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THE SULFOMUCOPOLYSACCHARIDES FROM A MAST CELL TUMOR OF THE MOUSE

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Abstract—Sulfomucopolysaccharides prepared from a murine mast cell tumor were fractionated by chromatography on Ecteola, by precipitation with cetyltrimethylammonium bromide, and by paper chromatography. The crude extract of murine sulfomucopolysaccharides, when extracted by the same procedure that was used to extract bovine sulfomucopolysaccharides, was associated with nitrogen-rich material, unlike the comparable bovine preparation. The nitrogen-rich heparin-containing material, which was precipitable with 0·25 M NaCl and strongly adsorbed to Ecteola, was immobile upon paper chromatography. Examination of material prepared in the same way from bovine lung, guinea pig lung, a canine mastocytoma, and the lung and kidney of the rat, showed that only the tissues of the rat, like the murine mast cells, contained an immobile component. Purified murine sulfomucopolysaccharide showed a greater affinity for histamine and 5-hydroxytryptamine than did bovine sulfomucopolysaccharide. It is suggested that the relatively greater affinity of the murine sulfomucopolysaccharide for amino-containing compounds explains the high concentration of nitrogen in crude extracts of the murine sulfomucopolysaccharide.

SEVERAL lines of the P-815 mast cell tumor of the mouse, which have been in culture for four years, have retained their capacity to synthesize heparin[1] as well as histamine and 5-hydroxytryptamine.[2, 3] That the crude sulfomucopolysaccharide contained heparin was shown[4] by its anticoagulant activity, metachromasia, content of ester-sulfate and glucosamine, the chromophore obtained after its reaction with carbazole, and its precipitation by quaternary salts such as cetyltrimethylammonium bromide (Cetavlon) and by 4-amino-4′-chlorodiphenyl, which precipitates only highly sulfated polysaccharides.[5] On paper chromatography, the sulfomucopolysaccharide that was extracted from cells grown in ^{35}S-sulfate was resolved into three radioactive and metachromatic components;[4] the heterogeneity of the sulfomucopolysaccharides extracted from the original P-815 tumor was shown in other laboratories by paper chromatography[6] and by chromatography on Ecteola,[7] although homogeneous material was obtained by fractionation with Cetavlon.[8]

The presence of three components in the murine extract seemed of particular interest for two reasons. First, the concentration of one of these was correlated with the concentration of histamine and especially of 5-hydroxytryptamine. Second, one of the

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components was not present in a commercial preparation of bovine lung heparin.[4, 9] Since heparin in lung is in mast cells, these observations suggested that the heparin[5] in murine and bovine mast cells may differ. A comparison of the sulfomucopolysaccharides extracted from the murine mast cell tumor with those extracted from bovine lung and the organs of some other mammals is the subject of this paper.

METHODS

Analytical methods. Sulfate was determined[10] after hydrolysis[11] in 5·0 N HCl in a sealed tube for 24 hr in a boiling water bath. The carbazole[12] and orcinol[13] reactions and methods for measuring nitrogen,[14] hexosamine,[15] acetate,[16] and anticoagulant activity[4] have been described. Histamine and 5-hydroxytryptamine were determined by bioassay.[4]

Materials. Sulfomucopolysaccharides from the mouse were extracted from X-1 and X-2 cells,[4] variants of the P-815 mastocytoma.[17] These cells were grown as ascitic tumors in mice; cells from over 500 mice were used. To obtain radioactive sulfomucopolysaccharides, 1 mc of ^{35}S-sulfate was injected intraperitoneally 24 hr before the the animals were killed. Bovine sulfomucopolysaccharides were prepared from bovine lung obtained from the slaughterhouse, and canine sulfomucopolysaccharides from a solid mastocytoma of the dog, kindly provided by Dr. William G. Clark of the Veterans Administration Hospital, Sepulveda, California. Commercial bovine lung heparin was bought from the Nutritional Biochemical Corporation. Heparin from swine intestinal mucosa was a gift of Dr. Floyd C. McIntire of the Abbott Laboratories.

Extraction of crude sulfomucopolysaccharides. Sulfomucopolysaccharides from the mouse, rat, cow, and dog were prepared by a method[4, 18, 19] that consisted of digestion with pancreatin, dialysis, removal of residual protein by treatment with trichlorotrifluorethane (Genetron 113), and precipitation with acetone.

The dialysis membrane (Arthur H. Thomas Company) that was used to prepare sulfomucopolysaccharides, even after heating for three days in water at 80° and repeated washing in 1 M NaCl and water, yielded on mock-dialysis in distilled water or salt solutions, nondiffusible material that formed precipitates upon the addition of acetone, Cetavlon, or 4-amino-4′-chlorodiphenyl, all of which precipitate heparin. The concentrated material was not metachromatic, it did not contain hexosamines or carbazole-reacting materials, and it did not adsorb to Ecteola. However, after hydrolysis it formed a white precipitate with barium chloride and it analyzed as inorganic sulfate. Accordingly, it was necessary to correct for the contribution of this material to the analytical results obtained with preparations of sulfomucopolysaccharides, and a control was run consisting of a dialysis bag of the same size, treated in the same way, and containing the same substances as those used for the preparation of sulfomucopolysaccharides, lacking only the tissue.

Fractionation with Ecteola. The methods previously described[20, 21] were used with several modifications. Alkali-washed columns (17 × 2 cm) of Ecteola, a cellulose anion-exchange resin, were equilibrated with 0·01 M sodium phosphate, pH 6·0, before the introduction of the crude extracts, which were dissolved in the phosphate solution. The column was then washed with 250 ml of the phosphate solution, and a gradient elution was begun. The reservoir consisted of 0·5 M or 1·0 M NaCl in the phosphate solution; the mixing chamber contained 500 ml of phosphate solution. Flow

rate was adjusted to 5 to 10 ml/hr. The murine sulfomucopolysaccharide was detected by measuring radioactive sulfate in a liquid scintillation counter. The sulfomucopolysaccharide of beef lung was measured by the carbazole reaction; a commercial preparation of bovine lung heparin was used as standard. Eluates were dialyzed and then concentrated *in vacuo*.

Despite prolonged washing of the Ecteola with 0·1 N NaOH, NaCl washings from the Ecteola alone were found to contain material that reacted with orcinol. These washings were free of sulfate, hexosamine, metachromatic activity, and carbazole-reacting material.

Fractionation with Cetavlon. The method of Korn[8] was used. Sulfomucopolysaccharides were precipitated by the addition of an excess of a 2% aqueous solution of Cetavlon in varying concentrations of NaCl at room temperature. The precipitates were collected by centrifugation, dissolved in 2 M NaCl, and excess Cetavlon removed by precipitation with KCNS. Excess KCNS was removed from the solution containing the sulfomucopolysaccharides by dialysis, and the nondiffusible material concentrated *in vacuo*.

Paper chromatography. Whatman 3MM paper was used in a solvent consisting of 0·04 M ammonium formate–2-propanol (65:35, v/v).[4] This solvent is like that used by Spolter and Marx,[6] except that they adjusted the pH. After development, papers were stained[4] with azure A to show metachromasia and, in some instances, with acridine orange or alcian blue. Radioactivity on the developed paper chromatograms was measured with an end-window Geiger-Müller counter. The total counts for all peaks were summed and the percentage radioactivity for each peak calculated.[4]

Conductivity measurements. All measurements were made at 25° with a Wheatstone bridge. The 1-ml cell had a constant of 10·93. This method[22] of measuring interaction of sulfomucopolysaccharides with other substances is more sensitive and more accurate than equilibrium dialysis and, furthermore, it obviates consideration of the Donnan equilibrium.

RESULTS

Fractionation with Ecteola

Figure 1 shows the resolution of crude murine mast cell ^{35}S-sulfomucopolysaccharides into two apparently separate fractions: one was eluted with a gradient of 0·0 to 0·5 M NaCl, the other soon after a 0·5 to 1·0 M NaCl gradient was begun. Together these fractions consisted of 55 per cent of the radioactivity of the starting material. The remainder of the material could be eluted with either 5·0 M ammonium formate or 1 M NaOH. The product formed by the reaction of all fractions with the carbazole reagent showed maximum absorption at 535 mμ, as did commercial preparations of heparin.

Figure 2 shows the chromatogram on Ecteola of the crude sulfomucopolysaccharides of bovine lung, prepared by the same procedure as that used for the preparation of the murine extract. The fractions, which were located by the carbazole reaction, showed a series of small peaks. All the material that was eluted by 0·0 to 0·5 M NaCl gradient was combined and kept separate from that eluted by the 1·0 M NaCl gradient. These two fractions, which showed maximum absorption at 535 mμ in the carbazole reaction, formed 88 per cent of the starting material. Treating the column with 5 M ammonium formate or 1 M NaOH failed to elute more carbazole-reacting material.

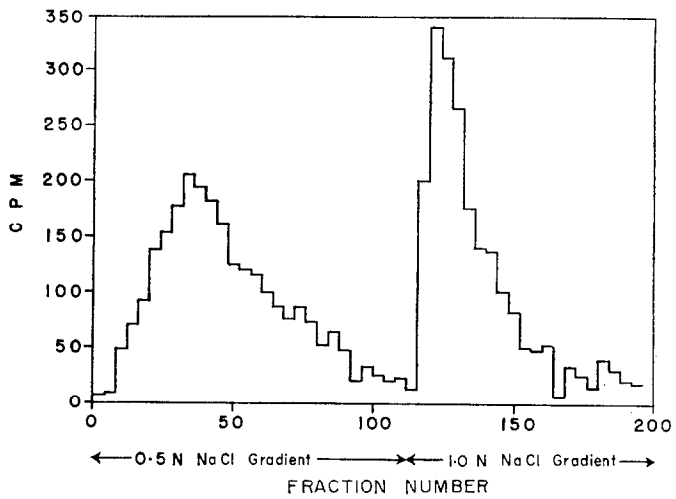

FIG. 1. Fractionation on Ecteola of a crude extract of ^{35}S-sulfomucopolysaccharides from murine neoplastic mast cells. Radioactivity in each 5-ml fraction was measured in a liquid scintillation counter. The elution was carried out in phosphate buffer with NaCl in gradients of 0 to 0·5 M and 0·5 M to 1·0 M.

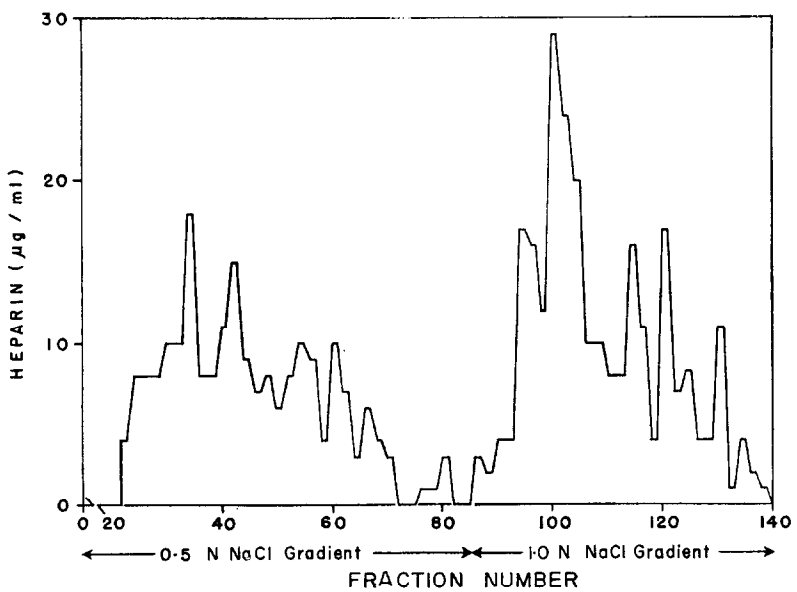

FIG. 2. Fractionation of Ecteola of a crude extract of sulfomucopolysaccharides from bovine lung. Each tube was reacted with carbazole and the resultant color read in a spectrophotometer at 535 mμ against a standard solution of a commercial preparation of bovine lung heparin. The elution was carried out as described in Fig. 1.

Precipitation with 0·25 M NaCl and with Cetavlon in NaCl

Crude sulfomucopolysaccharides of murine, bovine, and canine origin and Ecteola fractions were separately dissolved in water and brought to 0·25 M NaCl *without* the addition of Cetavlon. Precipitated material was collected by centrifugation. Under these conditions, only 4 per cent of the crude bovine extract, none of the canine extract, but 40 per cent of the crude murine extract, was precipitated (Table 1). All the material eluted from Ecteola with 5·0 M ammonium formate was precipitated by 0·25 M NaCl. These precipitates contained hexosamine and showed maximum absorption at 535 mμ in the carbazole reaction. None of the material eluted from Ecteola with NaCl contained material that was precipitable with 0·25 M NaCl alone.

To the supernatant solution remaining after removal of the material that precipitated in 0·25 M NaCl, sodium chloride was added to make the solution 1 M with respect to NaCl, and Cetavlon was added. The precipitate was collected by centrifugation. The supernatant solution was diluted with an equal volume of water to yield a solution of 0·5 M NaCl. The materal precipitating with Cetavlon in 0·5 M NaCl was collected, and the supernatant solution was diluted to 0·25 M NaCl. The sulfomucopolysaccharides were recovered by the procedure described in Methods.

TABLE 1. FRACTIONATION OF SULFOMUCOPOLYSACCHARIDES WITH SODIUM CHLORIDE ALONE AND WITH CETAVLON

After the solution was brought to 0·25 M NaCl, any precipitate that formed was collected by centrifugation, and the clear solution was brought to 1·0 M NaCl. Cetavlon was added and material precipitating with Cetavlon in 1·0 M NaCl was collected. The supernatant solutions, still containing Cetavlon, were then diluted with water and precipitates collected at 0·5 M and 0·25 M NaCl. The sulfomucopolysaccharides were recovered from the Cetavlon–sulfomucopolysaccharide complex and counted in a liquid scintillation counter.

Material	Per cent precipitated			
	Without Cetavlon	With Cetavlon		
	0·25 M NaCl	1·0 M	0·50 M	0·25 M
Murine mast cell				
Crude extract	40	53	6	1
Ecteola fractions				
0·5 M NaCl	0	85	12	3
1·0 M NaCl	0	94	5	1
5·0 M ammonium formate	100	0	0	0
Bovine lung				
Crude extract	4	90	2	4
Ecteola fractions				
0·5 M NaCl	0	77	18	5
1·0 M NaCl	0	96	2	2
Canine mastocytoma				
Crude extract	0	94	3	3

Table 1 shows that, after the addition of Cetavlon in 1·0 M NaCl, an additional 53 per cent of the crude murine sulfomucopolysaccharide was precipitated, leaving only 7 per cent in solution. From the crude extracts of bovine and canine tissue, 90 per cent and 94 per cent, respectively, were precipitated in Cetavlon–1·0 M NaCl. Dilution of the supernatant solutions to 0·5 M NaCl and then to 0·25 M NaCl caused precipitation of residual sulfomucopolysaccharides from these crude extracts, amounting to a total

of 7 per cent from the murine extract and of 6 per cent from both the bovine and canine extracts.

Similar results were obtained on the material that was eluted from Ecteola in 1·0 M NaCl: 94 per cent of the sulfomucopolysaccharides from the murine cells and 90 per cent from the bovine tissue were precipitated in Cetavlon–1·0 M NaCl, the remainder of the sulfomucopolysaccharides precipitating on dilution of the Cetavlon-containing solution to 0·5 M NaCl and 0·25 M NaCl.

The material that was eluted from Ecteola with 0·5 M NaCl behaved differently. Only 85 per cent of this fraction from the murine cells and 77 per cent from the bovine tissue precipitated in Cetavlon–1·0 M NaCl. Most of the remaining murine and bovine sulfomucopolysaccharides (12 per cent and 18 per cent respectively) was precipitated when the solution containing Cetavlon was diluted to 0·5 M NaCl.

Paper chromatography

The crude extract of murine ^{35}S-sulfomucopolysaccharides was resolved into three radioactive and metachromatic components, R_f 0·00, 0·76, and 0·94 (Table 2). Of the radioactivity in the crude extract, 50 per cent was immobile (i.e. R_f 0·00).

All the material that was precipitated from the crude murine extract at 0·25 M NaCl alone was immobile, as was the material that was eluted from Ecteola with 5·0 M ammonium formate.

TABLE 2. PAPER CHROMATOGRAPHY OF ^{35}S-SULFOMUCOPOLYSACCHARIDES IN 0·04 M AMMONIUM FORMATE–2-PROPANOL (65:35 v/v)

After the chromatograms were developed, radioactivity was measured with a Geiger–Muller tube. The total counts for all peaks were summed and the percentage of radioactivity under each peak calculated. Areas of radioactivity corresponded to areas showing metachromasia with azure A.

Fraction	R_f values 0·00	0·76	0·94
Crude extract	50%	10%	40%
0·25 M NaCl precipitate	100	0	0
Cetavlon–1 M NaCl precipitate	5	30	65
Ecteola fractions			
0·5 M NaCl	2	0	98
1·0 M NaCl	0	35	65
5·0 M ammonium formate	100	0	0

The material precipitating with Cetavlon in the presence of 1·0 M NaCl had only a trace of the immobile material; the bulk of the fraction migrated to R_f 0·94 and the rest to R_f 0·76. The Ecteola fraction that was eluted with 0·5 M NaCl showed only a trace of the immobile component; 98 per cent of it migrated to R_f 0·94 and none to R_f 0·76. The Ecteola fraction that was eluted with 1·0 M NaCl contained material that migrated to both R_f 0·76 and 0·94.

Table 3 shows that all other extracts examined, with the exception of those from the rat, lacked the immobile component. The crude extracts from the rat contained relatively less of the immobile component than did the corresponding extract from murine mast cells (cf. Table 2).

Analysis of some of the fractions

The crude murine extract had 13·1% nitrogen and 4·8% ester-sulfate, values that compare with 4·6 and 19·7, respectively, in the crude bovine extract (Table 4). This nitrogen-rich material in the murine extract was precipitable with 0·25 M NaCl and eluted from Ecteola with 5·0 M ammonium formate. The Cetavlon–1 M NaCl precipitate of the murine sulfomucopolysaccharides showed values for nitrogen (2·51

TABLE 3. PAPER CHROMATOGRAPHY OF SULFOMUCOPOLYSACCHARIDES IN 0·04 M AMMONIUM FORMATE–2-PROPANOL (65:35 V/V)

After the chromatograms were developed, the sulfomucopolysaccharides were demonstrated by staining the paper with azure A. The chromatogram of the ^{35}S-sulfomucopolysaccharides from rat were, in addition, counted with a Geiger-Muller tube. The total counts for all peaks were summed and the percentage of radioactivity under each peak calculated.

Material	R_f values 0·00	0·76	0·94
Bovine lung			
Crude extract	—	+	+
Cetavlon–1 M NaCl precipitate	—	+	+
Ecteola fractions			
0·5 M NaCl	—	—	+
1·0 M NaCl	—	+	+
Commercial heparin			
Bovine lung	—	+	+
Swine intestinal mucosa	—	+	+
Guinea pig lung			
Crude extract	—	—	+
Rat: crude extracts			
Lung (%)	15	0	85
Kidney (%)	10	90	0
Canine mastocytoma			
Crude extract		R_f = 0·25	

per cent) and sulfate (24·2 per cent) that were similar to those obtained with the comparable fraction from bovine lung (1·93 per cent and 22·3 per cent respectively). Acetate could not be detected in either of these Cetavlon-purified fractions. The Ecteola fractions of the murine preparations had anticoagulant activity similar to the comparable bovine fractions but lower than those of two commercial samples; not enough material was obtained from the NaCl eluates of Ecteola to measure nitrogen and ester-sulfate.

Reaction of sulfomucopolysaccharides with amines

The murine and bovine sulfomucopolysaccharides, purified by six successive precipitations with Cetavlon–1 M NaCl, were compared with respect to their interaction with histamine. Table 5 shows that the specific conductance of histamine and murine sulfomucopolysaccharide together (5·7 mhos $\times$ 10^4) is less than the specific conductance of the sum of each (4·3 + 1·8 = 6·1 mhos $\times$ 10^4), implying an interaction.[22]

TABLE 4. CONTENT OF NITROGEN AND ESTER-SULFATE AND ANTICOAGULANT ACTIVITY OF SOME SULFOMUCOPOLYSACCHARIDES*

Material	N	Ester-sulfate (%)	Anticoagulant activity (units/mg)
Murine mast cell			
Crude extract	13·1	4·8	
0·25 M NaCl precipitate	13·0	7·7	
Cetavlon–1 M NaCl precipitate	2·51	24·2	
Ecteola fractions			
0·5 M NaCl			10
1·0 M NaCl			23
5·0 M ammonium formate	12·6	6·8	
Bovine lung			
Crude extract	4·62	19·7	
Cetavlon–1 M NaCl precipitate	1·93	22·3	
Ecteola fractions			
0·5 M NaCl			6
1·0 M NaCl			25
Commercial heparin			
Bovine lung		27·1	100
Swine intestinal mucosa	(1·47)	29·9	(158)

* Numbers within parentheses were obtained from the Abbott Laboratories.

TABLE 5. SPECIFIC CONDUCTANCE OF SULFOMUCOPOLYSACCHARIDES AS INFLUENCED BY HISTAMINE

The conductance of histamine alone and each sulfomucopolysaccharide alone and with histamine was measured at 25° with a Wheatstone bridge. The 1-ml cell had a constant of 10·93.

	Specific conductance mhos × 10^4		Δ mhos × 10^4
	Alone	With histamine	
Histamine (10 mg/ml)	4·3		
Murine extract (1 mg/ml)	1·8	5·7	−0·4
Bovine lung extract (1 mg/ml)	1·7	5·9	−0·1
Commercial bovine lung heparin (1 mg/ml)	2·1	6·4	0·0

TABLE 6. AMOUNTS OF 5-HYDROXYTRYPTAMINE AND SULFATE IN PRECIPITATES OF 5-HYDROXYTRYPTAMINE WITH MURINE AND BOVINE SULFOMUCOPOLYSACCHARIDES

One ml of a solution of 5-hydroxytryptamine (5 mg/ml) was added to 1·0 ml of a solution of each of the sulfomucopolysaccharides (1 mg/ml) which had been purified with Cetavlon in 1·0 M NaCl; 5-hydroxytryptamine and ester-sulfate were measured on the precipitate which had been collected by centrifugation.

	5-Hydroxytryptamine (μmole)	sulfate	Ratio: sulfate/5-hydroxy-tryptamine
Murine precipitate	0·61	0·36	0·59
Bovine precipitate	0·16	0·32	2·0

A smaller difference obtained in analogous measurements with the comparable extract from bovine lung; no difference occurred when commercial bovine heparin was used.

Attempts to carry out similar experiments with 5-hydroxytryptamine resulted in the formation of a precipitate immediately upon the addition of the amine (5 mg/ml) to the murine or bovine extracts (1 mg/ml); this did not occur with commercial heparin from bovine lung. The precipitates of the murine and bovine heparins were analyzed for their content of ester-sulfate and 5-hydroxytryptamine (Table 6). The total mass of the precipitate obtained with the murine preparation was larger than that obtained with the bovine preparation, as was the molar ratio of 5-hydroxytryptamine to ester-sulfate.

DISCUSSION

The whole crude extract of sulfomucopolysaccharides of the mast cell tumor of the mouse differed from the comparable extract prepared from bovine lung in four significant ways. First, the crude murine extract contained nearly three times as much nitrogen as did the crude bovine extract (Table 4). Second, on chromatography of the crude extracts on Ecteola, 45% of the crude murine sulfomucopolysaccharide was eluted with 5·0 M ammonium formate to yield nitrogen-rich material; there was no comparable fraction obtained from the crude bovine extract. Third, in 0·25 M NaCl, nitrogen-rich material (Table 4) was precipitated from the murine extract but not from the bovine extract. Finally, on paper chromatography the crude murine extract showed (Table 2), in addition to two mobile components with R_f values of 0·76 and 0·94 (both of which were found in the bovine extract; Table 3), a third component that was immobile in the solvent system used. The nitrogen content of this immobile component could not be determined since it could not be eluted from the chromatogram, but it is probably the nitrogen-rich material, for paper chromatography of fractions of the crude material showed that only the two nitrogen-rich fractions—the ammonium formate eluate from Ecteola and the 0·25 M NaCl precipitate—were immobile.

The high percentage of nitrogen in this fraction suggests that it contains sulfomucopolysaccharide associated with a peptide or peptides. It has been noted before that preparations of sulfomucopolysaccharides, even highly purified commercial preparations including the bovine and swine heparins used in this study, contain nitrogenous material other than hexosamine.[11] This inference was based not only on the presence of nonhexosamine, ninhydrin-reacting substances in hydrolysates of heparin but on the demonstration of histidine in these hydrolysates. The present work suggests that the murine mast cell contains a sulfomucopolysaccharide that is distinguished by its association with a large amount of protein. This difference between the mouse and cow recalls another species difference recently elucidated: that the chondroitin sulfate-protein complexes from shark contain about half as much protein as the complexes from mammalian tissues.[23]

Aside from this nitrogen-rich component, the other sulfomucopolysaccharides in the murine mast cells showed no obvious differences from the bovine extract either by chromatography on Ecteola and paper or by content of nitrogen, ester-sulfate, and anticoagulant activity. Especially noteworthy was the finding that the Cetavlon–1 M NaCl precipitates of the murine and bovine sulfomucopolysaccharide had identical R_f values on paper (Tables 2 and 3) and very similar percentages of nitrogen and

ester-sulfate (Table 4). Notwithstanding these similarities, this murine fraction differed from the comparable bovine fraction, as manifest by its greater affinity for histamine (Table 5) and 5-hydroxytryptamine (Table 6). The high affinity of this purified murine sulfomucopolysaccharide for amino-containing compounds may explain the high content of nitrogenous material in the impure murine extract.

An interaction of heparin and histamine has been consistently observed in other laboratories,[24–26] but the interaction of heparin with 5-hydroxytryptamine, previously noted by Keller,[24] was not confirmed by Sanyal and West.[25] The contradictory evidence may be attributable to differences between the heparins that were used, for in the experiments reported here, a commercial preparation of bovine lung heparin did not react with amines, in contrast to the bovine lung material prepared in this laboratory. The different affinities of the sulfomucopolysaccharides for the amines cannot be explained by any obvious chemical differences, such as the content of sulfate groups (Table 4), which have been implicated as a site of interaction of sulfomucopolyscacharides and amines.[26, 27] Analogously, the capacities of sulfomucopolysaccharides to form complexes with streptomycin were not related to any obvious chemical property of the polymers.[28] It has been suggested that the interaction of sulfomycopolysaccharides and amines may be modified by the steric configuration of the sulfate and carboxyl groups, the number of carboxyl groups, the extent of branching, polymerization, and intramolecular reactions.[27, 29]

That the fine structure of a sulfomucopolysaccharide may differ among species is suggested by differences between the chondroitin sulfates prepared from shark and from mammalian tissues.[23, 30, 31] Although similar evidence of species differences among heparins has not been unequivocally obtained, it is known that preparations from different species differ not only in anticoagulant activity, but by paper chromatography as well.[6, 32, 33] Such differences have also been shown in this study (Tables 2 and 3). The sulfomucopolysaccharide from the canine mastocytoma had an R_f value of 0·25 differing from that of any other species examined. The component at R_f 0·76, which was present in bovine lung, was lacking in the lungs of guinea pig and rat (though present in the kidney of the rat). The immobile component was present only in the lung and kidney of the rat and in the murine cells. In like fashion, Spolter and Marx,[6] using a solvent system that differed from the present only in pH, showed that sulfomucopolysaccharides prepared from both the original murine mast cell tumor and the rat contained immobile material that was not present in sulfomucopolysaccharides extracted from the sheep and cow. It may be pertinent that the mouse and rat are also distinguished from other species in having 5-hydroxytryptamine in their mast cells.[2]

The relative proportion of the components resolved by paper chromatography varied in this murine tumor from time to time and from one cell line to the other, the original tumor showing a relatively small proportion of the immobile component and a relatively high proportion of the component at R_f 0·76. The latter was present in both the fraction that was eluted from Ecteola with 1·0 M NaCl and that precipitated in Cetavlon–1 M NaCl (Tables 2 and 3). It was not present in the fraction that was eluted from Ecteola with 0·5 M NaCl; this fraction was composed almost exclusively of the material with R_f 0·94 (Tables 2 and 3).

The fraction that was eluted from Ecteola with 1·0 M NaCl had greater anticoagulant activity than did the fraction that was eluted with 0·5 M NaCl. Ringertz[7] also observed that the fraction of sulfomucopolysaccharide eluted from Ecteola at a high

concentration of salt has greater anticoagulant activity (and a higher content of sulfate) than that eluted with low salt concentration. It may be inferred that the relatively greater anticoagulant activity of the 1·0 M NaCl eluate is due to the presence of the R_f 0·76 component.

Another difference between these eluates from Ecteola was seen after the addition of Cetavlon in 1·0 M NaCl. Under these conditions, a greater percentage of the sulfomucopolysaccharides was precipitated from the 1·0 M NaCl eluate than from the 0·5 M NaCl eluate (Table 1), an observation further suggesting that the latter eluate contains a less highly sulfated polysaccharide.[8]

The anticoagulant activities of the fraction prepared from bovine lung by chromatography on Ecteola were much lower than the anticoagulant activity of a commercial bovine lung heparin (Table 4). A possible cause of this is the nondialyzable, orcinol-positive material that was continually shed from Ecteola despite prolonged washing (see Methods). Others have shown that Ecteola,[35] like cellulose paper,[35] continues to release polysaccharide even after repeated washings.

Another source of artifact, which is not often considered, is the dialysis membrane. Although heated at 80° for three days and repeatedly washed in 1 M NaCl and water, it continued to elaborate nondialyzable, sulfate-containing material that was precipitable under conditions that precipitate heparin (see Methods). This material is almost certainly responsible for the high values of ester-sulfate previously reported in the murine mast cell tumor.[20] It has recently been shown that a dialysis membrane releases material that antagonizes insulin.[37]

Clearly implicit in the results obtained from the varied fractionation procedures used in this work is the difficulty in obtaining a heparin that is homogeneous, even by paper chromatography in a single solvent system, which is not a rigorous criterion. These results, besides showing the inadequacy of present methods, emphasize the existence of a group of heparins. These heparins differ not only among species but several are simultaneously present in the same cell.

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