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ISOLATION AND PHARMACOLOGICAL ACTIVITIES OF HEPARIN AND OTHER SULFATED MUCOPOLYSACCHARIDES FROM THYMUS*

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Abstract—The anticoagulant, antithrombic, antilipemic and antihemolytic activities of heparin, chondroitin sulfates A, B and C, and heparitin sulfate from beef thymus are reported. The heparin fraction extracted from beef thymus was more active on the release of lipoprotein lipase (LPL) than on the coagulation process (APTT) when compared with commercial heparin prepared from beef or pork intestinal mucosa. The physical and clinical properties of the different mucopolysaccharide fractions are also reported. All the results obtained suggest that heparin is a family of compounds, some with a prevailing activity on hemocoagulation, others on the lipoprotein lipase system, and others on the cytolytic system of complement.

Heparin has been widely used in medicine to prevent either initiation or progression of venous thromboembolism. Among several pharmacological actions, heparin exhibits potent anticoagulant, antilipemic, antithrombic, antihemostatic and antihemolytic activities [1, 2]. The main commercial sources of heparin for pharmaceutical use are beef lung and pork and beef intestinal mucosa. Differences in the pharmacological activities of the commercial preparations have been reported and a correlation drawn with the source from which the heparins were derived [3–7]. Recently, we have been able to demonstrate that heparin has a characteristic distribution in animal tissues and is present in relatively large amounts in thymus from eight mammalian species [8, 9].

Mondola *et al.* [10, 11] demonstrated in beef thymus of fetal and calf origin, the presence of a factor, probably protein in nature, with a potent effect on lipid metabolism in rats. Since it has been shown that commercial heparin has a fat-clearing activity, it was of interest to isolate the heparin and also the other sulfated mucopolysaccharides§ from thymus to study their possible roles in lipid metabolism as well as in the coagulation process.

In the present work, heparin, heparitin sulfate and chondroitin sulfates A, B and C have been isolated from beef thymus, and their anticoagulant, antithrombic, antilipemic and antihemolytic activities have been determined.

MATERIALS AND METHODS

Materials and enzymes. Chondroitin sulfates A, B and C, and chondroitinases AC and ABC were purchased from Miles Laboratories (Elkhart, IN, U.S.A.) Heparin was supplied by the Sigma Chemical Co. (St. Louis, MO, U.S.A.), LAOB Laboratories (São Paulo, Brasil), Opocrin Laboratories (Modena, Italy), Inorp Laboratories (Buenos Aires, Argentina) and The Upjohn Co. (Kalamazoo, MI, U.S.A.). Heparitin sulfate and heparinases and heparitinases from *F. heparinum* cells were prepared by methods described previously [12–14]. Agarose was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). 1,2-Diaminoethane and 1,3-diaminopropane were purchased from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), ampholine (pH 3.5 to 5.0) from LKB Bromma (Sweden) and Ediol from CalBiochem (La Jolla, CA, U.S.A.). Superase, a proteolytic enzyme preparation from a strain of spore-forming bacillus, was purchased from Chas. Pfizer & Co. (New York, NY, U.S.A.).

Extraction of heparin and other sulfated mucopolysaccharides. Beef thymus was obtained immediately after death. The tissues were ground and homogenized with 5 vol. of petroleum ether at room temperature for 5 hr. The suspension was centrifuged and the precipitate was treated with 10 vol. of acetone. After standing overnight at 4°, the suspension was again centrifuged, and the precipitate was washed once with acetone and dried under vacuum. The mucopolysaccharides were extracted from the dry tissues after proteolysis. One kg of dry tissue was suspended in 10 liters of a buffered solution, pH

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§ Mucopolysaccharide is also known as glycosaminoglycan; chondroitin sulfate A (ChSA) as chondroitin 4-sulfate; chondroitin sulfate C (ChSC) as chondroitin 6-sulfate; chondroitin sulfate B (ChSB) as dermatan sulfate; and heparitin sulfate (HTS) as heparan sulfate.

8, and incubated with 1 g of superase under a layer of toluene. After 5 hr at 40°, another 1 g of superase was added and the mixture was incubated for another 5 hr at 40° to ensure complete proteolysis. The mother liquid of the incubation mixture was tested for anticoagulant activity by the USP method; it had a potency of about 2 I.U./ml. The supernatant fraction containing the mucopolysaccharides was then precipitated by addition of 2 vol. of acetone at 4° overnight. The precipitate formed was collected by centrifugation, washed once with acetone, dried under vacuum, and subjected to further purification. To remove contamination of nucleic acids, the precipitate was dissolved in water, trichloroacetic acid was added to the mixture to a final concentration of 10%, the mixture was kept in the cold for 30 min. The precipitate formed was removed by centrifugation, and 2 vol. of methanol was added to the supernatant fraction which was maintained at 4° overnight. The precipitate formed was collected by centrifugation, washed with acetone, and dried under vacuum. Alternatively, the nucleic acids were removed by treatment with 0.1 M $MnCl_2$ at 60° for 30 min, and the precipitate formed was removed by centrifugation. The mucopolysaccharides were then converted to sodium salts by treatment with 0.1 M Na_2HPO_4, pH 8.5. The precipitate formed was removed by centrifugation, and the mucopolysaccharides were precipitated from the solution by addition of 2 vol. of methanol, collected by centrifugation, washed with acetone, and dried under vacuum.

Heparin was separated from the other sulfated mucopolysaccharides according to a procedure [9] modified from Scott *et al.* [15] as follows: 1 g of the sulfated mucopolysaccharide mixture was dissolved in 100 ml of water, 20 g of anhydrous potassium acetate was added, and the mixture was shaken to ensure complete dissolution of the salt. The pH was adjusted to 5.7 and the solution was maintained for 2 hr at 60°. About 1 g of celite was added to the solution, which was then filtered at 60°. The filtrate was maintained at 4° for 24–48 hr to ensure complete precipitation of heparin. The precipitate formed was collected by filtration through a pad of celite in a Buchner funnel at 4°. The pad was washed once with cold 2 M potassium acetate. The heparin fraction was eluted from the celite pad with water at room temperature and precipitated from the solution by addition of 2 vol. of methanol. After standing at 4° overnight, it was collected by centrifugation and dried. To the supernatant fraction remaining after the precipitation of heparin with potassium acetate, 2 vol. of methanol was added. The precipitate formed was collected by centrifugation and dried. This method has the advantage of separating heparin from the other sulfated mucopolysaccharides. Only heparin precipitates with potassium acetate, while all the other sulfated mucopolysaccharides remain in the supernatant fraction free of heparin.

Heparitin sulfate, chondroitin sulfate B, and chondroitin sulfate AC present in the supernatant fraction were fractionated by large scale agarose gel electrophoresis in 0.05 M 1,3-diaminopropane–acetate buffer, pH 9.0, as described previously [12].

Identification and quantitation of sulfated mucopolysaccharides. The sulfated mucopolysaccharides were identified and quantified by a combination of agarose gel electrophoresis and enzymatic degradation with specific mucopolysaccharidases as reported previously [16–18]. Mucopolysaccharide quantitation was performed by densitometry of the agarose slides after gel electrophoresis in 0.05 M 1,3-diaminopropane–acetate buffer, pH 9.0, and toluidine blue staining [19]. The error of the method was in the order of ± 4.5 per cent. The extinction coefficients of the mucopolysaccharides were calculated using standards of chondroitin sulfate A, chondroitin sulfate B, heparitin sulfate, and heparin. Molecular weight determinations were performed by polyacrylamide gel electrophoresis according to Dietrich and Nader [12], modified from Hilborn and Anastassiadis [20]. Electrofocusing was performed according to Nader *et al.* [21]. Assays of chondroitinases AC and ABC were performed as described by Yamagata *et al.* [22], heparinase as described by Dietrich *et al.* [13] and heparitinases as described by Silva *et al.* [14]. Paper chromatography of the products formed after enzymatic degradation was performed in isobutyric acid–1 M NH_4OH (5:3, v/v). The products formed were visualized with the use of an ultraviolet lamp and by silver nitrate or toluidine blue staining [12]. Aminosugars were measured after acid hydrolysis (4 M HCl for 6 hr at 100° in sealed tubes) by a modified Elson–Morgan reaction [23]. The percentage of glucosamine and galactosamine in the hydrolysates was estimated as described previously [24]. Uronic acid was measured by a modification of the carbazole reaction according to Sajdera as described in Ref. 25. Total sulfate was measured after acid hydrolysis (8 M HCl for 6 hr at 100° in sealed tubes) as described by Dodgson [26] and Nader and Dietrich [27].

Pharmacological assays. Anticoagulant activity was assayed in whole plasma according to the United States Pharmacopea against the Third International Standard of Heparin from the WHO International Laboratory for Biological Standards. Determination of activated partial thromboplastin time (APTT) was performed as described by Basu *et al.* [28], and determination of anticomplement activity as inhibition of immune hemolysis (CH_{50}) (described by Basevi and Furlan [29]) was modified from Mayer [30]. Lipoprotein lipase (LPL)-releasing activity was determined in post-heparin (or other sulfated mucopolysaccharides) rat plasma. Different dilutions prepared in saline of the samples to be tested were administered to groups of Wistar rats by intravenous injection through the tail vein, and exactly 10 min after, under ether anesthesia, the blood was collected by cardiac puncture with a syringe bathed in a 20% sodium citrate solution. The blood was drawn into a graduated cylinder containing sodium citrate to a final concentration of 1.2%, and the plasma was separated by centrifugation. Lipoprotein lipase activity was then assayed in this plasma using Ediol as a substrate. The LPL and APTT units determined *in vivo* were obtained from the average of three different dose–response curves corresponding to three different doses of the samples of sulfated mucopolysaccharides (SMPS) using five individual rats per dose [31].

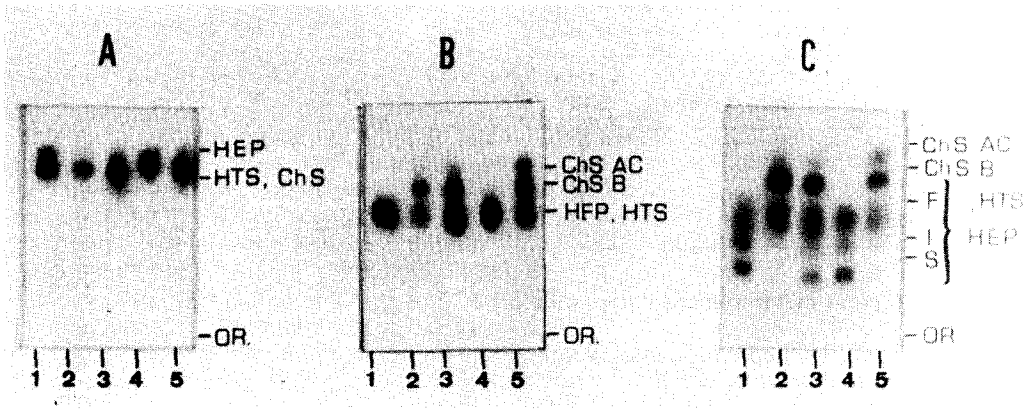

Fig. 1. Agarose gel electrophoresis of sulfated mucopolysaccharides (SMPS) from beef thymus extracted by potassium acetate. Five-microliter aliquots (about 10 μg) containing the precipitate (1) or the supernatant fraction (2) obtained by the potassium acetate procedure, the total SMPS extract from beef thymus (3), commercial heparin preparation from beef mucosa (4), or sulfated mucopolysaccharides standard mixture of chondroitin sulfate AC (ChSAC), chondroitin sulfate B (ChSB), heparitin sulfate (HTS) and heparin (HEP) (5) were applied on 5 × 7 cm (0.2 cm thick) agarose gel slabs (0.5% agarose in each individual buffer) at 1 cm from the negative electrode. Or = origin. Panel A: 0.06 M sodium barbital buffer, pH 8.6. The agarose gel was subjected to electrophoresis for 45 min at 100 V at 5°. The sulfated mucopolysaccharides were fixed in the gel with cetavlon and stained with toluidine blue as referred to in Materials and Methods. Panel B: 0.05 M 1,3-diaminopropane acetate buffer, pH 9.0. The agarose gel was subjected to electrophoresis for 1 hr at 100 V at 5° and the SMPS were fixed and stained as described in A. Panel C: Discontinuous agarose gel electrophoresis in barium-diaminopropane buffer. The SMPS were applied to an agarose gel slab prepared in 0.04 M barium acetate, pH 5.8, and subjected to electrophoresis (10 min, 75 V) in a chamber prepared with the same buffer at 5°. The whole gel slab was then transferred to another chamber, prepared with the diamino-propane acetate buffer, and maintained at 5° for 15 min. The current (100 V) was then applied in the same direction (toward the positive electrode) for 90 min or until the dye indicator (cresol red) migrated 4.5 cm from the origin. After electrophoresis, the gel was then fixed and stained as described in A. The heparin was fractionated into three different components, namely, slow-moving heparin (S), intermediate heparin (I) and fast-moving heparin (F), in this discontinuous buffer system.

RESULTS

SMPS composition of beef thymus. Figure 1 illustrates the electrophoretic behavior in three different buffer systems of the crude preparation of SMPS from beef thymus, as well as the precipitate and the supernatant fractions obtained after treatment with potassium acetate. It is clear from the analysis of these electropherograms that the crude preparation was a mixture of three main types of SMPS—heparin, heparitin sulfate, and chondroitin sulfate B—with traces of chondroitin sulfate AC, which can be distinguished by their different electrophoretic mobil-

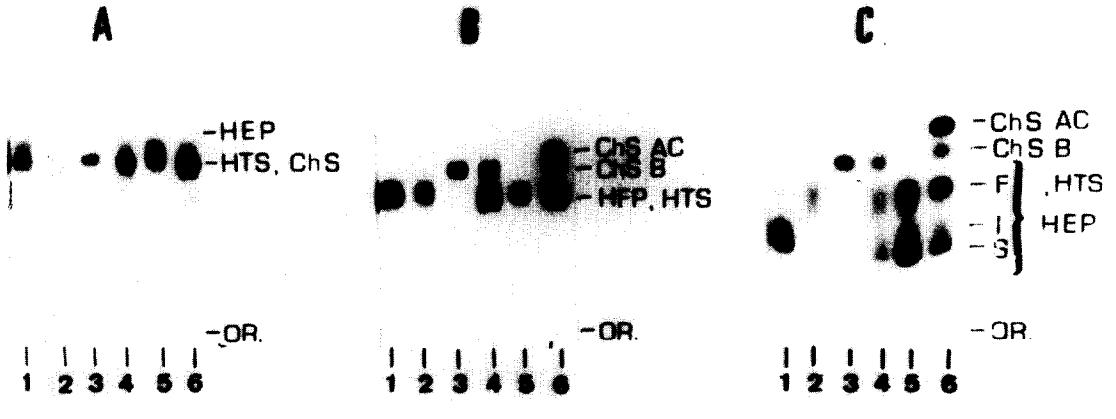

Fig. 2. Agarose gel electrophoresis of the purified fractions of sulfated mucopolysaccharides (SMPS) from beef thymus. The experiment was performed as described in the legend to Fig. 1 except that the purified sulfated mucopolysaccharide fractions were used as indicated: (1), heparin from beef thymus; (2) heparitin sulfate from beef thymus; (3) chondroitin sulfate B from beef thymus; (4) total sulfated mucopolysaccharides from beef thymus; (5) commercial heparin preparation from beef mucosa; and (6) sulfated mucopolysaccharide standard mixture of chondroitin sulfate AC (ChSAC), chondroitin sulfate B (ChSB), heparitin sulfate (HTS), and heparin (HEP). Or = origin.

Table 1. Sulfated mucopolysaccharide composition of beef thymus

	SMPS (μg/g tissue)	% of total SMPS
Heparin	274	36
Heparitin sulfate	182	24
Chondroitin sulfate B	289	38
Chondroitin sulfate AC	15	2

ities and their different metachromatic colors with toluidine blue.

The fraction that precipitated with potassium acetate in the cold behaved in all three buffer systems as heparin did. It showed the highest migration rate in barbital buffer, the higher affinity for the diamine, and was fractionated into three main components by the discontinuous buffer system (barium/diaminopropane). On the other hand, the supernatant fraction was composed mainly of heparitin sulfate and chondroitin sulfate B with trace amounts of chondroitin sulfate AC. The heparitin sulfate was then fractionated from chondroitin sulfate by large scale agarose gel electrophoresis. Figure 2 shows the electrophoretic migration of the purified fractions in agarose, using the three different buffers.

The percentage, as well as the total amount, of each SMPS present in beef thymus is shown in Table 1. Heparin, as previously shown [8, 9], was present in high amounts in beef thymus and constituted 36 per cent of the total SMPS.

Pharmacological properties of the SMPS from beef thymus. The antilipemic activity of the mucopolysaccharides is related to their lipoprotein lipase-releasing activity (LPL). LPL activity of the crude SMPS preparation from beef thymus, as well as of the various purified fractions, was determined along with the anticoagulant activity *in vivo* and *in vitro*, Table 2 summarizes these results. Heparin, among the SMPS, had the highest activity with respect to activation of the LPL and of the coagulation process (APTT and I.U.). The ratio of LPL/APTT for thymus heparin was about 3.3, whereas the heparins extracted from the various intestinal mucosae had a ratio of about 1.9 and from the lung, about 4.3. There was a slight difference between their anticoagulant potencies, expressed as I.U./mg, but this difference does not explain the difference between the ratios of LPL/APTT.

The heparitin sulfate and chondroitin sulfate B from thymus had negligible effects on both LPL and APTT when compared to that of heparin.

The test for the hemolytic C complement activity depends upon the ability to induce hemolysis of erythrocytes sensitized with optimal amounts of anti-erythrocyte antibodies. The relationship between the amount of C complement present and the proportion of cells lysed followed a sigmoidal curve. In this way, the CH_{50}, or 50 per cent hemolytic unit of C complement, designates the dilution of serum that will lyse 50 per cent of the indicator erythrocytes under defined conditions. Again, the highest immune hemolysis inhibiting activity (CH_{50}) was found for heparin. Commercial heparin preparations, except for the lung preparation, showed a

Table 2. Pharmacological data of the sulfated mucopolysaccharides from beef thymus and from commercial preparations*

	I.U./mg†	LPL/mg‡	APTT/mg‡	LPL/APTT	CH_{50}/mg‡
Total thymus SMPS	37.0 ± 3.1	6.4 ± 0.5	1.8 ± 0.2	3.55	16.6 ± 0.6
Thymus heparin	133.0 ± 8.0	38.0 ± 3.2	11.6 ± 1.0	3.28	56.2 ± 1.4
Thymus heparitin sulfate	4.1 ± 0.4	1.5 ± 1.5	2.0 ± 0.2	0.75	ND
Thymus chondroitin sulfate B	0.7 ± 0.1	≤0.25	≤0.25		ND
Commercial heparins					
Beef intestinal mucosa (LAOB)	157.0 ± 7.0	34.3 ± 2.5	16.9 ± 1.3	2.03	ND
Pork intestinal mucosa (Opocrin)	158.0 ± 6.9	33.0 ± 2.6	18.5 ± 1.5	1.78	37.5 ± 1.8
Pork intestinal mucosa (Sigma)	160.0 ± 5.3	34.1 ± 2.7	17.5 ± 1.4	1.95	36.5 ± 1.1
Beef lung (Inorp)	135.0 ± 4.2	44.3 ± 3.3	10.3 ± 0.8	4.30	60.0 ± 1.8
Commercial chondroitin sulfate B (Miles)	ND	≤0.25	≤0.25		1.1 ± 0.1
Pancreas heparitin sulfate	ND	≤0.25	0.4 ± 0.1		1.5 ± 0.1

* Abbreviations: I.U., anticoagulant activity measured *in vivo* by the United States Pharmacopea method; LPL, lipoprotein lipase activity measured *in vivo*; APTT, activated partial thromboplastin time measure *in vivo*; CH_{50}, anticomplement activity or antihemolytic activity; and ND, not determined.

† Average of five experiments.

‡ Average of three experiments.

Table 3. Chemical and physical data of purified sulfated mucopolysaccharides from thymus and from commercial preparations

Compound	Molar proportions				Molecular weight	
	Glucosamine	Galactosamine	Uronic acid	Total sulfate	Mode value	Range
Thymus heparin	1.0	0	1.60	2.9	15,000	(4,100–54,000)
Thymus heparitin sulfate	1.0	0	1.39	1.6	4,800 11,000	(2,000–68,000)
Thymus chondroitin sulfate B	0	1.0	0.61	1.2	26,000	(7,300–47,000)
Commercial heparins						
Pork intestinal mucosa	1.0	0	1.70	2.5	14,000	(4,100–54,000)
Beef lung	1.0	0	1.80	2.9	14,000	(4,000–53,000)
Commercial chondroitin sulfate B						
Pork skin	0	1.0	0.55	0.93	19,000	(9,500–29,000)
Heparitin sulfate B						
Beef lung	1.0	0	1.63	1.1	25,000	(10,000–35,000)
Heparitin sulfate						
Beef pancreas	1.0	0	2.10	1.6	40,000	(16,000–100,000)

lower CH_{50} activity, as well as the other sulfated mucopolysaccharides.

Chemical composition and physical data of the SMPS from beef thymus. Table 3 shows the molar proportions of hexosamine, uronic acid, and total sulfate of each SMPS obtained from thymus compared to commercial heparin and other standard sulfated mucopolysaccharides. The analytical data confirmed the electrophoretic behavior of the SMPS. The fraction with a migration similar to that of the standard heparins used in these experiments showed hexosamine/uronic acid/sulfate ratios similar to those of the commercial heparin preparations. The presence of galactosamine as the only aminosugar in the fraction that migrated as did chondroitin sulfate B, together with the molar ratio of uronic acid to hexosamine of about 0.5, which is indicative of the presence of iduronic acid residues in the molecule, characterize the fraction as chondroitin sulfate B.

Figure 3A shows polyacrylamide gel electrophoresis of each individual SMPS extracted from thymus. They exhibited different electrophoretic mobilities and different degrees of polydispersity. The calculated average molecular weight of these compounds

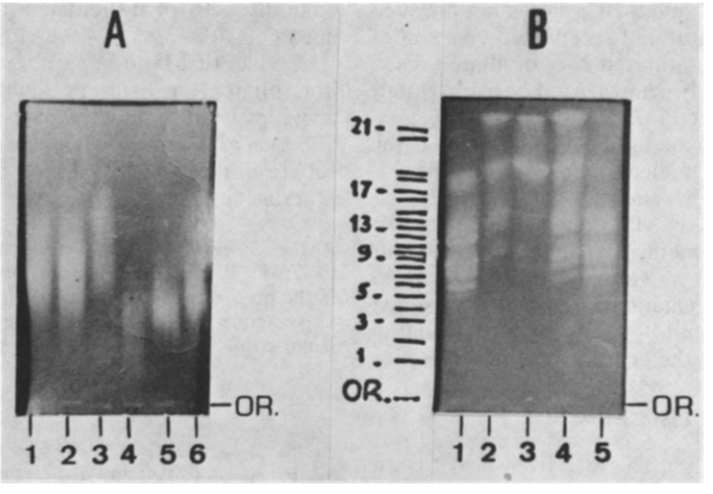

Fig. 3. Polyacrylamide gel electrophoresis and electrofocusing of the purified sulfated mucopolysaccharides (SMPS) extracted from beef thymus. Panel A: Polyacrylamide gel electrophoresis. One-microliter aliquots (15–30 μg) of SMPS were applied to the polyacrylamide gel and subjected to electrophoresis at 2 V/cm for 30 min in 0.06 M sodium barbital buffer, pH 8.6. The gel was then stained with toluidine blue (0.1% in 1% acetic acid) and destained with 1% acetic acid. Key: (1) heparin from beef thymus; (2) commercial heparin preparation from beef mucosa; (3) heparitin sulfate from beef thymus; (4) standard heparitin sulfate from beef pancreas; (5) chondroitin sulfate B from beef thymus; and (6) standard chondroitin sulfate B from pork mucosa. Panel B: Electrofocusing. One-microliter aliquots (15–30 μg) of SMPS were applied to the polyacrylamide gel containing 2% ampholyte (pH 3.5 to 5.0) at 1 cm from the negative end and subjected to a potential of 15 V/cm for 6 hr. The gel slab was then stained with toluidine blue (0.1% in 1% acetic acid) and destained with 1% acetic acid. Key: (1) heparin from beef thymus; (2) heparitin sulfate from beef thymus; (3) chondroitin sulfate B from beef thymus; (4) total sulfated mucopolysaccharide extracted from beef thymus; and (5) commercial heparin preparation from beef mucosa.

is shown in Table 3. Differences in molecular weight were not observed for the thymus heparin when compared to the commercial heparin preparations. On the other hand, both chondroitin sulfate B and heparitin sulfare from thymus showed an average, and a range of molecular weights different from the standards used. Furthermore, two species of heparitin sulfate with different molecular weights were present in these preparations. This has also been observed for heparitin sulfates of other tissues such as lung, spleen and heart [12, 19].

Among the SMPS, only heparin gives a characteristic pattern of fractionation in electrofocusing [21, 32]. All the commercial heparins give at least twenty-one fractions with different molecular weights. Figure 3B shows the electrofocusing of heparin, heparitin sulfate, and chondroitin sulfate B extracted from thymus. It is clear that only the heparin fraction shows the typical band formation, or the fingerprint of heparin.

Enzymatic degradation and identification of the SMPS from beef thymus. The SMPS from beef thymus were further characterized by degradation with specific mucopolysaccharidases. The heparin fraction was degraded by heparinase but not by heparitinase, chondroitinase AC, or chondroitinase ABC. The degradation products formed were trisulfated disaccharide and pentasulfated tetrasaccharide, which are typical of a heparin preparation [33]. The chondroitin sulfate B was degraded by chondroitinase ABC, but not by chondroitinase AC, heparitinases, or heparinase; it showed the formation of unsaturated 4-sulfate disaccharide by the action of chondroitinase ABC. The heparitin sulfate was degraded by heparitinases but not by heparinase or chondroitinases AC and ABC. The main products formed were unsaturated acetylated-nonsulfated disaccharide and unsaturated *N*-*O*-disulfated disaccharide as previously shown for heparitin sulfate from beef lung tissue [13].

The identification of the SMPS reported in this paper was based on electrophoretic migration in three different buffer systems, precipitation in agarose gel slabs with cetyltrimethylammonium bromide, characteristic metachromatic colors with toluidine blue, molecular weight, pattern of focalization, susceptibility to enzymatic degradation with mucopolysaccharidases, and by the types of degradation products formed by the action of the enzymes.

DISCUSSION

Sulfated mucopolysaccharides from beef thymus consist of a mixture of heparin, heparitin sulfate, and chondroitin sulfate B. The mixture had a ratio of LPL/APTT of about 3.5, which means that the mixture was more active on LPL than on APTT. These results indicate that the factor found in beef thymus by Mondola *et al.* [10, 11] is indeed the mixture of sulfated mucopolysaccharides reported in this paper.

The heparitin fraction extracted from thymus had a ratio of LPL/APTT of about 3.3, whereas the commercial heparin extracted from intestinal mucosa had a ratio of about 1.9, and lung heparin had a ratio of about 4.3. In previous works [34, 35], we were able to demonstrate that heparins extracted from bovine pancreas are more active on LPL release than on APTT at the same doses expressed in I.U., whereas other preparations (e.g. pig intestinal mucosa or beef intestinal mucosa) show an inverse correlation. Such behavior supports the hypothesis that two types of heparin exist, with different active sites and different affinities for antithrombin III and LPL and, therefore, presumably with different physiological roles. *In vitro*, i.e. in the absence of the LPL releasing system, heparin is completely available for antithrombin III, whereas *in vivo* there will be a competition between the two systems. Such a competition agrees with the existence of a high affinity of heparin for LPL and a low affinity for antithrombin III [36].

The greatest effect on inhibition of immune hemolysis was by the heparin fraction. Once again we have to consider the specificity of the heparin molecule itself on the phenomena. Peculiarities of the structure besides charge seem to be important features for a particular molecule to have a significant antihemolytic activity, since other sulfated mucopolysaccharides that contain a lower degree of sulfation and different types of hexosamine and uronic acid have a negligible effect on hemolysis.

These results once again suggest that heparin is a family of compounds that differ not only in their molecular weights but also in their biological activities. In particular, it seems justified to think that this family includes substances with a prevailing effect on either hemocoagulation, on lipoprotein lipase release, or on the cytolytic system of complement. It may therefore be possible to employ different heparin fractions in the treatment of thromboembolic diseases, of dislipidemia and of some immunocomplex diseases.

The fact that both heparitin sulfate and chondroitin sulfate B possess negligible LPL and APTT activities is an argument against the speculation of Silbert *et al.* [37] on the possible role of the heparitin sulfate in mechanisms of fibrinolysis, lipolysis, and atherogenesis.

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REFERENCES

1. L. B. Jaques, *Prog. med. Chem.* **5**, 139 (1967).
2. R. A. Bradshaw and S. Wessler (Eds.), *Advances in Experimental Medicine and Biology*, Vol. 52. Plenum Press, New York (1975).
3. P. L. Walton, C. R. Ricketts and D. R. Baugham, *Br. J. Haemat.* **12**, 310 (1966).
4. E. Novak, N. C. Sekhar, N. W. Dunham and L. L. Coleman, *Clin. Med.* **79** (7), 22 (1972).
5. T. W. Barrowcliffe, E. A. Johnson, C. A. Eggleton and D. P. Thomas, *Thromb. Res.* **12**, 27 (1977).
6. D. A. Lane, I. R. Macgregor, R. Michalski and V. V. Kakkar, *Throm. Res.* **12**, 257 (1978).
7. S. Wessler and S. N. Gitel, *Blood* **53**, 525 (1979).
8. H. B. Nader, A. H. Straus, H. K. Takahashi and C. P. Dietrich, in *Proceedings of the Fifth International Symposium on Glycoconjugates* (Eds. R. Schauer, P.

Boer, E. Buddecke, M. F. Kramer, J. F. G. Vligenthart and H. Wiegandt), p. 714. Georg Thieme Publishers, Stuttgart (1979).
9. H. B. Nader, H. K. Takahashi, A. H. Straus and C. P. Dietrich, *Biochim. biophys. Acta* **627**, 40 (1980).
10. P. Mondola, M. D'Errico and C. Falconi, *Boll. Soc. ital. Biol. sper.* **52**, 318 (1976).
11. P. Mondola, M. D'Errico and C. Falconi, *Boll. Soc. ital. Biol. sper.* **52**, 323 (1976).
12. C. P. Dietrich and H. B. Nader, *Biochim. biophys. Acta* **343**, 34 (1974).
13. C. P. Dietrich, M. E. Silva and Y. M. Michelacci, *J. biol. Chem.* **248**, 6408 (1972).
14. M. E. Silva, C. P. Dietrich and H. B. Nader, *Biochim. biophys. Acta* **437**, 129 (1975).
15. J. E. Scott, T. E. Stacey and M. J. Tigwell, *Biochem. J.* **108**, 50P (1968).
16. C. P. Dietrich and S. M. C. Dietrich, *Analyt. Biochem.* **46**, 209 (1972).
17. C. P. Dietrich and S. M. C. Dietrich, *Analyt. Biochem.* **70**, 645 (1976).
18. P. Bianchini, H. B. Nader, H. K. Takahashi, B. Osima, A. H. Straus and C. P. Dietrich, *J. Chromat.* **196**, 455 (1980).
19. C. P. Dietrich, L. O. Sampaio and O. M. S. Toledo, *Biochem. biophys. Res. Commun.* **71**, 1 (1976).
20. J. C. Hilborn and P. A. Anastassiadis, *Analyt. Biochem.* **39**, 88 (1971).
21. H. B. Nader, N. M. McDuffie and C. P. Dietrich, *Biochem. biophys. Res. Commun.* **57**, 488 (1974).
22. T. Yamagata, H. Saito, O. Habuchi and S. Suzuki, *J. biol. Chem.* **243**, 1523 (1968).
23. C. J. M. Rondle and W. T. J. Morgan, *Biochem. J.* **61**, 586 (1955).
24. C. P. Dietrich, H. B. Nader and P. A. S. Mourão, *Biochem. Med.* **8**, 371 (1972).
25. N. Di Ferrante, B. L. Nichols, P. V. Donnelly, G. Neri, R. Hargovcic and R. K. Berglund, *Proc. natn. Acad. Sci. U.S.A.* **68**, 303 (1971).
26. K. S. Dodgson, *Biochem. J.* **78**, 312 (1961).
27. H. B. Nader and C. P. Dietrich, *Analyt. Biochem.* **78**, 112 (1977).
28. D. Basu, A. Gallus, J. Hirsh and J. Cade, *New Engl. J. Med.* **287**, 324 (1972).
29. A. Basevi and S. Furlan, *G. Mal. infett. parassit.* **8**, 480 (1956).
30. M. M. Mayer, in *Experimental Immunochemistry* (Ed. E. A. Kabat), 2nd Edn, p. 133. Charles C Thomas, Springfield, IL (1971).
31. P. Bianchini, G. Guidi and B. Osima, *Biochem expl. Biol.* **10**, 243 (1972).
32. N. M. McDuffie, C. P. Dietrich and H. B. Nader, *Biopolymers* **14**, 1473 (1975).
33. M. E. Silva and C. P. Dietrich, *J. biol. Chem.* **250**, 6841 (1975).
34. P. Bianchini, B. Osima, V. Rimini and M. Macchi, in *Haemostasis and Thrombosis* (Eds. Neri, Serneri and Prentice), pp. 495–500. Academic Press, New York (1979).
35. P. Bianchini, in *Heparin: Structure, Cellular Functions, and Clinical Applications* (Ed. N. M. McDuffie), p. 99. Academic Press, New York (1979).
36. T. C. Laurent, A. Tengblad, L. Thunberg, M. Höök and U. Lindahl, *Biochem. J.* **175**, 691 (1978).
37. J. E. Silbert, H. K. Kleinman and C. K. Silbert, in *Advances in Experimental Medicine and Biology*, Vol. 52 (Eds. R. A. Bradshaw and S. Wessler), pp. 51–60. Plenum Press, New York (1975).