Age-related Changes in the Structure of the Proteoglycan Subunits from Human Articular Cartilage*

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The proteoglycan of articular cartilage provides this tissue with its elastic properties, and the structure of these molecules plays a major role in determining the resilience of the tissue to compression. In the present paper we have shown that the structure of the proteoglycan subunits in human articular cartilage changes considerably between the fetus and the mature adult. These changes occur gradually and are essentially complete by the end of growth. With increasing age the following changes were most pronounced: 1) a decrease in the proteoglycan content of the cartilage, 2) a decrease in the size of the proteoglycan subunit, 3) an increase in keratan sulfate relative to chondroitin sulfate, 4) an increase in 6-sulfation relative to 4-sulfation along the chondroitin sulfate chains, 5) an increase in protein relative to glycosaminoglycan, and 6) a decrease in serine and glycine and an increase in arginine content of the core protein. The susceptibility of the proteoglycan to proteolytic degradation by pepsin also showed age-related variations, but papain always produced the same degradation products. Irrespective of age, the majority of the proteoglycan subunits possessed the ability to interact with hyaluronic acid. Thus, although large structural changes take place in the glycosaminoglycan attachment region of the proteoglycan, the hyaluronic acid-binding region would appear to be relatively invariable. Decreases in the concentration, size, and charge of the proteoglycan would be expected to lower the elastic properties of the older cartilage.

The two major functions of articular cartilage are to act as a shock absorber during weight bearing and as a bearing surface during motion (Mankin, 1974), and these properties are related to the structure of the extracellular matrix of the cartilage. There are two major macromolecular components of this matrix—collagen and proteoglycan. Collagen provides the tissue with its tensile strength, whereas the proteoglycan is responsible for the elastic properties of the cartilage (Kempson et al., 1976), such as its resilience to compression.

The proteoglycan is composed of large subunits of high anionic charge, whose size is further increased by their specific interaction with hyaluronic acid to form multimolecular aggregates (Hardingham and Muir, 1972). The proteoglycan subunits consist of a central protein core along which chondroitin sulfate and keratan sulfate chains are arranged (Heinegård and Axelsson, 1977). Sulfate and carboxyl groups present on the glycosaminoglycan chains are the major contributors to the high anionic charge of the molecule. In bovine nasal and porcine laryngeal cartilages the subunit has been shown to consist of two distinct functional regions (Heinegård and Hascall, 1974a; Hardingham et al., 1976). One terminus of the core protein, comprising about one third of its length, is devoid of glycosaminoglycan chains and is maintained in a specific globular conformation through disulfide bonds. This region is responsible for the specific interaction with hyaluronic acid. The remainder of the core protein contains the glycosaminoglycan chains which appear to be arranged in groups of variable number and composition (Heinegård and Hascall, 1974b; Roughley, 1977). The size of the proteoglycan aggregate is determined by the length of the hyaluronic acid chain and the steric hindrance between the glycosaminoglycans of adjacent proteoglycan subunits (Hardingham and Muir, 1973; Hascall and Heinegård, 1974b). A typical aggregate may contain in excess of 50 proteoglycan subunits on a single hyaluronic acid molecule. It is the large size of the proteoglycan and its high charge that are mainly responsible for its contribution to the elastic properties of cartilage. The large size helps immobilize the proteoglycan within the matrix, and the high charge gives rise to strong water-binding and electrostatic interactions (Hascall, 1977).

The contribution of the proteoglycan to cartilage function will be impaired by parameters that decrease its size or charge. For example, decreases in size may be brought about by the lack of a functional hyaluronic acid-binding region or the action of degradative enzymes, and decreases in charge may be brought about by the core proteins being less glycosylated or the glycosaminoglycan chains themselves being less sulfated or carboxylated. Further, variations in the concentration of the proteoglycan within the cartilage, or in the interaction between proteoglycan and collagen, will also be expected to affect cartilage elasticity. This paper describes the changes that take place in the structure of the proteoglycan subunit in human cartilage from fetal development to the mature adult and discusses the possible relationship of these changes to the functional properties of the cartilage.

**EXPERIMENTAL PROCEDURES**

**Materials**—Guaniinium chloride, cesium chloride, and toluidine blue were obtained from BDH Chemicals, Ltd. Phenylmethylsulfonyl fluoride, iodoacetic acid, papain, bacterial collagenase, and hyaluronic acid (from human umbilical cord) were from Sigma Chemical Co. Pepain was from Worthington Biochemical Corp. Chondroitinase ABC and its disaccharide degradation products of chondroitin sulfate were from Miles Laboratories, Inc. Acrylamide, N,N'-methylenebis-acrylamide, and Coomassie brilliant blue G250 were from Eastman Kodak Co. Thin layer chromatography plates were obtained from Mandel Scientific Co. Pepstatin was a gift from the United States-Japan Medical Science Program.

**Source of Tissue**—Human articular cartilage was obtained at autopsy, usually within 12 h of death. Individuals were chosen in whom...
there was no evidence of connective tissue involvement of malignancy, no joint damage due to trauma, and no evidence of articular cartilage erosion.

Adult material consisted of all the cartilage covering the subchondral bone of the distal femur. Cartilage was chosen that appeared macroscopically normal, and any small areas of local degeneration were discarded. Fetel (25 weeks' gestation) and newborn material consisted of tissue from the epiphyses of both the distal femur and the proximal tibia, with care being taken to exclude the growth plates. Ossific nuclei were dissected from the cartilage and discarded. In all cases, except the old adult cartilage, the specimens under investigation were from a single individual. The old adult cartilage consisted of pooled specimens from three persons of the same age.

Cartilage samples were stored at -20°C prior to extraction of the proteoglycan.

Extraction of Proteoglycan—Cartilage slices were extracted with 10 volumes of 4 M guanidinium chloride, 0.1 M sodium acetate, pH 6.0, containing 1 mM concentrations of each of EDTA, iodoacetic acid, and phenylmethylsulfonyl fluoride, and 5 μg/ml of pepstatin, as inhibitors of metallo, thiol, serine, and carboxyl proteinases. Extraction was for 70 h at 4°C. The extracts were filtered, and the residue was washed with the extraction medium. CsCl was added to the filtrate to give a density of 1.50 g/ml, and guanidinium chloride was added so that its concentration remained at 4 M. The extracts were centrifuged at 10°C for 48 h at 37,000 rpm (100,000 × g) in a Sorvall T8652 rotor. The gradient was fractionated and the fractions were assayed for density, uronic acid, and absorbance at 280 nm. The fractions were divided into three pools according to their density; pool D1 contained fractions having a density greater than 1.54 g/ml. Pool D2 contained fractions having a density between 1.54 and 1.46 g/ml, and pool D3 contained fractions having a density less than 1.46 g/ml. D1 is expected to contain most of the proteoglycan subunit content of the extract, D2 is expected to contain low density proteoglycan and hyaluronic acid, and D3 is expected to contain most of the protein content of the extract. The pooled material was dialyzed two times against water and then subjected to chromatography on Sepharose 4B or Sepharose 2B (Fig. 3).

Chondroitinase Degradation—Chondroitinase ABC digestion was performed by a method based on that of Hascall and Heinegard (1974a). The proteoglycan (D1) was digested in 0.1 M sodium acetate, 0.1 M Tris/HCl, pH 7.3, at a concentration of 8 mg/ml. Chondroitinase ABC (5 units/ml) was then added at a concentration of 10 μg/ml of D1, and the mixture was incubated at 4°C for 4 h. Then iodoacetic acid was added to a concentration of 15 μM, and the mixture was left at 25°C for a further 20 h. After dialysis against water and potassium acetate, the solution was exhaustively dialyzed against water and then freeze-dried. Under these conditions the aggregation of bovine nasal cartilage proteoglycan with hyaluronic acid was eliminated.

Agarose/Polyacrylamide Gel Electrophoresis—The proteoglycan (D1) and its proteolytic degradation products were subjected to electrophoresis in agarose/polyacrylamide gels composed of 0.6% agarose and 1.2% polyacrylamide (McDevitt and Muir, 1971). The sample (20 μl) for electrophoresis consisted of the proteoglycan (2 mg/ml in water), chondroitin sulfate (1 mg/ml in water), and 50% containing 1 ml containing 0.01 M sodium phosphate buffer (52:32:16) and then with butanol/acidic acid/1 M ammonium hydroxide (2:3:1). The degradation products were visualized by irradiation with UV light and eluted from the cellulose by incubation in 0.5 ml of water at 4°C for 16 h. 0.2 ml of the resulting solution was assayed for disaccharide content by the method of Koseki et al. (1973), and the total column volume was determined by the elution of glucuronic acid standards on a gel filtration column.

Polyacrylamide/SDS Gel Electrophoresis—The proteoglycan (D1) was subjected to electrophoresis in 10% polyacrylamide gels. Gel preparation, buffer systems, and electrophoresis conditions were essentially as described by Laemmli and Favre (1973). Samples of 20 μl, composed of D1 (2 mg/ml) and sample buffer in a ratio of 1:1, were loaded on the gel. Staining was performed at 25°C with 0.1% Coomassie brilliant blue G250 in methanol/acetic acid/water (5:2:3), and destaining was at 40°C with methanol/formic acid/water (50:1:6).
RESULTS

Extraction of Cartilage—In the fetal and newborn specimens, the cartilage under extraction consisted of all the tissue in the epiphysis above the growth plate. The cartilage was dissected free from ossific nuclei prior to extraction. In early fetal tissue the ossific nucleus was not observed, whereas in the newborn there was a well-defined ossification center. Juvenile cartilage consisted of the full thickness of tissue above the developing ossific nucleus, and the thickness of cartilage, therefore, decreased with the age of the child. In the adult, all the tissue above the subchondral bone was removed. Even though subchondral bone formation had ceased, it was observed that the thickness of the overlying cartilage still decreased with age. In all cases the tissue appeared to be histologically normal, with no visible signs of degeneration. In juvenile and fetal tissues, there was no obvious demarcation between the tissue destined to become the articular cartilage of the adult and the deeper tissue destined to be involved in ossification.

The percentage of uronic acid extracted from the cartilage decreased with the age of the person (Table I). Nearly 90% of the uronic acid could be extracted from the fetal cartilage, whereas less than 60% was extractable from adult cartilage. There was little change in the percentage of extractable uronic acid from the adult tissues. The amount of uronic acid remaining within the cartilage did not vary much between newborn and adult specimens, although it was lower in the fetal tissue. Thus, the total quantity of uronic acid-containing material present in the cartilage would appear to exhibit an overall decrease with age up to the end of growth and then remain relatively constant.

The susceptibility of the tissue residue to degradation by either collagenase or papain varied considerably with age—the older specimens being much more resistant to proteolytic degradation. This trend occurred as a gradual change through the entire age group under investigation and is probably related to the degree of cross-linking of the collagen fibrils. The fetal cartilage could be fully solubilized by either enzyme within 4 h, whereas with the adult material a 48-h combined incubation period was necessary. In all cases, except the fetal tissue, solubilization of the tissue residue resulted in a homogeneous solution. However, with the fetal material the clear solution also contained a gel-like phase that was easily separable by centrifugation. This material was mainly protein in composition and did not contain hydroxyproline or significantly high alanine. The material does not, therefore, appear to be derived from collagen or elastin, but is fairly resistant to proteolysis by papain or collagenase.

The tissue components extracted with 4 M guanidinium chloride were subjected to CsCl density gradient centrifugation under dissociative conditions and divided into three fractions (Table II). The fraction of highest density, D1, is expected to contain most of the proteoglycan subunits, and the yield of this fraction decreased with the age of the specimen. In the newborn this fraction accounted for over 6% of the wet weight of the cartilage, whereas in the adult the value was less than 2%. The fraction of lowest density, D3, which is expected to be mainly protein, showed no marked age-related trend. It accounted for about 3.5% of the wet weight of the cartilage in the fetal, neonatal, and adult cartilages, but there was slightly lower occurrence in the juvenile cartilages. The intermediate fraction, D2, which is expected to contain mainly low density proteoglycans and hyaluronic acid showed a slight trend toward increasing yield with age. It accounted for about 0.5% of the wet tissue weight in the fetus and about 1% in the adult. These trends, therefore, suggest that the extractable protein shows little variation with age, whereas the extractable proteoglycan decreases significantly as age increases up to 20 years, and the proportion of proteoglycan present as low density, protein-rich material increases with the age of the specimen.

Chemical Composition of the Proteoglycan Subunit—The chemical composition of the proteoglycan subunit (D1) (Table III) showed age-related trends. The protein concentration of D1 doubled between fetal life and maturity: D1 from the fetus contains about 6% protein, whereas D1 from the 58-year-old contains about 12% protein. This is in agreement with the observed trend with age toward a greater proportion of lower buoyant density proteoglycans. In contrast, the amount of uronic acid present in D1 decreased with age from a value of about 24% at birth to about 16% at maturity. This change is mirrored by a decrease in galactosamine content with age,

![Table I](attachment:image1.png)

![Table II](attachment:image2.png)

![Table III](attachment:image3.png)

III) was estimated by summation of the amino acid residues (excluding cysteine/cystine), but is not corrected for any destruction of the residues that may occur on hydrolysis. The ratio of chondroitin sulfate to keratan sulfate (Table IV) was calculated from the ratio of galactosamine to glucosamine, as these residues are mutually exclusive components of the repeating disaccharide sequence of their respective glycosaminoglycans.
indicating that total chondroitin sulfate at maturity is about
two-thirds that at birth. The amount of glucosamine in D1
increased with age from about 2% at birth to about 7% at
maturity. Thus, while the chondroitin sulfate content of D1
decreases with age, the keratan sulfate content would appear
to increase about 4-fold between birth and maturity.

The changes outlined above, when expressed relative to one
another (Table IV), give a clearer indication of the magnitude
of the compositional changes occurring in the D1 preparations.
It is reasonable to assume that the molar ratio of galactosa-
mine to glucosamine reflects the ratio of chondroitin  sulfate
and keratan sulfate. The 4-sulfated disaccharide was not detected
at any age, and the nonsulfated disaccharide was always present
in polyacrylamide gels, there was no evidence of proteins
containing the proteoglycan.

Another change encountered during aging was in the position
of sulfation along the chondroitin sulfate chains. By
degradation of D1 with chondroitinase ABC, and subsequent
separation of the resulting disaccharides by thin layer chro-
matography, it is possible to evaluate the content of nonsul-
fated, 4-sulfated, 6-sulfated, and 4,6-disulfated galactosamine
residues. The 4,6-disulfated disaccharide was not detected
at any age, and the nonsulfated disaccharide was always present
as a minor component ranging from about 10% in the fetus
to 5% at maturity. The 4-sulfated disaccharide and the 6-sulfated
disaccharide showed considerable variation with age (Table
IV). In the fetus the two sulfation positions were present in
equimolar amounts, whereas during growth the proportion of
6-sulfation increased until by maturity 6-sulfation was about
25 times more abundant than 4-sulfation. The nonsulfated
disaccharide characteristic of hyaluronic acid could not be
detected at any age in the D1 preparations. Thus, by this
technique there does not appear to be significant contamina-
tion of the D1 preparations with hyaluronic acid.

The amino acid analyses of the D1 preparations (Table V)
are typical of hyaline cartilage proteoglycan, with serine,
glutamate, proline, and glycine being the predominant amino
acids. There were several age-related changes in amino acid
abundance, of which the most marked were a decrease in
serine and glycine and an increase in arginine and tyrosine
with age. There were also smaller increases in lysine, phenyl-
alanine, methionine, valine, alanine, and proline with age, and
decreases in glutamate and histidine. There was little change
in the abundance of aspartate, threonine, leucine, and isoleu-
cine. At no age could hydroxyproline or hydroxylysine be
detected by amino acid analysis, indicating that collagen was
not present as a contaminant of the D1 preparations. Further,
when the D1 preparations were subjected to electrophoresis
in polyacrylamide gels, there was no evidence of proteins
entering the gel, as expected if extraneous protein were con-
taminating the proteoglycan.

The changes in amino acid composition occurred gradually
between birth and 20 years, as was observed previously for
the changes in protein, uronic acid, and hexosamine composi-
tion, and for the variation in sulfate position. Thus, it appears
that the composition of the proteoglycan subunits present in
D1 shows little variation between 25 weeks’ gestation and
birth, then undergoes significant changes in protein and gly-
cosaminoglycan content during the first two decades of life,
and finally shows little further variation during aging of the
adult.

Size and Aggregation of the Proteoglycan Subunit—The
proteoglycan subunits present in D1 showed a trend toward
decreased viscosity with age (Table VI), which is consistent
with a decrease in the average hydrodynamic size of the
subunit. This is borne out by the elution profiles from Seph-
aro 2B (Fig. 1), which indicated that the proteoglycan
subunits of D1 were more retarded in the older specimens. In
the fetal and newborn preparations the elution position was
very close to the void volume ($K_v = 0.04$) and the partition
coefficient increased during development of the juvenile. This
was most marked up to about 12 years ($K_v = 0.23$), and after

TABLE IV

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<tr>
<th>Age (years)</th>
<th>Galactosamine/glucosamine</th>
<th>Chondroitin-6-sulfate/chondroitin-4-sulfate</th>
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TABLE V

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TABLE VI

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<th>Specific viscosity</th>
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In this age the increase was slower (at 58 years, $K_{av} = 0.29$), although the polydispersity of the included peak did continue to increase in the adult.

In no case was uronic acid detected in the region of the total column volume, indicating that small chondroitin sulfate-containing fragments typical of proteolytic degradation of the proteoglycan were absent from the preparations. In the fetal, neonatal, and juvenile specimens no distinct void volume peak was observed. However, in the adult specimens void volume peaks were observed, and this was most prominent in the oldest specimen. After reduction and alkylation of D1 from the 58 year old the distinct void volume peak disappeared, indicating that this sample probably contained traces of hyaluronic acid. Similarly, the elevated specific viscosity of this preparation was more analogous with values for the other adult specimens after reduction and alkylation. Reduction and alkylation of the younger specimens did not alter their elution profiles on Sepharose 2B, indicating that the proteoglycan subunits were themselves large enough to elute near the column void volume, and that hyaluronic acid was not a contaminant of these D1 preparations. The concentration of hyaluronic acid in articular cartilage is thought to increase during aging (Thonar et al., 1978; Bayliss and Ali, 1978b), and this increased abundance may account for its trace detection in the oldest D1 preparation.

Addition of hyaluronic acid to D1 in a weight ratio of 1:50, respectively, caused a large increase in specific viscosity (Table VII). The resulting viscosity showed little variation between the newborn and adult preparations, although with the fetal preparation it was of a slightly lower value. On Sepharose 2B, the majority of the uronic acid-containing material from all the hyaluronic acid/D1 mixtures was eluted at the void volume (Fig. 1). Thus, all the D1 preparations were capable of aggregating with hyaluronic acid, and the extent of this aggregation appeared to be independent of the age of the specimen.

The electrophoretic mobility of D1 in agarose/polyacrylamide gels also showed variations with age (Fig. 2). In the fetal and newborn specimens a single zone of staining was observed ($R_s = 0.54$). In the young juvenile two zones of slightly greater electrophoretic mobility ($R_s = 0.57, 0.62$) were present, both being stained to a similar intensity. In older age groups three zones became apparent ($R_s = 0.59, 0.64, 0.69$), with the one of intermediate mobility being stained with the greatest intensity. The trend toward increased mobility with age is compatible with the decreased size of the D1 preparations observed on Sepharose 2B. However, the appearance of multiple electrophoretic zones in the older preparations indicates that more than one polydisperse component may be present, in comparison to the fetal preparations where a single polydisperse component may occur.

**Proteolytic Degradation of the Proteoglycan Subunits**

The D1 preparations were subjected to degradation by papain. This proteinase is capable of cleaving adjacent to every chondroitin sulfate chain in the proteoglycan subunit from bovine nasal cartilage (Roughley, 1978). When the degradation products were subjected to electrophoresis in agarose/polyacrylamide gels, a single band was observed and, irrespective of the age of the preparation, its mobility was identical to that of chondroitin sulfate prepared from bovine nasal cartilage ($R_s = 1.0$). Thus, all the preparations were degraded in a similar manner by papain, suggesting that the peptide sequences surrounding the chondroitin sulfate chains do not vary markedly with age. When the degradation products were subjected to chromatography on Sepharose 4B (Fig. 3), a single, symmetrical, included peak was obtained with no material being eluted near the void volume or total column volume. The partition coefficient of this peak exhibited only a small variation with age ($R_s$ ranges from 0.62 to 0.68) indicating that the chondroitin sulfate chain length may decrease slightly from the fetus to the adult.
In contrast, the degradation by pepsin yielded much larger fragments. This proteinase has been shown to produce multichain glycosaminoglycan peptides containing both chondroitin sulfate and keratan sulfate from bovine nasal cartilage proteoglycan (Roughley, 1978). Large degradation products were obtained for all the D1 preparations with uronic acid eluting predominantly near the void volume on Sepharose 4B (Fig. 3). The profile showed little change with the age of the specimen, and material was never eluted near the total column volume. Electrophoresis in agarose/polyacrylamide gels (Fig. 4) showed that the degradation products produced by pepsin were always less mobile than chondroitin sulfate, irrespective of the age of the specimen. The pattern of staining obtained with toluidine blue indicated that the types of degradation products were dependent on the age of the specimen. The fetal and neonatal preparations showed three zones of staining (Res = 0.68, 0.78, 0.83) with the more mobile zone being slightly more intensely stained. The 2 year old showed four zones of staining (Res = 0.64, 0.69, 0.79, 0.84) with the two zones of least mobility being more strongly stained. Older specimens also showed four bands (e.g. at 58 years, Res = 0.68, 0.74, 0.84, 0.88), although their mobilities were gradually increasing with age. The staining intensity of the two most mobile bands decreased with age, and in the adult these components were barely detectable. The change in degradation product produced by pepsin between fetal/neonatal and older specimens occurred at the same time as the D1 samples themselves were undergoing changes with respect to electrophoretic mobility (Fig. 2). This may indicate that the arrangement of glycosaminoglycan chains, or even the structure of the core protein, changes significantly during early development and thereafter only minor structural changes occur.

**DISCUSSION**

The proteoglycans of hyaline cartilage are heterogeneous with respect to size and composition. Such heterogeneity may be ascribed to either synthetic or degradative changes. Synthetic heterogeneity may include variations in core protein structure (Hopwood and Robinson, 1975) and variations in the type and position of glycosylation (Hoffman et al., 1974), whereas degradative heterogeneity is mainly the result of proteolytic activity within the glycosaminoglycan attachment region of the core protein (Hardingham et al., 1979). It is possible that the extracted proteoglycan from human articular cartilage contains all types of heterogeneity, although the existence of genetically distinct core proteins is still a debatable point. The contribution from each type of heterogeneity will probably depend on the metabolic state of the tissue and hence the age of the specimen.

The trends observed in the amino acid composition of the proteoglycan at different ages show some analogies with those described by Rosenberg et al. (1976) for bovine articular cartilage proteoglycans of different sizes. Rosenberg et al. ascribed their observations to the enrichment of the hyaluronic acid-binding region in proteoglycan preparations of smaller size. Such an interpretation could also account for the variations in amino acid composition observed with the decreasing size of the human articular cartilage proteoglycan as the age of the specimen increases. This hypothesis requires that all proteoglycans possess an invariable hyaluronic acid-binding region (Heinegård and Hascall, 1974a; Hardingham et al., 1976), and that heterogeneity arises by variations within the glycosaminoglycan attachment region (Heinegård, 1977).

The existence of distinct proteoglycan populations has been suggested by a number of workers (Hopwood and Robinson, 1975; Stanescu et al., 1977). If discrete proteoglycan populations are due to distinct core proteins, one would expect that their proteolytic degradation would yield different degradation products if the proteinase in use is capable of only limited proteolytic cleavages. Such a proteinase is pepsin, which produced only glycosaminoglycan clusters from the human proteoglycan, irrespective of age. However, the pattern of degradation products observed on agarose/polyacrylamide gel electrophoresis did vary with age. Further, the changes in pattern for the pepsin degradation products and the intact proteoglycans occurred at the same ages. These data would be consistent with the occurrence of different core proteins in the older specimens, with each core possessing characteristic sites for glycosylation and proteolytic cleavage.

It is interesting to note that at no age is the amino acid composition of the human proteoglycan identical to that of the proteoglycan from bovine nasal cartilage (Heinegård, 1977), bovine articular cartilage (Rosenberg et al., 1976), porcine laryngeal cartilage (Hardingham and Muir, 1974), or chick growth plate cartilage (Dickson and Roughley, 1978), even though the different species may possess similar glycosaminoglycan compositions. Similarly, at no age is the pattern of degradation products produced by pepsin identical for human, bovine, and chick proteoglycans (Roughley, 1978; Roughley and Dickson, 1979). This suggests that, even though the proteoglycans from different species may possess similar glycosaminoglycan compositions, there may be variations in core protein sequences between the species. Small variations in amino acid sequences may not result in marked differences in amino acid composition but may significantly affect the site of proteolytic cleavage for an enzyme such as pepsin. Larger variations in core protein sequences may also affect the arrangement of the glycosaminoglycan chains along the core proteins and give rise to differing structural features.

Irrespective of age, single chondroitin sulfate chains are also the only degradation products produced by the action of papain on the human proteoglycans. A similar result was observed for the proteoglycan of bovine cartilage (Roughley, 1978) and of chick cartilage (Roughley and Dickson, 1979). Thus, irrespective of age or species, papain always produced the same degradation product suggesting that the amino acid sequences along which it cleaves may be common to all species. The amino acid composition of all cartilage proteogly-
cans is rich in serine, glycine, glutamate, proline, and leucine, and Isemura and Ikenaka (1975) have shown that these amino acids form the linkage sequences for chondroitin sulfate chains in bovine tracheal cartilage. The observed action of papain can therefore be explained if the linkage sequences are conserved between species and papain cleaves along these sequences. Thus, in any species, age variations in amino acid composition would be expected to occur in the more remote sequences between the chondroitin sulfate chains.

The major structural variations observed in the human proteoglycan subunits during development and aging were with respect to size and glycosaminoglycan composition. Similar observations have been reported in canine articular cartilage by Bayliss et al. (1978) and in human articular cartilage by Bayliss and Ali (1978a, 1978b) and Venn (1978). Age-dependent changes in sulfation position have also been reported in human cartilages by Matthews and Glagov (1966) and by Mason and Wusteman (1970). The variations in sulfation position begins at birth and continues through early development but is essentially complete by the end of growth, as observed by Bjelle (1975). This decrease in 4/6-sulfation with age cannot be extrapolated back to early fetal life where tissue differentiation may result in the occurrence of the opposite trend (Mathews and Glagov, 1966; Robinson and Dorfman, 1969). The proportions of chondroitin sulfate and keratan sulfate are also changing most rapidly prior to the end of growth. Even in the fetus small amounts of glucosamine could be detected, although keratan sulfate was not detected histochemically in human cartilages prior to birth (Mason, 1971). Changes in proteoglycan structure, other than 4/6-sulfation and keratan sulfate/chondroitin sulfate ratio, were also shown to be essentially complete by the end of growth.

The changes described in proteoglycan structure may not take place uniformly over the whole cartilage, but may be subject to variations depending on anatomical site and depth. Sweet et al. (1976, 1978) have shown that proteoglycan size and buoyant density are greater in the superficial regions compared to the deeper regions of immature calf articular cartilage. Further, keratan sulfate content is greatest in regions of maximum contact on the femoral condyles (Sweet et al., 1977). Although Sweet et al. observed no change in chondroitin sulfate chain length with depth, Jones and Lemp perg (1978) reported that chondroitin sulfate molecular weight was decreased with depth in old human femoral head cartilage. It thus seems likely that the changes in proteoglycan structure outlined in this paper occur first in the deeper zones of the cartilage and at the sites of maximum weight bearing. It is possible that these changes are initiated not only by aging but also by a response of the chondrocytes to increased load during growth.

Before concluding, it should be pointed out that this paper investigates the proteoglycan from only a limited number of individuals, and as such we do not claim that all individuals of a particular age will possess identical proteoglycan compositions. Indeed, it is most probable that the age-related changes described in this paper will occur at slightly different rates in each individual. Further, the changes in proteoglycan structure refer only to the DI fraction of the extractable proteoglycan, and at present there is no data available to determine whether the nonextractable proteoglycan behaves in a similar manner. Although the generalized age-related trends were inferred from a relatively small data base, we have no reason to believe that these conclusions are not valid for all normal cartilage. Neither further unpublished work by ourselves nor the data reported from the literature are in disagreement with these conclusions.

It is finally worthwhile to indicate what effect the changes in proteoglycan structure during development and aging might have on the functional properties of the articular cartilage. Up to the end of growth there is a marked decrease in total proteoglycan content, in the size of the monomer, and in the amionic charge on the monomer. These changes will probably result in decreased cartilage elasticity during growth. After growth there is little further change in proteoglycan structure or content. However, the cartilage was observed to thin with further maturation, and this would result in less matrix being available for an equivalent weight in the old adult relative to the young adult. In turn, this may result in the chondrocytes of the older tissue being subjected to increased loads, and such increased loads (Mitchell and Cruess, 1977) may make the tissue more susceptible to degenerative changes with increased age.

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Proteoglycans from Human Articular Cartilage 223
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Age-related changes in the structure of the proteoglycan subunits from human articular cartilage.

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