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Research Article

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Regional Distribution of Acid Mucopolysaccharides in the Kidney

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ABSTRACT Kidneys from 20 dogs were dissected into cortical and medullary components and analyzed for acid mucopolysaccharide content. Heparitin sulfate accounted for approximately 80% of cortical acid mucopolysaccharide, 10% was chondroitin sulfate B, and 10% was low molecular weight hyaluronic acid. Medullary tissue exhibited a 4- to 5-fold higher concentration of acid mucopolysaccharide than did cortical tissue, and the dominant compound was moderately highly polymerized hyaluronic acid. While chondroitin sulfates A and (or) C were not detected in this study, the presence of minor amounts of these substances could not be excluded. A model experiment indicated that hyaluronic acid retards sodium diffusion, apparently due to its viscous properties rather than its electronegativity.

INTRODUCTION

The major acid mucopolysaccharide (AMPS) component of normal urine and that from patients is thought to be chondroitin sulfate A (3–5). While it is generally recognized that urinary mucopolysaccharides may reflect the turnover of connective tissue ground substance, it is important to measure the contribution from kidney mucopolysaccharides to the total found in urine.

Beef renal papillae have been analyzed and shown to contain 10–20 mg of AMPS/g of dry weight (6). On the basis of paper electrophoresis and susceptibility to testicular hyaluronidase these workers concluded that hyaluronate, chondroitin sulfate A and (or) C, and chondroitin sulfate B were present. Investigators examining pooled whole rat kidneys provided chemical evidence that a heparin-like substance and hyaluronic acid are the main AMPS constituents (7). Since only about 10% of the hexosamine was galactosamine, they concluded that rat kidneys do not contain significant amounts of chondroitin sulfates.

In this laboratory the dog renal medulla was shown to be much richer in acid mucopolysaccharides than cortical tissue, the major medullary AMPS resembling hyaluronic acid (HA), the predominant cortical AMPS resembling heparitin sulfate (HS) (1, 2). The present study extends our previous data concerning the identification of AMPS components and outlines the compositional pattern in medulla and cortex of canine kidneys. The proportion of renal medulla occupied by intertubular connective tissue stroma was measured by a planimetric method making it possible to calculate the approximate tissue mucopolysaccharide concentrations. This provided a quantitative basis for constructing a laboratory model to evaluate the effect of a single mucopolysaccharide on sodium flux in the extratubular portion of the medulla of the kidney.

METHODS

Tissue preparation. 20 normal dog kidneys were analyzed for acid mucopolysaccharides in 10 separate pools of variable size. Kidneys were dissected into medullary and cortical portions, cut into 0.5-cm fragments, and weighed. The wet tissue was extracted for 24 hr with two changes of acetone at 4°C and then with three changes

Preliminary reports of parts of this study have appeared in abstract form (1, 2).

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of 2:1 chloroform:methanol (approximately 10 volumes/volume of tissue). The dried tissue was weighed, and this weight recorded as lipid-free dry weight. After suspension in pH 7.0 phosphate-saline buffer, the dry sample was homogenized in a Waring Blendor. Collagenase,[1] 1.0 mg/ml, was added and the suspension incubated with stirring at 37°C for approximately 8 hr and then dialyzed (4°C) against a 0.05 M Tris buffer, pH 8.0. The proteolytic enzyme Pronase[2] was added at a concentration of approximately 1.0 mg/ml and incubated with stirring for 6–8 hr at 37°C.

Isolation of hyaluronate and sulfated mucopolysaccharide fractions. The solubilized tissue was dialyzed against distilled water and a crude mixture of mucopolysaccharides isolated by addition of cetylpyridinium chloride (CPC) and sulfate ion (8). The CPC-polyanion precipitate was washed three times with distilled water, dried, and dissolved in methanol. The sodium salt of the mixed acid mucopolysaccharides was precipitated by adding 10% sodium acetate in methanol. Hyaluronic acid was then separated from sulfated mucopolysaccharides (SO_4-MPS) by precipitating the latter with CPC from a solution of 0.2 M sodium sulfate. The hyaluronate portion was precipitated by dilution of the supernatant fluid remaining after removal of SO_4-MPS. HA and SO_4-MPS were individually recovered from their CPC complexes as indicated above. Sulfated mucopolysaccharides were further fractionated with either varying concentrations of ethanol in the presence of acetic acid and calcium acetate (9) or with CPC in the presence of 0.4 M $AlCl_3$.

When fractionating the sulfated mucopolysaccharide mixture with ethanol, the mixture was brought to a concentration of 5% calcium acetate in 0.25 M glacial acetic acid. Ethanol was added to a concentration of 23% by volume. This stood at 4–6°C overnight and the first precipitate was harvested and identified as the 0–23% fraction. The supernatant fluid was brought to 50–52% ethanol and the remaining polysaccharide precipitated as the 23–52% fraction. In some instances the mucopolysaccharides were treated for approximately 2 hr with both ribonuclease and deoxyribonuclease, dialyzed, and the polysaccharide reisolated.

Fractionation of sulfated polysaccharides with CPC in the presence of aluminum chloride was suggested by our previous experience with a polymer chromatography system in which it seemed clear that the CPC complex of chondroitin sulfate B (CS-B) was insoluble in 0.4 M aluminum chloride, whereas most other mucopolysaccharide CPC complexes were soluble (11). Consequently an alternative procedure for fractionating the mixed sulfated mucopolysaccharides consisted of making the mixture 0.4 mole/liter with aluminum chloride and adding an excess of CPC in the presence of small amounts of sulfate ion. The MPS-CPC complex which precipitated under these conditions could be washed and reisolated as the sodium salt. Upon analysis this fraction was nearly all (over 90%) CS-B. The MPS in the supernatant fraction could be recovered by 1:10 dilution of the sample which led to precipitation of the CPC complexes of the remaining mucopolysaccharides. Analysis of this fraction revealed a form of heparitin sulfate (HS). Representative samples of sulfated mucopolysaccharide mixtures were also fractionated by the column method of Schiller, Slover, and Dorfman (10).

Analytical procedures. Tentative identification of isolated mucopolysaccharides was accomplished by polymer chromatography using six solvent systems on paper as reported earlier (11). Routine uronic acid measurements were made with a borate modification (12) of the carbazole procedure supplemented where appropriate by measurement with the Dische (13) and orcinol procedures (14). Hexosamine was measured by a modification of the Elson-Morgan procedure (15) and sulfate by a method employing barium chloranilate (16). In each case the analysis was done after a 16 hr hydrolysis of the polymerized sample with 4.0 N HCl in a sealed tube at 100°C. Before chromatographic examination or colorimetric measurement, hexosamine samples from the hydrolysate were passed through a Boas colume (17). The distinction between glucosamine and galactosamine was made using two chromatographic systems (18, 19). The distinction between glucuronic acid and iduronic acid was made by chromatography of the respective lactones after hydrolysis with formic acid (20). Protein was measured by a modification of the Lowry procedure (21) as well as by ultraviolet methods. Hexose was measured by an anthrone method (22).

Viscosity measurements were made employing Ostwald viscometers with buffer flow times of 60–70 sec and an upper bulb capacity of 3.0 ml. Relative viscosity (η_r) was measured as the ratio of the flow time of polymerized mucopolysaccharide to the flow time of a sample after incubation with testicular hyaluronidase (23). Intrinsic viscosity ($[\eta]$) was calculated as relative viscosity -1 divided by the concentration of mucopolysaccharide in g/100 ml. These measurements were carried out at extreme dilutions and provide a measure of the molecular weight of carbohydrate macromolecules (24).

Histological procedures were performed on a few samples of normal dog kidney, using Bouins' fixative and 10% formalin containing 1% CPC (25). Sections were dehydrated in alcohol, cleared with aniline oil and acetone, and embedded in paraffin. Paraffin sections were cut at 4 μ and stained with both the Masson trichrome procedure and with a PAS-alcian blue method. To determine the volume of renal medulla occuplied by interstitial material, eight sections of renal medulla were cut at right angles to the plane of the tubules. Photomicrographs of the sections were enlarged to 11 × 14 inches. Four areas from each photo were chosen at random and projected on a sheet of paper of known area and the tubular images were drawn. The surface area occupied by tubules in these 32 drawings was determined by planimetry and subtracted from the known area to determine interstitial area which was expressed as a percentage of total area.

[1] Worthington Biochemical Corp., Freehold, N. J.

[2] Calbiochem, Los Angeles, Calif.

TABLE I

Distribution of Hyaluronic Acid and Sulfated Mucopolysaccharide in the Kidney

Sample	Total AMPS*		Hyaluronate		Sulfated AMPS
			Proportion of total AMPS	[η]‡	Proportion of total AMPS
	mg/g wet wt	*mg/g dry wt*	%		%
Medulla	1.27 ± 0.10§	8.90 ± 0.70	68.4 ± 5.0	17.2 ± 1.9	31.6 ± 5.0
Cortex	0.33 ± 0.01	1.35 ± 0.20	11.3 ± 2.2	9.2 ± 1.8	88.7 ± 2.2

* AMPS, acid mucopolysaccharide.

‡ [η], intrinsic viscosity.

§ Values are recorded as a mean ± SEM.

To measure the effect of macromolecular sugars on the diffusion rate of sodium, we used two 13.0-ml chambers separated by a Visking cellophane dialysis membrane. The sodium-rich side was mixed by bubbling air through the chamber and the sodium-poor side was connected to a 1000 ml reservoir by a pumping system that changed the chamber contents every 4 min. Hyaluronic acid and Dextran [3] were added in separate experiments to the sodium-rich chamber, and the decreasing sodium concentration was measured as a function of time while it diffused towards the sodium-poor reservoir. Sodium was measured by flame photometry.

RESULTS

Chemical fractionation studies. The hyaluronic acid and sulfated mucopolysaccharide fractions of the renal medulla and cortex are quantitatively distributed as illustrated in Table I. It is apparent that the total mucopolysaccharide is more concentrated in the renal medulla than in the cortex. On a dry weight basis the dog renal medulla actually had approximately six times as much acid mucopolysaccharide per gram of dry weight as the accompanying cortex. When these mucopolysaccharides are divided with CPC in the presence of 0.2 M sodium sulfate into a hyaluronate and sulfated polysaccharide fraction, it is apparent that nearly 70% of the medullary MPS is hyaluronate, whereas only approximately 11% of the mucopolysaccharide in the cortex is hyaluronate. It is noteworthy that the intrinsic viscosity of hyaluronate isolated from the medulla is nearly twice that in the cortex, indicating a higher molecular weight for the medullary material.

Although viscosity measurements on material identified as hyaluronate by virtue of the solubility of its CPC complex in 0.2 M sodium sulfate pro-

[3] Dextran-2000, mol wt = 2×10^6 was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

TABLE II

Characterization of Hyaluronic Acid Fraction from Cortex and Medulla of Dog Kidneys

Material analyzed	Preparation	Protein	c/o	Polymer chromatog.*	Hexosamine chromatog.		Uronic acid chromatog.		Molar ratios		
					Gm	Galm	Gluc	Id	Hexosamine	Uronic acid	Sulfate
		%									
Cortex "HA"	isolated twice	24.2	1.09	HA	+‡	Tr.			1.00	0.97	0.20
Medullary "HA"	isolated twice	4.8	1.15	HA	+	Tr.			1.00	0.99	0.12
Medullary "HA"	isolated three times		1.41		+	0	+	0	1.00	1.06	0.06

c/o, carbazole (Dische):orcinol ratio; Gm, glucosamine; Galm, galactosamine; Gluc, glucuronic acid; Id, iduronic acid; Tr, trace.

* Ref. No. 11.

‡ + = major component.

vided strong evidence that the mucopolysaccharide was HA, more definitive identification procedures were carried out. These fractions were identified by the paper polymer chromatography systems as hyaluronate, and carbazole-orcinol ratios were compatible with this identification (Table II). Hexosamine identified in the preparations was glucosamine and the uronic acid was glucuronic acid. The molar ratios of hexosamine and uronic acid were approximately 1.0 and in each case essentially no sulfate was found.

Tables III and IV present data supporting the

TABLE III

Characterization of Sulfated Mucopolysaccharides after Ethanol Fractionation

Material analyzed	Preparation*	Fraction of total sample	c/o	Polymer chromatog.	Hexosamine chromatog.		Uronic acid chromatog.		Molar ratios			Relative amounts of SO_4-MPS‡	
					Gm	Galm	Gluc	Id	Hexosamine	Uronic acid	Sulfate	HS	CSB
		%	%		%								%
Cortex–SO_4-MPS	0–23 EtOH	16.4	0.90	"CS-B"+"HS"	50	50	Tr.	+	1.00	1.15	1.40		
	23–52 EtOH	83.6	1.71	"HS" ?CSA	95	5	+	Tr.	1.00	1.17	1.02	87.4	12.4
Medullary–SO_4-MPS	0–23 EtOH	5.7	0.65	"CS-B"	25	75			1.00	1.16	1.80		
	23–52 EtOH	94.3	1.31	"HS"+"CSB"	40	60	+	+	1.00	1.12	1.34	39.2	60.8

See Table II for abbreviations.

* Preparation, 0–23% EtOH refers to AMPS fraction having lowest alcohol solubility, while the 23–52% fraction required a higher ethanol concentration to cause precipitation of the calcium salt.

‡ Estimation of relative amounts of CS-B and HS was based on semiquantitative estimate of relative amounts of the two hexosamines and uronolactones seen on paper chromatography.

TABLE IV

Characterization of Sulfated Mucopolysaccharides after Fractionation with CPC and Aluminum Chloride

Preparation No.	Starting material	CPC-$AlCl_3$ fraction	Proportion of total	c/o	Polymer chromatog.	Hexosamine chromatog.		Uronic acid chromatog.	
						Gm	Galm	Gluc	Id
			%			%			
69	Cortex, SO_4-AMPS	$AlCl_3$-Ppt.* "CS-B"	7.4	0.69	CS-B	5	95	Tr.	+
		$AlCl_3$-Super‡ "HS"	71.0	1.89	HS	100	0	+	0
		Not recovered	20.8						
87	Cortex, SO_4-AMPS	$AlCl_3$-Ppt. "CS-B"	8.6		CS-B	20	80	0	+
		$AlCl_3$-Super	70.0		HS				
		Not recovered	21.4		—				
75	Cortex, SO_4-AMPS	$AlCl_3$-Ppt. "CS-B"	5.7	0.77		25	75		
		$AlCl_3$-Super "HS"	64.4	2.16	HS	95	5		
		Not recovered	29.9						
88	Medulla, SO_4-AMPS	$AlCl_3$-Ppt. "CS-B"	48.0		CS-B	0	100	0	+
		$AlCl_3$-Super "HS"	52.0						
73	Medulla, SO_4-AMPS	$AlCl_3$-Ppt. "CS-B"	53.0						

* $AlCl_3$-Ppt. refers to AMPS fraction which is precipitated from 0.4 M $AlCl_3$ by CPC as "CS-B," and shows appropriate analyses.

‡ $AlCl_3$-Super refers to AMPS fraction which is not precipitated from 0.4 M $AlCl_3$ by CPC, and is identified as heparitin sulfate, "HS."

characterization of sulfated mucopolysaccharide fractions resulting from the ethanol fractionation on one hand and the CPC–aluminum chloride fractionation procedure on the other. It is apparent in Table III that nearly 90% of the sulfated MPS from renal cortex was HS and the remainder CS-B. The fractions derived from medullary sulfated polysaccharides show approximately 40% HS and 60% CS-B. Material recorded as "CS-B" was so identified by paper polymer chromatography (11). On hydrolysis of the "CS-B," approximately 50% of the hexosamine from the cortical fraction and 75% of that from the medullary fraction were identified as galactosamine, and iduronolactone was the uronic acid derivative found by chromatography.

Unfortunately it was not possible to obtain ethanol fractions that were not mixtures and for that reason the ethanol fractionation studies were supplemented by a CPC–aluminum chloride procedure. The polysaccharide precipitating with CPC in the presence of 0.4 M AlCl, CS-B, represented 7–9% of the total cortical polysaccharide, in fair agreement with the results (12.4%) by the ethanol fractionation procedure (see Table III, two right-hand columns). In the case of the medullary polysaccharides, it was evident that approximately one-half of the total sulfated polysaccharide precipitated as CS-B. Identification of the CS-B was supported by a carbazole-orcinol ratio of 0.69 and a typical paper chromatographic pattern. Virtually all of the detectable hexosamine was identified by chromatography as galactosamine and the lactone derivative of the uronic acid was iduronolactone. Identification of the MPS remaining soluble in the supernatant as heparitin sulfate was supported by a carbazole-orcinol ratio approaching 2.0 and appropriate polymer chromatograms. The hexosamine in this fraction was glucosamine, the only uronic acid detected was glucuronic acid, and the sulfate-hexosamine ratio was 1.4:1. Information concerning the purity of fractionated SO_4-MPS characterized as HS is given in Table V. Samples were essentially protein free, had no detectable hexose, could be eluted from Dowex 1 × 2 Cl^- with 1.25 M NaCl, and had a carbazole-orcinol (C/O) ratio of about 2.0.

TABLE V

Characterization of Heparitin Sulfate from Dog Kidney

Source of SO_4-MPS	Fractionation method	Hexose content*	Protein content	Glucosamine	c/o
			%	%	
Dog cortex	Column‡	0	1.3	>90	1.88
Dog cortex	AlCl-Super	0	0.7	>95	2.16

* Anthrone reactions for hexose were carried out on the same volume of sample used for the carbazole and orcinol reactions.

‡ This fraction was eluted from a Dowex 1 × 2 Cl^- column with 1.25 M NaCl as suggested by Schiller (10), desalted by passage through a Sephadex G-15 column, precipitated with CPC, and converted to the sodium salt with methanolic sodium acetate.

TABLE VI

Composition of Unfractionated Mucopolysaccharides from Dog Renal Cortex and Medulla, and Urine

Source	Hexosamine	Uronic acid	Sulfate	Type of hexosamine*	
				Gm	Galm
Dog kidney					
Cortex	1.00‡	0.81	1.97	+	Tr
Medulla	1.00	1.10	1.10	+	Tr
Urine	1.00	1.35	1.36	0	+

* Hexosamine from MPS preparations were separated by paper chromatography using butanol:pyridine:water (6:4:3) (19) and a ninhydrin method to identify the resolved components (26).

‡ Values represent the molar ratios of the AMPS constituents measured, taking hexosamine as unity.

These data, taken together, suggest that most of the mucopolysaccharide found in the dog renal cortex is heparitin sulfate, with perhaps as much as 10% chondrotin sulfate B and 10% hyaluronic acid. In the medulla it would appear that the major mucopolysaccharide constituent is moderately highly polymerized hyaluronic acid and that the residual sulfated mucopolysaccharide is evenly divided between heparitin sulfate and chondroitin sulfate B.

As indicated earlier, most evidence suggests that the major mucopolysaccharide isolated from human urine is chondroitin sulfate A (CS-A). The present data provide no support for the presence of CS-A within the architecture of the kidney itself. In an attempt to see whether the same dichotomy held for the dog, 24-hr collections of urine were made; the animal was sacrificed and the kidneys removed and dissected. The urinary mucopolysaccharides were isolated by a cetylpyridinium chloride method previously described (5) and the renal MPS were isolated as noted above. The mixtures of mucopolysaccharides from dog

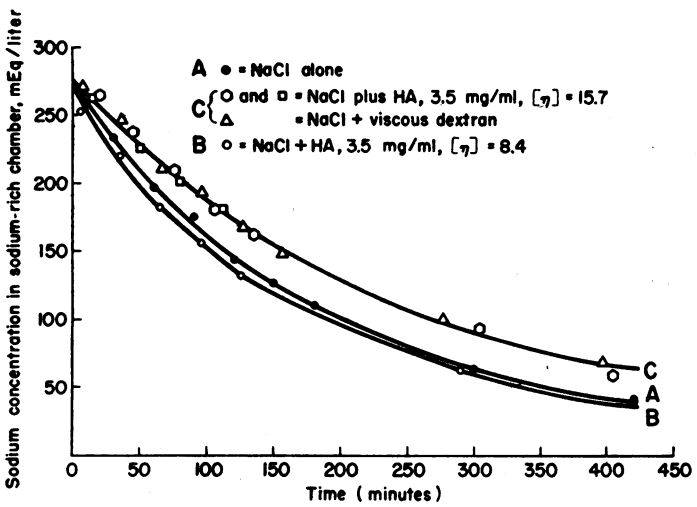

FIGURE 1 Hyaluronate retardation of sodium diffusion. Curve *A* describes the rate of sodium loss from a standard sodium-rich chamber in the absence of carbohydrate additives. The same curve was demonstrated in two additional experiments. Only viscous preparations of hyaluronate retarded sodium diffusion (curve *C*). Two experiments with viscous HA were carried out, and points from both fall along same line, curve *C*. The same concentration of a lower molecular weight preparation, (thus less viscous) had no effect (curve *B*). A viscous preparation of an uncharged polysaccharide, dextran, behaved like viscous HA. The points in the sodium die-away curve in the presence of viscous dextran fell along curve *C*.

cortex, dog medulla, and dog urine were subjected to hydrolysis with 4.0 N HCl for 16 hr and sulfate and hexosamine analysis carried out. In addition, hexosamine chromatography was performed. As noted earlier with respect to fractionated renal mucopolysaccharide specimens, the major hexosamine found in dog kidney was glucosamine. On the other hand, we found no evidence of glucosamine in the polysaccharide isolated from dog urine; all of the hexosamine had the chromatographic mobility of galactosamine. From these studies it would appear that in the dog as in the human, the contribution from the normal kidney to the mucopolysaccharides found in the urine must be virtually insignificant (see Table VI).

Physiologic observations. Sections were made in both a longitudinal and horizontal orientation with respect to the renal pyramids. The Masson trichrome stain disclosed strikingly large amounts of loosely arranged fine collagenous fibrils lying between tubular structures. Alcian blue staining of such sections revealed punctate and linear aggregations of dye between the tubules. PAS staining was primarily restricted to basement membranes around the tubules. Alcian blue staining of the intertubular stroma is compatible with localization of anionic macromolecules of the acid mucopolysaccharide type, although not specific for these substances.

Planimetric studies of 32 areas of renal medulla revealed a mean value of 43.4% of tissue cross-section, hence, volume, occupied by "interstitial materials" (mean = 43.4%, SD = ± 7.1%). Since shrinkage artifacts related to fixation and dehydration are likely to exert a disproportionate effect on the water-rich extratubular space, the 43.4% figure should be regarded as a minimum value. If one assumes that the tissue density of tubular and interstitial tissue is similar, then the data from Table I may be used to calculate approximate tissue concentrations of the different classes of acid mucopolysaccharides. Hyaluronic acid, if confined to the interstitial space, would be found at tissue concentrations in excess of 2.0 mg/ml, in a range commonly found in the synovial fluid of joints and bursae (27).

While the high concentration of hyaluronic acid in the extratubular stroma of the medulla undoubtedly contributes a resilient gelatinous support for delicate tubular structures, it seemed possible that this diffusely distributed anionic macromolecule might serve as a retention lattice for the sodium gradient known to be present in this portion of the kidney (28). To measure the effect of hyaluronic acid on sodium movement in an aqueous milieu, we calibrated the rate at which sodium (275 mEq/liter) diffused across a standard cellophane membrane. Curve A (Fig. 1) illustrates the die-away curve of sodium in a test chamber over 7 hr. As illustrated by curve B, hyaluronic acid of low molecular weight ($[\eta]=8.4$) had no retarding effect on sodium efflux in concentrations ranging from 1.0 to 3.5 mg/ml. On the other hand, moderately highly polymerized hyaluronate ($[\eta]=15.7$) at 3.5 mg/ml slowed sodium loss via transmembrane diffusion (curve C). This latter HA preparation contributed not only an anionic lattice to the sodium-rich chamber, but substan-

tial viscosity as well. Fig. 1 indicates that a dextran solution prepared to a η_{sp} similar to the HA in curve C, duplicated the sodium retarding effect of the more highly polymerized (thus viscous) hyaluronate preparation.

DISCUSSION

Careful studies by Allalouf, Ber, and Sharon established that mucopolysaccharide mixtures isolated from whole rat kidneys contained primarily glucosamine as the hexosamine component (7). The minor amount of galactosamine led them to conclude that rat kidneys do not contain significant amounts of chondroitin sulfates. Glucosamine in renal MPS was believed derived mostly from a heparin-like substance and to a lesser extent from hyaluronic acid. Earlier studies by Farber, Cohn, and Kastor of beef and rabbit renal papillae did not detect a heparin-like material, but suggested that CS-A, CS-C, and to a lesser extent, CS-B accounted for the SO_4-MPS isolated (6). While it is conceivable that a marked species difference might explain these divergent results, the fact that the hexosamines in beef and rabbit renal MPS were not identified leaves the exact identity of the compounds from these sources in doubt.

Our analyses of canine renal AMPS are in general agreement with Allalouf's data from rat kidneys. Their analyses, carried out on whole rat kidneys, suggested that hyaluronic acid accounted for 15–20% of the total renal acid mucopolysaccharides, while our measurements indicate that HA accounts for 35% of canine renal AMPS. Our data shows, in addition, that most of the renal HA is found in the medulla. Where the latter authors chose to identify the major glucosamine-containing MPS as heparin-like, we interpreted our data as evidence for the presence of heparitin sulfate. Paper chromatograms supported HS rather than heparin, and carbazole-orcinol ratios were close to 2.0 (as for HS) rather than 4.0 which our analyses show for heparin. In addition, the sulfate-hexosamine ratio was low, (1.02 : 1.00 in our best preparation) more in keeping with HS than heparin (29). The present data confirm the presence of HA and identify small amounts of CS-B in canine renal tissue.

Separate analyses of cortical and medullary tissue showed clearly that the proportions of HA (and its $[\eta]$), HS, and CS-B differ in these two regions. It is noteworthy that medullary HA, if uniformly distributed in extratubular water, would approach the concentrations found in joint fluid. Isolation procedures may be attended by unsuspected losses of hyaluronate, and molecular weight estimations represent only a lower limit due to the likelihood of degradation of the polymer during isolation. It is possible that both the concentration and the molecular weight are actually higher than our data indicate. In this connection it should be noted that the difference in $[\eta]$ of HA from cortex and medulla could be explained by hyaluronidase localization in the cortex, leading to degradation of HA during isolation, instead of reflecting a different molecular weight at these two tissue sites.

It is unclear whether the acid mucopolysaccharide (HA) is in the medulla to provide mechanical protection for tubular structures, as in Wharton's jelly of the umbilical cord, or whether it functions as an exchange resin providing a chemical scaffold for the varying sodium concentrations involved in the countercurrent system. Our data support the concept that hyaluronate slows the translocation of sodium, presumably by virtue of its gel-like consistency rather than its electronegativity. The role of CS-B and HS, present in substantial quantities in the medulla and much more electronegative than HA, remains to be studied as do the effects of a multitude of other substances known to reside in the interstitium.

That renal tissue should be heavily endowed with HS in addition to lesser amounts of HA and CS-B is not surprising, since the kidney is highly vascular and these compounds have previously been isolated from blood vessels (30, 31). The latter studies, however, show that aorta, pulmonary artery, and coronary artery also contain chondroitin sulfate A and (or) C in amounts accounting for half or more of the isolated MPS, a feature not shared with renal tissue. Kidney AMPS composition may be somewhat analogous to lung parenchyma which is a source for both HS and CS-B, as well as heparin, and to liver tissue which contains HS in addition to heparin. In a similar vein, we have found no published data supporting a substantial content of CS-A and (or) CS-C in lung or liver. It is possible that HS is characteristic of small blood vessels in view of its prominence in these tissues with an extensive microvasculature.

Isolation of CS-B from a mixture of AMPS was greatly facilitated by utilizing CPC in the presence of 0.4 M aluminum chloride and provides a successful tactic for separating CS-B and HS.

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