^{35}S-Glycosaminoglycan and ^{35}S-Glycopeptide Metabolism by Diabetic Glomeruli and Aorta

DAVID M. BROWN, DAVID J. KLEIN, ALFRED F. MICHAEL, AND THEODORE R. OEGEMA

SUMMARY

^{35}S-glycosaminoglycan metabolism by glomeruli isolated from streptozotocin-diabetic and control rats was studied in vivo and in vitro. Total ^{35}S-glycosaminoglycan synthesis and retention in the matrix by diabetic glomeruli was reduced while degradation was increased. ^{35}S-glycosaminoglycan content of isolated GBM was similarly decreased. Whereas ^{35}S-glycosaminoglycan content of glomeruli and GBM was decreased after in vitro incubation with $^{35}SO_4$, a larger proportion of total ^{35}S-glycosaminoglycans was found in the incubation medium from diabetic glomeruli. Both control and diabetic glomeruli synthesize ^{35}S-labeled glycopeptides, the quantity from diabetic glomeruli being reduced. Aorta from $^{35}SO_4$-injected diabetic rats also synthesized reduced quantities of ^{35}S-glycosaminoglycans. There were no preferential metabolic alterations of species of ^{35}S-glycosaminoglycans by diabetic glomerull or aortas. These studies suggest that synthesis of ^{35}S-glycosaminoglycans and ^{35}S-glycopeptides by diabetic glomeruli are altered by disturbances of both synthetic as well as degradative pathways. An alteration of ^{35}S-glycosaminoglycans interaction with matrix components in diabetes is postulated. DIABETES *31*:418–425, May 1982.

The morphologic basis for diabetic nephropathy in experimentally induced diabetes has been well described.[1,2] Until recently, the search for alterations in the biochemistry of the glomerulus in diabetes has focused upon the nature of the collagen-like components of the glomerular basement membrane.[3–10] Histologic,[11] immunohistochemical,[12] and biochemical[13] demonstrations of glycosaminoglycans and proteoglycans in the glomerulus have led to speculation that these large-molecular-weight, highly negatively charged molecules with properties of large volume domains may influence glomerular filtration and macromolecular processing.[14,15] We have initiated an investigation of the effects of experimental diabetes mellitus upon glomerular glycosaminoglycan and sulfoglycopeptide metabolism using in vivo $^{35}SO_4$ labeling techniques[16] with comparisons using in vivo aortic and in vitro glomerular studies.

From the Departments Pediatrics, Laboratory Medicine and Pathology, and Orthopedic Surgery and Biochemistry, University of Minnesota, Minneapolis, Minnesota.

Address reprint requests to David M. Brown, M.D., Pediatric Nephrology, Box 491 Mayo, University of Minnesota, 420 Delaware Street S.E., Minneapolis, Minnesota 55455.

Received for publication 28 September 1981 and in revised form 11 January 1982.

MATERIALS AND METHODS

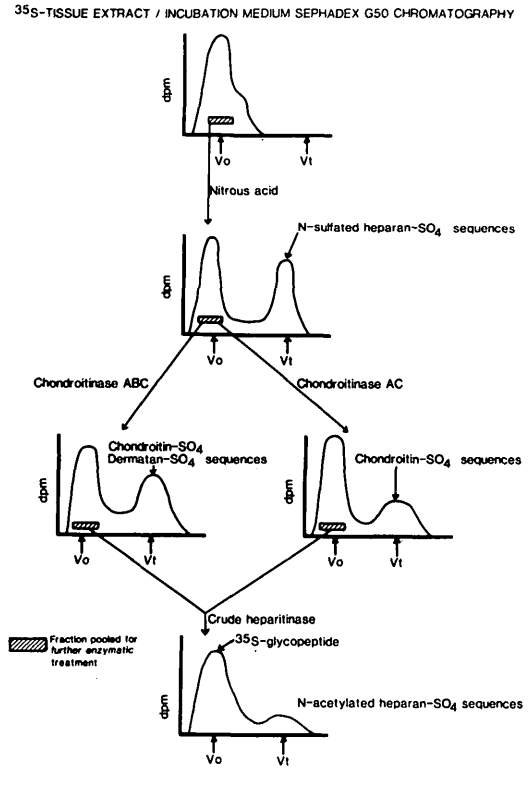
Male Sprague-Dawley rats weighing 100–120 g were made diabetic (nonfasting plasma glucose greater than 400 mg/dl) with streptozotocin as previously described.[17] Experiments were conducted 1 mo after induction of diabetes and included age-matched controls.[18] Preparations enriched in glomerular basement membrane (GBM)[19] were isolated by a modification[16] of the techniques of Carlson et al.[20]

In vivo studies were performed by killing animals and isolating glomeruli 2, 4, or 8 h after the intraperitoneal injection of 1 mCi per 250 g body weight of carrier free $^{35}SO_4$ (New England Nuclear, Boston, Massachusetts). Kidneys from 40 diabetic and 40 control rats were studied at each time period. Abdominal aortas from the same groups of diabetic and control rats were manually cleaned of adipose tissues, rinsed with 0.9% NaCl before processing, and finely minced for isolation of glycosaminoglycans. Plasma samples were obtained at each time period for determinations of ^{35}S levels. In vitro studies were performed on glomeruli isolated from 20 diabetic and 20 control rats by incubation with $^{35}SO_4$, 20 μCi/ml (20 ml/incubation) of RPMI 1640 medium (Bio-Rad Laboratories, Richmond, California) in which $MgCl_2$ was substituted for $MgSO_4$ and to which was added penicillin and streptomycin (1%) and glutamine (0.36 mM). Glomeruli were incubated at 37°C for an initial recovery period of 2 h after which $^{35}SO_4$ was added and incubation continued for an additional 24 h. Previous studies[16] with this system have shown that the peak uptake of $^{35}SO_4$ by glo-

meruli after in vivo treatment was 4 h whereas the peak was reached at 24 h after in vitro incubation.

The distribution of the ^{35}S-glycosaminoglycan subspecies and the demonstration of ^{35}S-glycopeptides in whole glomeruli, in isolated GBM, and in the in vitro incubation media was determined using a modification of the methods of Conrad[21] and Brown[16] (Figure 1). In this method the various tissue fractions are papain digested, followed by trichloroacetic acid (TCA) precipitation, chloroform-MeOH lipid extraction, and extensive dialysis, leaving behind only ^{35}S-labeled polysaccharides with short peptide chains attached.[22] This material is then subject to sequential chemical and enzymatic digestion followed by Sephadex G-50 chromatography. Nitrous acid sensitive material is N-sulfated heparan sulfate. The presence of dermatan sulfate is determined by sensitivity to chondroitinase ABC with lack of sensitivity to chondroitinase AC, while chondroitin sulfate is sensitive to both these enzymes. Crude heparitinase[23] would allow all of the ^{35}S-glycosaminoglycan subspecies to be retarded by the Sephadex G-50 column, including the ^{35}S-labeled N-acetylated heparan sulfate, which had thus far been untouched in the sequential scheme. In addition, samples from in vivo experiments were treated with keratanase (keratan sulfate 4-galactopyranosyl-glycanohydrolase from *Pseudomonas*, Sp. I.F.O. 13309, Miles Laboratory, Elkhart, Indiana), using 0.1 U of enzyme in 1 ml 0.05 M Tris-HCl buffer, pH 7.4, 37°C, 8 h. None of the ^{35}S-labeled substances were degraded by keratanase. Papain undigested non-TCA precipitable, nondialyzable ^{35}S-labeled material insensitive to the above series of treatments is defined as ^{35}S-glycopeptides. This material has been previously demonstrated by others using similar methods in rat GBM,[22] human kidney tumor cells,[24] and in chick embryos with $^{35}SO_4$.[25] Ascertainment of completeness of degradative procedures was described previously.[16] The N-sulfate fraction includes free sulfate released by the nitrous acid procedure as well as di- and tetrasaccharides cleaved from highly N-sulfated regions. The percent of N-sulfated regions of heparan sulfate is overestimated by these methods since heavily N-sulfated regions may contain up to four sulfate residues per disaccharide repeating unit as compared with one sulfate residue per repeating unit in predominantly N-acetylated regions.

FIGURE 1. Degradative sequence for ^{35}S-glycosaminoglycan and -glycopeptide identification.



RESULTS

In vivo ^{35}S-glycosaminoglycan and ^{35}S-glycopeptide metabolism by glomerular fractions. Glomeruli from diabetic rats studied in vivo contained 75% less $^{35}SO_4$ in glycosaminoglycans and glycopeptides at the time of maximum labeling (Table 1). Reduced uptake by glomerular fractions was mainly responsible for this difference due to the low relative incorporation of $^{35}SO_4$ into GBM (approximately 1% of the total $^{35}SO_4$ uptake). The reduced labeling of ^{35}S-glycosaminoglycan and ^{35}S-glycopeptide in whole glomeruli from diabetics examined in vivo as compared with controls was present at all time periods examined (Figure 2) despite lack of a significant difference in plasma ^{35}S levels (Figure 3). In addition, when total ^{35}S-glycosaminoglycans were compared, the increment between 2 and 4 h in controls was

FIGURE 2. Glomerular (excluding GBM fractions) ^{35}S-glycosaminoglycan and ^{35}S-glycopeptide metabolism in vivo.

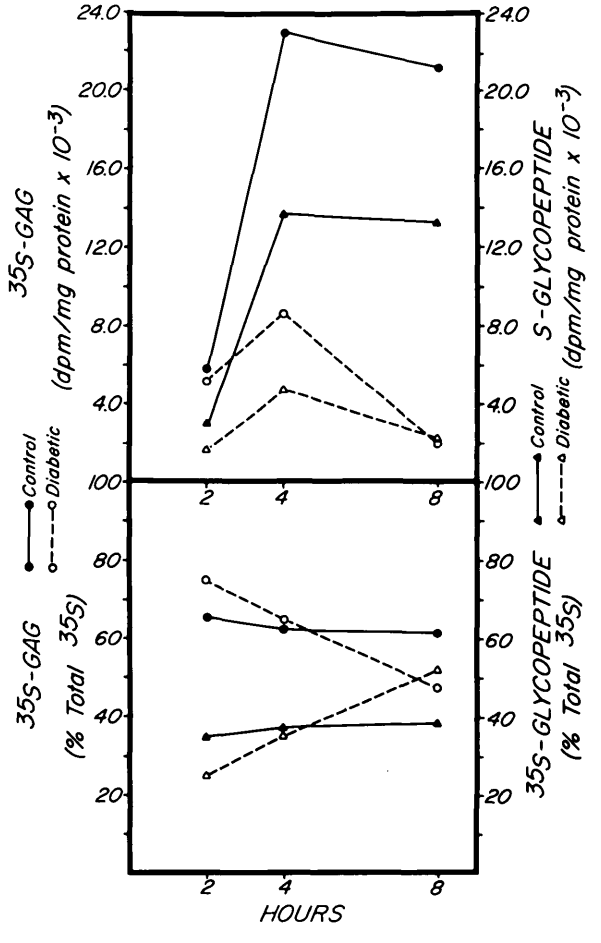


TABLE 1
Uptake of $^{35}SO_4$ into glomerular glycosaminoglycans (GAG) and glycopeptides (GP) of diabetic and control rats in vivo* and in vitro†

	Total ^{35}S-GAG;-GP (dpm)	Glomeruli × 10^6	dpm/10^6 Glomeruli	Dry wt. GBM (mg)	dpm/mg GBM	Protein GBM (mg)	dpm/mg GBM Protein
In vivo							
Control GBM	2,596	43.8	59	34.8	75	17.5	148
Glomerular fractions‡	306,228		6,991		8,800		17,499
Total ^{35}S-GAG all fractions			7,050		8,875		17,647
Diabetic GBM	2,118	54.2	39	35.6	60	18.5	114
Glomerular fractions	94,914		1,751		2,666		5,130
Total ^{35}S-GAG all fractions			1,790		2,726		5,244
In Vitro							
Control GBM	2,365			26.3	90		
Glomerular fractions	23,987				912		
Control medium	9,654				367		
Total ^{35}S-GAG all fractions					1,369		
Diabetic GBM	1,716			29.9	57		
Glomerular fractions	8,804				294		
Diabetic medium	16,168				541		
Total ^{35}S-GAG all fractions					892		

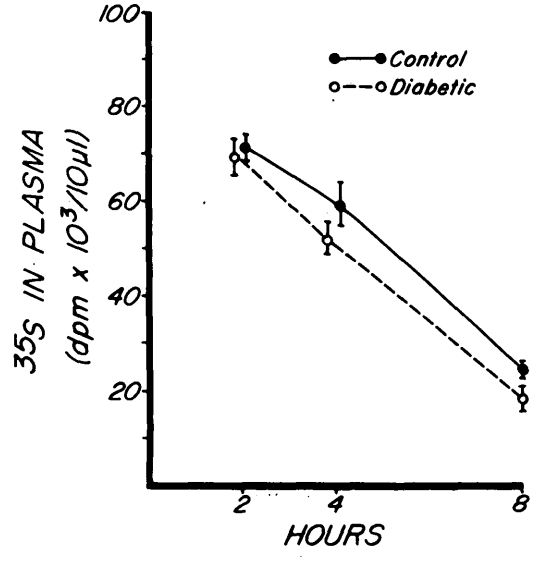
* Diabetic and control rats were injected with 1 mCi/250 g body wt intraperitoneally and killed after 4 h.
† Isolated glomeruli from diabetic and control rats were incubated in 0.14 mM Na $^{35}SO_4$ RPMI medium for 24 h; 0.6 × 10^6 glomeruli were used at each incubation.
‡ Glomerular fractions corresponding to H_2O, DNase, and deoxycholate-derived fractions.

288% and in diabetics 68% followed by a 7% decrease between 4 and 8 h in controls versus a 77% decrease in diabetic glomeruli. The metabolic turnover of ^{35}S-glycopeptides had a similar pattern, the increment in control glomeruli between 2 and 4 h of 343% versus 173% in diabetics followed by a decrease between 4 and 8 h of 2% in controls compared with a 31% decrease in diabetic glomeruli. Thus, in diabetic glomeruli in vivo the rate of synthesis (as judged by the magnitude of uptake of $^{35}SO_4$) of ^{35}S-glycosaminoglycans and ^{35}S-glycopeptides by diabetic glomeruli was decreased, while the rate of disappearance of incorporated label was increased, suggesting either decreased retention of labeled material in tissue or increased degradation in addition to the decreased synthesis.

The majority of the total ^{35}S from both the diabetic and control glomeruli peak excluded from Sephadex G-50 chromatographic columns consisted of ^{35}S-glycosaminoglycans at all time periods (Figure 4A). Relatively larger proportions of the chromatographically included ^{35}S-labeled material (Figure 4B) was attributed to ^{35}S-glycopeptides. Thus, as determined by the specific degradative procedures, at 2, 4, and 8 h, 85.3%, 87.4%, and 77.2%, respectively, of the total ^{35}S-labeled material from control glomeruli and 89.3%, 83.0%, and 72.4%, respectively, from diabetic glomeruli that were excluded from the chromatographic column was ^{35}S-glycosaminoglycans. During corresponding times, 68.3%, 68.2%, and 60.2%, respectively, of the chromatographically included ^{35}S-labeled material from control glomeruli and 49.9%, 56.0%, and 71.0% from diabetic glomeruli was attributable to ^{35}S-glycopeptides, defined as nondegradable ^{35}S-labeled material. Thus, relatively small amounts of the ^{35}S-glycosaminoglycans had low molecular weights to permit their inclusion in Sephadex G-50 columns. The percent distributions of these glycosaminoglycans in chondroitin sulfate, dermatan sulfate, N-sulfated, and N-acetylated heparan sulfate in diabetic and control glomeruli (excluding the GBM fractions) after in vivo (Table 2) or in vitro (Table 3) (whole glomeruli, GBM fractions, and incubation medium) treatment were similar.

Diabetic GBM fractions isolated after in vivo $^{35}SO_4$ tissue labeling showed a substantial decrease in total ^{35}S-glycosaminoglycans at all time periods examined, as was the case in whole glomeruli (Figure 5). The absolute amount of HNO_2-degradable ^{35}S-heparan sulfate was also substantially decreased at each of these times (Figure 6). Despite this decrease in total ^{35}S-heparan sulfate synthesis, ^{35}S-labeled diabetic GBM had a proportionately equal susceptibility to HNO_2 degradation, thus indicating an equivalent proportion of N-sulfation of heparan sulfate. This implies that a decrease in total uptake of $^{35}SO_4$ by diabetic GBM is responsible for the decrease in N-sulfated heparan sulfate and not a selective block in heparan sulfate synthesis, although fur-

FIGURE 3. ^{35}S in plasma.



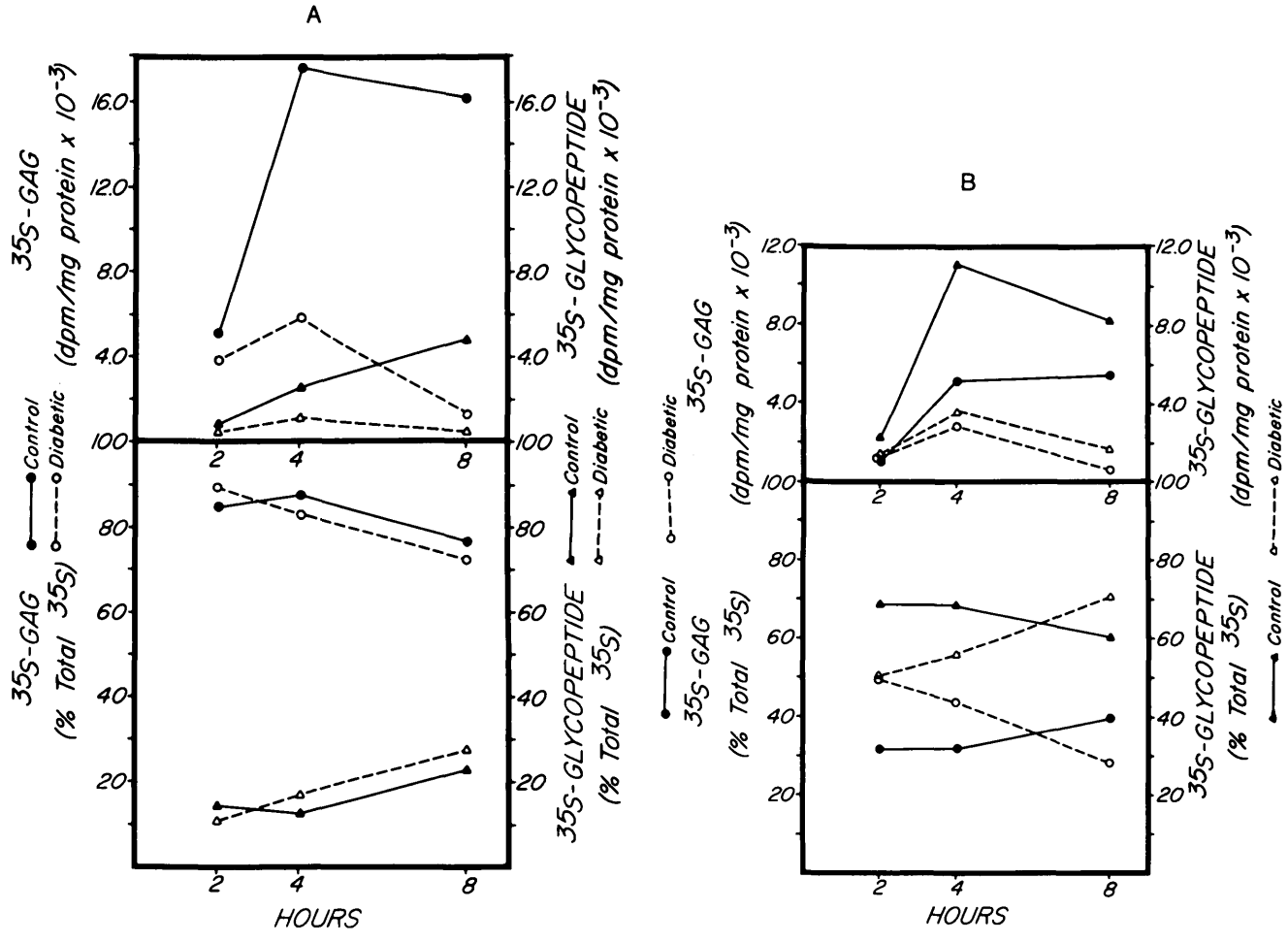


FIGURE 4. Distribution of ^{35}S-glycosaminoglycan and -glycopeptide from glomeruli labeled in vivo by Sephadex G-50 chromatography. (A) Proportions in excluded chromatographic volume; (B) proportions in included chromatographic volume.

ther identification of ^{35}S-glycosaminoglycan subspecies in GBM from in vivo studies was not possible due to the relatively low uptake of $^{35}SO_4$. The peak HNO_2-degradable ^{35}S-glycosaminoglycans at 4 h in diabetic GBM was 56.5% of control between 4 and 8 h, HNO_2-degradable ^{35}S-glycosaminoglycans decreased by 56.2% in control GBM, while there was an 83.3% decrement in the diabetic GBM. In fact, by 8 h, ^{35}S-glycosaminoglycans attributable to N-sulfated heparan sulfate in diabetic GBM were almost at baseline. Thus, as was the case with total ^{35}S-glycosaminoglycans in whole glomeruli, the amount of ^{35}S-glycosaminoglycans attributable to N-sulfated heparan sulfate in GBM was decreased due to decreased synthesis, decreased tissue retention, or accelerated degradation.

TABLE 2
Percentage distribution of ^{35}S in glycosaminoglycans and glycopeptides in whole glomeruli minus GBM fractions: in vivo synthesis*

Time (h)	Chondroitin sulfate	Dermatan sulfate	Heparan sulfate			^{35}S-glycopeptides†
			Percent	Percent N-sulfated	Percent N-acetylated	
Control						
2	11.7	7.4	46.4	89.2	10.8	34.5
4	13.4	6.2	43.0	90.2	9.8	37.4
8	9.7	8.1	43.9	93.6	6.4	38.3
Diabetic						
2	14.4	14.2	46.4	84.1	15.9	25.1
4	9.2	4.9	50.6	83.8	16.2	35.3
8	8.1	6.7	32.5	82.2	17.8	52.6

* Experimental conditions described in Table 1 and the text.
† Glycosaminoglycans that are HNO_2-resistant and that remain in void volume from the G-50 Sephadex column or other high-molecular-weight peaks that do not move further into the column after enzyme digestion.

TABLE 3
Distribution of ^{35}S-GAG in diabetic glomeruli after in vitro labeling*

Glomerular fraction	Chondroitin SO_4	Dermatan SO_4	N-Sulfated heparan	N-acetylated heparan SO_4 plus ^{35}S-glycopeptides†
Control GBM	21.6	15.2	31.4	31.8
Glomerular fractions‡	39.2	15.9	27.8	17.1
Incubation medium	40.0	1.6	43.6	14.8
Percent distribution of ^{35}S-GAG in all glomerular fractions plus medium	38.6	9.2	36.1	16.1
Diabetic GBM	20.8	12.0	15.4	51.8
Glomerular fractions	29.3	11.9	31.1	27.7
Incubation medium	28.9	6.0	35.7	29.4
Percent distribution of ^{35}S-GAG in all glomerular fractions plus medium	28.5	7.1	36.2	28.2

* Experimental conditions as described in Table 1 and in the text.
† The ^{35}S-labeled material from the combined excluded and included fractions of the Sephadex G-50 chromatography that were HNO_2-resistant and that did not move further into the column after enzyme digestion, which did not permit distinction between N-acetylated heparan sulfate and sulfated glycopeptides.
‡ Explanation in Table 1.

In vitro ^{35}S-glycosaminoglycan metabolism by glomerular fractions. Incorporation of $^{35}SO_4$ into total glycosaminoglycans plus glycopeptides by diabetic glomeruli in vitro was decreased (Table 1) and was reflected in decreased ^{35}S in glomerular fractions and in GBM (showing a 36% decrease in $^{35}SO_4$ uptake on a dpm/mg GBM basis). However, in the case of diabetic glomeruli, larger absolute amounts (541 versus 367 dpm/mg GBM for controls) and a larger proportion (60.6%) of the total ^{35}S-incorporated material were found in the incubation medium as compared with the control glomerular medium (26.1%), thus suggesting both decreased synthesis and altered metabolism of ^{35}S-incorporated material by diabetic glomeruli.

When the ^{35}S-labeled material from the incubation medium of in vitro incubations of control and diabetic glomeruli were each separated by Sephadex G-50 chromatography (Figure 7), a larger proportion of the total ^{35}S-labeled material in the incubation medium from diabetic glomeruli was found in the void volume, although the patterns of HNO_2 sensitivity were similar. Thus, the majority of the ^{35}S-labeled polysaccharide found in diabetic glomerular fractions in vitro represented ^{35}S-glycosaminoglycans. This observation is consistent with decreased tissue retention or increased degradation of ^{35}S-glycosaminoglycans by diabetic glomeruli.

As in the in vivo studies, the percent of the $^{35}SO_4$ label attributable to the various glycosaminoglycan subspecies in the glomeruli of diabetic and control animals was equivalent, although the proportion of total ^{35}S-glycosaminoglycan in chondroitin sulfate and dermatan sulfate was higher in in vitro incubations (Table 1) than in the in vivo study (Table 2).

Aorta ^{35}S-glycosaminoglycan metabolism. Total ^{35}S-glycosaminoglycan synthesis by diabetic aorta in vivo was markedly decreased at all labeling times (by 67.0%, 78.2%, and 74.0% at 2, 4, and 8 h, respectively) (Figure 8) with proportionate decreases of all ^{35}S-glycosaminoglycan components. ^{35}S-heparan sulfate was highly N-sulfated at all time periods (68.5%, 55.1%, and 95.2% in controls and 86.1%, 95.7%, and 62.1% in diabetics), and greater than 97% of all the ^{35}S-labeled material from aortas was degraded by se-

FIGURE 5. Total ^{35}S-glycosaminoglycan plus ^{35}S-glycopeptide in GBM fraction labeled in vivo.

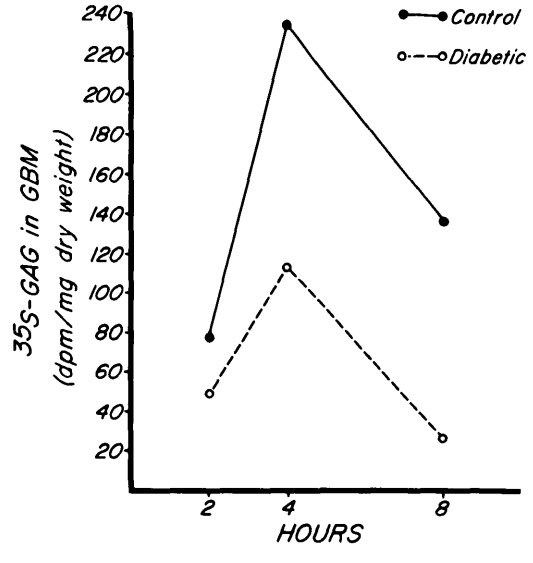
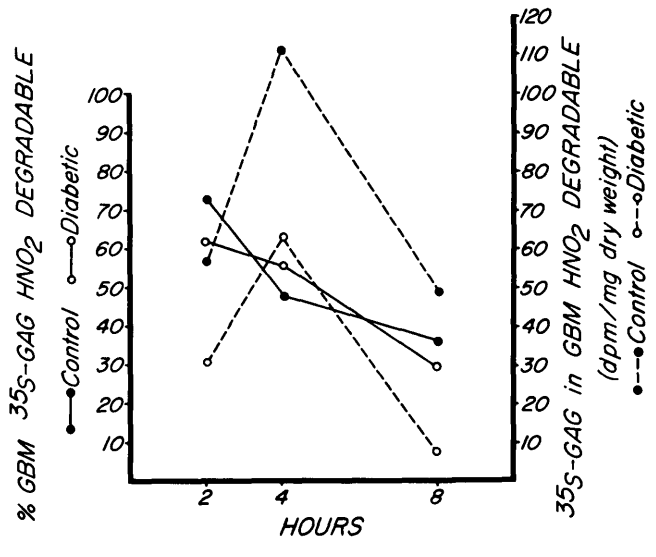


FIGURE 6. HNO_2 sensitivity of ^{35}S-glycosaminoglycan plus -glycopeptide in GBM fraction labeled in vivo.



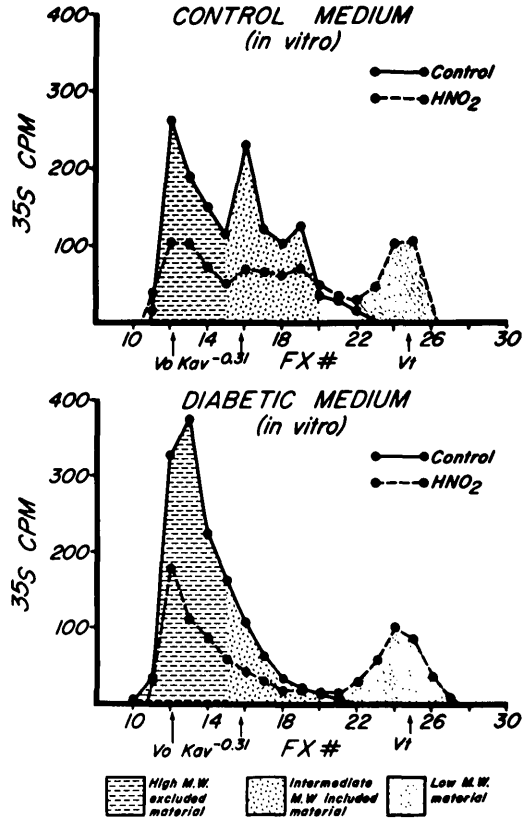
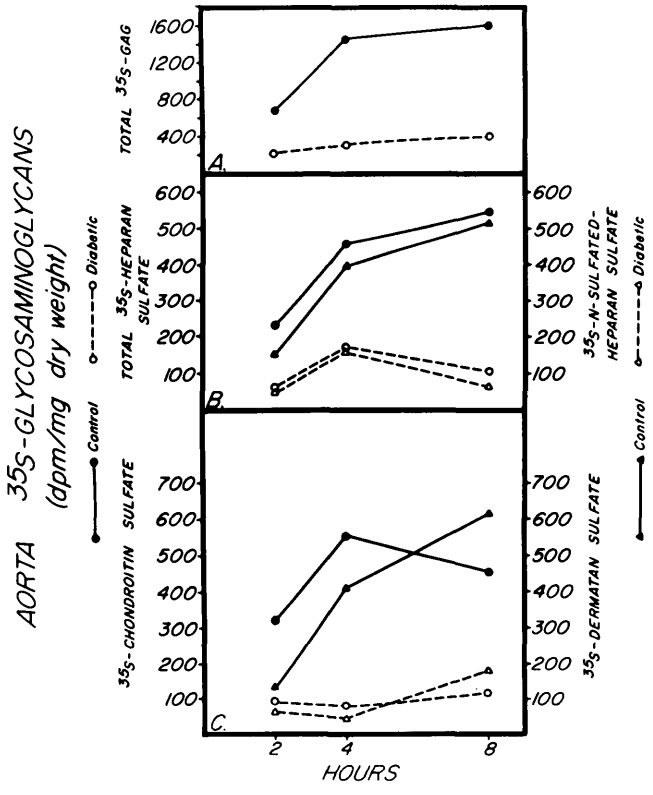


FIGURE 7. Sephadex G-50 chromatography of ^{35}S-labeled material from incubation medium of glomeruli labeled in vitro.

FIGURE 8. Aorta ^{35}S-glycosaminoglycan metabolism in vivo. A: Total ^{35}S-glycosaminoglycans; B: total and N-sulfated ^{35}S-heparan sulfate; C: ^{35}S-chondroitin sulfate and ^{35}S-dermatan sulfate.



quential degradative procedures, thus suggesting the presence of minimal ^{35}S-glycopeptides in this tissue.

DISCUSSION

Proteoglycans are large-molecular-weight (approximately 2.5×10^6 daltons) compounds containing a protein core covalently linked to large carbohydrate moieties known as glycosaminoglycans, which constitute 90% of the weight of the molecule.[26] Heparan sulfate proteoglycans have been isolated from basement membranes of murine sarcoma cells[27] and from liver plasma membranes[28] that have 50% and 25% carbohydrate content and molecular weights of $0.5–1 \times 10^6$ and 8×10^4 daltons, respectively. These protein polysaccharide compounds are known to interact with other connective tissue components such as fibronectin[29] and collagen-like basement membrane components.[14] The present study revealed a decrease in the amount of total ^{35}S-labeled glycosaminoglycan and ^{35}S-glycopeptide in diabetic glomeruli, GBM, and aorta at all time periods studied in vivo. In vitro data supported these conclusions, showing 75% less ^{35}S-incorporated material in the diabetic glomeruli at the time of maximum labeling. They furthermore revealed an increased proportion of ^{35}S-labeled material in the incubation medium from diabetic glomerular incubations. The increased ^{35}S-glycosaminoglycans and ^{35}S-glycopeptides found in the medium may have been due to a diminished ability of the normally highly negatively charged proteoglycans from interacting with other connective tissue components in the diabetic glomerulus.[14,29] This altered interaction may be due to a change in quantities or charge distribution of proteoglycans, to cleavage of covalent bonds involved in proteoglycan linkage to other matrix components, or to altered collagen synthesis[4,30] or other connective tissue components in diabetic glomeruli.

Increased turnover of ^{35}S-labeled glycosaminoglycans may lead to their decreased retention in diabetic glomeruli. The latter mechanism is supported by the increase in the ^{35}S-glycosaminoglycan disappearance rates in diabetic glomeruli (Figure 2). Although little is known about proteoglycan degradative processes in glomeruli, acid hydrolases have been found to degrade glycosaminoglycans, and the levels of these enzymes in the plasma, urine, and kidneys are altered in experimental diabetes.[31,32] Plasma activities of some acid hydrolases have been found to be increased in diabetes despite depression of renal homogenate tissue activity. That the disorder of glycosaminoglycan metabolism present in the glomeruli of experimentally diabetic animals might be a more generalized phenomenon is revealed by a similar decrease in aortic incorporation of $^{35}SO_4$ label into glycosaminoglycan.

The functional role of proteoglycans in normal and diabetic glomeruli remains uncertain. Recent studies with kidneys perfused with heparitinase, which selectively degrades heparan sulfate, showed that the permeability of GBM is altered by removal of the heparan sulfate.[33] This suggests that abnormalities in glycosaminoglycan metabolism may alter the GBM filtration barrier resulting in the proteinuria that is characteristic of the diabetic nephropathy early in its course.[34,35] Proteoglycans have a variety of functions and properties that could be influenced by abnormalities in glycosaminoglycan metabolism found in the experimental diabetic glomerulopathy. These include alteration of

collagen fibrillogenesis,[36] cation binding,[37] inhibition of platelet aggregation,[38] and the unique property of occupancy of a large molecular volume domain due to the configuration of the intact molecular macro aggregates.[39] Abnormal glomerular mesangial macromolecular processing in diabetes[40] may result from disturbances in glomerular proteoglycan metabolism. Altered glycosaminoglycan metabolism in the diabetic aorta may be important in the pathogenesis of atherosclerosis in that it has been shown that low density lipoproteins bind to glycosaminoglycans.[41]

The present study has confirmed that the glomerulus contains relatively large amounts of ^{35}S-glycopeptides[22] similar to those demonstrated in both kidney tumor cells[24] and fetal lung.[25] A sulfated glycoprotein associated with basement membranes has recently been described and has been postulated to interact with extracellular matrix material.[42] The locations and functions of these sulfated glycoproteins is not known, although it has been speculated that they may serve important functions in cell surface properties. Interestingly, in this study little ^{35}S-glycopeptide has been found in aorta. As was the case with glomerular ^{35}S-glycosaminoglycans, synthesis of glomerular ^{35}S-glycopeptides was decreased and degradation increased in diabetes. It is possible that these ^{35}S-glycopeptides represent an alternate pathway of acid glycopeptide metabolism. There was no evidence in the present study that diabetes selectively altered glomerular ^{35}S-metabolism, as the magnitudes of the decreased synthesis and increased degradation of both ^{35}S-glycosaminoglycans and ^{35}S-glycopeptides in diabetes were similar.

Recent work with antisera to defined aorta-derived proteoglycans has suggested that there are unique characteristics of glomerular endothelial cell-derived and epithelial cell-derived proteoglycans,[12] each with unique specificity for GBM components. Thus GBM-related proteoglycans derived from glomerular endothelial and epithelial cells each may have unique chemical characteristics that are differentially influenced by the diabetic state. Further definition of proteoglycan metabolism in diabetic kidneys will necessitate a search for the localization of the various proteoglycan subspecies at different filtration sites in the GBM and within other glomerular structures.

ACKNOWLEDGMENTS

The authors are grateful for the technical expertise of Audrey Bernstein and for the manuscript preparation by Mary Jo Jansen and Marshall Hoff. The work was supported in part by grants from the NIH (AM-17697, HL-21612), the Juvenile Diabetes Foundation, and the Viking Children's Fund.

REFERENCES

[1] Steffes, M. W., Brown, D. M., Basgen, J. M., Matas, A., and Mauer, S. M.: Glomerular basement membrane thickness following islet transplantation in the diabetic rat. Lab. Invest. *41*:116–18, 1979.

[2] Steffes, M. W., Brown, D. M., Basgen, J. M., and Mauer, S. M.: Amelioration of mesangial volume and surface alterations following islet transplantation in diabetic rats. Diabetes *29*:509–15, 1980.

[3] Spiro, R. G.: Search for a biochemical basis of diabetic microangiopathy. Diabetologia *12*:1–14, 1976.

[4] Brownlee, M., and Spiro, R. G.: Glomerular basement membrane metabolism in the diabetic rat. In vivo studies. Diabetes *28*:121–25, 1979.

[5] Haft, D. E., and Reddi, A. S.: Glucosyltransferase activity in kidney fractions of normal and streptozotocin-diabetic rats. Biochim. Biophys. Acta *584*:1–10, 1979.

[6] Chang, A. Y., and Noble, R. E.: 5-Hydroxymethylfurfural-forming proteins in the renal glomeruli of control and streptozotocin-diabetic rats. Life Sci. *26*:1329–33, 1980.

[7] Taylor, S. A., Price, R. G., Kang, S. S., and Yudkin, J.: Modification of the glomerular basement membrane in sucrose-fed and streptozotocin-diabetic rats. Diabetologia *19*:364–72, 1980.

[8] Cohen, M. P., Urdanivia, E., Surma, M., and Ciborowski, C. J.: Nonenzymatic glycosylation of basement membranes. In vitro studies. Diabetes *30*:367–71, 1981.

[9] LePape, A. L., Buitton, J.-D., and Muh, J.-P.: Modification of glomerular basement membrane cross-links in experimental diabetic rats. Biochem. Biophys. Res. Commun. *100*:1214–21, 1981.

[10] Cohen, M. P., Dasmahapatra, A., and Wu, V.-Y.: Deposition of basement membrane in vitro by normal and diabetic rat renal glomeruli. Nephron *27*:146–51, 1981.

[11] Kanwar, Y. S., and Farquhar, M. G.: Presence of heparan sulfate in the glomerular basement membrane. Proc. Natl. Acad. Sci. USA *76*:1303–1307, 1979.

[12] Klein, D. J., Oegema, T. R., Eisenstein, R., Furcht, L., Michael, A. F., and Brown, D. M.: Glomerular localization of proteoglycans by immunohistochemistry. Fed. Proc. *40*:830, 1981.

[13] Kanwar, Y. S., and Farquhar, M. G.: Isolation of glycosaminoglycans (heparan sulfate) from glomerular basement membranes. Proc. Natl. Acad. Sci. USA *76*:4493–97, 1979.

[14] Parthasarathy, N., and Spiro, R. G.: Characterization of the glycosaminoglycan component of the renal glomerular basement membrane and its relationship to the peptide portion. J. Biol. Chem. *56*:507–13, 1981.

[15] Kanwar, Y. S., Hascall, V. C., and Farquhar, M. G.: Partial characterization of newly synthesized proteoglycans isolated from the glomerular basement membrane. J. Cell Biol. *90*:527–32, 1981.

[16] Brown, D. M., Michael, A. F., and Oegema, T. R.: Glycosaminoglycan synthesis by glomeruli in vivo and in vitro. Biochim. Biophys. Acta *674*: 96–104, 1981.

[17] Mauer, S. M., Sutherland, D. E. R., Steffes, M. W., Leonard, R. J., Najarian, J. S., Michael, A. F., and Brown, D. M.: Pancreatic islet transplantation: effects on the glomerular lesions of experimental diabetes in the rat. Diabetes *23*:748–53, 1974.

[18] Blau, E. B., and Michael, A. F.: Rat glomerular basement membrane composition and metabolism in aminonucleoside nephrosis. J. Lab. Clin. Med. *77*:97–109, 1971.

[19] Houser, M. T., Scheinman, J. I., Basgen, J., Steffes, M. W., and Michael, A. F.: Heterogeneity of isolated glomerular basement membrane. J. Clin. Invest. In press.

[20] Carlson, E. C., Brendel, K., Hjelle, J. T., and Meezan, E.: Ultrastructural and biochemical analyses of isolated basement membranes from kidney glomeruli and tubules and brain and retinal microvessels. J. Ultrastruct. Res. *62*:26–53, 1978.

[21] Conrad, G. W., Hamilton, C., and Haynes, E.: Differences in glycosaminoglycans synthesized by fibroblast-like cells from chick cornea, heart, and skin. J. Biol. Chem. *252*:6861–70, 1977.

[22] Lemkin, M. C., and Farquhar, M. G.: Sulfated and non-sulfated glycosaminoglycans and glycopeptides are synthesized by kidney in vivo and incorporated into glomerular basement membranes. Proc. Natl. Acad. Sci. USA *78*:1726–30, 1981.

[23] Linker, A., and Hovingh, P.: Heparinase and heparitinase from flavobacteria. Methods Enzymol. *38*:902–11, 1972.

[24] Heifetz, A., and Prager, M. D.: The effect of butyrate on sulfated glycoprotein biosynthesis by human kidney tumor cells. J. Biol. Chem. *256*: 6529–32, 1981.

[25] Heifetz, A., and Lennarz, W. J.: Synthesis of a novel class of glycoproteins in embryonic liver and lung. J. Biol. Chem. *254*:6119–27, 1979.

[26] Hascall, V. C.: Interaction of cartilage proteoglycans with hyaluronic acid. J. Supromol. Struct. *7*:101–20, 1977.

[27] Hassell, J. R., Robey, P. G., Barrach, H.-J., Wilczek, J., Rennard, S. I., and Martin, G. R.: Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc. Natl. Acad. Sci. USA *77*:4494–98, 1980.

[28] Kjellen, L., Pettersson, I., and Hook, M.: Cell-surface heparan sulfate: an intercalated membrane proteoglycan. Proc. Natl. Acad. Sci. USA *78*:5371–75, 1981.

[29] Perkins, J. E., Tae, H. Ji, and Hynes, R. O.: Cross-linking of fibronectin to sulfated proteoglycans at the cell surface. Cell *16*:941–52, 1979.

[30] Cohen, M. P., and Vogt, C.: Evidence for enhanced basement membrane synthesis and lysine hydroxylation in renal glomerulus in experimental diabetes. Biochem. Biophys. Res. Commun. *49*:1542–46, 1972.

[31] Fishima, H., and Tarui, S.: β-glycosidases and diabetic microangiopathy. 1. Decreases of β-glycosidase activities in diabetic rat kidney. J. Biochem. (Tokyo) *79*:265–70, 1976.

[32] Chang, A. Y.: Acid glycohydrolase in Chinese hamster with spontaneous diabetes. 1. Depressed levels of renal α-galactosidase and β-galactosidase. Biochim. Biophys. Acta *522*:491–502, 1978.

[33] Kanwar, Y. S., Linker, A., and Farquhar, M. G.: Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion. J. Cell Biol. *86*:688–93, 1980.

[34] Lopes-Virella, M. F., Virella, G., Rosebrock, G., Sgel, J., Gonzalez, J., and Colwell, J.: Early diagnosis of renal malfunction in diabetes. Abnormal

proteinuria revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Diabetologia *16*:165–71, 1979.

[35] Carrie, B. J., and Myers, B. D.: Proteinuria and functional characteristics of the glomerular barrier in diabetic nephropathy. Kidney Int. *17*:669–76, 1980.

[36] Oegema, T. R., Laidlow, J., Hascall, V. C., and Dziewiatkowski, D. D.: The effect of proteoglycans on the formation of fibrils from collagen solutions. Arch. Biochem. Biophys. *170*:698–709, 1975.

[37] Harris, G. S.: A relationship among arterial wall acid mucopolysaccharides, cation binding and arterial reactivity. Circ. Res. *26* (Suppl. II):91–97, 1970.

[38] Klein, K., Sinzinger, H., Silberbauer, K., Stachelberger, H., and Leithner, C.: Influence of proteoglycans (PG) and glycosaminoglycans (GAG) on ADP-collagen- and thrombin-induced platelet aggregation. Artery *8*:410–15, 1980.

[39] Comper, W. D., and Laurent, T. C.: Physiological function of connective tissue polysaccharides. Physiol. Rev. *58*:255–315, 1978.

[40] Bower, G., Brown, D. M., Steffes, M. W., Vernier, R. L., and Mauer, S. M.: Studies of the glomerular mesangium and the juxtaglomerular apparatus in the genetically diabetic mouse. Lab. Invest. *43*:333–41, 1980.

[41] Vijayagopal, P., Srinivasan, S. R., Radhakrishnamurthy, B., and Berenson, G. S.: Interaction of serum lipoproteins and a proteoglycan from bovine aorta. J. Biol. Chem. *256*:8234–41, 1981.

[42] Carlin, B., Jaffe, R., Bender, B., and Chung, A. E.: Entactin, a novel basal lamina-associated sulfated glycoprotein. J. Biol. Chem. *256*:5209–14, 1981.