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Comparative study on glycosaminoglycans synthesized in peripheral and peritoneal polymorphonuclear leucocytes from guinea pigs

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Glycosaminoglycans synthesized in polymorphonuclear (PMN) leucocytes isolated from blood (peripheral PMN leucocytes) and in those induced intraperitoneally by the injection of caseinate (peritoneal PMN leucocytes) were compared. Both peripheral and peritoneal PMN leucocytes were incubated in medium containing [^{35}S]-sulphate and [^{3}H]glucosamine. Each sample obtained after incubation was separated into cell, cell-surface and medium fractions by trypsin digestion and centrifugation. The glycosaminoglycans secreted from peripheral and peritoneal PMN leucocytes were decreased in size by alkali treatment, indicating that they existed in the form of proteoglycans. Descending paper chromatography of the unsaturated disaccharides obtained by the digestion of glycosaminoglycans with chondroitinase AC and chondroitinase ABC identified the labelled glycosaminoglycans of both the cell and the medium fractions in peripheral PMN leucocytes as 55–58% chondroitin 4-sulphate, 16–19% chondroitin 6-sulphate, 16–19% dermatan sulphate and 6–8% heparan sulphate. Oversulphated chondroitin sulphate and oversulphated dermatan sulphate were found only in the medium fraction. In peritoneal PMN leucocytes there is a difference in the composition of glycosaminoglycans between the cell and the medium fractions; the cell fraction was composed of 60% chondroitin 4-sulphate, 5.5% chondrotin 6-sulphate, 16.8% dermatan sulphate and 13.9% heparan sulphate, whereas the medium fraction consisted of 24.5% chondroitin 4-sulphate, 28.2% chondroitin 6-sulphate, 33.7% dermatan sulphate and 10% heparan sulphate. Oversulphated chondroitin sulphate and oversulphated dermatan sulphate were found in the cell, cell-surface and medium fractions. On the basis of enzymic assays with chondro-4-sulphatase and chondro-6-sulphatase, the positions of sulphation in the disulphated disaccharides were identified as 4- and 6-positions of *N*-acetylgalactosamine. Most of the ^{35}S-labelled glycosaminoglycans synthesized in peripheral PMN leucocytes were retained within cells, whereas those in peritoneal PMN leucocytes were secreted into the culture medium. Moreover, the amount of glycosaminoglycans in peritoneal PMN leucocytes was significantly less than that in peripheral PMN leucocytes. Assay of lysosomal enzymes showed that these activities in peritoneal PMN leucocytes were 2-fold higher than those in peripheral PMN leucocytes.

Abbreviations used: PMN leucocytes, polymorphonuclear leucocytes; ΔDi-4S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulpho-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulpho-D-galactose; ΔDi-0S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose; ΔDi-diS$_E$, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4,6-di-*O*-sulpho-D-galactose.

Previous investigations on glycosaminoglycans in PMN leucocytes have been exclusively performed with peripheral leucocytes; Olsson & Gardell (1967) reported that chondroitin 4-sulphate was the predominant component of glycosaminoglycans in human PMN leucocytes, and Murata (1974) and Murata *et al.* (1974) reported that glycosaminoglycans in human and bovine PMN

leucocytes contained chondroitin 4-sulphate as the main constituent. Human PMN leucocytes have also been demonstrated to contain the proteochondroitin sulphate (Olsson, 1969*d*).

It is well known that glycosaminoglycans synthesized in PMN leucocytes are retained within intracellular granules (Horn & Spicer, 1964; Olsson, 1969*a*,*b*; Parmley *et al.*, 1979), which contain various hydrolytic enzymes (Olsson, 1969*c*). It is considered that one of the functions of glycosaminoglycans and proteoglycans in granules consists in the binding and storage of cationic proteins of enzymic and non-enzymic nature (Zeya & Spitznagel, 1971), but detailed roles are not yet clear.

Our previous study (Hasumi & Mori, 1980) on glycosaminoglycans was undertaken with peritoneal PMN leucocytes from guinea pigs. During that study we observed that peritoneal PMN leucocytes differed from peripheral PMN leucocytes in the biosynthesis of glycosaminoglycans. The present paper describes the difference in the composition and kinetics of synthesis between glycosaminoglycans synthesized in peripheral PMN leucocytes and those synthesized in peritoneal PMN leucocytes.

Experimental

Materials

Fischer's medium was obtained from Nissui Seiyaku, Tokyo, Japan, foetal-calf serum (lot no. 99861) from M. A. Bioproducts, Walkersville, MD, U.S.A., streptomycin sulphate from Meiji Seika, Tokyo, Japan, penicillin from Banyu Pharmaceutical Co., Tokyo, Japan, D-[6-^{3}H]glucosamine hydrochloride (22.6Ci/mmol) from The Radiochemical Centre, Amersham, Bucks., U.K., inorganic [^{35}S]sulphate (carrier-free) from Japan Atomic Energy Institute, Tokyo, Japan, and 0.25% trypsin (Ca^{2+}- and Mg^{2+}-free) from Chiba Serum Institute, Chiba, Japan. Chondroitinase AC (chondroitin AC lyase, EC 4.2.2.5), chondroitinase ABC (chondroitin ABC lyase, EC 4.2.2.4), chondro-4-sulphatase (EC 3.1.6.9), chondro-6-sulphatase (EC 3.1.6.10), ΔDi-4S, ΔDi-6S and ΔDi-0S were purchased from Seikagaku Kogyo, Tokyo, Japan. ΔDi-diS_E was prepared by the digestion of chondroitin sulphate E, which was isolated from cuttlefish (Kawai *et al.*, 1966), with chondroitinase ABC. Pronase E [10^7PU/g (1PU ≡ 1mmol of tyrosine/min)] was obtained from Kaken Kagaku, Tokyo, Japan, Sepharose 6B, Ficoll 400 and Dextran T500 were from Pharmacia Fine Chemicals, Uppsala, Sweden, Toyo filter paper (no. 51A) was from Toyo Roshi, Tokyo, Japan, Conray 400 was from Diichi Seiyaku, Tokyo, Japan, and Shodex Standard P-82 (M_r markers) was from Showa Denko, Tokyo, Japan.

Preparation of peripheral and peritoneal PMN leucocytes

Peripheral PMN leucocytes were isolated by the method of Böyum (1968). In brief, blood was withdrawn from the heart of a guinea pig into a syringe containing 1ml of 2% (w/v) $K_2EDTA,2H_2O$ in saline (0.9% NaCl). Dextran T500 (2%, w/v) was added to the blood in the syringe. The syringe was gently shaken and was kept for 30min at room temperature. The supernatant was layered on 15ml of Ficoll/Conray solution [9% (w/v) Ficoll/33.4% (w/v) Conray 400 (12:5, v/v)] and was centrifuged at 500*g* for 30min at 25°C. The cells obtained were washed with saline. Peritoneal PMN leucocytes were isolated from guinea pigs as described previously (Kakinuma, 1968; Hasumi & Mori, 1980). Male guinea pigs were injected intraperitoneally with 30ml of 1% (w/v) caseinate in sterile saline. After 16h, the guinea pigs were further injected with 50–80ml of sterile saline with the use of a large needle. The peritoneal exudate was ejected through the needle and centrifuged at 200*g* for 5min at 4°C. The supernatant was decanted and the cells were suspended in 10ml of saline. The suspension was layered on 15ml of Ficoll/Conray solution and was centrifuged as described above. The cells obtained were washed with saline. Giemsa's stain demonstrated that more than 90% of both cells were PMN leucocytes.

Incubation and labelling of leucocytes

Peripheral and peritoneal PMN leucocytes were separately suspended in Fischer's medium containing foetal-calf serum (15%, v/v), streptomycin (100μg/ml) and penicillin (100units/ml). The suspension was placed in a 35mm Falcon plastic dish (2.5ml/dish, 2.5×10^7 cells or 1.0×10^7cells/dish) and incubated under CO_2/air (1:19) at 37°C. After 24h, the medium was replaced by fresh medium and 25μCi of [^{35}S]sulphate or D-glucosamine was added to each dish. The incubation was carried out for 1–9h.

Separation of cell, medium and cell-surface fractions

The incubation medium (medium fraction) was removed from each dish and the cells on the dish were treated with 0.25% trypsin (Ca^{2+}- and Mg^{2+}-free) in phosphate-buffered saline (0.137M-NaCl/0.01M-sodium/potassium phosphate buffer, pH7.4) for 10min at 37°C. The supernatant (cell-surface fraction) and the residue (cell fraction) were separated by centrifugation.

Extraction of glycosaminoglycans and enzymic digestion

Extraction of glycosaminoglycans from each fraction, chondroitinase AC and chondroitinase ABC digestion of glycosaminoglycans and chondro-4-sulphatase and chondro-6-sulphatase digestion of unsaturated disaccharides were performed as described previously (Takasu *et al.*, 1982).

Gel chromatography

Gel chromatography was carried out on a Sepharose 6B column (1.67cm × 62.5cm) equilibrated with 50mM-Tris/HCl buffer, pH7.6, containing 0.15M-NaCl. Fractions of volume 1.7ml were collected.

Descending paper chromatography

The unsaturated disaccharides obtained by digestion with chondroitinase AC and chondroitinase ABC were spotted on to chromatography paper (20cm × 40cm). After a desalting in butan-1-ol/ethanol/water (13:8:4, by vol.) for 48h, descending paper chromatography was carried out in butan-1-ol/acetic acid/1M-ammonia (2:3:1, by vol.) for 24h at room temperature (Schuchman & Desnick, 1981). The resulting paper was cut into 1cm bands and the segments were extracted with water.

Enzyme assay

After peripheral PMN leucocytes (1.0×10^7 cells/dish) or peritoneal PMN leucocytes (1.0×10^7 cell/dish) had been incubated for 24h, each sample was separated into the cell and the medium fractions by centrifugation (2000*g* for 5min). The activities of the lysosomal enzymes acid phosphatase (EC 3.1.3.2), *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30), β-D-glucuronidase (EC 3.2.1.31) and cathepsin D (EC 3.4.23.5) were measured by the modified method described previously (Avila & Convit, 1973). In brief, the activities of acid phosphatase, *N*-acetyl-β-D-glucosaminidase and β-D-glucuronidase were determined in 50mM-sodium acetate buffer, pH4.0, with *p*-nitrophenyl phosphate (disodium salt) (Wako Pure Chemical Industries, Osaka, Japan), *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide (Seikagaku Kogyo, Tokyo, Japan) and *p*-nitrophenyl β-D-glucuronide (Sigma, St. Louis, MO, U.S.A.) as substrates respectively. Cathepsin D activity was assayed at pH3.6 with 0.37mM denatured haemoglobin (Miles Laboratories, Elkhart, IN, U.S.A.) as substrate in 0.1M-sodium acetate buffer.

Assay for radioactivity and uronic acid

The radioactivity was measured with an Aloka liquid-scintillation counter, with 5ml of Bray's solution containing 0.5ml of the sample. After the glycosaminoglycans in peripheral PMN leucocytes (1.0×10^9 cells) and peritoneal PMN leucocytes (1.0×10^9 cells) had been extracted as described above, the glycosaminoglycan content was determined as uronic acid content measured by the modified carbazole reaction (Bitter & Muir, 1962).

Results

Biochemical characterization of labelled glycosaminoglycans synthesized in peripheral and peritoneal PMN leucocytes

After both peripheral and peritoneal PMN leucocytes had been incubated with [^{35}S]sulphate, the medium fractions obtained were dialysed. The dialysed medium fraction was subjected to Sepharose 6B gel chromatography (Figs. 1*a* and 1*d*). ^{35}S-labelled glycosaminoglycan-containing materials were eluted as a single peak near the void volume. After material in the main peak had been treated with alkali, the material was rechromatographed (Figs. 1*b* and 1*e*). The elution profiles were different from those before alkali treatment, the alkali-treated material being retarded. Approx. 90% of the alkali-treated material was degraded by chondroitinase ABC (Figs. 1*c* and 1*f*). This indicated that the secreted glycosaminoglycans existed in the form of proteoglycan.

Glycosaminoglycans synthesized in both preparations of PMN leucocytes showed no significant difference in this respect and in M_r; in both cases glycosaminoglycans and proteoglycans exhibited average M_r values of 25000 and 300000 respectively (Fig. 1).

Composition of labelled glycosaminoglycans in peripheral and peritoneal PMN leucocytes

In order to determine the composition of the labelled glycosaminoglycans synthesized in peripheral and peritoneal PMN leucocytes, descending paper chromatography of the unsaturated disaccharides obtained by the digestion of the glycosaminoglycans with chondroitinase AC and chondroitinase ABC was carried out as illustrated in Fig. 2. It is noted that the radioactivity corresponding to the spot of ΔDi-diS_E was found in the medium fraction from peripheral PMN leucocytes and in the cell, cell-surface and medium fractions from peritoneal PMN leucocytes. The cell fraction from peripheral PMN leucocytes, however, contained no radioactivity corresponding to the spot of ΔDi-diS_E. After the spot corresponding to ΔDi-diS_E had been extracted with water, the extract was digested with chondro-6-sulphatase and chondro-4-sulphatase, in that sequence (Table 1). The radioactivity of the extract was transferred to the spot of ΔDi-4S as a result of the digestion with chondro-6-sulphatase (first treatment). When the spot of ΔDi-

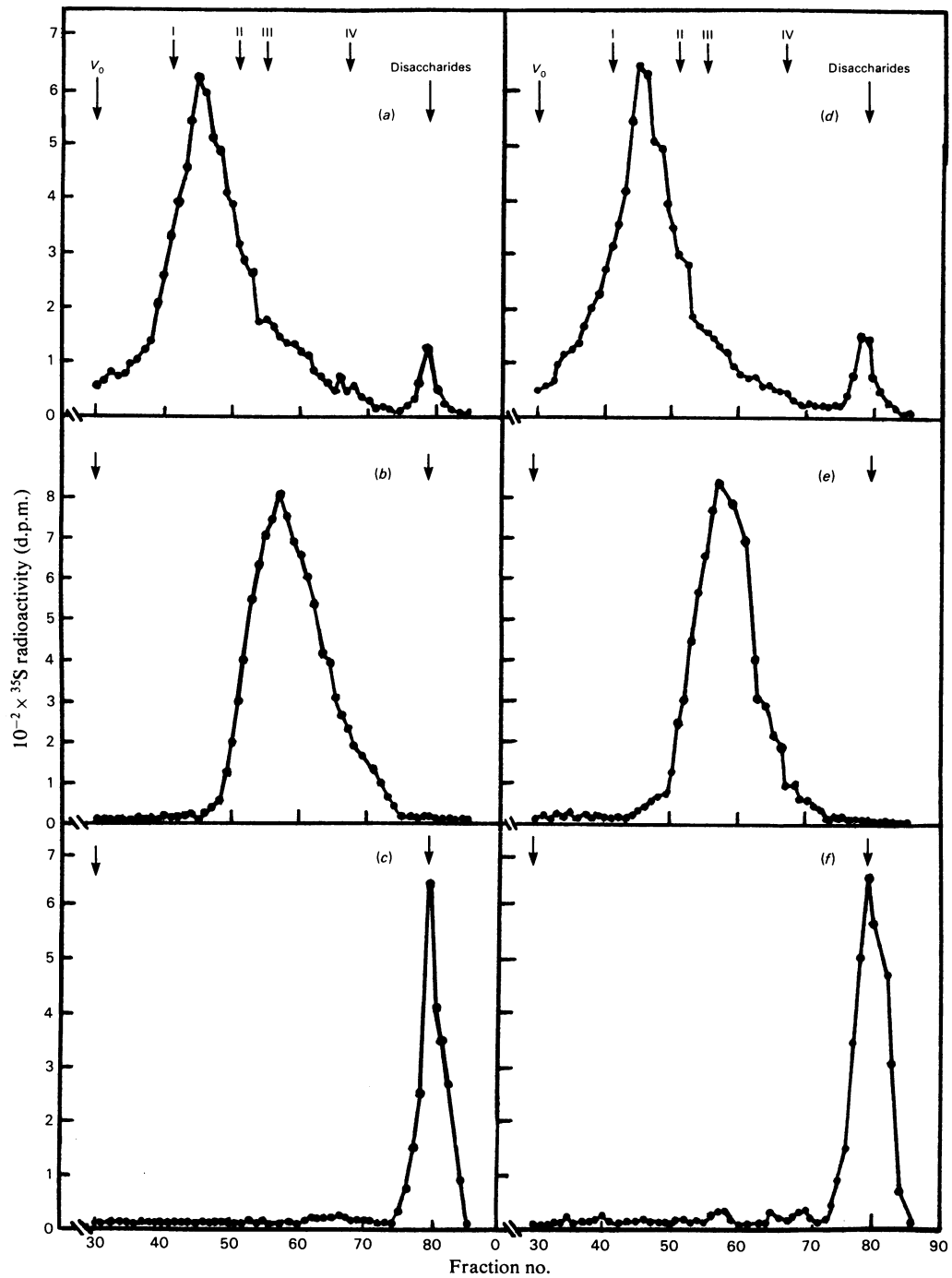

Fig. 1. *Elution profiles on Sepharose 6B of* ^{35}S*-labelled proteoglycans from peripheral and peritoneal PMN leucocytes*
The ^{35}S-labelled proteoglycans released into medium by peripheral and by peritoneal PMN leucocytes were applied to a column (1.67cm × 62.5cm) of Sepharose 6B and eluted with 50mM-Tris/HCl buffer, pH 7.4, containing 0.15M-NaCl (*a*, peripheral-PMN-leucocyte glycosaminoglycans; *d*, peritoneal-PMN-leucocyte glycosaminoglycans). Void-volume (V_0) material from both (*a*) and (*d*) was treated with 0.5M-NaOH for 30h at 4°C and rechromatographed (*b* from *a*; *e* from *d*). The material in the main peaks of (*b*) and (*e*) was digested with chondroitinase ABC in 50mM-Tris/HCl buffer, pH 7.4, and chromatography was carried out under the same condition (*c* from *b*; *f* from *e*). M_r markers are shown by the arrows (I, 400000; II, 100000; III, 50000; IV, 10000). The recovery of the radioactivity after all gel filtrations was more than 90%.

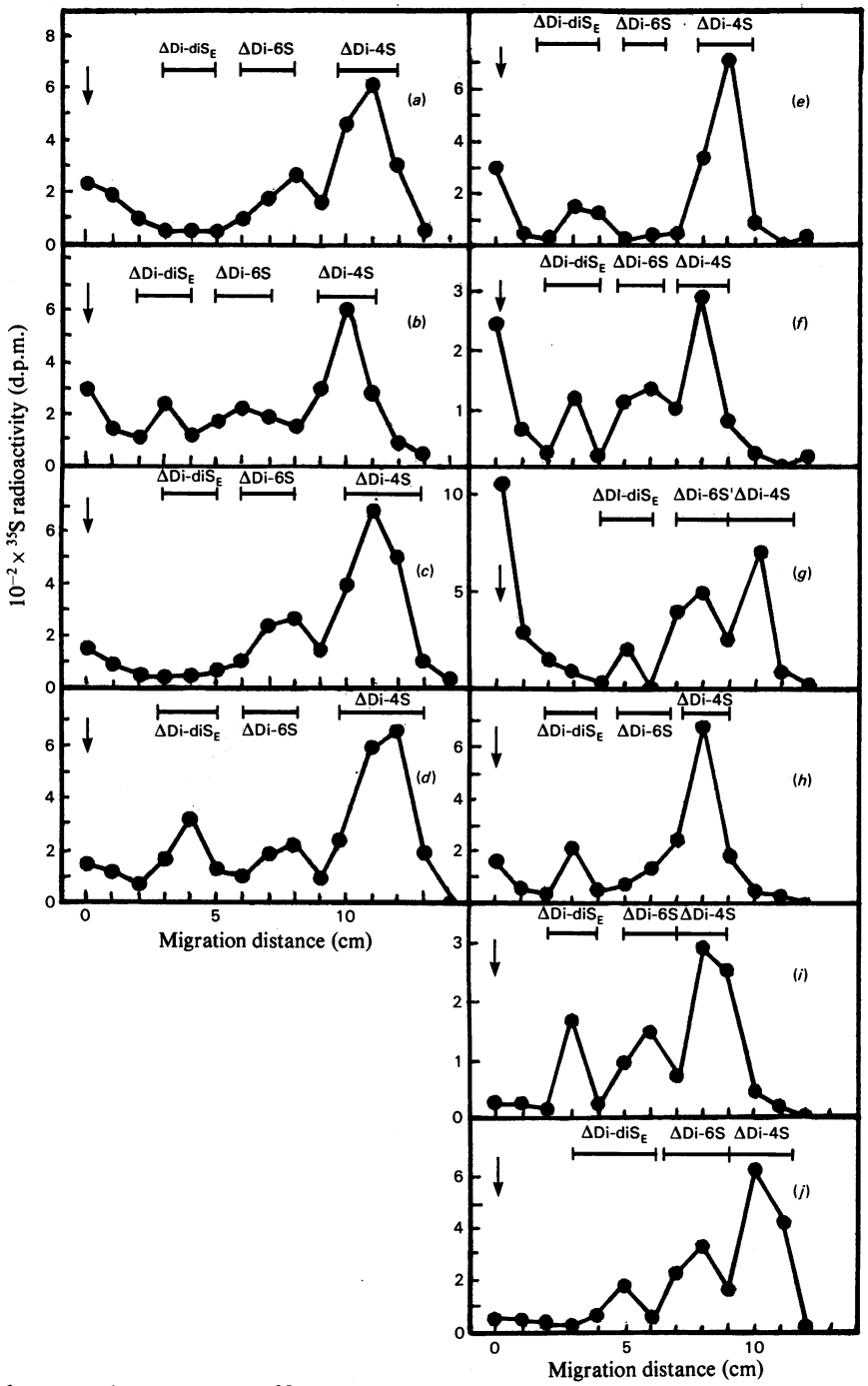

Fig. 2. *Descending paper chromatography of ^{35}S-labelled disaccharides obtained by the digestion of ^{35}S-labelled glycosaminoglycans with chondroitinase AC and chondroitinase ABC*

The ^{35}S-labelled glycosaminoglycans isolated from the cell, cell-surface and medium fractions of peripheral and peritoneal PMN leucocytes were digested with chondroitinase AC and chondroitinase ABC. The digests were applied to Toyo no. 51A filter paper. After a desalting ^{35}S-labelled unsaturated disaccharides were separated by the descending paper chromatography in butan-1-ol/acetic acid/1 M-ammonia (2:3:1, by vol.). The paper was cut into 1 cm bands and the segments were extracted with water. ^{35}S radioactivity in the extract was determined. Peripheral PMN leucocytes: chondroitinase AC digestion, (*a*) cell fraction, (*b*) medium fraction; chondroitinase ABC digestion, (*c*) cell fraction, (*d*) medium fraction. Peritoneal PMN leucocytes: chondroitinase AC digestion, (*e*) cell fraction, (*f*) cell-surface fraction, (*g*) medium fraction; chondroitinase ABC digestion, (*h*) cell fraction, (*i*) cell-surface fraction, (*j*) medium fraction.

Table 1. *Digestion of ^{3}H-labelled unsaturated disulphated disaccharides with chondro-4-sulphatase and chondro-6-sulphatase*
Peripheral PMN leucocytes (1.0×10^7 cells) and peritoneal PMN leucocytes (2.5×10^7 cells) were incubated in medium containing D-[^{3}H]glucosamine (25 μCi/dish) for 9h. The ^{3}H-labelled glycosaminoglycans isolated from peripheral and peritoneal PMN leucocytes as described in the Experimental section were digested with chondroitinase ABC and subjected to descending paper chromatography under the same conditions as indicated in the legend to Fig. 2. After the spot corresponding to ΔDi-diS$_E$ in the peripheral-PMN-leucocyte medium fraction and the peritoneal-PMN-leucocyte cell, cell-surface and medium fractions had been extracted with water, the extract was digested with chondro-6-sulphatase (first treatment). Next, a portion of the extract of ΔDi-4S in the first treatment was digested with chondro-4-sulphatase and subjected to descending paper chromatography again (second treatment). The data below indicate the movement of ^{3}H-labelled unsaturated disulphate in the peritoneal-PMN-leucocyte medium fraction after chondro-4-sulphatase and chondro-6-sulphatase digestion. Digestion of the spot corresponding to ΔDi-diS$_E$ in the other fractions with chondro-6-sulphatase and chondro-4-sulphatase indicated the same movement as described below (results not shown).

	First treatment (^{3}H d.p.m.)			Second treatment (^{3}H d.p.m.)
	ΔDi-diS$_E$	ΔDi-6S	ΔDi-4S	ΔDi-4S
No enzyme	693	10	20	–
Chondro-6-sulphatase	28	9	557	–
No enzyme	–	–	–	459
Chondro-4-sulphatase	–	–	–	39

Table 2. *Composition of ^{35}S-labelled glycosaminoglycans in peripheral and peritoneal PMN leucocytes*
The data are calculated from the descending paper chromatography of chondroitinase AC- or chondroitinase ABC-digested glycosaminoglycans as illustrated in Fig. 2. The values for chondroitin 4-sulphate and chondroitin 6-sulphate are indicated as percentages of total chondroitin sulphate. The other values given are the percentages of total labelled glycosaminoglycans. All values given are the averages for three experiments.

	Glycosaminoglycan composition (%)			
	Chondroitin sulphate (% of ΔDi-diS$_E$)			
PMN leucocytes	4-Sulphate	6-Sulphate	Dermatan sulphate (% of ΔDi-diS$_E$)	Heparan sulphate
Peripheral				
Cell fraction	73.5		18.6	7.9
	(0)		(0)	
	78.6	21.4		
Medium fraction	79.5		16.0	4.5
	(7.9)		(6.0)	
	68.6	23.5		
Peritoneal				
Cell fraction	70.3		16.8	12.9
	(6.7)		(12.8)	
	85.5	7.8		
Cell-surface fraction	70.3		26.0	3.7
	(9.7)		(9.6)	
	54.7	35.6		
Medium fraction	56.3		33.7	10.0
	(6.4)		(10.7)	
	43.6	50.0		

4S obtained by the first treatment was extracted with water and digested with chondro-4-sulphatase (second treatment), the radioactivity of ΔDi-4S was almost completely lost. This identified the unsaturated disaccharides extracted as ΔDi-diS$_E$. The existence of ΔDi-diS$_E$ containing 4,6-disulphated galactosamine residues suggests that both preparations of PMN leucocytes synthesized oversulphated glycosaminoglycans during incubation.

The composition of the labelled glycosaminoglycans was calculated from the results of the descending paper chromatography illustrated in Fig. 2, and is shown in Table 2. Glycosaminoglycans in the cell and medium fractions from peripheral PMN

leucocytes were found to be of the same composition, except for oversulphated glycosaminoglycans, which were observed only in the medium fraction. The main component of labelled glycosaminoglycans in the cell and medium fractions from peripheral PMN leucocytes was chondroitin 4-sulphate. In contrast, the composition of the labelled glycosaminoglycans in peritoneal PMN leucocytes differed among the three fractions; the proportion of chondroitin 4-sulphate in the total glycosaminoglycans was 60% in the cell fraction compared with 24.5% in the medium fraction from the peritoneal PMN leucocytes, and the proportion of chondroitin 6-sulphate was 5% in the cell fraction compared with 28% in the medium fraction. The chondroitin 4-sulphate/chondroitin 6-sulphate in the peritoneal PMN leucocytes was 11.9 in the cell fraction and 0.9 in the medium fraction. The ratio in peripheral PMN leucocytes was 3.7 in the cell fraction and 2.9 in the medium fraction.

Distribution of labelled glycosaminoglycans synthesized in peripheral and peritoneal PMN leucocytes and amounts of glycosaminoglycans in both PMN leucocyte preparations

Peripheral and peritoneal PMN leucocytes were

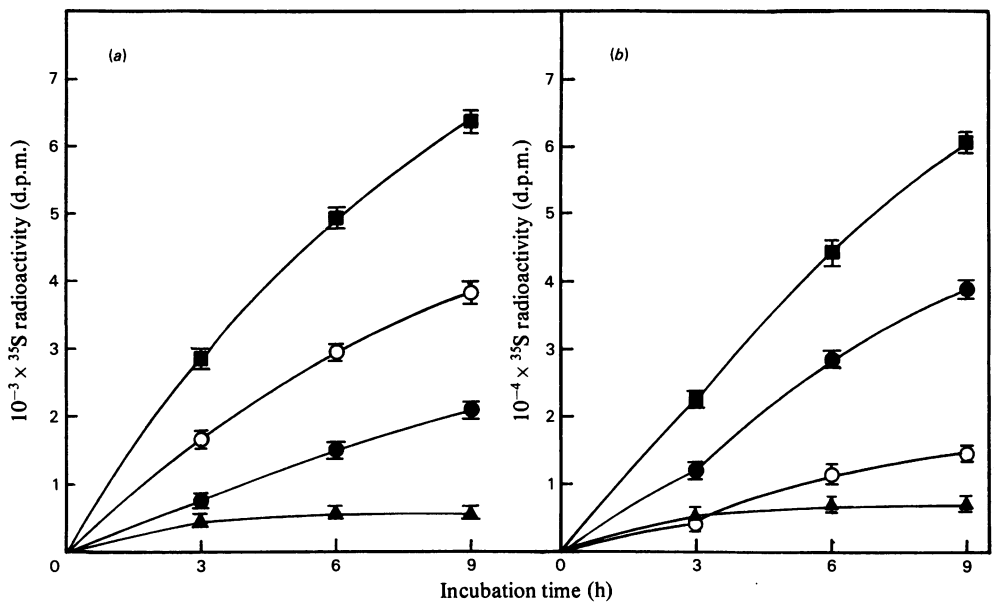

Fig. 3. *Incorporation of [^{35}S]sulphate into glycosaminoglycans in peripheral and peritoneal PMN leucocytes as a function time*
Peripheral PMN leucocytes (1.0×10^7 cells/dish) and peritoneal PMN leucocytes (2.5×10^7 cells/dish) were maintained in monolayer culture for 24h. After replacement of culture medium, 25μCi of [^{35}S]sulphate was added and the cells were incubated for the periods of time indicated. At the end of each incubation, the ^{35}S-labelled glycosaminoglycans were isolated. The radioactivity of the labelled glycosaminoglycans was determined as described in the Experimental section. (*a*) Peripheral PMN leucocytes; (*b*) peritoneal PMN leucocytes. ■, Total; ●, medium fraction; ○, cell fraction; ▲, cell-surface fraction. Each point represents the mean ± S.D. for four experiments.

Table 3. *Activities of lysosomal enzymes in peripheral and peritoneal PMN leucocytes*
After peripheral PMN leucocytes (1.0×10^7 cells) and peritoneal PMN leucocytes (1.0×10^7 cells) had been maintained in culture medium for 24h, the cell and the medium fractions were separated by centrifugation. The activities of the lysosomal enzymes acid phosphatase, *N*-acetyl-β-D-glucosaminidase, β-D-glucuronidase and cathepsin D in the cell and the medium fractions were measured as described in the Experimental section. All values represent the averages for three dishes.

PMN leucocytes	Acid phosphatase (μmol of *p*-nitrophenol/h per 10^7 cells)	*N*-Acetyl-β-D-glucosaminidase (μmol of *p*-nitrophenol/h per 10^7 cells)	β-D-Glucuronidase (μmol of *p*-nitrophenol/h per 10^7 cells)	Cathepsin D (μmol of tyrosine/h per 10^7 cells)
Peripheral				
Cell fraction	20.70	0.77	0.52	0.49
Medium fraction	0.35	0.10	0.04	0.48
Peritoneal				
Cell fraction	39.62	1.24	1.19	1.23
Medium fraction	0.45	0.18	0.05	0.52

incubated for various times in the culture medium containing [^{35}S]sulphate in order to compare the kinetics of glycosaminoglycan synthesized in peripheral PMN leucocytes (1.0×10^7 cells) with that in peritoneal PMN leucocytes (2.5×10^7 cells) (Fig. 3). It is noteworthy that 60% of glycosaminoglycans synthesized in peripheral PMN leucocytes was retained intracellularly whereas 80% of glycosaminoglycans synthesized in peritoneal PMN leucocytes was released into the medium during the incubation; the proportions of labelled glycosaminoglycans in the cell-surface fractions were similar for the two leucocyte preparations. The total radioactivity of glycosaminoglycans synthesized in both preparations of PMN leucocytes increased approximately linearly throughout the incubation, indicating that the glycosaminoglycans were being synthesized at fairly constant rates in both preparations. The difference in the distributions of labelled glycosaminoglycans among the fractions obtained from peripheral and peritoneal PMN leucocytes was confirmed by measuring the amount of glycosaminoglycans in peripheral and peritoneal PMN leucocytes as described in the Experimental section: the amount in peritoneal PMN leucocytes (21.6 μg as uronic acid/10^9 cells) was less than that in peripheral PMN leucocytes (48.0 μg as uronic acid/10^9 cells).

Activities of lysosomal enzymes

The activities of lysosomal enzymes were measured and are shown in Table 3. The activities of lysosomal enzymes in peritoneal PMN leucocytes were 2-fold higher than those in peripheral PMN leucocytes.

Discussion

Human PMN leucocytes have previously been demonstrated to contain proteochondroitin sulphate of low protein content and relatively small molecular mass (Olsson, 1969*a*). In the present investigation, PMN leucocytes isolated from guinea pigs released proteoglycans into the medium when they were incubated with radioactive precursors. The labelled proteoglycans synthesized exhibited an average M_r of 300000, and they were found to possess chondroitin sulphate side chains (M_r 25000) when assessed by Sepharose 6B gel filtration before and after hydrolysis with alkali.

Some papers have reported that glycosaminoglycans from human and bovine peripheral PMN leucocytes were composed of mainly chondroitin 4-sulphate with small amounts of heparan sulphate and hyaluronic acid (Olsson & Gardell, 1967; Murata, 1974; Murata *et al.*, 1974). In contrast, glycosaminoglycans in leukaemic PMN leucocytes were composed of chondroitin 4-sulphate, hyaluronic acid and chondroitin sulphate isomers containing oversulphated chondroitin sulphate (Murata, 1980). In the present work the cell fraction in peripheral PMN leucocytes consisted of 73.5% chondroitin sulphate, 18.6% dermatan sulphate and 7.9% heparan sulphate, but no oversulphated glycosaminoglycans were found. The absence of oversulphated chondroitin sulphate from peripheral PMN leucocytes is consistent with the observation by Murata (1980), in which normal PMN leucocytes were found to be lacking in oversulphated glycosaminoglycans. However, small amounts of oversulphated chondroitin sulphate and oversulphated dermatan sulphate were found in the medium fraction. On the other hand, oversulphated chondroitin sulphate was found in the cell, cell-surface and medium fractions of peritoneal PMN leucocytes. Digestion of glycosaminoglycans with chondroitinase AC and chondroitinase ABC indicated that disulphated disaccharides were derived from chondroitin sulphate and dermatan sulphate. Moreover, on the basis of the enzymic assay with chondro-4-sulphatase and chondro-6-sulphatase, the positions of sulphation in the disulphated disaccharides obtained were identified as the 4- and 6-positions of *N*-acetylgalactosamine. This suggests that peripheral and peritoneal PMN leucocytes are capable of synthesizing both oversulphated proteochondroitin sulphate and oversulphated proteodermatan sulphate. Similarly, Kolset *et al.* (1983) reported that the monocytes obtained during prolonged culturing on plastic acquired the ability to synthesize glycosaminoglycans containing 4,6-disulphated *N*-acetylgalactosamine units. Another noteworthy finding in the experiment is that peritoneal PMN leucocytes have greatly accelerated secretion of chondroitin 6-sulphate. This result conformed to the observation that glycosaminoglycans synthesized in mitogenically activated lymphocytes are different from those synthesized in non-stimulated cells (Hart, 1982); activated lymphocytes have accelerated secretion of glycosaminoglycans, which appear to be more highly sulphated than those of non-stimulated cells, and to be increased in the relative proportion of both cell-associated and cell-secreted chondroitin 6-sulphate in the total glycosaminoglycans. Furthermore, Vannucchi *et al.* (1982) reported a change in the glycosaminoglycans involved in the activation of PMN leucocytes in adhesion culture. These reports and our results suggest that PMN leucocytes possess an ability to synthesize various kinds of glycosaminoglycans in response to changes in situation.

Thus we compared the biosynthesis and secretion of glycosaminoglycans in peripheral PMN leucocytes with those in peritoneal PMN leuco-

cytes. Most of the glycosaminoglycans synthesized in peripheral PMN leucocytes were accumulated within the cells, whereas glycosaminoglycans synthesized in peritoneal PMN leucocytes were released into the culture medium. Also, the amount of glycosaminoglycans in peritoneal PMN leucocytes was significantly less than that in peripheral PMN leucocytes. It is considered that the difference in the kinetics of synthesis and amount of glycosaminoglycans in PMN leucocytes is due to the induction of PMN leucocytes from blood vessel to peritoneal cavity by injection of caseinate. On the other hand, the activity of lysosomal enzymes in peritoneal PMN leucocytes was lower than that in peripheral PMN leucocytes. The significance of the existence of glycosaminoglycans in PMN leucocytes is not yet clear.

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