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Uptake and degradation of hyaluronan in lymphatic tissue

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Afferent lymph vessels entering popliteal lymph nodes of sheep were infused with [^{3}H]acetyl-labelled hyaluronan of high M_r (4.3×10^6–5.5×10^6) and low M_r (1.5×10^5). Analysis of efferent lymph and of residues in the nodes showed that hyaluronan presented by this route is taken up and degraded by lymphatic tissue. Labelled residues isolated in node extracts by gel chromatography and h.p.l.c. included *N*-acetylglucosamine, acetate, water and a fraction provisionally identified as *N*-acetylglucosamine 6-phosphate. Between 48 and 75% of the infused material was unrecovered, and had been presumably eliminated through the bloodstream as diffusible residues. Rates of degradation reached as high as 43 μg/h in a node of 2 g wt. infused with 56 μg/h. Some HA passed into efferent lymph and some was detected in the nodes, but fractions of $M_r > 1 \times 10^6$ were not found in either. It is concluded that the amounts and M_r values of hyaluronan released from the tissues into peripheral lymph can be significantly underestimated by analysis of efferent lymph, i.e. lymph that has passed through lymph nodes. A substantial role in the normal metabolic turnover of at least one major constituent of intercellular matrix and connective tissue may now be added to the established functions of the lymphatic system.

INTRODUCTION

Much of the normal turnover of hyaluronan (HA) in the circulation [1,2] can be attributed to the input from lymph [3,4]. The HA content of lymph varies according to the site of collection, but it is consistently higher than that of blood plasma and also shows a higher average M_r, a difference attributed to faster elimination of the larger polymers when they enter the circulation [4]. In turn, HA in lymph shows a lower average M_r than in the matrix of many of the tissues from which it is derived.

It might be argued that the largest polymers of HA cannot escape from the tissues. If any do so, however, they would be more rapidly removed if lymph nodes absorb HA in the manner exemplified by hepatic endothelial cells, in which it has been shown that the affinity of HA for its binding site increases with increasing M_r of the polymer [5]. It is not known whether lymph nodes metabolize it or absorb it at all, since all studies of HA in the lymphatic system have been done to date only on lymph that has passed through at least one node. Isotopic label has been found in the nodes after injection of labelled HA into the synovial cavity of joints [6], and also after intravenous injection of enough to reverse the normal lymph/plasma concentration gradient [7]; but the isotopic label in the nodes was not identified as intact HA in either instance.

We now present evidence that lymph nodes can extract and metabolize HA presented through the afferent lymph pathways, and that its larger polymers are more rapidly removed.

MATERIALS AND METHODS

Preparation and analysis of ^{3}H-labelled HA

[^{3}H]Acetyl-labelled HA was prepared as described previously [1]. Purity of the labelled material was > 99% as determined by chromatography in Sephadex G-50 before and after degradation with *Streptomyces hyaluroliticus* hyaluronidase (EC 4.2.2.1) (2000 turbidity-reducing units/mg; Calbiochem–Behring, La Jolla, CA, U.S.A.), which is specific for HA [8]. Four preparations were used in this study, as follows: preparation A, 4.56×10^5 d.p.m./μg, M_w 5.5×10^6, M_n 4.6×10^4; preparation B, 4.60×10^5 d.p.m./μg, M_w 4.3×10^6, M_n 3.3×10^5; preparation C, 2.15×10^5 d.p.m./μg, M_w 5.3×10^6, M_n 1.6×10^5; preparation D, 2.15×10^5 d.p.m./μg, M_w 1.5×10^5, M_n 6.1×10^4. To obtain a product (preparation D) of low M_r and specific radioactivity identical with that of preparation C, a portion of the latter was degraded by sonication at 4 °C. A 6 ml portion of the stock solution was exposed in a flat-bottomed vessel of 15 mm diameter to a field of 20000 Hz for seven 1 min periods, with 1 min intervals to maintain cooling. The field was generated by the tuned micro-tip of a Sonifier (Branson Sonic Power Co., Danbury, CT, U.S.A.). The treated fraction was mixed with undegraded material of the same batch, as indicated below. All infused material was prepared and diluted in pyrogen-free aqueous 0.15 M-NaCl of pharmacopoeia grade (designated below as iso-osmotic saline).

Abbreviations used: HA, hyaluronan, hyaluronate.

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Perfusion of lymph nodes

All studies were performed in 2-year-old Merino ewes with the preparation described by Hall & Morris [9]. In brief, the efferent and one of the several afferent vessels of the popliteal lymph node were cannulated and the incision was repaired. On recovery, the animals were allowed to eat and drink freely. One day later, the afferent vessel was perfused with iso-osmotic saline delivered through a Gilson Minipuls II pump at a rate varied in different experiments between 0.9 and 1.4 ml/min. In the popliteal node there are multiple afferent lymph vessels but the efferent is usually single; a second efferent vessel found in one preparation was ligated. 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, Vic., Australia) added to the saline solution. Perfusion with isotopically labelled HA was then commenced, and the efferent lymph was collected in sterile tubes containing heparin (50 i.u.) at half-hourly intervals after the HA solution entered the afferent lymph vessel. At the end of each study, the perfused and contralateral popliteal nodes were removed within 5 min, weighed, and stored at −70 °C until analysed. Residual dye was seen in the lymph nodes but no leakage was detected. The deadspace volume of the infusion line was measured and the mean perfusion rate was calculated from the measured loss from the perfusate reservoir. At appropriate stages NaN_3 was added as an antimicrobial agent to a final concentration of 0.02% (w/v).

Further preparation of lymph

Cell-free lymph was separated by centrifugation at 400 $g_{av.}$ for 10 min at 4 °C for determination of radioactivity and for chromatography. Cell pellets were twice resuspended in 2.0 ml of iso-osmotic NaCl/phosphate buffer, pH 7.4 [10], recovered by centrifugation, suspended in 0.1 ml of water and dissolved in 1.0 ml of NCS tissue solubilizer (Amersham International, Amersham, Bucks., U.K.).

Extraction of lymph nodes

Each node was dispersed in chilled 0.15 M-NaCl/phosphate buffer (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), at 4.8 ml/g, for 2.5 min in an Omnimix tissue homogenizer (Sorvall, Boston, MA, U.S.A.) at setting 3. Samples (0.2 ml) were heated in 10 ml of Fluorosol scintillant/solubilizer (National Diagnostics, Somerville, NJ, U.S.A.) at 50 °C for 2 h; on cooling, 1.0 ml of Triton X-100 (New England Nuclear, Boston, MA, U.S.A.) was added with 0.4 ml of water, and the digest was left to stand for 48 h before scintillation counting. After an interval of 36 h at 4 °C and three cycles of freezing and thawing, the remainder was centrifuged at 90000 g ($r_{av.}$ 6.4 cm) for 2 h at 4 °C. The layer of fat was removed with 0.5 ml of the aqueous layer and extracted for 24 h in 10 ml of chloroform/methanol (2:1, v/v). Then 0.4 vol. of 0.15 M-NaCl was added and lipids were recovered for scintillation counting as described previously [1]. After removal of the remaining aqueous layer for further analysis, the residue was drained and extracted in 3 ml of Triton X-100 at ambient temperature for 48 h. The extract was centrifuged in a Beckman Microfuge for 6 min and a measured volume of the supernatant (approx. 2.5 ml) was added to 10 ml of toluene scintillant, followed by 0.2 ml of water and sufficient Triton X-100 (2–3 ml) to clear the resulting turbidity before the radioactivity was counted.

Chromatography

Samples (1 ml) were applied to Sephadex G-50 columns (1.6 cm × 35 cm) and 2.0 ml samples to Sephacryl S-1000 columns (1.6 cm × 63 cm), eluted with 0.15 M-NaCl/phosphate buffer, pH 7.25, at 9.0 and 10.0 ml/h respectively and recovered in 1.0 ml and 2.0 ml fractions respectively. Triton X-100 (0.2%, w/v) was added to the buffer for elution from Sephacryl S-1000. Both gels (Pharmacia, Uppsala, Sweden) were of superfine grade. Macromolecular fractions of the eluates were analysed for labelled HA content as follows. A sample diluted to 1.0 ml in 0.15 M-NaCl was incubated at 37 °C for a total of 18 h with 0.2 ml portions of *Streptomyces* hyaluronidase (20 turbidity-reducing units) in 50 mM-phosphate buffer, pH 6.8, added initially and after 3 h. The fraction resistant to degradation was estimated after further chromatography in Sephadex G-50. A sample of authentic labelled HA was included in each batch.

Metabolites of low M_r were recovered by gel chromatography, and identified by h.p.l.c. in an Aminex HPX-87H pre-packed column (Bio-Rad Laboratories, Richmond, CA, U.S.A.) at 70 °C, in conjunction with a Waters model 510 liquid chromatograph (Millipore, Milford, MA, U.S.A.). Samples (0.2 ml) were eluted in 5 mM-H_2SO_4 at 0.6 ml/min. Sources of reference standards for chromatography were as follows: sodium [^{3}H]acetate and ^3H_2O from Amersham International; *N*-acetyl-α-D-glucosamine 1-phosphate and *N*-acetyl-D-glucosamine 6-phosphate from Sigma Chemical Co., St. Louis, MO, U.S.A.; other saccharides from BDH Chemicals, Poole, Dorset, U.K.

Measurement of HA concentrations and M_r distribution

Concentrations were measured with a specific binding assay [11] modified for biological fluids [12]. M_r distribution of HA in lymph and of the infused labelled material was determined in terms of M_w and M_n [13]. Other comparisons of M_r were made by chromatography in Sephacryl S-1000 with calibration data for HA kindly provided by Dr. Kirsti Granath (Pharmacia).

Measurement of radioactivity

Packard 460D and Beckman LS3801 scintillation counters were used, with appropriate quench corrections. Samples were added as indicated above to Fluorosol scintillant/solubilizer, or to a toluene-based scintillant containing 2,5-diphenyloxazole (Packard, Downers Grove, IL, U.S.A.) (6.0 g/l) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (New England Nuclear, Boston, MA, U.S.A.) (0.25 g/l) when used alone, and 5.5 g of 2,5-diphenyloxazole/l and 0.15 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l when diluted 7:3 (v/v) with the non-ionic detergent Teric X10 (ICI Australia, Melbourne, Vic., Australia). For calculation of recovery in chromatography, radioactivities were taken to be significant when > 3 S.D. above the mean background (n = 10).

RESULTS

Recovery of labelled material from efferent lymph

Six studies were performed to encompass varied inputs of labelled HA in terms of amount and M_r (Table 1). The outflow volume was consistently greater than the rate of infusion, presumably owing to inflow from the intact afferents. It varied widely among the several animals but much less so in the individuals, with the exception of one that moved and squatted frequently (Expt. 3). During the first hour of infusion the extraction of labelled material was $\geqslant 99\%$ in all but Expts. 5 and 6, when a high proportion of small polymers was infused and total HA input was raised to 23 and 56 μg/h respectively. The content of labelled material in efferent lymph (Fig. 1) increased steadily after 1 h, except in Expt. 3, where the flow rate also varied erratically. The extraction rate nevertheless remained high at all times.

Analysis of labelled material in efferent lymph

Little or no activity was found in the cell deposits recovered by centrifugation. Twenty samples of the supernatant were examined by chromatography in Sephadex G-50. The labelled content was found entirely at V_0 in the first hour of infusion (Expt. 5). A smaller and variable proportion (2 to 32 %) was found at V_t thereafter. In one example of the latter, the peak of this fraction was shown by h.p.l.c. to contain [^{3}H]acetate and ^3H_2O (results not shown). The highest half-hour output of these metabolites was 28000 d.p.m., equivalent to 0.13 μg of HA and representing 9% of the labelled material in the terminal efferent lymph of Expt. 5. In nine samples taken after 3.5 h of infusion, between 96 and 100% of the material eluted at V_0 was degraded by *Streptomyces* hyaluronidase, which identified it as HA and indicated that there was almost no re-utilization of the acetyl moiety in synthesis of other macromolecules comparable with that seen in other studies [1,2]. The maximum estimated output of intact ^{3}H-labelled HA in lymph was 7.5 μg/h in the second last interval in Expt. 6.

Comparison of M_r values of labelled HA in infusate and outflow

Nine samples of efferent lymph collected in Expts. 5 and 6 after 3.5 h of infusion were examined by chromatography in Sephacryl S-1000 as illustrated below. In view of their relatively small content of metabolites, no preliminary separation was done. The major modal value of M_r ranged between 50000 and 100000 in individual samples. In contrast with the perfused material, no material with $M_r > 950000$ was found in any fraction.

Residual labelled content of lymph nodes

A small amount of ^{3}H was found in the lipid fraction in each instance. A little was detected in the solid deposit remaining after ultracentrifugation but was not identified. As in efferent lymph, the largest amounts were found in the aqueous fraction as labelled macromolecular material (Table 2), which proved to be entirely HA. The labelled water-soluble material of low M_r was relatively more abundant than in the supernatant of the final efferent lymph samples. In h.p.l.c. (Fig. 2) a fraction that was eluted as *N*-acetylglucosamine 6-phosphate and an unidentified fraction were found in all extracts, together with tritiated *N*-acetylglucosamine, acetate and water in one, water in two others and acetate in the fourth.

Proportion of infused material recovered from lymph and nodes

This is summarized in Table 3, with estimates of the amounts presumed to be metabolized in the nodes. These constituted between 63 and 77% of the amounts infused and for the most part were not recovered in nodes or lymph. The proportion retained as HA in the node or passed through to efferent lymph appeared largely independent of the rate of infusion.

M_r of labelled HA residues in lymph nodes

Samples from the aqueous fractions of the lymph-node extracts of Expts. 5 and 6 were compared with the

Table 1. Infusion of lymph nodes with [^{3}H]acetyl-labelled HA: rates of input and recovery of ^{3}H

The popliteal lymph node was infused at constant rate with an iso-osmotic saline solution of [^{3}H]acetyl-labelled HA through tubing placed in one of the afferent lymph vessels, as described in the Materials and methods section. All outflow was recovered from a single efferent vessel. Variation in the volume outflow and in recovery of the infused material as ^{3}H is illustrated in Fig. 1. The node was excised and weighed immediately after trimming away attached fat.

		Infusion of labelled material				Efferent lymph outflow	
				Infused [^{3}H]acetyl-labelled HA			
Expt. no.	Node wt. (g)	Time (h)	Flow rate (ml/h)	Total (μg)	Prepn.	Flow rate (mean) (ml/h)	Recovery of infused ^{3}H in lymph (%)
1	1.52	3.5	0.90	5.6	A	1.2	1
2	1.74	4.0	0.89	14.4	A	7.3	1
3	1.23	5.0	0.95	7.6	B	4.1	8
4	1.50	5.0	0.95	12.6	B	5.0	1
5	1.07	4.0	1.23	93	C/D	3.7	7
6	2.15	4.1	1.44	232	C/D	6.1	7

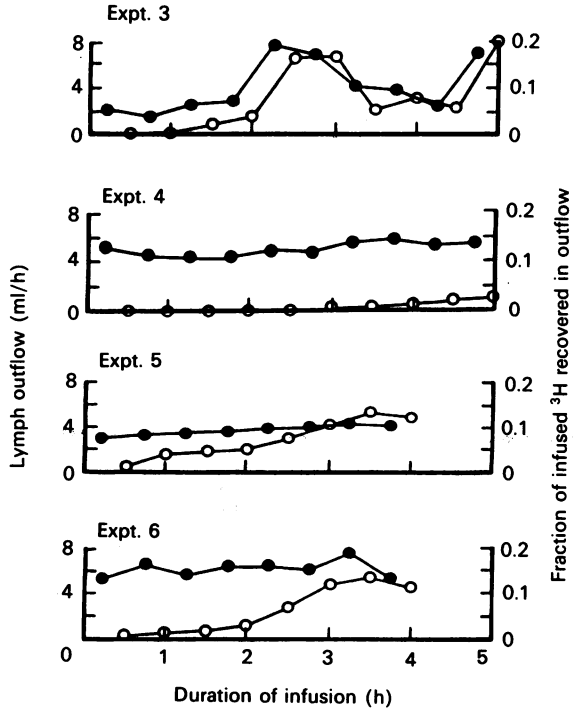

Fig. 1. Flow rate and ^{3}H content of efferent lymph during infusion of [^{3}H]acetyl-labelled HA through the afferent lymph pathway

Labelled HA in iso-osmotic saline was infused through an afferent lymph vessel to the popliteal node as described in the Materials and methods section. The input of labelled HA and flow rate (Table 1) were held constant in each study. The outflow of efferent lymph (●), and recovery of ^{3}H in efferent lymph (○), the latter expressed as a fraction of that infused in each half-hour interval, are shown for Expts. 3 to 6 from above downwards. Expts. 1 and 2 (not shown) showed outflow patterns similar to that of Expt. 4. Although the fractions were small, the levels of radioactivity were easily discernible above background and rose gradually as illustrated. The variations in Expt. 3 were associated with frequent movement and changes in posture.

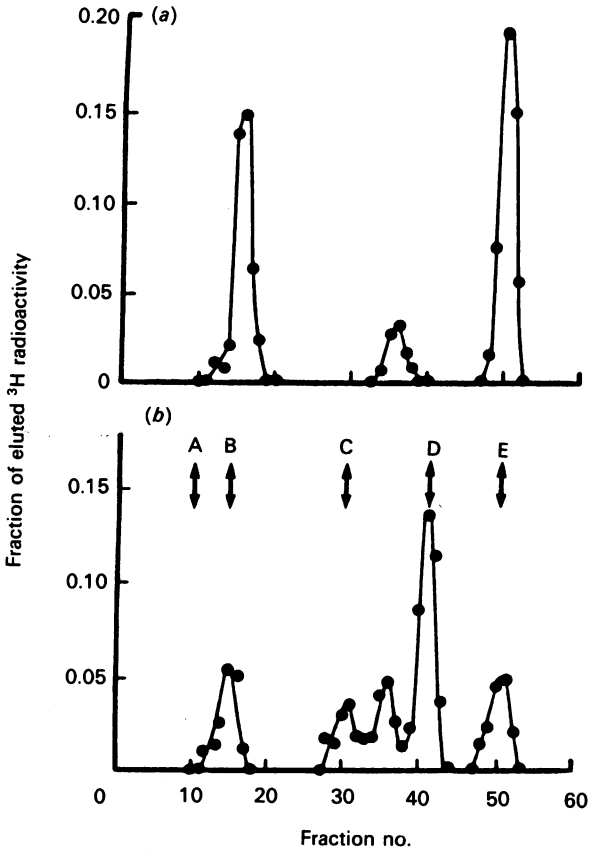

Fig. 2. H.p.l.c. analysis of labelled metabolites recovered from lymph nodes

The peaks of the labelled fractions from the aqueous lymph-node extracts, which were eluted in the second zone just before V_t in Sephadex G-50 (see Table 2), were subjected to h.p.l.c. as described in the Materials and methods section. Panel (*a*), Expt. 3; panel (*b*), Expt. 5. Standards are shown as follows: A, *N*-acetyl-α-D-glucosamine 1-phosphate; B, *N*-acetyl-D-glucosamine 6-phosphate; C, *N*-acetyl-D-glucosamine; D, [^{3}H]acetate; E, ^3H_2O. The other extracts also contained material eluted in position A (7 and 36%) and between C and D, followed by tritiated water in one and acetate in the other.

Table 2. Analysis of ^{3}H radioactivity in lymph nodes

Each node was dispersed in 0.15 M-NaCl/phosphate buffer. One sample was dissolved for determination of radioactivity. The aqueous phase of the remainder was separated from cell debris and lipid by ultracentrifugation at 113000 $g_{av.}$ for 120 min. Radioactivity in lipid was $\leqslant 1.4 \times 10^3$ d.p.m./g wet wt. of tissue, and in the cell debris $\leqslant 81 \times 10^3$ d.p.m./g ($\leqslant 2.6$% of the total recovered). The aqueous phase was fractionated by chromatography in Sephadex G-50, which yielded a distinct zone eluted at V_0 and another just before and overlapping V_t, as determined with ^3H_2O. The pooled V_0 fractions were digested with *Streptomyces* hyaluronidase to determine the content of labelled HA. Details are given in the Materials and methods section. The aqueous phase was also analysed by h.p.l.c. and chromatography in Sephacryl S-1000 (Figs. 2 and 3).

			Aqueous phase of buffer extracts			
Expt. no.	Node wt. (g)	10^{-5} × Radioactivity in whole node (d.p.m./g)	10^{-5} × Radioactivity (mean ± S.D., $n = 3$) (d.p.m./g)	Recovery of ^{3}H in Sephadex G-50		Degradation of V_0 fraction by hyaluronidase (%)
				V_0 (%)	V_t (%)	
3	1.2	8.3	7.1 ± 0.34	76	24	97
4	1.5	18.8	20.1 ± 1.59	71	29	98
5	1.1	42.4	42.7 ± 0.76	78	22	98
6	2.2	41.9	43.2 ± 1.79	90	10	99

Table 3. Recovery and estimated metabolism of infused [^{3}H]acetyl-labelled HA

Recovery of labelled HA was calculated from the proportions isolated as polymers in Sephadex G-50 from the node extracts and efferent lymph. The labelled content of unfractionated lymph samples was assumed to be entirely polymers. The error in this assumption is slight (see the Results section). Recovery is expressed as a percentage of the total HA infused. The unrecovered fraction was presumably through the bloodstream as small metabolites. HA output in efferent lymph, which includes endogenous sources, was estimated from a single half-hour sample in each study. The estimated rate of metabolism is an average for the period of infusion, derived from the sum of data in columns 5 and 6, and the rate of infusion of [^{3}H]acetyl-labelled HA.

Expt. no.	[^{3}H]Acetyl-labelled HA infused (μg/h)	Recovery as ^{3}H-labelled HA in		Recovery as ^{3}H-labelled HA metabolite in lymph node (%)	Unrecovered ^{3}H (%)	Estimated rate of metabolism of ^{3}H-labelled HA (μg/h)	HA content of efferent lymph (μg/h)
		Lymph (%)	Node (%)				
3	1.5	8	19	6	66	1.1	23.0
4	2.5	1	37	15	48	1.6	5.1
5	23	7	18	5	70	17	7.5
6	56	7	17	2	75	43	22.4

infused material by chromatography in Sephacryl S-1000. Very little of the lymph-node residue was found to correspond with the high-M_r ($> 1 \times 10^6$) fractions in the infusate (Fig. 3). The degradation of the high-M_r HA polymers caused by the lymph-node extraction (Fig. 3) was insufficient to explain their scarcity in the lymph-node residue.

Adsorption of HA by plastic

The maximum internal surface area of the polyvinyl tubing used in these studies was 0.8 cm^2. In other studies in this laboratory (results not shown), adsorption of HA by polystyrene was found to be 0.03 μg/cm^2, and by polyvinyl polymers 0.014 μg/cm^2, after 24 h exposure at 37 °C in a similar concentration.

DISCUSSION

We have demonstrated that ^{3}H-labelled HA is efficiently absorbed in the lymph node when perfused through afferent lymph vessels. The isolation of labelled degradation products shows, moreover, that HA is effectively metabolized in lymphatic tissue.

We have also found that very large polymers of HA, similar in size to those found normally in many tissues, appear to be degraded more rapidly than smaller polymers perfused concurrently. The M_r distribution of HA residues in the node is consistent with partial depolymerization as the first step in cellular degradation. It seems unlikely, however, that the large polymers are simply degraded in their passage through the node, since no mammalian hyaluronidase apart from the testicular enzyme shows appreciable activity in the pH of extracellular fluid, and it is difficult to envisage sufficient free-radical activity in normal tissue. Regardless of the initial steps, the lymph node has a considerable capacity for further degradation of HA. The estimates given here can only represent a fraction of the total capacity, since only one of the usual four or five afferents was perfused and the input from each was restricted to a portion of the node, which was outlined by residual dye. Comparison of infusion and efferent flow rates (Table 1) shows that a major part of the node was still perfused with lymph from the other afferents.

It follows from these findings that the HA content of efferent lymph may not indicate correctly the amount of HA that escapes from tissues through peripheral lymphatics, or its polymer distribution. This conclusion is supported by current studies on the M_r distribution of endogenous HA in afferent and efferent lymph. The former has a much higher M_r than the latter (T. C. Laurent, W. G. Kimpton, R. N. P. Cahill & J. R. E. Fraser, unpublished work).

The striking effect of polymer size on HA absorption in the lymph node is similar to that observed in isolated hepatic endothelial cells, in which it has been shown that HA is taken up through specific binding sites [5], rather than by indiscriminate endocytosis of fluid. Non-specific elimination of HA at the rates observed in our last two studies would require fluid endocytosis of approx. 1 ml/h (calculated as the product of the fraction degraded and rate of infusion; Tables 1 and 2).

The detection of ^{3}H-labelled *N*-acetylglucosamine, acetate, water and lipid indicates degradative pathways in the lymph node similar to those in the liver [1]. The identification of *N*-acetylglucosamine 6-phosphate must be regarded as provisional, since it rests on h.p.l.c. alone. Nevertheless, the point of elution is distinct from that of the 1-phosphate, which has been tentatively identified as a metabolic residue of HA in fetal livers (J. R. E. Fraser, L. B. Dahl, W. G. Kimpton, R. N. P. Cahill, T. J. Brown & N. Vakakis unpublished work), and phosphorylation in the C-6 position is a prerequisite for deacetylation [14]. There was no hyaluronidase-resistant product [1,2] that would indicate re-utilization of phosphorylated *N*-acetylglucosamine in synthesis of immunoglobulins or other glycoproteins, but the level of activity in this pathway may depend heavily on immunological stimulation.

The recovery of radioactivity from the node and efferent lymph did not account for the amount infused. It is unlikely that so much intact HA was lost through blood capillary walls, unless they are much more permeable than elsewhere. For example, renal excretion of labelled HA in mature animals [1,2] is limited to HA with an $M_r < 25000$. Moreover, the largest polymers of HA disappeared most rapidly. The blood circulation through popliteal lymph nodes is in the order of 24 ml/h per g node wt. [15], which is much higher than

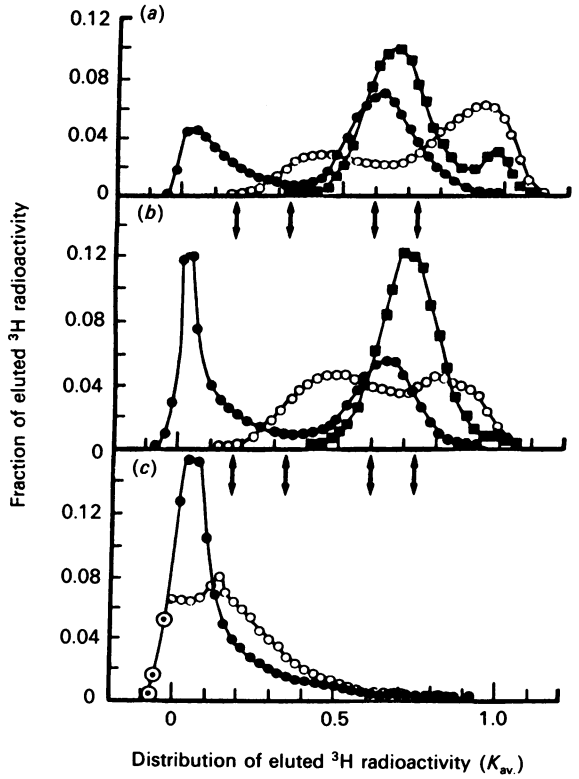

Fig. 3. M_r values of infused [^{3}H]acetyl-labelled HA compared with labelled residues in efferent lymph and lymph nodes

The various samples were prepared and fractionated in Sephacryl S-1000, as described in the Materials and methods section, to show the M_r distribution of labelled material. Panels (*a*) and (*b*) show data respectively from Expts. 5 and 6; ● indicates the infused mixture of HA polymers, ■ the content of the final efferent lymph sample, and ○ the aqueous phase of the lymph-node extract. No prior separation of HA from metabolites was done in the lymph and node extracts. The amounts of ^{3}H radioactivity in the infusion, lymph and node can be compared from the data of the Tables and Fig. 1. Panel (*c*) shows the effect of the lymph-node extraction method on the M_r of the labelled HA preparation C: ● before treatment and ○ after treatment. The arrows indicate, from the left, M_r values of 5×10^6, 1×10^6, 1×10^5 and 5×10^4. Polymers of $M_r > 10^6$ constituted 32% of the infused HA in Expt. 5, and 52% in Expt. 6. Mean recovery from the columns was 98% (S.D. 5.7%).

in most parts of the body and could maintain low extracellular concentrations of labelled water or acetate by effectively increasing their extracellular volume of distribution 6-fold or more.

Preliminary comparisons of HA in afferent and efferent lymph have shown a distinctively higher M_r in afferent lymph, but the differences in concentration observed to date have not always been as large as the present findings would imply. Input of HA to lymph from blood plasma can be excluded by its much lower plasma concentration. Two other explanations can be proposed. Firstly, it may be that small polymers of HA are synthesized in the node and released in efferent lymph. The outflow of some intact HA regardless of the rate of input or absorption suggests that there are channels through the node where this could occur. Secondly, it is likely that proteoglycan subunits and their chondroitin sulphate residues, which bind to the same receptor in liver endothelial cells [5], are also present in the peripheral lymph. Since the restricted distribution of dye tracers seen on the lymph-node surface in these and other studies [6] indicates a limited mixing of the lymph that enters by each afferent, the infused HA in this study would not be fully exposed to competition for binding sites with other glycosaminoglycans in afferent lymph. Nevertheless, it is clear that the lymph nodes can remove a significant amount of incoming HA from peripheral lymph, and it follows that estimates of tissue HA turnover based on the content of thoracic duct and other efferent lymph samples must now be considered conservative.

The recognized functions of the lymphatic system fall into three categories: the removal of micro-organisms, other foreign substances and particulate matter; induction and propagation of immune responses; and maintenance of balance in the exchange of fluid between the blood and tissues. The present findings add to these the uptake and metabolic degradation of HA and, possibly, of other tissue constituents released during normal turnover of connective tissue and intercellular matrix.

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