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Changes in glycosaminoglycan biosynthesis during differentiation *in vitro* of human monocytes

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Monocytes isolated from human blood were maintained *in vitro* on plastic culture dishes. After 3–4 days, adherent cells displayed morphological changes previously attributed to differentiation of the cells into histiocytes. ^{35}S-labelled glycosaminoglycans were isolated after incubation of the cells with inorganic [^{35}S]sulphate. Polysaccharide recovered from the culture medium after labelling from day 0 to day 2 or from day 5 to day 7 *in vitro* was ~90% galactosaminoglycan (resistant to deamination by HNO_2), irrespective of labelling period. Whereas day-0–2 material was extensively degraded to disaccharide on incubation with the bacterial eliminase chondroitinase AC, a significant portion, about 30%, of the day-5–7 material resisted degradation under the same conditions. The resistant portion was readily depolymerized by treatment with chondroitinase ABC and may be dermatan sulphate. Paper electrophoresis and paper chromatography of the disaccharides obtained by eliminase digestion identified the day-0–2 labelled galactosaminoglycan as chondroitin 4-sulphate. In contrast, the corresponding day-5–7 material yielded ~20% disulphated disaccharide, both on digestion with chondroitinase AC and on subsequent enzymic degradation of the chondroitinase AC-resistant fraction. Further treatment of the disulphated disaccharide with chondro-4-sulphatase and chondro-6-sulphatase indicated that both sulphate groups were located on the *N*-acetylgalactosamine residue. In accordance with these findings, the day-5–7 polysaccharide showed a higher negative charge density than the day-0–2 material on ion-exchange chromatography. It is concluded that the novel properties acquired by the monocyte during prolonged culturing on plastic include the ability to synthesize glycosaminoglycan(s) containing 4,6-disulphated *N*-acetylgalactosamine units.

Glycosaminoglycans are known to be produced by a large variety of animal cells (Kraemer, 1979). These polysaccharides exhibit marked structural diversity, which is due both to the occurrence of distinctly different classes of molecules and to structural variability (microheterogeneity) within each polysaccharide class (Lindahl & Höök, 1978; Rodén, 1980). Attempts have been made to correlate polysaccharide structure with various expressions of cell function. Numerous reports have thus appeared concerning the effects of culture conditions or growth properties of cells *in vitro* on the production and structure of glycosaminoglycans (for references, see Kraemer, 1979). In some cases, specific correlations have been observed, for instance, a decreased sulphation of heparan sulphate resulting from viral transformation (Underhill & Keller, 1975; Winterbourne & Mora, 1978). Moreover, it has been suggested that the exposure of glycosaminoglycans on the cell surface of macrophages is specifically modulated in response to various functional stimuli (Cappelletti *et al.*, 1980). In the present paper we show that differentiation *in vitro* of human monocytes is accompanied by the appearance of an oversulphated galactosaminoglycan containing 4,6-di-*O*-sulphated *N*-acetylgalactosamine residues.

Abbreviations used: 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.

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Experimental

Materials

Heparin (Stage 14) from pig intestinal mucosa was obtained from Inolex Pharmaceutical Division, Park Forest South, IL, U.S.A., and was purified as described previously (Lindahl *et al.*, 1965). Dermatan sulphate isolated from heparin by-products (pig intestinal mucosa) was given by Dr. L. Rodén, University of Birmingham, Birmingham, AL, U.S.A., chondroitin sulphate from bovine nasal septa by Dr. Å. Wasteson, University of Uppsala, Uppsala, Sweden, and hyaluronic acid from rooster combs by Dr. T. C. Laurent, University of Uppsala, Uppsala, Sweden. ^{3}H-labelled chondroitin sulphate (200×10^3–300×10^3 c.p.m./μg of hexuronic acid) and dermatan sulphate (200–300×10^3 c.p.m./μg of hexuronic acid) were prepared by partial *N*-deacetylation (hydrazinolysis), followed by re-*N*-acetylation with [^{3}H]acetic anhydride (Höök *et al.*, 1982). Mono- and di-*O*-sulphated hexuronosyl-2,5-anhydro[1-^{3}H]mannitol disaccharides were prepared from heparin, and separated into mono-*O*- and di-*O*-sulphated species by preparative paper electrophoresis, as described by Thunberg *et al.* (1982). The unsaturated disaccharides, ΔDi-4S and ΔDi-6S, isolated after digestion of chondroitin sulphate with chondroitinase, were obtained from Seikagaku Fine Chemicals, Tokyo, Japan.

Inorganic [^{35}S]sulphate (carrier-free) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Papain and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Bacterial 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) and chondro-6-sulphatase (EC 3.1.6.10) were from Seikagaku. Penicillin and streptomycin (Gibco Bio-Cult, Paisley, Renfrewshire, Scotland); Percoll, Sephadex gel (Pharmacia Fine Chemicals, Uppsala, Sweden), and Whatman DEAE-cellulose (DE-52; Whatman Biochemicals, Maidstone, Kent, U.K.) were from the commercial sources indicated.

Methods

Hexuronic acid was determined by the carbazole method (Bitter & Muir, 1962). Radioactivity was determined as described previously (Lindahl *et al.*, 1976). Paper chromatography was carried out by using Whatman 3MM paper in acetic acid/n-butanol/1 M-NH_3, 3:2:1 (by vol.). For additional separation methods see the legends to the Figures.

Cell-culture experiments. Human monocytes were isolated and cultured as follows (Pertoft *et al.*, 1980). Blood was drawn from healthy donors into Vacutainer tubes containing polystyrene beads (Becton and Dickinson, Grenoble, France) and defibrinated at room temperature for 20 min. A stock solution of Percoll was made up from 9 vol. of Percoll and 1 vol. of 1.5 M-NaCl; before use, this solution (6 vol.) was diluted with 0.15 M-NaCl (4 vol.). Defibrinated blood (7 ml) was layered on top of 4 ml of the diluted Percoll solution in a 12 ml conical polystyrene tube (AB Cerbo, Stockholm, Sweden) and centrifuged at 1000 *g* at room temperature for 20 min in a swing-out rotor. A clear supernatant of serum was collected and used in the cell cultures (see below). Mononuclear cells at the serum/Percoll interphase were collected with a Pasteur pipette, diluted with F10 medium and centrifuged for 10 min at 100 *g*. The cell pellet was resuspended in F10 medium supplemented with 20% autologous serum and 100 units of penicillin and streptomycin/ml. The cells were seeded in 16 mm Costar wells (Costar, Broadway, Cambridge, MA, U.S.A.) at a density of 2×10^6 cells/well (1 ml of medium) and incubated at 37°C in an atmosphere of CO_2/air (19:1). After 2 h, non-adherent cells were removed by washing the wells with phosphate-buffered saline (0.14 M-NaCl/2 mM-KCl/8 mM-Na_2HPO_4/1.5 mM-KH_2PO_4, pH 7.4). Adherent cells, monocytes (Pertoft *et al.*, 1980), were reincubated in 1 ml of F10 medium containing antibiotics as described above and 20% autologous serum. During extended culture periods the medium was changed every second day.

For biosynthetic labelling of glycosaminoglycans the cultured cells were washed with sulphate-depleted F10 medium ($MgCl_2$ substituted for $MgSO_4$) and incubated in this medium (1 ml) supplemented with 20% autologous serum. Inorganic [^{35}S]sulphate (50 μCi) was added to each well and the cells were incubated for 48 h. Labelling was initiated either directly after establishing the cultures (yielding day-0–2 polysaccharide) or after a 5-day pre-incubation period (day-5–7 polysaccharide). After completed ^{35}S incorporation, the spent culture media (and, in some experiments, the cell fractions) were digested with papain in the presence of 0.5 mg of carrier chondroitin sulphate (Lindahl *et al.*, 1973), and labelled polysaccharide was isolated by gel chromatography on Sephadex G-50 in 1 M-NaCl. Effluent fractions containing labelled material, excluded from the gel, were pooled and desalted by dialysis against distilled water.

Degradation of polysaccharides. Polysaccharides containing *N*-sulphated glucosamine residues, i.e. heparin or heparan sulphate, were depolymerized to oligosaccharides by treatment with HNO_2 at pH 1.5 (Shively & Conrad, 1976). Galactosaminoglycans were degraded by incubation with chondroitinase ABC in 0.05 M-Tris/HCl, pH 8.0, containing 0.05 M-sodium acetate and 0.05 mg of bovine serum albumin/ml, or by incubation with chondroitinase AC in 0.05 M-Tris/HCl, pH 8.0, containing 0.05 M-sodium acetate, 0.05 M-NaCl and 0.1 mg of bovine

serum albumin/ml (Yamagata *et al.*, 1968). To the incubation mixtures (generally 0.5ml) were added 0.5mg of unlabelled polysaccharide (chondroitin sulphate or dermatan sulphate) and 0.05 unit of enzyme. The samples were incubated for 15h. Unsaturated disaccharides resulting from chondroitinase digestions were further degraded by incubation with chondro-4-sulphatase or chondro-6-sulphatase (Yamagata *et al.*, 1968). Samples were dissolved in 50μl of the buffer used for digestions with chondroitinase AC and incubated with 0.01 unit of enzyme at 37°C for 45min.

Results

During 1 week in culture the number of cells attached to the plastic substratum generally decreased to about 50% of the initial value. Within this period of time the remaining cells displayed a 2–3-fold increase in cellular diameter, which became evident after about 4 days in culture. This change in morphology, as well as in a number of functional properties (see the Discussion section), have been reported by others and interpreted as an expression of differentiation of the monocyte *in vitro* into a macrophage-like cell.

The amounts of [^{35}S]glycosaminoglycan recovered after a 48h labelling period amounted to about 3×10^3 c.p.m./10^6 cells seeded, with no significant difference in yield between day-0–2 and day-5–7 material. Pilot studies showed that approx. 75% of the total labelled polysaccharide was released into the culture medium. Additional preliminary experiments (S. O. Kolset & L. Kjellén, unpublished work) indicated that the labelled macromolecules were largely released as proteoglycans that could be cleaved to single polysaccharide chains by treatment with alkali. The present work was restricted to such molecules, isolated after proteolytic treatment of the culture medium.

Structure of carbohydrate backbone

Treatment of the day-0–2 or day-5–7 material with HNO_2 resulted in degradation of less than 10% of the total labelled polysaccharides, as determined by gel chromatography of the products on Sephadex G-50 (results not shown). The material susceptible to HNO_2, presumably heparan sulphate, was not further investigated. Digestion of the HNO_2-resistant portion of the day-0–2 polysaccharide with chondroitinase AC resulted in extensive degradation of the material to disaccharides (Fig. 1*a*). In contrast, a significant portion, about 30%, of the corresponding day-5–7 material resisted chondroitinase AC digestion and remained excluded from Sephadex G-50, the susceptible portion again appearing as disaccharide (Fig. 1*b*). Unlabelled chondroitin sulphate from bovine nasal cartilage, added to the day-5–7 material in excess amounts as an internal control, was quantitatively degraded. The two degradation patterns in Figs. 1(*a*) and 1(*b*) were highly reproducible with different batches of day-0–2 and day-5–7 polysaccharide respectively.

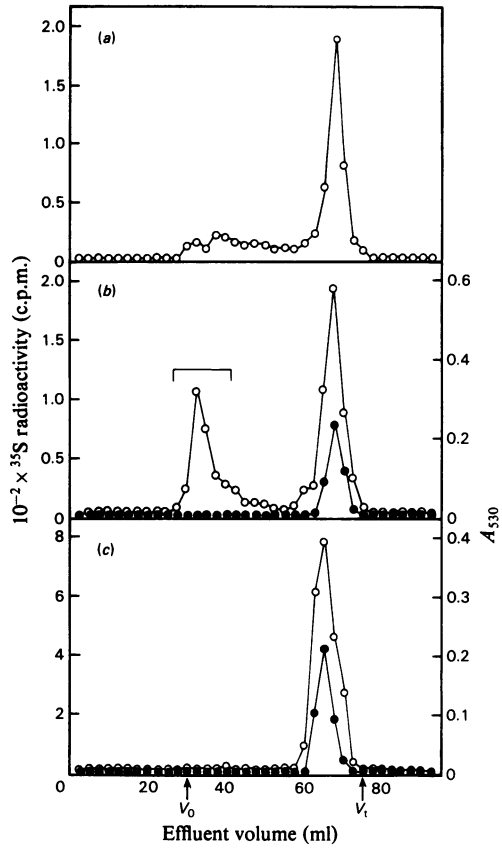

Fig. 1. *Digestion of ^{35}S-labelled monocyte glycosaminoglycans with bacterial eliminases*

Polysaccharides (about 30×10^3 c.p.m. of ^{35}S) from day-0–2 (*a*) or from day-5–7 (*b*) cultures were incubated with chondroitinase AC. The resistant portion of the day-5–7 material (indicated by the horizontal bracket in *b*) was further digested with chondroitinase ABC (*c*). All digestions were performed with 0.5mg of chondroitin sulphate as internal control (shown in *b* and *c* only). The digested samples were applied to a column (1cm × 95cm) of Sephadex G-50, and eluted with 0.2M-NH_4HCO_3 at a rate of about 5ml/h. Effluent fractions were analysed for radioactivity (○), and for hexuronic acid by the carbazole method (●; A_{530}). Fractions recovered for further analysis were desalted by freeze-drying. Before enzyme digestion, samples were treated with HNO_2 in order to eliminate any heparan sulphate present; the resulting degradation products were removed by gel chromatography on Sephadex G-50 as described above. For additional information, see the text.

The chondroitinase AC-resistant portion of the day-5–7 material was readily depolymerized by chondroitinase ABC, yielding disaccharide exclusively as product (Fig. 1*c*). The simplest explanation for these findings implied that the day-0–2 material consisted of chondroitin sulphate, with D-glucuronic acid as the only hexuronic acid component, whereas the day-5–7 material included, in addition, L-iduronic acid-containing sequences that were susceptible to degradation by chondroitinase ABC but not by chondroitinase AC (see Yamagata *et al.*, 1968). [Co-polymeric galactosaminoglycans (dermatan sulphate) containing both D-glucuronic acid and L-iduronic acid residues in the same polysaccharide chain have been demonstrated in a variety of species and tissues (see Rodén, 1980).] However, this interpretation is open to some doubt, since repeated chondroitinase AC digestion of once-resistant day-5–7 material (indicated by the horizontal bracket in Fig. 1*b*) shifted an additional 30% of the label into the disaccharide region (result not shown). In control experiments with mixtures of polysaccharide standards, chondroitin sulphate was completely degraded during a single incubation with chondroitinase AC, whereas dermatan sulphate remained polymeric, regardless of the proportions of the two glycosaminoglycans (Fig. 2). The identification of dermatan sulphate in the day-5–7 material must be regarded as tentative.

Location of sulphate groups

Paper electrophoresis of the disaccharide obtained by chondroitinase AC digestion of day-0–2 material (see Fig. 1*a*) showed essentially a single ^{35}S-labelled component that migrated like a mono-*O*-sulphated disaccharide standard (Fig. 3*a*). This component co-migrated with the disaccharide ΔDi-4S on paper chromatography (result not shown). It is therefore concluded that the day-0–2 polysaccharide was mainly chondroitin 4-sulphate. The

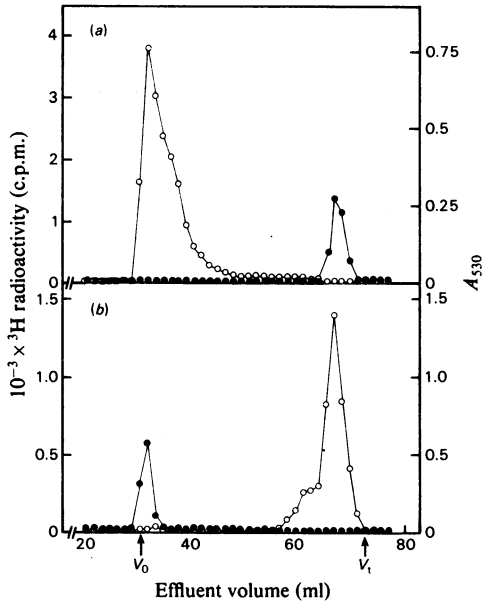

Fig. 2. *Digestion of reference glycosaminoglycans with chondroitinase AC*

Mixtures of (*a*) ^3H-labelled dermatan sulphate (○; 100×10^3 c.p.m.; about 1.5 μg of polysaccharide) with unlabelled chondroitin sulphate (●; 1 mg); and of (*b*) ^3H-labelled chondroitin sulphate (○; 100×10^3 c.p.m.; about 1.5 μg of polysaccharide) with unlabelled dermatan sulphate (●; 1 mg) were incubated with chondroitinase AC as described in the Experimental section. The digestion products were analysed by gel chromatography on Sephadex G-50, as described in the legend to Fig. 1; effluent fractions were analysed for radioactivity (○) and for hexuronic acid (●).

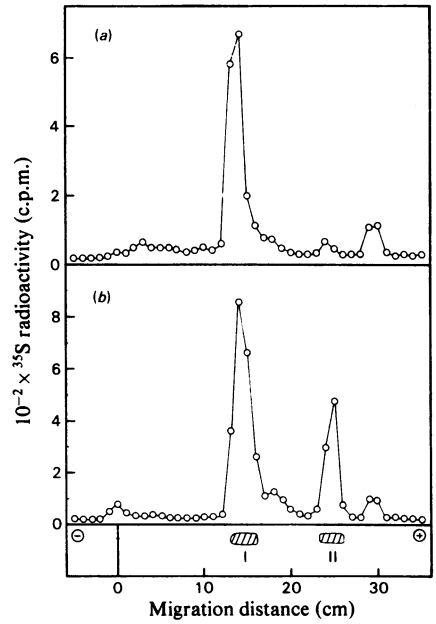

Fig. 3. *Paper electrophoresis of ^{35}S-labelled disaccharides formed by digesting monocyte glycosaminoglycans with chondroitinase AC*

Disaccharides (about 5×10^3 c.p.m. of ^{35}S) recovered from (*a*) day-0–2 polysaccharide (see Fig. 1*a*) and from (*b*) day-5–7 polysaccharide (see Fig. 1*b*) were freeze-dried, dissolved in water and applied to Whatman 3MM paper. The samples were separated by high-voltage electrophoresis in 1.6 M-formic acid, pH 1.7, at 40 V/cm for 80 min. The standards shown below the tracings are: (I) monosulphated and (II) disulphated hexuronosyl-2,5-anhydro-[1-^3H]mannitol disaccharides.

disaccharides released by chondroitinase AC from the day-5–7 material (Fig. 1*b*) yielded a more complex electrophoresis pattern, with significant amounts of di-*O*-sulphated disaccharide (Fig. 3*b*). The difference in disaccharide composition between day-0–2 and day-5–7 material was consistent; although the di-*O*-sulphated disaccharide invariably accounted for less than 10% of the total label in day-0–2 material, it generally (as in Fig. 3*b*) amounted to about 30% of the label in day-5–7 material (corresponding, on a molar basis, to about 20% of the total disaccharide), and in some preparations to as much as 50% of the ^{35}S. The chondroitinase AC-resistant fraction of the day-5–7 material showed the same ratio of mono-*O*-sulphated/di-*O*-sulphated disaccharide units (after degradation with chondroitinase ABC; see Fig. 1*c*) as did the chondroitinase AC-susceptible portion.

The mono-*O*-sulphated disaccharide obtained by chondroitinase AC digestion of day-5–7 material was identified by paper chromatography as ΔDi-4S, similar to the corresponding day-0–2 component. The disulphated disaccharide was isolated by preparative paper electrophoresis and further characterized by digestion with chondrosulphatases. After incubation for 45 min with a mixture of chondro-4-sulphatase and chondro-6-sulphatase the disaccharide was virtually completely desulphated (Fig. 4*d*), suggesting that both sulphate groups had been located on the *N*-acetyl-D-galactosamine unit. Attempts to corroborate this conclusion by separately incubating the disaccharide with each sulphatase were complicated by apparent cross-contamination of the two enzyme preparations. Prolonged incubation (6 h) of the disaccharide with either sulphatase thus resulted in essentially complete release of the label as inorganic [^{35}S]sulphate, without any significant accumulation of mono-[^{35}S]sulphated disaccharide (result not shown; same as in Fig. 4*d*). Nevertheless, brief incubation (45 min) of the disaccharide with the alleged chondro-6-sulphatase preparation produced about equal amounts of labelled inorganic sulphate and monosulphated disaccharide, presumably ΔDi-4S (Fig. 4*c*). In contrast, corresponding treatment with the 4-sulphatase preparation resulted in only partial degradation of the disulphated disaccharide, yielding inorganic [^{35}]sulphate in excess of mono[^{35}]sulphated disaccharide (Fig. 4*b*). The most likely explanation for this finding is that the monosulphated disaccharide, ΔDi-6S, was desulphated by the contaminating 6-sulphatase along with its formation, by 4-desulphation, from the disulphated disaccharide. Taken together, these results indicate that the disulphated disaccharide is identical with 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4,6-di-*O*-sulpho-D-galactose (ΔDi-diS$_E$ in the terminology of Suzuki *et al.*, 1968). Since this disaccharide had been released from the glycosaminoglycan by the action of chondroitinase AC, the unsaturated hexuronic acid residue would correspond to a D-glucuronic acid unit in the intact polysaccharide chain.

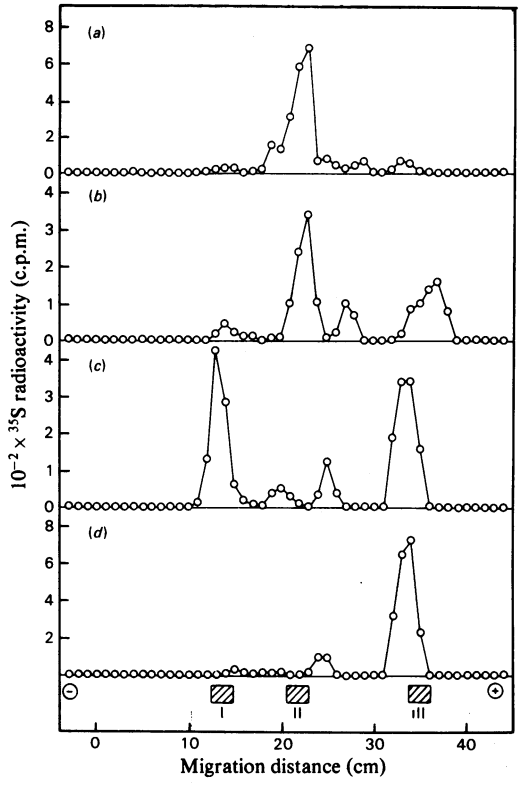

Fig. 4. *Digestion with chondrosulphatases of disulphated disaccharide obtained from day-5–7 material*
Disulphated ^{35}S-labelled disaccharide was isolated from a chondroitinase AC digest of day-5–7 polysaccharide, by gel chromatography (Fig. 1*b*) followed by preparative paper electrophoresis (Fig. 3*b*). The disaccharide was incubated with chondrosulphatases for 45 min, as described in the Experimental section: (*a*) mixture of chondro-4-sulphatase and chondro-6-sulphatase, heat-inactivated enzymes; (*b*) chondro-4-sulphatase; (*c*) chondro-6-sulphatase; (*d*) mixture of chondro-4-sulphatase and chondro-6-sulphatase (active enzymes). The products were analysed by high-voltage paper electrophoresis, as described in the legend to Fig. 3. The standards I and II are as in Fig. 3; standard III is inorganic [^{35}S]sulphate. The ^{35}S-labelled component migrating between the disulphated disaccharide and inorganic sulphate in (*b*–*d*) has not been identified. For additional information, see the text.

The presence of disulphated disaccharide units in monocyte galactosaminoglycans was reflected by the overall charge density of the molecule, as shown

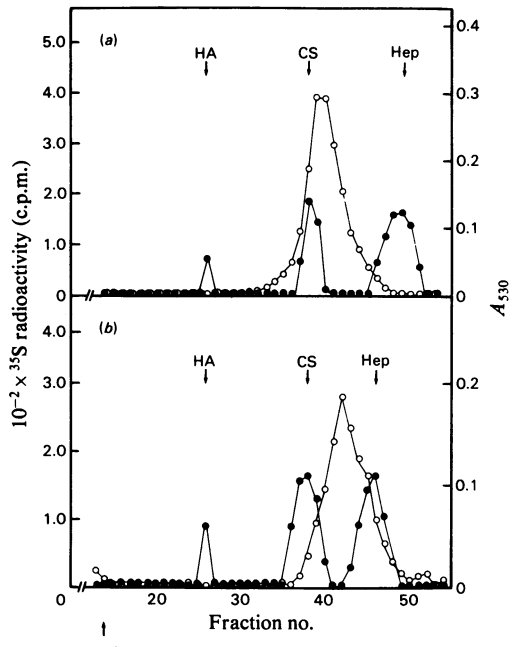

Fig. 5. *Ion-exchange chromatography on DEAE-cellulose of ^{35}S-labelled polysaccharides from human monocytes*
Samples (about 3×10^3 c.p.m.) of (*a*) day-0–2 polysaccharide, and (*b*) day-5–7 polysaccharide were applied to a column (1 cm × 7.5 cm) of Whatman DEAE-cellulose DE-52 along with internal standards of hyaluronic acid (HA, 0.25 mg), chondroitin sulphate (CS, 1 mg) and heparin (Hep, 2 mg). The column was eluted at a rate of about 6 ml/h with a gradient (beginning at arrow) extending from 0.05 M- to 1.5 M-LiCl in 0.05 M-acetate buffer, pH 4.0. Fractions (3 ml) were collected and analysed for radioactivity (○) and for hexuronic acid (●).

by ion-exchange chromatography on DEAE-cellulose. Whereas the day-0–2 material essentially co-chromatographed with a chondroitin sulphate internal standard (sulphate/hexosamine molar ratio ~1.0; Fig. 5*a*), the day-5–7 material was more retarded and showed significant overlap with the heparin reference (Fig. 5*b*). This pattern was consistently repeated with polysaccharides isolated from six different batches of cells from different donors; furthermore, it was observed not only with polysaccharide secreted into the culture medium, but also with the fraction that remained associated with the cells (result not shown).

Discussion

Oversulphated chondroitin sulphate, containing 4,6-di-*O*-sulphated *N*-acetyl-D-galactosamine units adjacent to D-glucuronic acid residues, was first found in squid cartilage (Suzuki *et al.*, 1968), but has since been detected also in vertebrate species. Analogous sequences with L-iduronic acid substituting for the D-glucuronic acid units were detected in dermatan sulphate from hagfish (*Myxine glutinosa*) skin (Akiyama & Seno, 1981). Both types of structures have recently been claimed to occur in glycosaminoglycans produced by cells *in vitro*. A chondroitinase AC-resistant (but ABC-susceptible) polysaccharide [thus presumably (see the Results section) a dermatan sulphate] containing 4,6-disulphated *N*-acetylgalactosamine residues was produced by peritoneal macrophages from the guinea-pig (Takasu *et al.*, 1982). Similarly, oversulphated chondroitin sulphate was synthesized by differentiated bone-marrow-derived mast cells (Razin *et al.*, 1982), and by cultured chick-embryo chondrocytes (Kim & Conrad, 1982). Interestingly, in some of the cultures in the latter study the ability to synthesize the disulphated disaccharide unit appeared to be correlated with the differentiation state of the cells. Moreover, a regulatory role for cyclic AMP-dependent protein kinase was postulated in relation to the formation of *N*-acetylgalactosamine 4,6-disulphate residues by foetal-calf articular cartilage *in vitro* (Speight *et al.*, 1981). The detection of such residues as a minor component in various mammalian tissues (see Razin *et al.*, 1982) indicates that the synthesis of oversulphated galactosaminoglycan is not restricted to conditions *in vitro*.

In the present study, human monocytes were found to produce glycosaminoglycan containing 4,6-di-*O*-sulphated *N*-acetylgalactosamine residues in response to the conditions of cultivation *in vitro*. Such residues occurred in unsaturated disaccharides formed by the action of the bacterial eliminase chondroitinase AC, and would therefore have been located between D-glucuronic acid units in the intact polymer, i.e. chondroitin sulphate. In addition, indications for the occurrence of similarly oversulphated dermatan sulphate were obtained on the basis of the differential effects of chondroitinases AC and ABC. However, the identification of dermatan sulphate remains tentative (see the Results section), pending the actual identification of L-iduronic acid residues.

A number of investigators have noted morphological and functional changes in human monocytes under conditions *in vitro*. Most strikingly, the cells become larger, increasing their diameter up to 2–3-fold (Johnson *et al.*, 1977; Zuckerman *et al.*, 1979). Moreover, they increase their synthesis of 5′-nucleotidase and lysozyme (Johnson *et al.*, 1977), develop functional complement receptors (Newman *et al.*, 1980) and decrease their capacity to produce H_2O_2 to a level comparable with that of macro-

phages (Nakagawara *et al.*, 1981). Throughout this process, which occurs within a period of about 4–5 days, the cells retain their ability to phagocytose (Zuckerman *et al.*, 1979). The overall change of the cell mimics the transition taking place during the differentiation of monocytes into macrophages, and has therefore been regarded as an expression of a differentiation process *in vitro*. The cells studied in the present investigation conformed to this pattern, as judged from the conspicuous increase in size observed after about 4 days in culture. Whether the formation of oversulphated galactosaminoglycan is yet another marker of differentiation, or rather a fortuitous finding without any apparent relation to the functional state of the cell, remains to be established. However, results of preliminary studies (S. O. Kolset, U. Lindahl & R. Seljelid, unpublished work) pointing to a functional correlation may be noted. Monocytes cultured on fibronectin-coated dishes thus did not show the morphological changes displayed by cells on uncoated plastic, nor did they produce such large amounts of oversulphated galactosaminoglycan.

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References

Akiyama, F. & Seno, N. (1981) *Biochim. Biophys. Acta* **674**, 289–296

Bitter, T. & Muir, H. M. (1962) *Anal. Biochem.* **4**, 330–334

Cappelletti, R., Del Rosso, M., Vannucchi, S., Cella, C., Fibbi, G. & Chiarugi, V. P. (1980) *J. Reticuloendothel Soc.* **27**, 383–391

Höök, M., Riesenfeld, J. & Lindahl, U. (1982) *Anal. Biochem.* **119**, 236–245

Johnson, W. D., Mei, B. & Cohn, Z. A. (1977) *J. Exp. Med.* **146**, 1613–1626

Kim, J. J. & Conrad, H. E. (1982) *J. Biol. Chem.* **257**, 1670–1675

Kraemer, P. M. (1979) in *Surfaces of Normal and Malignant Cells* (Hynes, R. O., ed.), pp. 149–198, John Wiley and Sons, Chichester

Lindahl, U. & Höök, M. (1978) *Annu. Rev. Biochem.* **47**, 385–417

Lindahl, U., Cifonelli, J. A., Lindahl, B. & Rodén, L. (1965) *J. Biol. Chem.* **240**, 2817–2820

Lindahl, U., Bäckström, G., Jansson, L. & Hallén, A. (1973) *J. Biol. Chem.* **250**, 7234–7241

Lindahl, U., Jacobsson, I., Höök, M., Bäckström, G. & Feingold, D. S. (1976) *Biochem. Biophys. Res. Commun.* **70**, 492–499

Nakagawara, A., Nathan, C. F. & Cohn, Z. A. (1981) *J. Clin. Invest.* **68**, 1243–1252

Newman, S. L., Musson, R. A. & Henson, P. M. (1980) *J. Immunol.* **125**, 2236–2244

Pertoft, H., Johnsson, A., Wärmegård, B. & Seljelid, R. (1980) *J. Immunol. Methods* **33**, 221–229

Razin, E., Stevens, R. L., Akiyama, F., Schmid, K. & Austen, K. F. (1982) *J. Biol. Chem.* **257**, 7229–7236

Rodén, L. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, J., ed.), pp. 267–371, Plenum Press, New York

Shively, J. E. & Conrad, H. E. (1976) *Biochemistry* **15**, 3932–3942

Speight, G., Handley, C. J. & Lowther, D. A. (1981) *Biochim. Biophys. Acta* **672**, 89–97

Suzuki, S., Saito, H., Yamagata, T., Anno, K., Seno, N., Kawai, Y. & Furuhashi, T. (1968) *J. Biol. Chem.* **243**, 1543–1550

Takasu, Y., Hasumi, F. & Mori, Y. (1982) *Biochim. Biophys. Acta* **716**, 316–323

Thunberg, L., Bäckström, G. & Lindahl, U. (1982) *Carbohydr. Res.* **100**, 393–410

Underhill, C. B. & Keller, J. M. (1975) *Biochem. Biophys. Res. Commun.* **63**, 448–453

Winterbourne, D. J. & Mora, P. T. (1978) *J. Biol. Chem.* **253**, 5109–5120

Yamagata, T., Saito, H., Habuchi, O. & Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1523–1535

Zuckerman, S. H., Ackerman, S. K. & Douglas, S. D. (1979) *Immunology* **38**, 401–411