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The Excretion and Degradation of Chondroitin 4-Sulphate Administered to Guinea Pigs as Free Chondroitin Sulphate and as Proteoglycan

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The excretion and degradation was studied of ^{35}S-labelled 4-chondroitin sulphate injected into guinea pigs in the form of proteoglycan isolated from cartilage and in the form of free chondroitin 4-sulphate prepared from the same proteoglycan by proteolysis. When the proteoglycan was injected there was a delay of about 15–20min before significant amounts or radioactivity were excreted, whereas after injection of chondroitin 4-sulphate a considerable amount of radioactivity was excreted within 10min and a much higher proportion of the radioactive dose was excreted in 1h or 24h compared with the proteoglycan. In both cases, however, a major part of the radioactivity was not excreted even in 24h. Sterile conditions were used to collect the radioactive material directly from the bladder. When chondroitin 4-sulphate was injected, the molecular sizes of injected and excreted materials were similar, as assessed by gel chromatography on Sephadex G-200, whereas when proteoglycan was injected the molecular size of the excreted labelled material was similar to that of the chondroitin 4-sulphate chains in the original proteoglycan. In neither case did the size of the excreted labelled material change with time over 1h, and low-molecular-weight labelled material was virtually absent. In contrast, when urine was collected for 24h without preservative the labelled material in it was extensively degraded after either the proteoglycan or chondroitin 4-sulphate had been given. Chondroitin 4-sulphate became similarly degraded when incubated with non-sterile urine, but not when the urine was passed through a bacterial filter, suggesting that degradation was caused by contaminating micro-organisms in the experiments in which urine was collected for 24 h. It is concluded that chondroitin 4-sulphate chains of about 18000molecular weight can be excreted readily as such, whereas intact proteoglycans must be degraded to free glycosaminoglycans first, although both are taken up by the tissues more rapidly than they are excreted.

Normal urine contains small amounts of glycosaminoglycan which is mainly chondroitin sulphate (Di Ferrante & Rich, 1957; Varadi *et al.*, 1963). In previous work on the metabolism and excretion of chondroitin sulphate, the free glycosaminoglycan has been used rather than the proteoglycan, the form in which it occurs in the tissues. When it was injected in large amounts, chondroitin sulphate was recovered in the urine (Smith & Kerby, 1960), whereas when small amounts of ^{35}S-labelled chondroitin sulphate or homogenized ^{35}S-labelled cartilage were injected into rats, most of the radioactivity excreted in 24h was in the form of inorganic sulphate (Dziewiatkowski, 1956). Kaplan & Meyer (1962), were unable to recover significant amounts of chondroitin 4- or 6-sulphate in the urine when it was injected into human subjects or dogs.

In the present study the metabolism and excretion are compared of exogeneous labelled chondroitin 4-sulphate administered either as the intact proteoglycan or as free chondroitin sulphate prepared from the same proteoglycan by proteolysis.

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Materials and Methods

Reagents

All reagents were of analytical grade, with the exception of 9-aminoacridine hydrochloride, used to prepare glycosaminoglycans and proteoglycans. Acetone and ethanol were R.R. grade (James Burrough Ltd., London S.E.11, U.K.). Sterile sodium [^{35}S]sulphate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Determination of hexuronic acid

Hexuronic acid was determined by the carbazole reaction (Dische, 1947) as described by Bitter & Muir (1962). Glucuronolactone was used as a standard.

Preparation of ^{35}S-labelled proteoglycan and glycosaminoglycan

Proteoglycan labelled with ^{35}S was prepared by incubating pig laryngeal-cartilage slices with sodium [^{35}S]sulphate as described by Hardingham & Muir (1972). The specific radioactivity of the purified proteoglycan was 98000c.p.m./mg of uronic acid. Labelled glycosaminoglycan was prepared from this

by digestion with papain, also as described by Hardingham & Muir (1972). In addition, compounds of higher specific radioactivity were kindly given by Dr. T. E. Hardingham. The specific radioactivities of this proteoglycan and glycosaminoglycan were 150000 and 255000c.p.m./mg of uronic acid respectively.

Injection of ^{35}S-labelled proteoglycan and glycosaminoglycan and collection of excreted radioactive materials

Series I: bladder irrigation. Eight female Hartley guinea pigs weighing 400–500g were sedated by intraperitoneal injection of pentobarbitone sodium B.Vet.C. (Nembutal; Abbot Laboratories Ltd., Queenborough, Kent, U.K.) at a dose of 2.4mg/100g body wt. A fine Neoplex tube (diam. 15mm) was passed into the bladder, which was irrigated with 2ml of sterile distilled water. Irrigation fluid was collected by gentle suprapubic pressure to empty the bladder. Additional surgical anaesthesia was provided by the use of anaesthetic diethyl ether, while an incision was made in the neck and the jugular vein dissected. Solutions containing 0.5mg of ^{35}S-labelled proteoglycan or glycosaminoglycan/ml were made up in 0.15M-NaCl. Doses of 0.5 or 0.25mg of proteoglycan or glycosaminoglycan were injected into the jugular vein, under direct vision, with a fine needle (Gillette no. 17: 25 S.W.G. × 15/16in). The bladder was then irrigated with 2ml of sterile water by the method described above at intervals of 10min up to 1h afterwards, except with two animals receiving proteoglycan, when collections were made at 15min intervals (see Fig. 1). The animals were all sedated throughout this period. The radioactivity measurement and gel chromatography of excreted material were carried out as soon as possible, otherwise they were stored at 4°C where necessary.

Series II: urine collections. Twelve female Hartley guinea pigs weighing 400–500g were injected intravenously with ^{35}S-labelled proteoglycan or glycosaminoglycan as described above, except that ether anaesthesia alone was used during dissection of the jugular vein. Doses of 0.1, 0.25 and 0.5mg of proteoglycan (0.5mg/ml in 0.15M-NaCl) or 0.1 and 0.2mg of glycosaminoglycan (0.2mg/ml in 0.15M-NaCl) were used. After recovering from anaesthesia the animals were placed in metabolism cages, in which urine was collected separately from faeces by means of a funnel containing a light-weight ball in the neck. Urine was collected, without preservative, for 24h after the injection.

Determination of radioactivity of excreted ^{35}S-labelled material

Samples of bladder irrigation fluid (series I) and urine (series II) were made up to known volumes and centrifuged, and 0.5ml portions of clear supernatant were each taken into a volume of 15ml of scintillation fluid consisting of 80g of naphthalene, 4g of 2,5 bis-(t-butylbenzoxazol-2-yl)thiophen and 520ml of toluene made up to 1 litre with 2-methoxyethanol (Hardingham & Phelps, 1968). Radioactivity was measured in a Packard Tri-Carb model 3320 liquid-scintillation spectrometer. The radioactivity of each sample was counted in the absence and in the presence of quenching, and counts were corrected according to the calculated efficiency, which varied between 75 and 80%. The background count for each urine or irrigation fluid was obtained by counting the apparent radioactivity of the sample obtained from the animal before the labelled material was injected. These background counts were then subtracted after correction for quenching from the corrected counts for each sample, and the results expressed in terms of the radioactivity in the total volume of the sample. At least 1000 counts above the background value were recorded from each sample. The maximum counting error was ±3.2%.

Gel chromatography of injected and excreted material

The number-average molecular weight of ^{35}S-labelled glycosaminoglycan prepared as described above was determined by gel chromatography on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) by the method of Wasteson (1969). Samples (1ml) containing 1mg of glycosaminoglycan were applied to a column (55cm × 1.1cm) of Sephadex G-200 and eluted with 0.2M-sodium acetate buffer, pH6.8, at a flow rate of 5ml/h at 4°C. Fractions (0.85ml) were collected and the uronic acid content and radioactivity were determined. The elution profile of uronic acid coincided exactly with that of radioactivity. Proteoglycan from pig laryngeal cartilage was eluted in the void volume.

Samples of bladder irrigation fluid (series I) representing material excreted at 10min intervals during 1h were centrifuged, and 1ml of each clear supernatant was applied to the same column of Sephadex G-200, which was eluted in the same way with 0.2M-sodium acetate buffer, pH6.8, and the radioactivity was determined on 0.5ml of each eluent fraction of volume 0.85ml. The irrigation fluid from animal IB, collected for 45min, and from animal IC, collected for 1h, were separately pooled and dialysed under positive pressure by using a Diaflo UMO2 membrane. The radioactivity in the diffusates and dialysis residues was determined and samples of the latter were applied to the column.

Urine was collected for 24h (series II) and was dialysed under negative pressure through previously heated dialysis tubing to decrease porosity (Callanan *et al.*, 1957), except in three experiments where the urine was not dialysed. The radioactivity of diffusates

and dialysis residues was determined. Samples (1 ml) of dialysis residues from animals IIC, IIG and IIH were each chromatographed on the same column of Sephadex G-200 as described above, and 0.5 ml of each of the eluent fractions was taken for the determination of radioactivity.

Incubation of glycosaminoglycan with urine in vitro

Two 1 ml samples of bladder irrigation fluid were obtained by catheterization of a normal female guinea pig as described above. Sterile water was used for irrigation, the tubing used was sterilized by boiling for 30 min before use and the fluid was collected in sterile disposable Universal containers (Sterilin Ltd., Richmond, Surrey, U.K.). A further sample of irrigation fluid, obtained under similar sterile conditions, was filtered under positive pressure through a Millipore filter (13 mm diam., 0.45 μm pore size; Millipore Ltd., Wembley, Middlesex, U.K.). Samples (1 ml) of urine collected from normal animals by catheterization as described above were also filtered in the same way to remove bacteria, whereas other samples were used directly. To each 1 ml sample of irrigation fluid or urine was added 0.4 ml of a solution of ^{35}S-labelled glycosaminoglycan containing 1 mg of uronic acid/ml. The solutions were incubated at 37°C for 24 h, after which samples (0.5 ml) were taken from each solution for gel chromatography on Sephadex G-200 as described above, and the radioactivity of the eluent fractions was determined.

Results

Series I

Recovery of radioactivity in the bladder at 10 min intervals for 1 h after intravenous injection of ^{35}S-labelled proteoglycan or glycosaminoglycan. There

Table 1. *Series I: proportion of the injected radioactivity in bladder irrigation fluid of guinea pigs 1 h after the intravenous injection of proteoglycan or chondroitin sulphate (molecular weight 18000) and the molecular size of ^{35}S-labelled material appearing in the fluid at 10 min intervals*

Experimental details are given in the text.

Animal	Amount of material injected (mg)	Total radioactivity of combined samples of irrigation fluid (% of injected radioactivity)	Time of collection of sample for gel chromatography (min after injection)	$K_{av.}$	Number-average molecular weight
		Proteoglycan			
A	0.25		0–10	0.48	14500
			10–20	0.45	16000
		14.3			
B	0.50	8.5	0–45*	0.45	16000
C	0.50	9.1	0–60*	0.44	16000
D	0.50		20–30	0.44	16500
			30–40	0.43	16500
			40–50	0.40	18000
		9.7			
		Glycosaminoglycan			
E	0.25		0–10	0.45	16000
			10–20	0.45	16000
			20–30	0.42	17000
		40.5			
F	0.25		0–10	0.42	17000
			10–20	0.42	17000
			20–30	0.40	18500
		40.2			
G	0.25	30.1 (in 30 min)	0–10	0.47	15000
			10–20	0.45	16000
H	0.25		0–10	0.40	18000
			10–20	0.39	19000
		36.2			

* Pooled dialysed samples (see the text).

was a marked difference in the total radioactivity excreted during 1h after proteoglycan or glycosaminoglycan was injected. When ^{35}S-labelled proteoglycan was injected only about 8–14% of the radioactive dose was excreted, whereas when ^{35}S-labelled glycosaminoglycan was injected 36–40% was excreted (Table 1). In the case of animal IG as much as 30.1% of the radioactive dose was excreted in the first 30min, after which the experiment was stopped because there was blood in the irrigation fluid, which may have resulted from damage by the catheter to the bladder mucosa. Fig. 1 shows the proportion of the radioactivity excreted during successive intervals of 10 or 15min (animals B and C; Fig. 1) after intravenous injection of labelled proteoglycan and glycosaminoglycan. It is notable that, in all four animals given labelled glycosaminoglycan, most of the excreted radioactivity appeared in the first 30min, whereas with animals IB, IC and ID, which were given proteoglycan, there was a delay of at least 15min before significant radioactivity appeared in the irrigation fluid.

Gel chromatography of radioactive material excreted within 1 h of the injection of ^{35}S-labelled proteoglycan and glycosaminoglycan. The ^{35}S-labelled material excreted after the injection of labelled proteoglycan was retarded on Sephadex G-200, although the original proteoglycan itself was totally excluded from the gel. The elution profiles, however, were symmetrical and the number-average molecular weight was calculated to be 14500–18500 (Wasteson, 1969), which corresponded to the number-average molecular weight of 18000 for the glycosaminoglycan obtained by papain digestion of the original proteoglycan (Fig. 2*a*). Moreover, the molecular size of material excreted by different animals during successive 10min intervals after injection of radioactive compounds did not change with time, as shown in Table 1.

In contrast with proteoglycans, when ^{35}S-labelled glycosaminoglycan was injected the molecular sizes of injected and excreted material were similar. The molecular weight of the radioactive material in the bladder irrigation fluid was thus calculated to be 15000–19000 (Table 1), compared with 18000 for the injected glycosaminoglycan. The excreted materials showed symmetrical elution profiles and again there was no significant change of molecular size with time after injection, nor was there a significant difference between animals. The elution profiles of the original glycosaminoglycan that was injected and of the material in the irrigation fluid obtained from animal IE at 0–10, 10–20 and 20–30min after the injection are shown in Fig. 2.

On no occasion after the injection of either proteoglycan or glycosaminoglycan was there evidence for the presence of inorganic sulphate or material of low molecular weight in the excreted material, as shown by elution behaviour on Sephadex G-200. In two experiments where irrigation fluid was dialysed under positive pressure (animals IB and IC) only 9 and 1% respectively of the radioactivity appeared in the diffusate.

Series II

Recovery of radioactivity in urine collected for 24h after the intravenous injection of ^{35}S-labelled proteoglycan and glycosaminoglycan. The amount of radioactive material excreted in the second series of experiments, in which urine was collected for 24h after the injection of either proteoglycan or glycosaminoglycan, varied much more from animal to animal.

Exactly how much more labelled material was excreted after the first hour during the subsequent 23h was difficult to determine because of the much greater variation between animals in these experiments. Three animals (IIB, IID and IIE) excreted amounts of radioactivity comparable with that voided in 1h after the injection of proteoglycan, whereas the others excreted somewhat larger amounts in 24h than had animals of series I during 1h. On the other hand, when glycosaminoglycan was administered, less radioactivity was excreted by three animals (IIG, IIH and IIM) in 24h than by animals in series I after 1h. Owing to variation between animals it was therefore possible only to conclude that after the first hour not much more ^{35}S-labelled material was excreted during the following 23h, and again when ^{35}S-labelled proteoglycan was injected less of the radioactive dose was recovered in the urine than when ^{35}S-labelled glycosaminoglycan was given (Table 2).

Gel chromatography of labelled material in the urine excreted during 24h. In all six animals that had been given ^{35}S-labelled proteoglycans a large part of the ^{35}S-labelled material excreted in 24h was diffusible on dialysis, representing between 52.5 and 79.5% of the radioactivity in the urine (Table 2). The non-diffusible radioactive material in the urine from animal IIC was polydisperse and retarded on Sephadex G-200, being mainly eluted near the bed volume. The urine from three of the animals given glycosaminoglycan (IIG, IIH and IIJ) likewise contained 71.5, 86.5 and 78.5% diffusible material (Table 2). The labelled material in dialysed urine from animals IIG and IIH gave asymmetrical elution profiles with a maximum near the bed volume in both cases (Fig. 3). After the injection of either labelled proteoglycan or glycosaminoglycan, only a small amount of radioactive material of larger molecular size was demonstrable in urine, either by dialysis or gel chromatography. Most of the labelled material was highly degraded and probably consisted of inorganic sulphate and small oligosaccharides.

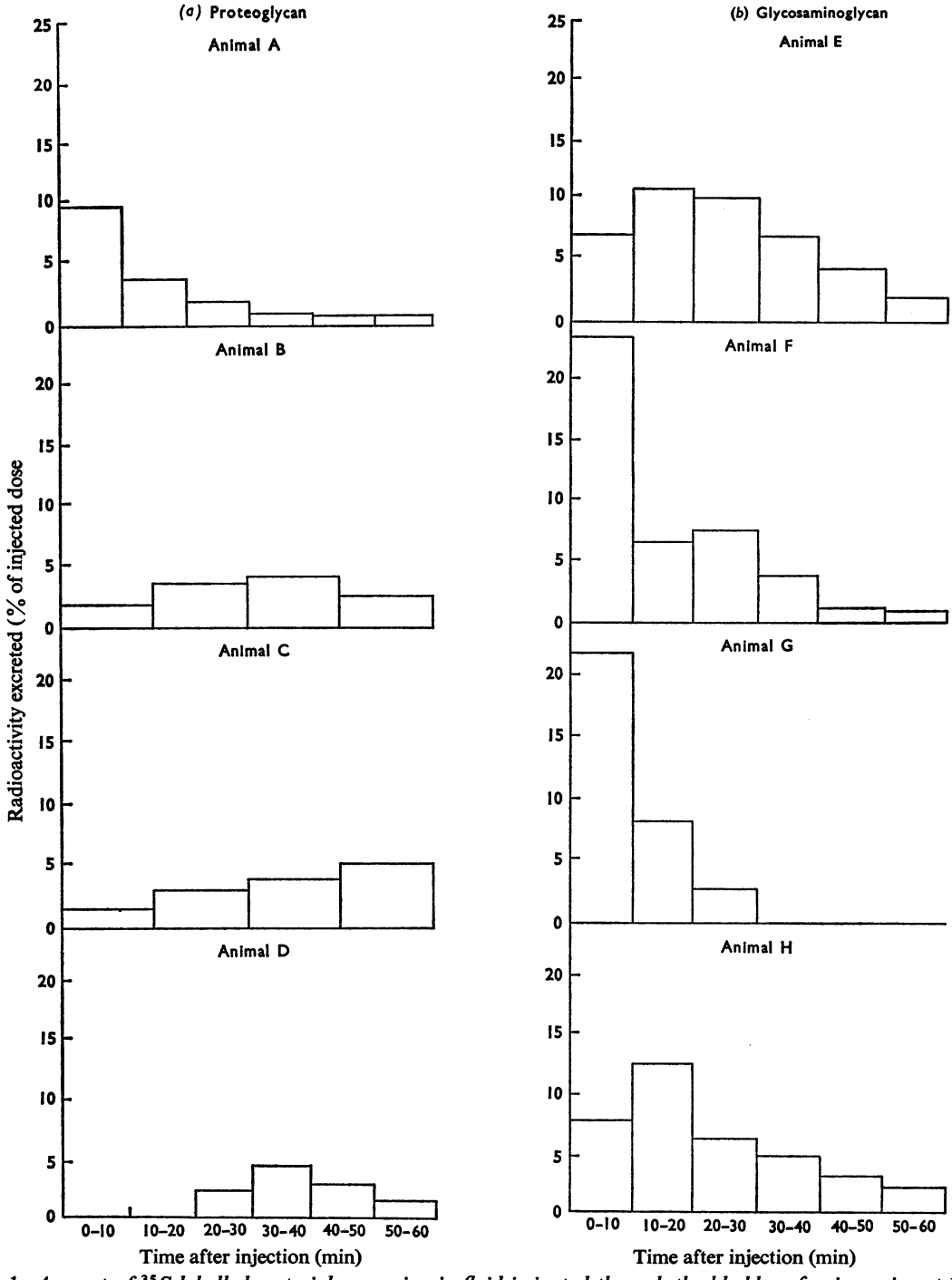

Fig. 1. *Amount of ^{35}S-labelled material appearing in fluid irrigated through the bladder of guinea pigs at 10 min intervals after the injection of ^{35}S-labelled chondroitin sulphate proteoglycan (a) or glycosaminoglycan (b)*

Experimental details are given in the text. In experiments with animals B and C the bladder was irrigated at 15 min intervals.

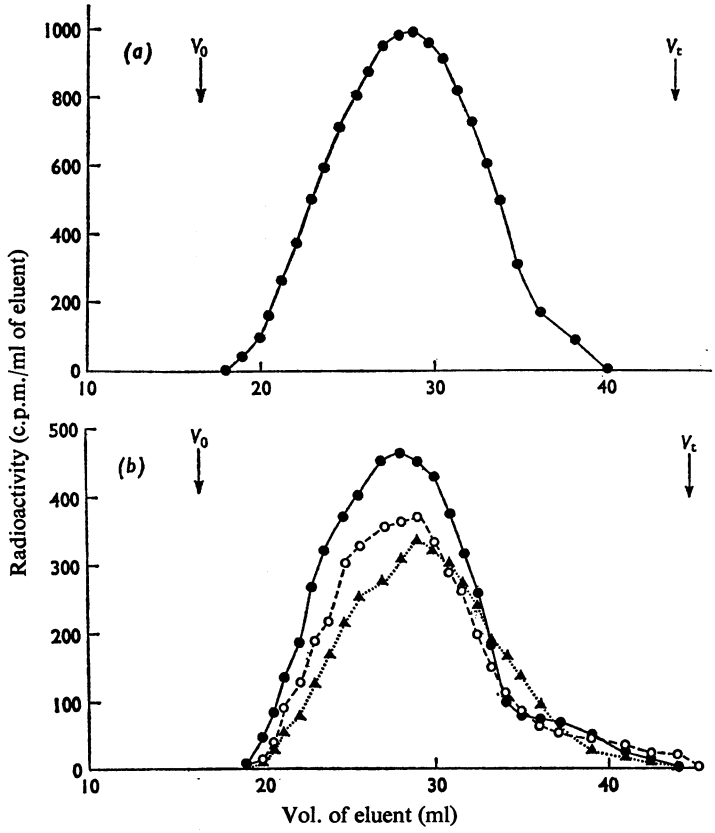

Fig. 2. *Gel chromatography of glycosaminoglycan on Sephadex G-200*

Samples (1 ml) were applied to a column (55 cm × 1.1 cm) of Sephadex G-200 and eluted with 0.2 M-sodium acetate buffer, pH 6.8, at a rate of 5 ml/h. Fractions (0.85 ml) were collected and the radioactivity of each was measured. (*a*) ^{35}S-labelled glycosaminoglycan obtained by papain digestion of ^{35}S-labelled proteoglycan from pig cartilage (for details see the text), 0.1 mg of which was applied to the column; (*b*) ^{35}S-labelled material present in the bladder irrigation fluid obtained from guinea pig IE after the following intervals of time: ▲, 0–10 min; o, 10–20 min; ●, 20–30 min. V_0 and V_t mark the void volume and total volume of the column respectively.

Incubation of normal urine with glycosaminoglycan in vitro

The possibility that the difference in the molecular size of material excreted by animals in experiments of series I compared with those of series II was due to bacterial contamination of urine was investigated by incubating chondroitin sulphate in normal guinea-pig urine without preservative. After 24 h at 37°C the chondroitin sulphate was found to be partially degraded, being retarded on Sephadex G-200 to give a bimodal elution profile (Fig. 4*b*). The elution position of the minor component of larger size corresponded to that of the glycosaminoglycan (Fig. 4*a*), whereas the major component was eluted at the bed volume and was therefore of low molecular weight. On the other hand, when glycosaminoglycan was incubated with urine from which bacteria had been removed by filtration through a Millipore membrane only a small amount of degraded material was evident, most of the material being eluted in the position of the original glycosaminoglycan (Fig. 4*c*). It therefore appears that in experiments of series II the excreted glycosaminoglycans were degraded by contaminating bacteria in the urine during the 24 h period of collection.

Discussion

The results show that free chains of chondroitin 4-sulphate itself or that liberated *in vivo* from the injected proteoglycan may be excreted without further breakdown. No partial degradation products were

Table 2. *Series II: proportion of the injected radioactivity appearing in urine of guinea pigs 24h after intravenous injection of proteoglycan or glycosaminoglycan and the behaviour on dialysis and column chromatography of ^{35}S-labelled material in the urine*

Experimental details are given in the text. V_t is the total volume of the column.

Animal	Amount of material injected (mg)	Total radioactivity in urine after 24h (% of injected radioactivity)	Radioactivity of diffusible material (% of total radioactivity in urine)	Elution on Sephadex G-200
		Proteoglycan		
A	0.50	19.4	53.5	
B	0.50	10.5	69.9	
C	0.25	20.1	62.2	Polydisperse at V_t
D	0.25	10.1	52.5	
E	0.10	8.7	58.5	
F	0.10	17.7	79.5	
		Glycosaminoglycan		
G	0.20	20.5	71.5	Polydisperse at V_t
H	0.20	31.4	85.5	Polydisperse at V_t
J	0.20	41.5	78.5	
K	0.20	37.2		
L	0.10	57.7		
M	0.10	28.6		

evident in the bladder irrigation fluid, nor did the molecular size of the excreted radioactive material decrease with time. Since the chain lengths of the injected chondroitin 4-sulphate prepared by papain digestion of the proteoglycan showed a normal distribution about a mean, which coincided with the distribution of size of the radioactive material in the irrigation fluid, shorter chains do not appear to have been preferentially excreted.

In contrast, since proteoglycans are of high molecular weight, the constituent glycosaminoglycans have first to be released before they can be excreted. Presumably, catheptic enzymes, which may be present in the blood or in the kidneys, slowly attack the protein moiety of proteoglycans. This would account for the delay of 15–20min after the injection of ^{35}S-labelled proteoglycan before appreciable amounts of radioactivity appeared in the bladder irrigation fluid with all the animals except IA, whereas after the injection of ^{35}S-labelled chondroitin 4-sulphate a large proportion of the excreted radioactivity appeared during this initial period (Fig. 1). As the proportion of the radioactive dose excreted after either 1h or 24h was two to four times greater when chondroitin 4-sulphate was injected than when the proteoglycan was given (Tables 1 and 2), proteoglycans may be taken up more readily by the tissues than glycosaminoglycans.

When bladder irrigation fluid was collected at short intervals the glycosaminoglycans were not degraded, whereas there was extensive degradation when urine was collected over 24h without preservative. Since this appeared to be caused by the action of micro-organisms in unsterilized urine, normal bacterial contamination of urine may explain some of the variable results obtained by other workers. Dziewiatkowski (1956) found that when ^{35}S-labelled chondroitin sulphate was injected into rats 37–62% of the radioactivity appeared as inorganic sulphate in the urine, although when large doses were given some of the excreted radioactive material was similar to chondroitin sulphate on paper chromatography and electrophoresis. Similar results were obtained by Danishefsky & Eiber (1959) when ^{35}S-labelled heparin was injected into dogs; much more of the radioactivity was diffusible on dialysis when small doses were given compared with large ones. Dohlman (1956) found that when ^{35}S-labelled chondroitin sulphate was injected into rats the proportion of the diffusible radioactivity increased with time, although it was not all inorganic sulphate. All these investigators suggested that there was some sulphatase activity towards glycosaminoglycans in mammalian tissues. The doses used by Dziewiatkowski (1956) and Dohlman (1956) were much larger per unit of body weight than the amounts used in the present study. Nevertheless, even with these much lower doses, which were 10–1000-fold less, there was no detectable

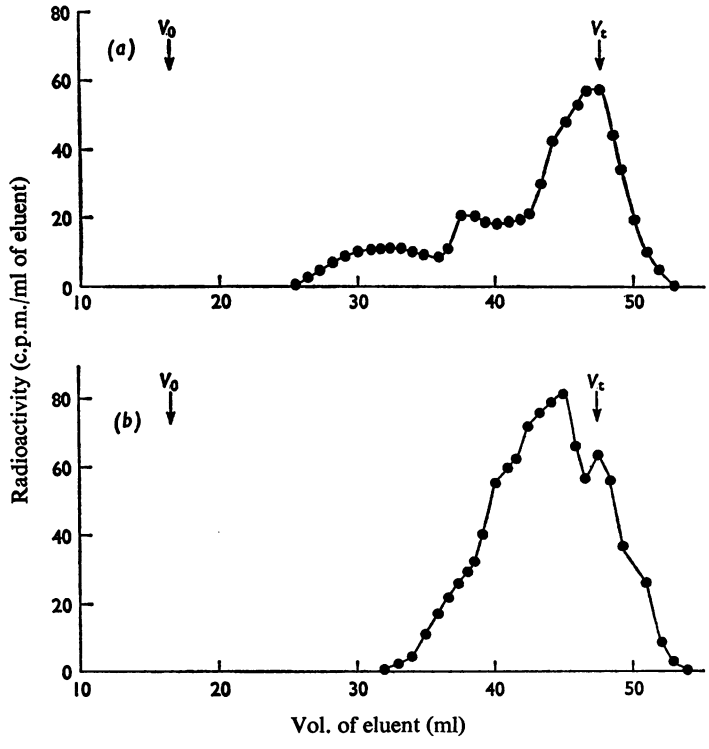

Fig. 3. *Gel chromatography of radioactive material in dialysed urine of guinea pigs on Sephadex G-200*

The conditions of gel chromatography were as described in Fig. 2, 1 ml of urine being applied to the column. The gel chromatography was carried out on radioactive material in dialysed urine from (*a*) animal IIG and (*b*) animal IIH.

degradation of chondroitin sulphate during 1 h when sterile conditions of collection were used. In contrast with the present results, Kaplan & Meyer (1962) recovered almost none of the injected chondroitin 4-sulphate in human subjects, or chondroitin 6-sulphate in dogs when the amounts given were small, even though chloroform was used as a preservative in the urine. There appears to be no explanation for the discrepancy with the present results. Kaplan & Meyer (1962) did, however, recover about one-third of dermatan sulphate when given in similar amounts as chondroitin 4-sulphate. The present study shows that there is little degradation of chondroitin 4-sulphate in the circulation, and, even though hyaluronidase has been identified in plasma (Bollet, 1969), the non-specific inhibitor that is present in serum (Mathews & Dorfman, 1955) may render it inactive.

A large part of the injected radioactivity when either proteoglycan or glycosaminoglycan was given never appeared in the urine even after 24 h (Table 2). Since glycosaminoglycans (Kaplan & Meyer, 1962) or labelled proteoglycans (P. A. Revell, unpublished work) are cleared from the circulation within 4 h and 60–90 min respectively, they must be taken up by the tissues as well as excreted in the urine. Once inside the cell it is possible that the material has to be completely degraded and the radioactive label converted into inorganic [^{35}S]sulphate before it emerges again into the circulation. Since this would equilibrate with the pool of inorganic sulphate in the body and therefore partly be used for the synthesis of new proteoglycan, some would remain in the body for appreciable lengths of time. This might explain why a considerable proportion of the injected radioactivity was not excreted even after 24 h.

On the other hand, cathepsin D, which is the principal protease involved in the breakdown of connective tissue (Woessner, 1967), particularly cartilage, *in vitro* (Weston *et al.*, 1969), causes only a partial breakdown of cartilage proteoglycans to relatively large-molecular-weight products (Morrison, 1970). *In vivo*, however, the amount of glycosaminoglycans in normal urine is small and much less than the probable turnover of proteoglycans (Leaback, 1970).

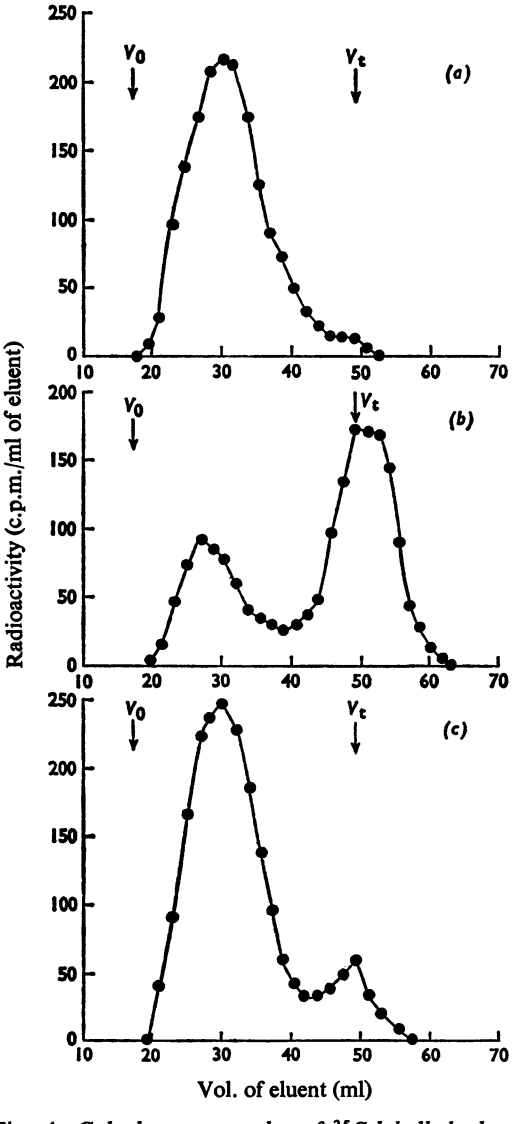

Fig. 4. *Gel chromatography of ^{35}S-labelled glycosaminoglycan from pig cartilage on Sephadex G-200*

The conditions of gel chromatography were the same as described in Fig. 2, except that 0.5ml samples were applied to the column. The gel chromatography was carried out on ^{35}S-labelled glycosaminoglycan (*a*) before and (*b*) after incubation with guinea-pig urine for 24h at 37°C, and (*c*) after incubation with guinea-pig urine previously sterilized through a Millipore membrane of pore size 0.45μm.

Presumably therefore the initial breakdown products of proteoglycans are taken up by the cells of the body and degraded to low-molecular-weight products no longer identifiable as originating from proteoglycans. The work of Neufeld and co-workers (Fratantoni *et al.*, 1968, 1969) provides some support for this suggestion, because fibroblasts derived from patients with mucopolysaccharidoses, and who had as a consequence accumulated labelled glycosaminoglycans, secreted labelled material as low-molecular-weight products when treated with 'corrective' factors. These factors, which are lacking in pathological cells, appear to be degradative enzymes that are specific for those glycosaminoglycans which accumulate in different types of mucopolysaccharidosis (Kresse *et al.*, 1971; Matalon *et al.*, 1971). However, the pathological glycosaminoglycans are considerably degraded (Knecht *et al.*, 1967; Dean *et al.*, 1971), and, since chondroitin sulphate chains may be excreted intact even though much larger than pathological glycosaminoglycans, it is improbable that the latter accumulate in the body because they are insufficiently broken down to be excreted in the urine: rather it seems that extensive degradation is necessary for their removal from the cells that contain them.

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