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Sulfated and nonsulfated glycosaminoglycans and glycopeptides are synthesized by kidney *in vivo* and incorporated into glomerular basement membranes

([^{35}S]sulfate/[^{3}H]glucosamine/heparan sulfate/hyaluronic acid/chondroitin sulfate)

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ABSTRACT The biosynthesis of glycosaminoglycans (GAG) and glycopeptides was studied in rat kidney cortex, glomeruli, and isolated glomerular basement membranes (GBM). Rats were given four intraperitoneal injections of [^{35}S]sulfate and [^{3}H]glucosamine (over 10 hr) and sacrificed 14 hr after the last injection. Fractions of kidney glomeruli and purified GBM were prepared. The percent of the label incorporated into specific GAG or into glycopeptides was determined by selective degradative techniques in conjunction with gel filtration chromatography using the methods of Hart [Hart, G. W. (1976) *J. Biol. Chem.* 251, 6513–6521; Hart, G. W. (1978) *Dev. Biol.* 62, 78–98]. After digestion with Pronase and chromatography on Sephadex G-50, ≈68% of the total ^{35}S radioactivity and 10–15% of the total ^{3}H radioactivity incorporated into cortex, glomeruli, or GBM was found in the GAG fraction, and the remainder (≈32% of ^{35}S radioactivity and 85–90% of the ^{3}H radioactivity) was found in glycopeptide fractions. Treatment of GAG fractions isolated from the three sources (cortex, glomeruli, and GBM) with nitrous acid (which degrades heparan sulfates) indicated that the majority (85%, 65%, and 87%) of the ^{35}S radioactivity as well as the majority (60%, 50%, and 91%) of the ^{3}H radioactivity from all three sources was degraded by this treatment. When nitrous acid-resistant GAG from GBM were subjected to digestion with *Streptomyces* hyaluronidase (which degrades hyaluronic acid), ≈6% of the ^{3}H-labeled material was sensitive to this treatment. The remaining ^{35}S- and ^{3}H-labeled GAG isolated from GBM were digested with chondroitinase ABC (which degrades chondroitin sulfates A and C and dermatan sulfate). Although the ratios of the types of GAG synthesized by all three sources were similar, in GBM the ratios of ^{35}S- to ^{3}H-labeled GAG and of ^{3}H-labeled glycopeptides to ^{3}H-labeled GAG were higher (2.5 times) than those found for glomeruli. The data demonstrate the synthesis of both sulfated and nonsulfated GAG by rat kidney cortex and glomeruli and their transport to and incorporation into the GBM. Heparan sulfate is the major GAG synthesized by glomeruli, but the glomeruli also synthesize smaller amounts of hyaluronic acid and chondroitin sulfates, which are in part incorporated into GBM. In addition, the renal cortex and the glomeruli synthesize glycopeptides, some of which are sulfated, and incorporate them into GBM.

Recently glycosaminoglycans (GAG) were detected by cytochemical procedures (1) in glomerular basement membranes (GBM) and subsequently isolated from purified GBM fractions (2). Evidence has been presented that these GAG may play a role in creating the normal restrictive permeability properties of the GBM to plasma and other proteins, because increased permeability of the GBM to native ferritin (3) and ^{125}I-labeled bovine serum albumin (unpublished data) was seen after removal of GAG by enzyme digestion *in situ*. To be able to assess the importance of GAG in renal physiology and pathology, it is essential to obtain information on the nature, amount, biosynthesis, and turnover of GAG in normal glomeruli *in vivo*, subjects about which virtually nothing is known.

In this paper we have used double-labeling with [^{35}S]sulfate and [^{3}H]glucosamine *in vivo* and specific degradative techniques followed by analysis of the degradation products. The results indicate that, under the conditions of our experiments, GAG were synthesized by glomeruli and transported to the GBM. We have also obtained a more comprehensive inventory of the glycoconjugates (GAG plus glycopeptides) present in the GBM than was available previously, and we have compared this inventory to that obtained for glomeruli and whole kidney cortex in the same experiments.

MATERIALS AND METHODS

Labeling of Kidneys. Rats (125 g, Charles River) were given intraperitoneally a total of 2.5 mCi of $Na_2{}^{35}SO_4$ (New England Nuclear, 540–940 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels) and 0.4 mCi of D-[6-^{3}H]glucosamine (Amersham, 38 Ci/mmol) per animal (four injections over a 10-hr period) and sacrificed 14 hr after the last injection.* Kidneys were flushed with normal saline prior to sacrifice and frozen at −20°C. Twelve kidneys were used for each glomerular analysis and 48 for GBM analysis.

Isolation of Glomeruli and GBM. Cortices were dissected from the labeled kidneys, and a sample was homogenized and set aside for glycoconjugate analysis. The remaining cortex was used to isolate glomeruli by the method of Krakower and Greenspon (5). The purity of the glomerular fraction was monitored as described (2). Yields ranged between 10,000 and 20,000 glomeruli per kidney (25,000–49,000 glomeruli per g of cortex). A sample of the glomeruli was set aside for glycoconjugate analysis and the GBM were isolated from the remaining glomeruli according to a modification (2) of the method of Meezan *et al.* (6). In this procedure the glomeruli are first osmotically lysed, then digested with deoxyribonuclease (100 units/ml in 1 M NaCl), and finally treated with 1% deoxycholate. This protocol gives morphologically pure GBM, free from cellular contamination (1, 6).

Determination of Specific Activity. Protein in samples of cortex, glomeruli, and GBM preparations was measured by a fluorescamine assay (7) (using IgG or albumin as a reference standard) after acid hydrolysis (in 1 M HCl at 110°C for 24 hr). ^{3}H or ^{35}S radioactivity was measured in aliquots of the same preparation after digestion with Pronase and dialysis of the di-

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

* A 24-hr time point was selected after the work of Barry and Bowness (4), who demonstrated the incorporation of satisfactory amounts of [^{35}S]sulfate and [^{14}C]glucose into kidney GAG *in vivo* after this interval. Multiple injections (instead of a single injection) were used because this resulted in higher specific radioactivities of the glycoconjugates.

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gest. The specific radioactivity of the glycoconjugates thus obtained is given as cpm/mg of protein.

Glycoconjugate Analysis. Cortex (0.3–0.4 g), glomeruli (derived from 3.5–4.0 g of cortex), and GBM (derived from ≈16.0 g of cortex) were each treated with predigested Pronase (1.5 mg/ml, Sigma, protease VI in 5.0 ml of 0.2 M Tris·HCl buffer, pH 8.0) for 48 hr at 50°C. Fresh enzyme was added after 24 hr, making a final volume of 10 ml. Pronase digestion produces essentially single polysaccharide chains each attached to a small peptide moiety. The Pronase digest was dialyzed against double-distilled water for 48 hr (to remove low molecular weight digestion products and unincorporated label).

The distribution of label in glycopeptides and in each species of GAG was determined according to the procedure of Hart (8, 9) with minor modifications. This protocol involves selective degradative techniques, in conjunction with gel filtration chromatography, to characterize the digested moieties and the material resistant to chemical or enzymatic treatment. In the first step, the entire Pronase digest obtained from each source (cortex, glomeruli, and GBM) was loaded on a Sephadex G-50 column (1 × 200 cm) in order to separate labeled GAG and glycopeptides. Elution was with 0.1 M ammonium acetate in 20% (vol/vol) ethanol; 1.2-ml fractions were collected and 0.2 or 0.4-ml aliquots of each fraction were analyzed for radioactivity. GAG standards and labeled GAG eluted as a sharp peak with blue dextran in the void volume; glycopeptides eluted over a broad range in the retarded fractions (see Fig. 1).

The fractions containing GAG were pooled, lyophilized, and subjected to a series of chemical and enzymatic degradative procedures, each followed by gel filtration: (*i*) The GAG fraction was subjected to nitrous acid oxidation for 3 hr at room temperature (9), a procedure that selectively and completely degrades *N*-sulfated GAG (heparan sulfates) (10–12). This chemical digest was then chromatographed on a Sephadex G-50 fine column (1 × 100 cm) to separate nitrous acid-resistant GAG from degradation products. Elution was with 0.1 M ammonium acetate in 20% ethanol; 1.75-ml fractions were collected, and 0.5-ml aliquots were sampled for radioactivity. (*ii*) The residual GAG fraction was pooled and treated with *Streptomyces* hyaluronidase (Miles; 5 turbidity-reducing units/ml in 3.0 ml of 0.02 M sodium acetate buffer, pH 5.0) for 24 hr at 37°C with fresh enzyme added after 12 hr. This enzyme specifically degrades hyaluronic acid (13). The enzyme-resistant GAG and hyaluronidase digestion products were separated by gel filtration as described above in step *i*. (*iii*) GAG resistant to these chemical and enzymatic treatments were pooled and digested with chondroitinase ABC (Miles; 0.17 units/ml in 3.0 ml of 0.01 M Tris·HCl buffer, pH 8.0, for 24 hr at 25°C), an enzyme that degrades chondroitin 4-sulfate, dermatan sulfate, and chondroitin 6-sulfate [formerly called chondroitin sulfates A, B, and C, respectively (14); hereafter referred to as chondroitin sulfates]. The digest was rechromatographed; any residual enzyme-resistant material present eluted in the void volume. The amounts of nitrous acid, hyaluronidase, and chondroitinase used were sufficient to degrade 2 mg of the appropriate substrates (8, 9).

The combined sequence of these four chemical and enzymatic treatments each followed by gel filtration gave a total profile of the incorporation of [^{35}S]sulfate and [^{3}H]glucosamine into glycoconjugates in the renal cortex, glomeruli, and GBM.

RESULTS

Specific Radioactivities. The specific radioactivities (cpm/mg of protein) for ^{3}H and ^{35}S incorporated into glycoconjugates (GAG and glycopeptides) in cortex, glomeruli, and GBM are given in Table 1. The data show that there is a slight enrichment of ^{35}S radioactivity in glomeruli compared to cortex and that the specific ^{35}S radioactivity in GBM is only 11% of that in glomeruli. Cortex is more enriched in ^{3}H-labeled glycoconjugates than either glomeruli or GBM are. The specific ^{3}H radioactivity of the GBM is ≈28% of that in the glomeruli.

Table 1. Specific radioactivity of glycoconjugates in kidney cortex, glomeruli, and GBM

Kidney fraction	Specific activity of glycoconjugates, cpm/mg protein	
	^{35}S	^{3}H
Cortex	11,270	8273
Glomeruli	13,720	4694
GBM	1,520	1333

Five rats were given [^{35}S]sulfate and [^{3}H]glucosamine *in vivo*. The cortices were pooled, glomeruli were isolated therefrom, and purified GBM were prepared as described in text. Specific radioactivity was determined in samples of cortices (0.076 g of cortex), glomeruli (derived from 1.35 g of cortex), and GBM (derived from 2.70 g of cortex), after digestion with Pronase and dialysis as described in text. Protein was determined by fluorometric assay of hydrolyzed protein.

Total GAG and Glycopeptides. Typical column profiles from cortex, glomeruli, and GBM labeled *in vivo* with [^{35}S]sulfate and [^{3}H]glucosamine, treated with Pronase, and chromatographed on Sephadex G-50 are illustrated in Fig. 1. The percent radioactivity present in the GAG and glycopeptide fractions is given in Table 2. The data show that cortex, glomeruli, and GBM all incorporated [^{35}S]sulfate and [^{3}H]glucosamine into both GAG and glycopeptides. In all cases the majority (66–69%) of the ^{35}S label was incorporated into GAG and the remainder (31–34%) into glycopeptides. In the case of [^{3}H]glucosamine, the situation was reversed: the majority (80–90%) of the label was incorporated into glycopeptides and the remainder (10–20%) into GAG. Total radioactivity incorporated into macromolecules ranged between 6000 (GBM) and 125,000 (cortex) cpm for [^{35}S]sulfate and between 5100 (GBM) and 121,000 (cortex) cpm for [^{3}H]glucosamine.

Table 2. Distribution of radioactivity incorporated from [^{35}S]sulfate and [^{3}H]glucosamine into kidney cortex, glomeruli, and GBM

Kidney fraction	Radioactivity, %	
	^{35}S	^{3}H
Cortex		
GAG	68	16
Glycopeptides	32	84
Glomeruli		
GAG	66	17
Glycopeptides	34	83
GBM		
GAG	69	7
Glycopeptides	31	93

Cortex, glomeruli, and GBM were isolated from kidneys labeled *in vivo* with [^{35}S]sulfate and [^{3}H]glucosamine. GAG and glycopeptides were separated by gel filtration as described in the text. In the starting preparations, the average ^{35}S radioactivities were 123,100 cpm for cortex and 22,760 cpm for glomeruli; the corresponding values for ^{3}H were 120,800 cpm for cortex and 10,750 cpm for glomeruli. Total radioactivities in the GBM preparation were 5895 cpm for ^{35}S and 5040 cpm for ^{3}H. The percents given represent the averages of three experiments for cortex and glomeruli and data from one experiment for GBM. The percent of total radioactivity in GAG = [(total cpm in GAG)/(total cpm in GAG + total cpm in glycopeptides)] × 100. The percent of total radioactivity in glycopeptides = [(total cpm in glycopeptides)/(total cpm in GAG + total cpm in glycopeptides)] × 100.

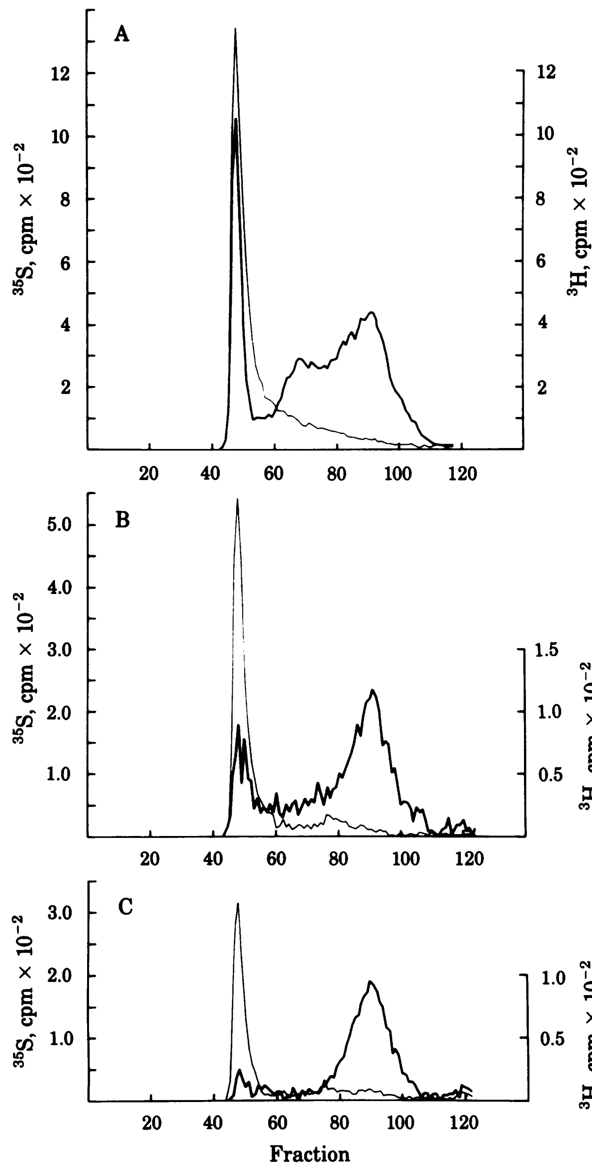

FIG. 1. Separation of GAG and glycopeptides by gel filtration on Sephadex G-50 (1 × 200 cm) after Pronase digestion. Representative column profiles are shown from cortex (*A*), glomeruli (*B*), and GBM (*C*). Fractions (1.2 ml) were collected; 0.2-ml aliquots were taken for cortex and glomeruli and 0.4-ml aliquots for GBM. GAG eluted in fractions 44–56. Glycopeptides were retarded and eluted in fractions 57–110. Thin line, ^{35}S radioactivity; thick line, ^{3}H radioactivity.

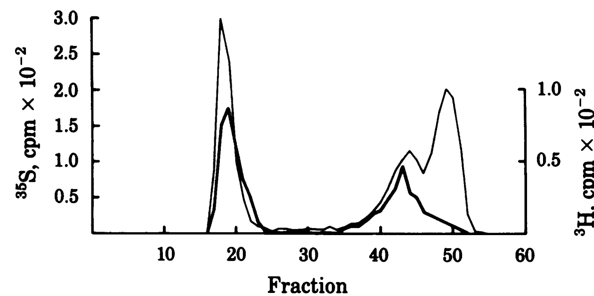

FIG. 2. Representative example from labeled glomeruli, showing separation of resistant GAG and degradation products after nitrous acid oxidation. Total GAG remaining after sampling for radioactivity (see Fig. 1*B*) were pooled, subjected to nitrous acid treatment, and rechromatographed on Sephadex G-50 (1 × 100 cm). Fractions (1.75 ml) were collected, and 0.5-ml aliquots were assayed for radioactivity. ^{35}S-Labeled digestion products eluted as two major peaks and ^{3}H-labeled eluted as one major peak. Labeled GAG resistant to nitrous acid eluted in the void volume (fractions 16–24). Thin line, ^{35}S radioactivity; thick line, ^{3}H radioactivity.

Relative Incorporation into GAG and Glycopeptides. Comparison of the amounts of ^{35}S and ^{3}H label in GAG in cortex, glomeruli, and GBM (Fig. 1) shows a 2.5 times higher ratio (^{35}S-GAG/^{3}H-GAG) in GBM compared to cortex. The data suggest either a higher amount of newly synthesized sulfated GAG in the GBM or a higher degree of sulfation of individual GAG species in GBM. When the ratio of ^{3}H-glycopeptides/^{3}H-GAG is compared for cortex, glomeruli, and GBM, this value is found to be 2.5 times greater for GBM, suggesting a relative enrichment of ^{3}H-glycopeptides in this structure.

Distribution of Label in Specific GAG. *Heparan sulfate.* When the GAG fractions that eluted in the void volume from the Sephadex G-50 column (Fig. 1) were subjected to nitrous acid oxidation and rechromatographed (Fig. 2), the majority of both the ^{35}S- and ^{3}H-GAG from all three sources was degraded; therefore, it consists of heparan sulfate. In the case of ^{35}S-GAG derived from cortex, glomeruli, and GBM, 85%, 68%, and 87%, respectively, of the label originally present in GAG eluted with the degradation products (Table 3), whereas the corresponding values were 51%, 60%, and 91% in the cases of ^{3}H-GAG.

Hyaluronic acid. When the GAG that were resistant to nitrous acid oxidation (void volume in Fig. 2) were treated with hyaluronidase and rechromatographed (Fig. 3), an average of 9%, 13%, and 6% of the ^{3}H-labeled GAG from cortex, glomeruli, and GBM was found to be sensitive to hyaluronidase (Table 3), indicating that small amounts of newly synthesized hyaluronic acid are found in all three sources.

Chondroitin sulfates. When GAG resistant to both nitrous acid and hyaluronidase (void volume in Fig. 3) were digested with chondroitinase ABC and rechromatographed (Fig. 4), averages of 10%, 16%, and 9% of the total ^{35}S-GAG and 14%, 20%, and 3% of the ^{3}H-GAG obtained from cortex, glomeruli, and GBM, respectively, were sensitive to this enzyme. This indicates that small amounts of newly synthesized chondroitin sulfates are found in all three sources.

Unidentified chemical- and enzyme-resistant material. Some undigested ^{35}S- and ^{3}H-labeled material remained (void volume in Fig. 4) after all these chemical and enzymatic treatments (Table 3). The nature of this residual material is not known but it could represent: (*i*) unusually large glycopeptide moieties that eluted with GAG on the first column (see Fig. 1); or (*ii*) keratan

Table 3. Distribution of radioactivity incorporated into GAG from [^{35}S]sulfate and [^{3}H]glucosamine

	^{35}S radioactivity, %			^{3}H radioactivity, %		
Type of GAG	Cortex	Glomeruli	GBM	Cortex	Glomeruli	GBM
Heparan sulfate	85	68	87	60	51	91
Hyaluronic acid	—	—	—	9	13	6
Chondroitin sulfates and dermatan sulfate	10	16	9	14	20	3
Unidentified enzyme-resistant material	5	15	4	14	4	0

Cortex, glomeruli, and GBM were prepared from kidneys labeled *in vivo* with [^{35}S]sulfate and [^{3}H]glucosamine. GAG were isolated from these preparations by specific digestion followed by gel filtration as described in the text. The figures for cortex and glomeruli are averages for three different preparations; those for GBM came from a single preparation. The percent of total GAG = (cpm in specific GAG/total cpm in GAG) × 100.

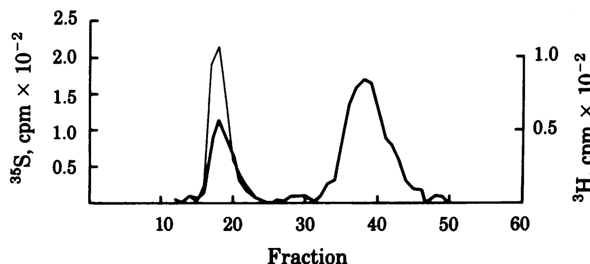

FIG. 3. Example from labeled glomeruli, showing separation of nitrous acid- and hyaluronidase-resistant GAG from hyaluronidase digestion products. Nitrous acid-resistant GAG remaining after aliquots had been removed for radioactivity assay (see Fig. 2) were pooled, digested with *Streptomyces* hyaluronidase, and rechromatographed on Sephadex G-50 as in Fig. 2. Fractions (1.75 ml) were collected and radioactivity was determined in 0.5-ml samples. ^{3}H-Labeled digestion products eluted in one major peak. Enzyme-resistant ^{35}S- and ^{3}H-GAG eluted in the void volume (fractions 16–24). Thin line, ^{35}S radioactivity; thick line, ^{3}H radioactivity.

sulfate [which is not degraded by any of the procedures applied (8)]. A similar unidentified chemical- and enzyme-resistant fraction has been found in other tissues (8, 15).

DISCUSSION

In this study we have labeled *in vivo*, biosynthetically, kidney cortex, glomeruli, and GBM with [^{35}S]sulfate and [^{3}H]glucosamine and have identified the radioactive macromolecules synthesized with these precursors. The results demonstrate the incorporation of label into several GAG species and into glycopeptides isolated from all three sources. In all cases, heparan sulfate is the major labeled GAG present, because the majority of both the ^{35}S and ^{3}H radioactivity is associated with this GAG (on the basis of susceptibility to nitrous acid oxidation). Small amounts of newly synthesized nonsulfated GAG (hyaluronic acid) and sulfated GAG other than heparan sulfate (i.e., chondroitin sulfates A or C or dermatan sulfate) are also present in these preparations (on the basis of susceptibility of ^{3}H-labeled material to *Streptomyces* hyaluronidase and on the basis of susceptibility of ^{35}S- and ^{3}H-labeled material to chondroitinase ABC, respectively). This biosynthetic profile correlates well with the compositional profile obtained previously by chemical analysis of GAG isolated from GBM in which heparan sulfate was found to be the major GAG present in GBM (2). The previous data (obtained by cellulose acetate electrophoresis) also suggested that a small amount of hyaluronic acid may be

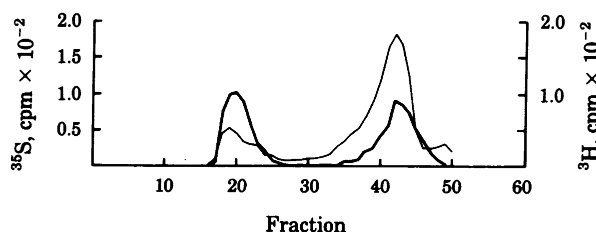

FIG. 4. Representative example from labeled cortex showing separation of GAG resistant to nitrous acid, hyaluronidase, and chondroitinase ABC from chondroitinase ABC digestion products. Hyaluronidase-resistant GAG, after sampling for radioactivity, were pooled, treated with chondroitinase ABC, and rechromatographed on Sephadex G-50 as in Fig. 2. Fractions (1.75 ml) were collected and 0.5-ml aliquots were taken for radioactivity assays. ^{35}S- and ^{3}H-labeled digestion products coeluted as one major peak. Unidentified material resistant to all three treatments eluted in the void volume (fractions 16–24). Thin line, ^{35}S radioactivity; thick line, ^{3}H radioactivity.

present in GBM (2), a finding that is confirmed by the present approach utilizing radiolabeling. The present work has detected a small amount of chondroitin sulfates or dermatan sulfate or both in GBM. On the basis of radiolabeling it appears to be a true component of GBM, although it constitutes a relatively small percentage ($\approx$9%) of the total ^{35}S-labeled GAG. Recent data obtained on newly synthesized proteoglycans extracted from ^{35}S-labeled GBM confirm the presence of chondroitin sulfates in GBM and indicate that heparan sulfate and chondroitin sulfates are located on different proteoglycans (16).

In addition to GAG synthesis, synthesis of glycopeptides by cortex and glomeruli and the incorporation of these products into the GBM *in vivo* has been demonstrated here. We have found that some of the glycopeptides are sulfated, as demonstrated by the fact that $\approx$1/3 of the ^{35}S label incorporated into the GBM was associated with glycopeptides. Although sulfated glycopeptides have been detected in other tissues, especially in brain (17), their presence in the glomerulus and in GBM has not previously been reported.

A surprising finding is that the profiles of labeled GAG extracted from cortex, glomeruli, and GBM are similar in that the major GAG synthesized are heparan sulfate, chondroitin sulfates, and hyaluronic acid, with heparan sulfate predominating in all three. One might expect relatively more label to be incorporated into chondroitin sulfates and hyaluronic acid in the whole cortex due to the large amount of interstitial connective tissue matrix present; however, such was not the case. At present it is not possible to determine whether the data reported reflect different amounts or different turnover rates for the types of GAG found in cortex and glomeruli. Further experiments—autoradiography for localization and double labeling at different time points to study turnover—should clarify this issue.

Notwithstanding the overall similarity of the type and ratios of labeled GAG found in cortex, glomeruli, and GBM (heparan sulfate $\gg$ chondroitin sulfates and hyaluronic acid), there are some distinct differences in the radioactivity distribution patterns obtained from the three sources. Notable were the findings that there was a 2.5 times higher ratio of ^{35}S-GAG/^{3}H-GAG in GBM compared to whole cortex. In addition, there was a 2.5 times higher ratio of ^{3}H-glycopeptides/^{3}H-GAG in the GBM as opposed to glomeruli or whole cortex. With the approach used we cannot determine the nature of the ^{3}H-labeled glycopeptide molecules. In principle, the label could be associated with either collagenous or noncollagenous components of the GBM. Because basement membrane collagens are present in a procollagen-like form (18), and if the situation is the same for basement membrane (types IV and V) as for interstitial (type I) collagen (19), the glucosamine label could be associated with the NH_2- or COOH-terminal extensions of the procollagen molecules. In addition, several noncollagenous glycoproteins (e.g., fibronectin, laminin) have been found to be associated with many basement membranes, including the GBM (20, 21).

Up to now there has been very little work on the synthesis of GAG in the kidney. The most extensive study carried out to date was that of Barry and Bowness (4), who compared the incorporation of label into GAG in kidney cortex with that in medulla after labeling *in vivo* with [^{35}S]sulfate and [^{14}C]glucose. They also found heparan sulfate to be the major GAG synthesized in medulla as well as cortex, with smaller amounts of hyaluronic acid, chondroitin sulfates, and dermatan sulfate detected. Cohen (22) has studied GBM synthesis *in vitro* in isolated glomeruli and has reported incorporation of [^{35}S]sulfate into uronic acid-containing material extracted (with 8 M urea) from GBM; however, she did not determine incorporation into individual GAG species or into glycopeptides.

A number of studies have been done on the synthesis of GAG and their assumed incorporation into basement membranes in other tissues, primarily embryonic tissues such as lens, neural tube, notochord (23), cornea (24), and salivary (25) and mammary (26) glands. All these tissues were found to synthesize various amounts of both heparan sulfate and chondroitin sulfate, and most also synthesized hyaluronic acid. In these embryonic systems a role has been postulated for basement membrane GAG in morphogenesis and development. In all cases the association between GAG and basement membranes was inferred from combined biosynthetic (autoradiographic) and cytochemical observations rather than demonstrated on isolated basement membrane fractions because satisfactory basement membrane fractions have not yet been prepared from these tissues.

Previous cytochemical studies have demonstrated that heparan sulfate is a major component of anionic sites localized in the laminae rarae (interna and externa) of GBM (27) because treatment of GBM with heparitinase (which specifically digests heparan sulfate) removes the anionic sites as detected by binding of cationized ferritin (1). The location of hyaluronic acid and chondroitin sulfates in GBM is not known. Treatment of GBM with hyaluronidase or chondroitinase ABC has no detectable effect on the anionic sites (with cationized ferritin used as a probe) (1). This could indicate that these GAG have a different location than heparan sulfate or, alternatively, that, although associated with the anionic sites, these GAG do not contribute significantly to their net charge. Accordingly, their removal would not interfere with cationized ferritin binding and would not be detected.

The finding that the specific radioactivity of the GBM for ^{35}S and ^{3}H is ≈11% and ≈28% of the corresponding values for glomeruli raises the question as to the location of the remaining non-GBM-associated glycoconjugates in the glomerulus. Assuming that minimal amounts of GAG and glycopeptides are lost during the isolation of GBM, there must be a considerable pool of newly synthesized GAG and glycopeptides associated with glomerular cells or cell surfaces whose distribution is not known at present. In the future it will be of interest to localize, identify, and characterize further these newly synthesized glomerular glycoconjugates.

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