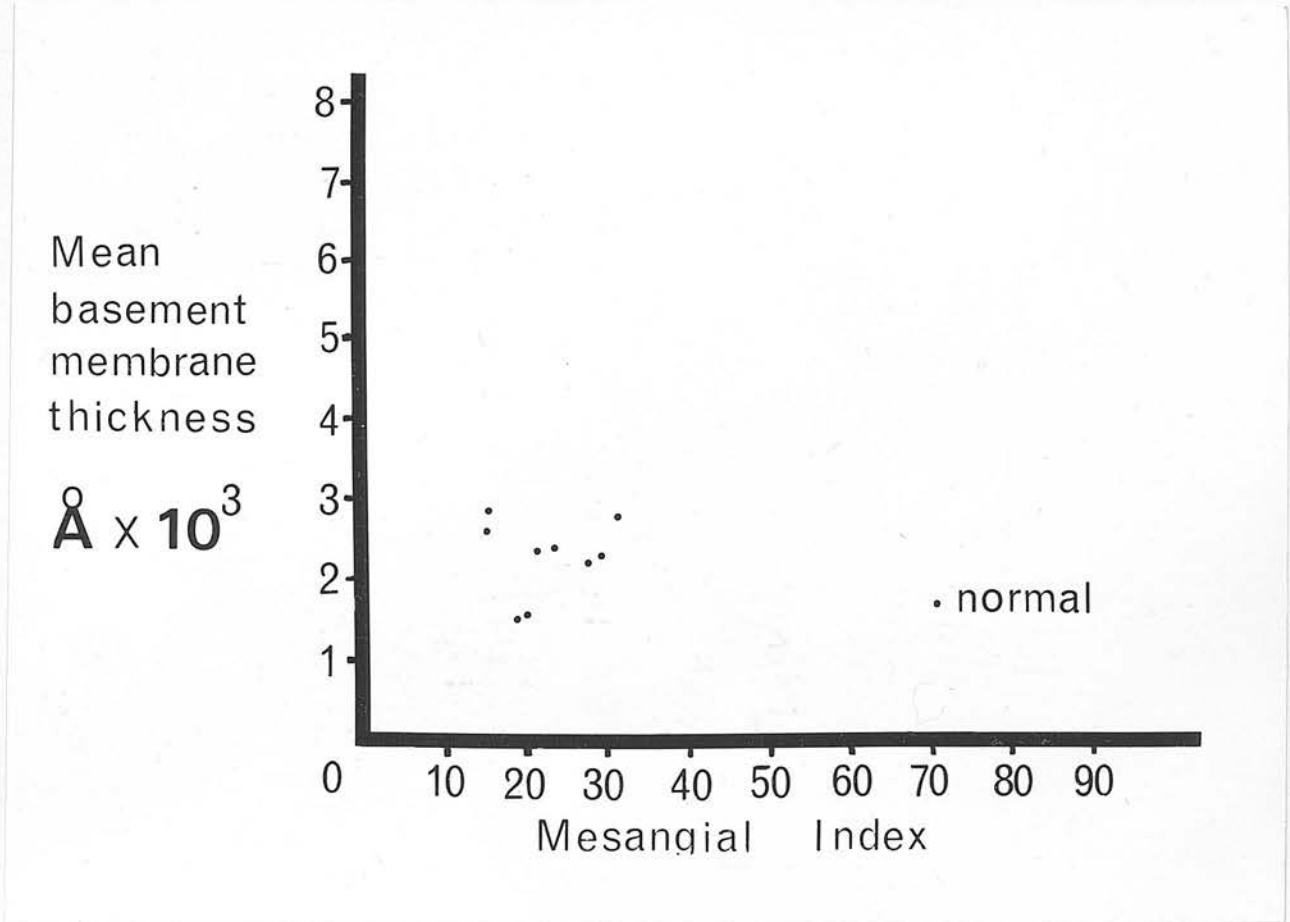


Fig. 7: Non-diabetic subjects. Mean values of basement membrane thickness ($\mathring{A} \times 10^3$) and mesangial index showing absence of positive correlation.

since the "Mesangial Index" represents the ratio of basement membrane substance to cytoplasm within the mesangium expressed as a percentage, it is clear that in all normal subjects so measured the ratio of basement membrane to cytoplasm is low. Electron micrographs of normal tissue are illustrated (Plates 1 (page 38) and 12).

Discussion:

Many reports of normal values of glomerular capillary basement membrane thickness in isolated cases (Bloom, Hartmann and Vernier, 1959; Bergstrand and Bucht, 1959; Fisher et al., 1964; Rosenbaum, Kattine and Gottsegen, 1963) are not comparable with the present series on account of inadequate documentation with reference to site and number of measurements made.

On the other hand two publications (Østerby-Hansen, 1965; Osawa, Kimmelstiel and Seiling, 1966) report careful studies in which the capillary basement membrane was measured by methods similar to the present series. However in both reports these authors measured the cross-section of the capillary loops on the basis that the peripheral basement membrane "is considered as that part which is beyond the first endothelial pore starting in the mesangial region" (Østerby-Hansen, 1965). Østerby-Hansen measured one glomerulus from each of three healthy subjects and found mean values of 2,500Å, 2,800Å and 2,850Å. Osawa et al. measured 26 glomeruli from 7 biopsies and found a mean value of 3,150Å.

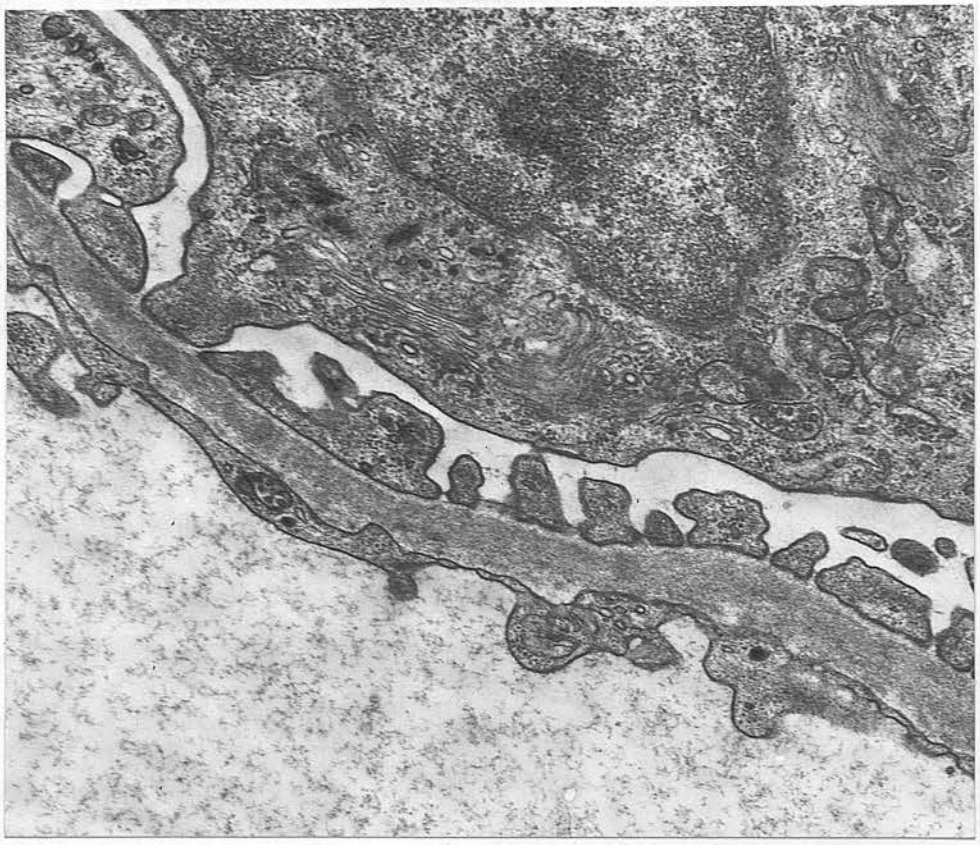


Plate 12: Glomerular capillary basement membrane.

Normal subject. Case 8. X 30,000.

The fact that the mean values for normal basement membrane thickness in this series are less than those recorded by others is probably the result of confining measurements to the peripheral half of the capillary loops (Chapter 4) and total avoidance of the proximity of the mesangial zone where the basement membrane tends, even in the normal, to be thicker. However, this arbitrary limitation of measurements was essential in avoiding bias in selecting the junction between the periphery and the mesangium. Despite the range of values in this series there was no positive correlation with age. Using this series as a yardstick for comparison, in any one subject peripheral basement membrane thickness should not be regarded as abnormal unless it exceeds 3,500Å a value two standard deviations greater than the mean thickness in this control series.

Yodaiken, Seftel, Kew, Lillenstein and Ipp (1969) have discussed in detail the potential errors in electron microscopy magnifications and their effects upon measurement of basement membrane thickness in muscle capillaries. Although they found variations in voltage fluctuations to cause errors of up to 30%, others (Reisner, 1965; Baker and Zeikler, 1965) have suggested that the error should be no greater than 10%. In the author's experience, the characteristics of the A.E.1. EM6 electron microscope, used exclusively in this study, are such that whereas errors may arise at the extremes of magnification, in the 6,000 to 13,000 times range of magnification used for measuring capillary

basement membrane thickness, the error was consistently less than 5%. Although lower magnifications were used to estimate the mesangial index, the method is independent of magnification.

Several workers, notably Siperstein, Unger and Madison (1969) have measured the thickness of basement membranes in muscle and other capillaries by placing a template over electron micrographs and obtaining readings at fixed points. Whereas this method is free of bias in selection of measuring points, the results obtained are largely of tangential measurements and thus represent abstract mathematical values rather than true cross-section thickness. In a recent study of the theoretical and practical considerations in the estimation of vascular basement membrane thickness (Williamson, Vogler and Kilo, 1969) the authors concluded that measurements were of value only if true cross-section readings were obtained. In this context, however, as mentioned earlier (Chapter 4) the electron microscopist has been given a unique advantage in measuring glomerular, as opposed to other capillaries.

CHAPTER 6.

Glomerular ultra-structure in secondary diabetics.

> ".... a man aged thirty-five years, strong, healthy and corpulent, accustomed to free living and strong corporeal exertions in pursuit of country amusements who in December, 1787, was seized with diabetes.... He gradually became emaciated and debilitated, and his urine was found to be sweet and to contain a substance which was fermentable with yeast....At necropsy the pancreas was full of calculi, which were firmly imparted in its substance".
>
> Thomas Cawley, 1788.

One of the outstanding problems concerning the pathogenesis of diabetic angiopathy is whether the lesions are a genetically determined and integral component of the idiopathic disorder or are due to one or more of the biochemical derangements of diabetes mellitus (Berson and Yalow, 1965). Many animal studies (Lukens and Dohan, 1946; Ricketts, Test, Pertersen, Lints, Tupikova and Steiner, 1959; Hausler, Sibay and Campbell, 1964; Gibbs, Wilson and Gifford, 1966; Beaser, Sak, Donaldson, McLauchlin and Somners, 1964; Bloodworth, 1965; Bloodworth, Engerman and Powers, 1969) have been undertaken in an attempt to settle this issue, yet the occurrence in man of secondary or pancreatic diabetes ideally lends itself to the investigation of this problem. Although diabetic glomerulosclerosis has occasionally been reported in secondary diabetics (Sprague, 1947; Duncan, MacFarlane and Robson,

1958; Becker and Miller, 1960; Deckert, 1960; Shapiro and Smith, 1966) and in lipo-atrophic diabetics (Hamwi, Kruger, Eymontt, Scarpelli, Gwinup and Byron, 1966; Marcus, 1966), on the basis of light microscopy study, the use of electron microscopy allows a more accurate assessment of the glomerular lesions.

This study reports the light and electron microscopic appearances of renal tissue obtained by biopsy from patients having secondary or pancreatic diabetes. Measurements of peripheral capillary basement membrane thickness in the glomeruli of subjects are statistically analysed and the cellular ultra-structure described and compared.

Material and Methods:

The secondary diabetic patients were unselected except that they were available for study and willing to have a renal biopsy performed (Tables 3 and 4). The clinical investigations and criteria which established the diagnosis of the primary pancreatic disease are set out in Table 3 .

No subject was hypertensive and, with the exception of Case 19 who had pyelonephritis, none had clinical or biopsy evidence of non-diabetic renal disease. There was no family history of diabetes in either group at the time of investigation, although two years later a sibling of Case 16 developed the disorder.

Renal tissue was obtained by percutaneous biopsy, using a Franklin-modified Vim-Silverman needle, except in the two

Case no.	Primary disease of pancreas	Clinical criteria confirming diagnosis of disease of pancreas	Duration of diabetic features	Diabetic regime
10	Recurrent acute pancreatitis	Acute onset of diabetic features immediately following attack of acute pancreatitis six years previously. Recurrent acute pancreatitis confirmed by estimation of serum amylase. Barium meal: large pancreatic cyst with anterior displacement of stomach. Tests of pancreatic exocrine function abnormal. Laparotomy: pancreatic fibrosis and pseudocyst formation.	6 years	Insulin therapy
11	Alcoholic pancreatitis	Chronic alcoholic. Drainage of pancreatic pseudocyst four years before onset of diabetic features. Chronic pancreatic fistula. Laparotomy: pancreatic fibrosis.	1 year	Insulin therapy
12	Hemochromatosis	Skin pigmentation, hepatomegaly, hypogonadism. Serum iron: 360 μg./100 ml. T.I.B.C.: 420 μg./100 ml. Sternal marrow biopsy: excess iron staining. Renal biopsy: iron stain in tubules. Liver biopsy: cirrhosis and iron deposition.	15 years	Insulin therapy
13	Chronic pancreatitis	Intermittent, recurrent upper abdominal pain. X-ray abdomen: calcification in head and body of pancreas. Five-day stool fat collection: 11 gm. per day. D-xylose excretion normal. Folic acid and Vitamin B_{12} absorption tests normal. Secretin and pancreozymin tests: reduction in pancreatic exocrine function.	3 years	Diet alone
14	Hemochromatosis	Skin pigmentation, hepatomegaly. Serum iron: 440 μg./100 ml. T.I.B.C.: 465 μg./100 ml. Sternal marrow biopsy: excess iron stain. Liver biopsy: cirrhosis and iron deposition.	3 months	Insulin therapy
15	Carcinoma of pancreas	Upper abdominal pain. Laparotomy: carcinoma of pancreas.	6 months	Diet alone
16	Chronic pancreatitis	Five-year history of intermittent abdominal pain and steatorrhea before investigation by laparotomy. Cholecystectomy and choledochotomy. Diabetes diagnosed at time of operation. Symptoms of abdominal pain continued following operation. Subsequent secretin and pancreozymin tests: marked reduction in pancreatic exocrine function.	10 years	Insulin therapy
17	Carcinoma of pancreas	Obstructive jaundice. Laparotomy: carcinoma of pancreas.	3 months	Diet alone
18	Chronic pancreatitis	Weight loss, diarrhea, steatorrhea. Five-day stool fat collection: 10 gm./day. D-xylose excretion normal. Folic acid and Vitamin B_{12} absorption tests normal. Secretin and pancreozymin tests: considerable reduction in pancreatic exocrine function. X-ray abdomen: extensive calcification in head and body of pancreas.	2 years	Diet and chlorpropamide
19	Chronic pancreatitis	Recurrent upper abdominal pain investigated by laparotomy one year before onset of diabetic features. Chronic calculus cholecystitis, pancreatic fibrosis found. Cholecystectomy and choledochotomy carried out. Subsequent secretin and pancreozymin tests: marked reduction in pancreatic exocrine function. Chronic urinary infection.	11 years	Insulin therapy

Table 3: Clinical data concerning secondary diabetics.

Secondary diabetic subjects: Renal biopsy findings on light and electron microscopy

Case no.	Age	Sex	Primary disease of pancreas	Renal biopsy findings on light microscopy	Electron microscopy measurements of peripheral glomerular capillary basement membrane thickness (Angstrom units) Glomerulus 1 Mean thickness	± 1 S.D.	Glomerulus 2 Mean thickness	± 1 S.D.	Glomerulus 3 Mean thickness	± 1 S.D.	Mean of all measurements obtained	± 1 S.D.
10	30	M	Recurrent acute pancreatitis	Early diffuse glomerulosclerosis	4,920	1,050	5,900	1,150	4,725	770	5,200	1,060
11	47	M	Alcoholic pancreatitis	Normal	4,075	520	4,125	485	4,170	670	4,140	690
12	48	M	Hemochromatosis	Diffuse glomerulosclerosis	3,740	710	3,750	720	3,600	730	3,700	730
13	50	F	Chronic pancreatitis	Normal	4,230	460	3,880	390	3,920	425	4,000	510
14	54	M	Hemochromatosis	Normal	4,145	390	3,985	300	3,765	450	3,880	525
15	56	M	Carcinoma of pancreas	Normal	4,000	450	3,770	375	3,785	415	3,800	470
16	57	F	Chronic pancreatitis	Normal	3,475	625	3,340	670	3,275	610	3,350	695
17	61	F	Carcinoma of pancreas	Normal	1,870	400	1,930	360	2,275	350	1,950	470
18	65	F	Chronic pancreatitis	Diffuse and early nodular glomerulosclerosis	5,420	610	4,965	520	5,020	640	5,000	730
19	68	M	Chronic pancreatitis	Interstitial fibrosis and round cell infiltration of renal parenchyma	2,215	320	2,090	290	2,420	480	2,125	700

Table 4: Secondary diabetics. Renal biopsy findings on light and electron microscopy.

patients having carcinoma of the pancreas, in which cases it was obtained by open biopsy at laparotomy. The specimens were immediately divided, cubes from either end being fixed in 1 per cent buffered osmium tetroxide and embedded in Araldite for examination by electron microscopy while the centre portion of each specimen was fixed in corrosive formol for examination by light microscopy. In each case at least three glomeruli were sectioned and the peripheral glomerular capillary basement membrane thickness measured as described in Chapter 4.

Results:

Light Microscopy:

The changes in the renal tissue of the secondary diabetics are shown in Table 2

Electron Microscopy:

In these secondary diabetics the mean glomerular capillary basement membrane thickness ranged from 1,950Å to 5,200Å (Table 4) the mean thickness for the group was 3,700Å. Although this group cannot be matched for age and sex with the normal subjects reported in the preceding chapter, the electron microscopy methods were identical and comparisons are valid (Fig. 8). Using the parametric data reported i.e. that in individuals, mean glomerular capillary basement membrane thickness in excess of 3,500Å exceeds the normal mean by 2 standard deviations, seven of these unselected secondary diabetics had abnormal thickening of the

glomerular capillary basement membrane. Applying the more valid non-parametric Mann-Whitney 'U' test (Chapter 4) the differences between the control and secondary diabetic groups are significant ($p < 0.01$). Since the 2 groups did not contain equal numbers the Kolmogorov-Smirnov test could not be applied. Also, there was absence of significant variance between glomeruli within biopsy samples which suggests that, for the subjects studied, the results obtained were representative of the glomeruli of each individual and that glomerular capillary thickening, when present, was diffusely distributed in the kidney. The differences in the results were not correlated to the patient's age (note that in Fig. 8 the results have been ranked according to age), duration of diabetic features or the severity of the diabetes. p.92

Sections from secondary diabetics are illustrated in Plates 13, 14, 15 and 16. In addition to the peripheral basement membrane thickening, all three cell types showed an increase in their cytoplasm so that they encroached upon the vascular and urinary spaces which thus appeared reduced in size. The cytoplasm was rich in mitochondria, RNA studded endoplasmic reticulum and Golgi zones suggesting increased cellular activity. Of those cases with significant peripheral basement membrane thickening, only Case 18 showed accumulation of basement membrane material in the axial zones encroaching on the endothelial and mesangial cells in the manner seen in idiopathic diabetes

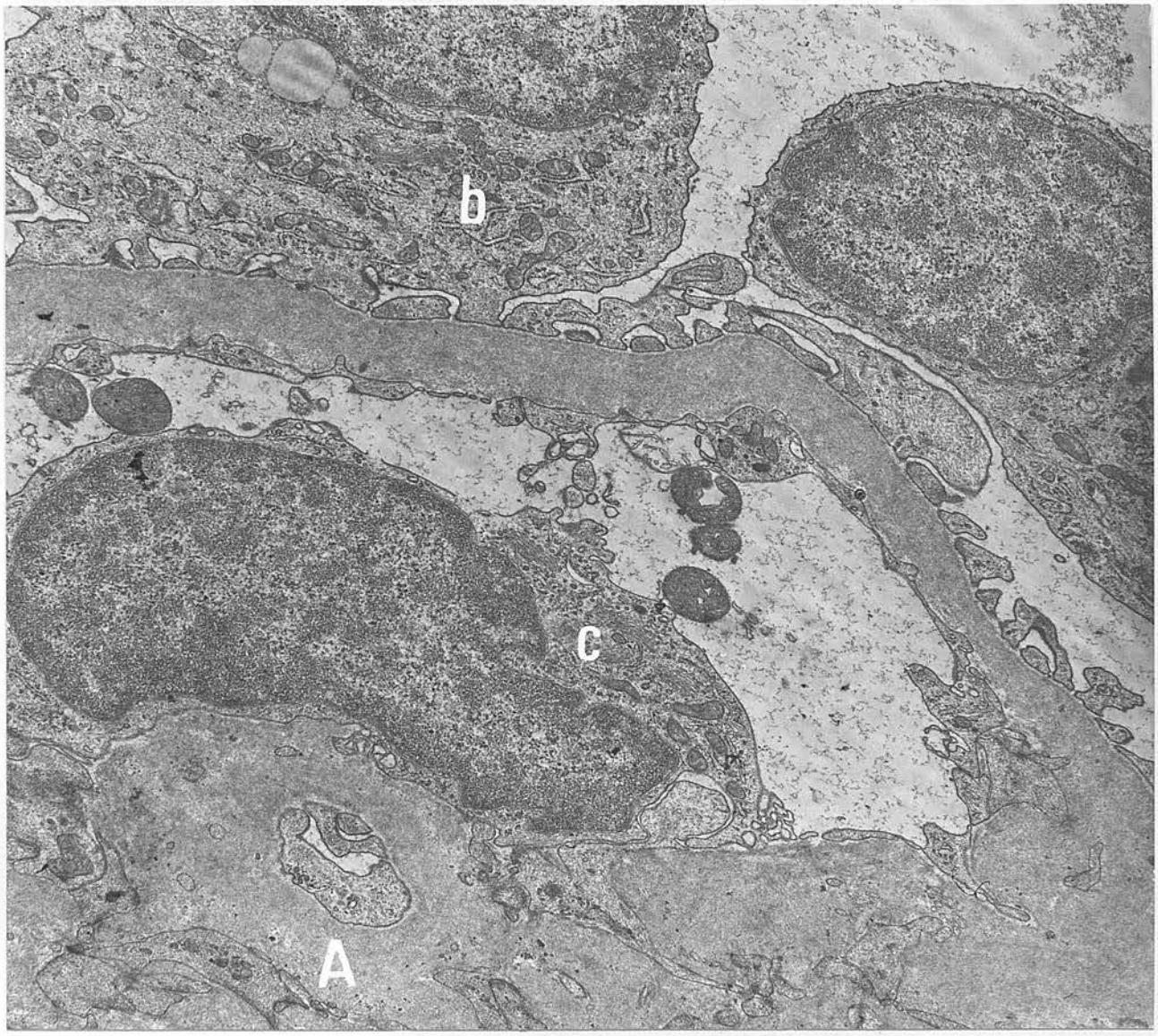


Plate 13: Basement membrane thiekening in a glomerular capillary loop from a patient having diabetes secondary to chronic pancreatitis. Mesangial zone (A) with adjacent endothelial cell (C). Epithelial cell (b) lies over free surface of the capillary. Case 18. X 18,000.

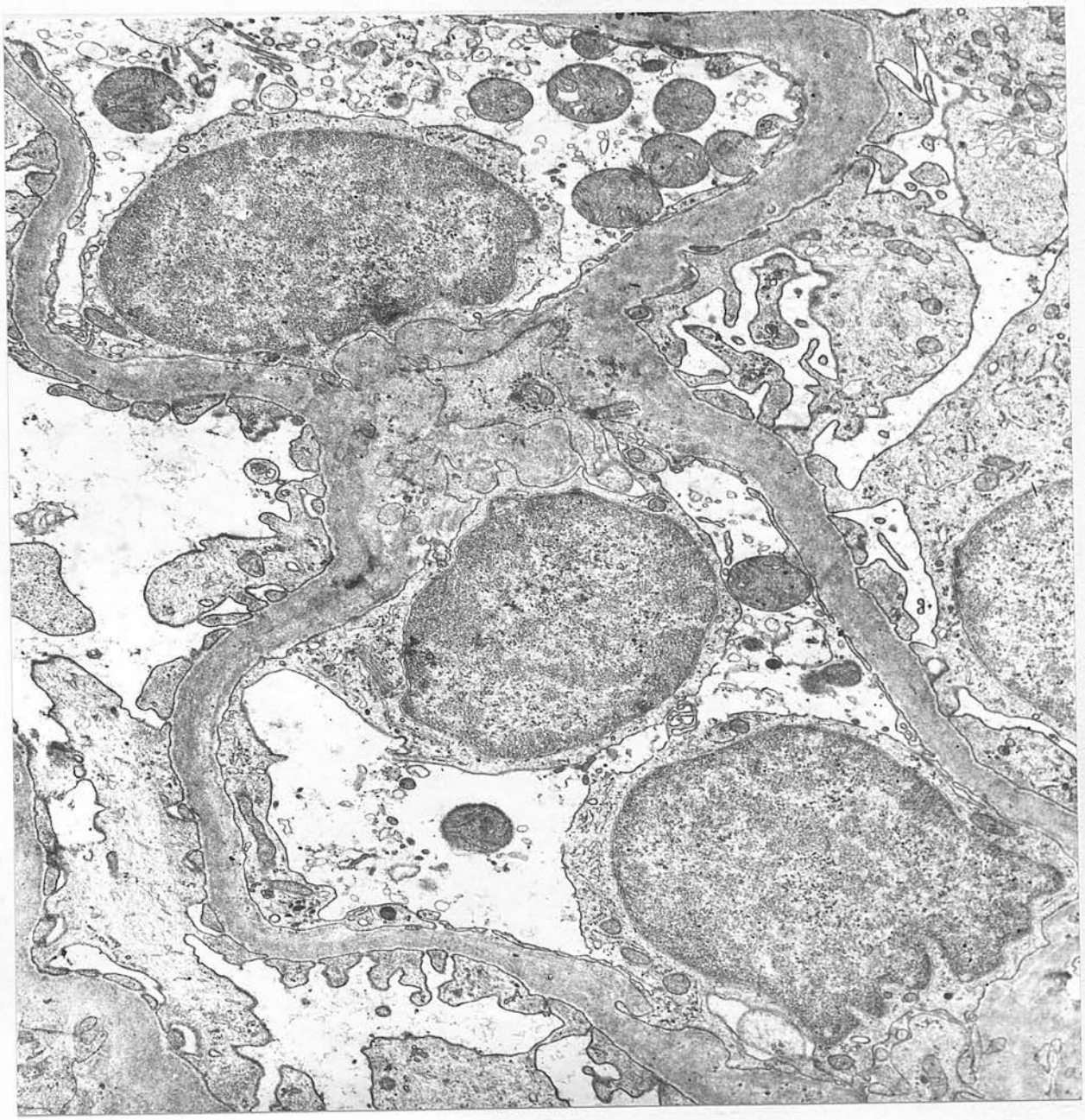


Plate 14: Glomerular capillary loops from patient having diabetes secondary to recurrent acute pancreatitis. Case 10. X 10,000.

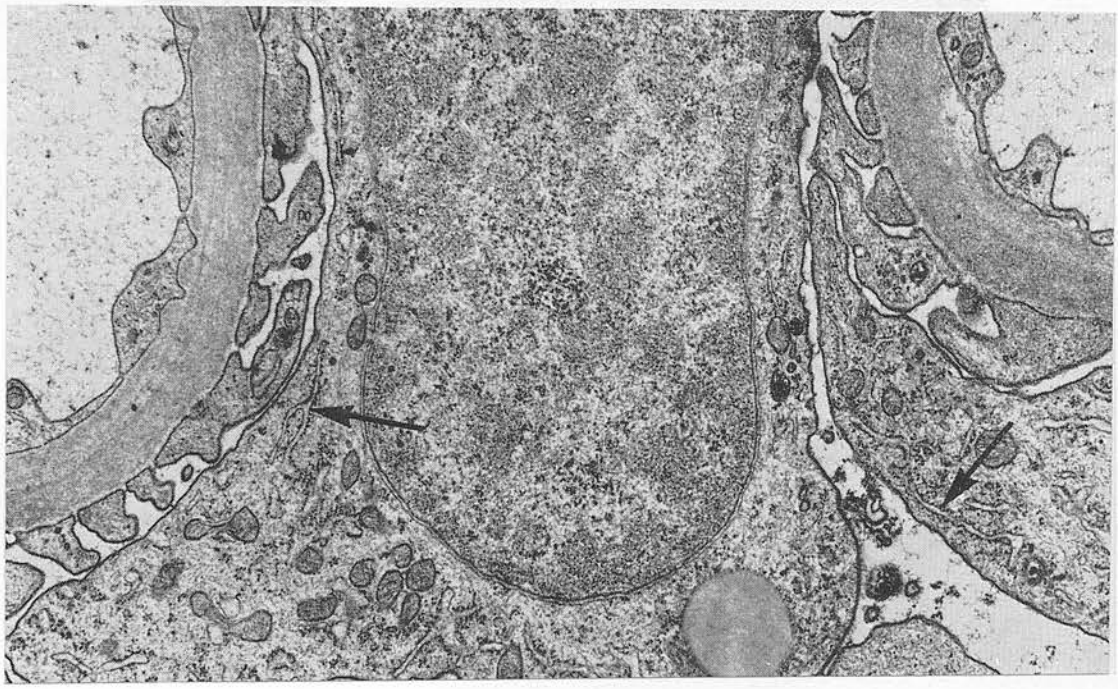


Plate 15: Peripheral glomerular capillary loops separated by epithelial cell showing numerous channels of RNA-studded endoplasmic reticulum (arrowed). Patient having diabetes secondary to recurrent acute pancreatitis. Case 10. X 20,000.

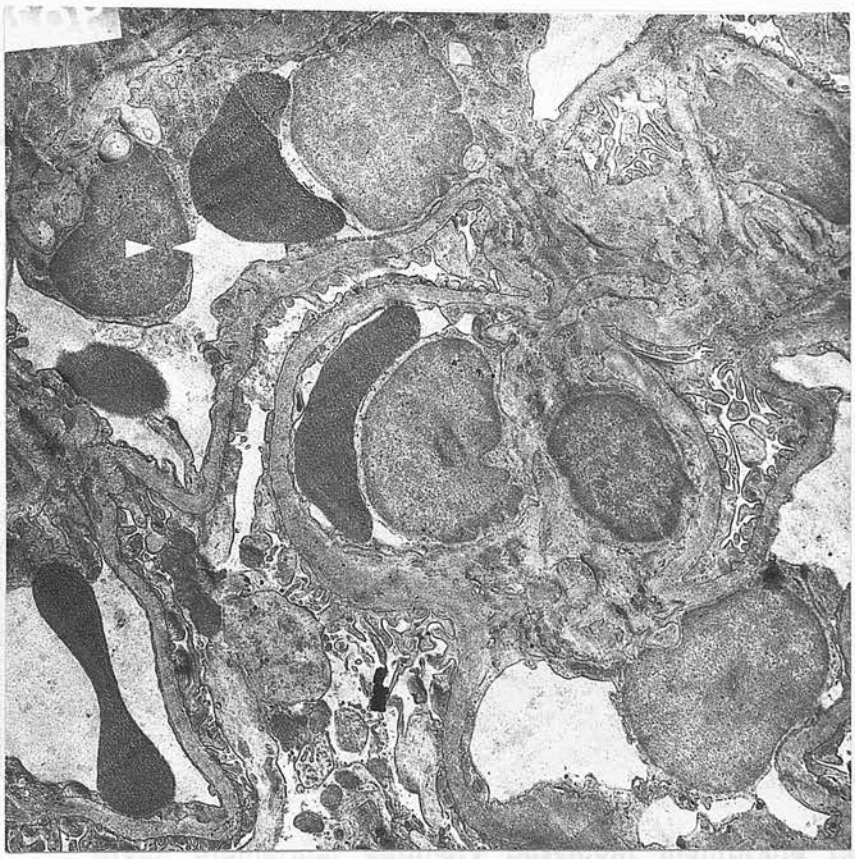
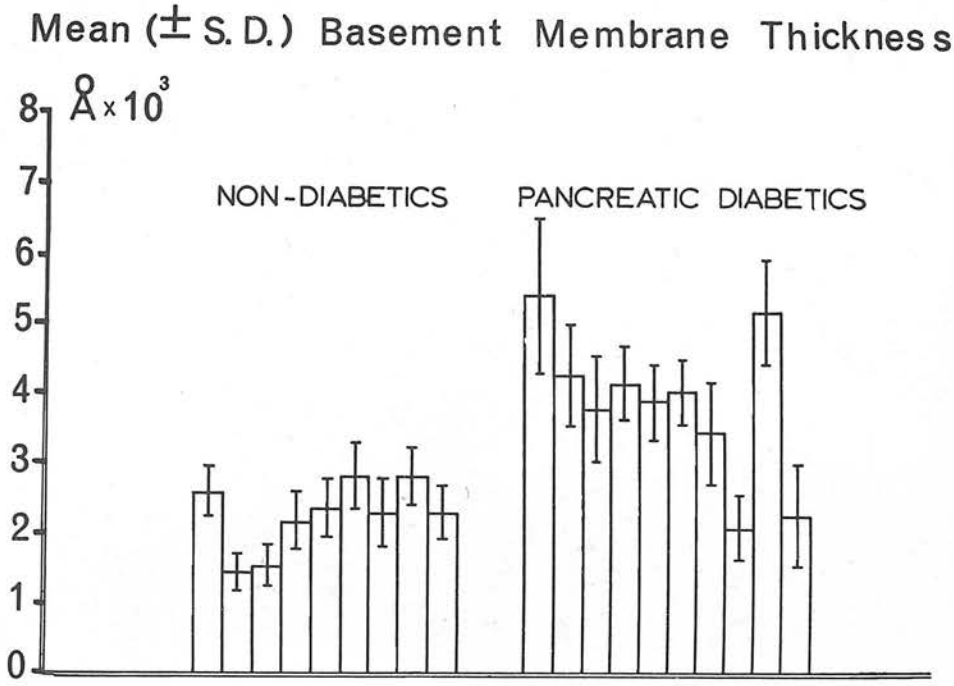


Plate 16: Lobule from patient having diabetes secondary to chronic pancreatitis. Thickened basement membrane. Mesangial and endothelial cells encroaching upon capillary lumena. Case 13. X 6,000.



Mean glomerular capillary basement membrane thickening ± S.D. in nondiabetics and pancreatic diabetics.

Fig. 8

(Chapters 8 and 9). In all the other secondary diabetics the axial zones contained loose-textured basement membrane material, as in normal subjects, and the mesangial and endothelial cells appeared either normal or hypertrophied (Plates 13, 14, 15 and 16).

Discussion:

In 1960 Becker and Miller studied by light microscopy the renal tissue of twenty-two deceased subjects who were known to have had haemochromatosis with secondary diabetes. In the thirteen with recognised diabetes of less than four years' duration no diffuse or nodular glomerulosclerosis was seen, but in seven of the nine known to have been diabetic for a longer time glomerular lesions attributable to diabetes were present.

The present investigation extends these observations to patients having other forms of pancreatic diabetes and, because the electron microscope was employed, changes in the glomeruli could be more accurately detected and measured than by light microscopy. However, Kimmelstiel (1966) has stated that in many electron microscopic studies of diabetic nephropathy the method of measuring glomerular capillary basement membrane thickness was inadequate, control data were absent and the results were not subjected to statistical analysis. In this study these deficiencies have, as far as possible, been overcome.

The glomerular capillary basement membrane was significantly thickened in seven of the ten unselected secondary

diabetics and the mean thickness of the group as a whole was significantly greater than that of the normal control subjects.

It is possible that these pancreatic diabetic patients were genetically predisposed to idiopathic diabetes. This could have contributed towards the development of diabetes, which might not otherwise have occurred, and could have been also directly or permissively responsible for any observed basement membrane thickening. Vallance-Owen (1966) has reported, however, that all the pancreatic diabetics he studied were synalbumin-negative, suggesting that diabetes secondary to pancreatic disease is not genetically determined. The possibility of genetic influence seems unlikely in the light of present concepts of diabetic inheritance (Clarke, 1966; Neel, 1970) and the high prevalence of glomerular lesions in these cases. But the absence of a family history of diabetes does not exclude the possibility that an individual is genetically diabetes-prone. At the time of biopsy Case No. 16 had a negative family history and two years later one of her siblings developed idiopathic diabetes. Thus there is reason to suspect that this patient was genetically diabetic, but the fact that there was no glomerular capillary basement membrane thickening further supports the view that the lesion is not genetically determined.

The variable extent, and even absence of glomerular lesions in this group of secondary diabetics is not unexpected. The findings are in accord with previous observations in long-standing

idiopathic diabetics where capillary basement membrane thickening may not be present in all cases (Chapter 9). Whereas published reports suggest that light microscopic evidence of glomerulosclerosis is less common in pancreatic than idiopathic diabetes, the findings reported here emphasise that the true prevalence of the glomerular lesions can be more accurately assessed by electron microscopy. Furthermore, diabetes of pancreatic origin is often a late event in a disease which may be fatal relatively soon thereafter so that there may not be enough time for the lesions revealed by the electron microscope to progress to the extent that they can be demonstrated by light microscopy.

The results of the present study suggest that the development of glomerular capillary basement membrane thickening in diabetes is not, as has been proposed, dependent on the presence of the genetically determined diathesis to idiopathic diabetes but is due to some metabolic derangement, possibly resulting from inadequate insulin action on the tissues, common to both primary and secondary diabetes. The possibility that some components of diabetic microangiopathy can occur only in and be due to some as yet undefined aspect of the idiopathic diabetic disorder is, however, not excluded by these observations (Chapter 11).

CHAPTER 7.

Glomerular ultrastructure in newly-diagnosed juvenile diabetics.

"Equivocal non-specific focal glomerular changes in pre-diabetes are insufficient evidence upon which to base a firm statement that basement membrane changes precede detectable metabolic changes".

Berkman and Rifkin, 1966.

Whereas the possibility of significant thickening in the glomerular capillaries of long-standing diabetics is beyond dispute, it is still uncertain whether this lesion arises before or after the onset of clinical diabetes. In recent years this aspect has been further confused by the introduction of the term "prediabetes"; an intermediate stage between normality and clinical diabetes. Prediabetes has been variously characterised as "loss of normal carbohydrate tolerance without signs or symptoms of overt diabetes" (Graef, 1962), and "the period from conception to the first demonstrable impairment of glucose tolerance in response to stress, steroid ingestion or glucose loading" (Conn and Fajans, 1961). Wisely, the British Diabetic Association advised against the use of the term (Fitzgerald and Keen, 1964).

In any event, the author felt that to study the possibility of the basement membrane lesion being present before the onset of clinical diabetes, the acute onset insulin-dependent type of diabetic might be a better focus of attention. These patients characteristically have a sudden onset of symptoms of only a few weeks duration before diagnosis. If no lesion were found at the

time of diagnosis, then basement membrane thickening in the so-called pre-diabetic phase would be unlikely.

Material and Methods:

Percutaneous renal biopsy samples were obtained from four newly diagnosed juvenile diabetics (Table 5). Each of the diabetics had been admitted to hospital following acute onset of symptoms of less than 10 weeks duration, and the biopsies were obtained before insulin therapy was started. The methods of preparation of tissue and measurement of basement membrane were as described in Chapter 4.

Case No.	Age	Sex	Peripheral glomerular capillary basement membrane thickness (Angstrom Units)		Mesangial Index	
			Mean of 3 glomeruli	± I.S.D.	Mean	± I.S.D.
21	17	F	1900	520	27	5
22	20	F	2980	440	33	3
23	20	M	2350	280	16	7
24	22	M	2760	385	25	9

Results:

The overall mean basement membrane thickness and mesangial index results are summarised in Table 5. There was no significant difference between the findings in these patients and the non-diabetic dontrols described in Chapter 5 (Fig. 9). Nor was there any significant abnormality noted in the epithelial, endothelial, or

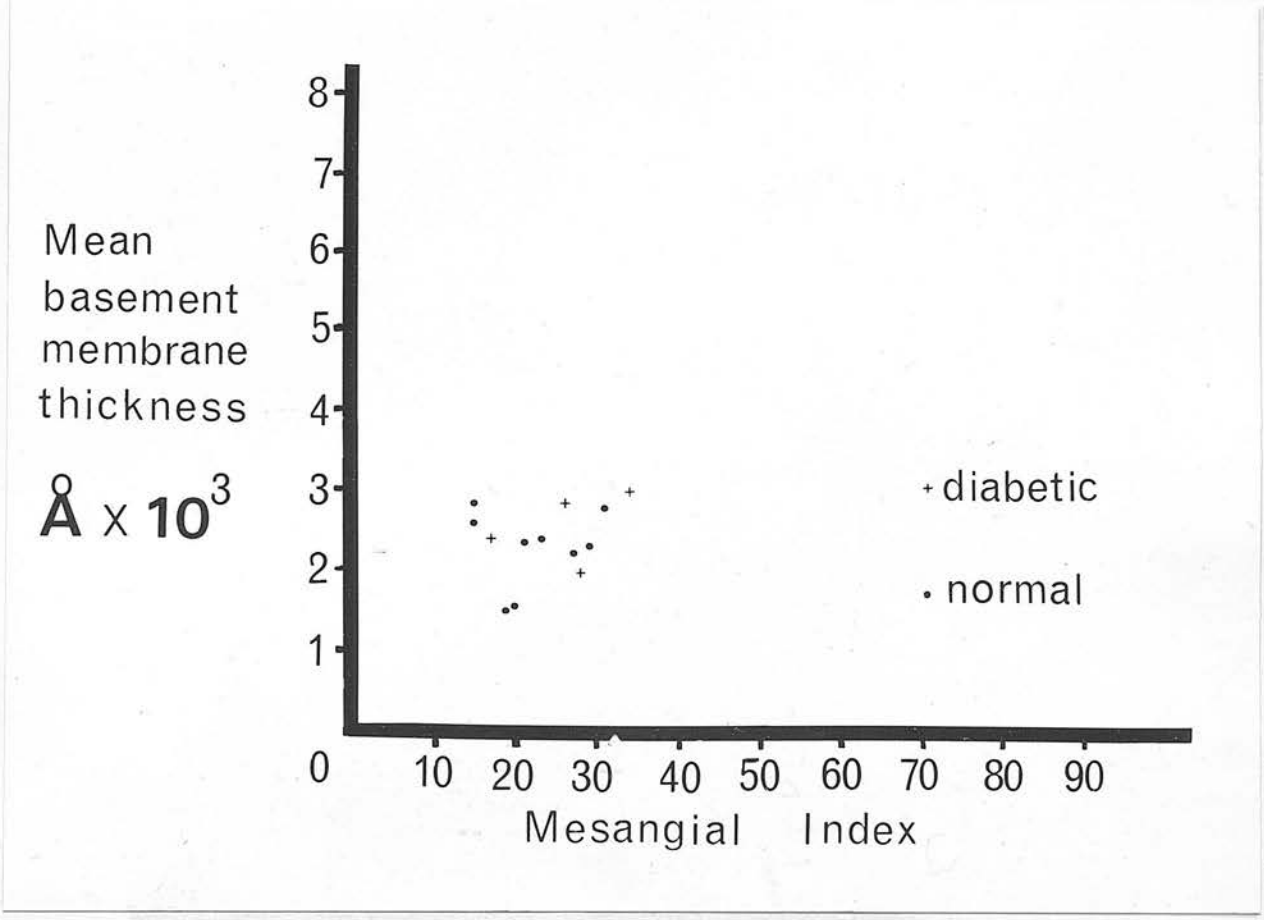


Fig. 9: Mean basement membrane thickness ($\mathring{A} \times 10^3$) and mesangial index in normal and newly diagnosed diabetic patients showing absence of significant difference between them.

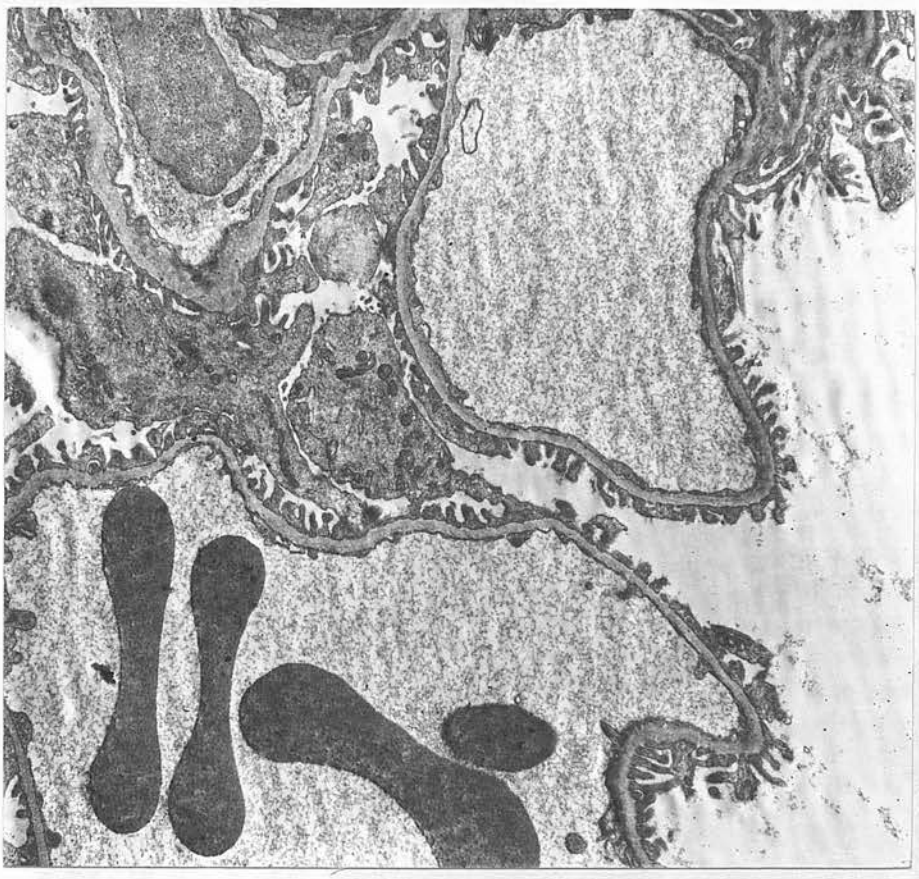


Plate 17: Peripheral glomerular capillary loops (one containing RBCs) from newly diagnosed diabetic. Case 21. X 6,000.

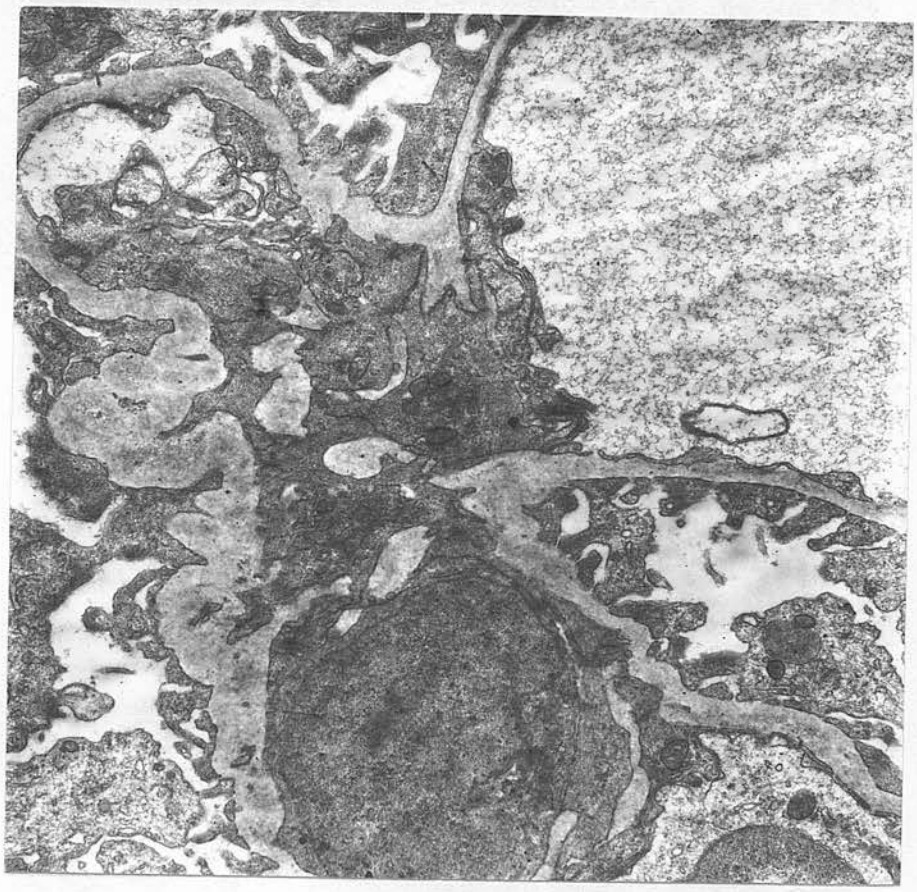


Plate 18: Mesangial zone from newly diagnosed diabetic. Apparent folding of basement membrane in tissue NOT cut in true cross section. Case 21. X 10,000.

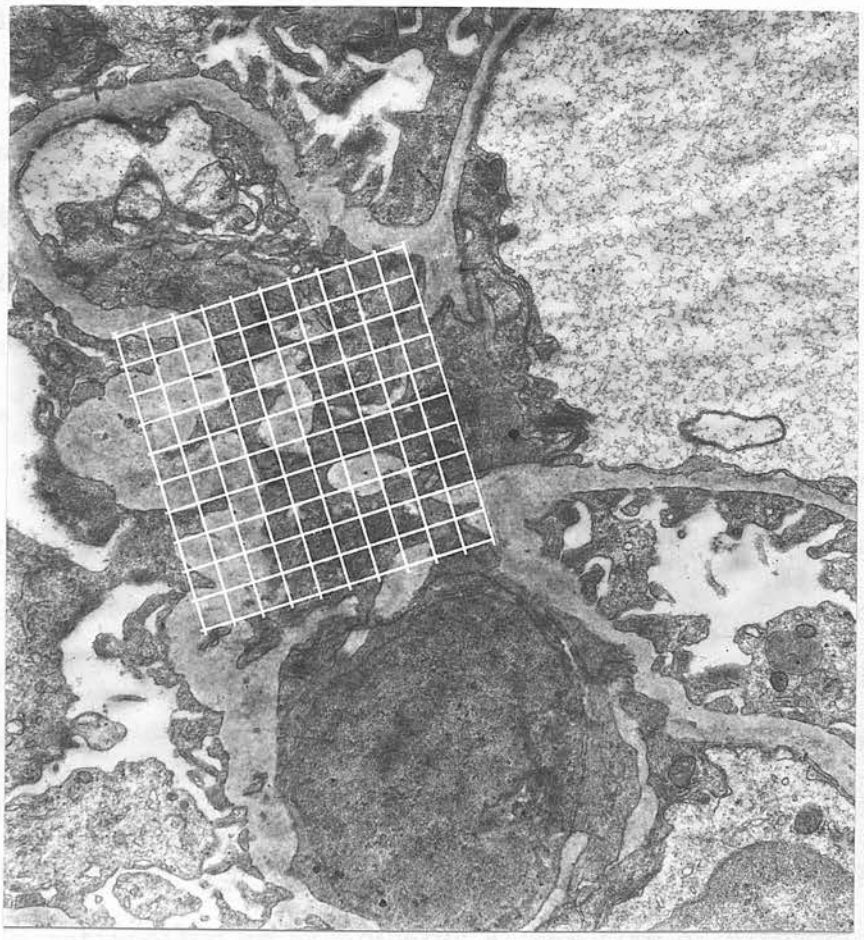


Plate 19: Same as Plate 18. With 100 square grid in mesangial zone.

mesangial cell ultrastructure (Plates 17 and 18).

Discussion:

Electron microscopy examination has been used in several studies in which the glomerular capillary basement membrane was reported to be thickened in recently diagnosed diabetic and pre-diabetic patients (Goetz, Hartmann and Lazarow, 1960; Daysog, Dobson and Brennan, 1961; Sabour, MacDonald and Robson, 1962; Rees, Camerini-Davalos, Caulfield, Lozano-Casteneda, Cattelier, Pometta, Cervantes-Amezcura, Krautthammer, and Marble, 1964). Although these findings have been cited in favour of a genetic basis for diabetic microangiopathy, the techniques used and control observations were often inadequate. In a careful study, Østerby-Hansen (1965) found no difference in glomerular capillary basement membrane thickness between recent diabetics and non-diabetics.

Although this is a small series of patients, the findings, both in terms of basement membrane thickness and mesangial index, support the view that morphological changes do not precede the onset of clinical juvenile diabetes. On the other hand, some patients who are found to be diabetic in later life may have diabetic retinopathy or nephropathy at the time of diagnosis. In such cases, however, the possibility that an undetected disturbance of glucose tolerance has been present for many years cannot be overlooked.

CHAPTER 8.

The effect of pituitary ablation on the renal arteriolar and glomerular lesions in diabetes.

"A suspicion that the abolition of pituitary function cannot prevent the progression of extensive diabetic angiopathy was illustrated by a case I published in 1953. This patient, who was born in 1915 and acquired diabetes at the age of 9, developed a Sheehan syndrome after delivery in 1945. In the same year 2nd degree retinopathy was diagnosed and then regressed.

From 1951 the patient received substitution therapy consisting of cortisone and thyroid. She died in uraemia in 1960. If the favourable effects of ablation of pituitary function were caused by hormonal changes, a protective influence upon vascular areas other than the retina would also have been expected after spontaneous cessation of pituitary function".

Jacob E. Poulsen, 1967.

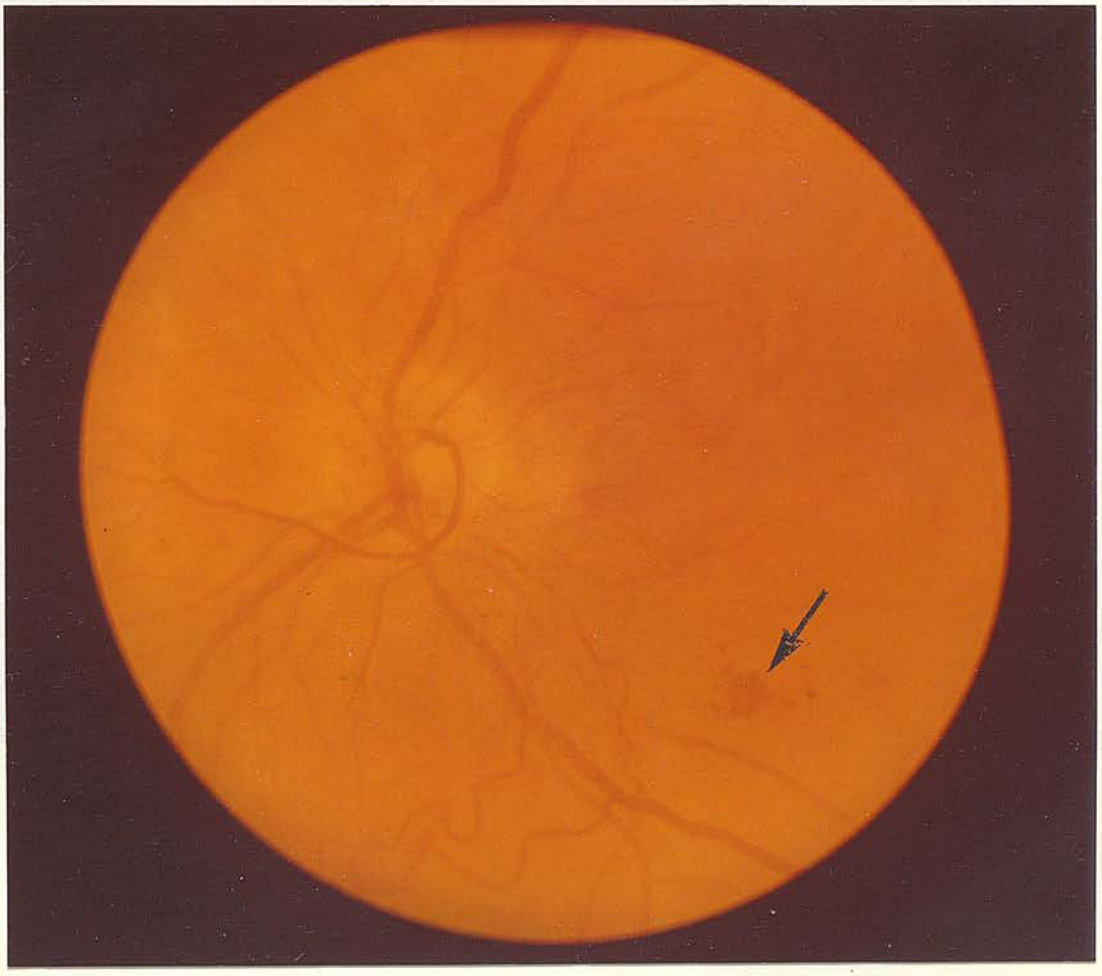
Although the studies of Houssay and Biasotti (1926) showed that some of the metabolic aspects of experimental diabetes mellitus are influenced by the anterior pituitary, the relationship of this gland to the pathogenesis of such specific complications of human diabetes as retinopathy and glomerulosclerosis remains uncertain. Improvement in retinopathy has occurred in many diabetic patients subjected to pituitary destruction, but it is not known whether the procedure simply ameliorates the vascular changes in the retina or favourably

influences the more widespread small blood vessel disorder. The light and electron microscopy findings in renal tissue obtained by biopsy from diabetic patients in whom the pituitary was ablated because of progressive retinopathy are described in this section.

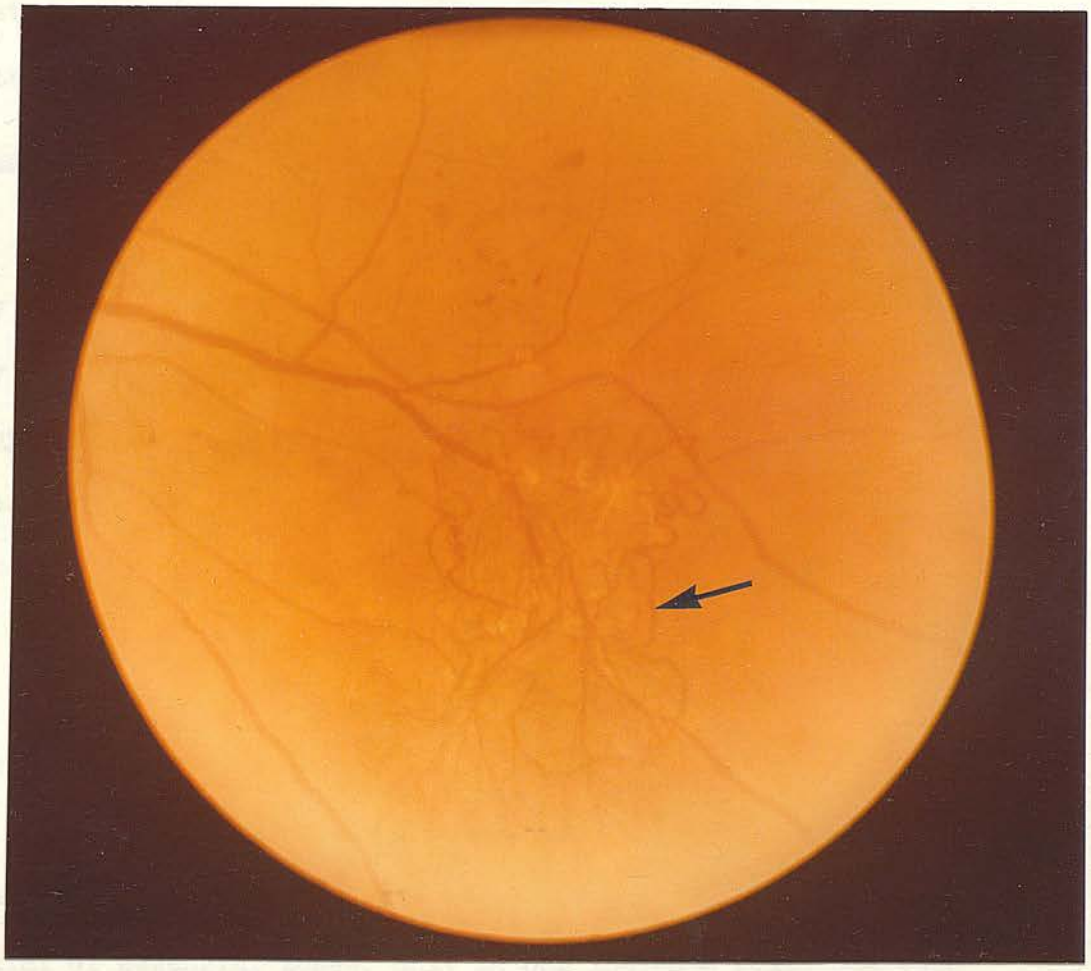
Material and Methods

The clinical data of the six patients studied are summarised in Table 6. All patients had long-standing, insulin-dependent diabetes and the progressive nature of their predominantly proliferative retinopathy had been confirmed by serial retinal photography (Colour plates 8 and 9). No patient was hypertensive, and there was no clinical or subsequent evidence by biopsy of pyelonephritis or other non-diabetic renal disease. Proteinuria was minimal except in Case 36 whose renal function was poor. Three patients were treated by pituitary stalk section and three by implantation of radioactive-90-Yttrium.

Renal tissue was obtained by percutaneous biopsy a few days before pituitary operation and repeated one to two years later. At each biopsy two cores of renal cortex were obtained; the centre portion of each was prepared for light microscopy study and cubes from either end were processed and embedded in Araldite for examination and measurement of the glomerular capillary basement membrane thickness by electron microscopy as previously described. Four glomeruli were examined from each biopsy sample, and the mean capillary basement membrane thickness was calculated with standard



Colour plate 8: Retinal photograph of proliferative diabetic retinopathy. Note large numbers of fine new vessels growing outwards from disc. Distended veins. Only one small patch of microaneurysms (arrowed).
Case 35.



Colour plate 9: Retinal photograph of proliferative diabetic retinopathy. Delicate meshwork of preretinal new vessels.

deviations both for individual glomeruli and the whole sample. Variance between glomeruli within samples and the differences between samples and groups of samples could thus be analysed statistically.

Results

The effectiveness of pituitary destruction was demonstrated by the fall in post-operative insulin requirements (Table 6) and by the need for endocrine replacement therapy with cortisone and thyroxine in all patients except Case 34. This patient's insulin dosage was not significantly altered and, before the second biopsy, steroid replacement therapy was withdrawn without adverse effect. A metopirone test showed appreciable anterior pituitary function.

The renal changes found on light microscopy are summarised in Table 7.

In all patients there was diffuse thickening of the glomerular capillaries. Although this was frequently most prominent in the axial or mesangial zones, only in one instance (Case 33) were early Kimmelstiel-Wilson nodules definitely seen. Because the degree of diabetic glomerulosclerosis is difficult to evaluate by the light microscope, only the measurements determined by electron microscopy have been used to compare the pre- and post-operative glomerular changes.

The arteriolar structure was normal in Case 35 only, and in Cases 31 to 34 there was variable afferent and efferent arteriosclerosis; Case 36, who had clinical evidence of renal decompensation, had advanced arteriolosclerosis, glomerular

Clinical data of the six patients studied

Case no.	Age (yrs.)	Sex	Duration of diabetes (yrs.)	Method of pituitary ablation	Preoperative data					Postoperative data			
					B.P.	Creatinine clearance	Proteinuria (24 hrs.)	Daily insulin requirements	Interval after pituitary surgery	B.P.	Creatinine clearance	Proteinuria (24 hrs.)	Daily insulin requirements
31	35	F	17	Stalk section	160/80	44 ml./min.	0.1 gm.	44 U.	1 yr.	150/90	60 ml./min.	0.1 gm.	24 U.
32	29	M	15	Stalk section	150/100	95 ml./min.	0.3 gm.	54 U.	18 mos.	180/110	70 ml./min.	1.2 gm.	36 U.
33	45	M	26	Stalk section	130/78	80 ml./min.	<0.1 gm.	80 U.	18 mos.	125/70	90 ml./min.	<0.1 gm.	24 U.
34	32	M	18	Implantation of Yttrium-90	130/90	170 ml./min.	3 gm.	70 U.	2 yrs.	200/105	42 ml./min.	2.3 gm.	60 U.
35	39	F	20	Implantation of Yttrium-90	150/80	125 ml./min.	<0.1 gm.	40 U.	16 mos.	140/80	110 ml./min.	<0.1 gm.	12 U.
36	53	M	12	Implantation of Yttrium-90	140/80	48 ml./min.	4.3 gm.	26 U.	2 yrs.	135/80	16 ml./min.	7.5 gm.	Nil

Table 6: Clinical data of 6 patients studied.

Renal changes seen on light microscopy

Case no.	Preoperative renal biopsy	Postoperative renal biopsy
31	Moderate diffuse glomerulosclerosis. Moderate arteriolosclerosis.	No change
32	Moderate diffuse glomerulosclerosis. Moderate arteriolosclerosis.	Advanced arteriolosclerosis
33	Marked diffuse glomerulosclerosis and early nodular glomerulosclerosis. Moderate arteriolosclerosis.	Mild diffuse glomerulosclerosis. No change in arteriolar lesions
34	Moderate diffuse glomerulosclerosis, hyalinization and moderate arteriolosclerosis.	Increased glomerular hyalinization and marked arteriolosclerosis
35	Early diffuse glomerulosclerosis. Minimal arteriolosclerosis.	No change
36	Marked diffuse glomerulosclerosis, glomerular hyalinization. Interstitial fibrosis. Marked arteriolosclerosis.	Increased glomerular hyalinization and arteriolosclerosis

Table 7: Renal changes on light microscopy.

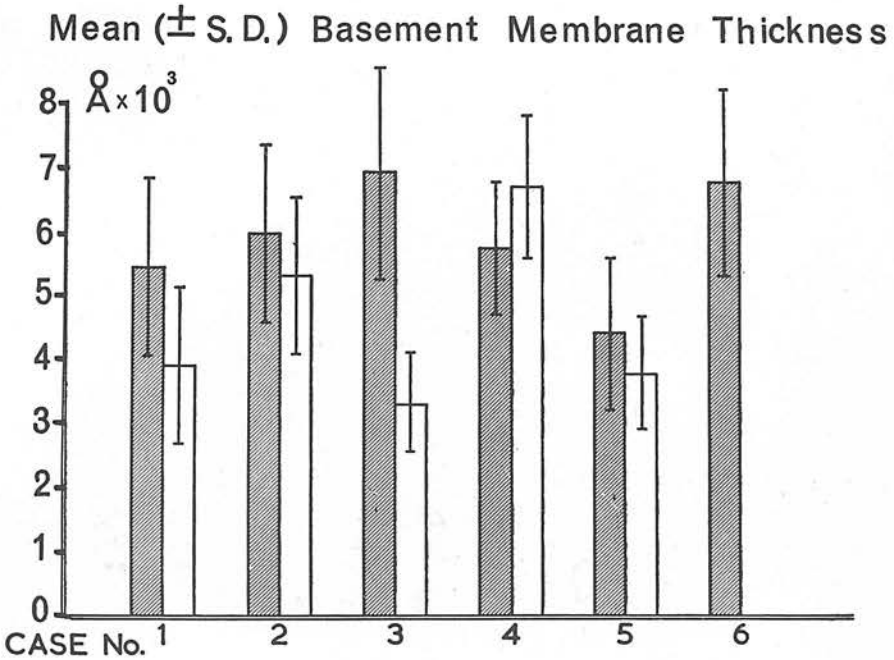
hyalinisation, interstitial fibrosis and tubular atrophy. No improvement was detected in the arteriolar lesions following pituitary surgery, and in Cases 32, 34 and 36 the changes had advanced. If Case 34, whose pituitary had not been adequately ablated is excluded, this finding suggests that the renal arteriolar lesion is unaffected by pituitary destruction.

Case 36 died of renal failure one year after the second biopsy. At post-mortem glomerular hyalinisation and advanced diffuse glomerulosclerosis were found (Colour plate 10, p118 see also colour plates 1-7 in Chapter 2). Exudative lesions were present in many glomeruli, and some of those less severely affected had typical Kimmelstiel-Wilson nodules which differed from the exudative lesions in their staining and situation. Arteries of all calibres had hyperplastic intimal fibrocellular thickening, and hyalinisation of the arteriolar walls was conspicuous. Despite these advanced lesions there were no changes in the heart consistent with hypertension.

The pre-operative measurements of the peripheral glomerular capillary loops are summarised in Table 8. The over-all mean glomerular capillary basement membrane thickness for individual patients ranged from 4,360 Å to 6,800 Å, the mean for the group being 5,600 Å. The mean thickness in the non-diabetic subjects similarly measured was 1,400 Å to 2,700 Å (Chapter 5). Thus there was diffuse thickening of the peripheral capillary walls in all. The relatively large standard deviations in these results (Table 8) indicate that the thickening of the basement membrane of the

Case no.	Peripheral glomerular capillary basement membrane thickness: Angstrom units										
	Glomerulus 1		Glomerulus 2		Glomerulus 3		Glomerulus 4		Total number of measure-ments	Over-all mean of all measure-ments	
	Mean	±1 S.D.	Mean	±1 S.D.	Mean	±1 S.D.	Mean	±1 S.D.			±1 S.D.
31	5,620	820	4,720	1,620	5,680	1,260	5,400	1,390	237	5,320	1,400
	3,670	1,100	3,300	835	4,360	1,460	3,560	430	273	3,780	1,200
32	5,850	1,625	5,650	1,420	6,730	1,460	5,800	1,030	131	5,870	1,430
	5,080	1,450	5,025	900	5,640	1,040	5,060	1,200	181	5,200	1,220
33	6,130	1,800	6,700	1,100	7,700	1,800	6,660	1,600	190	6,800	1,650
	2,625	700	3,230	680	3,310	525	3,380	670	102	3,200	700
34	5,840	1,000	5,375	1,100	5,600	825	5,720	750	134	5,625	1,020
	6,825	870	6,160	930	6,840	940	6,550	1,050	128	6,600	1,150
35	4,650	1,025	5,320	875	4,220	960	3,900	940	368	4,360	1,170
	3,330	670	3,760	700	3,850	675	3,875	855	608	3,650	875
36	7,420	1,270	6,330	1,310	6,480	1,450	6,275	1,525	154	6,680	1,470

Table 8: Peripheral glomerular capillary basement membrane thickness, in Angstrom units, before (upper figures) and after (lower figures) pituitary ablation, with the exception of case 36 where no post-operative data were obtained.



Mean glomerular capillary basement membrane thickness ± S.D. The hatched columns represent the pre-operative results and the clear columns the post-operative results for individuai patients.

Fig. 10:

glomerular capillaries occurred irregularly. But the absence of significant variance between the measurements from glomeruli from the same biopsy sample indicates also that the diabetic glomerular lesion occurs diffusely throughout the kidney and that the values in this study are representative of these changes.

The measurements made from the biopsies obtained one to two years after the pituitary surgery are also summarised in Table 8 and Fig. 10. Although adequate renal cortex was obtained from Case 36 for light microscopy examination, no glomeruli were found in the tissue prepared for electron microscopy. With the exception of Case 34, where the lesion had advanced, the post-operative mean basement membrane thickness was significantly less in the peripheral capillaries of the remaining patients when analysed as a group.

Thus comparing the pre- and post-operative results in Cases 31, 32, 33 and 35 as a group, using both the Mann-Whitney 'U' test and the Kolmogorov-Smirnov test the differences were significant ($p < 0.008$ and $p < 0.01$ respectively). However on comparing the results in individual patients, i.e. 4 pre-operative glomeruli with 4 post-operative glomeruli, the results were significant in Cases 31 and 33 only (See Figs. 11 and 12).

Comparison of the glomerular ultrastructure in the tissue obtained before and after pituitary ablation showed significant differences. Before surgery, in addition to irregular thickening of the peripheral capillary loops, there was variable accumulation of basement membrane material within the axial or mesangial zones of the glomerular tuft. The mesangial cells, which lie deep within

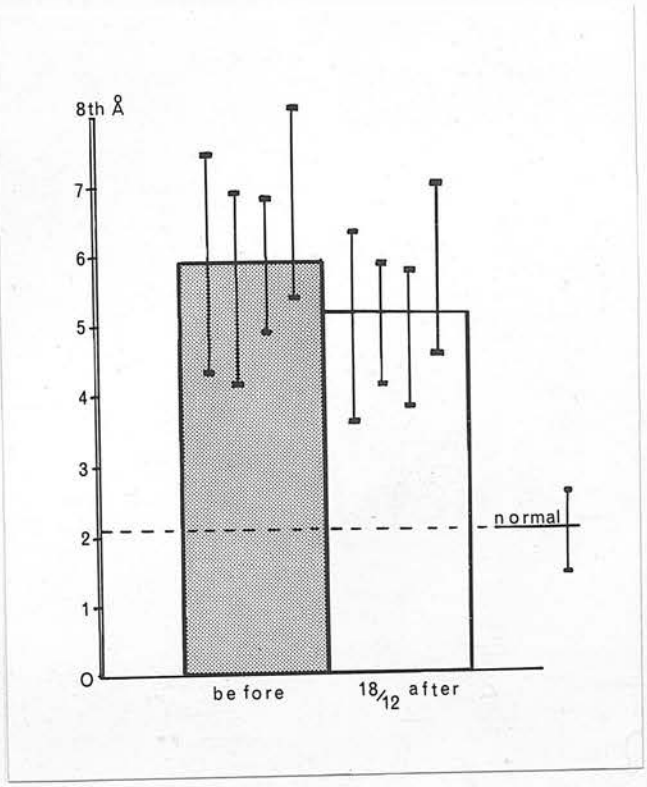


Fig. 11: Pre- and post-operative mean basement membrane thickness. Vertical lines represent standard deviations in 4 glomeruli from each biopsy. Lack of significant difference between pre- and post-operative results. Case 32.

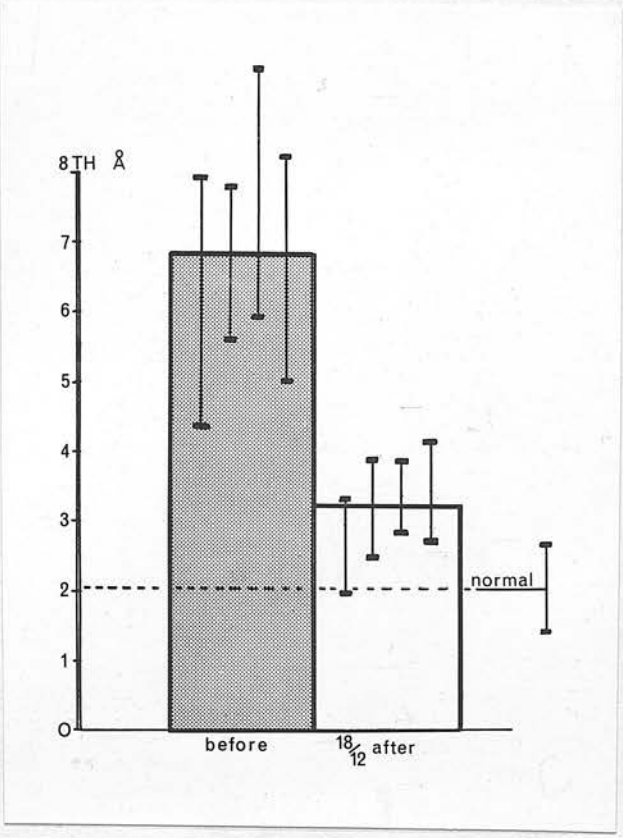
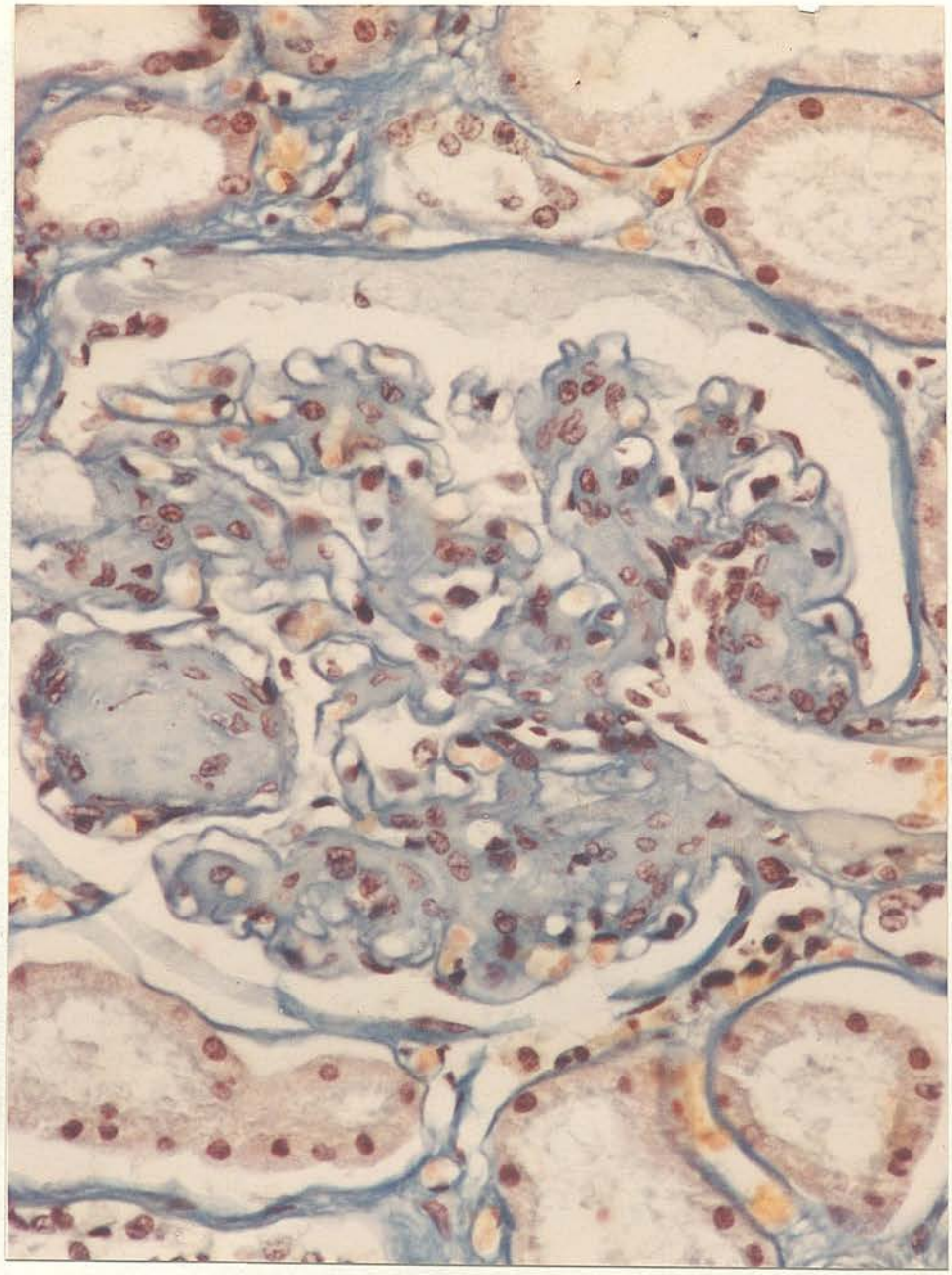


Fig. 12: As Fig. 11. Showing significant difference between pre- and post-operative results. Case 33.



Colour plate 10: Diffuse and nodular glomerulosclerosis. Case 36 at P.M. M.S.B. stain. X 500.

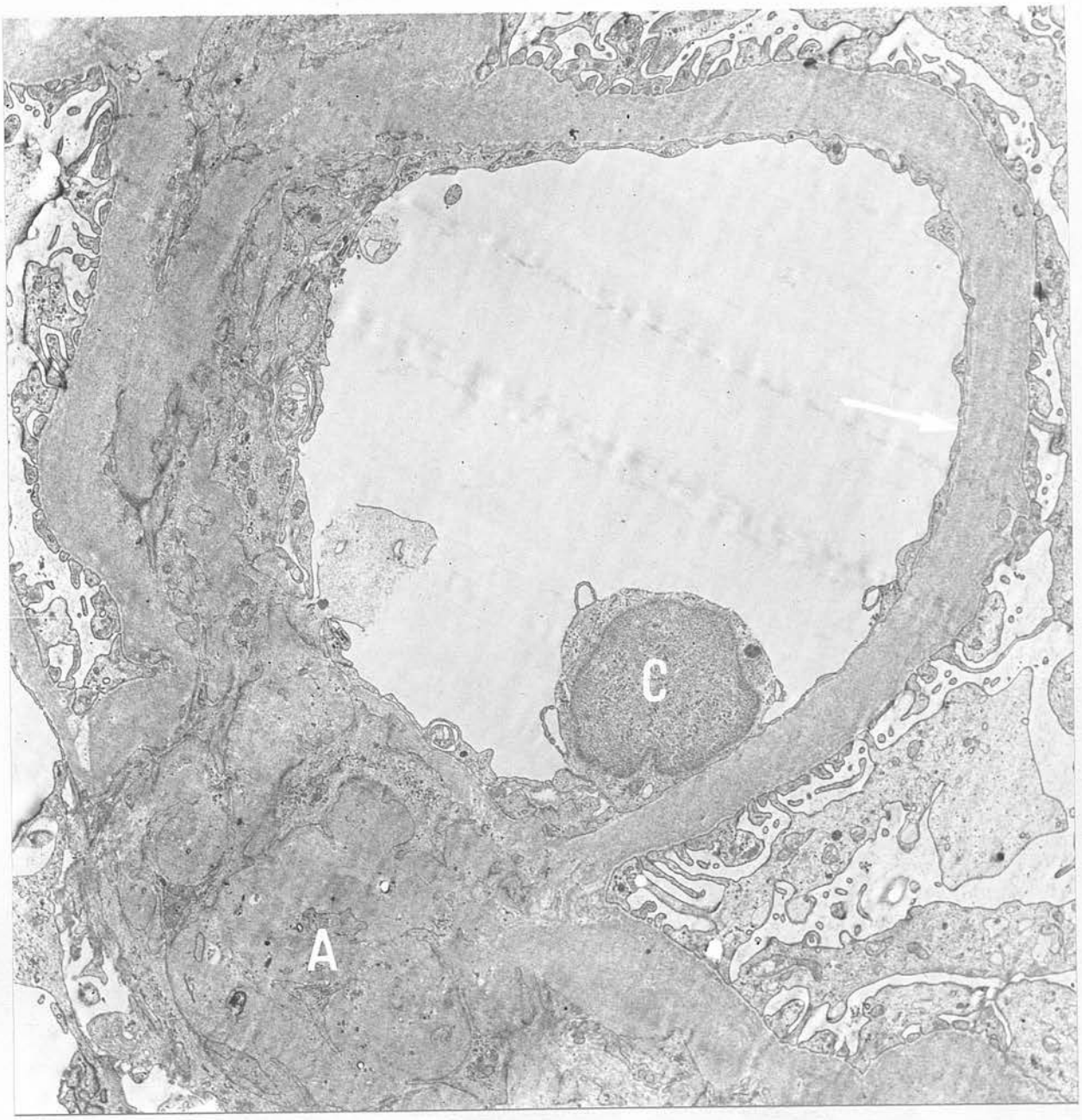


Plate 20: Glomerular capillary loop from pre-operative specimen. Basement membrane accumulation in the mesangium or axial zone (A) with compression of mesangial cytoplasm. Adjacent endothelial cell (C) and attenuated endothelial cytoplasm (arrowed). Case 31.

X 17,000.

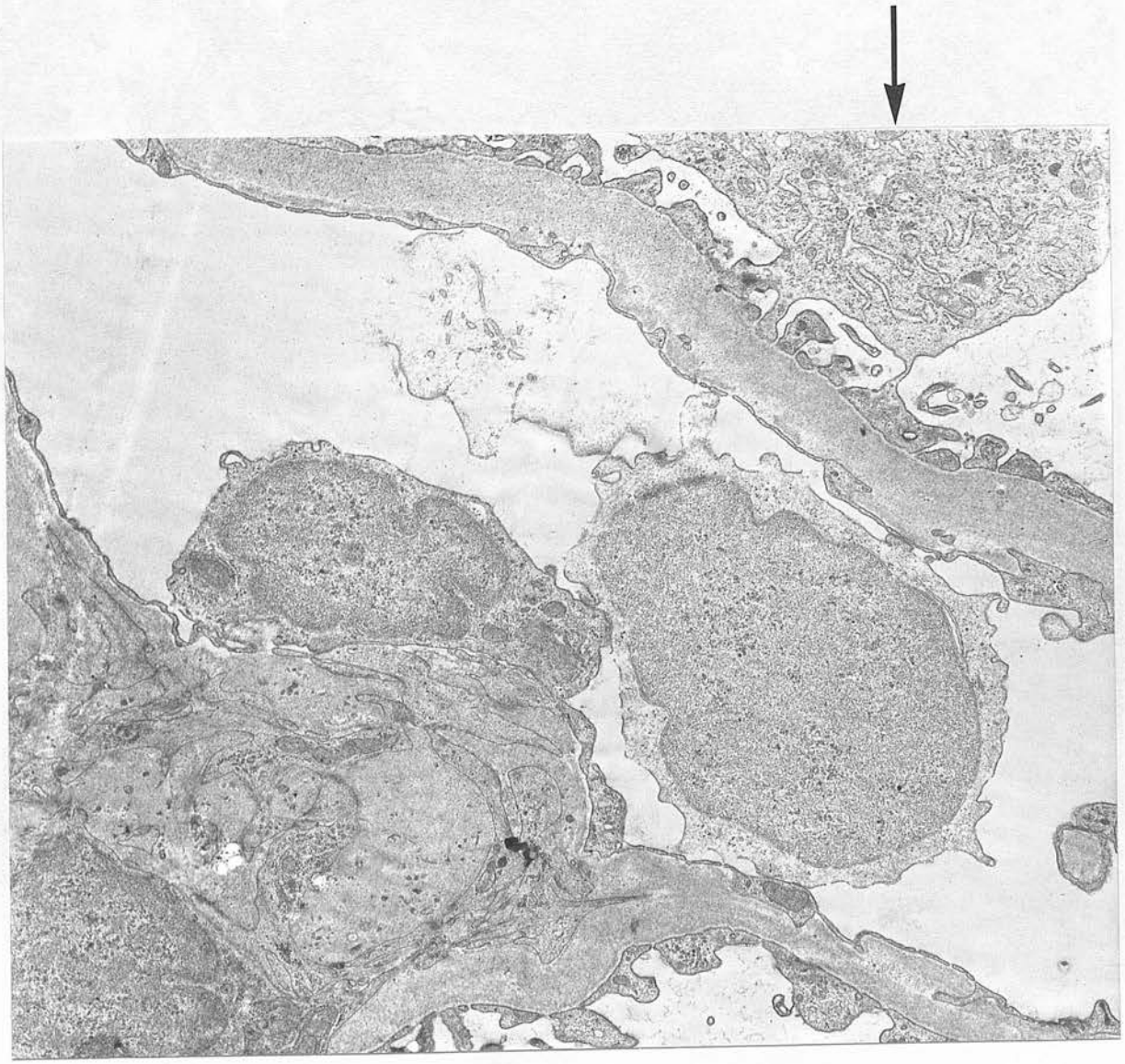


Plate 21: Glomerular capillary loops from a pre-operative specimen. Basement membrane accumulation in mesangium. Contrast attenuated cytoplasm with prominent epithelium (arrowed). Case 1. X 15,000.

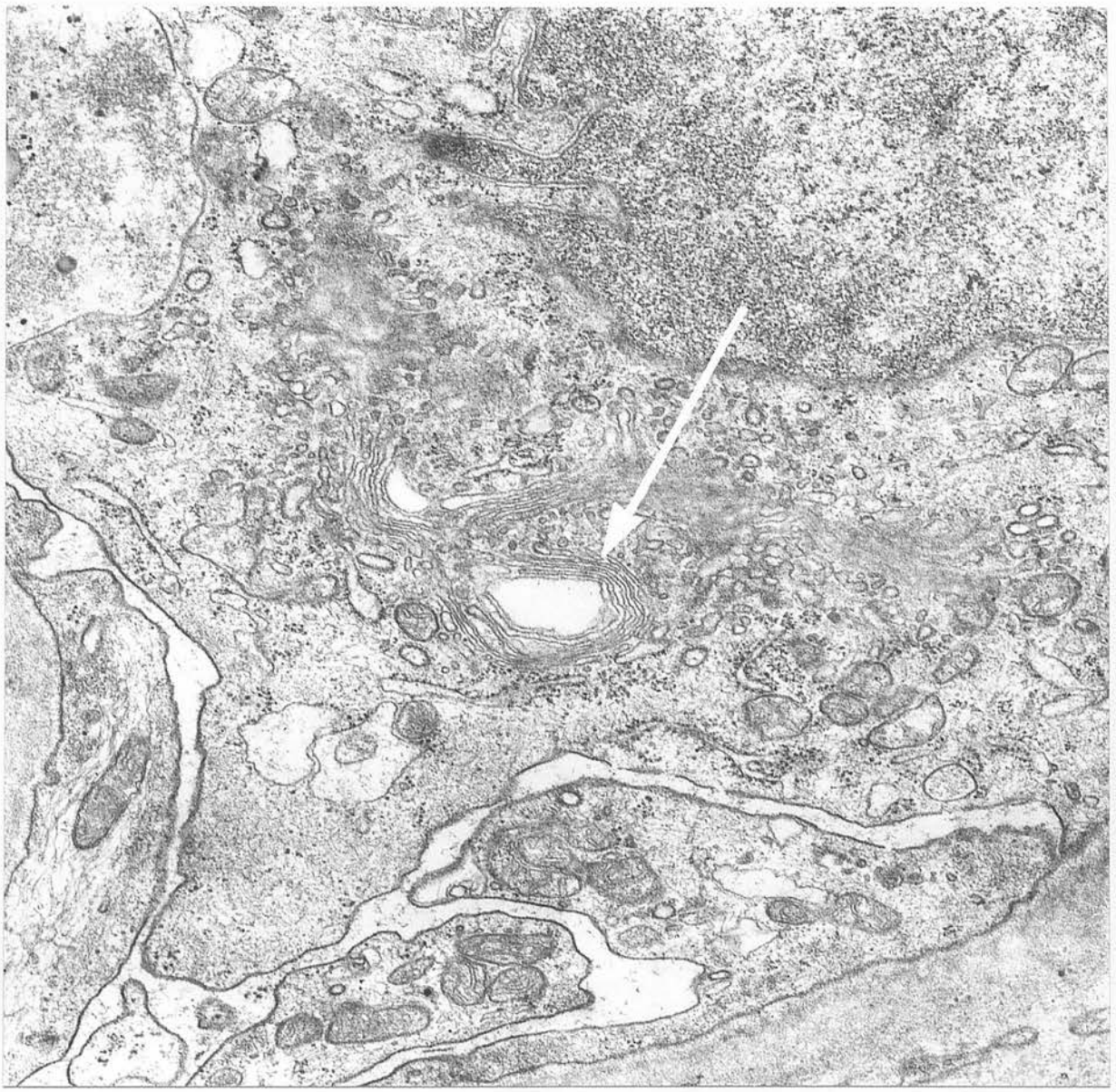


Plate 22: Epithelial cell showing cytoplasmic details including Golgi zones (arrowed). From a pre-operative specimen. Case 35. X 30,000.

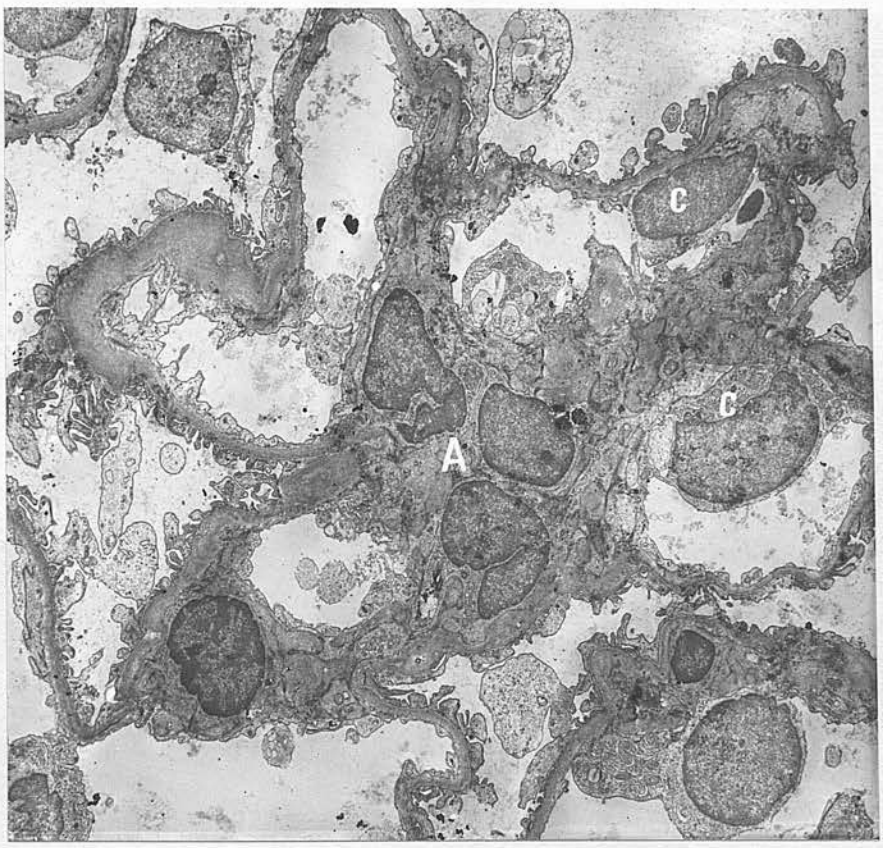


Plate 23: Post-operative specimen showing proliferation of cells in mesangium (A) and of endothelial cells (C). Case 35. X 3,500.

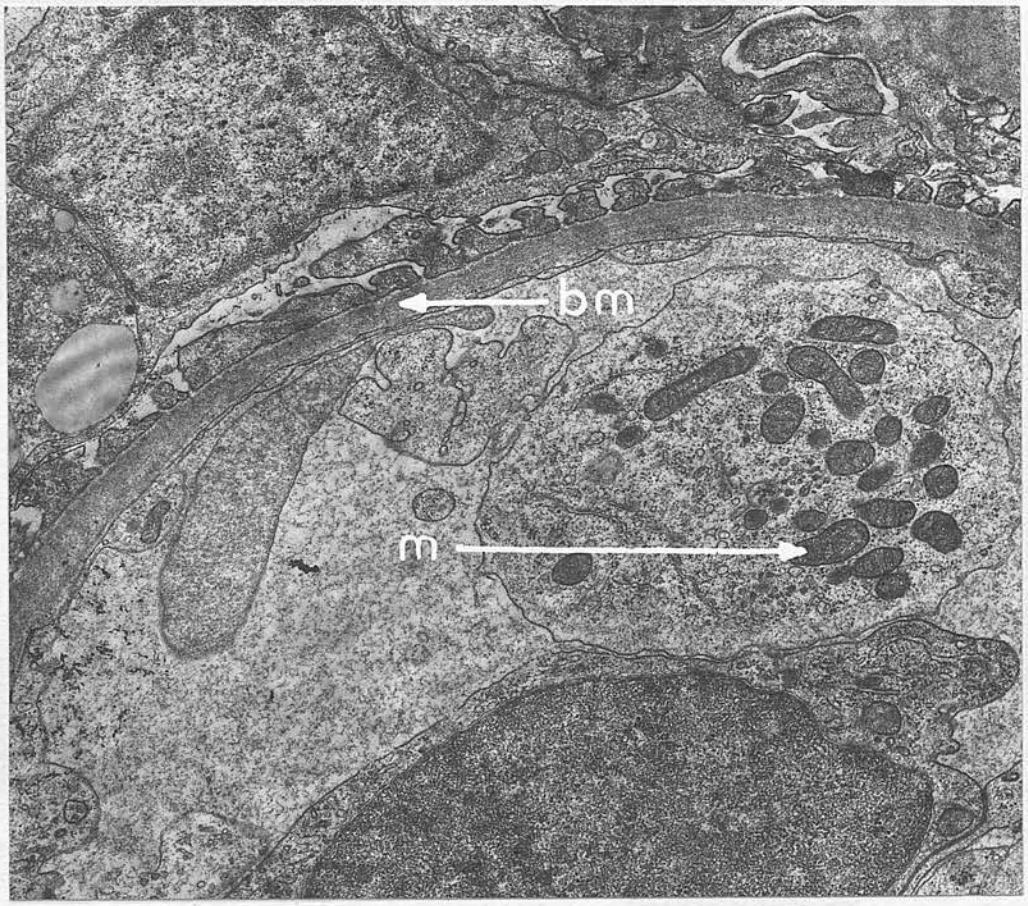


Plate 24: Post-operative specimen showing detail of basement membrane (bm) and numerous mitochondria in endothelium (m). Case 35. X 14,000.

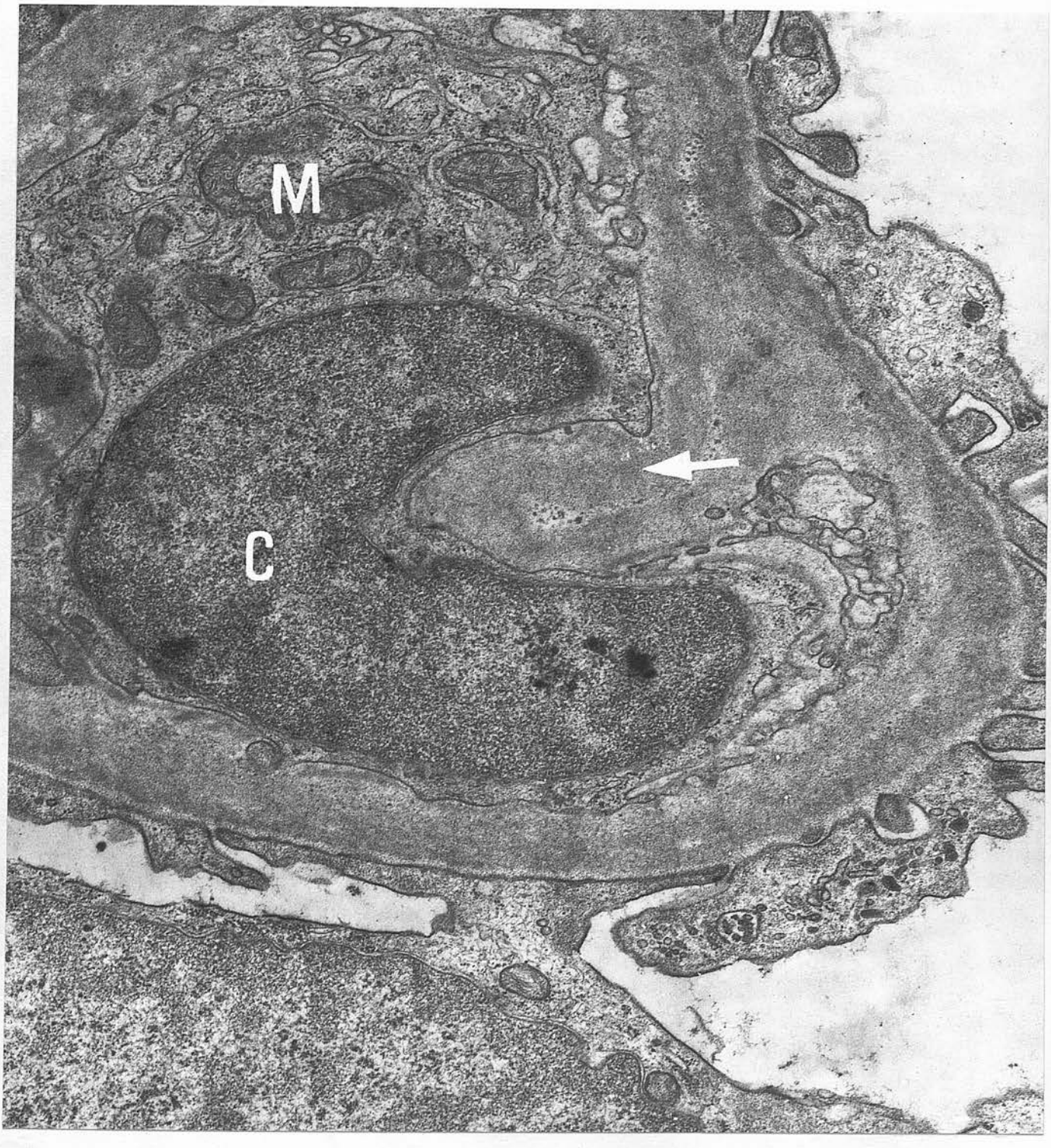


Plate 25: Endothelial cell with prominent cytoplasm containing many mitochondria occupying capillary lumen and engulfing basement membrane (arrowed). Post-operative specimen. Case 35. X 40,000.

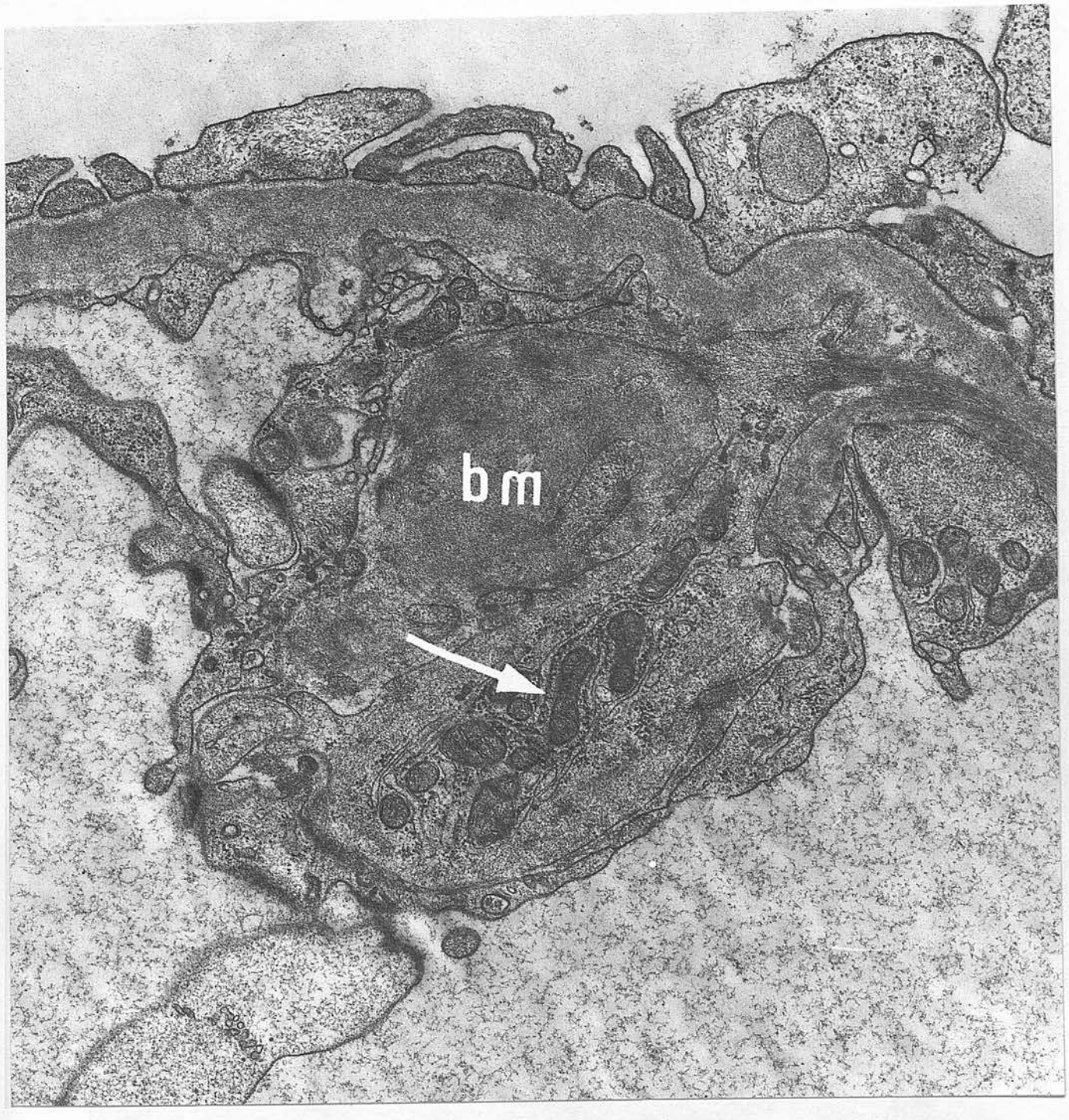


Plate 26: Peripheral capillary loop from post-operative specimen showing basement membrane substance (bm) within endothelial cytoplasm which also contains mitochondria and RNA-studded endoplasmic reticulum (arrowed). Case 35.

X 30,000.

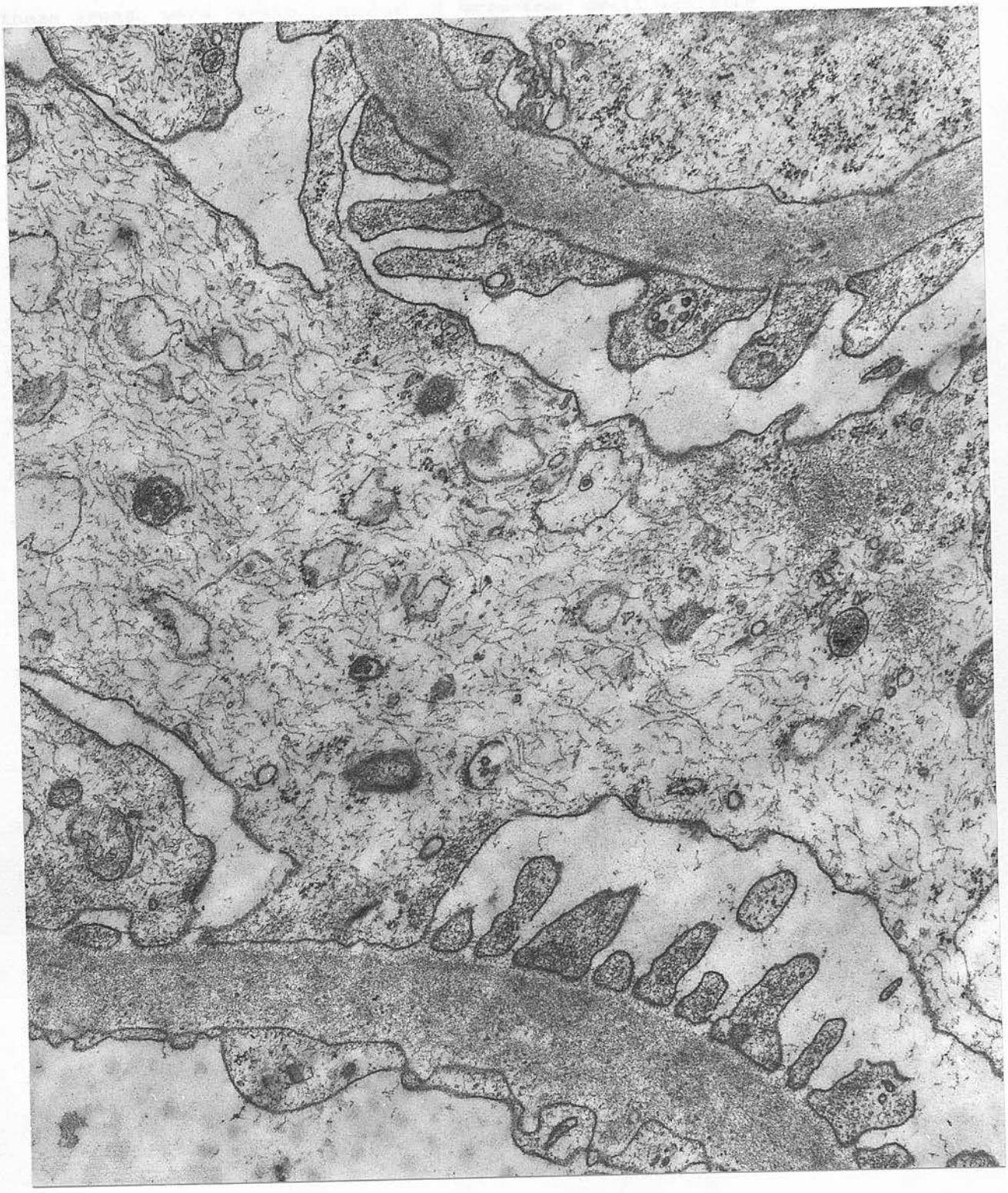


Plate 27: Epithelial cytoplasm showing vacuolation and lack of formed elements, in a post-operative specimen. Case 35. X 24,000.

these areas, were rarely composed of more than small isolated aggregates of attenuated cytoplasm, usually lacking in such formed elements as mitochondria, endoplasmic reticulum and Golgi zones; frequently the nuclei were crenated or fragmented (Plates 20 and 22). The endothelial cells, situated within the capillary lumen close to the mesangial zone, were only occasionally compressed within the basement membrane material; nevertheless, their cytoplasm was attenuated and lacking in formed elements (Plate 20). In contrast, the epithelial cells contained healthy nuclei and the cytoplasm, rich in mitochondria, RNA-studded endoplasmic reticulum and Golgi zones (Plate 22) encroached upon the urinary space in a manner similar to that found in other diabetics (Chapter 9) and patients having diabetes secondary to pancreatic disease (Chapter 6). The mitochondria were usually elongated and cylindrical; the endoplasmic reticulum extended throughout the cytoplasm and appeared to be organised into a system of channels with numerous ribosome particles studding their surface or occasionally lying freely in the ground substance.

In the tissue obtained after pituitary destruction there was striking restoration of the normal structure of the nuclei and cytoplasm of the mesangial cells (Plate 23). At the same time the cytoplasm of the endothelial cells was greatly increased. Frequently it was seen to engulf basement membrane substance both in the mesangial zones and peripheral capillary loops and in these areas was rich in mitochondria, RNA-studded endoplasmic reticulum

and Golgi zones (Plates 25 and 26). No definite difference in the epithelial structures was detected other than occasional areas where the cytoplasm appeared vacuolated (Plate 27).

Discussion

These findings, after successful pituitary ablation, of improvement in the glomerular capillary lesion without change in that of the arterioles suggest that the two may develop independently and not necessarily due to the same cause.

On electron microscopy, unequivocal thickening of the glomerular capillary basement membrane was found in association with abundant epithelial cytoplasm containing prominent mitochondria, RNA-studded or rough-surfaced endoplasmic reticulum and Golgi zones. Indeed, abundant and healthy epithelial cytoplasm would appear to be as characteristic of the diabetic capillary lesion as basement membrane thickening. The evidence that this prominent epithelium is concerned with increased intra-cellular production and deposition of basement membrane material is discussed in detail in Chapter 11.

In contrast to the epithelial cells the deep mesangial cells contained crenated nuclei and atrophic cytoplasm within irregular accumulations of basement membrane substance and the endothelial cytoplasm was attenuated and lacking in formed elements in the pre-operative biopsies. Farquhar (1964), from a study of the turnover of ferritin particles, suggested that the mesangial cells have a phagocytic function which distinguishes them from endothelial cells. She has further suggested that the increase in basement membrane

in the glomeruli of diabetics might be due to its defective removal by the mesangial cells. Whether the mesangial cell lesion is a consequence of the accumulation in the axial region of basement membrane which leads to their compression and dysfunction, or arises independently to cause further basement membrane accumulation and apparent compression is uncertain. In support of the latter view is the finding, in the biopsies obtained after pituitary ablation, of striking changes indicative of return of activity in the mesangial and endothelial cells in association with reduction in both peripheral and mesangial basement membrane substance, suggesting that the mesangial and endothelial cells of the glomerulus are functionally related and may share phagocytic activity. While these results support Farquhar's hypothesis concerning defective removal of basement membrane in diabetes, it is, however, improbable that alteration in mesangial and endothelial cell structure and function is the only or primary aetiological defect in diabetic nephropathy, since peripheral basement membrane thickening may be present without there being apparent abnormality in the mesangial or endothelial cells. Thus in glomeruli from patients having diabetes secondary to pancreatic disease, and in long-standing insulin-dependent diabetics without retinopathy (Chapter 9), significant peripheral basement membrane thickening was frequently evident despite healthy mesangial and epithelial cells.

It is therefore possible that in those diabetics who develop proliferative retinopathy and nephropathy some pituitary-dependent

factor inhibits the activity of the mesangial and endothelial cells (as is mirrored by changes in their morphology) which leads to alteration in the capillary basement membrane. Pituitary destruction would remove the depressant influence on the mesangial and endothelial cells, restore their structure and activity and thus, indirectly, cause some improvement in the capillary lesion. It must be stressed, however, that in the patients in this study the renal arteriolar lesions were either unchanged or had progressed after pituitary ablation. Thus all forms of diabetic microangiopathy are unlikely to be pituitary-dependent, and this may explain why Poulsen (1966) and others (Graef, 1966) reported that pituitary destruction does not beneficially influence the progressive clinical course of diabetic renal disease. If the latter is predominantly due to glomerular ischaemia and hyalinisation consequent upon the arteriolar lesion or pyelonephritis, then no improvement could be expected. Moreover, deterioration was not reversed in Case 36 who had advanced arteriolar and glomerular lesions before pituitary ablation. On the other hand some long-term clinical benefit might be obtained in diabetics whose pre-operative renal biopsy has revealed minimal arteriolar changes and the glomerular lesions are of the pituitary-dependent cellular and basement membrane type described above.

CHAPTER 9.

A comparison of renal biopsies in age-and duration-matched insulin dependent diabetics with, and without retinopathy.

> "It is quite clear that the longer diabetes exists, the worse its angiopathy becomes. This is one fact on which all clinicians and pathologists agree".
>
> A.R. Colwell, 1964.

The striking changes seen in mesangial cell morphology following successful pituitary ablation (Chapter 8) prompted the development of a technique for measuring mesangial morphology, namely the mesangial index. Since the aim of pituitary ablation is to improve diabetic retinopathy, it seemed that examination of renal tissue from long-standing diabetics without retinopathy might be of value in identifying morphological features of significance in preventing advanced diabetic small blood vessel disease.

In this section the electron microscopy findings in renal biopsy tissue from five long-standing insulin-dependent diabetics without either retinopathy or other clinical complications are compared with those of an equal number of age- and duration-matched patients having advanced proliferative retinopathy.

Material and Methods

The patients were selected on the basis of routine ophthalmoscopic examination while attending the diabetic clinics. Whereas advancing retinopathy is brought to the clinician's attention on account of the complaint of deteriorating vision, unfortunately careful searching is necessary in selecting long-standing diabetics

without retinopathy or such other clinical evidence of angiopathy as digital capillary disease. The clinical data concerning the patients studied are summarised in Table 9. Percutaneous renal biopsies were obtained and prepared as previously described (Chapter 4). At least three glomeruli from each biopsy sample were examined by electron microscopy and both peripheral basement membrane thickness and mesangial index were measured. Two of the biopsies from the group of patients having retinopathy were the pre-operative samples from cases described in Chapter 8 (Cases 31 and 32).

Results

In Table 10 and Figure 13 the results of the overall mean glomerular capillary basement membrane thickness and mesangial index are summarised. With the exception of Case 45 in whom the basement membrane thickness was not significantly outwith the normal range, all the remaining patients whether with or without retinopathy were found to have significant basement membrane thickening. Although the results in the retinopathy group exceeded the others, the differences were not significant. Thus in the Mann-Whitney 'U' test $p < 0.21$. On the other hand the mesangial index was significantly less in the group without retinopathy ($p < 0.01$ in both the Mann-Whitney 'U' and Kolmogorov-Smirnov tests).

The morphological features in the patients without retinopathy are demonstrated in plates 28 to 31 and in plates 3, 4, 6

pp 137 et seq. pp 41, 42 & 45

Case No.	Sex	Age (years)	Age of onset of diabetes	Duration of diabetes. (years)
Without Retinopathy				
41	F	34	6	28
42	M	24	9	15
43	M	28	9	19
44	M	36	19	17
45	M	57	34	23
Mean Values		35	15	20
Proliferative Retinopathy				
31	F	35	18	17
32	M	29	14	19
37	F	33	4	29
38	F	45	19	26
39	M	31	9	22
Mean Values		34	13	21

Table 9: Clinical data concerning idiopathic diabetics: Cases 41 - 45 without retinopathy. Cases 31, 32, 37, 38 & 39 having advanced retinopathy.

Case No.	Peripheral glomerular capillary basement membrane thickness (Angstrom Units) Mean of 3 glomeruli	± 1 S.D.	Mesangial Index Mean	± 1 S.D.
Without Retinopathy				
41	5640	850	40	12
42	5700	560	44	9
43	6540	1300	50	10
44	5780	990	41	8
45	3200	520	35	12
Mean values	5470	780	42	10
With Retinopathy				
31	5320	1400	72	14
32	5870	1430	66	11
37	6230	1320	58	9
38	6800	1600	59	14
39	7640	1260	71	11
Mean values	6370	1400	65	12

Table 10: Mean glomerular capillary basement membrane thickness (B.M.T.) ± I.S.D. and Mesangial Index ± I.S.D. in diabetics with, and without retinopathy.

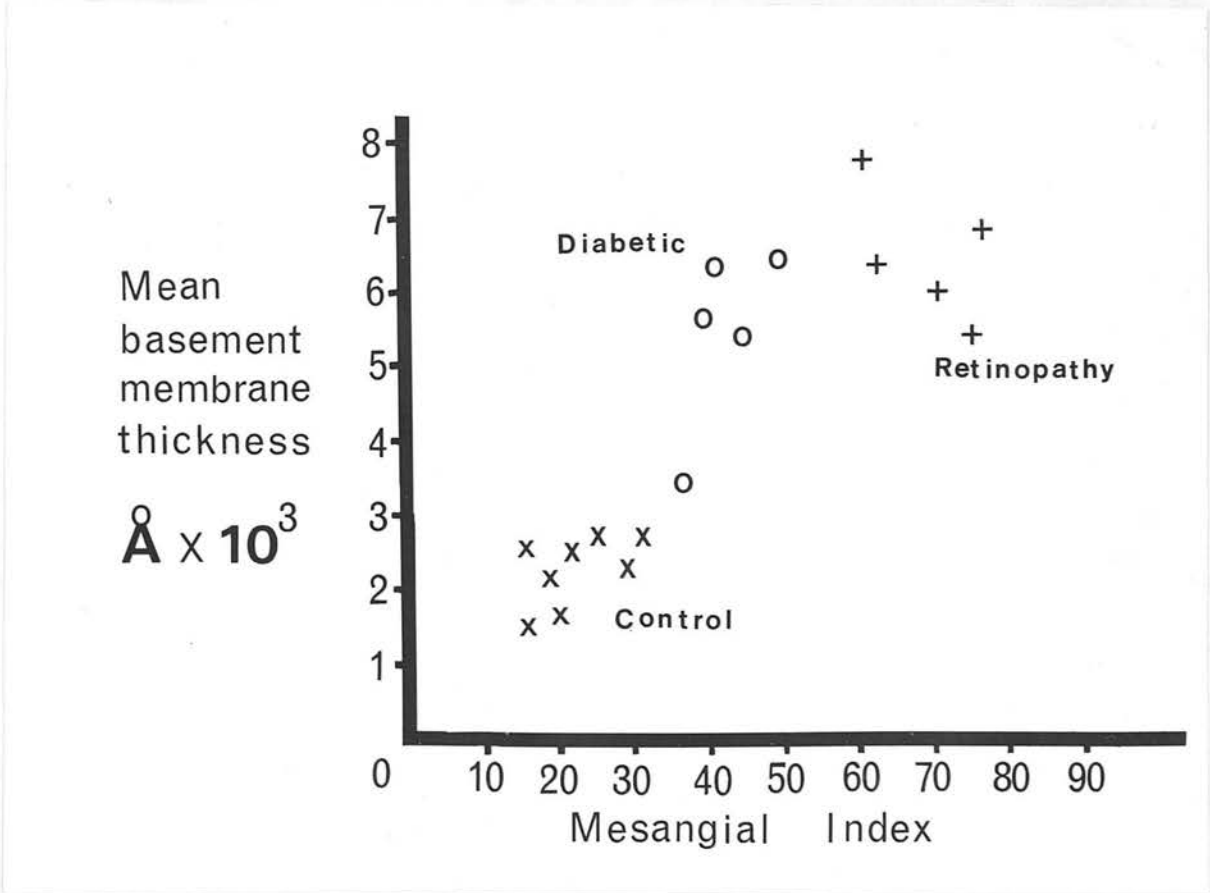


Fig. 13: Mean basement membrane thickness and mesangial index in diabetics without retinopathy (O) and with retinopathy (+).

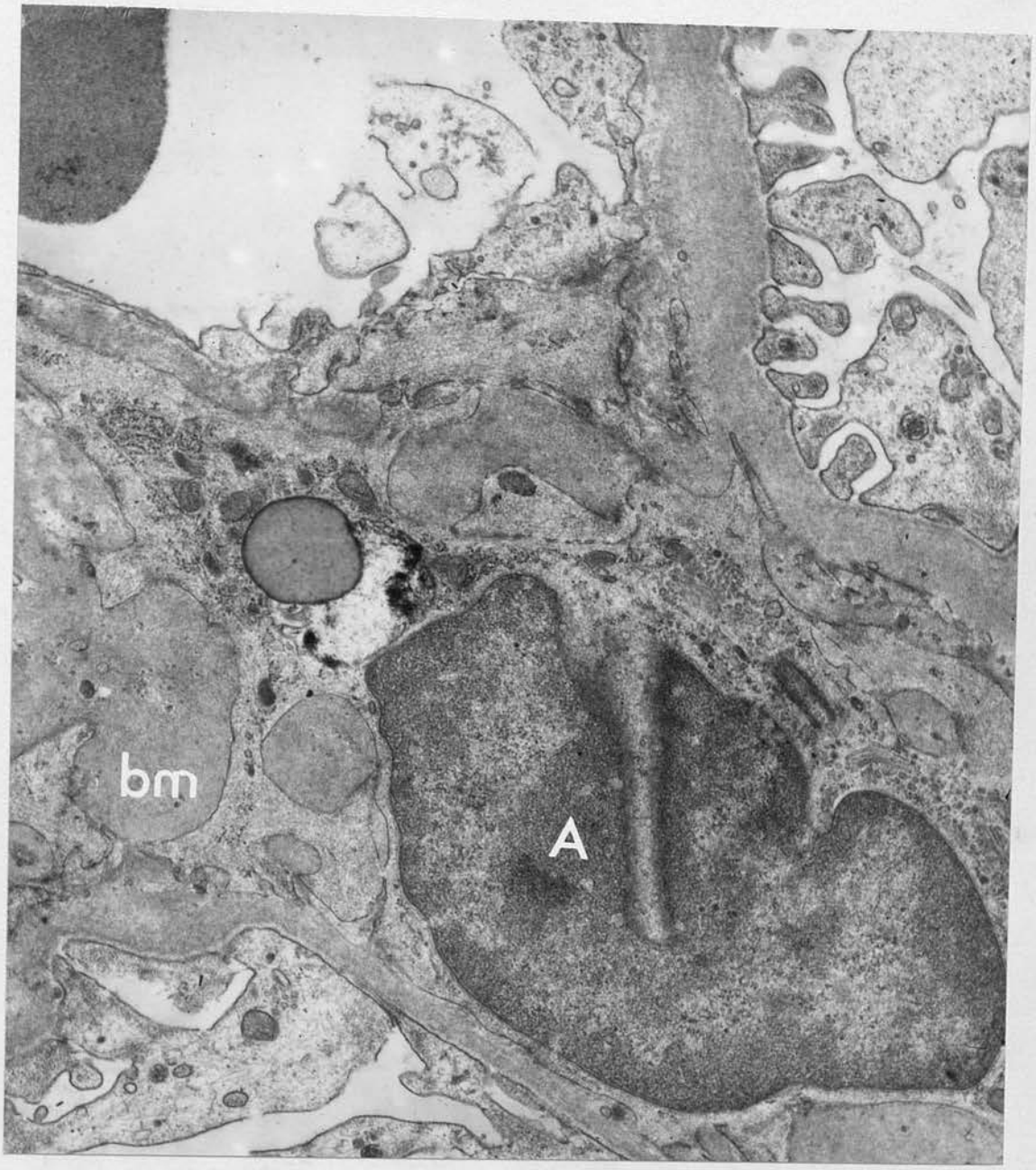


Plate 28: Mesangial zone showing mesangial cell (A) and loose-textured basement membrane (bm). Idiopathic diabetic without retinopathy. Case 42. X 20.000.

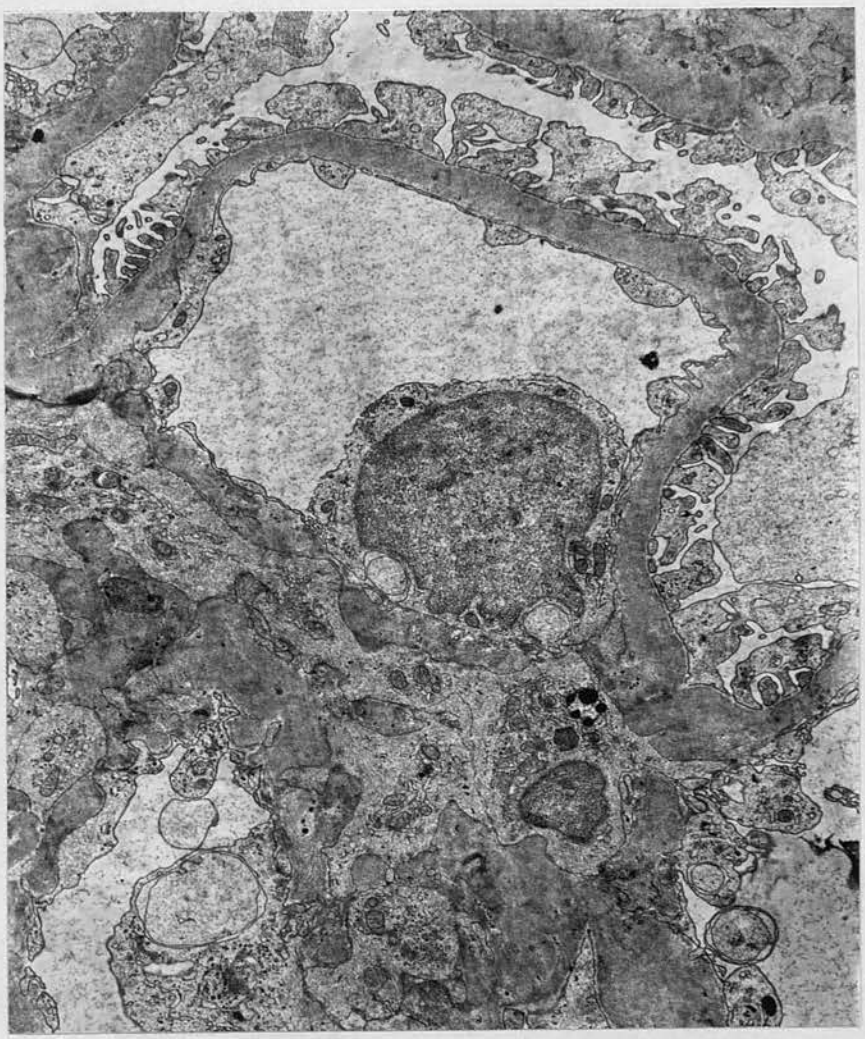


Plate 29: Capillary loop showing peripheral basement membrane thickening in idiopathic diabetic without retinopathy. Note healthy mesangial cytoplasm in lower half of plate. Case 43. X 8,000.

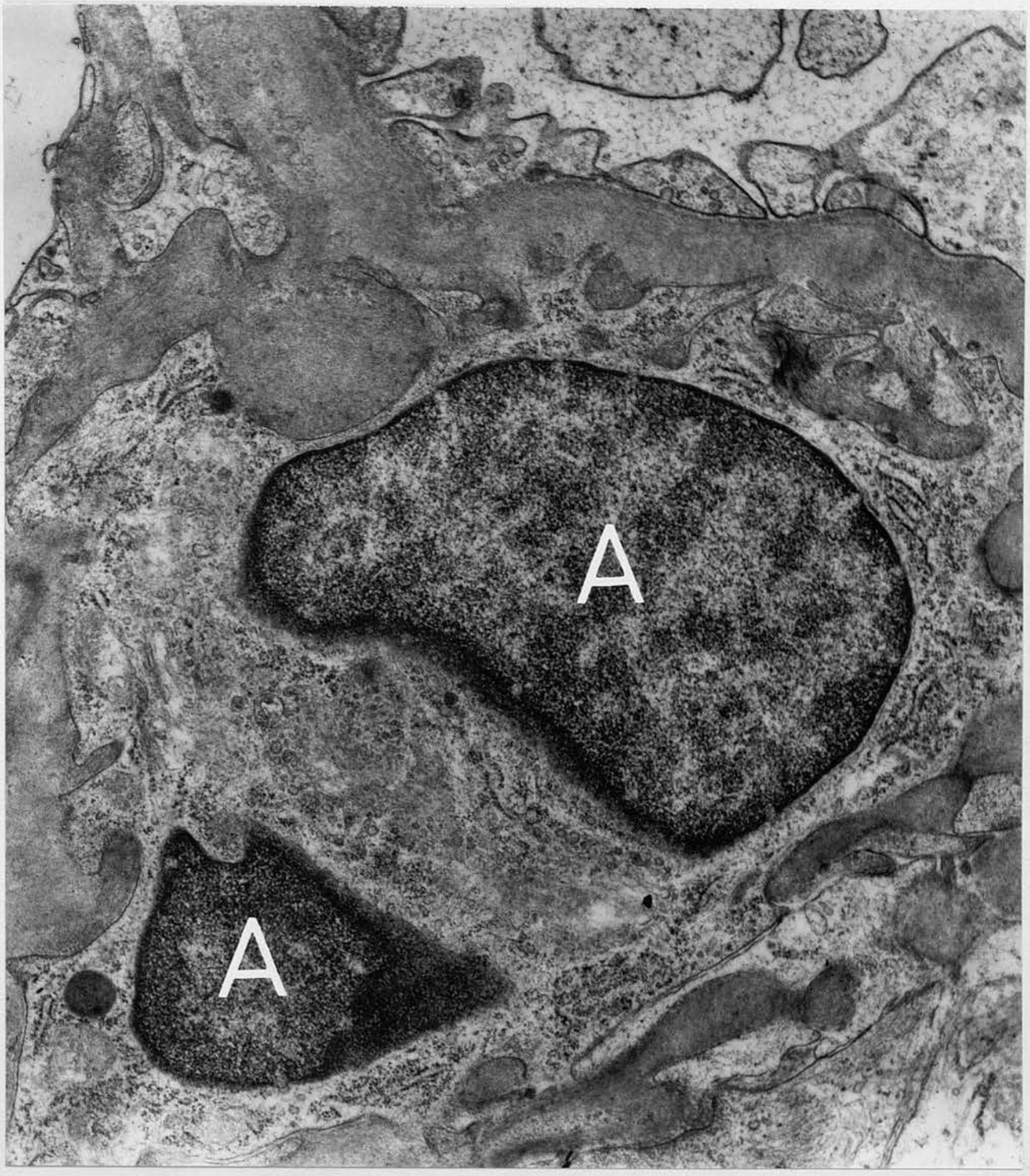


Plate 30: Healthy mesangial cell (A), (A), showing active cytoplasm containing well developed RNA-studded endoplasmic reticulum. Diabetic without retinopathy. Case 44. X 35,000.

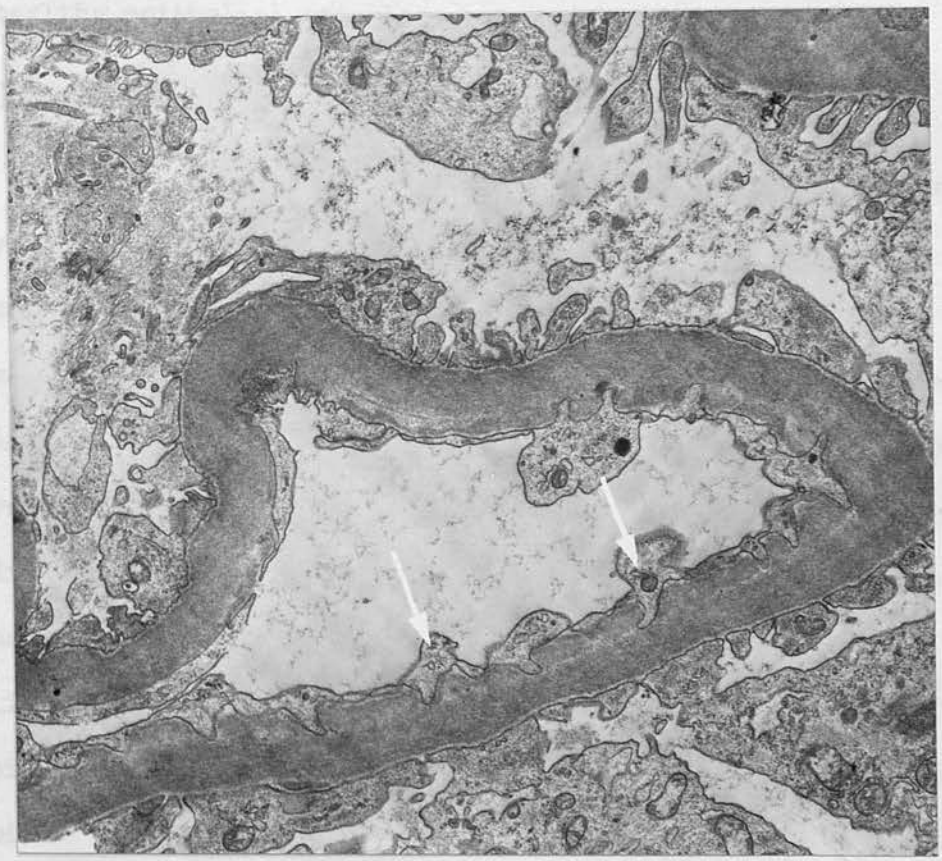


Plate 31: Capillary loop from idiopathic diabetic without retinopathy. Note indentation of thickened basement membrane in areas adjacent to endothelium (arrowed). Case 41. X 10,000.

(Chapter 3) and in plate 10 (Chapter 4). In all, well preserved mesangial and endothelial cell cytoplasm was a characteristic feature despite peripheral basement membrane thickening. This contrasted with accumulation of basement membrane material and attenuation of mesangial cytoplasm in those having retinopathy (Plates 32 and 33, and plates 5 and 7 in Chapter 3). However, in both groups healthy epithelial cytoplasm was prominent.

Discussion

That one of these 5 patients, having been diabetic for 23 years, was found to have glomerular capillaries within the normal range in gratifying evidence against the basement membrane lesion being an inevitable consequence of long-standing insulin dependence. Although the remaining patients without retinopathy had peripheral basement membrane thickening they were distinguished by having mesangial indices of 50 or less whereas those having retinopathy had mesangial indices of 60 or greater. On the limited basis of this data, in quantitative evaluation of renal biopsies the mesangial index discriminates better between those with and without retinopathy than measurement of basement membrane thickness.

Others have suggested that the mesangial and endothelial cells lay down excess basement in the mesangium and elsewhere (Bloodworth, 1963). Certainly the demonstration of healthy mesangial and endothelial cells, in the presence of peripheral basement membrane thickening could be interpreted in this way .

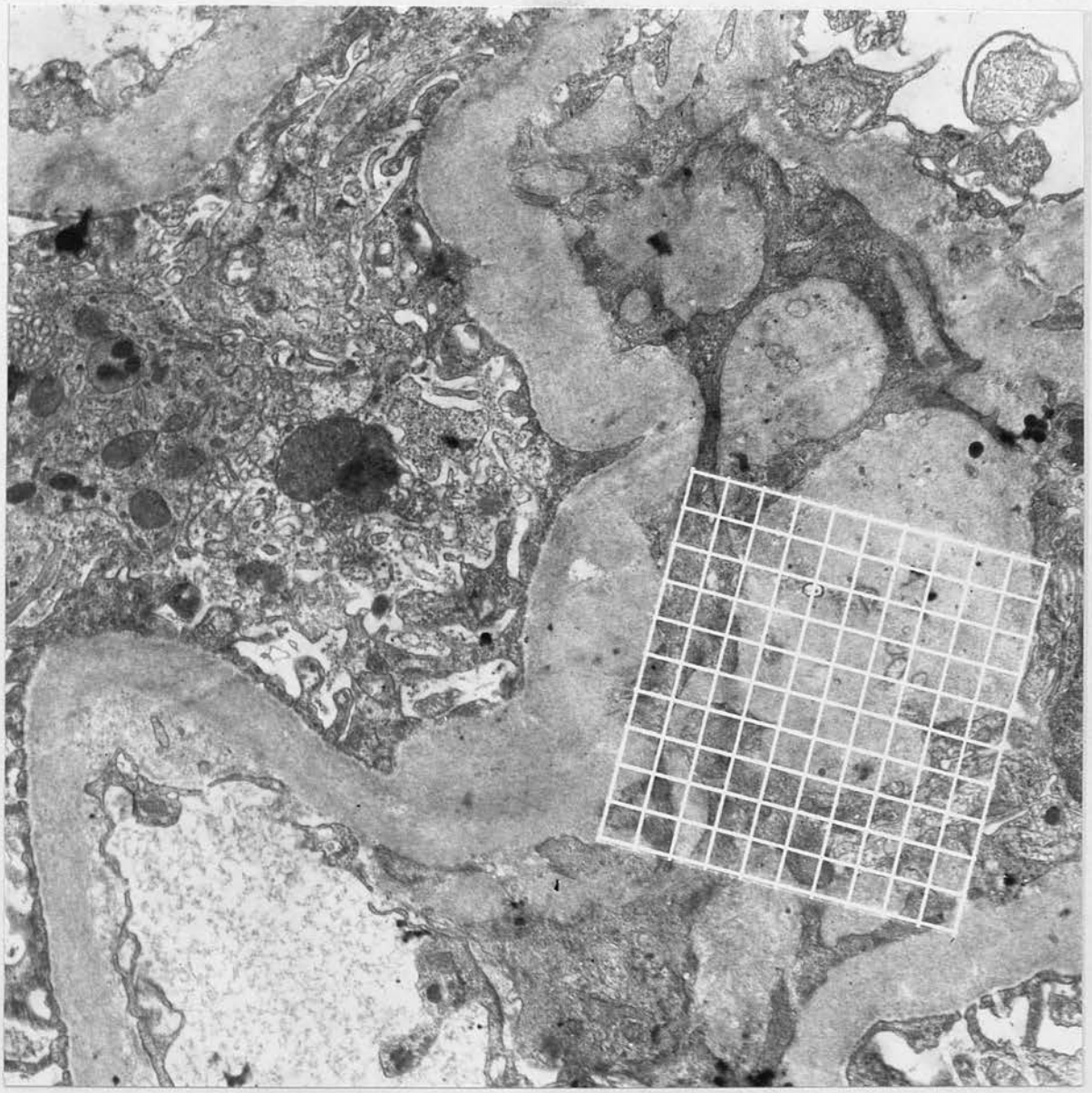


Plate 32: 100 square grid in mesangial zone of idiopathic diabetic having proliferative retinopathy. Note accumulation of basement membrane substance. Case 39.

X 14,000.

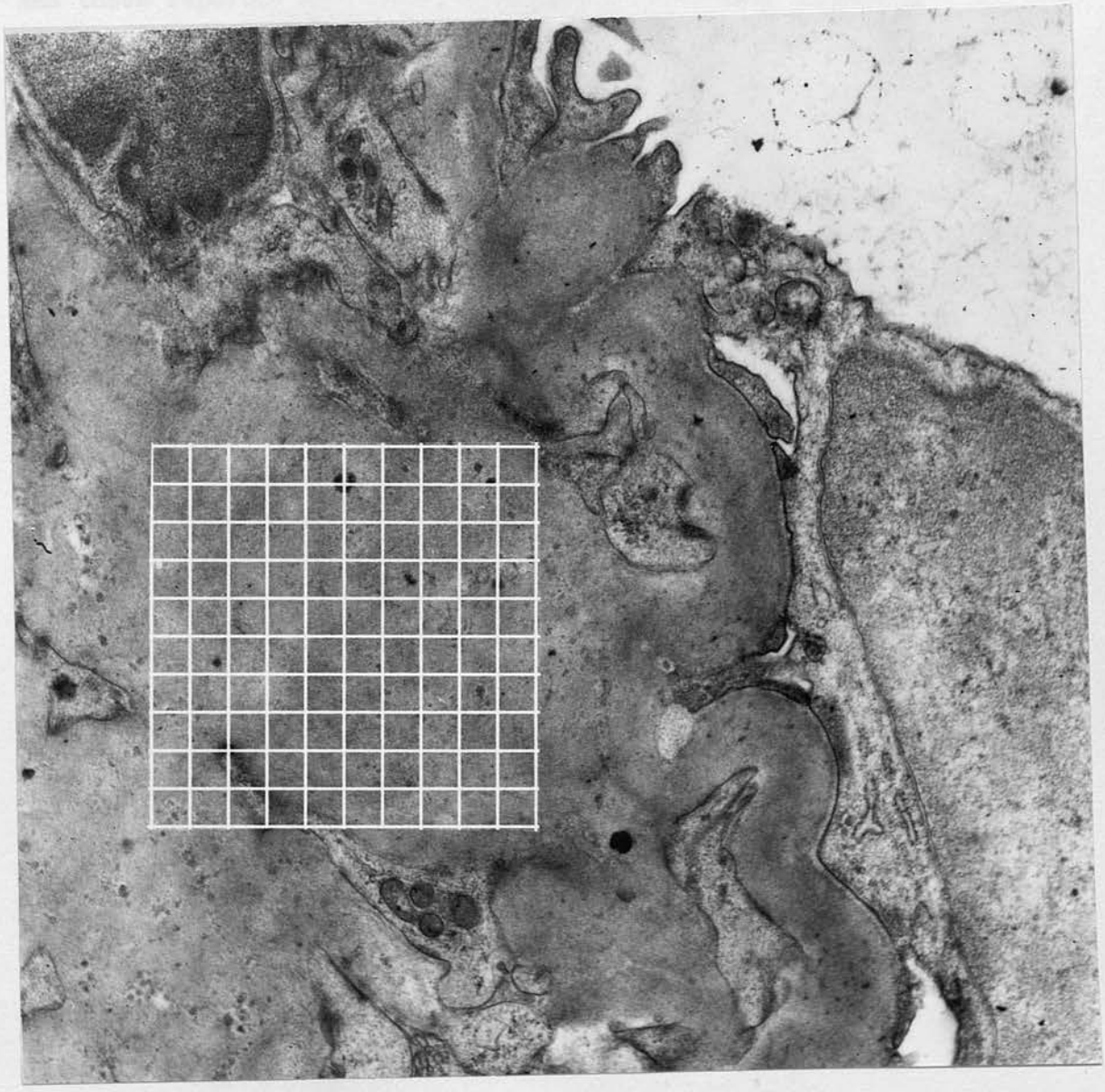


Plate 33: Accumulation of basement membrane in mesangial zone of idiopathic diabetic having retinopathy. Case 31 before pituitary ablation. X 14,000.

However, the findings reported here in those without retinopathy and those reported in Chapter 8 following successful pituitary ablation support the alternative view suggesting a phagocytitic or protective function for these cells (Farquhar, 1964). Whether a low mesangial index will prove to be of good prognostic significance in assessing diabetic patients has yet to be shown. On the other hand, a high mesangial index is the apparent quantitative expression of the renal findings in those with clinical evidence of advanced angiopathy. However this study, and all others so far reported, have failed to give any clear indication of reasons why some patients do, and others do not, develop advanced angiopathy.

CHAPTER 10.

Electron microscopy of the glomerulus in patients having hypertension or proteinuria.

Falstaff: "Sirra, you giant, what saies the doctor to my water?"

Page: "He said, Sir, the water it selfe was a good healthy water : but for the party that ow'd it, he might have more diseases than he knew for".

Henry IV, Part 2. Act 1, Scene II.

Of the clinical accompaniements of diabetic renal disease two in particular, hypertension and albuminuria, may have a special relevance to the basement membrane lesion.

Hypertension

Hypertension is a common consequence of a wide variety of renal diseases. Several authors (Lambie and MacFarlane, 1955; Lundbaek, 1957; Gellman et al., 1959; Hatch et al., 1961; Thomsen, 1965) have considered, on the basis of clinico-pathological studies, whether hypertension is of primary pathological significance in diabetes or a sequal to glomerulosclerosis. All demonstrated a variable relationship, none concluded that hypertension was of primary importance. Furthermore Bell (1953), in an examination of 1465 diabetic autopsies, showed that a large percentage had renal arteriolar lesions without evidence of hypertension in the heart.

So far the author has evaded this issue by confining renal biopsies, as reported in the preceding chapters, to normotensive patients. However the opportunity of examining by light and

electron microscopy renal biopsy tissue obtained from 2 young hypertensive non-diabetic patients without clinical or subsequent biopsy evidence of other underlying renal disease has demonstrated features which contrast sharply with the diabetic lesion.

The patients, Cases 50 and 51, were aged 33 and 36 years respectively. Both presented to the Southern General Hospital on account of severe headache and deteriorating vision, the first to a neurosurgeon and the second to a general physician.

Before treatment, Case 50 had a B.P. of 190/130 mm. mercury, E.C.G. and radiological evidence of left ventricular hypertrophy and bilateral papilloedema. I,V.P. showed normal renal outlines, but function was impaired: creatinine clearance 34 ml. per minute. The urine contained 400 mg. protein per 24 hours, but on microscopy no casts, R.B.Cs or pus cells. Urinary metadrenalines were normal. Selective aortography failed to show vascular stenosis. Light microscopy examination of a percutaneous renal biopsy showed marked hypertensive arteriolar changes, the glomeruli had either shrunken capillary tufts or variable hyalinisation. There was a fine interstitial fibrosis.

Case 51 had, before treatment a B.P. of 185/140 mm. mercury, bilateral papilloedema, E.C.G., but no radiological evidence of left ventricular hypertrophy. I.V.P. and renal aortography were normal. The endogenous creatinine clearance was 74 ml. per minute. The urine contained neither protein nor excess metadrenalines, and

microscopy was normal. Light microscopy of renal biopsy tissue was similar to that of Case 50 but showed only minimal glomerular hyalinisation and less interstitial fibrosis.

On electron microscopy of the glomeruli, the mesangial zones showed shrinkage and wrinkling of the capillary walls (Plate 34). The peripheral capillary loops, although retaining their fine detail of epithelial and endothelial structure were generally collapsed so that the lumenae were considerably narrowed and Bowman's space correspondingly enlarged (Plates 35 and 36). Although not subjected to the number of measurements applied to the other series, 50-70 measurements of peripheral capillary basement membrane thickness gave mean values of 4,750 Å and 5,280 Å in Cases 50 and 51 respectively.

These appearances of the capillary wall in hypertension contrast sharply with the diabetic having basement membrane thickening. Characteristically, the diabetic capillary loop is distended so that Bowman's space is reduced (See Plate 37 and Plate 10 on p.62, Chapter 4).

From these limited observations in hypertensive patients, apparent peripheral basement membrane thickening occurs in the context of wrinkled and shrunken capillary loops whereas in diabetes true basement membrane thickening occurs in generally distended capillaries which characteristically encroach on the urinary, and eventually through mesangial enlargement, on the capillary space. Thus these contrasting features are further evidence against diabetic glomerular capillary disease being

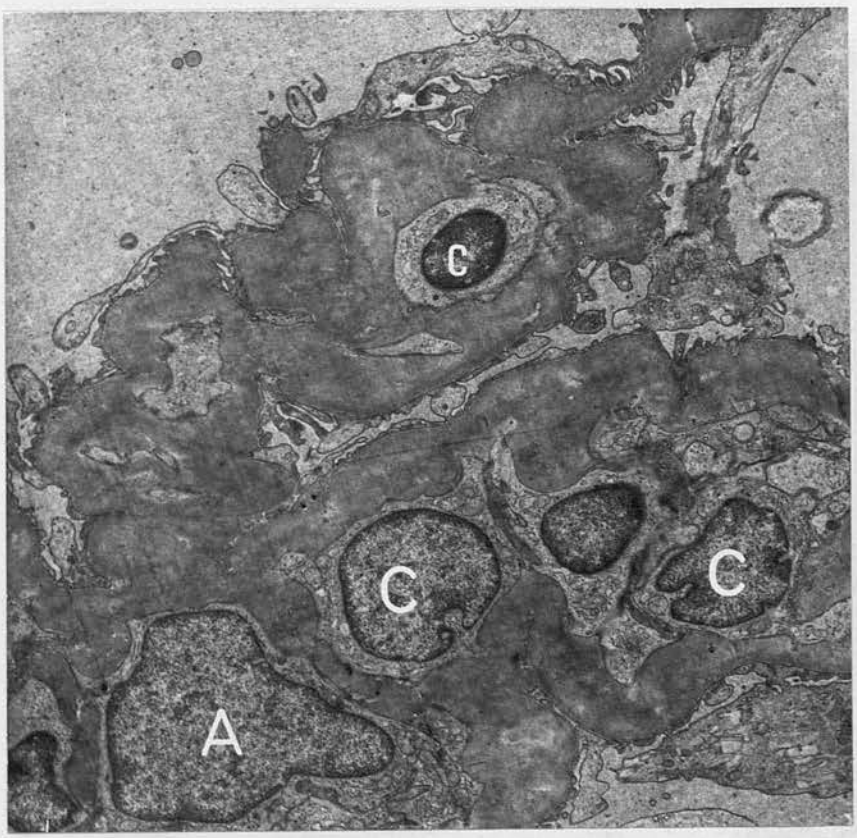


Plate 34: Hypertension without diabetes. Mesangial zone showing contracted tissue and apparent basement membrane accumulation around mesangial (A) and endothelial (C) cells. Case 50. X 4,000.

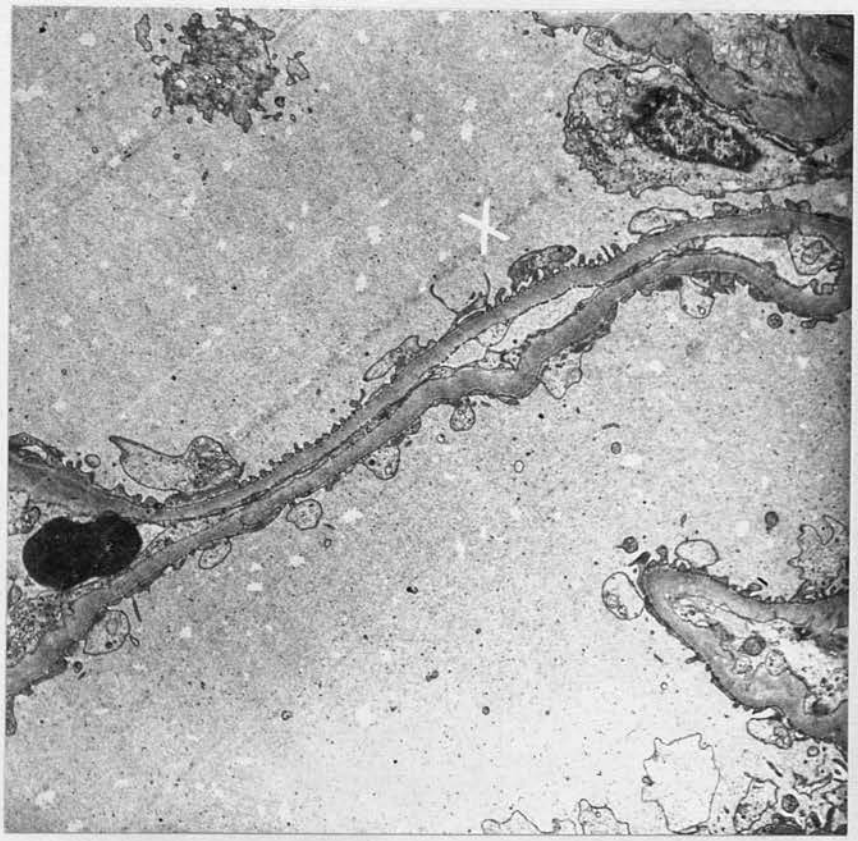


Plate 35: Capillary loop from hypertensive patient without diabetes. Note collapsed capillary and wide Bowman's space. See also plates 36, 37. Case 50.

X 4,000.

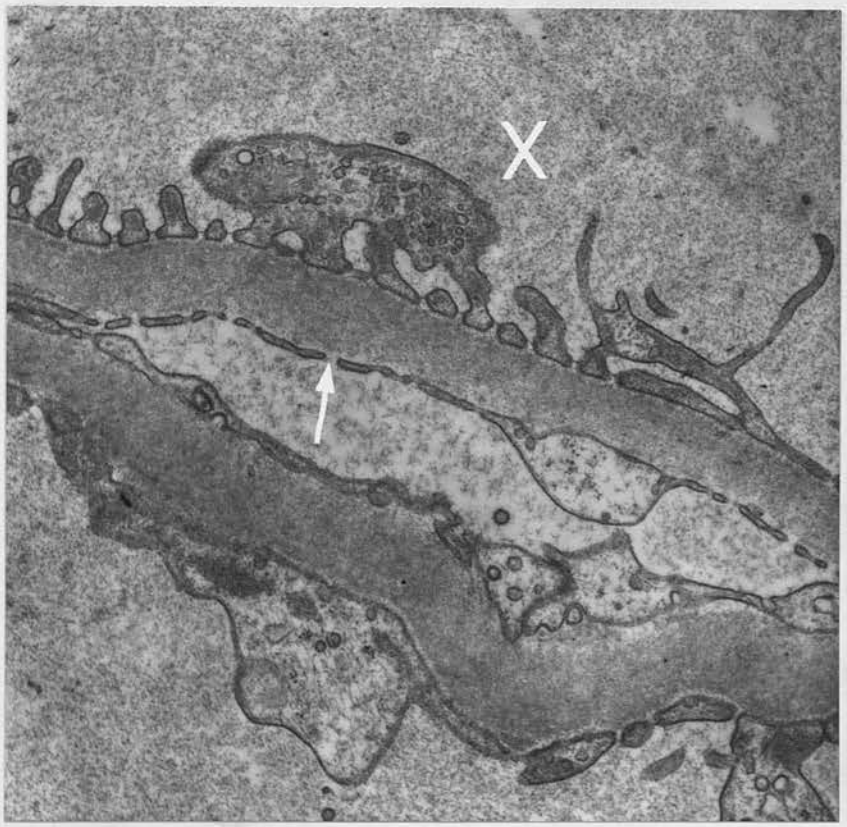


Plate 36: Detail of Plate 35. Note Bowman's space (X) and endothelial pores (arrowed) in narrow capillary lumen. Case 50. X 20,000.

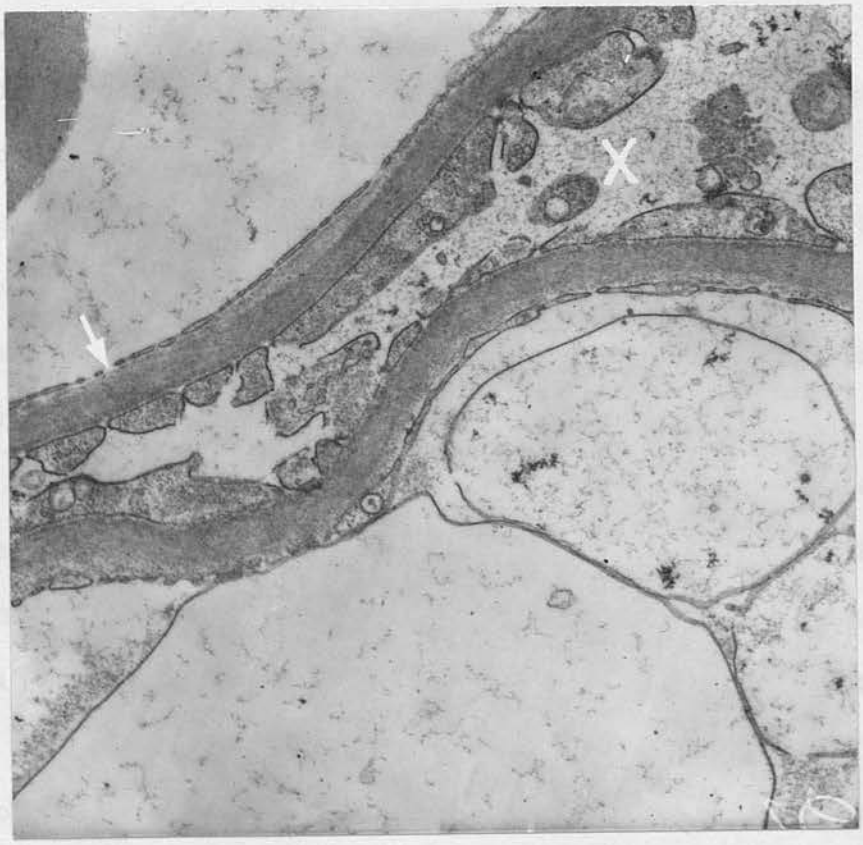


Plate 37: Capillary loop from idiopathic diabetic. Compare narrow Bowman's space (X) and dilated capillary loop showing endothelial pores (arrowed) with plates 35 & 36. For low power view of this plate see plate 11 (Chapter 4). Case 44. X 14,000.

secondary to either arteriolar involvement or hypertension, despite the fact that both the latter are common accompaniments of long-standing diabetes.

Albuminuria

Although differences have been demonstrated in the mesangial and endothelial appearances in the various groups of diabetics studied, two features, namely peripheral basement membrane thickening and well developed epithelial cytoplasm, have almost invariably been found in harmony.

Farquhar (1964), in a study of turnover of ferritin particles in nephrotic animals, has shown that although the basement membrane remains the principal filter the epithelial cytoplasm demonstrates increased pinocytic activity. Particles which have escaped abnormally through the basement membrane collect in small vesicles, aggregate into vacuoles and condense into 'adsorption droplets' or dense bodies. She has concluded that the glomerular epithelium functions to monitor the glomerular filtrate and to recover escaping macromolecules thus compensating for the defect in the basement membrane filter. Since albuminuria is characteristic of well established diabetic glomerulosclerosis one cannot overlook the possibility that the epithelial hypertrophy arises, as Farquhar has suggested, in response to increased basement membrane permiability to protein. In plates 38 and 39 (in plate 2, page 39 and plate 24, page 123) protein particles are evident within the epithelial cytoplasm. All of these patients had 24-hour urinary

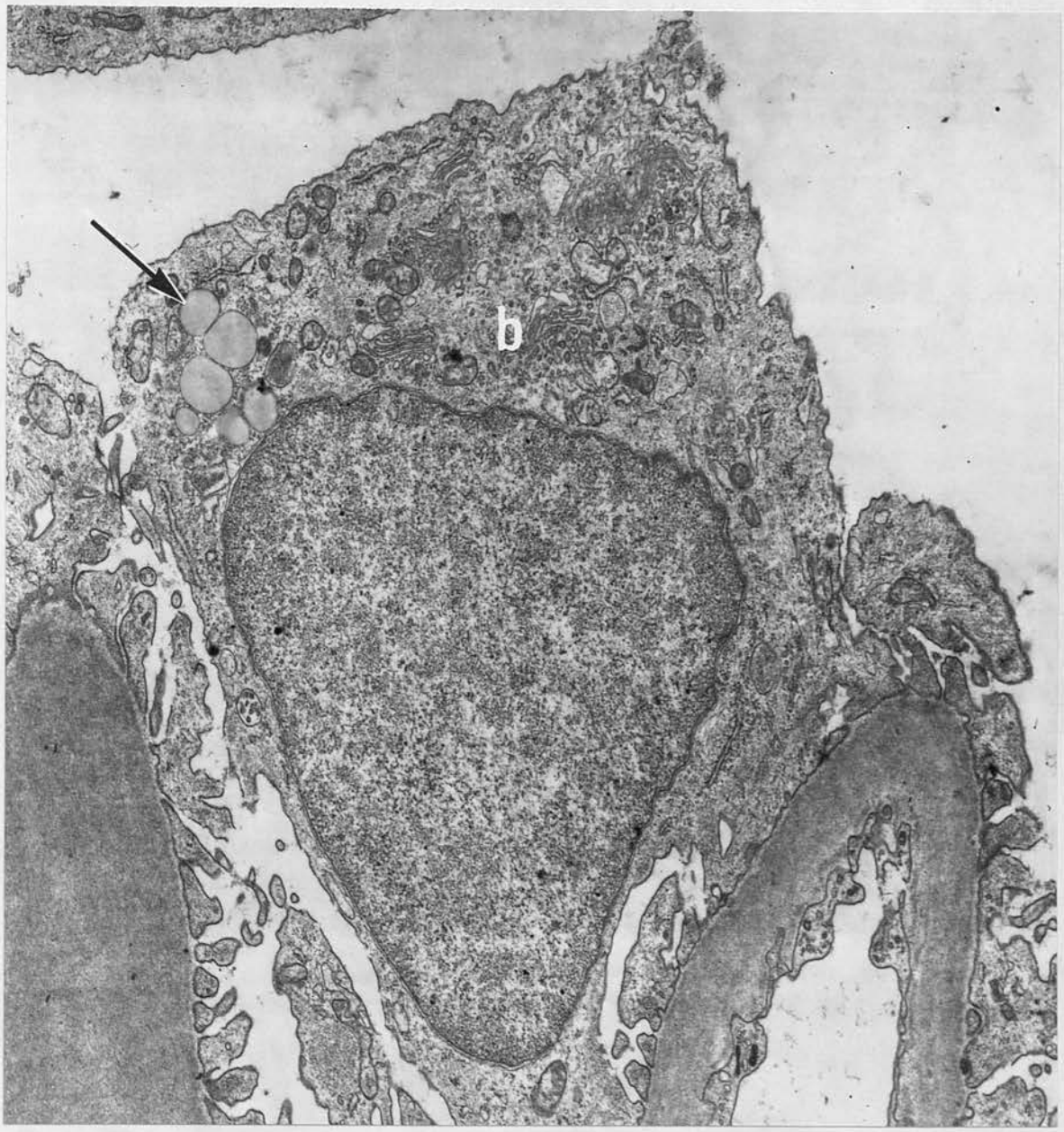


Plate 38: Epithelial cell from idiopathic diabetic without retinopathy. Note Golgi zones in cytoplasm (b) and protein particles (arrowed). Case 43. X 16,000.

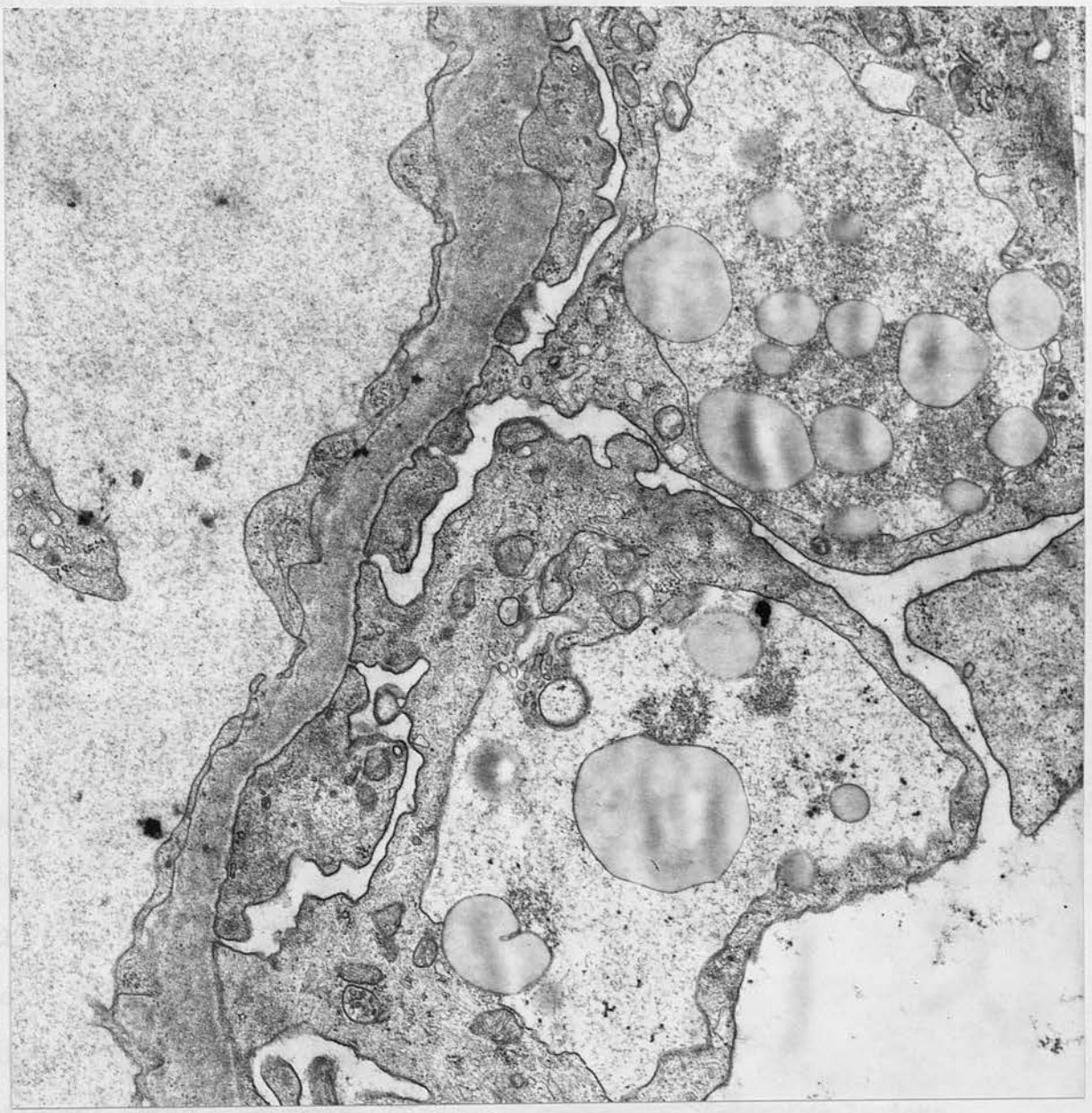


Plate 39: Peripheral capillary loop from idiopathic diabetic following pituitary ablation. Note numerous protein particles in epithelial cytoplasm.
Case 35. X 20,000.

protein levels of less than 0.1 gm. Whether protein particles trapped in the epithelial cytoplasm provides a substrate for the synthesis of the amino acid constituents of basement membrane glycoprotein is a subject of further speculation (Chapter 11).

Another aspect of the diabetic renal lesion, relevant to proteinuria, deserves emphasis. In none of the patients having unequivocal basement membrane thickening reported in the previous sections was there evidence of smudging of the epithelial foot processes or other degenerative changes as seen in non-diabetic glomerular capillary diseases (MacDonald, 1966). However, with the exception of Case 36 (Chapter 8) few had significant proteinuria. To compensate for this inadequacy renal biopsy tissue has been obtained from two diabetic patients having 24-hour urinary protein levels greater than 6.0 g. at the time of biopsy. The patients, Cases 48 and 49, were aged 67 and 53 years respectively. Case 48 had been controlled by diet, or diet and intermittent oral therapy for 15 years and Case 53 by diet alone for 8 years. Light microscopy examination of the renal biopsies showed advanced diffuse glomerulosclerosis and well developed hyalinisation of afferent arterioles, while in Case 48 occasional exudative lesions were also evident. Electron microscopy examination showed marked peripheral basement membrane thickening (mean thickness 8,200 Å and 7,650 Å in Cases 48 and 49 respectively). Yet the epithelium was well preserved and contained protein particles (Plates 40 and 41).

It is probable that proteinuria in the diabetic having advanced glomerular lesions reflects the fact that in the presence

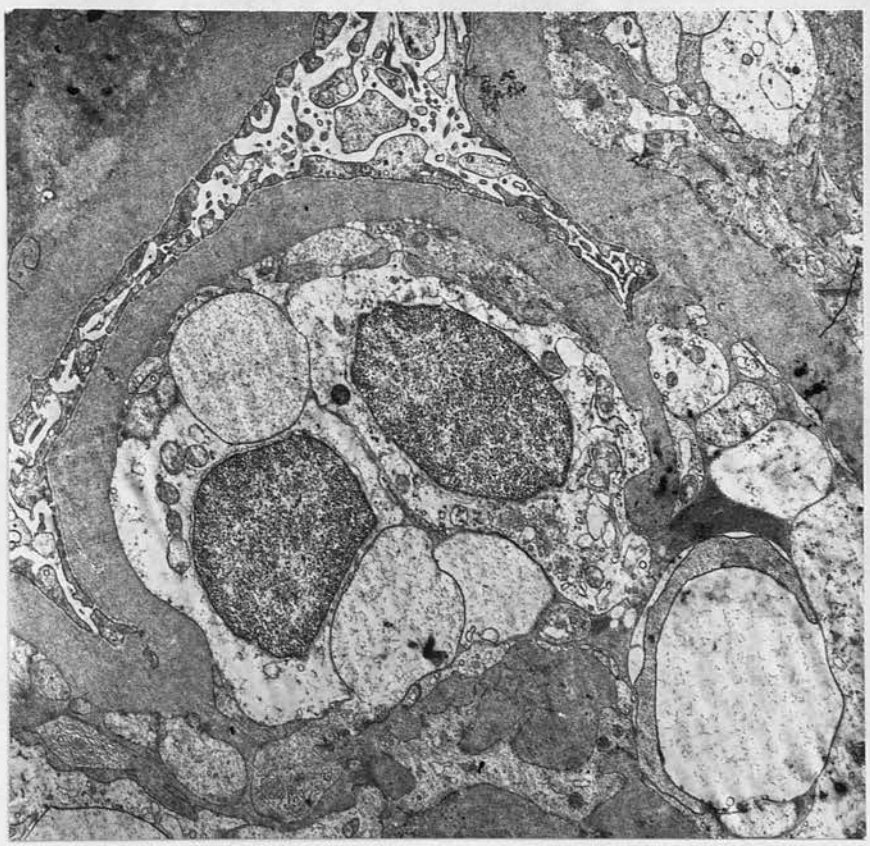


Plate 40: Capillary loops from diabetic patient having heavy proteinuria. Note diffuse basement membrane thickening, well preserved endothelial cell within capillary lumen and well preserved epithelial detail.

Case 48. X 5,000.

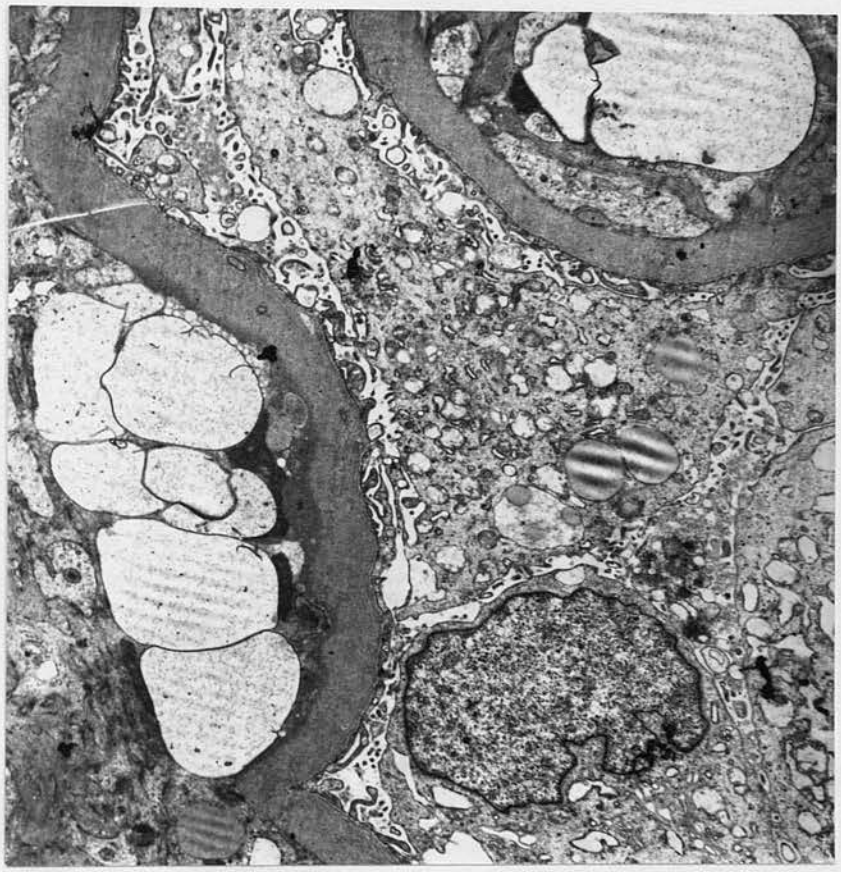


Plate 41: Case 48. Epithelial cell separating capillary loops. Well preserved cytoplasm containing protein particles.

X 4,000.

of a markedly abnormal basement membrane filter the amount of material passed exceeds the capacity of both the glomerular epithelium and tubules to deal with it (See Chapter 11).
On the other hand, however, absence of proteinuria need not be synonymous with a normal basement membrane. Also, if in the presence of proteinuria, a diabetic is found on electron microscopy to have marked smudging of the foot processes and other epithelial defects, such findings would raise the possibility of some other non-diabetic lesion. Unfortunately, the diabetic patient has no peculiar immunity to other diseases.

CHAPTER 11.

General Discussion

Including:

1. Clinical correlations, diagnosis and incidence of diabetic renal disease.

2. Factors of significance in pathogenesis.

3. The evolution of diabetic glomerular capillary disease with particular reference to the Kimmelstiel-Wilson nodule.

"I shall be quoting only myself, for the simple reason that in giving a line of thought, I can be more certain of what was passing through my mind, than what passed through the minds of others".

Claude Bernard.

1. Clinical Correlations, Diagnosis and Incidence:

Proteinuria, nephrotic oedema, hypertension and renal failure have been the accepted clinical features of diabetic kidney disease since Kimmelstiel and Wilson's description of the nodular glomerular lesion. Yet none of these features is confined to, or diagnostic of, diabetic arteriolosclerosis or glomerulosclerosis; in addition, such lesions may be advanced in patients with minimal clinical evidence of renal disease. Thus in the group of patients undergoing pituitary ablation for advancing retinopathy (Chapter 8) there were unequivocal glomerular capillary lesions yet only one patient (Case 36) had clinical evidence of renal decompensation.

Although it is generally believed that proteinuria is the first clinical warning of diabetic renal disease, in a large series studied by Bell (1953) it had been absent in 10 per cent. of those found at autopsy to have died of renal lesions. He also found that in more than half of those over the age of 60 years, proteinuria was due to cardiac failure or causes other than diabetic renal disease. Thomsen (1965) concluded that in the absence of cardiac failure, pyuria or keto-acidosis, proteinuria was a good

sign of diabetic glomerular damage yet 20 per cent. of his own series of cases, found at renal biopsy to have well-established glomerular lesions, were free of proteinuria. Although the constituents of urinary proteins have been clearly characterised in diabetics (Syllaba, 1969), studies of differential protein clearance by immunological or gel filtration methods have failed to show any consistent pattern of selectivity in patients having diabetic glomerulosclerosis (MacLean, Petrie and Robson, 1968). Absence of proteinuria may indicate either effective monitoring by the glomerular epithelium (Chapter 10) or reabsorption by the tubules (Hardwicke and Soothill, 1967). Thus assessment of diabetic renal disease on the basis of proteinuria may be unrealistic.

Several studies (Chapter 10, page 146) have shown a good correlation between hypertension and renal arteriolar lesions, yet in a large series Bell (1953) found that many normotensive diabetics had afferent and efferent arteriolosclerosis. Thomsen (1965) showed that renal lesions preceded hypertension and concluded that the diabetic changes were a cause rather than a consequence of hypertension. The findings reported in Chapter 10, contrasting the glomerular capillaries in hypertensive and diabetic patients, also suggest that hypertension is not of primary importance in the diabetic capillary lesion.

Renal failure in the diabetic may result from several factors (Fig. 1, page 10), thus it would be unwise to assume that such features in a diabetic were due to the glomerular capillary lesion.

In particular, the possibility of pyelonephritis should always be considered (Chapter 2, pages 31-33). On the other hand, the finding of diabetic retinopathy would be strong evidence in favour of diabetic glomerular capillary lesions also being present. Although it has been stated that retinopathy can occur without diabetic nephropathy, such statements have not been supported by renal biopsy data. For example, the findings reported in Chapter 9, showing definite glomerular capillary lesions in four of five patients having no evidence of retinopathy, are further evidence that light and electron microscopy examination of renal biopsy tissue is more reliable than other clinical methods currently available. Assessment of clinical progress by measurement of endogenous creatinine clearance may, in the diabetic, be unreliable since hyperglycaemia, glycosuria and keto-acidosis are sources of error in creatinine determination (Thomsen, 1965); moreover, it should not be forgotten that optimistic results may be obtained in the presence of proteinuria. Whereas deterioration in glomerular filtration is expected with increasing severity of glomerulosclerosis, Ditzel and Schwartz (1967) and others have found enhanced clearance of ^{57}Co-cyanocobalamin in early juvenile diabetics. Whether this represents increased capillary permeability or evidence of a greater filtration surface in the early stage of glomerular capillary basement membrane changes is uncertain. Although the advanced diabetic glomerular capillary lesion ultimately reduces the filtration surface, it is possible that the early lesion with

characteristically distended capillaries (Fig. 10, page 62) may permit enhanced glomerular filtration.

Since the clinical features of diabetic renal involvement are essentially non-specific, accurate diagnosis can be made only with the aid of percutaneous renal biopsy. Notably, elderly patients may have advanced diabetic nephropathy in the presence of a mild disturbance of carbohydrate metabolism, which may be overlooked because both age (Butterfield, Keen and Whichelow, 1967) and renal failure may raise the renal threshold for glucose. Conversely, pseudo-diabetic carbohydrate intolerance of uraemia (Cohen, 1962; Westervelt and Schreiner, 1962; Luke, Dinwoodie, Linton and Kennedy, 1964) or impaired tubular reabsorption of glucose in the nephrotic syndrome may be misinterpreted as indicative of diabetes mellitus (Churg, Grishman, Goldstein, Yunis and Porush, 1965).

Without serial renal biopsy examination the incidence and clinical course of diabetic kidney disease is difficult to evaluate. It is distressingly clear, however, that of those who develop diabetes in childhood, few escape renal involvement after 20 years of the disorder and that in this group renal failure is the major cause of death (Entmacher, Root and Marks, 1964; Warren, Le Compte and Legg, 1966). Many of those who develop diabetes in later life may have minimal renal involvement after a similar interval, while others, notably the elderly, may have well-established lesions at the time of diagnosis. Thus it

would be unrealistic to imagine a straightforward clinical picture of kidney disease in diabetes.

2. The Pathogenesis of Diabetic Glomerular Capillary Disease:

Genetic Factors

Many investigators have suggested that microangiopathy is not a complication of diabetes but rather an integral concomitant of the disease, perhaps genetically determined and expressed as an independent phenotype of the genetic potential. As discussed in Chapter 7 (page 103) many reports of diabetic lesions in newly diagnosed or "pre-diabetic" patients have been interpreted in this way. In addition to the previously mentioned statistical limitations of these studies, any theory concerning the cause of vascular lesions depends upon the clear demonstration that the lesions are not related to the complex metabolic abnormalities induced by insulin deficiency. Many animal experiments have been undertaken in the hope of producing diabetic glomerular lesions as a result of the metabolic disturbance of meta-hypophyseal or alloxan-diabetes. Although glomerulosclerosis has been reported in most of these studies, the lesions bear little relationship to those seen in human diabetes. However, in a well-controlled and exhaustive light and electron microscopy study, Bloodworth (1965) has demonstrated typical diffuse and nodular diabetic lesions in animals made diabetic and treated with insulin for up to six years. Diabetic lesions have also been demonstrated

convincingly in other sites (Bloodworth, Engerman and Powers, 1969).

The genetic problem is further confused by disagreement concerning diabetic inheritance. Although in the past regarded as a Mendelian recessive trait with incomplete penetrance (Steinberg, 1965), Clarke (1966) has pointed out that geneticists have tried to force heterogeneous and complicated human data in simple Mendelian ratios. Both Simpson (1964) and Clarke (1966) hold the view that diabetic inheritance is multifactorial, however Clarke admits that it is probably unnecessary to make too much of these distinctions particularly since the single gene hypothesis shades into multifactorial inheritance the lower the penetrance (Edwards, 1960). Neel (1964 and 1970) has pointed out that the variable clinical expression of the basement membrane lesions suggests that they are secondary to metabolic or other factors rather than genetically determined.

Despite the intellectual uncertainty surrounding the inheritance of diabetes or its vascular complications, the apparent lack of microangiopathy in patients having diabetes secondary to chronic pancreatitis, carcinoma of the pancreas or haemochromatosis has also been cited in favour of a genetic cause of diabetic microantiopathy. Yet there have been several isolated reports of diabetic lesions in such patients (See Chapter 6, page 82). Furthermore, significant glomerular capillary basement

membrane thickening was demonstrated in secondary diabetics compared with healthy controls (Chapter 6). While these findings suggest that glomerular capillary disease in diabetes is due to a metabolic derangement common to idiopathic and secondary diabetes, the possibility that certain components of diabetic small blood vessel disease can occur in and be due to some as yet undefined aspect of the idiopathic disorder cannot be overlooked (vide infra).

Metabolic factors

The frequently suggested possibility that in diabetes the renal vascular lesions result from deposition on their walls of protein or other abnormal substances has been repeatedly investigated as new techniques of study become available (Gordon, 1964; Frankl, 1964). However, in a critical review, Winzler (1964) concluded that it was uncertain whether elevated levels of glycoproteins in the serum of diabetics were a cause, a passive concomitant, or a result of small blood vessel disease.

Likewise in the many studies made of plasma lipids (Syllaba, 1969) in relation to small blood vessel disease and atheroma in diabetes, the general conclusion that their alteration could be a consequence of nephropathy cannot be overlooked (Adlersberg, Wang, Rifkin, Berkman, Ross and Weinstein, 1956).

Nonetheless, the possibility that the renal small blood vessel changes might be a morphological consequence of the bio-

chemical alterations of diabetes, though long suspected, has been supported by many recent studies. Robb-Smith (1957) and many others since have established the high protein content of glomerular capillary basement membrane; the use of PAS staining has repeatedly demonstrated the presence of carbohydrate. The composition of the glycoprotein complexes of which the basement membrane is largely composed has been studied in detail by Lazarow and Speidel (1964). They showed that although diabetic glomeruli contained a great excess of glycoprotein by comparison with those of normal humans, on hydrolysis of the carbohydrate content to simple sugars there was no qualitative difference between the two groups.

Although increased synthesis of glycoprotein might appear to be a paradox in a disorder characterised by impaired carbohydrate metabolism, Spiro (1963) has postulated a biochemical basis for such a possibility. He found that the production of the glucosamine component of glycoprotein along non-insulin dependent pathways was increased in liver slices from insulin-deficient alloxan-diabetic rats. Spiro (1967) has further characterised the carbohydrate and other constituents of glomerular basement membrane, while Walker and Patrick (1968) have confirmed, in the alloxan-diabetic rabbit, that in insulin deficiency glucose is preferentially utilised in the synthesis of glucosamine. Studies by Winegrad and Burden (1966) of l-xylose metabolism in relation to the synthesis of glycoproteins is further evidence favouring increased basement membrane synthesis in diabetes.

The possible relationship of these observations to the findings reported in this study is discussed in a later section.

Immunological factors

Because diffuse and nodular diabetic glomerulosclerosis were not reported until many years after the discovery of insulin it has been suggested that the lesions might be an immunological response to exogenous insulin. However, it would also be necessary to postulate an autoimmune response to endogenous insulin to explain the common finding of diabetic glomerular lesions in patientspatients who have never received insulin. Blumenthal et al. (1964) have reported binding of fluorescein-conjugated insulin by the nodular diabetic glomerular lesion, and have reported similar results in rabbits immunised with beef insulin and Freund's adjuvant mixtures. Burkholder (1965) studied the localisation of plasma proteins and fixation of guinea-pig complement in the lesions of diabetic glomerulosclerosis, but cautiously concluded that tissue fixation of insulin is not necessarily of pathological significance.

Indeed the immunology of glomerular capillary basement membrane is both complex and obscure. Cruikshank (1964) has emphasised the difficulties of localising antigens native to glomerular capillary basement membrane. Berson and Yalow (1965) who were among the first to explore this subject, observed that to date no experiment adequately incriminated exogenous or endogenous insulin.

Anterior pituitary and adrenal cortex

The treatment of diabetic retinopathy by pituitary destruction has been extensively observed and studied. Thus, Bradley, Rees and Fager (1965) reviewed 387 reported cases of hypophysectomy for diabetic retinopathy and concluded that neovascularisation (Colour plates 8 and 9, pages 107 and 108) in particular was significantly benefited by the procedure. Joplin, Fraser, Hill, Oakley, Scott and Doyle (1965) have reported favourably on their extensive experience of pituitary ablation and Oakley and Joplin (1968) have also reviewed the clinical, therapeutic and pathological aspects of diabetic retinopathy and pituitary ablation. Poulsen (1966) and others (Graef, 1966), however, have reported that pituitary destruction has no beneficial effect on the progressive clinical course of diabetic renal disease. Yet these conclusions are based largely upon tests of renal function and although Isaacs, Pazianos and Greenberg (1969) obtained renal biopsies from six of their patients, none have examined such tissue by electron microscopy. In the series reported in Chapter 8, pituitary ablation did not appear to benefit either arteriolar or advanced glomerular lesions. However, successful pituitary ablation was followed by significant reduction in the thickness of glomerular capillary basement membrane and restoration of previously atrophic endothelial and mesangial cells. Whereas it would be unwise to draw firm conclusions from the limited data available, it is possible that the anterior pituitary influences certain aspects of diabetic capillary, but not arteriolar, disease.

Misinterpretation of some similarities between experimental cortisone-induced glomerular lesions and human diabetic glomerulosclerosis has led to the belief that the adrenal cortex influences the pathogenesis of the diabetic lesion. However, in a comprehensive study of urinary and plasma corticosteroids, including measurements of hydrocortisone turnover rates and aldosterone secretion, Rifkin et al. (1958) found no significant differences in these parameters in diabetic patients with and without microangiopathy. Many others have confirmed these findings though Lentle and Thomas (1964) found differences between controls, diabetics with, and diabetics without small blood vessel disease when plasma and urinary corticosteroids were measured following administration of ACTH or dexamethasone.

Effect of duration, severity and control

Clinical experience with individual patients suggests that fluctuations in "control" may be associated with exacerbations and remissions of retinopathy and neuropathy (Dollery and Oakley, 1965). However, these observations are difficult to reconcile with others of clinical and histological evidence of advanced small blood vessel disease in patients with such mild diabetes that it almost escapes clinical recognition. Nor is micro-angiopathy an inevitable consequence of long-standing insulin dependence. Thus, Case 45, reported in Chapter 9, was found to have neither retinopathy nor glomerular capillary lesions after 23 years of insulin dependence. Nonetheless, it has been

established beyond doubt that duration of diabetes is the single most significant factor in determining the severity of diabetic small blood vessel disease (Berkman and Rifkin, 1966).

Assessment of the influence of "severity" and "control" of diabetes is almost impossible in the absence of universally acceptable definitions of these terms. Also, both may reflect unknown factors beyond the influence of present day therapy. Following an exhaustive analysis of over 300 publications on the subject of diabetic control in relation to complications, Knowles (1965) was unable to reach a conclusion. He noted, with interest, that few of the individual authors had been unable to do so. Moreover, the demonstration of an association between poor control and severity of renal or other complications does not prove a cause and effect relationship. The possibility that diabetics having complications are more difficult to control than those without such lesions cannot be overlooked.

3. The evolution of diabetic glomerular capillary disease with particular reference to the Kimmelstiel-Wilson nodule.

Although the diffuse and nodular diabetic glomerular lesions have been considered individually in Chapter 2, on reviewing the features seen on electron microscopy it was suggested that several observers (page 47) doubted their separate identity. Thus, many believed that the diabetic nodule represented centrolobular exaggeration of the diffuse lesion. On the contrary, Kimmelstiel (1966) suggested that the primary defect was in the

mesangium, that the mesangial lesion could occur without evidence of basement membrane thickening in the peripheral capillaries and that any lesion in the periphery was secondary to mesangial pathology. More recently, Iidaka, McCloy and Kimmelstiel (1968) have attempted to support this conclusion by a quantitative estimation of mesangial area by light microscopy and camera Lucida examination of glomeruli in autopsy tissue. Although an absolute increase in mesangial cells is postulated in diabetic glomerulosclerosis, no evidence is available concerning the clinical details of the cases studied, nor is it clear whether the patients died of renal disease or other causes.

Despite these opposing views, the Kimmelstiel-Wilson nodule remains for the light microscopist the undisputed hallmark of diabetes in the kidney. Like all great discoveries it remains untarnished by time. However, its significance as a primary factor in pathogenesis of diabetic small blood vessel disease is less certain. In the author's view this subject is further confused on two counts. Firstly there is a tendency to equate diffuse glomerulosclerosis with peripheral glomerular lesions and the nodule with the mesangial lesion. Yet diffuse glomerular lesions of light microscopy terminology involve both the peripheral and mesangial parts of the glomerular capillaries and the nodule may obliterate a whole lobule or several lobules (Colour plate 3, page 21). Secondly, misunderstanding of the

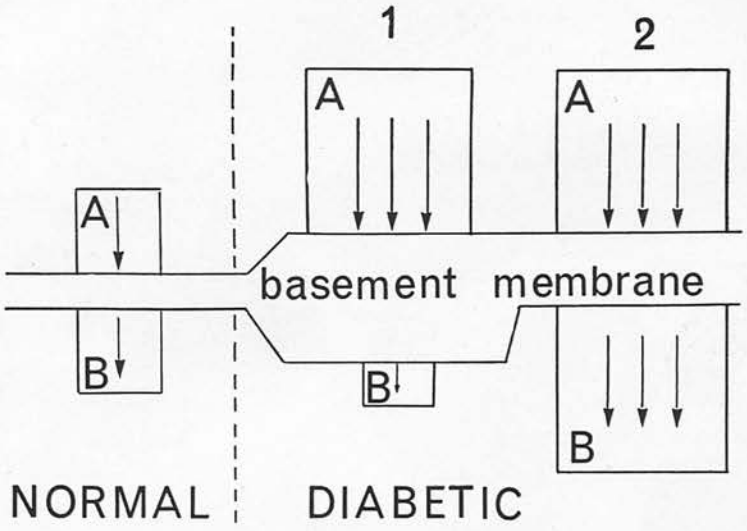
pathology of diabetic nephropathy arises from the continued use of the term glomerulosclerosis. This implies a sclerotic lesion with hyalinisation, whereas the changes revealed by the electron microscope in the cellular ultrastructure and capillary basement membrane are inadequately designated by such nomenclature.

On the basis of the observations reported in this study it is probable that the pathogenesis of diabetic glomerular capillary disease is more complex than either of the previously mentioned opposing views. The following tentative hypothesis is suggested. The integrity of the basement membrane probably depends on the function of the cells of the glomerular tuft; thus alterations in the membrane structure may be due to dysfunction in these cells rather than to primary metabolic changes within the membrane itself. In biopsies revealing thickening of the glomerular capillary basement membrane, the epithelial cytoplasm was abundant and contained prominent mitochondria, RNA-studded or rough-surfaced endoplasmic reticulum and Golgi zones. Since there is considerable evidence (Farquhar, Wissig and Palade, 1961; Andres, Morgan, Hsu, Rifkind and Seegal, 1962; Kurtz and Feldman, 1962; Vernier, 1964; Lee, Blasey, Goldstein and Pierce, 1969) that the epithelial cells are responsible for the synthesis of the basement membrane, it is possible that the prominent epithelium in diabetic renal tissue reflects increased intracellular production and deposition of basement membrane material. The biochemical basis for such a

possibility was discussed in the previous section.

The appearances of the mesangial and endothelial cells were more variable. Whereas they were essentially intact in patients having secondary diabetes and in long-standing idiopathic diabetics without retinopathy, they were crenated and atrophic and largely replaced by accumulations of basement membrane material in diabetics having progressive proliferative retinopathy. Since the mesangial and endothelial cells were most defective in patients having the greatest amount of basement membrane, both in terms of peripheral thickening and mesangial accumulation, it is unlikely that these cells are responsible for the excess basement membrane production as Kimmelstiel (1966) suggested. However, if these cells have, as Farquhar (1964) has suggested, a phagocytic function then the increase in basement membrane in the glomeruli of some diabetics might be due to its defective removal by the mesangial cells, besides excess epithelial production. The findings in patients following successful pituitary surgery and in long-standing diabetics without retinopathy support this view. These observations are summarised diagrammatically in Fig. 14.

Thus the diabetic glomerular capillary lesions may represent varying degrees of epithelial production or impaired mesangial turnover of basement membrane. It would appear that the cells may be influenced by the metabolic defects of diabetes and by anterior pituitary function, but the complex interrelations are



Diagrammatic representation of basement membrane turnover. On left, normal production by epithelial cells (A) and removal by mesangial and endothelial cells (B). On right, the diabetic: 1. before pituitary surgery showing increased production of basement membrane by the epithelial cells (A) and diminished removal by the mesangial and endothelial cells (B); 2. after pituitary surgery showing restoration of mesangial and endothelial function (B) and some reduction in basement membrane thickening.

Fig. 14.

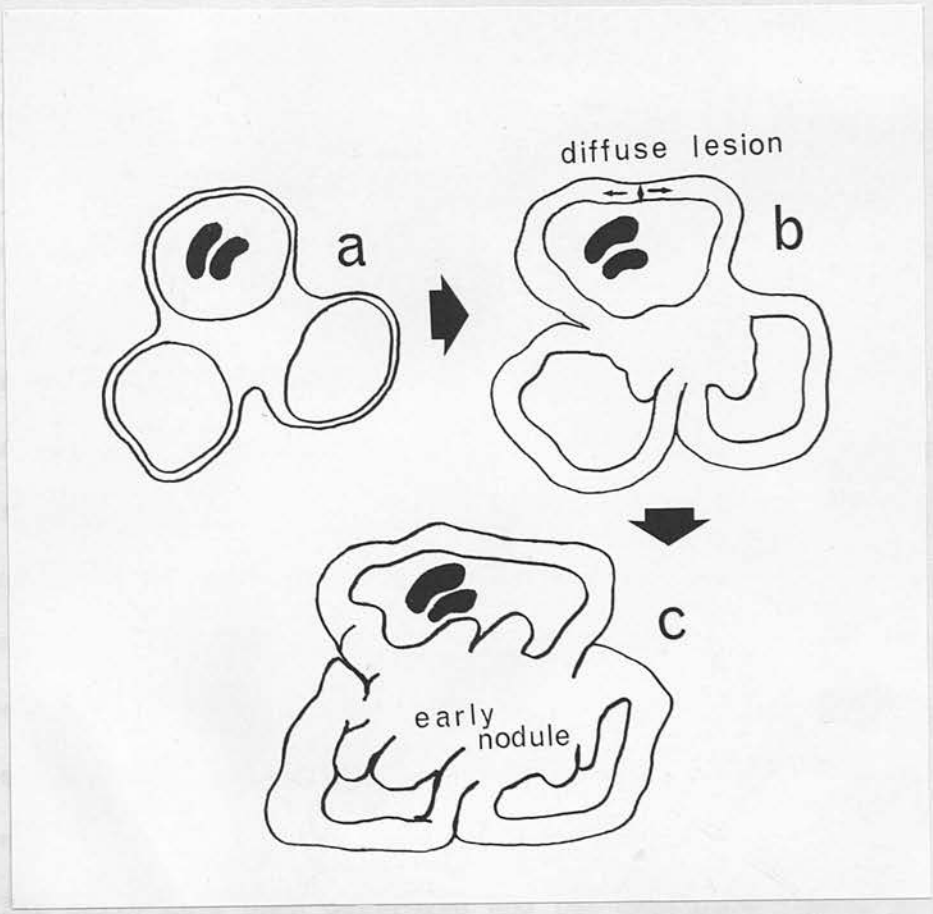


Fig. 15: Diagram of increase in basement membrane material. a Normal lobule. b Diffuse enlargement of capillary wall. c Mesangial enlargement and capillary obliteration.

far from clear. Although the light microscopist has been reluctant to regard the diffuse diabetic lesion as a specific entity, MacDonald (1966) has shown, however, that other non-diabetic causes of diffuse glomerular lesions can be distinguished by the electron microscope. In membranous glomerulonephritis the basement membrane is not only thickened, but composed of degenerate material with additional components of degenerate epithelium and endothelium. In early amyloid, apparent basement membrane thickening is due to deposition of abnormal protein in the membranes. Diabetes is unique in that there is an increase in the amount of basement membrane material without alteration in its fine structure.

In assessing renal biopsies from diabetic patients, peripheral basement membrane thickening, associated with epithelial hypertrophy and normal mesangial indices is apparently the earliest lesion. As the lesion advances, the mesangial index increases, indicating basement membrane accumulation in the mesangial zone with atrophy and crenation of mesangial and endothelial cells. At this stage diffuse glomerular capillary disease will be evident on light microscopy (Fig. 15). The nodule usually arises only when the mesangial cells have been destroyed and the capillary lumina of the lobule are obliterated. Since there is usually significant arteriolar involvement by the time that the nodule develops, it may be, as Kimmelstiel and Wilson (1936) originally suggested, that the nodule is the end product of a combined arteriolar and capillary lesion. However, for the individual patient, a low mesangial index, or healthy

mesangial and endothelial cells, and absence of significant peripheral basement membrane thickening would appear to be synonymous with well-being. On the other hand, a high mesangial index, whatever its pathogenesis, is a warning that diabetic renal disease is well established.

Although this study has provided a method of evaluating on a quantitative basis diabetic renal biopsies it has so far failed to indicate the specific factors responsible for accelerated microangiopathy in some diabetics and relative freedom from such complications in others. At least it has shown that small blood vessel disease in the diabetic kidney cannot be consolidated into a simple straightforward picture. The individual contributions of metabolic, genetic, endocrine, immunological or other factors remain in doubt. Thus the persuasive advocacy of a particular theory, however plausible, would be unwise without critical and balanced examination of the logical alternatives. Whereas there are always those who believe and expect natural phenomena to be simple, there are many areas of medicine which, with greater understanding, become increasingly complex; hypertension and blood coagulation are obvious examples. Despite the fact that a specific diabetic microangiopathy is an established concept, it remains an invitation to enquiry. The ultimate solution is unlikely to be either simple or immediately available. Unfortunately, for many diabetics it is already overdue.

CHAPTER 12.

Conclusions.

Although the nodular glomerular lesion of Kimmelstiel and Wilson remains for the light microscopist the hallmark of diabetes in the kidney, usually it is found only in association with the more common diffuse glomerular changes, and with afferent and efferent arteriolosclerosis. Accompanying changes in the tubules and interstitial tissue which often in the past have been interpreted as chronic healed pyelonephritis probably represent the end result of diabetic vascular lesions.

Electron microscopy examination has shown that the characteristic diabetic defect in the glomerular capillaries is thickening of their walls with excessive basement membrane material which probably represents varying degrees of its epithelial production or impaired mesangial turnover. The absence of glomerular lesions in newly diagnosed juvenile diabetics and the demonstration of basement membrane thickening in secondary diabetics suggests that the diabetic lesion is not, as has been proposed, dependent on the presence of the genetically determined diathesis to idiopathic diabetes but is due to some metabolic derangement common to both primary and secondary diabetes. The possibility that some components of diabetic microangiopathy can occur only in and be due to some as yet undefined aspect of the idiopathic disorder is, however, not excluded by these observations. Indeed it is probable that diabetic nephropathy results from the development of several separate though interrelated lesions each of which may be variously influenced by the metabolic disturbance,

the inherited diabetic diathesis or pituitary activity. Although the idea of a specific diabetic microangiopathy is now widely accepted, this simple concept probably conceals the complexity of the factors concerned in the pathogenesis of the various arteriolar and capillary lesions.

Almost 2,000 years ago Aretaeus the Cappadocian suspected that diabetes was a pernicious affliction affecting the kidney. Although Kimmelstiel and Wilson have confirmed this suspicion, progress in our understanding of the problem, with few exceptions, has been unremarkable.

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Mr. Paul Kinnear, M.Sc., Department of Psychology, the University of Aberdeen, has guided and advised the author in statistical methods appropriate to electron micrographs obtained from biopsy samples. The expert technical assistance of Dr. B.K. Patnaik, Ph.D., during two years of the early phase of this study, is gratefully acknowledged. Through the initiative of Dr. A. Dick, Senior Consultant Pathologist, Southern General Hospital, Glasgow, the author has not only obtained facilities in his department, but

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APPENDIX 1

M.S.B. (Martius, Scarlet Blue) stain for fibrin. (Lendrum et.al., 1962)

Fixation: Primary or Secondary formol-sublimate for 24 hours upwards.

Sections: Thin (3-4 micron) paraffin sections.

Technique:

1. Sections are taken to water.
2. Stain nuclei with celestine blue for 20 minutes, followed by haemalum for 10 minutes.
3. Rinse in tap water.
4. Differentiate nuclei in 0.25% HCL in 70% alcohol.
5. Wash in tap water.
6. Rinse in 95% alcohol and stain with 0.5% martius yellow in 95% alcohol containing 2% phosphotungstic acid for 2 mins.
7. Rinse in distilled water and stain in 1% brilliant crystal scarlet 6R in 2.5% of acetic acid for 10 mins.
8. Rinse in distilled water and treat with 1% phosphotungstic acid to fix and differentiate the red stain for up to 5 mins.
9. Rinse with distilled water and stain 0.5% of soluble blue in 1% acetic acid for up to 10 mins.,
10. Rinse in 1% acetic acid, blot, dehydrate in absolute alcohol clear in Xylene and mount in a synthetic resin medium.

RESULTS:

Nuclei	:	Black
Erythrocytes	:	Yellow
Fibrin	:	Red
Connective Tissue	:	Blue

APPENDIX 2

1. Electron microscopy methods of fixing and embedding Renal Tissue for Electron Microscopy.

FIXATIVE: 2% Osmium tetroxide in Sörensen Buffer.

1.	Fixation	: 2 hours at 4°C
2.	10% Alcohol	: 3 x 15 min. changes (Washing free of fixative)
3.	Absolute Alcohol	: 3 x 30 min. changes (Dehydration)
4.	Epoxy propane	: 2 x 15 min. changes (Antemedium)
5.	Araldite	: 12 to 24 hours at room temp. to impregnate.
6.	Embed in Araldite	: 48 hours at 56°C

7. Transfer blocks to 37°C incubator for 3 hours to allow slow cooling, this prevents air bells forming around tissue.

ARALDITE EMBEDDING MEDIUM:

Solution A:	Araldite - 100 ml. Hardener - 100 ml.
Solution B:	Accelerator - 1 ml. n-butyl Phthalate - 4 ml.

Take 19 ml. of solution 'A', add 1 ml. of solution 'B'. Mix on rotary mixer overnight at room temperature. Store at 4°C.

APPENDIX 2

2. Staining Methods for Ultrathin Araldite Sections

STAINING:

1.	Uranylacetate in 50% alcohol	: 10 mins.
2.	50% alcohol	: 20 secs.
3.	Distilled Water	: 10 secs.
4.	Lead Citrate	: 2 mins.
5.	0.02N. NAOH	: 20 secs.
6.	Distilled Water	: 10 secs.

STAIN PREPARATION:

Uranyl Acetate: Saturated solution in 50% alcohol immediately before use.

REYNOLDS LEAD CITRATE SOLUTION:

Add:	Lead nitrate	: 1.33 gms.
	Sodium Citrate	: 1.76 gms.
	Distilled Water	: 30 ml.

to a 50 ml., volumetric flask, shake vigorously for 1 minute. All to stand for 30 mins. with intermittent shaking. This is to ensure complete conversion of lead nitrate to lead citrate.
Add 8 ml. N. NAOH and dilute to 50 ml. with distilled water and mix by inversion. The ph should be 12.

SORVALL® MT-1

"PORTER-BLUM" 1/40 to 1/2 MICRON

ULTRA-MICROTOME

Ivan Sorvall, Inc. takes pride in presenting herein the details of its two Ultra-Microtomes, the "Porter-Blum" models MT-1 and MT-2. The MT-1 has been known and used for many years by the world's leading microtomists. The simplicity of operation and reliability of this instrument have made the name "Porter-Blum" the most respected in the field. Its sectioning range of 1/40 to 1/2 micron has made it the ideal ultra-microtome for either research or teaching. Consequently, the MT-1 is to be found in more electron microscopy labs than any other ultra-microtome. Thousands of MT-1's are in daily use throughout the world.

SORVALL® MT-2

"PORTER-BLUM" 100A to 4 MICRONS

ULTRA-MICROTOME

Carrying the many proven design principles of the MT-1 much further, the MT-2 was developed. More than half a decade of experienced research, as well as many, many more years of ultra-microtome design and manufacturing "know-how," have been built into the MT-2. We do not hesitate to proclaim it as a "new dimension in ultra-microtomy" in fact, and "an extension of the operator's will" in use. We invite you to compare the "Porter-Blums," which users say are the finest available, with any other instruments of their kind anywhere.

SORVALL® MT-1 "PORTER-BLUM" ULTRA-MICROTOME

1/40 to 1/2 MICRON

Uniform, High-Quality Sections · Alternate Thin and Thick Sections · Simplicity of Operation · Cuts Wide Variety of Materials · New, Modern Accessories · Moderate Cost

The dependability and simplicity of the "Porter-Blum" MT-1 in precision sectioning have been recognized for many years. Its convenient cutting range, and ability to cut thin and thick sections alternately when desired — as well as its budget price — make it the ideal instrument for many microtomists working with light *and* electron microscopy. The MT-1 is truly a dual-purpose ultra-microtome.

The principle of the MT-1 is mechanical. The specimen is advanced toward a fixed knife (glass or diamond) by turning the manually-operated handwheel. One full turn of 360° moves the cantilever arm through one complete cutting cycle. Advancement of the specimen may be made in increments as small as 0.025 micron.

Operating problems, such as static friction for instance, have been reduced in the MT-1 almost to the point of complete elimination. This is because the specimen-end of the cantilever arm follows a parallelogram which avoids stop and reversal movements. The continuous circuit described by the specimen permits by-passing the knife on the return portion of the cutting cycle, thereby eliminating all possibility of damage to specimen or knife.

The MT-1 has been used extensively for teaching purposes in many laboratories. Also, nearly all the significant achievements credited to advanced microtomy techniques during the past several years have been facilitated by this instrument.

Following the established SORVALL policy of "continuing design," we have developed several new accessories (see pg. 8) for the MT-1. These additional optional items all may be added to existing instruments, thus providing the user with convenient, economical means for updating his present MT-1.

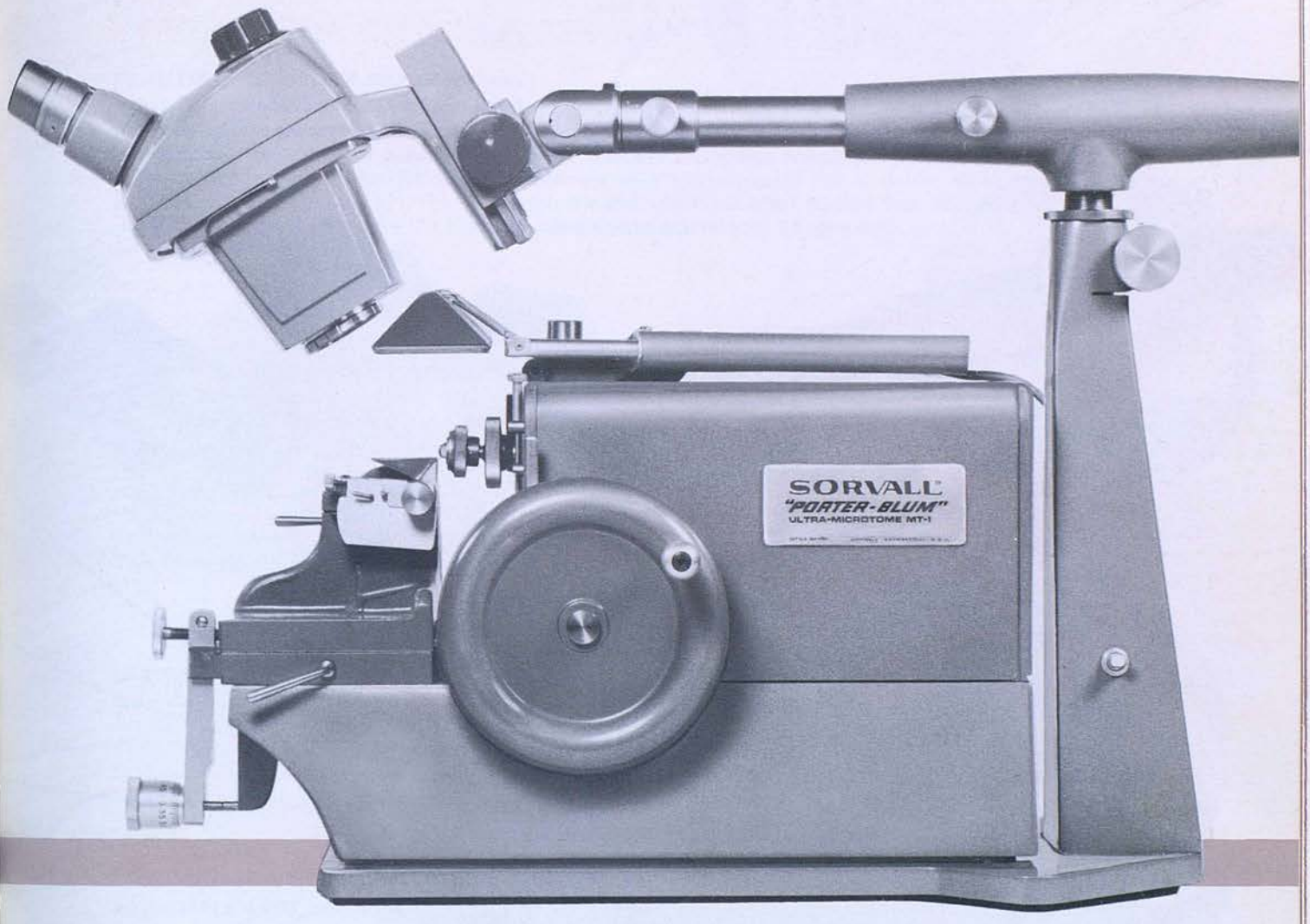
The MT-1 is surpassed in quality of result, operating precision, and ease of use only by the SORVALL "Porter-Blum" MT-2 Ultra-Microtome (see pg. 10).

FEATURES

- 1/40 to 1/2 micron cutting range
- Alternate thin and thick sectioning
- Sections wide variety of hard and soft materials
- Cuts high-quality uniform serial sections
- Accepts glass or diamond knives
- Six sizes of Specimen Holders optional
- Dial-control tissue thickness selector
- Mechanical advance
- Calibrated knife holders
- Specimen arm lock for trimming

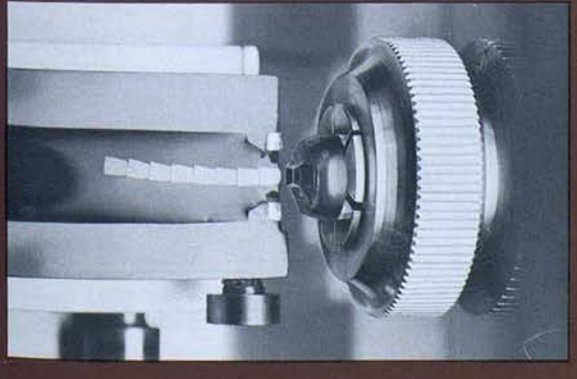
1

2



- Accommodates different types of stereomicroscopes
- New, pivoting telescopic stereomicroscope mount and base plate (optional)
- New, adjustable cold-light source (optional)
- Trouble-free operation — minimum maintenance
- Compact, integrated design — readily portable
- Simple to operate
- No special installation required

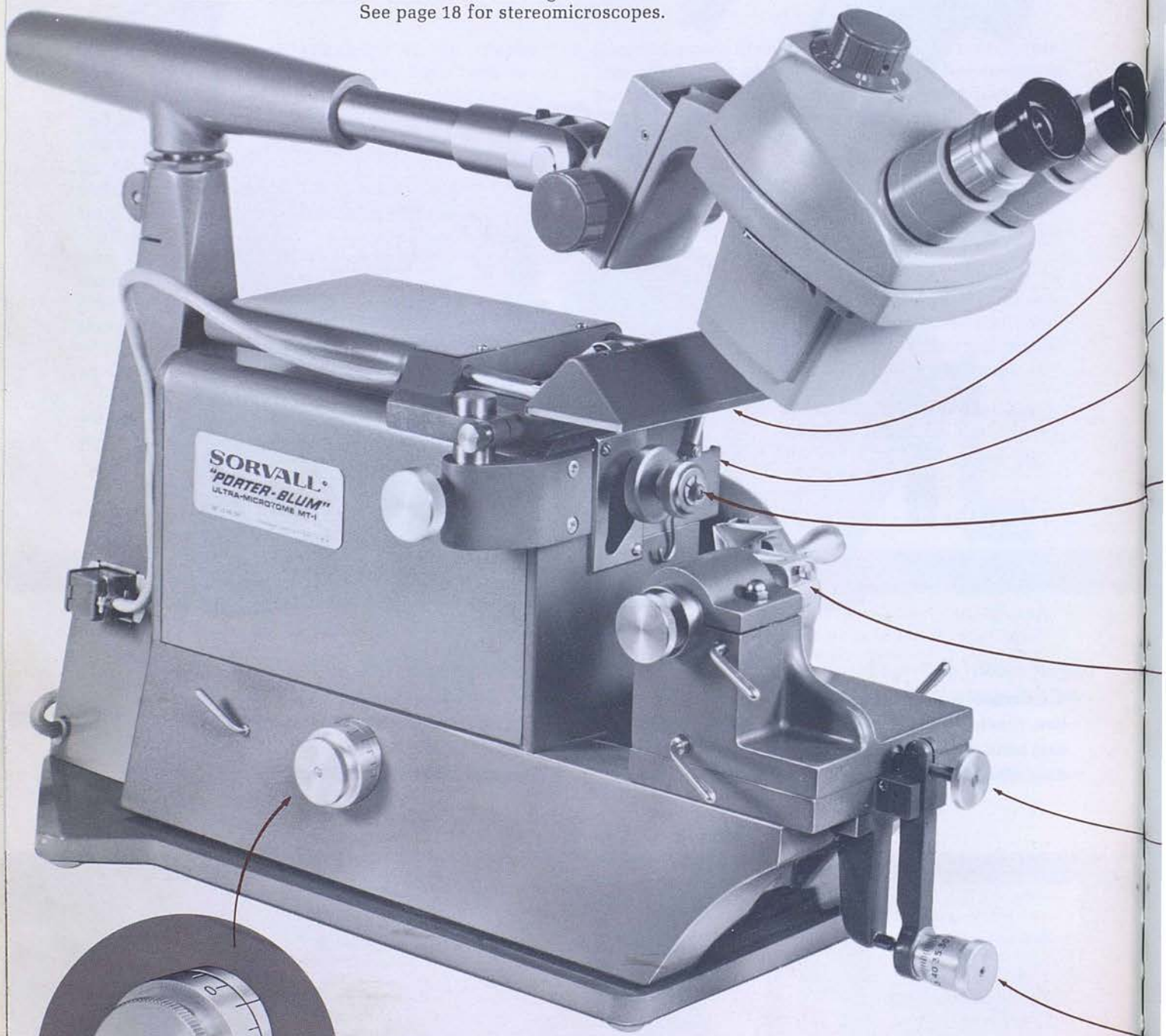
3

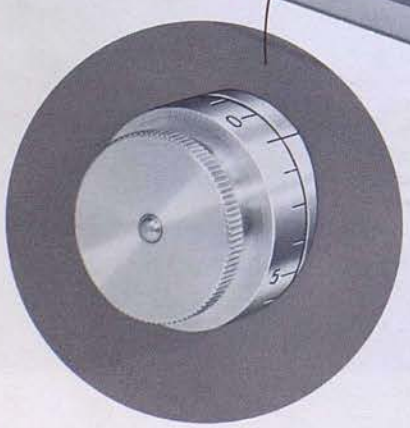


SORVALL® MT-1 "PORTER-BLUM" ULTRA-MICROTOME

PIVOTING TELESCOPIC MOUNT & BASE PLATE (Optional) A convenient, functional mount for AO and B&L stereomicroscopes (adaptable to certain other stereomicroscopes). Permits the swinging of the microscope out of the way when trimming specimen block, or changing specimen holders, etc. Combined with sturdy base-plate which is the ideal platform for the MT-1. Vertical post of microscope mount includes Switch and Ballast for Cold Light Source.

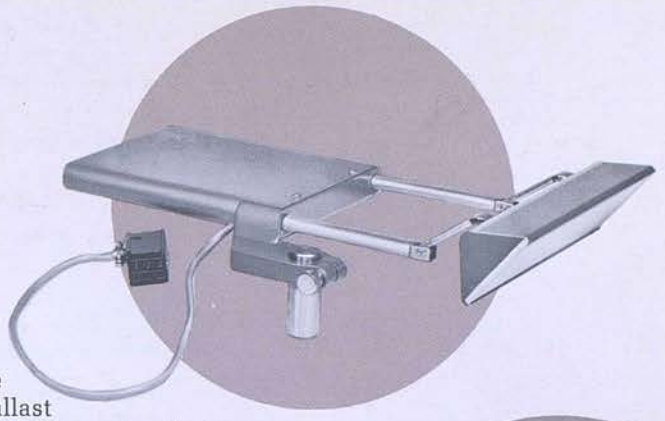
See page 18 for stereomicroscopes.



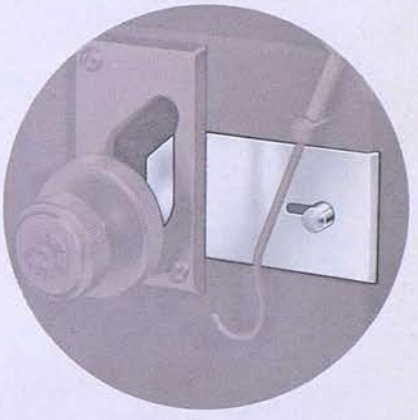


SECTION THICKNESS SELECTOR An easy-to-use control that enables the operator to dial the section thickness he requires from 1/40 to 1/2 micron. The Selector is conveniently located on the left side of the microtome base.

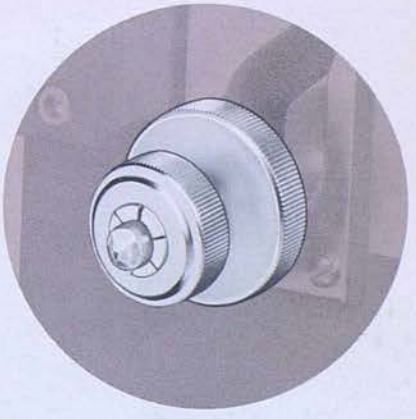
The SORVALL "Porter-Blum" MT-1 Ultra-Microtome may be operated on ordinary work surfaces; NO SPECIAL MOUNTINGS ARE REQUIRED.



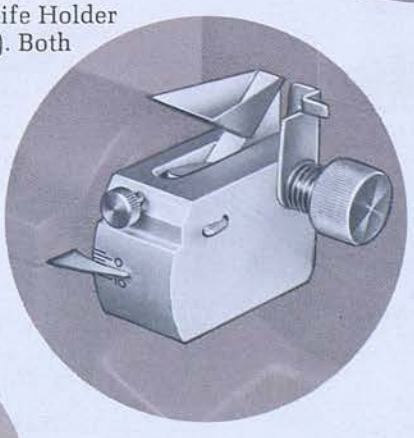
ADJUSTABLE COLD LIGHT SOURCE (Optional)
A new Cold Light Source which adjusts to the operator's convenience has been designed for the MT-1. It may be operated with the Switch and Ballast incorporated in the optional Telescopic Mount, or with a separate Light Switch Box (see pg. 8) if the Telescopic Mount is not ordered. A Mounting Post attached to the Light Source fits into a bracket on the left of the standard MT-1.



SPECIMEN BY-PASS SLIDE By setting the Specimen By-Pass Slide to the "in" position, the cycle of the Cantilever Arm is completed *without the specimen contacting the knife.* If the operator is cutting ultra-thin sections, and then desires thicker sections, he sets the Specimen By-Pass Slide to "in," turns the handwheel until the specimen has advanced the required distance, presses the Slide release button, and cuts the thick section. He may then continue cutting ultra-thin sections of the thickness for which the Tissue Thickness Selector was originally set.

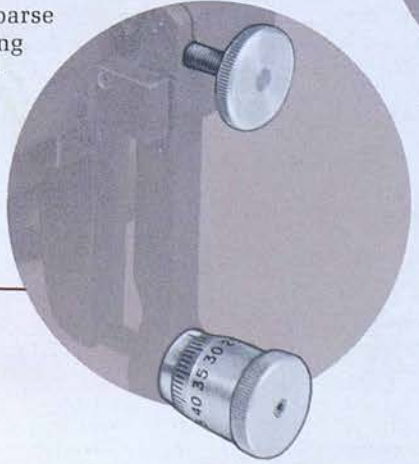


SPECIMEN HOLDERS A 5/16″ Collet-Type Specimen Holder is supplied with the MT-1. 7/32″ and 3/16″ Collet-Type, and a Vise-Type Holder for flat embeddings, are available as optional accessories (see pg. 8). Firm, "finger-tight" adjustment is sufficient to clamp specimens in these holders ready for trimming or sectioning. Various size inexpensive Aluminum Specimen Holders also available.



ADJUSTABLE KNIFE HOLDERS The basic MT-1 is fitted with an Adjustable Glass Knife Holder Assembly. A Diamond Knife Holder Assembly is available as an optional accessory (see pg. 8). Both Assemblies permit lateral movement of knives while maintaining the operator's pre-selected knife angle. 12° vertical orientation scales and Knife Angle Indicators are provided on both types of Assembly.

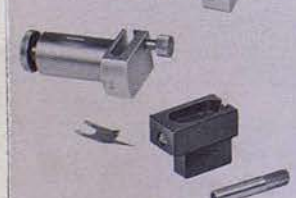
COARSE ADJUSTMENT THUMB SCREW
For making the initial, coarse adjustments in positioning the Knife Stage preparatory to sectioning. Permits bringing the Knife almost to the section position quickly and easily.



FINE ADJUSTMENT MICROMETER Graduated in divisions of 1μ, this micrometer control allows the Knife Stage to be advanced with the utmost precision and positioned exactly, prior to the start of sectioning. Together with the lateral movement possible with the Adjustable Knife Holder Assembly, the optimum knife position may be easily and accurately obtained.

SORVALL®

MT-1 "PORTER-BLUM" ULTRA-MICROTOME OPTIONAL ACCESSORIES



MT-1350 PIVOTING TELESCOPIC MOUNT AND BASE PLATE for factory-modified AO and B&L Stereomicroscopes, complete with Switch and Ballast for MT-1400 Cold Light Source.

For 115 volts, 60 cycles, AC, single phase; 3-wire cord and NEMA standard parallel blade molded cap with U-shaped ground to fit Hubbell receptacle No. 5261 or equivalent.

MT-1350A PIVOTING TELESCOPIC MOUNT AND BASEPLATE, same as above, but for 220 volts, 50 cycles, AC, single phase; 3-wire cord and NEMA standard tandem blade molded cap with U-shaped ground to fit Hubbell receptacle No. 5664 or equivalent.

MT-1400 ADJUSTABLE COLD LIGHT SOURCE complete with Jones Plug (to be used with MT-1350 or MT-1350A). Can also be used with MT-1411 (MT-1411A) Light Switch Box if Mount and Base Plate are not ordered.

MT-1411 LIGHT SWITCH BOX equipped with Switch and Ballast.

For 115 volts, 60 cycles, AC, single phase; 3-wire cord and NEMA standard parallel blade molded cap with U-shaped ground to fit Hubbell receptacle No. 5261 or equivalent.

MT-1411A LIGHT SWITCH BOX, same as above, but for 220 volts, 50 cycles, AC, single phase; 3-wire cord and NEMA standard tandem blade molded cap with U-shaped ground to fit Hubbell receptacle No. 5664 or equivalent.

MT-1131 COLLET-TYPE HOLDER, 5/16″ for specimens embedded in Nos. 0 and 00 gelatin capsules.

MT-1308 COLLET-TYPE HOLDER, 7/32″, for specimens embedded in Nos. 1 and 2 gelatin capsules.

MT-1161 COLLET-TYPE HOLDER, 3/16″, for specimens embedded in Nos. 3, 4 and 5 gelatin capsules.

MT-1197 VISE-TYPE HOLDER for flat embeddings (Ref. Recent Developments in Methacrylate Embedding, Emil Borysko, J. Biophysic. and Biochem. Cytol., July 25, 1956, Vol. 2 #4, pp 15-20).

MT-1189 SPECIMEN TRIMMING BLOCK for holding MT-1 and MT-2 Specimen Holders.

MT-1450 GLASS KNIFE HOLDER (for 1″ triangular Glass Knives) with 12° vertical orientation scale, complete with MT-1318 Knife Angle Indicator and MT-2801 Wrench.

MT-1449 GLASS KNIFE HOLDER ASSEMBLY with 12° vertical orientation scale, complete with Stainless Steel Drum (for 1″ triangular Glass Knives), MT-1318 Knife Angle Indicator and MT-2801 Wrench.

MT-1451 DIAMOND KNIFE BOAT HOLDER with 12° vertical orientation scale and MT-1318 Knife Angle Indicator (accepts MT-2595 and MT-2802 Diamond Knife Boats or DuPont Diamond Knife).

MT-1452 DIAMOND KNIFE BOAT HOLDER ASSEMBLY with 12° vertical orientation scale complete with Stainless Steel Drum and MT-1318 Knife Angle Indicator (accepts MT-2802 Diamond Knife Boat, Rondikn or DuPont Diamond Knife).

MT-2802 DIAMOND KNIFE BOAT ASSEMBLY complete with MT-28024 Locking Wrench. (Will hold 3 mm. x 5 mm. rectangular shank diamond knives as manufactured by I.V.I.C. & GE FE RI.) Fits MT-1451 Boat Holder.

MT-2805 STAINLESS STEEL TROUGHS (box of 12)

PE-60902 FLUORESCENT REPLACEMENT BULB for Cold Light Source, 4 watts.

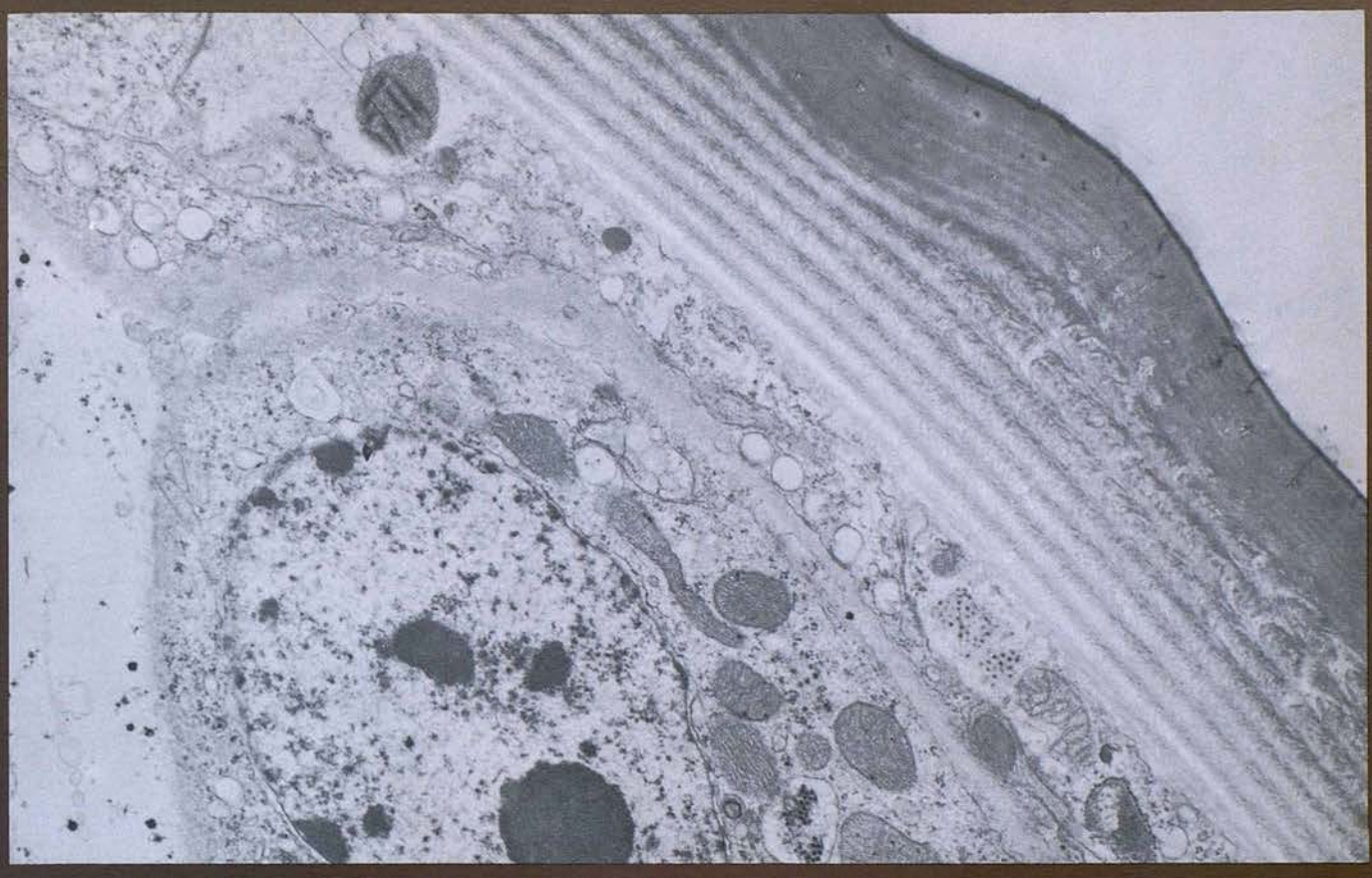
MT-2838 Set of thirty Aluminum Specimen Holders complete with protective plastic guards and storage case.

Five sizes available, any size Holders or combinations of sizes may be selected to make up 30-piece set.

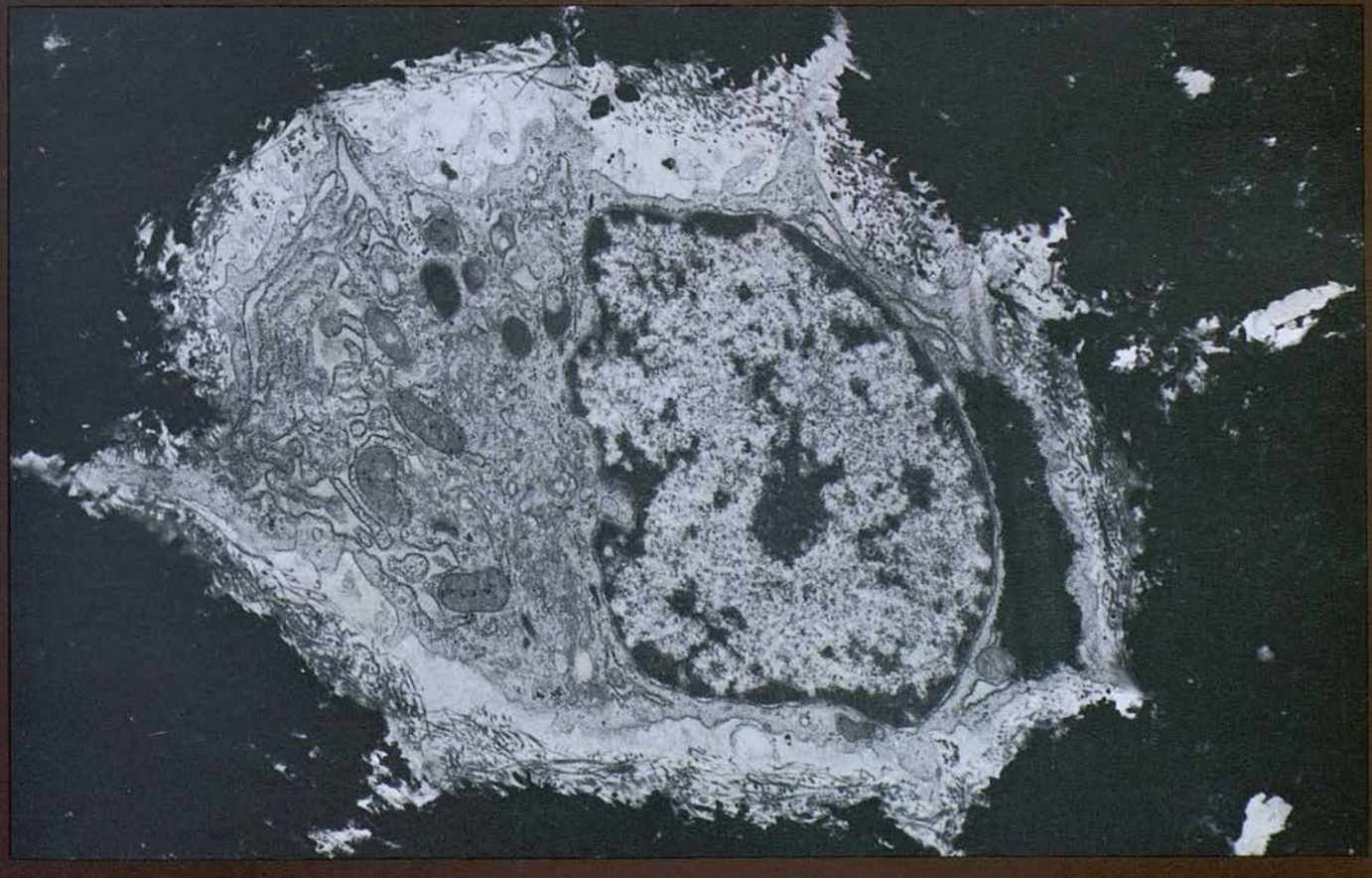
MT-1500 Adapter for MT-1 Ultra-Microtome to accept Aluminum Specimen Holders.

See pp. 14 and 18 for Stereomicroscopes, Spot Illuminator and Glass Knife Pliers.

The Micrographs reproduced in this brochure have been taken from sections cut with SORVALL Porter-Blum Ultra-Microtomes in the laboratories of Dr. Keith Porter, Biological Laboratories, Harvard University, Cambridge, Massachusetts.



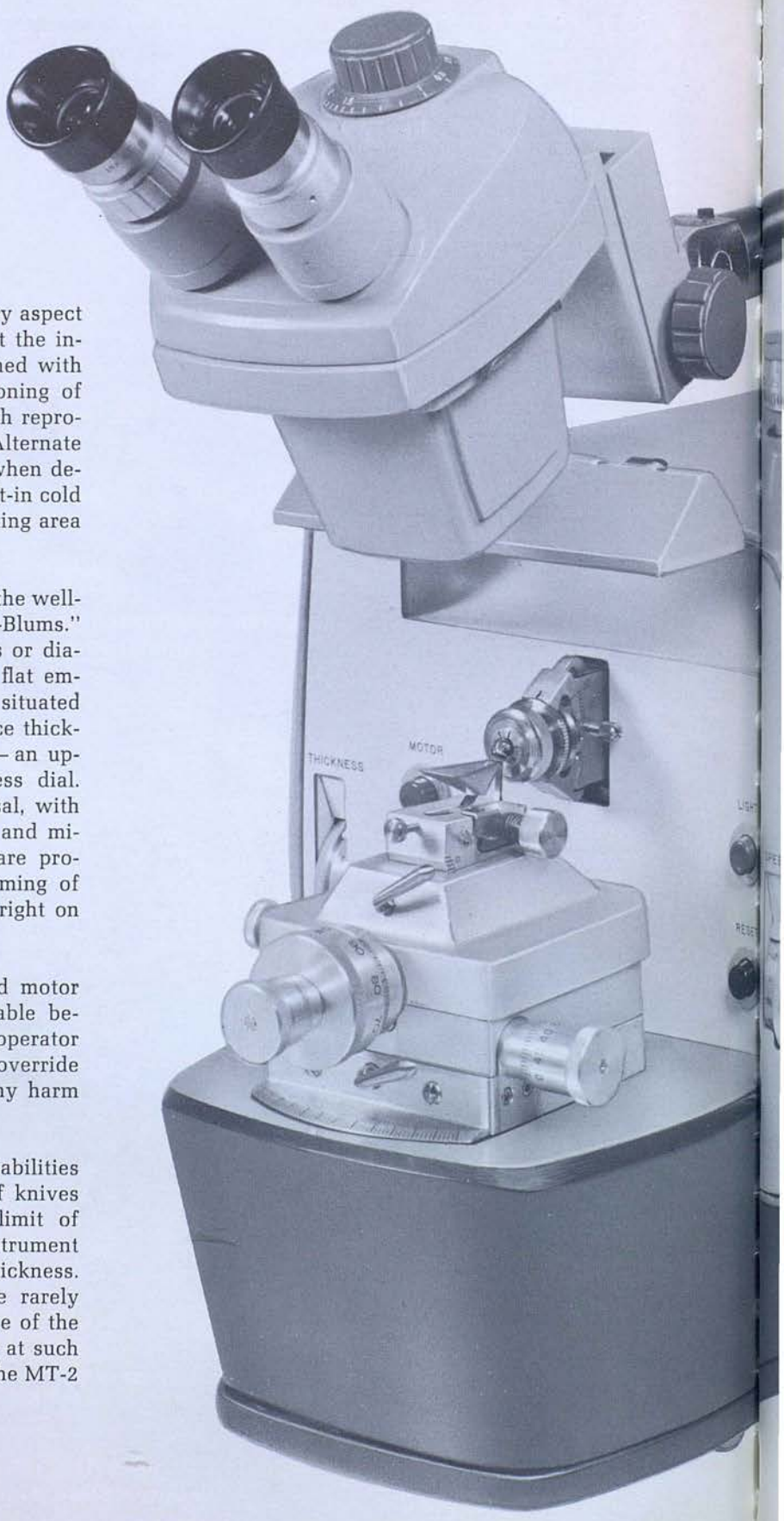
ELECTRON MICROGRAPH OF AN APHID CUTICLE
MAGNIFICATION 15,600X



ELECTRON MICROGRAPH OF AN OSTEOCYTE IN TIBIA OF A 50 gm RAT
MAGNIFICATION 11,000X

SORVALL® MT-2 "PORTER-BLUM"

"Tomorrow's Ultra-Microtome Today"



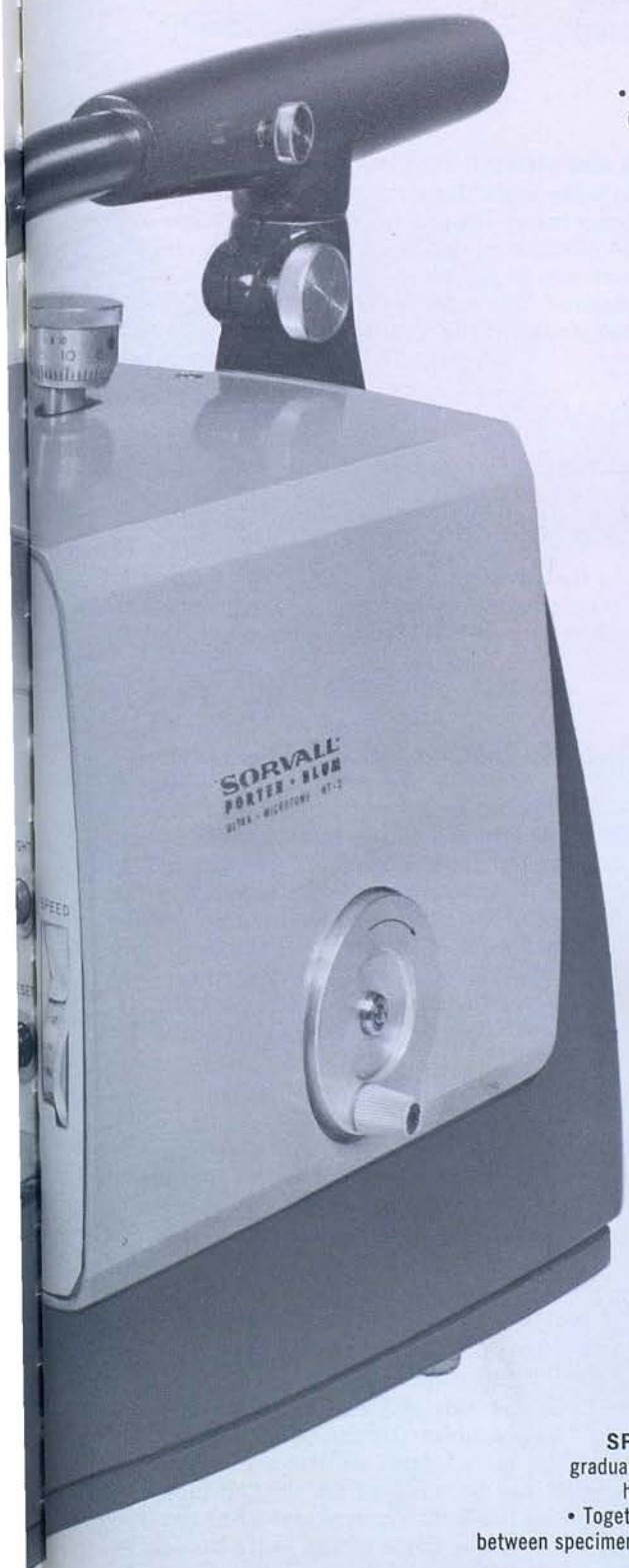
The superiority of the "Porter-Blum" MT-2 in every aspect is evident from the moment the operator looks at the instrument. Integrated, single-unit design is combined with logical placement of all controls. Uniform sectioning of the highest quality is possible with the MT-2, with reproducibility of sections an automatic achievement. Alternate thin and thick sectioning is easily accomplished when desired. A pivoting stereomicroscope mount and built-in cold light source for wide angle illumination of the cutting area are basic features.

The advance system is mechanical, and follows the well-established, proven principles of previous "Porter-Blums." The specimen is advanced to a fixed knife. Glass or diamond knives may be used. Specimens, round or flat embeddings, are held securely in adjustable holders situated at the end of a cantilever arm. Advance, and hence thickness of sections, is determined by two controls — an upper pivot control and a panel-mounted thickness dial. Movement of the knife stage assembly is universal, with coarse and fine forward and reverse adjustment, and micrometer lateral adjustment. Orientation scales are provided for additional convenience. Precision trimming of specimens prior to sectioning may be performed right on the MT-2.

The drive system is motor and/or manual, and motor speeds which are set by dial are infinitely variable between .09 mm and 3.2 mm per second. If the operator should elect to use the motor drive, he may still override it by turning the handwheel manually without any harm to the instrument.

It should be pointed out that the sectioning capabilities of the MT-2 are limited only by the suitability of knives currently available. The stated thin-sectioning limit of 100A is in this respect somewhat arbitrary. The instrument itself is capable of performing well below this thickness. However, sections of such extreme thinness are rarely satisfactory for study. Nevertheless, it is indicative of the overall refinement of the MT-2 that it can operate at such fine tolerances, and it is one of the many reasons the MT-2 is years ahead of any competitive instrument.

ULTRA-MICROTOME 100A to 4 MICRONS



MECHANICAL ADVANCE • Continuously variable following the proven "Porter-Blum" principle of mechanical advance of specimen to fixed knife • Automatic and manual drive • Positive operator control of sectioning at all times • Unaffected by voltage fluctuations that can destroy accuracy of thermal advance systems • No stress or "bending" principles, or other design compromises • Automatic reset to start position with facility for selecting any intermediate position on advance screw • Constant, positive-contact, specimen arm suspension for maximum accuracy of operation • Specimen arm universally mounted on hardened steel edges • Massive, special alloy steel specimen arm highly resistant to thermal changes

UNIVERSAL KNIFE STAGE ASSEMBLY • Stage Assembly pivots on sectioning point through calibrated scale 30° either side of central position • Separate micrometer controls on upper and middle stages for forward, backward, and lateral travel • Upper stage micrometer calibrated in increments of 1μ — lateral micrometer calibrated in increments of 10μ • Universal movement of Stage Assembly and vertical rotation of knife holder, permit positioning of knife at any point in relation to the specimen • Equally simple knife positioning for trimming the block and sectioning • Specimen trimming to any logical conformation • Tripod support pads for each stage ensure maximum stability • Accepts holders with 12° vertical orientation scale for glass and diamond knives

MOTOR DRIVE FOR AUTOMATIC OPERATION • Smooth, built-in motor drive permits stepless selection of cutting speeds from .09mm to 3.2mm per second • Specimen arm return is three times faster than cutting portion of cycle — production is speeded up without sacrificing sectioning quality • Variable speeds provide from .4 section to 15 sections per minute • Motor drive may be overridden with handwheel at any time during automatic operation • Vibrationless operation through unique mounting arrangement • Pushbutton starting on front control panel

HANDWHEEL FOR MANUAL OPERATION • For precise manual trimming • Personalizes instrument for individual sectioning requirements • Slower or faster cutting speeds without setting speed control

KNIVES • Diamond • Glass

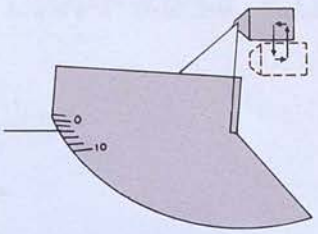
TRIMMING • Microtome itself is used as a refined tool for symmetrically-perfect specimen trimming — of great importance to serial and precision sectioning

SECTIONING • 100A to 4μ — continuously variable thickness selection (By-pass facility for thicker sections) • Large area specimens sectioned with ease • Upper thickness micrometer for micro adjustment — panel thickness control dial for macro adjustment — ensure maximum accuracy and fingertip simplicity of exact thickness setting • Section thinness is limited only by knives currently available • Highest uniformity in serial sectioning available anywhere

ALTERNATE THIN & THICK SECTIONS • Pushbutton by-pass facility enables operator to increase thickness of sections — instrument then returns automatically to original setting

SPECIMEN HOLDERS • Collet-type and vise-type holders with reference scale in 2° graduations • 360° rotation of holder around central axis • 20° rotation action of specimen holder mount on vertical plane • Maximum versatility in orienting specimen to knife • Together with Universal Stage Assembly, provides optimum sectioning point relationship between specimen and knife • Various size inexpensive Aluminum Specimen Holders also available

Schematic of Specimen Movement During Cutting Cycle

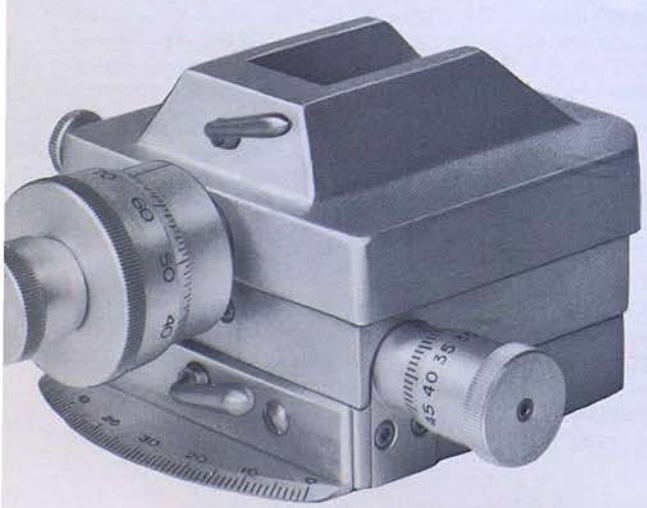


SPECIMEN MOVEMENT CYCLE The cantilever arm is withdrawn .6 mm straight back from the knife on the return stroke as shown in the accompanying schematic drawing. This prevents section pickup and assures accurate, uniform alignment of the block and knife on each cutting stroke. The upward movement of the specimen during the return stroke is faster than its downward movement during the cutting stroke, thus reducing the delay between cuts.

ILLUMINATING AND VIEWING SYSTEM A built in fluorescent lamp provides wide angle illumination of the knife trough which is necessary for judging section thickness and quality. The position of this lamp is adjustable, and the entire lamp can be retracted into the mirotome panel when desired. The lamp is turned on and off with a push button switch at the right side of the front panel.
See page 18 for stereomicroscopes.

ADVANCE SYSTEM/SECTION THICKNESS Two separate controls determine the specimen advance on each cutting cycle — the upper thickness (pivot) control, and the thumb-operated thickness dial on the front panel. The total advance per cycle is the product of these two settings. Any section thickness within the range of 100A to 4μ may be obtained. As the advance is mechanical (see schematic drawing pg. 17), section thickness does not depend on the speed of the cutting cycle as it does in thermal advance systems.

SPECIMEN HOLDERS Specimen holders of the collet-type for 5/16″, 7/32″, and 3/16″ diameter specimen blocks, and a vise-type for flat embeddings, mount on the front end of the cantilever arm and can be interchanged easily. The 5/16″ collet-type chuck is supplied with the MT-2; others are optional. Provision is made for rotation of the holder around the axis of the arm, and for tilting the holder to assure a proper vertical alignment of the front face of the specimen. Calibrated scales are provided for both of these movements. Various size inexpensive Aluminum Specimen Holders also available.



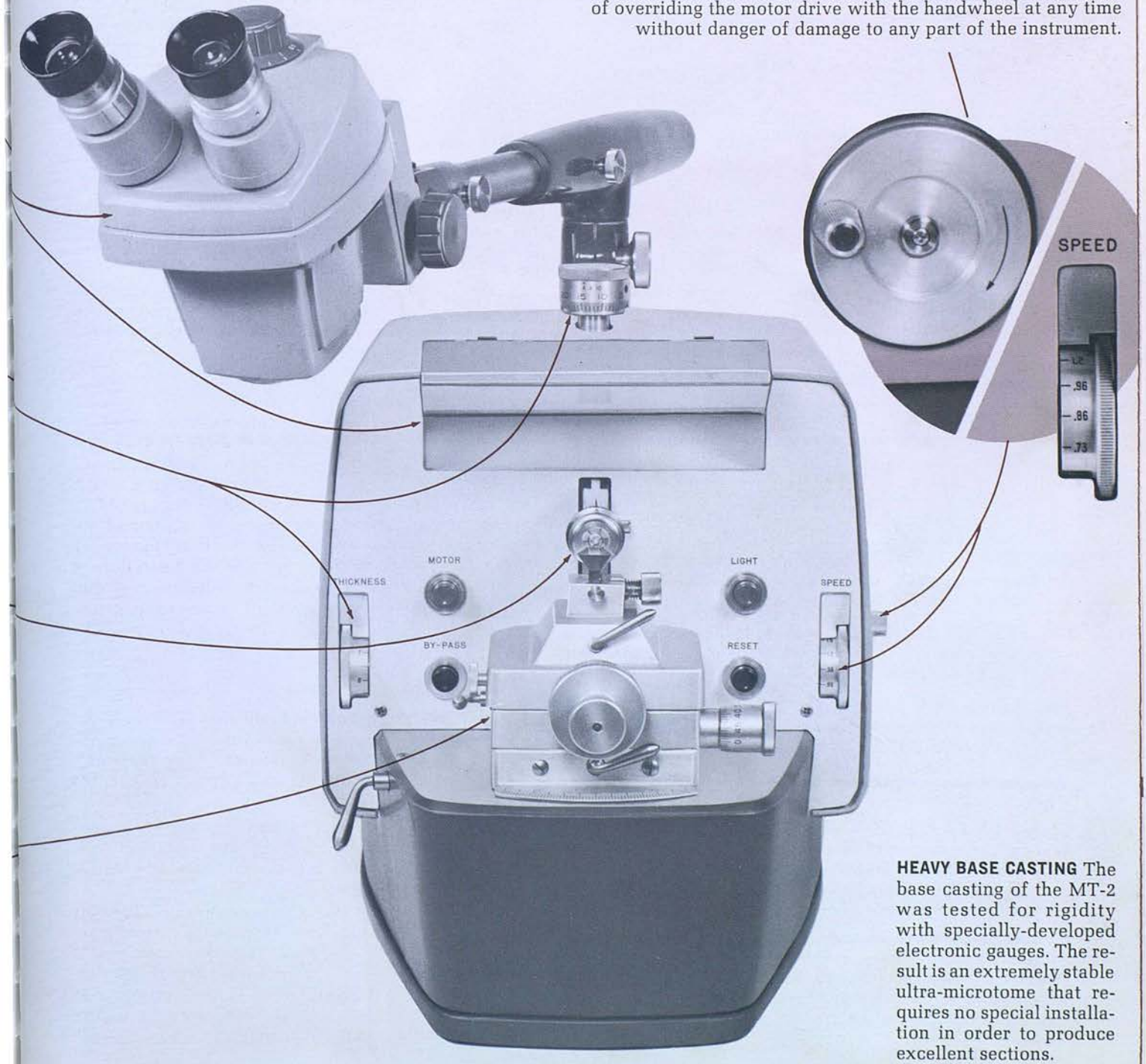
KNIFE STAGE ASSEMBLY The universal knife stage system is designed to provide all movements necessary for simple and accurate alignment of the knife edge with respect to the specimen. When desired, the entire knife stage is easily removed from the table of the microtome.

The entire knife stage rotates around a vertical axis that passes through the point of contact of the knife and specimen. This rotation is calibrated through 30° in each direction, and can be locked firmly in place in any position.

Both lateral and forward movements of the knife stage assembly are accomplished with micrometers (Upper stage micrometer calibrated in increments of 1μ — lateral micrometer calibrated in increments of 10μ.) that can be released and locked independently. The forward advance has both a coarse and a fine control. A scale is provided to indicate the angle of the knife holder, so that, if desired, a previous setting can be reproduced quickly and accurately.

SORVALL® MT-2 "PORTER-BLUM" ULTRA-MICROTOME

MOTOR/MANUAL DRIVE SYSTEM AND SPEED CONTROL Speed range is continuously variable by dial from .09mm to 3.2mm. The motor drive system, pushbutton started from the front panel, drives the microtome through a vibration-free coupling to the handwheel. Thus motor drive duplicates hand operation, but assures a more uniform and repeatable operation than possible by hand. An important feature in the motor drive system is the possibility of overriding the motor drive with the handwheel at any time without danger of damage to any part of the instrument.



HEAVY BASE CASTING The base casting of the MT-2 was tested for rigidity with specially-developed electronic gauges. The result is an extremely stable ultra-microtome that requires no special installation in order to produce excellent sections.

ORVALL®

MT-2 "PORTER-BLUM" ULTRA-MICROTOME OPTIONAL ACCESSORIES

MT-2080 COLLET-TYPE HOLDER, 5/16″, with reference scale in 5° graduations for specimens embedded in Nos. 0 and 00 gelatin capsules.

MT-2081 COLLET-TYPE HOLDER, 7/32″, with reference scale in 5°graduations for specimens embedded in Nos. 1 and 2 gelatin capsules.

MT-2082 COLLET-TYPE HOLDER, 3/16″, with reference scale in 5° graduations for specimens embedded in Nos. 3, 4 and 5 gelatin capsules.

MT-2083 VISE-TYPE HOLDER for flat embeddings with reference scale in 5° graduations complete with MT-2081 Wrench.

MT-2575 GLASS KNIFE HOLDER with 12° vertical orientation scale.

MT-2585 DIAMOND KNIFE BOAT HOLDER with 12° vertical orientation scale (accepts MT-2802 Diamond Knife Boat, Rondikn or DuPont Diamond Knife).

MT-2802 DIAMOND KNIFE BOAT assembly complete with MT-28024 Locking Wrench. (Will hold 3 mm. x 5 mm. rectangular shank diamond knives as manufactured by I.V.I.C. & GE FE RI.)

MT-2805 STAINLESS STEEL TROUGHS (box of five).

PE-60902 FLUORESCENT REPLACEMENT BULB for Cold Light Source, 4 watts.

PM-61058 PHILLIPS SCREW DRIVER.

PE-61050 LITTELFUSE, 1/2 amp., 250 v.

MT-2813 TRIMMING BLOCK for holding all MT-2 Specimen Holders.

MT-2838 Set of thirty Aluminum Specimen Holders complete with protective plastic guards and storage case.

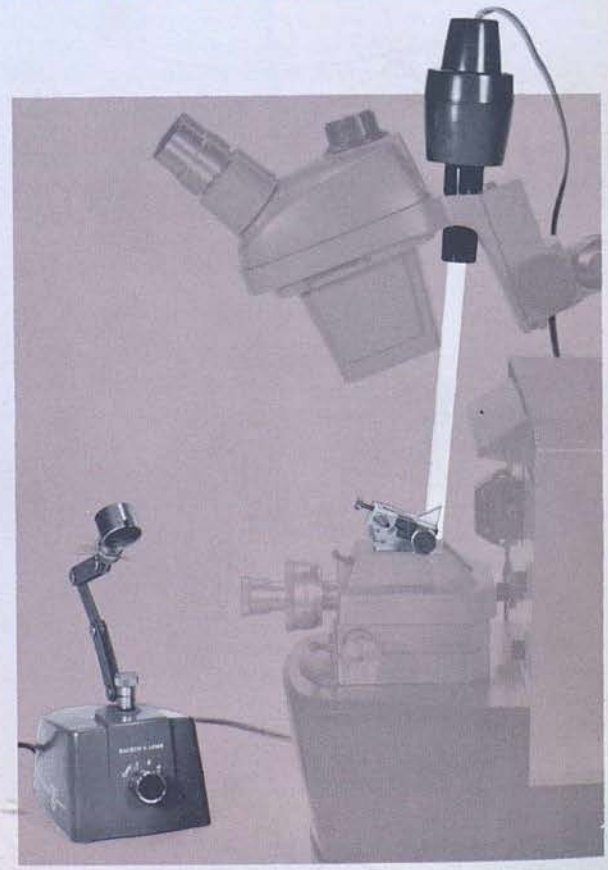
Five sizes available, any size Holders or combinations of sizes may be selected to make up 30-piece set.

See page 18 for Stereomicroscopes.

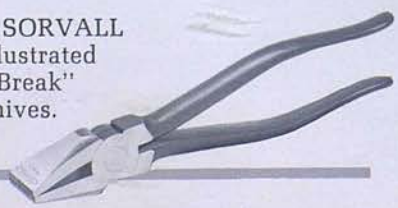
-2184 B&L SPOT ILLUMINATOR — Incandescent light source for knife edge examination, complete with lamp bracket and control unit. For 115 volts, 60 cycles, AC.

2184A B&L SPOT ILLUMINATOR — same as above but for 220 volts, 50 cycles, AC. A step-down transformer is provided for 220 volts operation.

This incandescent light permits convenient inspection of the edges of glass or diamond knives — a useful accessory for users having B&L Stereomicroscopes.



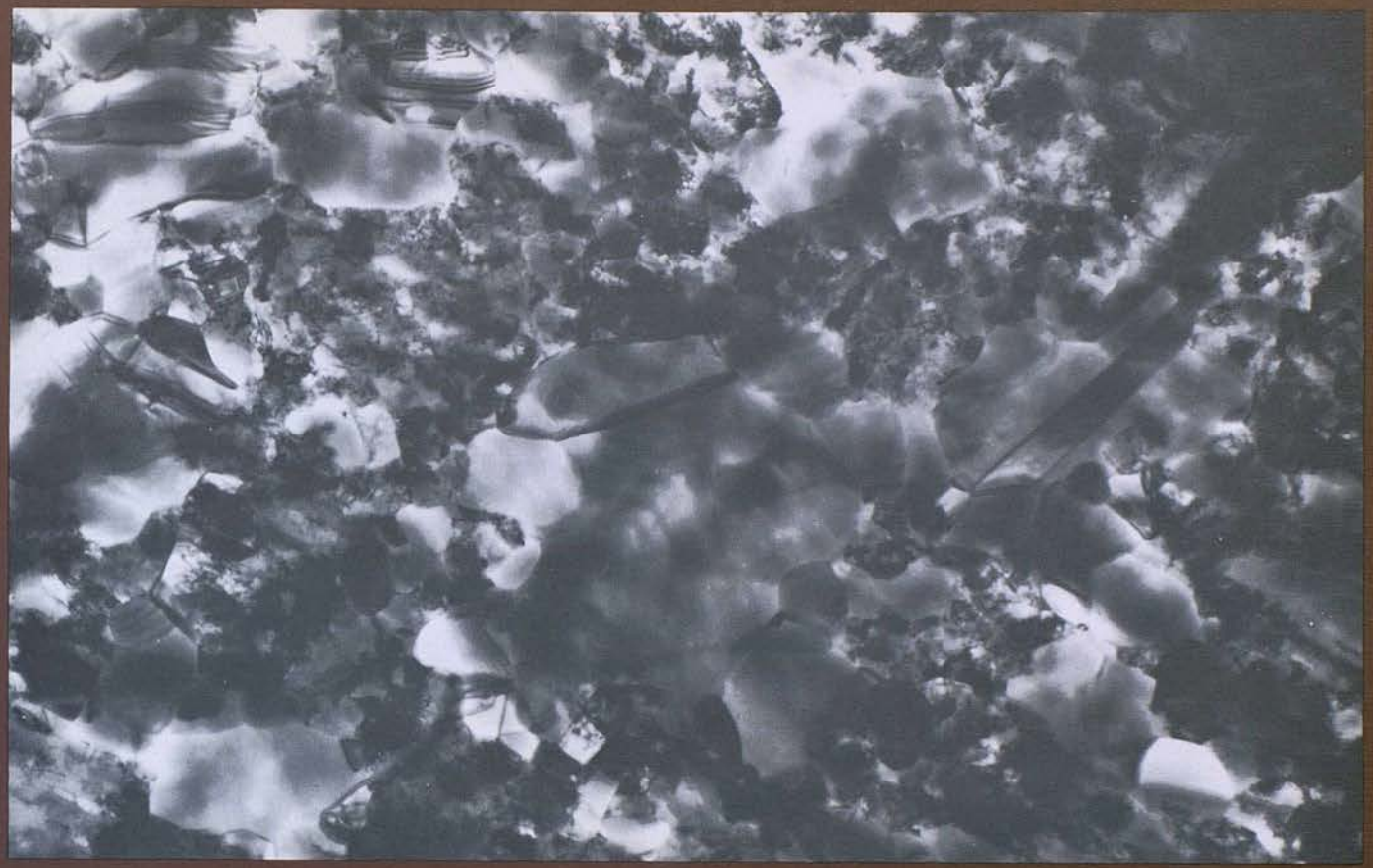
-2812 GLASS KNIFE PLIERS — SORVALL modified, complete with illustrated instructions for the "Free Break" method of making glass knives.



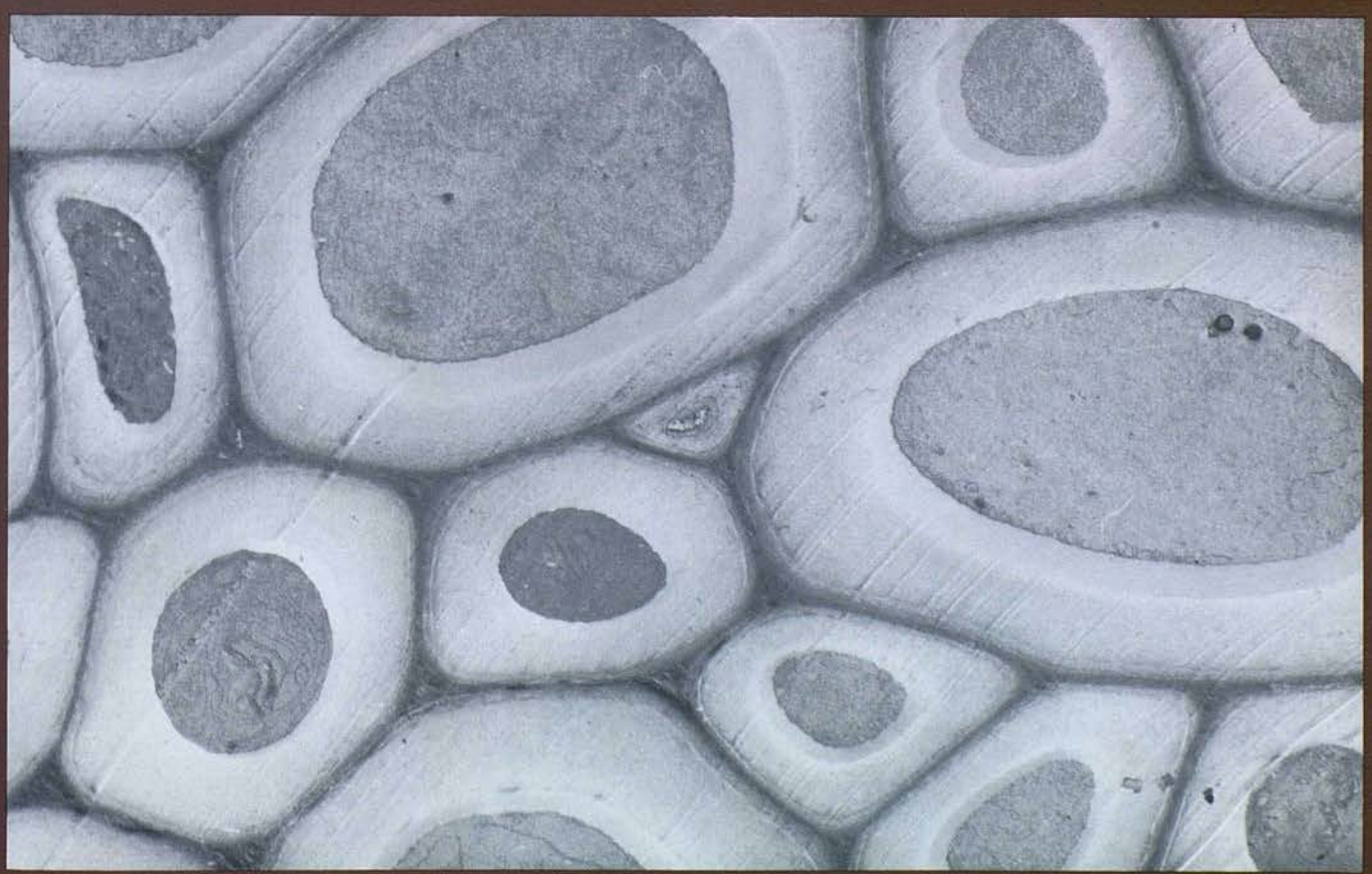
These pliers are specially machined, heavy glaziers pliers which provide the necessary pressure points for the accurate breaking of glass from which to obtain knives.

NOTE: The Spot Illuminator and Glass Knife Pliers are equally useful with either the MT-1 or the MT-2.

The Micrographs reproduced in this brochure have been taken from sections cut with SORVALL Porter-Blum Ultra-Microtomes in the laboratories of Dr. Keith Porter, Biological Laboratories, Harvard University, Cambridge, Massachusetts.



ELECTRON MICROGRAPH OF COPPER SECTIONED WITH A DIAMOND KNIFE. THICKNESS APPROXIMATELY 50 μ
MAGNIFICATION 30,000X

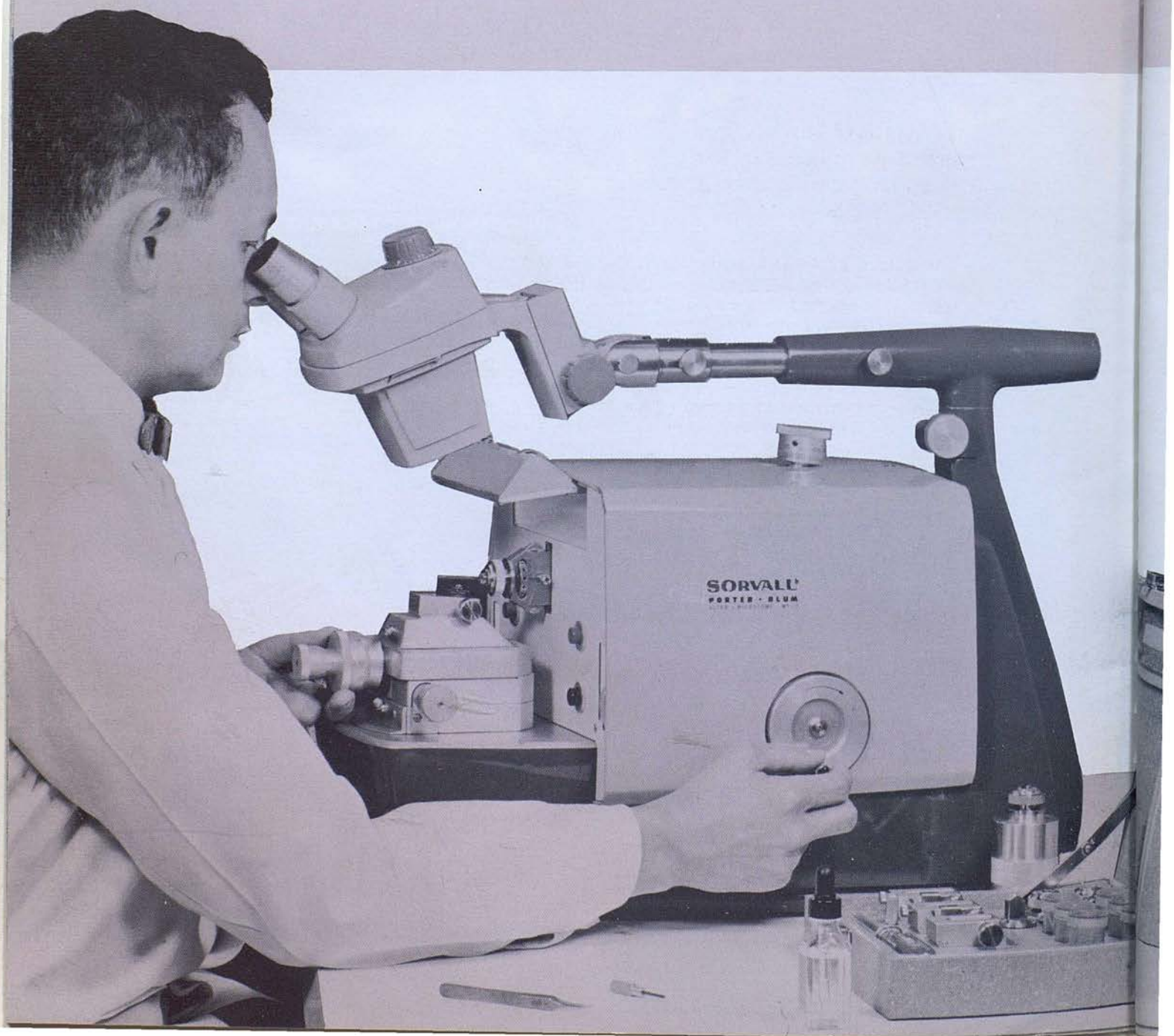


ELECTRON MICROGRAPH OF LOCUST WOOD (ROBINEA PSEUDOACACIA) (SOME KNIFE MARKS VISIBLE)
MAGNIFICATION 7,700X

SORVALL® "PORTER-BLUM" ULTRA-MICROTOMES

Accustomed as today's microtomist is to the availability of several precision instruments with which to pursue his specialty, it is surprising to note that true ultra-microtome development is but two decades old. Speed of sectioning was first thought to be the answer to thin sectioning. This was a theory which unfortunately did not prove itself in practical application. Such high-speed cutting made harvesting from the resultant "burst" of sections all but impossible or, at best, extremely difficult. Gradually, persons in different parts of the world developed other principles involving different theories of mechanical and thermal advance, and cutting actions. It is fair to say that all systems have some disadvantages, but it is the belief of Ivan Sorvall, Inc. that the "Porter-Blum" has far fewer than any other. It is less affected by external conditions than are systems employing cutting cycles dependent upon gravitational force.

"Directness" and "simplicity" characterize the "Porter-Blum" design. Mechanical advance of the specimen is achieved by mounting a cantilever arm on offset pivots on a vertical pivot arm. The relationship of these pivots is variable in the MT-2 and fixed in the MT-1. The lower end of the pivot arm rests in a lead screw. The magnitude of advance of the cantilever arm, and hence the specimen, depends upon

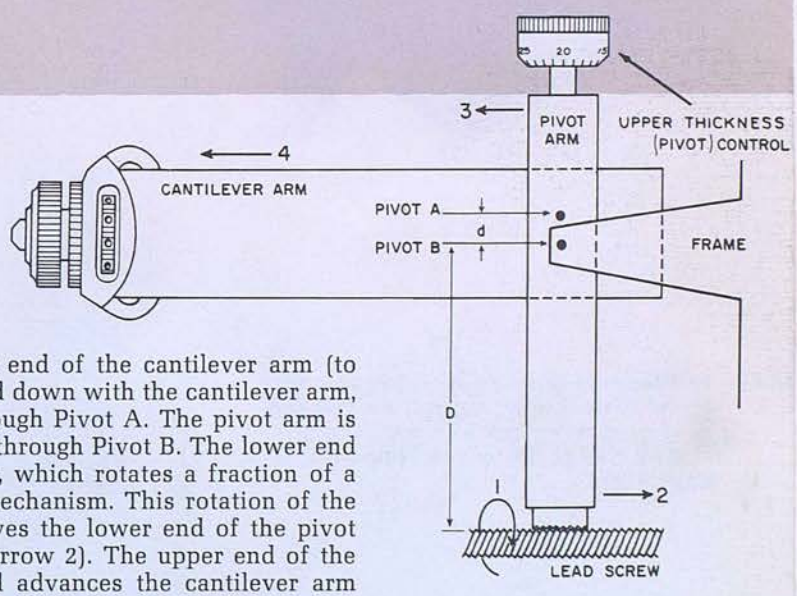


the ratio of the cantilever arm pivots one to the other and the amount the lead screw is rotated.

Naturally, many factors constrain to affect section uniformity and quality obtainable from any microtome. However, given precision engineering and use of the best available materials, stable environmental conditions in the area where the instrument will be used, together with operator compatibility and/or motor drive, the mechanical advance system as developed in the "Porter-Blums" is unsurpassed for simplified production of useable sections.

Every component in the "Porter-Blums" is designed to bring maximum accuracy to bear upon the optimum sectioning point. This is true both of the MT-1 and of the MT-2, but this component co-ordination is particularly apparent in the results achievable with the latter. SORVALL even designed special gauges with which to establish the rigidity of the MT-2 base casting, and the instrument as a whole was bench-tested to the equivalent of ten years of use.

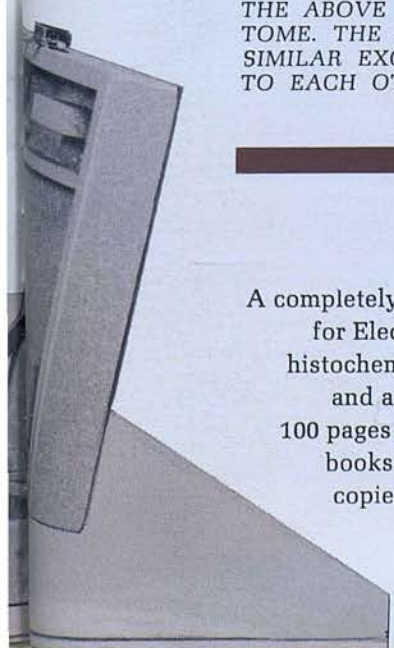
Either "Porter-Blum" Ultra-Microtome will, within its respective sectioning ranges, meet the needs of the most exacting microtomist. It is, of course, up to the individual operator to determine which "Porter-Blum" will best meet his particular sectioning requirements.



Schematic of Cantilever Arm Mounting — Advance System for MT-2 Ultra-Microtome

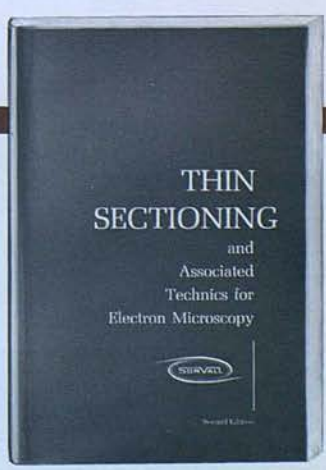
The specimen holder is mounted on the end of the cantilever arm (to the left in the diagram), and moves up and down with the cantilever arm, which is connected to the pivot arm through Pivot A. The pivot arm is connected to the frame of the microtome through Pivot B. The lower end of the pivot arm rests on the lead screw, which rotates a fraction of a turn with each cycle of the microtome mechanism. This rotation of the lead screw (arrow 1 in the diagram) moves the lower end of the pivot arm toward the rear of the microtome (arrow 2). The upper end of the pivot arm moves forward (arrow 3) and advances the cantilever arm (arrow 4). The magnitude of this advance depends both on the amount of rotation of the lead screw and on the ratio of the spacing (d) between the two pivots to the distance (D) between Pivot B and the lead screw. The spacing between the two pivots (d) is determined by the setting of the pivot control, on the top of the pivot arm.

THE ABOVE APPLIES TO THE MT-2 "PORTER-BLUM" ULTRA-MICROTOME. THE ADVANCE SYSTEM FOR THE MT-1 "PORTER-BLUM" IS SIMILAR EXCEPT THAT THE PIVOTS ARE FIXED IN RELATIONSHIP TO EACH OTHER INSTEAD OF BEING VARIABLE AS IN THE MT-2.

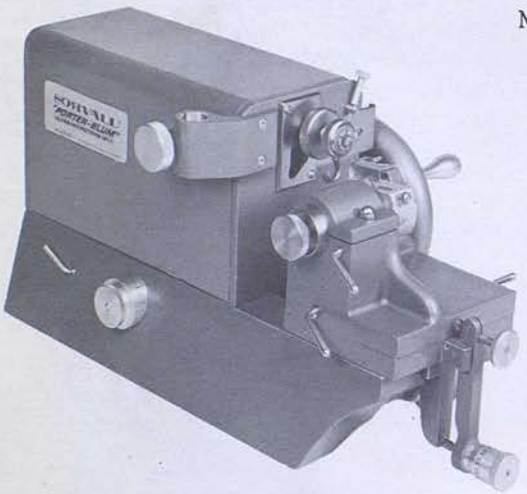


THIN SECTIONING & ASSOCIATED TECHNICS FOR ELECTRON MICROSCOPY

A completely revised edition of "Thin Sectioning & Associated Technics for Electron Microscopy" is now available. It includes a section on histochemical methods which can be applied to electron microscopy, and a greatly expanded bibliography. "Thin Sectioning" has over 100 pages and is bound in soft cover. One of these highly informative books is supplied with each "Porter-Blum" purchased. Additional copies may be obtained from Ivan Sorvall, Inc. at $3.00 per copy.



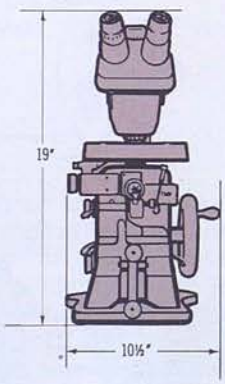
SORVALL® MT-1 SPECIFICATIONS

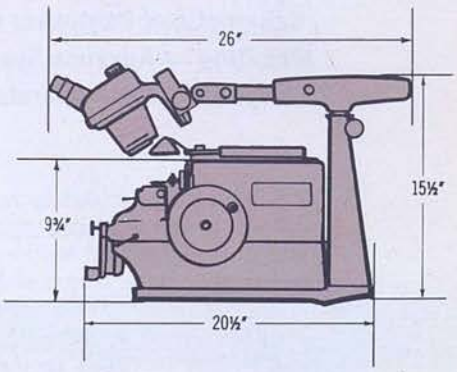


MT-1 SORVALL "PORTER-BLUM" ULTRA-MICROTOME, Basic Instrument, with mechanical advance, sectioning range variable between 1/40 to 1/2 micron, By-Pass Slide for thicker sections, Bracket for Light Source, Cantilever Arm Lock, Fine Adjustment Micrometer (graduated in 1μ divisions) for advancing Upper Stage, 5/16″ Collet-Type Specimen Holder, Glass Knife Holder Assembly with 12° vertical orientation scale and MT-2801 Wrench, 2 Glass Knives (samples)*, 2 Stainless Steel Troughs, Dust Cover, "Thin Sectioning" booklet and Operating Instructions.

Net weight (Basic Unit) 32 lbs. Shipping weight 48 lbs.

NOTE: 1″ Triangular Glass Knives made according to the "Free Break" method are now supplied as samples only with each new MT-1 or MT-2. See pg. 14 for description of Glass Knife Pliers.





STEREOMICROSCOPES

MT-1253A
AMERICAN OPTICAL SPENCER CYCLOPTIC STEREOMICROSCOPE, factory modified, Series 55 F-2, with wide field 15x eyepieces and 1.33x body magnification. Total magnification 20x.

MT-1253B
AMERICAN OPTICAL SPENCER CYCLOPTIC STEREOMICROSCOPE, factory modified, Series 55 M-2, with wide field 15x eyepieces and Magni-Changer for 10x, 15x, 20x, 30x and 40x magnifications.

MT-1253C
BAUSCH & LOMB STEREOMICROSCOPE, factory modified, Model KF, with wide field 10x eyepieces and 2x fixed power pod. Total magnification 20x.

MT-1253D
BAUSCH & LOMB STEREOMICROSCOPE, factory modified, Model KV, with wide field 15x eyepieces and variable power pod. Magnifications continuously variable between 10x and 45x.

Models MT-1253C and MT-1253D are as shown above. The stereomicroscopes shown on SORVALL Ultramicrotomes in this brochure are earlier models.

NOTE: For the MT-2 the above Stereomicroscopes require the use of the MT-2900 Pivoting Telescopic Mount and Base Plate.
For the MT-1 the above Stereomicroscopes require the use of the MT-1350 Pivoting Telescopic Mount and Base Plate.

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Glomerular Ultrastructure in Secondary Diabetics and Normal Subjects

J. T. Ireland, M.B., M.R.C.P. (Ed.), B. K. Patnaik, M.B., Ph.D., and L. J. P. Duncan, M.B., B.Sc., F.R.C.P. (Ed.), Edinburgh, Scotland

SUMMARY

Renal biopsy tissue obtained from nine nondiabetic subjects and ten patients having diabetes secondary to primary pancreatic disease was studied by light and electron microscopy. The technic for measurement of the basement membrane thickness at the periphery of the glomerular capillaries is described. In seven of the secondary diabetics the capillary basement membrane thickness exceeded 3,500 Å, a width greater than the mean value in the healthy control group by more than two standard deviations. Changes in cellular ultrastructure were also observed. These findings suggest that microangiopathy occurs more frequently in secondary diabetics than has hitherto been conceded and support the view that at least one of the primary causes of basement membrane changes is the metabolic disturbance consequent upon inadequate insulin activity. DIABETES *16*:628-35, September, 1967.

One of the outstanding problems concerning the pathogenesis of diabetic angiopathy is whether the lesions are a genetically determined and integral component of the idiopathic disorder or are due to one or more of the biochemical derangements of diabetes mellitus.[1] Many animal studies[2-7] have been undertaken in an attempt to settle this issue, yet the occurrence in man of secondary or pancreatic diabetes ideally lends itself to the investigation of this problem. Although diabetic glomerulosclerosis has occasionally been reported in secondary diabetics,[8-12] and in lipoatrophic diabetics,[13,14] on the basis of light microscopy study, the use of electron microscopy allows a more accurate assessment of the glomerular lesions. This method has been used in several studies in which the glomerular capillary basement membrane was reported to be thickened in recently diagnosed diabetic and prediabetic patients.[15-18] Although these findings have been cited in favor of a genetic basis for diabetic microangiopathy, the technics used and control observations were often inadequate.[19] In a careful study, Osterby-Hansen[20] found no difference in glomerular capillary basement membrane thickness between recent diabetics and nondiabetics.

This study reports the light and electron microscopic appearances of renal tissue obtained by biopsy from patients having secondary or pancreatic diabetes and from nondiabetics. Measurements of peripheral capillary basement membrane thickness in the glomeruli of subjects in both groups are statistically analyzed and the cellular ultrastructure described and compared.

MATERIAL AND METHODS

The nine nondiabetic control subjects were all healthy except Case 9, who was included because he had chronic pancreatitis without abnormality of glucose tolerance (table 1). The secondary diabetic patients were unselected except that they were available for study and willing to have a renal biopsy performed (table 2). The clinical investigations and criteria which established the diagnosis of the primary pancreatic disease are set out in table 3.

No subject was hypertensive and, with the exception of Case 9 who had pyelonephritis, none had clinical or biopsy evidence of nondiabetic renal disease. There was no family history of diabetes in either group at the time of investigation, although two years later a sibling of Case 16 developed the disorder. The two groups could not be matched for age and sex since few patients have diabetes secondary to pancreatic disease, and the opportunities of obtaining renal biopsies from healthy subjects are limited.

Renal tissue was obtained by percutaneous biopsy, using a Vim-Silverman needle, except in the two patients having carcinoma of the pancreas, in which cases it was obtained by open biopsy at laparotomy. The

From the Diabetic and Dietetic Department, Royal Infirmary of Edinburgh, and the Department of Pathology, University of Edinburgh, Edinburgh, Scotland.

TABLE 1

Nondiabetic subjects: Clinical data, light and electron microscopy findings

Case no.	Age	Sex	Clinical status	Light microscopy findings in renal biopsies	Electron microscopy measurement of peripheral glomerular capillary basement membrane thickness (Angstrom units)							
					Glomerulus 1		Glomerulus 2		Glomerulus 3		Mean of all measurements obtained	
					Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.		± 1 S.D.
1	15	M	Normal	Normal	2,275	350	2,680	290	2,575	315	2,540	350
2	17	M	Normal	Normal	1,450	225	1,420	250	1,370	210	1,400	250
3	19	F	Normal	Normal	1,200	300	1,525	225	1,550	215	1,450	300
4	19	M	Normal	Normal	2,055	370	1,975	280	2,310	355	2,150	380
5	38	F	Normal	Normal	1,980	335	2,300	250	2,550	285	2,310	400
6	41	M	Normal	Normal	2,570	350	2,815	400	2,775	430	2,750	450
7	44	M	Normal	Normal	2,240	420	2,410	470	2,130	420	2,260	470
8	52	M	Normal	Normal	2,725	400	2,650	330	2,600	360	2,700	400
9	78	M	Chronic pancreatitis	Normal	1,875	340	2,340	275	2,510	325	2,210	390

TABLE 2

Secondary diabetic subjects: Renal biopsy findings on light and electron microscopy

Case no.	Age	Sex	Primary disease of pancreas	Renal biopsy findings on light microscopy	Electron microscopy measurements of peripheral glomerular capillary basement membrane thickness (Angstrom units)							
					Glomerulus 1		Glomerulus 2		Glomerulus 3		Mean of all measurements obtained	
					Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.	Mean thickness	± 1 S.D.		± 1 S.D.
10	30	M	Recurrent acute pancreatitis	Early diffuse glomerulosclerosis	4,920	1,050	5,900	1,150	4,725	770	5,200	1,060
11	47	M	Alcoholic pancreatitis	Normal	4,075	520	4,125	485	4,170	670	4,140	690
12	48	M	Hemochromatosis	Diffuse glomerulosclerosis	3,740	710	3,750	720	3,600	730	3,700	730
13	50	F	Chronic pancreatitis	Normal	4,230	460	3,880	390	3,920	425	4,000	510
14	54	M	Hemochromatosis	Normal	4,145	390	3,985	300	3,765	450	3,880	525
15	56	M	Carcinoma of pancreas	Normal	4,000	450	3,770	375	3,785	415	3,800	470
16	57	F	Chronic pancreatitis	Normal	3,475	625	3,340	670	3,275	610	3,350	695
17	61	F	Carcinoma of pancreas	Normal	1,870	400	1,930	360	2,275	350	1,950	470
18	65	F	Chronic pancreatitis	Diffuse and early nodular glomerulosclerosis	5,420	610	4,965	520	5,020	640	5,000	730
19	68	M	Chronic pancreatitis	Interstitial fibrosis and round cell infiltration of renal parenchyma	2,215	320	2,090	290	2,420	480	2,125	700

specimens were immediately divided, cubes from either end being fixed in 1 per cent buffered osmium tetroxide and embedded in Araldite for examination by electron microscopy while the center portion of each specimen was fixed in corrosive formol for examination by light microscopy. In each case at least three glomeruli were sectioned in ultramicrotomes and thin sections transferred to copper grids for examination in an A.E.I. (Metropolitan Vickers) E.M.6 electron microscope. Areas of glomerular tufts were examined and photographed at magnifications of 800 to 2,500 times (figure 1) and at higher magnifications of 2,500 to 20,000 times. The latter were used to measure basement membrane thickness and study glomerular morphology. The observer did not know whether the biopsy being examined had been obtained from a healthy control or a secondary diabetic subject.

The areas of glomeruli examined were unselected, apart from suitability for photographic reproduction. The method of measuring the thickness of capillary loops is illustrated in figure 2. Because, in both normal and diabetic subjects, the capillary basement membrane

TABLE 3

Case no.	Primary disease of pancreas	Clinical criteria confirming diagnosis of disease of pancreas	Duration of diabetic features	Diabetic regime
10	Recurrent acute pancreatitis	Acute onset of diabetic features immediately following attack of acute pancreatitis six years previously. Recurrent acute pancreatitis confirmed by estimation of serum amylase. Barium meal: large pancreatic cyst with anterior displacement of stomach. Tests of pancreatic exocrine function abnormal. Laparotomy: pancreatic fibrosis and pseudocyst formation.	6 years	Insulin therapy
11	Alcoholic pancreatitis	Chronic alcoholic. Drainage of pancreatic pseudocyst four years before onset of diabetic features. Chronic pancreatic fistula. Laparotomy: pancreatic fibrosis.	1 year	Insulin therapy
12	Hemochromatosis	Skin pigmentation, hepatomegaly, hypogonadism. Serum iron: 360 μg./100 ml. T.I.B.C.: 420 μg./100 ml. Sternal marrow biopsy: excess iron staining. Renal biopsy: iron stain in tubules. Liver biopsy: cirrhosis and iron deposition.	15 years	Insulin therapy
13	Chronic pancreatitis	Intermittent, recurrent upper abdominal pain. X-ray abdomen: calcification in head and body of pancreas. Five-day stool fat collection: 11 gm. per day. D-xylose excretion normal. Folic acid and Vitamin B_{12} absorption tests normal. Secretin and pancreozymin tests: reduction in pancreatic exocrine function.	3 years	Diet alone
14	Hemochromatosis	Skin pigmentation, hepatomegaly. Serum iron: 440 μg./100 ml. T.I.B.C.: 465 μg./100 ml. Sternal marrow biopsy: excess iron stain. Liver biopsy: cirrhosis and iron deposition.	3 months	Insulin therapy
15	Carcinoma of pancreas	Upper abdominal pain. Laparotomy: carcinoma of pancreas.	6 months	Diet alone
16	Chronic pancreatitis	Five-year history of intermittent abdominal pain and steatorrhea before investigation by laparotomy. Cholecystectomy and choledochotomy. Diabetes diagnosed at time of operation. Symptoms of abdominal pain continued following operation. Subsequent secretin and pancreozymin tests: marked reduction in pancreatic exocrine function.	10 years	Insulin therapy
17	Carcinoma of pancreas	Obstructive jaundice. Laparotomy: carcinoma of pancreas.	3 months	Diet alone
18	Chronic pancreatitis	Weight loss, diarrhea, steatorrhea. Five-day stool fat collection: 10 gm./day. D-xylose excretion normal. Folic acid and Vitamin B_{12} absorption tests normal. Secretin and pancreozymin tests: considerable reduction in pancreatic exocrine function. X-ray abdomen: extensive calcification in head and body of pancreas.	2 years	Diet and chlorpropamide
19	Chronic pancreatitis	Recurrent upper abdominal pain investigated by laparotomy one year before onset of diabetic features. Chronic calculus cholecystitis, pancreatic fibrosis found. Cholecystectomy and choledochotomy carried out. Subsequent secretin and pancreozymin tests: marked reduction in pancreatic exocrine function. Chronic urinary infection.	11 years	Insulin therapy

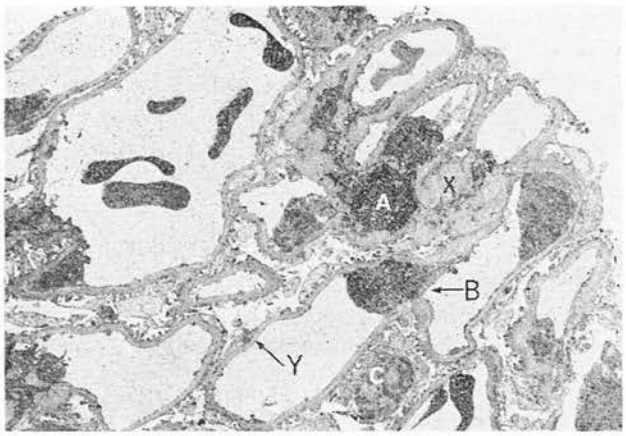

FIG. 1. Part of a glomerular tuft from a normal subject. The axial zone contains mesangial cells (A) and endothelial cells (B). Epithelial cells (C) lie over the free surfaces of the capillary loops. Apparent basement membrane thickening (X) resulting from tangential sectioning in the axial zone and normal basement membrane (Y) in the peripheral loops which have been cut in true cross section. Case 7. X 2,500.

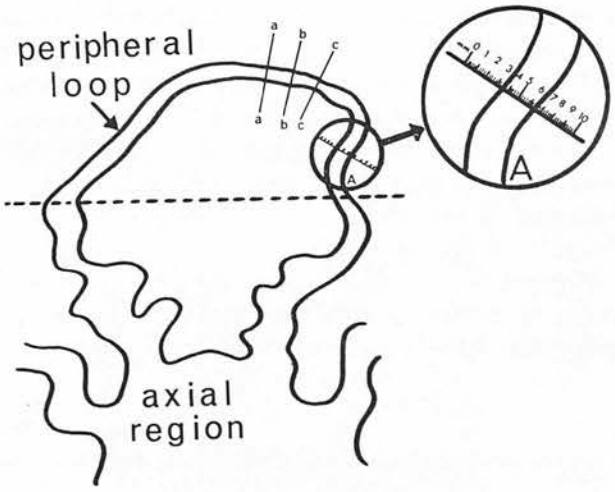

FIG. 2. Diagram showing method of measuring basement membrane thickness in the peripheral half of the loop. A magnifying micrometer (A) is placed at right angles to the plane of the basement membrane at regular intervals represented by lines aa, bb and cc and the thickness of the basement membrane measured between the bases of the epithelial foot processes and the endothelial membrane.

becomes irregular and tortuous as it nears the point of attachment of the capillary (the axial or mesangial zone), cross section measurements were made only in the peripheral half of the loops where the capillaries face Bowman's space. Furthermore, these measurements were restricted to those parts of the peripheral capillary loops which had been cut in true cross section, i.e., where either the epithelial foot processes had been sectioned longitudinally or the pores in the endothelial cytoplasm occurred as spaces (figure 3). The electron microscope magnifications were accurately calibrated and the capillary basement membrane thickness measured on negatives using a micrometer. From examination of at least ten capillary loops from random areas of each glomerulus approximately 250 measurements were obtained from each biopsy, and thus the risk of significant error from inadequate sampling was kept reasonably low. The mean basement membrane thickness and standard deviations were calculated both for individual glomeruli and the sum of the total measurements in each biopsy sample. Variance between glomeruli within samples or the differences between samples or groups of samples could thus be analyzed statistically by application of such nonparametric methods as the Mann-Whitney "U" test and by analysis of variance. Differences at the 1 per cent level were regarded as significant.

The large number of photographs obtained also provided an opportunity to study the cellular morphology in the normal control and the secondary diabetic subjects.

RESULTS

Light microscopy

No abnormalities were seen in the glomeruli, tubules and arterioles in the biopsies obtained from the healthy control subjects. The changes in the renal tissue of the secondary diabetics are shown in table 2.

Electron microscopy

Figure 1 shows part of a glomerular tuft of a normal subject. The axial zones lie close to the origin of the capillaries from the afferent arteriole; the peripheral capillary loops branch out from the axial zones and face Bowman's space. In the axial zones are the cell bodies of the endothelium and cells of an additional type known as either mesangial, deep or third cells. These cells occupy the centers of the zones and are separated from the endothelium by loose textured basement membrane substance (figure 4). However, even in normal subjects, the glomerular capillaries are extremely tortuous so that the three-dimensional distribution of the cells of the axial zone is difficult to visualize on electron microscopy of sectioned material. Without multiple serial sections, the size of the axial zones and their cellular complement cannot be estimated accurately. The peripheral capillary loops, however, have only three distinct components: the endothelium, the basement membrane, and the epithelium

(figure 3). The basement membrane is the only continuous layer; the endothelium is composed of the attenuated periphery of the endothelial cells and is perforated by large pores; the epithelium is attached to the basement membrane by a system of foot processes arising from the epithelial cells which lie over the free surface of the capillary loops. These unique features of the epithelium and endothelium of the peripheral loops, relevant to the special function of glomerular capillaries, provide the electron microscopist with a simple means of distinguishing whether or not capillaries have been cut in true section or tangentially and thus allow the thickness of the basement membrane to be accurately measured.

The mean glomerular capillary basement membrane thickness of the nondiabetic control subjects ranged from 1,400 to 2,750 Å, the mean value for the group being 2,200 Å (table 1). However, the mean basement membrane thickness varied considerably between individuals so that in any one subject this measurement should not be judged abnormal unless it exceeds 3,500 Å, a value two standard deviations greater than the mean thickness in the controls. In the secondary diabetics the mean thickness ranged from 1,950 to 5,200 Å (table 2); the mean thickness for the group was 3,700 Å which is significantly greater than the mean of the controls ($p < 0.01$). The absence of significant variance between glomeruli within biopsy samples suggests that for each of the subjects studied, whether healthy or a secondary diabetic, the results obtained were representative of the glomerular population of

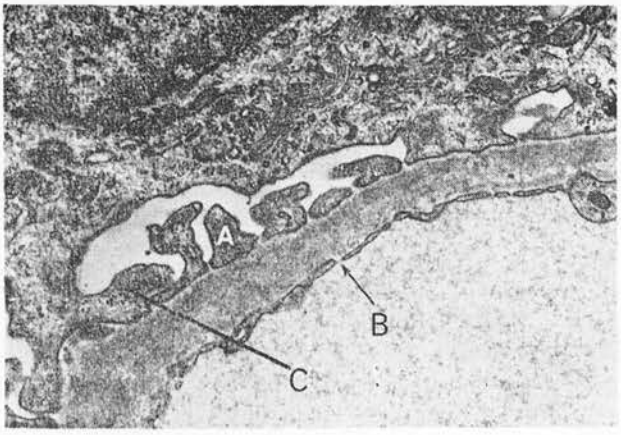

FIG. 3. Detail of glomerular peripheral capillary loop from a normal subject showing epithelial foot processes (A) and pores in endothelial cytoplasm (B) cut in true cross section. Tangential cutting (below line C) with loss of endothelial detail and apparent basement membrane thickening. Case 8. X 30,000.

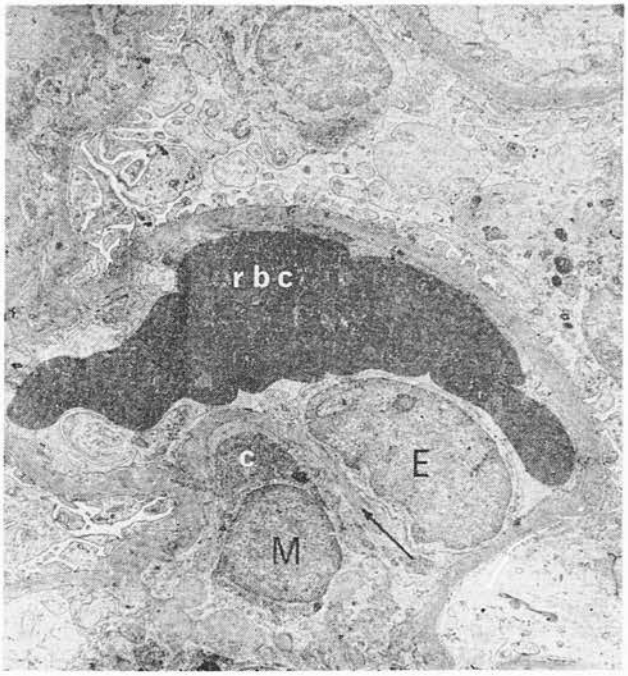

FIG. 4. Glomerular capillary loop containing red blood cells (rbc) from a patient having diabetes secondary to hemochromatosis. In the axial zone a mesangial cell (M) with cytoplasm containing many mitochondria (c) is separated from an endothelial cell (E) by basement membrane substance (arrowed). Case 12. X 5,000.

that individual and that glomerular capillary thickening, when present, was diffusely distributed in the kidney. The differences in the results were unrelated to the patient's age, duration of the diabetic features or the severity of the diabetes.

Sections from secondary diabetics are illustrated in figures 4, 5, 6 and 7. In addition to the peripheral basement membrane thickening, all three cell types showed an increase in their cytoplasm so that they encroached upon the vascular and urinary spaces which thus appeared reduced in size. The cytoplasm was rich in mitochondria, RNA studded endoplasmic reticulum and Golgi zones suggesting increased cellular activity. Of those cases with significant peripheral basement membrane thickening, only Case 18 showed accumulation of basement membrane material in the axial zones encroaching on the endothelial and mesangial cells in the manner seen in idiopathic diabetes (Ireland, Patnaik and Duncan).[21] In all the other secondary diabetics the axial zones contained loose-textured basement membrane material, as in normal subjects, and the mesangial and endothelial cells appeared either normal or hypertrophied (figures 4, 6 and 7).

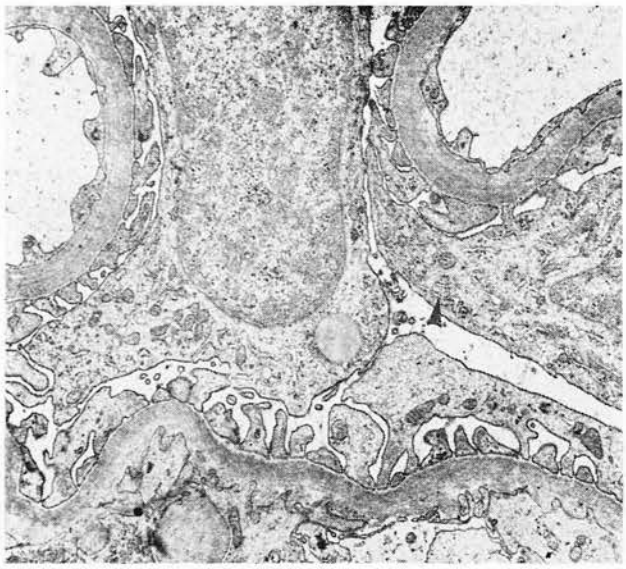

FIG. 5. Peripheral glomerular capillary loops from a patient having diabetes secondary to recurrent acute pancreatitis showing thickening of the basement membrane and epithelial cytoplasm containing numerous channels of RNA-studded endoplasmic reticulum (arrowed) and many mitochondria. Case 10. X 6,000.

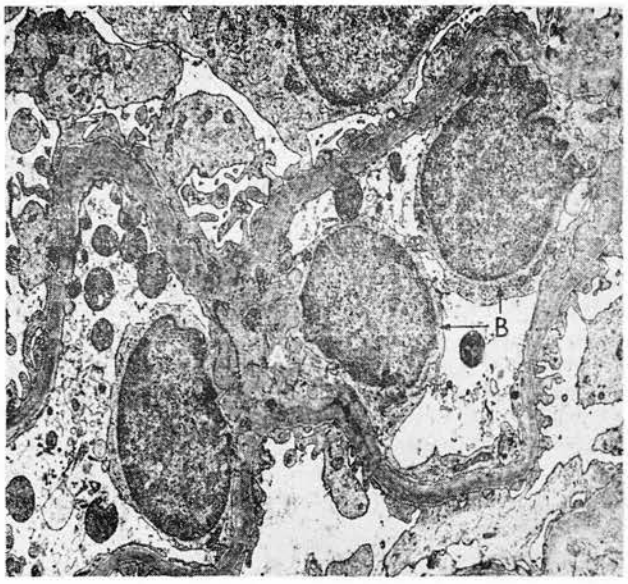

FIG. 6. Glomerular capillary loops from patient having diabetes secondary to recurrent acute pancreatitis showing axial zone (A) and prominent endothelial cells (B). Case 10. X 4,000.

DISCUSSION

In 1960 Becker and Miller studied by light microscopy the renal tissue of twenty-two deceased subjects who were known to have had hemochromatosis with secondary diabetes.[9] In the thirteen with recognized diabetes of less than four years' duration no diffuse or nodular glomerulosclerosis was seen, but in seven of the nine known to have been diabetic for a longer time glomerular lesions attributable to diabetes were present.

The present investigation extends these observations to patients having other forms of pancreatic diabetes and, because the electron microscope was employed, changes in the glomeruli could be more accurately detected and measured than by light microscopy. However, Kimmelstiel, in a recent editorial,[19] has stated that in many electron microscopic studies of diabetic nephropathy the method of measuring glomerular capillary basement membrane thickness was inadequate, control data were absent and the results were not subjected to statistical analysis. In this study these deficiencies have, as far as possible, been overcome.

The fact that the mean values reported in this study of basement membrane thickness in both the normal and diabetic subjects are less than those recorded by others[20,22-24] is probably due to our having confined measurements to the peripheral half of the capillary loop and to areas where the membrane was cut in true cross section.

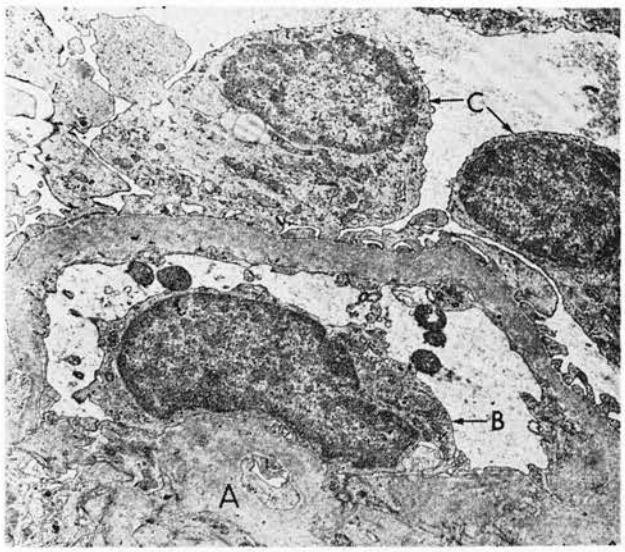

FIG. 7. Basement membrane thickening in a glomerular capillary loop from a patient having diabetes secondary to chronic pancreatitis. The axial zone, (A) with an adjacent endothelial cell (B), consists of excess basement membrane material. Epithelial cells (C) lie over the free surface of the capillary. Case 18. X 7,500.

The glomerular capillary basement membrane was significantly thickened in seven of the ten unselected

secondary diabetics and the mean thickness of the group as a whole was significantly greater than that of the normal control subjects.

It is possible that these pancreatic diabetic patients were genetically predisposed to idiopathic diabetes. This could have contributed towards the development of diabetes, which might not otherwise have occurred, and could have been also directly or permissively responsible for any observed basement membrane thickening. Vallance-Owen[25] has reported, however, that all the pancreatic diabetics he studied were synalbumin-negative, suggesting that diabetes secondary to pancreatic disease is not genetically determined. The possibility of genetic influence seems unlikely in the light of present concepts of diabetic inheritance[26] and the high prevalence of glomerular lesions in our cases. But the absence of a family history of diabetes does not exclude the possibility that an individual is genetically diabetes-prone. At the time of biopsy Case No. 16 had a negative family history and two years later one of her siblings developed idiopathic diabetes. Thus there is reason to suspect that this patient was genetically diabetic, but the fact that there was no glomerular capillary basement membrane thickening further supports the view that the lesion is not genetically determined.

The variable extent, and even absence of glomerular lesions in this group of secondary diabetics is not unexpected. The findings are in accord with previous observations in long-standing idiopathic diabetics where capillary basement membrane thickening may not be present in all cases.[27] Whereas published reports and our own studies suggest that light microscopic evidence of glomerulosclerosis is less common in pancreatic than idiopathic diabetes, the findings reported here emphasize that the true prevalence of the glomerular lesions can be more accurately assessed by electron microscopy. Furthermore, diabetes of pancreatic origin is often a late event in a disease which may be fatal relatively soon thereafter so that there may not be enough time for the lesions revealed by the electron microscope to progress to the extent that they can be demonstrated by light microscopy.

The results of the present study suggest that the development of glomerular capillary basement membrane thickening in diabetes is not, as has been proposed, dependent on the presence of the genetically determined diathesis to idiopathic diabetes but is due to some metabolic derangement, possibly resulting from inadequate insulin action on the tissues, common to both primary and secondary diabetes. The possibility

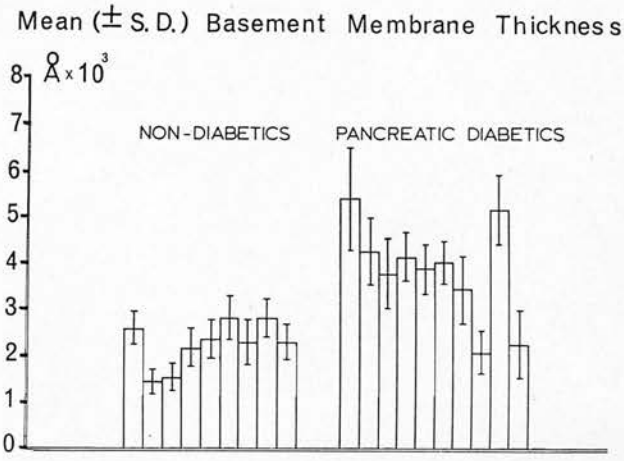

FIG. 8. Mean glomerular capillary basement membrane thickening ± S.D. in nondiabetics and pancreatic diabetics.

that some components of diabetic microangiopathy can occur only in and be due to some as yet undefined aspect of the idiopathic diabetic disorder is, however, not excluded by these observations (Ireland, Patnaik and Duncan).[21]

ACKNOWLEDGMENT

We are grateful to G. L. Montgomery, Professor of Pathology, for his encouragement and advice and for making available the facilities of his department. We are also indebted to Mr. P. Kinnear, M.Sc., for statistical analysis of the data. The illustrations were prepared by the Medical Photography Department of the University of Edinburgh. Generous financial support was provided by Pfizer Ltd., the Lawson Tait Memorial Trust and The British Diabetic Association.

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Effect of Pituitary Ablation on the Renal Arteriolar and Glomerular Lesions in Diabetes

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SUMMARY

Renal tissue of six diabetics having proliferative retinopathy was obtained before and one to two years after pituitary surgery and examined by light and electron microscopy. Successful pituitary ablation was followed by significant reduction in the thickness of the glomerular capillary basement membrane, restoration of the atrophic endothelial and mesangial cells to their normal appearance, persistence of the morphological features suggesting overactivity of the epithelial cells and no improvement in the arteriolar lesion.

These observations and changes suggest that diabetic nephropathy results from the development of several separate though inter-related lesions each of which may be variously influenced by the metabolic disturbance, the inherited diabetic diathesis or pituitary activity. Hypophysectomy will not alter the progressive clinical course of diabetic kidney disease if significant renal arteriosclerosis is present. DIABETES *16*:636-42, September, 1967.

Although the studies of Houssay showed that some of the metabolic aspects of experimental diabetes mellitus[1] are influenced by the anterior pituitary, the relationship of this gland to the pathogenesis of such specific complications of human diabetes as retinopathy and glomerulosclerosis remains uncertain. Improvement in retinopathy has occurred in many diabetic patients subjected to pituitary destruction, but it is not known whether the procedure simply ameliorates the vascular changes in the retina or favorably influences the more widespread small blood vessel disorder. This paper describes the light and electron microscopy findings in renal tissue obtained by biopsy from diabetic patients in whom the pituitary was ablated because of progressive retinopathy.

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MATERIAL AND METHODS

The clinical data of the six patients studied are summarized in table 1. All patients had long-standing, insulin-dependent diabetes and the progressive nature of their predominantly proliferative retinopathy had been confirmed by serial retinal photography. No patient was hypertensive, and there was no clinical or subsequent evidence by biopsy of pyelonephritis or other nondiabetic renal disease. Proteinuria was minimal except in Case 6 whose renal function was poor. Three patients were treated by pituitary stalk section and three by implantation of radioactive-90-Yttrium.

Renal tissue was obtained by percutaneous biopsy a few days before pituitary operation and repeated one to two years later. At each biopsy two cores of renal cortex were obtained; the center portion of each was prepared for light microscopy study and cubes from either end were processed and embedded in Araldite for examination and measurement of the glomerular capillary basement membrane thickness by electron microscopy as previously described.[2] Four glomeruli were examined from each biopsy sample, and the mean capillary basement membrane thickness was calculated with standard deviations both for individual glomeruli and the whole sample. Variance between glomeruli within samples and the differences between samples and groups of samples could thus be analyzed statistically.

RESULTS

The effectiveness of pituitary destruction was demonstrated by the fall in postoperative insulin requirements (table 1) and by the need for endocrine replacement therapy with cortisone and thyroxine in all patients except Case 4. This patient's insulin dosage was not significantly altered and, before the second biopsy, steroid replacement therapy was withdrawn without adverse effect. A metopirone test showed appreciable

anterior pituitary function.

The renal changes found on light microscopy are summarized in table 2.

In all patients there was diffuse thickening of the glomerular capillaries. Although this was frequently most prominent in the axial or mesangial zones, only in one instance (Case 3) were early Kimmelstiel-Wilson nodules definitely seen. Because the degree of diabetic glomerulosclerosis is difficult to evaluate by the light microscope, only the measurements determined by electron microscopy have been used to compare the pre- and postoperative glomerular changes.

The arteriolar structure was normal in Case 5 only, and in Cases 1 to 4 there was variable afferent and efferent arteriosclerosis; Case 6, who had clinical evidence of renal decompensation, had advanced arteriolosclerosis, glomerular hyalinization, interstitial fibrosis and tubular atrophy. No improvement was detected in

TABLE 1

Clinical data of the six patients studied

					Preoperative data					Postoperative data			
Case no.	Age (yrs.)	Sex	Duration of diabetes (yrs.)	Method of pituitary ablation	B.P.	Creatinine clearance	Proteinuria (24 hrs.)	Daily insulin requirements	Interval after pituitary surgery	B.P.	Creatinine clearance	Proteinuria (24 hrs.)	Daily insulin requirements
1	35	F	17	Stalk section	160/80	44 ml./min.	0.1 gm.	44 U.	1 yr.	150/90	60 ml./min.	0.1 gm.	24 U.
2	29	M	15	Stalk section	150/100	95 ml./min.	0.3 gm.	54 U.	18 mos.	180/110	70 ml./min.	1.2 gm.	36 U.
3	45	M	26	Stalk section	130/78	80 ml./min.	<0.1 gm.	80 U.	18 mos.	125/70	90 ml./min.	<0.1 gm.	24 U.
4	32	M	18	Implantation of Yttrium-90	130/90	170 ml./min.	3 gm.	70 U.	2 yrs.	200/105	42 ml./min.	2.3 gm.	60 U.
5	39	F	20	Implantation of Yttrium-90	150/80	125 ml./min.	<0.1 gm.	40 U.	16 mos.	140/80	110 ml./min.	<0.1 gm.	12 U.
6	53	M	12	Implantation of Yttrium-90	140/80	48 ml./min.	4.3 gm.	26 U.	2 yrs.	135/80	16 ml./min.	7.5 gm.	Nil

TABLE 2

Renal changes seen on light microscopy

Case no.	Preoperative renal biopsy	Postoperative renal biopsy
1	Moderate diffuse glomerulosclerosis. Moderate arteriolosclerosis.	No change
2	Moderate diffuse glomerulosclerosis. Moderate arteriolosclerosis.	Advanced arteriolosclerosis
3	Marked diffuse glomerulosclerosis and early nodular glomerulosclerosis. Moderate arteriolosclerosis.	Mild diffuse glomerulosclerosis. No change in arteriolar lesions
4	Moderate diffuse glomerulosclerosis, hyalinization and moderate arteriolosclerosis.	Increased glomerular hyalinization and marked arteriolosclerosis
5	Early diffuse glomerulosclerosis. Minimal arteriolosclerosis.	No change
6	Marked diffuse glomerulosclerosis, glomerular hyalinization. Interstitial fibrosis. Marked arteriolosclerosis.	Increased glomerular hyalinization and arteriolosclerosis

TABLE 3

Peripheral glomerular capillary basement membrane thickness, in Angstrom units, before (upper figures) and after (lower figures) pituitary ablation, with the exception of Case 6 where no postoperative data were obtained

Case no.	Peripheral glomerular capillary basement membrane thickness: Angstrom units										
	Glomerulus 1		Glomerulus 2		Glomerulus 3		Glomerulus 4		Total number of measurements	Over-all mean of all measurements	±1 S.D.
	Mean	±1 S.D.	Mean	±1 S.D.	Mean	±1 S.D.	Mean	±1 S.D.			
1	5,620	820	4,720	1,620	5,680	1,260	5,400	1,390	237	5,320	1,400
	3,670	1,100	3,300	835	4,360	1,460	3,560	430	273	3,780	1,200
2	5,850	1,625	5,650	1,420	6,730	1,460	5,800	1,030	131	5,870	1,430
	5,080	1,450	5,025	900	5,640	1,040	5,060	1,200	181	5,200	1,220
3	6,130	1,800	6,700	1,100	7,700	1,800	6,660	1,600	190	6,800	1,650
	2,625	700	3,230	680	3,310	525	3,380	670	102	3,200	700
4	5,840	1,000	5,375	1,100	5,600	825	5,720	750	134	5,625	1,020
	6,825	870	6,160	930	6,840	940	6,550	1,050	128	6,600	1,150
5	4,650	1,025	5,320	875	4,220	960	3,900	940	368	4,360	1,170
	3,330	670	3,760	700	3,850	675	3,875	855	608	3,650	875
6	7,420	1,270	6,330	1,310	6,480	1,450	6,275	1,525	154	6,680	1,470

the arteriolar lesions following pituitary surgery, and in Cases 2, 4 and 6 the changes had advanced. If Case 4, whose pituitary had not been adequately ablated is excluded, this finding suggests that the renal arteriolar lesion is unaffected by pituitary destruction.

Case 6 died of renal failure one year after the second biopsy. At postmortem glomerular hyalinization and advanced diffuse glomerulosclerosis were found. Exudative lesions were present in many glomeruli, and some of those less severely affected had typical Kimmelstiel-Wilson nodules which differed from the exudative lesions in their staining and situation. Arteries of all calibers had hyperplastic intimal fibrocellular thickening, and hyalinization of the arteriolar walls was conspicuous. Despite these advanced lesions there were no changes in the heart consistent with hypertension.

The preoperative measurements of the peripheral glomerular capillary loops are summarized in table 3. The over-all mean glomerular capillary basement membrane thickness for individual patients ranged from 4,360 Å to 6,800 Å, the mean for the group being 5,600 Å. The mean thickness in the nondiabetic subjects similarly measured was 1,400 Å to 2,700 Å.[2] Thus there was diffuse thickening of the peripheral capillary walls in all. The relatively large standard deviations in our results (table 3) indicate that the thickening of the basement membrane of the glomerular capillaries occurred irregularly. But the absence of significant variance between the measurements from glomeruli from the same biopsy sample indicates also that the diabetic glomerular lesion occurs diffusely throughout the kidney and that the values in this study are representative of these changes.

The measurements made from the biopsies obtained one to two years after pituitary surgery are also summarized in table 3. Although adequate renal cortex was obtained from Case 6 for light microscopy examination, no glomeruli were found in the tissue prepared for electron microscopy. With the exception of Case 4, where the lesion had advanced, the postoperative mean basement membrane thickness was significantly less in the peripheral capillaries of the remaining patients when analyzed as a group; though individual data were significant only in Cases 1 and 3.

Comparison of the glomerular ultrastructure in the tissue obtained before and after pituitary ablation showed significant differences. Before surgery, in addition to irregular thickening of the peripheral capillary loops, there was variable accumulation of basement membrane material within the axial or mesangial zones of the glomerular tuft. The mesangial cells, which lie deep within these areas, were rarely composed of more than small isolated aggregates of attenuated cytoplasm, usually lacking in such formed elements as mitochondria, endoplasmic reticulum and Golgi zones; frequently the nuclei were crenated or fragmented (figures 1 and 2). The endothelial cells, situated within the capillary lumen close to the mesangial zone, were only occasionally compressed within the basement membrane material; nevertheless, their cytoplasm was attenuated and lacking in formed elements (figure 2). In contrast, the epithelial cells contained healthy nuclei and the cytoplasm, rich in mitochondria, RNA-studded endoplasmic reticulum and Golgi zones (figure 3) encroached upon the urinary space in a manner similar to that found in other diabetics and patients having

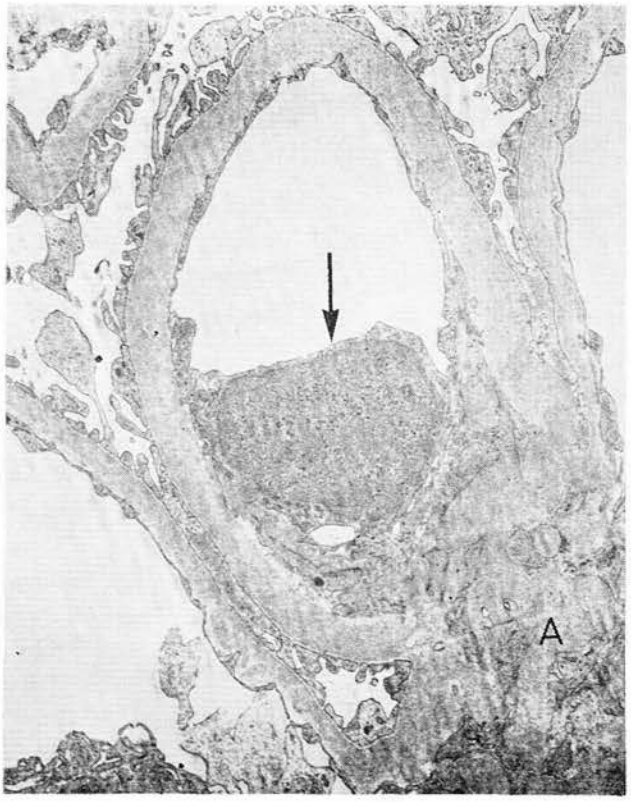

FIG. 1. Glomerular capillary loop from a preoperative specimen. Basement membrane accumulation in the axial zone (A) with compression of the mesangial cytoplasm and an adjacent endothelial cell with attenuated cytoplasm (arrowed). Case 1. X 6,000.

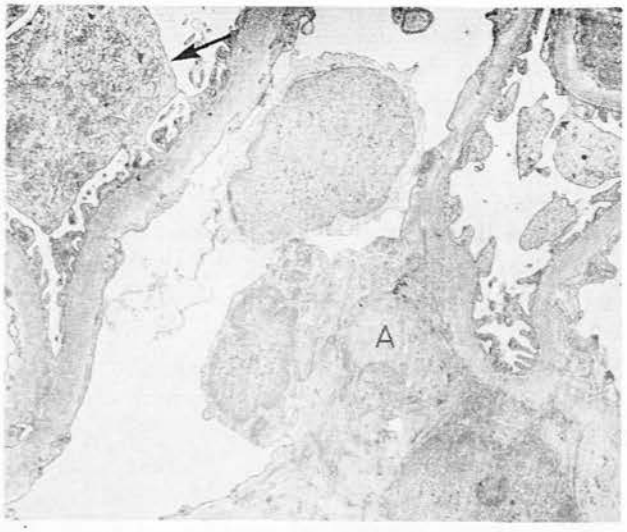

FIG. 2. Glomerular capillary loops from a preoperative specimen. Basement membrane accumulation in axial zone (A) with attenuated mesangial and endothelial cytoplasm in contrast to prominent epithelial cytoplasm (arrowed). Case 1. X 5,000.

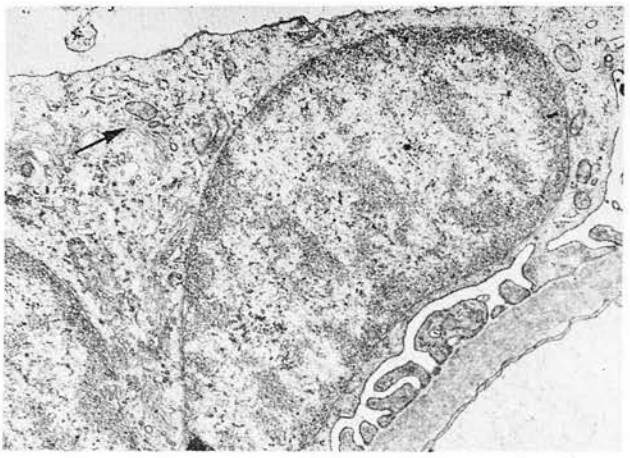

FIG. 3. Epithelial cell showing cytoplasmic details including several Golgi zones (arrowed) from a preoperative specimen. Case 5. X 15,000.

diabetes secondary to pancreatic disease.[2] The mitochondria were usually elongated and cylindrical; the endoplasmic reticulum extended throughout the cytoplasm and appeared to be organized into a system of channels with numerous ribosome particles studding their surface or occasionally lying freely in the ground substance.

In the tissue obtained after pituitary destruction there was striking restoration of the normal structure of the nuclei and cytoplasm of the mesangial cells (figure 4). At the same time the cytoplasm of the endothelial cells was greatly increased. Frequently it was seen to engulf basement membrane substance both in the mesangial zones and peripheral capillary loops

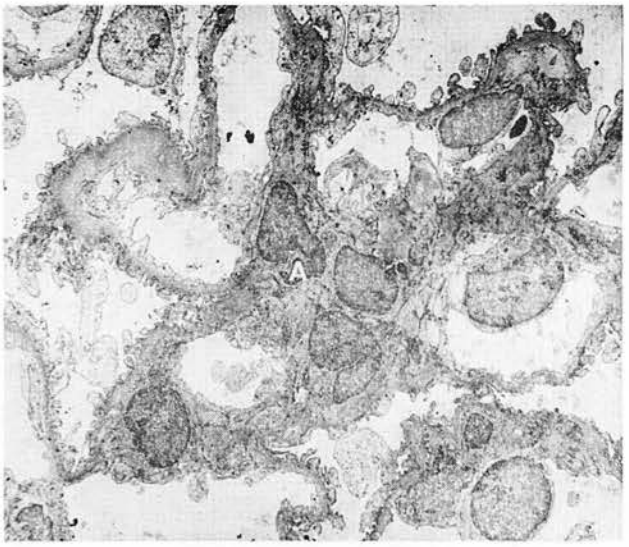

FIG. 4. Postoperative specimen showing proliferation of cells with prominent cytoplasm in the axial zone (A). Case 5. X 2,500.

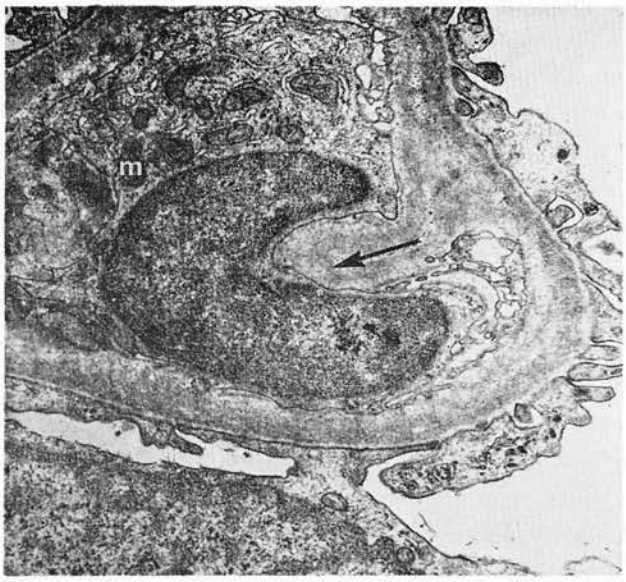

FIG. 5. Endothelial cell with prominent cytoplasm containing many mitochondria (m) occupying the capillary lumen and engulfing basement membrane (arrowed) from the periphery. Postoperative specimen. Case 5. X 14,000.

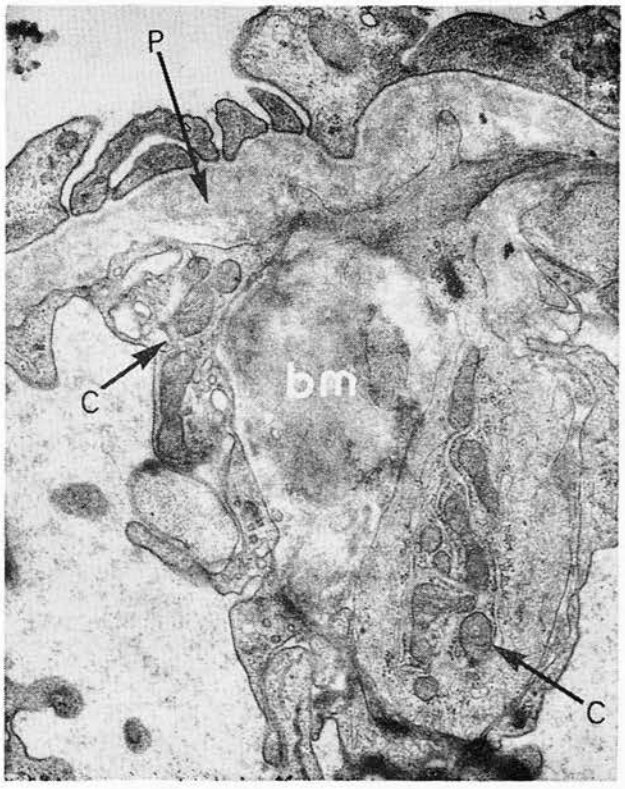

FIG. 6. Part of a peripheral capillary loop (P) from a postoperative specimen showing basement membrane substance (bm) within endothelial cytoplasm which also contains mitochondria and endoplasmic reticulum (C). Case 5. X 22,000.

and in these areas was rich in mitochondria, RNA-studded endoplasmic reticulum and Golgi zones (figures 5 and 6). No definite difference in the epithelial structures was detected other than occasional areas where the cytoplasm appeared vacuolated (figure 7).

DISCUSSION

Understanding of the pathogenesis of renal disease in diabetes has been obscured by disagreement regarding the diffuse and nodular glomerular lesions of light microscopy terminology and by uncertainty concerning their relationship to arteriolosclerosis and pyelonephritis, both of which can cause glomerular hyalinization and renal failure in diabetic patients. The demonstration in several studies[3] of diabetic kidneys of an association between arteriolosclerosis and diabetic glomerular changes might suggest that the latter were due to the arteriolar lesions. This seems unlikely, however, since arteriolosclerosis in the absence of diabetes usually leads to ischemic atrophy and hyalinization of the glomeruli and not to the diffuse or nodular capillary changes. The present findings, after pituitary ablation, of improvement in the glomerular capillary lesion without change in that of the arterioles suggest that the two may develop independently and not necessarily be due to the same cause.

Whether the diffuse and nodular glomerular lesions are separate entities remains undecided. Kimmelstiel[4] proposes that they may exist independently, while others[5-8] regard the nodules as being merely a more advanced stage of the diffuse basement membrane lesion. In this study early nodules were found in only one of the six diabetic patients having both advancing pro-

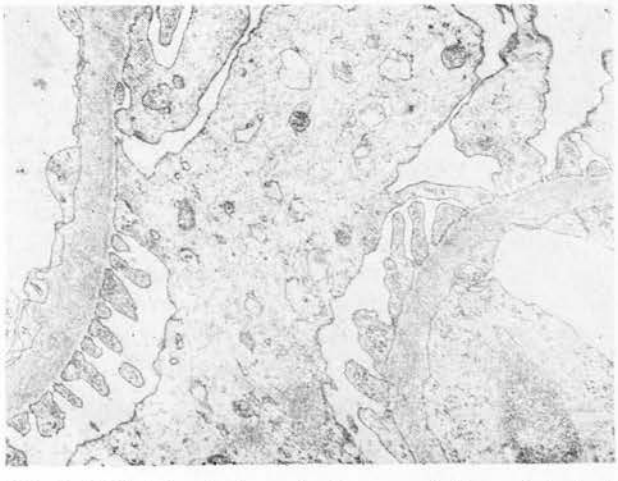

FIG. 7. Epithelial cytoplasm showing vacuolation and lack of formed elements in a postoperative specimen. Case 5. X 10,000.

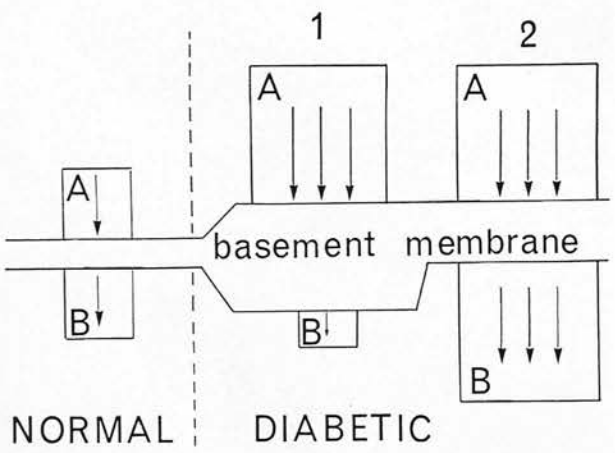

FIG. 8. Diagrammatic representation of basement membrane turnover. On left, normal production by epithelial cells (A) and removal by mesangial and endothelial cells (B). On right, the diabetic: 1. before pituitary surgery showing increased production of basement membrane by the epithelial cells (A) and diminished removal by the mesangial and endothelial cells (B); 2. after pituitary surgery showing restoration of mesangial and endothelial function (B) and some reduction in basement membrane thickening.

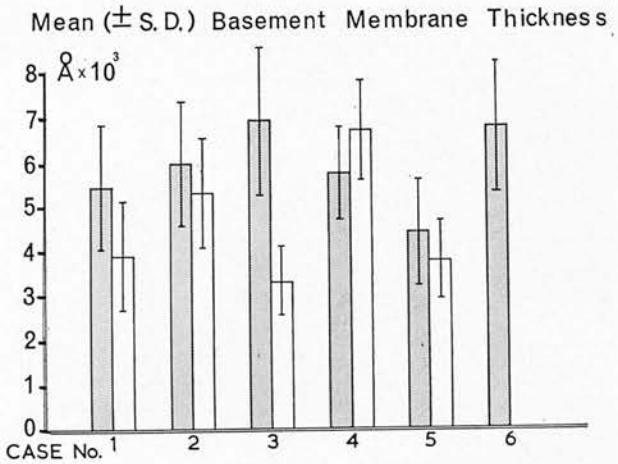

FIG. 9. Mean glomerular capillary basement membrane thickness ± S.D. The hatched columns represent the preoperative results and the clear columns the postoperative results for individual patients.

liferative retinopathy and considerable diffuse glomerular lesions. Nevertheless the nodule remains, for the light microscopist, the hallmark of diabetes since other causes of diffuse glomerular lesions cannot be distinguished by this means. Further misunderstanding of the pathology of diabetic nephropathy arises from the continued use of the light microscopy term glomerulosclerosis. This implies a sclerotic lesion with hyalinization, whereas the changes revealed by the electron microscope in the cellular ultrastructure and capillary basement membrane are inadequately designated by such nomenclature.

The observations made in this study suggest to us the following tentative hypothesis concerning the pathogenesis of glomerular capillary disease in diabetes. The integrity of the basement membrane probably depends on the function of the cells of the glomerular tuft; thus alterations in the membrane structure may be due to dysfunction of these cells rather than to primary metabolic changes within the membrane itself. In biopsies revealing thickening of the glomerular capillary basement membrane, the epithelial cell cytoplasm was abundant and contained prominent mitochondria, RNA-studded or rough-surfaced endoplasmic reticulum and Golgi zones. Since there is considerable evidence[9-12] that the epithelial cells are responsible for the synthesis of the basement membrane, it is possible that the prominent epithelium in diabetic renal tissue reflects increased intracellular production and deposition of basement membrane material. Although increased synthesis of glycoprotein, one of the main constituents of basement membrane, might appear to be a paradox in a disorder characterized by impaired carbohydrate metabolism, Spiro[13] has postulated a biochemical basis for such a possibility. He found that the production of the glucosamine component of glycoprotein along insulin-independent pathways was increased in liver slices from insulin-deficient alloxan-diabetic rats. Recent studies[14] of L-xylose metabolism in the glucuronic acid pathway of glucose metabolism and its relationship to the synthesis of the carbohydrate constituents of glycoprotein and mucoprotein may further explain the increase in basement membrane material in diabetes.

In contrast to the epithelial cells the deep mesangial cells contained crenated nuclei and atrophic cytoplasm within irregular accumulations of basement membrane substance and the endothelial cytoplasm was attenuated and lacking in formed elements in the preoperative biopsies. Farquhar,[12] from a study of the turnover of ferritin particles, suggested that the mesangial cells have a phagocytic function which distinguishes them from endothelial cells. She has further suggested that the increase in basement membrane in the glomeruli of diabetics might be due to its defective removal by the mesangial cells. Whether the mesangial cell lesion is a consequence of the accumulation in the axial region of basement membrane which leads to their compression and dysfunction, or arises independently to cause further basement membrane accumulation and apparent compression is uncertain. In support of the latter view

is the finding, in the biopsies obtained after pituitary ablation, of striking changes indicative of return of activity in the mesangial and endothelial cells in association with reduction in both peripheral and mesangial basement membrane substance, suggesting that the mesangial and endothelial cells of the glomerulus are functionally related and may share phagocytic activity. While our results support Farquhar's hypothesis concerning defective removal of basement membrane in diabetes, it is, however, improbable that alteration in mesangial and endothelial cell structure and function is the only or primary etiological defect in diabetic nephropathy, since peripheral basement membrane thickening may be present without there being apparent abnormality in the mesangial or endothelial cells. Thus in glomeruli from patients having diabetes secondary to pancreatic disease, we frequently found healthy mesangial and endothelial cells although the peripheral basement membrane was significantly thickened.[2]

It is therefore possible that in those diabetics who develop proliferative retinopathy and nephropathy some pituitary-dependent factor inhibits the activity of the mesangial and endothelial cells (as is mirrored by changes in their morphology) which leads to alteration in the capillary basement membrane. Pituitary destruction would remove the depressant influence on the mesangial and endothelial cells, restore their structure and activity and thus, indirectly, cause some improvement in the capillary lesion, as illustrated in figures 8 and 9. It must be stressed, however, that in the patients we studied the renal arteriolar lesions were either unchanged or had progressed after pituitary ablation. Thus all forms of diabetic microangiopathy are not pituitary-dependent, and this may explain why Poulsen[16] and others[17] reported that pituitary destruction does not beneficially influence the progressive clinical course of diabetic renal disease. If the latter is predominantly due to glomerular ischemia and hyalinization consequent upon the arteriolar lesion or pyelonephritis, then no improvement could be expected. On the other hand some long-term clinical benefit might be obtained in diabetics whose preoperative renal biopsy has revealed minimal arteriolar changes and the glomerular lesions are of the pituitary-dependent cellular and basement membrane type described above.

ACKNOWLEDGMENT

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The scope and limitations of renal biopsy in the assessment of diabetic microangiopathy

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THE concept of a specific diabetic microangiopathy is now well established. Moreover the general belief that diabetics are more prone than non-diabetics to atheroma and other less specific degenerative changes in larger blood vessels in no way detracts from the concept that it is involvement of small blood vessels, and of capillaries in particular, which characterises the diabetic lesion. Despite an extensive literature concerning diabetic renal involvement, disagreement regarding the diffuse and nodular glomerular lesions, and uncertainty about their relationship to pyelonephritis, arteriolosclerosis and other vascular lesions, have obscured the pathogenesis of renal disease in diabetes. It was anticipated that electron microscopy might resolve some of these difficulties by sharpening the focus on glomerular and other capillaries. Yet failure to apply reliable quantitative analysis of data in many studies using this method has only added further controversy. It is the purpose of this review to investigate the scope and limitations of light and electron microscopy examination of renal biopsy tissue in the management of diabetic patients and in the study of diabetic angiopathy.

Light microscopy of the glomerulus in diabetes

(i) *Nodular Lesion.* It is now agreed that the nodule is confined to, and pathognomonic of, diabetes mellitus (Berkman & Rifkin, 1966), and that early reports to the contrary were a consequence of mistaking either lobular nephritis or exudative glomerular lesions with the nodule or failure to detect clinically mild disturbances of carbohydrate metabolism in so-called non-diabetics. Kimmelstiel (1966) has stressed that confusion has arisen in the past through failure to define the morphology of the nodule. In considering its ultrastructure and pathogenesis (vide infra) it is worth emphasising its three main features: namely, that it is focal, centrolobular and has an accelular centre.

(ii) *Diffuse Lesion.* As a result of a careful clinicopathological study of biopsy material, Gellman, Pirani, Soothill, Muehrcke & Kark (1959) showed that diffuse glomerulosclerosis was twice as common as the nodule and that the severity of the features of renal failure and nephrotic syndrome in diabetes correlated with the extent of diffuse rather than nodular glomerular involvement. The diffuse diabetic lesion usually begins with thickening of the whole circumference of the wall of peripheral capillaries of the glomerular tuft and with increasing severity the lesion becomes diffuse within the glomerulus and generalized in the kidney. The light microscopist has been reluctant to regard the diffuse diabetic lesion as a specific entity because of difficulty in distinguishing between diffuse lesions due to diabetes and such other causes as membranous glomerulonephritis or amyloidosis. Some observers have made the paradoxical and confusing statement that the specific diabetic nodule arises in glomeruli involved by, and is a more advanced stage of, the non-specific diffuse lesion. To a large extent, the use of electron microscopy has resolved these difficulties (vide infra).

(iii) *Exudative Lesion.* The exudative lesion is the least significant of the three glomerular changes in diabetes. It occurs also in various non-diabetic glomerular disorders associated with renal failure, and in diabetics only in the late stages of nephropathy. The lesion results from non-specific glomerular damage but is of importance because it has been confused with the nodule leading to doubts that the latter is pathognomonic of diabetes. Conversely, steroid-induced lesions in the glomeruli of experimental animals which resemble the exudative lesion have been interpreted erroneously as specifically diabetic.

Arteriolar Lesions. There is efferent arteriolar involvement besides afferent arteriosclerosis in diabetes, indeed the efferent lesion is sufficiently characteristic of diabetes to be of aid to the light microscopist in concluding that associated glomerular disease is diabetic in origin. Although Blumenthal, Berns, Owens & Hirata (1964) have described a proliferative arteriolar lesion involving the endothelial cells and occurring more

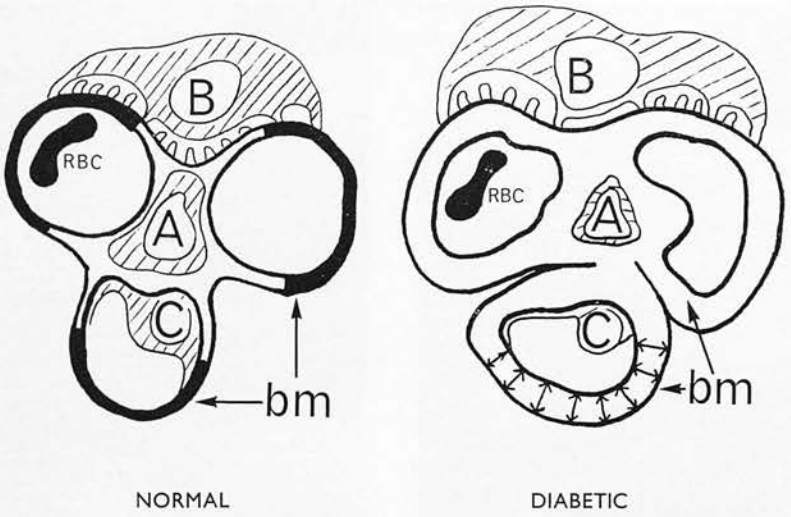

FIG. 1. Diagram of glomerular capillary lobule in normal and diabetic. A: Mesangial cell in centrolobular region. B: Epithelial cell in Bowman's Space, attached to capillary by foot processes. C: Endothelial cell within capillary lumen. RBC: Red blood cell with capillary lumen to illustrate scale of diagram. bm: Continuous basement membrane of capillary wall. In NORMAL, black areas indicate peripheral capillary wall where cross section measurements are obtained. In DIABETIC arrows indicate method of measuring basement membrane thickness at regular intervals.

commonly in the renal arterioles of diabetics than non-diabetics, this lesion has rarely been noted by other observers.

Tubules. Although various lesions may be found in the tubules of diabetics, few are of specific significance and in general they are secondary to glomerulosclerosis, ischaemia, pyelonephritis or long-standing electrolyte disturbance. Thus such changes are more marked in autopsy rather than biopsy tissue and in particular the 'Armanni-Ebstein lesion' which was a common post-mortem finding in the pre-insulin era, is now only found in those who die following uncorrected hyperglycaemia, acidosis and dehydration. Large, clear, 'empty' vacuoles may be found in the proximal convoluted and collecting tubules at biopsy in diabetics having severe potassium depletion and may be confused easily with the fine hydropic degeneration which accompanies glucose-induced osmotic diuresis.

Interstitial Tissue

It is generally believed that diabetics are more prone than others to infections in general and pyelonephritis in particular. In autopsies on diabetics, changes interpreted as chronic pyelonephritis were frequently reported to be found in association with diabetic arteriolosclerosis. Indeed, some observers were sufficiently impressed by the association to conclude that the diabetic lesions might be a consequence of chronic pyelonephritis. However, the more experienced observer now appreciates that most of the changes which resemble healed chronic pyelonephritis in the interstitial tissue and tubules are secondary to ischaemia and the glomerular lesions of diabetics. Heptinstall (1967) in a perceptive review of the limitations of diagnosis in chronic pyelonephritis, found it painfully obvious that many pathological processes can produce a similar end result.

Whereas renal biopsy is ideally suited to the assessment of specific diabetic renal involvement which is essentially diffuse, the procedure may be misleading in such a focal disorder as chronic pyelonephritis.

Electron microscopy of the glomerulus in diabetes.

Anatomical considerations.

The afferent arteriole divides into several branches to form the glomerular capillaries which are grouped into lobules (Fig. 1). Thus two or more capillaries become arranged about a centrolobular region known also as the axial zone, mesangium or intercapillary space, containing the axial, mesangial or deep endothelial cells (Fig. 1, Plate 1). These cells, surrounded by loose-textured basement membrane, may be compared, because of their situation, to the pericytes of other capillaries (Farquhar, 1964).

In the periphery of the capillary loops, the structure of the epithelium and endothelium provide the electron microscopist with a reliable method of

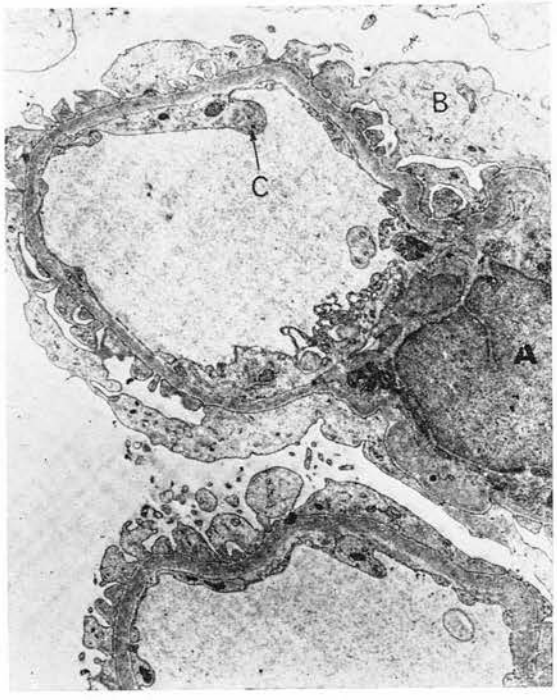

PLATE 1. Glomerular capillary loop in normal subject. A: Mesangial cell. B: Epithelial cell. C: Endothelial cell ×6,000.

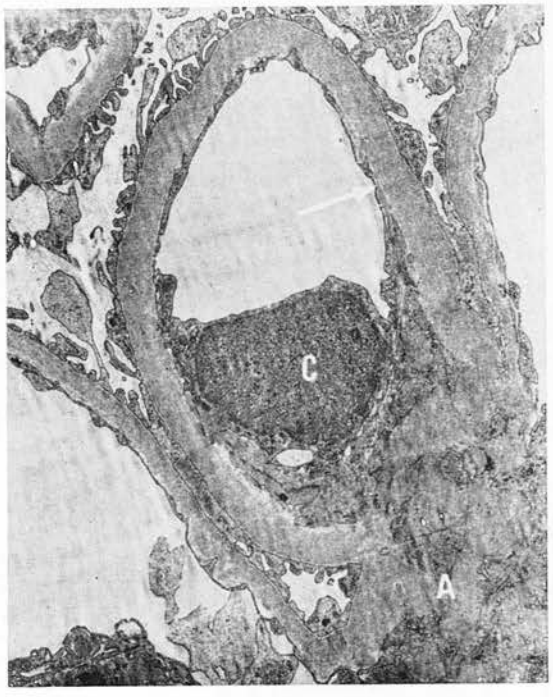

PLATE 2. Glomerular capillary loop from diabetic having diffuse glomerulosclerosis. A: Attenuated mesangial cell cytoplasm and accumulation of basement membrane substance. C: Endothelial cell. Arrow: Attenuated endothelial cytoplasm and thickened basement membrane of capillary wall ×6,000.

measuring the intervening basement membrane thickness. Thus, where either the epithelial foot processes are sectioned longitudinally, or where the pores in the endothelial cytoplasm occur as spaces, the examiner can be confident of having obtained a true cross-section of the capillary basement membrane (Fig. 1, Plate 4). Using such methods, studies of basement membrane thickness in normal subjects (Osterby-Hansen, 1965; Kimmelstiel, 1966; Ireland, Patnaik & Duncan, 1967a), have established reliable values with which the diabetic lesion may be compared by statistical analysis. However, examination of the anatomy of the glomerular lobule (Fig. 1) will show that such studies arbitrarily exclude the mesangial region and other cellular details, since the three dimensional distribution of the cells and basement membrane in the mesangium is difficult to visualise on electron microscopy of sectioned material. Without multiple serial sections, the size of the mesangial zone and its cellular complement cannot be accurately estimated.

Electron microscopy of the glomerulus in diabetes.

Early electron microscopy studies of renal biopsy tissue obtained from diabetic patients having clinical evidence of nephropathy, confirmed that

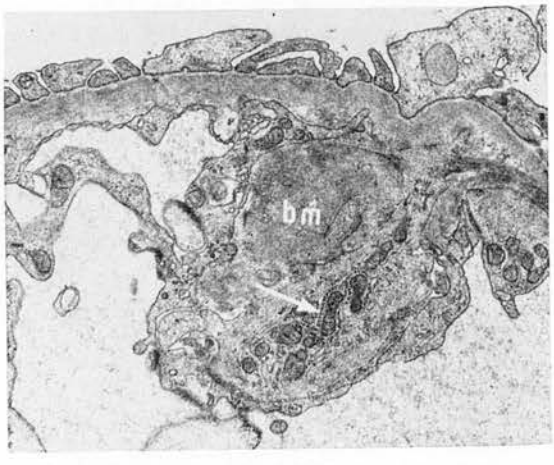

PLATE 3. Detail of capillary loop in biopsy from diabetic patient following successful pituitary ablation. Arrow: Endothelial cytoplasm showing numerous mitochondria, RNA studded endoplasmic reticulum and basement membrane (bm) being engulfed ×12,000.

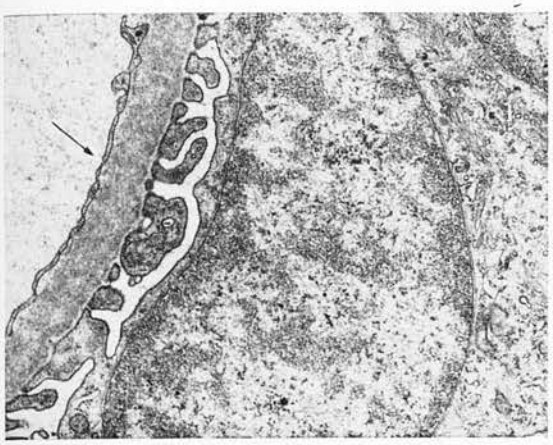

PLATE 4. Detail of capillary loop in diabetic. Arrow indicates attenuated endothelium. On outer aspect of capillary wall (right) epithelial cell attached by foot processes × 12,000.

diffuse glomerulosclerosis was due to marked thickening of the glomerular capillary basement membrane. The peripheral capillary loops were universally, though not uniformly affected, while the mesangial zones contained large accumulations of basement membrane material (Plate 2). Many subsequent reports confirmed these observations (MacDonald, 1966), and showed also that there was a generalized increase in quantity of basement membrane leading to folding and fusion of capillary loops, massive axial enlargement and capillary obliteration. Thus it appeared to most observers that the nodular lesion of light microscopy terminology represented focal, centrolobular exaggeration of diffuse basement membrane thickening. Yet Kimmelstiel (1966) strongly opposed this view by arguing that the nodule may arise without diffuse capillary thickening being present. This controversy will not be settled until reliable methods of quantitative analysis of the basement membrane in the mesangium besides the peripheral capillaries have been established.

It is probable that the pathogenesis of diabetic glomerular capillary disease is more complex than either of these opposing views, and that the basement membrane can be modified by altered function in the associated epithelial, endothelial and mesangial cells (Ireland, 1968). Thus the diabetic lesions may represent varying degrees of epithelial production or impaired mesangial turnover of basement membrane.

Renal biopsy in the diagnosis and clinical assessment of diabetic nephropathy.

Since the clinical features of diabetic renal involvement (proteinuria, nephrotic oedema, hypertension and renal failure) are essentially nonspecific, accurate diagnosis can be made only with the aid of percutaneous renal biopsy. Although it is generally believed that proteinuria is the first clinical warning of diabetic renal disease, many patients without proteinuria have been found at biopsy to have well established glomerular lesions (Thomsen, 1965). Moreover, preliminary studies of differential protein clearance by immunological or gel filtration methods have failed to show any consistent pattern in diabetic glomerulosclerosis. Likewise, tests of glomerular filtration, whether by measurement of endogenous creatinine clearance, ^{57}Co-cyanocobalamin or other methods may give misleading results in diabetes (Ireland, 1968).

Renal biopsy in the study of the pathogenesis of diabetic angiopathy

A fundamental problem concerning the pathogenesis of diabetic microangiopathy is whether the lesions are a genetically determined and integral component of the idiopathic disorder or due to one or more of the biochemical derangements of diabetes. Electron microscopy examination of renal biopsy tissue has been used in several studies in which glomerular capillary basement membrane was reported to be thickened in recently diagnosed and pre-diabetic patients. Although these findings have been quoted in favour of a genetic basis for diabetic microangiopathy, the methods used and control observations were often inadequate. Osterby-Hansen (1965), in a careful study, found no difference in peripheral glomerular capillary basement membrane thickness between recent diabetics and non-diabetics. The apparent lack of microangiopathy in patients having diabetes secondary to haemochromatosis, chronic pancreatitis or pancreatic carcinoma has also been cited in favour of a genetic cause of diabetic lesions. However, in addition to isolated reports of diabetic lesions in such patients, Ireland *et al.* (1967a) found significant glomerular capillary basement membrane thickening compared with healthy controls in ten patients having diabetes secondary to haemochromatosis, chronic pancreatitis or pancreatic carcinoma. Although these findings suggest that glomerular capillary disease in diabetes is due to a metabolic derangement common to idiopathic and secondary diabetes, the possibility that certain components of diabetic small blood vessel disease can occur in and be due to some as yet undefined aspect of the idiopathic disorder cannot be overlooked (Ireland *et al.*, 1967b).

The relationship of the anterior pituitary to the pathogenesis of diabetic angiopathy also remains uncertain. Although improvement in diabetic retinopathy has occurred in many patients subjected

to pituitary destruction, it is generally believed that the procedure has no beneficial effect on the progressive clinical course of diabetic renal disease. In a study of serial renal biopsies obtained from patients before and after pituitary ablation, Ireland *et al.* (1967b) found that the renal arteriolar lesion was unaffected or deteriorated. However, successful pituitary ablation was followed by significant reduction in the thickness of glomerular capillary basement membrane and restoration of previously atrophic endothelial (Plate 3) and mesangial cells. Although it would be unwise to draw firm conclusions from the limited data available, it is possible that the anterior pituitary influences certain aspects of diabetic capillary, but not arteriolar, disease.

Although the duration of diabetes is the single most significant factor in determining the severity of diabetic nephropathy (Berkman & Rifkin, 1966), the absence of a straightforward clinical course leads to many exceptions. Thus some insulin-dependent diabetics of more than 20 years' standing have no significant renal involvement found on light and electron microscopy examination of renal biopsies, while others, notably the elderly, may have well established lesions at the time of diagnosis of diabetes (Ireland, 1968).

Poor diabetic control is frequently cited as a cause of diabetic vascular lesions. However the demonstration of an association between poor control and severity of renal or other complications does not prove a cause and effect relationship, since the possibility that diabetics having complications are more difficult to control than those without such lesions cannot be overlooked.

Conclusions

The value of data based on renal biopsy samples is limited unless it can be shown that the findings are representative of the individual's glomerular population. Moreover, lesions which cannot be resolved by the light microscope must be assessed by reliable quantitative methods when the electron microscope is used to reveal their fine detail. Such methods have been applied to the estimation of basement membrane thickness in the periphery of glomerular capillaries. The results indicate that when basement membrane thickening occurs it does so diffusely, thus renal biopsy is of value in assessing peripheral capillary basement membrane thickness in diabetes. However, such methods arbitrarily exclude the mesangial region and other cellular details. It is probably for this reason that controversy concerning the relationship between diffuse and nodular glomerular lesions continues. None the less the nodule remains for the light microscopist the hallmark of diabetes in the kidney. Most studies of biopsy tissue have shown that the nodule is infrequently found and usually occurs in association with the more common diffuse changes and with afferent and efferent arteriosclerosis. Thus in the majority of biopsies electron microscopy examination is necessary to distinguish between diabetic and other causes of diffuse capillary changes.

Accompanying lesions in the tubules and interstitial tissue, which often in the past have been interpreted as chronic healed pyelonephritis probably represent the end result of diabetic vascular lesions. Whereas renal biopsy is pertinent to the assessment of specific diabetic renal involvement which is essentially diffuse, the procedure is of little value in such a focal disorder as chronic pyelonephritis.

Whether the basement membrane lesion in diabetes is a consequence of a metabolic, genetic, immunological or other defect is uncertain, but it is possible that the lesion represents altered basement membrane turnover secondary to the influence of one or more of such defects upon the associated epithelial, endothelial and mesangial cellular function. Until reliable methods are available for assessing possible alterations in these cells, besides the basement membrane, the pathogenesis of diabetic microangiopathy will remain uncertain.

Although the concept of a specific diabetic microangiopathy is widely accepted, with the increasing application of electron microscopy and other new techniques, it is difficult to escape the conclusion that this simple concept conceals the complexity of the factors concerned in the pathogenesis of the various arteriolar and capillary lesions.

Acknowledgments

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DIABETES

Kidney changes in diabetes

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IN 1936, Kimmelstiel and Wilson described spherical hyaline masses in the glomeruli of eight patients who died of renal failure following illnesses characterised by albuminuria, nephrotic oedema and hypertension. The glomerular lesions were attributed to diabetes mellitus because seven of the patients were known to have had the disorder. Although many would accord Kimmelstiel and Wilson a place in medical history on account of their description of a new syndrome, the fundamental importance of their observation lay in the interest it stimulated in the possibility of a specific small blood vessel disorder in diabetes. The subsequent demonstration, in autopsies on diabetics, of diffuse glomerular lesions (Fahr, 1942), and of associated hyaline arteriolosclerosis (Bell, 1952), together with renewed interest in diabetic retinopathy (Ballantyne and Loewenstein, 1944), led to the concept of a specific and widespread diabetic microangiopathy (Lundbaek, 1954).

The general belief that diabetics are more prone than non-diabetics to atheroma and other non-specific degenerative changes in larger blood vessels in no way detracts from the concept that it is involvement of small blood vessels, and of capillaries in particular, which characterises the diabetic lesion. Yet disagreement regarding the diffuse and nodular diabetic glomerular lesions of light microscopy terminology and uncertainty concerning their relationship to arteriolosclerosis and pyelonephritis, both of which can cause renal failure in diabetic patients (**Fig. 1**), have obscured the pathogenesis of renal disease in diabetes. Some of these difficulties have been overcome by the use of electron microscopy, which has shown, by sharpening the focus on glomerular and other capillaries, that homogenous thickening of their basement membranes distinguishes diabetes from other causes of capillary disease. As a result "basement membrane thickening" has become the new catch-phrase of diabetic vascular pathology. Whether this lesion is a morphological consequence of a metabolic or genetic defect, or an immunological response to exogenous insulin or other antigens remains undecided. That the problem is of more than academic interest is emphasised by the fact that more than half of those who develop diabetes in childhood die of renal failure or its consequences.

The gross appearance of the diabetic kidney is unremarkable, though it may be slightly enlarged (Rifkin *et al.*, 1952). On light microscopy examination lesions of the following structures may be found:

1. Arteries of the kidney.
2. Afferent and efferent arterioles.
3. Glomeruli.
4. Tubules.
5. Interstitial tissue.

The characteristics of the lesions will be described and discussed before reviewing their pathogenesis and associated clinical features.

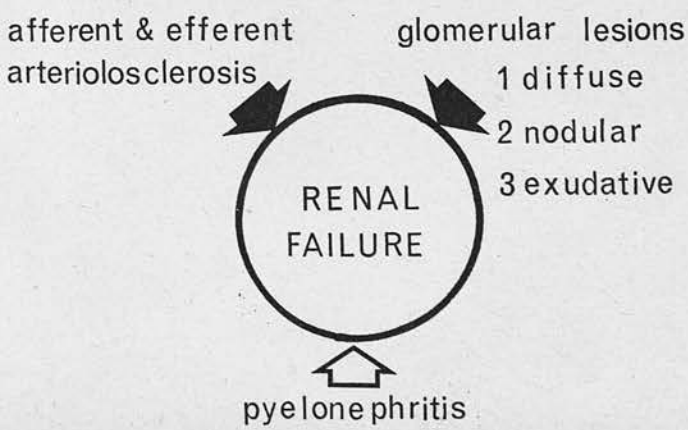


Fig. 1. *Diabetic nephropathy.*

Arteries

Although atheroma is said to be more common in diabetics there is no accurate documentation of any increase in its incidence in the larger renal vessels of diabetics in autopsy or other studies of age-matched subjects. On the other hand, diffuse intimal fibrosis in these vessels, which is believed to be almost universal after the age of 50, has been found to be more frequent and advanced in autopsies on diabetics by comparison with non-diabetics (Bell, 1952; Warren *et al.*, 1966).

Arterioles

It was Bell (1953) who established the importance of hyaline arteriolar lesions in diabetes. He emphasised that there was efferent arteriolar involvement besides afferent arteriolosclerosis in diabetes and showed that although hypertension was common in the 1,465 diabetics examined by him at autopsy, a large percentage without hypertension had these lesions. Indeed efferent arteriolar involvement is sufficiently characteristic of diabetes to be of aid to the light microscopist in concluding that associated glomerular disease is diabetic in origin.

Blumenthal *et al.* (1962) have described a proliferative arteriolar lesion with excessive deposition of PAS-positive material, involving the endothelial cells and occurring

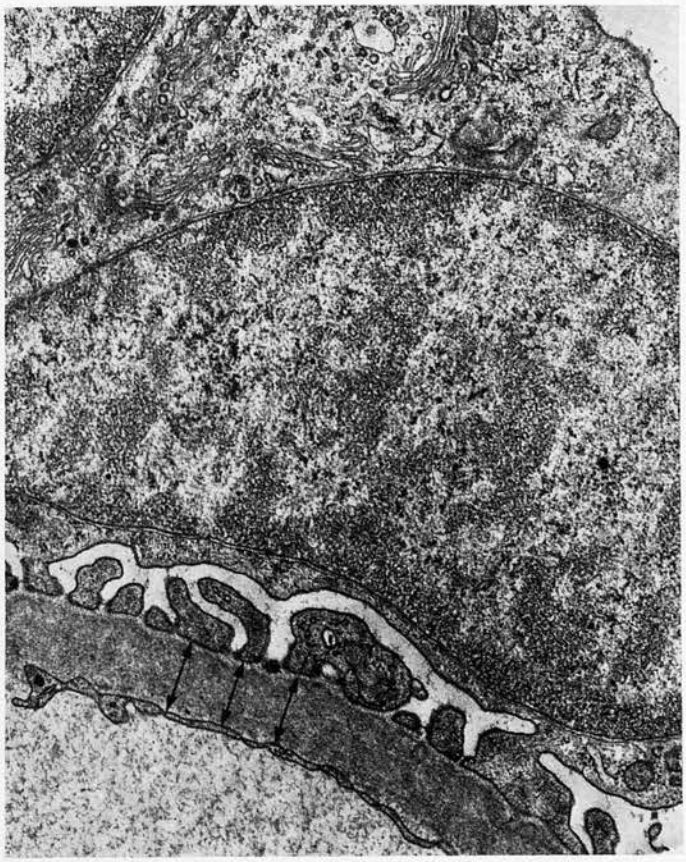


Plate 1 (above). *Peripheral glomerular capillary wall from an idiopathic diabetic. Cross-section measurements of the basement membrane thickness are obtained at regular intervals (arrowed). Epithelial cell (above) attached by foot processes.* × 20,000.

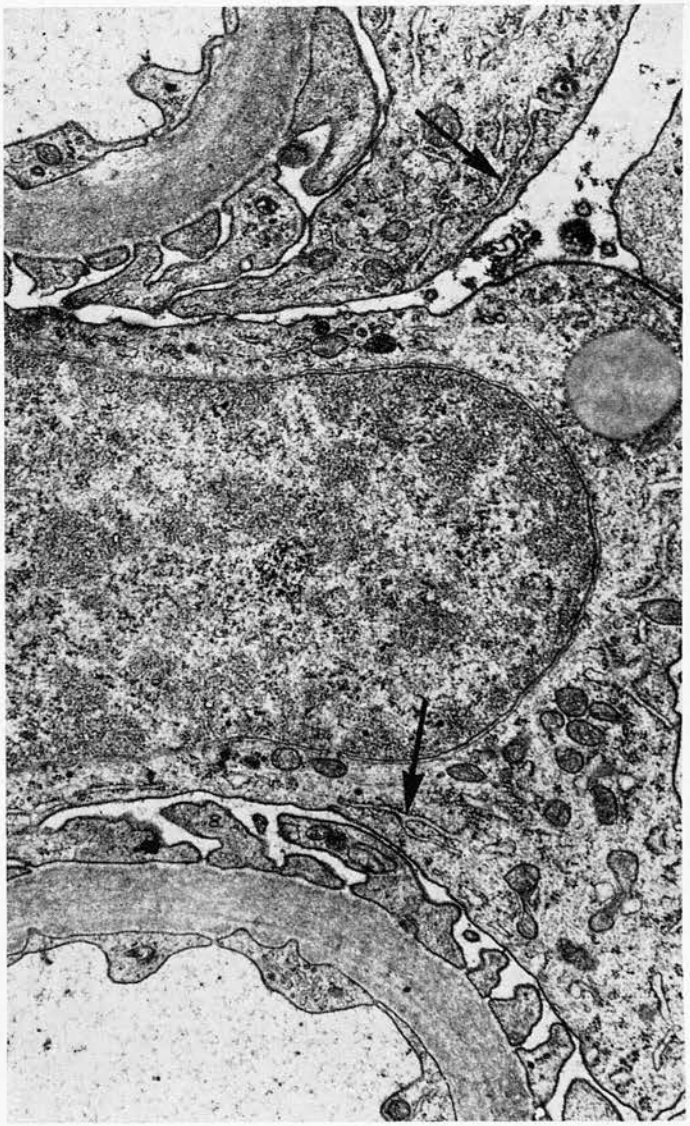


Plate 2 (right). *Peripheral glomerular capillaries from patient having diabetes secondary to chronic pancreatitis. Thickened capillary walls (above and below) separated by epithelial cell having prominent mitochondria and RNA-studded endoplasmic reticulum (arrowed).* × 20,000.

Plate 3 (below). *Diffusely thickened glomerular capillary loop from idiopathic diabetic. Mesangial region (A) with excess basement membrane. Epithelial cytoplasm (b), above and below. Attenuated endothelial cell and cytoplasm (C).* × 6,000.

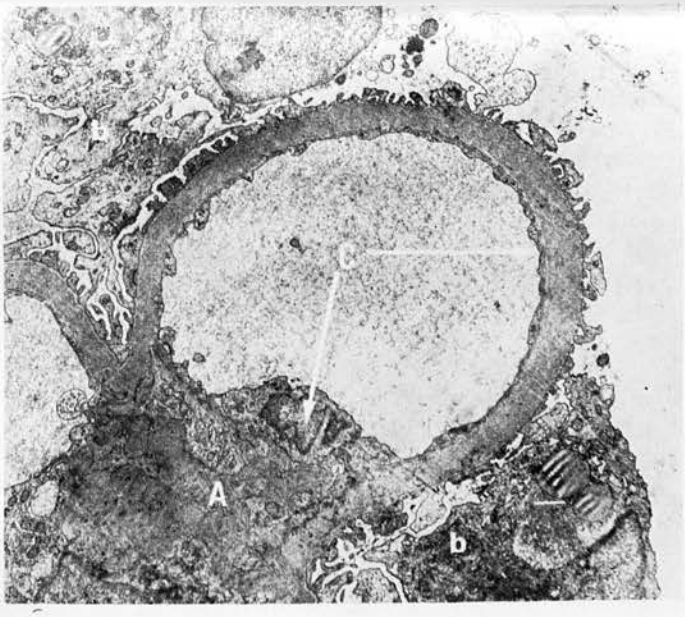
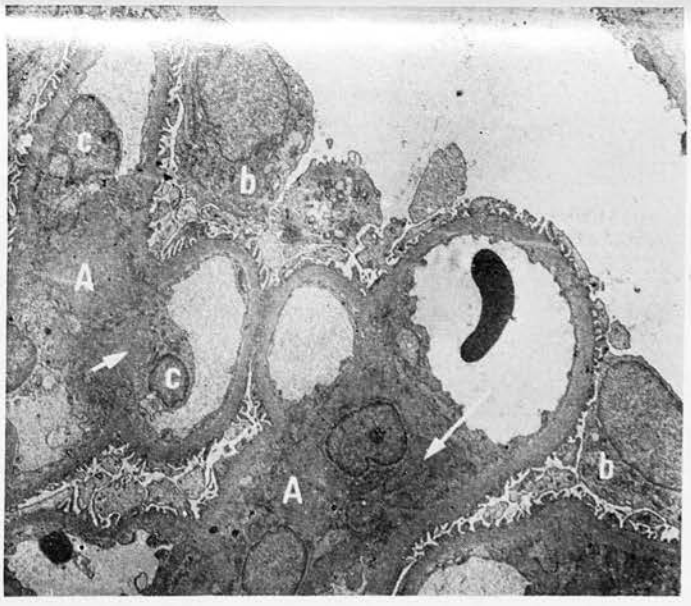


Plate 4 (below). *Diffuse thickening of basement membrane of glomerular capillaries from idiopathic diabetic. Mesangial regions (A) having excess basement membrane (arrowed). Epithelial cytoplasm (B). Endothelium (C).* × 2,000.



more commonly in the renal arterioles of diabetics than non-diabetics. The same workers have reported similar lesions in many other sites and have suggested an immunological relationship to exogenous and endogenous insulin; yet this lesion has rarely been noted by other observers (Berkman and Rifkin, 1966).

Glomerulus

Light microscopy

Under the general heading of diabetic glomerulosclerosis the following lesions have been recognised by light microscopy:

(i) Nodular
(ii) Diffuse
(iii) Exudative lesions.

Nodular glomerular lesions: Kimmelstiel (1966) has emphasised that in the past confusion has arisen through failure to define the morphology of this lesion. Within an affected glomerulus, which may be normal in size or enlarged, nodules occupy the centres of single or multiple peripheral glomerular lobules. The fully developed lesion may be an almost spherical, homogenous, vacuolated, fibrillar or lamellar mass often having a patent or distended capillary running over its surface. The histological lesion most commonly confused with the diabetic nodule is the lobular form of glomerulonephritis. However, the latter can usually be distinguished because the lobules are more uniformly affected, the peripheral capillary is less distinct and the afferent arteriole is not involved as it is in diabetes.

It is now agreed that the nodule is confined to, and pathognomic of, diabetes mellitus (Rifkin *et al.*, 1962) and that early reports to the contrary were a consequence of mistaking either lobular nephritis or exudative glomerular lesions with the nodule, or of failure to detect clinically mild disturbances of carbohydrate metabolism in so-called non-diabetics. In considering the ultrastructure and pathogenesis of the nodular lesion (*vide infra*) it is worth emphasising its three main features: namely, that it is focal, centrolobular and has an acellular centre.

Diffuse glomerular lesion: Although diffuse diabetic glomerulosclerosis was first described by Fahr (1942), the lesion received little attention until Gellman *et al.* (1959), as a result of a careful clinico-pathological study of biopsy material, showed that it was twice as common as the nodular lesion and that the severity of the features of renal failure and nephrotic syndrome correlated with the extent of diffuse rather than nodular glomerular involvement.

The diffuse diabetic lesion usually begins with thickening of the whole circumference of the wall of peripheral capillaries of the glomerular tuft (Gellman *et al.*, 1959). With increasing severity the lesion becomes diffuse within the glomerulus and generalised in the kidney. Further progression leads to narrowing of the capillary lumina and eventually to complete hyalinisation of the glomerulus. Gellman emphasised that nodules occur only in glomeruli involved by the diffuse process; nonetheless he regarded the two lesions as separate entities whereas many other observers (Farquhar *et al.*, 1959; MacDonald, 1966; Bloodworth, 1963) believe that the nodule simply represents a more advanced stage of the diffuse lesion. Bell (1953) recognised the separate identity of the diffuse and nodular lesions, but also described an intermediate stage, chiefly affecting the centrolobular region which he termed "diffuse intercapillary glomerulosclerosis."

Being unable to distinguish between diffuse lesions due to diabetes and such other causes as membranous glomerulonephritis or amyloidosis, the light microscopist has been reluctant to regard the diffuse diabetic lesion as a specific entity. This uncertainty has led many observers to make the paradoxical and confusing statement that the specific diabetic nodule arises in glomeruli involved by, and is a more advanced stage of, the non-specific diffuse lesion. The application of examination by electron microscopy as well as light microscopy has, to a large extent, resolved these difficulties (*vide infra*).

Bell (1953) first emphasised the parallel association between the severity of afferent arteriolosclerosis and diffuse and nodular glomerular involvement. This has led some to conclude that the diabetic glomerular lesions might be a consequence of the arteriolar disease. This seems unlikely, however, since arteriolosclerosis in the absence of diabetes usually leads to ischaemic atrophy of the glomerular tuft with thickening of the capillary wall at the hilus and shrinkage of the remaining vessels instead of the diffuse and nodular changes.

Exudative glomerular lesion: The exudative lesion is the least significant of the three glomerular changes in diabetes. It also occurs in various non-diabetic glomerular disorders associated with renal failure, and in diabetics only in the late stages of nephropathy. Also known as "hyaline-fibrinoid," "acellular hyaline," "fibrin cap" and "capsular drop," the exudative lesion usually consists of rounded and crescentic deposits of either homogenous or vacuolated, intensely acidophilic material without nuclei. It may form a cap over a glomerular capillary loop or may be attached to the inside of Bowman's space, and represents a mixture of various proteins and fibrinoid which has leaked into the capsular space. Alternatively the material may be within the glomerular capillary lumen.

The lesion represents non-specific glomerular damage and it is only of importance because it has been confused with the Kimmelstiel-Wilson nodule leading to doubts that the latter is pathognomonic of diabetes. Conversely, steroid-induced lesions in the glomeruli of experimental animals which resemble the exudative lesion have been interpreted erroneously as specifically diabetic (Bloodworth and Hamwi, 1956).

Electron microscopy

The application of electron microscopy has led to many recent advances in our knowledge of the normal anatomy of the glomerulus and of its appearance in diabetes. Accordingly the structure of the glomerular capillaries will first be reviewed with particular reference to those details relevant to diabetes.

Normal anatomy: The afferent arteriole divides into several branches to form the glomerular capillaries which are grouped into lobules **(Fig. 2).** Thus two or more capillaries become arranged about a centrolobular region known also as the axial zone, the mesangium or the intercapillary space. The central zone lies closest to the origin of the capillaries from the afferent arteriole; the peripheral capillary loops branch out from the central zone to face Bowman's space. The capillary walls are composed of a homogenous basement membrane separating the endothelium from the epithelial cells. The endothelial nuclei usually lie in the proximity of the central or axial zones which contain a third type of cell known, because of their situation, as axial, mesangial or deep endothelial cells. **(Fig. 2).** The mesangial cells are separated from the endo-

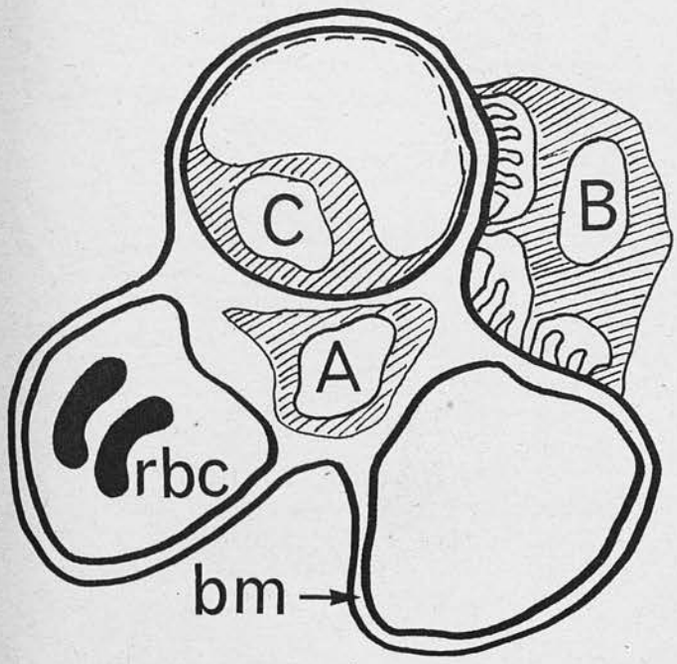


Fig. 2. *Diagram of glomerular capillary lobule.*
A *Mesangial cell in centrolobular region.* **B** *Epithelial cell in Bowman's space.* **C** *Endothelial cell within capillary.* **rbc** *Red blood cells within capillary lumen.* **bm** *Continuous basement membrane of capillary wall.*

thelial cells by loose-textured basement membrane and and have been likened, on account of their situation, to the pericytes in other capillaries (Farquhar and Palade, 1962). However, even in normal subjects, the glomerular capillaries are extremely tortuous, thus the three-dimensional distribution of the cells in the axial or mesangial zone is difficult to visualise on electron microscopy of sectioned material.

In contrast, the peripheral capillary loops have only three distinct components: the endothelium, the basement membrane and the epithelium. The basement membrane is the only continuous layer; the endothelium is composed of the attenuated periphery of the endothelial cells and is perforated by large pores; the epithelium lies over the free surface of the capillaries and is attached to the basement membrane by a system of foot processes **(Plate 1** *page* 782). These unique features of the epithelium and endothelium of the peripheral loops, relevant to the special function of the glomerulus, are of advantage to the electron microscopist in assessing basement membrane thickness. Thus, where either the epithelial foot processes are sectioned longitudinally or where the pores in the endothelial cytoplasm occur as spaces, the examiner can be confident of having obtained a true cross section of the capillary basement membrane **(Plate 1).** Using this method, measurement of sufficient capillary loops to obtain statistically significant data has revealed a wide range in normal basement membrane thickness; overall mean values of 2250Å to 3000Å having been reported (Osterby-Hansen, 1965; Osawa *et al.*, 1966; Ireland *et al.*, 1967a).

The glomerulus in diabetes: Early electron microscopy studies of renal biopsy tissue obtained from diabetic patients having clinical evidence of nephropathy, confirmed that diffuse glomerulosclerosis was due to marked thickening of the glomerular capillary basement membrane (Farquhar *et al.*, 1959). The peripheral capillary loops were universally, though not uniformly affected, while the axial zones contained large accumulations of basement membrane material **(Plates 3 and 4).** Many subsequent reports confirmed these observations (Bloodworth, 1963; MacDonald, 1966), and showed also that there was a generalised increase in quantity of basement membrane leading to folding and fusion of capillary loops, massive axial enlargement and capillary obliteration **(Fig. 3).** Thus it appeared to most observers that the nodular lesion of light microscopy terminology represented focal, centrolobular exaggeration of diffuse basement membrane thickening. Yet Kimmelstiel (1966) strongly opposed this view by arguing that the nodule may arise from the centrolobular (intercapillary) zone without diffuse capillary thickening being present.

It is probable that the pathogenesis of diabetic glomerular capillary disease is more complex than either of these opposing views, and that the basement membrane can be modified by altered function in the associated epithelial, endothelial and mesangial cells. There is considerable evidence that the basement membrane is synthesised by the epithelial cells (Farquhar, 1964). In diabetics with significant basement membrane thickening these cells have abundant cytoplasm containing prominent RNA-studded or rough-surfaced endoplasmic reticulum **(Plates 1 and 2)** suggesting increased intracellular production and deposition of basement membrane. Farquhar (1964), from a study of the turnover of ferritin particles, has demonstrated that the mesangial cells have a phagocytic function and she suggests that the centrolobular accumulation of basement membrane may represent its diminished removal by defective mesangial cells. This view is supported by the fact that the mesangial cells are atrophic in massive centrolobular accumulations of basement membrane and by the absence of cells from the centres of nodular lesions on light microscopy examination.

Thus the diabetic lesions may represent varying degrees of epithelial production or impaired mesangial turnover of basement membrane. Preliminary studies of these cellular functions in relation to the glomerular capillary basement membrane have been made in idiopathic and

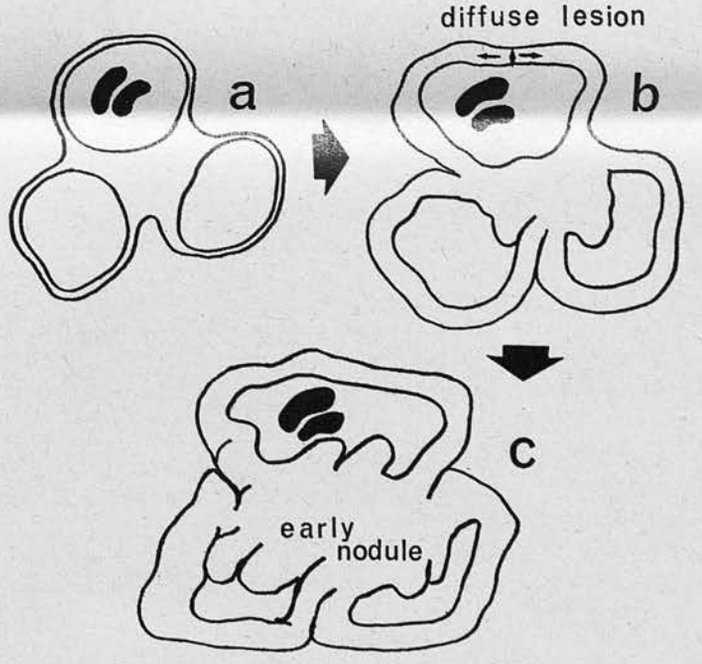


Fig. 3. *Diagram of increase in basement membrane material.*
a *Normal lobule.* **b** *Diffuse enlargement of capillary wall.* **c** *Mesangial enlargement and capillary obliteration.*

secondary diabetics (Ireland *et al.*, 1967b) and in the experimental animal (Walker, 1968). It would appear that the cells may be influenced by the metabolic defects of diabetes and by anterior pituitary function, but the complex interrelations are far from clear. Both epithelial hyperactivity and mesangial dysfunction may be present in patients shown to have diffuse glomerulosclerosis, whereas the nodule usually arises only when the mesangial cells have been destroyed and the capillary lumina obliterated. MacDonald (1966) has shown that other non-diabetic causes of diffuse glomerulosclerosis can be distinguished from the diffuse diabetic lesion. The latter is unique in that there is an increase in the amount of basement membrane material without alteration in its fine structure.

Tubules

Although various lesions may be found in the tubules of diabetics, few are of specific significance and in general they are secondary to glomerulosclerosis, ischaemia, pyelonephritis or long-standing electrolyte disturbance.

PAS-positive thickening of both the proximal and distal tubular basement membrane has been reported frequently in diabetics leading some to regard this as further evidence of "widespread basement membrane disease in diabetes." However there are no reports of data comparing thickness in diabetic and non-diabetic tubular tissue examined by electron microscopy; furthermore, the PAS-positive changes may also be found in the tubules in the presence of other diseases causing ischaemia or in pyelonephritis.

The "Armanni-Ebstein lesion", or "glycogen nephrosis", first described in 1877, consists of glycogen-laden vacuoles in the tubules of the cortico-medullary region. It was a common post-mortem finding in the pre-insulin era and is still occasionally found in those who die following uncorrected hyperglycaemia, acidosis and dehydration. Severe electrolyte and circulatory distances in profound diabetic keto-acidosis may also cause acute tubular necrosis.

Large, clear, "empty" vacuoles may be found in the proximal convoluted and collecting tubules at biopsy in diabetics having severe potassium depletion. Electron microscopy examination shows degeneration of the epithelial cytoplasm, and in diabetics the lesion may be confused easily with the fine hydropic degeneration which accompanies glucose-induced osmotic diuresis.

Interstitial tissue

Pyelonephritis

Many believe that diabetics are more prone than others to infections in general and pyelonephritis in particular. In autopsies on diabetics, changes interpreted as chronic pyelonephritis were frequently reported to be found in association with diabetic arteriolosclerosis and glomerulosclerosis. Indeed, some observers were sufficiently impressed by the association to conclude that the diabetic lesions might be a consequence of chronic pyelonephritis. However, the more experienced observer now appreciates that most of the changes which resemble healed chronic pyelonephritis in the interstitial tissue and tubules are secondary to ischaemia and the glomerular lesions of diabetes.

Kimmelstiel (1961) was one of the first to doubt the validity, in the presence of ischaemia, of accepting many of the criteria upon which the diagnosis of healed chronic pyelonephritis might otherwise be made. He concluded that pronounced lymphocytic and plasma cell infiltration of the intertubular tissue, periglomerular fibrosis and increased connective tissue probably resulted from ischaemia alone. Heptinstall (1967), in a perceptive review of the limitations of diagnosis in chronic pyelonephritis, found it painfully obvious that many pathological processes can produce a similar end result. He stressed that the most certain evidence in favour of an infective cause of interstitial damage is the presence of associated inflammatory changes in the pelvi-calyceal system.

Nonetheless, there are several factors which may predispose the diabetic to urinary tract infection. These include the effect of diabetic autonomic neuropathy on bladder function favouring stasis, the possibility of catheterisation still being practised in the management of keto-acidosis, and that infection is more likely in renal tissue affected by such vascular lesions as arteriolosclerosis and glomerulosclerosis.

Attempts to assess in diabetics the incidence of acute and chronic pyelonephritis on the basis of quantitative bacteriological examination of the urine, radiology, renal biopsy or other methods have produced conflicting results. Whereas renal biopsy is ideally suited to the assessment of specific diabetic renal involvement which is essentially diffuse, the procedure may produce misleading results in such a focal disorder as chronic pyelonephritis. Reviewing many studies based on quantitative examination of the urine, Thomsen (1965) concluded that, with the exception of the elderly diabetic female, urinary infection was not more frequent in diabetic patients. Velsgaard (1966) also found a significantly higher incidence of infection in diabetic women but no significant difference between diabetic and non-diabetic men.

Nevertheless, in diabetics having glomerulosclerosis and renal failure, the possibility that the latter is being aggravated by either acute or chronic pyelonephritis **(Fig. 1)** should always be considered, particularly since infection is more amenable to treatment than the diabetic lesion.

Renal papillary necrosis

This usually results from acute infection in which the tips of medullary tissue become necrotic. In diabetics the lesion may be exacerbated by ischaemia due to disease of the long thin vasa recta though compression of the submucosal vessels by infective oedema may further compromise papillary blood supply. Radiological examination is of value in making the diagnosis by demonstrating a "moth-eaten" appearance of the calyces as contrast media penetrates necrotic tissue, while the "ring-shadow" corresponding to necrotic papillae is believed to be pathognomonic. With improved antibiotic therapy the condition is reported in diabetics less frequently, though phenacetin abuse is becoming increasingly recognised as an alternative cause in non-diabetics.

Finally it should not be overlooked that diabetics are by no means immune to renal tuberculosis or any other non-diabetic renal disease.

CLINICAL CORRELATIONS, DIAGNOSIS AND INCIDENCE

Proteinuria, nephrotic oedema, hypertension and renal failure have been the accepted clinical features of diabetic kidney disease since Kimmelstiel and Wilson's description of the nodular glomerular lesion. Yet none of these features is confined to, or diagnostic of, diabetic arteriolosclerosis or glomerulosclerosis; moreover, such lesions may be advanced in patients with minimal clinical evidence of renal disease (Ireland *et al.*, 1967b).

Although it is generally believed that proteinuria is the first clinical warning of diabetic renal disease, in a large series studied by Bell (1953) it had been absent in 10 per cent. of those found at autopsy to have died of renal lesions. He also found that in more than half of those over the age of 60 years, proteinuria was due to cardiac failure or causes other than diabetic renal disease. Thomsen (1965) concluded that in the absence of cardiac failure, pyuria or keto-acidosis, proteinuria was a good sign of diabetic glomerular damage yet 20 per cent. of his own series of cases, found at renal biopsy to have well-established glomerular lesions, were free of proteinuria. Preliminary studies of differential protein clearance by immunological or gel filtration methods have failed to show any consistent pattern of selectivity in patients having diabetic glomerulosclerosis (MacLean *et al.*, 1968).

Several studies have shown a good correlation between hypertension and renal arteriolar lesions, yet in a large series Bell (1953) found that many normotensive diabetics had afferent and efferent arteriolosclerosis. Thomsen (1965) showed that renal lesions preceded hypertension and concluded that the diabetic changes were a cause rather than a consequence of hypertension.

Renal failure in the diabetic may result from several factors **(Fig. 1)** though in biopsy data it correlates best with the severity of glomerular hyalinisation. Assessment of clinical progress by measurement of endogenous creatinine clearance may, in the diabetic, be unreliable since hyperglycaemia, glycosuria and keto-acidosis are sources of error in creatinine determination (Thomsen, 1965); moreover, it should not be forgotten that optimistic results may be obtained in the presence of proteinuria. Whereas deterioration in glomerular filtration is expected with increasing severity of glomerulosclerosis, Ditzel and Schwartz (1967) and others have found enhanced clearance of ^{57}Co-cyanocobalamin in early juvenile diabetics. Whether this represents increased capillary permeability or evidence of a greater filtration surface in the early stage of glomerular capillary basement membrane changes **(Fig. 3b)** is uncertain.

Since the clinical features of diabetic renal involvement are essentially non-specific, accurate diagnosis can be made only with the aid of percutaneous renal biopsy. Notably, elderly patients may have advanced diabetic nephropathy in the presence of a mild disturbance of carbohydrate metabolism, which may be overlooked because both age (Butterfield *et al.*, 1967) and renal failure may raise the renal threshold for glucose. Conversely, pseudo-diabetic carbohydrate intolerance of uraemia or impaired tubular reabsorption of glucose in the nephrotic syndrome may be misinterpreted as indicative of diabetes mellitus.

Without serial renal biopsy examination the incidence and clinical course of diabetic kidney disease is difficult to evaluate. It is distressingly clear, however, that of those who develop diabetes in childhoood, few escape renal involvement after 20 years of the disorder and that in this group renal failure is the major cause of death (Warren *et al.*, 1966). Many of those who develop diabetes in later life may have minimal renal involvement after a similar interval, while others, notably the elderly, may have well-established lesions at the time of diagnosis. Thus it would be unrealistic to imagine a straightforward clinical picture of kidney disease in diabetes.

THE PATHOGENESIS OF DIABETIC GLOMERULAR CAPILLARY DISEASE

Genetic factors

One of the outstanding problems concerning the pathogenesis of diabetic microangiopathy is whether the lesions are a genetically determined and integral component of the idiopathic disorder or due to one or more of the biochemical derangements of diabetes. Electron microscopy has been used in several studies in which the glomerular capillary basement membrane was reported to be thickened in recently diagnosed and pre-diabetic patients (Goetz *et al.*, 1960; Daysog *et al.*, 1961; Rees *et al.*, 1964). Although these findings have been quoted in favour of a genetic basis for diabetic microangiopathy, the methods used and control observations were often inadequate. Osterby-Hansen (1965), in a careful study, found no difference in glomerular capillary basement thickness between recent diabetics and non-diabetics. Many animal experiments have been undertaken in the hope of producing diabetic glomerular lesions as a result of the metabolic disturbance of meta-hypophyseal or alloxan-diabetes. Although glomerulosclerosis has been reported in most of these studies, the lesions bear little relationship to those seen in human diabetes. However, in a well-controlled and exhaustive light and electron microscopy study, Bloodworth (1965) has demonstrated typical diffuse and nodular diabetic lesions in animals made diabetic and treated with insulin for up to six years.

The apparent lack of microangiopathy in patients having diabetes secondary to chronic pancreatitis, carcinoma of the pancreas or haemochromatosis has also been cited in favour of a genetic cause of diabetic microangiopathy. Yet there have been several isolated reports of diabetic lesions in such patients (Duncan *et al.*, 1958; Deckert, 1960; Shapiro and Smith, 1966), and in an electron microscopy study of 10 patients having diabetes secondary to haemochromatosis, chronic pancreatitis or pancreatic carcinoma, Ireland *et al.* (1967a) found significant glomerular capillary basement membrane thickening compared with heathy controls. While these findings suggest that glomerular capillary disease in diabetes is due to a metabolic derangement common to idiopathic and secondary diabetes, the possibility that certain components of diabetic small blood vessel disease can occur in and be due to some as yet undefined aspect of the idiopathic disorder cannot be overlooked (Ireland *et al.*, 1967b).

Metabolic factors

The frequently suggested possibility that in diabetes the renal vascular lesions result from deposition on their walls of protein or other abnormal substances has been repeatedly investigated as new techniques of study become available (Gordon, 1964). However, in a critical review, Winzler (1964) concluded that it was uncertain whether elevated levels of glycoproteins in the serum of diabetics were a cause, a passive concomitant, or a result of small blood vessel disease.

Likewise in the many studies made of plasma lipids in relation to small blood vessel disease and atheroma in diabetes, the general conclusion that their alteration could be a consequence of nephropathy cannot be overlooked (Adlersberg *et al.*, 1956).

Nonetheless, the possibility that the renal small blood vessel changes might be a morphological consequence of

the biochemical alterations of diabetes, though long suspected, has been supported by many recent studies. Robb-Smith (1957) and many others since have established the high protein content of glomerular capillary basement membrane; the use of PAS staining has repeatedly demonstrated the presence of carbohydrate. The composition of the glycoprotein complexes of which the basement membrane is largely composed has been studied in detail by Lazarow and Speidel (1964). They showed that although diabetic glomeruli contained a great excess of glycoprotein by comparison with those of normal humans, on hydrolysis of the carbohydrate content to simple sugars there was no qualitative difference between the two groups.

Although increased synthesis of glycoprotein might appear to be a paradox in a disorder characterised by impaired carbohydrate metabolism, Spiro (1963) has postulated a biochemical basis for such a possibility. He found that the production of the glucosamine component of glycoprotein along non-insulin dependent pathways was increased in liver slices from insulin-deficient alloxan-diabetic rats. Studies by Winegrad and Burden (1966) of *l*-xylose metabolism in the glucuronic acid pathway of glucose metabolism in relation to the synthesis of glycoproteins is further evidence favouring increased basement membrane synthesis in diabetes. The epithelial cell hypertrophy seen in genetic and secondary diabetes (Ireland *et al.*, 1967b) is consistent with such a hypothesis since the epithelium is known to be responsible for basement membrane synthesis. However the evidence that defective mesangial cells impede the clearing of basement membrane and other proteins (Farquhar, 1964) suggests that in those diabetics having the latter lesion, basement membrane accumulation may result from its diminished turnover besides increased synthesis.

Immunological factors

Because diffuse and nodular diabetic glomerulosclerosis were not reported until many years after the discovery of insulin it has been suggested that the lesions might be an immunological response to exogenous insulin. However, it would also be necessary to postulate an autoimmune response to endogenous insulin to explain the common finding of diabetic glomerular lesions in patients who have never received insulin. Blumenthal *et al.* (1964) have reported binding of fluorescein-conjugated insulin by the nodular diabetic glomerular lesion, and have reported similar results in rabbits immunised with beef insulin and Freund's adjuvant mixtures. Burkholder (1965) studied the localisation of plasma proteins and fixation of guinea-pig complement in the lesions of diabetic glomerulosclerosis, but cautiously concluded that tissue fixation of insulin is not necessarily of pathological significance.

Indeed the immunology of glomerular capillary basement membrane is both complex and obscure. Cruikshank (1964) has emphasised the difficulties of localising antigens native to glomerular capillary basement membrane. Berson (1965), who was one of the first to explore this subject, observed that to date no experiment adequately incriminated exogenous or endogenous insulin.

Anterior pituitary and adrenal cortex

Although the classic studies of Houssay showed that some of the metabolic aspects of experimental diabetes are influenced by the anterior pituitary, the relationship of this gland to the pathogenesis of specific diabetic complications remains uncertain. Improvement in diabetic retinopathy has occurred in many patients subjected to pituitary destruction, but it is not known whether the procedure simply improves the vascular changes in the retina or favourably influences small blood vessel disease elsewhere. Poulsen (1966) and others (Graef, 1966) have reported that pituitary destruction had no beneficial effect on the progressive clinical course of diabetic renal disease. In a study of serial renal biopsies obtained from patients before and after pituitary ablation, Ireland *et al.* (1967a) found that the renal arteriolar lesion was unaffected. However, successful pituitary ablation was followed by significant reduction in the thickness of glomerular capillary basement membrane and restoration of previously atrophic endothelial and mesangial cells. Thus it is possible that the anterior pituitary influences certain aspects of diabetic capillary, but not arteriolar, disease; however, it would be unwise to draw firm conclusions from the limited data available.

Misinterpretation of some similarities between experimental cortisone-induced glomerular lesions and human diabetic glomerulosclerosis has led to the belief that the adrenal cortex influences the pathogenesis of the diabetic lesion. However, in a comprehensive study of urinary and plasma corticosteroids, including measurements of hydrocortisone turnover rates and aldosterone secretion, Rifkin *et al.* (1958) found no significant differences in these parameters in diabetic patients with and without microangiopathy. Many others have confirmed these findings though Lentle and Thomas (1964) found differences between controls, diabetic with, and diabetics without small blood vessel disease when plasma and urinary corticosteroids were measured following administration of ACTH or dexamethasone.

Effect of duration, severity and control

Although some insulin-dependent diabetics of more than 20 years' standing have no significant renal involvement found on light and electron microscopy examination of renal biopsies (MacDonald and Ireland, 1964), it has been established beyond doubt that duration of diabetes is the single most significant factor in determining the severity of diabetic nephropathy (Berkman and Rifkin, 1966).

Assessment of the influence of "severity" and "control" of diabetes is almost impossible in the absence of universally acceptable definitions of these terms. Following an exhaustive analysis of over 300 publications on the subject of diabetic control in relation to complications, Knowles (1965) was unable to reach a conclusion. He noted, with interest, that few of the individual authors had been able to do so. Moreover, the demonstration of an association between poor control and severity of renal or other complications does not prove a cause and effect relationship. The possibility that diabetics having complications are more difficult to control than those without such lesions cannot be overlooked.

CONCLUSION

Although the nodular glomerular lesion of Kimmelstiel and Wilson remains for the light microscopist the hallmark of diabetes in the kidney, it is found only in association with the more common diffuse glomerular changes, and with afferent and efferent arteriolosclerosis. Accompanying changes in the tubules and interstitial tissue which often in the past have been interpreted as chronic healed

pyelonephritis probably represent the end result of diabetic vascular lesions. Electron microscopy examination has shown that the characteristic diabetic defect in the glomerular capillaries is thickening of their walls with excessive basement membrane material which probably represents imbalance in the function of the associated epithelial, endothelial and mesangial cells. Although the pathogenesis of these various changes remains unknown, it is probable that diabetic nephropathy results from the development of several separate though interrelated lesions each of which may be variously influenced by the metabolic disturbance, the inherited diabetic diathesis or pituitary activity. Although the idea of a specific diabetic microangiopathy is now widely accepted, this simple concept probably conceals the complexity of the factors concerned in the pathogenesis of the various arteriolar and capillary lesions.

Almost 2,000 years ago Aretaeus the Cappadocian, who gave diabetes mellitus its name, suspected that the disorder was a pernicious affliction affecting the kidney. One cannot contemplate, without dismay, the sombre truth that the insulin era has afforded ample opportunity to witness, but so far not to avert diabetic nephropathy.

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