The Catabolism of Intravenously Injected Heparan *N*-[³⁵S]Sulphate in the Rat

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The metabolic fate of heparan *N*-[³⁵S]sulphate was studied in rats. Heparan sulphate was obtained from either bovine aorta or lung and labelled with ³⁵S by desulphation and subsequent resulphation *in vitro*. Experiments in which heparan *N*-[³⁵S]sulphate was administered intravenously to either free-range or wholly anaesthetized rats with ureter cannulae established that substantial desulphation occurs *in vivo*, with elimination of inorganic [³⁵S]sulphate in urine. Oligosaccharides labelled with ³⁵S, possible intermediates in heparan sulphate degradation, could not be detected in urine or blood. The general distribution of radioactivity after administration of heparan *N*-[³⁵S]sulphate, as demonstrated by whole-body radioautography, suggested that desulphation was not restricted to one organ in particular. Support for this view was obtained in experiments in which heparan *N*-[³⁵S]sulphate was administered to animals after the removal of kidneys, liver, spleen, pancreas or gastrointestinal tract. In all cases inorganic [³⁵S]sulphate was still produced. The ability of rats to desulphate heparan *N*-[³⁵S]sulphate was progressively impaired by increasing concentrations of heparin administered simultaneously. It was concluded that heparan sulphate is metabolized at a number of sites in the body by a sequence of degradative events leading to the formation of inorganic sulphate. It is also concluded that at least some of these events are common to heparan sulphate and heparin.

Heparan sulphate is produced by most mammalian cells and is known to be associated with cell surfaces (Kraemer, 1971). It is widely distributed in various tissues and organs of the body (Dietrich *et al.*, 1976), and its presence has been reported in plasma (Calatroni *et al.*, 1969) and urine (Linker & Terry, 1963). The sites and mechanisms for degrading heparan sulphate *in vivo* are not established, although fibroblasts in culture are capable of degrading a proteoglycan rich in heparan sulphate with liberation of inorganic sulphate after absorptive pinocytosis of the macromolecule (Kresse *et al.*, 1975). Endoglycosidases that degrade heparan sulphate to oligosaccharides have been reported in a variety of tissues and cell types, including rat liver (Höök *et al.*, 1975) and human skin fibroblasts (Klein *et al.*, 1976). Further degradation of the oligosaccharides, which is defective in the fibroblasts of patients suffering from some forms of mucopolysaccharidosis, is thought to proceed by the sequential action of specific exoglycosidases and sulphatases (Dorfman & Matalon, 1976).

The purpose of the present study was to investigate the distribution and metabolic fate of heparan *N*-[³⁵S]sulphate in the rat, after intravenous administration. In particular it was hoped to establish whether heparan sulphate and the anti-coagulant heparin share a common degradative pathway, as would be suggested by their many structural

similarities (Kresse, 1973). Heparan sulphate labelled in the sulphamino group was chosen, since reports (Friedman & Arsenis, 1974) on the properties of heparin sulphamidase, for which heparan sulphate is also a substrate (Kresse, 1973), suggest that total *N*-desulphation is unlikely to take place without extensive degradation of the carbohydrate chain. Thus it was unnecessary to obtain more general labelling of the molecule by using techniques *in vivo* which would lead to lower specific radioactivities in the heparan sulphate.

Materials and Methods

There are as described by Wood *et al.* (1973, 1976), with the addition of the following.

Materials

Unlabelled heparin (mucus; 177 units of anti-coagulant activity/mg assayed as in British Pharmacopoeia), was obtained from Evans Medical Co., Liverpool, U.K., and *N*-[³⁵S]sulphated heparin (pig mucus; 113 units of anti-coagulant activity/mg; specific radioactivity 17.8 μ Ci/mg) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Before use, the labelled heparin was freed from contaminating radioactive materials of low molecular weight by gel chromatography on a column (550 mm $\times$ 9 mm) of Sephadex G-25 (Pharmacia,

London W5, U.K.) from which the heparin was eluted (with water) at the void volume. Heparan sulphate was obtained from bovine aorta by papain digestion (Scott, 1960), after which the soluble extracts were precipitated at 4°C by addition of 3 vol. of ethanol and 1 vol. of ethanol saturated with sodium acetate trihydrate. The precipitated material was collected by centrifuging (1000g for 10 min), dissolved in 0.1 M-sodium phosphate buffer, pH 7.3 (0.25 ml of buffer/g original wet wt. of tissue), and nucleic acid was depolymerized by incubation at 37°C for 16 h after addition of ribonuclease and deoxyribonuclease (both from bovine pancreas; BDH Chemicals, Poole, Dorset, U.K.; 19 and 450 Kunitz units*/mg of glycosaminoglycan respectively) and manganese acetate (to 1 mM).

Residual protein was removed by passage through a mixed bed of cation-exchange resins and the glycosaminoglycans were isolated from the soluble digest by ion-exchange chromatography on Dowex 1 (X2; Cl⁻ form; 200–400 mesh; Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K.) (Mason & Wusteman, 1970). The fraction eluted from this column by 1.25 M-NaCl was subjected to digestion by testicular hyaluronidase (10 units/mg of glycosaminoglycan; from sheep testis; BDH) in 0.1 M-sodium acetate/acetic acid buffer, pH 5.0, containing 0.15 M-NaCl, at 37°C for 16 h. The digest was loaded on a column of Dowex 1 (X2; Cl⁻ form) (5 ml bed volume/mg of glycosaminoglycan), which was washed exhaustively with 0.8 M-NaCl, then eluted with 2.5 bed volumes each of 1.0 M-NaCl and 1.25 M-NaCl. The major glycosaminoglycan fraction isolated (92% of recovered uronic acid) was eluted by 1.0 M-NaCl and had a mobility on cellulose acetate electrophoresis of 0.68 relative to that of chondroitin 4-sulphate from whale cartilage (Seikagaku Kogyo, Tokyo, Japan). This preparation of heparan sulphate contained, per mol of hexosamine (exclusively glucosamine by amino acid analysis), 1.01 mol of uronic acid, 0.22 mol of amino acid residues, 0.55 mol of total ester sulphate [measured by turbidimetry (Dodgson & Price, 1962) after hydrolysis at 110°C for 5 h in 1 M-HCl] and 0.35 mol of *N*-sulphate as determined by the method of Lagunoff & Warren (1962). A portion of this preparation of heparan sulphate was partially desulphated by autohydrolysis at 55°C and resulphated on the amino group, both as described by Levy & Petracek (1962). To ensure that no under- or over-sulphated heparan sulphate was used, the product was again fractionated on Dowex 1 (X2; Cl⁻ form; Mason & Wusteman, 1970) when 56% of the recovered uronic acid was eluted by 0.8–1.0 M-NaCl. This preparation of heparan *N*-

[³⁵S]sulphate had a specific radioactivity of 0.57 μCi/μmol of uronic acid. Its electrophoretic mobility on cellulose acetate was unchanged and a serine content of 0.054 mol/mol of glucosamine (compared with 0.051 for its unlabelled precursor) indicated that covalently bound peptide had not been lost during the labelling process.

In earlier animal experiments a second preparation of heparan sulphate was also used. This was obtained from bovine lung by a procedure similar to that described above, but the product differed from the aortic heparan sulphate in that it was eluted from Dowex 1 (X2; Cl⁻ form) by 1.0–1.25 M-NaCl and had an electrophoretic mobility on cellulose acetate of 0.78 relative to that of chondroitin 4-sulphate. It had a number-average mol.wt. of 15000 (determined by osmometry) and a weight-average mol.wt. of 21000 (determined by sedimentation-equilibrium centrifugation; Wusteman & Davidson, 1975). Gel chromatography on Sephadex G-100 indicated that this preparation of heparan sulphate was of lower mol.wt. than that obtained from the aorta which, from its elution profile (see Wasteson & Wessler, 1971) was estimated to be in the range of 25000–27000. The heparan sulphate obtained from the lung contained 0.95 mol of uronic acid, 0.21 mol of amino acid residues, 0.90 mol of total ester sulphate and 0.50 mol of *N*-sulphate, all per mol of hexosamine. After de-*N*-sulphation and resulphation the product had a specific radioactivity of 0.75 μCi/μmol of uronic acid. Unless otherwise stated, the aortic heparan *N*-[³⁵S]sulphate was used in the experiments described in the Results section.

Gel chromatography

Samples (0.5 ml) of the injected solution, urine or plasma were applied to a column (550 mm × 9 mm) of Sephadex G-100 (Pharmacia) and eluted with 0.5 M-NaCl at a flow rate of 8 ml/h. Fractions (1 ml) were collected and 0.5 ml portions were removed, added to 5 ml of PCS Solubilizer (Amersham/Searle, The Radiochemical Centre) and radioactivity was measured by liquid-scintillation counting in a Corumatic 200 instrument (International Chemical and Nuclear Corp., Hersham, Surrey, U.K.). Tissue samples were counted for radioactivity after adding a weighed portion (approx. 100 mg) to 2 ml of Soluene 350 (Packard Instrument Co., Downers Grove, IL, U.S.A.) and digesting at 50°C for 16 h.

Results

Experiments with free-range animals

After intravenous injection of heparan *N*-[³⁵S]-sulphate to rats (three male and three female at doses of 0.34–0.64 mg/kg body wt.), 48.3–97.5% of the radioactivity was excreted in the urine and faeces

* One Kunitz unit produces an increase in A_{260} (DNA) or A_{300} (RNA) of 0.001/min at 25°C when the enzyme is incubated in acetate buffer, pH 5.0, in the presence of highly polymerized nucleic acid.

over 24 h. Of the radioactive dose, 1.5–25% was excreted in the faeces. The elution profiles on Sephadex G-100 of urine collected over 6 h showed two distinct radioactive peaks (see Fig. 1). One radioactive peak, a polymeric component, corresponded to heparan N -[^{35}S]sulphate, and a component of low molecular weight was identified as inorganic [^{35}S]sulphate by its elution profile, precipitation by Ba^{2+} ions and its electrophoretic mobility. After 6 h, inorganic [^{35}S]sulphate was the major radioactive component in urine. Although these experiments showed that circulating heparan sulphate can be extensively N -desulphated in the rat, the wide variability in the recoveries of the total radioactivity made it necessary to use wholly anaesthetized rats

with cannulated ureters to eliminate retention of urine in the bladder and thus obtain a more accurate time course of heparan sulphate degradation and excretion.

Experiments with cannulated animals

The recoveries of radioactivity in urine and bile after intravenous injection of heparan N -[^{35}S]sulphate are shown in Table 1. Analysis of the urine samples of Sephadex G-100 showed that, during the first 2 h after administration, all the radioactivity in urine was associated with macromolecular material. After 2 h, inorganic [^{35}S]sulphate appeared in the urine, and its proportion of the total radioactivity in urine increased steadily during the subsequent 4 h. Some radioactivity (up to 7.7% of the dose) was excreted in bile, but polymeric material was not detected.

Tissue distribution of radioactivity after administration of heparan N -[^{35}S]sulphate

Whole-body radioautograms obtained from animals killed between 1 h and 6 h after injection of heparan N -[^{35}S]sulphate at a dose of 20 mg/kg body wt. showed a general distribution of radioactivity with no significant intracellular accumulation of radioisotope above the concentration in the blood. The only regions showing concentration of ^{35}S were the lumen of the stomach and intestine and the renal pelvis.

The apparent lack of accumulation of ^{35}S in the major organs was confirmed by a series of experiments in which anaesthetized adult rats were injected intravenously with heparan N -[^{35}S]sulphate (1.57–4.05 mg/kg). Urine was collected, the animals were killed at various times after injection (see Table 2) and the radioactive contents of selected organs and body fluids were determined. The results summarized in Table 2 suggest that an appreciable proportion of the injected radioactivity is to be found only in the liver and kidneys. Even these results do not, however, prove that intracellular accumulation takes place in these organs. When considered as

Fig. 1. Gel chromatography on Sephadex G-100 of urine collected 3–6 h after injection of heparan N -[^{35}S]sulphate into wholly anaesthetized rats with cannulated ureters. The column was eluted with 0.5 M-NaCl. Elution profiles are shown of the labelled metabolites after injection of heparan N -[^{35}S]sulphate at a dose of 1.6 mg/kg body wt. alone (○), and simultaneously with heparin at a dose of 8.0 mg/kg body wt. (●). For comparison the elution profile of the injected heparan sulphate dissolved in rat urine is shown (▲). The void volume of the column (550 mm × 9 mm) is indicated by the arrow.

Table 1. Excretion of radioactivity from rats with cannulated ureters after intravenous injections of heparan N -[^{35}S]sulphate. The results are expressed as percentage of injected dose. Values in parentheses are inorganic [^{35}S]sulphate determined by gel chromatography on Sephadex G-100. When measuring total plasma radioactivity, a plasma volume of 4 ml/100 g wt. was assumed. * indicates that the sample injected was derived from heparan sulphate of bovine lung, not aorta.

Dose (mg/kg body wt.)	Radioactivity present in							Plasma 6h after injection
	Urine						Bile 0–6h	
	0–1 h	1–2h	2–3h	3–4h	4–5h	5–6h		
0.51*	26.6 (0)	11.7 (0)	2.7 (0.8)	0.8 (0.6)	0.6 (0.5)	0.3 (0.3)	0.4 (0.4)	—
1.7	25.6 (0)	15.7 (0)	13.3 (1.2)	2.3 (0.6)	2.1 (0.8)	1.4 (0.8)	6.4 (6.4)	0.8
2.5*	25.8 (0)	26.3 (0)	10.3 (1.4)	3.2 (1.6)	1.6 (1.3)	0.8 (0.7)	3.3 (3.3)	2.1

Table 2. *Distribution of radioactivity in the organs of rats with cannulated ureters after intravenous injection of heparan N-[³⁵S]sulphate*

The results are expressed as percentage of injected dose. Values in parentheses are the specific radioactivities (c.p.m./100mg wet wt. of tissue). In determining total plasma radioactivity a plasma volume of 4 ml/100g body wt. was assumed. * indicates that the sample injected was derived from heparan sulphate of bovine lung, not aorta.

Dose (mg/kg body wt.)	Time after injection (min)	Radioactivity present in						
		Plasma	Urine	Liver	Kidney	Spleen	Small intestine	Lung
4.05	80	29.1	5.9	3.27 (176)	2.68 (2227)	0.03 (102)	0.26 (113)	0.17 (273)
3.70	125	24.7	40.4	5.63 (200)	0.60 (375)	0.06 (135)	0.52 (101)	0.16 (187)
2.50*	360	2.1	68.0	3.70 (722)	1.67 (1350)	0.15 (600)	—	0.47 (470)
1.57	360	5.2	41.5	6.89 (148)	1.13 (188)	0.25 (40)	0.43 (60)	0.87 (112)

radioactivity per g of tissue, the accumulation in the liver is no greater than that of other organs, and the values for kidney radioactivity must include a substantial contribution from the radioactive components in the process of urinary excretion. Other tissues examined included thymus, lymph nodes, salivary glands, heart and stomach. All were labelled to some extent, with specific radioactivities (expressed per g wet wt. of tissue) in the same range as observed for the organs, other than kidney, listed in Table 2.

Effect of the removal of certain organs on the metabolism of heparan N-[³⁵S]sulphate

In an attempt to assess their contribution to the catabolism of heparan N-[³⁵S]sulphate *in vivo*, certain organs were removed or eliminated from the circulation of wholly anaesthetized rats. Plasma and urine (where possible) were collected and analysed by chromatography on Sephadex G-100 6h after intravenous administration of heparan N-[³⁵S]sulphate. After this period the plasma of a renalectomized rat which had been given a dose of 1.6mg/kg body wt. was found to contain 26% of the injected radioactivity, of which 11% was inorganic [³⁵S]sulphate. The comparable values for five control animals range between 40.5 and 78.5% of the injected radioactivity recovered in urine plus plasma, of which 5.6–10.6% was inorganic [³⁵S]sulphate. Thus though renalectomy resulted in greater uptake of radioactivity by the tissues, no significant decrease in production of inorganic [³⁵S]sulphate resulted. It was concluded that the kidneys were not involved to a major extent in the catabolism of heparan sulphate. In a similar manner it was found that removal of the spleen did not decrease urinary excretion of inorganic [³⁵S]sulphate after intravenous administration of heparan N-[³⁵S]sulphate at doses of 0.3–0.9mg/kg body wt. For technical reasons hepatectomy alone is not possible in the rat, and it is necessary to remove the gastrointestinal tract and accessory organs in addition to the liver. After removal of the gastrointestinal tract, pancreas and spleen, but not the liver, urinary excretion of the inorganic [³⁵S]sulphate was decreased

(from 4.1% of injected radioactivity to 2.3%, both at a dose of 0.9mg/kg body wt.), but still appreciable.

With the liver also removed from the circulation it was found that heparan N-[³⁵S]sulphate at a dose of 1.6mg/kg body wt. was still desulphated to a significant extent, giving rise to inorganic [³⁵S]sulphate in urine and plasma which accounted for 2.1% of the injected radioactivity (control values 3.4–7.6 in five rats). The radioactivity remaining in the plasma after 6h was also high (34.7% compared with control values of 0.2–16.7%), whereas, in contrast, removal of the other organs (except for the liver) had resulted in a decrease in plasma radioactivity (from 9.1% in the control to 1.3%). Though the liver is clearly not essential for the desulphation of circulating heparan sulphate, it seems to have a role in its uptake by the tissues. However, these data do not allow quantitative assessment of the contribution of any particular organ, and certainly do not exclude the possibility that organs such as liver, spleen, kidney and gastrointestinal tract are all involved to some extent in the degradation of heparan N-[³⁵S]sulphate *in vivo*. Collectively these results suggest many similarities to the catabolism of heparin, which is known to take place at many sites *in vivo* (Stau *et al.*, 1973). It was decided therefore to establish if heparin decreased the extent of heparan sulphate metabolism when the two polymers were administered simultaneously.

Effect of heparin on the metabolism of heparan N-[³⁵S]sulphate

The effect of heparin on heparan N-[³⁵S]sulphate degradation was studied by comparing the radioactive contents of plasma and urine obtained from control animals receiving heparan N-[³⁵S]sulphate alone and test animals receiving heparan N-[³⁵S]sulphate together with various amounts of heparin. Control animals (two) with ureter cannulae received heparan N-[³⁵S]sulphate (1.6mg/kg body wt.) intravenously. Urine collected in two fractions (0–3h and 3–6h) and plasma (obtained after 6h) were analysed for radioactivity both quantitatively and qualitatively

Table 3. *Effect of heparin on the desulphation of heparan N -[^{35}S]sulphate (1.6 mg/kg body wt.) in rats with cannulated ureters*

The results are expressed as percentage of injected dose. Values in parentheses are inorganic [^{35}S]sulphate determined by gel chromatography on Sephadex G-100. In determining total plasma radioactivity a plasma volume of 4 ml/100 g body wt. was assumed.

Heparin (mg/kg body wt.)	Radioactivity present in urine		Radioactivity present in plasma 6h after injection
	0-3 h	3-6 h	
0.0	55.2 (2.0)	8.6 (4.6)	9.0 (1.0)
0.0	42.0 (0.0)	9.8 (4.3)	16.7 (1.6)
2.0	54.4 (0.0)	11.2 (1.1)	21.3 (0.5)
4.0	50.8 (0.0)	10.2 (0.9)	36.1 (0.0)
8.0	55.2 (0.0)	8.0 (0.2)	29.8 (0.0)

Table 4. *Effect of heparan sulphate on the desulphation of N -[^{35}S]sulphated heparin (1.6 mg/kg body wt.) in rats with cannulated ureters*

The results are expressed as percentage of injected dose. Values in parentheses are inorganic [^{35}S]sulphate determined by gel chromatography on Sephadex G-100.

Heparan sulphate (mg/kg body wt.)	Radioactivity present in urine		Radioactivity present in plasma after injection
	0-3 h	3-6 h	
0.0	35.5 (1.0)	4.8 (3.1)	0.2
0.0	31.7 (0.9)	3.2 (3.0)	1.9
2.0	47.5 (1.2)	7.3 (3.9)	1.5
2.0	33.5 (1.5)	2.6 (2.4)	0.9
8.0	40.4 (0.9)	2.8 (2.1)	1.3
16.0	32.3 (0.6)	2.7 (2.0)	1.4

(Fig. 1). Test animals (three) were treated similarly, except that various doses of heparin (2-8 mg/kg body wt.) were administered intravenously 4 min before the administration of the heparan N -[^{35}S]sulphate. The results (see Table 3) showed two striking differences between test and control animals. Firstly, the amounts of radioactivity in the plasma after 6 h in test animals (21.3-36.1% of the injected dose) were substantially greater than those recorded in control animals (9.0 and 16.7% of the injected dose). Further, in test animals receiving heparin at 4 and 8 mg/kg, inorganic [^{35}S]sulphate could not be detected in the plasma. Secondly, the proportion of inorganic [^{35}S]sulphate in the 3-6 h urines was progressively decreased by increasing doses of heparin from 44 and 54% in the controls to 10, 9 and 3% in test animals receiving heparin at 2, 4 and 8 mg/kg respectively.

Subsequent experiments in which the metabolism

of N -[^{35}S]sulphated heparin was studied in the presence and absence of heparan sulphate are recorded in Table 4. Control animals (two) with ureter cannulae received N -[^{35}S]sulphated heparin (1.6 mg/kg body wt.) intravenously. Urines, collected in two fractions (0-3 and 3-6 h) and plasma (obtained after 6 h) were analysed for radioactivity both quantitatively and qualitatively (Fig. 2). Test animals were treated similarly except that heparan sulphate (2.0-16.0 mg/kg body wt.) was administered intravenously 4 min before the administration of N -[^{35}S]sulphated heparin. The results showed that, unlike heparan sulphate, heparin is cleared rapidly from the circulation, since both the radioactivity in plasma after 6 h and the urinary excretion of N -[^{35}S]sulphated heparin were low. The simultaneous administration of unlabelled heparan sulphate had no effect on either of these quantities.

Experiments with isolated perfused rat liver

Although the whole-body radioautograms and the determinations of tissue specific radioactivities obtained after intravenous administration of heparan N -[^{35}S]sulphate showed no accumulation of radioactivity in the liver, these experiments do not exclude this organ as a possible site of metabolism. Clearly, the extent of accumulation of radioisotope only reflects the relative rates of uptake and secretion and gives no indication of the turnover of labelled material in the liver or any other organ. The capacity

Fig. 2. *Gel chromatography on Sephadex G-100 of urine collected 3-6 h after injection of N -[^{35}S]sulphated heparin into wholly anaesthetized rats with cannulated ureters* The column was eluted with 0.5 M-NaCl. Elution profiles of the labelled metabolites after injection of N -[^{35}S]sulphated heparin at a dose of 1.6 mg/kg body wt. alone (○), and simultaneously with heparan, sulphate at a dose of 16.0 mg/kg body wt. (●). For comparison the elution profile of the injected heparin dissolved in rat urine is shown (▲). The void volume of the column (550 mm × 9 mm) is indicated by the arrow.

of the liver to metabolize heparan sulphate cannot be investigated by perfusion of heparinized whole blood because of the inhibitory effect of heparin. For this reason, isolated rat liver (approx. 13.8 g) was perfused for 6 h with defibrinated rabbit blood (100 ml) containing heparan N -[^{35}S]sulphate (from bovine lung; 1.4 mg/100 ml of perfusate). After 6 h, 19% of the radioactivity had been removed from the perfusate and was hence bound by the liver. Analysis of the plasma by gel chromatography on Sephadex G-100 showed, however, no degradation or desulphation of the remaining heparan N -[^{35}S]sulphate.

Metabolism of heparan N -[^{35}S]sulphate by rat blood

For the reasons detailed above it was not possible to assess the degradation of heparan N -[^{35}S]sulphate in heparinized whole blood. To avoid the inhibitory effect of heparin, potassium oxalate was used as an alternative anti-coagulant. At the concentration used oxalate was found not to prevent extensive degradation of heparan N -[^{35}S]sulphate by a crude rat spleen extract (F. S. Wusteman, unpublished work). Blood (8.5 ml) was collected into 1.5 ml of 0.1 M-potassium oxalate. Heparan N -[^{35}S]sulphate (360 μg) was added and the mixture incubated at 37°C for 6 h. The plasma obtained by centrifuging (1500 g for 5 min) was subjected to chromatography on Sephadex G-100. The elution profile showed the presence of unchanged heparan N -[^{35}S]sulphate only.

Discussion

The desulphation of heparan N -[^{35}S]sulphate in the rat is in some aspects comparable with the catabolism of another glycosaminoglycan, chondroitin 4-sulphate (Wood *et al.*, 1973). Unchanged polymer is initially excreted in the urine, but after 3 h most of the radioactivity in urine is in the form of inorganic [^{35}S]sulphate. A further parallel exists in the absence of detectable oligosaccharide sulphates in the circulation or in the urine, which suggests that cellular uptake of both polymers is followed by total removal of N -sulphate groups in the lysosomes. However, the catabolism of the two glycosaminoglycans differs in at least one important respect; whereas intravenously administered chondroitin sulphate accumulates in the liver almost exclusively and is degraded there, heparan sulphate is still desulphated after removal of the liver as well as kidneys, spleen and much of the gastrointestinal tract. Moreover, since free inorganic sulphate re-enters the perfusate during metabolism of chondroitin sulphate by isolated rat liver, its absence after 6 h perfusion with labelled heparan sulphate must mean that N -desulphation has not taken place. The uptake of some radioactivity by the perfused liver could be due to the

reversible binding of intact heparan sulphate to liver cells, as reported by Kjellen *et al.* (1977), but may also involve uptake by liver lysosomes and partial degradation by endoglycosidase (Höök *et al.*, 1975) yielding products still too large to escape from the lysosome. This negative result does not necessarily exclude the liver as a possible site of desulphation *in vivo* over longer periods of time. Moreover, in the preparation of defibrinated blood, the leucocytes and other cells are removed and some of them may aid the degradation of heparan sulphate by the liver. On the other hand, the failure of the isolated perfused liver to desulphate heparan sulphate is consistent with similar results obtained for heparin (Di Ferrante & Popenoe, 1966). A further similarity to the fate of heparin is suggested by the inhibitory effect of this glycosaminoglycan on the uptake and desulphation of heparan N -[^{35}S]sulphate (though heparan sulphate does not appear to inhibit the catabolism of heparin). Several explanations for these results are possible. The more rapid uptake of heparin from the circulation implies that the affinity of the metabolizing cells may be considerably greater than for heparan sulphate, a conclusion supported by the results of Kjellen *et al.* (1977), working with rat liver cells (these authors also note that heparin competes successfully with heparan sulphate for its binding sites, though the converse does not happen).

Another possible explanation stems from studies *in vitro* with a heparan sulphate endoglycosidase from rat spleen (M. Perry, unpublished work). This endoglycosidase is similar to that reported in liver (Höök *et al.*, 1975) in that it does not degrade heparin significantly. Although heparin is not a substrate for this enzyme, it totally inhibits the degradation of heparan sulphate when the two glycosaminoglycans are present in equal concentrations. Presumably, a preliminary endoglycosidase degradation is a prerequisite for extensive desulphation (Dorfman & Matalon, 1976), so competition for this enzyme(s) within the lysosome could explain the inhibitory effect of heparin.

Collectively, the results show that intravenously administered heparan N -[^{35}S]sulphate, like heparin, is distributed to a number of sites in the rat. Further, inhibitory studies *in vitro* and *in vivo* suggest that at least some of these sites are common to both polymers. Since there is no evidence that heparin metabolism is abnormal in the mucopolysaccharidoses (Muir, 1973), there must be some degradative process which is not shared by heparin and heparan sulphate. This could be the intracellular digestion resulting when heparan sulphate in the form of undegraded proteoglycan is taken up by pinocytosis, a process described by Kresse *et al.* (1975) for bovine arterial fibroblasts.

Any heparan sulphate not so degraded at its point of origin could be released into the circulation after

proteolytic attack. Since there is no evidence of degradation in the blood itself, it can either be excreted by ultrafiltration in the kidneys (Wasteson & Wessler, 1971) or undergo degradation, as does heparin, at sites, such as the vascular endothelium (Hiebert & Jaques, 1976), which are widespread in the body.

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