Inhibition of hyaluronan uptake in lymphatic tissue by chondroitin sulphate proteoglycan

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Afferent lymph vessels entering the popliteal lymph nodes of sheep were infused with [^3H]acetyl-labelled hyaluronan [HA; M_r of $(0.85\text{--}1.2) \times 10^5$] for up to 4 h at a rate of 17.4–23.1 $\mu\text{g/h}$. As much as 22.8 μg (99%) of infused [^3H]HA was taken up by the node per h and degraded. During this interval it was observed that infused HA polymers of higher M_r were absorbed by the node to a greater degree than those of lower M_r . When proteoglycan monomer (PG; M_r 5×10^5 ; 400 μg of hexuronic acid/h) was infused concurrently with [^3H]HA, the absolute amount of radioactivity appearing in efferent lymph (i.e. labelled material not absorbed by the node) increased, whereas the amount of labelled metabolites of low M_r was reduced considerably. During this period the M_r distribution of labelled HA in efferent outflow reverted to that of the infused material within 30–60 min. Our findings suggest that PG subunits and their chondroitin sulphate chains compete with HA for uptake into the peripheral lymph node of sheep. This indicates that PG, chondroitin sulphate and HA share the same pathway of elimination in this tissue, and is consistent with the view that the lymph node is involved in the metabolic turnover of normal intracellular matrix.

INTRODUCTION

Glycosaminoglycans (GAGs) such as hyaluronan (HA), chondroitin sulphate (CS), dermatan sulphate and chondroitin sulphate proteoglycan (PG), which are major constituents of the intracellular matrix and connective tissue, have been demonstrated to be taken up by cells within the liver and subsequently degraded into low- M_r products (Wood *et al.*, 1976; Fraser *et al.*, 1981; Ericksson *et al.*, 1983). Furthermore it has been shown *in vitro* that CS and HA are bound to the same receptor on liver endothelial cells. The binding affinity for the receptor is related to the degree of polymerization of the GAG. This is thought to be simply due to the increased probability of binding due to the repetitive sequence within the GAG chain (Laurent *et al.*, 1986). Laurent *et al.* (1986) also showed that CS can displace receptor-bound HA, and that it has a binding affinity which is 3-fold greater than that of HA of similar size.

We have recently demonstrated *in vivo* (Fraser *et al.*, 1988) that peripheral lymph nodes are involved in the normal metabolic turnover of HA. It was shown that HA of two sizes (M_r 1.5×10^5 and 5×10^6) is actively taken up by lymph nodes in proportion to the degree of polymerization, and metabolized to *N*-acetylglucosamine, acetate, water and probably *N*-acetylglucosamine 6-phosphate. The apparently preferential uptake of high- M_r HA indicated that cellular uptake, as for the liver, is via specific binding sites.

In this study we present evidence which shows that [^3H]HA uptake by lymph nodes *in vivo* is inhibited by unlabelled CS PG monomer and by CS chains.

MATERIALS AND METHODS

Perfusion of lymph nodes

All studies were performed in 2-year-old merino ewes. The efferent and one of the several afferent vessels of the

popliteal lymph node were cannulated and the incision was repaired as described by Hall & Morris (1965). On recovery, the animals were allowed to eat and drink freely. One day later, the afferent vessel was perfused (Fig. 1) with iso-osmotic saline delivered through a Gilson Minipuls II pump at a rate which varied in different experiments between 0.99 and 1.16 ml/h.

Fig. 1. Schematic diagram of perfusion of sheep popliteal lymph node

The efferent and one of several afferent vessels of the popliteal lymph node of sheep were cannulated. The afferent vessel was perfused with saline and Patent Blue V dye before and after each experiment to confirm the patency of the perfusion pathway. Efferent lymph, which includes input from the afferents as well as the infused solution, was collected at 30 min intervals.

Abbreviations used: HA, hyaluronan; PG, proteoglycan; UA, hexuronic acid; CS, chondroitin sulphate; GAG, glycosaminoglycan; GdnHCl, guanidinium chloride.

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In the popliteal node there are multiple afferent lymph vessels, but usually only one efferent vessel. The outflow collected from the node thus included input from the intact afferents as well as the infused solution. The patency of the perfusion pathway was verified by pulses of Patent Blue V dye (May and Baker, Melbourne, Australia) added to the saline solution. Perfusion with [^3H]HA (17.4–23.1 μg of HA/ml) was then started, and continued for up to 4 h (stage 1). This was followed by infusion of [^3H]HA in the presence of untreated or reduced and alkylated PG [M_r 5×10^5 as determined by use of HA standards, 400 μg of hexuronic acid (UA)/ml] for up to 4 h or in the presence of CS chains (M_r 14.9×10^3 ; 400 μg of UA/ml) for 3 h (stage 2). The efferent lymph was collected in sterile 10 ml tubes, containing crystalline EDTA as anticoagulant, at half-hourly intervals. At the end of each study, the perfused popliteal nodes were removed within 5–10 min, trimmed of fat, weighed and stored at -20°C until analysed. After collection, NaN_3 was added as an antimicrobial agent to a final concentration of 0.02 % (w/v).

Recovery of ^3H -labelled material from lymph nodes

Each node was dissected into small pieces and chilled phosphate-buffered saline (0.15 M- Na_2HPO_4 titrated to pH 7.25 with 0.15 M- NaH_2PO_4 and mixed with 2 vol. of 0.15 M- NaCl) was added to give a total volume of 10 ml. This preparation was then dispersed with 2 cycles of 20 s, with a 2 min interval to maintain cooling, in an Omnimix tissue homogenizer (Sorvall, Boston, MA, U.S.A.) at setting 1.5. Duplicate portions of approx. 0.2 ml (determined by weight) were solubilized by the addition of 1.0 ml of NCS tissue solubiliser (Amersham). After 1 h at room temperature, the lymph node extract was heated at 50°C for 2 h or until completely solubilized. On cooling, 10 ml of scintillation cocktail [Teric/toluene (7:3, v/v)] was added with 0.5 ml of water and the digest was kept in the dark for 48 h before scintillation counting.

The remainder of the homogenate was centrifuged at 4°C for 1.5 h at 105000 g (r_{av} 5.9 cm) after an interval of at least 48 h in darkness and four cycles of freezing and thawing. The layer of fat was removed by aspiration and the remaining aqueous layer was separated from the pellet for further analysis.

Preparation of [^3H]HA

HA labelled with ^3H in the acetyl configuration was prepared from human synovial cultures as described by Fraser *et al.* (1981). The purity of the HA preparation was determined by digestion with *Streptomyces hyalurolyticus* hyaluronidase (hyaluronate lyase; EC 4.2.2.1; 2000 turbidity-reducing units/mg; Calbiochem-Behring, La Jolla, CA, U.S.A.), which is specific for HA (Ohya & Kaneko, 1970). The labelled material was shown to be > 99 % susceptible to degradation by the enzyme as determined by Sephadex G-50 gel chromatography. Four preparations were used in this study, as shown in Table 1.

HA stock solution was prepared containing high- M_r (5×10^6) molecules which were degraded to give the desired M_r by sonication at 4°C . The stock solution (6 ml) was exposed in a flat-bottomed vessel of 15 mm diam. to a field of 20000 Hz for seven (preps. A, and B) and six (prep. C) cycles of 1 min, with 1 min intervals to maintain cooling. The field was generated by the tuned

Table 1. HA preparations used

HA prep.	$10^{-5} \times \text{Activity}$ (d.p.m./ μg of HA)	$10^{-4} \times M_r$	Expts.
A	5.283	9	1, 2 and 3
B	4.384	8.5	4
C	5.283	12	5
D	1.813	10.5	6 and 7

microtip of a Sonifier (Branson Sonic Power Co., Danbury, CT, U.S.A.). All infused material was prepared and diluted in pyrogen-free aqueous NaCl (0.15 M) of pharmacopoea grade.

Preparation of PG monomers

Cartilage was excised from bovine femoral condyles and was then cut into small pieces. Throughout this procedure, the cartilage was kept in chilled iso-osmotic NaCl solution.

The tissue was then extracted at 4°C in 2 vol of 4 M-guanidinium chloride (GdnHCl) in 50 mM-sodium acetate, pH 5.8, with constant slow stirring for 24 h in the presence of: EDTA (10 mM), NaN_3 (0.02 %, v/v), benzamidine hydrochloride (5 mM) and soybean trypsin inhibitor at 2 $\mu\text{g}/\text{ml}$ (type II-S, Sigma). A further 3 vol. of 4 M-GdnHCl in 50 mM-sodium acetate, pH 5.8, was added (containing only 0.02 % NaN_3) and extraction was carried out for a further 72 h. The extract was filtered and tissue was again extracted in 3 vol. of 4 M-GdnHCl in 50 mM-sodium acetate, pH 5.8, in the presence of 0.02 % (v/v) NaN_3 for 72 h at 4°C .

The extract was finally separated from the residue by centrifugation at 20000 g for 30 min at 4°C in a Beckman J2-21 centrifuge using a JA-14 rotor. The supernatant was kept at -20°C until processed further.

The PG was purified by CsCl density-gradient centrifugation in 4 M-GdnHCl at a density of 1.545. The extract was centrifuged in 30 ml polycarbonate tubes at 182000 g (r_{av} 6.34 cm) for 22 h at 15°C (60 Ti rotor, Beckman LC-65 ultracentrifuge). The lower 10 ml was fractionated and dialysed extensively against water, lyophilized and stored at -20°C .

Prepared PG was subjected to digestion with *Proteus vulgaris* chondroitinase ABC (chondroitin ABC lyase; EC 4.2.2.4) and shown to be > 88 % degraded as determined by gel chromatography on Sephacryl S-1000 in 4 M-GdnHCl.

For perfusion expts. 1–5, the PG preparation was diluted to 400 μg of UA/ml in pyrogen-free aqueous iso-osmotic saline, and sterilized by filtration through a 0.2 μm pore size porous filter.

Preparation of reduced and alkylated PG monomer

Purified PG monomer was brought to 4 M-GdnHCl in 0.1 M-Tris/HCl, pH 9. The solution was purged with N_2 and dithiothreitol was added to a concentration of 10 mM. Reduction was allowed to proceed at 37°C for 4 h. The reduced PG was then alkylated with 40 mM-iodoacetic acid for 20 h at 20°C . The solution was dialysed extensively against iso-osmotic (0.9 %) NaCl (Sajdera & Hascall, 1969). The purified PG (M_r approx. 5×10^5) was infused into the lymph node with labelled HA at a rate of approx. 400 μg of UA/h for expt. 6.

Preparation of CS peptide

Lyophilized PG (100 mg), prepared from bovine cartilage as described above, was dissolved in 20 ml of buffer containing 2 mM-CaCl₂, 0.02% EDTA and 50 mM-Tris/HCl, pH 7.4. To this was added 10 mg of trypsin (Trypsin IX; EC 3.4.21.4; Sigma). After incubation for 21 h at 37 °C, trichloroacetic acid was added at a concentration of 5% (v/v) and chilled to precipitate proteins, which were then pelleted by centrifugation at 1500 g for 15 min in a bench-top centrifuge. The resulting supernatant was dialysed extensively against water, followed by cetylpyridinium chloride (0.1%, v/v) precipitation and ethanol precipitation (4 washes) at -20 °C overnight.

For perfusion expt. 7, the CS preparation (M_r 14950) was diluted to 400 µg of UA/ml in iso-osmotic saline and sterilized by filtration through a 0.2 µm pore size porous filter.

Chromatography

Samples of efferent lymph and of lymph node aqueous extract were applied to Sephadex G-50 (1.6 cm × 36 cm) (1 ml samples) and to Sephacryl S-1000 (1.6 cm × 65 cm) (2 ml samples) columns and eluted with phosphate-buffered saline, pH 7.25, at 13 ml/h and recovered in 1.0 ml and 2.0 ml fractions respectively. Teric (0.2%, w/v) was added to the buffer for elution from Sephacryl S-1000. Recovery of applied radioactivity was always > 95%. All gels (Pharmacia, Uppsala, Sweden) were of superfine or fine grade.

The addition of PG to [³H]HA (3%, w/w) of $M_r > 5 \times 10^6$ in phosphate-buffered saline, pH 7.25, produced limited aggregation as shown by Sepharose CL-2B (1.6 cm × 64 cm column) gel chromatography (results not shown). The elution of ³H radioactivity (K_{av}) was not altered by inclusion of PG and there was only a 3% shift of PG towards V_0 as measured by the Dimethylmethylene Blue binding assay (Farndale *et al.*, 1986) with some modifications (Carroll, 1987). The reduced and alkylated PG was inactive in HA binding.

Determination of M_r of HA and PG preparations was by Sephacryl S-1000 gel chromatography, with cali-

bration data for HA kindly provided by Dr. Kirsti Granath (Pharmacia AB, Uppsala, Sweden).

Sephacryl S-300 in 1.0 M-NaCl/10 mM-Tris/HCl, pH 7.6, was used for GAG sizing (column size 1.6 cm × 65 cm). Sizing of PG was determined by gel chromatography on Sephacryl S-1000 (column size 1.6 cm × 65 cm) in 4 M-GdnHCl/10 mM-Tris/HCl, pH 7.6, which had previously been calibrated with HA standards.

Measurement of radioactivity

Packard 460CD and Beckman LS3801 scintillation counters were used, with appropriate quench corrections. Samples were added to a toluene-based scintillant containing 2,5-diphenyloxazole (PPO; Packard, Downers Grove, IL, U.S.A.), 5.5 g/l and *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP; New England Nuclear), 0.15 g/l diluted 7:3 (v/v) with the non-ionic detergent Teric X-100 (ICI Australia, Melbourne, Australia). For calculation of recovery in chromatography, radioactivity was taken as significant when > 3 s.d. above the mean background ($n = 10$).

RESULTS

Recovery of ³H from efferent lymph

Seven separate experiments were performed to determine the effect of PG and CS on [³H]HA uptake by the lymph node (Table 2). The efferent lymph flow rate was consistent in each experiment but varied widely in different animals (1.44–9.98 ml/h).

[³H]HA was infused into the lymph node at a rate that ranged from 17.4 to 23.1 µg/h in different experiments. After 2 h in expts. 1–4 and after 4 h in expt. 5, [³H]HA infusion was continued but in the presence of untreated PG monomer (approx. 400 µg of UA/h) for 2 h and 3 h respectively. After 3 h in expts. 6 and 7, [³H]HA infusion was continued but in the presence of reduced and alkylated PG (approx. 400 µg of UA/h) and CS (approx. 400 µg of UA/h) respectively.

During infusion of HA alone (stage 1), between 61 and 99% of labelled HA was taken up by the node. In the

Table 2. Infusion of lymph nodes with [³H]HA in the presence and absence of PG rates of input and recovery of ³H

Afferent lymph vessels entering popliteal lymph nodes were infused for up to 4 h with [³H]HA at a flow rate that varied in different experiments from 0.99 to 1.16 ml/h (approx. 20 µg of HA/ml). Flow rates given are means ± s.d. of ≥ 8 determinations. This was followed by infusion of HA in the presence of untreated PG (expts. 1–5) monomer, of reduced and alkylated PG (expt. 6) monomer ($M_r 5 \times 10^6$ as determined by use of HA standards; approx. 400 µg of UA/h), or of CS (M_r 14950; approx. 400 µg of UA/h) for 2–3 h. Total radioactivity in efferent lymph collected and in lymph node aqueous extract was determined.

Expt. no.	Node wt. (g)	Efferent lymph flow rate (ml/h)	Recovery of ³ H in efferent flow (%) during infusion of:		³ H in aqueous lymph extract (%)	Total infused ³ H not recovered (%)
			HA	HA + PG		
1	3.09	9.98 ± 1.42	6.4	22.4	5.9	79.4
2	3.47	7.46 ± 0.38	0.01	10.3	2.9	91.9
3	2.17	1.44 ± 0.54	3.6	42.9	4.5	71.6
4	2.56	4.38 ± 0.54	25.9	62.3	13.8	41.7
5	1.92	5.70 ± 0.84	31.6	70.2	11.1	41.0
6	5.55	8.83 ± 0.49	12.8	51.3	12.0	51.9
7	1.89	4.87 ± 1.23	38.9	78.0	9.0	27.6

Fig. 2. Uptake of HA by lymph node in the presence and absence of PG

HA (○) was infused into an afferent vessel of a popliteal lymph node at a rate of approx. 1.0 ml/h (20 µg of HA/ml) for 120 min (a, expt. 3) or 240 min (b, expt. 6) as described in the Materials and methods section. This was immediately followed by infusion of HA (approx. 20 µg/h) in the presence of untreated PG (approx. 400 µg of UA/h) for 120 min (a) or in the presence of reduced and alkylated PG (approx. 400 µg of UA/h) for 180 min (b) (●). Efferent lymph outflow was collected at 30 min intervals and the amount of ³H recovered in the efferent lymph outflow was determined. Wet weights of the nodes were 2.17 g (a) and 5.55 g (b). ■, Lymph outflow rate (ml/h).

presence of PG (stage 2) the amount extracted by the lymph node was greatly reduced (Table 2). The percentage of infused ³H recovered in efferent lymph ranged from 0.01 % (expt. 2) to 38.9 % (expt. 7) as shown in Table 2. In stage 2, when the lymph node was infused with [³H]HA in the presence of PG or CS, the total amount recovered in efferent lymph increased rapidly within approx. 1 h from 10.3 % (expt. 2) to 78.0 % (expt. 7). A small amount of ³H (2.9–13.8 %) was also found in the node after extraction (Table 2). A high proportion of infused ³H was not recovered in nodes or lymph (Table 2), and was presumably released into the bloodstream as low-*M_r* metabolites (Fraser *et al.*, 1988). Expts. 1–7 provide further evidence that lymph nodes take up HA. Since free radicals do not degrade HA sufficiently for it to be removed by the blood circulatory system (C. Tzaicos, J. R. E. Fraser, E. Tsotsis & W. G. Kimpton, unpublished work), it seems more likely that the [³H]HA must first be taken up and degraded by the lymph node before it enters the bloodstream.

Fig. 2 shows examples from two separate experiments of the amount of ³H recovered in efferent lymph for each 30 min interval throughout the entire experiment. During

Fig. 3. Sephacryl S-1000 gel chromatography of infused [³H]HA and of ³H activity recovered from the lymph node and efferent lymph

Sephacryl S-1000 gel chromatography equilibrated in phosphate-buffered saline (pH 7.2) and 0.2 % Teric (v/v) was carried out on: efferent lymph after 210 min of [³H]HA infusion (stage 1; ○); efferent lymph after 240 min of [³H]HA plus PG infusion (stage 2; ●); original [³H]HA preparation before infusion (■); and aqueous phase of lymph node buffer extract (▲). [³H]HA was infused at approx. 20 µg/h and PG at approx. 400 µg of UA/h (expt. 5). The node was removed immediately after the completion of the experiment, homogenized in phosphate-buffered saline, and the aqueous extract was recovered after centrifugation at 105 000 *g*. Samples were applied and eluted, and activity determined as described in the Materials and methods section. *M_r* values are given at the top of the Figure.

[³H]HA infusion alone (stage 1), the proportion of the infused ³H activity recovered in the efferent lymph became fairly stable. On introduction of PG (stage 2) there was a sharp rise in recovery of ³H in all experiments.

Analysis of ³H-labelled metabolites in efferent lymph and lymph node extract

Cell-free efferent lymph at various time intervals (expt. 5) was examined by gel chromatography in Sephacryl S-1000 (Fig. 3) and Sephadex G-50 (Fig. 4). The *M_r* distribution of the labelled material in the infusate and efferent lymph was compared by Sephacryl S-1000 gel chromatography. During the infusion of [³H]HA alone, the labelled polymers in the efferent lymph were of distinctly lower *M_r* than in the infusate. During the concurrent infusion of PG, the polymer distribution in the efferent lymph approximated more closely to that of the infusate (Fig. 3). Furthermore, there was a substantial amount of low-*M_r* products (28.4 %), presumably mainly water and possibly some acetate (Fraser *et al.*, 1988), in efferent lymph from stage 1, with a much smaller proportion (8.8 %) in efferent lymph from stage 2 of the experiment (Fig. 4).

Fig. 3 shows that [³H]HA in the efferent lymph had a

Fig. 4. Sephadex G-50 chromatography of infused [^3H]HA and ^3H activity recovered from the lymph node and efferent lymph

Sephadex G-50 gel chromatography in phosphate-buffered saline (pH 7.2) was carried out on: (a) efferent lymph after 210 min of [^3H]HA infusion (stage 1); (b) efferent lymph after 240 min of [^3H]HA infusion (stage 1) followed by 60 min of [^3H]HA plus PG infusion (stage 2); (c) efferent lymph after 240 min of [^3H]HA infusion (stage 1) followed by 150 min of [^3H]HA plus PG infusion (stage 2); and (d) aqueous phase of lymph node buffer extract. [^3H]HA was infused at approx. 20 $\mu\text{g}/\text{h}$ and PG at approx. 400 μg of UA/h (expt. 5). The node was removed immediately after the completion of the experiment, homogenized in phosphate-buffered saline, and the aqueous extract was recovered after centrifugation at 105000 g . Samples were applied and eluted, and activity was determined as described in the Materials and methods section. Values in parentheses are percentage recoveries of ^3H .

lower M_r than the infused [^3H]HA. When [^3H]HA alone was infused, the proportion of low- M_r products (eluting near V_t) was much greater, as is shown in Fig. 4.

Analysis of labelled residues in the lymph nodes showed that infused HA was degraded (Fig. 3) and that

a large proportion (29%) was eluted close to V_t on Sephadex G-50 gel chromatography (Fig. 4). Further analysis of the lymph node extract by Sephacryl S-1000 gel chromatography, to distinguish the larger fractions, showed that all the [^3H]HA infused was extensively degraded (Fig. 3). It should be noted that there was no degradation of HA by the lymph node extraction procedure.

In order to determine whether there was a significant aggregation of infused [^3H]HA (approx. M_r 1×10^4) and PG *in vivo*, a sample of the efferent lymph collected after 150 min of concurrent infusion of [^3H]HA and PG was compared with the infused [^3H]HA with PG and with [^3H]HA alone by gel chromatography on Sepharose CL-4B equilibrated and eluted in phosphate-buffered iso-osmotic saline, pH 7.2. There was no accelerated elution of [^3H]HA from the efferent sample, indicating that no significant degree of aggregation had occurred during passage through the node (results not shown).

DISCUSSION

At present there is no evidence that PG occurs in peripheral lymph, but during normal turnover of PG from skin and joints some must escape into peripheral lymph as is the case for HA. Some PG is normally found in synovial fluid (Silpananta *et al.*, 1967), and it is almost certainly present in increased amounts in arthritis.

When [^3H]HA was infused into the lymph node together with PG or CS, [^3H]HA uptake by the node was inhibited by up to 70% and 78% respectively. Although *in vitro* there was a small amount of aggregation of HA with the PG used in this study, this did not account for the enhanced escape of HA from the node. The use of reduced and alkylated PG (inactive in HA binding) in expt. 6 and CS chains in expt. 7 gave similar results.

HA and PG appear to share the same receptor in hepatic endothelial cells, as suggested by the partial inhibition of HA binding by HA oligosaccharides and CS (Smedsrød *et al.*, 1984) and by the inhibition of CS binding by HA (Smedsrød *et al.*, 1985). These results also indicate an increased affinity of high- M_r HA for the cell surface. Laurent *et al.* (1986) found the binding sequence of HA to be the size of an octasaccharide. They also proposed that the increased probability of binding of high- M_r HA to liver endothelial cells is simply due to the repetitive sequence in the GAG chain; binding of 1 mol of octasaccharide to each receptor will therefore lead to a more rapid uptake of high- M_r material.

Binding affinity for CS is even greater than that for HA, given polymers of equal M_r (Laurent *et al.*, 1986). If the same is true for HA binding sites in the lymph node, it may simply be a matter of competition between the two for cellular uptake. On the other hand, it is possible that there is no common binding site in lymph nodes but that competition is due to interference arising from quantitative limitations in the generation of primary lysosomes or in regeneration of the plasma membrane binding sites. Assuming the same class of cell is responsible for uptake of both HA and PG, it is possible that competition is not specific but due to steric exclusion.

Even though the amount of [^3H]HA taken up was much less in the presence of PG, a proportion of infused HA was still unaccounted for and was presumably also degraded to low- M_r metabolites (acetate, water) as shown previously (Fraser *et al.*, 1988).

The specificity of HA binding may vary from site to site and according to the function of HA within the site. In some cell types in the body, the specificity of binding sites for HA may extend to include CS (McGuire *et al.*, 1987). In HA-PG aggregation, HA plays a unique role in which strict specificity of the binding site is essential. However, in catabolism of HA, the specificity of the receptor linked to intracellular degradation in the lymph node may also extend to CS at least, as is the case in liver endothelial cells.

The results in this study confirm previous findings in this laboratory (Fraser *et al.*, 1988) that HA is absorbed into the lymph node when it enters through afferent vessels. Furthermore, analysis of the efferent lymph and lymph node residues showed that the HA of larger M_r is preferentially taken up and subsequently degraded by the lymph node. Fraser *et al.* (1988) have shown that HA of high M_r ($> 4 \times 10^6$) was preferentially extracted compared with that of low M_r (1.5×10^5). In this study it is shown that the rate of uptake is still directly related to polymer size in the lower M_r range. The effect of polymer size on HA absorption in the lymph node suggests that cellular uptake occurs through specific binding sites and not through indiscriminant endocytosis. This system is similar to that observed in hepatic endothelial cells *in vitro* (Laurent *et al.*, 1986). The effect of polymer size on [3 H]HA absorption in the lymph node is consistent with the binding characteristics of HA observed with hepatic endothelial cells *in vitro*, where a common binding site is shared by HA and CS. It seems likely that lymph node cells may carry a similar receptor for HA, which is coupled to internalization and subsequent metabolic breakdown, and sharing specificity with CS. A number of studies (Revell & Muir, 1972; Wood *et al.*, 1973, 1975, 1976; Fraser *et al.*, 1984; Laurent & Fraser, 1986; Kirch *et al.*, 1987) have been carried out on the turnover and catabolism of various PGs and GAGs, but all were carried out with isolated livers or with material injected into the bloodstream of an animal. Receptors play an important role in the uptake and metabolism of specific molecules (Dautry-Varsat & Lodish, 1984). This is especially evident when the molecule is present at a very low concentration and with a vast excess of unrelated molecules such as in the lymphatic system (Tengblad *et al.*, 1986).

The lymphatic system is involved in a wide range of functions including the uptake and degradation of extraneous macromolecules. The present results support previous findings in our laboratory (Fraser *et al.*, 1988) that the functions of the lymphatic system may include a

major role in the normal metabolic turnover of HA, and suggest that this function may also extend to PG, another common constituent of intracellular matrix.

This work was supported by grants from the National Health and Medical Research Council of Australia.

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