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Biosynthesis and metabolism *in vivo* of intervertebral-disc proteoglycans in the mouse

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1. The synthesis and turnover *in vivo* of ^{35}S-labelled proteoglycans in mouse cervical, thoracic and lumbar intervertebral discs, and in costal cartilage, was investigated after intraperitoneal injection of [^{35}S]sulphate. 2. Intervertebral discs and costal cartilage synthesize similar amounts of ^{35}S-labelled proteoglycans per μg of DNA. 3. Discs and cartilage all synthesize a major proteoglycan species (approx. 85%) of large hydrodynamic size and a minor species (approx. 15%) of small size. 4. Both proteoglycans carry chondroitin sulphate chains. Keratan sulphate was not found associated with either species. 5. The total ^{35}S-labelled proteoglycan pool had a metabolic half-life ($t_{\frac{1}{2}}$) of 10–12 days in discs, and 17 days in cartilage. The extractable major and minor species turned over at similar rates. Those proteoglycans left in the tissue after 29 days turn over very slowly. 6. Approx. 50% of the major ^{35}S-labelled proteoglycan species formed mixed aggregates with hyaluronic acid and rat chondrosarcoma proteoglycan. The ability to form aggregates did not decrease up to 45 days after synthesis. 7. Of the heterogeneous population of proteoglycans comprising the major species, those remaining in the tissue 9 days after synthesis were of smaller average hydrodynamic size and had shorter chondroitin sulphate side chains than the average size at the time of synthesis. 8. With increasing time after synthesis, proteoglycans were less readily extracted from the tissue by 4.0 M-guanidinium chloride than at the time of synthesis.

The intervertebral discs are cartilage-like tissues located between the hyaline cartilage endplates of the vertebrae. They have two parts, an outer fibrous section, the annulus fibrosus, and a gelatinous central section, the nucleus pulposus. The major components of both parts of the disc, in varying ratios, are collagen (Herbert *et al.*, 1975; Osebold & Pedrini, 1976; Eyre & Muir, 1977) and proteoglycans (Heinegård & Gardell, 1967). Several studies have been performed on the structure of disc proteoglycans (for references, see McDevitt, 1981). They show certain homologies with the structure of hyaline-cartilage proteoglycans, but are of smaller hydrodynamic size than the latter and are generally less capable of forming aggregates with hyaluronic acid (Emes & Pearce, 1975; Stevens *et al.*, 1979*a*).

Biosynthetic studies (Oegema *et al.*, 1979; McDevitt *et al.*, 1981) suggest that disc proteoglycans may be synthesized as relatively large molecules compared with the hydrodynamic size of proteoglycans extracted from the tissue and characterized by chemical methods. In such studies the radioactively labelled proteoglycans represent newly synthesized molecules, and the chemically characterized proteoglycans represent mature molecules in the tissue. Thus questions arise as to whether newly synthesized disc proteoglycans have the same structural characteristics as newly synthesized hyaline-cartilage proteoglycans and whether they are subsequently processed to give molecules of a different character. In the study reported below, the properties of newly synthesized proteoglycans in discs at different levels in the vertebral column in the CBA mouse were compared with those of newly synthesized proteoglycans in hyaline costal cartilage. The biochemistry of normal mouse disc components is of particular interest in order to provide comparative data for studies on a mutant strain in which genetically determined disc degeneration occurs (Mason & Palfrey, 1977).

Methods

Labelling procedure in vivo

Male CBA mice (30 days old) from an inbred colony were injected intraperitoneally without anaesthetic with 0.25ml doses of between 1 and 20mCi of $Na_2{}^{35}SO_4$ (5mCi/μg of sulphur, carrier free; Amersham International)/kg body wt. Then 3h later an injection of 1mg of Na_2SO_4 in 0.5ml of sterile saline was given as a chase. Animals were killed by asphyxiation with CO_2 after chase times from 15min to 45 days. Immediately after death, hair was removed from the abdominal skin with a commercial depilatory cream and the skin excised. The whole of the costal cartilage was dissected out. The intervertebral discs (C2–C7, T1–T12, L1–L6) were excised individually under a dissecting microscope and the discs from each region pooled.

Assessment of total incorporation of [^{35}S]sulphate into glycosaminoglycans

Skin was digested in 1ml of 0.1M-potassium phosphate buffer, pH6.2, containing 5mM-EDTA, 5mM-cysteine hydrochloride, 1.0M-potassium acetate and 60 units of twice-recrystallized papain (Sigma), and cartilage and discs were digested in 0.5ml of the buffer with 30 units of enzyme (Scott, 1960). Portions of each digest were chromatographed with 1mg of carrier RCS-D1 proteoglycan (see below) on PD10 columns (9ml; Sephadex G-25; Pharmacia), equilibrated with 4.0M-guanidinium chloride/0.05M-sodium acetate, pH5.8, to separate ^{35}S-labelled glycosaminoglycans from unincorporated [^{35}S]sulphate (Mason *et al.*, 1982). Another portion (0.1ml) was dialysed exhaustively against distilled water at 4°C and dried at 60°C over P_2O_5, *in vacuo*, before fluorimetric analysis of DNA (Setaro & Morley, 1976).

Extraction of tissues

Costal-cartilage slivers and intervertebral discs were extracted in 0.5ml of 4.0M-guanidinium chloride/0.05M-sodium acetate, pH5.8, containing proteinase inhibitors for 24h, at 1°C, with gentle shaking. The inhibitors used were 1mM-sodium iodoacetate, 100mM-6-aminohexanoic acid, 5mM-benzamidine hydrochloride, 10mM-disodium EDTA, 1mM-phenylmethanesulphonyl fluoride and 1mg of soya-bean trypsin inhibitor/litre (Pearson & Mason, 1979). Extracts were separated by centrifugation in an Eppendorf Microfuge (2min, 16000*g*) and the residues digested with 30 units of papain (see above). The digests were clarified by centrifugation (2min, 16000*g*). Unincorporated ^{35}S radioactivity was removed by PD10-column chromatography; 1mg of reduced and alkylated RCS-D1 proteoglycan was co-chromatographed with each sample to maximize recovery of ^{35}S-labelled proteoglycans.

In some experiments, utilizing a 1h chase, a sequential extraction was used in which tissues were extracted initially with 0.5M-guanidinium chloride/0.05M-sodium acetate, pH5.8, containing proteinase inhibitors (1°C, 24h) before extraction with 4.0M-guanidinium chloride/0.5M-sodium acetate, pH5.8, and subsequent papain digestion.

Preparation of carrier proteoglycan

Natural proteoglycan aggregates (aA1) and proteoglycan monomer (D1) were isolated from Swarm rat chondrosarcoma (RCS) as described by Faltz *et al.* (1979) and Oegema *et al.* (1975) respectively). RCS-D1 was reduced and alkylated by the method of Heinegård (1977).

Gel-permeation chromatography

Chromatography gels were obtained from Pharmacia. Columns (Omnifit, Cambridge, U.K.) packed with (*a*) Sepharose CL2B (150cm × 0.6cm), (*b*) Sepharose CL6B (100cm × 0.6cm) or (*c*) Sephadex G-50 (fine grade) (150cm × 0.6cm) were equilibrated with 4.0M-guanidinium chloride/0.05M-sodium acetate, pH5.8 (dissociative, *a*), 0.5M-sodium acetate, pH5.8 (associative, *a* and *b*), or 50mM-Tris/HCl buffer, pH7.2 (*c*). Extracts containing ^{35}S-labelled proteoglycan (1000–5000d.p.m.) and 1mg of carrier proteoglycan were applied in 0.8ml (*a*, *c*) or 0.5ml (*b*) and eluted (2.5ml/h) with the same buffers; 0.8ml (*a*, *c*) or 0.5ml (*b*) fractions were collected and analysed for radioactivity. Hexuronic acid in associative-column fractions was monitored by an automated carbazole assay (Heinegård, 1973).

Analytical procedures

Extracts containing ^{35}S-labelled proteoglycans were treated with 0.05M-NaOH containing 1.0M-$NaBH_4$ (45°C, 18h) to release glycosaminoglycan chains (Carlson, 1968), before chromatography on Sepharose CL6B to determine chain size (Wasteson, 1971).

^{35}S-labelled proteoglycans or ^{35}S-labelled glycosaminoglycans were digested with either chondroitinase ABC or AC (Miles Biochemicals) to give their specific ^{35}S-labelled chondroitin 4-sulphate or chondroitin 6-sulphate unsaturated disaccharide repeat units (Saito *et al.*, 1968). The disaccharides were separated from one another by cellulose t.l.c. and counted for radioactivity (Mason *et al.*, 1982). Other digests were chromatographed on columns of Sepharose CL6B or Sephadex G-50.

^{35}S-labelled glycosaminoglycans or chondroitinase digests thereof in 50mM-Tris/HCl buffer, pH7.2, were digested with keratanase (Miles Biochemicals) for 26h at 37°C (Nakazawa & Suzuki, 1975): 0.05 unit of enzyme was added at 0, 4, 18 and 22h. Control experiments showed that corneal keratan

sulphate (Miles Biochemicals) was completely excluded from a Sephadex G-50 column both before and after treatment with the alkaline borohydride reagent and/or chondroitinase ABC. After subsequent digestion with keratanase (4×0.05 unit), 0.5 mg was completely degraded to di- and tetrasaccharides and traces of higher saccharides, which were eluted in the included volume of the column. An automated anthrone assay (Heinegård, 1973) was used to monitor column fractions.

Histology

Intervertebral discs were fixed in buffered 10% formalin and paraffin sections prepared by routine methods. Sections were stained with haematoxylin and eosin. The discs are highly cellular, have clearly distinguishable nucleus pulposus and annulus fibrosus and are virtually free of any adjacent tissue.

Results and discussion

Synthesis of ^{35}S-labelled glycosaminglycans in mouse connective tissues

Preliminary experiments showed that the amount of [^{35}S]sulphate (d.p.m.) incorporated into glycosaminoglycans increased in a nearly linear manner with increasing doses of [^{35}S]sulphate in all tissues.

A dose of 20 mCi of $Na_2{}^{35}SO_4$/kg was used in all subsequent experiments. This gave an incorporation of about 2×10^4 d.p.m. of ^{35}S-labelled glycosaminoglycan/μg of DNA into intervertebral discs from all three spinal regions and costal cartilage. Synthesis in these tissues was approx. 20–30 times greater than in the skin.

Hydrodynamic properties of ^{35}S-labelled proteoglycans

After a 3 h pulse with [^{35}S]sulphate, the intervertebral discs and costal cartilage were removed after chase times of 15 min, 1, 4, 8 and 16 h, 1, 3, 6, 9, 17, 29 and 45 days. The 4.0 M-guanidinium chloride extracts of the tissues were examined by dissociative Sepharose CL2B chromatography. At all time points two ^{35}S-labelled peaks were observed (Fig. 1, continuous line). Exact quantification of the two peaks was impeded at late chase times, partly by an increase in $K_{av.}$ of the major peak from 0.35 to 0.45, resulting in poorer chromatographic resolution, and partly by metabolic turnover and isotope decay, resulting in decreased ^{35}S radioactivity, particularly under the minor peak ($K_{av.}$ 0.75). However, during the period 15 min to 45 days after synthesis, the contribution of the minor peak to the total ^{35}S radioactivity in the extract increased from approx. 15% to not more than 20%, so, although the major component undergoes considerable turnover (see below), there is no convincing evidence for the accumulation in the tissues of small fragments from it.

For thoracic intervertebral discs, extracts for every chase time were chromatographed and showed that the change in $K_{av.}$ of the major peak occurred between 9 and 17 days after synthesis. ^{35}S-labelled glycosaminoglycan chains released from proteoglycans by alkaline borohydride cleavage were examined by Sepharose 6B chromatography. All chase times were examined for the thoracic disc, and selected time points in other tissues. An increase in $K_{av.}$ values occurred between 9 and 17 days after synthesis (Fig. 2) and coincided with the increase in $K_{av.}$ of the ^{35}S-labelled proteoglycan over the same period.

Aggregation properties of ^{35}S-labelled proteoglycans

After addition of RCS-aA1 proteoglycan (1 mg) and hyaluronate (0.01 mg, from umbilical cord; Sigma) to samples of 4.0 M-guanidinium chloride extracts, they were dialysed to associative conditions against 100 vol. of 0.5 M-sodium acetate, pH 5.8 (4°C, 18 h), and chromatographed on associative Sepharose CL2B columns. Approx. 50–60% of the ^{35}S radioactivity was eluted in the void volume of the column (Fig. 1, broken line). The void-volume peak represents the ability of ^{35}S-labelled proteoglycans to form aggregates with hyaluronic acid, because it is not present under dissociative conditions (Fig. 1, continuous line) and was greatly diminished in experiments when exogenous hyaluronate was omitted. In the latter, extracts of tissues, chased for 1 h, were dialysed to associative conditions with reduced and alkylated RCS-D1 proteoglycan (1 mg). Only 15% of the ^{35}S-labelled proteoglycan in the non-diffusible material was eluted in the void volume of the Sepharose CL2B column (results not shown), and probably represents aggregates formed solely between mouse proteoglycan and endogenous hyaluronate, rather than proteoglycan–proteoglycan interactions by any other mechanism. A dissociation constant, K_d, of 10^{-8} M has been reported for the interaction between cartilage-type proteoglycans and hyaluronate (Nieduszynski *et al.*, 1980). Although dissociation constants for mouse proteoglycans are unknown, they are likely to be of a similar order. If this is so, as the concentration of mouse proteoglycans in tissue extracts is very low, it would be expected that they would show only minimal aggregation with endogenous hyaluronate in the absence of aggregating carrier.

There is no difference between newly synthesized ^{35}S-labelled proteoglycans and those remaining in the tissue 45 days after synthesis in their ability to form mixed aggregates with hyaluronate and rat chondrosarcoma proteoglycans (Fig. 1).

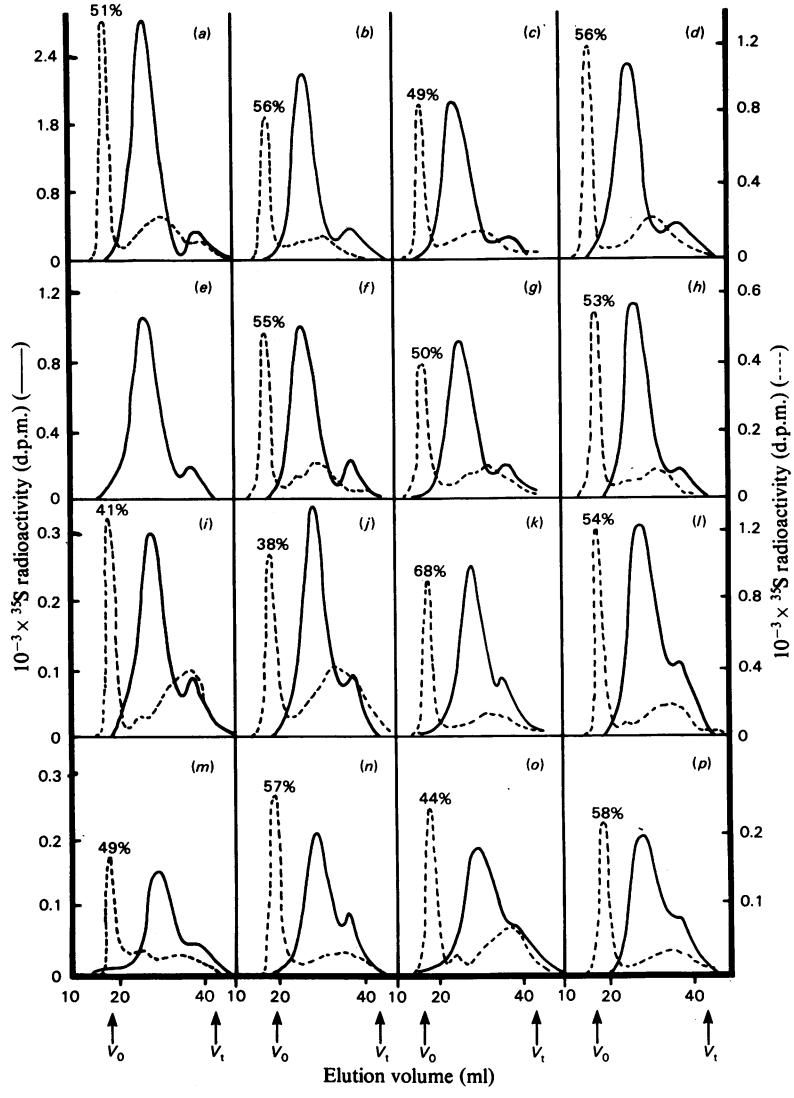

Fig. 1. *Sepharose CL2B chromatography of ^{35}S-labelled proteoglycans from intervertebral discs and costal cartilage*
Dissociative Sepharose CL2B (——) and associative Sepharose CL2B (----) profiles of ^{35}S-labelled proteoglycans in 4.0M-guanidinium chloride extracts of mouse tissues at 15min (*a–d*), 5 days (*e–h*), 29 days (*i–l*) and 45 days (*m–p*) after synthesis. (*a*), (*e*), (*i*), (*m*), Cervical discs; (*b*), (*f*), (*j*), (*n*), thoracic discs; (*c*), (*g*), (*k*), (*o*), lumbar discs (*d*), (*h*), (*l*), (*p*), costal cartilage. V_0, column void volume; V_t, column total volume. Percentages shown are amounts of aggregate.

Turnover of ^{35}S-labelled disc proteoglycans

The total ^{35}S-labelled proteoglycan remaining in the discs at various chase times was calculated from the sum of that found in the 4.0M-guanidinium chloride extract and in the papain digest of the residue and is shown as a semi-logarithmic plot in Fig. 3(*a*). During the first 24h of the chase, no decrease in ^{35}S-labelled proteoglycan was detected (results not shown). Between 1 and 29 days the ^{35}S radioactivity in the discs decreased with a half-life ($t_{\frac{1}{2}}$) of about 10.5 days in the thoracic and lumbar discs, and 12.5 days in the cervical discs. After 29 days, ^{35}S-labelled proteoglycans remaining in the tissue have a very slow turnover rate.

The ^{35}S-labelled proteoglycan extracted from the tissue represents two distinct ^{35}S-labelled proteoglycans (Fig. 1). For the thoracic disc the proportion of total ^{35}S-labelled proteoglycan extracted with 4.0M-guanidinium chloride remained fairly

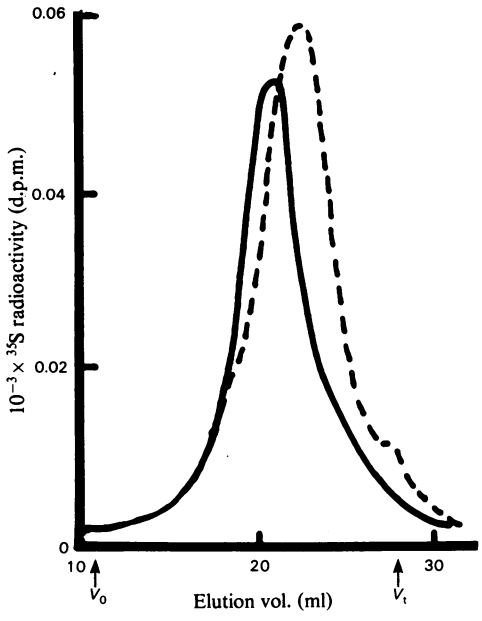

Fig. 2. *Sepharose 6B chromatography of ^{35}S-labelled glycosaminoglycans in thoracic discs*

^{35}S-labelled glycosaminoglycans were released by alkaline-borohydride treatment from 4.0M-guanidinium chloride extracts of thoracic discs 9 days (——) and 17 days (– – – –) after synthesis. V_0, column void volume; V_t, column total volume.

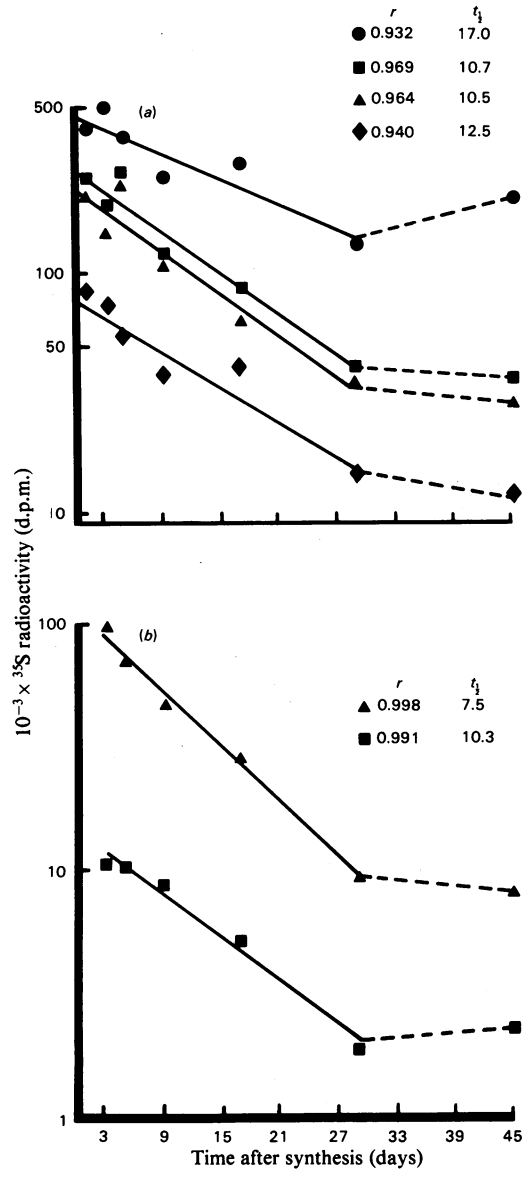

Fig. 3. *Turnover of ^{35}S-labelled proteoglycans in intervertebral discs and costal cartilage*

(*a*) Semi-logarithmic plots of total ^{35}S radioactivity remaining in costal cartilage (●), cervical (◆), thoracic (▲) and lumbar (■) discs at increasing chase times after synthesis. (*b*) Semi-logarithmic plots of the ^{35}S radioactivity remaining in the major (▲) and minor (■) proteoglycan peaks of 4.0M-guanidinium chloride extracts of mouse thoracic discs at increasing chase times after synthesis. All values are corrected for decay of the radioisotope within the time course of the experiment. Lines between 1 and 29 days (*a*) and 3 and 29 days (*b*) were plotted by linear regression analysis; correlation coefficients (r) and half-lives ($t_{\frac{1}{2}}$; days) are shown.

constant from 3 days chase time onwards (Fig. 4). The half-lives for the major and minor components were 7.5 days and 10.3 days respectively (Fig. 3*b*).

The proportion of ^{35}S-labelled proteoglycans that could be extracted with 4.0M-guanidinium chloride was lower at late chase times than at early chase times (Fig. 4). This change was more pronounced in the costal cartilage than in the intervertebral discs. This phenomenon may reflect increasing entrapment of proteoglycans within the collagen network of the extracellular matrix. Nevertheless, no changes in the rate of turnover of ^{35}S-labelled proteoglycans were observed between 1 and 29 days, suggesting that the molecules are still accessible to catabolic processes during this time.

Characterization of ^{35}S-labelled proteoglycans

Discs and cartilage from 1h chase experiments were extracted sequentially with 0.5M-guanidinium chloride, pH5.8, and 4.0M-guanidinium chloride, pH5.8, which solubilized about 20% and 50% of the total ^{35}S-labelled macromolecules respectively. Dissociative Sepharose CL2B chromatography profiles of the 0.5M-guanidinium chloride disc extracts showed two partially resolved components, the more

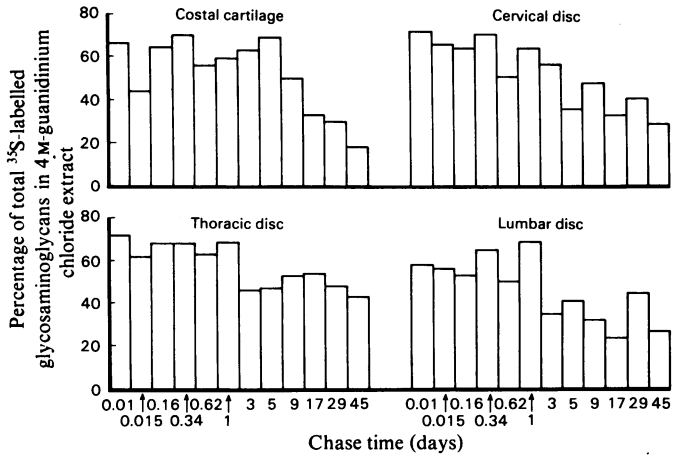

Fig. 4. *Percentage of the total ^{35}S-labelled proteoglycans that can be extracted with 4.0 M-guanidinium chloride at increasing chase times after synthesis*

retarded being more prominent (Fig. 5). The latter peak presumably contains smaller proteoglycans, including the minor species seen in direct 4 M-guanidinium chloride extracts (Fig. 1). Natural proteoglycan aggregates were not solubilized by 0.5 M-guanidinium chloride, since no void-volume ^{35}S radioactivity was detected after associative Sepharose CL2B chromatography of untreated extracts with reduced and alkylated RCS-D1 carrier proteoglycan (results not shown).

The ^{35}S-labelled glycosaminoglycan chains of proteoglycans in the 0.5 M-guanidinium chloride extract were cleaved from core protein by an alkaline borohydride reagent and chromatographed on a Sepharose 6B column before and after digestion with chondroitinase ABC or AC (Fig. 6). A small amount of ^{35}S-labelled glycosaminoglycan was not completely degraded by chrondroitinase AC, whereas chondroitinase ABC degraded all the glycosaminoglycan to disaccharides. This indicates that 0.5 M-guanidinium chloride extracts a small amount of proteoglycan with glycosaminoglycan chains containing iduronate residues.

^{35}S-labelled proteoglycans in the sequential 4.0 M-guanidinium chloride extract were eluted as a single peak from dissociative Sepharose CL2B columns (Fig. 5). After alkaline cleavage their glycosaminoglycan components were eluted from a Sepharose 6B column (results not shown) with $K_{av.}$ 0.52, corresponding to a molecular weight of 18000 (Wasteson, 1971). The ^{35}S-labelled glycosaminoglycan chains were completely excluded from Sephadex G-50 columns, but after treatment with chondroitinase ABC all ^{35}S radioactivity moved into the column (Fig. 7). Most was recovered in the total column volume (V_t) and represents disaccharide repeat units; a small proportion was eluted just ahead of the V_t and probably constitutes the reducing end of chondroitin sulphate chains, since chondroitinase ABC does not remove the sulphated disaccharide adjacent to the linkage region of the glycosaminoglycan (Oike *et al.*, 1980).

The absence of any ^{35}S radioactivity from the void volume (V_0) of the column after chondroitinase-ABC treatment suggests that the glycosaminoglycan chains of mouse discs and costal cartilage were entirely composed of chondroitin sulphate. To test whether keratan sulphate was present, the neutralized alkaline-borohydride-treated fractions were digested with keratanase. There was no retention of ^{35}S radioactivity on the column, even though the enzyme was shown to be fully active against corneal keratan sulphate (see the Methods section). Chondroitinase-ABC digests were also analysed for their specific chondroitin sulphate disaccharide composition by t.l.c. Less than 4% of the ^{35}S radioactivity in the digest was recovered from the baseline of the chromatogram, confirming the absence of keratan sulphate chains from these proteoglycans. Chondroitinase 6-sulphate accounted for 20–30% and chondroitin 4-sulphate for 70–80% of the ^{35}S radioactivity in both discs and cartilage.

General discussion

Both mouse discs and costal cartilage synthesize two species of extractable proteoglycans with different sizes but similar turnover rates. The minor species, a small, non-aggregating, proteoglycan, has not previously been described in intervertebral discs.

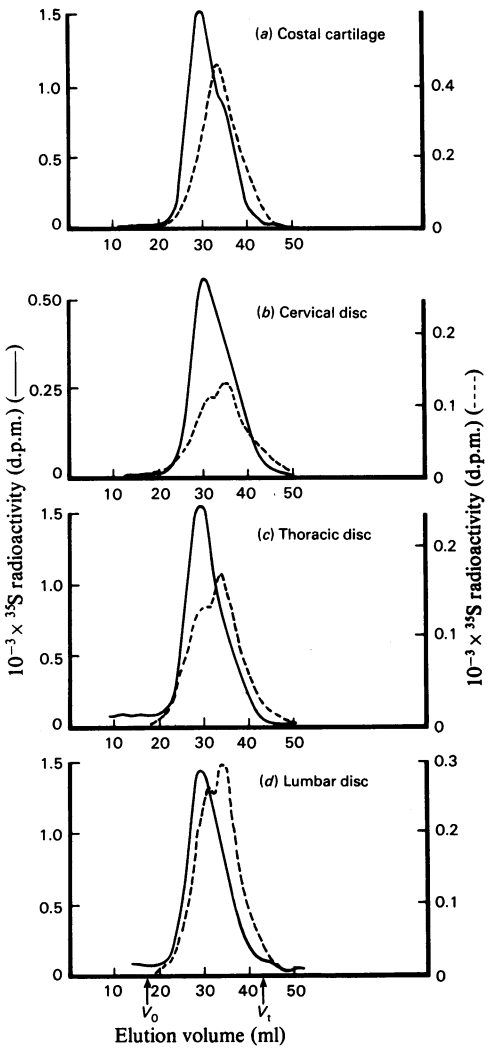

Fig. 5. *Dissociative Sepharose CL2B chromatography of ^{35}S-labelled proteoglycans in sequential extracts of intervertebral discs and costal cartilage*

^{35}S-labelled proteoglycans extracted by 0.5 M-guanidinium chloride (----) and sequential 4.0 M-guanidinium chloride extracts (——) of (*a*) costal cartilage, (*b*) cervical disc, (*c*) thoracic disc and (*d*) lumbar disc. V_0, column void volume; V_t, column total volume.

It may be analogous to the small proteoglycan reported to be present in cartilage and undifferentiated mesenchymal tissue (Kimata *et al.*, 1978; Stanescu & Sweet, 1981; Heinegård *et al.*, 1981).

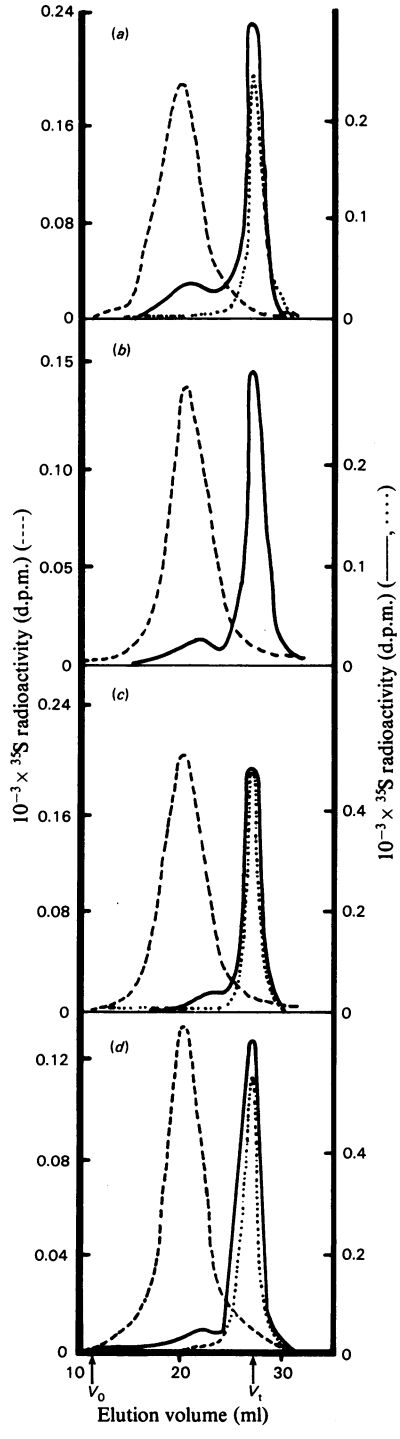

Fig. 6. *Analysis of ^{35}S-labelled glycosaminoglycans in 0.5 M-guanidinium chloride extracts of mouse tissues*

Sepharose 6B profiles of ^{35}S radioactivity present in 0.5 M-guanidinium chloride extracts of (*a*) costal cartilage, (*b*) cervical discs, (*c*) thoracic discs and (*d*) lumbar discs after (----) alkaline-borohydride treatment alone, (——) alkaline-borohydride/chondroitinase AC treatment, (····) alkaline-borohydride/chondroitinase-ABC treatment. V_0, column void volume; V_t, column total volume.

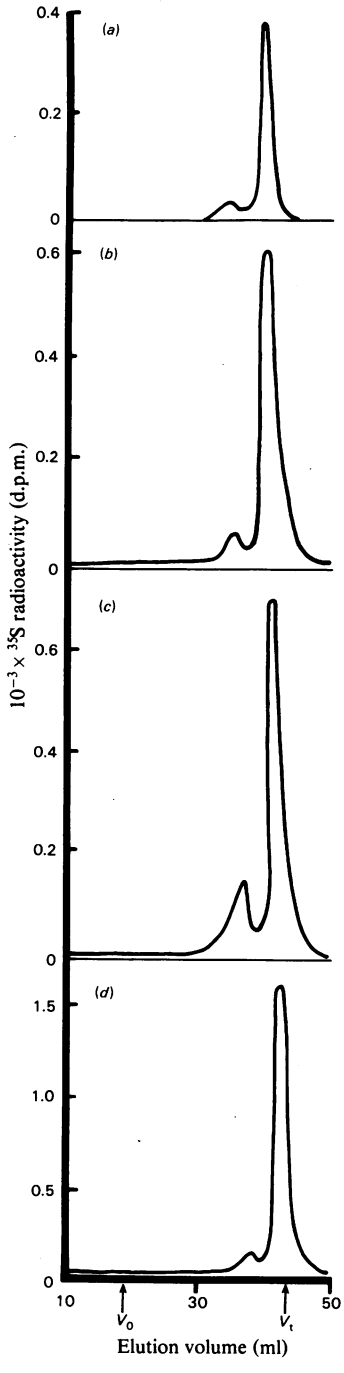

Fig. 7. *Analysis of ^{35}S-labelled glycosaminoglycans in sequential 4.0M-guanidinium chloride extracts of mouse tissues*

Sephadex G-50 profiles of ^{35}S-labelled digestion products of sequential 4.0M-guanidinium chloride extracts of (*a*) costal cartilage, (*b*) cervical discs, (*c*) thoracic discs and (*d*) lumbar discs after alkaline-borohydride/chondroitinase-ABC treatment. V_0, column void volume; V_t, column total volume.

The major proteoglycan ($K_{av.}$ on Sepharose CL2B 0.35–0.45) has glycosaminoglycan chains composed solely of chondroitin sulphate. No evidence was obtained for the presence of keratan sulphate. The mouse disc proteoglycan therefore differs in composition from those of pig, human, dog, rabbit and guinea pig, which contain both chondroitin sulphate and keratan sulphate chains (Stevens *et al.*, 1979*a*; Oegema *et al.*, 1982; Ghosh *et al.*, 1977; Lohmander *et al.*, 1973). The major mouse proteoglycan is probably analogous to that designated type I by Heinegård (1981).

The average hydrodynamic size of the major proteoglycan species was smaller at 17 days and later after synthesis than it was at the time of synthesis. The smaller size is due, at least in part, to these proteoglycans having shorter chondroitin sulphate side chains than the average for the whole population at the time of synthesis. Although exoglycosidase action leading to decreased chain length *in situ* in the matrix cannot be excluded, it is more likely that the smaller proteoglycans were synthesized as such and remain in the tissue for a longer time than larger molecules. The size of the major proteoglycan remaining in discs and costal cartilage 45 days after synthesis is the same as for mouse articular-cartilage proteoglycans (Rostand *et al.*, 1982).

The aggregation properties of proteoglycans did not change during the chase period, there being no evidence for either maturation of the hyaluronate-binding region in the 24h after synthesis as proposed by Oegema *et al.* (1979, 1982) or for its selective degradation in the matrix at later times (Stevens *et al.*, 1979*b*; McDevitt *et al.*, 1981).

Between 1 and 29 days after synthesis there is a pool of molecules with an average $t_{\frac{1}{2}}$ of 10½–12½ days for discs and 17 days of cartilage. The latter value agrees well with that found by Bostrom (1952) for rat costal cartilage proteoglycans. There is probably some variation in turnover of different proteoglycans within this 'fast' pool, as shown by the shorter $t_{\frac{1}{2}}$ for the major extractable proteoglycan (7½ days) compared with that of the minor species (10½ days) or the total ^{35}S-labelled proteoglycan (10½ days) in thoracic discs. The small amounts of ^{35}S-labelled proteoglycans remaining in the tissue 29 days after synthesis have very slow turnover, but would nevertheless accumulate over a period of time and may constitute a prominent component of adult mouse discs. The half-lives for mouse disc and costal-cartilage proteoglycans are shorter than the values reported for these molecules in the same tissues in guinea pigs, rabbits and dogs (Davidson & Small, 1963; Lohmander *et al.*, 1973; Maroudas, 1975; McDevitt *et al.*, 1981; Oegema *et al.*, 1982) after labelling procedures *in vivo*. This difference may be due to a faster turnover rate in small species

such as mouse and rat (Bostrom, 1952). However, direct comparison of our results with previously published data is difficult, since most reports were based on calculation of specific radioactivities, whereas ours are calculated from total undegraded ^{35}S-labelled proteoglycan remaining in the tissue at given times after synthesis. There is general agreement that disc proteoglycans turn over more rapidly than do hyaline-cartilage proteoglycans in the same species and that not all molecules within a tissue turn over with exactly the same half-life.

The average size of newly synthesized mouse disc proteoglycans is the same as for those in the costal cartilage and similar to that of ^{35}S-labelled proteoglycans synthesized in rabbit discs *in vivo* (Oegema *et al.*, 1982). Other metabolic labelling experiments show that proteoglycans synthesized *in vitro*, in human nucleus pulposus (Oegema *et al.*, 1979), or *in vivo*, in dog nucleus pulposus (McDevitt *et al.*, 1981), are of larger size than the proteoglycans extracted from the same tissues and analysed by uronic acid profiles of gel-filtration-column eluates. Similar chemical investigations have shown that most of the proteoglycans in pig and human intervertebral discs are of smaller hydrodynamic size than hyaline-cartilage proteoglycans (Stevens *et al.*, 1979*a*). Stevens *et al.* (1979*b*) examined discs for enzymes which decreased the size of proteoglycans after their synthesis, but no such activity was detected. An alternative proposal would be that the major proteoglycan species is synthesized as a heterogeneous population of molecules of predominantly large hydrodynamic size. If within this group the largest proteoglycans turned over rapidly but the smaller ones more slowly, there would be a tendency for the latter to accumulate in the tissue and become a prominent component. This component would become the one primarily detected in chemical analyses. Furthermore, the differences in size of disc and hyaline-cartilage proteoglycans in some species may arise because of slower turnover of large proteoglycans in the latter tissue, compared with the former. The overall size of cartilage proteoglycans which accumulate in the tissue and are detected by chemical analysis would then be greater than for disc proteoglycans.

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References

Bostrom, H. (1952) *J. Biol. Chem.* **196**, 477–481
Carlson, D. (1968) *J. Biol. Chem.* **243**, 616–626
Davidson, E. A. & Small, W. (1963) *Biochim. Biophys. Acta* **69**, 445–452
Emes, J. H. & Pearce, R. H. (1975) *Biochem. J.* **145**, 549–556
Eyre, D. R. & Muir, I. H. (1977) *Biochim. Biophys. Acta* **492**, 29–42
Faltz, L. L., Reddi, A. H., Hascall, G. K., Martin, D., Pita, J. C. & Hascall, V. C. (1979) *J. Biol. Chem.* **254**, 1375–1380
Ghosh, P., Taylor, T. F. K. & Braund, K. G. (1977) *Gerontology* **23**, 99–109
Heinegård, D. (1973) *Chem. Scr.* **4**, 199–201
Heinegård, D. (1977) *J. Biol. Chem.* **252**, 1980–1989
Heinegård, D. (1981) *Glycoconjugates; Proc. Int. Symp. Glycoconjugates 6th* p. 358
Heinegård, D. & Gardell, S. (1967) *Biochim. Biophys. Acta* **148**, 164–171
Heinegård, D., Paulsson, M., Inerot, S. & Carlstrom, C. (1981) *Biochem. J.* **197**, 355–366
Herbert, C. M., Lindberg, K. A., Jayson, M. I. V. & Bailey, A. J. (1975) *J. Mol. Med.* **1**, 79–91
Kimata, K., Oike, Y., Ito, K., Karasawa, K. & Suzuki, S. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1431–1439
Lohmander, S., Antonopoulos, C. A. & Friberg, U. (1973) *Biochim. Biophys. Acta* **304**, 430–448
Maroudas, A. (1975) *Philos. Trans. R. Soc. London, Ser. B* **271**, 293–313
Mason, R. M. & Palfrey, A. J. (1977) in *Scoliosis; Proc. 5th Symp.* (Zorab, P. A., ed.), pp. 349–367, Academic Press, London
Mason, R. M., Kimura, J. H. & Hascall, V. C. (1982) *J. Biol. Chem.* **257**, 2236–2245
McDevitt, C. A. (1981) in *Tissue Repair and Regeneration* (Glynn, L. E., ed.), vol. 3, pp. 111–143, Elsevier/North-Holland Biomedical Press, Amsterdam
McDevitt, C. A., Billingham, M. E. J. & Muir, I. H. (1981) *Semin. Arthritis Rheum.* **11**, 17–18
Nakazawa, K. & Suzuki, S. (1975) *J. Biol. Chem.* **250**, 912–917
Nieduszynski, I. A., Sheehan, J. K., Phelps, C. F., Hardingham, T. E. & Muir, I. H. (1980) *Biochem. J.* **185**, 107–114
Oegema, T. R., Hascall, V. C. & Dziewiatkowski, D. D. (1975) *J. Biol. Chem.* **250**, 6151–6159
Oegema, T. R., Bradford, D. S. & Cooper, K. M. (1979) *J. Biol. Chem.* **254**, 10579–10581
Oegema, T. R., Cooper, K. M., Kraft, E. L. & Bradford, D. S. (1982) *Proc. Annu. Meet. Orthop. Res. Soc. 28th* p. 49
Oike, Y., Kimata, K., Shinomura, T., Nakazawa, K. & Suzuki, S. (1980) *Biochem. J.* **191**, 193–207
Osebold, W. R. & Pedrini, V. (1976) *Biochim. Biophys. Acta* **434**, 390–405
Pearson, J. P. & Mason, R. M. (1979) *Biochim. Biophys. Acta* **583**, 512–526
Rostand, K. S., Baker, J. R., Caterson, B. & Christner, J. E. (1982) *J. Biol. Chem.* **257**, 703–719
Saito, H., Yagamata, Y. & Suzuki, S. (1968) *J. Biol. Chem.* **243**, 1530–1542
Scott, J. E. (1960) *Methods Biochem. Anal.* **8**, 146–196
Setaro, F. & Morley, C. D. G. (1976) *Anal. Biochem.* **71**, 313–317
Stanescu, V. & Sweet, B. M. (1981) *Biochim. Biophys. Acta* **673**, 101–113
Stevens, R. L., Ewins, R. J. F., Revell, P. A. & Muir, H. (1979*a*) *Biochem. J.* **179**, 561–572
Stevens, R. L., Dondi, P. G. & Muir, H. (1979*b*) *Biochem. J.* **179**, 573–578
Wasteson, A. (1971) *J. Chromatogr.* **59**, 87–93