Disturbances in Glomerular Basement Membrane Glycosaminoglycans in Experimental Diabetes

VAN-YU WU, BRYAN WILSON, AND MARGO PANUSH COHEN

SUMMARY

Glycosaminoglycans (GAGs) were purified from basement membranes isolated from glomeruli of control and streptozocin-induced diabetic rats and were quantitatively analyzed with a recently described competitive binding assay that is specific for and sensitive to microgram amounts of chondroitin and heparan sulfate. Total GAG content in glomeruli from diabetic rats and in the basement membranes prepared from these samples (17.22 ± 1.45 and 6.56 ± 0.49 $\mu g/10^5$ glomeruli, respectively) was significantly less than that found in comparable control preparations (43.71 ± 3.35 and 16.05 ± 1.41 $\mu g/10^5$ glomeruli, respectively). The portion of total GAG in the water-soluble fraction recovered after osmotic lysis of isolated glomeruli was also markedly decreased in diabetic samples (26.11 ± 4.55 vs. 3.30 ± 0.32 $\mu g/10^5$ glomeruli, control vs. diabetic). Treatment of lysed glomeruli with the ionic detergent deoxycholate, required for liberation of the extracellular matrix from plasma membrane lipoproteins and purification of the insoluble glomerular basement membrane (GBM), resulted in solubilization of ~10% of the water-insoluble GAG in control samples but >50% in diabetic membranes. Heparan sulfate comprised >90% of the GAGs in both control and diabetic GBM, defined as the water- and detergent-insoluble matrix. The findings clearly demonstrate that the GAG content of GBM is diminished in experimental diabetes and provide evidence that the reduction in GBM anionic sites associated with diabetes derives from a decrease in the constituent GAGs of this extracellular matrix. The results further suggest that the interaction between GBM and populations of GAG associated with the surface of plasma membranes of adjacent cells is disturbed in diabetes. *Diabetes* 36:679–83, 1987

From the Department of Medicine, University of Medicine and Dentistry of New Jersey, Newark, New Jersey.

Address correspondence and reprint requests to Margo P. Cohen, MD, Division of Endocrinology and Metabolism, University of Medicine and Dentistry of New Jersey, Medical Science Bldg. I-588, 100 Bergen Street, Newark, NJ 07103.

Received for publication 11 August 1986 and accepted in revised form 9 December 1986.

Glycosaminoglycans (GAGs) constitute a class of polysaccharides present in connective tissues and on cell surfaces, usually in covalent association with protein to form proteoglycans (for reviews, see refs. 1–3). They consist of a carbohydrate backbone, containing alternating uronic acid and hexosamine residues, and possess a high net negative charge, largely conferred by the presence of hexosamine-linked or ester sulfate groups, which influences the electrochemical properties of tissues. In this regard, GAGs of the renal glomerular basement membrane (GBM) are of particular interest, because they constitute anionic sites that regulate the charge-selective nature of the glomerular filtration barrier (4–8) and have been implicated in the pathogenesis of the increased glomerular permeability occurring in diabetes and other proteinuric states (9). The findings that incorporation of [^{35}S]sulfate into basement membrane proteoglycans is diminished in diabetes (10–14) and that removal of heparan sulfate by an in situ enzymatic digestion leads to a dramatic increase in the permeability of GBM to ferritin (7) and ^{125}I-labeled albumin (8) lend support to the hypothesis that alterations in basement membrane GAGs contribute to the defective function of the glomerular filtration barrier in diabetes. It is not clear, however, whether such changes are qualitative or quantitative, because there are conflicting reports regarding the amount of measurable uronic acid or proteoglycan, the latter determined by ELISA with antibody directed against core protein, in basement membranes from diabetic subjects or experimental animals compared with controls (10–12,14,15). Furthermore, reduced radioactive sulfate incorporation may reflect decreased GAG production or undersulfation of GAG chains in diabetes; there is some support for the latter possibility in at least two reports, which describe fewer sulfate groups in the heparan sulfate proteoglycan isolated from liver plasma membranes of streptozocin-induced diabetic (STZ-D) rats (16) and in the [^{35}S]-heparan sulfate prepared from intestinal epithelial cells of diabetic animals (17).

We recently developed a competitive binding assay for sulfated GAG that can accurately and reproducibly measure these constituents in GBM derived from small amounts of tissue. The assay is sensitive to microgram quantities of chondroitin and heparan sulfate and is specific for heparan sulfate, the predominant GAG species in GBM, in chondroitinase-treated samples (18). In our study, we used this assay to address the question of whether there is a quantitative change in the amount of heparan sulfate in GBM isolated from diabetic animals.

MATERIALS AND METHODS

Experimental animals and tissue preparation. Age-matched male white rats (Taconic Farms, NJ), fed standard laboratory chow and provided water ad libitum, were used in all experiments. Diabetes was induced by a single injection of STZ, 65 mg/kg body wt, into the tail vein. Control and diabetic animals were maintained for 21–28 days after induction of diabetes, when they were killed in a CO_2 chamber. Kidneys were quickly removed from each rat, placed in cold 0.85% NaCl, and processed for glomerular isolation. For each experiment, renal cortex from 3 to 4 rats within the same experimental group was pooled for isolation of glomeruli, which was accomplished by differential sieving through a series of stainless steel meshes (19). This method yields preparations virtually free of tubular elements.

The glomeruli were osmotically lysed, in the cold, by stirring in distilled water containing protease inhibitors (20 mM EDTA, 1 mM benzamidine hydrochloride, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM EGTA, and 10 mM *N*-ethylmaleimide). Because the efficiency of lysis may vary among cell types, the preparations were monitored microscopically at the end of the stirring period to ensure that lysis was complete and was comparable in control and diabetic glomeruli. The insoluble material, representing basement and cell membranes, was collected by centrifugation, treated twice with 4% deoxycholate (DOC) containing 1 mM PMSF to liberate plasma membrane lipoproteins, and then washed three times with distilled water to remove adherent extraction fluid. Treatment with DOC is a traditional step in basement membrane purification that removes cells and cell debris and separates plasma membrane lipoproteins from the insoluble matrix (20,21). It is also required for dissociation of basement membrane GAGs from cell membrane lipids and for their subsequent measurement in the assay described below. However, DOC solubilizes a fraction of total extracellular matrix GAGs that probably is bound to adjacent cell membranes (22). The DOC extracts were therefore also analyzed for GAG content.

Isolation of GAGs. For isolation of GAGs, lyophilized samples of the basement membranes prepared as described above were digested with 2% papain (wt/vol) overnight at 60°C in a solution of 0.05 M sodium acetate buffer, pH 5.5, containing 5 mM EDTA and 5 mM cysteine. Undigested material was removed by centrifugation, and the supernatants were made 1% in cetylpyridinium chloride (CPC) to precipitate the GAGs. The CPC-precipitated material was dissolved in 2 M NaCl and reprecipitated with 2 vol absolute ethanol. After resolubilization, residual protein and nucleic acids were removed by precipitation with 10% trichloroacetic acid (TCA). The supernatant was lyophilized and extracted with absolute ethanol to remove TCA and again dried. After addition of water, the GAGs in the aqueous solution were precipitated with 1% CPC, again dissolved in 2 M NaCl, and reprecipitated with ethanol, followed by washing once with chloroform:methanol (1:2 vol:vol) and three times with 95% ethanol. The final GAG precipitate was dried and reconstituted in a small volume of distilled water. The DOC extracts were also lyophilized, extracted with absolute ethanol to remove the detergent, and then papain digested and subjected to CPC precipitation. This CPC-precipitated material was further purified with salt solubilization, ethanol reprecipitation, and chloroform:methanol and ethanol washing and then dried and reconstituted in distilled water. Intracellular contents, recovered after osmotic lysis of whole glomeruli, were treated in the same fashion following CPC precipitation.

Competitive binding assay. Quantitative analysis of GAG in these samples followed the described procedure (18), modified as indicated below to increase sensitivity, with heparan sulfate labeled with tritium by borohydride reduction and unlabeled standard purified from porcine renal cortex. The purity of the latter has been repeatedly documented with cellulose acetate electrophoresis, resistance to chondroitinase ABC digestion, sensitivity to nitrous acid treatment, and gel filtration (18,23). [^{3}H]heparan sulfate (10^6 cpm/mg) was incubated in the presence of zinc acetate (2 mg/ml), normal human serum (1:10,000 final dilution), and varying amounts of unlabeled standard or basement membrane samples in a total volume of 1 ml. The precipitated complexes were removed by centrifugation. Radioactivity in the supernatants was measured in a liquid scintillation counter after addition of 10 ml Bray's solution. In this assay, unlabeled heparan sulfate competes with radiolabeled material for complexation with serum lipoproteins in a dose-dependent and saturable manner, allowing construction of standard curves from which the amount of GAG in unknown samples can be calculated. Both the decrease in counts in the precipitate and the increase in counts in the supernatant are proportional to the amount of added cold heparan sulfate (18). Because equivalent amounts of chondroitin sulfates A, B, and C, but not hyaluronate, compete in the assay, aliquots of the samples were first measured without prior treatment to determine total sulfated GAG, followed by chondroitinase ABC (Sigma, St. Louis, MO) digestion to specifically determine heparan sulfate (18,23). Chondroitinase digestion was performed with 2–5 U of enzyme in 0.01 M Tris buffer, pH 7.4, overnight at 37°C. Preliminary assessment of the GAG content in each of the membrane preparations and soluble fractions was performed to ensure that subsequent aliquots taken for quantitative measurement were appropriate for the limits of the assay system. Because maximum displacement under the described conditions occurs with 0–2 μg of unlabeled

TABLE 1
Experimental animal data

	Control	Diabetic
Body wt (g)	276 ± 8	215 ± 10*
Kidney wt (g)	2.49 ± 0.13	2.86 ± 0.16*
Kidney wt/g body wt	9.02 ± 0.23	13.30 ± 0.69*

*Significantly different from controls at $P < .05$.

standard, all aliquots contained between 0 and 2 μg of GAG, which yielded results falling within the linear portion of the displacement curve.

RESULTS

All STZ-D rats were markedly hyperglycemic with mean blood glucose concentrations >18 mM when killed and manifested typical insulin-deficient diabetes. Diabetes developed within 3 days after STZ injection and persisted until death in all injected animals. Body weights in diabetic rats were significantly less than those of control animals; as previously noted (24), kidney weights were increased in diabetic animals, and renal cortical mass was preserved despite loss of body weight (Table 1).

Analysis of basement membrane GAG, isolated from glomeruli and subjected to osmotic lysis followed by DOC extraction, was performed with three separate preparations of control or diabetic tissue, each derived from glomeruli isolated from renal cortex pooled from three to four rats in the respective experimental groups. At least three different aliquots, each performed in duplicate or triplicate, of all basement membranes, DOC extracts, and water-soluble fractions from each preparation were assayed for GAG content in the competitive binding assay. The intra-assay coefficient of variation was <5%, and the interassay variation for membranes assayed from the same pool was <10%. Results were calculated as μg GAG/100,000 glomeruli. The GAG isolates in both control and diabetic samples were devoid of DNA, documented by complete resistance of the material to treatment with DNase.

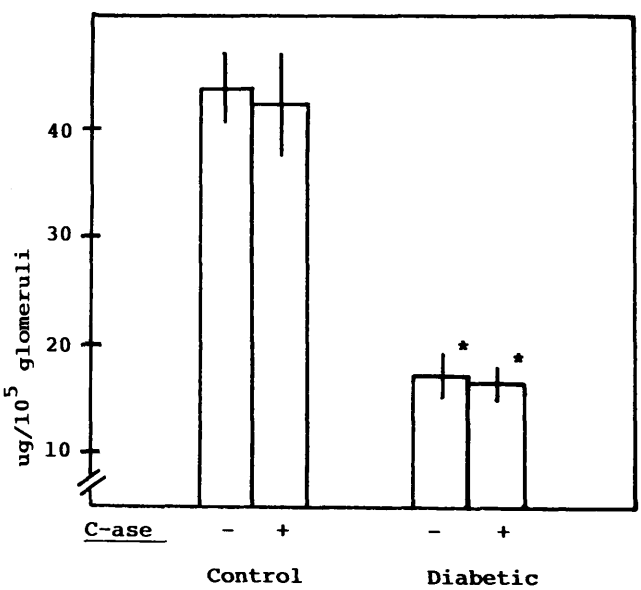

FIG. 1. Glycosaminoglycan (GAG) contents of control and diabetic glomeruli. Results represent total basement membrane GAG (DOC-insoluble matrix plus DOC- and water-soluble fractions) expressed as μg/10⁵ glomeruli. Dry weights of osmotically lysed glomeruli (before DOC treatment) in control preparations were 40.4, 27.4, and 25.5 mg; in diabetic samples, dry weights were 25.9, 21.1, and 24.9 mg. At least 3 aliquots performed in duplicate from each fraction in each preparation from both experimental groups were used for GAG assay; results are given as means ± SE of determinations. Aliquots of each sample were also taken for chondroitinase ABC digestion, followed by measurement in duplicate in the competitive binding assay. Chondroitinase (C-ase)-resistant material represents heparan sulfate. *$P < .05$ compared with control.

TABLE 2
Solubilization by deoxycholate (DOC) of glycosaminoglycans in control and diabetic glomeruli

Fraction	Control	Diabetic
DOC insoluble	16.05 ± 1.41	6.56 ± 0.49
DOC soluble	1.55 ± 0.51	7.46 ± 0.43
Solubilized (%)	8.7 ± 3.0	54.4 ± 3.5

Results expressed as μg/10⁵ glomeruli and represent means ± SE of assays performed in duplicate with each preparation in both experimental groups. DOC extraction was performed on material collected after osmotic lysis of the glomerular isolates.

Total glomerular GAG, representing the sum of measurements in the DOC-insoluble basement membranes plus the DOC- and water-soluble fractions, was significantly less in diabetic than in control preparations (Fig. 1). At least 90% of the total GAG was heparan sulfate, documented by subjecting portions of the GAG isolated from each of the membrane preparations and soluble fractions to digestion with chondroitinase ABC to distinguish enzyme-sensitive (chondroitin sulfates) from enzyme-resistant (heparan sulfate) species (Fig. 1). Thus, heparan sulfate is the predominant species of GAG in renal glomeruli, and the proportion of heparan versus chondroitin sulfate in diabetic samples does not differ from that in glomeruli from control animals.

Treatment with DOC results in the solubilization of some membrane-associated GAG, with recovery in the DOC extract as discussed above. This represented ~9% of the total, a value compatible with previous results in normal preparations (22; Table 2). In contrast, ~50% of the membrane GAG in diabetic samples was recovered in the DOC-soluble fraction, indicating that the relative proportions of membrane-associated GAG liberated by the ionic detergent are quite different in control versus diabetic preparations.*

GBM purified from diabetic rats contained significantly less GAG than did that prepared from control animals (6.56 ± 0.49 vs. 16.05 ± 1.41 μg/10⁵ glomeruli, respectively) (Fig. 2). More than 90% of the GAG in this DOC-insoluble matrix was heparan sulfate, documented by demonstration of resistance to chondroitinase ABC digestion and sensitivity to treatment with nitrous acid. These results confirm that heparan sulfate is the predominant species of GAG in GBM and indicate that the proportion of heparan versus chondroitin sulfate is unchanged in GBM from rats with experimental diabetes compared with controls.

Basement membrane is insoluble in water, neutral buffer, and dilute acid, and the material obtained after osmotic lysis therefore represents intracellular contents. GAG content in this material was significantly diminished in diabetic compared with control glomeruli (Fig. 3). Values were essentially

*The term *membrane-associated GAG* includes cell and basement membrane–associated populations. Because the lamina densa of the GBM is bordered on both sides by the less electron-dense laminae rarae, which are closely approximated to and have interactions with adjacent cell surfaces, sharing of certain basement membrane and cell surface components may occur. Thus, the distinction between proteoglycans and attachment proteins of the cell surface versus the extracellular matrix is not as clear-cut as previously thought. However, it is recognized that some of the GAGs solubilized by DOC derive from cell surfaces that do not abut onto the GBM.

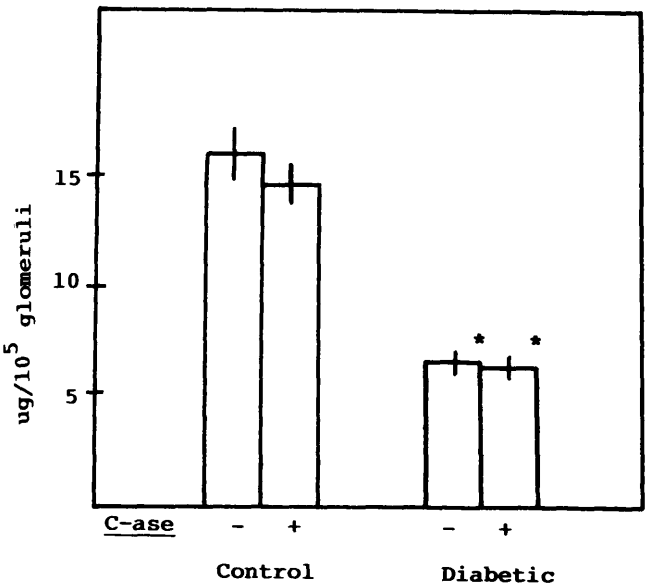

FIG. 2. Glycosaminoglycan (GAG) contents of control and diabetic glomerular basement membrane. Results represent GAG content measured in DOC-insoluble extracellular matrix prepared from glomerular samples described in Fig. 1 legend and expressed as μg/10⁵ glomeruli. Chondroitinase (C-ase)-resistant material represents heparan sulfate, confirmed by cellulose acetate electrophoresis and susceptibility to nitrous acid treatment. *$P < .05$ compared with control.

the same with or without prior chondroitinase treatment in both experimental groups. Thus, reduction in the GAG of diabetic basement membrane appears to correspond with a reduction in synthesis and/or an increase in intracellular degradation.

DISCUSSION

The experiments reported herein utilized a recently developed competitive binding assay for sulfated GAG, which is specific for heparan sulfate in chondroitinase-treated samples, to accurately and reproducibly quantitate these constituents in GBM purified from control and STZ-D rats. The results confirm, as previously reported (25–28), that heparan sulfate is the principle species, and clearly demonstrate that the GAG content of GBM is diminished in experimental diabetes. This decreased heparan sulfate content occurred after a duration of diabetes comparable with that in previous experiments in which a reduction of [^{35}S]sulfate incorporation, both in vitro (10) and in vivo (11), into basement membrane of diabetic glomeruli was observed. Although reduced radiolabeled sulfate incorporation in those experiments occurred despite apparent absence of significant change in the uronic acid content of the diabetic samples compared with controls, the method used in this study for quantification of GAG is more sensitive and accurate than the carbazole reaction and allows actual measurement of GAG rather than a monosaccharide unit. Furthermore, our experiments demonstrate that the GAG content of the intracellular material released with osmotic lysis is significantly decreased in diabetic glomeruli. Together with the results of a previous study showing that the in vivo turnover of ^{35}S-labeled GAG is unchanged in diabetes, although ^{35}S incorporation is reduced (11), these findings strongly suggest that a decrease in GAG production is responsible for the decrease in anionic sites of GBM associated with diabetes. Although subtle differences in the degree of sulfation of GAGs do not appear to alter their ability to compete in the binding assay (18), our results do not exclude the possibility that such differences exist between the heparan sulfate of normal versus diabetic GBM. Differences in the size of constituent GAG chains would also not be detected in this assay.

With autopsy specimens, Parthasarathy and Spiro (15) reported that, although the sulfate content of GAG isolated from GBM of diabetic subjects was lower than that of nondiabetic samples, the reduction was proportional to the diminution in uronic acid found in diabetic specimens, and hence there was no difference in sulfate-to-uronate ratios in diabetic versus nondiabetic preparations. Kanwar et al. (13) found that ^{35}S incorporation into proteoglycans of GBM in rats with STZ-D of 7 mo duration was 30–40% that of control, but that the molecular weights of the ^{35}S-labeled proteoglycans (assessed by Sepharose CL-6B chromatography) and the electrophoretic mobility of the constituent GAG chains (assessed by cellulose acetate electrophoresis after alkaline borohydride treatment of the proteoglycans) in diabetic samples were similar to those of controls. In EHS tumors transplanted to diabetic mice, Rohrbach et al. (14) reported significantly less heparan sulfate proteoglycan, measured by ELISA, than in tumors grown in control animals, but the size and net charge of intact proteoglycan molecules (assessed by Sepharose CL-4B and DEAE-cellulose chromatography) from diabetic and control mice were similar, and they contained GAG chains of comparable length. These results are compatible with our own, even though there are substantial differences in methodology, sample derivation, and duration of diabetes among the various studies. On the other hand, Kjellen et al. (16) found that cell membrane heparan sulfate proteoglycans isolated from livers of STZ-D rats and analyzed by anion-exchange chromatography had a reduced net negative charge compared with that in heparan sulfate proteoglycans from control rats. This was ascribed to a reduction in the number of *N*-

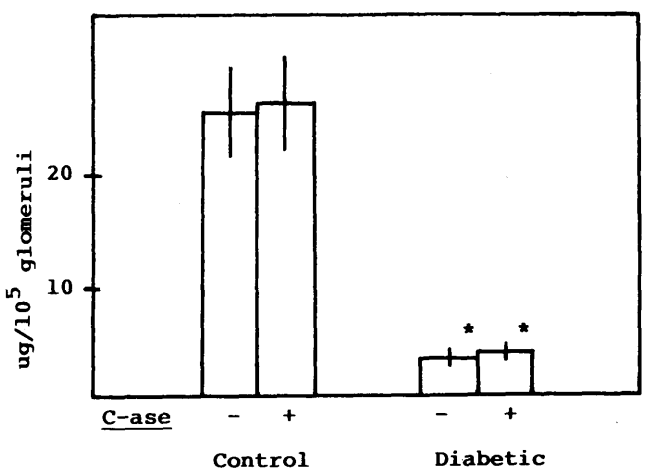

FIG. 3. Intracellular GAG contents. Results expressed as μg/10⁵ glomeruli and represent means ± SE. Intracellular contents represent material recovered after osmotic lysis of isolated glomeruli, with isolation of GAGs as described in text. *$P < .05$ compared with control.

linked sulfate groups, determined by selective nitrous acid deamination. However, the elution profiles on anion-exchange chromatography and after nitrous acid degradation of the diabetic samples differed only minimally from those of controls, suggesting the degree of undersulfation is modest. The consequences of such a reduction in charge, if present, on structural or intermolecular organizational properties of extracellular matrix GAGs are unknown, and it is possible that even a subtle change has profound impact.

The marked increase in the proportion of GAG released by detergent treatment of osmotically lysed glomeruli in diabetic samples is particularly interesting with respect to the way GAGs are handled and incorporated into the GBM and to potential defects in the organization of the extracellular matrix in diabetes. We have suggested there are two populations of GAG in the GBM: one that is intercalated with adjacent cell membranes and another that remains as an integral component of the matrix after detergent extraction (22). The dramatic increase in percent DOC solubility, coupled with the striking reduction in GAG content in the insoluble matrix, suggests that cell surface–associated proteoglycans are poorly incorporated into, or have compromised interaction with, the insoluble matrix in diabetes. Because this increased solubility is associated with a reduction in total glomerular GAG content, it cannot be argued that abnormal cross-linking of the GAG into the GBM is the only defect in diabetes. On the other hand, because there is a severe reduction in the absolute amount of heparan sulfate in the insoluble membrane, relative differences in the component composition of the insoluble matrix clearly must exist in experimental diabetes. The reason for faulty incorporation of GAGs into insoluble GBM must await better definition of the normal molecular interaction between basement membrane and plasma membrane–associated GAGs and between GAGs and other GBM components, as well as of the nature and origin of various proteoglycans in different regions of the glomerular extracellular matrix.

ACKNOWLEDGMENTS

We thank Marsha Taylor for secretarial assistance.

This work was supported by NIH Grants AM-32447 and EY-05441.

REFERENCES

1. Comper WD, Laurent TC: Physiologic function of connective tissue polysaccharides. *Physiol Rev* 58:255–315, 1978
2. Lindahl V, Hook M: Glycosaminoglycans and their binding to biological macromolecules. *Annu Rev Biochem* 47:385–417, 1978
3. Hook M, Kjellen L, Johansson S, Robinson J: Cell surface glycosaminoglycans. *Annu Rev Biochem* 53:847–69, 1984
4. Caulfield JP, Farquhar MG: Distribution of anionic sites in glomerular basement membrane: their possible role in filtration and attachment. *Proc Natl Acad Sci USA* 73:1646–50, 1976
5. Kanwar YS, Farquhar MG: Anionic sites in glomerular basement membrane: in vivo and in vitro localization to the laminae rarae by cationic probes. *J Cell Biol* 81:137–53, 1979
6. Brenner BM, Hostetter TH, Humes HD: Molecular basis of proteinuria of glomerular origin. *N Engl J Med* 298:826–33, 1978
7. Kanwar YS, Linker A, Farquhar MG: Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycan (heparan sulfate) by enzyme digestion. *J Cell Biol* 86:688–93, 1980
8. Kanwar YS, Rosenzweig LJ: Clogging of the glomerular basement membrane. *J Cell Biol* 93:489–94, 1982
9. Martinez-Hernandez A, Amenta P: The basement membrane in pathology. *Lab Invest* 48:656–77, 1983
10. Cohen MP, Surma ML: [^{35}S]-sulfate incorporation into glomerular basement membrane glycosaminoglycans is decreased in experimental diabetes. *J Lab Clin Med* 98:715–22, 1981
11. Cohen MP, Surma ML: Effect of diabetes on in vivo metabolism of ^{35}S-labeled glomerular basement membrane. *Diabetes* 33:8–12, 1984
12. Rohrbach DH, Hassell JR, Kleinman HK, Martin GR: Alterations in the basement membrane (heparan sulfate) proteoglycan in diabetic mice. *Diabetes* 31:185–88, 1982
13. Kanwar YS, Rosenzweig LJ, Linker A, Jakubowski ML: Decreased de novo synthesis of glomerular proteoglycans in diabetes: biochemical and autoradiographical evidence. *Proc Natl Acad Sci USA* 80:2272–75, 1983
14. Rohrbach DH, Wagner CW, Star VL, Martin GR, Brown KS: Reduced synthesis of basement membrane heparan sulfate proteoglycan in streptozotocin-induced diabetic mice. *J Biol Chem* 258:11676–77, 1983
15. Parthasarathy N, Spiro RG: Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 31:738–41, 1982
16. Kjellen L, Bielefeld D, Hook M: Reduced sulfation of liver heparan sulfate in experimentally diabetic rats. *Diabetes* 32:337–42, 1983
17. Levy P, Picard J, Bruel A: Evidence for diabetes-induced alterations in the sulfation of heparan sulfate intestinal epithelial cells. *Life Sci* 35:2613–20, 1984
18. Wu VY, Cohen MP: A competitive binding assay for measurement of heparan sulfate in tissue digests. *Anal Biochem* 139:218–23, 1984
19. Cohen MP, Klein CV: Glomerulopathy in rats with streptozotocin diabetes: accumulation of the glomerular basement membrane analogous to human diabetic nephropathy. *J Exp Med* 149:623–31, 1979
20. Carlson EC, Brendel K, Hjelle JT, Meezan E: Ultrastructural and biochemical analyses of isolated basement membrane from kidney glomeruli and brain and retinal microvessels. *J Ultrastruct Res* 62:26–53, 1978
21. Cohen MP, Surma ML: Renal glomerular basement membrane: in vivo biosynthesis and turnover in normal rats. *J Biol Chem* 1767–70, 1980
22. Wu VY, Chiu, I-IC, Cohen MP: Quantitative analysis of glomerular basement membrane glycosaminoglycans and evidence for their binding to adjacent cell membranes. *Anal Biochem* 145:57–62, 1985
23. Wu VY, Cohen MP: Platelet factor 4 binding to glomerular microvascular matrix. *Biochim Biophys Acta* 797:76–82, 1984
24. Beyer-Mears A, Ku L, Cohen MP: Glomerular polyol accumulation in diabetes and its prevention by oral Sorbinil. *Diabetes* 33:604–607, 1984
25. Kanwar YS, Farquhar MG: Isolation of glycosaminoglycans (heparan sulfate) from glomerular basement membranes. *Proc Natl Acad Sci USA* 76:4493–97, 1979
26. Kanwar YS, Farquhar MG: Presence of heparan sulfate in the glomerular basement membrane. *Proc Natl Acad Sci USA* 76:1303–307, 1979
27. Brown DM, Michael AF, Oegema TR: Glycosaminoglycan synthesis by glomeruli in vivo and in vitro. *Biochim Biophys Acta* 674:96–104, 1981
28. Linker A, Hovingh P, Kanwar YS, Farquhar MG: Characterization of the heparan sulfate isolated from dog GBM. *Lab Invest* 44:560–65, 1981