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Significance of fibrils in the formation of the Kimmelstiel-Wilson nodule

Tadashi Yasuda[1], Hirokazu Imai[1], Yasushi Nakamoto[1], Akira B. Miura[1], and Shiegki Inomata[2]

[1] Third Department of Internal Medicine,
[2] First Department of Internal Medicine, Akita University School of Medicine, 1-1-1 Hondo, Akita 010, Japan

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Summary. The pathogenesis of the nodular lesion in diabetic glomerulosclerosis is described in association with fibrils. Thirteen diabetic patients with glomerular nodular lesions and 9 diabetics without the nodules were examined by electron microscopy using periodic acid-thiocarbohydrazide-silver proteinate staining. In cases of nodular glomerulosclerosis, abundant fibrillar structures mixed with electron-dense material were detected within the nodule and the mesangial matrix. They were also occasionally observed along the subendothelial space of the glomerular capillary walls. On the cross-section, these fibrils, including the lucent periphery, were 34 nm wide. Immunohistologically, collagen V and collagen VI were detected in nodular lesions. In contrast, in cases of the diffuse type of glomerulosclerosis, the widened mesangium was composed of dense material, which resembled the original mesangial matrix. The above fibrils were not detected in the mesangium. These findings suggest that the accumulation of the peculiar fibrils in the glomerular mesangium is a major pathogenic factor in the formation of Kimmelstiel-Wilson nodules.

Key words: Kimmelstiel-Wison nodule – Fibrils – Periodic acid-thiocarbohydrazide-silver proteinate stain – Collagen V – Collagen VI

Introduction

The nodular lesion, which was first termed intercapillary glomerulosclerosis by Kimmelstiel and Wilson (1936), has been regarded as the most conspicuous and ominous lesion in diabetic nephropathy. However, the pathological significance of the nodule is still a matter of investigation.

In renal amyloidosis (Dikman et al. 1977), light chain nephropathy (Gallo et al. 1980), lobular glomerulonephritis (Alpers and Biava 1989) nodules form which are sometimes reminiscent of Kimmelstiel-Wilson nodules. In those glomeruli, fibrillar structures have been identified and several studies have reported on diabetic patients having fibrils in their glomeruli (Sohar et al. 1970; Hsu and Churg 1979). Therefore, the possibility that the diabetic nodule is caused by the accumulation of fibrils must be investigated. However, it is usually difficult to distinguish fibrils from the mesangial matrix by conventional staining of lead citrate and uranium acetate. In order to enhance the density of the mesangial matrix, we applied periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) staining to renal tissue. As one of its chief advantages the stain provides and effective visualization of both the mesangial matrix and fibrillar structures. The purpose of the present paper is to evaluate the fibrils in relation to nodule formation.

Patients and methods

Thirteen diabetics with glomerular nodular lesions and 9 patients without nodules were examined. The study population consisted of 7 women and 15 men, ranging in age from 34 to 68 years (mean 49). All patients met clinical diagnostic criteria for non-insulin-dependent diabetes mellitus. Patients with coincidental renal diseases were excluded from this study. Renal biopsy specimens were taken from 1982 to 1989 at Akita University Hospital and other affiliated hospitals. Biopsies were performed only after informed consent had been obtained from patients. The clinical characteristics of the patients are summarized in Table 1. In 2 patients, nephrotic range proteinuria was documented. Five patients had functional renal impairment at the time of the renal biopsy, with serum creatinine greater than 1.4 mg/dl.

The kidney biopsy specimens were immediately divided into three portions for light, immunofluorescent, and electron microscopy. The portion for light microscopy was fixed in formalin, embedded in paraffin and sectioned at 1–2 μm. The sections were stained with haematoxylin- and eosin, periodic acid-Schiff, Heidenhain's azocarmine aniline blue, and Jones' periodic acid silver methenamine. On light microscopy, the diffuse and nodular lesions were graded on a scale of 0 to IV according to the criteria of Gellman et al. (1959), and the extent of arteriolar hyalinosis was classified according to the criteria described by Nakamoto et al. (1980).

Correspondence to: T. Yasuda

Table 1. Characteristics of patients at renal biopsy

Case no.	Age (years)/ sex	Disease duration (years)	Urinary protein	BUN/Cr (mg/dl)	C_{Cr} (ml/min)	HbA_{1c} (%)
1.	45/M	Unclear	+++	19/0.9	69	7.8
2.	68/M	23	+++	22/2.8	19	6.1
3.	60/M	5	+++	17/1.1	49	5.5
4.	34/M	13	+++	21/1.4	32	11.8
5.	53/F	12	+++	25/1.5	26	12.1
6.	60/F	4	−	21/0.6	63	7.6
7.	55/M	16	+++	18/0.9	79	11.2
8.	50/M	15	−	20/1.0	99	5.7
9.	53/M	21	+	10/0.8	93	10.4
10.	45/M	14	+++	20/1.6	40	8.2
11.	54/M	16	+	17/0.7	98	5.8
12.	49/M	4	−	17/1.0	80	5.4
13.	64/M	8	++	17/0.8	117	8.2
14.	61/M	5	−	13/0.8	ND	5.3
15.	41/F	11	++	21/1.1	47	13.7
16.	48/M	18	−	16/1.0	79	4.7
17.	56/M	15	−	21/1.2	70	6.9
18.	49/F	10	−	22/0.4	119	6.6
19.	62/F	13	+	14/0.7	113	5.9
20.	64/M	10	+	21/1.4	52	6.7
21.	52/F	6	+	16/0.6	63	12.6
22.	46/F	1	−	12/0.6	ND	6.6

Urinary protein was graded on a scale of − to +++: +++, more than 3 g/day; ++, 1–3 g/day; +, less than 1 g/day
ND, Not done; C_{Cr}, creatinine clearance; HbA_{1C}, haemagobin Alc

For immunofluorescent microscopy, the tissues were frozen at −70° C, and then cut at 4 μm in a cryostat. They were routinely studied by the direct method of using monospecific and heavy chain specific antisera against IgG, IgA, IgM, kappa and lambda light chains, C3, C1q, and fibrinogen. In some cases, formalin-fixed and paraffin-embedded sections were deparaffinized and treated with 0.1% trypsin, then stained, by an indirect method using primary monoclonal antisera for collagen I, III, IV, VI (Fuji, Japan), polyclonal antisera for laminin (Chemicon, USA), collagen V (LSL, Japan). Subsequently, fluorescein isothiocyanate-labelled anti-mouse antibodies (Tago, USA) and anti-rabbit antibodies (E.Y. Labs, USA) were used as secondary antibodies.

The portion for electron microscopy was fixed in 2.5% glutaraldehyde buffered with 0.2 M cacodylate (pH 7.4), post-fixed with 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. PA-TCH-SP staining was carried out according to Thiery's method (1967), with minor modifications. Thin sections were mounted on gold grids covered with collodion membranes, oxidized in 1% periodate for 30 min, rinsed in distilled water, and exposed for 1 h to 1% thiocarbohydrazide in 5% acetic acid solution. The sections were then rinsed in 5% acetic acid and distilled water. After rinsing, they were treated for 2 h at 50° C with 2% silver proteinate buffered with borate (pH 9.2) as a control, periodate oxidation was omitted from the procedure. Without the periodic acid treatment, staining with thiocarbohydrazide and silver proteinate showed no enhancement.

Results

The histological grade as examined by light microscopy is summarized in Table 2. Thirteen patients had nodular lesions in the glomeruli (patients 1–13). The remaining 9 patients showed only diffuse mesangial expansion (patients 14–22). Capillary microaneurysm was detected in 5 patients.

Fluorescent microscopy was performed on 16 biopsies. Mild segmental deposits of IgM were demonstrated in 1 patient. In the remaining patients, glomerular capillary walls showed a weak deposition of IgG in a linear pattern. There were no findings characteristic of light chain nephropathy.

The specimens from the paraffin-embedded block showed depositions of various collagens in the nodules (Fig. 1). Collagen IV, and laminin were found rather in the peripheral portion of the nodules. In the central area of the nodule, collagen V and VI were found in moderate intensity. Collagen I and III were only faintly detected in the glomeruli.

Electron microscopy with PA-TCH-SP staining was carried out in all biopsy specimens. Compared with conventional uranium acetate and lead citrate staining, the PA-TCH-SP stain vividly enhanced the electron density of the glomerular basement membrane and the mesangial matrix.

The specimens for electron microscopy contained nodular lesions in 6 of 13 instances. Electron microscopically, nodular lesions consisted of two different components: the part of the electron-dense mesangial matrix, and the less dense aggregated fibrillar structures (Fig. 2). A higher magnification disclosed that fibrils were surrounded by rarefaction. Measurements of fibril diameters, including the lucent periphery, showed a thickness of 34 nm (Fig. 2, inset). The fibrils infiltrated into the mesangial matrix as well as in the nodular lesion (Fig. 3A, B). On occasions, they accumulated on the

Table 2. Pathological findings in kidney biopsy

Case no.	Histological grade			Electron microscopy	
	DL	NL	AH	Fibrils	Collagen
1.	IV	III	III	++	−
2.	IV	III	III	++	+
3.	IV	III	III	++	−
4.	IV	II	I	++	−
5.	IV	II	III	++	+
6.	II	II	III	+	+
7.	III	II	II	++	+
8.	III	II	II	++	+
9.	III	I	III	++	−
10.	IV	I	III	++	+
11.	II	I	III	+	+
12.	III	I	I	+	+
13.	III	I	II	+	+
14.	III	0	I	−	−
15.	III	0	III	−	−
16.	III	0	III	+	+
17.	II	0	I	−	−
18.	II	0	II	−	−
19.	II	0	I	−	+
20.	II	0	II	−	+
21.	II	0	II	−	−
22.	II	0	I	−	+

DL, Diffuse lesion; NL, nodular lesion; AH, arteriolar hyalinosis; ++, global, abundant; +, segmental, sparse; −, not detected

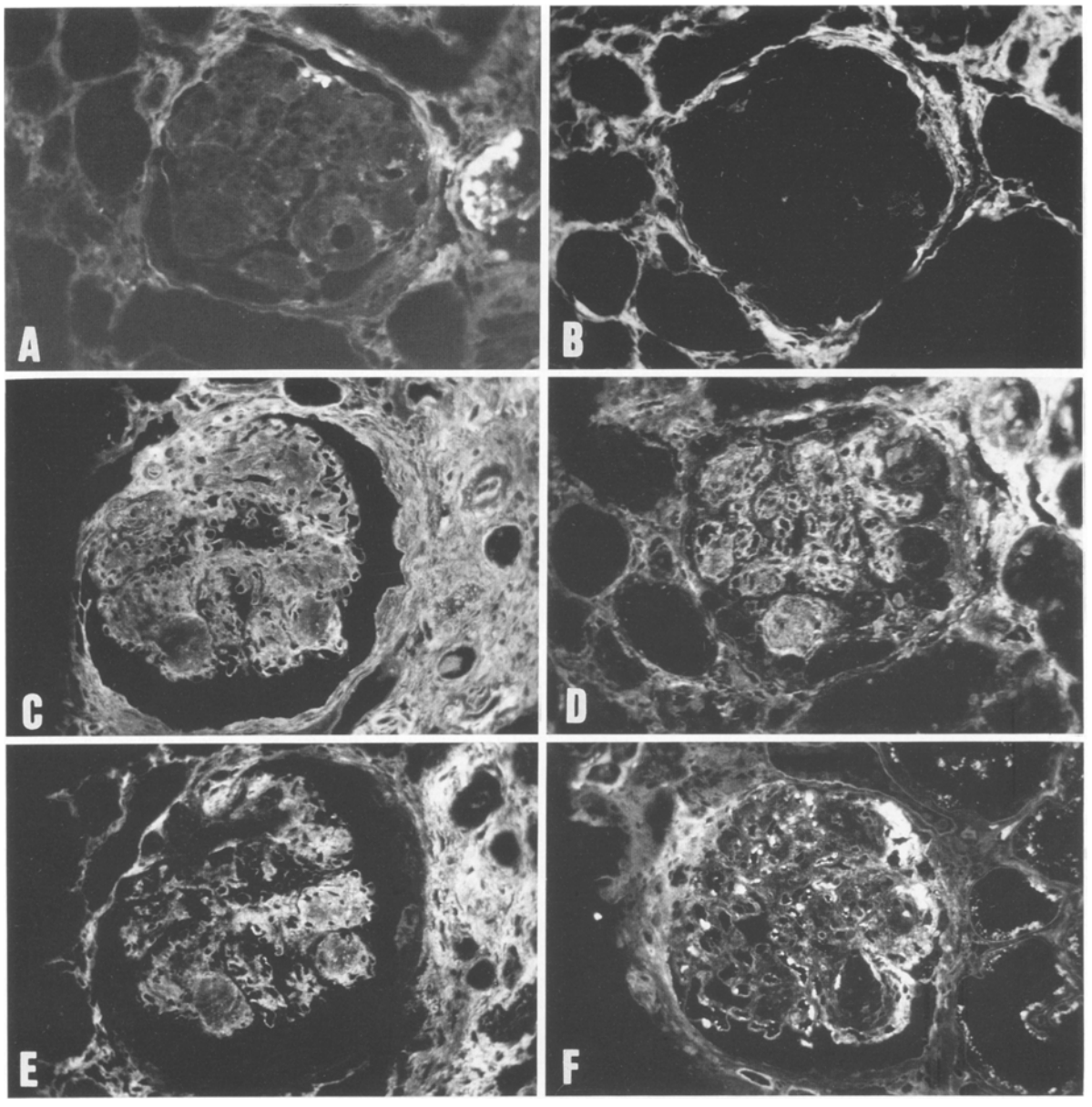

Fig. 1 A–F. Immunohistological staining of nodular lesions. **A** Nodules do not contain collagen I. **B** Collagen III is scarcely found in glomerulus. **C** The periphery of the nodules stain for collagen IV. **D** Collagen V is evenly distributed within the nodules. **E** collagen VI is present in somewhat peripheral areas. **F** Laminin is also found in the periphery of the nodules

subendothelial spaces of glomerular capillary walls (Fig. 4).

In the patients without nodular lesions, diffuse mesangial expansion was mostly due to increased accumulation of electron-dense material that was not distinguished from the original mesangial matrix (Fig. 5). The fibrils were detected in the mesangium of only 1 patient in this group (Table 2).

Discussion

The morphological changes commonly seen in renal biopsies from diabetic patients have been well documented (Bloodworth 1978; Heptinstall 1983). These abnormalities consist of diffuse mesangial widening, a thickening of the basement membrane, arteriolar hyalinosis, and nodular formation.

The pathogenesis of nodular formation was initially explained as the result of either an accumulation of hyaline material (Farquhar et al. 1959) or an increase in substances which closely resemble the normal mesangial matrix (Dachs et al. 1964). Later, Bloodworth (1978) suggested that large nodules more than 40 μm in diameter were formed by the organization of glomerulocapillary microaneurysms. He also suggested that an unknown injury or stimulus could cause disruption of anchor points and that the rupture of these anchors would produce a microaneurysm.

Nakamoto et al. (1980) supported this theory by indicating an intermediate form between the capillary micro-

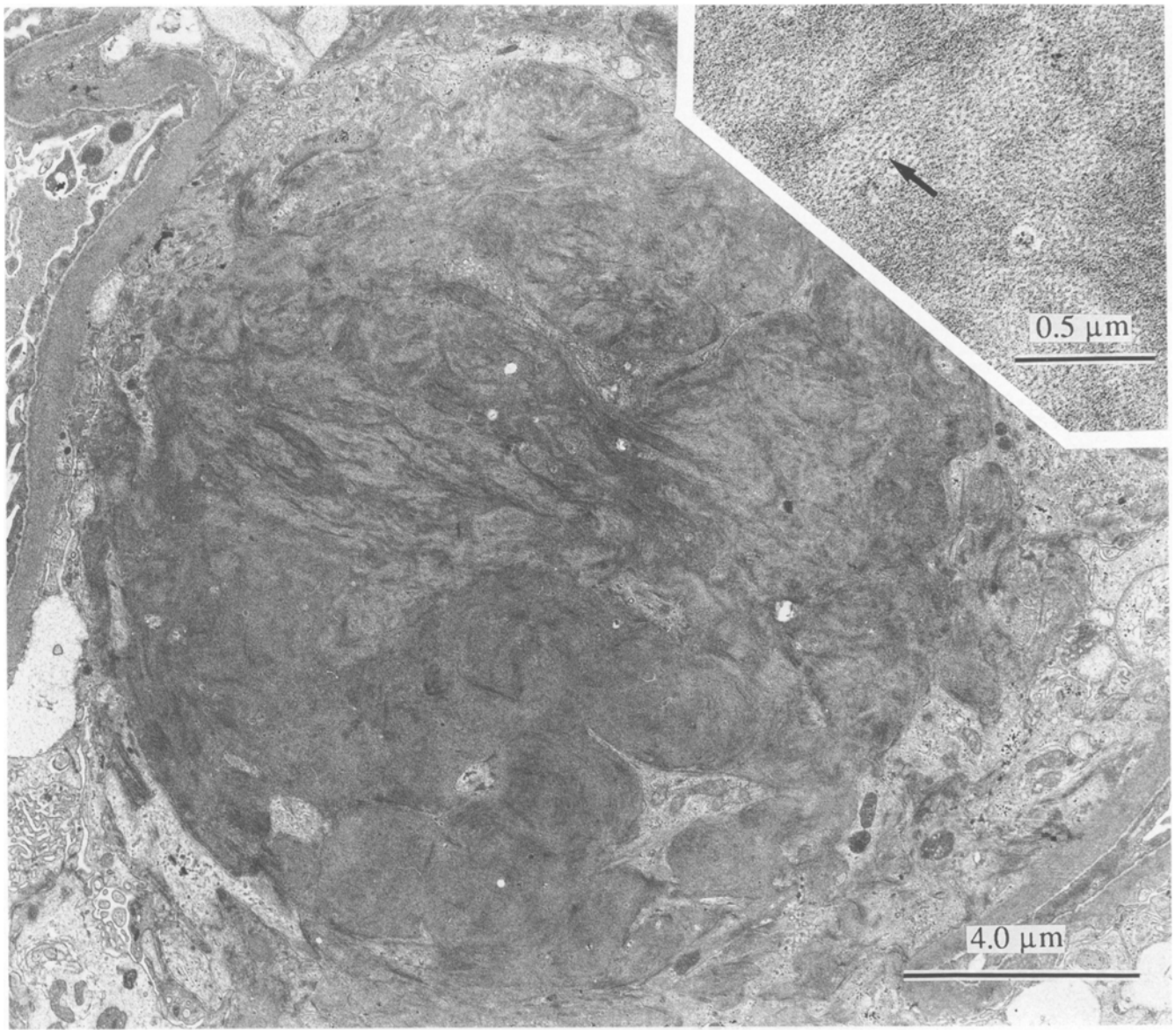

Fig. 2. Electron micrograph of a nodular lesion. Two different parts of the nodule are clearly visible. Lower part of the nodule is, as a whole, electron dense and shows an irregular increase of density. Upper portion exhibits rather lucent fibrillar structure. Case no. 3. Periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) stain, ×8,700. *Inset:* High magnification electron micrograph showing abundant fibrils in a cross-section and a longitudinal section. The diameter of the fibril is approximately 34 nm, and has a core 12 nm wide (*arrow*). Case no. 3. PA-TCH-SP stain, ×42,500

aneurysm and Kimmelstiel-Wilson nodules. They also speculated that the segmentally turbulent intraglomerular circulation might play an important role in the progression of diabetic glomerulosclerosis (Nakamoto et al. 1990). However, Saito et al. (1988) regarded mesangiolysis as an initial lesion occurring in glomeruli in the process of diabetic nodule formation, and they believe that the lamination of the nodule is the result of the compression of the lysed mesangial matrix by the recanalized capillaries.

We observed a large number of peculiar fibrils in diabetic nodules when they stained with PA-TCH-SP. These fibrils were also present within the mesangial matrix in areas other than the nodules. Furthermore, in some cases fibrils were detected on the subendothelial spaces of capillary walls. However, most of the diffuse lesions without nodules did not contain these fibrils. The matrix appeared denser and was likely to be composed of packed thin filaments.

In our study, nodular lesions consisted mainly of two distinct components: the electron-dense matrix and lucent fibrils. The electron-dense matrix using the PA-TCH-SP stain had a close resemblance to the original mesangial matrix. This component corresponds well to collagen IV found in immunohistological study. A recent study has shown that the normal mesangial matrix has a structure of fibrillar networks (Mundel et al. 1988; Kritz et al. 1990a, b; Naramoto et al. 1991). However, we could not visualize the fine structure of the electron-dense matrix because of relatively low resolution. Never-

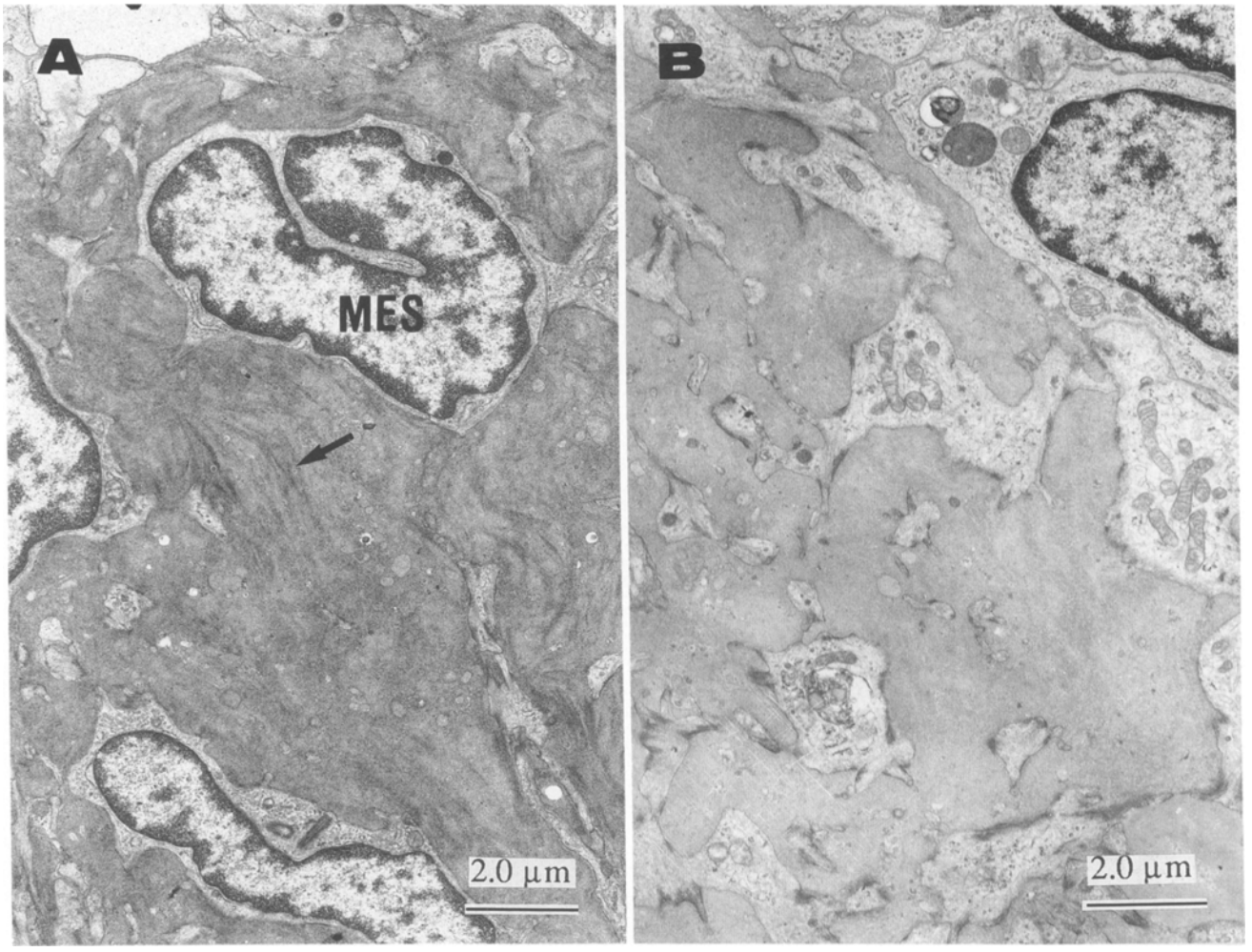

Fig. 3. A Electron micrograph in the mesengial matrix outside of the nodule. The *arrows* denote electron dense strands and show the same density as the original mesangial matrix. Other parts are composed of less electron dense fibrils. *MES*, Mesangial cell. Case no. 1. PA-TCH-SP stain, ×7,700. **B** Case 1. By uranium acetate and lead citrate staining, the mesangial matrix, as a whole, shows an unclear appearance. ×8,800

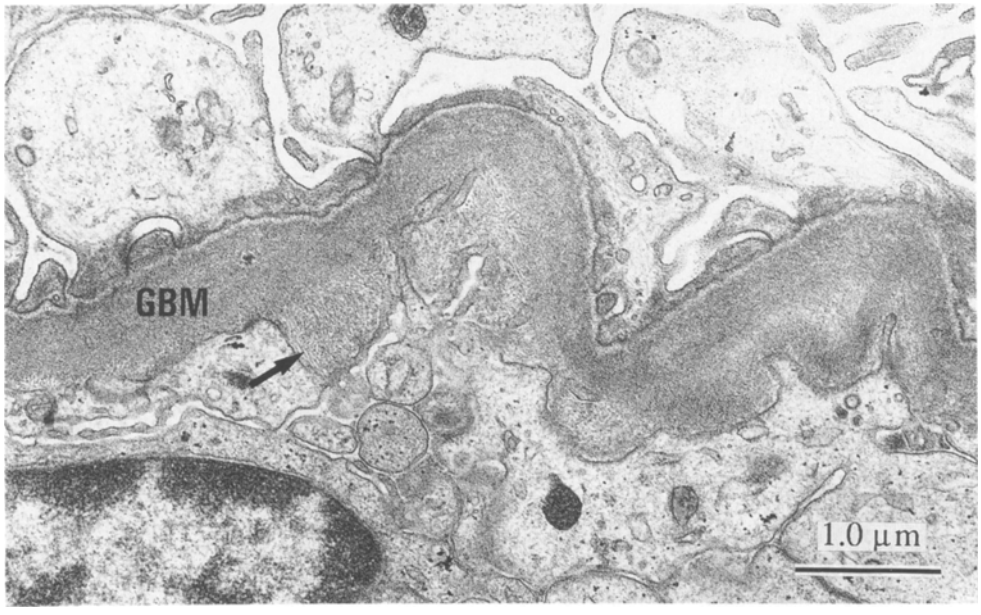

Fig. 4. Electron micrograph of a glomerular capillary wall. The fibrils are also detected on the subendothelial space of the capillary loop. *GBM*, Glomerular basement membrane Case no. 1. PA-TCH-SP stain, ×20,300

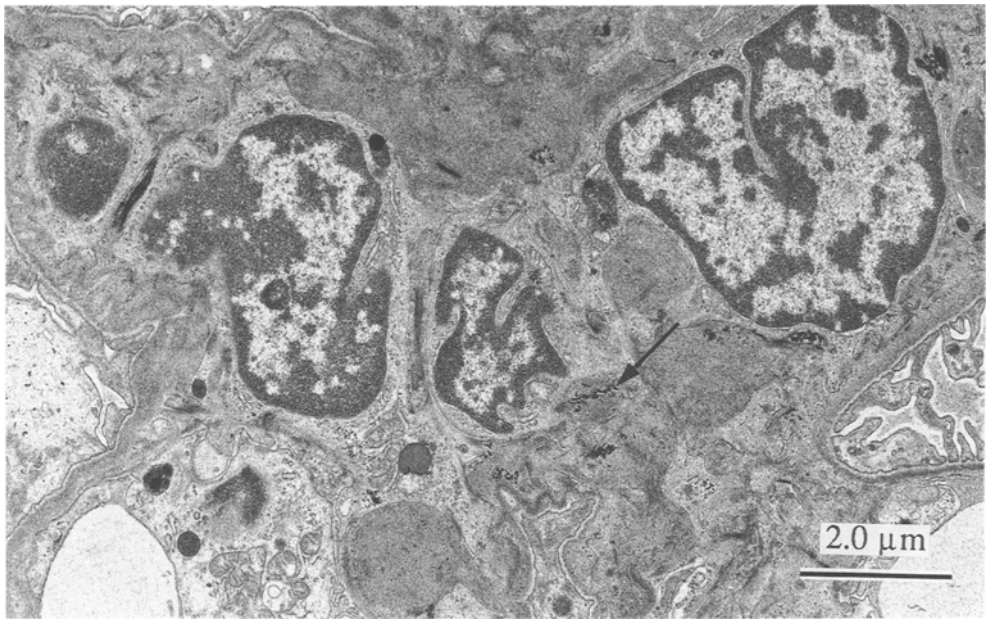

Fig. 5. Diffuse mesangial expansion without a nodule does not contain lucent fibrils. The matrix is likely to be composed of thin packed filaments. The extremely dense strands are interstitial collagen fibres (*arrow*). Case 19. PA-TCH-SP stain, ×10,700

theless thicker fibrils (34 nm wide) exhibited a conspicuous appearance. The fibril had a core 12 nm wide and had a lucent periphery. These were easily distinguished from interstitial collagen because the latter were extremely dense according to the PA-TCH-SP stain (Fig. 4). These peculiar fibrils seem to be superimposed on an electron-dense matrix. A recent immunohistochemical study (Nerlich and Schleicher 1991) showed that in nodular glomerulosclerosis, the main component found in the nodule was collagen V. In our study, collagen VI was similarly found in the nodule, but this was present in peripheral areas.

These components, however, do not correspond well to the thicker fibrils described above, because collagen V and VI could be found in a relatively early stage of diffuse glomerulosclerosis. Therefore we cannot exclude the possibility that further components could be found in the nodules and could well correspond to the fibrils.

We would like to emphasize the importance of the presence of the fibrils in the nodular lesion, since nodule formation is seen in other glomerulopathies. These include renal amyloidosis (Dikman et al. 1977), light chain nephropathy (Gallo et al. 1980), lobular glomerulonephritis (Alpers and Biava 1989), primary glomerular fibrosis (Ikeda et al. 1990; Yasuda et al. 1991), and other cases having amyloid-like fibrillar structures (Rosenmann and Eliakim 1977). It is of a great interest that these renal lesions are commonly rich in fibrils. In diabetic nephropathy, however, fibrillar structures are not usually observed since conventional staining cannot delineate the fibrils consistently. In reviews of the literature, some authors have noticed that nodular lesions often exhibit fibrillar appearances (Dachs 1964; Bloodworth 1978). Sohar et al. (1970) were the first to draw attention to the contribution of fibrillar deposition to the pathogenesis of vascular complications in diabetic patients. Hsu and Churg (1979) also described a certain type of microfibrils present in the mesangium of diabetic glomerulosclerosis. Those authors, however, did not consider the fibrils to be a component of nodular lesions. From the fact that the fibrils appear to be closely related to nodular lesions, it is conceivable that the accumulation of fibrils could be a cause of nodule formation. Of note is the fact that these fibrils were also present along the glomerular capillary walls, suggesting that they are not produced by mesangial cells.

In conclusion, the Kimmelstiel-Wilson nodule is composed of peculiar fibrils which are mixed with an increased original mesangial matrix. Taking other nodule-forming diseases into consideration, it seems likely that the accumulation of these fibrils is a major pathological process in the formation of the Kimmelstiel-Wilson nodule. It is also demonstrated that in nodular glomerulosclerosis, various collagen are overproduced. With respect to microaneurysm and mesangiolysis, we regard them as by-products of this process.

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