Metabolism of Macromolecular Heparin in Mouse Neoplastic Mast Cells

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1. Polysaccharide in a heparin-producing mouse mastocytoma was pulse-labelled *in vivo* with [^{35}S]sulphate, and after various periods of time was isolated from subcellular fractions. Such fractions were recovered from tissue homogenates by consecutive centrifugations at 1000g for 10 min, 20000g for 20 min and 100000g for 1 h. Initially, the ^{35}S -labelled polysaccharide formed occurred principally in the second centrifugal fraction (20000g precipitate), with smaller amounts in the first (granular) and third (microsomal) fractions. Analysis for glycosyltransferase activity confirmed that glycosaminoglycans were formed chiefly in particles sedimenting at 20000g. Molecules of this newly synthesized polysaccharide were considerably larger than those of commercially available heparin, as judged from gel chromatography. 2. Within the first hour after injection of [^{35}S]sulphate, most of the labelled polysaccharide was redistributed from the second to the first centrifugal fraction. During, and possibly also after, this shift, the macromolecular polysaccharide was degraded, ultimately to the size of commercial heparin. The degradation process appeared complete 6 h after injection of [^{35}S]sulphate. 3. Particulate subcellular fractions were incubated with macromolecular [^{35}S]heparin and the products were analysed by gel chromatography. Significant degradation of the substrate occurred only with the second centrifugal fraction. Further characterization of this fraction, by density-gradient centrifugation in iso-osmotic colloidal silica, revealed a single visible band of particles, at approximately the same density as lysosomes. This band contained all the β -glucuronidase, ^{35}S -labelled endogenous polysaccharide and heparin-degrading enzyme present in the second fraction.

Heparin is a glycosaminoglycan endowed with unique biological properties, the best-known of which are its anticoagulant and lipolysis-stimulating activities (Ehrlich & Stivala, 1973). The polysaccharide is synthesized by mast cells (Selye, 1965), and possibly by basophilic leucocytes (Olsson *et al.*, 1970), and is stored within the cells in granules containing, in addition, protein and vasoactive amines.

Most glycosaminoglycans occur in the native state as proteoglycans, composed of several polysaccharide chains covalently linked to a common polypeptide core (Lindahl & Rodén, 1972). Attempts to isolate a heparin proteoglycan were unsuccessful, yielding essentially single polysaccharide chains, with mol.wts. ranging from 7000 to about 25000 (Lindahl, 1970; Ögren & Lindahl, 1971; Jansson *et al.*, 1975). However, in rodent tissues heparin has been shown to occur as a multichain macromolecular species (mol.wt. up to 1000000) quite different from 'conventional' proteoglycans (Horner, 1971; Ögren & Lindahl, 1971); in this rodent heparin molecule, the individual polysaccharide chains are joined in a so far unknown manner,

possibly by a polysaccharide core (Horner, 1971). Horner (1972) suggested that macromolecular heparin is a precursor form, since lipoprotein lipase-releasing activity appeared only on depolymerization. Intracellular degradation of macromolecular heparin was implied from the results of pulse-labelling *in vivo* of mouse neoplastic mast cells with [^{35}S]sulphate (Ögren & Lindahl, 1971). Since that time an enzyme has been isolated (from the same tissue) which degrades macromolecular heparin to single polysaccharide chains, similar in size to commercially available heparin (Ögren & Lindahl, 1975a). The liberated polymer molecules were resistant to further degradation. The enzyme (hereafter termed 'heparin-liberating enzyme') was tentatively identified as an endoglucuronidase. Evidence has been obtained suggesting that the formation and degradation of macromolecular heparin may also occur in mammals other than rodents (see Jansson *et al.*, 1975).

The present investigation was undertaken to obtain further information about the intracellular metabolism of the newly synthesized heparin. The results show that degradation of the polysaccharide

occurs in a particulate fraction that sediments after 20 min at 20000g; the degradation products are largely transferred to particles that sediment after 10 min at 1000g. The heparin-liberating enzyme is localized in particles in the 20000g precipitate, which share some properties with lysosomes.

A preliminary report of some of this work has already appeared (Ögren & Lindahl, 1975b).

Experimental

Materials

The transplantable mouse mastocytoma used in this study has been described previously (Ögren & Lindahl, 1971).

Sodium [^{35}S]sulphate (carrier-free) and UDP-[^{14}C]glucuronic acid (287 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. UDP-N-acetylglucosamine, phenyl β -D-glucuronide, bovine serum albumin and D-glucuronolactone were from Sigma Chemical Co., St. Louis, MO, U.S.A. Pronase (lot 45550) was from Calbiochem, Los Angeles, CA, U.S.A. Papain was crystallized from a crude preparation as described by Kimmel & Smith (1954) and was used as a suspension containing 24 mg of protein/ml.

Heparin (stage 14, isolated from pig intestinal mucosa), obtained from Inolex Pharmaceutical Div., Park Forest South, IL, U.S.A., was purified by repeated precipitation with cetylpyridinium chloride from 1 M-NaCl, essentially as described by Lindahl *et al.* (1965). High-molecular-weight mouse mastocytoma heparin, labelled *in vivo* with [^{35}S]sulphate, was prepared as described by Ögren & Lindahl (1975a).

Sephacrose 4B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Colloidal silica sol, Ludox HS (40%, w/w, in deionized water), was purchased from E. I. duPont de Nemours, Wilmington, DE, U.S.A. Polyvinylpyrrolidone (mol. wt. 40000) was a product of A. H. Thomas Co., Philadelphia, PA, U.S.A. Triton X-100 was obtained from Rohm and Haas, Philadelphia, PA, U.S.A.

Analytical methods

Uronic acid was determined by the carbazole method of Bitter & Muir (1962), with D-glucuronolactone as standard. Protein was measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard. β -Glucuronidase activity was measured as described by Allison & Sandelin (1963), with phenyl β -D-glucuronide as substrate.

Radioactivity was determined with Instagel (Packard Instrument Co., Downers Grove, IL, U.S.A.) as scintillation medium in either a Beckman LS 250 or a Packard Tri-Carb 2002 liquid-scintillation spectrometer.

Gel chromatography was performed at 4°C on

columns (1.2 cm $\times$ 95 cm) of Sepharose 4B, eluted with 1 M-NaCl in 0.05 M-Tris/HCl, pH 8.0, at a rate of 7–8 ml/h. Effluent fractions (about 2.5 ml) were collected and analysed for radioactivity.

Preparation of subcellular fractions from mouse mastocytoma

Tumour-bearing mice were killed by cervical dislocation. Tumours (each about 1 g wet wt.) were then immediately dissected out, carefully separated from other tissues and transferred to a Dounce homogenizer, chilled in an ice bath. Cold 0.25 M-sucrose in 0.05 M-Tris/HCl, pH 7.4, was added (2 ml/g of tissue) and the tissue was then homogenized with five strokes of the loose-fitting pestle A and eight strokes of the tight-fitting pestle B. This treatment resulted in disruption of practically all cells, as checked in a light microscope. All subsequent steps were carried out at 4°C. The homogenate was centrifuged in a Sorvall RC2B refrigerated centrifuge at 1000g for 10 min; the resulting pellet was washed once with 1 ml of buffered sucrose/g of original tumour tissue. The pooled supernatants were then centrifuged in the same apparatus at 20000g for 20 min. Finally, microsomal and cell-sap fractions were recovered from the 20000g supernatant by centrifugation in a Beckman L3-40 preparative ultracentrifuge at 100000g for 1 h. The various subcellular preparations are referred to below as 1000g fraction, 20000g fraction, 100000g fraction and 100000g supernatant respectively.

Isolation of glycosaminoglycan from subcellular fractions

Subcellular fractions were prepared as described above from 10g (wet wt.) of mastocytoma tissue, and were then digested with Pronase as described by Ögren & Lindahl (1971). Polysaccharide was isolated from the filtered digests by repeated precipitation with cetylpyridinium chloride from 0.8 M-NaCl (Ögren & Lindahl, 1971).

Glycosaminoglycan, labelled *in vivo* with [^{35}S]sulphate, was isolated from subcellular fractions as follows. Tumour-bearing mice were injected intraperitoneally with 75 μCi of carrier-free [^{35}S]sulphate in 0.2 ml of modified Ringer solution (ACO, Stockholm, Sweden), and were then killed by cervical dislocation after various periods of time. The times of injection were chosen so that all tumours were of approximately similar size when harvested. Subcellular fractions were prepared, as described above, from homogenates of three tumours, each homogenate corresponding to a specific labelling period. After the addition of carrier heparin (1 mg to the particulate fractions and 3 mg to the 100000g supernatant), the various fractions were subjected

to proteolysis and polysaccharide was isolated, as described above.

Incorporation of [^{14}C]glucuronic acid into endogenous glycosaminoglycan

Samples of the 20000g and 100000g fractions were washed twice in 0.25M-sucrose and were then suspended in 0.25M-sucrose/0.05M-Hepes [2-(N-2-hydroxyethyl)piperazin-N'-yl]ethanesulphonic acid] buffer, pH7.3, to yield a protein concentration of 40mg/ml. Samples (20 μl) of these enzyme preparations were analysed for glucuronosyltransferase activity in incubation mixtures, containing (per ml of 0.05M-Hepes, pH7.3) 10 μCi of UDP-[^{14}C]glucuronic acid, 2.5 μmol of UDP-N-acetylglucosamine, 10 μmol of MgCl_2 , 10 μmol of MnCl_2 , 5 μmol of CaCl_2 and 10mg of enzyme protein (Lindahl *et al.*, 1973; the UDP-N-acetylglucosamine concentration was chosen to give maximal polymerase activity); the final incubation volumes were 75 μl . After incubation at 37°C for 60min the mixtures were heated at 100°C for 3min and digested overnight at 55°C with Pronase (10 μl of a solution containing 10mg of enzyme/ml). The digests were centrifuged, and were then analysed for labelled polysaccharide by the method of Wasteson *et al.* (1973). Samples (50 μl) of the supernatants were applied to strips of Whatman 3MM filter paper; the strips were dried and subsequently washed for 30min in each of five changes of 1% (w/v) cetylpyridinium chloride in 0.05M-NaCl. ^{14}C -labelled polysaccharide was measured by liquid-scintillation counting, as described by Wasteson *et al.* (1973).

Heparin-liberating enzyme in subcellular fractions

The 1000g and 20000g fractions were tested for heparin-liberating enzyme as follows. Portions (1ml) of each pellet, corresponding to approximately equal amounts of tumour tissue, were suspended in 2ml of 0.25M-sucrose/0.05M-Tris/HCl, pH7.4. Samples (0.9ml) of the suspensions and of a 20000g supernatant were mixed with 0.1ml of 0.5M-sodium acetate buffer, pH5.0 (pH optimum determined for the isolated enzyme; Ögren & Lindahl, 1975a); samples (0.5ml) were frozen and thawed and were then incubated with macromolecular ^{35}S -labelled heparin (3×10^4 c.p.m.) at 37°C in a stirred water bath. After 24h the incubation mixtures were heated at 100°C for 5min and were then diluted with an equal volume of 4M-NaCl, containing 0.02M-cysteine hydrochloride and 0.02M-EDTA. Papain (0.1ml) was added and the samples were digested at 65°C for 30min, boiled, clarified by centrifugation and analysed by gel chromatography.

Density-gradient centrifugation of 20000g fraction

Each of three tumour-bearing mice was injected with [^{35}S]sulphate, as described above; 4h later the

mice were killed and the tumours were removed and homogenized. The 20000g fraction was prepared and subjected to density-gradient centrifugation in the iso-osmotic colloidal silica (Ludox HS)/polyvinylpyrrolidone medium (PEL-1.055) described by Wolff & Pertoft (1972). The 20000g fraction, suspended in 5ml of 0.25M-sucrose, was layered in 1ml portions on top of the gradient solution in five centrifuge tubes (each containing 11.5ml of gradient medium) and was then centrifuged at 30000rev./min (80000g) in a Beckman L4 ultracentrifuge (rotor 30.2) for 20min. Fractions (about 1.5ml) were collected and their density was measured (Miller & Gasek, 1960). They were then mixed with Triton X-100 (final concentration 0.1%, v/v) and finally analysed for radioactivity and for β -glucuronidase activity after freezing and thawing of the samples. Before analysis for heparin-liberating enzyme, fractions were pooled as indicated in Fig. 4 and washed free of gradient medium by three successive centrifugations at 20000g for 20min with 0.25M-sucrose/0.05M-Tris/HCl, pH7.4. The pellets obtained were suspended in 1ml of 0.15M-NaCl/0.05M-sodium acetate buffer, pH5.0; portions (0.5ml) were frozen, thawed and then incubated with [^{35}S]heparin, as described above.

Results

Subcellular distribution of glycosaminoglycans labelled in vivo with [^{35}S]sulphate

After intraperitoneal injection of inorganic [^{35}S]sulphate, the amount of radioactivity rapidly increased in the tumour tissue, reaching a peak value of about 2×10^7 c.p.m./g wet wt. of tissue in about 30min. During the following hour the ^{35}S concentration steeply declined to a value less than 5% of the peak value; this amount of radioactivity was then maintained throughout the 24h observation period. The amount of ^{35}S -labelled polysaccharide steadily increased during this period, from about 1.5×10^5 c.p.m./g of tissue 15min after injection to about 1×10^6 c.p.m./g of tissue obtained 24h after injection.

The distribution with time of ^{35}S -labelled polysaccharide between the various subcellular fractions is illustrated in Fig. 1. Immediately after injection of [^{35}S]sulphate most of the ^{35}S -labelled polysaccharide was found in the 20000g fraction. During the first hour after injection the amount of ^{35}S -labelled polysaccharide in the 20000g fraction (and in the 100000g fraction) decreased to less than one-third of the initial value, whereas that in the 1000g fraction increased. The total amounts of ^{35}S -labelled polysaccharide found in cell homogenates were similar (2×10^5 c.p.m./g of tissue) at 30min after injection and at 1h after injection. The dramatic shift of labelled material from the 20000g fraction to the 1000g fraction during the second

Fig. 1. Distribution of ^{35}S -labelled polysaccharide between subcellular fractions of mastocytoma at different times after injection of tumour-bearing mice with $[^{35}\text{S}]$ sulphate

○, 1000g fraction; △, 20000g fraction; ●, 100000g fraction; ▲, 100000g supernatant; □, 20000g supernatant (applies only to the 24h sample, which was not fractionated beyond the 20000g centrifugation). For experimental details, see the text.

Table 1. Occurrence of glycosaminoglycan and glucuronosyltransferase in subcellular fractions of neoplastic mast cells

The glycosaminoglycan content of each fraction was determined by the carbazole reaction for uronic acid, after isolation of polysaccharide as described in the Experimental section. Polymerase activity is indicated by the amount of radioactivity incorporated into endogenous polysaccharide during incubation of the respective subcellular fraction with UDP- $[^{14}\text{C}]$ glucuronic acid and UDP-*N*-acetylglucosamine (see the Experimental section; incubations were carried out in duplicate).

Subcellular fraction	Glycosaminoglycan content (μg of uronic acid/10g of tissue)	Polymerase activity (c.p.m. of $[^{14}\text{C}]$ -glucuronic acid incorporated into polysaccharide/mg of protein)
1000g fraction	460	—
20000g fraction	520	2300, 2240
100000g fraction	73	4140, 4220
100000g supernatant	53	—

half-hour period after the injection therefore indicates an actual redistribution of ^{35}S -labelled polysaccharide between these fractions. Similar results were obtained in three separate series of experiments. A tendency towards reversal of this process with time was noted, the ratio of ^{35}S -labelled polysaccharide in the two fractions being close to unity 24h after the injection (Fig. 1). The amounts of labelled polysaccharide in

the 100000g pellet and supernatant fractions remained low throughout the experiment.

The results of the radiochemical experiments can be related to the subcellular distribution of the unlabelled bulk polysaccharide (Table 1). The polysaccharide recovered from the various subcellular fractions together accounted for 85% of the total sulphated glycosaminoglycan of the mastocytoma tissue. The major portion (90%) of the total polysaccharide occurred in particles sedimenting at 20000g, and was about evenly distributed between the 1000g and the 20000g fractions. These relations are in fair agreement with the subcellular distribution of ^{35}S -labelled polysaccharide when recorded 24h after injection of $[^{35}\text{S}]$ sulphate (Fig. 1).

Fig. 1 shows that the amounts of ^{35}S -labelled polysaccharide in the 20000g and the 100000g fractions decreased in parallel during the first hour after $[^{35}\text{S}]$ sulphate injection; thus no redistribution of labelled material from the latter to the former fraction was observed. As ^{35}S was initially incorporated mostly into the 20000g fraction, probably most of the polysaccharide produced by the tumour cells was actually formed in this fraction rather than in the 100000g ('microsomal') fraction. This conclusion was supported by experiments in which each of the two fractions was incubated with UDP- $[^{14}\text{C}]$ glucuronic acid in the presence of unlabelled UDP-*N*-acetylglucosamine. The data given in Table 1, expressed as amounts of ^{14}C -labelled polysaccharide formed/mg of protein, must be considered in relation to the total protein contents of the fractions; the overall polymerase activity then appears to be higher in the 20000g than in the 100000g fraction. Although these results may not be directly applicable to the situation *in vivo*, they clearly demonstrate that the ability to synthesize glycosaminoglycans is not restricted to the microsomal fraction.

Molecular size of glycosaminoglycans labelled in vivo with $[^{35}\text{S}]$ sulphate

The molecular size of ^{35}S -labelled glycosaminoglycans was investigated by gel chromatography on Sepharose 4B. The gel chromatograms illustrated in Fig. 2 refer to the preparations isolated from the 20000g fraction at various times after injection of the mice with $[^{35}\text{S}]$ sulphate. The material obtained after 30min showed a major peak of high-molecular-weight labelled material, with smaller amounts of more retarded components. With increasing time a progressive shift towards lower molecular weight was observed; the elution pattern of ^{35}S -labelled polysaccharide isolated 6h or more after injection was similar to that of the bulk polysaccharide of the tissue (see Ögren & Lindahl, 1971).

The corresponding elution patterns of ^{35}S -labelled polysaccharide preparations isolated from the 1000g fraction (not shown in the Figure) essentially

Fig. 2. Gel chromatography on Sepharose 4B of ^{35}S -labelled polysaccharide isolated from 20000g fraction of mastocytoma at different times after injection of tumour-bearing mice with ^{35}S sulphate

Polysaccharide was isolated from the 20000g fraction, as described in the Experimental section. $\circ$, 30 min, $\bullet$, 1 h, $\triangle$, 2 h, or $\blacktriangle$, 6 h after the injection of ^{35}S -sulphate.

conformed to those illustrated in Fig. 2; however, the polysaccharide obtained 30 min after injection of ^{35}S sulphate contained relatively more low-molecular-weight components than the corresponding material from the 20000g fraction. The ^{35}S -labelled polysaccharide preparations obtained from the 10000g supernatant were all retarded equally on gel chromatography, irrespective of time of isolation.

Subcellular location of mastocytoma heparin-liberating enzyme

Subcellular fractions were tested for heparin-liberating enzyme by incubation with macromolecular ^{35}S heparin, followed by gel chromatography on Sepharose 4B (see the Experimental section). Incubation of the substrate with the 1000g fraction (Fig. 3) or with the 20000g supernatant (not shown) did not result in any significant degradation. By contrast, appreciable breakdown of the ^{35}S -labelled polysaccharide was noted after incubation with the 20000g fraction. Although these results cannot be quantitatively evaluated in terms of enzyme activity, they suggest that the heparin-liberating enzyme occurs preferentially in the 20000g fraction. The low activity of the 1000g fraction should probably not be ascribed to inhibitory effects of endogenous heparin (Ögren & Lindahl,

Fig. 3. Gel chromatography on Sepharose 4B of ^{35}S -labelled polysaccharide obtained by incubation of macromolecular ^{35}S heparin with subcellular mastocytoma fractions

Macromolecular ^{35}S heparin was incubated with $\circ$, 1000g fraction, $\bullet$, 20000g fraction, or $\triangle$, 20000g fraction, heated at 100°C for 5 min, as described in detail in the Experimental section.

Fig. 4. Density-gradient centrifugation in colloidal silica of 20000g fraction prepared 4h after injection of tumour-bearing mice with ^{35}S sulphate

Fractions about 7.5 ml, obtained by combining corresponding 1.5 ml fractions from five separate tubes) were analysed for: $\bullet$, ^{35}S , $\blacktriangle$ (E_{435}), β -glucuronidase activity, and $\circ$, density. Before analysis for heparin-liberating enzyme (see the text) the fractions were combined in three groups as indicated by the horizontal bars.

1975a), as the incubated amount of 1000g fraction contained less heparin than that of 20000g fraction.

The particles in the 20000g fraction were characterized further by gradient centrifugation in colloidal silica. A single distinct visible band was obtained, corresponding to a density of 1.01–1.05 g/ml (Fig. 4). This band contained all the β -glucuronidase and ^{35}S recovered in the 20000g fraction. Further, all the heparin-liberating enzyme of the 20000g fraction was associated with the banded particles (i.e. the middle group of Fig. 4), as shown by incubation with macromolecular [^{35}S]heparin and subsequent gel chromatography.

Discussion

The present investigation was initiated to gain a more detailed insight, at the molecular and subcellular level, into the mechanisms involved in the intracellular metabolism of heparin. This process was studied by pulse-labelling *in vivo* of polysaccharide in mouse mastocytoma; the glycosaminoglycan produced by this tissue is 80–90% heparin (Ögren & Lindahl, 1971; Helting *et al.*, 1972). A rapid and seemingly transient labelling of the mastocytoma pool of inorganic sulphate occurred after a single injection of carrier-free [^{35}S]sulphate into tumour-bearing mice. However, significant amounts of inorganic [^{35}S]sulphate apparently remained (or were regenerated) in the tissues for several hours, as the incorporation of label into mastocytoma glycosaminoglycans continued beyond the pulse period; hence the distribution of labelled polysaccharide between subcellular fractions (Fig. 1) would not obey strict pulse-chase kinetics. Interpretation of these results is further complicated by the metabolic heterogeneity of the cell population in the tumour tissue. Previous studies by Pertoft (1970), with the same mastocytoma as was used in the present investigation, showed the presence of at least four fractions of mast cells, some of which differed markedly with regard to heparin-synthesizing capacity. The relative amounts of the various cell types in tumours at different stages of development is unknown; it is noteworthy that the tumour approximately doubles its size in 24h. From these considerations it is evident that the results obtained, bearing on the degradation and subcellular distribution of heparin, should be evaluated with some caution. However, the following conclusions seem to be justified.

Heparin is synthesized in subcellular particles, a major fraction of which may be recovered by centrifugation at 20000g. The assembly of an individual polysaccharide molecule probably occurs within a few minutes or less; studies on the cell-free biosynthesis of glycosaminoglycans suggest that

both polymer formation (Richmond *et al.*, 1973) and sulphation (DeLuca *et al.*, 1973; Höök *et al.*, 1975) are rapid processes. The product is a complex macromolecule, with a molecular weight considerably exceeding that of commercially available heparin (Ögren & Lindahl, 1971). Within the first hour after biosynthesis the macromolecular heparin is transferred to particles sedimenting at 1000g; alternatively, the particles containing the newly synthesized polysaccharide become modified, and acquire altered sedimentation properties. In association with this shift, the macromolecular heparin is degraded, ultimately to the size of commercial heparin (Ögren & Lindahl, 1975a). The degradation process appears to be essentially complete within 6h after biosynthesis (this period of time, as indicated by gel-chromatography patterns of the kind shown in Fig. 2, may, however, have been overestimated owing to the contribution of high-molecular-weight ^{35}S -labelled polysaccharide formed after the pulse period).

The particles containing heparin-liberating enzyme were characterized further by density-gradient centrifugation in colloidal silica. The low density thus recorded is similar to that observed for lysosomes in the same medium (H. Pertoft, personal communication). The similarities to lysosomes also include the presence of β -glucuronidase. However, additional work is required to establish the true nature of these particles and their functional relationship to other cell organelles, particularly the mast-cell granules serving as place of storage for the final heparin product.

The occurrence of heparin-liberating enzyme is not restricted to neoplastic mast cells, as similar enzymes have been demonstrated in small intestine and spleen from both rat and man (Horner, 1972; S. Ögren, unpublished work). Further, heparin isolated from bovine liver capsule showed the structural properties expected (i.e. a molecular weight similar to commercial heparin and lack of a carbohydrate-protein linkage region) for the single-chain product released from macromolecular heparin by heparin-liberating enzyme (Jansson *et al.*, 1975). Macromolecular heparin has been isolated not only from rodents but also from monkey and man (Horner, 1970). It therefore seems plausible that the intracellular events described in the present paper may have bearing on the metabolism of heparin in general.

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