Variations in the composition of bovine hip articular cartilage with distance from the articular surface

Ahnders FRANZÉN, Sven INEROT, Sven-Olof HEJDERUP and Dick HEINEGÅRD
Department of Physiological Chemistry 2, University of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden

(Received 15 September 1980/Accepted 17 February 1981)

Punch biopsies of bovine hip articular cartilage was sectioned according to depth and the proteoglycans were isolated. The mid-sections of the cartilage contained more proteoglycans than did either the superficial or the deepest portions of the cartilage. The most superficial 40 μm of the cartilage contained relatively more glucosaminoglycans compared with the remainder of the cartilage. The proteoglycans recovered from the surface 200 μm layer contained less chondroitin sulphate, were smaller and almost all of these molecules were able to interact with hyaluronic acid to form aggregates. From about 200 μm and down to 1040 μm from the surface, the proteoglycans became gradually somewhat smaller, probably owing to decreasing size of the chondroitin sulphate-rich region. The proportion of molecules that were able to interact with the hyaluronic acid was about 90% and remained constant with depth. The proteoglycans from the deepest layer near the cartilage–bone junction contained a large proportion of non-aggregating molecules, and the average size of the proteoglycans was somewhat larger. The alterations in proteoglycan structure observed with increasing depth of the articular cartilage beneath the surface layer (to 200 μm) are of the same nature as those observed with increasing age in full-thickness articular cartilage. The articular-cartilage proteoglycans were smaller and had much higher keratan sulphate and protein contents than did molecules isolated from bovine nasal or tracheal cartilage.

Cartilage has a high elasticity and resilience, largely because of the high content of proteoglycans in the tissue (Kempson *et al.*, 1971; Harris *et al.*, 1972; Scott, 1975; Kempson, 1975). The proteoglycans consist of a central protein core and a large number of charged and extended polysaccharide side chains. The proteoglycans therefore will occupy a very large domain. Several proteoglycans bind to one hyaluronic acid molecule, forming a very large proteoglycan aggregate. Part of the proteoglycan protein core is the hyaluronic acid-binding region, containing little or no polysaccharide. Another portion of the protein core, containing most of the keratan sulphate chains, constitutes the keratan sulphate region, probably interspaced between the hyaluronic acid-binding region and the part containing the chondroitin sulphate chains (Heinegård & Axelsson, 1977; Hascall & Heinegård, 1979).

The quantity of glycosaminoglycans is highest in the mid-portions of the cartilage and lower both towards the surface and towards the osteochondral junction (Stockwell & Scott, 1967; Maroudas *et al.*, 1969, 1973; Lipshitz *et al.*, 1976; Maroudas, 1979). It has also been shown that the glycosaminoglycan

glucosamine/galactosamine ratio increases with increasing depth in the cartilage (Stockwell & Scott, 1967). Such information, when related to the current model of proteoglycan structure, may indicate that the size of the proteoglycans is smaller in the deeper portions of the cartilage.

The present investigation was initiated to study the changes in articular-cartilage proteoglycan structure with distance from the articular surface.

Materials and methods

Chemicals

Aristar-grade HCl was obtained from BDH, Poole, Dorset, U.K. Papain and diphenylcarbamoyl chloride-treated trypsin were from Sigma Chemical Co., St. Louis, MO, U.S.A. Sepharose CL 2B, Sepharose 2B and Sephacryl S-200 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Tissue-Tec II (CM-cellulose) was obtained from Miles Laboratories, Naperville, IL, U.S.A. The other chemicals used were all of analytical grade. High-molecular-weight rooster comb hyaluronic acid (Healon) was a gift from Pharmacia. This prepara-

tion of hyaluronate chromatographs in the void volume of a Sepharose 2B column.

Tissue preparation

Femoral-head articular cartilages from three different 2-year-old bulls were used. Within 10 min after the death of the animals, their left hip joints were carefully opened and the femoral head was divided from the rest of the femur and removed. The synovial fluid was blotted off the articular surface with soft tissue paper. By using a specially made punch of diameter 20 mm, a cartilage-bone cylinder was prepared from the most weight-bearing area of the cartilage, just dorsal and lateral to ligamentum teres femoris. The specimen was immediately removed from the punch, frozen in liquid N₂ and transported to the laboratory immersed in liquid N₂. The cartilage-bone cylinder was longitudinally divided into small pieces with a cross-sectional area of about 3 mm². The surface of each of these pieces had only a minor curvature and was for practical purposes considered to have no curvature. Each piece was mounted to an aluminium cylinder with the aid of Tissue-Tec and fitted to a freeze-microtome. All operations were done with the tissue pieces frozen at -30°C. Serial sections of 20 µm thickness were prepared from the articular-cartilage surface to the cartilage-bone junction. Each cartilage piece yielded 62 sections. Cartilage from three animals was pooled separately into two sets of seven groups, with distances from the articular surface of 0-40, 40-240, 240-440, 440-640, 640-840, 840-1040 and 1040-1240 µm for animals A and C and 0-40, 40-120, 120-200, 200-280, 280-360, 360-440 and 440-1240 µm for animal B. Special care was taken to ensure that the cartilage specimens did not thaw during preparation and sectioning, to minimize degradation of the proteoglycans.

Histological investigation

A small cartilage-bone cylinder of diameter 2 mm was taken adjacent to the preparative punch biopsy, for histological investigation after staining with hematoxylin/eosin.

Preparation of proteoglycans

Proteoglycans were extracted from each pool of sectioned cartilage from animals A and B with 3 ml of 4 M-guanidinium chloride/0.05 M-sodium acetate, pH 5.8, containing the proteinase inhibitors EDTA (0.01 M), benzamidine hydrochloride (0.005 M) and 6-aminohexanoic acid (0.1 M) (Oegema *et al.*, 1975). Extraction was done for 24 h at 4°C on a shaker. Extracts and residues were separated by filtration through a nylon sieve, which was rinsed twice with 0.5 ml of the 4 M-guanidinium chloride solution used for extraction. The washings were pooled with the original extract and a sample of each pool was

removed for determination of extraction yield. The residues were stored frozen until processed further.

The extracts were dialysed against 10 vol. of 0.05 M-sodium acetate, pH 5.8, containing the proteinase inhibitors described above. The density of the dialysis residue was adjusted to 1.64 g/ml by addition of solid CsCl. The solutions (7 ml/tube) were then centrifuged at 4°C for 60 h in an MSE 65 ultracentrifuge in the 10 × 10 ml titanium angle rotor at 35 000 rev./min (85 000 g, r_{av} 6.44 cm). After termination of the centrifugation, the tubes were frozen in an absolutely vertical position by carefully immersing them in liquid N₂. While still frozen the tubes were cut with a saw in a special guide to give the bottom two-fifths (the A1 fraction) and the top three-fifths (the A2 fraction). The densities of the fractions were determined with a 300 µl constriction pipette as a pycnometer. The fractions were then dialysed against one change of 0.5 M-sodium acetate and then extensively against distilled water, and then freeze-dried.

Agarose/polyacrylamide-gel electrophoresis

Analytical agarose/polyacrylamide-gel electrophoresis of the reduced and alkylated A1 fractions from the different layers was carried out as described by McDevitt & Muir (1971). The gels were scanned by measuring the A_{520} .

Column chromatography

Analytical columns (0.3 cm × 140 cm) of Sepharose CL 2B and Sephacryl S-200 were eluted with 0.5 M-sodium acetate, pH 7.0, at 4°C. Usually 150 µg of proteoglycan or digest thereof was applied. The uronic acid content of each fraction was determined by an automated version of the carbazole method (Heinegård, 1973). Alternatively about 25 µg of proteoglycan was chromatographed on a Sepharose 2B column (0.5 cm × 140 cm), eluted with 0.25 M-Na₂SO₄ at 15°C with a constant-flow infusion pump. The effluent was continuously monitored for A_{206} with a Uvicord S monitor. At this wavelength the absorbance for protein is about 6 times that of glycosaminoglycans on a dry-weight basis.

The ratios of proteoglycan aggregates to monomers in the A1 fractions were determined after chromatography on Sepharose CL 2B. To ensure binding of all proteoglycan monomers capable of interaction, high-molecular-weight hyaluronic acid (1% of proteoglycan dry wt.) was added to the A1 fractions before chromatography (Hascall & Heinegård, 1979).

The size distribution of the proteoglycan monomers was determined by chromatography on Sepharose CL 2B and Sepharose 2B after reduction and alkylation of the A2 preparations (Heinegård, 1977).

Typically 25–150 μg of fraction A1 was dissolved in 150 μl of 4 M-guanidinium chloride/0.05 M-Tris/HCl/0.005 M-dithiothreitol, pH 7.6, and incubated at 37°C for 5 h. Iodoacetic acid (20 μl of a 0.1 M solution) was then added and the sample was incubated for 12 h at room temperature in the dark. Reduction and alkylation prevent aggregate formation by proteoglycan monomers (Heinegård, 1977).

The size distribution of the chondroitin sulphate chains was determined by chromatography on Sephacryl S-200 after digestion of 150 μg of fraction A1 with 1 μl of a suspension of crystalline papain (25 mg/ml) at 65°C for 3 h in 150 μl of 0.005 M-sodium phosphate buffer, pH 7.0, containing 0.005 M-EDTA and 0.005 M-cysteine hydrochloride.

Isolation and determination of glycosaminoglycans

Extraction yields were determined as follows. The residues and a known volume of the extracts from animal A were each dialysed against water and freeze-dried. The samples were then suspended in 500 μl of the buffer described above and digested with 2 μl of the suspension of crystalline papain at 65°C for 15 h. The released glycosaminoglycans were then isolated from the glycopeptide fraction by Ecteola-cellulose ion-exchange chromatography (Axelsson & Heinegård, 1975). Glycosaminoglycans were eluted with 2 M-HCl. Total tissue glycosaminoglycan content and distribution was measured after papain digestion of the sections from animal C with 15 μl of the papain suspension for 72 h at 65°C. Glycosaminoglycans were isolated as described above.

Chemical methods

Hexosamine contents of fractions were determined by using a Durrum automatic amino acid analyser after hydrolysis of samples in 4 M-HCl in sealed tubes under argon at 100°C for 10 h. Hydrolysis for determination of amino acids was with 6 M-HCl at 110°C for 24 h in sealed tubes under argon. The amino acids were then determined by using the amino acid analyser. Hydroxyproline

was determined as described by Stegemann & Stalder (1967).

Results and discussion

Morphology of tissue specimens

The articular cartilage showed no macroscopic sign of degeneration. The histology of the tissue biopsy showed three major regions. The region nearest to the surface extended from 0 to 40 μm and contained fibres and discoidal cells arranged parallel to the articular surface. The second region in the middle of the cartilage extended from 40 to 1040 μm and had an appearance typical for cartilage, with few cells and an abundant intercellular matrix. The region (200 μm) just above the cartilage–bone junction (1040–1240 μm from the cartilage surface) contained spheroidal cells and represents the proliferation area of the cartilage bone junction. The thickness of the articular cartilage measured in the microscope was 1250–1300 μm , in good agreement with the thickness obtained from the sum of the sections prepared for chemical investigation.

Distribution of glycosaminoglycans

Available data indicate that the collagen content of hip articular cartilage is constant at different depths of the cartilage (Lipshitz *et al.*, 1975; Maroudas, 1979). It was therefore assumed that the collagen content was constant and that the ratio of glycosaminoglycan hexosamine to hydroxyproline can be taken to indicate the proteoglycan content of the sections. One set of sections from animal C was digested with papain, and weighed samples from the digests were taken for hydroxyproline and glycosaminoglycan determinations. The higher content of glycosaminoglycans in the mid-sections compared with sections both from the surface and from the osteochondral junction (Table 1) corroborates previous data (Stockwell & Scott, 1967; Maroudas *et al.*, 1969; Lipshitz *et al.*, 1976; Maroudas, 1979). The content of glycosaminoglycan glucosamine, probably indicating keratan sulphate, varied only

Table 1. *Glycosaminoglycan contents of sections from articular cartilage from animal C*

Glycosaminoglycans are quantified as hexosamine of isolated glycosaminoglycans and collagen as hydroxyproline. Ratios are w/w.

Section	Glycosaminoglycan hexosamine hydroxyproline	Glycosaminoglycan galactosamine hydroxyproline	Glycosaminoglycan $\frac{\text{glucosamine}}{\text{galactosamine}}$
0–40 μm	0.21	0.14	0.54
40–240 μm	0.34	0.25	0.35
240–440 μm	0.50	0.35	0.43
440–640 μm	0.50	0.36	0.39
640–840 μm	0.49	0.34	0.45
840–1040 μm	0.44	0.30	0.45
1040–1240 μm	0.27	0.18	0.47

Table 2. Glucosamine/galactosamine ratio of glycosaminoglycan fractions of proteoglycan aggregate (A1) fraction, extract and extraction residue, and glycosaminoglycan extraction yields (animal A)
Abbreviation: n.d., not determined.

Section	Glucosamine/galactosamine in glycosaminoglycan fraction			Extraction yield (% of total)	Glucosamine/protein in A1 fraction	Galactosamine/protein in A1 fraction
	Extract	Residue	A1 fraction			
0–40 μ m	0.81	0.74	n.d.	98.6	n.d.	n.d.
40–240 μ m	0.25	0.40	0.49	97.4	0.31	0.62
240–440 μ m	0.28	0.57	0.39	99.1	0.36	0.93
440–640 μ m	0.37	0.60	0.46	97.6	0.38	0.82
640–840 μ m	0.33	0.51	0.61	98.8	0.40	0.65
840–1040 μ m	0.41	0.48	0.66	96.3	0.36	0.55
1040–1240 μ m	0.32	0.55	0.48	96.5	0.43	0.89

Table 3. Amino acid composition of the proteoglycans (A1 fractions) from the various tissue sections (animal A)
Values are expressed as residues per 1000.

Amino acid	Section ...	40–240 μ m	240–440 μ m	440–640 μ m	640–840 μ m	840–1040 μ m	1040–1240 μ m
Asp		81	78	79	79	81	82
Thr		57	56	54	54	53	57
Ser		154	144	154	156	169	156
Glu		131	146	147	152	136	149
Pro		70	74	71	76	60	74
Gly		150	163	179	169	181	151
Ala		66	62	63	68	57	62
Cys		8	6	4	6	3	2
Val		50	47	44	42	48	51
Met		4	2	2	3	0	0
Ile		30	28	25	24	21	25
Leu		62	63	60	58	60	66
Tyr		19	24	21	21	19	21
Phe		28	30	27	26	22	24
His		21	16	15	15	20	15
Lys		35	35	29	27	40	34
Arg		34	27	26	23	30	31

little, although the surface layer contained relatively more.

Extraction of proteoglycans

Extraction yields (animal A) were much higher (96–99%; Table 2) than those reported previously from other types of cartilage (for references see Hascall & Heinegård, 1979). Possibly the smaller pieces of cartilage obtained by sectioning result in improved extraction.

Corroborating previous results (Inerot *et al.*, 1978), the residues from all tissue samples contained relatively more glucosaminoglycans than did the extracts. The ratio of glucosamine/galactosamine in the glycosaminoglycan fractions (Table 1) was very high in the 0–40 μ m section in both the extract and the residue. It is likely that this layer of the cartilage may contain relatively more keratan sulphate or heparan sulphate. Stockwell & Scott

(1967) showed that the surface layer of human articular cartilage had a higher content of glycosaminoglycan glucosamine, mainly in the sulphated-polysaccharide fraction. The second pool of slices, corresponding to 40–240 μ m depth, showed the lowest ratio. The relative glucosaminoglycan content, however, increased somewhat with further depth of the cartilage.

Composition of A1 fractions

Contents of hexosamines, amino acid and protein of A1 fractions (density 1.71 g/ml) from animal A were determined. The glucosamine/galactosamine ratios were higher than for the corresponding extracts, but still tended to increase with depth of the cartilage. The layer from the cartilage–bone junction, however, again had a lower ratio (Table 2). The relative content of glucosamine was much

higher than that observed from bovine nasal and tracheal cartilage (Hascall & Heinegård, 1974), indicating a higher relative content of keratan sulphate, corroborating data from other articular cartilages (Inerot *et al.*, 1978). The amino acid compositions of the A1 fractions were very similar (Table 3). Protein contents of the fractions were determined as the sum of the amino acids. The ratio of hexosamines to protein was calculated (Table 2). Unfortunately the amount of material recovered from the A1 fraction from the top section was not sufficient for further analysis. The keratan sulphate/protein ratio (determined as glucosamine/protein) increased somewhat with distance from the cartilage surface. The chondroitin sulphate/protein ratio (determined as galactosamine/protein) was comparatively low in the A1 fraction recovered from 40 to 240 μm . The A1 fraction from the section 240–440 μm had a higher relative chondroitin sulphate content than any of the other fractions. The chondroitin sulphate/protein ratio gradually decreased with increasing depth of the cartilage. The sample near the cartilage–bone junction had a higher relative content of galactosamine. In summary, the composition of the A1 fractions recovered from sections at 240–1040 μm showed that the relative contents of chondroitin sulphate decreased, the contents of protein increased and the contents of keratan sulphate remained relatively constant. The decreased chondroitin sulphate content was not due to decreasing size of the chains, as discussed below. The 40–240 μm -section A1 fraction contained the least chondroitin sulphate and probably also less keratan sulphate than the other fractions analysed.

Gel chromatography

The size of the proteoglycan monomers from the various layers from animal A was determined by Sepharose-2B chromatography of the reduced and alkylated A1 fractions. The uronic acid (glycosaminoglycan) tracings of the chromatograms (Fig. 1) showed that the size distribution of the proteoglycans in the top fraction analysed (40–240 μm) differed, in that these molecules were on the average considerably smaller than those of the deeper layers. The proteoglycan monomers recovered from the layer 240–440 μm were on average somewhat larger than any of the others. The tracings indicate that the average size of the proteoglycan monomers decreases (see Table 4), in the samples from 240 and down to 1040 μm from the cartilage surface. All the samples showed approximately the same polydispersity, except for the much less polydisperse sample from the surface layer (40–240 μm). The gel-chromatographic data are consistent with changes in the composition of the proteoglycan

Fig. 1. Sepharose CL 2B chromatograms of proteoglycan monomers (reduced and alkylated A1 fractions from animal A)

The column (0.3 cm $\times$ 140 cm) was eluted with 0.5 M-sodium acetate, pH 7.0, in 300 μl fractions. The numbers indicate depth of cartilage layers from the surface. Abbreviations: V_0 , void volume; V_t , total column volume.

Table 4. *Relative proportion of proteoglycans in fractions 27–36 and 37–51 in the chromatograms in Fig. 1*

Distance from articular surface (μm)	Uronic acid (% of total in chromatogram)	
	Fraction 27–36	Fraction 37–51
40–240	0	100
240–440	32	68
440–640	39	61
640–840	21	79
840–1040	25	75
1040–1240	35	65

monomers discussed above. The top fraction analysed differs markedly, whereas the proteoglycans from the other sections show a tendency to a continuous change in the various parameters analysed. In order to determine the size of the proteoglycan monomers from the surface layer (0–40 μm) and to characterize the change in size of the proteoglycans in the uppermost 440 μm sections, the reduced and alkylated A1 fractions from the layers 0–40, 40–120, 120–200, 200–280, 280–360, 360–440 and 440–1240 μm (animal B) were separately chromatographed on Sepharose 2B. Because of the small amount of sample available, the procedure for analysis had to be altered. Instead of monitoring proteoglycans by uronic acid analysis, their A_{206} was continuously measured in column effluents, as discussed in the Materials and methods section. The chromatograms (Fig. 2) reveal that the proteoglycans in the uppermost 200 μm are distinctly smaller than those of the deeper layers and that the proteoglycans in the most superficial layer are of similar nature to those in the nearest layers.

It was considered that the changing size of the proteoglycan monomer may be due to changes in the size of the chondroitin sulphate chains. Therefore the size distribution of the chondroitin sulphate chains was determined by chromatography on Sephacryl S-200 of papain digests of the A1 fractions (animal A) (Fig. 3). The chromatograms, however, show that the size distribution of the chondroitin sulphate chains recovered from all the layers was very similar. Therefore other compositional parameters of the proteoglycan monomers were responsible for the variations observed.

The proteoglycan variability may be due to a changing proportion of aggregating and non-aggregating proteoglycans (Heinegård & Hascall, 1979). Therefore the A1 fractions (animal A) were chromatographed on Sepharose 2B after preincubation with hyaluronate to ensure that all proteoglycan monomers capable of interacting with hyaluronic acid would chromatograph in the void volume. The chromatograms (Fig. 4) indicate that

Fig. 2. *Sepharose 2B chromatograms of proteoglycan monomers (reduced and alkylated A1 fractions from animal B)*

The column (0.5 cm $\times$ 140 cm) was eluted with 0.25 M- Na_2SO_4 in 740 μl fractions. The A_{206} of the effluent was monitored with a LKB Uvicord S instrument. The numbers indicate depth of cartilage layers from the surface. The total-volume peak contains guanidinium chloride and dithiothreitol from reduction.

Fig. 3. *Sephacryl S-200* chromatograms of papain-digested A1 fractions from the layers indicated from animal A

The column (0.3 cm × 140 cm) was eluted with 0.5 M-sodium acetate, pH 7.0, in 300 μl fractions. The numbers indicate depth of cartilage layers from the surface.

Fig. 4. *Sepharose CL 2B* chromatograms of A1 fractions from the different layers from animal A

Before chromatography the samples were incubated with 1% (w/w) of high-molecular-weight hyaluronic acid (Healon). The column (0.3 cm × 140 cm) was eluted with 0.5 M-sodium acetate, pH 7.0, in 300 μl fractions. The numbers indicate depth of cartilage layers from the surface.

Fig. 5. Agarose/polyacrylamide-gel electrophoresis of reduced and alkylated A1 fractions from different layers.

The spectrophotometric scans at 520 nm are shown. The front marker (Bromophenol Blue) is indicated by an un-numbered arrow in each scan. The migration rate relative to that of Bromophenol Blue for the two or three components is indicated above the respective peaks. The numbers in μm indicate depth of cartilage layers from the surface.

the relative contents of aggregating proteoglycans did not change from the layers at 240–1040 μm . The top layer analysed (40–240 μm) contained only molecules capable of interacting with hyaluronic acid. The sample prepared from the proliferating zone near the cartilage–bone junction, on the other hand, contained a somewhat higher proportion of non-aggregating proteoglycans, possibly owing to changes in the cartilage matrix during cell proliferation and subsequent calcification.

Agarose/polyacrylamide-gel electrophoresis

On agarose/polyacrylamide-gel electrophoresis, cartilage proteoglycans migrate as two main components (Roughley & Mason, 1976). The scans of the agarose/polyacrylamide gel of the proteoglycans in the most superficial layer (40–240 μm) (Fig. 5) showed the two components. The scans of the preparations from the other sections showed only the two major bands. The proportion of these two bands changed so that the slower-migrating proteoglycan were somewhat more enriched with increasing depth of the cartilage (Fig. 5). The relative migration rate also decreased for both components in the deeper layers (840–1240 μm). These data indicate that there is more than one type of proteoglycan present in the articular cartilage. The smaller is enriched in the superficial layers.

General discussion

The data presented indicate that the proteoglycans isolated from the layers 0–200 μm differed from proteoglycans of deeper layers. They had a different composition, were of smaller size and all of them aggregated with hyaluronic acid. It should be pointed out, however, that there was an overlap in the chromatographic behaviour (Figs. 2 and 3). Therefore it is possible that the type of proteoglycans present in the most superficial layers (0–200 μm) may well have been present among the proteoglycans from the other layers, but then as one of several components. A small-molecular-size proteoglycan has previously been demonstrated in associative extracts of calf articular cartilage (Lipshitz *et al.*, 1976). The alterations in proteoglycan composition observed with increasing depth from 240 to 1040 μm indicate that the proteoglycan structure changes gradually from molecules containing a large chondroitin sulphate-rich region to molecules containing a smaller proportion of this region. It appears that in the intermediate sections of the cartilage the same large proportion of the molecules contained the hyaluronic acid-binding region and the keratan sulphate-rich region. The molecules found in the layer near the cartilage–bone junction had a different composition and contained a much larger proportion of non-aggregating proteo-

glycans. It is possible that some degradation of these molecules may have occurred *in vivo* and may be caused by proteinases released from the bone. The data presented indicate that the changes in proteoglycan structure with increasing age reported previously (Inerot *et al.*, 1978) are not due to losses of superficial layers of the articular cartilage. In comparison with proteoglycans isolated from nasal or tracheal cartilage of bulls of the same age, the articular-cartilage molecules are considerably smaller and have much higher content of keratan sulphate, corroborating previous data (for references see Muir, 1979). The molecules from the three tissues show essentially the same ability to interact with hyaluronic acid and form aggregates (Hascall & Heinegård, 1974; Heinegård & Hascall, 1979). These observations are consistent with a model where the hyaluronic acid-binding region and the keratan sulphate-rich region are constant, but the chondroitin sulphate-rich region varies (Heinegård, 1977).

It should be stressed that each set of data shown in the present paper were obtained from one punch biopsy from one animal. Therefore inter-individual variability does not obscure structural differences between layers. Other sets of chromatograms obtained with samples from another animal were essentially the same, indicating that the differences observed were not unique for one animal.

Grants were obtained from the Swedish Medical Research Council (13P-5739 and 13X-05668), Alfred Österlunds stiftelse, Kocks Stiftelser, Swedish Insurance Companies Fund for Medical Research, Konung Gustav V:s 80-års fond and the Medical and Odontological Faculties, University of Lund.

References

- Axelsson, I. & Heinegård, D. (1975) *Biochem. J.* **145**, 491–500
- Harris, E. D., Jr., Parker, H. G., Radin, E. L. & Krane, S. M. (1972) *Arthritis Rheum.* **15**, 497–503
- Hascall, V. C. & Heinegård, D. (1974) *J. Biol. Chem.* **249**, 4232–4241
- Hascall, V. C. & Heinegård, D. (1979) in *Glycoconjugate Research* (Gregory, J. & Jeanloz, R., eds.), vol. 1, pp. 341–374, Academic Press, New York
- Heinegård, D. (1973) *Chem. Scr.* **4**, 199–201
- Heinegård, D. (1977) *J. Biol. Chem.* **252**, 1980–1989
- Heinegård, D. & Axelsson, I. (1977) *J. Biol. Chem.* **252**, 1971–1979
- Heinegård, D. K. & Hascall, V. C. (1979) *J. Biol. Chem.* **254**, 927–934
- Inerot, S., Heinegård, D., Audell, L. & Olsson, S. E. (1978) *Biochem. J.* **169**, 143–156.
- Kempson, G. E. (1975) in *Dynamics of Connective Tissue Macromolecules* (Burleigh, P. M. C. & Poole, A. R., eds.), pp. 277–305, North-Holland Publishing Co., Amsterdam and Oxford
- Kempson, G. E., Spivey, C. J., Swanson, S. A. V. & Freeman, M. A. R. (1971) *J. Biomech.* **4**, 597–609
- Lipshitz, H., Etheredge, R. & Glimcher, M. (1975) *J. Bone Jt. Surg. Am. Vol.* **57**, 527–534
- Lipshitz, H., Etheredge, R. & Glimcher, M. (1976) *J. Bone Jt. Surg. Am. Vol.* **58**, 1149–1153
- Maroudas, A. (1979) in *Adult Articular Cartilage* (Freeman, M. A. R., ed.), 2nd edn., pp. 215–226, Pitman Medical Publishing Co., Tunbridge Wells
- Maroudas, A., Muir, H. & Wingham, J. (1969) *Biochim. Biophys. Acta*, **177**, 492–500
- Maroudas, A., Evans, H. & Almeida, L. (1973) *Ann. Rheum. Dis.* **32**, 1–9
- McDevitt, C. A. & Muir, H. (1971) *Anal. Biochem.* **44**, 612–622
- Muir, H. (1979) in *Adult Articular Cartilage* (Freeman, M. A. R., ed.), 2nd edn., pp. 145–214, Pitman Medical Publishing Co., Tunbridge Wells
- Oegema, T. R., Hascall, V. C. & Dziewiatkowski, D. D. (1975) *J. Biol. Chem.* **250**, 6151–6159
- Roughley, P. J. & Mason, R. J. (1976) *Biochem. J.* **157**, 357–367
- Scott, J. E. (1975) *Philos. Trans. R. Soc. London Ser. B*, **271**, 235–242
- Stegemann, H. & Stalder, K. (1967) *Clin. Chim. Acta* **18**, 267–273
- Stockwell, R. A. & Scott, J. E. (1967) *Nature (London)* **215**, 1376–1378