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Research Article

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ABSTRACT Heparin as measured by azure A metachromasia and anticoagulant activity has been extracted with 1 M NaCl from ^{35}S-labeled human lung fragments or dispersed human lung cells enriched for mast cells. The ^{35}S-labeled metachromatic material in the 3 M NaCl eluate from Dowex-1 chromatography of the extract from lung fragments exhibited an average mol wt of 20,000 by Sepharose 4B gel filtration. The ^{35}S-labeled metachromatic material with the charge characteristics of commercial porcine heparin on DEAE cellulose chromatography was entirely heparin by the criteria of resistance to degradation by chondroitin ABC lyase and complete degradation by purified heparinase. Antithrombin affinity chromatography of purified heparin with an anticoagulant activity of 137 U/mg, revealed that the one-third that was bound and eluted had a 273 U/mg sp act, whereas the unbound activity was 31 U/mg. Thus, the previously observed heterogeneity of commercial porcine heparin for binding to human antithrombin was also observed with human heparin. The mast cell-enriched human lung cell preparations yielded [^{35}S]mucopolysaccharides with an average mol wt of 60,000 by Sepharose 4B gel filtration. Approximately 30% of this fraction was degraded by chondroitin ABC lyase, and the residual 70% was degraded by purified heparinase. When the chondroitin ABC lyase-resistant fraction was subjected to alkali degradation the average mol wt was reduced to 20,000. The calculated human lung mast cell heparin content of 2.4–7.8 $\mu g/10^6$ cells gave a ratio to histamine on a weight basis similar to that of intact lung fragments, thereby implying that heparin in the lung fragments was largely restricted to the mast cells.

INTRODUCTION

Commercial heparin obtained from porcine intestine and that obtained from beef lung are comprised of glycosaminoglycans of ≅10,000 and 13,000 mol wt, respectively (1). The anticoagulant function of heparin depends upon an ability to accelerate antithrombin function (2, 3), and resides predominantly in those subfractions with antithrombin-binding activity (4, 5). As the antithrombin used for binding was human, the possibility existed that lack of species concordance contributed to the apparent heterogeneity in binding and function observed with porcine heparin (4, 5).

Human tissue heparin-like activity, defined only by the presence of anticoagulant function and metachromasia, has been observed in mucopolysaccharide-containing fractions extracted from lung (6, 7), liver (8), skin (9), and blood (10, 11). However, in no instance has the material been assessed for binding to human antithrombin. In the present studies, human lung heparin was isolated and defined by anticoagulant activity, azure A metachromasia, susceptibility to degradation by purified heparinase, and resistance to degradation by chondroitin ABC lyase. The human heparin purified from lung fragments had an average mol wt of 20,000 and approximately one-third bound to human antithrombin, a result analogous to the binding heterogeneity observed with porcine heparin. In addition, heparin with an average mol wt of 60,000 was isolated from dispersed enriched populations of human

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lung mast cells and shown to be composed of 20,000 mol wt glycosaminoglycan side chains.

METHODS

Whale cartilage chondroitin 4-sulfate, mol wt 25,000– 50,000, shark cartilage chondroitin 6-sulfate, mol wt 40,000–80,000, chondroitin ABC lyase from *Proteus vulgaris*, and porcine skin hyaluronic acid (Miles Laboratories, Inc., Elkart, Ind.); atropine sulfate, bovine albumin, elastase type I, histamine diphosphate, and porcine intestinal heparin, 170 U/mg (Sigma Chemical Co., St. Louis, Mo.); Sepharose 4B and Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.); Dowex 1-X2 (100–200 mesh, chloride form) and DEAE-cellulose (Bio-Rad Laboratories, Richmond, Calif.); azure A (Fisher Scientific Co., Fair Lawn, N. J.); carbazole (Eastman Kodak Co., Rochester, N. Y.); carrier-free $H_2{}^{35}SO_4$ (New England Nuclear, Boston, Mass.); chymopapain, collagenase type II, and deoxyribonuclease type I (Worthington Biochemical Corp., Freehold, N. J.); pronase B grade (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.); gelatin (Difco Laboratories, Detroit, Mich.); mepyramine maleate (Merck, Sharp & Dohme, Canada Ltd., Montreal, Quebec); Eagle's basal medium with Hanks' balanced salt solution (Microbiological Associates, Walkersville, Md.); and metrizamide (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) were purchased from the manufacturers. Antithrombin was isolated from human plasma by DEAE cellulose and heparin-Sepharose affinity chromatography (12, 13), and coupled to Sepharose 4B by cyanogen bromide activation (14). Approximately 3 mg of commercial heparin were bound per ml of packed gel. Purified heparinase prepared from *Flavobacterium heparinum* was obtained from Dr. A. Linker (University of Utah, Salt Lake City, Utah) (15).

The standard method of dialysis and concentration of human lung heparin between chromatography steps or before enzyme incubations used 40 vol of distilled water for 18 h followed by lyophilization; recovery of the heparin as measured by azure A metachromasia was 85–90%. Radioactivity was determined with a liquid scintillation spectrometer (Mark III liquid scintillation system, Searle Radiographics Inc., Des Plaines, Ill.). Histamine was measured by bioassay on the atropinized guinea pig ileum (16).

Total mucopolysaccharide was determined by measurement of uronic acid content (17). During extraction and purification procedures heparin was estimated by change in adsorbance at 510 after formation of a heparin-azure A metachromatic complex with commercial porcine heparin as a standard (18, 19). It required 25–30 times as much chondroitin 4-sulfate, dermatan sulfate, or chondroitin 6-sulfate to equal the metachromasia of heparin under the conditions specified. Thus, the selectivity and sensitivity of the azure A assay for heparin was appropriate for its use in isolating heparin from the limited amounts of starting material. The anticoagulant activity of heparin was determined by its ability to accelerate the neutralization of thrombin by antithrombin (4).

Labeling and extraction of human lung tissue and human lung mast cells. Normal human lung obtained from the tissue resected during the surgical excision of a carcinoma of the bronchus was cut into 250-mg fragments and washed three times in Tyrode's buffer (20). For radiolabeling, 1 g of tissue was placed in 2.5 ml Eagle's basal medium with Hanks' balanced salt solution with 0.05 mCi of [^{35}S]sulfate. After incubation for 18 h at 37°C under ambient air with 5.0% CO_2 tension (Hotpack Carbon Dioxide Incubator, Hotpack Corp. Philadelphia, Pa.), the lung fragments were washed three times in Tyrode's buffer and extracted by freeze-thawing six times in 1 M NaCl. Residual lung fragments were treated with 0.5 M NaOH for 18 h at 22°C to solubilize remaining glycosaminoglycans by β-elimination (21).

To obtain mast cells, the human lung fragments were enzymatically digested in a four-step procedure with chymopapain, pronase, collagenase, and elastase (22, 23). The dispersed cells obtained at each step were pooled, and the mast cells were concentrated to 40–70% purity by isopyknic and velocity gradient sedimentation (24, 25). The purity was defined by phase microscopy after staining with toluidine blue (26) and by electron microscopy (25). The mast cell-rich cell suspension at a concentration of 5×10^6–1×10^7 cells/ml was incubated in Eagle's basal medium with Hanks' balanced salt solution with 0.1 mCi [^{35}S]sulfate in a final vol of 5–10 ml for 18 h at 37°C under 5.0% CO_2 tension, washed three times in Tyrode's solution, and extracted by freeze-thawing six times in 1 M NaCl.

RESULTS

Isolation and purification of heparin from human lung fragments. The extracts from 55, 82, and 200 g of human lung fragments, designated preparations 1, 2, and 3, respectively, were each applied directly to a 2 × 20-cm column of Dowex-1 equilibrated in 1 M NaCl and eluted in stepwise fashion with 1, 3, and 4 M NaCl (2, 27). Portions of each fraction were assessed for ^{35}S, protein (28), uronic acid, metachromasia, and anticoagulant activity. The 1 M NaCl effluent contained more than 99% of ^{35}S, histamine, and total protein, but was without azure A metachromasia and anticoagulant activity. The material eluted by 3 M NaCl contained uronic acid, <0.1% of the total protein, <0.003% of the ^{35}S, and exhibited all of the azure A metachromasia and anticoagulant activity. Based upon metachromasia, the heparin content in the 3 M NaCl extract from the three separate lung fragment preparations was 4.5, 22.5, and 44.7 μg/g lung tissue. Histamine content in the 1 M NaCl effluent in the same three lung preparations was 2, 8, and 20 μg/g lung tissue. The 4 M NaCl eluate had minimal ^{35}S and no metachromasia or anticoagulant activity. Treatment of residual lung fragments with 4 M guanidinium chloride and 0.5 M NaOH failed to yield additional azure A-positive material but did free additional uronic acid-containing materials. A portion of the 3 M NaCl eluates from Dowex-1 obtained from the three different lung preparations and containing 140, 920, and 4,474 μg of heparin by metachromasia, respectively, was filtered in 2 M NaCl on a 1 × 60-cm column of Sepharose 4B at a flow rate of 2 ml/h. 1-ml fractions were collected, and portions were assayed for ^{35}S, uronic acid, anticoagulant activity, and metachromasia. Heparin, as determined by metachromasia, filtered as a single broad peak, with an estimated average mol wt of 20,000 (Fig. 1). Peak anticoagulant activity corresponded to peak metachromasia. Glycosaminoglycans, as determined by uronic acid content, were found only between fractions 20 and 34, whereas ^{35}S appeared in two peaks. The initial ^{35}S

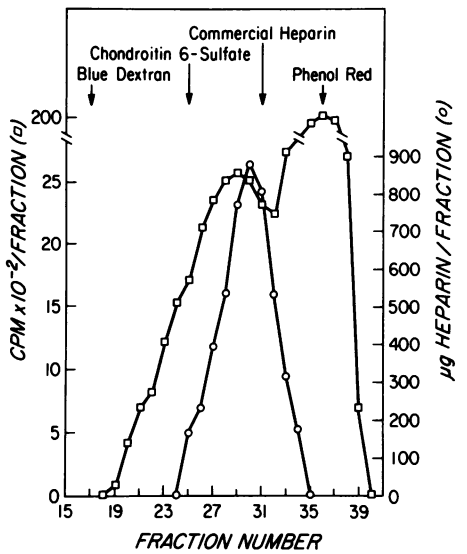

FIGURE 1 Sepharose 4B gel filtration of human lung fragment mucopolysaccharides obtained in the 3 M eluate from Dowex-1 chromatography of preparation 3. The positions of blue dextran, chondroitin 6-sulfate (average mol wt = 60,000), commercial heparin (average mol wt = 12,000), and phenol red, which were filtered separately on the same column, are shown.

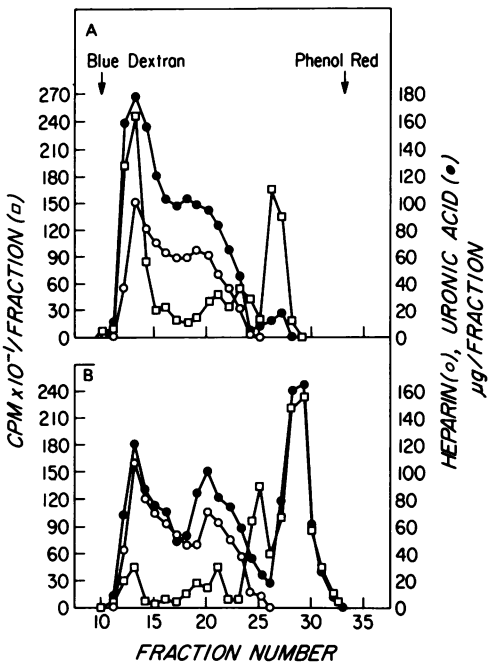

FIGURE 2 Effect of treatment with chondroitin ABC lyase on Sephadex G-50 gel filtration of human lung tissue mucopolysaccharides obtained from Sepharose 4B filtration of preparation 3. A, shows untreated material and B, the effect of chondroitin ABC lyase degradation. The positions of blue dextran and phenol red, which were filtered separately on the same column, are shown.

peak filtered with an average mol wt of 20,000–40,000 and the second, which contained low molecular weight materials, was not further characterized. A pooled sample of the first peak of the [^{35}S]mucopolysaccharides, fractions 20–34, was treated with 0.5 M NaOH at 22°C for 14 h to disrupt any xylosyl-seryl proteoglycan bonds (21). After this treatment, the [^{35}S]glycosaminoglycans filtered on Sepharose 4B in a single peak that was coincident with metachromasia and uronic acid and had an average mol wt of 20,000.

A pooled sample of the first peak of the [^{35}S]mucopolysaccharides (Fig. 1) containing 1,046 μg heparin by azure A metachromasia was incubated with 4 U of chondroitin ABC lyase in 0.25 ml of 0.25 M Tris buffer, made 0.3 M Na acetate, 0.25 M NaCl, and 5 mg/100 ml bovine serum albumin, pH 8.0, for 90 min at 37°C (enriched Tris buffer) (29); a replicate portion was treated under the same conditions with buffer alone. Both treated and untreated samples were then filtered on a 1 × 60-cm column of Sephadex G-50 equilibrated in H_2O at a flow rate of 3 ml/h, and 1-ml fractions were collected. After exposure to chondroitin ABC lyase, 90% of ^{35}S and 25% of uronic acid-containing material was degraded as indicated by a shift in filtration pattern, while the heparin, as defined by azure A metachromasia, was unaffected (Fig. 2). For preparation 3, the specific activity of the chondroitin ABC lyase-resistant mucopolysaccharides was 4.8 cpm/μg heparin by azure A metachromasia. Preparations 1 and 2 had more radioactivity but less endogenous heparin in the lung extract. A sample from preparation 1 containing 800 cpm and with a 12 cpm/μg heparin sp act by azure A metachromasia was incubated with 250 μg of purified heparinase for 90 min in 0.1 ml of 0.1 M acetate buffer, pH 7.0, at 30°C, and a replicate sample was incubated in buffer alone. Degradation was assessed by filtration on a 1 × 60-cm column of Sephadex G-50 equilibrated in H_2O with a flow rate of 2.5 ml/h, and 1-ml fractions were collected. More than 70% of the heparinase-treated material was degraded when compared to the untreated sample (Fig. 3). This pattern is similar to that obtained when 250 μg of commercial heparin was treated in the same fashion.

To isolate the heparin directly, a portion of the 3 M NaCl eluate from Dowex-1 chromatography of preparation 3 containing 2,092 μg of heparin as defined by metachromasia was applied to a 1 × 5-cm column of DEAE-cellulose equilibrated in 0.01 M sodium acetate buffer with 0.1 M LiCl, pH 5.5. The column was eluted with a logarithmic gradient from 0.1 to 1.0 M LiCl, followed by a second logarithmic gradient from 1.0 to 2.0 M LiCl (30). Fractions of 2 ml each were collected at a flow rate of 25–30 ml/h, and portions of each were assayed for radioactivity, uronic acid content and

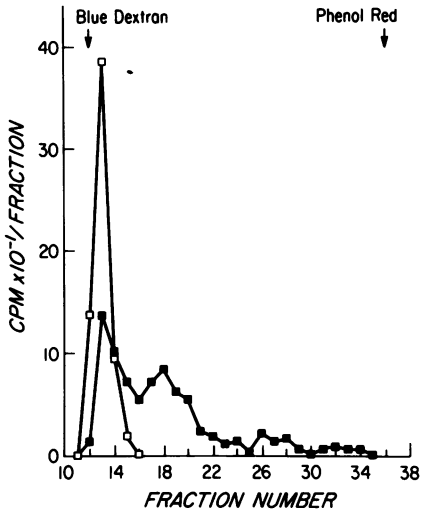

FIGURE 3 Sephadex G-50 gel filtration of chondroitin ABC lyase-resistant [^{35}S]mucopolysaccharides of preparation 1 before (□) and after (■) treatment with heparinase. The positions of blue dextran and phenol red, which were filtered separately on the same column, are shown.

metachromasia (Fig. 4). The ^{35}S-labeled material filtered in two distinct peaks with most appearing coincident with the effluent and the remainder eluting with azure A-positive fractions 87–111. Uronic acid was found in fractions 47–75 and 87–111. 51 μg of [^{35}S]mucopolysaccharides by uronic acid, containing 800 cpm, from pooled DEAE fractions 47–75, and 4.4 μg, containing 100 cpm, from pooled fractions 87–111 were

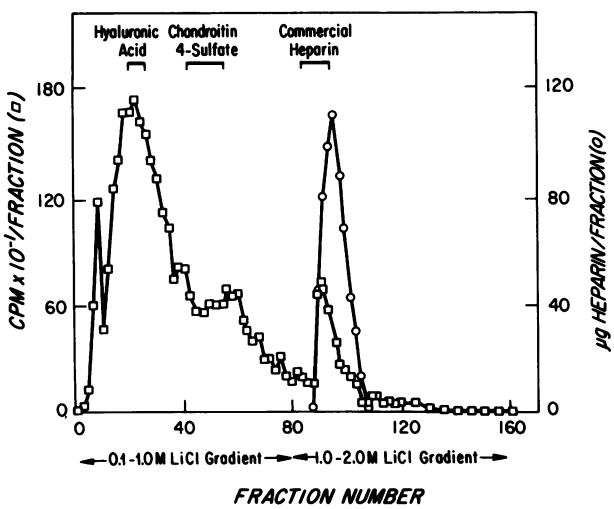

FIGURE 4 DEAE-cellulose chromatography of human lung fragment mucopolysaccharides from preparation 3 contained in the 3 M NaCl eluate from Dowex-1 chromatography. Determinations in every other tube are shown. The column was previously standardized with chondroitin 4-sulfate (3 mg), commercial heparin (3 mg), and hyaluronic acid (2 mg).

each incubated with 1 U of chondroitin ABC lyase in enriched Tris buffer for 90 min at 37°C. Sephadex G-50 gel filtration of treated and untreated material, performed as described for Fig. 2, showed that 90% of the [^{35}S]mucopolysaccharides from fractions 47–75 were degraded, whereas none was degraded in the pool from fractions 87–111. Based on azure A metachromasia, the specific activity of the heparin in the latter was 4.3 cpm/μg. This material was completely degraded by heparinase under the conditions described for Fig. 3. The total recovery of heparin after Sepharose 4B gel filtration of the original 3 M NaCl Dowex-1 eluate was 75–90% for three human lung preparations as defined by the chondroitin ABC lyase-resistant, heparinase-sensitive, azure A-positive material, and 70 and 74% in two preparations obtained by DEAE cellulose chromatography and defined by azure A metachromasia.

Specific anticoagulant activity and affinity for antithrombin. The heparin in the azure A-positive peak fractions 87–111 (Fig. 4) was divided into five 5-ml pools consisting of 1 ml from each of fractions 87–91, 92–96, 97–101, 102–106, and 107–111. After dialysis and lyophilization, each pool was assayed for heparin by uronic acid, radioactivity and anticoagulant activity (Table I). Specific anticoagulant activity increased sequentially in the five pools from 27 U/mg to 179 U/mg when compared to a heparin standard of known U. S. Pharmacopeia potency (4), while the specific radioactivity of [^{35}S]heparin/mg was highest in pool 1 (Table I).

Pools 1 and 2 with the lowest functional activity, and pools 3–5 with the highest functional activity were separately combined (Table II). Each composite pool was applied to 1 × 2-cm Sepharose-antithrombin column equilibrated in 0.1 M NaCl. The columns were washed with three bed volumes of equilibrating buffer, and eluted with 1 M NaCl. Approximately 19% of the heparin defined by uronic acid content in pools 1–2

TABLE I
Specific Anticoagulant Activity of Human Lung Heparin after DEAE-Cellulose Chromatography: Relationship to Incorporated ^{35}S

Pool fraction	DEAE fractions	Total heparin*	^{35}S*	Anticoagulant activity*
		mg/pool	*cpm/mg*	*U/mg*
1	87–91	0.125	5,920	27
2	92–96	0.164	3,600	85
3	97–101	0.180	1,744	133
4	102–106	0.096	1,250	169
5	107–111	0.056	1,750	179

* Each value is the mean of three determinations. Counts per minute were determined 3 wk after the data presented in Fig. 4.

TABLE II

Antithrombin-Sepharose 4B Affinity Chromatography of Human Lung Heparin from DEAE-Cellulose: Determination of Specific Anticoagulant Activity in Bound and Unbound fractions

Pool fractions	Heparin*	Anticoagulant activity*
	mg	*U/mg*
1–2		
Initial activity	0.180	60
After chromatography		
Unbound	0.096	8
Bound	0.035	187
3–5		
Initial activity	0.252	137
After chromatography		
Unbound	0.117	31
Bound	0.083	273

* Each value is the mean of three determinations.

bound to the antithrombin. After elution with 1 M NaCl it had a sp act of 187 U/mg heparin as compared to 8 U/mg heparin for the material that failed to bind. Approximately 33% of the heparin defined by uronic acid content in pools 3–5 bound to the antithrombin and had a specific activity of 273 U/mg heparin, whereas 31 U/mg heparin was recovered in material that failed to bind. The material in the wash between the collection of unbound and bound heparin was not quantitated because of dilution and it is assumed that this accounts for the loss of ≅20% of the material applied.

Heparin in mast cell-enriched cell suspensions. The extracts from three different preparations of ^{35}S-labeled dispersed human lung cells of 40–70% mast cell purity and containing 5.0, 6.4, and 8.0 × 10^6 mast cells were chromatographed over Dowex-1 in a manner identical to that used to separate human lung fragment mucopolysaccharides from histamine, protein, and free ^{35}S. The 3 M NaCl Dowex-1 eluate from three preparations contained 1,290, 1,800, and 4,521 cpm of [^{35}S]mucopolysaccharides and 13.5, 28.2, and 62.4 μg of heparin by metachromasia representing 2.7, 4.4, and 7.8 μg of heparin/10^6 mast cells. The 1 M NaCl Dowex-1 effluent contained 1.0, 2.3, and 2.8 μg of histamine/10^6 mast cells. In contrast, the 3 M NaCl Dowex-1 eluate from 2.6 × 10^6 lung cells consisting of <4% mast cells, had no detectable metachromasia and the 1 M NaCl Dowex-1 effluent had no measurable histamine. The entire 3 M NaCl Dowex-1 eluate of each of the three mast cell preparations was filtered separately in 2 M NaCl on a 1 × 60-cm column of Sepharose 4B at a flow rate of 2 ml/h and 1-ml fractions were assayed for ^{35}S. There was insufficient material to assay fractions by uronic acid content or metachromasia. As shown in Fig. 5A for the

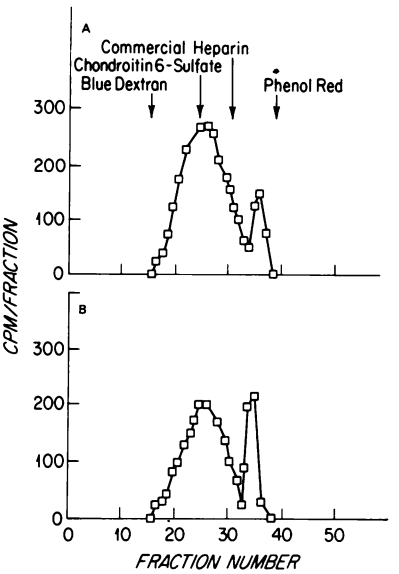

FIGURE 5 Sepharose 4B gel filtration of [^{35}S]mucopolysaccharides in the 3 M NaCl eluate from Dowex-1 chromatography of the extract from mast cell-rich cell suspensions. A, shows untreated material and B, the effect of chondroitin ABC lyase degradation.

preparation exhibiting the most radioactivity, the ^{35}S-labeled material presented as a broad peak that preceded a commercial heparin marker and was estimated to have an average mol wt of 60,000. The second smaller peak of low molecular weight was not characterized further. Fractions 15–34, which contained [^{35}S]mucopolysaccharide with an estimated average mol wt of 60,000, were pooled, dialyzed, and lyophilized. A portion of this pool was treated with 1 U of chondroitin ABC lyase in enriched Tris buffer for 90 min at 37°C. Rechromatography over the same Sepharose 4B columns demonstrated (Fig. 5B) that more than 70% was resistant to degradation by chondroitin ABC lyase, and the remainder appeared in a second peak of glycosaminoglycan degradation products.

Chondroitin ABC lyase-resistant material in fractions 15–34 (Fig. 5B) was pooled, dialyzed, and lyophilized. One-third was subjected to β-elimination with 0.5 M NaOH and another one-third to degradation with heparinase. When the portion treated with 0.5 M NaOH for 14 h at 22°C was rechromatographed on the same Sepharose 4B column, a predominant peak containing 74% of the [^{35}S]mucopolysaccharide filtered with an approximate mol wt of 20,000, followed by a peak of lower molecular weight. The second one-third was mixed with 250 μg of commercial heparin in 0.1 ml of 0.1 M Na acetate buffer, pH 7.0, filtered on a 1 × 60-cm column of Sephadex G-50 in distilled H_2O at a flow rate of 2.5 ml/h, and fractions of 0.67 ml collected. Fractions 20–49 were pooled, lyophilized, resus-

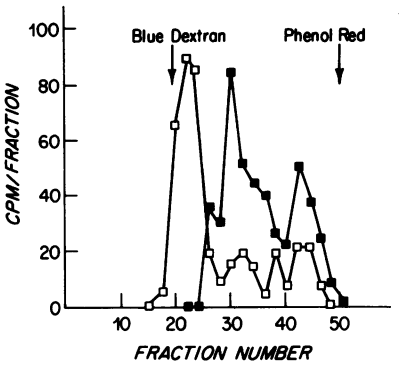

FIGURE 6 Sephadex G-50 gel filtration of chondroitin ABC lyase-resistant mucopolysaccharides obtained by Dowex-1 chromatography and Sepharose 4B gel filtration of the extract from mast cell-enriched cell suspensions before (□) and after (■) treatment with heparinase. The positions of blue dextran and phenol red, which were filtered separately on the same column, are shown.

pended in 0.1 ml of the above buffer and incubated with 250 μg of purified heparinase for 90 min at 30°C. Chromatography of this reaction mixture showed that the ^{35}S-labeled mucopolysaccharide was degraded when compared to untreated material (Fig. 6). The 250 μg of commercial heparin added as an internal standard was also degraded as assessed by a shift of the uronic acid from the excluded to the included fractions.

DISCUSSION

Human heparin, previously recognized only on the basis of metachromasia and anticoagulant activity (6–11), has been isolated from human lung and characterized in terms of size, charge, enzyme susceptibility, and specific functional activity. Human lung fragments were labeled with ^{35}S and extracted in 1 M NaCl, and the [^{35}S]mucopolysaccharides were separated from free counts and protein by chromatography on Dowex-1. The metachromatic material in the 3 M eluate fraction exhibited an average mol wt of 20,000 by Sepharose 4B gel filtration (Fig. 1) and the charge characteristics of commercial heparin on DEAE cellulose chromatography (Fig. 4). The glycosaminoglycan heparin isolated by DEAE cellulose chromatography was free of contaminating glycosaminoglycans, as there was no degradation by chondroitin ABC lyase and full degradation of the material by purified heparinase. When two DEAE fractions of this human heparin were subjected to antithrombin-affinity chromatography, the bound and eluted material exhibited a specific anticoagulant activity of 187 and 273 U/mg, whereas the unbound material had an activity of only 8 and 31 U/mg, respectively (Table II). Hence the partitioning of heparin on antithrombin cannot be ascribed to lack of species concordance.

To demonstrate that heparin was associated with mast cells, human lung fragments were enzymatically digested and the dispersed mast cells were concentrated to 40–70% (25). The [^{35}S]mucopolysaccharides extracted in 1 M NaCl and eluting from Dowex-1 in 3 M NaCl exhibited an average mol wt of 60,000 by Sepharose 4B gel filtration (Fig. 5A). Approximately 30% of this fraction was degraded by treatment with chondroitin ABC lyase (Fig. 5B), and the residual 70% was susceptible to degradation by purified heparinase (Fig. 6). As the degradation of heparitin sulfates by purified heparinase during two sequential 24-h digestions yields almost entirely products excluded on Sephadex G-50 (31), the degradation in 1.5 h to products that are entirely included on Sephadex G-50 gel filtration indicates that the chondroitin ABC lyase-resistant sulfated mucopolysaccharide was entirely heparin. When the chondroitin ABC lyase-resistant material was subjected to alkali treatment under conditions known to cleave xylosyl-seryl linkages in proteoglycans, the predominant radiolabeled peak was reduced to an estimated average mol wt of 20,000. This fact suggests that the mast cell-enriched preparations contain a small proteoglycan with an average mol wt of 60,000 and that the glycosaminoglycan side chains were similar in size to those isolated directly from the human lung fragments. The ratio of histamine to heparin by weight was 1:2.2, 1:2.9, and 1:2.2 for the three human lung fragment preparations and 1:1.9, 1:2.2, and 1:3.1 for the three preparations of dispersed lung cells obtained from additional lung specimens. Since the mast cell is the only defined source of lung histamine, the consistency in these ratios suggests that heparin was derived from the same cell. Basophils have not been observed by electron microscopic examination of dispersed human lung cells, or in the mast cell-enriched preparations (25); further, heparin is not a major mucopolysaccharide of this cell, being absent in guinea-pig basophils (32) and consisting of <20% of the mucopolysaccharide obtained from human or rat leukemic basophils (33, 34). The content of histamine in human lung mast cells ranged from 1.0 to 2.8 μg/10^6 cells, as observed previously (25), and of heparin from 2.4 to 7.8 μg/10^6 cells.

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