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Glomerular microfibrils in renal disease: A comparative electron microscopic study

HEY-CHI HSU and JACOB CHURG

Division of Renal Pathology, Department of Pathology, Mount Sinai School of Medicine, New York, New York

Glomerular microfibrils in renal disease: A comparative electron microscopic study. Microfibrils are a common component of connective tissue that have been described only rarely in the renal glomerulus. Structurally, microfibrils are fibrotubules with an average diameter of 12 nm, a lucid core, and a dark periphery. High resolution electron microscopy, including stereo microscopy performed on renal biopsy tissues, demonstrated the presence of microfibrils under the endothelium of the capillary walls and in the mesangium in several glomerular diseases. These diseases were characterized by widening of lamina rara interna or separation of the endothelium from the basement membrane, among them transplant glomerulopathy, focal segmental glomerulosclerosis (including a case associated with Marfan syndrome), preeclamptic toxemia, and less frequently hemolytic-uremic syndrome and malignant hypertension. The number of microfibrils generally correlated with the degree of subendothelial widening.

Microfibrilles glomérulaires dans les maladies rénales: Étude comparative en microscopie électronique. Les microfibrilles sont les constituants communs du tissu de soutien et ont été rarement décrits dans le glomérule rénal. Du point de vue structural les microfibrilles sont des fibrotubules d'un diamètre moyen de 12 nm, avec un centre transparent et une périphérie sombre. La microscopie électronique à haute résolution, comprenant la stéréo microscopie, montre, sur des biopsies rénales, la présence de microfibrilles sous l'endothélium des parois capillaires et dans le mesangium, dans plusieurs lésions glomérulaires caractérisées par l'élargissement de la lamina rara interna ou la séparation de l'endothélium d'avec la membrane basale. Les lésions en cause étaient la glomérulopathie du rein transplanté, la glomérulosclérose segmentaire et focale (y compris un cas associé à un syndrome de Marfan), la toxémie pré-éclamptique et, moins souvent, le syndrome hémolytique et urémique et l'hypertension maligne. Le nombre de microfibrilles est généralement proportionnel à l'élargissement sous-endothélial.

Among the various formed elements of the extracellular connective tissue, microfibrils represent a recent addition to the classical structures of the light microscopists, such as collagen, reticulum and elastic fibers, and basement membranes. In contrast to the rather thick (up to 100 nm) unit of collagen fibrils with conspicuous axial periodicity, structures classified as microfibrils by electron microscopists are thin aperiodic fibrils of less than 20 nm in diameter. They are quite ubiquitous [1] and are found in association with all the elements mentioned above. Recent studies have brought out the existence of three types of microfibrils [2]: large, 18 to 20 nm in diameter, which are susceptible to collagenase digestion and are believed to be precursors of unit collagen fibers; small, about 10 nm in diameter, first described as a component of the elastic tissue; and "thin filaments," 3 to 5 nm, which may be related to basement membranes. It is now realized that "small microfibrils" occur also independently of elastin and are found in a variety of tissues, including the renal glomerulus. They have a characteristic tubular structure and differ in chemical composition from collagen and from elastin but are similar in some respects to the basement membrane. This report will deal with the occurrence of the small microfibrils (thereafter referred to as S-microfibrils [SMF] or just microfibrils) in several glomerular diseases affecting the subendothelial space of the glomerular capillaries.

Methods

A total of 72 renal biopsy specimens in 58 patients were studied: 10 cases (20 biopsies) of transplant glomerulopathy (TGP), 25 cases (26 biopsies) of focal segmental glomerulosclerosis (including a case of Marfan syndrome), 10 cases (13 biopsies) of hemolytic-uremic syndrome, 11 cases (11 biopsies) of malignant hypertension, and 2 cases (2 biopsies) of preeclamptic toxemia. The clinical data in these cases are summarized in Table 1.

Renal tissue for electron microscopy was fixed in 2% solution of glutaraldehyde (J. T. Baker Chemical Co.) in 0.2 M phosphate buffer (pH, 7.4) followed by 1% buffered osmium tetroxide, dehy-

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Table 1. Clinical data and microfibril deposits[a]

Disease[b]	Sex (*M:F*)	Age, *yr (average & range)*	Duration[c] *onset to biopsy (average & range)*	Incidence of proteinuria (nephrotic syn)	Incidence of hematuria	Incidence of hypertension & average BP (in affected pts)	Incidence of renal insuf. & creatinine level	Incidence of microfibrils & amount *(average & range)*
TGP	7:3	29 (17–58)	22 mo (3–96)	9/10 (NS, 1)	0/10	8/10 150/100	10/10 Cr, 4–14	10/10 2+ (1+–3+)
FSG	10:14	26 (9–57)	7 pts, <3 mo 15 pts, >2 yr	24/24 (NS, 17)	3/24 (Micro)	11/24 150/90	7 pts; Cr, <1 13 pts; Cr, 1–2 4 pts; Cr, 2.1–5.5	22/24 1+ (0–3+)
Marfan syn	1 F	9	5 mo	NS	None	Normal	Cr, 0.6	4+
Preeclamp. toxemia	2 F	19 39	3 wk 4 wk	3+ 3+	None	150/90 160/100	Cr, 0.9 Cr, 1.1	3+ 1+
HUS	5:5	6 (0.5–22)	8 wk 1 wk–8 mo	10/10 (1–4+)	9/10 (6 Micro) (3 Gross)	7/10 (severe in 4) (190/130, 1 adult) (150/90, 150/100, 150/110; 3 child. <7 yr.o.)	8/10 (at bx) Cr, 2.5–6.8	4/10 2 pts, 3+ 2 pts, ±
Malig. HT	6:5	46 (32–59)	6 pts, 2–8 mo 2 pts, 2–3 yr 3 pts, 8–18 yr	11/11 (tr.–3+)	8/11 (Micro)	11/11 225/125 (av.) (190/100–280/180)	Cr, 11.5 (av.) (3.5–25.0)	2/11 2 pts, ±

[a] Abbreviations are: TGP, transplant glomerulopathy; FSG, focal segmental glomerulosclerosis; Preeclamp, preeclamptic; HUS, hemolytic-uremic syndrome; Malig. HT, malignant hypertension; av, average; bx, biopsy; Cr, creatinine; F, female; M, male, MF, microfibrils; Micro, microscopic; NS, nephrotic syndrome; Pts = Patients; syn = syndrome; tr = trace; ± to 4+ = degree of change.

[b] The incidence and the amount of microfibrils were the highest in TGP, in preeclamptic toxemia, and in Marfan syndrome with FSG. The incidence was high in FSG but the amount lower than it was in TGP. No correlation was noted in any group between the amount of MF and patients' *sex, age, degree of proteinuria,* presence or absence of *NS* or of *hematuria,* or the degree of *renal insufficiency.* In the group of FSG there was a correlation between the amount of MF and the presence of *hypertension*, but since hypertension in turn was related to the duration of illness, the significance of this datum is not clear.

[c] A degree of correlation between the duration and the amount of microfibrils was seen in the group of FSG: 5 out of 7 patients with recent onset (4 wk or less) had 0 or ± deposits of microfibrils; but 12 out of 15 patients with duration of 2 yr and over had 1+ to 3+ MF deposits. In HUS, all patients showing MF ($N = 4$) had the disease for at least 6 wk; moreover 1 patient with 4 biopsies showed progressive increase over a period of 25 mo. In TPG, increase of MF was observed in 5 patients with multiple biopsies over the span from 3 to 33 mo but this was not consistent.

drated in graded ethanols, and embedded in Epon 812. Thin sections (less than 30 nm in thickness) were cut with a diamond knife on a LKB microtome and stained with uranyl acetate and lead citrate, with the procedure of Venable and Coggeshall [3]. Three to five glomeruli were examined in each biopsy specimen in a Philips 300 electron microscope. For comparison, small pieces from biopsy samples of human skin were processed and examined in a similar manner.

Measurement of the size of the microfibrils (SMF) was made on micrographs enlarged 2.5 times from the original negative films taken at magnification of ×42,000, and assisted by a 7X magnifier with a built-in millimeter scale.

Results

An ultrastructural comparison was performed on the microfibrils of the connective tissue of the skin, arterial intima of small renal arteries, and the glomeruli and interstitium of the kidneys in various diseases. In all locations, the microfibrils formed a haphazard interwoven pattern, usually in close association with collagen fibrils and elastic fibers (Figs. 1 and 2, A to C). The microfibrils were quite long, but could not be measured accurately because of their curvature and the comparative thinness of the sections. In all the examined locations, microfibrils were morphologically similar. In longitudinal sections, the central axis was lucent and the periphery dense, so that the fibril often appeared as two, parallel, occasionally beaded lines (Fig. 2, A to C). Sometimes there was a suggestion of branching, but based on analysis of stereo pairs, this proved to be due to crossing and overlapping of fibrils. On cross-section, microfibrils were round, with a lucid core of about 6 to 7 nm in diameter surrounded by a denser periphery 2.5 to 3.0 nm thick. At higher magnifi-

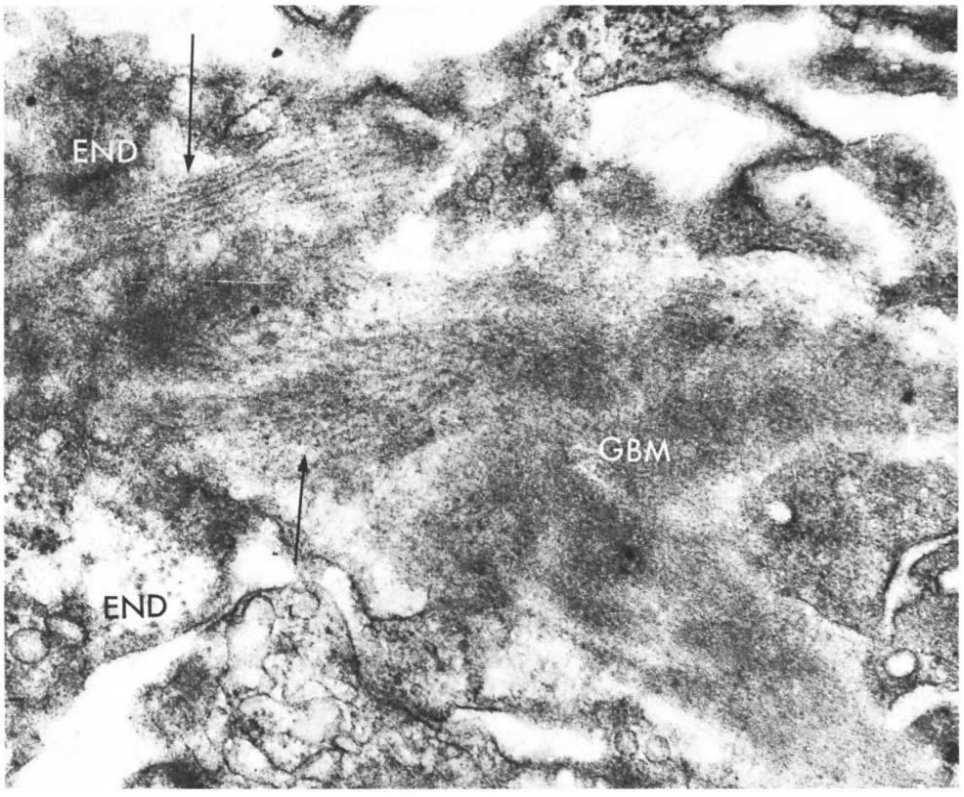

Fig. 1. *Tangential section of an approximately normal glomerular basement membrane (GBM) (case of mild nephrosclerosis) showing bundles of fibrils* (arrows) *close to the endothelium (END).* The fibrils measure approximately 10 nm in diameter and show beaded periodicity. (×42,000)

cations, the tubular wall showed eight to ten fine, dark, regularly spaced dots. The dots measured roughly 2 to 3 nm in diameter and could also be discerned in longitudinal sections (Figs. 2A, 4B, and 6). They are probably an indication of an infrastructure but their nature is presently unknown. The diameter of the microfibrils, when measured at a constant magnification of ×735,000, fell in a quite narrow range of 11 to 13 nm, with an average of 12 nm. In the arterial intima, glomerular basement membrane (GBM), and the mesangium, the microfibrils were often associated with amorphous material morphologically indistinguishable from basement membrane (Fig. 2, A and B), and also with "thin filaments" 3 to 5 nm thick.

Subendothelial changes of the glomerular basement membrane. Widening of the subendothelial aspect of the glomerular basement membrane (lamina rara interna) in various glomerular diseases was sometimes uniform but more often irregular, varying from glomerulus to glomerulus and from one capillary loop to the next, but generally involving most of the glomeruli. Within individual capillaries, there was a tendency to form protrusions and scalloped edges directed towards the endothlium (Fig. 5, A and B). The widened zone was often nonhomogeneous, varying from electron-lucent to moderately dense. On light microscopy, it tended to stain weakly with PAS and could also be identified as a pale zone in 1-μ plastic section stained with toluidine blue. Closer inspection of electron micrographs revealed several types of fine structures: very thin filaments, about 3 to 5 nm in diameter, mostly present in the lucent areas; basement membrane-like amorphous material of moderate density; and microfibrils (SMF) (Fig. 4). In addition, an apparently newly formed thin basement membrane sometimes paralleled the endothelium (Figs. 3 to 5). The concentration of these structures generally corresponded to the degree of subendothelial widening.

Microfibrils in renal disease. The glomerular microfibrils in the peripheral capillary walls were demonstrated in all ten cases of chronic renal allograft rejection showing transplant glomerulopathy. Transplant glomerulopathy (TGP) is defined as a

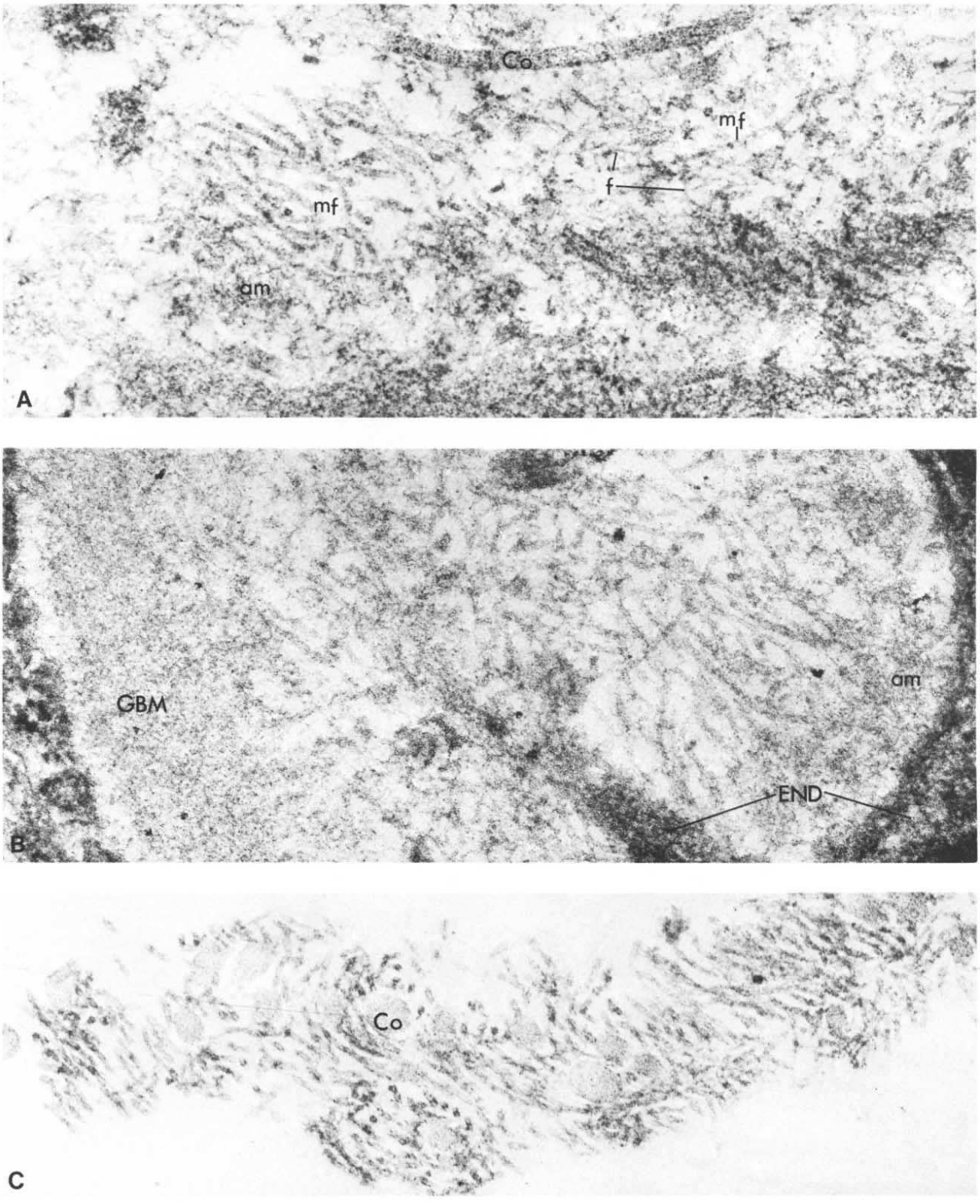

Fig. 2. *Microfibrils in various locations showing* **A** *subendothelium of a cutaneous vessel in a case of vasculitis,* **B** *widened subendothelial zone of glomerulus in a case of Marfan syndrome,* **C** *dermis in a case of scleredema of Buschke*. Note also the presence of fine filaments (f) and amorphous material (am). Co = collagen fibril, GBM = glomerular basement membrane, END = endothelial cell of glomerulus. (×105,000)

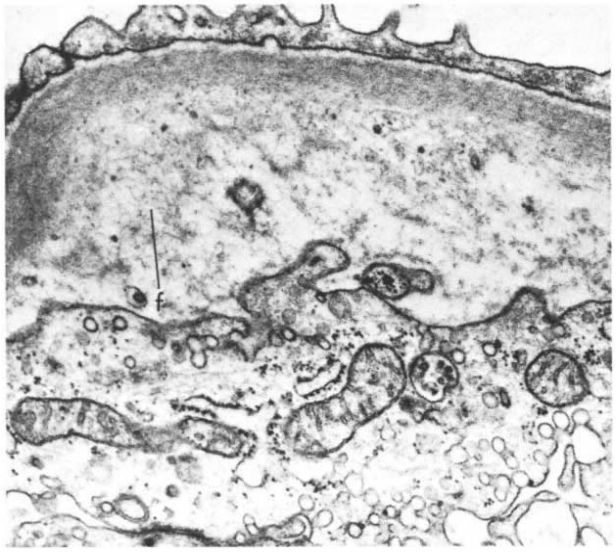

Fig. 3. *Part of a glomerular capillary wall in a case of focal sclerosis.* Within the widened subendothelial zone, there are many interconnecting fine filaments (f), but very few microfibrils. (×20,000)

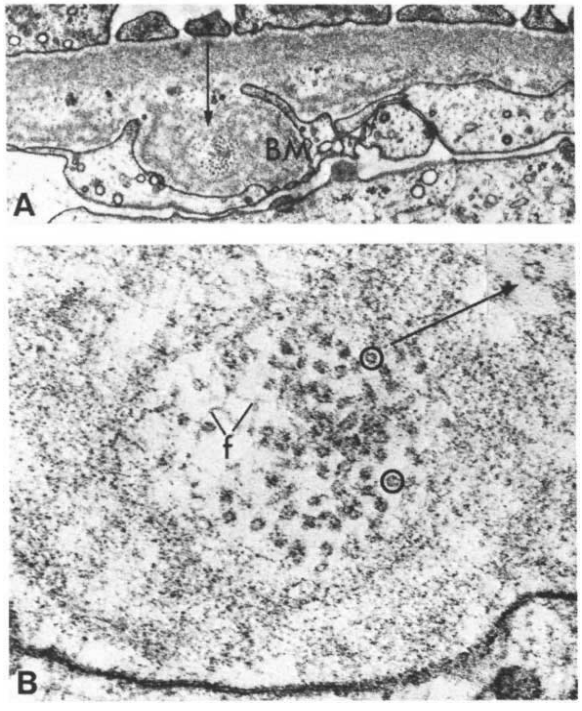

Fig. 4. *Glomerular capillary wall in a case of renal allograft rejection with sclerotic lesions in the glomeruli and nephrotic syndrome:* **A.** *Nodular subendothelial thickening of the basement membrane, containing basement membrane-like material (BM) and microfibrils in the center* (arrow) *(×6,800).* **B.** *Higher magnification of the nodular area showing a bundle of microfibrils in crosssection together with some fine filaments.* Dark dots along the rim of the microfibril are visible in the circled crosssections (×37,000) *Inset* (right upper corner): *higher magnification of a single microfibril in crosssection* (arrow) *(×105,000).*

glomerular abnormality characterized by widening of the subendothelial space of the capillary wall, often with detachment of the endothelium and wrinkling of the basement membrane. The widened subendothelial space contains, as a rule, various extracellular elements of connective tissue, such as microfibrils and thin filaments, apparently newly formed subendothelial basement membrane, in some instances, mesangial cells and matrix, and occasionally, fibrin strands (Hsu et al, in preparation). A degree of mesangial thickening and areas of segmental capillary collapse, and sclerosis are frequently present. Superimposed upon these changes, there may be diffuse or focal mesangial cellularity, subendothelial or subepithelial electron-dense deposits of immunoglobulins, capsular adhesions and crescents, and other changes similar to those seen in glomerulonephritis. Transplant glomerulopathy is usually associated with vascular damage of the type seen in chronic allograft rejection, and is probably itself the result of such rejection. Of the 15 cases of chronic allograft rejection studied by us, ten showed "pure" transplant glomerulopathy, one had, in addition, hemolytic-uremic syndrome with severe mesangiolysis.[1] The remaining four bore some resemblance to glomerulonephritis (recurrent or denovo). Only the cases of pure transplant glomerulopathy are included in this analysis. Microfibrils were usually located in the lucent subendothelial zone of the GBM, or in the lucent zone between the lamina densa of the GBM and the newly formed thin basement membrane, or in the irregular protrusions (Fig. 4). Microfibrils were fairly numerous in all cases and especially prominent in cases associated with segmental areas of sclerosis.

Microfibrils were seen in 23 out of 25 cases of focal segmental glomerulosclerosis associated with proteinuria and nephrotic syndrome, including a case of Marfan syndrome. In six of the cases, only an occasional microfibril could be discerned; in nine, they were more obvious; in six, they were fairly numerous, and in the case of Marfan syndrome, they were abundant (Fig. 2B). In general, the amount of microfibrils correlated with the severity and extent of the subendothelial widening (Figs. 5, A to D). Microfibrils frequently extended into (or

[1]Mesangiolysis is defined as degeneration and necrosis of mesangial cells and dissolution of mesangial matrix [8]. It is typically seen in envenomation by certain poisonous snakes (Habu snake of Okinawa), but is also seen on occasion in hemolytic-uremic syndrome, malignant hypertension, transplant rejection, and other glomerular diseases.

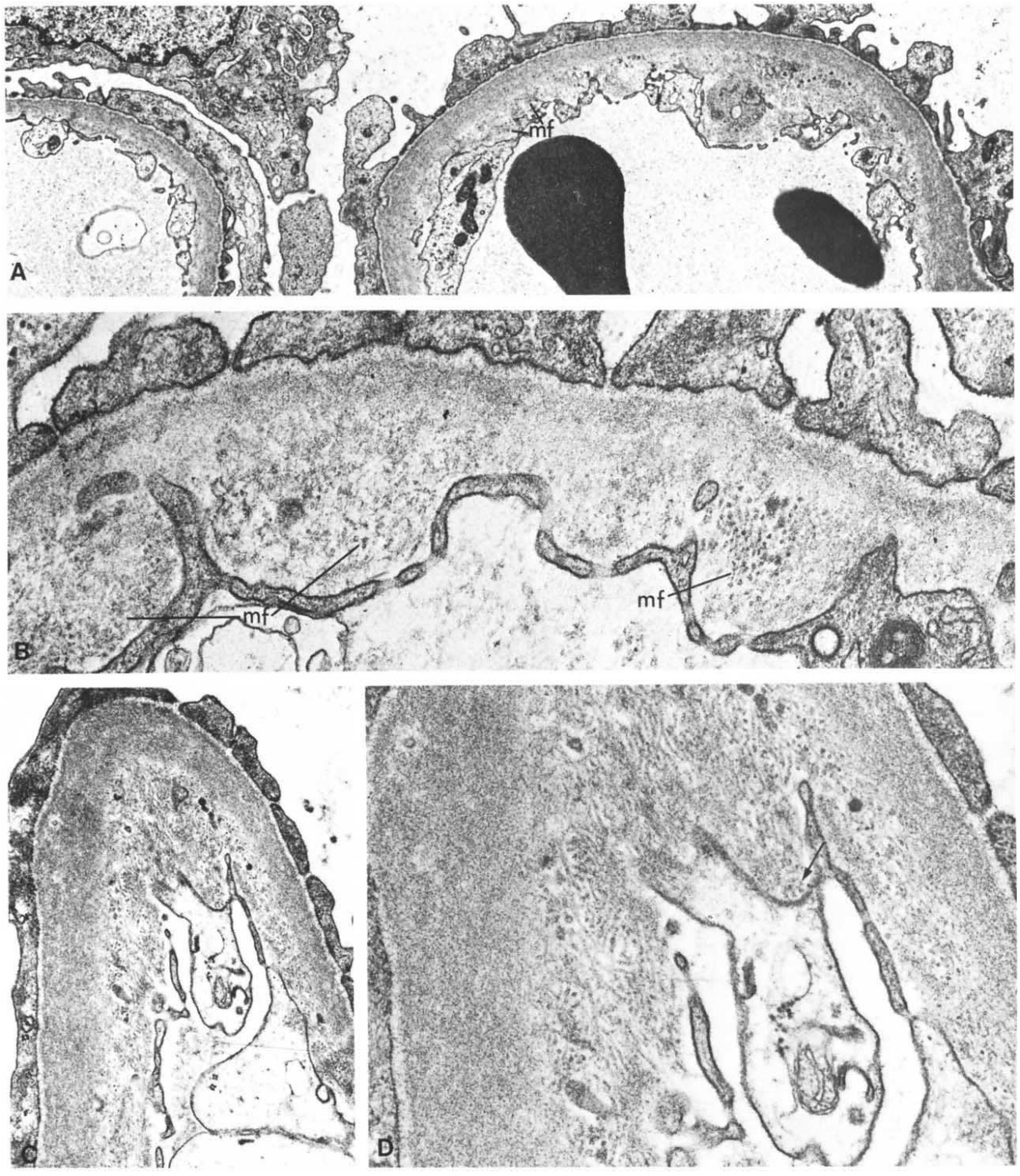

Fig. 5. A *Case of benign recurrent hematuria*. A few microfibrils (mf) in crosssection are seen in the widened subendothelial zone (×9,250). **B** *Case of focal sclerosis*. This shows rather numerous crosssections of microfibrils (mf) in the subendothelial space (×50,000). **C and D** *Case of focal sclerosis*. Numerous microfibrils are in the subendothelial space. Note close approximation of microfibrils to the endothelial cells (*arrow*) (C, ×20,000; D, ×50,000).

from) the adjacent mesangium. Focal podocyte degeneration and detachment [4, 5] was seen in 11 cases of focal segmental sclerosis. In eight of these, there was deposition of new basement membrane-like material between the podocyte and GBM. Microfibrils in this location were seen on very rare occasions, and then only few in number.

In the two cases of preeclamptic nephropathy [6], the amount of microfibrils correlated again with the severity of the subendothelial widening. In one

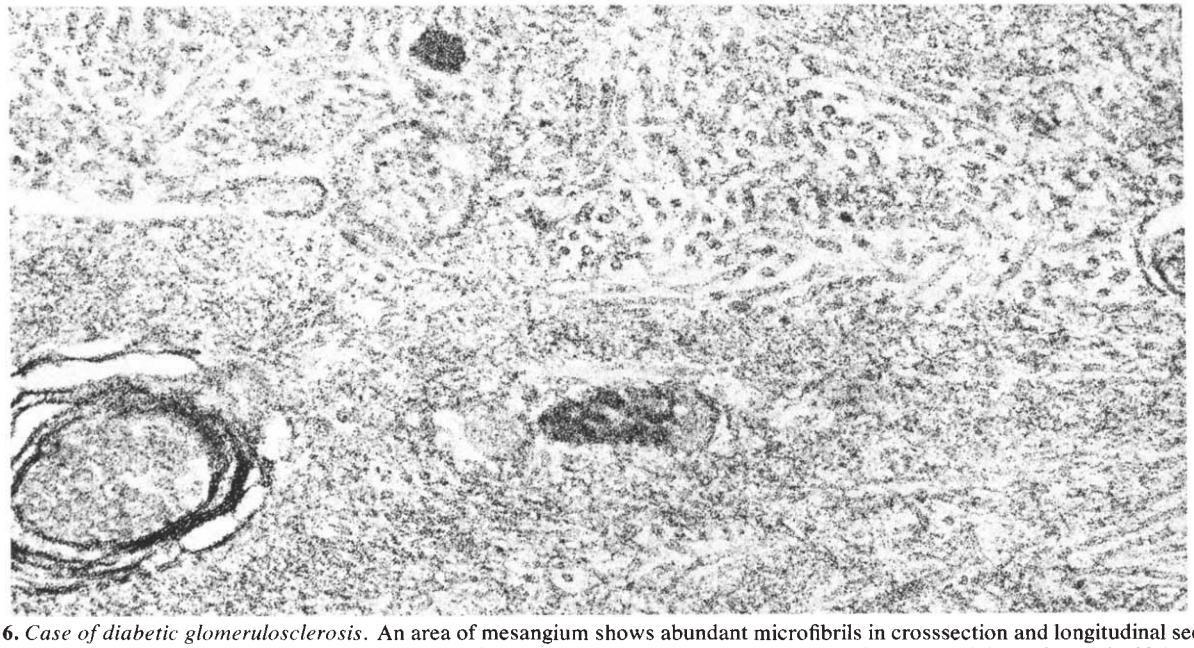

Fig. 6. *Case of diabetic glomerulosclerosis.* An area of mesangium shows abundant microfibrils in crosssection and longitudinal section. In the lower half of the picture, the microfibrils are mixed with amorphous material representing mesangial matrix (×105,000).

case, numerous microfibrils in the GBM gradually blended with both, the newly formed amorphous basement membrane-like material beneath the endothelium and with the mesangial matrix.

Of the ten cases of hemolytic-uremic syndrome, microfibrils were found in four; in two, they were numerous and were accompanied by irregular but generally considerable widening of the lamina rara interna; the other two cases showed only a few microfibrils. The latter were seen also in the edematous mesangial matrix, usually only in small numbers. In malignant hypertension, microfibrils were seldom found, being present in minimal amounts in only 2 out of 11 cases. Microfibrils were prominent in the mesangium in mesangiocapillary glomerulonephritis and especially in diabetic glomerulosclerosis (Fig. 6). Study of the mesangial microfibrils is being continued and will be reported at a later date.

Discussion

Present observations again confirm the close association of microfibrils with basement membranes and with connective tissue fibers as reported by Low [7] and by Haust [1]. From our comparative and stereo electron microscopic study, we conclude that microfibrils of the mesangium [8] and of the GBM are morphologically identical with those of the connective tissues [1, 2, 7, 9, 10, 11]. On chemical analysis, the microfibrils consist of a glycoprotein, which differs considerably in its composition from collagen and from elastin [11, 12]. It also differs from the collagenous component of the basement membrane, but is similar to its noncollagenous glycoprotein, particularly in its high content of dicarboxylic and aromatic aminoacids, and cystine [11, 12].

Microfibrils occur in the connective tissue in locations subject to stress, particularly at the interface with lining epithelial or endothelial cells: in the walls of capillaries and larger blood vessels; in tendons, ligaments, epineurium, perineurium, skin, and periodontal membrane [13]. They are often associated with elastin, contributing to the formation of elastic fibers, but also occur independently of the latter. At the dermoepidermal junction, microfibrils tend to aggregate into bundles known as oxytalan fibers [14], which are in direct contact with the basal layer of the epidermis. Microfibrils are also frequently associated with basement membranes. They are numerous in and around the alveolar basement membrane of the lung [7] and the trophoblastic basement membranes of the placenta [15], but are rather scanty in the normal glomerular basement membranes. They were first described in the glomerulus by Farquhar, Wissig, and Palade [16] who found them in the subendothelial spaces of the capillary walls. Farquhar also demonstrated their presence in the mesangium, their tubular nature [17, 18], and their occurrence in diabetes mellitus [19]. They were noted in the glomeruli in renal allograft rejection by Olsen, Bohman, and Petersen [20]. The fibrillar composition of the mesangial matrix was also discussed by Suzuki et al [8]. Both the capillary

wall and the mesangium of the glomerulus represent areas of stress.

Based on examination of sequential biopsy specimens, it appears that microfibrils in the areas of lucent subendothelial thickening are initially in direct contact with the endothelium, but later become surrounded by newly formed basement membrane-like material. The amount of the GBM microfibrils generally correlates with the severity of the subendothelial thickening, but also with the type of the disease. They are frequent in cases of transplant glomerulopathy, especially in those with focal sclerotic lesions in the glomeruli, in preeclamptic nephropathy, and in focal segmental glomerulosclerosis but are less common in hemolytic-uremic syndrome and in malignant hypertension. It is uncertain whether these differences are due to the nature of the disease process or to its duration. Their abundance in a case of Marfan's syndrome may, in some way, be related to the basic defect of the connective tissue. The gradual incorporation of microfibrils into the new basement membrane beneath the endothelium and into the mesangial matrix also indicates their possible contributory role to the development and function of these glomerular structures. This point is partially supported by Kewley, Steven, and Williams [21] who in an immunofluorescent study demonstrated the presence of microfibrillar protein in the human GBM. Recent studies suggest that normal basement membrane consists of a network of filaments approximately 1.6 nm in diameter with interstices of approximately 3 nm in diameter [22]. It is not clear at the moment how the microfibrils may fit into this arrangement.

Acknowledgments

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Reprint requests to Dr. Jacob Churg, Division of Renal Pathology, Department of Pathology, Mount Sinai School of Medicine, New York, New York 10029, USA

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