

Immunohistochemical Localization of Extracellular Matrix Components in Human Diabetic Glomerular Lesions

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The immunohistochemical localization of the extracellular matrix was examined in 31 cases with different degrees of human diabetic nephropathy using antisera to human collagen types I, III, IV, V, fibronectin, laminin, and basement-membrane-associated heparan sulfate proteoglycan (HSPG). In normal glomeruli, HSPG was predominantly localized in the glomerular basement membrane and in the mesangium, and to minor extent in the basement membranes of tubules and Bowman's capsule. Collagen IV and laminin were distributed in glomerular basement membrane and mesangium in minor amounts. Interstitial collagens usually do not occur within glomeruli except for collagen V which has a light microscopic glomerular distribution similar to collagen IV. In diabetic diffuse glomerulosclerosis, the enlarged mesangial matrix showed an increased staining reaction for collagen IV, V, laminin, and fibronectin whereas the staining pattern of HSPG was markedly reduced. Early, small nodular lesions in diabetic glomeruli were similarly positive for most of the basement membrane components, whereas HSPG remained absent. With an increase in the diameter of the noduli, however, the staining reaction for all basement membrane components diminished, whereas interstitial collagens V and III, but not collagen I, were present in these noduli in substantial amounts. These initial studies provide evidence that the changes in the glomerular matrix in diabetic nephropathy may be divided into distinct and progressing stages of lesions. The reduced amount of HSPG even in slight, early lesions may represent the morphologic correlate to the impaired filter function of the glomerular basement membrane. (Am J Pathol 1991; 139:889–899)

The glomerular basement membrane (GBM) is a unique extracellular matrix that forms an essential part of the glomeruli, playing an important role in glomerular filtration. It consists of a highly crosslinked network of collagen IV, laminin, heparan sulfate proteoglycan (HSPG), and many other minor glycoproteins.^{1–5} Studies carried out by Farquhar and colleagues^{6,7} resulted in a concept of the structural and functional architecture of the GBM. It is composed of a backbone of collagen IV that forms a highly compact meshwork and possibly plays a vital role in the size-selective sieving properties of the ultrafiltration unit. The proteoglycan-containing layer provides a viscous, negatively charged screen in front of the lamina densa. In this location, the HSPG seems to be ideally suited to play a major role in filtration and in cell attachment. Based on its known properties, the HSPG would be expected to retard filtration of macromolecules by both electrostatic repulsion and steric hindrance.

Recognition that the GBM undergoes alterations in diabetes that may impair the function of the renal filtration barrier has stimulated interest in the search for the underlying biochemical changes. Thickening of the GBM is a characteristic feature of diabetic nephropathy.⁸ Biochemical alterations of GBM subsequent to increased nonenzymatic glycation of structural proteins and the formation of advanced glycation end products have been proposed in the genesis of an altered size-selective area.^{9,10} The pathogenetic significance of these biochemical changes is not yet understood. Other observations of changes of GBM biochemistry in diabetes include an increased synthesis of basement membrane components^{10,11} and decreased rates of incorporation of sulfate into glycosaminoglycans.^{12–16} Further studies have demonstrated decreased amounts of HSPG in the diabetic GBM relative to total protein.^{9,17,18} These findings have led to speculation that derangement of base-

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ment membrane component synthesis and (or) turnover may be involved in the pathologic process. In particular, it would be interesting if the anionic sites of GBM are decreased locally in diabetes. Although, there are studies performed with diabetic rats¹⁹ showing disturbed anionic sites in GBM by the use of cationic dyes, knowledge on the distribution of HSPG in GBM of diabetic patients is scarce.

In the present work, we have investigated the immunolocalization of the basement membrane components collagen IV, laminin and HSPG, of fibronectin and the interstitial collagens I, III, and V in 31 diabetic patients with different degrees of diabetic nephropathy.

Materials and Methods

Tissue

Renal tissue specimens were obtained from 31 patients with clinically manifest diabetes mellitus. The study group is characterized in detail in Table 1. In three cases, biopsy samples could be examined, in 28 further cases kidney tissue was removed at autopsy 6 to 12 hours after death. In 12 cases, additional sufficient clinical data, especially serum-creatinine, creatinine-clearance, and urinary protein excretion were reported for evaluation of the renal function. These data were used to find out whether a correlation between glomerular structure and renal function exists. Control tissue comprised 10 kidney specimens obtained at autopsy from young, previously healthy accident victims.

For the histologic analysis, tissues were fixed in 4 to 8% buffered formalin for 24 hours followed by routine processing and embedding in paraffin. From each block, 2-μm serial sections were cut and placed on silanized slides.

Antibodies

Anti-human placental collagen IV and anti-mouse laminin antibodies were purchased from Eurodiagnostics (Leiden, NL), anti-human fibronectin was from Dako (Ham-

burg, FRG). Anti-porcine HSPG was made by immunizing rabbits with purified HSPG from extracts of isolated porcine glomeruli.²⁰ The antiserum was characterized by enzyme immunoassay, immunoprecipitation, and immunohistologic methods specifically recognizing the core protein of HSPG from porcine and human glomerular basement membrane without cross-reaction with other basement membrane proteins like laminin, fibronectin or collagen IV. Previous immunohistochemical studies on tissue sections from several human organs revealed prominent staining of glomerular and other capillary basement membranes.²¹ Furthermore, antibodies against the interstitial collagens I, III and V were prepared according to Timpl et al.²² Briefly, antisera against collagen I, III and V were adsorbed on columns containing immobilized collagen I, III and V, respectively. No cross reactivity was found when antibodies were tested by ELISA.

Immunohistochemical Staining

Formalin-fixed and paraffin-embedded tissue sections were deparaffinized and incubated with 0.4% pepsin as described for the immunohistochemical enhancement of staining of basement membrane components.^{21,23} For the staining of collagen I, enzymatic pretreatment with 0.2% trypsin (Sigma, Deisenhofen, FRG) was used. Previous control experiments with frozen sections had confirmed specificity. The sections were first incubated with the specific primary antiserum. Subsequently, avidin-biotin-complexed specific anti-rabbit antibodies (Vector, Burlingame, USA) were used as secondary antibodies. The avidin-biotin complexes of the secondary antibodies were visualized with aminoethylcarbazole, and sections were evaluated by routine light microscopy.²⁴ For doublestaining procedures, additionally the alkaline phosphatase-anti-alkaline phosphatase (APAAP)-method (Vector, Burlingame, CA) was used.²⁵

Results

Diabetic Nephropathy

In nine patients (two patients with insulin-dependent, seven patients with non-insulin-dependent diabetes mel-

Table 1. Study Population

	n	Age (yr)	Sex f/m	Duration of diabetes (yr)
IDDM	9	39-73 ($\bar{x} = 64$)	5/4	11-38 ($\bar{x} = 19$)
NIDDM	22	49-89 ($\bar{x} = 69$)	10/12	5-28 ($\bar{x} = 14$)
Total	31	39-89 ($\bar{x} = 67$)	15/16	5-38 ($\bar{x} = 16$)

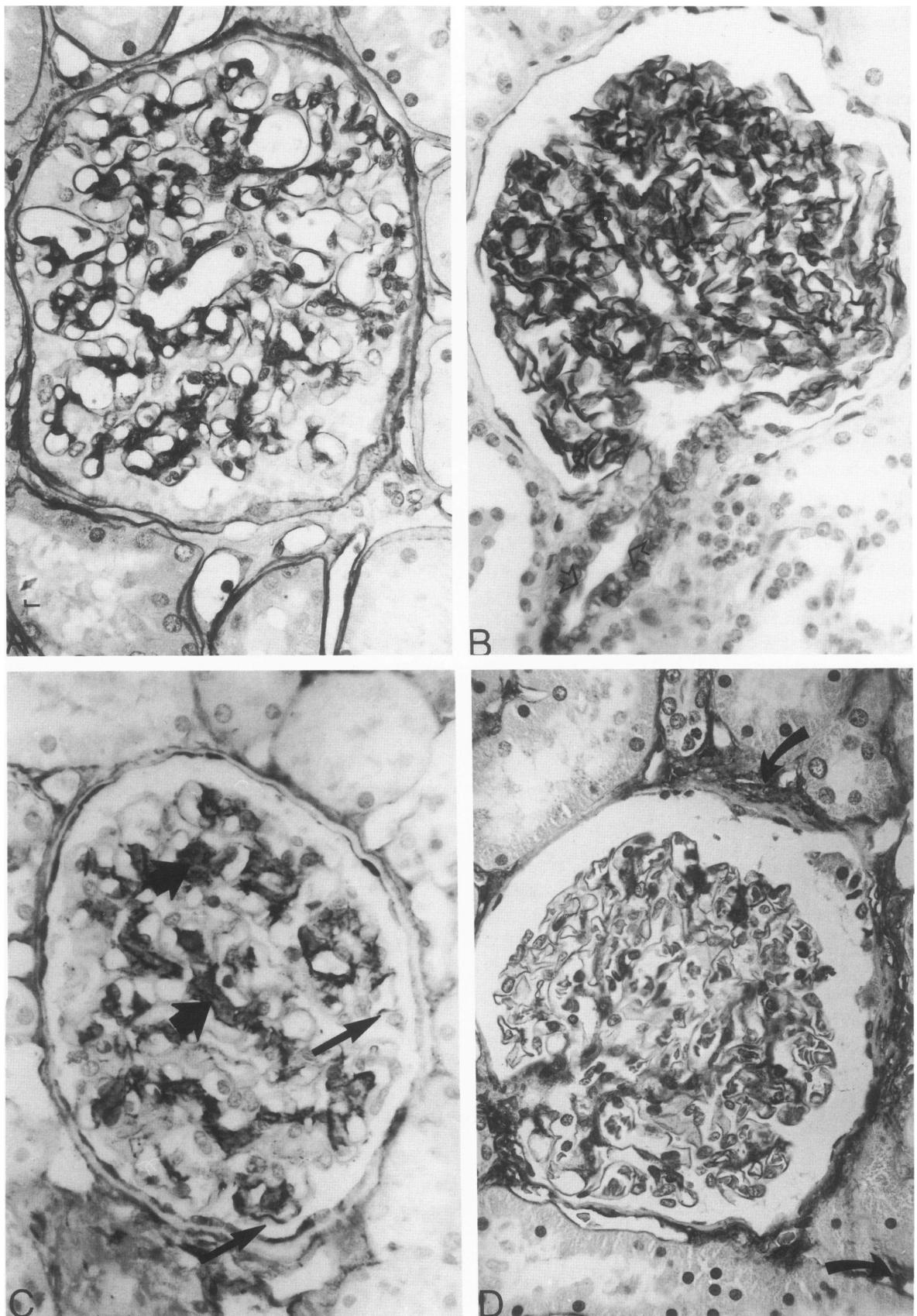


Figure 1. Distribution of BM-components and interstitial collagens in normal human glomeruli. **A:** Collagen IV is present in the glomerular BM and in mesangial matrix. **B:** HSPG shows a strong glomerular staining intensity when compared to arteriolar BM (→). **C:** Fibronectin has a predominantly mesangial distribution (→) with a fragmented pattern in glomerular BM (→). **D:** Collagen V is present along the glomerular BM, in mesangial matrix and the surrounding interstitium (→) (A-D, $\times 400$).

litus) only minor changes with only a slight increase of the mesangial matrix were found. In these patients, the capillary loops were unremarkable. In 11 further patients, diffuse glomerulosclerosis was present characterized by substantial mesangial thickening and rarification of capillary loops. In 11 patients, nodular lesions as typical for diabetic glomerulosclerosis (Kimmelstiel-Wilson) were present.

Immunohistochemical Findings in Normal Glomeruli

The normal glomerular BM showed a distinct, continuous staining for collagen IV and laminin, whereas staining for fibronectin was fragmentary (Figure 1A—D). Collagen V was found along the glomerular BM. The pattern for HSPG is continuous with high intensity of staining (as compared to the staining pattern of endothelial BM in small arterioles). A similar distribution was found in the mesangial matrix, again with intense staining for HSPG (Figure 1B). In addition, the mesangial matrix was rich in fibronectin. Interstitial collagens I and III were absent from mesangium and glomerular BM. The Bowman's capsule contained the various BM-components as well as small amounts of collagen I and III (Figure 3A).

Immunohistochemical Findings in Diffuse Glomerulosclerosis

Diabetic kidneys with slight changes showed only a minor increase in all BM-components tested except for HSPG, which was present in glomerular BM and in thickened mesangial matrix, although with decreased staining intensity. More pronounced diffuse glomerulosclerosis showed a further increase in BM-components with a special increase in collagen IV. Collagen V showed also increased staining pattern. In this instance, however, the

enlarged matrix lacked HSPG completely (Table 2). Minor amounts of HSPG could be found only in the GBM of the capillary loops in the periphery of glomeruli (Figure 2). Extensive diffuse glomerulosclerosis showed focal peripheral areas with positive staining for collagen III (Figure 3B—C).

Immunohistochemical Staining of Nodular Glomerulosclerosis

The composition of small nodular matrix enlargement resembles that of the diffuse glomerulosclerotic lesions. Again a marked increase in BM-collagen IV and in collagen V, and to a minor extent also for laminin and fibronectin, was found, whereas HSPG was not detectable (Table 2). In contrast, pronounced nodular lesions revealed a strong decrease in collagen IV, laminin, and fibronectin. These components were only present in the periphery of the noduli (Figure 4). In the hypocellular central areas of the noduli, only collagen V could be found (Figure 5D). In addition, peripheral areas of these noduli were also positive for collagen III (Figure 3D). Collagen I, which can only be found in the interstitium and the sclerotic parts of the Bowman's capsule, was not detectable in diffuse or in nodular lesions (Figure 5A, B).

Using doublelabelling procedures for collagens III and IV in those large nodules, the central portion of these noduli remained unstained for both components, although an overlapping, ringlike staining pattern for both components was found in the periphery of the noduli (not shown).

Correlation of Clinical Findings and Immunohistochemical Staining

In 12 cases in our study group sufficient additional clinical data were reported for the evaluation of the renal function.

Table 2. Composition of the Glomerular Matrix in Different Stages of Diabetes Mellitus

	Diffuse glomerular sclerosis		Nodular glomerular sclerosis	
	Early	Late	Early	Late
Collagen IV	↑	↑↑	↑↑	↓
Laminin	↑	↑	↑	↓
Heparan sulfate proteoglycan	↓→	↓↓	↓↓	↓↓
Fibronectin	↑	↑	↑	↓
Collagen V	↑	↑↑	↑↑	↑↑
Collagen III	—	(+)	(+)	—
Collagen I	—	—	—	—

↑ = increase, ↓ = decrease, → = no change, + = present, — = not detectable.

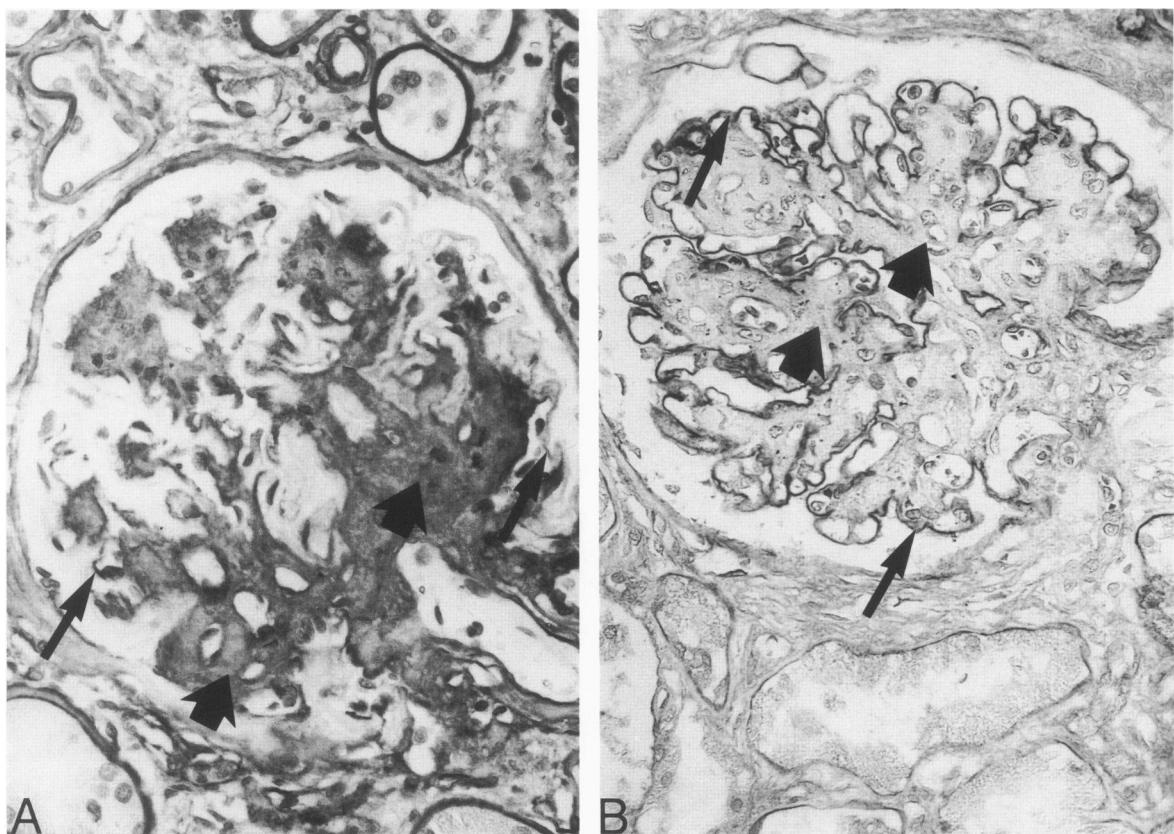


Figure 2. BM-components in diffuse glomerulosclerosis. The enlarged mesangial matrix (→) stains intensely for collagen IV (A), but not for HSPG (B). The glomerular BM (→) shows collagen IV and residual amounts of HSPG. (A, B, $\times 400$).

The patient-by-patient results are depicted in Table 3. A correlation of clinically evaluated renal function and the immunohistochemical composition of the glomeruli revealed in two patients a reduction in the glomerular HSPG-content without signs of significantly impaired filter function. In the other cases, no significant correlation existed between renal function impairment and matrix disarrangement, although all of them showed a marked decrease of glomerular HSPG staining.

Discussion

Our study investigated the participation of several intrinsic components of the normal human renal extracellular matrix in the matrix expansion of early and severe diabetic nephropathy. This is, to our knowledge, the first extensive study on the phenotypic expression of various BM-components and collagens throughout the course of diabetic nephropathy in human subjects.

Normal human glomerular BM and mesangial matrix contain several different BM-components in varying degrees.²¹ Thus, these structures are made up by collagen

IV and laminin in an almost even distribution, as well as by HSPG, which is present in a rather large amount, especially when compared with the surrounding BMs of the arterioles and tubuli. This matrix does not contain interstitial collagens I or III, which are only present in small amounts in the Bowman's capsule and the surrounding intertubular and interglomerular tissue.^{8,26,27} In our studies, collagen V was found along the glomerular BM confirming the findings of Roll et al.,²⁸ at variance with results reported by Martinez-Hernandez et al.²⁹ The reason for these differences are not obvious, but may be due to methodologic differences. Fibronectin, another ubiquitous component of the extracellular matrix is in the normal human glomerulus mainly present in the mesangium and to a far less degree and in a fragmented pattern in the glomerular BM. This result has also been shown by others.²⁷

Although it is assumed that collagen IV may play a vital role in the size-selective sieving properties of the ultrafiltration unit, the highly negatively charged HSPG may be important for the charge-selective filtration properties of the glomerular BM. Previous studies provided evidence that these charge-selective sieving properties,

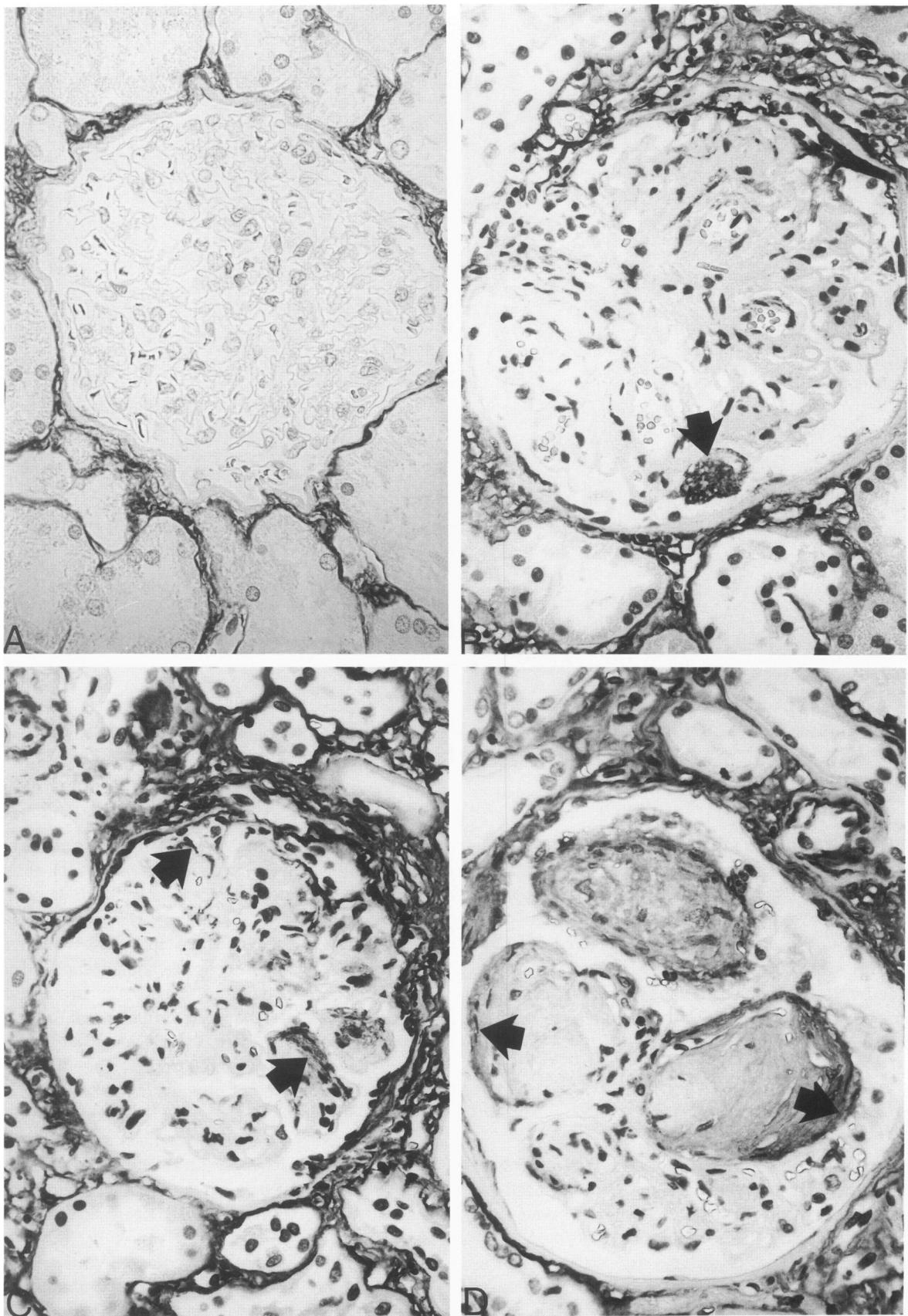


Figure 3. Distribution of collagen III in normal and diseased glomeruli. **A:** Normal glomeruli do not contain collagen III. **B:** In glomerulosclerosis focal deposits of collagen III are seen opposite to the vascular pole (→), whereas (**C**) extensive diffuse glomerulosclerosis shows multiple collagen III staining (→). **D:** In nodular glomerulosclerosis, focal areas in the periphery of the noduli stain positively for collagen III (→). (A-D, $\times 400$).

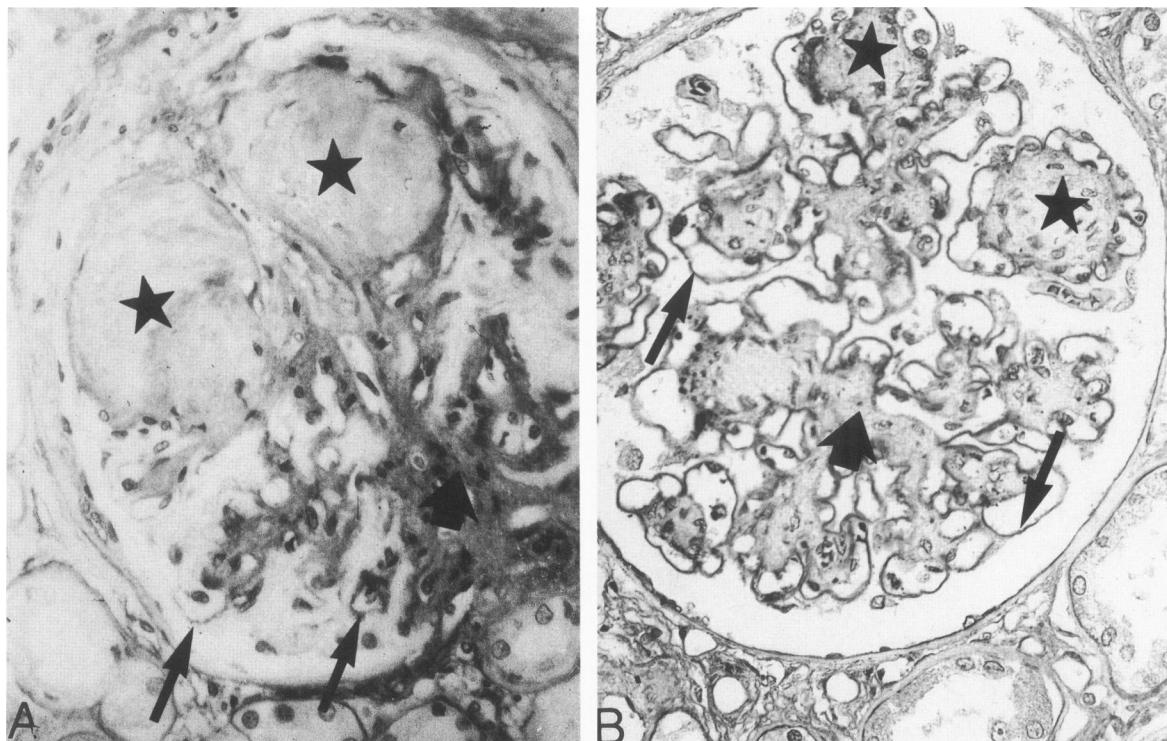


Figure 4. The extensive nodular lesion (*) lacks positive reaction for both collagen IV (A) and HSPG (B). Diffusely sclerotic parts of the glomerulus (→), however, still react with collagen IV, but not with HSPG. Only few peripheral capillary loops (→) show a preserved positive reaction for HSPG in the glomerular BM, although with markedly reduced intensity. (A, B, $\times 400$).

exerted by the HSPG, are involved in the renal filtration process. Thus, Farquhar and coworkers⁶ experimentally demonstrated, that polyanionic plasmaproteins like albumin easily penetrate the BM after the application of the heparan-sulfate-degrading enzyme heparitinase. In further experiments, Mynderse et al.³⁰ were able to induce a nephrotic syndrome in animals after the enzymatic *in vivo* degradation of HSPG. Further support for the role of the negatively charged HSPG is provided by the findings that the development of aminonucleoside nephrosis coincides with the loss of anionic sites from the glomerular basement membrane.³¹ In our initial experiments, we observed a marked reduction in the staining intensity for HSPG in those cases with diffuse and nodular glomerulosclerosis, whereas all other BM components tested were present to higher extent. Our observations of an increased deposition of collagen IV and laminin in diffuse and nodular glomerulosclerosis are well in accord with previous reports.^{8,27,32,33} Bruneval et al.,²⁷ however, mentioned that in their study on a group of four patients with late nodular glomerulosclerosis a heparan-sulfate-containing proteoglycan was detected in the widened mesangial areas, as it was seen for laminin and collagen IV. Since they did not characterize their antibody, it is not clear whether the antibody used by them may be the

same as used in our study, or if their antibody may have crossreacted with some other BM-component.

Our observations agree well with the known quantitative biochemical estimation of the glomerular HSPG content, which shows a marked decrease in diabetes.^{9,15,17,18} In an additional attempt to clarify the role of various BM-components and interstitial collagens in diabetic nephropathy, we were able to compare clinical parameters of renal function of 12 patients with the immunohistologic results (Table 3). Interestingly, in two patients a slight reduction in the staining intensity for HSPG could be found, even before a significant deterioration of renal function was observed. Those patients with a severe alteration of the kidney function showed marked decreases of glomerular HSPG staining properties.

In addition, we found that more pronounced diffuse and nodular glomerulosclerotic lesions demonstrate the occurrence of interstitial collagen III within the extended mesangial matrix, whereas collagen I was not found in the tissues tested. The occurrence of collagen III in these types of lesions showed a progressive character, which provides evidence that certain mesangial cells seem to change their pattern of expression, most probably as the consequence of chronic metabolic alteration.

Occurrence of collagen III in the mesangium of dia-

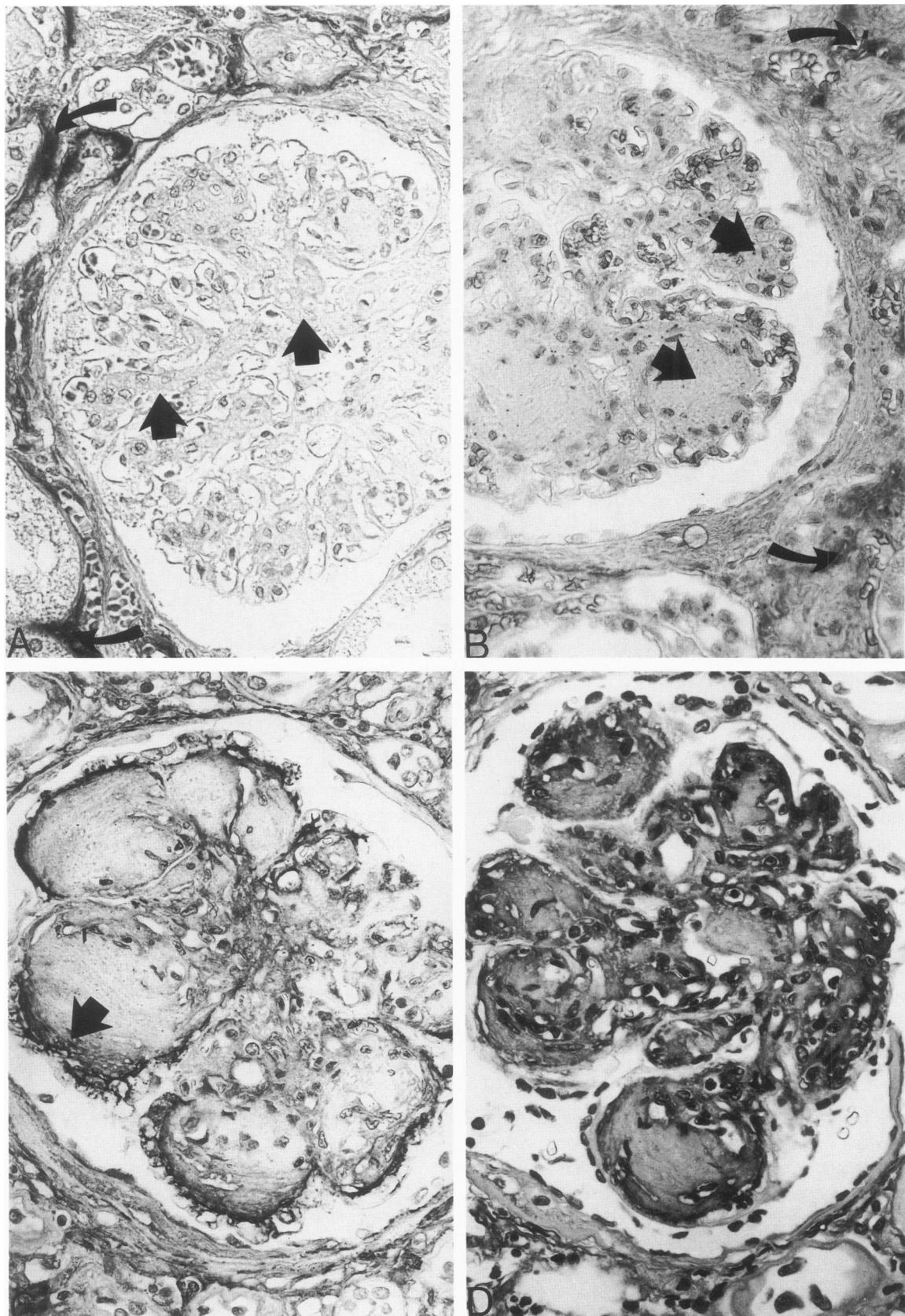


Figure 5. Pattern of the interstitial collagens in nodular glomerulosclerosis. Collagen I is not detected within diffuse (A) or nodular (B) lesions (►), but in the surrounding interstitium (→). Fibronectin (C) is present in the periphery of the noduli (►), as will as in the interglomerular interstitial space, whereas collagen V (D) can be found evenly distributed within the nodular lesion, in diffusely sclerotic mesangial areas and in the interstitial space surrounding the glomerulus. (A-D, $\times 400$).

Table 3. Correlation of Clinical and Pathologic Parameters

No.	Types of diabetes	Clinical parameters*				Histological type of lesion	Glomerular immunohistochemistry†						
		Serum creatinine (mg%)	Creatinine clearance (ml/min)	Urinary prot. excretion (g/24h)	HSPG		Collagen						
							IV	I	III	V	LM	FN	
1	NIDDM	1,2	110	Ø	Minimal DGS	→	→	Ø	Ø	→	→	→	
2	NIDDM	1,1	90	Ø	Minimal DGS	↓	→	Ø	Ø	→	→	→	
3	IDDM	1,6	120	Ø	Minimal DGS	↓	→	Ø	Ø	→	→	→	
4	NIDDM ^{a)}	1,6	75	2,0	Extensive DGS	↓↓	↑	Ø	(+)	↑	↑	↑	
5	NIDDM ^{a)}	1,5	70	2,6	Extensive DGS	↓↓	↑↑	Ø	Ø	↑↑	↑	↑	
6	NIDDM ^{a)}	1,8	55	3,5	Beginning NGS	↓↓	↑↑	Ø	+	↑↑	↑↑	↑	
7	NIDDM	1,9	40	5,2	Extensive DGS	↓↓	↑↑	Ø	(+)	↑↑	↑	↑↑	
8	IDDM	1,8	NR	4,0	Extensive DGS	↓↓	↑↑	Ø	+	↑↑	↑	↑	
9	IDDM	2,6	45	2,7	Extensive DGS	↓↓	↑↑	Ø	+	↑↑	↑↑	↑↑	
10	IDDM	2,2	NR	5,5	Extensive NGS	↓↓	↓	Ø	++	↑↑	↓	↓	
11	IDDM	2,5	NR	6,0	Beginning NGS	↓↓	↑↑	Ø	(+)	↑↑	↑↑	↑	
12	NIDDM	8,6	(on hemodialysis)		Extensive NGS	↓↓	↓	Ø	++	↑↑	↓	↓	

* NIDDM = non-insulin-dependent diabetes mellitus; IDDM = insulin-dependent diabetes mellitus; DGS = diffuse glomerulosclerosis; NGS = nodular glomerulosclerosis; NR = not reported.

† Staining intensities compared to normal renal tissue, ↑ = increased, ↑↑ = strongly increased; ↓ = decreased; ↓↓ = strongly decreased; → = no change; Ø = not detectable; (+) = isolated focal positive areas; + = focal positive; ++ = extensive positive; ^{a)} = biopsy.

LM = laminin, FN = fibronectin.

betic rats has also been reported by Abrass et al.³⁴ The influence of insulin on the switch in collagen-type expression they claimed in their study can, however, not be substantiated by our study, since we found no significant difference in the occurrence of collagen III within the mesangium between patients with insulin-dependent and non-insulin-dependent diabetes and no determination of blood-insulin levels were performed in our patients.

All types of lesions, especially late, nodular glomerulosclerotic lesions showed a marked increase in the deposition of collagen V. Since it is well known that mesangial cells are closely related ontogenetically to smooth muscle cells and since it is presumed that smooth muscle cells produce large amounts of collagen V and collagen III when compared with collagen I,³⁵ the observed change in the composition of the extracellular matrix in late nodular glomerulosclerosis may reflect a "dedifferentiation" of mesangial cells to smooth muscle type cells. Preliminary results from this laboratory showed that HSPG synthesis is decreased in cultured porcine mesangial cells by elevated glucose levels.³⁶ Although it cannot completely be excluded that fibroblast-type cells may

contribute to mesangial sclerosis, this is not likely since the sclerotic mesangium lacks collagen I.

Using doublestaining procedures, we observed late nodular glomerulosclerosis central areas, which stained only for collagen V but not for collagen III, IV, laminin, or fibronectin. We cannot exclude, that this central less-stained area may contain further components, usually present in the glomerular matrix, especially chondroitin-sulfate proteoglycan, which usually comprises 5 to 10% of the glomerular anionic sites.³⁷

Our findings corroborate and supplement the pathogenetic hypothesis of Rohrbach et al.¹⁵ According to their biochemical findings, they postulated that a reduction in the glomerular HSPG content may lead to an increased synthesis in BM components, resulting in a progressively thickened BM. A recurrent diabetic metabolic injury of the glomerular BM may thus lead to the multilaminated deposition of BM material, which lacks, however, HSPG. In addition to this hypothesis we have demonstrated that the late stage of diabetic glomerulopathy may lead to an altered expression of the mesangial cells with progressing sclerosis of the glomerulum. The occurrence of col-

lagen III in the mesangium reflects irreversibility. There are, however, marked differences between this process and reparative processes, like woundhealing,³⁸ since in diabetic glomerulosclerosis no collagen I can be detected. These findings underline the special properties of the mesangial matrix.

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References

- Martinez-Hernandez A, Amenta PS: The basement membrane in pathology. *Lab Invest* 1983, 48:656-677
- Timpl R, Dziadek M: Structure, development and molecular pathology of basement membranes. *Intl Rev Pathol* 1986, 29:1-112
- Abrahamson DR: Recent studies on the structure and pathology of basement membranes. *J Pathol* 1986, 149:257-278
- Timpl R: Recent advances in the biochemistry of glomerular basement membrane. *Kidney Int* 1986, 30:293-304
- Paulsson M: Non-collagenous proteins of basement membrane. *Coll Rel Res* 1987, 7:443-461
- Farquhar MG: The glomerular basement membrane: A selective macromolecular filter. *Cell Biology of Extracellular Matrix*. Edited by ED Hay. New York, Plenum Press, 1981, pp 335-349
- Stow JL, Sawada H, Farquhar MG: Basement membrane heparan sulfate proteoglycans are concentrated in the laminae rarae and in podocytes of the rat renal glomerulus. *Proc Natl Acad Sci USA* 1985, 82:3296-3299
- Falk RJ, Scheinman JI, Mauer SM, Michael AF: Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. *Diabetes* 1983, 32(Suppl. 2):34-39
- Schleicher E, Wieland OH: Changes of human glomerular basement membrane in diabetes mellitus. *J Clin Chem Clin Biochem* 1984, 22:223-227
- Brownlee M, Cerami A: The biochemistry of the complications of diabetes mellitus. *Annu Rev Biochem* 1981, 50:385-432
- Westberg NG, Michael AF: Human glomerular basement membrane: Chemical composition in diabetes mellitus. *Acta Med Scand* 1973, 39:194-198
- Kanwar YS, Rosenzweig LJ, Linker A, Jakubowski ML: Decreased de novo synthesis of glomerular proteoglycans in diabetes: Biochemical and autoradiographic evidence. *Proc Natl Acad Sci USA* 1983, 80:2272-2275
- Klein DJ, Brown DM, Oegema TR: Glomerular proteoglycans in diabetes: Partial structural characterization and metabolism of de novo synthesized heparan-³⁵SO₄ and dermatan-³⁵SO₄ proteoglycans in streptozotocin-induced diabetic rats. *Diabetes* 1986, 35:1130-1142
- Cohen MP, Klepser H, Wu V-Y: Undersulfation of glomerular basement membrane heparan sulfate in experimental diabetes and lack of correction with aldose reductase inhibition. *Diabetes* 1988, 37:1324-1327
- Rohrbach DH, Hassell JR, Kleinmann HK, Martin GR: Alterations in the basement membrane (heparan sulfate) proteoglycan in diabetic mice. *Diabetes* 1982, 31:185-188
- Brown DM, Klein DJ, Michael AF, Oegema TR: ³⁵S-Glycosaminoglycan and ³⁵S-glycopeptide metabolism by diabetic glomeruli and aorta. *Diabetes* 1982, 31:418-425
- Parthasarathy N, Spiro RG: Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 1982, 31:738-741
- Shimomura S, Spiro RG: Studies on macromolecular components of human glomerular basement membrane and alterations in diabetes: Decreased levels of heparan sulfate proteoglycan and laminin. *Diabetes* 1987, 36:374-381
- Rohrbach R: Reduced content and abnormal distribution of anionic sites (acid proteoglycans) in the diabetic glomerular basement membrane. *Virchows Arch Cell Pathol* 1986, 51:127-135
- Olgemöller B, Schleicher E, Nerlich A, Wagner EM, Gerbitz KD: Isolation, characterization and immunological determination of basement membrane-associated heparan sulfate proteoglycan. *Biol Chem Hoppe-Seyler* 1989, 370:1321-1329
- Schleicher E, Wagner EM, Olgemöller B, Nerlich A, Gerbitz KD: Characterization and localization of basement membrane-associated heparan sulfate proteoglycan in human tissues. *Lab Invest* 1989, 61:323-332
- Timpl R, Wick G, Gay S: Antibodies to distinct types of collagens and procollagens and their application in immunohistology. *J Immunol Meth* 1977, 18:165-175
- Barsky SH, Rao NC, Restrepo C, Liotta LA: Immunocytochemical enhancement of basement membrane antigens by pepsin: Applications in diagnostic pathology. *Am J Clin Pathol* 1984, 82:191-194
- Hsu SM, Raine L, Fanger H: A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Path* 1981, 75:734-739
- Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford AF, Stein H, Mason DY: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase. *J Histochem Cytochem* 1984, 32:219-225
- Remberger K, Gay S, Adelmann BC: Immunhistochemische Charakterisierung und Lokalisation unterschiedlicher Kollagentypen bei chronischen Nierenerkrankungen. *Verh Dtsch Ges Path* 1975, 60:314
- Bruneval P, Foidart JM, Nochy D, Camilleri JP, Bariety J: Glomerular matrix proteins in nodular glomerulosclerosis in

- association with light chain deposition disease and diabetes mellitus. *Hum Pathol* 1985, 16:477-484
28. Roll FJ, Madri JA, Albert J, Furthmayr H: Codistribution of collagen types IV and AB2 in basement membranes and mesangium of the kidney. *J Cell Biol* 1980, 85:597-616
 29. Martinez-Hernandez A, Gay S, Miller EJ: Ultrastructural localisation of type V collagen in rat kidney. *J Cell Biol* 1982, 92:343-349
 30. Mynderse LA, Hassel JR, Kleinmann HK, Martin GR, Martinez-Hernandez A: Loss of heparan sulfate proteoglycan from glomerular basement membrane of nephrotic rats. *Lab Invest* 1983, 48:292-302
 31. Caulfield JP, Farquhar MG: Loss of anionic sites from the glomerular basement membrane in aminonucleoside nephrosis. *Lab Invest* 1978, 39:505-512
 32. Gubler MC, Noel LH, Monnier F, Gros F, Wieslander J: Immunohistochemical study of extracellular matrix components in diabetic glomerulosclerosis. *Progress in Basement Membrane Research*. Edited by MC Gubler, M Sternberg. London, Paris, John Libbey, 1989, pp 201-204
 33. Bendayan M: Alteration in the distribution of type IV collagen in glomerular basal laminae in diabetic rats as revealed by immunocytochemistry and morphometrical approach. *Diabetologia* 1985, 28:373-378
 34. Abrass CK, Peterson CU, Rangi GJ: Phenotypic expression of collagen types in mesangial matrix of diabetic and non-diabetic rats. *Diabetes* 1988, 37:1695-1702
 35. Barnes MJ: Collagens of normal and diseased blood vessel wall. *Collagen*. Vol. I. Edited by M Nimni. New York, CRC Press, 1988, pp 275-290
 36. Olgemöller B, Schleicher ED, Schwaabe S, Gerbitz KD: Elevated glucose decreases the content of a basement membrane associated heparan sulfate proteoglycan in cultured mesangial and aortic smooth muscle cells. *Diabetologia* (submitted)
 37. Stow LJ, Soroka CJ, MacKay K, Striker L, Striker K, Farquhar MG: Basement membrane heparan sulfate proteoglycan is the main proteoglycan synthetized by glomerular epithelial cells in culture. *Am J Pathol* 1989, 135:637-646
 38. Kurkinen M, Vaheri A, Roberts PJ, Stenman S: Sequential appearance of fibronectin and collagen in experimental granulation tissue. *Lab Invest* 1980, 43:47-54