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A Comparative Study of the Proteoglycan of Growth Cartilage of Normal and Rachitic Chicks

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1. Proteoglycan was isolated from growth cartilage of normal and rachitic chicks. 2. The proteoglycan from normal cartilage showed differences in chemical composition and physical properties from a comparable fraction isolated from bovine nasal cartilage. 3. The proteoglycan from rachitic-chick cartilage was of smaller size than its normal counterpart, though of similar average chemical composition. 4. Differences between proteoglycan from normal and rachitic cartilages can be explained in terms of limited proteolytic cleavage.

During skeletal development, bone grows in length by a process involving a growth plate containing a temporary cartilage tissue; this process is termed endochondral ossification (Vaughan, 1975). Proliferating chondrocytes first lay down a cartilage matrix that consists primarily of collagen, proteoglycan and water. Near the mineralizing front these cells hypertrophy and their longitudinal septa calcify. The calcified cartilaginous matrix is resorbed and is replaced by a bone organic matrix that undergoes calcification. This matrix consists mainly of collagen, of a different constitutional type from the cartilage collagen (Toole *et al.*, 1972). The proteoglycan characteristic of cartilaginous tissues is absent from this new matrix, but small amounts of a chondroitin sulphate–sialoprotein complex (Herring, 1972) are present.

Vitamin D deficiency results in a disorder in the process of bone formation. Growth cartilage continues to be laid down, but there is a defect in the transition to calcified bone. As a consequence the growth plate becomes abnormally wide and the cells within this enlarged plate do not hypertrophy (Bisaz *et al.*, 1975). This disorder in bone formation was once thought to be a consequence of the disturbance in calcium and phosphate metabolism secondary to vitamin D deficiency, but there is increasing evidence for a direct, though as yet undefined, role of the vitamin in bone formation.

In order to devise therapeutic means for correcting abnormalities in the rate of bone formation or in the mechanisms by which bone is formed, it is necessary to have a much more extensive understanding of the biochemistry of this complex process and its physiological control. Useful information may be gained by a detailed examination of the structure, function and metabolism of the individual components of the tissue, and how they become altered by induced disorders of metabolism. Relatively little is known about the structure of the proteoglycan of growth cartilage isolated by techniques that result in its minimal degradation. This paper reports studies of the proteoglycan of the growth cartilage of chicks and the changes that occur in the vitamin D-deficient state. Besides providing an excellent model for studying growth-cartilage components in the normal and rachitic states, study of the chick cartilage has enabled us to compare the composition, structure and properties of various components with those in normal mammalian cartilage.

Methods

Chemicals were of analytical grade or the best grade commercially available.

Source of cartilage

Ranger cockerels (1 day old; Ross Poultry, Woodhall Spa, Lincs., U.K.) were maintained on a vitamin D-deficient diet (Barnes *et al.*, 1973) in a room lit solely by tungsten light. Normal chickens were given an oral dose of 200i.u. (5μg) of cholecalciferol [Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K.] in 0.5ml of arachis oil once every week. At 5 weeks the chickens were killed with chloroform and the legs dissected. Each tibia was cleaned of adhering muscle and periosteum, and its proximal and distal ends were split longitudinally to expose the cartilage growth plates. In rachitic animals the proximal growth plate was as much as 5–10mm wide, whereas in the vitamin D-replete animals it was about 3mm wide. The cartilage growth plate was dissected out by a procedure similar to that described by Toole *et al.* (1972) and the tissue immediately frozen for storage at −20°C.

Isolation of proteoglycan

Pooled growth cartilage was pulverized in a freezer mill (Spex Industries, Metuchen, NJ, U.S.A.) cooled with liquid N_2. A 2.0g portion of cartilage was extracted with 40ml of 4M-guanidinium chloride containing 0.05M-sodium acetate, pH5.0, and 0.4ml each of iodoacetic acid (100mM, in propan-2-ol), phenylmethane sulphonyl fluoride (100mM, in propan-2-ol) and EDTA (disodium salt; 100mM aqueous solution). The mixture was stirred continuously for 48h at 5°C and then centrifuged (MSE 65, 8×50 rotor) at 20000$g_{av.}$ ($r_{av.}$ 7.4cm) for 1h at 5°C. The supernatant was dialysed overnight at 5°C against 100ml of 0.05M-sodium acetate, pH6.8, containing 10ml of 100mM-EDTA. Overnight dialysis was repeated and CsCl added to raise the density of the solution to 1.70g/ml (Sajdera & Hascall, 1969). The solution was then centrifuged (MSE 65, 8×50 rotor) at 90000$g_{av.}$ ($r_{av.}$ 7.4cm) at 5°C for 65h.

The gel of protein that formed at the top of each tube was removed and the remaining solution fractionated. The fractions (2ml) were assayed for density, hexuronic acid content and A_{280}. Fractions of density greater than 1.72g/ml (A1), those of density between 1.72 and 1.62g/ml (A2), and those of density less than 1.62g/ml (A3) were each pooled. Nomenclature for density-gradient fractions is as described by Heinegård (1972). The gel and preparations A2 and A3 were dialysed exhaustively against water at 4°C, converted into their sodium salts, and then freeze-dried. Guanidinium chloride was added to preparation A1 to a final concentration of 4.0M, and the density adjusted to 1.5g/ml by the addition of CsCl. This solution was then centrifuged at 90000g as described above and fractionated according to density. Fractions of density greater than 1.56g/ml (A1D1), those of density between 1.56 and 1.46g/ml (A1D2), and those of density less than 1.46g/ml (A1D3) were each pooled. The material from each preparation was then isolated as its freeze-dried sodium salt.

Agarose/polyacrylamide-gel electrophoresis

Cylindrical gels containing 0.6% (w/v) agarose and 1.2% (w/v) acrylamide were prepared by a modification of the method of McDevitt & Muir (1971). Sample preparation and electrophoresis conditions were as described by Roughley & Barrett (1977). Gels were stained overnight at 20°C with Toluidine Blue (0.02% in 0.1M-acetic acid), then destained at 35°C with 3% (v/v) acetic acid.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed in slab gels containing a 7–15% (w/v) polyacrylamide gradient by using a sulphate/borate discontinuous buffer system described by Neville (1971). Sample preparation was as described by Roughley & Mason (1976), and the electrophoresis was performed at 20°C with a current of 40mA/slab. Slabs were stained at 55°C for 1h with Coomassie Brilliant Blue (0.1% in methanol/acetic acid/water, 5:2:3, by vol.), then destained at the same temperature with methanol/formic acid/water (30:1:69, by vol.).

Viscosity measurements

Proteoglycan (2mg/ml) and hyaluronic acid (1mg/ml; Miles Laboratories, Stoke Poges, Bucks., U.K.) were dissolved in 0.2M-sodium acetate, pH5.5. The flow rates for the samples and buffer were recorded at 25°C in a U-tube viscometer (buffer flow time 28.1s; Poulten, Selfe and Lee, Wickford, Essex, U.K.). For investigations of aggregation, hyaluronic acid (10μl) was added to the proteoglycan (0.5ml) in the viscometer and the flow time recorded after a constant value had been attained.

Column chromatography

Samples (10mg in 5ml of 0.2M-sodium acetate buffer, pH5.5) were applied to a column of Sepharose 2B (Pharmacia Products, London W5 5SS, U.K.; column bed dimensions 2.5cm×37cm, volume 180ml) and eluted with the same buffer at a flow rate of 20ml/h. Fractions (5ml) were collected and portions of these (0.5ml) taken for hexuronic acid analysis. On the basis of the hexuronic acid-elution profile (see Fig. 4) the following fractions were pooled: 13, 14, 15 (I); 18, 19, 20 (II); 23, 24, 25 (III); 28, 29, 30 (IV).

Cetylpyridinium chloride precipitation of proteoglycan fraction

Proteoglycan was isolated from the pooled fractions obtained from Sepharose 2B chromatography by precipitation in the presence of 1% (w/v) cetylpyridinium chloride. This precipitate was purified and converted into the potassium salt as described by Roughley & Barrett (1977).

Analytical methods

Analysis for hexuronic acid was performed by the method of Bitter & Muir (1962) with glucuronolactone standards. Samples for hexosamine analysis were hydrolysed with 4.0M-HCl at 100°C for 8h, and those for amino acid analysis with 6.0M-HCl at 105°C for 20h under vacuum. The acid was removed by evaporation in a vacuum desiccator over pellets of

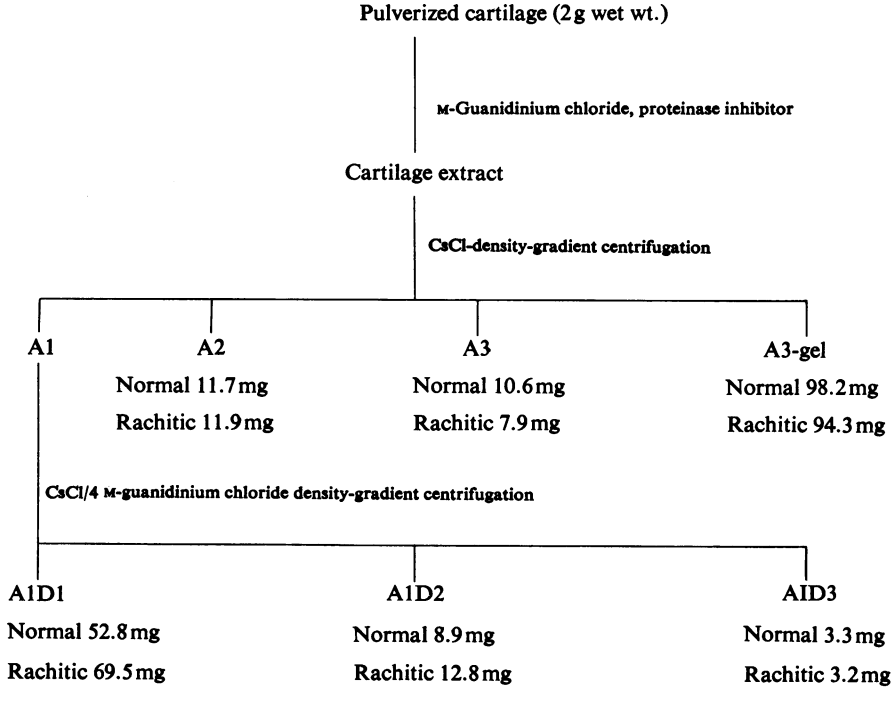
NaOH. Analysis for total glucosamine and galactosamine was done by a modification of the Elson–Morgan procedure (Neuhaus & Letzring, 1957). The hexosamine molar ratio was determined by separating the amino sugars on a Locarte amino acid analyser by elution with a citrate buffer, pH5.25. For amino acid analysis separation was performed with a three-buffer system (citrate, pH3.25 and 4.25, and borate, pH9.0). Analysis for sulphate was performed by the turbidimetric method of Dodgson (1961) after hydrolysis for 18h at 100°C with 4% (w/v) trichloroacetic acid. The reagent was prepared with gelatin from BDH, Poole, Dorset, U.K. Sialic acid was determined after hydrolysis at 80°C for 1h with 0.05M-H_2SO_4 by the method of Aminoff (1961) with *N*-acetylneuraminic acid standards.

Results

Yield and composition of preparations

The yields of preparations A2, A3, A3-gel and A1D3 from normal and rachitic-chick cartilage were similar (see Scheme 1) when expressed in relation to the wet weight of the cartilage. In contrast, the amount of preparations A1D1 and A1D2 were higher in the rachitic-chick cartilage than in the normal cartilage. The rachitic-chick cartilage also contained relatively large amounts of collagen; the residue remaining after extraction of the rachitic-chick cartilage contained 12.2mg of hydroxyproline, more than double that found in the residue from the normal tissue (5.8mg of hydroxyproline).

Preparation A1D1 from normal and rachitic chicks (A1D1-N and A1D1-R respectively) contained similar amounts of hexuronic acid, hexosamine and sulphate (Table 2) and these amounts were similar to those found in bovine nasal cartilage (A1D1-BNC). The two chick preparations also had a similar amino acid composition, but this differed from that of the bovine proteoglycan, most noticeably in the content of threonine (approx. 50% higher in the chick proteoglycan) and of lysine (approx. 50% higher in the bovine proteoglycan). The total peptide content of preparation A1D1-R was lower than that of preparation A1D1-N, and both were lower than that of the bovine proteoglycan. The chick proteoglycans also showed a slightly higher galactosamine/glucosamine molar ratio than that of the bovine material, the ratio being greatest in the rachitic-chick cartilage.



Scheme 1. *Isolation of proteoglycan from chick growth cartilage*
The yields quoted are weights after freeze-drying and air-equilibration. See the Methods section for details of experimental procedure.

Table 1. *Electrophoretic mobility of chick proteoglycan on agarose/polyacrylamide-gel electrophoresis*
Mobilities are quoted as R_x values relative to Bromophenol Blue. See the Methods section for details of experimental procedure: *denotes intense staining. Bovine nasal-cartilage preparation A1D1 has R_x values of 0.75 and 0.83.

Preparation	Cartilage from ...	R_x value: Normal chicks	R_x value: Rachitic chicks
A1D1		0.75*, 0.88, 0.98	0.76, 0.89, 0.99*
A1D2		1.00, 1.07	1.00, 1.08
A1D3		No staining	No staining
A2		0.74	0.90, 0.97
A3		No staining	No staining

Table 2. *Composition of proteoglycan from growth cartilage from normal (A1D1-N) and rachitic (A1D1-R) chicks and from bovine nasal cartilage (A1D1-BNC)*
Values for hexuronic acid, hexosamine, sulphate and peptide are expressed as a percentage of the freeze-dried air-equilibrated weights. Amino acid values are residues per 1000 amino acid residues. Abbreviation: N.D., not determined. Peptide represents the sum of the weights of the amino acids.

	A1D1-N	A1D1-R	A1D1-BNC
Hexuronic acid (%)	24.0	24.1	23.4
Hexosamine (%)	31.6	32.5	N.D.
Sulphate (%)	10.0	9.6	11.0
Galactosamine/glucosamine molar ratio	13.6	14.7	13.0
Peptide (%)	5.2	4.6	6.0
Asp	56	52	64
Thr	91	89	61
Ser	140	144	131
Glu	154	152	140
Pro	97	102	126
Gly	114	122	126
Ala	84	79	73
Val	59	63	59
Met	5	4	3
Ile	48	48	34
Leu	61	64	80
Tyr	18	16	19
Phe	36	35	36
Lys	11	9	19
Arg	26	23	28

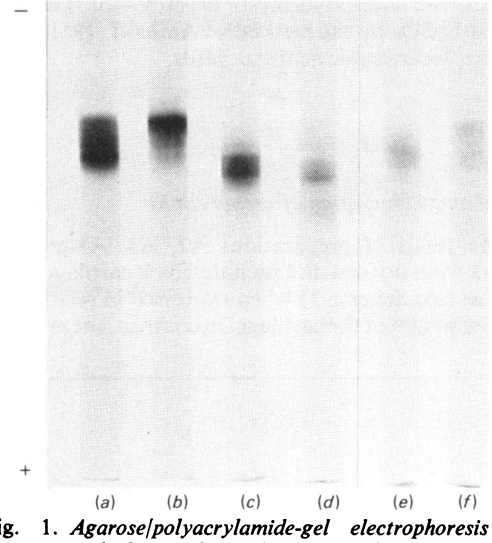


Fig. 1. *Agarose/polyacrylamide-gel electrophoresis of chick growth-cartilage proteoglycan*
The following preparations were studied: (*a*) A1D1-R; (*b*) A1D1-N; (*c*) A1D2-R; (*d*) A1D2-N; (*e*) A2-R; (*f*) A2-N. For details of experimental procedure see the Methods section.

Agarose/polyacrylamide-gel electrophoresis

Preparations A1D1-R and A1D1-N each show three zones of Toluidine Blue staining (Fig. 1) of comparable electrophoretic mobility (see Table 1). In preparation A1D1-N the major component was of lowest mobility, whereas in preparation A1D1-R the major component was of greatest mobility. The least mobile component has an electrophoretic mobility comparable with that of the least mobile of the two components of preparation A1D1-BNC. Preparation A1D2 showed two more mobile components in both samples from the rachitic and the normal birds, and preparation A2 contained the major components of preparation A1D1. No Toluidine Blue-staining components were observed in preparation A1D3 or A3.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

No differences in protein composition were found between preparations from rachitic and normal chicks when they were subjected to electrophoresis on sodium dodecyl sulphate/polyacrylamide gels (Fig. 2). Preparation A1D3 contained one major component, of mol.wt. 50000, which is comparable in size with the larger of the link proteins present in bovine nasal cartilage (mol.wts. 50000 and 46000). Preparation A3 contained two major components, of

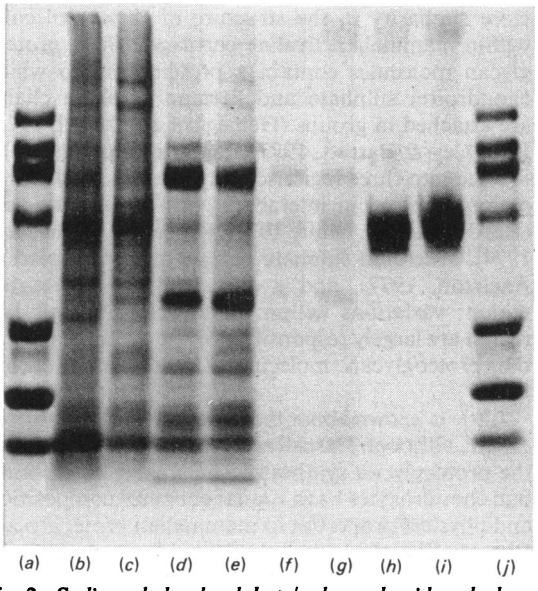


Fig. 2. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of chick growth-cartilage proteins*
(*a*) Standards; (*b*) A3-gel-N; (*c*) A3-gel-R; (*d*) A3-N; (*e*) A3-R; (*f*) A2-N; (*g*) A2-R; (*h*) A1D3-N; (*i*) A1D3-R; (*j*) standards. Standards used, with their molecular weights, were: phosphorylase A (100000), human transferrin (78000), bovine serum albumin (68000), carbonic anhydrase (29000), immunoglobulin G chains (50000 and 25000), soya-bean trypsin inhibitor (21000), cytochrome *c* (12750). See the Methods section for details of experimental procedure.

mol.wts. 68000 and 32000, as well as many minor components of low molecular weight. The 68000-mol.wt. component was assumed to be serum albumin. The major components were also present in preparation A2. A feature of the first associative ultracentrifugation was the appearance of a peak of 280nm-absorbing material near the middle of the gradient, with the maximum absorption occurring at a point corresponding to a density of 1.68g/ml in tissue extracts from both the rachitic and normal birds. This peak was contained in preparation A2, but electrophoresis provided no evidence of proteins other than small amounts of the major components of preparation A3. Examination of the u.v. spectrum of the A2 preparations revealed an absorption maximum around 257nm. The peak most probably relates to the presence of nucleic acids which would have a buoyant density in that region as well as a similar u.v. spectrum. The A3-gel preparation showed many components with molecular weights ranging from 10000 to over 100000, with the most prominent bands corresponding to mol.wts. 50000 and 14000.

Table 3. *Viscosity of proteoglycan in the absence and presence of hyaluronic acid*
The proteoglycan solutions were 2mg/ml in 0.2M-sodium acetate, pH5.5. Hyaluronic acid (10μl, 1mg/ml in the same buffer) was added to 500μl of proteoglycan solution. Measurements of relative viscosity were made at 25°C.

Fraction	Relative viscosity: No hyaluronic acid	Relative viscosity: +hyaluronic acid
Buffer	1.00	1.02
A1D1-BNC	1.49	1.86
A1D1-N	1.52	1.60
A1D1-R	1.34	1.44

Relative-viscosity studies

The relative viscosity of preparation A1D1-BNC was lower than that of preparation A1D1-N, but higher than that of preparation A1D1-R (Table 3). Addition of hyaluronic acid (0.1%) to buffer alone resulted in only a slight increase in the relative viscosity of the solution, whereas when an equivalent amount of hyaluronic acid was added to a solution of preparation A1D1-BNC there was a large increase in the relative viscosity of the solution. When hyaluronic acid was added to solutions of preparations A1D1-R or A1D1-N the increase in viscosity was considerably less, being approx. 25% of that observed with the bovine proteoglycan solution.

Sepharose 2B chromatography

On Sepharose 2B chromatography both preparations A1D1-R and A1D1-N gave components over a continuous range of sizes, eluted between V_0 and V_t. However, preparation A1D1-N showed a $K_{av.}$ of 0, whereas preparation A1D1-R had a $K_{av.}$ of 0.45. In comparison, preparation A1D1-BNC had an intermediate $K_{av.}$ of 0.27 (Roughley, 1977).

Four pools (each containing three fractions of total volume 15ml and representing proteoglycan molecules of various hydrodynamic sizes) were collected from the effluent of each column run (see Fig. 3). Proteoglycan was isolated from each pool by precipitation with cetylpyridinium chloride, as described in the Methods section, and was examined by agarose/polyacrylamide-gel electrophoresis and hexosamine analysis. Equivalent fractions from preparations A1D1-N and A1D1-R showed a similar electrophoretic mobility (results not shown), though the fraction of smallest size (IV) from rachitic-chick cartilage appeared to be of greater heterogeneity than its normal counterpart. The electrophoretic mobility of the fractions increased with decreasing hydrodynamic size of the constituent molecules, but

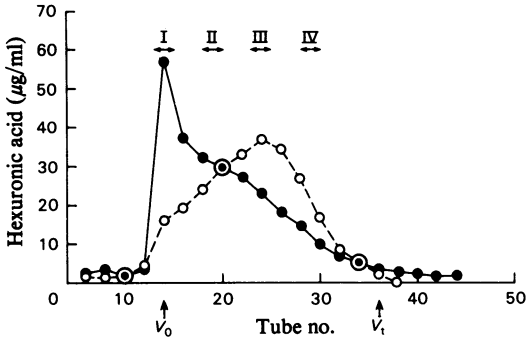


Fig. 3. *Chromatography of chick growth-cartilage proteoglycan on Sepharose 2B*

For details of experimental procedure see the Methods section. I, II, III and IV refer to fractions subjected to cetylpyridinium chloride precipitation for further investigation. ●, Normal chicks; ○, rachitic chicks.

Table 4. *Galactosamine/glucosamine molar ratios for chick A1D1 proteoglycan fractions isolated by Sepharose 2B chromatography*

Proteoglycan molecules were precipitated in the presence of 1% cetylpyridinium chloride as described in the Methods section.

	Galactosamine/glucosamine molar ratio	
Fraction	Normal	Rachitic
I	9.6	8.7
II	15.2	17.1
III	21.2	22.9
IV	20.5	12.0

with none of the fractions was there evidence of resolution into distinct bands (cf. Fig. 1). The galactosamine/glucosamine molar ratio of the fractions from normal cartilage increased with decreasing hydrodynamic size, with the intact proteoglycan possessing an intermediate value. The fractions from the rachitic-chick cartilage showed a similar, trend, except for that of smallest size, which had a ratio less than that of the intact proteoglycan (Table 4).

Discussion

Studies of proteoglycan isolated from bovine nasal cartilage (Hascall & Sajdera, 1970; Mayes *et al.*, 1973), pig laryngeal cartilage (Hardingham & Muir, 1974), bovine articular cartilage (Rosenberg *et al.*, 1976), dog epiphyseal cartilage (Lohmander & Hjerpe, 1975) and human epiphyseal cartilage (Stanescu & Maroteaux, 1975) provide evidence of a close similarity in the structure of these molecules within mammalian hyaline cartilages. Such proteoglycan molecules contain a protein core to which chondroitin sulphate and keratan sulphate chains are attached in groups (Heinegård & Hascall, 1974; Roughley & Barrett, 1977). The core protein can be divided into three regions: a glycosaminoglycan-free region involved in interaction with hyaluronic acid (Hardingham & Muir, 1974; Hascall & Heinegård, 1974), a keratan sulphate-rich region (Heinegård & Axelsson, 1977), and a chondroitin sulphate-rich region: variations within the structure of this last region are largely responsible for the heterogeneity of the proteoglycan molecules (Hardingham *et al.*, 1976).

Little is known about the structure of avian proteoglycan, although Hascall *et al.* (1976) have shown that the proteoglycan synthesized *in vitro* by chick limb-bud chondrocytes has a similar chemical composition and physical properties to mammalian proteoglycan. Our studies of the proteoglycan from 5-week-old normal chick growth cartilage indicate that its chemical composition is similar to that of mammalian proteoglycan with respect to hexuronic acid, sulphate and total hexosamine, though there is a slight decrease in the glucosamine and protein contents. However, these molecules differ in a number of respects from mammalian proteoglycan. There is a distinct difference between the amino acid composition of the core protein and that of bovine nasal-cartilage proteoglycan, this being most marked in the contents of lysine and threonine: a high threonine content was also recorded by Hascall *et al.* (1976) for the chick limb-bud proteoglycan. Mammalian proteoglycan shows two zones of Toluidine Blue staining on agarose/polyacrylamide-gel electrophoresis (Roughley & Mason, 1976; Stanescu *et al.*, 1977), whereas the chick proteoglycan shows one major band, of similar mobility to the less-mobile band of bovine nasal cartilage, and two more mobile minor bands. On Sepharose 2B chromatography the chick proteoglycan is eluted mainly at the void volume, whereas that of bovine nasal cartilage is more retarded (Roughley, 1977). It therefore appears that the proteoglycan of normal chick epiphyseal cartilage is of larger average hydrodynamic size than its mammalian counterpart. Further, unlike the proteoglycan from bovine nasal cartilage, that from the chick shows little increase in viscosity when hyaluronic acid is added, implying that either the hyaluronic acid-binding region is absent from many of the molecules or it is only partially functional. This would lead us to expect that within the cartilage the molecules do not exist in the form of large aggregates, and this view is supported by our observation of relatively large amounts of what appears to be link protein in preparation A3 after ultracentrifugation under associative conditions. In contrast, Hascall *et*

al. (1976) showed that about 75% of the A1-proteoglycan from chick limb-bud chondrocytes was in an aggregated form. It is unlikely that our result has arisen from degradation of the proteoglycan during the milling procedure, as there is no evidence for small uronic acid-containing fragments on Sepharose 2B chromatography (cf. Lohmander & Hjerpe, 1975).

The A1D1 proteoglycan of rachitic-chick cartilage is of similar chemical composition to that of normal cartilage. The agarose/polyacrylamide-gel-electrophoresis patterns of these preparations both show three zones of Toluidine Blue staining. However, that of greatest mobility is most prominent in the preparation from rachitic chicks, whereas the most prominent component in the normal chick preparation corresponds to the zone of lowest mobility. The hexuronic acid profile on Sepharose 2B implies that proteoglycan from the rachitic chick is of smaller average hydrodynamic size than that from the normal tissue, and this is supported by the relative-viscosity measurements.

By resolution of the A1D1 preparation from normal cartilage on Sepharose 2B we were able to obtain subpopulations of proteoglycan molecules whose galactosamine/glucosamine molar ratio increased with decreasing hydrodynamic size. This implies that the smaller proteoglycan molecules contain a greater proportion of chondroitin sulphate relative to keratan sulphate, which is in direct contrast with the trend observed with the proteoglycan from bovine nasal cartilage (Rosenberg *et al.*, 1976) and pig laryngeal cartilage (Hardingham *et al.*, 1976). Thus if the glycosaminoglycan chain length is constant, it appears that chick growth-cartilage proteoglycan does not contain a keratan sulphate-rich region in the position postulated by Heinegård & Axelsson (1977).

The corresponding subpopulations from the rachitic-chick A1D1 preparation showed a similar trend with decreasing hydrodynamic size, except for the fraction containing the smallest molecules, which showed a marked decrease in its galactosamine/glucosamine molar ratio. This difference was also reflected in the greater heterogeneity observed in this subpopulation on agarose/polyacrylamide-gel electrophoresis, and can be explained by the presence of small proteoglycan molecules containing relatively large amounts of keratan sulphate within the A1D1 preparation isolated from rachitic-chick cartilage.

The differences that we have observed in the chemical and physical properties of the rachitic-chick cartilage proteoglycan can be explained in terms of either limited proteolytic degradation or an altered pattern of synthesis by the chondrocytes. Without further experimental work it is not possible to determine which is correct. However, circumstantial evidence makes the hypothesis of limited proteolysis more plausible. In rickets the large proteoglycan molecules characteristic of normal cartilage may be synthesized initially, but then later undergo proteolysis to produce two major fragments, the smaller of these having a greater keratan sulphate/chondroitin sulphate ratio than the larger. The absence of small glycosaminoglycan–peptide from the rachitic tissue suggests that this process is fairly specific and takes place at only a limited number of sites. Metalloproteinases, active at neutral pH, are known to be present within the extracellular matrix of cartilage (Sapolsky *et al.*, 1976), and such enzymes cleave proteoglycan into large fragments (P. J. Roughley & A. Sellers, unpublished work). Further, in the rachitic state, growth cartilage continues to be laid down but is not calcified or resorbed, and the width of the growth plate is consequently increased. Thus many of the proteoglycan molecules that we have studied in the rachitic-chick cartilage are likely to have been present for a greater time than their normal counterparts, and accumulation in this manner would enhance the probability of cleavage by extracellular enzymes.

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