

Biosynthesis of chondroitin sulphate by a Golgi-apparatus-enriched preparation from cultures of mouse mastocytoma cells

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Mouse mastocytoma cells grown in suspension culture produce chondroitin 4-sulphate. A Golgi-apparatus-enriched fraction from these cells was prepared and examined for chondroitin-synthesizing activity. When Golgi-apparatus-enriched fractions were incubated with UDP-[¹⁴C]glucuronic acid and UDP-*N*-acetylgalactosamine, they demonstrated a greater than 13-fold increase in chondroitin-synthesizing activity over cell homogenates. Similar incubations with the addition of a pentasaccharide from chondroitin sulphate resulted in a greater than 40-fold increase in [¹⁴C]glucuronic acid-incorporating activity over cell homogenates. Other membrane fractions had much less activity, suggesting that the Golgi apparatus is the most active location for chondroitin biosynthesis. Products of the incubations indicated the formation of [¹⁴C]chondroitin glycosaminoglycan on endogenous primers and formation of [¹⁴C]-hexasaccharide and somewhat larger [¹⁴C]oligosaccharides on exogenous pentasaccharide acceptors. There was, however, a significant amount of large [¹⁴C]-chondroitin glycosaminoglycan formed on pentasaccharide, indicating that some pentasaccharide did serve as a true primer for polysaccharide synthesis.

It is now generally accepted that the Golgi apparatus is the major intracellular location for incorporation of the terminal sugar portions of various glycoproteins. Thus Golgi-apparatus-enriched preparations from several sources have been shown to contain most of the *N*-acetylglucosaminyl-, galactosyl-, sialyl- and fucosyl-transferase activities (Fleischer *et al.*, 1969; Schachter *et al.*, 1970; Morré, 1971; Wagner & Cynkin, 1971; Letts *et al.*,

1974). Indirect evidence has been obtained from electron microscopy and radioautography (Peterson & Leblond, 1964; Godman & Lane, 1964; Neutra & Leblond, 1966) to suggest that sulphation of the glycosaminoglycan portion of proteoglycans also takes place in this organelle. However, synthesis of glycosaminoglycans by the Golgi apparatus has not been established. Direct demonstration would require the isolation and use of Golgi-apparatus preparations from cells that were capable of synthesizing glycosaminoglycans.

Abbreviations used: ΔDi-OS, 2-acetamido-2-deoxy-3-*O*-(β-D-gluc-4-enopyranosyluronic acid)-D-galactose; ΔDi-4S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluc-4-enopyranosyluronic acid)-4-*O*-sulpho-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluc-4-enopyranosyluronic acid)-6-*O*-sulpho-D-galactose; Di-OS, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, or *N*-acetylchondrosine; Di-4S, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-4-*O*-sulpho-D-galactose, or *N*-acetylchondrosine 4-sulphate; Di-6S, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-6-*O*-sulpho-D-galactose, or *N*-acetylchondrosine 6-sulphate; Mes, 4-morpholine-ethanesulphonic acid.

We have shown that certain neoplastic mouse mast cells grown in suspension culture synthesize chondroitin 4-sulphate (Lewis *et al.*, 1973a), and a microsomal preparation from these cells has been utilized to demonstrate cell-free synthesis of this glycosaminoglycan from sugar nucleotide precursors (Lewis *et al.*, 1973b). We have also obtained a Golgi-apparatus-enriched preparation from suspension cultures of these cells and have described the galactosyl-glycoprotein transferase activity in some detail (Freilich *et al.*, 1975a, 1977). The Golgi-apparatus-enriched preparation has now been used to determine whether or not this organelle is the main site of chondroitin-synthesizing activity. This

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Golgi-apparatus-enriched preparation has also been used to describe some of the characteristics of chondroitin synthesis on exogenous acceptors.

Experimental procedures

Materials

UDP-[¹⁴C]glucuronic acid, UDP-[¹⁴C]galactose and [³H]leucine were purchased from New England Nuclear. Non-radioactive UDP-glucuronic acid was purchased from Sigma Chemical Co. UDP-*N*-acetylgalactosamine was synthesized as described previously (Silbert, 1964). Chondroitinase ABC, chondro-6-sulphatase, chondro-4-sulphatase, chondroitin 6-sulphate, chondroitin 4-sulphate and various disaccharides produced by degradation with chondroitinase ABC (Δ Di-OS, Δ Di-4S, Δ Di-6S) were purchased from Miles Laboratories. Hexasaccharide, pentasaccharide and tetrasaccharide from chondroitin 6-sulphate or chondroitin 4-sulphate were prepared as previously described (Rodén *et al.*, 1972; Silbert & Reppucci, 1976). *N*-Acetylchondrosine 4-sulphate (Di-4S) and *N*-acetylchondrosine 6-sulphate (Di-6S) were prepared by isolation of the disaccharide products after degradation of chondroitin 4-sulphate tetrasaccharide and chondroitin 6-sulphate tetrasaccharide by chondroitinase ABC. *N*-Acetylchondrosine was prepared as described previously (Silbert & Reppucci, 1976).

Methods

The mast cells utilized for these studies were originally derived from a Dunn-Potter P815 mouse mastocytoma. Maintenance of cell cultures and techniques for large-volume production with harvests as great as 14 ml of packed cells from a 12-litre spinner culture have been described (Lewis *et al.*, 1973a). Cell cultures of 250 ml were labelled with [³H]leucine as previously described and were pooled with 12-litre spinner cultures to provide [³H]protein for an estimate of protein content in the subsequent subcellular fractions. A Golgi-apparatus-enriched fraction and a 'mixed membrane' fraction from 8 ml of packed cells were prepared through intermediate 'Golgi-apparatus-rich' and 'membrane' stages as previously described (Freilich *et al.*, 1977). 'Membrane fraction II' as previously described was fractionated further. This fraction was added to 2.1 M-sucrose buffer sufficient to make an overall concentration of 1.9 M. This was layered on to 2.1 M-sucrose with a layer of 1.7 M-sucrose placed over it and centrifuged at 105 000 *g* for 75 min, resulting in a band at the 1.7 M/1.9 M interface and a smaller band at the 1.9 M/2.1 M interface. The bands at the 1.7 M/1.9 M and the 1.9 M/2.1 M interfaces were designated 'mixed membrane I' and 'mixed membrane II' respectively. The Golgi-apparatus-

enriched band, mixed membrane I band and mixed membrane II band were centrifuged at 105 000 *g* for 30 min. The major portion of each resulting pellet was utilized for electron microscopy as described previously (Freilich *et al.*, 1977). The Golgi-apparatus-enriched pellet demonstrated fields containing many Golgi stacks and parallel cisternae in addition to irregular membrane-bound spaces representing dilations of individual cisternae (Freilich *et al.*, 1977). The mixed membrane I and mixed membrane II fractions exhibited a mixture of vesicles appearing to be plasma membrane and/or smooth endoplasmic reticulum plus many free ribosomes. No rough endoplasmic reticulum or structures resembling Golgi cisternae could be found.

Galactosyltransferase activity was assayed with ovalbumin, desialylated degalactosylated orosomucoid and *N*-acetylglucosamine as acceptor substrates as previously reported (Freilich *et al.*, 1975b). For the determination of chondroitin-synthesizing activity, a typical incubation mixture with Golgi-apparatus-enriched preparations or other fractions contained 50 mM-Mes/HCl buffer, pH 6.5, containing 10 mM-MnCl₂, 0.5 mM-UDP-[¹⁴C]glucuronic acid (5.5×10^6 c.p.m./ μ mol) and 1.6 mM-UDP-*N*-acetylgalactosamine, with or without 3 mM-pentasaccharide, plus various amounts of the cellular fraction described above in a total volume of 0.025 ml. Similar incubations with UDP-[¹⁴C]glucuronic acid (40×10^6 c.p.m./ μ mol) were conducted to obtain products for identification and characterization. Incubations were conducted for 1–3 h at 37°C. After the incubation, each entire reaction mixture was spotted on Whatman no. 4 paper and chromatographed with butanol/acetic acid/1 M-NH₃ (2:3:1, by vol.) for 18 h. In this system labelled glycosaminoglycans formed on endogenous primers remain fixed to the origin. They cannot be eluted with water, but can be removed by treatment with alkali or proteolytic enzymes (Silbert, 1964). Labelled oligosaccharides or polysaccharides formed by addition to exogenous pentasaccharide also remain at the origin, but can be quantitatively removed by washing with water (Silbert & Reppucci, 1976). After chromatography, origins were washed with water and analysed for radioactivity. After this, the material remaining at the origins was incubated with 0.5 M-NaOH overnight at room temperature and samples were analysed.

¹⁴C-labelled material formed on endogenous primers and on exogenous oligosaccharides was subjected to degradation with chondroitinase ABC (Saito *et al.*, 1968). Products were chromatographed in the above chromatography system, and the resulting disaccharides were identified by their chromatographic behaviour and their susceptibilities to degradation by chondrosulphatases. Gel filtration of labelled oligosaccharides was per-

formed on a 1 cm × 60 cm column of Sepharose 6B and on a 2.7 cm × 120 cm column of Sephadex G-25, with 0.1 M-LiCl as eluent. Fractions from the former column were subjected to degradation with chondroitinase ABC, and the products were identified by chromatography as above.

Uronic acid-containing material was assayed by the modified carbazole method of Bitter & Muir (1962). Radioactivity was assayed with a liquid-scintillation spectrometer or with a low-background (0.5 c.p.m.) gas-flow planchette counter.

Results

Incorporation of [¹⁴C]glucuronic acid on to endogenous chondroitin or on to exogenous chondroitin oligosaccharides with the various subcellular fractions is summarized in Table 1. Incorporations were linear between 1 h and 3 h time points for all fractions. Results for 3 h incubations are shown. On the basis of [³H]protein or Lowry protein content, the final Golgi-apparatus-enriched fraction was enriched 13-fold compared with the homogenate for incorporation on to endogenous acceptor. However, this incorporation is a function of the presence of endogenous acceptors and therefore is not a true reflection of enzyme activity. Furthermore, the amount of incorporation on to endogenous acceptors was small, so that enrichments are only approximate.

Use of an oligosaccharide acceptor is a better means for determination of enzymic activity, since it provides a defined higher concentration of acceptor. Thus all fractions exhibited a much greater incorporation on to exogenous pentasaccharide than on to endogenous acceptor. On the basis of [³H]protein or Lowry protein content, the final Golgi-apparatus-enriched fraction was enriched more than 40-fold compared with the homogenate for incorporation on to exogenous pentasaccharide. The yield of chondroitin-synthesizing activity in Golgi-apparatus-enriched fractions, as measured by addition to oligosaccharides, was 16% of the activity in the starting homogenate. The final mixed membrane I and mixed membrane II fraction had much less chondroitin-synthesizing activity than had the final Golgi-apparatus-enriched fraction. The galactosyltransferase activities and 5'-nucleotidase activities of the fractions are shown in Table 1 for comparison and have been published previously (Freilich *et al.*, 1977). The galactosyltransferase activity for each fraction closely paralleled the chondroitin-synthesizing activity whereas the 5'-nucleotidase activity did not. Some of the chondroitin-synthesizing activity and galactosyltransferase activity was discarded in the first pellet, which contained intact cells and cell fragments. Activity was also lost between the bands of the various den-

Table 1. Formation of [¹⁴C]chondroitin and [¹⁴C]oligosaccharide with mastocytoma-cell fractions
Experimental details are given in the text.

Fraction	Protein (mg)		Incorporation of [¹⁴ C]glucuronic acid on to				Enzyme enrichment			
	Total	In assay	Endogenous acceptors (c.p.m.)	(nmol/mg of protein)	Exogenous pentasaccharide (c.p.m.)	(nmol/mg of protein)	Endogenous acceptor	Exogenous pentasaccharide	Galactosyltransferase*	5'-Nucleotidase
Homogenate	1540	0.36	170	0.09	4300	2.2	1	1	1	1
Golgi-apparatus-rich Membrane I	690	0.13	105	0.15	3300	4.6	1.6	2.1	1.2	1
Membrane II	153	0.105	125	0.21	5100	8.8	2.3	4	4.6	0.9
Pellets	390	0.24	180	0.14	4600	3.5	1.5	1.6	1.0	1.2
Golgi-apparatus-enriched	6	0.04	255	1.2	20200	92	13	42	59	12
Mixed membrane I	4	0.11	120	0.2	4700	7.7	2.2	3.5	1.7	8
Mixed membrane II	<0.5	0.015	<40	<0.5	1100	14	<6	6	<3	—

* Average of activity with three different acceptors (Freilich *et al.*, 1977).

sity-gradient centrifugations and in the supernatants of the final sedimentations. Final yield of chondroitin-synthesizing activity in the Golgi-apparatus-enriched pellet closely paralleled the final yield of galactosyltransferase activity.

Incubation with UDP-[^{14}C]glucuronic acid (40×10^6 c.p.m./ μmol) for 3 h with 0.002 ml of Golgi-apparatus-enriched fraction containing $40 \mu\text{g}$ of protein resulted in incorporation of 1.9×10^3 c.p.m. of [^{14}C]glucuronic acid on to endogenous material and 149×10^3 c.p.m. of [^{14}C]glucuronic acid on to exogenous pentasaccharide. Products of the incorporations were [^{14}C]chondroitin on endogenous acceptors and [^{14}C]chondroitin oligosaccharides on exogenous acceptors. The disaccharide products of chondroitinase ABC degradation of [^{14}C]labelled material formed with the Golgi-apparatus-enriched fraction are shown in Fig. 1. Degradation of the [^{14}C]labelled material formed on endogenous acceptors resulted in [^{14}C] $\Delta\text{Di-OS}$ as

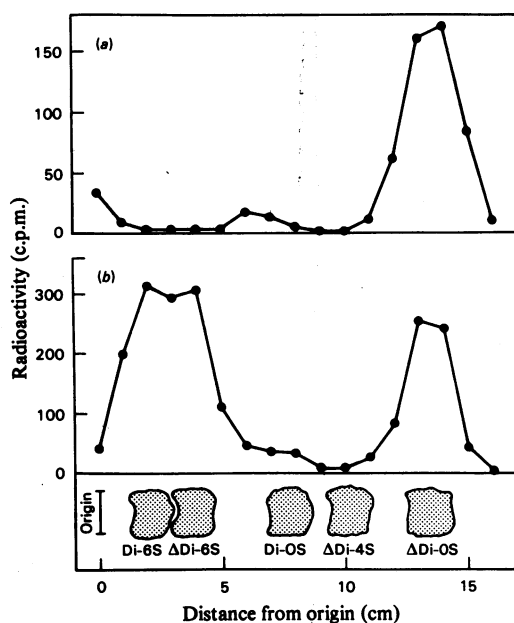


Fig. 1. Chondroitinase degradation products of [^{14}C]chondroitin and [^{14}C]oligosaccharides

Samples of (a) [^{14}C]labelled endogenous material and (b) [^{14}C]labelled oligosaccharides were degraded with chondroitinase ABC together with carrier chondroitin 6-sulphate. Products were chromatographed together with added $\Delta\text{Di-OS}$, Di-OS , $\Delta\text{Di-4S}$ and Di-6S as described in the Experimental procedures section. Strips (1 cm) were eluted and assayed for [^{14}C] radioactivity. Disaccharides were located by u.v. absorption and determination by the carbazole method.

the main product (Fig. 1a). Pentasaccharides obtained from both chondroitin 4-sulphate and chondroitin 6-sulphate were also used as acceptors of [^{14}C]glucuronic acid from UDP-[^{14}C]glucuronic acid. There was equal incorporation with each of these. Degradation of the [^{14}C]labelled material formed with either of these exogenous pentasaccharide preparations resulted in a mixture of [^{14}C]Di-6S, [^{14}C]Di-OS, [^{14}C] $\Delta\text{Di-6S}$ and [^{14}C] $\Delta\text{Di-OS}$ (Fig. 1b). The identity of the disaccharides was confirmed by their susceptibility or lack of susceptibility to degradation by chondro-6-sulphatase and chondro-4-sulphatase (Saito *et al.*, 1968).

The presence of saturated disaccharides such as [^{14}C]Di-6S or [^{14}C]Di-OS reflects the non-reducing end of the newly formed material, whereas unsaturated [^{14}C] $\Delta\text{Di-OS}$ or [^{14}C] $\Delta\text{Di-6S}$ is a product of the rest of the newly synthesized chains. Therefore the ratio of the unsaturated disaccharides to saturated disaccharides is an indication of chain length. Also, the relative amounts of labelled 6-sulphated disaccharides is inversely proportional to the size of the newly synthesized chains. Thus the material formed on endogenous primers was shown to be [^{14}C]chondroitin of substantial size, whereas the addition to pentasaccharide was much less in extent of polymerization. These results are similar in most respects to those previously reported with oligosaccharide acceptors and a chondroitin-synthesizing system from chick-embryo epiphyseal cartilage (Silbert & Reppucci, 1976).

No [^{14}C]Di-4S or [^{14}C] $\Delta\text{Di-4S}$ was found. Since the initial chondroitin 4-sulphate from which the pentasaccharide was prepared contained about 20% chondroitin 6-sulphate, it was clear that the incorporation was on to pentasaccharide 6-sulphate that was present in the pentasaccharide 4-sulphate preparation. Pentasaccharide with a non-reducing terminal *N*-acetylgalactosamine 4-sulphate thus did not act as an acceptor.

Chromatography on Sepharose 6B of the [^{14}C]labelled exogenous oligosaccharides is shown in Fig. 2(a). Most appeared in a large peak in the area of hexasaccharide and slightly larger oligosaccharides. However, in addition to this main peak, about one-quarter of the incorporation radioactivity appeared in the area of the chondroitin sulphate standard (fractions 24–32 and 33–42). Rechromatography of this material (Figs. 2b and 2c) indicated that approx. 6–8% of the products were as large as the chondroitin sulphate standard (mol.wt. range 40000–80000). This indicates that some pentasaccharide acted as a primer for synthesis of [^{14}C]chondroitin of substantial size. Total formation of the largest [^{14}C]chondroitin (Fig. 2b) was more than 5 times the amount of [^{14}C]chondroitin formed on endogenous acceptors. Rechromatography of the main peak (fractions 43–46 and 47–51) on Sepha-

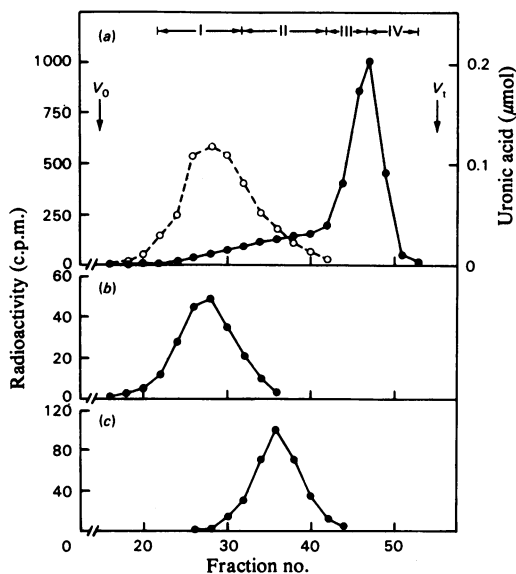


Fig. 2. Sepharose 6B chromatography of $[^{14}\text{C}]$ oligosaccharides

Oligosaccharides were chromatographed together with a chondroitin 6-sulphate standard (molecular-weight range 40000–80000) on a column (1 cm \times 60 cm) of Sepharose 6B. Blue Dextran and Phenol Red were used for determination of V_0 and V_t respectively. The eluent was 0.1 M-LiCl, and 1.0 ml fractions were collected. (a) Fractions were analysed for ^{14}C (●) and uronic acid (○). (b) Rechromatography of fractions 24–32. (c) Rechromatography of fractions 33–42.

dex G-25 is shown in Fig. 3, and confirms the size as a mixture of hexasaccharide and larger oligosaccharides.

Degradation of the larger chondroitin-like material with chondroitinase ABC is shown in Fig. 4. The site of chondroitin chain initiation will yield $\Delta\text{Di-6S}$, the non-reducing end of the chondroitin chain will yield either Di-OS or *N*-acetylgalactosamine, and the remainder of the chain will yield $\Delta\text{Di-OS}$. The amount of $\Delta\text{Di-6S}$ or Di-OS plus *N*-acetylgalactosamine relative to $\Delta\text{Di-OS}$ is a measure of chain length (Silbert, 1978). As shown in Fig. 4, almost all the products after digestion with chondroitinase ABC co-chromatographed with $\Delta\text{Di-OS}$. There was a small amount of radioactive material that co-chromatographed with $\Delta\text{Di-6S}$, but it was not sufficient to show on the graph, nor for further identification. The $\Delta\text{Di-OS}/\Delta\text{Di-6S}$ ratio was at least 50:1, which was consistent with the size of the $[^{14}\text{C}]$ chondroitin demonstrated by chromatography on Sepharose 6B (Fig. 2). There was some

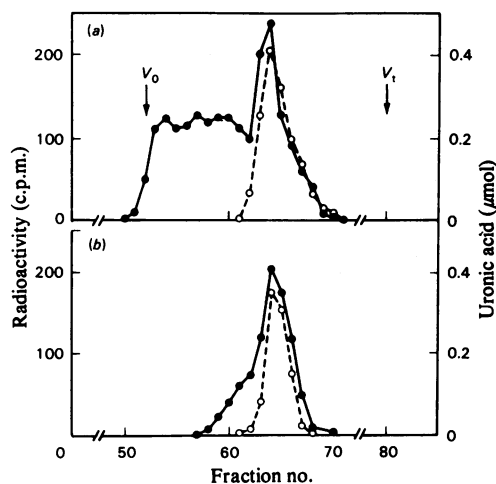


Fig. 3. Sephadex G-25 chromatography of $[^{14}\text{C}]$ oligosaccharides

Fractions 43–46 (a) and 47–51 (b) from the Sepharose 6B chromatogram (Fig. 2) were chromatographed together with hexasaccharide on a column (2.7 cm \times 120 cm) of Sephadex G-25. The eluent was 0.1 M-LiCl. Fractions of volume 5 ml were collected and analysed for radioactivity (●) and uronic acid (○).

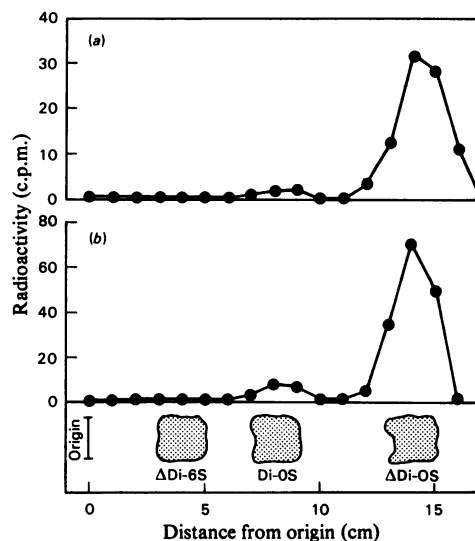


Fig. 4. Chondroitinase degradation products of $[^{14}\text{C}]$ -chondroitin formed on pentasaccharide primer. Samples of the $[^{14}\text{C}]$ chondroitin from Fig. 2, namely fractions 24–32 (a) and 33–42 (b) were degraded with chondroitinase ABC, chromatographed and assayed as indicated in Fig. 1.

material found in the region of Di-OS, but it was insufficient for further characterization. The amounts were high relative to the amount of the [^{14}C]chondroitin and high relative to the small amount of $\Delta\text{Di-6S}$, suggesting that it may be a contaminant. A similar peak was found in other chromatograms and was usually slightly out of position with Di-OS standards (Fig. 1a).

Discussion

Chondrocytes in culture have been shown to require several minutes after a pulse of [^3H]serine before formation of [^3H]proteoglycan is maximal (DeLuca *et al.*, 1978). In contrast, [^{35}S]sulphate is incorporated more rapidly into glycosaminoglycan, suggesting that the [^3H]protein portion is synthesized first with subsequent addition of the [^{35}S]glycosaminoglycan portion. These findings are consistent with other work that has demonstrated the biosynthesis of chondroitin sulphate glycosaminoglycan on chondroitin primers attached to a protein core (Richmond *et al.*, 1973). Moreover, earlier work with puromycin (Telser *et al.*, 1965) indicated that the glycosaminoglycan portions of proteoglycans were added subsequent to formation of the protein core.

It has been shown by radioautography that ^{35}S and ^3H appear in the Golgi region of chondrocytes within 5–10 min after injection of [^{35}S]sulphate, [^3H]glucose or [^3H]galactose into animals (Peterson & Leblond, 1964; Neutra & Leblond, 1966) and within 3 min after incubation of [^{35}S]sulphate with cartilage fragments (Godman & Lane, 1964). The appearance of labelled proteins in the Golgi apparatus after injection of labelled amino acids takes longer. These results suggest that sulphation and perhaps glycosaminoglycan synthesis take place in the Golgi apparatus. However, other loci for glycosaminoglycan synthesis have been suggested. Microsomal material from chick-embryo cartilage has been separated into membrane fractions enriched for rough and smooth endoplasmic reticulum, and enzymic activities involved in chondroitin sulphate glycosaminoglycan synthesis have been assayed (Horwitz & Dorfman, 1968). It was concluded from this work that the elongation of glycosaminoglycans was a gradual process occurring as the incomplete proteoglycan molecules moved through the rough and the smooth endoplasmic reticulum to the Golgi apparatus. However, the methods reported did not provide for a Golgi-apparatus-enriched fraction, so that the activities could have been due to fragments of Golgi apparatus distributed through all of the fractions.

We have attempted to answer the question of locale for chondroitin synthesis by using chondroitin sulphate-synthesizing cells that provided a better opportunity than cartilage cells for obtaining

Golgi-apparatus-enriched fractions. The mastocytoma cells used for this work can be grown in large amounts in suspension culture and can be ruptured gently because of the absence of extracellular matrix. In contrast with the use of cartilage as a cell source, this permits an easier preparation of fractions with recognizable Golgi-apparatus elements. Use of these Golgi-apparatus-enriched preparations has now demonstrated that they have a marked enrichment for chondroitin-synthesizing activity. All the other recognizable membrane fractions, which would include endoplasmic reticulum and plasma membrane, had little of this activity. Moreover, the chondroitin-synthesizing activity closely paralleled the galactosyltransferase activity, which is generally considered to be a biochemical marker for the Golgi apparatus (Fleischer *et al.*, 1969; Morr , 1971). The chondroitin-synthesizing activity did not parallel the 5'-nucleotidase, which is considered to be a more general membrane marker. Other markers were not examined because of severe limitations in the amounts of material available for the assays.

It appears from the present work that chondroitin glycosaminoglycan formation occurs primarily in the Golgi apparatus. Since sulphation accompanies or follows chondroitin synthesis (DeLuca *et al.*, 1973), it is probable that sulphation also occurs in the Golgi apparatus. Formation of the galactosylgalactosylxylosyl linkage between protein and glycosaminoglycan was not examined.

We have previously studied the addition of sugars on to endogenous chondroitin primer and on to exogenous pentasaccharides by using microsomal preparations from chick-embryo cartilage (Richmond *et al.*, 1973). Although polymerization occurred readily on endogenous material, there was no synthesis of long-chain polymer on oligosaccharide acceptors; only a few sugar residues could be added to each oligosaccharide molecule. We have now performed similar experiments with the Golgi-apparatus-enriched preparations from mastocytoma cells. Most of the addition to exogenous oligosaccharides was similar to that found with the cartilage microsomal system. However, unlike the results obtained with the cartilage system, there was a significant amount of formation of large-sized polymers. The explanation for this is not apparent. One possibility may be fixation of some of the exogenous primer to the site of polymerizing enzymes, thus approximating the fixation that is present with endogenous primers. Whatever the mechanism, the polymerization on oligosaccharides with the mast-cell Golgi-apparatus-enriched system is the first demonstration of cell-free glycosaminoglycan polymer formation on a defined acceptor.

The mast-cell chondroitin sulphate is all chondroitin 4-sulphate (Lewis *et al.*, 1973a,b); no

6-sulphated material is made by these cells. Nevertheless, pentasaccharide ending in *N*-acetylgalactosamine 4-sulphate was inactive as an acceptor of [¹⁴C]glucuronic acid, whereas the pentasaccharide ending in *N*-acetylgalactosamine 6-sulphate was a good acceptor. A similar phenomenon was previously reported in the cartilage microsomal system (Rodén *et al.*, 1972; Silbert & Reppucci, 1976), suggesting that this may be a general phenomenon for all chondroitin sulphate-synthesizing systems. Therefore the specific formation of chondroitin 4-sulphate or chondroitin 6-sulphate appears to be unrelated to any difference in the glucuronyltransferase involved in the formation of these two compounds.

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