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Endocytosis and degradation of chondroitin sulphate by liver endothelial cells

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Intravenously administered chondroitin sulphate, chemically labelled by [^{3}H]acetylation of partially deacetylated polysaccharide, was taken up and degraded by the non-parenchymal cells of the liver. Studies using primary monolayer cultures of pure Kupffer cells, liver endothelial cells and parenchymal cells revealed that [^{3}H]chondroitin sulphate was taken up and degraded by the liver endothelial cells only. Binding studies at 4°C with [^{3}H]chondroitin sulphate and ^{125}I-chondroitin sulphate proteoglycan indicated that the glycosaminoglycan and the proteoglycan are recognized by the same binding sites on the liver endothelial cells. The ability of hyaluronic acid to compete with the labelled ligands for binding suggested that the binding site is identical with the recently described hyaluronate receptor on the liver endothelial cells [Smedsrød, Pertoft, Eriksson, Fraser & Laurent (1984) *Biochem. J.* **223**, 617–626]. Fluorescein-labelled chondroitin sulphate proteoglycan accumulated in perinuclear vesicles of the liver endothelial cells, indicating that the proteoglycan is internalized and transported to the lysosomes. The finding that [^{3}H]chondroitin sulphate and ^{125}I-chondroitin sulphate proteoglycan were degraded by the liver endothelial cells to low-molecular-mass radioactive products suggested that both the polysaccharide chain and the core protein were catabolized by the cells.

Chondroitin sulphate, a member of the family of glycosaminoglycans, consists of repeating disaccharide units of glucuronic acid and *N*-acetylgalactosamine, the latter being *O*-sulphated in the 4- and/or 6-positions (Rodén, 1980). Several chains of chondroitin sulphate covalently linked to a core protein form chondroitin sulphate–proteoglycans, which are major components of the connective tissue.

The normal catabolic pathways of chondroitin sulphate are poorly understood (Rodén, 1980). Nevertheless, previous work has shown that the liver is the main site of uptake and degradation of intravenously injected peptide-linked and free chains of chondroitin sulphate (Wood *et al.*, 1973, 1976). The cell type responsible for the clearance of this polysaccharide has not yet been identified. Therefore, in the present study, a recently developed technique to establish pure cultures of intact Kupffer cells, liver endothelial cells and parenchymal cells from a single rat liver (Smedsrød & Pertoft, 1985), was employed to determine the cell type capable of endocytosis and breakdown of chondroitin sulphate and chondroitin sulphate proteoglycan. The present results along with a previous report on the endocytosis of hyaluronic acid (Smedsrød *et al.*, 1984) suggest that the liver endothelial cells may play a central role in the normal turnover of connective-tissue polysaccharides.

Materials and methods

Chemicals, animals and tissue-culture medium

[^{3}H]Acetic anhydride (500Ci/mol) and Na^{125}I were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Collagenase (type V), bovine serum albumin (fraction V), dinitrophenylalanine, Hepes, Tes, Tricine and sodium dodecyl sulphate were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Pronase (B-grade) was from Calbiochem, Los Angeles, CA, U.S.A., and chondroitinase ABC (EC 4.2.2.4) was from Seikagaku, Tokyo, Japan. Percoll, Sephadex G-25 (PD-10 columns), G-100, G-200, and Blue Dextran were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Fluorescein isothiocyanate was obtained from Fluka A.G., Buchs, Switzerland. Male Sprague–Dawley rats weighing about 200g were from Anticimex, Stockholm, Sweden. Cul-

ture medium (RPMI 1640) and the supplements L-glutamine, gentamicin and fungizone (final concentrations in medium, 2mM, 200μg/ml and 50μg/ml respectively), were from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. Chondroitin sulphate and chondroitin sulphate proteoglycan (A1D1) from bovine nasal cartilage were kindly given by Dr. Å. Wasteson, University of Uppsala, Uppsala, Sweden, and Dr. B. Caterson, University of Morgantown, Morgantown, WV, U.S.A., respectively.

Radiolabelling of ligands

Labelling of chondroitin sulphate with ^3H was accomplished by chemical [^3H]acetylation of partially deacetylated polysaccharide as described by Höök *et al.* (1982). The final product had a specific radioactivity of 5.6×10^4 c.p.m./μg of uronic acid (preparation I) and 2.6×10^5 c.p.m./μg of uronic acid (preparation II). Digestion of [^3H]chondroitin sulphate with chondroitinase ABC as described by Kolset *et al.* (1983) resulted in a quantitative conversion of the starting material into a labelled product that was eluted in the position of a disaccharide on gel chromatography on a Sephadex G-50 column. Hence, it is concluded that the labelling procedure did not significantly alter the structure of the polysaccharide, and, furthermore, the label was associated with the polysaccharide and not with any remnant peptide residues that may be attached to the chondroitin sulphate chains, even after the removal of the core protein. Labelling of chondroitin sulphate proteoglycan with ^{125}I was performed with Iodo-beads (Pierce Chemical Co., Rockford, IL, U.S.A.). The resulting specific radioactivity of the proteoglycan was 9×10^4 c.p.m./μg (preparation I) and 8×10^5 c.p.m./μg (preparation II). ^3H Radioactivity was measured in a Beckman LS 5800 liquid-scintillation system (Beckman Instruments, Irvine, CA, U.S.A.). ^{125}I Radioactivity was measured in a Packard 5260 Auto-Gamma scintillation spectrometer.

Conjugation of chondroitin sulphate proteoglycan with fluorescein

The proteoglycan (20mg/ml) was incubated with fluorescein isothiocyanate (50μg/ml) in 0.1M-carbonate/bicarbonate buffer, pH9, at 4°C for 18h. Dye that had not reacted was removed by gel filtration through a PD-10 column in 0.13M-NaCl/0.02M-phosphate buffer, pH7.4. Sterile filtered fluoresceinated chondroitin sulphate proteoglycan was stored at 4°C.

Preparation of rat liver cells after injection of [^3H]chondroitin sulphate

At 15min after an intravenous administration of [^3H]chondroitin sulphate (preparation I, 5×10^6 c.p.m. in 0.5ml of 0.9% NaCl) the liver was dispersed by collagenase perfusion as described by Öbrink (1982). The resulting suspension of liver cells was divided into two parts, which were subjected to differential centrifugation and Pronase treatment respectively (see Scheme 1 below).

Differential centrifugation. One part of the suspension of collagenase-dispersed liver cells was diluted to 100ml with Buffer 3 (Öbrink, 1982; 137mM-NaCl/5.6mM-KCl/0.65mM-$MgSO_4$/1.2mM-$CaCl_2$/10mM-Hepes/bovine serum albumin (15g/l), pH7.4). After passage through a nylon filter (mesh width 50μm; Monyl; Züricher Beutelfabrik AG, Rüschlikon, Switzerland) to remove aggregated cells and tissue debris, 15ml of the suspension (denoted Fraction 1D in Scheme 1) were saved for analytical density-gradient centrifugation, 2ml for estimation of cell number and 2ml for measurement of ^3H radioactivity. The remaining 81ml were centrifuged for 2min at 70*g*. The supernatant was saved and the pellet was washed twice by resuspension in 75ml of Buffer 3, followed by centrifugation for 2min at 70*g*. The final pellet was resuspended in 22ml of Buffer 3 (denoted Fraction 2D in Scheme 1). A 15ml portion of this suspension was analysed by density-gradient centrifugation, and the remaining 7ml were used to determine the number of cells and the amount of ^3H radioactivity. A suspension enriched in non-parenchymal cells (Fraction 3D in Scheme 1) was prepared, by centrifugation at 400*g* for 6min, of the supernatant from the first centrifugation of Fraction 1D. The pellet was resuspended in 22ml of Buffer 3, and the resulting Fraction, 3D, was analysed as described for Fractions 1D and 2D.

Pronase treatment. The remaining part of the dispersed liver was diluted to 100ml with a buffer, pH7.6, containing 68mM-NaCl, 6.4mM-KCl, 1.1mM-KH_2PO_4, 0.7mM-Na_2SO_4, 30mM-Hepes, 30mM-Tes, 36mM-Tricine and 52.5mM-NaOH (Seljelid & Smedsrød, 1980). The suspension was filtered through gauze and incubated with Pronase (0.15%, w/v) at 37°C for 30min. After passage through a nylon filter (mesh width 50μm) and subsequent centrifugation at 400*g* for 6min, the pellet was suspended in 22ml of Buffer 3. This suspension (denoted 'Fraction P' in Scheme 1 below) was analysed in the same manner as Fraction 1D, 2D and 3D (see above).

Analytical density-gradient centrifugation

Portions (15ml each) of Fractions 1D, 2D, 3D, and P respectively (see Fig. 1 below), were layered on top of 75ml of preformed iso-osmotic (10–80%, w/v) Percoll gradients and centrifuged at 800*g* for 30min in a swing-out rotor at 4°C (Pertoft *et al.*, 1979). Fractions (approx. 5ml) were collected from the top of the tubes by displacing the gradients

with 60% (w/v) sucrose. The densities of the fractions were measured in organic density columns (Miller & Gasek, 1960).

Isolation and cultivation of parenchymal and non-parenchymal cells

The preparation and characterization of isolated liver cells without the use of Pronase has recently been described by Smedsrød & Pertoft (1985). The cells obtained were purified with less than 5% cross-contamination. The Kupffer cells were cultivated on glass or plastic; the liver endothelial cells and the parenchymal cells were cultivated on supports coated with fibronectin. The cells were grown in Costar dishes (Costar, Cambridge, MA, U.S.A.) on growth areas of 2 and 9.6 cm^2 respectively. The growth densities of cells were: Kupffer cells, 7×10^4 cells/cm^2; liver endothelial cells, 2×10^5 cells/cm^2; and parenchymal cells, 1×10^5 cells/cm^2. Studies on binding and endocytosis were carried out in the presence of 1% (w/v) bovine serum albumin.

Results

Interaction of liver cells with circulating [^{3}H]chondroitin sulphate

In order to identify the cell type(s) responsible for the clearance and subsequent degradation of chondroitin sulphate in the liver, a rat was injected with [^{3}H]chondroitin sulphate and parenchymal and non-parenchymal cells were separated after collagenase perfusion of the liver, as outlined in Scheme 1. Differential centrifugation of the cell suspension obtained after collagenase perfusion (Fraction 1D) resulted in partial separation of the larger (30–40 μm) often binucleated parenchymal cells (Fraction 2D) and the smaller (10 μm) non-parenchymal cells (Fraction 3D). Pronase treatment by the method of Seljelid & Smedsrød (1980) selectively destroyed the parenchymal cells and thus produced a preparation enriched in non-parenchymal cells (Fraction P). As shown in Fig. 1, the amount of ^{3}H radioactivity recovered in the fractions correlated well with the number of non-

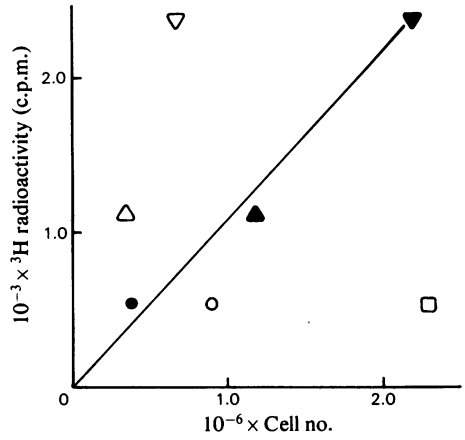
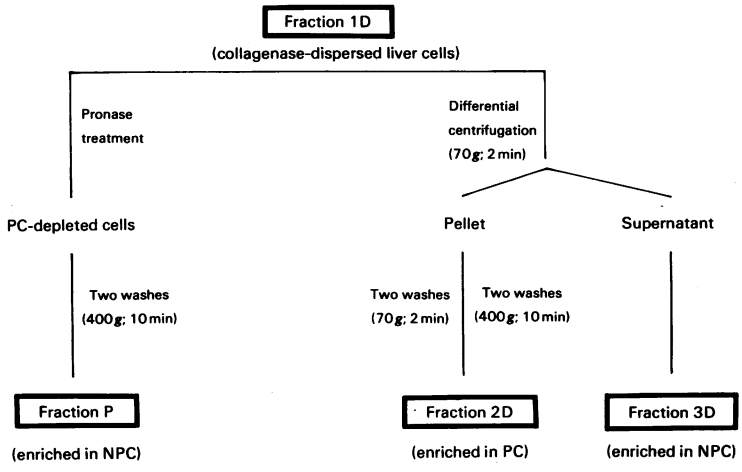


Fig. 1. *Correlation between the content of [^{3}H]chondroitin sulphate and the number of parenchymal or non-parenchymal cells*

The amount of ^{3}H radioactivity in 1 ml of Fractions 1D (○, ●), 2D (□), 3D (△, ▲) and P (▽, ▼) respectively (see Scheme 1) was determined and plotted against the density (no./ml) of parenchymal cells (open symbols) and non-parenchymal cells (closed symbols) respectively.



Scheme 1. *Procedure used for the preparation of fractions enriched in parenchymal (PC) and non-parenchymal (NPC) cells after intravenous administration of [^{3}H]chondroitin sulphate*

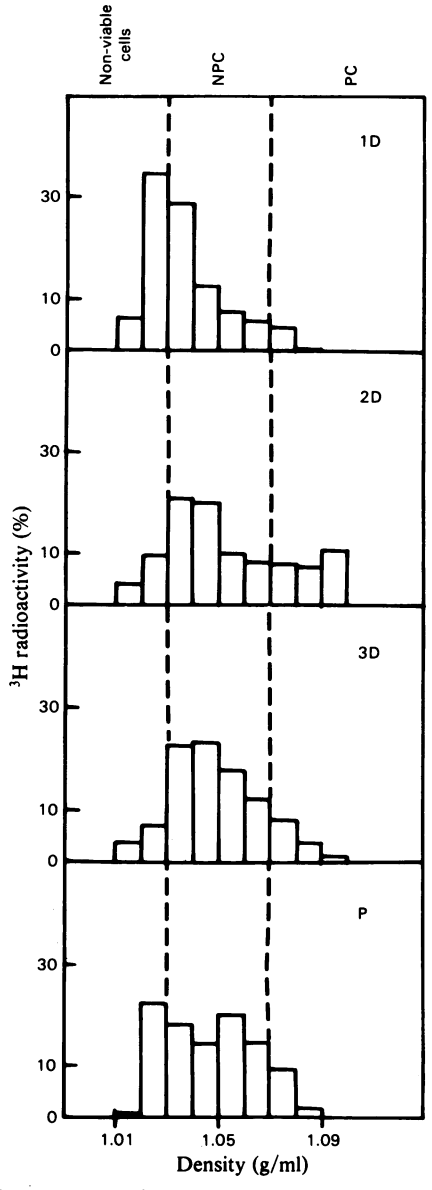


Fig. 2. *Density-gradient centrifugation of liver-cell fractions from a rat injected with [^{3}H]chondroitin sulphate*
Portions (15ml each) of Fractions 1D, 2D, 3D and P (see Scheme 1) respectively, prepared from the liver of a rat 15min after intravenous injection of [^{3}H]chondroitin sulphate (preparation I), were layered on top of gradients of Percoll. After centrifugation, fractions (approx. 5ml) were collected and analysed for density and ^{3}H radioactivity as described in the Materials and methods section. Fraction 1D: 13.5×10^6 parenchymal cells (PC); 5.7×10^6 non-parenchymal cells (NPC). Fraction 2D: 34.5×10^6 parenchymal cells; non-parenchymal cells not counted. Fraction 3D: 5.3×10^6 parenchymal cells; 18×10^6 non-parenchymal cells. Fraction P: 10.5×10^6 parenchymal cells; 33×10^6 non-parenchymal cells.

parenchymal cells, indicating that the latter group of cells was responsible for the uptake of [^{3}H]chondroitin sulphate.

Further purification of the four cell fractions was achieved by density-gradient centrifugation in Percoll. After centrifugation, parenchymal cells were recovered at a buoyant density of 1.07–1.09g/ml, non-parenchymal cells at a buoyant density of 1.03–1.07g/ml, and non-viable cells and cell debris on top of the gradient (Pertoft *et al.*, 1979). As shown in Fig. 2, most of the ^{3}H radioactivity of all four fractions was present in the non-parenchymal cell fraction.

A sample from Fraction 3D was analysed by gel chromatography on Sephadex G-200. As shown in Fig. 3, the injected [^{3}H]chondroitin sulphate was eluted at a later position than the starting material and had hence been subjected to degradation. This result may indicate that the non-parenchymal cells have the ability to degrade chondroitin sulphate. Alternatively, the [^{3}H]chondroitin sulphate could have been subjected to degradation before uptake by the non-parenchymal cells.

Degradation in vitro of [^{3}H]chondroitin sulphate by isolated liver endothelial cells

The ability of cultured liver cells to degrade [^{3}H]chondroitin sulphate was investigated. Cul-

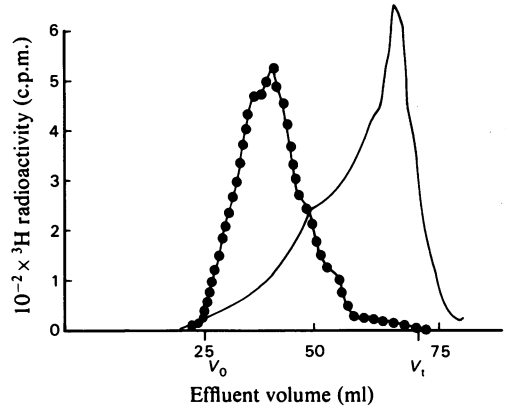


Fig. 3. *Degradation of [^{3}H]chondroitin sulphate by rat liver cells in vivo*
A sample (2ml) of Fraction 1D (see Scheme 1) from the liver of a rat injected with [^{3}H]chondroitin sulphate (preparation I) was digested with papain and analysed by gel chromatography on a column of Sephadex G-200, eluted with 0.2M-NaCl at a flow rate of 4.5ml/h. Fractions (1.5ml) were collected and analysed for ^{3}H radioactivity. Dextran Blue and dinitrophenylalanine were used as visual markers for the void (V_0) and the total (V_t) volume respectively. ●, Elution profile of [^{3}H]chondroitin sulphate before injection; ——, elution profile of ^{3}H-labelled material recovered from liver cells after injection.

tures of liver endothelial cells, Kupffer cells or parenchymal cells were incubated with [^{3}H]chondroitin sulphate for 6 or 24h at 37°C. The media were collected and centrifuged for 5min at 400*g* to remove detached cells. Gel chromatography on Sephadex G-100 of the media (Fig. 4) revealed that the liver endothelial cells were capable of degrading the added [^{3}H]chondroitin sulphate, whereas neither the parenchymal cells nor the Kupffer cells showed this activity. After 6h of incubation with cultures of liver endothelial cells, a large proportion of the polysaccharide had been degraded to low-M_r products that were eluted in a broad peak close to the total volume (Fig. 4*d*). Prolonged incubation (24h) resulted in a more complete degradation, yielding a peak that contained homogeneously sized degradation products.

To determine the extent of extracellular degradation, spent media from 12h-old liver-endothelial-cell cultures were collected, incubated with [^{3}H]chondroitin sulphate for another 12h at 37°C, and finally subjected to gel chromatography on Sephadex G-100. The resulting chromatogram (not shown) indicated that no degradation of the glycosaminoglycan had taken place in the conditioned medium.

Binding of [^{3}H]chondroitin sulphate and ^{125}I-chondroitin sulphate proteoglycan to liver endothelial cells

By incubating monolayers of liver endothelial cells at 4°C, internalization is prevented, and any ligand associated with the cells can be taken as bound to the cell surface. The binding at 4°C of [^{3}H]chondroitin sulphate to cultures of liver endothelial cells could be largely inhibited by including, in the incubation medium, excess amounts of unlabelled chondroitin sulphate, chondroitin sulphate proteoglycan or hyaluronic acid, whereas ovalbumin, which has been previously shown to be taken up in a specific manner by these cells (Smedsrød *et al.*, 1982), had virtually no inhibitory effect (Table 1).

The binding of ^{125}I-chondroitin sulphate proteoglycan could be largely inhibited by the presence of excess amounts of unlabelled chondroitin sulphate or hyaluronic acid (Table 1). Ovalbumin did not significantly inhibit the binding of ^{125}I-chondroitin sulphate proteoglycan.

Uptake and degradation of chondroitin sulphate proteoglycan in liver endothelial cells

As revealed in Fig. 5, fluorecein-labelled chondroitin sulphate proteoglycan, incubated with cultures of liver endothelial cells for 12h at 37°C, was accumulated in perinuclear vacuoles. No uptake was observed when this ligand was incubated with parenchymal cells or Kupffer cells *in vitro*.

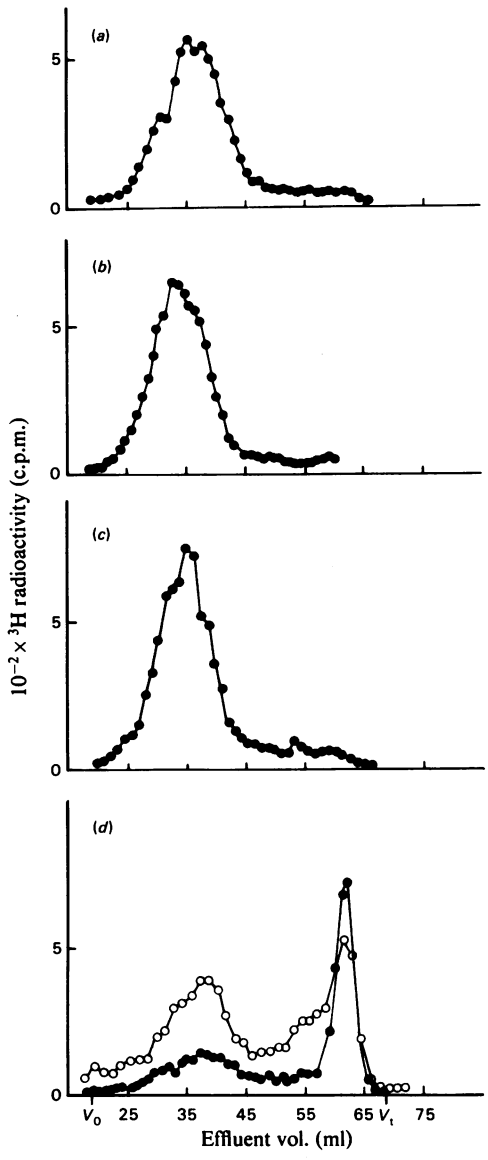


Fig. 4. *Degradation of [^{3}H]chondroitin sulphate by rat liver cells in vitro*

Cultures (2cm^2 growth area) of purified populations of rat liver cells were incubated at 37°C with approx. 10000c.p.m. of [^{3}H]chondroitin sulphate (preparation II). After 6 or 24h, media were collected and analysed by gel chromatography on a column of Sephadex G-100 eluted with 1M-NaCl at a flow rate of 6ml/h. Fractions (1ml) were collected and analysed for ^{3}H radioactivity. Dextran Blue and dinitrophenylalanine were used as markers for the void (V_0) and the total (V_t) volume respectively. (*a*) Incubation in culture medium without cells for 24h; (*b*) incubation with Kupffer cells (1.4×10^5 cells/culture) for 6h; (*c*) incubation with parenchymal cells (2×10^5 cells/culture) for 6h; (*d*) incubation with liver endothelial cells (4×10^5 cells/culture) for 6h (○) or 24h (●).

Table 1. *Specificity of binding of [^{3}H]chondroitin sulphate and ^{125}I-chondroitin sulphate proteoglycan at 4°C to cultivated liver endothelial cells*

Confluent monolayers of liver endothelial cells (9.6cm^2 growth area containing approx. 2×10^6 cells) were incubated with [^{3}H]chondroitin sulphate or ^{125}I-chondroitin sulphate proteoglycan (20ng/ml) at 4°C without, or in the presence of, unlabelled chondroitin sulphate, chondroitin sulphate proteoglycan, hyaluronic acid or ovalbumin in a volume of 500μl/dish. After 24h the cultures were washed to remove unbound label. Radioactivity remaining in the dishes was determined after solubilization with 1% (w/v) sodium dodecyl sulphate containing 0.3M-NaOH.

Inhibitor added	Concn. (μg/ml)	Bound radioactivity (%)* [^{3}H]Chondroitin sulphate	Bound radioactivity (%)* ^{125}I-chondroitin sulphate proteoglycan
None	—	100	100
Chondroitin sulphate	0.1	34	82
	1.0	23	67
	10.0	18	49
Chondroitin sulphate proteoglycan	0.1	23	24
	1.0	15	6
	10.0	10	3
Hyaluronic acid	0.1	45	77
	1.0	38	32
	10.0	28	30
Ovalbumin	2500.0	95	84

* The amount of radioactivity bound in the presence of inhibitor is expressed as a percentage of the amount bound in the absence of inhibitor. The percentages bound to cell-free substrate were 25% ([^{3}H]chondroitin sulphate) and 2.5% (^{125}I-chondroitin sulphate proteoglycan).

Table 2. *Degradation of ^{125}I-chondroitin sulphate proteoglycan by cultivated liver endothelial cells*

Confluent monolayers of liver endothelial cells (2cm^2 growth area containing approx. 0.4×10^6 cells) were incubated with ^{125}I-chondroitin sulphate proteoglycan at 37°C in a volume of 200μl of medium/dish. After 18h the media were collected and chromatographed on PD-10 columns and eluted with phosphate-buffered saline (see Table 1). Fractions (0.5ml) were collected and analysed for ^{125}I radioactivity. Low-M_r material eluted in the total volume (V_t) represented degraded material. The label remaining in the dishes after three successive washings were determined after solubilization with 1% (w/v) sodium dodecyl sulphate containing 0.3M-NaOH.

Expt.	Amount of label added (c.p.m.)	Degradation (material eluted in V_t) (%)	Amount remaining in the dishes (%)
Cell-free control	37000	4	1
Liver endothelial cells	2500	60	4
	37000	56	3

Gel chromatography, on a Sephadex G-25 column, of culture medium from liver endothelial cells incubated for 18h at 37°C with ^{125}I-chondroitin sulphate proteoglycan showed that 56–60% of the added ^{125}I radioactivity was eluted in the total volume of the column (Table 2), indicating that the protein core of the proteoglycan was degraded by the cells to low-M_r products.

Discussion

It has been previously reported that intravenously administered preparations of peptide-free and peptide-bound chondroitin sulphate are taken up and degraded by the liver (Wood *et al.*, 1973, 1976). In the present study, two lines of evidence suggest that injected [^{3}H]chondroitin sulphate is cleared mainly by the non-parenchymal liver cells. First, proportionality was found between the number of non-parenchymal cells and the amount of radioactivity in preparations of liver cells obtained after Pronase treatment or differential centrifugation (Fig. 1). In contrast, no such correlation was found for the parenchymal cells. Second, Percoll separations of these preparations revealed that the radioactivity co-separated with the non-parenchymal cells (Fig. 2). The demonstration of low-M_r ^{3}H-labelled products in the recovered non-parenchymal cells (Fig. 3) suggests degradation of the sequestered ligand by these cells. These results indicate that the non-parenchymal cells are responsible for the clearance by the liver

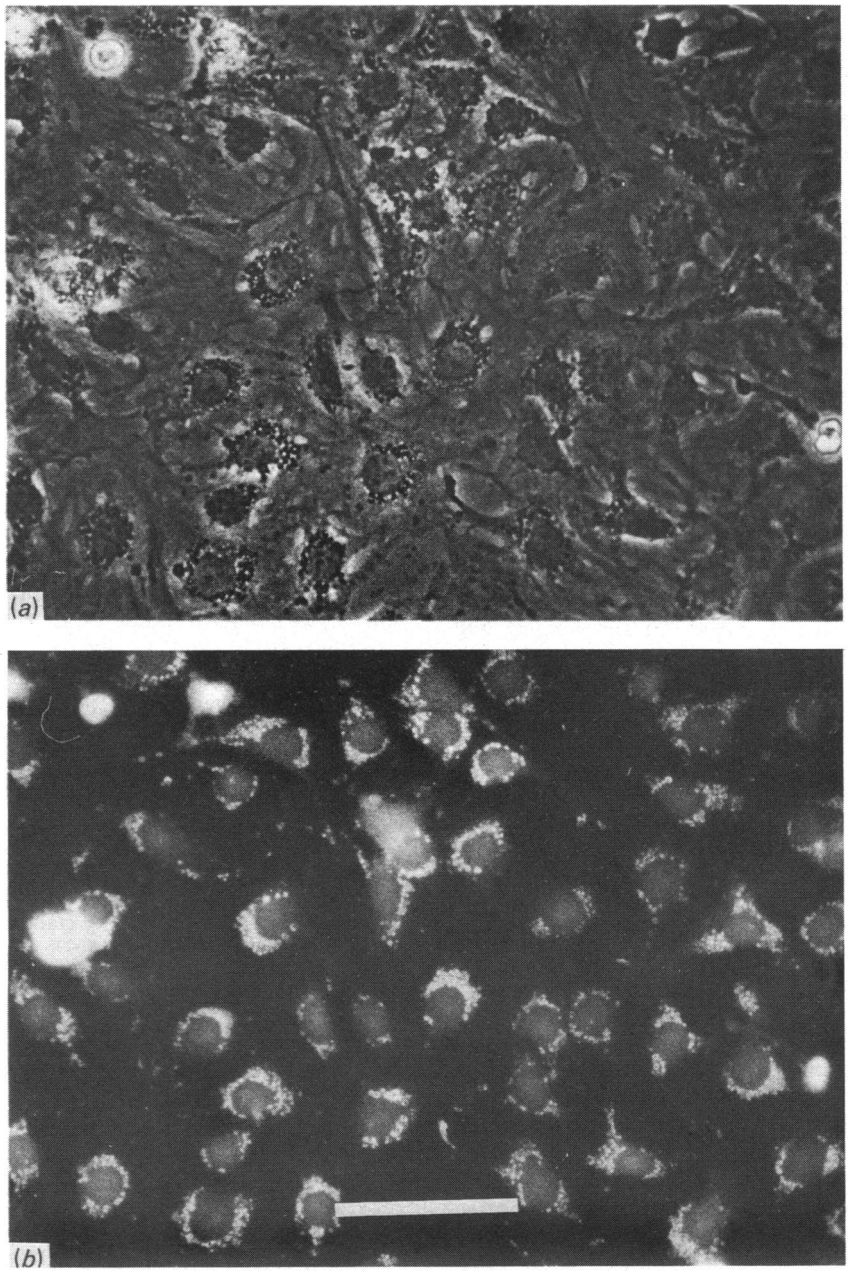


Fig. 5. *Uptake of fluorescein-labelled chondroitin sulphate proteoglycan by cultured liver endothelial cells*
Monolayers of liver endothelial cells were incubated with fluorescein-labelled chondroitin sulphate proteoglycan (0.1 mg/ml) for 12 h and prepared for microscopic examination. (*a*) Phase-contrast image; (*b*), fluorescence image. The bar represents 50 μm.

of intravenously administered [^{3}H]chondroitin sulphate.

Monolayers of liver endothelial cells, but not cultures of Kupffer cells or parenchymal cells, had the ability to degrade [^{3}H]chondroitin sulphate (Fig. 4). The uptake of fluorescein-labelled proteoglycan (Fig. 5) was observed in the liver endothelial cells only. These findings *in vitro* suggest that the liver endothelial cells are the site of both uptake and breakdown in the liver of circulating chondroitin sulphate.

W. Eskild, B. Smedsrød & T. Berg (unpublished

work) showed that endocytic antagonists, such as NH_3 and monensin, effectively inhibit the uptake and degradation of [^{3}H]chondroitin sulphate by preparations of liver endothelial cells *in vitro*. This finding, along with the observations that conditioned medium from cultures of liver endothelial cells does not degrade the polysaccharide, and that fluorescein-labelled proteoglycan (Fig. 5) accumulates in the liver endothelial cells in perinuclear vesicles, probably secondary lysosomes, indicate that chondroitin sulphate, as well as chondroitin sulphate proteoglycan, are endocytosed and then degraded intralysosomally.

The binding of ^{125}I-chondroitin sulphate proteoglycan to cultivated liver endothelial cells could be inhibited by the presence of unlabelled chondroitin sulphate and, conversely, the proteoglycan could effectively inhibit the binding of [^{3}H]chondroitin sulphate, indicating that the binding site recognizes the polysaccharide part of the proteoglycan (Table 1). Moreover, the finding that hyaluronic acid, but not ovalbumin, could inhibit the binding of both [^{3}H]chondroitin sulphate and ^{125}I-chondroitin sulphate proteoglycan indicates that the binding sites are identical with the recently reported receptors on the liver endothelial cells for the endocytosis of hyaluronic acid (Smedsrød *et al.*, 1984). In the same report it was noted that the binding of [^{3}H]hyaluronic acid could be inhibited by the presence of unlabelled chondroitin sulphate, but was unaffected by the presence of ovalbumin, thus giving support to the notion of a common receptor on the liver endothelial cells for the two polysaccharides. Since ovalbumin had no inhibitory effect, it is concluded that the receptor which recognizes this and other glycoproteins with terminal mannose and *N*-acetylglucosamine (Praaning-van Dalen *et al.*, 1982) is not involved in the binding of chondroitin sulphate by the liver endothelial cells.

Saito & Uzman (1971*a*) studied the uptake of [^{35}S]chondroitin sulphate by the Chinese-hamster cell line A_{12} VIII and found that subsequent to internalization the polysaccharide was transported back to the medium by a process termed 'reversal pinocytosis'. Moreover, degradation of the polysaccharide was not detected, and heparin and poly-L-lysine, but not hyaluronic acid, could inhibit the uptake (Saito & Uzman, 1971*b*). Since our results show that the uptake of chondroitin sulphate by liver endothelial cells leads to degradation of the ligand, and further, that hyaluronic acid and chondroitin sulphate compete for the same binding sites, it appears that the uptake of chondroitin sulphate by the A_{12} VIII cells represent a type of interaction that is different from the one presently described.

It has been previously reported that cultivated fibroblasts are capable of specific uptake and degradation of [^{35}S]proteoglycans which contain chondroitin sulphate (Kresse *et al.*, 1975; Kresse & Truppe, 1978; Prinz *et al.*, 1978; Truppe & Kresse, 1978). These reports suggest that proteoglycans and glycosaminoglycans are recognized by two different receptor species. Since the present results suggest the presence of common binding sites on the liver endothelial cells for chondroitin sulphate proteoglycan and protein-free chains of chondroitin sulphate, it appears that different mechanisms are responsible for the uptake of the two macromolecules by fibroblasts and liver endothelial cells.

It has been suggested that the degradation of chondroitin sulphate proteoglycan is initiated by a limited proteolysis of the core protein, with the production of non-aggregating species that may be eliminated from the connective tissue by diffusion into the circulatory system (Wasteson *et al.*, 1972; Sandy *et al.*, 1978). In support of this hypothesis, chondroitin sulphate has been demonstrated in the blood plasma of normal individuals (Calatroni *et al.*, 1969). This finding, along with those of the present study and a recent report from this laboratory (Smedsrød *et al.*, 1984) that chondroitin sulphate and hyaluronic acid compete for the same receptors on the liver endothelial cells, suggest that these cells may play a central role in the catabolism of the two glycosaminoglycans.

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