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Glycosaminoglycans in rat mucosal mast cells

Lennart ENERBÄCK*, Svein O. KOLSET,†‡ Marion KUSCHE,† Anders HJERPE§ and Ulf LINDAHL†

**Department of Pathology II, University of Göteborg, Sahlgren Hospital, 413 45 Göteborg, Sweden, †Department of Medical and Physiological Chemistry, Swedish University of Agricultural Sciences, The Biomedical Centre, 751 23 Uppsala, Sweden, §Department of Pathology II, Karolinska Institute F-42, Huddinge University Hospital, 141 86 Huddinge, Sweden, and ‡Department of Tumor Biology, Norwegian Cancer Society, University of Tromsø, 900 1 Tromsø, Norway*

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Rats were infected with the nematode *Nippostrongylus brasiliensis*, resulting in an approx. 5-fold increase in the number of mucosal mast cells and the histamine content of the intestinal (jejunum) wall. After injection of the infected animals with inorganic [^{35}S]sulphate, a similar increase in the yield of labelled intestinal glycosaminoglycans was observed, compared with uninfected control rats. Autoradiography showed a highly selective labelling of the numerous mucosal mast cells and of the few connective-tissue mast cells in the subserosal region of the bowel. Analysis of the labelled polysaccharide from the infected animals showed that almost 60% of this material consisted of oversulphated galactosaminoglycan, whereas heparin-related polysaccharides accounted for only 13%. The galactosaminoglycan contained 4-monosulphated and 4,6-disulphated *N*-acetylgalactosamine residues in approx. 5:1 molar ratio, both being linked to D-glucuronic acid residues; the occurrence of L-iduronic acid units could not be excluded. No significant difference in structure was found between this polysaccharide and the corresponding component isolated from uninfected rats. It is concluded that the major polysaccharide produced by rat mucosal mast cells *in vivo* is an oversulphated galactosaminoglycan rather than heparin.

Tissue mast cells and basophilic leucocytes of blood share fundamental properties such as histamine-storing capacity and the occurrence of metachromatic cytoplasmic granules and membrane IgE receptors. The metachromatic staining with cationic dyes at low pH indicates the presence of sulphated glycosaminoglycans, generally assumed to be heparin; however, various findings indicate that the two cell types may contain different polysaccharides. Although cells and tissues such as peritoneal mast cells (Yurt *et al.*, 1977), rat skin (Horner, 1971), human (Thunberg *et al.*, 1980; Metcalfe *et al.*, 1980*a*) and mouse (Ögren & Lindahl, 1971) mastocytoma, bovine liver capsule (Jansson *et al.*, 1975) and human lung mast cells (Metcalfe *et al.*, 1979) all contain heparin, the polysaccharide in normal guinea-pig basophilic leucocytes (Orenstein *et al.*, 1978) and in rat (Metcalfe *et al.*, 1980*b*) and human (Olsson *et al.*, 1970) leukaemic basophils was identified as predominantly dermatan sulphate and/or chondroitin sulphate. Moreover, tissue mast cells display heterogeneity with respect to polysaccharide content. A specific type of mast cell in the intestinal mucosa of rodents differs from the classical connective-tissue mast cell with regard to morphology, dye binding, amine content and functional properties (Enerbäck, 1981). The dye-binding properties of the mucosal mast cells suggested that they contain a polysaccharide with a lower degree of sulphation than the heparin of the connective-tissue mast cell (Enerbäck, 1966*a*; Tas & Berndsen, 1977; Wingren & Enerbäck, 1983). Heterogeneity within the mast-cell system is thus becoming increasingly evident (Bienenstock *et al.*, 1983) and may have important functional implications.

In the present study we have attempted to identify the glycosaminoglycan produced by the mucosal mast cell. For this purpose we took advantage of the fact that these cells proliferate in the response to infections with the nematode *Nippostrongylus brasiliensis* (Taliaferro & Sarles,

1939), resulting in a dramatic parallel increase in the number of mucosal mast cells and the histamine content of the tissue. The mast-cell proliferation occurs within a well-defined time interval, allowing the labelling of newly formed mast-cell polysaccharide with [^{35}S]sulphate.

Experimental

Materials

Hyaluronic acid from rooster combs was given by Dr. T. C. Laurent, University of Uppsala, Uppsala, Sweden, and chondroitin sulphate from bovine nasal septa was from Dr. Å. Wasteson, University of Uppsala, Uppsala, Sweden. Heparin (stage 14) from pig intestinal mucosa was purchased from Inolex Pharmaceutical Division, Park Forest South, IL, U.S.A., and purified as described by Lindahl *et al.* (1965). Mono- and di-*O*-sulphated hexuronosyl-2,5-anhydro[1-^{3}H]mannitol disaccharides were prepared from heparin and separated as described by Thunberg *et al.* (1982). The unsaturated disaccharides, ΔDi-4S and ΔDi-6S, isolated from chondroitin sulphate after digestion with chondroitinase were purchased from Seikagaku Fine Chemicals, Tokyo, Japan, whereas the disulphated disaccharide, ΔDi-di-4,6S, was prepared from a squid cartilage polysaccharide (Hjerpe *et al.*, 1982).

$Na_2{}^{35}SO_4$ (sp. radioactivity 800Ci/mol) was obtained from New England Nuclear, Dreieich, West Germany. Pronase, bovine serum albumin, histamine dihydrochloride and *o*-phthalaldehyde were from Sigma Chemical Co., St. Louis, MO, U.S.A. Toluidine Blue (CI no. 52040) was purchased from Merck, Darmstadt, West Germany, and berberine sulphate (CI no. 75160) from Fluka A.G., Buchs, Switzerland. Nuclear research emulsion (type L4) was obained from Ilford Ltd. (Ilford, Essex, U.K.), and D 19B developer and F-34 X-ray fixer were purchased from Eastman–Kodak (Rochester, NY, U.S.A.). Pentanesulphonic acid was obtained from Waters Associates, Milford, MA, U.S.A. Sephadex G-50 gel was from Pharmacia Fine Chemicals, Uppsala, Sweden, and Whatman DEAE-cellulose (DE-52) from Whatman Biochemicals, Maidstone, Kent, U.K. Chondroitinase ABC (chondroitin ABC lyase, EC 4.2.2.4) and chondroitinase AC (chondroitin AC lyase, EC 4.2.2.5) were purchased from Seikagaku Fine Chemicals, and chondro-4-sulphatase (EC 3.1.6.9) and chondro-6-sulphatase (EC 3.1.6.10) were obtained from Sigma Chemical Co.

Animals and experimental design

Male Sprague–Dawley rats were obtained from Anticimex AB, Stockholm, Sweden, and were 8–9 weeks old at the start of the experiment. They were infected with *Nippostrongylus brasiliensis* (Nawa & Miller, 1978) by subcutaneous injection, in the lateral back region, of 6000 stage-3 larvae (L_3). Age-matched uninfected control rats received sham injections with 0.9% NaCl, but were otherwise treated identically. During a 4-day period, 10–13 days after infection, when mucosal mast cells of nematode-infected rats proliferate and increase greatly in number (Miller & Jarret, 1971; Wingren *et al.*, 1983) the rats were injected with $Na_2{}^{35}SO_4$ (750μCi) intraperitoneally four times daily. After another 10 days (day 23 after infection) they were killed by decapitation, and tissue samples were taken for analysis.

Demonstration and quantification of mucosal mast cells

Small tissue samples from the mid-portion of the duodenum and jejunum were fixed in an isoosmotic formaldehyde/acetic acid mixture, embedded in paraffin wax, sectioned and stained with 0.5% Toluidine Blue at pH0.5 (Enerbäck, 1966*a*,*b*). Mucosal mast cells were counted per unit length of mucosa (Wingren *et al.*, 1983). Staining with Alcian Blue in the presence of $MgCl_2$ was performed as described by Scott & Dorling (1965), and staining with berberine was performed as described by Wingren & Enerbäck (1983).

For tissue autoradiography, paraffin sections (4μm thick) were coated with a fine-grain nuclear track emulsion by using the dipping technique and were then kept in darkness in a refrigerator for 1–3 weeks. The autoradiograms were developed for 3–4min and fixed for 10min, followed by washing in tap water. The coated sections were post-stained with 0.5% Toluidine Blue at pH4 (0.1M McIlvaine buffer) for 30s and mounted under cover slips in glycerol.

Isolation of mucosal [^{35}S]glycosaminoglycan

Whole small intestine carefully freed from mesenterial connective tissue and fat was used for the preparation. Samples (6–8g) of rat intestine were digested with 100mg of Pronase in 20ml of 0.1M-Tris/HCl, pH8.0, containing 2mM-$CaCl_2$, for 20h at 55°C. To the digests were added 4M-NaOH (final concn. 0.5M) and 20mg of $NaBH_4$, and the mixtures were kept at 4°C for 24h. After neutralization the products were filtered and separated by ion-exchange chromatography, as described in the legend to Fig. 2.

Analytical methods

Hexuronic acid was determined by the carbazole method of Bitter & Muir (1962), and radioactivity was determined in a Packard model 2450 liquid-

scintillation spectrometer. For determination of histamine contents, samples (75–150mg) from the mid-portion of the duodenum and jejunum were homogenized in 0.4M-$HClO_4$, followed by neutralization of the extract and precipitation of $KClO_4$. Histamine was purified by ion-pair chromatography in a reverse-phase h.p.l.c. system, on a C18 column, with 15mM-citrate buffer, pH3, containing 5% methanol as the mobile phase and 5mM-pentanesulphonic acid as counter-ion. Histamine was detected and assayed with a two-step post-column derivative-formation procedure, with *o*-phthalaldehyde as a fluorescent reagent (Allenmark *et al.*, 1985).

^{35}S-labelled glycosaminoglycans were identified by their susceptibility to selective chemical or enzymic depolymerization. Heparin-related polysaccharides (heparin or heparan sulphate) were degraded by deaminative cleavage with HNO_2 at pH1.5 (Shively & Conrad, 1976), whereas galactosaminoglycans were depolymerized by digestion with the bacterial eliminases, chondroitinase AC or ABC (Kolset *et al.*, 1983). Formation of degradation products was detected by gel chromatography on Sephadex G-50, as described by Kolset *et al.* (1983). Disaccharides obtained by eliminase digestion were separated further by high-voltage paper electrophoresis at pH1.7 (Kolset *et al.*, 1983), or by a slight modification of the h.p.l.c. technique described by Hjerpe *et al.* (1982). To obtain optimal resolution of the monosulphated disaccharides ΔDi-4S and ΔDi-6S, the column was eluted with 20mM-Na_2SO_4 in 10mM-sodium acetate, pH5.0; under these conditions disulphated disaccharides were retained by the resin. Separation of monosulphated (unresolved) and disulphated disaccharides was achieved in a separate run, elution being performed with 50mM-Na_2SO_4 in 10mM-sodium acetate, pH5.0. Samples of ^{35}S-labelled disaccharides were mixed with unlabelled internal reference standards, the elution positions of which were determined by continuously recording the A_{231} of the column effluents. Digestions of disaccharides with chondro-4-sulphatase and chondro-6-sulphatase were performed essentially as described by Kolset *et al.* (1983), except that the incubation time was extended to 16h.

Results

The nematode-infected rats killed 23 days after the infection appeared healthy and did not exhibit any gross or microscopic changes of the bowel. However, there was a pronounced (approx. 5-fold) increase in mucosal mast cells, and a parallel increase in the histamine content of the gut, as compared with control tissue (Table 1). The mucosal mast cells were found mainly in the lamina propria of the mucosa and, contrary to the findings during the early phase of the infection, rarely in the epithelium or the muscular coat. Very few connective-tissue mast cells (< 1% of the total number of mast cells) were found in the serosal or subserosal layers. Unlike the mucosal mast cells, the connective-tissue mast cells showed strong fluorescence after staining of the tissue with berberine. These results are in agreement with previous findings in this strain of rats, showing a peak increase of mucosal mast cells and of histamine content at days 12–14 after the infection, with concomitant elimination of worms from the bowel (Wingren *et al.*, 1983).

The granules of the mucosal mast cells, like those of the few connective-tissue mast cells in the subserosal region, stained strongly with Toluidine Blue at pH0.5 and with Alcian Blue in 0.8M-$MgCl_2$, while all other structures remained unstained (Fig. 1*a*). Autoradiography initiated 10 days after injection of the infected rats with inorganic [^{35}S]sulphate showed an accumulation of

Table 1. *Mucosal mast cells, histamine contents and ^{35}S-labelled glycosaminoglycans in the jejunum of nematode-infected rats*
Four rats infected with *N. brasiliensis* and four uninfected control rats were injected with inorganic [^{35}S]sulphate as described in the Experimental section. Where appropriate, values are given as means ± S.E.M. The values for histamine and [^{35}S]glycosaminoglycan contents refer to wet weight of tissue. The composition of the glycosaminoglycan preparations is expressed as percentage of total radioactivity; the values given represent averages for two to four preparations, as indicated in parentheses.

Animals	No. of mast cells/unit length of mucosa	Histamine (μg/g of tissue)	[^{35}S]Glycosaminoglycans			
			10^{-3} × Total (c.p.m./g of tissue)	Heparin/heparan sulphate (%)	Galactosamino-glycans (%)	Unidentified (%)
Infected	651 ± 10	20.4 ± 2.0	599 ± 89	13 (n = 3)	57 (n = 4)	30
Controls	138 ± 16	4.5 ± 1.5	117 ± 12	28 (n = 2)	53 (n = 2)	19
Infected / Controls	4.7	4.5	5.1			

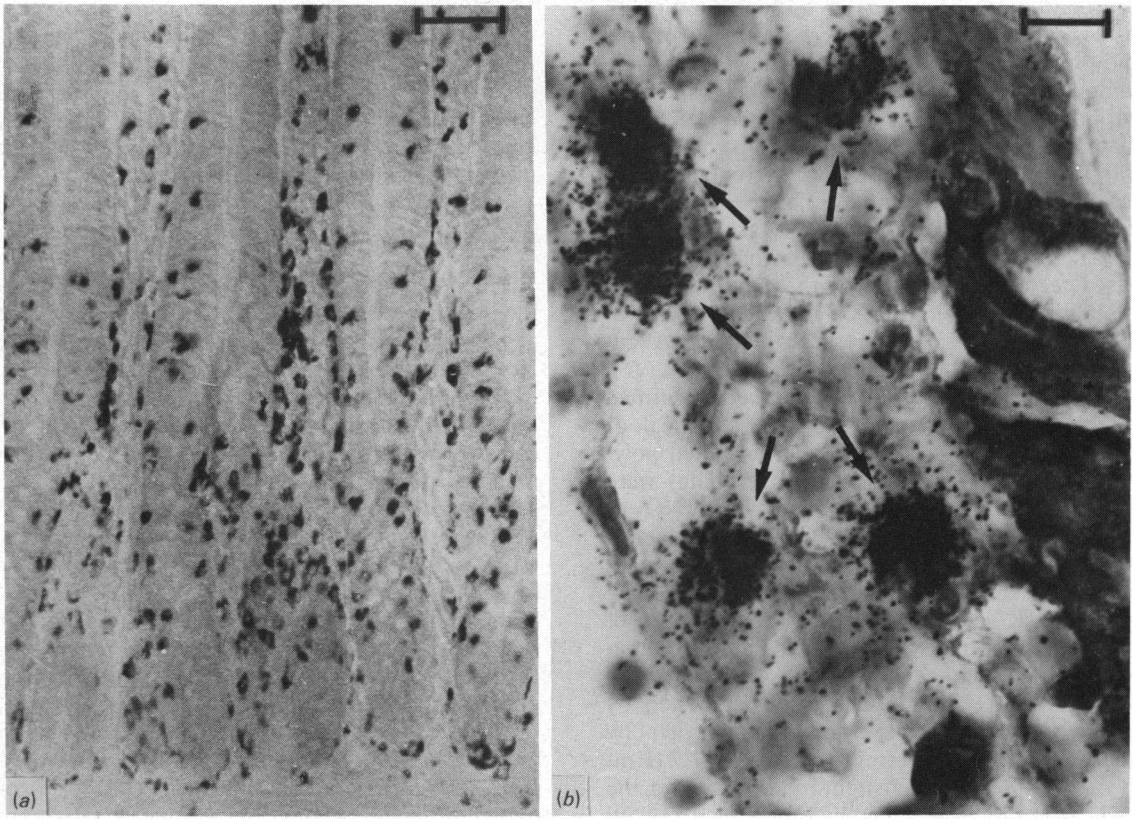

Fig. 1. *Photomicrographs of mucosal mast cells of rats infected with N. brasiliensis*
(*a*) Section stained with 0.5% Toluidine Blue at pH 0.5 showing selective staining of numerous mucosal mast cells and unstained surrounding epithelium and stroma. The bar represents 150 μm. (*b*) Autoradiogram showing accumulation of silver grains above metachromatically stained mast cells (arrows) of a rat injected with inorganic [^{35}S]sulphate. Exposure time was 14 days and the section was post-stained with 0.5% Toluidine Blue at pH 4. The bar represents 15 μm.

silver grains above the mast cells (Fig. 1*b*). The background grain density was low, and unselective with regard to other tissue structures.

The biosynthesis of glycosaminoglycans in the intestinal wall was studied by isolating polysaccharides labelled *in vivo* with inorganic [^{35}S]sulphate. Intestinal tissue was subjected to proteolytic digestion, followed by alkali treatment, and labelled polysaccharide was isolated by ion-exchange chromatography on DEAE-cellulose. One of the resulting separations is illustrated in Fig. 2, which shows a broad and apparently heterogeneous peak of labelled material, which partly overlaps those of the chondroitin sulphate and heparin internal standards. It is noted that a major portion of the material emerged at a retarded elution position in relation to chondroitin sulphate, and should thus contain on average more than one sulphate group per disaccharide unit. Essentially similar patterns were reproduced with four different preparations from intestinal tissue of infected rats, and did not differ significantly from the corresponding chromatograms pertaining to uninfected control animals. However, the total yield of labelled polysaccharide from the infected animals exceeded that of the controls by a factor of 5-fold, and thus closely matched the increase in histamine contents and mast-cell number induced by the infection (Table 1).

The composition of the labelled polysaccharide preparations was estimated by selective degradation with HNO_2, which cleaves *N*-sulphated polysaccharides (heparin or heparan sulphate), or with bacterial eliminases known to depolymerize galactosaminoglycans. Less than one-third of the [^{35}S]glycosaminoglycan obtained from control animals consisted of heparin (or heparan sulphate), as indicated by the modest effect of HNO_2 (Table 1), and this value was even lower (13%) for the material from infected animals. In contrast, between 50 and 60% of both types of preparations were consistently degraded by chondroitinase

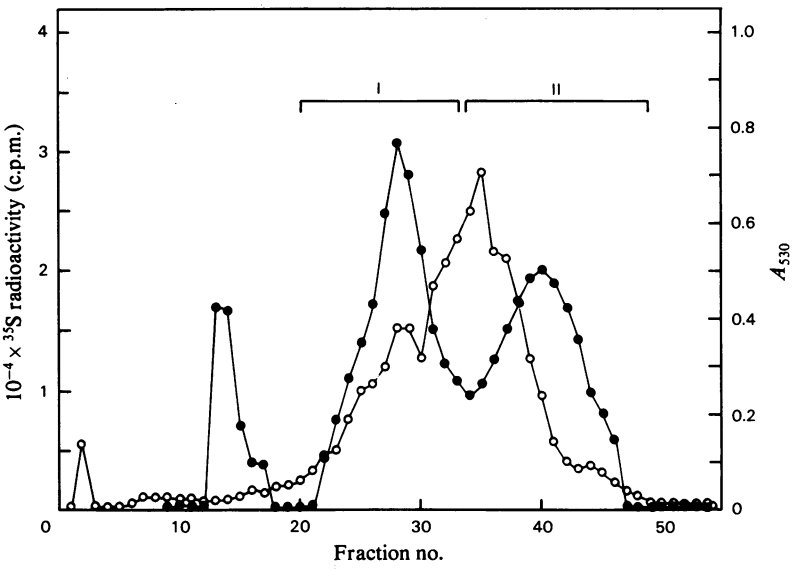

Fig. 2. *Ion-exchange chromatography on DEAE-cellulose of ^{35}S-labelled glycosaminoglycans from rat small intestine*
A sample of small intestine was prepared from a rat injected with inorganic [^{35}S]sulphate after infection with *N. brasiliensis* (see the Experimental section). After proteolytic digestion and alkali treatment the digest was mixed with 0.25mg of hyaluronic acid, 1mg of chondroitin 4-sulphate and 2mg of heparin, and applied to a column (1cm × 6cm) of DEAE-cellulose (Whatman DE-52). After extensive washing of the column with 0.05M-LiCl in 0.05M-sodium acetate buffer, pH4.0, elution was performed (starting at fraction no. 1) with a 160ml gradient of 0.05–1.5M-LiCl in acetate buffer. Fractions (approx. 3ml) were collected and analysed for ^{35}S radioactivity (○) and for hexuronic acid (carbazole reaction) (●). Fractions I and II, indicated by the brackets, were pooled, dialysed against water and analysed further as described in the text.

ABC, thus indicating that the polysaccharides consisted mainly of galactosaminoglycan. Attempts at further identification showed that only one-quarter to one-third of the galactosaminoglycans was susceptible to digestion with chondroitinase AC, which specifically degrades chondroitin sulphates (containing D-glucuronic acid units), but not dermatan sulphate (containing L-iduronic acid units). However, these results alone cannot be taken as evidence for the occurrence of dermatan sulphate, since galactosaminoglycan preparations may resist chondroitinase AC digestion in spite of a high glucuronic acid content (Yanagashita *et al.*, 1979). The actual content of chondroitin sulphate may thus be higher than indicated by these experiments.

The retarded elution position on ion-exchange chromatography (Fig. 2) suggested the occurrence of 'oversulphated' galactosaminoglycan containing more than one sulphate residue per disaccharide unit. Effluent fractions were combined as indicated in Fig. 2, and the two pools (I and II) were digested with chondroitinase ABC. Analysis of the digestion products by paper electrophoresis showed large amounts of disulphated disaccharides (Fig. 3). Calculations based on the ratios of monosulphated/disulphated disaccharides indicated that the latter component accounted for 15% and 45% of the chondroitinase-susceptible labelled material in fractions I and II respectively. The disulphated disaccharide units were consistently found in all polysaccharide preparations investigated, with no significant difference between material obtained from infected and control animals. However, the material shown in Fig. 3 was selected for its particularly high sulphate content; in general, the disulphated disaccharide unit accounted for 15–20% of the unfractionated galactosaminoglycan preparations. Digestion with chondroitinase AC or chondroitinase ABC yield about the same proportion of disulphated disaccharide.

The oversulphated galactosaminoglycan was characterized further by h.p.l.c. of the disaccharides obtained by digestion with chondroitinase ABC. The monosulphated disaccharide was thus directly identified as ΔDi-4S, only trace amounts of the 6-sulphated isomer being detected (Fig. 4*a*). Fig. 4(*b*) shows the same sample, fractionated under conditions which lead to separation between the mono- and di-sulphated disaccharides (but not to separation of the two monosulphated components). After exhaustive digestion with chondro-4-sulphatase and chondro-6-sulphatase the two disaccharide peaks were quantitatively eliminated and replaced by a single peak at the elution

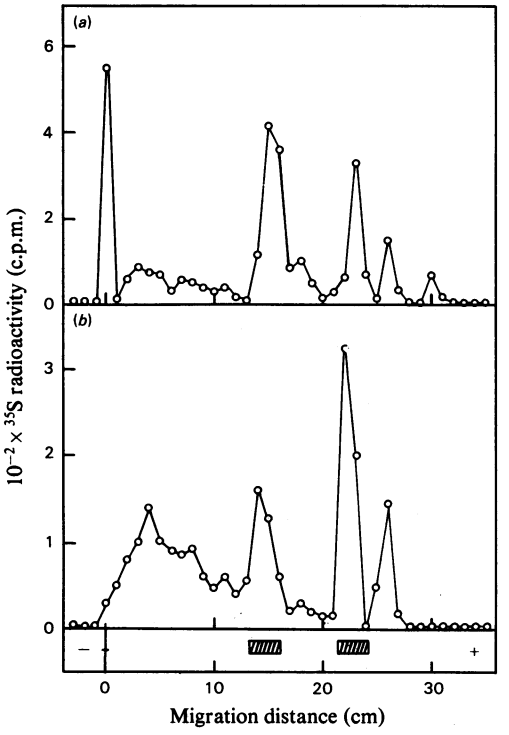

Fig. 3. *Paper electrophoresis of disaccharides formed by digestion of* [^{35}S]*galactosaminoglycans with chondroitinase ABC*

Samples (approx. 10^5 c.p.m.) of fractions I(*a*) and II(*b*) obtained by ion-exchange chromatography were digested with 0.05 unit (μmol/min) of chondroitinase ABC in 50μl of 0.05M-Tris/HCl, pH 8.0, containing 0.05M-sodium acetate and 50μg of bovine serum albumin/ml. After 15h at 37°C, 5μl of the incubation mixture was applied to Whatman 3MM paper and subjected to high-voltage electrophoresis in 1.6M-formic acid, pH 1.7, at 40V/cm for 80min. The standards shown below the tracings are: (I) monosulphated and (II) disulphated hexuronosyl-2,5-anhydro[1-^{3}H]mannitol disaccharides. The material migrating slower than the monosulphated standard should represent glycosaminoglycans resistant to chondroitinase, i.e. heparin-related components and some unidentified material (Table 1).

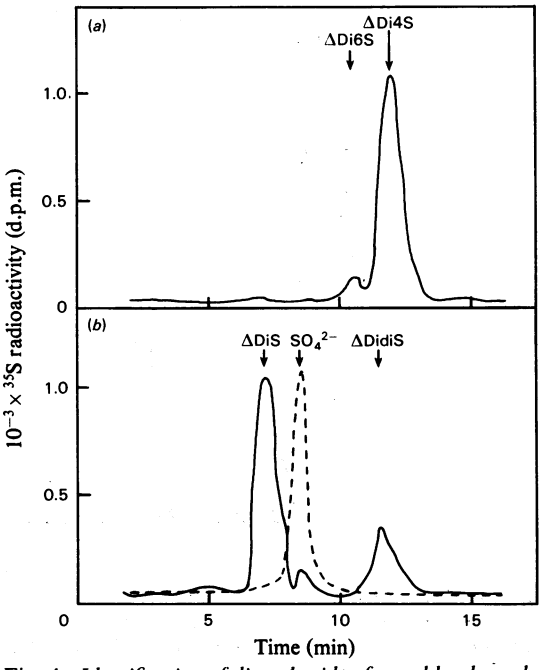

Fig. 4. *Identification of disaccharides formed by degradation of* [^{35}S]*galactosaminonglycan with chondroitinase ABC*

^{35}S-labelled intestinal polysaccharide (unfractionated), isolated from an infected rat, was digested with chondroitinase ABC as described in the legend to Fig. 3. The resulting disaccharides were isolated by gel chromatography on Sephadex G-50 in 1M-NaCl, and were then desalted by passage through Sephadex G-15, equilibrated with 0.2M-NH_4HCO_3, followed by freeze-drying. Samples (approx. 10^5 c.p.m.) were mixed with disaccharide internal standards (ΔDi-4S, ΔDi-6S, ΔDi-di-4,6S) and separated by h.p.l.c. on a column (250mm × 4.6mm) of Hypersil APS, eluted with 20mM-Na_2SO_4 in 10mM-acetate buffer, pH 5.0, to separate monosulphated disaccharides (*a*), or with 50mM-Na_2SO_4 in 10mM-acetate buffer to separate monosulphated from disulphated disaccharides (*b*). The dashed line refers to a sample incubated with chondro-4-sulphatase and chondro-6-sulphatase (see the Experimental section) before chromatography. The elution positions of the disaccharide internal standards (detected by their A_{231}) are indicated by the arrows.

position of inorganic [^{35}S]sulphate. The complete desulphation of the disulphated disaccharide by these enzymes, without any accumulation of enzyme-resistant monosulphated disaccharide, strongly indicates that the two sulphate groups were located at C-4 and C-6 of the *N*-acetylgalactosamine residue.

Discussion

The identification of mucosal mast cells as a specific subpopulation, differing from the classical mature connective-tissue mast cell, rests on a number of criteria, as has been previously discussed (Enerbäck, 1981). The latter occur in the connective tissues of many sites, including the outer part of the gut wall. Mucosal mast cells are found in the lamina propria of the intestinal mucosa and have been studied mostly in rodents. They are smaller than connective-tissue mast cells and contain fewer, often very large, granules of more variable shape (Enerbäck & Lundin, 1974; Enerbäck, 1966*a*). Their cell surface is devoid of microvilli, and the nuclei show irregular indentations (Enerbäck & Lundin, 1974). Mucosal mast

cells contain less histamine per cell (Enerbäck & Wingren, 1980; Befus *et al.*, 1982) and have been shown to contain a distinct chymotrypsin-like serine proteinase (Woodbury *et al.*, 1978; Woodbury & Miller, 1982), designated as rat mast-cell protease II, which is different from the chymotrypsin-like enzyme of connective-tissue mast cells. Mucosal mast cells, unlike the connective-tissue mast cells, are not stimulated to secretion by polyamines such as Compound 48/80 and polymyxin, but show instead an inverse proliferative response (Enerbäck & Lundin, 1974; Enerbäck & Löwhagen, (1979).

The most distinctive feature of mucosal mast cells is the dye-binding properties of their granules, which suggest the occurrence of a glycosaminoglycan that is less sulphated than heparin. This suggestion is supported by the following histochemical evidence. Firstly, staining with Alcian Blue and $MgCl_2$ (Scott & Dorling, 1965) shows a lower critical electrolyte concentration for granules of mucosal mast cells than for those of connective-tissue mast cells, suggesting the presence of a less-sulphated glycosaminoglycan in the former cells (Miller & Walshaw, 1972). A second piece of evidence comes from spectroscopic studies of glycosaminoglycan–Toluidine Blue complexes *in situ*, showing absence of typical heparin spectra for mucosal mast cells, but instead spectra suggesting the presence of a less-sulphated polysaccharide (Tas & Berndsen, 1977). A final piece of evidence derives from recent studies with the weakly fluorescent dye berberine. This dye forms a strongly fluorescent complex with heparin in mast cells and has been used for the quantification of mast-cell heparin by cytofluorimetry (Enerbäck, 1974; Enerbäck *et al.*, 1976). Unlike connective-tissue mast cells of the rat, mucosal mast cells do not exhibit fluorescent berberine binding (Wingren & Enerbäck, 1983). Taken together, these findings strongly suggest that the granules of mucosal mast cells contain not heparin but a glycosaminoglycan of lower sulphate content.

In the present investigation we attempted to identify the glycosaminoglycan produced by mucosal mast cells, by using labelling of the polysaccharide with [^{35}S]sulphate *in vivo*. Being aware of the inherent difficulties in ascribing a product of biosynthesis *in vivo* to a particular cell type, we took advantage of the selective proliferation of mucosal mast cells that occurs after infecting rats with the nematode *Nippostrongylus brasiliensis*. The closely similar increment in number of mucosal mast cells, histamine content of the tissue and formation of ^{35}S-labelled polysaccharide (Table 1) provides strong circumstantial evidence that the latter components were indeed derived from the mucosal mast cells. Moreover, their granules, increasing strongly in quantity during the nematode infection, and those of the very few connective-tissue mast cells, were the only structures showing the typical dye-binding properties of sulphated glycosaminoglycans. Most conclusively, autoradiography showed a highly selective ^{35}S-labelling of the abundant mucosal and the few connective-tissue mast cells.

The major labelled polysaccharide product isolated from the gut tissue was identified as an oversulphated galactosaminoglycan containing 4,6-disulphated *N*-acetylgalactosamine units. On the average, one out of five disaccharide units was disulphated; however, the polysaccharide preparation was heterogeneous and contained fractions in which almost half of the disaccharide residues carried two sulphate groups. Although the structure of the polysaccharide backbone was not established with certainty, part of the disulphated disaccharide units apparently contained glucuronic acid units; the galactosaminoglycan may therefore with some justification be referred to as an oversulphated chondroitin sulphate (sometimes denoted chondroitin sulphate E). In addition to this polysaccharide species (and some unidentified labelled material; see Table 1), the intestinal tissue also contained a minor proportion of labelled heparan sulphate or heparin. The source of this material is unclear; unlike the galactosaminoglycan, it did not increase in parallel with the number of mucosal mast cells and histamine content in response to the nematode infection (Table 1).

Insofar as the classification of mast cells may depend on their glycosaminoglycan contents, the demonstration of an oversulphated galactosaminoglycan in mucosal mast cells places this cell type in the same category as basophilic leucocytes (Orenstein *et al.*, 1978; Metcalf *et al.*, 1980*b*), whereas it clearly distinguishes the mucosal mast cell from the heparin-containing connective-tissue mast cells. A similarly oversulphated galactosaminoglycan was synthesized by cultured murine (i.e. rat and mouse) bone-marrow-derived mast cells (Razin *et al.*, 1982). To complicate the picture further, rat serosal mast cells, which normally synthesize a heparin proteoglycan, will produce oversulphated chondroitin sulphate *in vitro* when provided with an exogenous artificial inducer of polysaccharide-chain elongation (*p*-nitrophenyl β-D-xyloside) (Stevens & Austen, 1982; Stevens *et al.*, 1983). Thus also heparin-producing mast cells may be equipped with the enzymic machinery required for the formation of oversulphated galactosaminoglycan. Interestingly, intestine of various non-murine species, such as cow and pig, contain heparin (Nader *et al.*, 1980), thus conceivably pointing to a common functional role for the two polysaccharides.

The ability to produce oversulphated galactosaminoglycan is not restricted to mast cells, but is also expressed by a limited number of other, mammalian and non-mammalian, cell types (for references see Kolset *et al.*, 1983). Human monocytes cultured *in vitro* display striking changes in morphology and in functional properties, which are accompanied by a modulation of polysaccharide biosynthesis, from the production of chondroitin 4-sulphate to the formation of a galactosaminoglycan with 15–20% 4,6-disulphated disaccharide units (Kolset *et al.*, 1983). This transition depends on contact between the cells and foreign surfaces such as plastic or glass, and is prevented when the cells are maintained on a substrate coated with fibronectin or fibrin (Kolset *et al.*, 1984). Although the resemblance between this differentiation, or activation, process and the proliferative response of mucosal mast cells to nematode infection may appear tenuous, the two phenomena share two common features: the dependence on a foreign agent and the locally increased concentration of oversulphated galactosaminoglycan. Speculative as it may be, these similarities raise the possibility that the polysaccharide is functionally committed to specific types of inflammatory reactions.

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