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Isolation of glycosaminoglycans (heparan sulfate) from glomerular basement membranes

(rat kidney/anionic sites/cellulose acetate electrophoresis)

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ABSTRACT **Glycosaminoglycans were isolated from purified fractions of glomerular basement membranes and partially characterized by chemical analysis and cellulose acetate electrophoresis. Basement membranes were prepared by detergent treatment of rat glomeruli and subjected to digestion with papain and Pronase. Glycosaminoglycans were isolated from the digests by precipitation with cetyl pyridinium chloride and ethanol. Results of cellulose acetate electrophoresis of the isolated glycosaminoglycan fraction revealed the presence of one major and one minor spot. The major spot was identified as heparan sulfate because it comigrated with the heparan sulfate standard and was sensitive to heparinase and to nitrous acid oxidation but insensitive to chondroitinase ABC and to testicular or leech hyaluronidase. The minor spot was tentatively identified as hyaluronic acid based on its migratory behavior and sensitivity to leech and testicular hyaluronidase. The chemical composition of the isolated glycosaminoglycan was typical of that of heparan sulfate (high carbazole/orcinol ratio, high sulfate content, absence of galactosamine). The data support and confirm the cytochemical data obtained previously [Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. USA* 76, 1303–1307] demonstrating that heparan sulfate is the only sulfated glycosaminoglycan detectable in the glomerular basement membrane. The present results suggest that in addition to sulfated glycosaminoglycan some nonsulfated glycosaminoglycan (hyaluronic acid) may also be present in the glomerular basement membrane.**

Anionic sites have been detected in the glomerular basement membrane (GBM) by use of various cationic probe molecules (1–4). Recently we have presented cytochemical evidence (based on removal with specific enzymes) that the sites consist of glycosaminoglycans (GAG) rich in heparan sulfate (5). Because GAG have not been detected previously in isolated GBM, it became important to check their presence by appropriate biochemical procedures. We here report the results of studies in which we have prepared isolated GBM, extracted GAG therefrom, and identified the type of GAG present. The results confirm the cytochemical findings reported previously and indicate that GAG are present in the GBM and that they consist in large part of heparan sulfate.

MATERIALS AND METHODS

Materials. Chondroitinase ABC (*Proteus vulgaris*) was purchased from Miles, leech hyaluronidase from Biotrics, Inc. (Boston, MA), and Pronase (*Streptomyces griseus*, grade B) from Calbiochem. Testicular hyaluronidase (type VI), papain (type IV, *Papaya latex*), chondroitin sulfates (types A, B, and C), hyaluronic acid (grade I), and cetyl pyridinium chloride were obtained from Sigma. Crude heparinase (*Flavobacterium heparinum*) and heparan (heparitin) sulfate (bovine lung) were generous gifts of Alfred Linker and Martin Mathews, respectively. Alcian blue 8GX was obtained from Matheson; and horse spleen ferritin, 2× crystallized (cadmium free), was from Calbiochem. Cationized ferritin was prepared as described (4).

Isolation of Glomeruli. Kidneys were obtained from decapitated rats (150–200 g, both male and female), frozen at −20°C in normal saline, and stored for 24 hr to several weeks. Twelve to fifteen kidneys were removed at a time, and glomeruli were isolated therefrom by the technique of Krakower and Greenspon (6), carried out at 4°C. The efficiency of the glomerular isolation was monitored by examining a droplet of the suspension under a dissecting microscope. Any preparation found to contain tubular or interstitial fragments was resuspended in normal saline and centrifuged at 75 × *g* for 1–2 min, and the sediment was reexamined for contaminating tissue fragments. This process was repeated until all detectable contaminants were removed and the preparation consisted of virtually 100% glomeruli of which ≈85% were free of Bowman's capsule and ≈15% were still encapsulated (Bowman's capsule present). The glomeruli thus isolated were pooled and stored at −20°C in normal saline.

Isolation of GBM. Basement membrane fractions were prepared from the isolated glomeruli by the method of Meezan *et al.* (7) with minor modifications. Briefly, the glomeruli were hypotonically lysed in 0.05% sodium azide for 2 hr, digested with deoxyribonuclease (100 units/ml in 1 M NaCl) for 2 hr, and subsequently treated with 1% deoxycholate for 3 hr, all procedures being carried out at 4°C. The GBM fractions thus obtained were washed twice with distilled water and once with 0.15 M NaCl. An aliquot of each fraction was processed for electron microscopic examination in order to check for contamination by non-GBM (cell) components and to assess the preservation of the anionic sites and of their characteristic distribution pattern by using cationized ferritin (4). The remainder of each GBM fraction was lyophilized.

Extraction of GAG from GBM. In general, the method followed was that used for the isolation of GAG from bovine lung by Linker and Hovingh (8, 9). Isolated GBM (≈50–100 mg) were suspended in 50 ml of 0.1 M acetate buffer (pH 5.5), containing 1 mM EDTA and cysteine. Crystalline papain (10 mg, 10.8 units/mg) was added, and the suspension was incubated at 60°C for 24 hr. The pH was then raised to 7.3 with a few crystals of Tris base, Pronase (50 mg, 89,600 PUK/g) was added, and the digestion was continued for another 24 hr at 37°C. The suspension was then centrifuged at 10,000 × *g* for 15 min. The sediment was saved for electron microscopic ex-

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Abbreviations: GAG, glycosaminoglycans; GBM, glomerular basement membrane(s).

amination for ferritin-binding studies, whereas the supernatant was used for the isolation of GAG. Cetyl pyridinium chloride was added to the supernatant (to a final concentration of 10%) to precipitate the GAG. The mixture, which contained a flocculent precipitate, was dialyzed against distilled water (10 changes of 6 liters each) for 48 hr at 22°C to remove the excess cetyl pyridinium chloride. The turbid suspension thus obtained was centrifuged at 10,000 × *g* for 15 min at 22°C. The supernatant was discarded; the sediment was dissolved in 2–3 ml of 2 M NaCl, 2–4 vol of absolute ethanol were added (to precipitate the GAG), the resultant hazy suspension was centrifuged (10,000 × *g* for 15 min at 22°C), and the sediment was dissolved in distilled water. This isolated, crude GAG fraction was stored at −20°C.

In order to assess the efficiency of the extraction procedure applied, individual GAG standards (1 mg/ml each) were subjected to the same digestion and extraction procedures as the isolated GBM and the percent recovery was determined.

Enzyme Digestion Procedures. Aliquots of 50 μl of the isolated GAG fraction (each containing 9–14 μg of GAG) were mixed with equal volumes of enzyme solutions prepared as follows: heparinase (2.0 mg/ml) in 0.1 M sodium acetate (pH 7.0) (8, 9); chondroitinase ABC (5.0 units/ml) in 0.1 M Tris·HCl (pH 8.0) (10); testicular hyaluronidase (10,000 units/ml) in 0.1 M NaCl/acetate buffer (pH 5.4) (11); leech hyaluronidase (3 mg/ml) in citrate/phosphate buffer (pH 5.4) (11). All of these GAG/enzyme mixtures were incubated at 37°C for 60 min, except for that with heparinase, which was incubated at 30–35°C for 2 hr. In addition, another 50-μl aliquot of the isolated GAG was mixed with 50 μl of nitrous acid [prepared by mixing an equal vol of 5% sodium nitrite and concentrated (33%) acetic acid] and incubated at room temperature for 30 min (12).

The specificity of the enzymes was tested on GAG standards (1.0 mg/ml) by mixing them with an equal volume of the enzyme solutions and incubating them under similar conditions. Each enzyme was found to have the expected (see ref. 13) polysaccharide specificities. Testicular hyaluronidase and chondroitinase ABC are active on chondroitin, chondroitins 4- and 6-sulfate, and hyaluronic acid. The latter enzyme is also active on dermatan sulfate. Leech hyaluronidase is specific for hyaluronic acid, and crude heparinase digests all GAG (8, 9). After nitrous acid oxidation, staining of heparan sulfate and heparin is virtually abolished and, in addition, in our hands staining of hyaluronic acid is also somewhat reduced. The enzymatic digests of the GAG standards and of the isolated GAG fractions were electrophoresed along with untreated GAG standards.

Electrophoresis. Individual GAG were identified by cellulose acetate electrophoresis as described by Linker and Hovingh (9). Electrophoresis was carried out at 4°C either in 0.1 M barium acetate or 0.2 M calcium acetate (pH 7.0) at 5 mA per strip for 2 hr, with strips of Sepraphore X (Gelman Instrument, Ann Arbor, MI) in a TLE-double chamber Desaga unit (Heidelberg, West Germany). After electrophoresis, the strips were stained by immersion in alcian blue solution (0.1% alcian blue in 0.1% acetic acid) for 5 min and subsequently destained with 10% acetic acid. For quantitative determination of the GAG, the strips were cleared with Sepra clearing solution (Gelman Instrument Co.), dried at room temperature, and scanned on a microdensitometer (Mark III CS, Joyce-Loebl, Princesway, England). The density of the spots was compared with that of GAG standards.

Analytical Procedures. Uronic acid was determined by the carbazole (14) and orcinol (15) procedures, total hexosamine and galactosamine by the method of Blumenkrantz and Asboe-Hansen (16), and *N*-sulfated hexosamine by the method of Dische and Borenfreund (17). Sulfate content was determined by the benzidine reaction (18) and sialic acid by the Warren assay (19).

RESULTS

Yields. The procedures for the extraction of GAG were carried out twice on pooled GBM isolated from 700 (extraction I) and 500 (extraction II) rat kidneys. The cortical mass, which represented the starting material of the two isolations, was 450 g and 350 g, and the dry weights of the GBM fractions obtained therefrom were 80 and 50 mg, respectively. The recoveries of various standards were as follows: chondroitin A, B, and C, 87%, 89%, and 79%, respectively; heparan sulfate, 85%; and hyaluronic acid, 36%.

Electron Microscopy of GBM Fractions. GBM isolated by the method of Meezan *et al.* (cf. ref. 20) (which represents the starting material for the extraction procedure) consists of intact tubes of basement membrane devoid of attached cellular elements. We have previously demonstrated (4) that the anionic sites are preserved in freshly isolated GBM and have a distribution very similar to that found *in situ*, as shown by the binding pattern of cationized ferritin. Fig. 1 shows that the anionic sites are also preserved in GBM isolated after freezing and thawing the kidney.

Electron microscopic examination of the sediment obtained after digestion of isolated GBM with papain and Pronase revealed no recognizable remaining GBM elements. This is in keeping with the finding that prolonged digestion of bovine GBM with Pronase at 37°C causes complete solubilization of the GBM (21).

Chemical and Biochemical Analyses. The analytical data obtained on the isolated GAG fraction are given in Table 1. The uronic acid content of the isolated GAG, as determined by the carbazole reaction, was 36% (Extraction I) and 41% (Extraction II); the carbazole to orcinol ratios were 1.73 and 2.14, respectively. The total hexosamine content was ≈23% and 26% and the *N*-sulfated hexosamine was 24% and 27%, suggesting that all the hexosamine was *N*-sulfated. No galactosamine could be

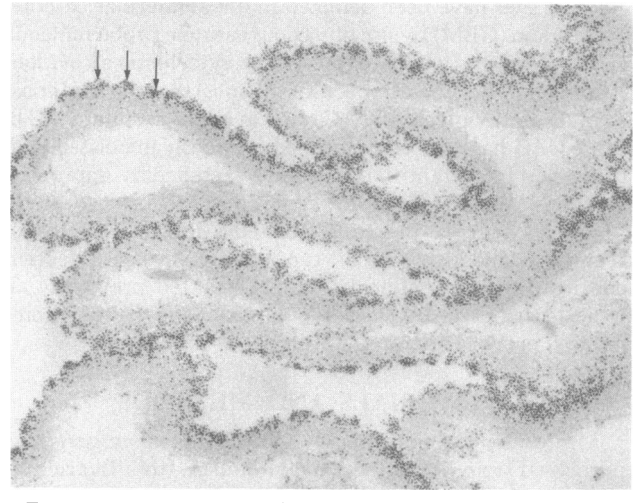

FIG. 1. Loops of isolated GBM incubated with cationized ferritin to label the anionic sites. Cationized ferritin molecules are seen binding to the GBM at regular intervals (arrows) of ≈60 nm, indicating that the sizes are still preserved after freezing and thawing of the whole kidneys. Cationized ferritin molecules bind only to the outer or exposed side of the GBM loops because the loops consist of intact, closed tubes and the tracer does not have access to the inner or unexposed side of the GBM. Disruption of the loops [e.g., by sonication (4)] leads to decoration of the sites on both sides of the GBM. (×44,000).

Table 1. Partial characterization of glycosaminoglycans isolated from glomerular basement membranes

	Extraction I	Extraction II
Total yield of GAG	798 μg	366 μg
Uronic acid content		
Carbazole	285 μg (36%)	150 μg (41%)
Orcinol	165 μg (21%)	70 μg (19%)
Carbazole/orcinol	1.73	2.14
Hexosamines		
Total	180 μg (23%)	95 μg (26%)
N-Sulfated hexosamine	195 μg (24%)	100 μg (27%)
Galactosamine	ND	ND
Sulfate content	105 μg (14%)	54 μg (15%)
Sialic acid	ND	ND

Dry weights of the GBM, which represented the starting material for the two extractions, were 80 and 50 mg, respectively. Values shown for total yield of GAG are based on comparative densitometer readings with heparan sulfate standards. Numbers given in parentheses represent the percent of the total GAG extracted from GBM based on heparan sulfate standards. ND, not detected.

detected, indicating that glucosamine was the only amino sugar present. The sulfate content was 14% and 15% in the two extractions. No sialic acid was detected, indicating that no contaminating sialoproteins were present in the isolated GAG fractions.

Electrophoretic Results. The electrophoretic patterns obtained on the isolated GAG fractions were similar in both extractions. After electrophoresis in 0.2 M calcium acetate buffer [which is the best system for differentiating different types of GAG from one another (8)] followed by staining with alcian blue, only two spots were detected—one major and the other minor (Fig. 2). The migration of the major spot corresponded to that of the heparan sulfate standard whereas the minor spot remained close to the origin; its mobility corresponded to that of the hyaluronic acid standard.

When samples were treated with chondroitinase ABC or with testicular or leech hyaluronidase prior to electrophoresis in the calcium acetate system, no effect was seen on the major spot, but the minor spot was no longer present (Fig. 3). If samples were digested with crude heparinase prior to electrophoresis, the major spot was no longer discernible; if they were treated with nitrous acid, the major spot was barely detectable (Fig. 3). The major spot can be reliably identified as heparan sulfate because it (*i*) has the same electrophoretic mobility as the heparan sulfate standard in the calcium acetate system, (*ii*) it is no longer demonstrable after digestion with crude heparinase, (*iii*) it is barely demonstrable after nitrous acid oxidation, and (*iv*) it is unaffected by treatment with chondroitinase ABC or testicular or leech hyaluronidase, which together remove all GAG except heparan sulfate and heparin.* The identification of the weak, minor spot is somewhat more problematic, but based on its electrophoretic mobility, which is similar to that of hyaluronic acid, and on its digestion by leech hyaluronidase (which specifically removes hyaluronic acid), it appears to consist of hyaluronic acid.

The amount of heparan sulfate present in the GAG fraction isolated from the GBM (calculated by comparing the intensity of the spot measured in a microdensitometer to that obtained with a heparan sulfate standard of known concentration) represented 1% (Extraction I) and 0.75% (Extraction II) of the dry weight of the GBM. It was impossible to obtain an accurate estimate of the minor spot tentatively identified as hyaluronic acid because of its faint staining and tendency to streak. More efficient extraction and labeling procedures will be required for the definitive identification and reliable quantitation of the minor spot.

DISCUSSION

In this investigation we have isolated GAG from GBM fractions prepared from rat glomeruli and have partially characterized the extracted GAG. The results obtained indicate that the major

* To confirm the identification of the major spot, we sent an aliquot of the GAG fraction obtained from Extraction II to Alfred Linker, who subjected it to digestion with purified heparitinase (which specifically digests heparan sulfate). This treatment completely removed the major spot, thereby identifying it as heparan sulfate.

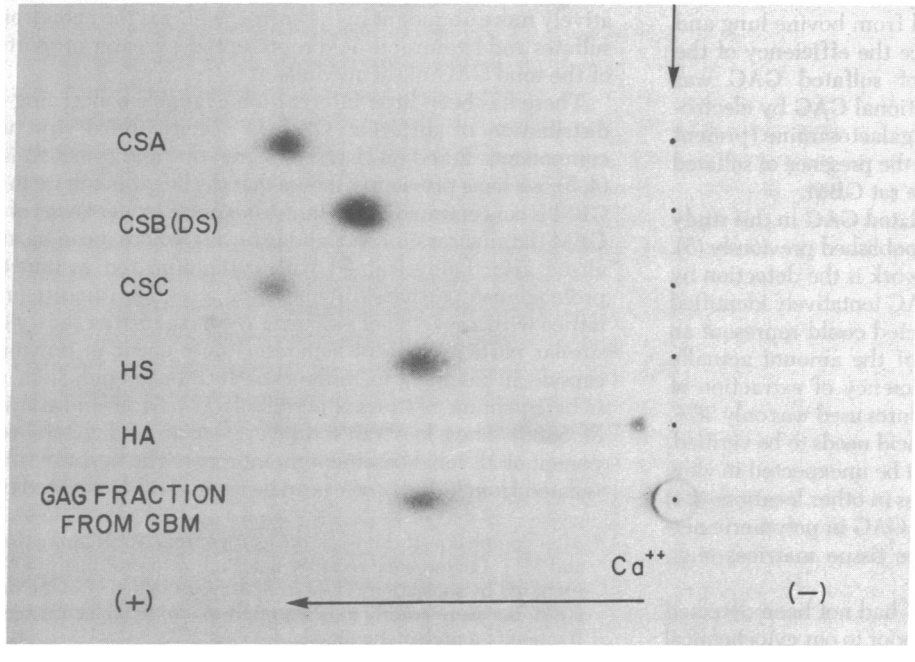

FIG. 2. Cellulose acetate electrophoresis in 0.2 M calcium acetate (pH 7.0) of GAG fraction isolated from the GBM. Two spots are seen: a major spot corresponding in mobility to that of heparan sulfate and a minor one close to the origin corresponding in location to the hyaluronate standard. CSA, chondroitin 4-sulfate; CSB, dermatan sulfate; CSC, chondroitin 6-sulfate; HS, heparan sulfate; HA, hyaluronate.

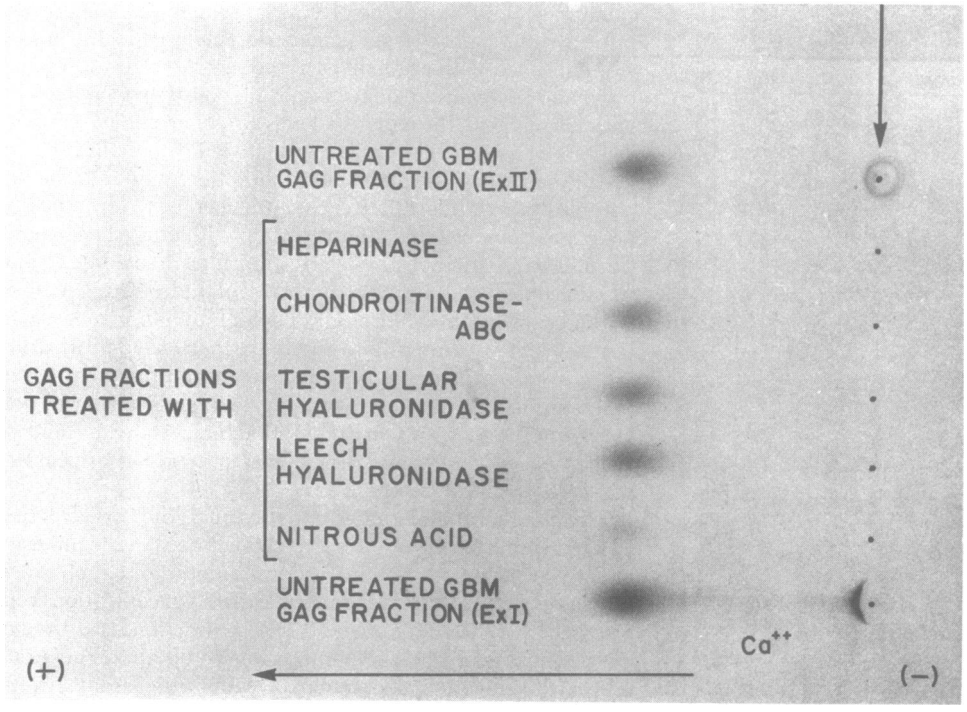

FIG. 3. Cellulose acetate electrophoresis in 0.2 M calcium acetate buffer of the isolated GAG fraction after treatment with GAG-degrading enzymes and nitrous acid. The minor spot is susceptible to all the GAG-degrading enzymes used. The major spot is susceptible to the treatment with heparinase, but is not susceptible to digestion with chondroitinase ABC and leech or testicular hyaluronidases. The spot is barely perceptible after treatment with nitrous acid.

GAG present is heparan sulfate; it constitutes ≈1% of the dry weight of the GBM. The conclusion that the isolated GAG consists mainly of heparan sulfate is based on the combined results obtained by electrophoresis and various analytical procedures. Electrophoresis of the isolated GAG on cellulose acetate reveals the presence of a single, major spot that comigrates with heparan sulfate (in an appropriate buffer system) and that is absent after digestion of the isolated GAG with specific enzymes or treatment with nitrous acid. A weak, minor spot was also detected, which was tentatively identified (based on its removal with specific enzymes) as hyaluronic acid. No other GAG was found. The analytical data are consistent with the electrophoretic findings indicating that heparan sulfate is the major GAG present: the sulfate content (14–15%), degree of sulfation of hexosamine residues, the carbazole and orcinol values, and the absence of galactosamine are in keeping with the data obtained previously for heparan sulfate isolated from bovine lung and aorta (8) and rat kidney (22, 23). Since the efficiency of the isolation procedure for extraction of sulfated GAG was ≈80–90%, the inability to detect additional GAG by electrophoresis together with the absence of galactosamine (present in chondroitin sulfates) render unlikely the presence of sulfated GAG other than heparan sulfate in the rat GBM.

The analytical data obtained on isolated GAG in this study fully confirm the cytochemical results published previously (5). An additional finding in the present work is the detection by electrophoresis of a small amount of GAG tentatively identified as hyaluronic acid. The amount detected could represent an underestimate (by as much as 2/3) of the amount actually present in the GBM because the efficiency of extraction of hyaluronic acid by the isolation procedures used was only 36%. Clearly, the localization of hyaluronic acid needs to be verified, but its presence in the GBM would not be unexpected in view of its presence in basement membranes in other locations (24) and its usual association with sulfated GAG in polymeric networks of proteoglycans in connective tissue matrices [e.g., cartilage (25, 26)].

As discussed previously (4, 5), GAG had not been detected in GBM fractions (see refs. 27 and 28) prior to our cytochemical results.† However, GAG have been repeatedly isolated and partially characterized from whole homogenates of kidneys from several species: rat (22, 23), bovine (29), human (30, 31), guinea pig (32), and rabbit (32) kidneys. In all cases heparan sulfate was the major GAG present, with smaller amounts of dermatan sulfate, hyaluronic acid, and chondroitin sulfates (A and C) usually being detected. The most extensive studies to date on GAG from rat kidney are those of Barry and Bowness (33), who isolated and characterized GAG from renal homogenates (after radiolabeling *in vivo*). They determined that heparan sulfate is the principal GAG in both cortex and medulla and found, in addition, that the renal heparan sulfate has a half-life comparable to that in other tissues (2–6 days). These findings are not restricted to the rat; in other species [dog (34) or rabbit (35)], as in the rat, heparan sulfate was present in high concentration in both renal cortex and medulla, but it was relatively more abundant in the cortex, whereas the chondroitin sulfates and hyaluronic acid represented a greater proportion of the total GAG in the medulla.

There has been little information available concerning the distribution of different GAG in specific renal structural components. Based on electron microscopy and cytochemistry (4, 5), we have previously shown that the heparan sulfate in the GBM is concentrated in the inner and outer lighter layers of the GBM (lamina rara interna and externa), where it can be visualized after being stained with ruthenium red as punctate proteoglycan particles distributed in a regular (quasiregular) lattice with a spacing of ≈60 nm. We have further shown that similar particles rich in heparan sulfate occur in Bowman's capsule, in basement membranes of the tubular epithelium, and in basement membranes of peritubular capillaries and arterioles (4, 5, 36). Thus, heparan sulfate appears to be a general component of all renal basement membranes. The heparan sulfate isolated from kidney cortex can be assumed to be derived from

† After completion of this work, we learned from Robert Spiro that he and M. J. Levine have recently detected hexuronic acids in GBM prepared by sonication. They were difficult to detect in the intact GBM, but were readily demonstrated in a high molecular weight fraction of a proteolytic digest.

all the basement membranes mentioned in addition to the GBM, whereas that isolated from the medulla could come from basement membranes of the kidney tubules and small blood vessels (capillaries and arterioles). We have also shown (36) that in the kidney cortex, as in other organs, chondroitin sulfate occurs in the peritubular, interstitial connective tissue matrix, in association with collagen fibrils, a finding that, in view of the greater abundance of extracellular matrix in the medulla, could explain the larger quantities of chondroitin sulfate found therein.

The demonstration of heparan sulfate in basement membranes is a novel finding because this GAG has been assumed to be associated primarily with cell surfaces in the kidney (34, 35) and elsewhere (25, 26, 37). In the kidney they have been assumed to be located along the surfaces of the tubule epithelium and to play a role in binding cations. Thus, the question immediately arises—does the major portion of the heparan sulfate present in kidney occur only along those portions of the cell facing basement membranes? Or, is some of the heparan sulfate associated with the remaining cell surfaces—i.e., the luminal and lateral cell fronts? Or, alternatively, is some of it associated with other connective tissue elements? At present these questions cannot be answered, but it can be concluded that the high concentrations of heparan sulfate noted in highly vascular tissues with abundant basement membranes such as the lung (8, 38) and kidney must be related, at least in part, to its presence in vascular basement membranes.

Another, still unanswered question is what is the function of the heparan sulfate in basement membranes? At present this question cannot be satisfactorily answered because of our state of relative ignorance on the biological functions of heparan sulfate. The available analytical data indicate that heparan sulfate is not a single compound, but constitutes a family of closely related polymers that differ in size, charge, and charge distribution in different tissues and even in the same tissue (8). Heparan sulfate, at least that isolated from the rat kidney (39), resembles heparin in possessing antithrombotic properties, but it has a lower sulfate content and less anticoagulant activity than heparin. Moreover, in apparent contrast to heparin, heparan sulfate does not exist as an unconjugated polysaccharide in tissue; like other sulfated GAG, it occurs in proteoglycans covalently bound to proteins (8, 25, 26). Among the possible functions of heparan sulfate in the GBM about which we have speculated elsewhere (4, 5) are its possible participation in creation of the glomerular charge barrier and its antithrombotic effects. The potential role of sulfated GAG in morphogenesis has been repeatedly discussed (40–42).

In summary, the presence of GAG consisting in large part of heparan sulfate in the GBM has been confirmed by isolation and characterization of GAG from purified GBM. Based on the evidence available, it appears that heparan sulfate is widely distributed in basement membranes. Its role in renal physiology and pathology remains to be elucidated.

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1. Caulfield, J. P. & Farquhar, M. G. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1646–1650.
2. Caulfield, J. P. & Farquhar, M. G. (1978) *Lab. Invest.* **39,** 505–512.
3. Kanwar, Y. S. & Farquhar, M. G. (1978) *Kidney Int.* **14,** 713 (abstr.).
4. Kanwar, Y. S. & Farquhar, M. G. (1979) *J. Cell Biol.* **81,** 137–153.
5. Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1303–1307.
6. Krakower, C. A. & Greenspon, S. A. (1951) *Arch. Pathol.* **51,** 629–639.
7. Meezan, E., Hjelle, J. T., Brendel, K. & Carlson, E. C. (1975) *Life Sci.* **17,** 1721–1732.
8. Linker, A. & Hovingh, P. (1973) *Carbohydr. Res.* **29,** 41–62.
9. Linker, A. & Hovingh, P. (1972) *Methods Enzymol.* **28,** 902–911.
10. Saito, H., Yamagata, T. & Suzuki, S. (1968) *J. Biol. Chem.* **243,** 1536–1542.
11. Wight, T. W. & Ross, R. (1975) *J. Cell Biol.* **67,** 660–674.
12. Cifonelli, J. A. (1968) *Carbohydr. Res.* **8,** 233–242.
13. Toole, B. P. (1976) in *Neuronal Recognition,* ed. Barondes, S. H. (Plenum, New York), pp. 275–329.
14. Bitter, T. & Muir, H. M. (1962) *Anal. Biochem.* **4,** 330–334.
15. Brown, A. H. (1946) *Arch. Biochem.* **11,** 269–278.
16. Blumenkrantz, N. & Asboe-Hansen, G. (1976) *Clin. Biochem.* **9,** 269–274.
17. Dische, Z. & Borenfreund, E. (1950) *J. Biol. Chem.* **184,** 517–522.
18. Terho, T. T. & Hartiala, K. (1971) *Anal. Biochem.* **41,** 471–476.
19. Warren, L. L. (1959) *J. Biol. Chem.* **232,** 1971–1975.
20. Carlson, E. C., Brendel, K., Hjelle, J. T. & Meezan, E. (1978) *J. Ultrastruct. Res.* **62,** 26–53.
21. Spiro, R. G. (1972) in *Glycoproteins: Their Composition, Structure and Function,* ed. Gottschalk, A. (Elsevier, Amsterdam), pp. 964–999.
22. Allalouf, D., Ber, A. & Sharon, N. (1964) *Biochim. Biophys. Acta* **83,** 278–287.
23. Seno, N., Ariizumi, K., Nagase, S. & Anno, K. (1972) *J. Biochem.* **72,** 479–481.
24. Cohn, R. H., Banerjee, S. D. & Bernfield, M. R. (1977) *J. Cell Biol.* **73,** 464–478.
25. Comper, W. D. & Laurent, T. C. (1978) *Physiol. Rev.* **58,** 255–315.
26. Lindahl, U. & Hook, M. (1978) *Annu. Rev. Biochem.* **47,** 385–417.
27. Spiro, R. G. (1967) *J. Biol. Chem.* **242,** 1915–1922.
28. Kefalides, N. A. & Denduehis, B. (1969) *Biochemistry* **8,** 4613–4621.
29. Murata, K. (1976) *Connect. Tiss. Res.* **4,** 131–140.
30. Murata, K. (1975) *Clin. Chim. Acta* **63,** 157–169.
31. Murata, K. & Horiuchi, Y. (1978) *Nephron* **20,** 111–118.
32. Dietrich, C. P., Sampio, L. O., Toledo, O. M. S. & Cassaro, C. M. F. (1977) *Biochem. Biophys. Res. Commun.* **75,** 329–336.
33. Barry, D. N. & Bowness, J. M. (1975) *Can. J. Biochem.* **53,** 713–720.
34. Castor, C. W., Greene, J. A., Prince, R. K. & Hazelton, M. J. (1968) *J. Clin. Invest.* **47,** 2125–2132.
35. Van Praag, D., Stone, A. L., Richter, A. J. & Farber, S. J. (1972) *Biochim. Biophys. Acta* **273,** 149–156.
36. Farquhar, M. G. & Kanwar, Y. S. (1979) in *Immune Mechanisms in Renal Diseases,* eds. Michaels, A. F. & Cummings, N. (Academic, New York), in press.
37. Kraemer, P. M. (1971) *Biochemistry* **10,** 1445–1451.
38. Brody, J. S. & Vaccaro, C. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 215–233.
39. Allalouf, D., Ber, A., Rechnic, J. & Sharon, N. (1967) *Thromb. Diath. Haemorrh.* **17,** 264–272.
40. Hay, E. D. & Meier, S. (1974) *J. Cell Biol.* **62,** 889–898.
41. Trelstad, R. L., Hayashi, K. & Toole, B. P. (1974) *J. Cell Biol.* **62,** 815–830.
42. David, G. & Bernfield, M. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 786–790.