Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6499–6503, September 1986
Cell Biology

Xylosylated-proteoglycan-induced Golgi alterations

(glomerular epithelial cells/sulfated glycosaminoglycan/basement membrane)

YASHPAL S. KANWAR*, LIONEL J. ROSENZWEIG†, AND MICHAEL L. JAKUBOWSKI*

*Department of Pathology, Northwestern University Medical School, Chicago, IL 60611; and †Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108

Communicated by Emanuel Margoliash, May 27, 1986

ABSTRACT **The effect of *p*-nitrophenyl β-D-xylopyranoside on the Golgi apparatus and proteoglycans (PG) of the renal glomerulus was investigated in an isolated kidney organ perfusion system and monitored by utilizing [^{35}S]sulfate as the PG precursor. By electron microscopy, a selective intracytoplasmic vesiculization of Golgi apparatus of visceral epithelium was observed in the β-xyloside-treated kidneys. Electron microscopic autoradiography revealed most grains localized to the intracytoplasmic Golgi-derived vesicles, while very few grains were associated with the extracellular matrix membranes. Biochemically, a 2.3-fold increase in cellular matrix and a reduction by a factor of 1.7 in extracellular matrix of [^{35}S]sulfate incorporation was observed. Besides a larger macromolecular form (K_{avg} = 0.25; M_r = 130,000), lower molecular weight PGs were recovered in the cellular (K_{avg} = 0.46, M_r = 30,000) and matrical (K_{avg} = 0.42, M_r = 45,000) compartments after xyloside treatment. The xyloside treatment increased the incorporated radioactivity, mostly included in free glycosaminoglycans and small PGs, in the media fraction by 3.8-fold. These data indicate that xyloside induces a dramatic imbalance in the *de novo*-synthesized PGs of cellular and extracellular compartments and that cellular accumulation of xylosylated (sulfated) PGs selectively alters the Golgi apparatus of the glomerular epithelial cell, the cell that actively synthesizes PGs.**

The proteoglycans (PGs) are comprised of glycosaminoglycan (GAG) chains covalently bound by *O*-glycosidic linkage to the core protein via galactosylgalactosylxylosylserine (for review, see ref. 1). The GAG chains are transferred onto the core protein in a stepwise manner and are initiated by UPD-D-xylose:core-protein xylosyltransferase followed by additional transferases for completion of these chains. In the final step, these chains are *O* or *N*-sulfated in the Golgi saccules (1, 2). The addition of GAG chains onto the core protein can be competitively inhibited by the presence of D-xylose or β-xyloside in the culture medium, because the xyloside itself acts as an initiator of chain formation, bypassing the requirement for the receptor core protein and xylosyltransferase. Not incorporated onto the core protein, these xyloside-initiated chains are discharged readily into the medium. Thus, in the presence of xyloside, an incomplete macromolecular form of PG occurs along with a net "stimulation" of "free" GAG chain synthesis. Such intriguing biochemical effects of xyloside on PG synthesis have been investigated in a wide variety of systems (3–10). However, the effect of this increased synthesis and intracellular accumulation of sulfated GAG chains on the cellular organelles remains undocumented. We report discrete cellular changes in the renal epithelium that may be induced by intracellular accumulation of sulfated GAG chains under the influence of xyloside.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Radiolabeling of Glomerular Cells and Matrices and Preparation of Electron Microscopic Autoradiograms. Glomerular cells and matrices were radiolabeled under sterile conditions in an *ex vivo* organ perfusion system as detailed in our previous publications (11, 12). [^{35}S]sulfate (Amersham) was the precursor for labeling of glomerular PGs or GAGs. Satisfactory labeling was achieved by constantly recirculating [^{35}S]sulfate (500 μCi/ml; 1 Ci = 37 GBq; specific activity, >1300 Ci/mmol) contained in a chemically defined perfusion medium (11, 12). To perturb the cellular synthesis of PGs/GAGs, the perfusate included 2.5 mM of *p*-nitrophenyl β-D-xylopyranoside (Sigma). The kidneys from 1- to 7-hr intervals were processed only for biochemical determination of incorporated radioactivity, and each kidney at the final time point of 7 hr was additionally processed for electron microscopy and autoradiography (11, 12).

Isolation and Characterization of PGs from Cells, Matrices and Media. After 1–7 hr of radiolabeling, the kidneys were perfused with Krebs–Ringer bicarbonate containing 1% bovine serum albumin and a mixture of protease inhibitors (10 mM 6-aminohexanoic acid, 5 mM benzamidine-hydrochloride, and 1 mM phenylmethylsulfonyl fluoride) at pH 7.0, and glomeruli were isolated by the sieving technique (11–13). The cellular PGs were extracted for 12 hr at 4°C with 1% deoxycholate containing 10 mM sodium EDTA, sodium acetate, and the mixture of protease inhibitors at pH 5.8. The solubilized cellular PGs were separated from the glomerular extracellular matrices (GEMs) by sedimenting the latter at ×3000 rpm in an IEC (International Equipment) refrigerated centrifuge. The sedimented GEMs were extracted with 4 M guanidine-hydrochloride containing 10 mM sodium EDTA and sodium acetate at pH 5.9 for 48 hr at 4°C in the presence of protease inhibitors (11–13). The unextracted residue was pelleted by centrifugation at ×10,000 rpm in a Beckman microfuge. The residue was then reextracted with 0.5 M sodium hydroxide at 60°C for 3 hr to solubilize the remaining GAGs. GEM-associated PGs were designated to be in the guanidine-hydrochloride and the cellular PGs were designated to be in the deoxycholate extract. All the extracts were extensively dialyzed against distilled water containing 1 mM phenylmethylsulfonyl fluoride and diisopropyl fluorophosphate at 4°C. The PGs/GAGs from the media fractions (xyloside and control) were isolated by applying them to a Sephadex G-50 column and purified by DEAE-Sephacel chromatography (13, 14). Aliquots were assayed for protein contents and total radioactivities. Techniques for characterization of PGs/GAGs (11–13) and determination of molecular weight (15–17) have been previously reported.

Abbreviations: GEM, glomerular extracellular matrix; GBM, glomerular basement membrane; HS-PG, heparan sulfate-proteoglycan; CS-PG, chondroitin sulfate proteoglycan; PG, proteoglycan; GAG, glycosaminoglycan.

RESULTS

General Morphology and Autoradiography of the Renal Glomerulus. The renal glomerulus is a network of capillaries (Fig. 1*A*) made up of epithelial, endothelial, and mesangial cells—and their cytosecretory products [basement membrane (GBM) and mesangial matrix; the latter two together are referred to as extracellular matrices (GEM)]. Heparan sulfate-proteoglycan (HS-PG) is regarded as the major PG of the GEMs, although minute amounts of chondroitin sulfate-proteoglycan (CS-PG) have also been reported (18, 19).

The general architecture of the glomerulus remained intact with no observable deterioration in the morphology resulting from perfusion with or without xyloside over a 7-hr period. Changes occurred mainly in the visceral epithelium in the form of cytoplasmic vesiculation (Fig. 1*A*). Devoid of clathrin-coat, the vesicles measured 10–100 nm and were distributed throughout the cytoplasm, appearing to originate from the terminal saccules of the Golgi apparatus. Besides this cytoplasmic vesiculation, no alterations in the mitochondria, rough endoplasmic reticulum, or nucleus were recognized. No vesicles associated with the plasmalemma were seen. Foot processes, emanating from the visceral epithelium, had a regular interdigitating arrangement with intact intervening slit diaphragms, and these processes remained firmly attached to the peripheral aspect of the GBM. No cytoplasmic changes were seen in the control kidneys. The endothelial and mesangial cells were unremarkable, and the morphology of the extracellular matrices—i.e., GEM—was not significantly altered; the electron density of the GBM was as usual with epithelial and endothelial components firmly adherent, and the mesangial matrix maintained its normal appearance and relationship to the mesangial cells.

By tissue autoradiography, a drastic increase in the [^{35}S]sulfate-associated silver grains was observed over the glomerular epithelium of the xyloside-treated kidneys (Fig. 1*B*). These grains were distributed throughout the cytoplasm but, for the most part, were excluded from the nucleus. They were associated with intracytoplasmic vesicles and Golgi saccules—the region where sulfotransferases seem to be localized and sulfation probably occurs. The autoradiographic grains were found in the Golgi saccules of the endothelial and mesangial cells; however, the experimental and control groups did not differ appreciably.

The dramatic increase in the autoradiographic grains over the epithelium after xyloside treatment meant that a differential accumulation of xylosylated-sulfated products had occurred. Conversely, a significant decrease in the autoradiographic grains over the GBM was seen (Fig. 1*B*), thereby indicating a dramatic reduction in the incorporation of *de novo*-synthesized PGs/GAGs into the extracellular matrical compartments.

Characterization of Glomerular and Media PGs/GAGs. Biochemical studies were done to ascertain the degree of

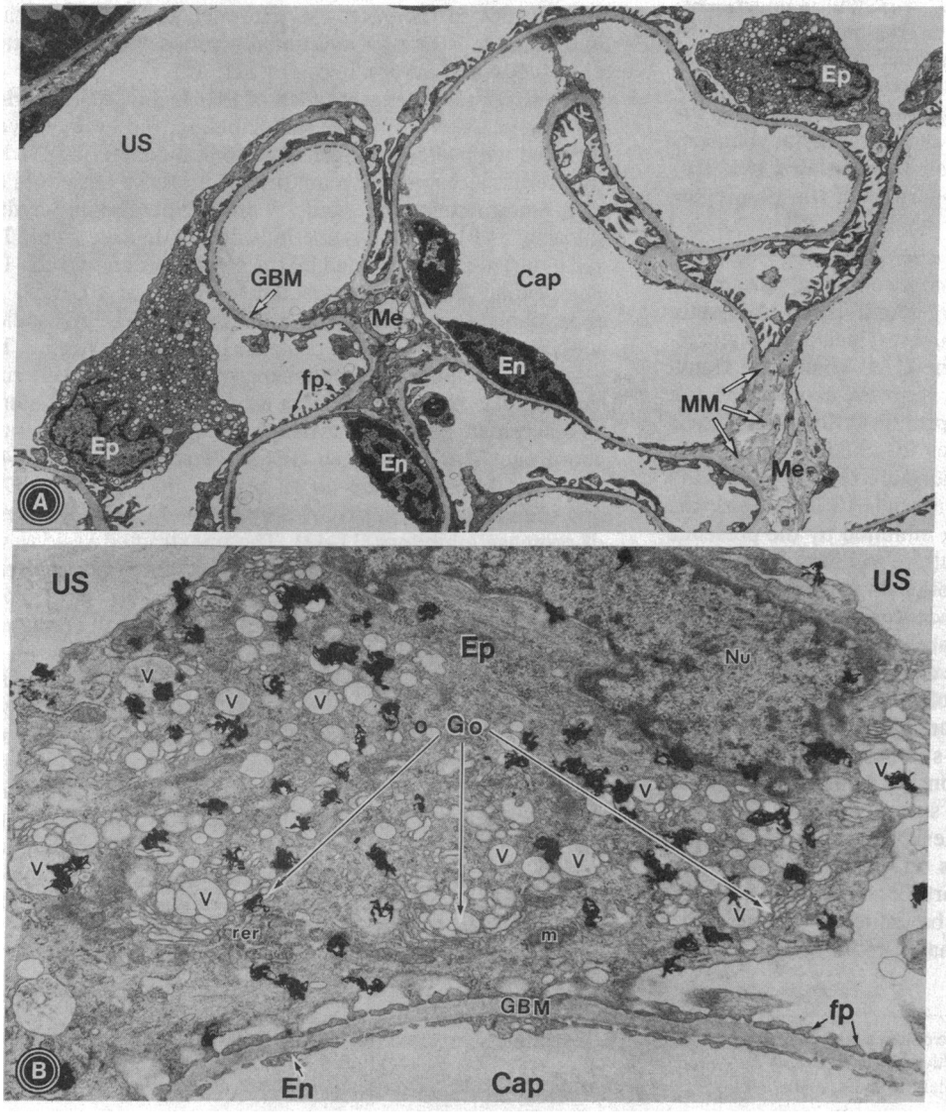

FIG. 1. (*A*) Electron micrograph of glomerular capillaries (Cap) from kidney treated with 2.5 mM xyloside for 7 hr in an isolated organ perfusion system. A uniform intracytoplasmic vesiculization of the visceral epithelium (Ep) is observed. The epithelial foot processes (fp) are normally arranged on the peripheral aspect of glomerular basement membrane (GBM). No changes are observed in the endothelium (En), mesangium (Me), basement membrane (GBM), and mesangial matrix (MM). (*B*) Electron micrograph of the glomerular visceral epithelium (Ep) of the kidney radiolabeled with [^{35}S]sulfate in the presence of xyloside. The vesicles (V) are distributed throughout the cytoplasm and more so in the vicinity of the Golgi (Go) complexes. The autoradiographic grains are either associated with vesicles or Golgi saccules. The rough endoplasmic reticulum (rer), mitochondria (m), nucleus (Nu), foot processes (fp), and endothelium (En) appear normal. No grains are seen over the basement membrane (GBM) in this micrograph. US, urinary space. (*A*, ×2800; *B*, ×10,500.)

Table 1. Radioactivity in glomerular extracellular matrices, cells, and media fractions

	Control	Xyloside
Glomerular cells		
Total, dpm	≈0.67 × 10^6	≈1.53 × 10^6
Peak A, %	100	≈10
Peak B, %	—	≈90
Extracellular matrix		
Total, dpm	≈0.72 × 10^6	≈0.43 × 10^6
Peak A, %	100	≈35
Peak B, %	—	≈65
Media fractions		
Total, dpm	≈14.55 × 10^6	≈55.48 × 10^6
Peak A, %	≈25	≈10
Peak B, %	≈75	≈90

imbalance, in the *de novo*-synthesized PGs, between cellular and extracellular compartments and to document that the synthesis of intracellular xylosylated GAGs/PGs had indeed been stimulated. Thus, the radioactivities incorporated into cells, matrices, and media were determined, and GAGs/PGs in the respective compartments were characterized.

The total incorporated radioactivities associated with cells and matrices were, respectively, ≈0.67 × 10^6 and ≈0.72 × 10^6 dpm per kidney in controls, and ≈1.53 × 10^6 and ≈0.43 × 10^6 dpm after xyloside treatment for 7 hr (Table 1); efficiency of extraction was >96%. A time-dependent trend of relatively higher [^{35}S]sulfate radioactivity per mg of protein was observed in the cellular compartment over the 1–7 hr treatment with xyloside (Fig. 2).

The "cellular" PGs in controls (Fig. 3*a*) eluted on Sepharose CL-6B as a single peak of radioactivity with K_{avg} value

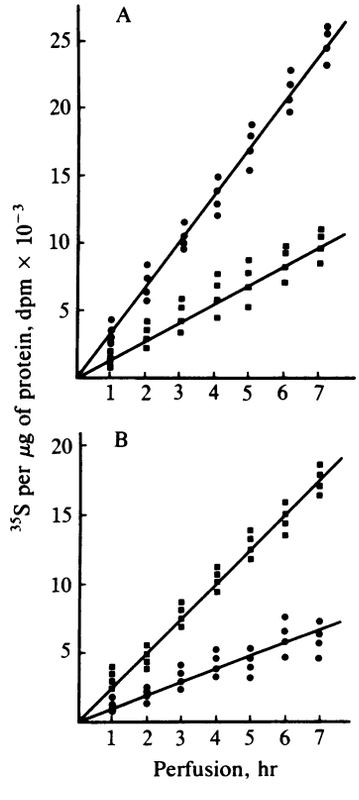

FIG. 2. Incorporation of [^{35}S]sulfate into the PGs/GAGs of cellular (*A*) and extracellular matrices (*B*) from *ex vivo*-perfused normal (■) and xyloside (●)-treated kidneys. Each point represents labeled isolates from glomerular cellular or extracellular compartments of a single perfused kidney.

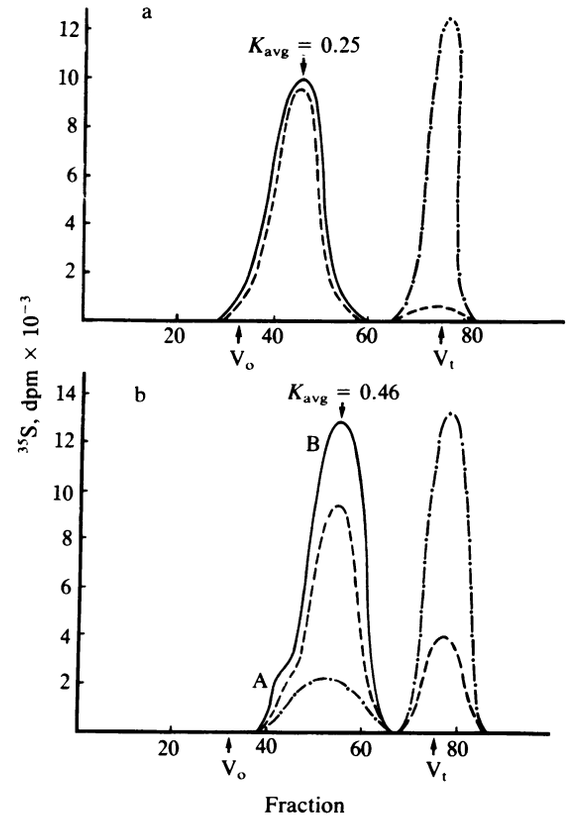

FIG. 3. Sepharose CL-6B chromatograms of aliquots of cellular extracts of the glomeruli isolated from normal (*a*) and xyloside-treated (*b*) kidneys (peaks A and B). (—), untreated; (---), chondroitinase ABC treated; (—·—), nitrous acid treated. V_o = void volume, V_t = total volume.

of 0.25, a value that corresponds to a M_r of 130,000–150,000, as estimated in our previous publications (11–13). Chondroitinase ABC digestion and nitrous acid treatment released ≈5% and ≈95% of the radioactivity from the PG peak into the V_t (V_t = total bed volume) fractions, respectively. The cellular PGs extracted from xyloside-treated kidneys (Fig. 3*b*) eluted as two peaks with ≈10% of radioactivity included in peak A (K_{avg} = 0.25) and ≈90% in peak B (K_{avg} = 0.46). As calculated previously, the latter peak probably includes very small sized PGs or only the free GAG chains with M_r of 25,000–30,000. Chondroitinase ABC treatment released ≈20% and ≈25% of radioactivities from peaks A and B into the V_t fractions, respectively.

The "matrix" PGs from controls (Fig. 4*a*) eluted on Sepharose CL-6B as a single peak of radioactivity with a K_{avg} value of 0.25. Like cellular PGs, chondroitinase ABC digestion and nitrous acid treatment released ≈5% and ≈95% of the radioactivities from the PG peak into V_t fractions, respectively. The matrix PGs extracted from kidneys treated with xyloside for 7 hr (Fig. 4*b*) eluted as distinct peaks with ≈35% of radioactivity included in peak A (K_{avg} = 0.25) and ≈65% in peak B (K_{avg} = 0.42). The latter peak reflects synthesis of a PG with an estimated M_r of ≈45,000. Chondroitinase ABC digestion released ≈25% and ≈20% of the radioactivities from peaks A and B into the V_t fractions, respectively. Nitrous acid treatment released ≈75% and ≈80% of the radioactivities from peaks A and B into the V_t fractions, respectively. These results indicate that both peaks A and B contain *de novo*-synthesized chondroitinase ABC sensitive PGs after xyloside treatment.

Total incorporated radioactivities in "media" fractions in the control and xyloside-treated groups were ≈14.55 × 10^6 dpm and ≈55.48 × 10^6 dpm, respectively, indicating that

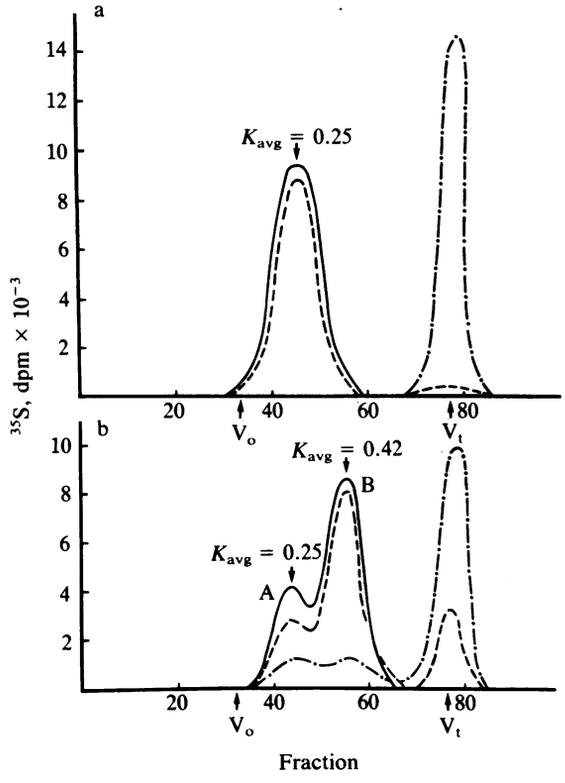

FIG. 4. Sepharose CL-6B chromatograms of aliquots of extracellular matrix extracts of the glomeruli isolated from normal (*a*) and xyloside-treated (*b*) kidneys (peaks A and B); (—), untreated; (---), chondroitinase ABC treated; (—·—), nitrous acid treated. V_o = void volume, V_t = total volume.

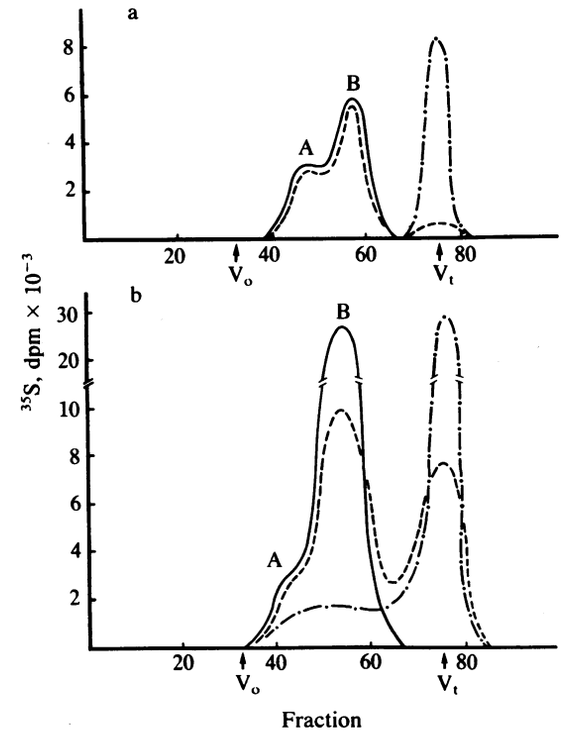

FIG. 5. Sepharose CL-6B chromatograms of aliquots of media fractions recovered after Sephadex G-50 and DEAE-Sephacel chromatography from control (*a*) and xyloside (*b*) experiments; (—), untreated; (---), chondroitinase-ABC treated; (—·—), nitrous acid treated.

substantial amounts of *de novo*-synthesized PGs and GAGs are discharged into the medium. In controls (Fig. 5*a*), the fractions chromatographed on Sepharose CL-6B column eluted as two peaks with K_{avg} values of ≈0.25 and ≈0.47, respectively. The peak A (K_{avg} = ≈0.25) represents a macromolecular form of PG, while peak B (K_{avg} = ≈0.47) represents free GAG chains. Treatment with chondroitinase ABC and nitrous acid indicated that ≈95% of the radioactivity in control medium was associated with HS-PGs (peak A) or GAGs (peak B). The media from xyloside-treated kidneys (Fig. 5*b*) also eluted as two peaks with similar K_{avg} values except that peak B contained more than 90% of the incorporated radioactivity, suggesting the highly accentuated synthesis of free GAG chains. Treatment with chondroitinase ABC and nitrous acid released ≈35% and ≈65% of the incorporated radioactivities into the V_t fractions, respectively, indicating that media fractions contained approximately 35% of the *de novo*-synthesized chondroitin sulfate GAG chains in the presence of xyloside.

DISCUSSION

Results of this investigation imply an explicit structural-biochemical relationship between observed changes in the organelles of glomerular cells under the influence of xyloside. These ultrastructural changes included selective alterations in the visceral epithelium of the glomerulus—intracytoplasmic vesiculization with a dramatic increase in autoradiographic grains associated with Golgi-derived vesicles coupled with a decrease over the GEMs. Concomitant biochemical changes included stimulation of *de novo* synthesis of free GAG chains recovered in both cellular and media fractions and reduction in the [^{35}S]sulfate incorporation into the macromolecular form of GEM PG. Additional *de novo* synthesis of chondroitin sulfate GAG/PG was observed in cellular, matrical, and media fractions. Although an accentuated cellular synthesis of free GAG chains was observed, the decreased incorporation into the extracellular matrical compartment suggests that incomplete or aborted macromolecular forms of PGs might not be properly inserted into the GEMs.

The available data indicate that cellular changes were preferentially localized to epithelial cells rather than mesenchymal-derived endothelial or mesangial cells. The reason for the selective targeting of epithelial cells is obscure and requires much further investigation since xyloside has been shown to affect the *de novo* synthesis of PGs in a wide variety of cells, irrespective of epithelial or mesenchymal origin (3–10). These reports emphasized biochemical aspects and generally ignored the cellular changes, which remain undocumented.

The cellular and biochemical changes reported here suggest an involvement of the Golgi system. The heightened *de novo* synthesis of GAGs indicates a stimulatory effect of xyloside, and the vesiculation of Golgi saccules is, most likely, related to an osmotic effect caused by the accumulation of a high concentration of hydrophilic sulfate radicals incorporated into GAG chains. The large amount of GAG-associated radioactivity in the cellular compartment indirectly indicates a high concentration of cellular sulfate, which could induce swelling and vesiculation of the Golgi saccules due to imbibition of water molecules. Whether the increased synthesis of GAG chains, or alternatively, proliferative vesicular response of the Golgi-saccular elements, initiates events could not be ascertained in our organ perfusion system. But, previously reported observations (13) (in short-term experiments) of similar biochemical alterations in the absence of morphologic changes suggest that Golgi-saccule vesiculization is not the primary event but probably the reflection of an osmotic effect. A similar vesicular-osmotic effect on the Golgi saccules has been seen in cells treated with

the sodium-selective monovalent ionophore monensin (20, 21). Monensin apparently causes swelling of the Golgi saccules by interfering with the membrane proton-gradient. The secretory processes are thus slowed, while the proximal biosynthesis of proteins or glycoproteins in the rough endoplasmic reticulum remains unperturbed. Xyloside itself may cause some disturbance in the sodium gradient across the plasma bilayer that could also explain our observations.

Xyloside not only seemed to stimulate free GAG chain formation, but also may have affected the *de novo* synthesis of PGs, which were of smaller molecular weight, especially those recovered in the extracellular matrical compartment (peak B, Fig. 4*b*). Peak B, like peak A, contained a mixture of HS-PG (≈80%) and CS-PG (≈20%), indicating that xyloside effects may not be CS-PG specific as has been suggested (3–5). Xyloside may compete with the native acceptor for initiation of both the heparan and chondroitin sulfate chains and thus inhibit their addition onto the core protein. The effect of xyloside on the synthesis of core protein of glomerular PGs is difficult to assess in our system because of technical limitations in peptide chain labeling. However, from experiments in other systems—e.g., chicken chondrocytes and limb bud mesenchymal cells—where no perturbation in peptide chain synthesis was observed (3–5), core-protein synthesis would seem not to be affected. Therefore, the decreased matrical synthesis of HS-PG can be best explained by the competitive action of xyloside at the heparan sulfate receptor on the core protein at the initiation site rather than by any change in the core-protein synthesis itself.

That xyloside indeed perturbs the biosynthesis of HS-PG of the GBM may have importance in future biological investigations. HS-PG has been implicated in charge-selective permeability properties of the GBM (22–24) and also protects the GBM from "clogging" (25) with circulating plasma proteins because of its strong electronegativity and hydrophilicity. This evidence comes from *in situ* experiments where the physiologic determinants of permselectivity—e.g., pressure gradients and blood flow—may have been inadequately controlled. Since xyloside can selectively inhibit the synthesis of GBM PGs, these experiments can be run *in vivo* to elucidate the biological functions of HS-PG under normal hemodynamic conditions. Similarly, the role of HS-PG in trapping of immune-complexes (25–28) within the GEMs can be explored *in vivo*. It is also conceivable that long-term *in vivo* treatment with xyloside may add insights concerning the HS-PG influence on the GEM assembly process. An associated loss of HS-PG with thickening of GBM in diabetic nephropathy (11) and lamination and splitting of tubular basement membrane in renal polycystic disease (29) are observations worth mentioning in this regard. The xyloside-induced cellular changes may also have value in studying Golgi functions in simpler biological systems, such as primary cultures of pituitary and pancreatic cells where it has been suggested that the sulfated PGs are involved in the concentration of various peptide hormones and exocrine secretory proteins before discharge into the extracellular compartment. These hypotheses can probably be tested in given cells altered primarily in posttranslational modification processes. In summary, the glomerular cellular and extracellular changes observed in the presence of xyloside that are described in this communication may stimulate further investigations into the basic biological processes of various cell types.

We are thankful to Ms. Jeannette Sanders for her excellent secretarial assistance. This work was supported by a research grant from the National Institutes of Health (AM 28492). Y.S.K. is the recipient of a Research Career Development Award from the National Institutes of Health (AM 01018).

1. Hascall, V. C. (1981) *Biol. Carbohydr.* **1**, 1–49.
2. Young, R. W. (1973) *J. Cell. Biol.* **57**, 175–189.
3. Okayama, M., Kimata, K. & Suzuki, S. (1973) *J. Biochem.* **74**, 1069–1073.
4. Schwartz, N. B., Galligani, L., Ho, P. & Dorfman, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4047–4051.
5. Lohmander, L. S., Hascall, V. C. & Caplan, A. I. (1979) *J. Biol. Chem.* **254**, 10551–10561.
6. Johnston, L. S. & Keller, J. M. (1979) *J. Biol. Chem.* **254**, 2575–2578.
7. Sudhakaran, P. R., Sinn, W. & von Figura, K. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 39–46.
8. Stevens, R. L. & Austen, F. K. (1982) *J. Biol. Chem.* **257**, 253–259.
9. Thompson, H. A. & Spooner, B. S. (1983) *J. Cell Biol.* **96**, 1443–1450.
10. Spooner, E., Gallagher, J. T., Krizsa, F. & Dexter, T. M. (1983) *J. Cell Biol.* **96**, 510–514.
11. Kanwar, Y. S., Rosenzweig, L. J., Linker, A. & Jakubowski, M. L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2272–2275.
12. Kanwar, Y. S., Veis, A., Kimura, J. H. & Jakubowski, M. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 762–766.
13. Kanwar, Y. S., Hascall, V. C., Jakubowski, M. L. & Gibbon, J. T. (1984) *J. Cell Biol.* **99**, 715–722.
14. Chang, Y., Yanagishita, M., Hascall, V. C. & Wight, T. N. (1983) *J. Biol. Chem.* **258**, 5679–5688.
15. Heinegard, D. & Hascall, V. C. (1974) *Arch. Biochem. Biophys.* **16**, 427–441.
16. Wateson, A. (1971) *J. Chromatogr.* **59**, 87–97.
17. Kimura, J. H., Hardingham, T. E. & Hascall, V. C. (1980) *J. Biol. Chem.* **255**, 7134–7143.
18. Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1303–1307.
19. Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4493–4497.
20. Tartakoff, A. M. & Vassali, P. (1977) *J. Exp. Med.* **146**, 1332–1345.
21. Tartakoff, A. M. & Vassali, P. (1978) *J. Cell Biol.* **79**, 694–707.
22. Rennke, H., Cotran, R. S. & Venkatachalam, M. A. (1975) *J. Cell Biol.* **67**, 638–646.
23. Venkatachalam, M. A. & Rennke, H. G. (1978) *Circ. Res.* **43**, 337–347.
24. Kanwar, Y. S., Linker, A. & Farquhar, M. G. (1980) *J. Cell Biol.* **86**, 688–693.
25. Kanwar, Y. S. & Rosenzweig, L. J. (1982) *J. Cell Biol.* **93**, 489–494.
26. Gallo, G. R., Caulin-Glaser, T. & Lamm, M. E. (1981) *J. Clin. Invest.* **67**, 1305–1313.
27. Border, W. A., Ward, H. J., Kamil, E. S. & Cohen, A. H. (1982) *J. Clin. Invest.* **69**, 451–461.
28. Gallo, G. R., Caulin-Glaser, T. C., Emancipator, S. N. & Lamm, M. E. (1983) *Lab. Invest.* **48**, 352–362.
29. Kanwar, Y. S. & Carone, F. A. (1984) *Kidney Int.* **26**, 35–42.